**Charles University in Prague**

Faculty of Science

Department of Cell Biology


# Identification of key regulators of gene expression in mammalian oocyte and embryo

**Mgr. Denisa Jansova**

PhD Thesis

Supervisor: Andrej Susor, Ph.D.

Institute of Animal Physiology and Genetics, ASCR v. v. i.

Prague, 2017

**Prohlášení:**
Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

I hereby declare that I wrote this thesis independently, using the cited literature. This work or a substantial part of it has not been submitted elsewhere to obtain any other academic degree.

Prague, 2017 Denisa Jansova

## Acknowledgement

First of all, I am deeply grateful to my supervisor: Andrej Susor, who gave me the opportunity to join the lab and supported me throughout my research, for his guidance, helpful ideas and considerable time spent discussing my project. I would like to thank technician assistants Jaroslava Supolikova and Marketa Hancova. Many thanks belong to recent members of our department for creating a friendly working environment. Finally, I would like to thank my family members and close friends for encouragement and patience. This study was supported by grant GAUK 227026 Grant Agency of the Charles university to D.J, by GACR 13-12291S to A.S and by Institutional Research Concept RVO67985904.

# Souhrn práce

Savčí oocyt je vysoce diferencovaná buňka, ze které po oplození vzniká embryo. Na konci růstové fáze se plně dorostlý oocyt stává transkripčně inaktivním. Během následujících fází vývoje oocytu, tedy během jeho zrání, oplození a následně během časného embryonálního vývoje, jsou využívány pouze transkripty nasyntetizované v růstové fázi oogeneze, které oocyt skladuje k pozdějšímu využití. Distribuce mRNA je úzce svázána s lokalizací a funkcí proteinu, který kóduje. Tohoto mechanismu regulace genové exprese využívají různé typy buněk. Zatím ovšem není mnoho známo o lokalizaci mRNA molekul a jejich translaci v savčím oocytu a časném embryu. Cílem této práce bylo detekovat celkový transkriptom a komponenty translačního aparátu v savčím oocytu a dvoubuněčném embryu a odhalit mechanismus regulující translaci, která je důležitá pro správný vývoj oocytu a embrya. Ukázali jsme, že jádro myšího i lidského oocytu obsahuje RNA molekuly a RNA vazebné proteiny. Po rozpadu jaderné membrány dochází k translaci v oblasti chromosómů. Předpokládáme, že molekuly mRNA, které jsou přítomny v jádře oocytu, jsou následně po rozpadu jaderné membrány zpřístupněny translačnímu aparátu a dochází k jejich translaci a vzniku proteinů, které jsou důležité pro správný průběh meiotického zrání oocytu a časného embryonálního vývoje. Tato translace je řízena skrze mTOR–eIF4F dráhu, která je aktivovaná po rozpadu jaderné membrány. Tyto výsledky nasvědčují tomu, že asymterická lokalizace RNA určuje načasování a lokalizaci translace v savčích oocytech.



# Abstract

Mammalian oocyte is a highly differentiated cell which gives rise to an embryo after fertilization. Importantly, fully-grown oocytes become transcriptionally inactive at the end of the growth phase. During following stages of development, i. e. meiotic maturation of the oocyte and early embryonic development, only transcripts previously synthesized and stored are used. The tight correlation between mRNA distribution and subsequent protein localization and function provides a mechanism of spatial and temporal regulation of gene expression used by various cell types. However, not much is known about mRNA localization and translation in the mammalian oocyte and early embryo. The aim of my thesis was to determine the localization of transcripts and components of translational machinery in the mammalian oocyte and embryo and to uncover the mechanisms of spatiotemporal regulation of translation as a prerequisite for correct oocyte and embryo development. We have shown that nuclei of both mouse and human oocytes contain RNA molecules and RNA binding proteins. Following the nuclear envelope breakdown (NEBD), translational hot-spots occur in the area surrounding the nuclear region. We suppose that mRNAs previously retained in the nucleus are released to the cytoplasm during NEBD and their subsequent translation gives rise to proteins essential for further meiotic progression and embryonic development. We have further shown that protein synthesis in the mentioned hot-spots is regulated via mTOR–eIF4F pathway, which is activated after NEBD. Taken together our results support the notion that asymmetric localization of RNA determines the timing and localization of translation in mammalian oocyte.

# Obsah

1 Introduction ..... 6
1.1 Maturation of mammalian oocyte ..... 6
1.2 Transcriptome of oocyte and embryo ..... 7
1.3 Regulation of gene expression and RNA storage ..... 8
1.4 Localization of RNAs in the right time and right the place ..... 10
1.5 Detection of RNA by in situ hybridization methods ..... 11
1.6 Translation in oocyte and embryo ..... 13
1.7 Regulation of cap dependent translation via mTOR pathway in oocyte and embryo ..... 14
2 Aims of the thesis ..... 18
3 Comments on publications ..... 19
3.1 Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway ..... 20
3.2 Translation in the mammalian oocyte in space and time ..... 21
3.3 Regulation of 4E-BP1 activity in the mammalian oocyte ..... 22
3.4 Transcriptome and translation in mammalian oocyte and embryo ..... 23
4 Conclusions of thesis ..... 40
5 Discussion ..... 41
6 References ..... 44
7 Abbreviations ..... 56
8 Curriculum vitae ..... 59
9 Supplementary material ..... 61

# 1 Introduction

## 1.1 Maturation of mammalian oocyte

The meiotic cell cycle, which is comprised of two consecutive M-phases, is crucial for the production of haploid germ cells (Marston and Amon, 2004). Oocyte maturation is a process, during which the oocyte attains the competence to be fertilized and undergone embryogenesis. When fully-grown oocytes are removed from their follicles, they can resume meiosis spontaneously under *in vitro* conditions. Mammalian oocytes are arrested in the dictyate stage of the first meiotic prophase, the so-called germinal vesicle (GV) stage. Meiotic maturation consists of two interlinked processes: cytoplasmic and nuclear maturation. Nuclear maturation covers chromatin changes from nuclear envelope breakdown (NEBD) through a reductive division called meiosis I (MI), followed by extrusion of the first polar body which leads to the formation of an oocyte arrested at metaphase of the second meiosis (MII) (Fulka et al., 1995) (Figure 1). Cytoplasmic

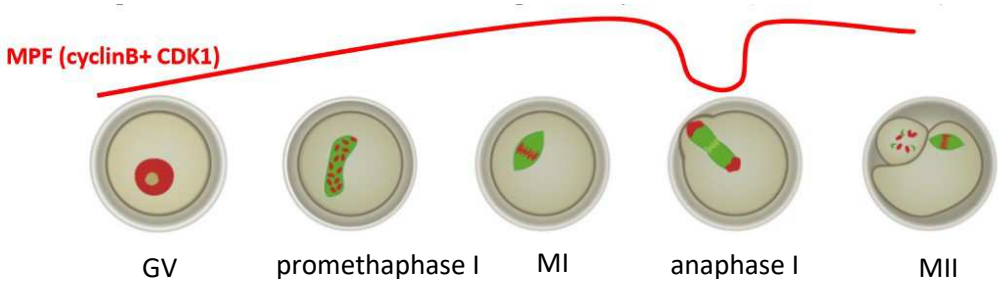



**Figure 1 – Maturation of an oocyte**

After hormonal stimulation or spontaneously in *in vitro* conditions meiotic resumption occurs: after germinal vesicle breakdown the spindle is formed (green), the oocyte reaches metaphase I and proceeds to anaphase I, the bivalents are separated and 1st polar body is extruded. MPF activity (cyclin B + CDK1; red line) grows and is maintained high until anaphase I, when the drop in cyclin B levels enable MI exit. (Adapted from archive of Alexandra Mayer)

maturation includes cytoplasmic changes, redistribution of organelles, cytoskeleton dynamics and molecular maturation (Ferreira et al., 2009). Trafficking of cytoplasmic organelles during maturation occurs through the actions of cytoskeletal microfilaments and microtubules and repositioning of the organelles depends on the needs of the cell during each stage of development. Upon NEBD, mitochondria move away from the perinuclear region, the Golgi apparatus is fragmented and aggregated in the central part of the oocyte, the endoplasmic reticulum (ER) localizes in cortical regions (FitzHarris et al., 2007) and microtubules are condensed around the chromosomes while microfilaments are densely accumulated in the subcortical region of the oocytes. At metaphase I, the Golgi apparatus is further fragmented and dispersed throughout the oocyte, the ER is asymmetrically distributed in the mature egg and cortical granules migrate towards the cortical cytoplasm and arrest in the cortex, while the large aggregates of intermediate filaments disperse into multiple small spots. In addition, microtubules are observed as fully organized meiotic spindles. Mitochondria in MI and MII oocytes become even more numerous and are dispersed in the ooplasm (Dumollard et al., 2006). At metaphase II, the first polar body is extruded, spindle is formed below the first polar body and intermediate filaments are evenly distributed throughout the cytoplasm (Brunet and Verlhac, 2011; Maro and Verlhac, 2002). Among the three types of cytoskeletal filaments, microtubules are more

directly involved in the processes of chromosome and organelle movement (Li et al., 2005; Sun and Schatten, 2006), and microfilaments are more directly involved in processes of chromosome migration, cortical spindle anchorage, polarity establishment and the first polar body emission during oocyte maturation (Azoury et al., 2009; Kim et al., 2000; Schuh and Ellenberg, 2008).

## 1.2 Transcriptome of oocyte and embryo

The gene expression in mammalian fully grown oocyte compared to somatic cell is regulated exclusively in the level of translation and utilization of stored RNAs (Curtis et al., 1995; Nothias et al., 1995). No transcriptional activity exists in fully grown oocyte and during maturation (De La Fuente et al., 2004). Unique strategies are set in place for the control of transcriptional silencing in pre-ovulatory oocytes. The duration of the period of maturation varies between mouse, rat, pig, bovine and human (Braude et al., 1988; Schultz, 1993). Inactivation of transcription in the oocyte depends on two different chromatin configurations, which seem to be important for the full meiotic and developmental competence. Chromatin in growing mouse oocytes is decondensed in configuration termed non-surrounded nucleolus (NSN, Figure 2) (Debey et al., 1993). In the second type when oocyte reaches fully grown state, the chromatin is highly condensed and surrounds the nucleolus (surrounded-nucleolus, SN, Figure 2). In addition, the transition into the SN configuration correlates with the timely progression of meiotic maturation (Debey et al., 1993) and with higher rates of blastocyst formation after *in vitro* fertilization of mouse oocytes (De La Fuente et al., 2004; Inoue et al., 2008; Zuccotti et al., 2002).

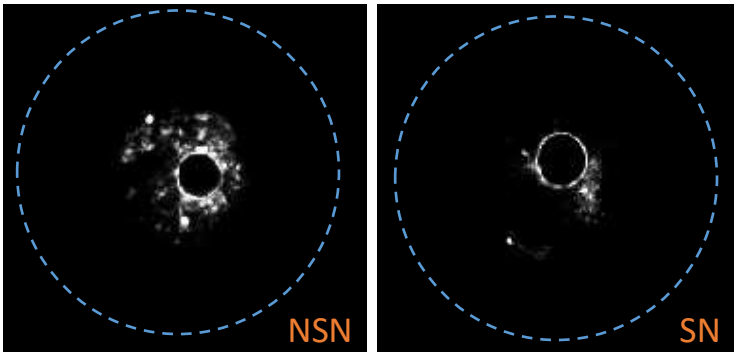



**Figure 2 – Mouse oocytes with two different types of chromatin organization**

(white DAPI; blue line indicates oocyte cortex), non-surrounded nucleolus and (NSN) surrounded nucleolus (SN)

Changes in mRNA abundance between GV-stage oocytes and their matured counterparts reflect the stability, utilization and degradation of the transcripts (Bachvarova, 1981; Curtis et al., 1995; Lin et al., 2009). Estimated 85 pg of mRNA is present in GV-stage of mouse oocyte, polyadenylated mRNA declines by about 50 pg during oocyte maturation. About half of it RNA undergoes deadenylation and the other half, about 30 % of the total mRNA, undergoes degradation (Bachvarova et al., 1985; Paynton et al., 1988). Cui et al., (2007) analyzed gene expression profiles of germinal vesicle (GV) and metaphase II (MII) stage oocyte. The genes that were up-regulated in GV oocytes were more likely to be involved in protein metabolism and modification, cell cycle, electron transport, fertilization or belong to the cytoskeletal protein family. The genes specifically upregulated in the MII oocytes

were more likely to be involved in DNA replication, amino acid metabolism or expression of G protein-coupled receptors and signaling molecules.

Fully grown and developmentally competent murine oocyte have been estimated to contain 200 times more RNA than a typical somatic cell (Sternlicht and Schultz, 1981). Only 15 % of all RNA produced by oocyte comprises heterogeneous RNA, 20 % 5S and tRNA and approximately 65 % comprises ribosomal RNA. Nevertheless the oocyte cytoplasm represents a rich source of messenger (Su et al., 2007) and noncoding RNAs (Karlic et al., 2017). The resources of transcripts accumulated in the egg during oogenesis support it during the whole oocyte maturation and even at the fisrt stages of early embryo development (Figure 3). The fate of oocyte mRNAs depends on association with different types of proteins that regulate the accessibility to initiation, degradation factors and ribosomes. In the mouse oocyte, new transcription period starts by minor gene activation of embryo during G2 in the zygote, the major activation occurs during the 2-cell stage (Schultz, 1993). Meiotic maturation triggers the degradation of maternal transcripts, which results in 90% decrease by the 2-cell stage embryo. Transcription from the newly formed zygotic genome, known as zygotic genome activation (ZGA) (Hamatani et al., 2004). Asymmetric maternal RNA sorting in early embryos might account for developmental polarity during the establishment and maintenance of the totipotency.

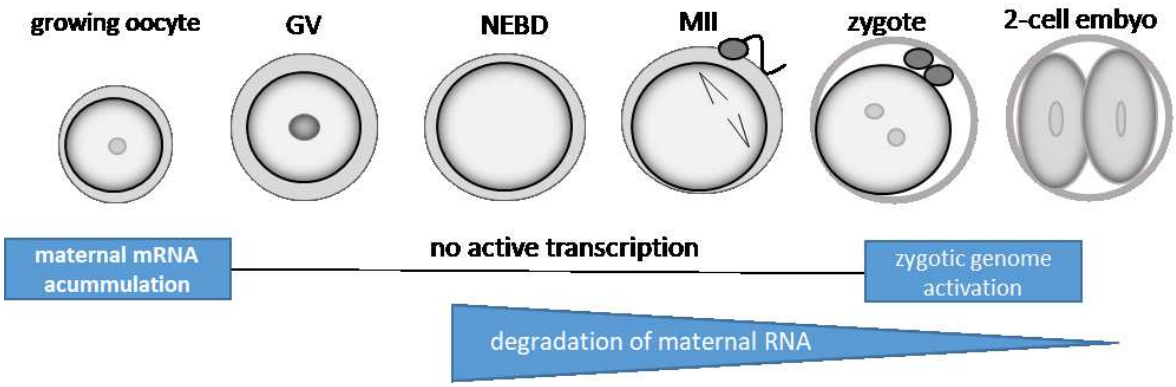



**Figure 3 – Scheme of summary transcription during maturation and early development**

Transcription drops substantially near or at the end of the growth phase. During meiotic maturation, which spans about 10 h (mouse), the oocyte enters M-phase, completes the first meiotic division MI, and then arrests at metaphase II. Transcription is not detectable during this time. Following fertilization (MII with sperm), weak promiscuous transcription can be detected during the first cell cycle (zygote), which spans about 18 h. The major activation of transcription occurs at 2-cell.

## 1.3 Regulation of gene expression and RNA storage

In the fully grown oocyte, which is transcriptionally inactive, gene expression is regulated post-transcriptionally. Stored transcripts are known as dormant mRNAs. In the right time during meiotic maturation specific maternal mRNAs are recruited to translation. The fate of different mRNAs is controlled via specific cis-motives (Vassalli and Stutz, 1995). Some transcripts are actively translated, while others are masked until their activation at respective developmental stage. Reversible cytoplasmic polyadenylation is the most common regulating mechanism of maternal RNA stability and activity of translation (Stutz et al., 1998). RNAs with long Poly(A) tails (average 100-200

nucleotides) are translationally active (Bachvarova et al., 1985; Lin et al., 2009). The reverse process used for regulation of translation is called cytoplasmic deadenylation (Huarte et al., 1992). On transcripts which contain less than 100 nucleotides of adenines RNA-binding proteins (RBPs) cannot bind to the mRNA, thus preventing their translation (Stebbins-Boaz et al., 1999). It seems that polyadenylation, deadenylation and degradation of RNAs in oocyte and embryo, correlate with translational efficiency. Deadenylation might lead to degradation of maternal transcripts which allows normal embryonic development (Paynton et al., 1988; Schultz, 1993).

Another possibility of degradation maternal RNA is elimination of mRNAs through the action of small silencing RNAs (Ghildiyal et al., 2010) or micro RNA (Hossain et al., 2012). A key function of miRNAs is to repress expression of their target genes through sequence complementation, which reduces the abundance of the target mRNAs and/or inhibits their translation (Bushati and Cohen, 2007).

The control role of translation relies on cis-regulating elements which are binding sites for trans-acting factors. The most common and studied cis-regulating RNA sequence is cytoplasmic polyadenylation element (CPE). CPE plays a specific role in cytoplasmic polyadenylation via RNA-binding proteins (RBPs) called CPE binding proteins (CPEBs). In mammals there are four identified members of this family: CPEB1-4 (Giangarrà et al., 2015). CPEB1 is a translational activator or repressor according to its phosphorylation state. The non-phosphorylated CPEB1 represses the translation initiation of CPE-containing mRNAs by recruiting other *trans*-acting factors such as Maskin and Pumilio (Stebbins-Boaz et al., 1999). On the contrary, the CPEB1 phosphorylation at S174 in *Xenopus* oocyte activates the polyadenylation of a CPE-containing mRNA by excluding the poly(A)-specific ribonuclease (PARN) from its 3′UTR (Kim and Richter, 2006).

CPEB1 has key role at onset of mammalian oocyte maturation (Komrskova et al., 2014). For instance, it was recently shown that CPEB1 activates the translation of *Dazl* mRNA (Chen et al., 2011) which in turn directs its own translation as well as that of an additional subset of mRNAs that are important for meiotic spindle assembly (e.g. *Bub1, Mad1, Mad2, Bub3, Cenpe*) (Eliscovich et al., 2008). CPEB1 in *Xenopus* oocyte stimulates translation of *Cpeb4* mRNA. In *Xenopus Leavis,* CPEB4 protein replace role of CPEB1 after meiosis I (Igea and Méndez, 2010). In somatic cell CPEB4 has role in cytokinesis during somatic cells mitosis (Novoa et al., 2010). However, the importance of the role of CPEB family – mediated translation in mammalian oocyte translation regulation is still not fully clear.

From the moment of creation of mRNA is "covered" by RBPs. As mentioned before, one of the most important regulators of translation are RBPs which might have alternative roles like splicing, export, localization, stabilization and degradation (Martínez-Salas et al., 2013). RBPs often interact with the untranslated region of mRNAs and associate with polyribosome, which are clusters of ribosomes bound to mRNA in act of translation (Rich et al., 1963). RBPs control translation efficiency of mRNAs by conventional cap dependent mechanism, which consists of an altered nucleotide at 5′-terminal 7-methyl-GTP (m7GTP) cap and is found on all eukaryotic mRNAs. Cap structure of mRNA is recognized by eukaryotic initiation factors (e.g. eIF4F complex). Capped and polyadenylated mRNAs have longer half-life (40 h) in oocytes than do uncapped mRNAs (6–10 h). The poly(A) tail promotes to stabilization of mRNA and also facilitate translation initiation (Drummond et al., 1985; Galili et al., 1988). On 3′ untranslated region (UTR) mRNA binds Poly(A) binding protein (PABP). The elongated 3′ poly(A) tail creates multiple binding sites for PABP, which binds to the

N-terminus of eIF4G1. Interaction of PABP and eIF4G1 promotes poly(A)-dependent translation initiation (Keiper and Rhoads, 1999).

## 1.4 Localization of RNAs in the right time and right the place

The spatial regulation of protein translation is an efficient way to create functional and structural asymmetries in cells. All RNAs are produced by transcription in the nucleus, where splicing also takes place. Processing may require transport of RNA between specific locations in the nucleus (Vargas et al., 2005). Once mature, most mRNAs are exported to the cytoplasm through the nuclear pores (Adams et al., 2014; Kelly and Corbett, 2009), however, some may be shuttled back into the nucleus (Hwang et al., 2007). Once in the cytoplasm, RNAs may be further transported to specific sites to carry out their functions, depending on the cell type, developmental stage, environmental signals or perturbations. The temporal and spatial distribution of localized RNAs is determined by intricate mechanisms that regulate their movement and anchoring. In the oocyte of *Drosophila* transport is influenced by actin and microtubule nets (Steinhauer and Kalderon, 2006). Polarized accumulation of RNA molecules was first visualized nearly 30 years ago, when *β-actin* mRNA was found to be asymmetrically localized within ascidian eggs and embryos (Jeffery et al., 1983). The fully grown oocytes of invertebrate and non-mammalian vertebrate species have asymmetrical distribution of organelles, localized RNAs and proteins, the polarity dictates the patterning of the future embryo (Holt and Bullock, 2009; King et al., 2005).

Subcellular mRNA trafficking has been demonstrated as a mechanism to control protein distribution. It is generally believed that most protein localization occurs subsequent to translation. Asymmetrically localized RNAs in the *Xenopus* and *Drosophila* oocyte are intensively studied (Holt and Bullock, 2009; King et al., 2005). Jambor et al., 2015 and Lécuyer et al., 2007 show that dozens of detected mRNAs were limited to small number of subcellular domains (e.g. spindle, nucleus, perinuclear region). Only few studies have focused on enrichment localization of maternal transcripts on spindle area on MII mouse oocyte (Romasko et al., 2013; VerMilyea et al., 2011). Spindle formation requires protein synthesis (Hashimoto and Kishimoto 1988), indicating a possible role for translational control by localized mRNAs in this process. The most prominently affected categories included proteins associated with the plasma membrane, chromatin/nucleus, signaling and as expected spindle/cytoskeletal functions. With reduced levels of mRNA, other functions were altered, like vesicle/endocytosis/protein transport, Golgi and endoplasmic reticulum, ubiquitination and protein degradation and RNA binding. In mice, few maternal factors have been identified that are essential for embryo viability (Tong et al., 2000) but mouse early embryos are thought to undergo regulative, rather than mosaic, development (Rossant and Tam, 2004). There is well studied mamalian maternal gene, which have role to embryo patterning, it is called Caudal type homeobox 2 (Cdx2). Cdx2 is the core transcription factor responsible for trophectoderm development. Cdx2 mutants die in the blastocyst stage as the trophectoderm is not properly specified and it fails to maintain epithelial integrity (Ralston and Rossant, 2008; Strumpf et al., 2005).

## 1.5 Detection of RNA by in situ hybridization methods

Fluorescent *in situ* hybridization (FISH) is a useful tool for visualization of RNA or DNA by in spatial context of cell. The basic principe of FISH consists of hybridization of probe with nucleic acid by Watson and Crick base paring. The protocol consists of four steps: fixation, premeabilization, hybridization and imaging. FISH has been widely used in cell and developmental biology research to study gene expression. This approach can address questions about RNA functions and potential activity. Recently, it is not much known about visualization of RNA in mouse oocyte and embryo. Older version of FISH used radiolabeled probes (Gall and Pardue, 1969). Radiolabeled methods were gradually replaced by hapten-containing probes (Rosner and Beddington, 1993.), whose presence is revealed by the binding of specific antibodies conjugated to alkaline phosphatase or horseradish peroxidase (HRP).

Robert Singer and colleagues pioneered single mRNA molecule imaging techniques (smFISH) (Long et al., 1995; Taneja et al., 1992). The key improvement was the replacement of long probes with 50 bp probes, that are complementary to sequential parts of the target mRNA and are each coupled to typically several fluorescent dyes at predefined positions (Figure 4A). Raj et al. 2008 modified the Singer method using a larger number (>30) of shorter oligonucleotides (20 bases) probes labeled with only a single fluorophore at their 3′ termini, each of which hybridized to a different portion of the target mRNA (Raj et al., 2008) (Figure 4B). Recently the synthesis of probes has become exponentially cheaper and more commercially available, fluorescent labels have become brighter, and image detection has become more sensitive (Coassin et al., 2014). This *in situ* technology provides direct detection of individual molecules of mRNA without amplification. FISH probes can be labeled with any number of different dyes, allowing for simultaneous detection of several mRNA targets. In the case of smFISH procedures are performed with fixed cells which facilitate the quantification and distribution of RNAs in steady state, but they do not provide dynamic. However hybridization typically takes from four hours to overnight, but Raj and colleagues developed a new technique called Turbo FISH that works far faster (Shaffer et al., 2013). By switching from formaldehyde fixation to methanol and increasing probe concentration, he reduced hybridization time to five minutes, on average, and to as little as 30 seconds. RNA cytoplasmic FISH (cFISH) was used for examine gene expression in whole mouse embryos (Gasnier et al., 2013) (Figure 4C). After hybridization of the probe to the target mRNA, the hapten is recognized by a specific antibody coupled to an HRP, which depends on the enzyme manufacturer, hapten-bound HRP then catalyzes the precipitation of tyramides that are tethered either to fluorophores, for direct detection, or alternatively to biotin, to proceed to an additional amplification step. cFISH can be combined with immunofluorescence to extend its labeling possibilities (Chazaud and Rossant, 2006). There are several studies which combine immunocytochemistry and RNA FISH together in somatic cells (Kochan et al., 2016, 2015; Toledano et al., 2012). These techniques simultaneously capture the location of cellular RNAs as well as RBPs to study the consequences of various stress conditions affecting mRNA translation initiation and protein synthesis. Kochan et al. (2015) describe a procedure that immunofluorescence (IF) technique with the smRNA FISH method based on the use of Stellaris probes. For signal enhancement several approaches of RNA FISH have been used, including branched DNA probes, quantum dots, and padlock-rolling circle amplification (RCA) (Kwon, 2013; Larsson et al., 2010; Wang et al., 2012). RCA is the only method so far capable of distinguishing single nucleotide allelic changes in transcripts. Briefly, reverse transcription is performed *in situ* on cells and tissue sections

to generate complementary DNA (cDNA), the mRNA is then degraded by ribonuclease H and padlock probes are hybridized to targeted cDNA with 5′ and 3′ arms circularized by a T4 DNA ligase. The circularized padlock probes serve as a template for RCA by Φ29 DNA polymerase, and then fluorophore-couple oligonucleotide probes specific for each padlock probe can be hybridized and visualized (Larsson et al., 2010) (Figure 4D).

To complete *in situ* detection methods I would like to quickly summarize information about detection of protein. Recently for a sensitive and specific detection of proteins, their posttranslational modifications and activation state as well as protein-protein interactions are detected by proximity ligation assay (PLA) (Weibrecht et al., 2010) (Figure 4E). The assay is based on the employment of proximity probes, composed by oligonucleotide-conjugated antibodies, to recognize a couple of specific targets. The binding of probes (only when they are in 40 nm close proximity) allows for the hybridization of the connector oligonucleotides, which will form circular DNA strands. These DNA circles can then be amplified by polymerase chain reaction and the addition of fluorescence-labelled oligonucleotides complementary to the amplification product allows the localized detection of individual or interacting proteins in cells and tissues (Söderberg et al., 2008). PLA approach has been already proven to be successful in mouse oocytes for detection of interaction Kinesin family member 4A (KIF4) protein with inner kinetochore protein CENP-C throughout meiosis (Camlin et al., 2017) and also several studies was published on early embryo development (Benesova et al., 2016; Roussis et al., 2016).

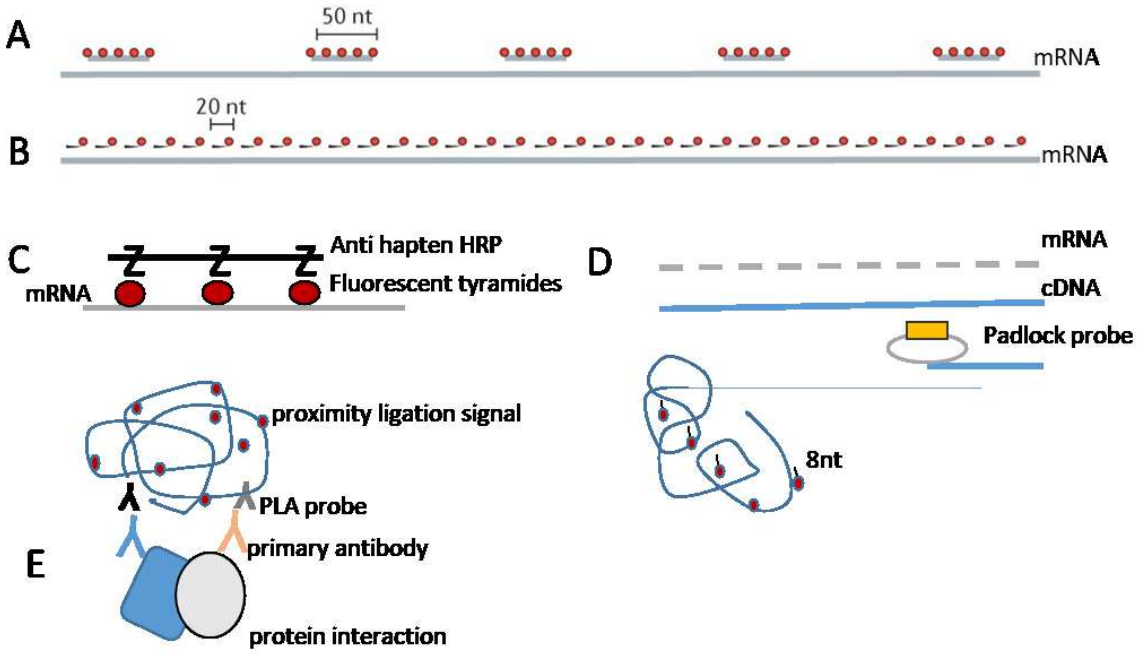



**Figure 4 – Summary of RNA FISH technique and proximity ligation assay**

**A)** Synthetized probes with incorporated labled nucleotide; **B)** sm FISH 20 nt probes labeled on 3′ termini; **C)** cytoplasmic FISH; **D)** detection by padlock probe and amplification signal by rolling circle amplification; **E)** detection interaction of proteins *in situ* by proximity ligation

## 1.6 Translation in oocyte and embryo

Translation is necessary for development control of cell cycle of oocyte. The overall translation gradually decreases during oocyte meiotic maturation (Schultz, 1993), but the activators of cap dependent translation become activated during this period, implying a role for translation of specific mRNAs to regulate meiosis (Ellederova et al., 2006; Tomek et al., 2002) (Figure 5). The basic translation machinery consists of ribosome, mRNA and translation factors. In the oocyte, maternal mRNAs can be translated in specific location to provide a spot of high protein concentration in the cell. Selective protein synthesis of oocyte determinants is governed temporally and spatially by both: specific repression and translation activation. Regulation of mRNA compartmentalization and local translation is a crucial mechanism of controlling gene expression and also allowing rapid changes in levels of proteins in specific locations. For a successful process of maturation and localization oocytes have to use post-translational regulation of synthesized proteins such as phosphorylation, dephosphorylation, ubiquitination and sumoylation (Shandala et al., 2001). Transcripts are translated into polypeptides by initiation, elongation, termination and ribosome recycling. Translational initiation is the rate-limiting and the most regulated step of translation (Gebauer and Hentze, 2004).

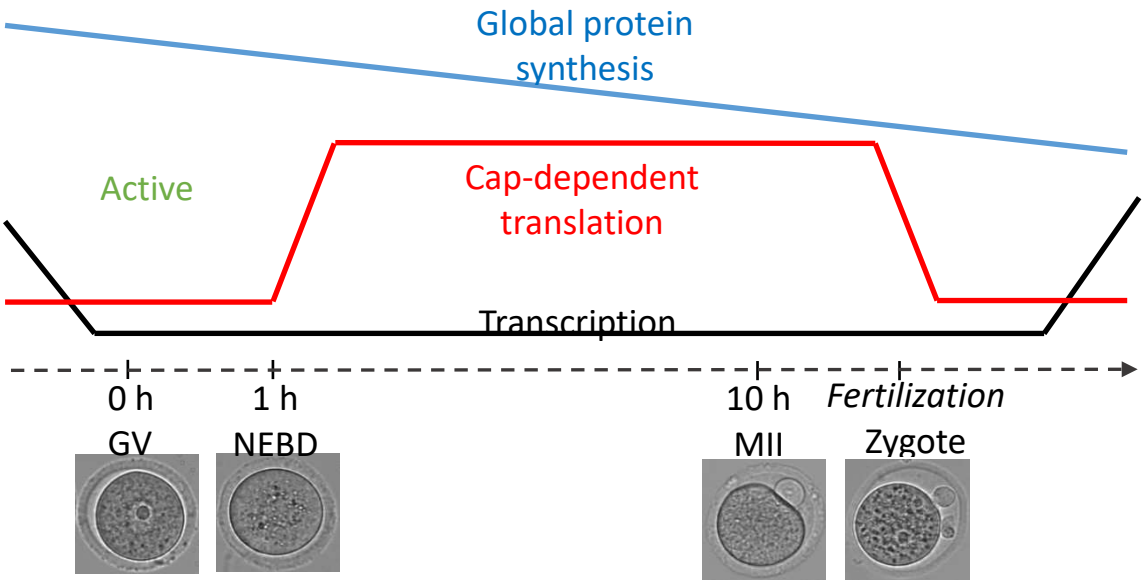



**Figure 5 – Scheme of protein synthesis during meiosis I and II in the mammalian oocyte**

The fully grown oocyte is transcriptionally silent and becomes active after fertilization (black line). Positive regulators of the cap dependent translational pathway become activated post NEBD and inactivated after fertilization (red line). Contrastingly, global protein synthesis gradually decreases during oocyte meiosis and early embryo development (blue line). Scheme is based on the studies of (Ellederova et al., 2006; Ellederová et al., 2008; Susor et al., 2008, 2015).

There are several recent reports using proteomic approaches to the study of ooctyes, including the exploration of bovine, pig and mouse oocyte proteomes (Ellederova et al., 2004; Memili et al., 2007; Vitale et al., 2007; Wang et al., 2010). The maternal protein compilation serves as an important tool for expanding our knowledge about regulation of multiple processes in mammalian reproduction. Vitale et al., (2007) used two-dimensional electrophoresis and mass spectrometry to identify 12 proteins that appeared to be differentially expressed between germinal vesicle (GV) and metaphase II (MII) murine oocytes. Calvert et al., (2003) identified 8 highly abundant heat shock proteins and related chaperones in the mature mouse egg by two-dimensional electrophoresis. Zhang et al., (2009) successfully identified 625 different proteins from mature mouse oocytes, where they compared the maternal proteins in oocyte with a recently published mouse embryo stem cell proteome and identified an overlap of 371 proteins. In 2010, Wang et al. published a characterization of mouse proteome including germinal vesicle, methaphase II oocytes and zygotes and embryonic stem cells. In that study it was found that GV oocytes overexpressed proteins related to metabolism and cytoskeleton. On the other hand, MII oocytes contained more proteins involved in RNA processing and Golgi transportation in comparison with zygotes which expressed more key factors involved in protein degradation. Interestingly, it was found that MII oocytes contained a group of mitochondrial protein which was totally absent in zygotes. Moreover, more than 70 transcription factors were expressed in mouse oocytes and zygotes. Zygotes express more key factor involved in protein degradation (e.g. ubiquitin C).

## 1.7 Regulation of cap dependent translation via mTOR pathway in oocyte and embryo

All eukaryotic nuclear-transcribed mRNAs possess 5′ terminal cap structure. Two macromolecular complexes that function in cap dependent translation initiation, the eIF4F and the 43S preinitiation complex, are the major target of the translation regulation. Cap dependent translation is initiated by assembly of the eIF4F complex consisting of eIE4E (cap binding protein), eIF4G1 (scaffolding protein), and eIF4A (helicase) on the 5' m7Gppp capping structure of the transcript. The eIF4F complex recruits the ribosomal complex to the ribosome recognition sequence in the 5' UTR for translation initiation. In this procedure, the most critical step is the association between mRNA bound to eIF4E with the eIF4G factor.

Protein synthesis in oocyte is regulated mostly at level of initiation of translation. This regulation occurs by both cis-regulatory elements, which are located in 5' and 3' UTRs, and transacting factors (Hershey, 1991). 5′ cap, secondary structures, multiple ORF (open reading frames), multiple initiation sites (upstream of AUG), IRES (internal ribosome entry sites, polyadenylation signals and motifs) are focal points of translation regulation (Figure 6). During translational initiation, an 80S ribosome assembles on the start codon of mRNA. Translational control is crucial also for proper embryonic development. First, early embryonic divisions are transcriptionally silent; one division in the mouse. It is believed that during mitosis, cap dependent translation is inhibited, so the ribosome accesses to mRNAs is independent of the cap-binding protein eIF4E. It is believed that under conditions of repressed cap dependent translation, a *cis*-regulatory element known as the internal ribosome entry site (IRES) recruits the ribosome to the mRNA through the IRES-*trans*-acting factors (ITAFs) (Pyronnet et al., 2000), it still requires other initiation factors (Gilbert et al., 2007).

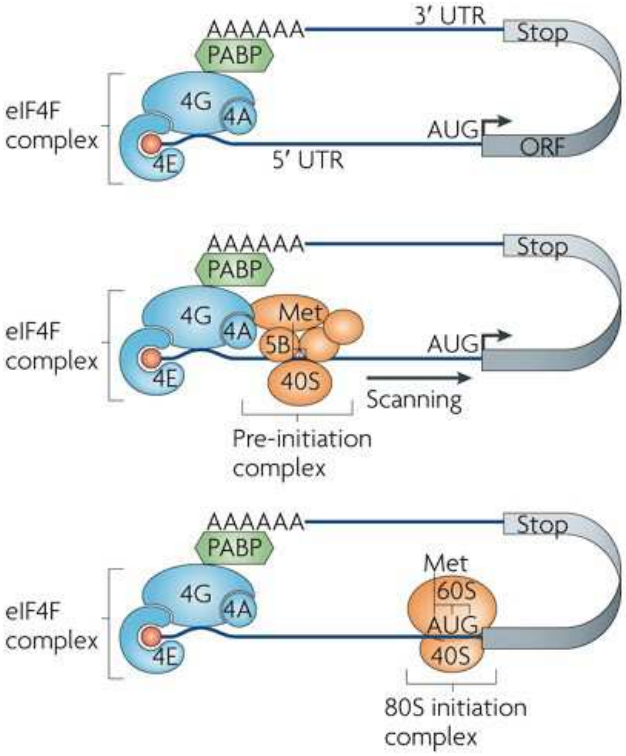



**Figure 6 – Cap dependent translation**

An initiation phase forming eIF4F complex, subsequent recruitment of the 43S pre-initiation complex (which includes the 40S ribosomal subunit). After scanning along the 5' UTR for an appropriate AUG start codon, the pre-initiation complex is then dissolved and the 60S ribosomal subunit joins the 40S subunit to form a translationally competent 80S ribosome. This process is facilitated by the factor eIF5B (5B) and initiates translation elongation. Scheme is adapted from Besse and Ephrussi, (2008).

The signaling pathway involving the mammalian target of rapamycin (mTOR) is the center of control of cap dependent translation in somatic cells and in oocyte (Jansova et al., 2017; Susor et al., 2015). mTOR is a conserved 289 kDa serine threonine kinase. Recent studies revealed that TOR is present in two distinct multiproteincomplexes called TORC1 and TORC2 (Dowling et al., 2010; Livingstone and Bidinosti, 2012; Mayer et al., 2014; Oh and Jacinto, 2011). Protein synthesis in oocytes is associated with mTORC1 signaling (Kogasaka et al., 2013; Romasko et al., 2013; Susor et al., 2015). mTORC1 interacts with raptor and is inhibited by rapamycin (Thoreen and Sabatini, 2009; Zheng et al., 1995). M-phase specific role for mTORC1 signaling have been demonstrated by (Yaba et al., 2008). Auto-phosphorylated form of mTOR has been localized in the midzone of microtubules and the midbody of mitotic apparatus in cancer cells (Vazquez-Martin et al., 2009). Translation initiation is a cellular response to both mitogenic stimulation and nutrient availability, important for cell growth, cell cycle progression and cell proliferation (Gingras et al., 2001). Murakami et al., (2004) showed that mTOR controls cell size and proliferation in early mouse embryos and embryonic stem cells, which might reflects its importance in cap dependent translation. mTOR mutant “flat-top” embryos failed to upregulate cell proliferation in the telencephalon, the anteriormost region of the forebrain.

Deletion of the C-terminal six amino acids of mTOR, which are essential for kinase activity, resulted in reduced cell size and proliferation arrest in embryonic stem cells (Murakami et al., 2004). Yang et al., (2009) showed by immunocytochemistry that mTOR mainly is located on nuclear membrane during GV stage, distributed with the chromosome after NEBD, and distributed with the spindle apparatus during MII stage. Experiments with rapamycin showed changes in distribution of mTOR and moreover they measured decrease of protein level (Yang et al., 2009). More detail was describe mTOR in cumulus cells and oocyte during maturation (Kogasaka et al., 2013). Spatiotemporal immunolocalization of mTOR kinase made by Kogasaka et al. 2013 confirmed that mTORC1 colocalized on the spindle, and phosphorylated mTOR was strongly expressed at spindle poles as well as the midbody in both cumulus cells and oocytes. The immunofluorescence intensity of phosphorylated mTOR increased during meiotic resumption and displayed a speckled localization pattern adjacent to the chromosomes, on the spindle poles, and on the midbody (Kogasaka et al., 2013). Similar pattern has been observed in components of the mTORC1 pathway as the upstream regulators phosphatydilinositol 3 kinase (PI3K), serine threonine kinase Akt (AKT), Tuberous sclerosis proteins 1/2 (TSC1/2), and mitogen activated protein kinase (MAPK) (Guertin and Sabatini, 2005; Sarbassov et al., 2005). It has been also described that p53, major checkpoint protein in cell lines, can inhibit mTOR activity (Feng et al., 2005).

In mouse oocytes, suppression of AKT activity delayed resumption of meiosis accompanied by a decrease in Cyclin-dependent kinase 1 (CDK1) activity. CDK1 or p34cdc2 kinase is the catalytic subunit of maturation-promoting factor (MPF), whose activity has been reported to be crucial for meiotic maturation of oocytes (Kalous et al., 2006). mTOR and CDK1 are important regulators in oocytes and cummular cells (Kogasaka et al., 2013).

The best characterized downstream effectors of mTOR include two signaling pathways that act parallel to control cap dependent translation: ribosomal protein S6 kinase1 pathway and eukaryotic translation initiation factor 4E-binding protein1 (4E-BP1). Importantly, the phosphorylation status of 4E-BPs regulates interaction with cap binding proteins. The 4E-BP family consists of three members: 4E-BP1, 4E-BP2 and 4E-BP3 (Pause et al., 1994). Nowadays, there is not much known about 4EBP1-3 in oocytes. They are studied mostly in somatic cells. These proteins undergo phosphorylation at seven sites, but only four are linked with mTOR signaling. In mouse these are T36, T45, T69 and S64. Upon phosphorylation of 4E-BP1 releases from eIF4E, allowing eIF4E to assemble with other translation initiation factors. Increased phosphorylation has also been shown during meiotic progression of mammalian oocytes (Ellederova et al., 2006; Tomek et al., 2002), and recently different phosphorylated forms of 4E-BP1 have been shown to co-localize with the meiotic spindle in mouse oocyte (Romasko et al., 2013). Hypophosphorylation 4E-BP1 leads to repression of translation, because eIF4E is bound with 4E-BP1 (Figure 7). Thoreen et al., (2012) described that eIF4F complex sensitive transcripts often possesses long highly structured 5'UTR or oligopyrimidine motif (TOP). The translation of TOP mRNAs is highly sensitive to stress and growth conditions, and behaves as an "all-or-none" phenomenon. Recent studies using high-resolution transcriptome-scale ribosome profiling have confirmed that the translation of TOP mRNAs is highly sensitive to mammalian target of mTOR inhibitors (Thoreen et al., 2012). TOP genes are mostly ribosomal proteins or elongation factors. This sequence is thought to serve as a *cis*-regulatory element which inhibits the binding of translational regulatory proteins

or the translational machinery itself. When a *trans*-factor binds to the TOP motif mRNAs under particular cellular conditions, it results in dissociation of bound ribosomes.

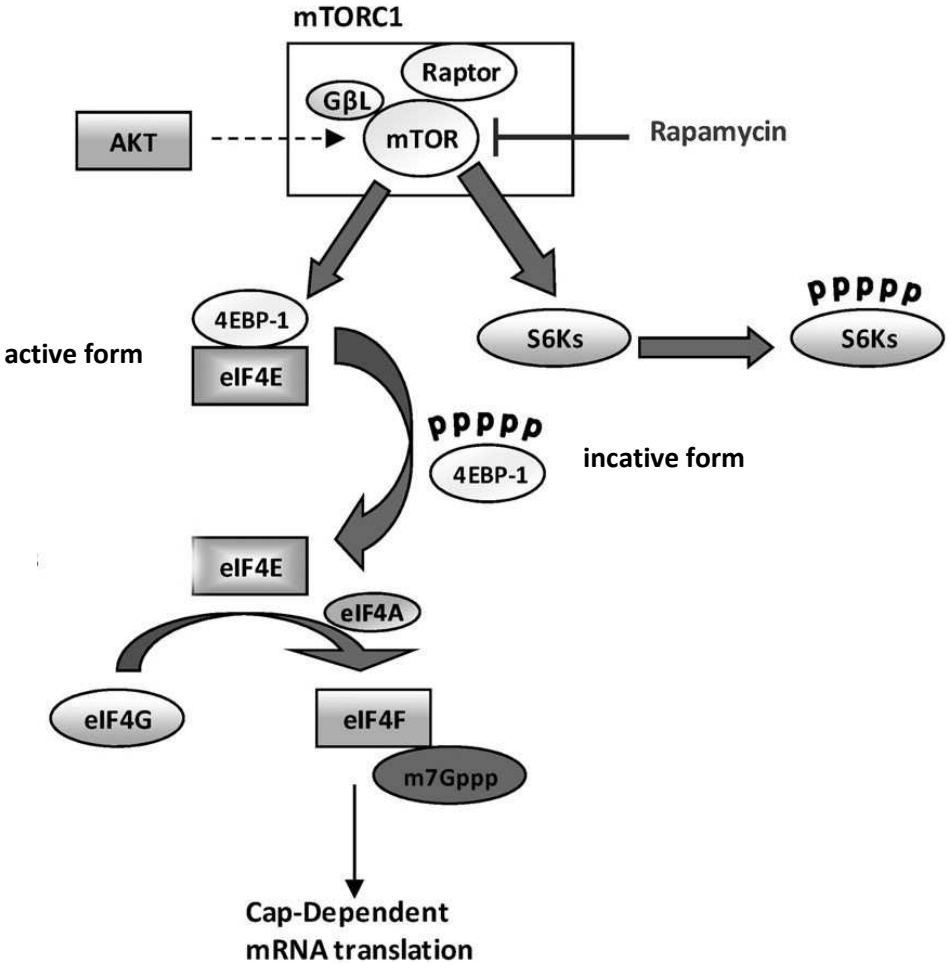



**Figure 7 – mTOR pathway and cap dependent translation**

In response to mTORC1-activating stimuli by AKT, mTORC1 docks to eIF3, localized at the 5′-cap, whereby it phosphorylates 4E-BP1 and S6K1, inducing 4E-BP1 release from eIF4E and S6K1 release from eIF3. Dissociation of 4E-BP1 enables eIF4G to dock to eIF4E, thus initiating assembly of the eIF4F complex. Upon release, S6Ks phosphorylates eIF4B, which induces eIF4B binding to eIF4A, an event that enhances eIF4A helicase activity. Rapamycin block activity of mTOR and consequently inhibit translation. Adapted from Li et al., (2011).

# 2 Aims of the thesis

**Hypothesis: Asymmetric localization of RNAs in oocyte determines temporal and spatial translation**

Aim 1: To detect localization of global RNA and specific RNAs in mammalian oocyte and early embryo.

Aim 2: To deremine localization of RNA binding proteins and components of translation machinery in mammalian oocyte and early embryo.

**Hypothesis: mTOR–eIF4F pathway has a role in temporal and spatial translational control of specific protein expression during mammalian oocyte development**

Aim 3: To detect global and specific translation in oocyte and early embryo.

Aim 4: To identify key regulators of 4E-BP1 and role of translation repressor 4E-BP1 in cap dependent translation in mammalian oocyte.

# 3 Comments on publications

During my PhD studies I have produced 4 publications in peer-reviewed journals. Based on rules of committee of Department of Developmental and Cell biology of Charles University my cumulative impact factor is **KIF = 6,648**. The 4th publication is in the preparation and will be submitted in the summer of 2017.

**3.1** Susor A, **Jansova D**, Cerna R, Danylevska A, Anger M, Toralova T, Malik R, Supolikova J, Cook MS, Oh JS, Kubelka M. Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway. **Nat Commun**. 2015 Jan; 6:6078. doi: 10.1038/ncomms7078. **IF: 11,329 (2015)**
D.J. performed fluorescent *in situ* hybridization of RNA, image analysis, gene expression analysis by real-time RT-PCR, immunocytochemistry and assisted with preparation of manuscript.

**3.2** Susor A, **Jansova D**, Anger M, Kubelka M. Translation in the mammalian oocyte in space and time. **Cell Tissue Res**. 2016 Jan; 363(1):69-84. doi: 10.1007/s00441-015-2269-6. Review **IF: 2,948 (2016)**
D.J. corrected text of manuscript and designed all schemes.

**3.3 Jansova D**, Koncicka M, Tetkova A, Cerna R, Malik R, Llano E, Kubelka M, Susor A. Regulation of 4E-BP1 activity in the mammalian oocyte, **Cell Cycle**, 2017. doi: 10.1080/15384101.2017.1295178. **IF: 3,53 (2017)**
D.J. designed and performed experiments (immunostaining and confocal microscopy, Western blot, measuring gene expression), analysed the data, wrote the manuscript.

**3.4 Jansova D**, Tetkova A, Koncicka M, Susor A. Transcriptome and translation in mammalian oocyte and embryo, **submitted in summer 2017, Sci. Rep.**
D.J. designed and performed experiments (excepts ReAsH), wrote the manuscript.

Listed publications are sorted chronologically.

## 3.1 Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway

To investigate the regulation of cap dependent translation we decided to perform a detailed analysis of the expression, localization and activation of the mTOR and eIF4F axis components. We raised the question whether downregulation of mTOR and the suppression of the formation of the eIF4F complex in maturing mouse oocytes effects oocyte development. We show that the downregulation of mTOR and the suppression of the formation of the eIF4F complex which is involved in the cap dependent translation by inhibitor of translation (4EGI), leads to significant defects in chromosome alignment and spindle morphology in metaphase I and metaphase II in cases 79 % ($p \leq 0{,}001$) oocytes compared to non-treated oocytes. Chromosomal spreads of inhibitor-treated oocytes show a 60% ($p \leq 0{,}001$) aneuploidy phenotype in MII oocytes compared to non-treated oocytes. On the other hand the disruption of mTOR/eIF4F signaling does not abolish meiosis I, in addition it does not visibly influence the overall translation. To explore the consequences we checked nascent proteosynthesis using the methionine analogue L-homopropargylglycine (HPG). In the GV oocyte, the translational activity signal was detected mainly in the perinuclear area. However, we detected two distinct areas with active translation in oocytes which underwent nuclear envelope breakdown. The first area was located in the immediate vicinity of chromosomes and we called it chromosomal translational area - CTA. This area was separated from another one by ER and a disrupted Lamin A/C structures. The second translation hot spot was found in the perispindular translation area (PTA). These regions of translational signal migrated with the spindle to the oocyte cortex and disappeared before extrusion of polar body. These findings suggest that the oocyte translates *de novo* proteins in distinct locations, which undergo remodeling shortly after NEBD and at cytokinesis (MII). Our results point out to NEBD as a crucial stage for regulation of translation of mRNAs through this pathway.

This idea confirmed our data of localization study of members of mTOR-eIF4F axis after 3 h post IBMX wash, there is also measured burst of translation in CTA. Our data indicated that phosphorylation form of mTOR(S2448), 4EBP1(T70) and eIF4E(S209) was localized at the CTA. Phosphorylated form of mTOR on S2448 was previously linked to the stimulation of translational activity (Navé et al., 1999) and phosphorylation status of 4EBP1(T70) again suggest to inactivation of translation repressor of initiation (Romasko et al., 2013), which confirmed stimulation of translational activity. Next, we analyzed by immunoblot eIF4E, phospho-eIF4E, mTOR, phospho-mTOR, phospho-S6K, S6K, phospho-RPS6, RPS6, phospho-eIF4G1, eIF4G1 and eIF4G2. Our data indicate that mTOR(S2448) become highly activated post NEBD which correlates with phosphorylation of the 4E-BP1 and activity of both factors decrease after fertilization. Similarly, substantial increase in phosphorylation of eIF4E(S209). In addition to elucidate in detail whether mTOR–eIF4F pathway regulates translation at NEBD we used Renilla luciferase (RL) assay. While translation of RL constructs after NEBD containing upstream non-TOP sequence (β-Actin) or mutated oligopyrimidine sequence (eEF2 $TOP^{M}$) did not change significantly in oocytes construct containing the canonical TOP sequence (eEF2TOP) was significantly translated post NEBD.

mTOR regulates transcript with TOP motif (Jefferies et al., 1997). This information lead us to select *Bub3, Nucleophosmin 1* and *Survivin*, which represent TOP mRNAs (Yamashita et al., 2008). We analyzed their protein expression by immunoblotting after 4EGI treatment, levels of selected proteins was around 30 % ($p \leq 0.001$). On the other hand the translation of mRNA with an internal ribosome entry site (IRES) motif (e.g. *Camk2a*;

Pinkstaff et al., (2001) was without effect. This experiments confirm that downregulation of mTOR–eIF4F translation in the oocyte does not influence the overall translational pattern, but suppress protein synthesis.

mRNA localization generally leads to targeted translation (Håvik et al., 2003), thus we decided to detect the poly(A)-RNA population by RNA FISH during maturation of oocyte. The poly(A)-RNA population is present in the nucleus of GV oocyte and in the vicinity of chromosomes matching post NEBD. Next we found that *Bub3*, *Npm1*, *Survivin* and *Dazl* mRNAs were localized in the nucleus, where *Camk2a*, *Mos* and *Gapdh* mRNAs were absent. Altogether, our findings indicate that a nuclear RNA population contributes to mammalian oocyte translational patterning and thus to the regulation of gene expression during the dynamic onset of meiosis. At the molecular level, we present an important function for the mTOR–eIF4F pathway in spatial translational control, suggesting a novel set of regulatory mechanisms ensuring specific gene expression at the right place and time in the mammalian oocyte.

## 3.2 Translation in the mammalian oocyte in space and time

This review is focused on the recently emerged findings on RNA distribution related to the temporal and spatial translational control of the meiotic progression of the mammalian oocyte. We utilized 202 research paper to write this review where we use compared studies in non-traditional model systems which are valuable in order to address dissimilarities and overlaps in transcriptome composition between model organisms, and are likely to provide important information regarding the components and mechanisms that may play critical regulatory roles in the fertility of nonmurine models, including the human. Post-transcriptional control of gene expression at the translational level has emerged as an important cellular function in normal development. Therefore, the regulation of gene expression in oocytes is controlled almost exclusively at the level of mRNA and protein stabilization and protein synthesis. We shed light on the propagation of genes expression by translation in vicinity of chromosomes during nuclear envelope break down. To elucidate mRNA localization in mammalian oocyte, we discuss our results from (Susor et al., 2015), how to explain retained Poly(A) RNA in nucleus of fully grown oocyte (Figure 8). We suppose that mRNA retention may serve as a way to orchestrate protein expression.

In this review we also focused on localization of global translation (Figure 9) and regulatory mechanisms of initiation of translation by the major trans-activating regulator of the 5‘ UTR of cap binding proteins which forming 4F complex. We found in mouse oocyte two distinct translational areas, chromosomal (CTA) and perispindular (PTA) are present. These areas are likely separated by ER and microfilament-rich structure. After the first polar body extrusion both translational areas disapear. These findings suggest that oocyte translates proteins *de novo* in distinct locations. The NEBD period appears important for translational reorganization and for timing of spindle assembly. Asymmetric localization of RNA determines the timing and localization of translation in mammalian oocyte.

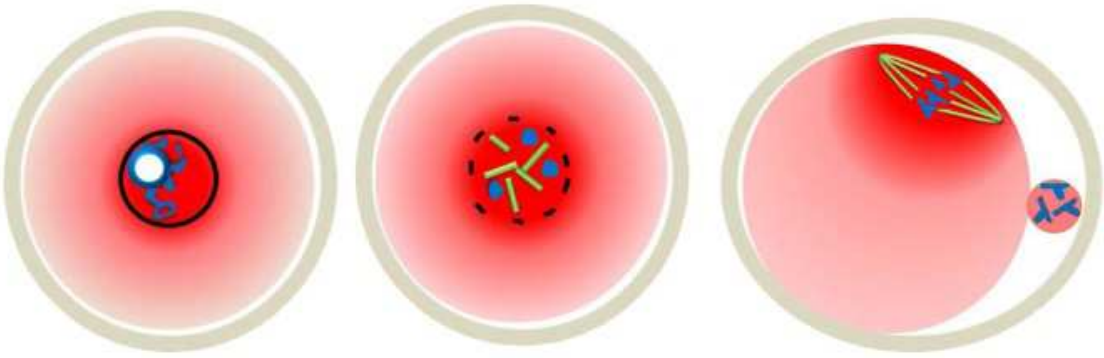


**Figure 8 – Scheme of localization of Poly(A) RNA population in mouse oocytes in the GV, NEBD and MII stages**

Nucleus of fully grown oocyte shows high poly(A) RNA signal that is retained in the nucleus and after nuclear envelope breakdown in the chromosomal area and disappears as the oocyte reaches MII stage. Adapted from (Susor et al., 2016).

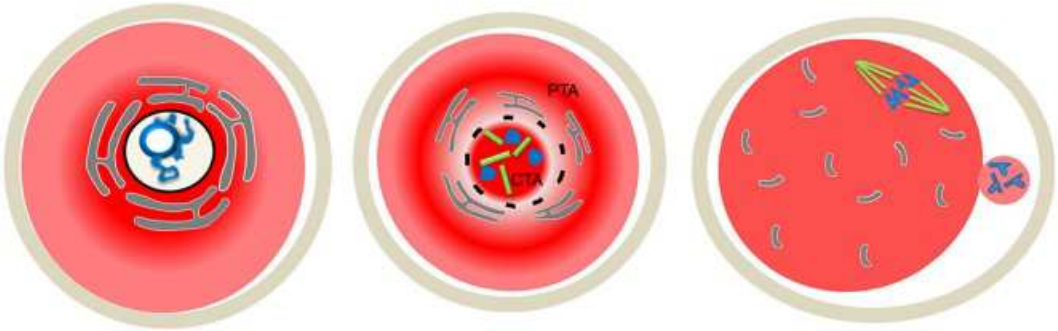



**Figure 9 – Scheme of in situ translation in the GV, NEBD and MII stages**

A strong translational signal is present in the perinuclear region in GV stage, two translational hotspots (CTA – Chromosomal Translational Area and PTA - Perispindular Translational Area) develop post NEBD that disappear after polar body extrusion in the MII stage. Adapted from (Susor et al., 2016).

## 3.3 Regulation of 4E-BP1 activity in the mammalian oocyte

We investigated role of translation repressor of cap dependent translation 4E-BP1 via mTOR and CDK1 signaling. This manuscript expands our previous findings from (Susor et al., 2015). We show that the mouse oocyte contains 3 forms of cap dependent translational repressor expressed on the mRNA level: 4E-BP1, 4E-BP2 and 4E-BP3. However, only 4E-BP1 is present as a protein in oocytes, it becomes inactivated by phosphorylation after nuclear envelope breakdown and as such it promotes cap dependent translation after NEBD. Phosphorylation of 4E-BP1 can be seen in the oocytes after resumption of meiosis but it is not detected in the surrounding cumulus cells, indicating that 4E-BP1 promotes translation at a specific cell cycle stage. Our immunofluorescence analyses of 4E-BP1 in oocytes during meiosis show an even localization of global 4E-BP1, as well as of its 4E-BP1 (T37/46) phosphorylated form. On the other hand, 4E-BP1 phosphorylated on S65 is localized at the spindle poles and 4E-BP1 phosphorylated on T70 localizes on the spindle. We further show that the main

positive regulators of 4E-BP1 phosphorylation after NEBD are mTOR and CDK1 kinases, but not PLK1 kinase. CDK1 exerts its activity toward 4E-BP1 phosphorylation via phosphorylation and activation of mTOR. Moreover, both CDK1 and phosphorylated mTOR co-localize with 4E-BP1 phosphorylated on T70 on the spindle at the onset of meiotic resumption. Expression of the dominant negative 4E-BP1 mutant adversely affects translation and results in spindle abnormality. Taken together, our results show that the phosphorylation of 4E-BP1 promotes translation at the onset of meiosis to support the spindle assembly and suggest an important role of CDK1 and mTOR kinases in this process. We also show that the mTOR regulatory pathway is present in human oocytes and is likely to function in a similar way as in mouse oocytes.

In this study we propose that localized translational regulation at the oocyte spindle regulated though an mTOR/CDK1 pathway might represent a mechanism which links spindle formation and function with the temporal and spatial regulation of the local transcriptome in the particular subcellular areas, which affects oocyte quality.

## 3.4 Transcriptome and translation in mammalian oocyte and embryo


### Abstract

The tight correlation between mRNA distribution and subsequent protein localization and function indicate a major role for mRNA localization within the cell. RNA localization followed by local translation presents a mechanism for spatial and temporal gene expression regulation utilized by various cell types. However not much is known about mRNA localization and translation in the mammalian oocyte and early embryo. Mammalian oocyte is differentiated cell, which gives foundation to embryo development importantly, fully-grown oocytes become transcriptionally inactive and only utilize transcripts previously synthesized and stored during earlier development. We found that an abundant RNA population is retained in the oocyte nucleus together with RNA binding proteins. Next, we characterized specific ribosome proteins which contribute to translation in the oocyte and embryo. By applying selected markers to mouse and human oocytes we found similar mechanism of RNA metabolism in both species. In conclusion we have visualized the location of both transcriptome and translation in the oocyte, which sheds some light on this *terra incognita* of these unique cell types from mouse and human.


### Introduction

Meiotic maturation in mammalian oocytes and oocyte-to-zygote transition proceed without transcription and depend entirely on the post-transcriptional regulation of maternal mRNAs. The overall translation gradually decreases during oocyte meiotic maturation (Schultz, 1993), but the activators of cap dependent translation become more activated during this period, implying a role for translation of specific mRNAs to regulate meiosis (Ellederova et al., 2006; Tomek et al., 2002). These mRNAs need to be recruited to translation and subsequently degraded in a tightly controlled temporal manner (Flemr et al., 2010). Spatial segregation of protein synthesis in cells involves the positioning of mRNAs according to where their protein products are required, and results in local or compartmentalized gene expression. mRNA localization can occur during specific stages in development. Surprisingly, little is known about mRNA localization and translation in the mammalian oocyte or early embryo.

Our previous study indicated that the oocyte nucleus contains an RNA population in fully grown oocyte that most likely contributes to translation in the vicinity of chromosomes after NEBD (Susor et al., 2015; Susor and Kubelka 2017). The localization

of mRNAs at the spindle is evolutionarily conserved between mammals and it is also seen in mitotic cells (Blower et al., 2007; Groisman et al., 2000). The differences of 384 mRNAs at meiotic spindle compared to other cortical regions were analyzed by microarray analysis (VerMilyea et al., 2011). The analyses of mRNA localization have been conducted in oocytes of *Drosophila* (Becalska and Gavis, 2009; Johnstone and Lasko, 2001), *Xenopus* (King et al., 2005; Kloc and Etkin, 2005) and mouse (Flemr and Svoboda, 2011). Localization of mRNA molecules within the cytoplasm provides a basis for cell polarization underlying developmental processes such as asymmetric cell division during meiosis or embryonic patterning. Fluorescence RNA *in situ* hybridization (FISH) has been widely used in cell and developmental biology research to study gene expression. This approach can address questions about RNA functions and potential activity. RNA FISH techniques is based on hybridization of multiple short, fluorescently labeled oligonucleotide probes on RNA (Raj et al., 2008). Many alternative FISH protocols have been developed for detecting mRNAs (Shaffer et al., 2013), and they mostly differ in the type of probe (Kwon, 2013). Visualization of mRNAs can be also performed by detection of mRNA-protein complexes.

Endogenous mRNA does not exist alone; they bind to number of proteins to form mRNA-protein complexes (Müller-McNicoll and Neugebauer, 2013). RNA-binding proteins (RBPs) and molecular motors mediate transport of mRNA on the cytoskeleton of cells, which cause the asymmetric distribution of RNA. RBPs are capable to regulate mRNA stability and translation. Some of RBPs such as CPEB, DAZL, eIF4E and 4E-BP1 post-transcriptionally regulate mRNA via binding to its 7-methylguanosine cap structure or 3′-untranslated regions (3′UTRs). Regulation of initiation of cap dependent translation is controlled via translational repressor protein 4E binding protein 1 (4E-BP1). Hierarchical phosphorylation of 4E-BP1 provides dissociation from eIF4E. In a previous study (Jansova et al., 2017) we localized 4E-BP1 enriched into nucleus of fully grown oocytes. A common mechanism regulating recruitment and stability of dormant maternal mRNAs is reversible polyadenylation that is controlled by cytoplasmic polyadenylation elements (CPEs) (Richter, 2007). CPEs are specific sequences in 3′UTRs of dormant maternal mRNAs that serve as the binding platform for the CPE-binding proteins (CPEBs), which control polyadenylation-induced translation. The family has four members. The most studied is CPE-binding protein 1 (CPEB1), which functions as a translational activator or repressor according to its phosphorylation state (Komrskova et al., 2014). Another RBPs that regulates RNA processing are known as heterogeneous nuclear ribonucleoproteins (hnRNPs) (Görlach et al., 1993). This family contains more than 20 members. The key characteristic of the hnRNPs is their nucleocytoplasmic shuttling (Piñol-Roma and Dreyfuss, 1992). The hnRNP proteins A1, A2/B1, A3 and A0 were initially considered as prime constituents of 40S heterogeneous nuclear ribonucleoprotein particles, which bind to and stabilize nascent pre-mRNA (Burd and Dreyfuss, 1994; Jean-Philippe et al., 2013). hnRNPA1 accumulates in cytoplasmic stress granules, in stress-activated cells, and is required for recovery from stress (Guil et al., 2006). Another RNA binding proteins are components of exon junction complex (EJC) which contains eIF4A3, a DEAD-box RNA helicase member of the eIF4A family of translation initiation factors (Chan et al., 2004). EJC proteins play important roles in postsplicing events including mRNA export, cytoplasmic localization, and nonsense-mediated decay (Chan et al., 2004; Wang et al., 2014). As mentioned above, 7-methylguanosine cap is required for stabilization and translation of the majority of mRNAs (Inoue et al., 1989; Ohno et al., 1987; Topisirovic et al., 2011). The presence of ribosomes is directly linked to protein synthesis during crucial periods of development and tightly connected with the developmental competence

of oocytes. It has been proposed by (Monti et al., 2013) that expression of mRNA of 27 ribosomal proteins has higher expressioned in developmentally competent oocytes compared to non-competent ones.

A remarkable feature of mammalian oocyte maturation is the significant elimination of rRNA and ribosomes (Clegg and Pikó, 1982). Moreover, genome-wide transcriptome analysis has shown that mRNAs coding for ribosomal proteins are degraded during maturation and after fertilization (Su et al., 2007; Zeng et al., 2004). Ribosomes are composed of two subunits: small 40S and large 60S subunits. The eukaryotic ribosome contains 4 RNAs and ~80 ribosomal proteins. In contact between two subunits is positioned RPL24 which has N-terminal domain in 60S (Ben-Shem et al., 2011), C-terminal part of RPL24 interact with the best characterized protein named as RPS6 (Krieg et al., 1988). The phosphorylation of RPS6 on five residues is response to mitogen and growth factor signaling (Rosner et al., 2011; Ruvinsky and Meyuhas, 2006). It has been long believed that RPS6 phosphorylation has an important function in the translational control of a subclass of mRNAs that harbor a 5′ tract oligopyrimidine (5′ TOP) sequence, and this level of regulation may imbue the ribosome with greater specificity (Meyuhas and Dreazen, 2009). Similalry as RPS6 also RPS14 play a role in regulation of the MDM2-p53 pathway (Kim et al., 2014; Zhou et al., 2013).

In this study we focused on visualization of transcriptome with connection to regulation and visualization of translation in mammalian oocyte and early embryo.

## Methods

Oocyte isolation and cultivation

Mouse ovaries were obtained from CD1 mice at least 6 weeks old which were stimulated to superovulate by intraperitoneal injection of 5 UI of pregnant mare serum gonadotropin (PMSG; Folligon, Merck Animal Health) 46 h prior to collection. Growing and fully grown GV oocytes were isolated subsequently into M2 medium (Millipore) supplemented with 100 μM of 3-isobutyl-1-methylxanthine (IBMX, Sigma Aldrich) used for preventing resumption of meiosis. Selected oocytes were stripped of cumulus cells and cultured in M16 medium (Millipore) without IBMX at 37 °C, 5% $CO_2$. To obtain zygotes females were mated to males after injection 5 UI of PMSG and subsequent (after 46 h) 5 UI of human chorionic gonadotropin (hCG; Merck Animal Health) injection. Zygotes were isolated 17 h after mating and cultured *in vitro* in M16 under mineral oil for 20 h, then 2-cell embryos were collected. All animal work was conducted according to Act No 246/1992 on the protection of animals against cruelty.

Immunocytochemistry

Oocytes were fixed 15 min in 4% paraformaldehyde (PFA, Sigma Aldrich) in PBS and permeabilized 10 min in 0.1% Triton X-100 in PBS with one drop of ActinGreen probe Phalloidin488 (Thermo Fisher). Then the oocytes were incubated overnight at 4 °C with primary antibodies diluted in PBS/ 0.2% normal bovine serum. The following antibodies were used in 1:150 dilution: rabbit anti-4E-BP1 (CST); rabbit anti-Ribosomal S14 (Santa Cruz); rabbit anti-Ribosomal S3 (CST); rabbit anti-RPL24 (Thermo Fisher); mouse anti-RPS6 (Santa Cruz); rabbit anti-CPEB4 (Thermo Fisher); mouse anti-hnRNPA1 (Sigma Aldrich); mouse anti-eIF4A3 (Abcam), rabbit anti-RPL7 (Abcam); mouse anti-m3Gcap/m7Gcap (Thermo Fisher). Mouse anti-5.8S rRNA antibody (Abcam), diluted 1:150, incubated at room temperature for 2 h. After washing in PBS for 2x15 min,

detection of the primary antibodies was performed by cultivation of the oocytes with relevant Alexa Fluor 488/594 conjugates (diluted 1: 250) for 1 h at room temperature. Oocytes were then washed 2x15 min in PBS and mounted using Vectashield Mounting Medium with DAPI (Vector Laboratories). Samples were visualized using Leica SP5 inverted confocal microscope in 16 bit depth. Images were assembled in Photoshop CS3 and quantified by Image J software (http://rsbweb.nih.gov/ij/).

Biological coating procedures for Nunc Lab-Tek II Chamber Slide System (Prepartion of slides for imaging)

The glass coverslip of well Lab-Tek II Chamber were coated at 37 °C for 2 h with poly-L-ornithine (Sigma Aldrich) diluted in RNase free water 1:250 and then overnight with laminin (Sigma Aldrich) diluted in PBS 1:1000 also at 37 °C.

RNA fluorescent in situ hybridization (FISH)

RNA FISH was performed with small changes according to Jansova, (2015). Oocytes were fixed for 10 min in 4% paraformaldehyde and permeabilized in 0.1% Triton X-100 in PBS with 40 units/20 µl of RNAseOut (Invitrogen), then mounted to pre-coated Nunc Lab-Tek II Chamber Slide System (Thermo Fisher) by 80% methanol pre-frozen to -20 °C. Oocytes were washed in the washing buffer A (Biosearch Technologies) and incubated overnight at 30 °C in hybridization buffer (Biosearch Technologies) with 75nM poly(dT) probe (Biotech Generi); Neat2 CalFluorRed610 (Biosearch Technologies); Dazl (Biosearch Technologies) and β-Actin labelled with Cy5 (Biotech Generi) (protected from light). Oocytes were then washed 3x in buffer A and 2x in 2xSSC (Sigma Aldrich). For visualization of chromatin structure the oocytes were incubated 1 min with 10nM DAPI (Sigma Aldrich) in 2xSSC; then washed 1x with 2xSSC and scanned in 2xSSC. For the negative control RNase A (Ambion) for 2 h at 37°C after the permeabilization step.

Rolling circle amplification (RCA) FISH

RCA FISH was performed according to Lee's et al. (2015) protocol with following changes: Oocytes were fixed 10 min in 4% PFA (Sigma Aldrich) and permeabilized in 0.1% Triton X-100 in PBS for 10 min and subsequently in 70% ethanol, pre-frozen to -20 °C, for 10 seconds. The whole transcriptome was converted into cDNA by M-MuLV reverse transcriptase (Enzymatics) and the reaction mix was prepared according to the mentioned protocol. The cDNA fragments were fixed to the cellular protein matrix using a nonreversible amine cross-linker (BS(PEG)9 (Sigma Aldrich) and circularized after degrading the RNA residues. The circular templates were amplified using RCA primers 100 µM (TCTTCAGCGTTCCCGA*G*A; * is phosphorothioate, Generi Biotech) complementary to the adapter sequence in the presence of aminoallyl-dUTP and stably cross-linked. For visualization of chromatin structure the oocytes were incubated 1 min with 10nM DAPI (Sigma Aldrich) in 2xSSC; then washed 1x with 2xSSC. We scanned the samples in 2xSSC.

*In situ* Proximity ligation assay (PLA)

Proximity ligation assay was performed according to manual instructions of PLA Duolink kit (Sigma Aldrich). Oocytes were fixed 15 min in 4% paraformaldehyde in PBS and permeabilized 10 min in 0.1% Triton X-100 in PBS. We added blocking solution (is contained in PLA Duolink kit) to each sample. Oocytes were incubated with primary antibodies at 4 °C overnight. The following antibodies were used: rabbit anti-RPL24

(Thermo Fisher) and mouse anti-RPS6 (Santa Cruz). After washing in PBS we washed the samples by buffer A (Sigma Aldrich). Then we incubated the samples with 40 µl reaction mixture which consists of 8 µl of PLA probe MINUS stock, 8 µl of PLA probe PLUS stock and 24 µl of PBS. The samples were incubated in a chamber for 1 h at 37 °C. We washed the slides by 1x Wash Buffer A for 6x2 min. Ligation was performed in 40µl reaction: 1 µl of Ligase to 39 µl of Ligation solution. Samples were incubated in ligation reaction mixture for 30 min at 37 °C. We washed the samples 6x2 min in Wash buffer A. To each sample 40 µl of amplification reaction (consists of 0.5 µl of Polymerase and 39.5 µl of amplification solution) was added and the samples were incubated for 100 min at 37 °C. Then the samples were washed in Wash buffer B (Sigma Aldrich) for 3x5 min and in 0.01x buffer B for 2 min. The samples were mounted by Vectashield Mounting Medium with DAPI (Vector Laboratories).

Live cell imaging of nascent translation (ReAsH method)

For the ReAsH method we used a plasmid provided by Robert Singer laboratory via Addgene (http://www.addgene.org/27123/). The growing oocytes were injected according to the protocol by Tetkova and Hancova, (2016) with a plasmid diluted to ~40 ng/µl into nuclei. Oocytes were incubated overnight in 1µM cycloheximide (CHX; Sigma Aldrich) in M16 medium to prevent translation. After CHX wash oocytes were incubated for 30 min in M16 supplemented with ReAsH dye (final concentration 20 µM, Thermo Fisher) and then transfered into 250 µM 2,3-dimercaptopropanol (BAL buffer, Thermo Fisher) in M16 and immediately scanned on confocal microscope Leica TCS SP5.

Statistical analysis

Mean and SD values were calculated using MS Excel, statistical significance of the differences between the groups was tested using Student's t-test (PrismaGraph5) and $P<0.05$ was considered as statistically significant.

## Results

### Detection of global transcriptome in oocyte and embryo

mRNA localization generally leads to target translation (Jambor et al., 2015). We asked how transcriptome is distributed in the oocyte. To visualize whole transcriptome we used rolling circle amplification method ((RCA; (Larsson et al., 2010; Lee et al., 2015)), containing reverse *in situ* transcription followed by hybridization of fluorescently labeled random hexamers. We detect evenly localized RNA in the cytoplasm with significant increase in the GV nucleus or 2-cell embryo nuclei (Fig 1). Treatment by RNase A erase fluorescence signal, which confirms its RNA origin in the rest of the experiments (Fig 1).

It is known that 70 % of transcriptome is polyadenylated (Clegg and Pikó, 1983), to detect this subpopulation we used fluorescently (CY5) labeled oligo-d(T) probe to detect RNA localization in the oocyte and 2-cell embryo. We observed similar localization of poly(A) RNA as with RCA method (Fig 1). Similarly degradation of RNA by RNase A treatment significantly decrease fluorescence signal (Fig 1). Next, we used for visualization of global transcriptome antibody against 5' UTR cap which binds to both m3G-cap and m7G-cap structures (Bochnig et al., 1987). Immunocytochemistry (ICC) of m3G-cap and m7G-cap shows similar RNA distribution in both stages as with RCA and RNA FISH methods (Fig 1). In addition using ICC with antibody against 5.8S ribosomal RNA (Elela and Nazar, 1997; Lerner et al., 1981), as a structural component of 40S subunit of ribosome we found strong fluorescence signal in the cytoplasm of oocyte and

embryo with significant decrease in the nucleoplasm of both stages (Fig 1). Treatment by RNase A in all experiments leads to the loss of fluorescent signal (Fig 1).

Using direct detection of whole transcriptome and by RCA, RNA FISH and ICC we were able to detect localization of various RNA types and markers in the mammalian oocyte and early embryo.

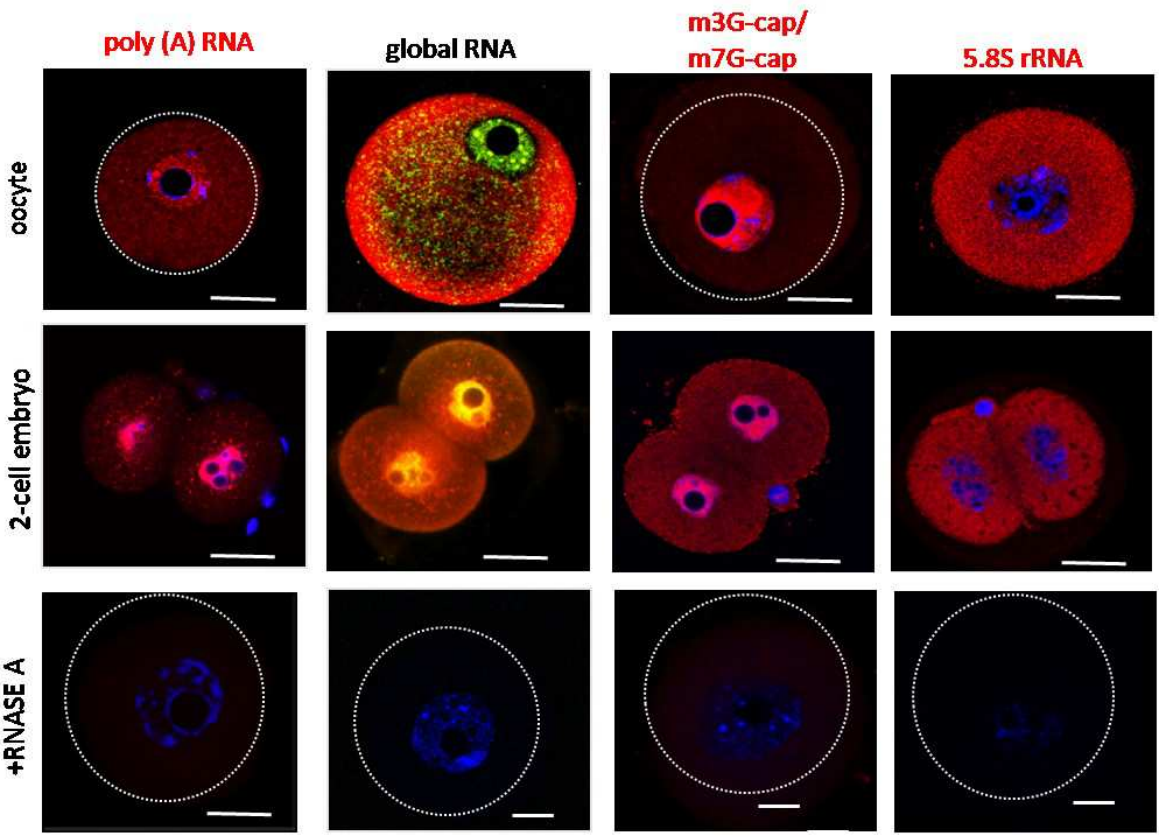



***Figure 1. Localization of transcriptome in oocyte and embryo***

Single Z from confocal images of GV (germinal vesicle) oocytes stages and 2-cell embryos. Rolling circle amplification FISH using random hexamers shows distribution of global RNA (red and green). RNA FISH detecting poly(A) RNA subpopulation (red). Antibody against m3G-cap/m7G-cap at the 5'UTR (red). Distribution of 5.8S rRNA in the oocyte and early embryo (red). White line indicates oocyte cortex, representative images of at least three independent experiments are shown. DNA stained by DAPI (blue); n≥3. As a negative control RNase A digestion was used after cell permeabilization step.

## Identification of specific RNAs in oocyte and embryo

Subcellular localization of specific RNA species might lead to their potential molecular role in such large cells. To visualize specific RNAs we used single molecular RNA FISH (smRNA FISH). First, we determine localization of Depleted in azoospermia-like RNA (*Dazl*) – a germ cell specific transcript. smRNA FISH shows even distribution of the mRNA in the cytoplasm and weak signal in the nucleoplasm of oocyte and early embryo (Fig 2). Mouse fibroblasts NIH3T3 and RNase A treatment shows no fluorescence signal (Fig 2) which supports specificity of our detection in the oocyte and early embryo. Another mRNA detected was β-*Actin* which shows presence in both subcellular compartments, nucleus and cytoplasm of the oocyte and dot-like structures

in the cytoplasm of the embryo (Fig 2). The localization in the NIH3T3 is mostly at the leading edge (Fig 2). Next we detected long noncoding RNA *Neat2* (Nuclear-Enriched Abundant Transcript 2) which is known to be localized in the nuclear speckles (Miyagawa et al., 2012). *Neat2* is exclusively localized in nucleus of GV oocyte and NIH3T3 however in embryo is localized in the cytoplasm (Fig 2). RNase A treatment shows no fluorescence signal (Fig 2) which supports specificity of our detection in the oocyte and early embryo. By detection of specific RNAs we show localization of mRNAs and lncRNA in the various cell types.

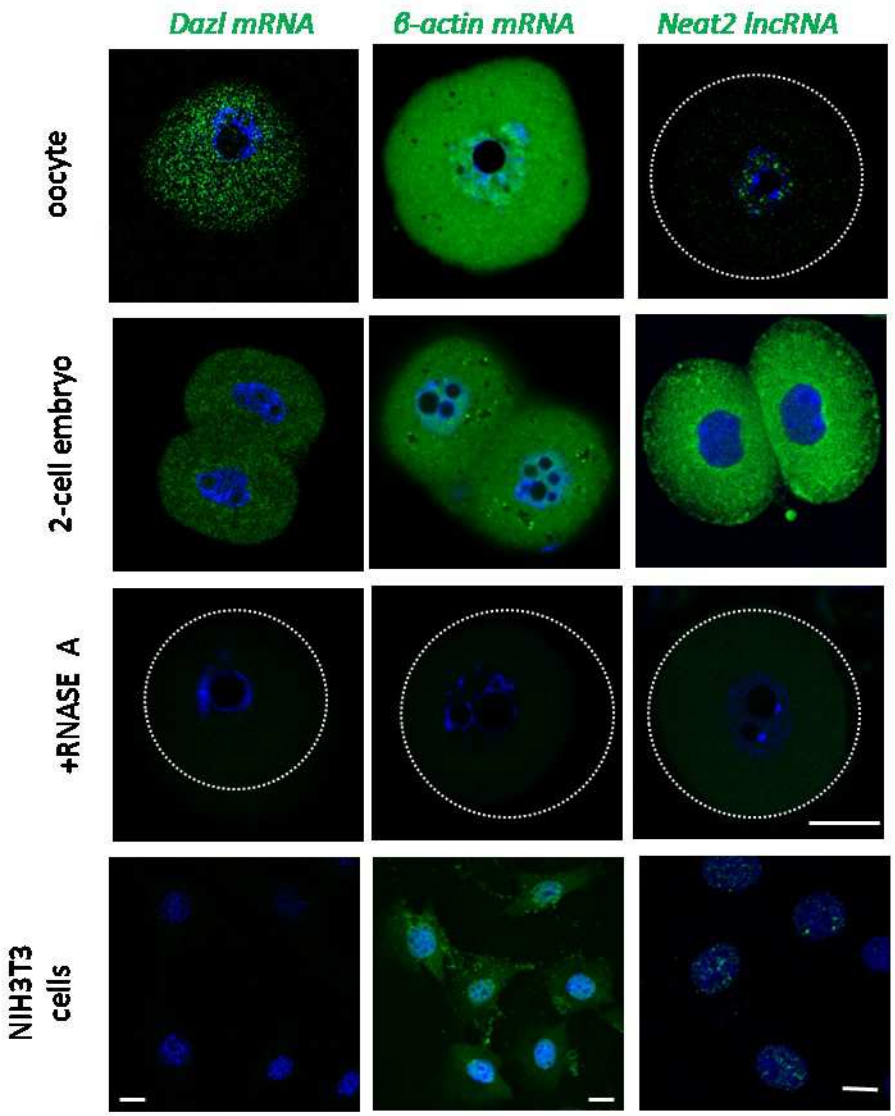
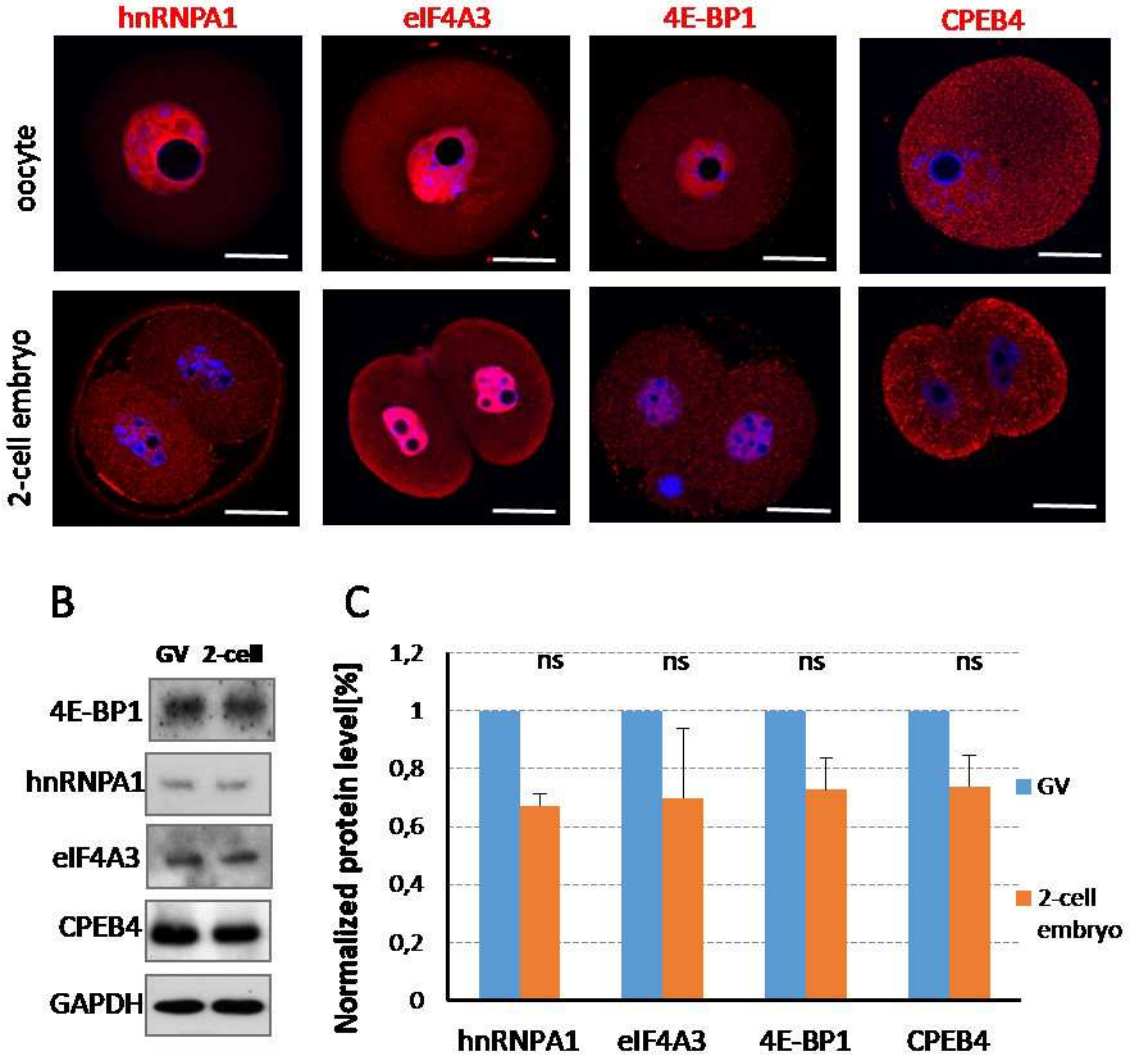


***Figure 2. Localization of specific RNAs in GV oocyte, 2-cell embryo and NIH3T3***
Confocal images of smRNA FISH for *Dazl* and B-*actin* mRNAs and *Neat2* lncRNA (green). White line indicates oocyte cortex, representative images of at least three independent experiments are shown. Representative images of at least three independent experiments are shown. DNA stained by DAPI (blue); n≥2. As a negative control RNase A digestion was used after cell permeabilization. Scale bar 20 μm.

## Expression and localization of RNA binding proteins in oocyte and 2-cell embryo

For RNA metabolism and localization RBPs are essential (Deshler et al., 1998; Tolino et al., 2012; Zhang et al., 2007). Firstly we analyzed localization of ubiquitously binding RBP, heterogeneous nuclear ribonucleoproteins (hnRNP) which participate in pre-mRNA processing and are important determinants of mRNA export localization, translation, and stability (Dreyfuss et al., 2002). Heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) is localized at cortex of the cytoplasm and in the nucleus of the oocyte (Fig 3A), however in the embryo cortical pattern disappears and is localized to whole volume of the cells (Fig 3A). Next we analyzed distribution of exon junction complex protein eIF4A3 which is deposited to mRNA during splicing and released during first round of translation (Chan et al., 2004; Shibuya et al., 2004). ICC shows that eIF4A3 localize mostly to the nucleus in oocyte and embryo (Fig 3A). 5'UTR binding protein 4E-BP1 which functions as a repressor of cap dependent translation (Gingras et al., 1999; Jansova et al., 2017; Romasko et al., 2013). 4E-BP1 shows granular structure in the cytoplasm with significant increase in the nucleoplasm (Supplemental Fig SI 3). RNase A treatment disrupts granular pattern in the oocyte (Supplemental Fig SI 3). Another targeted RBP is CPEB4 which is responsible for meiotic progression between MI and MII and regulates cytostatic factor in the Xenopus oocyte (Igea and Méndez, 2010). CPEB4 shows granular localization in the whole volume of the cells (Fig 3A). Next we used WB to validate antibodies and we quantified the expression levels of the analyzed RBPs (Fig 3B). Quantification of WB doesn't show decrease of level of all studied proteins in the embryo (Fig 3C).

***Figure 3. Localization and expression of RNA binding proteins in GV oocyte and 2-cell embryo***
**A)** Single Z of confocal images of oocytes and embryos probed with, hnRNPA1, eIF4A3, 4E-BP1 and CPEB4 (red). Representative images of at least three independent experiments are shown. DNA stained by DAPI (blue); n≥2. Scale bar 20 μm. **B**) Representative images from WB for hnRNPA1, eIF4A3, 4E-BP1, CPEB4 and loading control, GAPDH. n≥3. **C**) Quantification of hnRNPA1, eIF4A3, 4E-BP1 and CPEB4 expression in the oocyte and embryo. Data are represented as the mean±s.d.; values obtained for GV stage were set as 100%; asterisk denotes statistically significant differences (Student's t-test $P<0.005$); n=3.




**Localization of translational machinery**

To characterize the expression of ribosomal proteins in GV oocyte and 2-cell embryo, we selected components of 40S ribosomal subunit – ribosomal proteins S14 (RPS14) and S3 (RPS3) and phosphorylated RPS6 on S235/236; and components of 60S subunit – ribosomal protein L7 (RPL7) and L24 (RPL24). Both subunits form eukaryotic ribosome (Ben-Shem et al., 2011), a large molecular machine that catalyze the synthesis of proteins. ICC analysis shows that RPS14 is localized at the cortex of oocyte and embryo and in the nucleoplasm of oocyte (Fig 4A). However in the 2-cell embryo we found in the nucleoplasm and cytoplasm significant decrease of the fluorescence, we confirmed this result by immunoblot (Fig 4C). RPS3 protein is localized evenly in both compartments nucleus and cytoplasm in the oocyte and embryo (Fig 4A). The phosphorylation of RPS6 on S235/236 enhances its affinity for the cap structure, which strongly implies that RPS6 phosphorylation enhances mRNA translation initiation (Roux et al., 2007). Phosphorylated protein RPS6(S235/236) is localized in the whole cytoplasm with decreased presence in the nucleoplasm in the oocyte and embryo (Fig 4A). RPS6(S235/236) significantly decrease their expression in the 2-cell embryos (Fig 4C). Protein RPL7 is distributed

evenly in the cytoplasm of oocyte and embryo with high intensity in the nucleus of the oocyte (Fig 4A). We observed significant decrease presence of the protein in the nucleus of the oocyte (Fig 4A). RPL24 showed even distribution in the cytoplasm without presence in the nuclei (Fig 4A). By WB analysis we were able to detect and quantified all selected ribosomal components in the oocytes and embryos ($P<0.05$) (Fig 4B, C). By combination of ICC and WB analyses we were able to localize and quantify expression of the components of the eukaryotic ribosome in the oocytes and embryos.

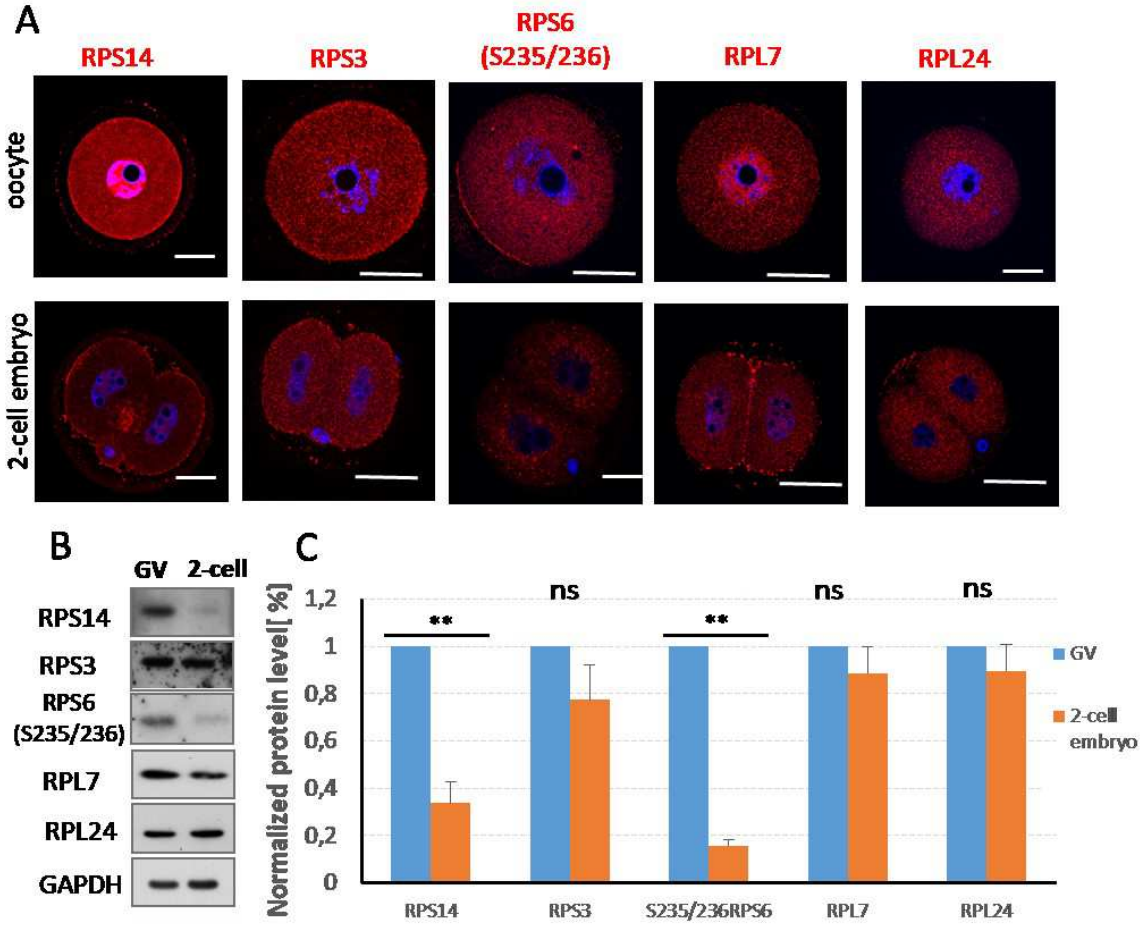



***Figure 4. Localization of ribosomal proteins and their expression***

**A)** Confocal images of oocyte and embryo probed with RPS14, RPS3, RPS6(S235/236), RPL7 and RPL24 antibodies (red). White line indicates oocyte cortex, representative images of at least three independent experiments are shown. DNA stained by DAPI (blue); n≥3. Scale bar 20µm. **B)** Representative images from WB for RPS14, RPS3, RPS6(S235/236), RPL7, RPL24 and loading control GAPDH.; n ≥3. **C)** Quantification of RPS14, RPS3, RPS6(S235/236), RPL7 and RPL24 expression in the oocyte and embryo. Data are represented as the mean±s.d.; values obtained for NEBD stage were set as 100%; asterisk denotes statistically significant differences (Student's t-test: $P<0.05$; n≥3).

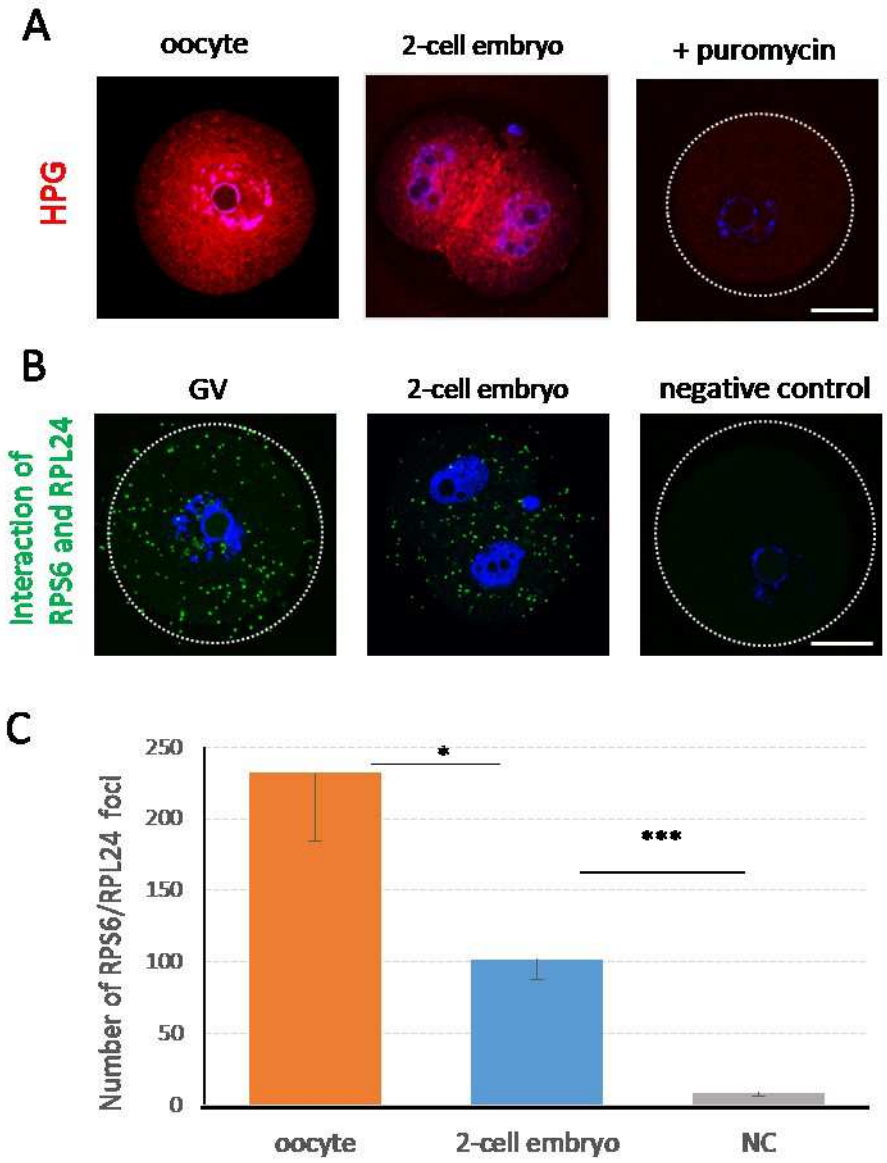
## Nascent translation in GV stage versus 2-cell embryo

To detect nascent translation *in situ* we used methionine analog L-homopropargylglycine (HPG) which incorporate to the translated proteins during short

30 min cultivation period following click-it protocol to fluorescently label HPG in the cell (Dieterich et al., 2010). We detected fluorescent signal of HPG in the whole oocyte increased in the perinuclear area (Fig 5A), while in 2-cell embryo we detected strong signal at dividing ridge of blastomeres. As expected, disruption of the ribosomes by puromycin decreased the intensity of HPG signal in GV oocyte (Fig 5A). To detect assembled 80S in the oocyte and embryo we used Duolink *in situ* proximity ligation assays (PLA) (Benesova et al., 2016; Söderberg et al., 2008) with RPL24 and RPS6 specific antibodies. The positive interaction of the RPL24 and RPS6 ribosomal proteins suggests completion of 80S ribosome and ongoing translation. We detect significant interactions of these two proteins in the cytoplasm of oocyte and 2-cell embryo (Fig 5B). Quantification of the RPL24 and RPS6 interaction foci shows 69% ($p \leq 0.05$) decrease of interaction in the embryo in comparison with oocyte (Fig 5B). Using only RPS6 antibody didn't show fluorescence signal in the negative control (Fig 5A).Combination of these methods allow us visualization of the *in situ* translation in the GV oocytes and 2-cell embryos.

***Figure 5. Detection of in situ translation in oocyte and embryo***

A) Single Z from confocal microscope shows HPG signal (red). Addition of Puromycin to the culture medium serves as a negative control of the HPG incorporation. White line indicates oocyte cortex, representative images of at least three independent experiments are shown. DNA stained by DAPI (blue). Scale bar 20µm. **B**) Fluorescent signal indicates RPL24/RPS6 interaction (green) in the oocyte and embryo and negative control with single RPS6 antibody. DNA stained by DAPI (blue); $n \geq 3$. Scale bar 20µm. **C**) Graph show quantification of RPL24/RPS6 interactions in the whole cell volumes. Values obtained for GV stage were set as 100%; asterisk denotes statistically significant differences (Student's t-test: $P \leq 0.05$).




**Localization of global RNA and RBPs in human oocyte**

To study similarity between mouse and human oocytes we analyzed localization of the global transcriptome by visualization of the poly(A) RNA population. We found that poly(A) RNA fluorescence signal is distributed evenly in the cytoplasm and nucleoplasm with presence of the abundant poly(A) RNA foci in the cytoplasm and nucleus of human oocyte (Fig 6A). Next we performed ICC labeling of RBPs, 4E-BP1 (green) and eIF4A3 (red) where we found similar localization as it is in the mouse oocyte (Fig 6B). 4E-BP1 is distributed in the cytoplasm and nucleoplasm. On the other hand eIF4A3 is localized in the nucleoplasm (Fig 6B). By detection of the transcriptome and RBPs we found similar localization of the selected transcriptome markers in both mammalian species.

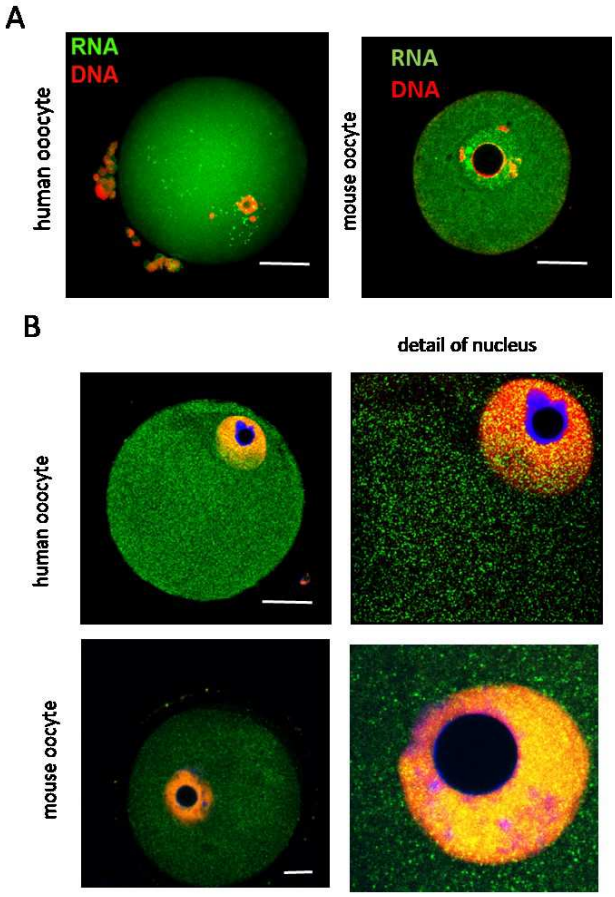



***Figure 6. Localization of poly(A) RNA population and RBPs in human and mouse oocytes.***
**A**) Single Z confocal images of GV (germinal vesicle) stage of human and mouse oocytes labeled by oligo(dT) probe to detect poly(A) RNA probe (green), DNA (red); n≥3. Scale bar 20µm. **B**) Confocal images from ICC shows localization of the 4E-BP1 (green) and eIF4A3 (red) in the human and mouse GV oocytes. DNA stained by DAPI (blue); n≥2. Scale bar 20µm.

## Visualization of translation of endogenous β-*actin* mRNA in live oocyte

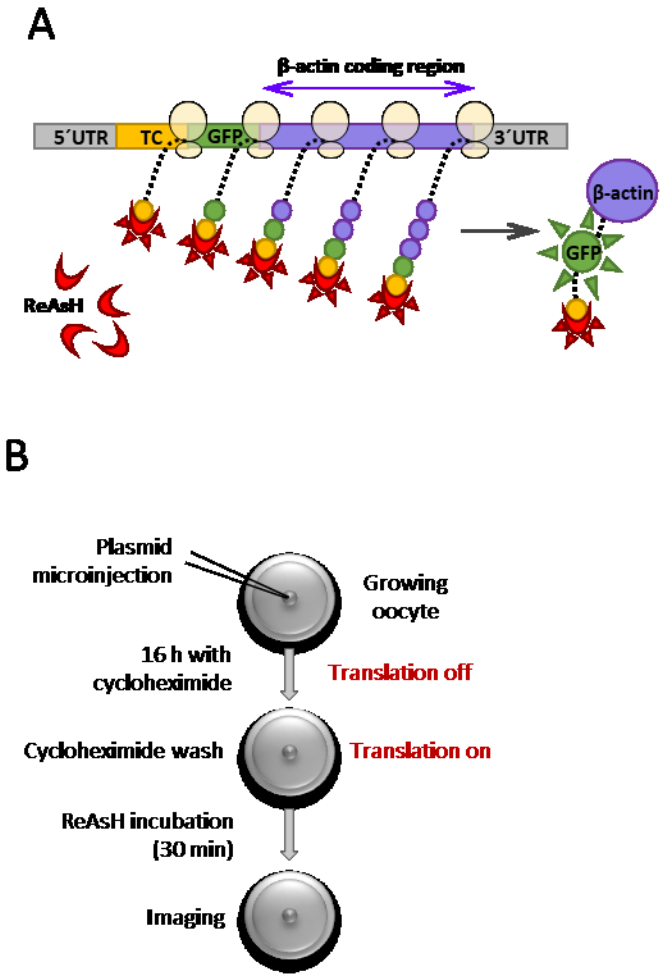
To detect *in situ* translation of the specific transcript (β-a*ctin*) we used ReAsH method (Fig 7A); (Machleidt et al., 2007). Plasmid coding tetra-cysteine (TC) domain at the 5'UTR of the β-actin ORF and EGFP at 3'UTR (Rodriguez et al., 2006) was injected to the nucleus of transcriptionally active oocyte (Fig 7B). Overnight cultivation of oocytes leads to transcription of the construct, which mimics endogenous *β-actin* mRNA. Next, oocytes were washed from cycloheximide (CHX) and cultured with ReAsH dye (Fig 7B) for 30 min. Following wash of the dye live oocytes were visualized by confocal microscope. We found translation of the β-actin in patches at the cortex of the oocyte where ReAsH and EGFP fluorescence is colocalized (Fig 7C, D). Quantification of the images shows 7-fold increase of fluorescence intensity of the ReAsH and EGFP in the patches at cortex (Fig 7D) in comparison with other areas of the cell and negative control

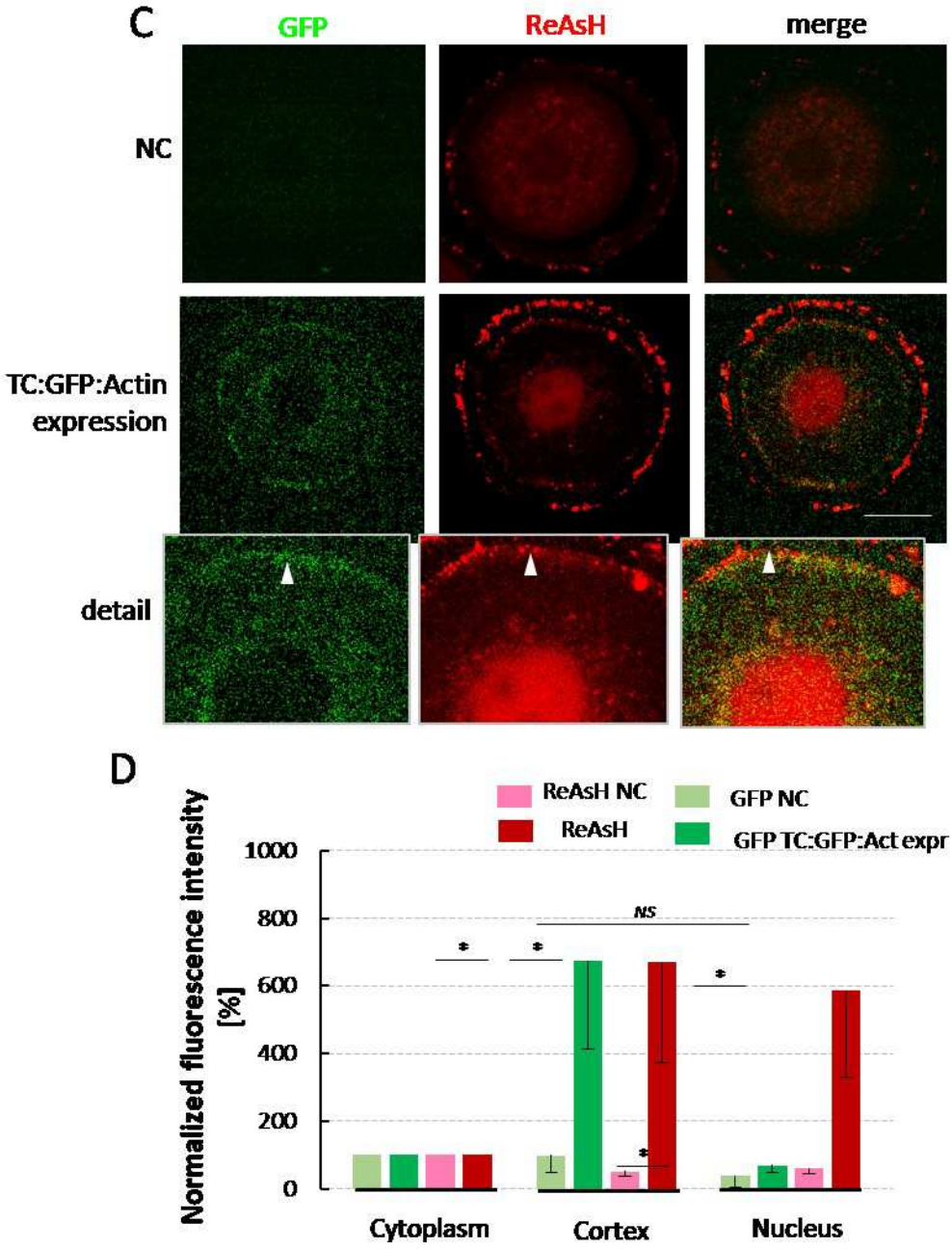
(no injected oocytes). However, we detect high presence of the ReAsH dye in the nucleus without EGFP (Fig 7B), which might suggest nonspecific incorporation or unknown process in the oocyte (Reid and Nicchitta, 2012a).

We were able to detect of the translation of the endogenous β-*actin* mRNA in the living oocyte which resemble known localization of filamentous actin in these cells (Supplemental Fig SI 7; (Azoury et al., 2009).

***Figure 7. Visualization of endogenous β-actin mRNA in oocyte.***

**A**) Scheme of the ReAsH protocol. **B**) Scheme of the experimental procedures to detect translation of the β-*actin* mRNA. **C**) Confocal images of negative control and microinjected oocyte with TC:GFP:Actin plasmid. ReAsH dye labels TC tag (red) of translated β-actin and EGFP (green). Arrowheads depict nascent translation of β-*actin RNA and EGFP,* n≥10. Scale bars 20μm. **D**) Quantification of the fluorescence intensity in the cytoplasm, cortex and nucleus. Data are represented as the mean±s.d.; values obtained for GV stage cytoplasm were set as 100%; asterisk denotes statistically significant differences (Student's t-test: *P value* ≤ *0.05*, bars with NS are not significant; n ≥ 3 independent experiments).






## Discussion

Post-transcriptional control of gene expression at the level of translation has been shown to be essential for regulating a number of cellular processes during development (Curtis et al., 1995; Jansova et al., 2017). This is especially true in mammalian oocytes which, after a transcriptionally active period during their growth, resume meiosis during a period of transcriptional quiescence with a store of maternally synthesized RNAs. Progression through meiosis and early embryogenesis is therefore regulated in the oocyte at the level of mRNA stabilization, translation and post-translational modification. In this study we detect global transcriptome in the mammalian GV oocyte and 2-cell embryo as a prerequisite for protein synthesis. Our results to locate the global transcriptome using

RCA, poly(A) and RNA antibodies subpopulation shows similar pattern of RNA distribution in the cytoplasm and in the nucleus of the transcriptionally inactive oocyte (Susor et al., 2015) and active 2-cell stage embryos (Schultz, 1993). Our results lead us to the conclusion that transcriptionally inactive nucleus might serve as a storage of the translationally dormant mRNAs which might be translated when nuclear envelope breakdown after resumption of meiosis. Our data from RNA FISH or immunocytochemistry are in positive correlation of the previously published result (Jansova et al., 2017; Susor et al., 2015). In addition to localization of global transcriptome in the nucleus, we detect presence in the nucleoplasm of specific mRNAs *Dazl* and β-*actin.* On the other hand *Neat2* lncRNA and other species of this class are known to be localized to the nuclei of other cell types (Cabili et al., 2015; Miyagawa et al., 2012). Despite of the published findings about translation in the nuclei of the cells (Belgrader et al., 1993; Reid and Nicchitta, 2012b), component of the translating ribosome 5.8S rRNA (Ford et al., 1999) is distributed in the cytoplasm, which suggest that translational dormancy of the RNA is present in the nucleus. In addition, translational repressor 4E-BP1 is active in the both stages (Jansova et al., 2017) and is also localized in the nuclei. Localization of the 4E-BP1 in the 2-cell embryo might have a role in the post-transcriptional regulation (Siemer et al., 2009), of the newly synthetized RNA upon embryonic gene activation (Hamatani et al., 2004). On the other hand eIF4A3 protein has nucleus localization signal sequence (Shibuya et al., 2006) which might be responsible for localization of the protein after the pioneer round of translation (Maquat et al., 2010) or they might be shuttled to the nucleus by another mechanism (Rebane et al., 2004). Key player in the polyadenylation of the maternal transcripts are CPEB proteins (Eliscovich et al., 2008; Groisman et al., 2000; Richter, 2007). Our previous results in mammalian oocyte (Komrskova et al., 2014) shows that CPEB1 is degraded after resumption of meiosis and Igea and Méndez, (2010) proposed that CPEB4 is accumulated during second meiotic division and thus substitute degraded CPEB1 in *Xenopus* oocyte. These authors have suggested a mechanism implying that CPEB4 replaces later polyadenylation events in the *Xenopus* oocyte. We found that CPEB4 is abundant in the oocyte prior to resumption of meiosis when CPEB1 is abundant and functional, which suggest different mechanism of the CPEBs in the mammalian oocyte.

To detect localization and expression of the translational machinery in oocytes and embryo we found that studied proteins are present in the cytoplasm and in the nucleus which suggest known mechanism of the ribosome biogenesis in the nucleus (David et al., 2012; Xue and Barna, 2012). We found that selected RBPs are expressed in the same level in the oocyte and embryo. However ribosomal components RPS14 and phosphorylation of RPS6(S235/236) shows significant reduction in the 2-cell embryos. On the other hand ribosomal components RPS3, RPL7 and RPL24 did not show significant changes in the embryos. It is accepted that maternal components (RNA, proteins, organelles) after fertilization become eliminated (Alizadeh et al., 2005; Su et al., 2007). Ellederova et al., (2006) and Susor et al., (2008) showed decrease of the global translation in the oocyte during meiosis and early embryo development, which supports our results generated by PLA where in the 2-cell embryos have 69% ($P \leq 0.05$) downregulated 80S assembly.

We propose that studied components of the translational machineries are essential for the oocyte and embryo development (Jansova et al., 2017; Romasko et al., 2013; Susor et al., 2015). Monti et al., (2013) show that RPL24 and RPS6 are transcribed at the end of oocyte's transcriptional activity and are essential for the oocyte to acquiring developmental competence. (Ellederova et al., 2006; Schultz et al., 1978; Tomek et al., 2002) also show that despite a decrease in overall protein synthesis in the mammalian oocyte during meiosis

there is a regulatory program that ensures temporal and spatial synthesis of specific proteins essential for meiotic progression and embryo development.

Although human oocyte is extremely valuable as the gold standard for assessing clinical relevance, using this cell is limited in several ways. By starting with the identification of localization of transcriptome and RBPs in the mouse oocyte and application of selected markers to human oocyte, we found similar localization of poly(A) RNA, 4E-BP1 and eIF4A3 in both mammalian species which might suggest similar RNA metabolism in human and mouse GV oocyte.

Our findings provide a fundamental insight into cellular architecture and metabolism of maternal RNAs in oocytes and embryos from two different mammalian species, mouse and human. We proposed view of localization of ribosomal protein, which revealing unique and unexpected roles for the translation machinery itself in directing fundamental aspects for oocyte and early embryo development.

# 4 Conclusions of thesis

- Nuclei of both mouse and human oocytes contain RNA and RNA binding proteins.
- RNA molecules retained in the nucleus are translated after the resumption of meiosis manifested by nuclear envelope breakdown.
- RNA metabolism in both human and mouse oocyte is regulated by similar mechanisms.
- Asymmetric localization of RNA regulates spatial and temporal translation in mammalian oocyte.
- Cap dependent translation is become highly active at the onset of meiosis and deactivated after fertilization.
- mTOR–eIF4F pathway is essential for meiotic spindle assembly and genome integrity of mouse oocyte.
- Cyclin-Dependent Kinase 1 regulates mTOR- eIF4F pathway in mouse oocyte.
- 4E-BP1 is responsible for regulation of translation at the meiotic spindle.
- Some components of translation machinery are degraded in early embryo, e.i RPS14 and RPS6.

# 5 Discussion

We unveil function of the mTOR–eIF4F pathway in temporal and spatial translational control, suggesting a novel set of regulatory mechanisms ensuring specific gene expression at the right place and time in the mammalian oocyte (Jansova et al., 2017; Susor et al., 2015). The positioning of mRNAs is required for the localized translation of proteins. mRNA localization can be more thermodynamically efficient for the cell rather than transporting proteins because fewer mRNA molecules needed to be mobilized (Weatheritt et al., 2014). Moreover it is also a very efficient way to orchestrate cellular processes during development of oocyte and embryo. Altogether, our findings indicate that a nuclear RNA population contributes to mammalian oocyte translational patterning and thus to the regulation of gene expression during the dynamic onset of meiosis. We show that nucleus of mouse GV oocyte contains large amount of polyadenylated RNA together with noncoding RNA and RNA binding proteins. We found that similar localization pattern persists in the human oocyte. Moreover, in this work is highlighted for the first time the importance of nuclear-retained specific mRNAs for the control and regulation of meiotic maturation. In our experiments it was found that mouse and human oocytes share similar mechanism of translation regulation, that suggest our localization both 4E-BP-1 (translation repressor) and eIF4A3 (initiation factor, EJC component) proteins mostly into nucleus and cytoplasm. In addition, retained transcripts in oocytes from both species are dormant and become translated after nuclear envelope breakdown. Inhibition of mTOR has been found to prevent dissociation of 4E-BP1 from the initiation factor eIF4E and to correlatively suppress a burst of cap dependent protein synthesis occurring at the resumption of meiosis. Our results show that this mechanism of RNA metabolism is important for meiotic spindle assembly.

Jambor et al., (2015) and Lécuyer et al., (2007) detected dozens of mRNAs asymmetrically localized in spindle, nucleus and perinuclear region. By modifying the protocol of RNA FISH (Raj et al., 2008; Shaffer et al., 2013) and RCA (Larsson et al., 2010) our experiments successfully allowed to visualize global population of RNA in the nucleus of GV oocyte.

We investigated the possible roles of Lamin A/C at translational chromosomal area during 2 hours post IBMX wash. Our data are consistent with localization by Dalton and Carroll, (2013) and FitzHarris et al., (2007). We detected Lamin A/C and ER around the forming spindle in the ICC experiments suggesting their value after NEBD for a proper cell division. Furthermore, both ER and Lamins are likely to be involved in the formation of the boundary between the two distinct translational areas and probably ensure physical separation of the chromosomes from the rest of the cytoplasm during early stages of meiosis after NEBD.

Our idea presents lamins and other structures as a semipermeable barrier which is important for maintenance of intracellular gradient in this large cell. Function of semipermeable barrier was previously known in mitotic cells (Schlaitz et al., 2013). The function of the semipermeable barrier is to retain nuclear components (e.g RNA, chromosomes) where multipolar spindle is formed. Nucleoplasm has a pivotal role in establishment of developmental competence, as shown in the experiments with nucleotransfer of using cytoplasts from fully gown oocytes by Polanski et al., (2005).

In those experiments nucleotransfer into GV cytoplast didn't yield successful reprograming events for development, however cytoplast from MII was competent to establish embryo.

Detection of nascent translation shows presence of increased translational activity at newly forming spindle. Therefore, in this work, it is proposed that local translation is crucial for synthesis of proteins essential for spindle assembly. Despite of local translation, it is important to note the contribution of transported of proteins from other cellular compartments. Our results indicate that mTOR-eIF4F axis has contribution to the local translation which is observed at the newly forming spindle in vicinity of chromosomes. These data are consistent with localization of abundant population of polyadenylated RNA. It is important to note that activity of mTOR-eIF4F is significantly increased after NEBD and a high decline was measured after fertilization, which positively correlate with CDK1 activity. In Jansova et al., (2017) we described that CDK1 (key component of MPF) has positive effect on the phosphorylation of mTOR at the onset of meiotic resumption. This way mTOR is activated with its subsequent function on cap dependent translation. Moreover my results did not detect 4E-BP1 phosphorylation in mitotically inactive cummular cells but it was detected in the oocyte entering to the prometaphase suggesting cell cycle dependent role of the mTOR-eIF4F axis. These findings support results from previous studies which describe significant increased activity of cap dependent translation at onset of mitosis (Gwinn et al., 2010; Heesom et al., 2001). However, is contradictory with earlier results (Pyronnet et al., 2000) which proposed inactivation of cap dependent translation upon entry to mitosis which might be explained by side effect of cell synchronization protocol. Downregulation of mTOR-eIF4F leading to chromosomal aberration (oocyte aneuploidy) supports our hypothesis about the role of mTOR-eIF4F pathway regulating translation at the vicinity of chromosomes during NEBD. Our hypothesis of role mTOR-eIF4F axis in the meiosis supports downregulation of axis which leads to the chromosomal aberration and results in the oocyte aneuploidy.

This thesis is also focused on detection of global and specific RNA, RNA binding proteins and ribosomal proteins in oocyte and two cell embryo. We confirmed that some of the ribosomal proteins, which are important for translation are degraded after fertilization. These results positively correlate with the decrease of global translation (Schultz, 1993).

We decided to study 4E-BP1, because it is translation repressor of cap dependent translation. We found that 4E-BP1 indeed binds to RNA as after adding RNase A, it lost its granular pattern in the oocyte.

We confirmed that fine-scale spatial and temporal control of translation can have more rapid and subtle effects on the microenvironment of the oocyte than transcriptional regulation.To conclude this thesis, we believe that experiments contribute to elucidate the molecular mechanisms of utilization of maternal transcriptome, which are necessary for regulation of translation and consequently for development of oocyte and early embryo. Moreover, this thesis proposes new molecular mechanisms of gene expression in this unique cell types. It is conceivable that in oocytes the RNA population retained in the nucleus might contribute to translation in dynamic area, where new spindle is assembled in the machinery, enabling the segregation of meiotic chromosomes. The Western blot data indicates that CDK1 influences the activity of mTOR in mouse oocytes suggesting that CDK1 acts indirectly on 4E-BP1 phosphorylation via mTOR activation. These results positively correlate with reprograming of cap dependent translation (Jansova et al., 2017;

Susor et al., 2015), which suggest it is an essential mechanism for successful progression through the cell cycle.

However, some points remain to be clarified. The fact that CDK1 is a positive regulator of 4E-BP1 during mouse oocyte maturation will add fuel to the complicated debate about the link between translational activity and the cell cycle. The investigation of oocyte and early embryo simultaneously rise a new interesting questions that can be solved in the future. Which mRNAs are actively translated in this spatial and temporal context? Further work remains to determine the function of RNP complexes in oocytes and their functional relationship to RNA stability.

# 6 References


Adams, R.L., Terry, L.J., Wente, S.R., 2014. Nucleoporin FG domains facilitate mRNP remodeling at the cytoplasmic face of the nuclear pore complex. Genetics 197, 1213–1224. doi:10.1534/genetics.114.164012

Alizadeh, Z., Kageyama, S.-I., Aoki, F., 2005. Degradation of maternal mRNA in mouse embryos: selective degradation of specific mRNAs after fertilization. Mol. Reprod. Dev. 72, 281–290. doi:10.1002/mrd.20340

Azoury, J., Verlhac, M.-H., Dumont, J., 2009. Actin filaments: key players in the control of asymmetric divisions in mouse oocytes. Biol. Cell Auspices Eur. Cell Biol. Organ. 101, 69–76.

Bachvarova, R., 1981. Synthesis, turnover, and stability of heterogeneous RNA in growing mouse oocytes. Dev. Biol. 86, 384–392.

Bachvarova, R., De Leon, V., Johnson, A., Kaplan, G., Paynton, B.V., 1985. Changes in total RNA, polyadenylated RNA, and actin mRNA during meiotic maturation of mouse oocytes. Dev. Biol. 108, 325–331.

Belgrader, P., Cheng, J., Maquat, L.E., 1993. Evidence to implicate translation by ribosomes in the mechanism by which nonsense codons reduce the nuclear level of human triosephosphate isomerase mRNA. Proc. Natl. Acad. Sci. U. S. A. 90, 482–486.

Benesova, V., Kinterova, V., Kanka, J., Toralova, T., 2016. Characterization of SCF-Complex during Bovine Preimplantation Development. PloS One 11, e0147096. doi:10.1371/journal.pone.0147096

Ben-Shem, A., Garreau de Loubresse, N., Melnikov, S., Jenner, L., Yusupova, G., Yusupov, M., 2011. The structure of the eukaryotic ribosome at 3.0 Å resolution. Science 334, 1524–1529. doi:10.1126/science.1212642

Blower, M.D., Feric, E., Weis, K., Heald, R., 2007. Genome-wide analysis demonstrates conserved localization of messenger RNAs to mitotic microtubules. J. Cell Biol. 179, 1365–1373. doi:10.1083/jcb.200705163

Bochnig, P., Reuter, R., Bringmann, P., Lührmann, R., 1987. A monoclonal antibody against 2,2,7-trimethylguanosine that reacts with intact, class U, small nuclear ribonucleoproteins as well as with 7-methylguanosine-capped RNAs. Eur. J. Biochem. 168, 461–467.

Braude, P., Bolton, V., Moore, S., 1988. Human gene expression first occurs between the four- and eight-cell stages of preimplantation development. Nature 332, 459–461. doi:10.1038/332459a0

Brunet, S., Verlhac, M.H., 2011. Positioning to get out of meiosis: the asymmetry of division. Hum. Reprod. Update 17, 68–75. doi:10.1093/humupd/dmq044

Burd, C.G., Dreyfuss, G., 1994. RNA binding specificity of hnRNP A1: significance of hnRNP A1 high-affinity binding sites in pre-mRNA splicing. EMBO J. 13, 1197–1204.

Bushati, N., Cohen, S.M., 2007. microRNA functions. Annu. Rev. Cell Dev. Biol. 23, 175–205. doi:10.1146/annurev.cellbio.23.090506.123406

Cabili, M.N., Dunagin, M.C., McClanahan, P.D., Biaesch, A., Padovan-Merhar, O., Regev, A., Rinn, J.L., Raj, A., 2015. Localization and abundance analysis of human lncRNAs at single-cell and single-molecule resolution. Genome Biol. 16, 20. doi:10.1186/s13059-015-0586-4

Calvert, M.E., Digilio, L.C., Herr, J.C., Coonrod, S.A., 2003. Oolemmal proteomics--identification of highly abundant heat shock proteins and molecular chaperones in

the mature mouse egg and their localization on the plasma membrane. Reprod. Biol. Endocrinol. RBE 1, 27.
Camlin, N.J., McLaughlin, E.A., Holt, J.E., 2017. Kif4 Is Essential for Mouse Oocyte Meiosis. PLoS ONE 12. doi:10.1371/journal.pone.0170650
Chan, C.C., Dostie, J., Diem, M.D., Feng, W., Mann, M., Rappsilber, J., Dreyfuss, G., 2004. eIF4A3 is a novel component of the exon junction complex. RNA 10, 200–209. doi:10.1261/rna.5230104
Chazaud, C., Rossant, J., 2006. Disruption of early proximodistal patterning and AVE formation in Apc mutants. Development 133, 3379–3387. doi:10.1242/dev.02523
Chen, J., Melton, C., Suh, N., Oh, J.S., Horner, K., Xie, F., Sette, C., Blelloch, R., Conti, M., 2011. Genome-wide analysis of translation reveals a critical role for deleted in azoospermia-like (Dazl) at the oocyte-to-zygote transition. Genes Dev. 25, 755–766. doi:10.1101/gad.2028911
Clegg, K.B., Pikó, L., 1983. Poly(A) length, cytoplasmic adenylation and synthesis of poly(A)+ RNA in early mouse embryos. Dev. Biol. 95, 331–341.
Clegg, K.B., Pikó, L., 1982. RNA synthesis and cytoplasmic polyadenylation in the one-cell mouse embryo. Nature 295, 342–345. doi:10.1038/295342a0
Coassin, S.R., Orjalo, A.V., Semaan, S.J., Johansson, H.E., 2014. Simultaneous detection of nuclear and cytoplasmic RNA variants utilizing Stellaris® RNA fluorescence in situ hybridization in adherent cells. Methods Mol. Biol. Clifton NJ 1211, 189–199. doi:10.1007/978-1-4939-1459-3_15
Crosetto, N., Bienko, M., van Oudenaarden, A., 2015. Spatially resolved transcriptomics and beyond. Nat. Rev. Genet. 16, 57–66. doi:10.1038/nrg3832
Cui, X.-S., Li, X.-Y., Yin, X.-J., Kong, I.K., Kang, J.-J., Kim, N.-H., 2007. Maternal gene transcription in mouse oocytes: genes implicated in oocyte maturation and fertilization. J. Reprod. Dev. 53, 405–418.
Curtis, D., Lehmann, R., Zamore, P.D., 1995. Translational regulation in development. Cell 81, 171–178.
Dalton, C.M., Carroll, J., 2013. Biased inheritance of mitochondria during asymmetric cell division in the mouse oocyte. J. Cell Sci. 126, 2955–2964. doi:10.1242/jcs.128744
David, A., Dolan, B.P., Hickman, H.D., Knowlton, J.J., Clavarino, G., Pierre, P., Bennink, J.R., Yewdell, J.W., 2012. Nuclear translation visualized by ribosome-bound nascent chain puromycylation. J Cell Biol 197, 45–57. doi:10.1083/jcb.201112145
De La Fuente, R., Viveiros, M.M., Burns, K.H., Adashi, E.Y., Matzuk, M.M., Eppig, J.J., 2004. Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. Dev. Biol. 275, 447–458. doi:10.1016/j.ydbio.2004.08.028
Debey, P., Szöllösi, M.S., Szöllösi, D., Vautier, D., Girousse, A., Besombes, D., 1993. Competent mouse oocytes isolated from antral follicles exhibit different chromatin organization and follow different maturation dynamics. Mol. Reprod. Dev. 36, 59–74. doi:10.1002/mrd.1080360110
Deshler, J.O., Highett, M.I., Abramson, T., Schnapp, B.J., 1998. A highly conserved RNA-binding protein for cytoplasmic mRNA localization in vertebrates. Curr. Biol. 8, 489–496. doi:10.1016/S0960-9822(98)70200-3
Dieterich, D.C., Hodas, J.J.L., Gouzer, G., Shadrin, I.Y., Ngo, J.T., Triller, A., Tirrell, D.A., Schuman, E.M., 2010. In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. Nat. Neurosci. 13, 897–905. doi:10.1038/nn.2580

Dowling, R.J.O., Topisirovic, I., Alain, T., Bidinosti, M., Fonseca, B.D., Petroulakis, E., Wang, X., Larsson, O., Selvaraj, A., Liu, Y., Kozma, S.C., Thomas, G., Sonenberg, N., 2010. mTORC1-mediated cell proliferation, but not cell growth, controlled by the 4E-BPs. Science 328, 1172–1176. doi:10.1126/science.1187532

Dreyfuss, G., Kim, V.N., Kataoka, N., 2002. Messenger-RNA-binding proteins and the messages they carry. Nat. Rev. Mol. Cell Biol. 3, 195–205. doi:10.1038/nrm760

Drummond, D.R., Armstrong, J., Colman, A., 1985. The effect of capping and polyadenylation on the stability, movement and translation of synthetic messenger RNAs in Xenopus oocytes. Nucleic Acids Res. 13, 7375–7394.

Dumollard, R., Duchen, M., Sardet, C., 2006. Calcium signals and mitochondria at fertilisation. Semin. Cell Dev. Biol., The Wnt Signaling Pathway and Embryonic PolarityCalcium Waves at Fertilization 17, 314–323. doi:10.1016/j.semcdb.2006.02.009

Elela, S.A., Nazar, R.N., 1997. Role of the 5.8S rRNA in ribosome translocation. Nucleic Acids Res. 25, 1788–1794. doi:10.1093/nar/25.9.1788

Eliscovich, C., Peset, I., Vernos, I., Méndez, R., 2008. Spindle-localized CPE-mediated translation controls meiotic chromosome segregation. Nat. Cell Biol. 10, 858–865. doi:10.1038/ncb1746

Ellederova, Z., Halada, P., Man, P., Kubelka, M., Motlik, J., Kovarova, H., 2004. Protein patterns of pig oocytes during in vitro maturation. Biol. Reprod. 71, 1533–1539. doi:10.1095/biolreprod.104.030304

Ellederova, Z., Kovarova, H., Melo-Sterza, F., Livingstone, M., Tomek, W., Kubelka, M., 2006. Suppression of translation during in vitro maturation of pig oocytes despite enhanced formation of cap-binding protein complex eIF4F and 4E-BP1 hyperphosphorylation. Mol. Reprod. Dev. 73, 68–76. doi:10.1002/mrd.20368

Feng, Z., Zhang, H., Levine, A.J., Jin, S., 2005. The coordinate regulation of the p53 and mTOR pathways in cells. Proc. Natl. Acad. Sci. U. S. A. 102, 8204–8209. doi:10.1073/pnas.0502857102

Ferreira, E.M., Vireque, A.A., Adona, P.R., Meirelles, F.V., Ferriani, R.A., Navarro, P. a. a. S., 2009. Cytoplasmic maturation of bovine oocytes: structural and biochemical modifications and acquisition of developmental competence. Theriogenology 71, 836–848. doi:10.1016/j.theriogenology.2008.10.023

FitzHarris, G., Marangos, P., Carroll, J., 2007. Changes in endoplasmic reticulum structure during mouse oocyte maturation are controlled by the cytoskeleton and cytoplasmic dynein. Dev. Biol. 305, 133–144. doi:10.1016/j.ydbio.2007.02.006

Flemr, M., Ma, J., Schultz, R.M., Svoboda, P., 2010. P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. Biol. Reprod. 82, 1008–1017. doi:10.1095/biolreprod.109.082057

Ford, C.L., Randal-Whitis, L., Ellis, S.R., 1999. Yeast proteins related to the p40/laminin receptor precursor are required for 20S ribosomal RNA processing and the maturation of 40S ribosomal subunits. Cancer Res. 59, 704–710.

Fulka, J., Moor, R.M., Fulka, J., 1995. Mouse oocyte maturation: meiotic checkpoints. Exp. Cell Res. 219, 414–419. doi:10.1006/excr.1995.1247

Galili, G., Kawata, E.E., Smith, L.D., Larkins, B.A., 1988. Role of the 3'-poly(A) sequence in translational regulation of mRNAs in Xenopus laevis oocytes. J. Biol. Chem. 263, 5764–5770.

Gall, J.G., Pardue, M.L., 1969. Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc. Natl. Acad. Sci. U. S. A. 63, 378–383.

Gasnier, M., Dennis, C., Vaurs-Barrière, C., Chazaud, C., 2013. Fluorescent mRNA labeling through cytoplasmic FISH. Nat. Protoc. 8, 2538–2547. doi:10.1038/nprot.2013.160
Gebauer, F., Hentze, M.W., 2004. Molecular mechanisms of translational control. Nat. Rev. Mol. Cell Biol. 5, 827–835. doi:10.1038/nrm1488
Ghildiyal, M., Xu, J., Seitz, H., Weng, Z., Zamore, P.D., 2010. Sorting of Drosophila small silencing RNAs partitions microRNA* strands into the RNA interference pathway. RNA 16, 43–56. doi:10.1261/rna.1972910
Giangarrà, V., Igea, A., Castellazzi, C.L., Bava, F.-A., Mendez, R., 2015. Global Analysis of CPEBs Reveals Sequential and Non-Redundant Functions in Mitotic Cell Cycle. PloS One 10, e0138794. doi:10.1371/journal.pone.0138794
Gilbert, W.V., Zhou, K., Butler, T.K., Doudna, J.A., 2007. Cap-independent translation is required for starvation-induced differentiation in yeast. Science 317, 1224–1227. doi:10.1126/science.1144467
Gingras, A.C., Gygi, S.P., Raught, B., Polakiewicz, R.D., Abraham, R.T., Hoekstra, M.F., Aebersold, R., Sonenberg, N., 1999. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev. 13, 1422–1437.
Gingras, A.C., Raught, B., Sonenberg, N., 2001. Regulation of translation initiation by FRAP/mTOR. Genes Dev. 15, 807–826. doi:10.1101/gad.887201
Görlach, M., Burd, C.G., Portman, D.S., Dreyfuss, G., 1993. The hnRNP proteins. Mol. Biol. Rep. 18, 73–78.
Groisman, I., Huang, Y.-S., Mendez, R., Cao, Q., Theurkauf, W., Richter, J.D., 2000. CPEB, Maskin, and Cyclin B1 mRNA at the Mitotic Apparatus: Implications for Local Translational Control of Cell Division. Cell 103, 435–447. doi:10.1016/S0092-8674(00)00135-5
Guil, S., Long, J.C., Cáceres, J.F., 2006. hnRNP A1 Relocalization to the Stress Granules Reflects a Role in the Stress Response. Mol. Cell. Biol. 26, 5744–5758. doi:10.1128/MCB.00224-06
Gwinn, D.M., Asara, J.M., Shaw, R.J., 2010. Raptor is phosphorylated by cdc2 during mitosis. PloS One 5, e9197. doi:10.1371/journal.pone.0009197
Hamatani, T., Carter, M.G., Sharov, A.A., Ko, M.S.H., 2004. Dynamics of Global Gene Expression Changes during Mouse Preimplantation Development. Dev. Cell 6, 117–131. doi:10.1016/S1534-5807(03)00373-3
Håvik, B., Røkke, H., Bårdsen, K., Davanger, S., Bramham, C.R., 2003. Bursts of high-frequency stimulation trigger rapid delivery of pre-existing alpha-CaMKII mRNA to synapses: a mechanism in dendritic protein synthesis during long-term potentiation in adult awake rats. Eur. J. Neurosci. 17, 2679–2689.
Heesom, K.J., Gampel, A., Mellor, H., Denton, R.M., 2001. Cell cycle-dependent phosphorylation of the translational repressor eIF-4E binding protein-1 (4E-BP1). Curr. Biol. 11, 1374–1379. doi:10.1016/S0960-9822(01)00422-5
Hershey, J.W.B., 1991. Translational Control in Mammalian Cells. Annu. Rev. Biochem. 60, 717–755. doi:10.1146/annurev.bi.60.070191.003441
Holt, C.E., Bullock, S.L., 2009. Subcellular mRNA Localization in Animal Cells and Why It Matters. Science 326, 1212–1216. doi:10.1126/science.1176488
Hossain, M.M., Salilew-Wondim, D., Schellander, K., Tesfaye, D., 2012. The role of microRNAs in mammalian oocytes and embryos. Anim. Reprod. Sci. 134, 36–44. doi:10.1016/j.anireprosci.2012.08.009

Huarte, J., Stutz, A., O'Connell, M.L., Gubler, P., Belin, D., Darrow, A.L., Strickland, S., Vassalli, J.D., 1992. Transient translational silencing by reversible mRNA deadenylation. Cell 69, 1021–1030.
Hwang, H.-W., Wentzel, E.A., Mendell, J.T., 2007. A hexanucleotide element directs microRNA nuclear import. Science 315, 97–100. doi:10.1126/science.1136235
Igea, A., Méndez, R., 2010. Meiosis requires a translational positive loop where CPEB1 ensues its replacement by CPEB4. EMBO J. 29, 2182–2193. doi:10.1038/emboj.2010.111
Inoue, A., Nakajima, R., Nagata, M., Aoki, F., 2008. Contribution of the oocyte nucleus and cytoplasm to the determination of meiotic and developmental competence in mice. Hum. Reprod. 23, 1377–1384. doi:10.1093/humrep/den096
Inoue, K., Ohno, M., Sakamoto, H., Shimura, Y., 1989. Effect of the cap structure on pre-mRNA splicing in Xenopus oocyte nuclei. Genes Dev. 3, 1472–1479.
Jambor, H., Surendranath, V., Kalinka, A.T., Mejstrik, P., Saalfeld, S., Tomancak, P., 2015. Systematic imaging reveals features and changing localization of mRNAs in Drosophila development. eLife 4. doi:10.7554/eLife.05003
Jansova, D., 2015. Single Molecule RNA FISH in the Mammalian Oocyte. Bio-protocol, 23 2015, e1666. doi:DOI: https://doi.org/10.21769/BioProtoc.1666
Jansova, D., Koncicka, M., Tetkova, A., Cerna, R., Malik, R., Del Llano, E., Kubelka, M., Susor, A., 2017. Regulation of 4E-BP1 activity in the mammalian oocyte. Cell Cycle Georget. Tex 1–13. doi:10.1080/15384101.2017.1295178
Jean-Philippe, J., Paz, S., Caputi, M., 2013. hnRNP A1: The Swiss Army Knife of Gene Expression. Int. J. Mol. Sci. 14, 18999–19024. doi:10.3390/ijms140918999
Jefferies, H.B., Fumagalli, S., Dennis, P.B., Reinhard, C., Pearson, R.B., Thomas, G., 1997. Rapamycin suppresses 5'TOP mRNA translation through inhibition of p70s6k. EMBO J. 16, 3693–3704. doi:10.1093/emboj/16.12.3693
Jeffery, W.R., Tomlinson, C.R., Brodeur, R.D., 1983. Localization of actin messenger RNA during early ascidian development. Dev. Biol. 99, 408–417.
Kalous, J., Solc, P., Baran, V., Kubelka, M., Schultz, R.M., Motlik, J., 2006. PKB/AKT is involved in resumption of meiosis in mouse oocytes. Biol. Cell Auspices Eur. Cell Biol. Organ. 98, 111–123. doi:10.1042/BC20050020
Karlic, R., Ganesh, S., Franke, V., Svobodova, E., Urbanova, J., Suzuki, Y., Aoki, F., Vlahovicek, K., Svoboda, P., 2017. Long non-coding RNA exchange during the oocyte-to-embryo transition in mice. DNA Res. Int. J. Rapid Publ. Rep. Genes Genomes. doi:10.1093/dnares/dsw058
Kelly, S.M., Corbett, A.H., 2009. Messenger RNA export from the nucleus: a series of molecular wardrobe changes. Traffic Cph. Den. 10, 1199–1208. doi:10.1111/j.1600-0854.2009.00944.x
Kim, J.H., Richter, J.D., 2006. Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. Mol. Cell 24, 173–183. doi:10.1016/j.molcel.2006.08.016
Kim, N.H., Cho, S.K., Choi, S.H., Kim, E.Y., Park, S.P., Lim, J.H., 2000. The distribution and requirements of microtubules and microfilaments in bovine oocytes during in vitro maturation. Zygote Camb. Engl. 8, 25–32.
Kim, T.-H., Leslie, P., Zhang, Y., 2014. Ribosomal proteins as unrevealed caretakers for cellular stress and genomic instability. Oncotarget 5, 860–871. doi:10.18632/oncotarget.1784

King, M.L., Messitt, T.J., Mowry, K.L., 2005. Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. Biol. Cell 97, 19–33. doi:10.1042/BC20040067

Kochan, J., Wawro, M., Kasza, A., 2016. IF-combined smRNA FISH reveals interaction of MCPIP1 protein with IER3 mRNA. Biol. Open 5, 889–898. doi:10.1242/bio.018010

Kochan, J., Wawro, M., Kasza, A., 2015. Simultaneous detection of mRNA and protein in single cells using immunofluorescence-combined single-molecule RNA FISH. BioTechniques 59, 209–212, 214, 216 passim. doi:10.2144/000114340

Kogasaka, Y., Hoshino, Y., Hiradate, Y., Tanemura, K., Sato, E., 2013. Distribution and association of mTOR with its cofactors, raptor and rictor, in cumulus cells and oocytes during meiotic maturation in mice. Mol. Reprod. Dev. 80, 334–348. doi:10.1002/mrd.22166

Komrskova, P., Susor, A., Malik, R., Prochazkova, B., Liskova, L., Supolikova, J., Hladky, S., Kubelka, M., 2014. Aurora kinase A is not involved in CPEB1 phosphorylation and cyclin B1 mRNA polyadenylation during meiotic maturation of porcine oocytes. PloS One 9, e101222. doi:10.1371/journal.pone.0101222

Krieg, J., Hofsteenge, J., Thomas, G., 1988. Identification of the 40 S ribosomal protein S6 phosphorylation sites induced by cycloheximide. J. Biol. Chem. 263, 11473–11477.

Kwon, S., 2013. Single-molecule fluorescence in situ hybridization: Quantitative imaging of single RNA molecules. BMB Rep. 46, 65–72. doi:10.5483/BMBRep.2013.46.2.016

Larsson, C., Grundberg, I., Söderberg, O., Nilsson, M., 2010. In situ detection and genotyping of individual mRNA molecules. Nat. Methods 7, 395–397. doi:10.1038/nmeth.1448

Lécuyer, E., Yoshida, H., Parthasarathy, N., Alm, C., Babak, T., Cerovina, T., Hughes, T.R., Tomancak, P., Krause, H.M., 2007. Global Analysis of mRNA Localization Reveals a Prominent Role in Organizing Cellular Architecture and Function. Cell 131, 174–187. doi:10.1016/j.cell.2007.08.003

Lee, J.H., Daugharthy, E.R., Scheiman, J., Kalhor, R., Ferrante, T.C., Terry, R., Turczyk, B.M., Yang, J.L., Lee, H.S., Aach, J., Zhang, K., Church, G.M., 2015. Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues. Nat. Protoc. 10, 442–458. doi:10.1038/nprot.2014.191

Lerner, E.A., Lerner, M.R., Janeway, C.A., Steitz, J.A., 1981. Monoclonal antibodies to nucleic acid-containing cellular constituents: probes for molecular biology and autoimmune disease. Proc. Natl. Acad. Sci. U. S. A. 78, 2737–2741.

Li, G.-P., Liu, Y., Bunch, T.D., White, K.L., Aston, K.I., 2005. Asymmetric division of spindle microtubules and microfilaments during bovine meiosis from metaphase I to metaphase III. Mol. Reprod. Dev. 71, 220–226. doi:10.1002/mrd.20255

Lin, A.C., Tan, C.L., Lin, C.-L., Strochlic, L., Huang, Y.-S., Richter, J.D., Holt, C.E., 2009. Cytoplasmic polyadenylation and cytoplasmic polyadenylation element-dependent mRNA regulation are involved in Xenopus retinal axon development. Neural Develop. 4, 8. doi:10.1186/1749-8104-4-8

Livingstone, M., Bidinosti, M., 2012. Rapamycin-insensitive mTORC1 activity controls eIF4E:4E-BP1 binding. F1000Research 1, 4. doi:10.12688/f1000research.1-4.v1

Long, R.M., Elliott, D.J., Stutz, F., Rosbash, M., Singer, R.H., 1995. Spatial consequences of defective processing of specific yeast mRNAs revealed by fluorescent in situ hybridization. RNA 1, 1071–1078.

Machleidt, T., Robers, M., Hanson, G.T., 2007. Protein labeling with FlAsH and ReAsH. Methods Mol. Biol. Clifton NJ 356, 209–220.
Maquat, L.E., Tarn, W.-Y., Isken, O., 2010. The Pioneer Round of Translation: Features and Functions. Cell 142, 368–374. doi:10.1016/j.cell.2010.07.022
Maro, B., Verlhac, M.-H., 2002. Polar body formation: new rules for asymmetric divisions. Nat. Cell Biol. 4, E281–E283. doi:10.1038/ncb1202-e281
Marston, A.L., Amon, A., 2004. Meiosis: cell-cycle controls shuffle and deal. Nat. Rev. Mol. Cell Biol. 5, 983–997. doi:10.1038/nrm1526
Martínez-Salas, E., Lozano, G., Fernandez-Chamorro, J., Francisco-Velilla, R., Galan, A., Diaz, R., 2013. RNA-Binding Proteins Impacting on Internal Initiation of Translation. Int. J. Mol. Sci. 14, 21705–21726. doi:10.3390/ijms141121705
Mayer, S., Wrenzycki, C., Tomek, W., 2014. Inactivation of mTor arrests bovine oocytes in the metaphase-I stage, despite reversible inhibition of 4E-BP1 phosphorylation. Mol. Reprod. Dev. 81, 363–375. doi:10.1002/mrd.22305
Memili, E., Peddinti, D., Shack, L.A., Nanduri, B., McCarthy, F., Sagirkaya, H., Burgess, S.C., 2007. Bovine germinal vesicle oocyte and cumulus cell proteomics. Reprod. Camb. Engl. 133, 1107–1120. doi:10.1530/REP-06-0149
Meyuhas, O., Dreazen, A., 2009. Ribosomal protein S6 kinase from TOP mRNAs to cell size. Prog. Mol. Biol. Transl. Sci. 90, 109–153. doi:10.1016/S1877-1173(09)90003-5
Miyagawa, R., Tano, K., Mizuno, R., Nakamura, Y., Ijiri, K., Rakwal, R., Shibato, J., Masuo, Y., Mayeda, A., Hirose, T., Akimitsu, N., 2012. Identification of cis- and trans-acting factors involved in the localization of MALAT-1 noncoding RNA to nuclear speckles. RNA 18, 738–751. doi:10.1261/rna.028639.111
Monti, M., Zanoni, M., Calligaro, A., Ko, M.S.H., Mauri, P., Redi, C.A., 2013. Developmental Arrest and Mouse Antral Not-Surrounded Nucleolus Oocytes. Biol. Reprod. 88, 2. doi:10.1095/biolreprod.112.103887
Müller-McNicoll, M., Neugebauer, K.M., 2013. How cells get the message: dynamic assembly and function of mRNA-protein complexes. Nat. Rev. Genet. 14, 275–287. doi:10.1038/nrg3434
Murakami, M., Ichisaka, T., Maeda, M., Oshiro, N., Hara, K., Edenhofer, F., Kiyama, H., Yonezawa, K., Yamanaka, S., 2004. mTOR Is Essential for Growth and Proliferation in Early Mouse Embryos and Embryonic Stem Cells. Mol. Cell. Biol. 24, 6710–6718. doi:10.1128/MCB.24.15.6710-6718.2004
Navé, B.T., Ouwens, M., Withers, D.J., Alessi, D.R., Shepherd, P.R., 1999. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. Biochem. J. 344, 427–431.
Nothias, J.Y., Majumder, S., Kaneko, K.J., DePamphilis, M.L., 1995. Regulation of gene expression at the beginning of mammalian development. J. Biol. Chem. 270, 22077–22080.
Novoa, I., Gallego, J., Ferreira, P.G., Mendez, R., 2010. Mitotic cell-cycle progression is regulated by CPEB1 and CPEB4-dependent translational control. Nat. Cell Biol. 12, 447–456. doi:10.1038/ncb2046
Oh, W.J., Jacinto, E., 2011. mTOR complex 2 signaling and functions. Cell Cycle Georget. Tex 10, 2305–2316. doi:10.4161/cc.10.14.16586
Ohno, M., Sakamoto, H., Shimura, Y., 1987. Preferential excision of the 5' proximal intron from mRNA precursors with two introns as mediated by the cap structure. Proc. Natl. Acad. Sci. U. S. A. 84, 5187–5191.

Pause, A., Belsham, G.J., Gingras, A.C., Donzé, O., Lin, T.A., Lawrence, J.C., Sonenberg, N., 1994. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5'-cap function. Nature 371, 762–767. doi:10.1038/371762a0

Paynton, B.V., Rempel, R., Bachvarova, R., 1988. Changes in state of adenylation and time course of degradation of maternal mRNAs during oocyte maturation and early embryonic development in the mouse. Dev. Biol. 129, 304–314.

Pinkstaff, J.K., Chappell, S.A., Mauro, V.P., Edelman, G.M., Krushel, L.A., 2001. Internal initiation of translation of five dendritically localized neuronal mRNAs. Proc. Natl. Acad. Sci. 98, 2770–2775. doi:10.1073/pnas.051623398

Piñol-Roma, S., Dreyfuss, G., 1992. Shuttling of pre-mRNA binding proteins between nucleus and cytoplasm. Nature 355, 730–732. doi:10.1038/355730a0

Polanski, Z., Hoffmann, S., Tsurumi, C., 2005. Oocyte nucleus controls progression through meiotic maturation. Dev. Biol. 281, 184–195. doi:10.1016/j.ydbio.2005.02.024

Pyronnet, S., Pradayrol, L., Sonenberg, N., 2000. A cell cycle-dependent internal ribosome entry site. Mol. Cell 5, 607–616.

Raj, A., van den Bogaard, P., Rifkin, S.A., van Oudenaarden, A., Tyagi, S., 2008. Imaging individual mRNA molecules using multiple singly labeled probes. Nat. Methods 5, 877–879. doi:10.1038/nmeth.1253

Ralston, A., Rossant, J., 2008. Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. Dev. Biol. 313, 614–629. doi:10.1016/j.ydbio.2007.10.054

Rebane, A., Aeb, A., Steitz, J.A., 2004. Transportins 1 and 2 are redundant nuclear import factors for hnRNP A1 and HuR. RNA 10, 590–599. doi:10.1261/rna.5224304

Reid, D.W., Nicchitta, C.V., 2012a. The enduring enigma of nuclear translation. J. Cell Biol. 197, 7–9. doi:10.1083/jcb.201202140

Reid, D.W., Nicchitta, C.V., 2012b. Primary role for endoplasmic reticulum-bound ribosomes in cellular translation identified by ribosome profiling. J. Biol. Chem. 287, 5518–5527. doi:10.1074/jbc.M111.312280

Rich, A., Warner, J.R., Goodman, H.M., 1963. The Structure and Function of Polyribosomes. Cold Spring Harb. Symp. Quant. Biol. 28, 269–285. doi:10.1101/SQB.1963.028.01.043

Richter, J.D., 2007. CPEB: a life in translation. Trends Biochem. Sci. 32, 279–285. doi:10.1016/j.tibs.2007.04.004

Rodriguez, A.J., Shenoy, S.M., Singer, R.H., Condeelis, J., 2006. Visualization of mRNA translation in living cells. J. Cell Biol. 175, 67–76. doi:10.1083/jcb.200512137

Romasko, E.J., Amarnath, D., Midic, U., Latham, K.E., 2013. Association of maternal mRNA and phosphorylated EIF4EBP1 variants with the spindle in mouse oocytes: localized translational control supporting female meiosis in mammals. Genetics 195, 349–358. doi:10.1534/genetics.113.154005

Rosner B,, Beddington RS, n.d. Whole-Mount in Situ Hybridization in the Mouse Embryo: Gene Expression in Three Dimensions. PubMed J.

Rosner, M., Fuchs, C., Dolznig, H., Hengstschläger, M., 2011. Different cytoplasmic/nuclear distribution of S6 protein phosphorylated at S240/244 and S235/236. Amino Acids 40, 595–600. doi:10.1007/s00726-010-0684-2

Rossant, J., Tam, P.P.L., 2004. Emerging asymmetry and embryonic patterning in early mouse development. Dev. Cell 7, 155–164. doi:10.1016/j.devcel.2004.07.012

Roussis, I.M., Guille, M., Myers, F.A., Scarlett, G.P., 2016. RNA Whole-Mount In situ Hybridisation Proximity Ligation Assay (rISH-PLA), an Assay for Detecting RNA-

Protein Complexes in Intact Cells. PLOS ONE 11, e0147967. doi:10.1371/journal.pone.0147967
Roux, P.P., Shahbazian, D., Vu, H., Holz, M.K., Cohen, M.S., Taunton, J., Sonenberg, N., Blenis, J., 2007. RAS/ERK Signaling Promotes Site-specific Ribosomal Protein S6 Phosphorylation via RSK and Stimulates Cap-dependent Translation. J. Biol. Chem. 282, 14056–14064. doi:10.1074/jbc.M700906200
Ruvinsky, I., Meyuhas, O., 2006. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. Trends Biochem. Sci. 31, 342–348. doi:10.1016/j.tibs.2006.04.003
Schlaitz, A.-L., Thompson, J., Wong, C.C.L., Yates, J.R., Heald, R., 2013. REEP3/4 ensure endoplasmic reticulum clearance from metaphase chromatin and proper nuclear envelope architecture. Dev. Cell 26, 315–323. doi:10.1016/j.devcel.2013.06.016
Schuh, M., Ellenberg, J., 2008. A New Model for Asymmetric Spindle Positioning in Mouse Oocytes. Curr. Biol. 18, 1986–1992. doi:10.1016/j.cub.2008.11.022
Schuh, M., Ellenberg, J., 2007. Self-Organization of MTOCs Replaces Centrosome Function during Acentrosomal Spindle Assembly in Live Mouse Oocytes. Cell 130, 484–498. doi:10.1016/j.cell.2007.06.025
Schultz, R.M., 1993. Regulation of zygotic gene activation in the mouse. BioEssays News Rev. Mol. Cell. Dev. Biol. 15, 531–538. doi:10.1002/bies.950150806
Schultz, R.M., LaMarca, M.J., Wassarman, P.M., 1978. Absolute rates of protein synthesis during meiotic maturation of mammalian oocytes in vitro. Proc. Natl. Acad. Sci. 75, 4160–4164.
Shaffer, S.M., Wu, M.-T., Levesque, M.J., Raj, A., 2013. Turbo FISH: A Method for Rapid Single Molecule RNA FISH. PLoS ONE 8. doi:10.1371/journal.pone.0075120
Shandala, T., Parkinson-Lawrence, E.J., Brooks, D.A., 2001. Protein: Cotranslational and Posttranslational Modification in Organelles, in: ELS. John Wiley & Sons, Ltd. doi:10.1002/9780470015902.a0005716.pub2
Shibuya, T., Tange, T.Ø., Sonenberg, N., Moore, M.J., 2004. eIF4AIII binds spliced mRNA in the exon junction complex and is essential for nonsense-mediated decay. Nat. Struct. Mol. Biol. 11, 346–351. doi:10.1038/nsmb750
Shibuya, T., Tange, T.Ø., Stroupe, M.E., Moore, M.J., 2006. Mutational analysis of human eIF4AIII identifies regions necessary for exon junction complex formation and nonsense-mediated mRNA decay. RNA 12, 360–374. doi:10.1261/rna.2190706
Siemer, C., Smiljakovic, T., Bhojwani, M., Leiding, C., Kanitz, W., Kubelka, M., Tomek, W., 2009. Analysis of mRNA associated factors during bovine oocyte maturation and early embryonic development. Mol. Reprod. Dev. 76, 1208–1219. doi:10.1002/mrd.21096
Söderberg, O., Leuchowius, K.-J., Gullberg, M., Jarvius, M., Weibrecht, I., Larsson, L.-G., Landegren, U., 2008. Characterizing proteins and their interactions in cells and tissues using the in situ proximity ligation assay. Methods San Diego Calif 45, 227–232. doi:10.1016/j.ymeth.2008.06.014
Stebbins-Boaz, B., Cao, Q., de Moor, C.H., Mendez, R., Richter, J.D., 1999. Maskin is a CPEB-associated factor that transiently interacts with elF-4E. Mol. Cell 4, 1017–1027.
Steinhauer, J., Kalderon, D., 2006. Microtubule polarity and axis formation in the Drosophila oocyte. Dev. Dyn. Off. Publ. Am. Assoc. Anat. 235, 1455–1468. doi:10.1002/dvdy.20770

Sternlicht, A.L., Schultz, R.M., 1981. Biochemical studies of mammalian oogenesis: kinetics of accumulation of total and poly(A)-containing RNA during growth of the mouse oocyte. J. Exp. Zool. 215, 191–200. doi:10.1002/jez.1402150209

Strumpf, D., Mao, C.-A., Yamanaka, Y., Ralston, A., Chawengsaksophak, K., Beck, F., Rossant, J., 2005. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Dev. Camb. Engl. 132, 2093–2102. doi:10.1242/dev.01801

Stutz, A., Conne, B., Huarte, J., Gubler, P., Völkel, V., Flandin, P., Vassalli, J.-D., 1998. Masking, unmasking, and regulated polyadenylation cooperate in the translational control of a dormant mRNA in mouse oocytes. Genes Dev. 12, 2535–2548.

Su, Y.-Q., Sugiura, K., Woo, Y., Wigglesworth, K., Kamdar, S., Affourtit, J., Eppig, J.J., 2007. Selective degradation of transcripts during meiotic maturation of mouse oocytes. Dev. Biol. 302, 104–117. doi:10.1016/j.ydbio.2006.09.008

Sun, Q.-Y., Schatten, H., 2006. Regulation of dynamic events by microfilaments during oocyte maturation and fertilization. Reproduction 131, 193–205. doi:10.1530/rep.1.00847

Susor, A., Jansova, D., Cerna, R., Danylevska, A., Anger, M., Toralova, T., Malik, R., Supolikova, J., Cook, M.S., Oh, J.S., Kubelka, M., 2015. Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway. Nat. Commun. 6, 6078. doi:10.1038/ncomms7078

Susor, A., Jelínková, L., Karabínová, P., Torner, H., Tomek, W., Kovárová, H., Kubelka, M., 2008. Regulation of cap-dependent translation initiation in the early stage porcine parthenotes. Mol. Reprod. Dev. 75, 1716–1725. doi:10.1002/mrd.20913

Susor, A., Kubelka, M., n.d. Translational regulation in the mammalian oocyte, in: Oocytes - Maternal Information and Functions.

Taneja, K.L., Lifshitz, L.M., Fay, F.S., Singer, R.H., 1992. Poly(A) RNA codistribution with microfilaments: evaluation by in situ hybridization and quantitative digital imaging microscopy. J. Cell Biol. 119, 1245–1260.

Tetkova, A., Hancova, M., 2016. Mouse Oocyte Isolation, Cultivation and RNA Microinjection. BIO-Protoc. 6. doi:10.21769/BioProtoc.1729

Thoreen, C.C., Chantranupong, L., Keys, H.R., Wang, T., Gray, N.S., Sabatini, D.M., 2012. A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 485, 109–113. doi:10.1038/nature11083

Thoreen, C.C., Sabatini, D.M., 2009. Rapamycin inhibits mTORC1, but not completely. Autophagy 5, 725–726.

Toledano, H., D'Alterio, C., Loza-Coll, M., Jones, D.L., 2012. Dual fluorescence detection of protein and RNA in Drosophila tissues. Nat. Protoc. 7, 1808–1817. doi:10.1038/nprot.2012.105

Tolino, M., Köhrmann, M., Kiebler, M.A., 2012. RNA-binding proteins involved in RNA localization and their implications in neuronal diseases. Eur. J. Neurosci. 35, 1818–1836. doi:10.1111/j.1460-9568.2012.08160.x

Tomek, W., Melo Sterza, F.A., Kubelka, M., Wollenhaupt, K., Torner, H., Anger, M., Kanitz, W., 2002. Regulation of translation during in vitro maturation of bovine oocytes: the role of MAP kinase, eIF4E (cap binding protein) phosphorylation, and eIF4E-BP1. Biol. Reprod. 66, 1274–1282.

Tong, Z.B., Gold, L., Pfeifer, K.E., Dorward, H., Lee, E., Bondy, C.A., Dean, J., Nelson, L.M., 2000. Mater, a maternal effect gene required for early embryonic development in mice. Nat. Genet. 26, 267–268. doi:10.1038/81547

Topisirovic, I., Svitkin, Y.V., Sonenberg, N., Shatkin, A.J., 2011. Cap and cap-binding proteins in the control of gene expression. Wiley Interdiscip. Rev. RNA 2, 277–298. doi:10.1002/wrna.52

Vargas, D.Y., Raj, A., Marras, S.A.E., Kramer, F.R., Tyagi, S., 2005. Mechanism of mRNA transport in the nucleus. Proc. Natl. Acad. Sci. U. S. A. 102, 17008–17013. doi:10.1073/pnas.0505580102

Vassalli, J.-D., Stutz, A., 1995. Translational Control: Awakening dormant mRNAs. Curr. Biol. 5, 476–479. doi:10.1016/S0960-9822(95)00095-9

Vazquez-Martin, A., Oliveras-Ferraros, C., Bernadó, L., López-Bonet, E., Menendez, J.A., 2009. The serine 2481-autophosphorylated form of mammalian Target Of Rapamycin (mTOR) is localized to midzone and midbody in dividing cancer cells. Biochem. Biophys. Res. Commun. 380, 638–643. doi:10.1016/j.bbrc.2009.01.153

VerMilyea, M.D., Maneck, M., Yoshida, N., Blochberger, I., Suzuki, E., Suzuki, T., Spang, R., Klein, C.A., Perry, A.C.F., 2011. Transcriptome asymmetry within mouse zygotes but not between early embryonic sister blastomeres. EMBO J. 30, 1841–1851. doi:10.1038/emboj.2011.92

Vitale, A.M., Calvert, M.E.K., Mallavarapu, M., Yurttas, P., Perlin, J., Herr, J., Coonrod, S., 2007. Proteomic profiling of murine oocyte maturation. Mol. Reprod. Dev. 74, 608–616. doi:10.1002/mrd.20648

Wang, F., Flanagan, J., Su, N., Wang, L.-C., Bui, S., Nielson, A., Wu, X., Vo, H.-T., Ma, X.-J., Luo, Y., 2012. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. J. Mol. Diagn. JMD 14, 22–29. doi:10.1016/j.jmoldx.2011.08.002

Wang, S., Kou, Z., Jing, Z., Zhang, Y., Guo, X., Dong, M., Wilmut, I., Gao, S., 2010. Proteome of mouse oocytes at different developmental stages. Proc. Natl. Acad. Sci. U. S. A. 107, 17639–17644. doi:10.1073/pnas.1013185107

Wang, Z., Murigneux, V., Le Hir, H., 2014. Transcriptome-wide modulation of splicing by the exon junction complex. Genome Biol. 15. doi:10.1186/s13059-014-0551-7

Weatheritt, R.J., Gibson, T.J., Babu, M.M., 2014. Asymmetric mRNA localization contributes to fidelity and sensitivity of spatially localized systems. Nat. Struct. Mol. Biol. 21, 833–839. doi:10.1038/nsmb.2876

Weibrecht, I., Leuchowius, K.-J., Clausson, C.-M., Conze, T., Jarvius, M., Howell, W.M., Kamali-Moghaddam, M., Söderberg, O., 2010. Proximity ligation assays: a recent addition to the proteomics toolbox. Expert Rev. Proteomics 7, 401–409. doi:10.1586/epr.10.10

Xue, S., Barna, M., 2012. Specialized ribosomes: a new frontier in gene regulation and organismal biology. Nat. Rev. Mol. Cell Biol. 13, 355–369. doi:10.1038/nrm3359

Yaba, A., Bianchi, V., Borini, A., Johnson, J., 2008. A putative mitotic checkpoint dependent on mTOR function controls cell proliferation and survival in ovarian granulosa cells. Reprod. Sci. Thousand Oaks Calif 15, 128–138. doi:10.1177/1933719107312037

Yamashita, R., Suzuki, Y., Takeuchi, N., Wakaguri, H., Ueda, T., Sugano, S., Nakai, K., 2008. Comprehensive detection of human terminal oligo-pyrimidine (TOP) genes and analysis of their characteristics. Nucleic Acids Res. 36, 3707–3715. doi:10.1093/nar/gkn248

Yang, C.R., Wei, Y.C., Zhang, Y., Zheng, K.J., Li, N., Yan, Y.Q., 2009. The expression and effect of mTOR during mouse oocyte maturation. PROG BIOCHEM BIOPHYS, 10 1334 – 1339.

Zeng, F., Baldwin, D.A., Schultz, R.M., 2004. Transcript profiling during preimplantation mouse development. Dev. Biol. 272, 483–496. doi:10.1016/j.ydbio.2004.05.018

Zhang, P., Ni, X., Guo, Y., Guo, X., Wang, Y., Zhou, Z., Huo, R., Sha, J., 2009. Proteomic-based identification of maternal proteins in mature mouse oocytes. BMC Genomics 10, 348. doi:10.1186/1471-2164-10-348

Zhang, Z., Wang, Y., Jiang, Y., Lin, P., Jia, X., Zou, Z., 2007. Ribosomal protein L24 is differentially expressed in ovary and testis of the marine shrimp Marsupenaeus japonicus. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 147, 466–474. doi:10.1016/j.cbpb.2007.02.013

Zheng, X.F., Florentino, D., Chen, J., Crabtree, G.R., Schreiber, S.L., 1995. TOR kinase domains are required for two distinct functions, only one of which is inhibited by rapamycin. Cell 82, 121–130.

Zhou, X., Hao, Q., Liao, J., Zhang, Q., Lu, H., 2013. Ribosomal Protein S14 Unties the MDM2-p53 Loop Upon Ribosomal Stress. Oncogene 32, 388–396. doi:10.1038/onc.2012.63

Zuccotti, M., Ponce, R.H., Boiani, M., Guizzardi, S., Govoni, P., Scandroglio, R., Garagna, S., Redi, C.A., 2002. The analysis of chromatin organisation allows selection of mouse antral oocytes competent for development to blastocyst. Zygote Camb. Engl. 10, 73–78.

# 7 Abbreviations

| | |
|---|---|
| 3'UTR | 3 untranslated region |
| 5'UTR | 5 untransalated region |
| 4E-BP1-3 | eukaryotic translation initiation factor 4E binding protein 1-3 |
| 4EGI | inhibitor eIF4E:eIF4G interaction |
| AKT | thymoma viral proto-oncogene 1 |
| AUG | intiation codon |
| BS(PEG)9 | PEGylated bis(sulfosuccinimidyl)suberate |
| CDK1 | Cyclin-depednent kinase 1 |
| cDNA | complementary DNA |
| Cdx2 | caudal type homeobox 2 |
| CENP-C | centromere protein C |
| cFISH | cytoplasmic fluorescent in situ hybridization |
| CPE | cytoplasmic polyadenylation element |
| CPEBs | Cytoplasmic polyadenylation element binding proteins |
| CPEB1 | Cytoplasmic polyadenylation element binding protein1 |
| CPEB4 | Cytoplasmic polyadenylation element binding protein4 |
| CST | Cell Signaling Technology |
| CTA | chromosomal translation area |
| CY5 | Cyanine 5 dye |
| DAPI | 4',6-Diamidino-2-Phenylindole, Dihydrochloride |
| DNA | Deoxyribonucleic acid |
| eEF2 | eukaryotic Elongation factor 2 |
| EJC | exon juction complex |
| elF4F complex | eukaryotic translation Initiation factor 4F complex |
| elF4G1 | eukaryotic translation Initiation factor 4G1 |
| elF5B | eukaryotic translation Initiation factor 5B |
| ER | endoplasmic reticulum |
| FISH | fluorescent in situ hybridization |
| G2 | gap 2 phase of cell cycle |
| GTP | guanosine-5'-triphosphate |
| GV | germinal vesicle oocyte |
| GVBD | germinal vesicle breakdown |
| hCG | human chorionic gonadotropin |
| hnRNP | heterogeneous nuclear ribonucleoproteins |
| hnRNPA1 | heterogeneous Nuclear Ribonucleoprotein A1 |
| HPG | homopropargylglycine |
| HRP | horseradish peroxidase |
| IBMX | 3-isobutyl-1-methylxanthine |
| ICC | immunocytochemistry |

| | |
|---|---|
| lncRNA | long non-coding RNAs |
| IRES | internal ribosome entry sites |
| ITAFs | internal initiation trans-acting factor |
| KIF4 | Kinesin family member 4 protein |
| m3Gcap/m7Gcap | 2,2,7-trimethylguanosine(m3G)-containing cap structure |
| m7GTP | 7-Methylguanosine 5′-triphosphate |
| MAPK | Mitogen-activated protein kinase |
| MDM2 | Mouse double minute 2 homolog |
| MI | metaphase I stage of oocyte |
| MII | metaphase II stage of oocyte |
| M-MuLV Reverse Transcriptase | Moloney Murine Leukemia Virus Reverse Transcriptase |
| MPF | Maturation-promoting factor |
| mRNA | messenger ribonucleic acid |
| mTOR | Mammalian Target Of Rapamycin |
| mTORC1 | Mammalian Target Of Rapamycin complex 1 |
| NEBD | nuclear envelope breakdown |
| NSN | non-surrounded-nucleolus |
| ORF | open reading frames |
| PABP | Poly(A)-binding protein |
| PARN | Poly(A)-Specific Ribonuclease |
| PBS | Phosphate Buffered Saline |
| PFA | Paraformaldehyde |
| PI3K | Phosphatidylinositol-4,5-bisphosphate 3-kinase |
| PLA | proximity ligation assay |
| PLK1 | Polo Like Kinase 1 |
| PMSG | pregnant mare's serum gonadotropin |
| Poly(A) | polyadenylated |
| PTA | perispindular translation area |
| RBPs | ribosome binding proteins |
| RCA | rolling circle amplification |
| ReAsH | resorufin arsenical hairpin binder |
| RL | Renilla luciferase |
| RNA | ribonucleic acid |
| RPL24 | 60S ribosomal protein L24 |
| RPS14 | 40S ribosomal protein S14 |
| RPS6 | 40S ribosomal protein S6 |
| rRNA | ribosomal ribonucleic acid |
| S174 | serine174 |
| S209 | serine209 |
| S2448 | serine2448 |
| S64 | serine64 |
| S65 | serine65 |
| S6K1 | Ribosomal protein S6 kinase beta-1 |

| | |
|---|---|
| smRNA FISH | single molecule RNA fluorescent in situ hybridization |
| SN | surrounded-nucleolus |
| T36 | threonine36 |
| T45 | threonine45 |
| T69 | threonine69 |
| TOP motif | terminal oligopyrimidine tract |
| tRNA | transfer RNA |
| UTR | untranslated region |
| ZGA | zygotic genome activation |

# 8 Curriculum vitae

Name: Mgr. Denisa Jansová
E-mail: jansova@iapg.cas.cz
Date of birth: 20th July 1988

**Education/Qualifications**

2013-present Charles University in Prague, Czech republic; Faculty of Science
A PhD student at the Developmental Biology Program
Research interest: Transcriptiome in the mammalian germ cells
PhD Thesis: Identification of key regulators of gene expression in mammalian oocyte and embryo

2011-2013 Charles University in Prague, Czech Republic; Faculty of Science
Department of Cell Biology, *Master´s study program*
Diploma Thesis: Transcriptional activity of the genes characterizing developmentally competent cytoplasm in bovine oocytes

2008-2011 Charles University in Prague, Czech Republic; Faculty of Science
Department of Cell Biology, *Bachelor´s study program*
Bachelor thesis: Actin and its regulation in clathrin endocytosis

**Other Experience/Presentation and conferences**

July 2017 RNA localization and local translation 2017, Barna, Italy, participant poster session: *RNA a translation machinery in oocyte and embryo*

November 2016 Super-Resolution Microscopy Studies, CEITEC, Brno, workshop

September 2016 RNA CLUB 2016, CEITEC Brno, Czech Republic, invited speaker, *RNA world in progress: Complex visualization of transcriptome in oocyte and embryo*

January 2016 *Analysis and processing next generation seguencing data, Prague CLIP lab,* Czech Republic, workshop

September 2015 EMBO conference „Protein Synthesis and Translational Control", Heidelberg, Germany, participant poster session: *Phosphorylation of 4E-BP1 regulates translation in mammalian germ cells*

September 2015 RNA Club 2015 Ceske Budejovice, Czech Republic, poster presentation: *Visualization of the RNA world in the mammalian oocyte*

April 2015 Processing and analysis of microscopical data in biomedicine, Institute of Molecular Biology, Prague, Czech Republic, workshop

September 2014 18th International Microscopy congress (Prague), Czech Republic, participant poster session, *Phosphorylation of 4E-BP1 promotes translation at the oocyte spindle*

May 2014 Summer school of microinjection and advance microscopy technique (CEITEC, Brno), Czech Republic, workshop

| | |
|---|---|
| May 2014 | XXII Cytoskeletal Club meeting, Vranovska Ves, Czech Republic |
| 2010-2011 | Charles University in Prague, Czech Republic; Faculty of Science,Departement of Cell Biology. *Searching of candidate genes by RNAi in Wnt signaling on C. elegans* |

**Publications**

Susor A, **Jansova D**, Cerna R, Danylevska A, Anger M, Toralova T, Malik R, Supolikova J, Cook MS, Oh JS, Kubelka M. Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway. Nat Commun. 2015 Jan 28;6:6078. doi: 10.1038/ncomms7078. **IF:11,329**

Susor A, **Jansova D**, Anger M, Kubelka M. Translation in the mammalian oocyte in space and time. Cell Tissue Res. 2016 Jan;363(1):69-84. doi: 10.1007/s00441-015-2269-6. Review **IF:2,948**

Nemcova L, **Jansova D**, Vodickova-Kepkova K, Vodicka P, Jeseta M, Machatkova M, Kanka J. Detection of genes associated with developmental competence of bovine oocytes. Anim Reprod Sci. 2016 Mar;166:58-71. doi: 10.1016/j.anireprosci.2016.01.004 IF: **1,377**

**Jansova D**, Koncicka M, Tetkova A, Cerna R, Malik R, Llano del E, Kubelka M, Susor A.. Regulation of 4E-BP1 activity in the mammalian oocyte, **Cell Cycle**, 2017. **IF: 3,53**

**Jansova D**, Konicka M, Tetkova A, Susor A. Transcriptome and translation in mammalian oocyte and embryo, submitted 2017, **Sci. Rep.**

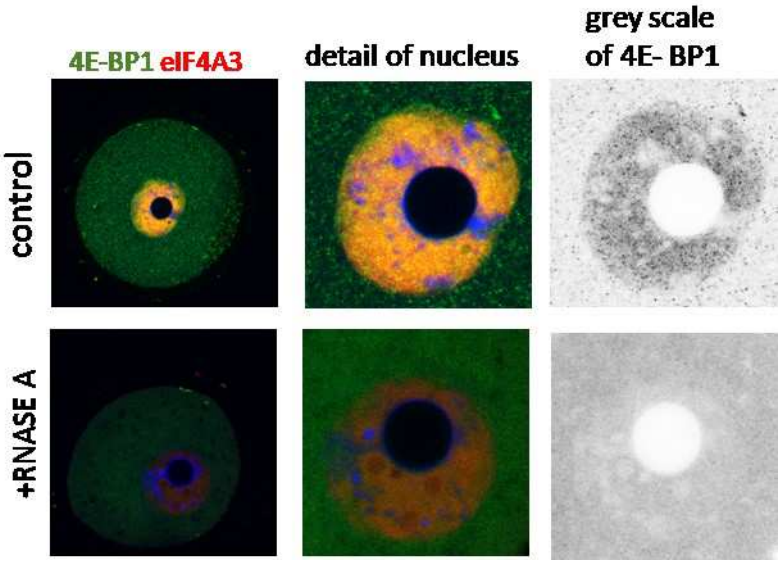
**Grants**

| | |
|---|---|
| 4/2015-12/2017 | GAUK 227026, student's grant of Charles Universtity: *RNA world in mammalian oocyte* |
| 4/2014-12/2014 | IGA, internal student's grant of the Institute of Animal Physiology and Genetics: *Localization specific mRNA in live oocyte* |

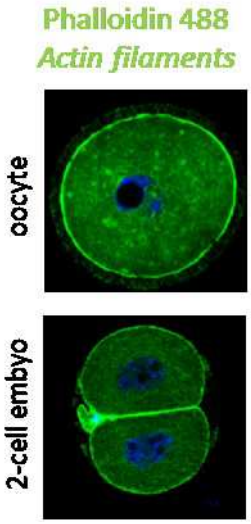
# 9 Supplementary material

1. Transcriptome and translation in mammalian oocyte and embryo, **supplement figure SI 3**
2. Transcriptome and translation in mammalian oocyte and embryo, **supplement figure SI 7**
3. Susor A, **Jansova D**, Cerna R, Danylevska A, Anger M, Toralova T, Malik R, Supolikova J, Cook MS, Oh JS, Kubelka M. Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway, **Nat Commun**, 2015.
4. Susor A, **Jansova D**, Anger M, Kubelka M. Translation in the mammalian oocyte in space and time, **Cell Tissue Res**, 2016 .
5. **Jansova D**, Koncicka M, Tetkova A, Cerna R, Malik R, Llano del E, Kubelka M, Susor A.. Regulation of 4E-BP1 activity in the mammalian oocyte, **Cell Cycle**, 2017.

SI 3


4E-BP1 eIF4A3
detail of nucleus
grey scale of 4E- BP1
control
+RNASE A

# SI 7



nature COMMUNICATIONS

ARTICLE

Received 3 Feb 2014 | Accepted 10 Dec 2014 | Published 28 Jan 2015

DOI: 10.1038/ncomms7078 OPEN

# Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR–eIF4F pathway

Andrej Susor[1], Denisa Jansova[1], Renata Cerna[1], Anna Danylevska[2], Martin Anger[1,2], Tereza Toralova[1], Radek Malik[3], Jaroslava Supolikova[1], Matthew S. Cook[4], Jeong Su Oh[5] & Michal Kubelka[1]

The fully grown mammalian oocyte is transcriptionally quiescent and utilizes only transcripts synthesized and stored during early development. However, we find that an abundant RNA population is retained in the oocyte nucleus and contains specific mRNAs important for meiotic progression. Here we show that during the first meiotic division, shortly after nuclear envelope breakdown, translational hotspots develop in the chromosomal area and in a region that was previously surrounded the nucleus. These distinct translational hotspots are separated by endoplasmic reticulum and Lamin, and disappear following polar body extrusion. Chromosomal translational hotspots are controlled by the activity of the mTOR–eIF4F pathway. Here we reveal a mechanism that—following the resumption of meiosis—controls the temporal and spatial translation of a specific set of transcripts required for normal spindle assembly, chromosome alignment and segregation.

[1] Institute of Animal Physiology and Genetics, ASCR, Rumburska 89, 277 21 Libechov, Czech Republic. [2] CEITEC-Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic. [3] Institute of Molecular Genetics, ASCR, Videnska 1083, 142 20 Prague, Czech Republic. [4] The Eli and Edythe Broad Center of Regeneration Medicine and Stem Cell Research, UCSF, San Francisco, California 94143, USA. [5] Department of Genetic Engineering, Sungkyunkwan University, Gyeonggi-do, Suwon 440-746, South Korea. Correspondence and requests for materials should be addressed to A.S. (email: susor@iapg.cas.cz).

Post-transcriptional control of gene expression at the level of translation has emerged as an important cellular function in normal development[1]. A hallmark of early development in mammals is the reliance on translation and utilization of stored RNA and proteins rather than *de novo* transcription of genes to sustain rapid development[1–3]. After a period of active transcription during growth, the nucleus (germinal vesicle, GV) of mammalian oocytes becomes transcriptionally inactive[4]. In the absence of transcription, the completion of meiosis and early embryo development in mammals relies significantly on maternally synthesized RNAs[1,5,6]. Therefore, regulation of gene expression in oocytes is controlled almost exclusively at the level of mRNA stabilization and translation. At the onset of the first meiotic division, nuclear envelope breakdown (NEBD) occurs, chromosomes condense and a bipolar spindle is formed from the microtubule organizing centres[7]. During meiosis I, the spindle migrates from the centre of the oocyte to the cortex, and the oocyte undergoes an asymmetric division resulting in a large egg competent for fertilization and a relatively small polar body. Proper positioning of the spindle during asymmetric cell division ensures correct partitioning of cellular determinants[8]. How these events are orchestrated remains unclear.

The early development of all animals is programmed by maternal RNAs and proteins deposited in the egg[1]. The localization of mRNA within a cell is an essential prerequisite for the correct propagation of genetic information and it is also a very efficient way to orchestrate cellular processes. In many species, including *Drosophila and Xenopus,* the synthesis of proteins is localized by compartmentalization of mRNAs[9–11]. This is critical for the determination of the animal and vegetal poles of *Xenopus* embryos, which requires accurate asymmetric distribution of several mRNAs[12]. However, little is known about the patterning of mammalian oocytes through localization of mRNAs, except for reported accumulation of RNA in the cortex of the oocyte[13,14].

Control of cap-dependent translation occurs mainly at the initiation step through the regulation of activity of the cap-binding protein complex eIF4F. This complex consists of three subunits: eIF4E, which specifically recognizes the cap structure, eIF4A helicase, and a bridging protein, eIF4G, responsible for eIF4F complex integrity[15]. The most important factor is probably the cap-binding protein, eIF4E. Its binding capacity is believed to be enhanced by the phosphorylation on S209, which correlates with an increase in translation[16–18]. EIF4E participates in the formation of the eIF4F complex, and it is also controlled via the regulatory proteins binding to eIF4E, the 4E-binding proteins (4E-BPs), which have to undergo phosphorylation to dissociate from eIF4E in such a way to enable its coupling with eIF4G and formation of the functional eIF4F complex[19]. EIF4E also stimulates eIF4A helicase activity[20], which is important for unwinding the mRNAs with long and highly structured 5′UTRs that have been previously reported to be translated in an eIF4E-dependent manner[21]. The kinase responsible for phosphorylating 4E-BPs on several sites is mTOR, which itself is regulated by the PI3K/Akt signalling pathway[18]. Two different mTOR complexes have been described that are associated with two different regulatory proteins, raptor and rictor. mTORC1 represents the complex of mTOR with raptor that is sensitive to rapamycin (Rap) and is responsible for 4E-BP1 and ribosomal protein S6 kinase (S6K) phosphorylation. Alternatively, mTORC2, the Rap-resistant mTOR–rictor complex, regulates cytoskeletal changes and Akt kinase phosphorylation[22]. Although Cdk1 kinase has been shown to phosphorylate 4E-BP1 on S65 and T70[23] and Plk1 seems to be responsible for the phosphorylation on S112[24], phosphorylation of these sites requires priming phosphorylation on T37 and T46, which is mediated by mTOR[19]. Increased phosphorylation of 4E-BP1 has also been shown during meiotic progression of mammalian oocytes[25,26], and recently different phosphorylated forms of 4E-BP1 have been shown to co-localize with the meiotic spindle in mouse oocytes[27]. In conclusion, mTOR appears to be of crucial importance for the formation of the active eIF4F complex, which stimulates the translation of eIF4E-sensitive mRNAs characterized by a 5′ terminal oligopyrimidine (TOP) motif[28].

We have used a molecular and biochemical approach to identify the previously uncharacterized *in situ* translation in mammalian oocytes. We show a direct link between localization of an enriched population of poly(A)-RNAs and active translation, as well as of active components of the mTOR–eIF4F regulatory pathway in the newly described and distinctly bordered areas around the chromosomes and spindle. They form shortly after NEBD and are likely to contribute to spindle formation as well as the fidelity of chromosome segregation. Together these findings suggest a spatiotemporally regulated translational control of chromosome segregation and functional spindle formation mediated by mTOR–eIF4F during meiotic progression of mammalian oocytes.

## Results

**Cap-dependent translation is essential for genomic stability.** Cap-dependent translation is known to be important during the G1/S transition in somatic cells, and it has also been shown to be involved in the regulation of meiotic progression in mammalian oocytes. The overall translation gradually decreases during oocyte meiotic maturation, but the activators of cap-dependent translation become activated during this period, implying a role for translation of specific mRNAs to regulate meiosis[25,26]. Here we show that the downregulation of mTOR and the supression of the formation of the eIF4F complex[28] (which is involved in the cap-dependent translation Supplementary Fig. 1a,b) in maturing mouse oocytes using a specific inhibitor of interaction between eIF4E and eIF4G1, 4EGI-1 (ref. 29) (4EGI), leads to 79% ($P<0.001$) of oocytes with significant defects in chromosome alignment and spindle morphology in metaphase I and II (Fig. 1a,b and Supplementary Fig. 2a,b,e), without blocking meiotic progression *per se* (Supplementary Fig. 2c,d). This in turn results in chromosome aneuploidy. Indeed, chromosomal spreads of inhibitor-treated oocytes revealed a 60% aneuploidy rate in MII oocytes (Fig. 1c,d).

Similar results were obtained using eIF4E (4E) or eIF4G1 (4G1) antibodies, as well as (Rap, an inhibitor of mTOR. Although the oocytes extruded a polar body and appeared normal (Supplementary Fig. 2d), abnormalities in spindle assembly and chromosome alignment were observed (Fig. 1a,b and Supplementary Fig. 2a,b,e). This phenotype was observed when oocytes were cultured in the presence of 4EGI (79%; $P<0.001$), Rap (68%; $P<0.001$) or microinjected with antibodies against eIF4E and eIF4G1 (76.5%; $P<0.001$). When global translation was disrupted by puromycin, oocytes progressed through metaphase I stage; however, cytokinesis was impaired and polar body extrusion did not occur[30]. Both 4EGI- and Rap-treated oocytes show no change in eIF2a phosphorylation (Supplementary Fig. 3), suggesting that such treatments do not induce a translational stress response[31]. Oocytes with a disrupted mTOR–eIF4F pathway are able to progress through meiosis I and extrude a first polar body, however, severe errors in chromosome segregation occur.

**The mTOR/4F axis is highly active at the onset of meiosis.** The mTOR–eIF4F pathway is responsible for the early recognition of capped mRNAs during translation initiation, and this interaction

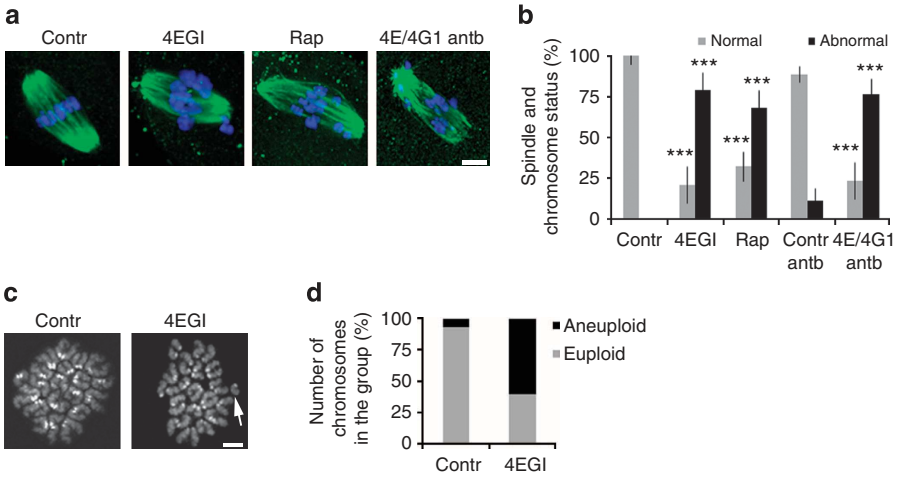



**Figure 1 | Disruption of the mTOR–eIF4F pathway affects genomic stability in meiosis I and impairs translation of specific mRNAs.** (**a**,**b**) Oocytes treated with 4EGI or Rap or microinjected with an eIF4E/eIF4G1 antibody cocktail show aberrant spindle formation. Data are represented as the mean ± s.d. Asterisks denote $P<0.001$; NS, non significant; according to a Student's *t*-test; $n\geq35$. Tubulin (green) and DAPI (blue). Scale bar, 5 μm. (**c**,**d**) Chromosomal spreads show aneuploidy and loose chromosomes/chromatids in oocytes with a downregulated 4F complex. Representative image from two independent experiments is shown ($n\geq14$); arrow denotes separated chromatid. Scale bar, 10 μm. See also Supplementary Figs 1–3.

is stabilized by eIF4G1 resulting in the activation of translation initiation. Interaction between eIF4E and eIF4G1 is mainly regulated by mTOR-mediated phosphorylation of 4E-BP1 (refs 19,32).

To better understand the observed phenotype of cap-dependent translational regulation we decided to perform a detailed analysis of the expression, localization, and activation of the mTOR and 4F pathway components. Our data show that the mTOR and 4F pathways become activated shortly (3 h post IBMX wash; PIW) after NEBD (Fig. 2a,b). We detected increased expression as well as phosphorylation-dependent activation of mTOR (Fig. 2a,b) with parallel the phosphorylation of its target substrate, 4E-BP1 (Fig. 2a,b). Similarly, substantial increase in eIF4E phosphorylation accompanied by increased expression levels and phosphorylation of eIF4G1 was observed after NEBD (Fig. 2a,b). These two proteins belong to the key translational factors that promote translation of specific mRNAs[28,33]. On the other hand, another mTOR substrate, S6K, which was shown previously to be involved in the regulation of proteosynthesis[34], became gradually dephosphorylated after NEBD (Fig. 2a,b). It should be noted that the expression level of the non-cap-dependent translation promoter[35,36] eIF4G2 was constant or even slightly decreased during oocyte maturation (Fig. 2a,b). The data suggest that the critical period for mTOR–eIF4F translational pathway activation is the time at or shortly after NEBD, with activation being maintained up to the MII stage. The translational complex becomes remodelled/deactivated after fertilization with parallel dephosphorylation of 4E-BP1 and eIF4E (Fig. 2a,b and Supplementary Fig. 10).

We next tested whether the activation of the mTOR–eIF4F pathway regulates translation of injected renilla luciferase (RL) reporters. Because it is known that the eIF4F complex promotes the translation of TOP RNAs[28], we microinjected the oocytes with reporter RNA: RL constructs containing an upstream non-TOP sequence (Actb), a mutated oligopirimidine sequence ($eEF2^{TOPM}$), or a canonical oligopirimidine sequence ($eEF2^{TOP}$). Firefly luciferase (FL) was used as a microinjection control. Oocytes injected with the reporter containing a canonical TOP sequence showed a 46% increase in RL signal ($P<0.01$) after NEBD. On the other hand, its translation was low before NEBD in the GV oocyte. The translation of the other reporters containing either non-TOP or mutated TOP sequences was unaffected after NEBD (Fig. 2c). These data suggest that the mTOR–eIF4F pathway becomes highly activated after NEBD and regulates mRNAs with TOP sequences.

***In situ* translation reveals two distinct hotspots after NEBD.** Using the methionine analogue homopropargylglycine (HPG; L-homopropargylglycine)[37] we analysed nascent proteosynthesis in the oocyte. Oocytes were exposed to HPG for a short cultivation period[36] (30 min), which facilitated incorporation into translated proteins and subsequent visualization using confocal microscopy. Our results showed that although the whole oocyte was translationally active, two distinct areas with different translation patterns could be identified after NEBD. In the GV oocyte, the translational activity appeared mainly in the perinuclear area (Fig. 3a). After NEBD, however, we detected two distinct areas with active translation. The first was located in the immediate vicinity of the chromosomes (from here on called chromosomal translational area—CTA), and the second was found in the perispindular area (from here on called perispindular translational area—PTA). (Fig. 3a,b and Supplementary Movie 1). Both the regions were separated by the cytoplasm with a decreased HPG signal. These regions of HPG signal migrated with the spindle to the oocyte cortex and disappeared after cytokinesis (polar body extrusion; MII) (Fig. 3a).

To elucidate the role of these distinctly defined translational regions further, we decided to characterize the localization/distribution of endoplasmic reticulum (ER), which was recently reported to be present at the perispindular area in both oocytes and somatic cells[38–40]. Interestingly, the ER-tracker revealed that the ER formed a circular structure between the CTA and PTA regions with overlaps on the PTA and surrounding cytoplasm (Fig. 3c and Supplementary Fig. 4). Immunostaining of Lamin A/C (LMN) revealed structures surrounding the CTA and present in the gap region with an absence of a nascent HPG translation signal (Fig. 3c and Supplementary Fig. 4). Surprisingly, although the nuclear membrane was already disassembled during NEBD, it appeared that its former structure was subsequently preserved by LMN fragments during pro-metaphase I (pro-MI) before

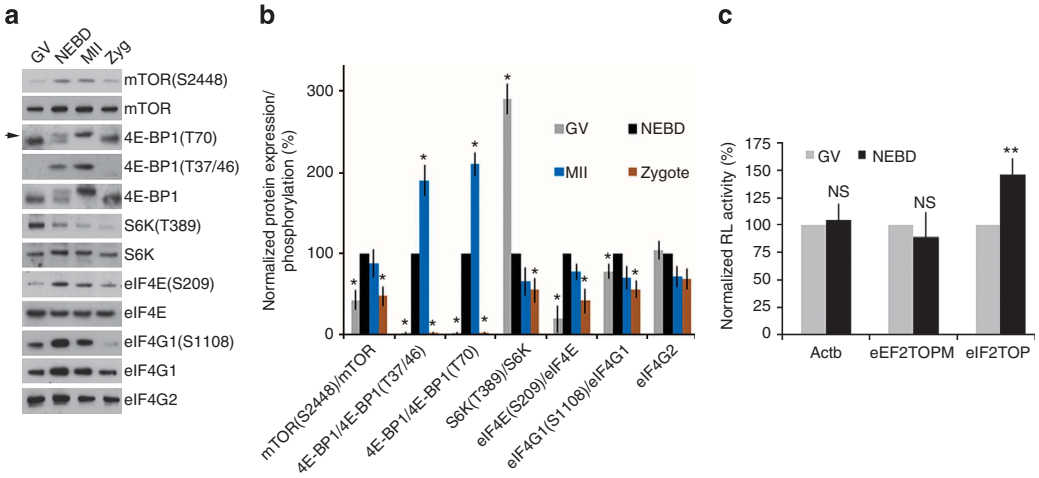



**Figure 2 | The mTOR-eIF4F translational pathway is highly active at the onset of meiosis and downregulated after fertilization.** (**a**,**b**) Immunoblot analysis of the key players of the mTOR-eIF4F pathway shows their upregulation after NEBD (3 h PIW). Ratios of the abundance of the phosphorylated form of mTOR, 4E-BP1, S6K, eIF4E end eIF4G1 are presented in the form of a bar chart. Data are represented as the mean ± s.d.; values obtained for NEBD stage were set as 100%; asterisk denotes statistically significant differences (Student's *t*-test: $P<0.05$); $n\geq3$; arrow denotes phospho-4E-BP1(T70). See also Supplementary Fig. 6. (**c**) RNA RL construct with TOP motif ($eEF2^{TOP}$) has increased translation after NEBD. Data are represented as the mean ± s.d. $^{**}P<0.01$, according to a Student's *t*-test. Data are representative of at least three independent experiments. Values obtained for GV stage were set as 100%. See also Supplementary Fig. 10.

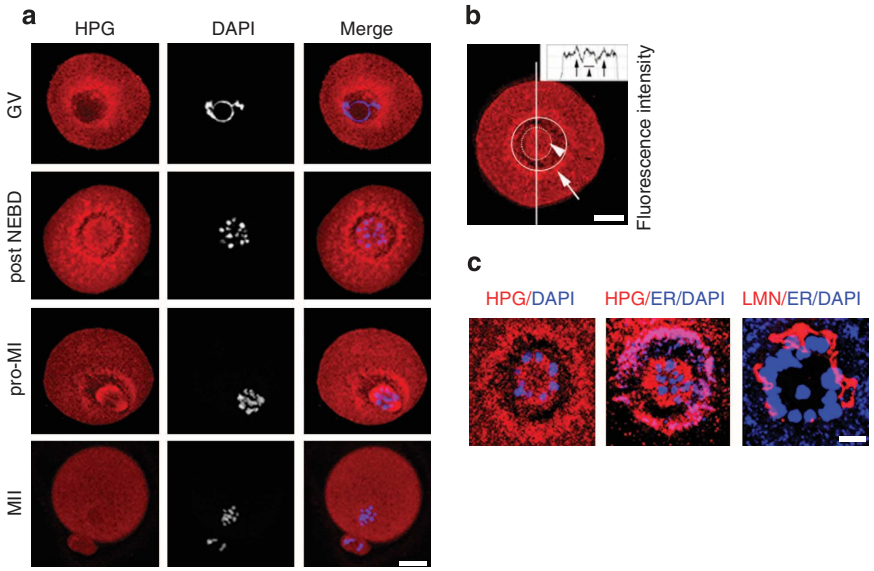



**Figure 3 | *In situ* translation shows two distinct hotspots in oocytes.** (**a**) Oocytes in different stages were cultured in the presence of HPG for 30 min. HPG (red); DAPI (blue). (**b**) NEBD oocytes cultured for 30 min in HPG. Histogram shows HPG intensity depicted along the white line. The arrowhead and arrow indicates the CTA (dot line) and PTA (uninterupted line), respectively; HPG (red). See also Supplementary Movie 1. (**c**) ER tracker shows perispindular localization of ER and overlaps with the PTA post NEBD. Polymerized LMN separates the CTA from the PTA. HPG, LMN, (red); ER tracker and DAPI (blue). Data are representative of at least two independent experiments; scale bar, ~10 μm.

disappearing in the MII stage. The localization of LMN staining between the CTA and PTA regions overlaps with ER-tracker localization (Fig. 3c). Disruption of the microfilament network by cytochalasin D abolished the observed translational pattern as well as LMN from the CTA cortex (Supplementary Fig. 5).

These findings suggest that the oocyte translates *de novo* proteins in distinct locations, which then undergo remodelling at or shortly after NEBD and at cytokinesis (MII). Both ER and LMN are likely to be involved in the formation of the boundary between the two distinct translational areas and probably ensure physical separation of the chromosomes from the rest of the cytoplasm during early stages of meiosis after NEBD. Since the period around NEBD appeared to be crucial both for the translational reorganization and for the timing of spindle assembly, further experiments focused on this stage.

**Components of the mTOR/4F axis are localized to the CTA.** Our results thus far led us to hypothesize that the observed phenotype developed due to the defects in the translation of specific mRNAs in specific subcellular compartments. To confirm this hypothesis and to determine whether the mTOR–eIF4F

pathway is involved in the CTA and PTA localized translation, we analysed the key components of this pathway at the time of NEBD. Because the mTOR–eIF4F pathway was activated at or shortly after NEBD (when the CTA became apparent), we analysed oocytes 3 h PIW for the presence and localization of eIF4E, phospho-eIF4E, mTOR, phospho-mTOR, phospho-S6K, mTOR's substrate 4E-BP1 and two differently phosphorylated forms of 4E-BP1.

Both mTOR and mTOR phosphorylated on S2448 (this modification of mTOR was previously linked to the stimulation of translational activity)[41,42] were localized predominantly at the CTA (Fig. 4a,b). However, the analysis of its substrate, 4E-BP1, showed an even distribution within the oocyte. Although its phosphorylated form (T37/46) was localized with a similar pattern asthat of the total protein, significantly higher intensity of the phospho-4E-BP1 signal could be seen in the vicinity of the chromosomes (Fig. 4b). Surprisingly, 4E-BP1 phosphorylated on T70 showed exclusive signal at the CTA (Fig. 4b). Consistent with immunoblot analysis data (Fig. 2a,b and Supplementary Fig. 6), 4E-BP1 was not phosphorylated at the GV stage. The immunofluorescence signal for eIF4E and eIF4E (S209) was localized evenly and it was also present in the vicinity of chromosomes after NEBD. eIF4E phosphorylated on S209 and S6K phosphorylated on T389 also showed an evenly distributed signal in the oocyte. However, in the case of eIF4E (S209), staining could be seen at the CTA and PTA (Fig. 4b,c). Furthermore, the presence of ribosomal protein 6 (RPS6), which has been known to upregulate mRNA translation and can be used as a marker for active translation[43], was found throughout the cytoplasm as well as at CTA and PTA (Fig. 4b).

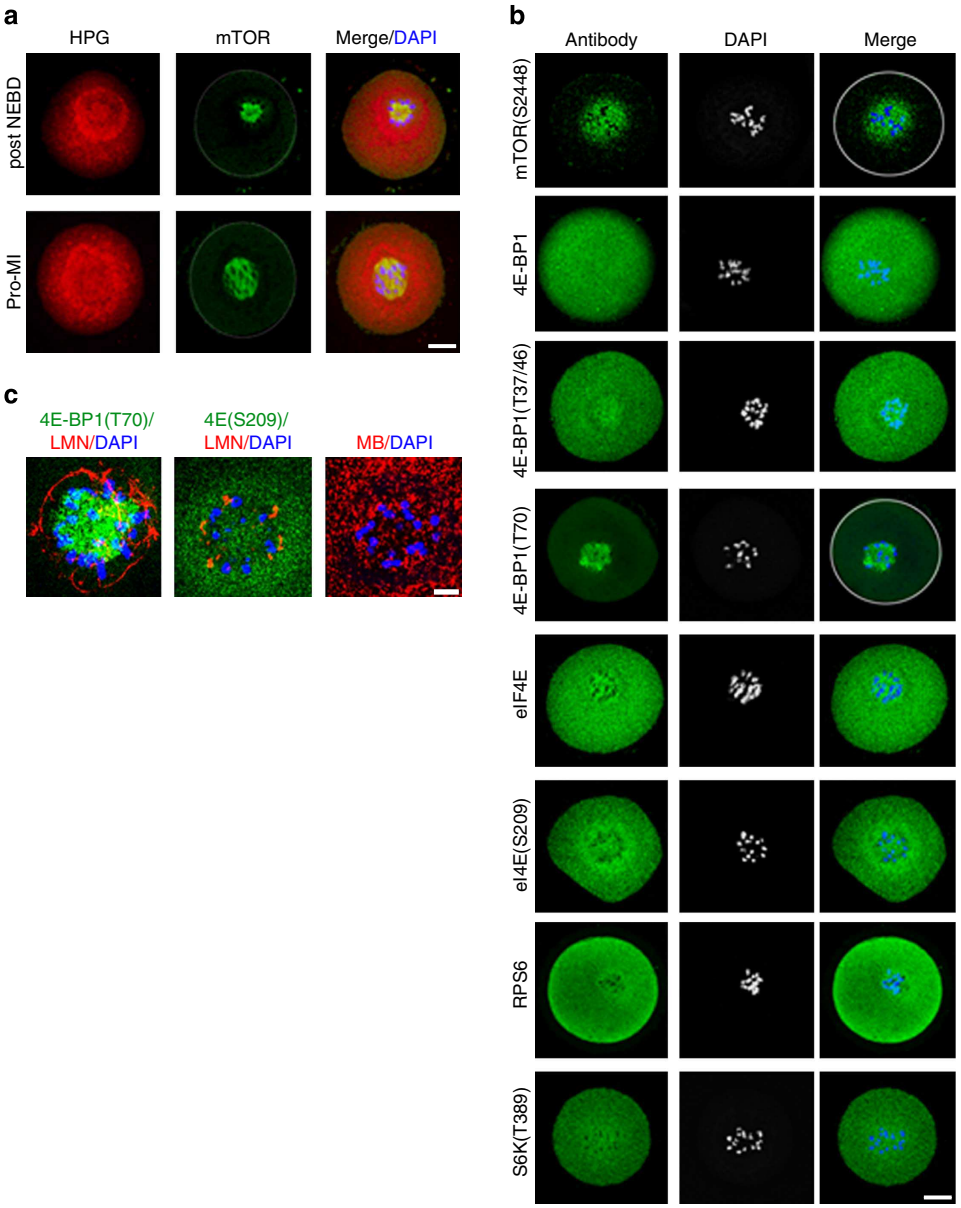



**Figure 4 | mTOR-eIF4F key players are localized at the CTA.** (**a**) mTOR (green) localizes with HPG signal (red) at the CTA. (**b**) Immunocytochemistry shows the localization of mTOR–eIF4F pathway components 2 h post NEBD. White line indicates oocyte cortex; representative images of at least three independent experiments are shown; scale bars, 20 μm. See also Supplementary Fig. 6. (**c**) ER tracker shows perispindular localization of ER and overlaps with the PTA post NEBD. Polymerized LMN separates CTA from PTA. HPG, LMN, MB (red); 4E-BP1(T70), eIF4E(S209) green; ER tracker and DAPI (blue). Data are representatives of at least two independent experiments; white line indicates oocyte cortex; scale bar, ~10 μm.

Additional experiments also show the localization of 4E-BP1(T70) and eIF4E(S209), as well as poly(A)-RNA at the CTA and/or PTA correlating with LMN localization (Fig. 4c and Supplementary Fig. 4). These data clearly demonstrate that the key components of the mTOR–eIF4F pathway are located at the CTA and PTA regions where translation is presumably increased.

**The mTOR–eIF4F pathway regulates translation at CTA**. To further elucidate the involvement of the mTOR/4E pathway in the regulation of the translation localized at the CTA and PTA regions, we performed additional experiments utilizing specific inhibitors of this pathway, 4EGI and Rap.

Incorporation of $^{35}$S-Methionine in the oocytes treated with 4EGI or Rap during 12 h in meiotic progression revealed no major effect on the overall protein synthesis (Fig. 5a,b). This indicates that the inhibition of the mTOR–eIF4F pathway likely affects translation of only a subset of mRNAs. This was also supported by the previously described experiment in which we analysed the translation of RL RNA reporter constructs microinjected into oocytes. While translation of RL constructs after NEBD containing upstream non-TOP sequence (Actb) or mutated oligopirimidine sequence (eEF2$^{TOPM}$) did not change significantly in oocytes treated with 4EGI or Rap, the translation of the construct containing the canonical oligopirimidine sequence (eEF2$^{TOP}$) was significantly decreased (Fig. 5c).

The timing of NEBD was similar to the control group in both the treatments (Supplementary Fig. 2c). When oocytes were cultured in the presence of HPG and treated with 4EGI or Rap, a significant decrease (~20%; $P<0.001$) in translation fluorescence signal could be seen within the CTA with no visible change in translation within the cytoplasm (Fig. 6a,b). Puromycin is a potent inhibitor of all translations, and treatment on oocytes resulted in the suppression of $^{35}$S-Methionine incorporation (Fig. 5a,b) as well as signal from HPG (~ 90%; $P<0.001$; Fig. 6a,b).

We further asked whether the downregulation of mTOR–eIF4F would also influence phosphorylation of the mTOR substrate 4E-BP1 on T70 (this modification was detected in our previous experiments to be present exclusively at CTA). Oocytes were cultured in the presence or absence of inhibitors for 3 h PIW and probed for phospho-4E-BP1 (T70). The immunofluorescence signal in the equatorial confocal image section was quantified at the CTA and PTA/cytoplasm. The phospho-4E-BP1 (T70) signal significantly decreased by 57% ($P<0.001$) in the presence of Rap but not 4EGI (Fig. 6c). Interestingly, fluorescence intensity of the PTA/cytoplasm did not change significantly between the groups (Fig. 6a,b). Further support of the effect of Rap brought the immunoblotting experiment showing substantially decreased phosphorylation of 4E-BP1 on T70 in oocytes treated with Rap, but not with 4EGI. On the other hand, 4EGI supressed the formation of the 4F initiation complex (Supplementary Fig. 1a,b) 4EGI. Supression of 4F complex formation did not show an effect on S6K phosphorylation, whereas a mild (30%) effect compared with Hela cells (100%) could be seen when Rap, an inhibitor of mTOR, was used (Supplementary Fig. 1c,d).

Although 4EGI and Rap should not decrease the overall protein synthesis and are supposed to inhibit only cap-dependent translation, we wanted to confirm this. It is known that the eIF4F complex promotes translation of RNAs containing[33,44] TOP. We selected three TOP RNAs, *Bub3, Npm1* (ref. 33) and *Survivin*[45], whose translation would be negatively affected by the disruption of the eIF4F complex. We analysed protein expression by immunoblotting and found that the translation of selected mRNAs was significantly downregulated (~70% of treated oocytes). On the other hand, translation of TUBA, GAPDH and eIF4E proteins was not influenced by the treatment (Fig. 6d,e and Supplementary Fig. 10). However, the translation of mRNA with an internal ribosome entry site motif for CAMK2A[46] increased 25%. Translation of BUB3 and NPM1 increased substantially after NEBD, however, the level of Survivin decreased at the MII stage (Fig. 6f,g and Supplementary Fig. 10). Although translation of specific transcripts was decreased in treated oocytes, their mRNA level was not affected (Supplementary Fig. 7) except for *Camk2a,* the mRNA level of which was significantly increased by 4EGI treatment.

These data demonstrate that although the downregulation of mTOR–eIF4F translation in the oocyte does not influence the overall translational pattern, protein synthesis at the CTA region is impaired.

**The oocyte nucleus stores a large pool of RNA**. We further determined the role of RNA localization in the translation detected at the CTA and PTA regions. Since it has been

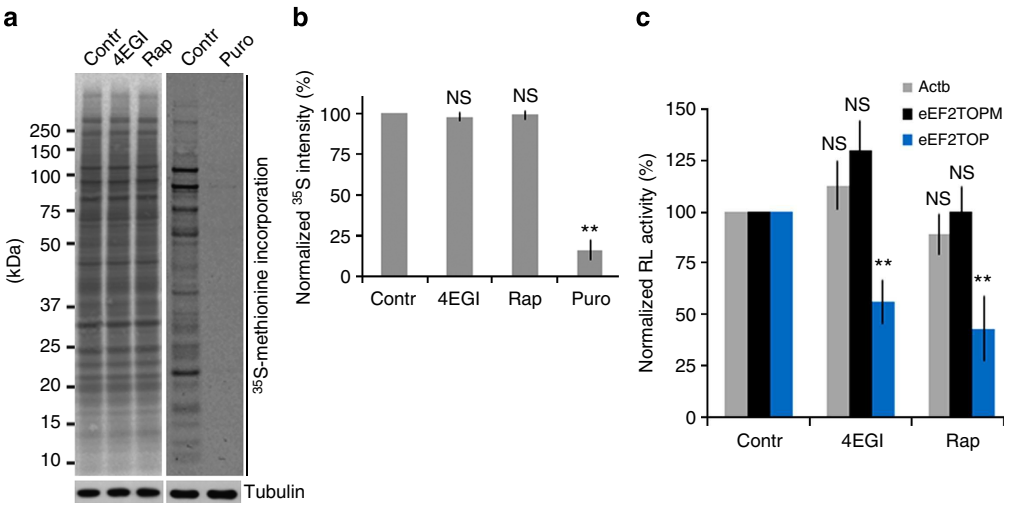



**Figure 5 | Downregulation of mTOR–eIF4F does not affect global translation, however, shows decreased level of candidate proteins.** (**a**,**b**) 4EGI or Rap treatments during meiotic progression do not affect the overall protein synthesis in the oocytes (data are represented as mean ± s.d.; $^{**}P<0.01$, according to Student's *t*-test; $n\geq3$). The overall translation was supressed by puro (puromycin). (**c**) Translation of TOP motive RL RNA reporter construct is affected in the oocytes with downregulated mTOR–eIF4F pathway (as mean ± s.d.; $^{**}P<0.01$; according to Student's *t*-test.

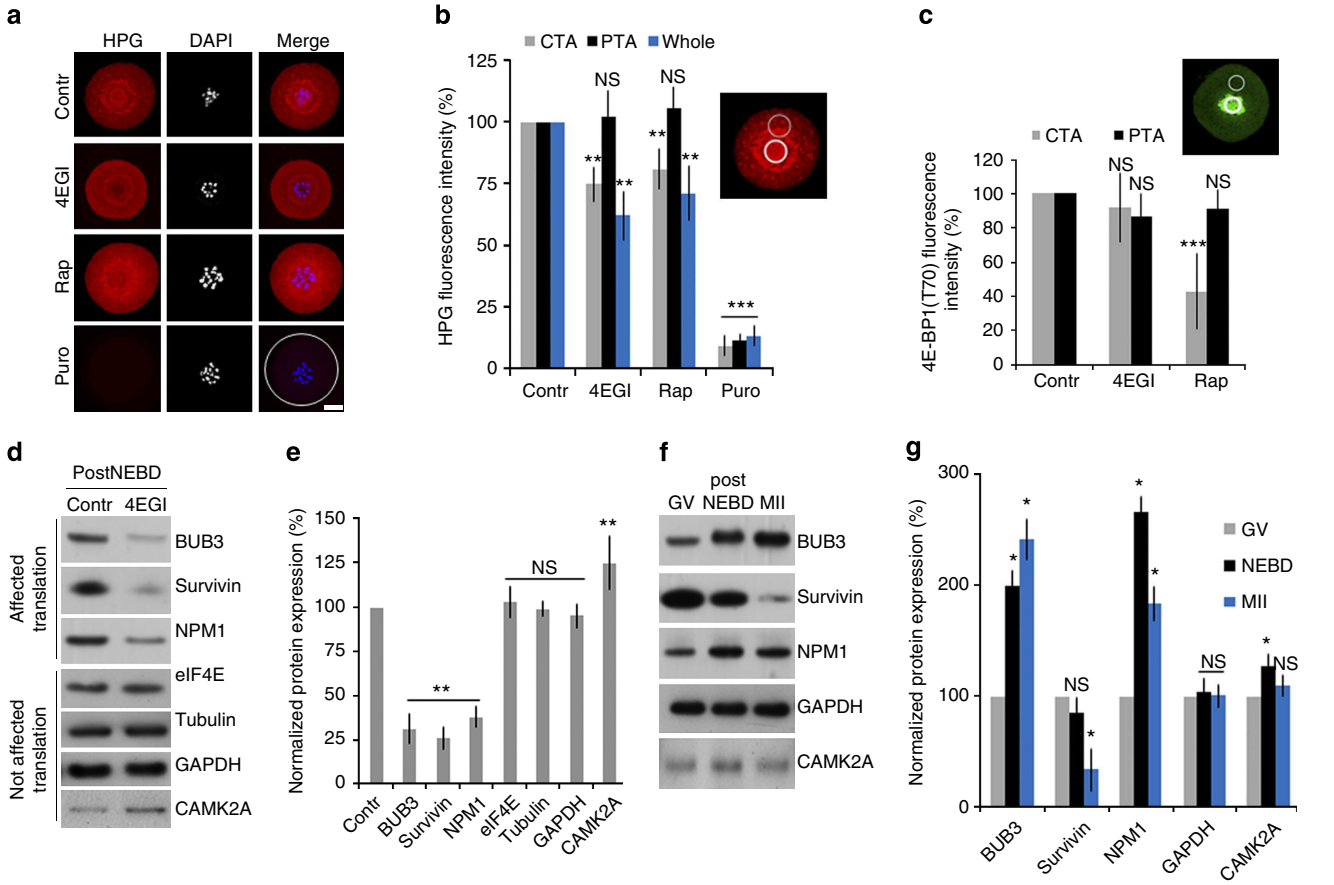



**Figure 6 | Downregulation of mTOR and 4F abolishes the translation at the CTA.** (**a**,**b**) Inhibition of mTOR or 4F decreases HPG fluorescence at the CTA, followed by quantification of HPG fluorescence (bold circle indicates measured area (CTA); thin circle (PTA)). Data are represented as mean ± s.d.; $^{**}P<0.01$ and $^{***}P<0.001$, according to a Student's *t*-test; $n\geq 20$. Scale bar, 35 μm. The overall translation was supressed by puromycin. (**c**) Measurement of phosphorylation intensity of the 4E-BP1 (T70) shows a decrease at the CTA in the presence of Rap ($^{***}P<0.001$; $n\geq 19$). 4EGI does not affect the phosphorylation intensity significantly ($P>0.1$; $n=20$). Bold circle indicates measurement at the CTA and thin circle at PTA/cytoplasm. Data represents the mean ± s.d.; asterisks denote statistically significant differences Student's *t*-test. (**d**,**e**) Downregulation of the 4F pathway results in decreased translation of selected mRNAs. Data are representative of at least three independent experiments. Values obtained for GV stage were set as 100%. Data are represented as the mean ± s.d. $^{**}P<0.01$, according to a Student's *t*-test. (**f**,**g**) Immunoblot analysis of the candidate proteins during meiotic maturation. Values obtained for GV stage were set as 100%, $n\geq 3$. Data represents the mean ± s.d.; asterisk denotes statistically significant differences Student's *t*-test: $P<0.05$. See also Supplementary Fig. 10.

shown that mRNA localization generally leads to targeted translation[47–49], we labelled the poly(A)-RNA population with an oligo dT probe to detect mRNA localization in the oocyte via fluorescence *in situ* hybridization (FISH). Surprisingly, we detected a strong signal of endogenous poly(A)-RNAs in the nucleus of the fully grown oocyte (Fig. 7a). RNase treatment resulted in a decrease in the FISH signal, while treatment with DNase did not abolish the poly(A)-RNA signal in the oocyte (Supplementary Fig. 8a). After NEBD, a strong signal corresponding to poly(A)-RNA could be detected in the vicinity of chromosomes matching precisely to the CTA region and to a lesser extent in the cytoplasm. In the pro-MI stage, the poly(A) signal was still present at the CTA (Fig. 7a). The poly(A)-RNA population in the nucleus of growing oocytes appeared diffuse in comparison to fully grown oocytes (Supplementary Fig. 8b).

To confirm the data from RNA FISH in live oocytes we performed experiments using a molecular beacon probe[50] (MB), which under *in vivo* conditions was able to hybridize to the poly(A) stretch of endogenous RNA. Oocytes in the GV stage were microinjected with a MB probe and the distribution of poly(A)-RNA was followed by live-cell imaging. The results obtained with the MB probe injected into live oocytes were consistent with RNA FISH results (Fig. 7b and Supplementary Movie 2). Furthermore, staining of the oocytes with the nucleic acid marker SYTO14 also showed the presence of fluorescence signals in the nucleus of oocytes and also in the vicinity of chromosomes during MI in a region consistent with the CTA (Supplementary Fig. 8c).

Finally, we also investigated, whether the nucleus of a fully grown oocyte contained specific mRNAs, especially those coding for proteins affected by the 4EGI inhibitor (Fig. 6d,e). We isolated RNA from the oocyte nuclei (Fig. 7c) and cytoplasms and performed PCR for selected RNAs known to be present in the nucleus[51]. Our data clearly showed the presence of *Bub3, Npm1, Survivin, Dazl* and *Pabn1l* mRNAs in both the nucleus and cytoplasm (Fig. 7c), while other transcripts, such as *Mos, Gapdh, Tuba, mTOR, Eif4e* and *Camk2a*, were present only in the cytoplasm and were excluded from the nucleus. We also looked for the presence of known transcripts localized to the nucleus such as non-coding RNAs (*Neat2, U2* and *U12)* and *Pabpnl1* mRNA[52,53] (Fig. 7c). The presence or absence of mRNAs in the

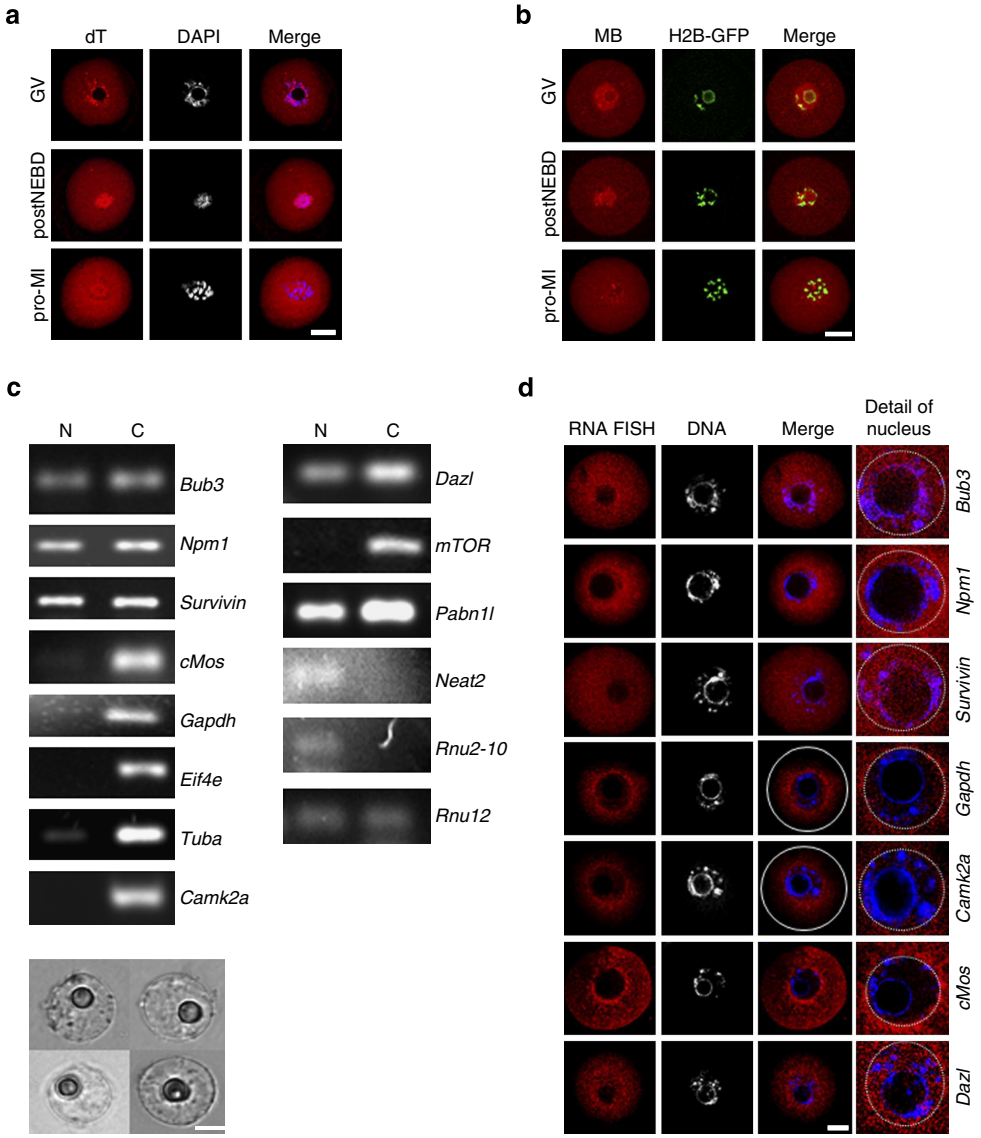



**Figure 7 | The oocyte nucleus stores a pool of RNA.** (**a**) RNA FISH shows the presence of a poly(A)-RNA population in the nucleus and in the vicinity of chromosomes in GV and NEBD stage oocytes. Poly(A) (red); DAPI (Blue). See also Supplementary Fig. 8. Scale bar, 20 μm. (**b**) MB shows the presence of poly(A)-RNA in the nucleus and in the vicinity of chromosomes in the GV oocyte and 2 h after NEBD. MB (red); H2B-GFP (green fluorescent protein; green). Scale bar, 20 μm. See also Supplementary Movie 2. (**c**) The oocyte nucleus contains specific mRNAs. RNA isolated from the nuclear (N) and cytosolic (C) fractions shows the presence or absence of specific transcripts by PCR. Data represents at least three independent experiments. Representative images of the isolated nuclei from mouse oocytes are shown. Scale bar, 10 μm. See also Supplementary Fig. 9 and Supplementary Table 1. (**d**) RNA FISH shows nuclear absence of *Camk2a, cMos, Gapdh* and nuclear presence of *Bub3, Nph1, Survivin, Dazl* mRNAs. The white line indicates the border of oocyte cortex; the white dashed line indicates the border of the nucleus; representative images of two independent experiments shown. Bar size, 20 μm.

oocyte nucleus was also visualized by single-molecule RNA FISH (Fig. 7d), and the results showed that while *Camk2a*, *Mos* and *Gapdh* mRNA were absent, *Bub3, Npm1, Survivin* and *Dazl* mRNAs were localized to the nucleus.

Taken together, our data indicate that the oocyte nucleus contains an RNA population that most likely contributes to translation in the vicinty of chromosomes after NEBD.

## Discussion

Post-transcriptional control of gene expression at the level of translation has been shown to be essential for regulating a number of cellular processes during development[1]. This is especially true in mammalian oocytes which, after a transcriptionally active period during their growth, resume meiosis during a period of transcriptional quiescence with a store of maternally synthesized mRNAs. Progression through meiosis is therefore regulated in the oocyte at the level of mRNA stabilization, translation and post-translational modification.

The importance of protein synthesis for meiotic and mitotic progression has been shown previously. Those published results revealed that protein synthesis is not required for NEBD in mouse oocytes, although the formation of the spindle and progression to metaphase II requires active protein synthesis[54]. This requirement for global translation has been attributed mainly to the activation of maturation-promoting factor, the key regulator of M-phase entry. However, the identity of the proteins, which need to be synthesized, and the spatiotemporal regulation of translation in the oocytes, is not entirely clear.

In this study we show that the disruption of mTOR–eIF4F signalling (playing a central role in the regulation of cap-dependent translation)[28,33,55] does not impair the oocyte meiotic progression to metaphase II. However, it leads to severe defects in the spindle morphology and chromosome alignment in metaphase I and II resulting in chromosomal aneuploidy. This suggests that activation of the mTOR–eIF4F signalling pathway is not required for maturation-promoting factor activation, but it is important for the synthesis of specific proteins that are required for the normal function of the spindle and proper distribution of the chromosomes during meiosis I. Disruption of the mTOR–eIF4F signalling pathway does not visibly influence the overall translation. Instead, we observe the downregulation of translation of a subset of specific mRNAs. This indicates that translation of the vast majority of mRNAs is regulated through other mechanisms[56]. It has been shown that translation of *Bub3, Npm1* and *Survivin* mRNAs is regulated by the 4F complex[33,45]. BUB3, NPM1 and Survivin play roles in spindle assembly and chromosome alignment and thus in the maintenance of genomic stability[57–60]. Both BUB3 and NPM1 are increasingly translated after NEBD; however, the translation of Survivin decreases in MII suggesting rapid protein turnover in the oocyte[61]. *Camk2a* mRNA with an internal ribosome entry site motif[45] revealed that its translation is not affected after inhibition of 4F formation, which positively correlates with the results obtained using a RL reporter of mRNAs without TOP or with mutated TOP motifs. On the other hand, *Camk2a* shows higher stability after 4EGI treatment suggesting that the active translation exerts a protective effect on mRNA from decay. Despite aberrant translation of selected transcripts, meiotic progression is unaffected probably due to the altered spindle assembly checkpoint regulatory mechanism in oocytes[62]. Consistent with this, defects in spindle morphology and chromosome alignment have been observed.

We show that activation of the key components of the mTOR–eIF4F pathway and translation of RNAs with a 5′ TOP motif after NEBD in oocytes and inactivation after fertilization (entry to interphase) that indicates a role in cell cycle progression. Furthermore, we have detected nascent translation with surprisingly precise localization of two particular 'translational hotspots'. These newly described areas with an increased level of translation, one in the vicinity of chromosomes and another around the spindle (perispindular area), have been designated as the CTA and PTA, respectively. We have further shown that both mTOR and phosphorylated (active) mTOR, as well as eIF4E and phospho-eIF4E, are predominantly localized to the CTA. Similar localization has also been observed for the mTOR direct target, 4E-BP1, with protein phosphorylation on T37/46. T70 phospho-4E-BP1 is present almost exclusively at the CTA and this phosphorylation is affected by the mTOR inhibitor Rap. Consistent with these results, the distribution of differently phosphorylated forms of 4E-BP1 and RPS6 during mouse oocyte meiotic progression has been recently described[27]. The phosphorylation of RPS6 contributes to the formation of translation initiation complexes and the formation of polysomes[63,64], and it correlates with an increase in translation of 5′TOP mRNA sequences[65,66], thus it is commonly used as a marker of active translation. Another branch of the mTOR pathway is S6K; however, S6K in our model system is already highly phosphorylated at the GV stage and then its phosphorylation significantly decreases during meiotic maturation. Our data positively correlate with data published previously[67] showing that during cell cycle progression the inactivation of S6K presumably serves to spare energy for costly cell cycle processes at the expense of ribosomal protein synthesis. Moreover, the gradual decrease in the S6K activity during oocyte maturation can also explain our previously published data[26], showing that the overall protein synthesis decreases during meiotic maturation of porcine oocytes while both eIF4E and 4E-BP1 become phosphorylated during this period. Upon treatment with Rap we observed only a minor (30%) decrease in S6K phosphorylation when compared with the control oocytes. A possible explanation for this rather unusual observation might be sequence divergence of the region encompassing the Ser/Thr phosphorylation site of S6K in oocytes compared with somatic cells, which could cause partial insensitivity to Rap treatment[68].

Our data, along with those published by others, indicate that the key components of the mTOR–eIF4F pathway (as markers of active cap-dependent translation) play an important role at the CTA, and that this localization is essential for translation of specific RNAs involved in the correct formation of the spindle and accurate positioning of chromosomes. This idea is also supported by our data showing that the inhibition of the mTOR–eIF4F pathway (either by 4EGI or Rap treatment) leads to an abolition of translation at the CTA.

The region between the CTA and PTA with diminished translation contains ER and LMNs. We hypothesize that this gap between the translational active areas is some sort of semipermeable membrane formed on the basis of microfilaments[69,70], ER[38–40], LMN and possibly other constituents. This structure becomes apparent in the fully grown GV oocyte[38,70] with the PTA. We also hypothesize that this structure plays a role after NEBD onset to prevent rapid escape of nuclear components (mRNAs, ncRNAs, nuclear proteins and chromosomes) to the cytoplasm of such a large cell ($\sim$70 μm) and/or to prevent the entry of the cytoplasmic elements into the CTA, successfully maintaining organelle compartmentalization. Spatial translational control may provide an important means to maintain and refine these patterns of expression over time. Indeed, the distribution of certain transcripts and proteins appears to be distinct. This may contribute to spindle and chromosome organization and play an important role in the maintenance of genomic stability.

Previously, it has been reported that an abundant RNA population with RNA-binding proteins is localized to the cortical region of the oocyte[13,14]. This would, however, suppose that the RNAs or their products have to undergo massive changes in localization to ensure non-erroneous regulation of all the morphological changes occurring during meiotic progression. Alternatively, our results reveal a markedly enriched population of poly(A)-RNAs present in the nucleus of the fully grown oocyte without significant subcortical enrichment. In addition, mTOR–eIF4F axis components are not enriched in the subcortical region. Using multiple independent methods, we document the presence of endogenous RNAs in the nucleus of the oocyte that persist after NEBD in the vicinity of the condensed chromosomes overlapping with the CTA region. We believe that the observed nuclear localization of RNAs is a mechanism to ensure temporal and spatial translation of mRNAs important for the onset and progression of the dynamic processes of meiosis, especially spindle assembly. The oocyte nucleus seems to serve as a reservoir of transcripts retained during the transcriptionally active phase, and this finding positively correlates with protein localization at the spindle/chromosome area during cell cycle[59,71,72]. This hypothesis is supported by our results showing the presence of selected transcripts in the nuclei of a fully grown oocyte. Importantly, it has been shown that RNA is not translated following injection into the nucleus, but it is translated after NEBD[73]. Oocytes before NEBD are unsuitable as recipients for nuclear transfer, leading to abnormal cell division[74,75]. Our research demonstrates that this could be caused by the fact that the nucleoplasm contains a rich RNA population that resembles a 'nuclear factor' essential to support oocyte maturation and early embryo development. The oocyte's nuclear transcriptome remains to be described. These results suggest that the function

of mRNA retention in the nucleus may be to sustain translational repression, and that their subsequent translation can be regulated in a spatiotemporal restricted manner in response to cell cycle events.

Preserving the localization of specific translational factors and RNAs in specific cell compartments (chromosomes and newly forming spindle) at the onset of meiosis contributes to a less error-prone cell cycle progression in such a large cell. Moreover, the preservation of LMN and ER structures after NEBD posibly contributes to cytoplasm fractionation and ensures organelle compartmentalization. It is well-known that the nucleus contains various RNA species (coding and non-coding) that might also contribute to localized translation after NEBD[51–53]. Understanding the mechanisms whereby mRNAs are localized and their translation is locally regulated thus promises to provide important insights into many aspects of cell physiology.

Major causes of human aneuploidy involve errors that arise during meiosis[76]. Our data suggest that misslocalization of specific transcripts within the oocyte and their aberrant translation could be another cause of aneuploidy. This work describes components that are potential clinically relevant targets.

Altogether, our findings indicate that a nuclear RNA population contributes to mammalian oocyte translational patterning and thus to the regulation of gene expression during the dynamic onset of meiosis. At the molecular level, we present an important function for the mTOR–eIF4F pathway in spatial translational control, suggesting a novel set of regulatory mechanisms ensuring specific gene expression at the right place and time in the mammalian oocyte.

## Methods

**Oocyte culture and microinjection.** GV oocytes were obtained from at least 6 week-old CD1 mice 46 h after injection of pregnant mare serum gonadotropin (PMSG). Oocytes were placed in M2 medium (Millipore) supplemented with 100 μM of IBMX ((3-isobutyl-1-methylxanthine, phosphodiesterase inhibitor; Sigma)) to prevent NEBD. Selected oocytes were denuded and cultured in M16 medium (Millipore) without IBMX at 37 °C, 5% $CO_2$. After IBMX wash (PIW) at least 90% of oocytes resume meiosis (NEBD) within 70 min. To obtain MII oocytes, hCG (Sigma) was administered 48 h after PMSG. Zygotes were obtained from the PMSG-primed females mated to males 17 h post hCG. Oocytes were microinjected by Narishige microinjector with ~5 pl of the solution containing 20–50 ng μl$^{-1}$ RNA per oocyte and cultured according to the protocol. Oocytes were treated with of 100 μM 4EGI (Calbiochem), 100 nM Rap, 3 μg ml$^{-1}$ CCD or 1 μg ml$^{-1}$ puromycin (Sigma). Dimethylsulphoxide was used as a control. All animal work was conducted according to Act No 246/1992 on the protection of animals against cruelty. Hela cells were cultured in DMEM F12 with 5% fetal bovine serum, 1%penicilin/streptomycin, 1% Glutamax and with presence or absence of 100 nM Rap for 3 h.

**Immunocytochemistry and fluorescent probe detection.** Oocytes were fixed in 4% paraformaldehyde (PFA) in PBS for 30 min, permeabilized for 15 min in PBS with 0.1% Triton X-100 and incubated overnight at 4 °C with primary antibodies (1:100) against 4E-BP1(T70), 4E-BP1(T37/46), S6K(T389; Cell Signaling Technology), RPS6 (Santa Cruz), LMN A/C or α-tubulin (Sigma). After washing, the oocytes were incubated for 1 h at room temperature with an Alexa Fluor conjugated antibodies (1:250; Molecular Probes). RNaseOut (500 U ml$^{-1}$; Invitrogen) was used in all the buffers. For nascent protein synthesis specific stage (GV-0 h, NEBD-2 h, pro-MI-7 h, MII-12 h) oocytes were cultured in the methionine-free medium (Gibco) supplemented with 1% dialyzed fetal bovine serum (10,000 MW; Sigma) and 50 μM HPG for 30 min[77]. HPG was detected by using Click-iT Cell Reaction Kit (Life Technologies). Chromosome spreads from mouse oocytes were prepared as previously described[78]. ER was detected by 1 μM ER-Tracker (Green dye and Blue-White DPX dye for double staining; Molecular Probes) in M16 for 1 h. DAPI was used for chromosome staining (Vectashield). Nucleic acids were labelled by 50 nM SYTO14 (Molecular Probes) in M16 for 20 min then fixed by PFA and imaged. Samples were visualized using an inverted confocal microscope in 16 bit depth (TCS SP5; Leica). Images were assembled in Photoshop CS3 and quantified by Image J software.

**Measurement of overall protein synthesis.** To measure the overall protein synthesis, 50 μCi of $^{35}$S-methionine[79] (Perkin Elmer) was added to methionine-free culture medium. Twenty-five oocytes per sample were labelled for 12 h, then lysed in SDS-buffer and subjected to SDS–polyacrylamide gel electrophoresis (PAGE). The labelled proteins were visualized by autoradiography on BasReader (Fuji) and quantified by Aida software (RayTest). Tubulin was used as a loading control.

**Immunoblotting.** Oocytes were lysed in 10 μl of 1 × Reducing SDS Loading Buffer (Cell Signaling Technology) and heated at 100 °C for 5 min. Proteins were separated by gradient 4–20% SDS–PAGE and transferred to Immobilon P membrane (Millipore) using a semidry blotting system (Biometra GmbH) for 25 min at 5 mA cm$^{-2}$. Membranes were blocked, depending on the used antibody, in 2.5 or 5% skimmed milk dissolved in 0,05% Tween-Tris-buffer saline (TTBS), pH 7.4 for 1 h. After a brief washing in TTBS, membranes were incubated at 4 °C overnight with the following primary antibodies with 1% milk/TTBS: mTOR(1:8,000), mTOR-S2448 (1:8,000), eIF4G1-S1108 (1:1,000), eIF4E-S209 (1:1,000), 4E-BP1 (1:500), 4E-BP1-T70 (1:500), 4E-BP1-T36/47 (1:500), eIF4G2 (1:500), eIF2a (1:500), eIF2a-S51 (1:500), S6K (1:2,000), S6K-T389 (1:500), Survivin (1:2,000), CAMK2A (1:1,000) from Cell Signaling Technology; eIF4G1 (1:500), eIF4E (1:500), BUB3 (1:500), from BD; NPM1 (1:500) from Life Technologies, α-Tubulin (1:7,500) from Abcam and GAPDH (1:30,000) from Sigma. Immunodetected proteins were visualized by ECL kit (Amersham), films were scanned using a GS-800 calibrated densitometer (Bio-Rad and quantified using Image J (http://rsbweb.nih.gov/ij) software.

**Live-cell imaging.** Oocytes 1–2 h after microinjection were transferred in M16 medium to Leica SP5 confocal microscope equipped with EMBL stage incubator and HCX PL APO 20 × /0.7 IMM CORR $\lambda_{BL}$ and HCX PL APO 40 × /1.1 Water corrected objectives. MB (2′OME-RNA: Cal Fluor Red 635-GCACGT-$(U)_{20}$–ACGTGC–3′BHQ2) probe (Biosearch Technologies) was injected in 20 μg μl$^{-1}$ concentration with non-polyadenylated H2B:GFP RNA[80]. Movie was assembled using Image J.

**Polymerase chain reaction.** RNA was extracted with RNeasy Plus Micro kit (Qiagen). Genomic DNA was depleted using columns (Supplementary Fig. 9). Primers were designed in two exons flanking introns (Supplementary Table 1). Reverse transcription with Sensiscript RT kit (Qiagen). PCR program used: 95 °C/30 s, (95 °C/30 s, 60 °C/90 s for *Rnu2-10* and *Mos*, for other genes 58 °C/90 s, 72 °C/90 s) × 35 cycles, 72 °C/5 min. Products were detected by electrophoresis on 1.2% agarose gel with ethidium bromide. RT–PCR was carried out on Rotor-Gene 3000 (Corbett Research) using OneStep RT–PCR Kit (Qiagene) and SybrGreen, data was analysed by internal software Rotor-Gene 3000. Reaction conditions were: RT 50 °C/30 min, initial activation 95 °C/15 min, (95 °C/15 s, annealing at a temperature specific for each set of primers (see Supplementary Table 1)/20 s, 72 °C/30 s) × 40 cycles, 72 °C/10 min.

**Dual-luciferase assay.** Oocytes were injected with 50 ng μl$^{-1}$ *in vitro* trascribed RNA (T7 mMessage, Ambion) from Renilla Luciferase constructs (RL; # 38234, 38235, 38236, Addgene[81]; pRL-EMCV[82]) with combination of injection amount control Firefly Luciferase (FL; # 18964; Addgene) in the presence of IBMX. Oocytes were cultured for 5 h with or without IBMX. At least five oocytes were lysed in 5 μl of Passive Lysis Buffer and stored at − 80 °C until luciferase activity was measured by the Dual-Luciferase Assay System (Promega) according to the manufacturer's instructions. Signal intensities were measured using a Glomax Luminometer (Promega). Activity of RL was normalized to that of FL luciferase.

**Chromosome spreads.** Zona pellucida was removed by Tyrode acid solution (polar bodies had become detached), washed with M2 medium and subsequently placed into hypotonic solution (1% fetal calf serum in deionized $H_2O$). Hypotonic treatment was carried out for < 1 min at room temperature. For fixation, oocytes were transferred into 50 μl drop of solution (0.1% paraformaldehyde, 0.2% Triton X-100, 1 mM dithiothreitol, adjusted to pH 9.2 with NaOH) in glass slide (Fisher Scientific). Fixation was allowed to proceed overnight at room temperature. Slides were dried and mounted in Vectashield with 4′,6-diamidino-2-phenylindole (DAPI), covered with a glass coverslip and kept at 4 °C. Samples were visualized using an inverted confocal microscope (TCS SP5; Leica) with × 63 objective.

**RNA FISH.** RNA FISH was performed according to ref. 83, briefly: 4% PFA fixed and permeabilized by 0.1% Triton X-100 in PBS with RnaseOut (Life Technologies), oocytes were washed with the washing buffer (15% formamide-Sigma, 2xSSC in RNAse free water) and hybridized with 100 nM probe in hybridization buffer (15% formamide-Sigma, 0.1% dextran sulfate, 1 mg ml$^{-1}$ *E.coli* tRNA-Roche, 2 mM Vanadyl-ribonucleoside complex-NEB, 2xSSC in RNAse free water), $dT_{(22)}$, *Bub3, Nph1, Survivin, Gapdh, Camk2a, cMos* and *Dazl* (Biosearch Technologies) labelled with Cy5 or Cal Fluor Red 635 fluorophores for single-molecule RNA FISH at 30 °C overnight. After three washes, the oocytes were mounted into a medium with DAPI (Vectashield). RNase A or DNase (25 μg ml$^{-1}$ for 30 min at 37 °C; Qiagen) was used after the permeabilization step in controls.

**Nuclei isolation.** Zona pellucida was removed using Tyrode acid solution (Sigma). The oocytes were disrupted in 100 µl Nuclei EZ lysis buffer (Sigma) and washed four times by centrifugation (2,000 *g* for 4 min at 4 °C). Nuclei sediment and cytoplasm fraction was collected and frozen.

**Immunoprecipitation.** Oocytes were lysed in lysis buffer containing 0.5% Triton X-100, 5 mM Tris, 1% deoxycholate sodium salt, 0.15 M NaCl, 1 mM $Na_3VO_4$, 4 mM protease inhibitors (Roche), pH 7.5. After centrifugation at 10,000 *g* for 10 min at 4 °C, the supernatants from 300 post-NEBD oocytes were incubated with 20 µl washed protein agarose beads (Sigma Aldrich) and agarose conjugated with eIF4E antibody (P-2, Santa Cruz) for 12 h at 4 °C. After centrifugation, the bead pellets were washed with ice cold lysis buffer for 5 min three times. Oocyte extracts incubated with resin without antibody was used as a negative control. The SDS-denatured agarose beads were separated by SDS–PAGE and analysed by immunoblotting.

**Statistical analysis.** Mean and s.d. values were calculated using MS Excel, statistical significance of the differences between the groups were tested using Student's *t*-test and $P<0.05$ was considered as statistically significant.

## References


1. Curtis, D., Lehmann, R. & Zamore, P. D. Translational regulation in development. *Cell* **81,** 171–178 (1995).
2. Raff, R. A., Colot, H. V., Selvig, S. E. & Gross, P. R. Oogenetic origin of messenger RNA for embryonic synthesis of microtubule proteins. *Nature* **235,** 211–214 (1972).
3. Capco, D. G. & Jeffery, W. R. Origin and spatial distribution of maternal messenger RNA during oogenesis of an insect, Oncopeltus fasciatus. *J. Cell Sci.* **39,** 63–76 (1979).
4. De La Fuente, R. *et al.* Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. *Dev. Biol.* **275,** 447–458 (2004).
5. Brandhorst, B. P. Informational content of the echinoderm egg. *Dev. Biol. (NY)* **1,** 525–576 (1985).
6. Nothias, J. Y., Majumder, S., Kaneko, K. J. & DePamphilis, M. L. Regulation of gene expression at the beginning of mammalian development. *J. Biol. Chem.* **270,** 22077–22080 (1995).
7. Schuh, M. & Ellenberg, J. Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. *Cell* **130,** 484–498 (2007).
8. Kusch, J., Liakopoulos, D. & Barral, Y. Spindle asymmetry: a compass for the cell. *Trends Cell Biol.* **13,** 562–569 (2003).
9. King, M. L., Messitt, T. J. & Mowry, K. L. Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. *Biol. Cell* **97,** 19–33 (2005).
10. Holt, C. E. & Bullock, S. L. Subcellular mRNA localization in animal cells and why it matters. *Science* **326,** 1212–1216 (2009).
11. Dubowy, J. & Macdonald, P. M. Localization of mRNAs to the oocyte is common in *Drosophila* ovaries. *Mech. Dev.* **70,** 193–195 (2013).
12. Nieuwkoop, P. D. Inductive interactions in early amphibian development and their general nature. *J. Embryol. Exp. Morphol.* **89,** 333–347 (1985).
13. Li, L., Baibakov, B. & Dean, J. A subcortical maternal complex essential for preimplantation mouse embryogenesis. *Dev. Cell* **15,** 416–425 (2008).
14. Flemr, M., Ma, J., Schultz, R. M. & Svoboda, P. P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. *Biol. Reprod.* **82,** 1008–1017 (2010).
15. Mader, S., Lee, H., Pause, A. & Sonenberg, N. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. *Mol. Cell. Biol.* **15,** 4990–4997 (1995).
16. Joshi, B. *et al.* Phosphorylation of eukaryotic protein synthesis initiation factor 4E at Ser-209. *J. Biol. Chem.* **270,** 14597–14603 (1995).
17. Minich, W. B., Balasta, M. L., Goss, D. J. & Rhoads, R. E. Chromatographic resolution of *in vivo* phosphorylated and nonphosphorylated eukaryotic translation initiation factor eIF-4E: increased cap affinity of the phosphorylated form. *Proc. Natl Acad. Sci. USA* **91,** 7668–7672 (1994).
18. Scheper, G. C. & Proud, C. G. Does phosphorylation of the cap-binding protein eIF4E play a role in translation initiation? *Eur. J. Biochem.* **269,** 5350–5359 (2002).
19. Gingras, A. C. *et al.* Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev.* **13,** 1422–1437 (1999).
20. Feoktistova, K. *et al.* Human eIF4E promotes mRNA restructuring by stimulating eIF4A helicase activity. *Proc. Natl Acad. Sci. USA* **110,** 13339–13344 (2013).
21. Koromilas, A. E., Lazaris-Karatzas, A. & Sonenberg, N. mRNAs containing extensive secondary structure in their 5′ non-coding region translate efficiently in cells overexpressing initiation factor eIF-4E. *Embo. J.* **11,** 4153–4158 (1992).
22. Jacinto, E. *et al.* Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat. Cell Biol.* **6,** 1122–1128 (2004).
23. Heesom, K. J., Gampel, A., Mellor, H. & Denton, R. M. Cell cycle-dependent phosphorylation of the translational repressor eIF-4E binding protein-1 (4E-BP1). *Curr. Biol.* **11,** 1374–1379 (2001).
24. Shang, Z. F. *et al.* 4E-BP1 participates in maintaining spindle integrity and genomic stability via interacting with PLK1. *Cell Cycle* **11,** 3463–3471 (2012).
25. Tomek, W. *et al.* Regulation of translation during *in vitro* maturation of bovine oocytes: the role of MAP kinase, eIF4E (cap binding protein) phosphorylation, and eIF4E-BP1. *Biol. Reprod.* **66,** 1274–1282 (2002).
26. Ellederova, Z. *et al.* Suppression of translation during *in vitro* maturation of pig oocytes despite enhanced formation of cap-binding protein complex eIF4F and 4E-BP1 hyperphosphorylation. *Mol. Reprod. Dev.* **73,** 68–76 (2006).
27. Romasko, E. *et al.* Association of maternal mRNA and phosphorylated EIF4EBP1 variants with the spindle in mouse oocytes: localized translational control supporting female meiosis in mammals. *Genetics* **195,** 349–355 (2013).
28. Thoreen, C. C. *et al.* A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* **485,** 109–113 (2012).
29. Moerke, N. J. *et al.* Small-molecule inhibition of the interaction between the translation initiation factors eIF4E and eIF4G. *Cell* **128,** 257–267 (2007).
30. Stern, S., Rayyis, A. & Kennedy, J. F. Incoporation of amino acids during maturation *in vitro* by the mouse oocyte: effect of puromycin on protein synthesis. *Biol. Reprod.* **7,** 341–346 (1972).
31. Oyadomari, S., Harding, H. P., Zhang, Y., Oyadomari, M. & Ron, D. De-phosphorylation of translation initiation factor 2α (eIF2α) enhances glucose tolerance and attenuates hepato-steatosis in mice. *Cell. Metab.* **7,** 520–532 (2008).
32. Gingras, A. C., Raught, B. & Sonenberg, N. Regulation of translation initiation by FRAP/mTOR. *Genes Dev.* **15,** 807–826 (2001).
33. Yamashita, R. *et al.* Comprehensive detection of human terminal oligo-pyrimidine (TOP) gene and analysis of their characteristics. *Nucleic Acids Res.* **36,** 3707–3715 (2008).
34. Fenton, T. R. & Gout, I. T. Functions and regulation of the 70 kDa ribosomal S6 kinases. *Int. J. Biochem. Cell Biol.* **43,** 47–59 (2011).
35. Gradi, A. *et al.* A novel functional human eukaryotic translation initiation factor 4G. *Mol. Cell Biol.* **18,** 334–342 (1998).
36. López de Quinto, S., Lafuente, E. & Martínez-Salas, E. IRES interaction with translation initiation factors: functional characterization of novel RNA contacts with eIF3, eIF4B, and eIF4GII. *RNA* **7,** 1213–1226 (2001).
37. Dieterich, D. C. *et al.* In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nat. Neurosci.* **13,** 897–905 (2010).
38. FitzHarris, G., Marangos, P. & Carroll, J. Changes in endoplasmic reticulum structure during mouse oocyte maturation are controlled by the cytoskeleton and cytoplasmic dynein. *Dev. Biol.* **305,** 133–144 (2007).
39. Dalton, C. M. & Carroll, J. Biased inheritance of mitochondria during asymmetric cell division in the mouse oocyte. *J. Cell Sci.* **126,** 2955–2964 (2013).
40. Schlaitz, A.-L., Thompson, J., Wong, C. C. L., Yates, 3rd J. R. & Heald, R. REEP3/4 ensure endoplasmic reticulum clearance from metaphase chromatin and proper nuclear envelope architecture. *Dev. Cell* **26,** 315–323 (2013).
41. Navé, B. T., Ouwens, M., Withers, D. J., Alessi, D. R. & Shepherd, P. R. Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *Biochem. J.* **344,** 427–431 (1999).
42. Chiang, G. G. & Abraham, R. T. Phosphorylation of mammalian target of rapamycin (mTOR) at Ser-2448 is mediated by p70S6 kinase. *J. Biol. Chem.* **280,** 25485–25490 (2005).
43. Dufner, A. & Thomas, G. Ribosomal S6 kinase signaling and the control of translation. *Exp. Cell Res.* **253,** 100–109 (1999).
44. Hornsten, O. & Meyuhas, E. in *Translational Control of Gene Expression* (eds Sonenberg, N., Hershey, J. W. B. & Mathews, M. B.) 671–693 (Cold Spring Harbor Laboratory Press, 2000).
45. Mamane, Y. *et al.* Epigenetic activation of a subset of mRNAs by eIF4E explains its effects on cell proliferation. *PLoS ONE* **2,** e242 (2007).
46. Pinkstaff, J. K., Chappell, S. A., Mauro, V. P., Edelman, G. M. & Krushel, L. A. Internal initiation of translation of five dendritically localized neuronal mRNAs. *Proc. Natl Acad. Sci. USA* **98,** 2770–2775 (2001).
47. Dollar, G., Struckhoff, E., Michaud, J. & Cohen, R. S. Rab11 polarization of the Drosophila oocyte: a novel link between membrane trafficking, microtubule organization, and oskar mRNA localization and translation. *Development* **129,** 517–526 (2002).
48. Håvik, B., Røkke, H., Bårdsen, K., Davanger, S. & Bramham, C. R. Bursts of high-frequency stimulation trigger rapid delivery of pre-existing alpha-CaMKII mRNA to synapses: a mechanism in dendritic protein synthesis during long-term potentiation in adult awake rats. *Eur. J. Neurosci.* **17,** 2679–2689 (2003).
49. Kugler, J. M. & Lasko, P. Localization, anchoring and translational control of oskar, gurken, bicoid and nanos mRNA during *Drosophila* oogenesis. *Fly (Austin)* **3,** 15–28 (2009).

50. Bratu, D. P., Catrina, I. E. & Marras, S. A. E. Tiny molecular beacons for *in vivo* mRNA detection. *Methods Mol. Biol.* **714,** 141–157 (2011).
51. Hutchinson, J. N. *et al.* A screen for nuclear transcripts identifies two linked noncoding RNAs associated with SC35 splicing domains. *BMC Genomics* **8,** 39 (2007).
52. König, H., Matter, N., Bader, R., Thiele, W. & Müller, F. Splicing segregation: the minor spliceosome acts outside the nucleus and controls cell proliferation. *Cell* **131,** 718–729 (2007).
53. Watson, A. J., Wiemer, K. E., Arcellana-Panlilio, M. & Schultz, G. A. U2 small nuclear RNA localization and expression during bovine preimplantation development. *Mol. Reprod. Dev.* **31,** 231–240 (1992).
54. Hashimoto, N. & Kishimoto, T. Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. *Dev. Biol.* **126,** 242–252 (1988).
55. Richter, J. D. & Sonenberg, N. Regulation of cap-dependent translation by eIF4E inhibitory proteins. *Nature* **433,** 477–480 (2005).
56. Merrick, W. C. Cap-dependent and cap-independent translation in eukaryotic systems. *Gene* **332,** 1–11 (2004).
57. Kalitsis, P., Earle, E., Fowler, K. J. & Choo, K. H. Bub3 gene disruption in mice reveals essential mitotic spindle checkpoint function during early embryogenesis. *Genes Dev.* **14,** 2277–2282 (2000).
58. Cuomo, M. E. *et al.* p53-Driven apoptosis limits centrosome amplification and genomic instability downstream of NPM1 phosphorylation. *Nat. Cell Biol.* **10,** 723–730 (2008).
59. Li, M. *et al.* Bub3 is a spindle assembly checkpoint protein regulating chromosome segregation during mouse oocyte meiosis. *PLoS ONE* **4,** e7701 (2009).
60. Van der Horst, A. & Lens, S. M. A. Cell division: control of the chromosomal passenger complex in time and space. *Chromosoma* **123,** 25–42 (2013).
61. Delacour-Larose, M., Molla, A., Skoufias, D. A., Margolis, R. L. & Dimitrov, S. Distinct dynamics of Aurora B and Survivin during mitosis. *Cell Cycle* **3,** 1418–1426 (2004).
62. Sebestova, J., Danylevska, A., Novakova, L., Kubelka, M. & Anger, M. Lack of response to unaligned chromosomes in mammalian female gametes. *Cell Cycle* **11,** 3011–3018 (2012).
63. Nielsen, P. J., Manchester, K. L., Towbin, H., Gordon, J. & Thomas, G. The phosphorylation of ribosomal protein S6 in rat tissues following cycloheximide injection, in diabetes, and after denervation of diaphragm. A simple immunological determination of the extent of S6 phosphorylation on protein blots. *J. Biol. Chem.* **257,** 12316–12321 (1982).
64. Duncan, R. & McConkey, E. H. Rapid alterations in initiation rate and recruitment of inactive RNA are temporally correlated with S6 phosphorylation. *Eur. J. Biochem.* **123,** 539–544 (1982).
65. Jefferies, H. B. *et al.* Rapamycin suppresses 5′TOP mRNA translation through inhibition of p70s6k. *EMBO J.* **16,** 3693–3704 (1997).
66. Peterson, R. T. & Schreiber, S. L. Translation control: connecting mitogens and the ribosome. *Curr. Biol.* **8,** 248–250 (1998).
67. Shah, O. J., Ghosh, S. & Hunter, T. Mitotic regulation of ribosomal S6 kinase 1 involves Ser/Thr, Pro phosphorylation of consensus and non-consensus sites by Cdc2. *J. Biol. Chem.* **278,** 16433–16442 (2003).
68. Kang, S. A. *et al.* mTORC1 phosphorylation sites encode their sensitivity to starvation and rapamycin. *Science* **341,** 1236566 (2013).
69. Yu, Y., Dumollard, R., Rossbach, A., Lai, F. A. & Swann, K. Redistribution of mitochondria leads to bursts of ATP production during spontaneous mouse oocyte maturation. *J. Cell. Physiol.* **224,** 672–680 (2010).
70. Yi, K. *et al.* Sequential actin-based pushing forces drive meiosis I chromosome migration and symmetry breaking in oocytes. *J. Cell Biol.* **200,** 567–576 (2013).
71. Amin, M. A., Matsunaga, S., Uchiyama, S. & Fukui, K. Nucleophosmin is required for chromosome congression, proper mitotic spindle formation, and kinetochore-microtubule attachment in HeLa cells. *FEBS Lett.* **582,** 3839–3844 (2008).
72. Yue, Z. *et al.* Deconstructing Survivin: comprehensive genetic analysis of Survivin function by conditional knockout in a vertebrate cell line. *J. Cell Biol.* **183,** 279–296 (2008).
73. Braddock, M. *et al.* Intron-less RNA injected into the nucleus of *Xenopus* oocytes accesses a regulated translation control pathway. *Nucleic Acids Res.* **22,** 5255–5264 (1994).
74. Gao, S. *et al.* Germinal vesicle material is essential for nucleus remodeling after nuclear transfer. *Biol. Reprod.* **67,** 928–934 (2002).
75. Polanski, Z., Hoffmann, S. & Tsurumi, C. Oocyte nucleus controls progression through meiotic maturation. *Dev. Biol.* **281,** 184–195 (2005).
76. Jones, K. T. & Lane, S. I. R. Molecular causes of aneuploidy in mammalian eggs. *Development* **140,** 3719–3730 (2013).
77. Dieterich, D. C. *et al. In situ* visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nat. Neurosci.* **13,** 897–905 (2010).
78. Hodges, C. A. & Hunt, P. A. Simultaneous analysis of chromosomes and chromosome associated proteins in mammalian oocytes and embryos. *Chromosoma* **111,** 165–169 (2002).
79. Susor, A. *et al.* Regulation of cap-dependent translation initiation in the early stage porcine parthenotes. *Mol. Reprod. Dev.* **75,** 1716–1725 (2008).
80. Bhattacharyya, T. *et al.* Mechanistic basis of infertility of mouse intersubspecific hybrids. *Proc. Natl Acad. Sci. USA* **110,** E468–E477 (2013).
81. Thoreen, C. C. *et al.* A unifying model for mTORC1-mediated regulation of mRNA translation. *Nature* **485,** 109–113 (2012).
82. Meijer, H. A. *et al.* Translational repression and eIF4A2 activity are critical for microRNA mediated gene regulation. *Science* **340,** 82–85 (2013).
83. Raj, A., van den Bogaard, P., Rifkin, S. A., van Oudenaarden, A. & Tyagi, S. Imaging individual mRNA molecules using multiple singly labeled probes. *Nat. Methods* **5,** 877–879 (2008).


## Acknowledgements

We thank Stepan Hladky, Michaela Rakocyova, Veronika Benesova and Adela Brzakova for assistance with experiments and Martin Bushell for kindly providing the plasmid. This work was supported by GACR 13-12291S to A.S.; MEYS Grant ED1.1.00/02.0068 to M.A.; GACR P502122201 to M.K. and M.A.; P502-10-0944 to M.K. Institutional Research Concept RVO67985904.

## Author contributions

A.S. and M.K. designed the experiments, carried out the data analysis and planed the project. A.S., D.J., R.C., T.T. and J.S. caried out most of the experiments; A.D. and M.A. caried out live-cell immaging; M.S.C. and J.S.O. performed experiments that contributes intellectually but did not result in figures; R.M. caried out Dual-luciferase assays; A.S. and M.K. wrote the manuscript.

## Additional information

**Supplementary Information** accompanies this paper at http://www.nature.com/naturecommunications

**Competing financial interests:** The authors declare no competing financial interests.

**Reprints and permission** information is available online at http://npg.nature.com/reprintsandpermissions/

**How to cite this article:** Susor, A. *et al.* Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR–eIF4F pathway. *Nat. Commun.* 6:6078 doi: 10.1038/ncomms7078 (2015).

Cell Tissue Res
DOI 10.1007/s00441-015-2269-6

REVIEW

# Translation in the mammalian oocyte in space and time

**Andrej Susor**[1] · **Denisa Jansova**[1] · **Martin Anger**[1,2] · **Michal Kubelka**[1]

Received: 30 April 2015 / Accepted: 3 August 2015


**Abstract** A hallmark of oocyte development in mammals is the dependence on the translation and utilization of stored RNA and proteins rather than the de novo transcription of genes in order to sustain meiotic progression and early embryo development. In the absence of transcription, the completion of meiosis and early embryo development in mammals relies significantly on maternally synthesized RNAs. Post-transcriptional control of gene expression at the translational level has emerged as an important cellular function in normal development. Therefore, the regulation of gene expression in oocytes is controlled almost exclusively at the level of mRNA and protein stabilization and protein synthesis. This current review is focused on the recently emerged findings on RNA distribution related to the temporal and spatial translational control of the meiotic progression of the mammalian oocyte.

**Keywords** Oocyte · Translation · RNA · RNP · Meiosis

## Introduction

The mammalian oocyte is a relatively large cell that undergoes two asymmetric divisions on the way to form a fertilizable egg. During the hiatus between maternal and zygotic genomic transcription, early development relies on post-transcriptional gene regulation.

After a period of active transcription during growth, the nucleus (germinal vesicle, GV) of mammalian oocytes becomes transcriptionally inactive (De La Fuente et al. 2004). In the absence of transcription, the completion of meiosis and early embryo development in mammals relies significantly on maternally synthesized RNAs and proteins (Brandhorst 1985; Curtis et al. 1995; Nothias et al. 1995). Therefore, the regulation of gene expression in oocytes is controlled almost exclusively at the level of mRNA translation and stabilization and posttranslational modifications of proteins.

In order to halve the chromosome number during meiosis, mammalian oocytes undergo two successive divisions without any intervening DNA replication. During resumption of meiosis, the nuclear envelope breaks down (NEBD), chromosomes condense and microtubule organizing centers (MTOC) form a bipolar spindle engaging all bivalent chromosomes into division (Schuh and Ellenberg 2007). At the end of meiosis I, the spindle migrates from the center of the oocyte to the cortex in preparation for the first asymmetric division. This period of 'meiotic maturation', completed by the spindle re-assembly and meiosis II arrest, is crucial for the formation of a fully-developed egg capable of being fertilized and generating viable offspring.

## Oocyte transcriptome

The absence of nuclear transcription between the resumption of meiotic maturation in oocytes and the activation of the embryonic genome emphasizes the critical role of the pre-existing stockpile of RNAs and proteins (Seydoux and Braun 2006). Oocytes of many species, both invertebrate and vertebrate, contain a large collection of localized

✉ Andrej Susor
susor@iapg.cas.cz

[1] Institute of Animal Physiology and Genetics, ASCR, Rumburska 89, 277 21 Libechov, Czech Republic

[2] CEITEC-Veterinary Research Institute, Hudcova 296/70, 621 00 Brno, Czech Republic

Published online: 04 September 2015

regulators in the form of proteins and translationally inactive maternal mRNAs.

In mammals, maternally-deposited transcripts were shown to be generally more conserved than those newly synthesized by the nascent embryo (King et al. 2005). Growing mouse oocytes are transcriptionally highly active, generating the large amount of maternal RNAs required for the oocyte-to-zygote transition while transcription is silenced (Stitzel and Seydoux 2007).

The last period of transcriptional activity in growing mouse oocytes seems to be important for the full developmental competence of the oocytes, as indicated by the results of analyses of the two categories of oocytes, usually obtained from ovarian antral follicles—NSN oocytes ("not surrounded nucleolus") and SN oocytes ("surrounded nucleolus"). SN-oocytes have higher meiotic and developmental competence than NSN-oocytes and correspond to a more advanced stage of oocyte development (Zuccotti et al. 2002, 2011; Inoue et al. 2008).

During the growth phase, oocytes synthesize and accumulate rRNA and tRNA; more than 60 % of the total amount of RNA in oocytes is rRNA. The fully grown oocytes are transcriptionally quiescent. An outcome of this would be that all the 47S rRNA would already have been processed to mature forms. Monti et al. (2013) show that expression of mRNA of 27 ribosomal proteins is higher in SN oocytes relative to NSN oocytes, suggesting that cytoplasm of these oocytes is not fully equipped for normal translation that could be the plausible cause of the two-cell developmental arrest of the NSN oocyte-derived embryos (Inoue et al. 2008). Indeed, it is intriguing that mouse oocytes destroy a large amount of rRNA and ribosomal protein mRNAs during maturation, which rise again to high levels upon fertilization and during early embryonic development. Then, in late two-cell stage, the zygotic genome activation becomes apparent (Schultz and Wassarman 1977; Lin et al. 2014), and genes involved in the ribosome biogenesis and assembly are included in the first gene activation burst (Zeng and Schultz 2005; Lin et al. 2014). It is possible that mammalian oocytes adjust their pool of rRNA and ribosomes to match the mRNA pool and protein requirements, with high levels during oocyte growth, a relatively quiescent MII stage accompanied by a global reduction in protein synthesis (Schultz and Wassarman 1977; Susor et al. 2008; Ellederová et al. 2008). The presence or absence of specific ribosomal proteins in the ribosome is known to control translation of specific subsets of mRNAs (Kondrashov et al. 2011; Kang et al. 2013). This suggests that the deficiency in maternal ribosomes might be the result of a low meiotic and developmental competence in the NSN oocyte (Inoue et al. 2008; Chen et al. 2013).

tRNA is the physical link between the nucleotide sequence of RNA and the amino acid sequence of proteins. tRNAs have also been reported to be the second most abundantly represented class of small RNAs in porcine oocytes and embryos (Yang et al. 2012).

In addition to mRNAs which are translated shortly after synthesis, the oocyte maternal transcriptome contains stable dormant maternal mRNAs, which are recruited later during meiotic maturation and/or during early embryogenesis. Selective polyadenylation and decapping are the two major mechanisms regulating the recruitment and stability of dormant maternal mRNAs (Bettegowda and Smith 2007; Ma et al. 2013). RNA molecules are rarely dispersed in cells, as their function usually requires the formation of various ribonucleoprotein (RNP) complexes (Balagopal and Parker 2009). Maternal mRNP composition can determine mRNA fate: localization, translational repression, level of translation or mRNA stability.

It is important to emphasize that the RNA transcripts are allocated in the oocyte as well as in the polar body. The symmetry of the RNA distribution between these cellular compartments has been studied using various quantification methods, with the results unambiguously showing that the RNA is equally distributed between the oocyte and the polar body relative to their volume. Differential gene enrichment analysis of all oocyte samples against all polar body samples revealed no genes that were differentially enriched between the two populations at any level of significance in the oocytes of the human (Reich et al. 2011) and the mouse (VerMilyea et al. 2011).

Thanks to RNA sequencing, the number of uncharacterized transcribed regions which have been identified is growing quickly. They can be divided into several groups, each of them likely to have different functions within the cell. Recent reports suggest, however, that the vast majority of mammalian transcriptome is non-coding (Mattick and Makunin 2006; Mercer et al. 2009).

MicroRNAs (miRNA) are a class of small non-coding RNAs, which are involved in the processes of translational repression and mRNA decay (Fabian et al. 2010). While it was initially postulated that mRNA levels did not change substantially in response to miRNAs, it was later shown that mRNA destabilization, prompted by deadenylation and decapping by the mRNA degradation machinery, is the main mode of mRNA regulation by mammalian miRNAs (Guo et al. 2010).

It was a surprise to learn that oocyte-specific targeting of the miRNA processing regulator, Dgcr8, which prevents the formation of mature miRNAs in oocytes, results in a normal transcriptome, and in oocytes undergoing normal meiotic maturation, fertilization, and embryonic development (Ma et al. 2010; Suh et al. 2010). It is possible that the loss of miRNA function in oocytes and early embryos is related to the loss of processing bodies (P-bodies) that occurs earlier during the GV stage and does not recover until the blastocyst stage (Flemr et al. 2010). P-bodies are distinctive foci in the cytoplasm of

eukaryotic cells which have a functioning role in mRNA decay and miRNA-mediated translational repression (Chan and Slack 2006; Parker and Sheth 2007; Jakymiw et al. 2007). Overexpressed ZAR1L, a P-body component in the somatic cells, forms cloud-like structures in the perinuclear cytoplasm of oocytes and cytoplasmic foci in two-cell embryos. Such structures were, however, absent in MI, MII oocytes, and zygotes (Hu et al. 2010). Swetloff et al. (2009) also reported that DCP1 foci localized with P-body marker LSM14A in the cytoplasm of GV oocytes and similarly to the structures visible after ZAR1L overexpression disappeared after NEBD. Since the structures were observed after overexpression of P-body components, they might represent an aggregation of overexpressed protein and might not be formed under physiological conditions.

Hundreds of conserved circRNAs were also reported in *C. elegans* oocytes and early embryos (Memczak et al. 2013). We might speculate that the model in which interaction of miRNAs and circRNA induces the inhibition of miRNA activity (Hansen et al. 2013; Memczak et al. 2013) is also taking place in mammalian oocytes and early embryos.

Endogenous short interfering RNAs trigger endonucleolytic cleavage of target mRNAs, mediated by AGO2. The catalytically inactive knock-in allele of Ago2 in oocytes, which disrupts the function of siRNAs, leads to severe defects in spindle formation and chromosome alignment resulting in the meiotic catastrophe. The transcriptome of these oocytes is widely perturbed and expression of the most abundant transposable element in mouse oocytes is increased (Stein et al. 2015). It is believed that, after fertilization, neither miRNAs nor siRNAs play a role in preimplantation development, since zygotic deletions of *Dgcr8*, *Dicer*, and *Ago2* do not have a phenotype until after embryo implantation (Bernstein et al. 2003; Morita et al. 2007; Suh and Blelloch 2011). Moreover, maternal deletion of *Dgcr8* has no phenotype until post-implantation, suggesting no essential role for maternal miRNAs throughout oocyte maturation or during the whole preimplantation development (Suh et al. 2010).

## RNA localization

mRNA localization linked to local translation is a fundamental mechanism for the successful propagation of genetic information to the next generation and is a very efficient way to orchestrate various cellular processes. Spatial segregation of protein synthesis in cells is crucially dependent on the correct positioning of mRNAs. This asymmetrical distribution of mRNA, termed mRNA localization, might be more thermodynamically efficient than transporting proteins because fewer RNA molecules need to be moved. It is also possible that spatially controlled translation offers a more subtle control of local protein activity (Weatheritt et al. 2014) in comparison to other means. Furthermore, proteins synthesized locally are structurally and functionally distinct from transported proteins; they are more likely to contain domains that promote protein–protein interactions, and are subject to a tighter regulation of expression and to more post-translational modifications than proteins that are not translated locally (Weatheritt et al. 2014).

Among the mechanisms by which intracellular mRNAs are targeted to specific compartments, there are two which are relatively well studied. Firstly, cotranslational enrichment of mRNAs encoding for secreted and membrane proteins. The mechanism involves a so-called "signal recognition particle" (SRP), which was found to bind the signal peptide of the nascent polypeptide, arrest translation, and then direct the mRNA–ribosome–nascent chain complex to the endoplasmic reticulum (ER) membrane for cotranslational translocation (Keenan et al. 2001; Ménétret et al. 2007; Cross et al. 2009; Saraogi and Shan 2011). Secondly, RNA localization sequence (zipcode) mediated intracellular localization of mRNAs, generally for the mRNAs encoding for cytosolic proteins, which lack the signal peptide. The localization of these mRNAs relies on one or multiple zipcode(s) on the RNA, often in the 3'UTR (Haim et al. 2007; Jambhekar and Derisi 2007; Kraut-Cohen and Gerst 2010).

Well-known examples of localized mRNAs are *oskar*, *bicoid*, *gurken* or *nanos*, which accumulate at the anterior or posterior poles of the *Drosophila* oocyte. This spatial separation is essential for patterning during embryogenesis (King et al. 2005). In a similar manner, the maternal transcripts *Vg1* and *VegT* encoding mesoderm-specifying factors localize to the vegetal cortex of late stage *Xenopus* oocytes (Holt and Bullock 2009).

In contrast, little is known about the localization patterns of mRNAs or RNPs in mammalian oocytes, with consequences for meiosis and early embryo development particularly for the maternal RNPs.

## RNA cytoplasmic structures

As mentioned above, most mRNAs require an association with protein factors to create functional RNPs in order to operate. Furthermore, their transport, translation, protection from degradation and anchoring, as well as repression or degradation of mRNAs is regulated by a number of adaptors and "RNA-binding proteins" (RBPs). A large proportion of mRNAs, together with RNPs and RBPs, are localized in specific cytoplasmic structures, usually awaiting further processing. A variety of mammalian RNA granules exhibiting an overlapping composition but possessing different structures and functions have been described. We will focus only on those with a connection to translational regulation.

**Subcortical complexes** The subcortical region of the mammalian oocyte is rich in structures involved in mRNA storage and metabolism. A subcortical RNP domain (SCRD) has been described in oocytes representing another maternal mRNA storage structure (Flemr et al. 2010). Subcortical complexes share components with P-bodies including several RNA-binding proteins, such as DDX6, YBX2 (MSY2), and CPEB. They localize to the cortex-forming transient RNP aggregates containing maternal mRNAs. Consistent with their function as a storage compartment, they detach from the cortex upon resumption of meiosis, relocate toward the center of the oocyte, and dissolve. These SCAs (subcortical aggregates) emerge during the formation of SN chromatin and disappear during meiotic maturation, resulting in a homogeneous subcortical structure. One of the components of the SCRD and SCAs is the translational repressor DDX6 (Rck/p45) that is implicated in the control of maternal mRNAs in lower organisms (Weston and Sommerville 2006). DDX6 orthologues are found in a number of different RNA granules regulating mRNA stability and translation throughout female germ cell development and in the soma (Weston and Sommerville 2006).

Apart from the SCRD and SCAs, the subcortical region of oocytes and early embryos is also the site of localization of the protein complex, subcortical maternal complex (SCMC). SCMC contains MATER, together with FILIA (KHDC3L), FLOPED (MOEP19), PADI6, and TLE6 proteins, and this complex has been shown to be essential for preimplantation embryo development beyond the two-cell stage (Ohsugi et al. 2008; Herr et al. 2008; Li et al. 2008, 2010, 2013; Zhu et al. 2015). The role of the SCMC complex in mRNA processing is not clear, although it is known that FLOPED is a putative RNA-binding protein (Pierre et al. 2007; Herr et al. 2008; Li et al. 2008). Absence of the SCMC significantly impedes development beyond the two-cell embryo, and it seems likely that defects arise earlier, as the progression from one- to two-cells is delayed and initial cell division is often asymmetrical. This could result from abnormalities in syngamy, mitotic spindle formation (loss of FILIA causes defects in spindle assembly (Flemr et al. 2010), cytokinesis or cell cycle progression and could affect later events in embryonic development including axis formation, a subject of intense investigative interest (Rossant and Tam 2004; Louvet-Vallée et al. 2005; Motosugi et al. 2005; Kurotaki et al. 2007; Bischoff et al. 2008). *Padi6* null females have impaired embryonic transcription and dysregulation of protein translation (Yurttas et al. 2008). The SCRD marker GW182 does not localize with the marker of SCMC (MATER), suggesting that the SCMC complex is possibly not associated with mRNA metabolism. Also, RNA staining does not co-localize with the SCMC and treatment with RNase does not disrupt the complex as determined by immunoprecipitation (Li et al. 2008). Nevertheless, specific transcripts could bind to the SCMC and given the prominent role of RNA localization in development (Strome and Lehmann 2007), this possibility warrants further investigation.

Polysomes are clusters of ribosomes bound with mRNA in the act of translation (Slayter et al. 1963). Early development is characterized by major fluctuations in the abundance of RNA (Bachvarova et al. 1985; Vallée et al. 2009), with specific waves of maternal RNA degradation (Schultz 2002; Schier 2007). However, measurements of global mRNA abundance provide only limited information about cells with significant mRNA populating in dormant form, since it does not identify mRNA that is stabilized/stored and thus not contributing to cellular function, or on its way to degradation, or translation. mRNAs engaged in translation are most likely bound to the ribosome-translational apparatus. Actively translated messengers are simultaneously linked to multiple ribosomal units (Hendler 1974; Hall et al. 1982, 1984; Vedeler et al. 1991), which is in turn a hallmark of active translation. Studies using ribosome profiling have already provided new insights into the identity and quantity of proteins that are produced by the oocyte (Scantland et al. 2011; Kronja et al. 2014).

## Localization of RNA to cellular compartments

There are a number of examples, both in vertebrate and non-vertebrate cells, of mRNA localization to various subcellular structures including nuclear membranes, ER, mitochondria and peroxisomes. The mechanism of targeting mRNAs to these membranes is described above and involves either cotranslational (SRP-pathway; Kraut-Cohen and Gerst 2010) or co-translational (zipcode based) targeting (Boylan et al. 2008; Eliscovich et al. 2008). However, not much is known about mRNA localization to the compartments or structures in the mammalian oocyte.

### Nuclear RNA retention

As mammalian oocytes acquire competence to resume and complete meiosis, the chromosomes cluster on the nuclear as well as the nucleolar membrane, which is manifested as a shift from NSN oocytes to SN oocytes. A general hypothesis in the field suggests that only the NSN oocytes actively synthesize rRNA, whereas the SN-type oocytes are transcriptionally silent (Bouniol-Baly et al. 1999; Pesty et al. 2007). Published results have shown that, irrespective of the functional status, the nucleolus of fully grown mouse oocytes contains snRNAs, small nuclear RNPs (Kopecny et al. 1996), but not rRNAs (Shishova et al. 2015). The maternal nucleolus contributes to the storage of maternal nucleoplasmic spliceosomal components before being diluted in the cytoplasm post-NEBD (Szöllösi et al. 1993; Vautier et al. 1994). These authors have

also shown that nuclear RNPs, released post-NEBD, reincorporate into both the male and female pronucleus.

Retention of mRNA within the nucleus has been described in plants and drosophila (Lécuyer et al. 2007; Boothby and Wolniak 2011; Boothby et al. 2013; Niedojadło et al. 2014; Göhring et al. 2014; Jambor et al. 2015). However, the first example of this phenomenon in mammalian oocytes was provided only recently when endogenous poly(A)-RNAs were seen in the nucleus of the fully grown mouse GV oocyte (Fig. 1; Susor et al. 2015). After NEBD, the poly(A)-RNA signal is stronger in the vicinity of the chromosomes and in the region of spindle formation, in contrast to the cytoplasm where the signal is significantly lower. Poly(A)-RNA is distributed diffusely in the nucleus of growing oocytes compared to fully grown oocytes, where the signal is clustered. Previous data have clearly shown that some transcripts, including *Bub3, Npm1, Survivin, Dazl* and *Pabn1* mRNAs, are equally distributed in both the nucleus and the cytoplasm, while other transcripts, such as *Mos*, *Gapdh*, *Tuba*, *mTOR*, *Eif4e* and *Camk2a*, were abundant in the cytoplasm and absent from the nucleus. Also, other transcripts known to be localized in the nucleus in somatic cells, such as non-coding RNAs (*Neat2*, *U2* and *U12*), as well as Pabpnl1 (Watson et al. 1992; König et al. 2007) mRNA, were found in the nucleus of mouse oocytes. Separate from the specific mRNAs detected in the oocyte nucleus mentioned above, a pool of poly(A) + mRNAs (as classified by Yang et al. (2011) according to the presence or absence of a poly(A) tail at their 3' ends) was also found to be localized in the nucleus; however, their identity has yet to be determined. The presence of mRNAs in the nucleus might be the result of the post-transcriptional retention of these mRNAs, or such mRNAs once exported from the nucleus might be imported back prior to NEBD. The mechanisms responsible for either of the events are currently unknown in mammalian oocytes; however, one possibility is that the pre-mRNA could be retained within the nucleus, which might be subsequently spliced in the dynamic process as described in the gametophyte by Boothby et al. (2013). This mechanism of action highlights an important paradigm for the essential role of the nuclear-retained stable RNA transcripts in regulating gene expression (Prasanth et al. 2005; Bhatt et al. 2012; Carrieri et al. 2015).

The above-mentioned examples suggest that nuclear mRNA retention may serve as a way to orchestrate protein expression in various cellular processes (Kambadur et al. 1998; Pilot et al. 2006; Brandt et al. 2006; Grosskortenhaus et al. 2006). This mechanism seems to be a relatively common form of post-transcriptional gene regulation. It is conceivable that in oocytes the RNA population retained in the nucleus might contribute to translation in the vicinity of the chromosomes after NEBD.

## Spindle RNA localization

Spindle formation requires protein synthesis (Hashimoto and Kishimoto 1988), indicating a possible role for translational control by localized mRNAs in this process. Spindle complexes are sites of targeted polysomal mRNA localization in diverse systems (Blower et al. 2007; Mili and Macara 2009), with the spindles preparing the chromosomes for division (Brunet and Verlhac 2011). The enrichment of various mRNAs on the meiotic spindle allows for the localized

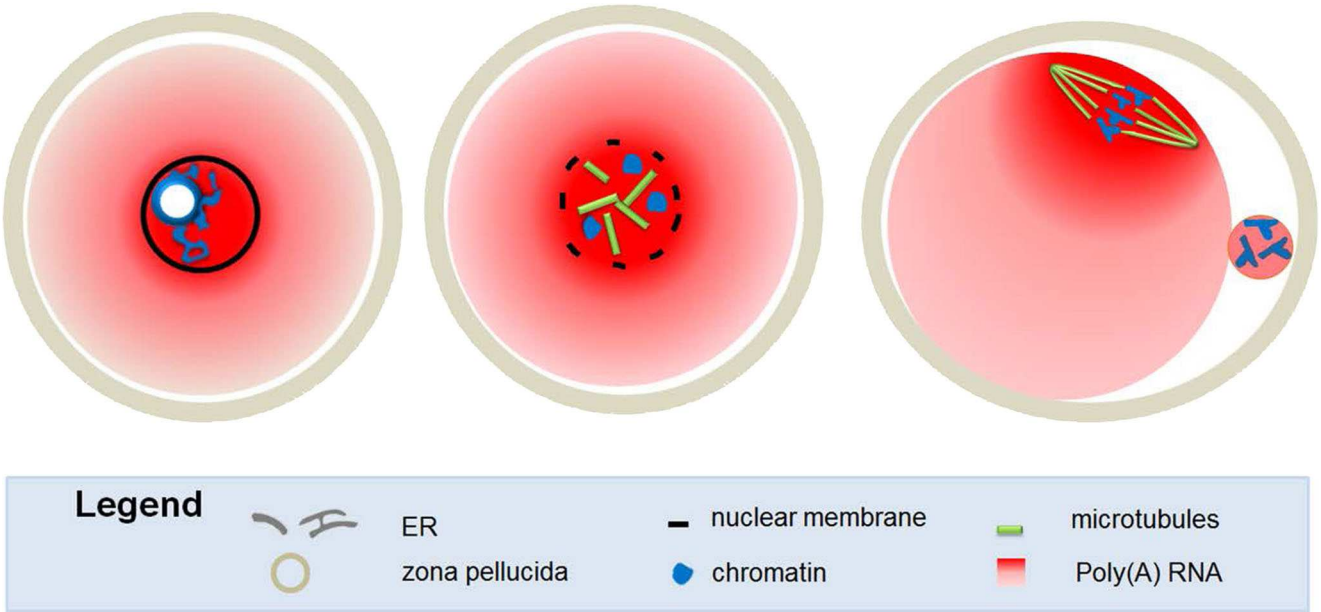



**Fig. 1** Scheme of localization of poly(A) RNA population in the mouse oocytes in the GV, NEBD and MII stages. Nucleus of the fully grown oocyte shows high poly(A) RNA signal that is retained in the chromosomal area and disappears as the oocyte proceeds through meiosis to M II stage

translation of specific proteins in the vicinity of the spindle and chromosomes (Susor et al. 2015). Among the processes which might be regulated in this manner are the control of the meiotic progression, spindle-dependent translation remodeling, and asymmetric division, typical for mammalian female meiosis (Vinot et al. 2004; Dumont et al. 2007). Experiments utilizing microsurgery techniques to study the differential abundance of mRNA in various compartments of meiotically maturing mouse oocytes revealed a significant abundance of specific mRNAs in the sample containing spindle (Romasko et al. 2013; VerMilyea et al. 2011). This suggests that by unknown mechanisms mRNAs in mammalian oocytes are not distributed equally within the whole cell volume but rather form distinguishable transcriptomes, one of which is linked to the spindle. Localized translational activation of CPE-regulated mRNAs on the meiotic spindle has also been reported in *Xenopus* oocytes (Eliscovich et al. 2008), where the subset of microtubule bound mRNAs was found to also be associated with polyribosomes (Blower et al. 2007).

Whether mRNAs are asymmetrically distributed prior to the resumption of meiosis is unknown. The global RNA population in the mouse oocyte is localized uniformly in the cytoplasm, although a strong signal of poly(A) + mRNAs in the nucleus has been reported recently (Susor et al. 2015). It is hypothesized that the RNA pool in the nucleus remains in the spindle formation area post-NEBD even after the dissolution of the barriers between the nucleus and the cytoplasm, providing a local pool of very specific transcripts supporting various spindle functions.

One can speculate that asymmetric targeting of developmentally regulated mRNAs to one spindle pole could be a mechanism by which asymmetry is established in the mouse oocyte after reaching spindle bipolarity, and it is worth noting the asymmetric localization of specific mRNAs. Another possibility is that mRNAs might not be enriched on spindles but their translation may be spatially restricted to microtubules where the key players of the cap-dependent pathway are localized. Localization of specific mRNAs to the spindle might be evolutionarily conserved between mammals and other vertebrates, possibly serving as the mechanism for enhancing protein localization. Examples of known localization of specific RNAs in the mouse oocyte are shown in Fig. 2.

## Translational regulation in the oocyte

Regulation of mRNA compartmentalization and local translation is a crucial mechanism of controlling gene expression and so allowing rapid changes in levels of proteins in specific locations. Three key aspects used by cells to control this process are the regulation of mRNA localization, translation, and stability. All steps of this process require a specific repertoire of proteins interacting with mRNA and forming RNPs.

Although the fundamental role of RNA localization has been firmly established, it remains to be elucidated how locally produced proteins are linked to various cellular processes. Such studies require techniques for tracing newly synthesized proteins from their sites of translation to the loci where they

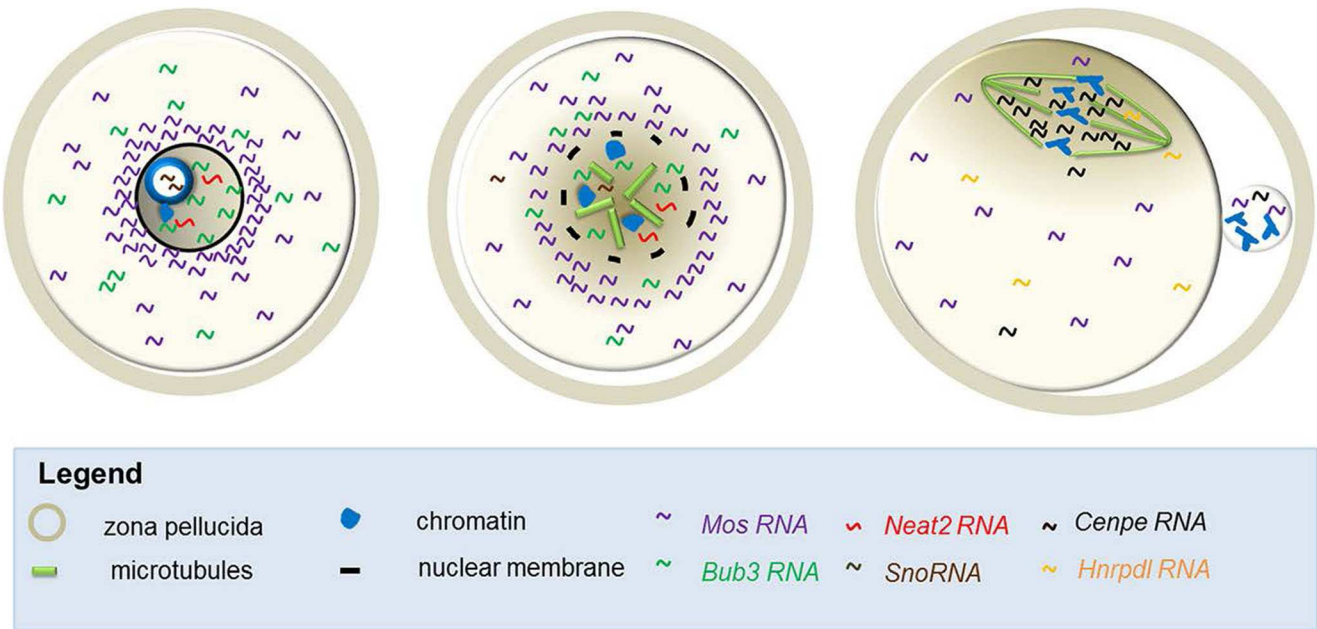



**Fig. 2** Example of localization of the specific RNAs in the mouse oocyte. The nucleus of the fully grown oocyte contains a pool of specific mRNAs and non-coding RNAs, such as *Neat2* mRNA or *Bub3* mRNA, the latter is also present in cytoplasm, while *Mos* mRNA is localized exclusively in cytoplasm. Spindle shows enrichment of *Cenpe* and *Hnrpdl* mRNAs to the spindle area. Scheme is based on the studies of Romasko et al. (2013), Susor et al. (2015), and VerMilyea et al. (2011)

are finally utilized. In mammalian oocytes, localized translation of maternal mRNAs is essential for meiotic progression (Susor et al. 2015). However, this requirement goes beyond meiosis, since genome transcription is not yet established during the first cleavage cycle of mouse embryos (Knowles et al. 2003), and therefore mRNA translation, rather than transcription, is essential for the regulation of these events (Paronetto et al. 2008).

Although some regulatory mechanisms exist during the elongation phase, the initiation of translation appears to be the rate-limiting step in the overall process and is influenced by cis-acting elements located primarily in the mRNA 5′ and 3′ untranslated regions—UTRs of maternal mRNAs, and trans-acting factors that bind to them (Hershey et al. 1996). These regions are therefore targets of at least two regulatory mechanisms. On the one hand, the translational activation of dormant mRNAs stored during oogenesis seems to be controlled by the de novo polyadenylation of the transcripts (Paynton and Bachvarova 1994; Gebauer et al. 1994; Vassalli and Stutz 1995). On the other hand, the phosphorylation/dephosphorylation changes in the number of different initiation factors and their regulators (such as eIF-2A, B, eIF-3, eIF-4 F, or 4E-BP1) are associated with changes in the rate of translation (Hershey 1991; Morley and Thomas 1991). The number, position, and combination of cis-acting elements and the presence of transacting factors offer a complex combinatorial system controlling mRNA localization, stability, and translation (Kim and Richter 2006; Racki and Richter 2006; Piqué et al. 2008; Belloc and Méndez 2008). The paradigm for temporal control of mRNA translation in maturing oocytes may apply to other developmental processes.

The major transacting regulator of the 5′UTR is the cap-binding protein complex, which is crucially important for the initiation of cap-dependent translation. A key player in the regulation of translation initiation is the mRNA cap-binding protein, eIF4E, which is the limiting component of the eIF4F initiation complex. eIF4E preferentially enhances the translation of a selective group of mRNAs with an extensive secondary structure on their 5′UTR. The main mechanisms regulating the availability of eIF4E for translation initiation (i.e. its binding to eIF4G allowing the formation of an active eIF4F complex) are both its phosphorylation by MAP kinase-integrating kinases Mnk1 and Mnk2 (Waskiewicz et al. 1997; Pyronnet 2000; Scheper et al. 2001; Ellederová et al. 2008), and its binding to and release from a family of translational repressors, the eIF4E binding proteins (4E-BPs). Hypophosphorylated 4E-BPs compete with eIF4G1 for a common binding site on eIF4E (Mader et al. 1995). The most studied from the 4E-BPs is 4E-BP1, which, in its unphosphorylated form, has been shown to bind to eIF4E preventing the formation of the active eIF4F complex. Upon phosphorylation, 4E-BP1 releases from eIF4E leaving it available for eIF4G binding (Sonenberg and Gingras 1998; Gingras et al. 1999). The major kinase responsible for phosphorylating 4E-BPs on several sites is mTOR, which itself is regulated by the PI3K/Akt signaling pathway (Scheper and Proud 2002).

Contrastingly, changes in the translation of mRNAs are frequently correlated with cytoplasmic changes in poly(A)-tail length occurring on the 3′-mRNA end, with increases in length generally correlating with translational activation. This phenomenon has been widely studied during the early development of higher eukaryotes (Puoti et al. 1997; Mendez et al. 2000), but has also been reported in somatic cells (Novoa et al. 2010). In vertebrate oocytes, the most studied cis-acting 3′UTR element is the cytoplasmic polyadenylation element (CPE) and its binding protein (CPEB) (Mendez and Richter 2001). CPEs and CPEBs play a major role in the meiotic maturation that is driven by cytoplasmic polyadenylation and sequential translational activation of dormant maternal mRNAs (Mendez et al. 2002; Richter 2007; Piqué et al. 2008; Villalba et al. 2011; Komrskova et al. 2014). CPEB is one of many RNA binding proteins which recognize either sequence motifs or secondary structures within 3'UTRs and regulate mRNA metabolism. CPEB null mutant females lack oocytes, thus demonstrating the essential role of CPEB in oogenesis (Tay and Richter 2001).

Other highly studied cis-acting motifs are AU-rich elements (AREs). In somatic cells, they regulate the stability and translation of up to 8 % of mammalian mRNAs (Bakheet et al. 2006). At least 24 ARE-binding proteins (AUBPs) have been identified (reviewed in Barreau et al. 2005), and 13 of them were shown to regulate ARE-mediated mRNA decay or translation (Garneau et al. 2007). The complex set of multiple translation-activating elements (TAEs) and translation-repressing elements (TREs) controlling translation during the meiotic maturation of oocytes, and during the early one-cell stage via a novel mode of translation control mediated primarily by the negative effects of TREs, has been described (Potireddy et al. 2010). A survey of the mammalian 3′ UTR database reveals that many mRNA encoding proteins crucial for developmental processes contain Musashi binding element (MBE) and CPE regulatory elements (Grillo et al. 2010). Studies of early mouse embryos revealed that only about one-third of the mRNA-expressed sequence tags analyzed contained CPE-like elements, indicating that elements other than CPE are involved in mRNA regulation after fertilization (Hwang et al. 2001; Evsikov et al. 2006). Most (86 %) of maternal mRNAs are preferentially translated in the egg containing known CPEs, but nearly half the maternal mRNAs (47.3 %) recruited at the late one-cell stage lacked a known CPE (Potireddy et al. 2010).

Although a fully grown mouse oocyte is able to resume meiosis without proteosynthesis, the formation of the spindle and the progression to metaphase II require active translation

(Schultz and Wassarman 1977; Hashimoto and Kishimoto 1988; Kanmera et al. 1995). These results suggest that the proteins synthesized shortly after NEBD play an important role in oocyte meiotic progression. The published studies show that, despite a decrease of overall protein synthesis in the mammalian oocyte during meiosis (Schultz et al. 1978; Tomek et al. 2002b; Ellederova et al. 2006), there is a regulatory program that ensures temporal and spatial synthesis of specific proteins essential for meiotic progression and embryo development. Alves et al. (2009) discovered that GCN2 is one of the four mammalian kinases that phosphorylate the alpha subunit of translation initiation factor 2 (eIF2a), resulting in protein synthesis repression (Moreno et al. 2012). High levels of eIF2a (S51) are found in the MII oocyte followed by a drastic decrease after fertilization (Alves et al. 2009). These results suggest that GCN2/eIF2a-mediated global translational repression control may contribute to the regulatory mechanisms operating during oocyte maturation (Schultz et al. 1978; Tomek et al. 2002b; Ellederova et al. 2006).

The actual proportion of genes expressed in oocytes has still not been determined, but it is clear that the translational activity and its regulation are crucial for oocyte development and maturation (MacNicol and MacNicol 2010). This requirement for global translation has been attributed mainly to the activation of a maturation-promoting factor, the key regulator of M-phase entry. However, the identity of the proteins which need to be synthesized and the means of spatiotemporal regulation of translation in the oocytes are not entirely clear. Inhibition of translation resulted in the blocking of development at the one-cell stage in embryos confirming that mRNA translation, rather than transcription, is also essential for the first mitotic cycle in pre-implantation embryos (Paronetto et al. 2008).

## Localized translation

Mammalian oocytes share many physiological mechanisms with lower eukaryotes (Curtis et al. 1995; Lipshitz and Smibert 2000) and also with neurones (Krichevsky and Kosik 2001; Smith et al. 2005). It is therefore not surprising that spatial mRNA translation also plays an important role during mammalian oocyte maturation (Romasko et al. 2013; Susor et al. 2015). Several reports (Krichevsky and Kosik 2001; Blower et al. 2007; Romasko et al. 2013; Susor et al. 2015) suggest the presence of localized translation ("translational hot-spots") within the oocytes. The translation of localized maternal mRNAs provides a high concentration of proteins at specific loci, while minimizing potential deficiencies related to limitations in the speed or degree of protein accumulation elsewhere within the ooplasm. Nuclear-retention of specific mRNAs in oocytes potentially serves as a limit to their translation in space and time in the vicinity of the chromosomes immediately after NEBD (Romasko et al. 2013; Susor et al. 2015). The abundance of mRNAs positively correlates with translation (Nakamura et al. 2004). The detection of nascent protein synthesis using the incorporation of the methionine analogue, L-homopropargylglycine (HPG) (Dieterich et al. 2010), is a useful tool to follow spatially determined translation in the cell. Recent results have shown that, although the whole oocyte was translationally active, in the GV oocyte, the translational activity appeared mainly in the perinuclear area and two distinct areas with different translation patterns could be identified after NEBD (Fig. 3). One was located in the immediate vicinity of the chromosomes at the newly forming spindle (chromosomal translational area, CTA), and the second in the perispindular area (perispindular translational area, PTA). Both regions were separated by cytoplasm with a decreased HPG signal. These regions of HPG signal migrated with the spindle–chromosome complex to the oocyte cortex and disappeared after the first polar body extrusion.

Translation at the PTA may synthetize proteins related to the ER and cytoplasm, when the spindlechromosome complex is translocated to the cell surface (FitzHarris et al. 2007; Dalton and Carroll 2013). The presence of mRNA encoding proteins associated with the CTA or PTA may also contribute to asymmetric spindle localization in the oocyte. The presence of mRNA encoding proteins related to ubiquitination would also be consistent with a local role for these proteins in controlling spindle formation and function (Mtango et al. 2012).

Translational control of mRNAs localized at the CTA could be facilitated by stage-dependent, spatially-restricted 4E-BP1 phosphorylation. Shortly after NEBD, the multipolar spindle forms in the cytoplasm at the same time as the CTA becomes distinguishable. During the MII stage, the CTA disappears, as does the ER-rich structure (FitzHarris et al. 2007; Zheng et al. 2013; Susor et al. 2015). Lamin A/C and B1 are supposed to dissipate in the prometaphase I stage; however, published data (Sanfins et al. 2004; Susor et al. 2015) indicate the partial retention of lamins in the membrane-like structures surrounding CTA after NEBD in the region previously occupied by the nuclear membrane. This suggests that such a structure is formed in order to prevent the rapid escape of nucleoplasm components (mRNAs, ncRNAs, nuclear proteins and chromosomes) into the cytoplasm of such a large cell and/or to prevent the entry of cytoplasmic elements into the CTA, and so successfully maintaining organelle compartmentalization. Indeed, the distribution of certain transcripts and proteins appears to be distinct. This may contribute to spindle and chromosome organization and play an important role in the maintenance of genomic stability. The results of Schlaitz et al. (2013) also indicate that clearance of ER from chromosomes is required for proper chromosome segregation during anaphase, potentially because membranes in the chromosome area may interfere with microtubule–kinetochore interactions

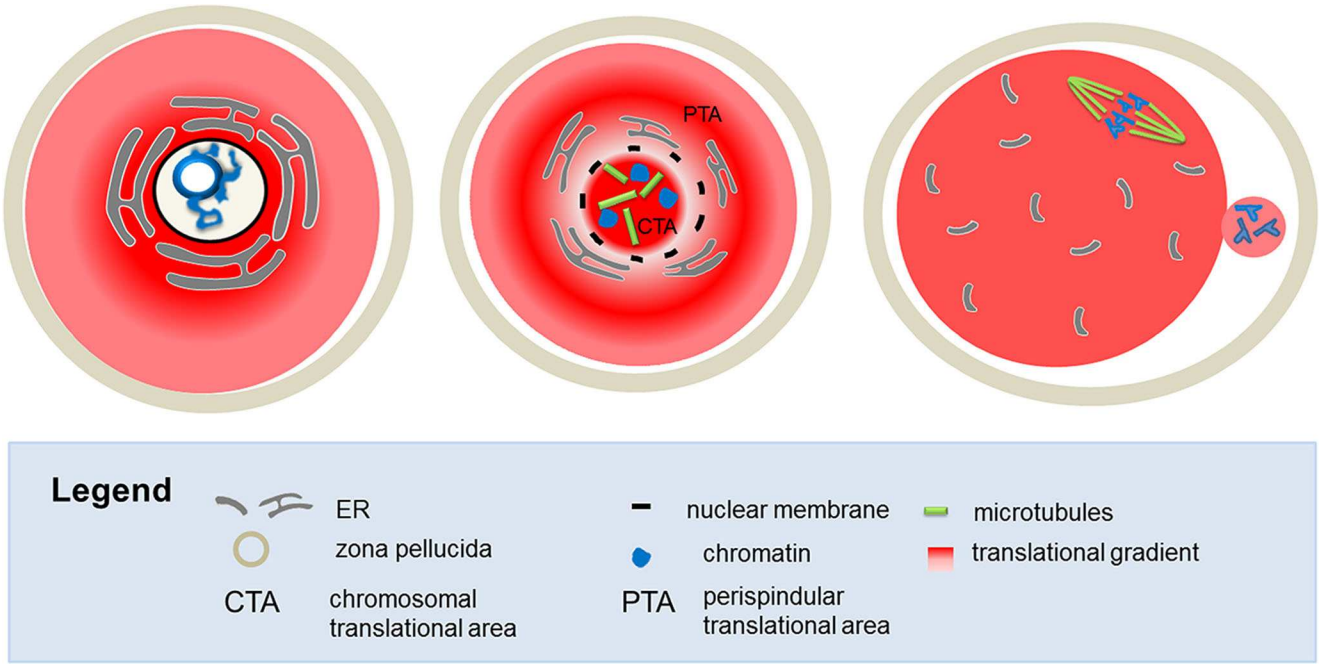



**Fig. 3** Scheme of in situ translation in the GV, NEBD and MII stage. Strong translational signal is present in the perinuclear region in GV stage, two translational hotspots (*CTA* chromosomal translational area, *PTA*, perispindular translational area) develop post-NEBD that disappear after polar body extrusion in the MII stage

and chromosome movements, as well as the correct separation of daughter cells. These findings suggest that the oocyte translates de novo proteins in distinct locations, which then undergo remodeling at, or shortly after, NEBD. Both ER and Lamin structures are likely to be involved in the formation of the boundary between the two distinct translational areas, and probably ensure the physical separation of the chromosomes from the rest of the cytoplasm during the early stages of meiosis after NEBD. The period around NEBD appears to be crucial both for translational reorganization and for the timing of spindle assembly.

Furthermore, immunofluorescence analysis of eIF4E phosphorylated at S209 (believed to be important for mRNA translation initiation) (Sonenberg et al. 1978; Gingras et al. 1999) in the mouse oocyte revealed the presence of phosphorylated eIF4E in the vicinity of chromosomes in NEBD stage oocytes. Interestingly, unlike the phosphorylated form of eIF4E, the presence of total eIF4E protein is not specifically localized. In addition, analyses of oocytes from other mammalian species (Tomek et al. 2002a, 2002b; Ellederova et al. 2006; Siemer et al. 2009) show a high increase in the activation of translation stimulator eIF4E after NEBD and an increased phosphorylation of the translational repressor 4E-BP1 corresponding to the inhibition of this repressor (Manzella et al. 1991).

Romasko et al. (2013) and Susor et al. (2015) also documented that, post-NEBD, the key translational regulator, 4E-BP1, undergoes dynamic and complex spatially regulated changes in the pattern of phosphorylation at sites that regulate its association with eIF4E and its ability to repress translation. Interestingly, 4E-BP1 becomes phosphorylated shortly post-NEBD (Susor et al. 2015). 4E-BP1 phosphorylation variants appear at the spindle (T37/46 and T70) and spindle poles (S65, S112) at different stages of meiosis. These results indicate that dynamic, spatially restricted patterns of inactive translational repressor 4E-BP1 promote the localized translation of specific mRNAs to support spindle formation, chromosome condensation and attachment, as well as chromosome segregation in meiotically dividing oocytes. Regulated 4E-BP1 phosphorylation at the spindle may help to coordinate spindle formation with progression through meiosis. Studies in *Xenopus* revealed enriched localization of mRNAs encoding spindle proteins to spindle microtubules (Brown et al. 2007; Blower et al. 2007). Metaphase spindle is a complex structure: proteomic studies of isolated spindles have identified more than 1200 spindle-associated proteins, of which almost 400 are specific to spindles (Sauer et al. 2005; Bonner et al. 2011), suggesting that a large array of proteins is needed to support spindle formation, maintenance, and function.

The dynamic spatial and temporal pattern of localization of phosphorylated 4E-BP1 at the spindle is indicative of a novel mechanism promoting localized protein production. In order to explain the mechanism of the above-mentioned processes, three models have been proposed: (1) after NEBD the newly forming spindle captures RNAs from nucleoplasm (Susor et al. 2015); (2) the forming spindle captures cytoplasmic RNAs, and (3) a combination of both. One can also speculate that the utilization and distribution of mRNAs in this region changes as the spindle/oocyte progress in meiosis I.

This mechanism may allow diverse cellular signals, e.g., mTOR or CDK1 (Heesom et al. 2001; Susor et al. 2015), to be integrated into the control of the timing of localized mRNA translation in support of spindle formation and meiotic progression. The regulation of 4E-BP1 phosphorylation at the spindle is likely to be temporally and mechanistically distinct from its regulation in the rest of the oocyte. Active AKT and mTOR (Kalous et al. 2006; Kogasaka et al. 2013) are localized at the newly forming spindle. Based on the results of Romasko et al. (2013) and Susor et al. (2015), there are differences between pro-metaphase, metaphase I and II in the state of phosphorylation of 4E-BP1 and in in situ translation (Fig. 4). These differences between MI and MII point to a possible functional heterogeneity of localized translation and 4E-BP1 in supporting the formation and function of spindles that participate during meiotic progression. Downregulation of the mTOR/eIF4F axis leads to aberrant meiotic progression and aneuploidy (Susor et al. 2015). It seems that the mTOR/eIF4F axis is likely to regulate mRNA translation in the oocyte and its depletion results in the decreased translation of the target mRNA group. This supports the hypothesis that spatial and temporal mRNA translation are crucial for correct chromosome segregation. An alternative hypothesis suggesting the influence of maternal age on global gene expression has been offered as an explanation for the age-related rise in aneuploidy (Jones et al. 2008). This suggests that local protein synthesis plays a role in successful spindle assembly. Future studies are needed to determine the exact role that in situ protein synthesis plays after NEBD in the propagation of genetic information/genomic stability.

A similar mechanism of localized translation is used in retinal growth cones and axons of developing neurons where the translation of specific mRNAs is initiated through a cap-dependent mechanism (Campbell and Holt 2003; Cox et al. 2008).

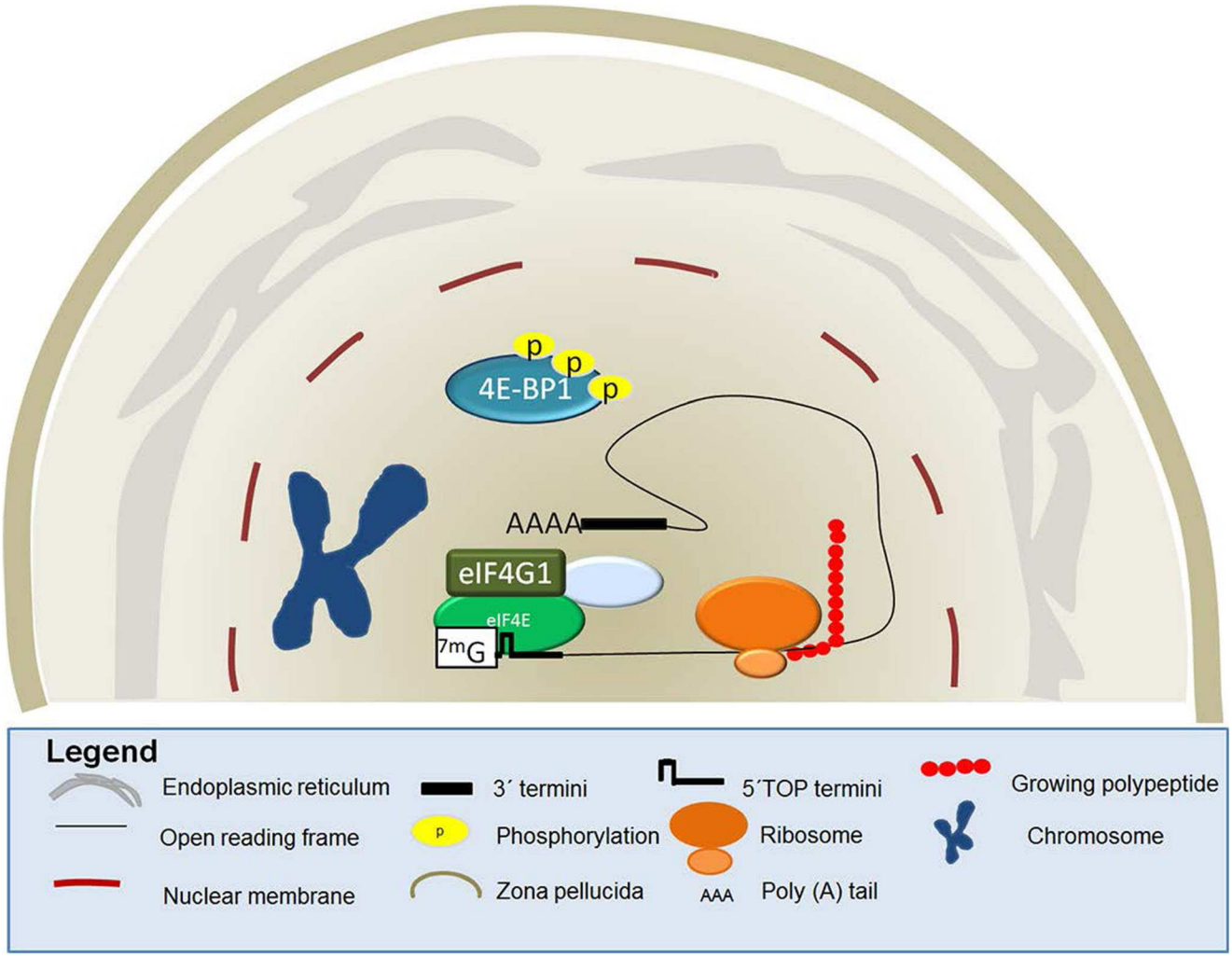



**Fig. 4** Scheme of translational repressor 4E-BP1 inactivation at the newly forming spindle post-NEBD. 4E-BP1 (eIF4E repressor) becomes hyperphosphorylated predominantly in the spindle area, and subsequently it is released from eIF4E, which is then available for its engagement in the cap-binding complex necessary for translational initiation of a given mRNA in the spatial and temporal manner

## Perspectives

The recent great expansion in the development of available/affordable bioinformatic, molecular and imaging methods/techniques (Bodenmiller et al. 2012; Shapiro et al. 2013; Di Palma and Bodenmiller 2015; Lee et al. 2015) brings closer the possibility of their application to such an exceptional cell as the mammalian oocyte. This suggests that in the near future we can expect a surge of detailed information about the localization of the number of RNPs as well as their RNA and protein content. The implementation of genetic and advanced imaging approaches (Buxbaum et al. 2015; Halstead et al. 2015) would certainly shine more light on the spatial and temporal translational regulation occurring in the mammalian oocyte.

Although we live in the ncRNA revolution era, up to now we have only very incomplete knowledge about the ncRNA world, i.e. about their content and their role in oocytes and early embryos. The role of long noncoding RNA (lncRNA) has been studied in the cells, and these studies suggest that they are involved in transcription, RNA processing, and translation (Cech and Steitz 2014). lncRNAs constitute one group of factors that can also explain local epigenetic alterations. The number of known lncRNAs is now rapidly increasing and the experimental evidence for epigenetic alterations mediated by long intergenic noncoding RNAs, a distinctive fraction of lncRNAs, is accumulating. For example, *HOTAIR* acts as a chromatin repressor acting on hundreds of promoters with the polycomb repressive complex 2 (Rinn et al. 2007; Gupta et al. 2010; Tsai et al. 2010). Antisense lncRNA-mediated translation may be another mechanism which maintains the synthesis of pro-survival proteins, such as UCHL1 (Carrieri et al. 2012). UCHL1 is an abundant protein in the oocyte (Susor et al. 2007) with a high physiological importance in the oocyte and the embryo (Susor et al. 2010; Mtango et al. 2012). When cells are treated with mTOR inhibitor, antisense Uchl1 relocalizes to the cytoplasm, triggering the binding of Uchl1 mRNA to polysomes and an increase in UCHL1 protein levels (Carrieri et al. 2012). Further work is needed in order to gain a better understanding of the role of these transcripts in the regulation of the fate of mRNA molecules and hence the possibility that they are vital for the developmental competence of embryos.

Comparative studies in non-traditional model systems are valuable in order to address dissimilarities and overlaps in transcriptome composition between model organisms, and are likely to provide important information regarding the components and mechanisms that may play critical regulatory roles in the fertility of nonmurine models, including the human.

We look forward to the new discoveries of the near future and the expansion of our knowledge of these enigmatic processes in this unique cell.

**Acknowledgments** This review was supported by GACR 13-12291S, GACR 15-22765S/502, GACR P502122201 and Institutional Research Concept RVO67985904.

## References

Alves VS, Motta FL, Roffé M et al (2009) GCN2 activation and eIF2alpha phosphorylation in the maturation of mouse oocytes. Biochem Biophys Res Commun 378:41–44. doi:10.1016/j.bbrc.2008.10.161

Bachvarova R, De Leon V, Johnson A et al (1985) Changes in total RNA, polyadenylated RNA, and actin mRNA during meiotic maturation of mouse oocytes. Dev Biol 108:325–331

Bakheet T, Williams BRG, Khabar KSA (2006) ARED 3.0: the large and diverse AU-rich transcriptome. Nucleic Acids Res 34:D111–114. doi:10.1093/nar/gkj052

Balagopal V, Parker R (2009) Polysomes, P bodies and stress granules: states and fates of eukaryotic mRNAs. Curr Opin Cell Biol 21:403–408. doi:10.1016/j.ceb.2009.03.005

Barreau C, Paillard L, Osborne HB (2005) AU-rich elements and associated factors: are there unifying principles? Nucleic Acids Res 33:7138–7150. doi:10.1093/nar/gki1012

Belloc E, Méndez R (2008) A deadenylation negative feedback mechanism governs meiotic metaphase arrest. Nature 452:1017–1021. doi:10.1038/nature06809

Bernstein E, Kim SY, Carmell MA et al (2003) Dicer is essential for mouse development. Nat Genet 35:215–217. doi:10.1038/ng1253

Bettegowda A, Smith GW (2007) Mechanisms of maternal mRNA regulation: implications for mammalian early embryonic development. Front Biosci 12:3713–3726

Bhatt DM, Pandya-Jones A, Tong A-J et al (2012) Transcript dynamics of proinflammatory genes revealed by sequence analysis of subcellular RNA fractions. Cell 150:279–290. doi:10.1016/j.cell.2012.05.043

Bischoff M, Parfitt D-E, Zernicka-Goetz M (2008) Formation of the embryonic-abembryonic axis of the mouse blastocyst: relationships between orientation of early cleavage divisions and pattern of symmetric/asymmetric divisions. Development 135:953–962. doi:10.1242/dev.014316

Blower MD, Feric E, Weis K, Heald R (2007) Genome-wide analysis demonstrates conserved localization of messenger RNAs to mitotic microtubules. J Cell Biol 179:1365–1373. doi:10.1083/jcb.200705163

Bodenmiller B, Zunder ER, Finck R et al (2012) Multiplexed mass cytometry profiling of cellular states perturbed by small-molecule regulators. Nat Biotechnol 30:858–867. doi:10.1038/nbt.2317

Bonner MK, Poole DS, Xu T et al (2011) Mitotic spindle proteomics in Chinese hamster ovary cells. PLoS ONE 6, e20489. doi:10.1371/journal.pone.0020489

Boothby TC, Wolniak SM (2011) Masked mRNA is stored with aggregated nuclear speckles and its asymmetric redistribution requires a homolog of mago nashi. BMC Cell Biol 12:45. doi:10.1186/1471-2121-12-45

Boothby TC, Zipper RS, van der Weele CM, Wolniak SM (2013) Removal of retained introns regulates translation in the rapidly developing gametophyte of Marsilea vestita. Dev Cell 24:517–529. doi:10.1016/j.devcel.2013.01.015

Bouniol-Baly C, Hamraoui L, Guibert J et al (1999) Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. Biol Reprod 60:580–587. doi:10.1095/biolreprod60.3.580

Boylan KLM, Mische S, Li M et al (2008) Motility screen identifies Drosophila IGF-II mRNA-binding protein–zipcode-binding protein

acting in oogenesis and synaptogenesis. PLoS Genet 4:e36. doi:10.1371/journal.pgen.0040036
Brandhorst BP (1985) Informational content of the echinoderm egg. Dev Biol (NY) 1:525–576
Brandt A, Papagiannouli F, Wagner N et al (2006) Developmental control of nuclear size and shape by Kugelkern and Kurzkern. Curr Biol 16:543–552. doi:10.1016/j.cub.2006.01.051
Brown KS, Blower MD, Maresca TJ et al (2007) Xenopus tropicalis egg extracts provide insight into scaling of the mitotic spindle. J Cell Biol 176:765–770. doi:10.1083/jcb.200610043
Brunet S, Verlhac MH (2011) Positioning to get out of meiosis: the asymmetry of division. Hum Reprod Update 17:68–75. doi:10.1093/humupd/dmq044
Buxbaum AR, Haimovich G, Singer RH (2015) In the right place at the right time: visualizing and understanding mRNA localization. Nat Rev Mol Cell Biol 16:95–109. doi:10.1038/nrm3918
Campbell DS, Holt CE (2003) Apoptotic pathway and MAPKs differentially regulate chemotropic responses of retinal growth cones. Neuron 37:939–952
Carrieri C, Cimatti L, Biagioli M et al (2012) Long non-coding antisense RNA controls Uchl1 translation through an embedded SINEB2 repeat. Nature 491:454–457. doi:10.1038/nature11508
Carrieri C, Forrest ARR, Santoro C et al (2015) Expression analysis of the long non-coding RNA antisense to Uchl1 (AS Uchl1) during dopaminergic cells' differentiation in vitro and in neurochemical models of Parkinson's disease. Front Cell Neurosci 9:114. doi:10.3389/fncel.2015.00114
Cech TR, Steitz JA (2014) The noncoding RNA revolution-trashing old rules to forge new ones. Cell 157:77–94. doi:10.1016/j.cell.2014.03.008
Chan S-P, Slack FJ (2006) microRNA-mediated silencing inside P-bodies. RNA Biol 3:97–100
Chen J, Torcia S, Xie F et al (2013) Somatic cells regulate maternal mRNA translation and developmental competence of mouse oocytes. Nat Cell Biol 15:1415–1423. doi:10.1038/ncb2873
Cox LJ, Hengst U, Gurskaya NG et al (2008) Intra-axonal translation and retrograde trafficking of CREB promotes neuronal survival. Nat Cell Biol 10:149–159. doi:10.1038/ncb1677
Cross BCS, McKibbin C, Callan AC et al (2009) Eeyarestatin I inhibits Sec61-mediated protein translocation at the endoplasmic reticulum. J Cell Sci 122:4393–4400. doi:10.1242/jcs.054494
Curtis D, Lehmann R, Zamore PD (1995) Translational regulation in development. Cell 81:171–178
Dalton CM, Carroll J (2013) Biased inheritance of mitochondria during asymmetric cell division in the mouse oocyte. J Cell Sci 126:2955–2964. doi:10.1242/jcs.128744
De La Fuente R, Viveiros MM, Burns KH et al (2004) Major chromatin remodeling in the germinal vesicle (GV) of mammalian oocytes is dispensable for global transcriptional silencing but required for centromeric heterochromatin function. Dev Biol 275:447–458. doi:10.1016/j.ydbio.2004.08.028
Di Palma S, Bodenmiller B (2015) Unraveling cell populations in tumors by single-cell mass cytometry. Curr Opin Biotechnol 31:122–129. doi:10.1016/j.copbio.2014.07.004
Dieterich DC, Hodas JJL, Gouzer G et al (2010) In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. Nat Neurosci 13:897–905. doi:10.1038/nn.2580
Dumont J, Petri S, Pellegrin F et al (2007) A centriole- and RanGTP-independent spindle assembly pathway in meiosis I of vertebrate oocytes. J Cell Biol 176:295–305. doi:10.1083/jcb.200605199
Eliscovich C, Peset I, Vernos I, Méndez R (2008) Spindle-localized CPE-mediated translation controls meiotic chromosome segregation. Nat Cell Biol 10:858–865. doi:10.1038/ncb1746
Ellederova Z, Kovarova H, Melo-Sterza F et al (2006) Suppression of translation during in vitro maturation of pig oocytes despite enhanced formation of cap-binding protein complex eIF4F and 4E-BP1 hyperphosphorylation. Mol Reprod Dev 73:68–76. doi:10.1002/mrd.20368
Ellederová Z, Cais O, Susor A et al (2008) ERK1/2 map kinase metabolic pathway is responsible for phosphorylation of translation initiation factor eIF4E during in vitro maturation of pig oocytes. Mol Reprod Dev 75:309–317. doi:10.1002/mrd.20690
Evsikov AV, Graber JH, Brockman JM et al (2006) Cracking the egg: molecular dynamics and evolutionary aspects of the transition from the fully grown oocyte to embryo. Genes Dev 20:2713–2727. doi:10.1101/gad.1471006
Fabian MR, Sonenberg N, Filipowicz W (2010) Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem 79:351–379. doi:10.1146/annurev-biochem-060308-103103
FitzHarris G, Marangos P, Carroll J (2007) Changes in endoplasmic reticulum structure during mouse oocyte maturation are controlled by the cytoskeleton and cytoplasmic dynein. Dev Biol 305:133–144. doi:10.1016/j.ydbio.2007.02.006
Flemr M, Ma J, Schultz RM, Svoboda P (2010) P-body loss is concomitant with formation of a messenger RNA storage domain in mouse oocytes. Biol Reprod 82:1008–1017. doi:10.1095/biolreprod.109.082057
Garneau NL, Wilusz J, Wilusz CJ (2007) The highways and byways of mRNA decay. Nat Rev Mol Cell Biol 8:113–126. doi:10.1038/nrm2104
Gebauer F, Xu W, Cooper GM, Richter JD (1994) Translational control by cytoplasmic polyadenylation of c-mos mRNA is necessary for oocyte maturation in the mouse. EMBO J 13:5712–5720
Gingras AC, Gygi SP, Raught B et al (1999) Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev 13:1422–1437
Göhring J, Jacak J, Barta A (2014) Imaging of endogenous messenger RNA splice variants in living cells reveals nuclear retention of transcripts inaccessible to nonsense-mediated decay in Arabidopsis. Plant Cell 26:754–764. doi:10.1105/tpc.113.118075
Grillo G, Turi A, Licciulli F et al (2010) UTRdb and UTRsite (RELEASE 2010): a collection of sequences and regulatory motifs of the untranslated regions of eukaryotic mRNAs. Nucleic Acids Res 38:D75–80. doi:10.1093/nar/gkp902
Grosskortenhaus R, Robinson KJ, Doe CQ (2006) Pdm and Castor specify late-born motor neuron identity in the NB7-1 lineage. Genes Dev 20:2618–2627. doi:10.1101/gad.1445306
Guo H, Ingolia NT, Weissman JS, Bartel DP (2010) Mammalian microRNAs predominantly act to decrease target mRNA levels. Nature 466:835–840. doi:10.1038/nature09267
Gupta RA, Shah N, Wang KC et al (2010) Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 464:1071–1076. doi:10.1038/nature08975
Haim L, Zipor G, Aronov S, Gerst JE (2007) A genomic integration method to visualize localization of endogenous mRNAs in living yeast. Nat Methods 4:409–412. doi:10.1038/nmeth1040
Hall C, Mahadevan LC, Whatley SA et al (1982) The polyadenylated RNA directing the synthesis of the rat myelin basic proteins is present in both free and membrane-bound forebrain polyribosomes. Biochem J 202:407–417
Hall C, Mahadevan L, Whatley S et al (1984) Characterization of translation products of the polyadenylated RNA of free and membrane-bound polyribosomes of rat forebrain. Biochem J 219:751–761
Halstead JM, Lionnet T, Wilbertz JH et al (2015) Translation. An RNA biosensor for imaging the first round of translation from single cells to living animals. Science 347:1367–1671. doi:10.1126/science.aaa3380
Hansen TB, Jensen TI, Clausen BH et al (2013) Natural RNA circles function as efficient microRNA sponges. Nature 495:384–388. doi:10.1038/nature11993

Hashimoto N, Kishimoto T (1988) Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. Dev Biol 126:242–252

Heesom KJ, Gampel A, Mellor H, Denton RM (2001) Cell cycle-dependent phosphorylation of the translational repressor eIF-4E binding protein-1 (4E-BP1). Curr Biol 11:1374–1379

Hendler RW (1974) Protein synthesis by membrane-bound polyribosomes. Biomembranes 5:147–211

Herr JC, Chertihin O, Digilio L et al (2008) Distribution of RNA binding protein MOEP19 in the oocyte cortex and early embryo indicates pre-patterning related to blastomere polarity and trophectoderm specification. Dev Biol 314:300–316. doi:10.1016/j.ydbio.2007.11.027

Hershey JW (1991) Translational control in mammalian cells. Annu Rev Biochem 60:717–755. doi:10.1146/annurev.bi.60.070191.003441

Hershey JW, Asano K, Naranda T et al (1996) Conservation and diversity in the structure of translation initiation factor EIF3 from humans and yeast. Biochimie 78:903–907

Holt CE, Bullock SL (2009) Subcellular mRNA localization in animal cells and why it matters. Science 326:1212–1216. doi:10.1126/science.1176488

Hu J, Wang F, Zhu X et al (2010) Mouse ZAR1-like (XM_359149) colocalizes with mRNA processing components and its dominant-negative mutant caused two-cell-stage embryonic arrest. Dev Dyn 239:407–424. doi:10.1002/dvdy.22170

Hwang SY, Oh B, Knowles BB et al (2001) Expression of genes involved in mammalian meiosis during the transition from egg to embryo. Mol Reprod Dev 59:144–158. doi:10.1002/mrd.1017

Inoue A, Nakajima R, Nagata M, Aoki F (2008) Contribution of the oocyte nucleus and cytoplasm to the determination of meiotic and developmental competence in mice. Hum Reprod 23:1377–1384. doi:10.1093/humrep/den096

Jakymiw A, Pauley KM, Li S et al (2007) The role of GW/P-bodies in RNA processing and silencing. J Cell Sci 120:1317–1323. doi:10.1242/jcs.03429

Jambhekar A, Derisi JL (2007) Cis-acting determinants of asymmetric, cytoplasmic RNA transport. RNA 13:625–642. doi:10.1261/rna.262607

Jambor H, Surendranath V, Kalinka AT et al (2015) Systematic imaging reveals features and changing localization of mRNAs in Drosophila development. eLife. doi:10.7554/eLife.05003

Jones GM, Cram DS, Song B et al (2008) Gene expression profiling of human oocytes following in vivo or in vitro maturation. Hum Reprod 23:1138–1144. doi:10.1093/humrep/den085

Kalous J, Solc P, Baran V et al (2006) PKB/AKT is involved in resumption of meiosis in mouse oocytes. Biol Cell 98:111–123. doi:10.1042/BC20050020

Kambadur R, Koizumi K, Stivers C et al (1998) Regulation of POU genes by castor and hunchback establishes layered compartments in the Drosophila CNS. Genes Dev 12:246–260

Kang SA, Pacold ME, Cervantes CL et al (2013) mTORC1 Phosphorylation Sites Encode Their Sensitivity to Starvation and Rapamycin. Science 341:1236566. doi:10.1126/science.1236566

Kanmera S, Sakakibara R, Ishiguro M (1995) Inhibition of polar body formation in mouse denuded oocytes cultured in vitro by protein synthesis inhibitors. Biol Pharm Bull 18:1255–1258

Keenan RJ, Freymann DM, Stroud RM, Walter P (2001) The signal recognition particle. Annu Rev Biochem 70:755–775. doi:10.1146/annurev.biochem.70.1.755

Kim JH, Richter JD (2006) Opposing polymerase-deadenylase activities regulate cytoplasmic polyadenylation. Mol Cell 24:173–183. doi:10.1016/j.molcel.2006.08.016

King ML, Messitt TJ, Mowry KL (2005) Putting RNAs in the right place at the right time: RNA localization in the frog oocyte. Biol Cell 97:19–33. doi:10.1042/BC20040067

Knowles BB, Evsikov AV, de Vries WN et al (2003) Molecular control of the oocyte to embryo transition. Philos Trans R Soc Lond B 358:1381–1387. doi:10.1098/rstb.2003.1330

Kogasaka Y, Hoshino Y, Hiradate Y et al (2013) Distribution and association of mTOR with its cofactors, raptor and rictor, in cumulus cells and oocytes during meiotic maturation in mice. Mol Reprod Dev 80:334–348. doi:10.1002/mrd.22166

Komrskova P, Susor A, Malik R et al (2014) Aurora kinase A is not involved in CPEB1 phosphorylation and cyclin B1 mRNA polyadenylation during meiotic maturation of porcine oocytes. PLoS ONE 9, e101222. doi:10.1371/journal.pone.0101222

Kondrashov N, Pusic A, Stumpf CR et al (2011) Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. Cell 145:383–397. doi:10.1016/j.cell.2011.03.028

König H, Matter N, Bader R et al (2007) Splicing segregation: the minor spliceosome acts outside the nucleus and controls cell proliferation. Cell 131:718–729. doi:10.1016/j.cell.2007.09.043

Kopecny V, Biggiogera M, Laurincik J et al (1996) Fine structural cytochemical and immunocytochemical analysis of nucleic acids and ribonucleoprotein distribution in nuclei of pig oocytes and early preimplantation embryos. Chromosoma 104:561–574

Kraut-Cohen J, Gerst JE (2010) Addressing mRNAs to the ER: cis sequences act up! Trends Biochem Sci 35:459–469. doi:10.1016/j.tibs.2010.02.006

Krichevsky AM, Kosik KS (2001) Neuronal RNA granules: a link between RNA localization and stimulation-dependent translation. Neuron 32:683–696

Kronja I, Whitfield ZJ, Yuan B et al (2014) Quantitative proteomics reveals the dynamics of protein changes during Drosophila oocyte maturation and the oocyte-to-embryo transition. Proc Natl Acad Sci U S A 111:16023–16028. doi:10.1073/pnas.1418657111

Kurotaki Y, Hatta K, Nakao K et al (2007) Blastocyst axis is specified independently of early cell lineage but aligns with the ZP shape. Science 316:719–723. doi:10.1126/science.1138591

Lécuyer E, Yoshida H, Parthasarathy N et al (2007) Global analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell 131:174–187. doi:10.1016/j.cell.2007.08.003

Lee JH, Daugharthy ER, Scheiman J et al (2015) Fluorescent in situ sequencing (FISSEQ) of RNA for gene expression profiling in intact cells and tissues. Nat Protoc 10:442–458. doi:10.1038/nprot.2014.191

Li L, Baibakov B, Dean J (2008) A subcortical maternal complex essential for preimplantation mouse embryogenesis. Dev Cell 15:416–425. doi:10.1016/j.devcel.2008.07.010

Li L, Zheng P, Dean J (2010) Maternal control of early mouse development. Development 137:859–870. doi:10.1242/dev.039487

Li L, Lu X, Dean J (2013) The maternal to zygotic transition in mammals. Mol Asp Med 34:919–938. doi:10.1016/j.mam.2013.01.003

Lin C-J, Koh FM, Wong P et al (2014) Hira-mediated H3.3 incorporation is required for DNA replication and ribosomal RNA transcription in the mouse zygote. Dev Cell 30:268–279. doi:10.1016/j.devcel.2014.06.022

Lipshitz HD, Smibert CA (2000) Mechanisms of RNA localization and translational regulation. Curr Opin Genet Dev 10:476–488

Louvet-Vallée S, Vinot S, Maro B (2005) Mitotic spindles and cleavage planes are oriented randomly in the two-cell mouse embryo. Curr Biol 15:464–469. doi:10.1016/j.cub.2004.12.078

Ma J, Flemr M, Stein P et al (2010) MicroRNA activity is suppressed in mouse oocytes. Curr Biol 20:265–270. doi:10.1016/j.cub.2009.12.042

Ma J, Flemr M, Strnad H et al (2013) Maternally recruited DCP1A and DCP2 contribute to messenger RNA degradation during oocyte maturation and genome activation in mouse. Biol Reprod 88:11. doi:10.1095/biolreprod.112.105312

MacNicol MC, MacNicol AM (2010) Developmental timing of mRNA translation–integration of distinct regulatory elements. Mol Reprod Dev 77:662–669. doi:10.1002/mrd.21191

Mader S, Lee H, Pause A, Sonenberg N (1995) The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. Mol Cell Biol 15:4990–4997

Manzella JM, Rychlik W, Rhoads RE et al (1991) Insulin induction of ornithine decarboxylase. Importance of mRNA secondary structure and phosphorylation of eucaryotic initiation factors eIF-4B and eIF-4E. J Biol Chem 266:2383–2389

Mattick JS, Makunin IV (2006) Non-coding RNA. Hum Mol Genet 15(Spec No 1):R17–29. doi:10.1093/hmg/ddl046

Memczak S, Jens M, Elefsinioti A et al (2013) Circular RNAs are a large class of animal RNAs with regulatory potency. Nature 495:333–338. doi:10.1038/nature11928

Mendez R, Richter JD (2001) Translational control by CPEB: a means to the end. Nat Rev Mol Cell Biol 2:521–529. doi:10.1038/35080081

Mendez R, Hake LE, Andresson T et al (2000) Phosphorylation of CPE binding factor by Eg2 regulates translation of c-mos mRNA. Nature 404:302–307. doi:10.1038/35005126

Mendez R, Barnard D, Richter JD (2002) Differential mRNA translation and meiotic progression require Cdc2-mediated CPEB destruction. EMBO J 21:1833–1844. doi:10.1093/emboj/21.7.1833

Ménétret J-F, Schaletzky J, Clemons WM et al (2007) Ribosome binding of a single copy of the SecY complex: implications for protein translocation. Mol Cell 28:1083–1092. doi:10.1016/j.molcel.2007.10.034

Mercer TR, Dinger ME, Mattick JS (2009) Long non-coding RNAs: insights into functions. Nat Rev Genet 10:155–159. doi:10.1038/nrg2521

Mili S, Macara IG (2009) RNA localization and polarity: from A(PC) to Z(BP). Trends Cell Biol 19:156–164. doi:10.1016/j.tcb.2009.02.001

Monti M, Zanoni M, Calligaro A et al (2013) Developmental arrest and mouse antral not-surrounded nucleolus oocytes. Biol Reprod 88:2. doi:10.1095/biolreprod.112.103887

Moreno JA, Radford H, Peretti D et al (2012) Sustained translational repression by eIF2α-P mediates prion neurodegeneration. Nature 485:507–511. doi:10.1038/nature11058

Morita S, Horii T, Kimura M et al (2007) One Argonaute family member, Eif2c2 (Ago2), is essential for development and appears not to be involved in DNA methylation. Genomics 89:687–696. doi:10.1016/j.ygeno.2007.01.004

Morley SJ, Thomas G (1991) Intracellular messengers and the control of protein synthesis. Pharmacol Ther 50:291–319

Motosugi N, Bauer T, Polanski Z et al (2005) Polarity of the mouse embryo is established at blastocyst and is not prepatterned. Genes Dev 19:1081–1092. doi:10.1101/gad.1304805

Mtango NR, Sutovsky M, Susor A et al (2012) Essential role of maternal UCHL1 and UCHL3 in fertilization and preimplantation embryo development. J Cell Physiol 227:1592–1603. doi:10.1002/jcp.22876

Nakamura A, Sato K, Hanyu-Nakamura K (2004) Drosophila cup is an eIF4E binding protein that associates with Bruno and regulates oskar mRNA translation in oogenesis. Dev Cell 6:69–78

Niedojadło J, Kubicka E, Kalich B, Smoliński DJ (2014) Poly(A) RNAs including coding proteins RNAs occur in plant Cajal bodies. PLoS ONE 9:e111780. doi:10.1371/journal.pone.0111780

Nothias JY, Majumder S, Kaneko KJ, DePamphilis ML (1995) Regulation of gene expression at the beginning of mammalian development. J Biol Chem 270:22077–22080

Novoa I, Gallego J, Ferreira PG, Mendez R (2010) Mitotic cell-cycle progression is regulated by CPEB1 and CPEB4-dependent translational control. Nat Cell Biol 12:447–456. doi:10.1038/ncb2046

Ohsugi M, Zheng P, Baibakov B et al (2008) Maternally derived FILIA-MATER complex localizes asymmetrically in cleavage-stage mouse embryos. Development 135:259–269. doi:10.1242/dev.011445

Parker R, Sheth U (2007) P bodies and the control of mRNA translation and degradation. Mol Cell 25:635–646. doi:10.1016/j.molcel.2007.02.011

Paronetto MP, Bianchi E, Geremia R, Sette C (2008) Dynamic expression of the RNA-binding protein Sam68 during mouse pre-implantation development. Gene Expr Patterns 8:311–322. doi:10.1016/j.gep.2008.01.005

Paynton BV, Bachvarova R (1994) Polyadenylation and deadenylation of maternal mRNAs during oocyte growth and maturation in the mouse. Mol Reprod Dev 37:172–180. doi:10.1002/mrd.1080370208

Pesty A, Miyara F, Debey P et al (2007) Multiparameter assessment of mouse oogenesis during follicular growth in vitro. Mol Hum Reprod 13:3–9. doi:10.1093/molehr/gal089

Pierre A, Gautier M, Callebaut I et al (2007) Atypical structure and phylogenomic evolution of the new eutherian oocyte- and embryo-expressed KHDC1/DPPA5/ECAT1/OOEP gene family. Genomics 90:583–594. doi:10.1016/j.ygeno.2007.06.003

Pilot F, Philippe J-M, Lemmers C et al (2006) Developmental control of nuclear morphogenesis and anchoring by charleston, identified in a functional genomic screen of Drosophila cellularisation. Development 133:711–723. doi:10.1242/dev.02251

Piqué M, López JM, Foissac S et al (2008) A combinatorial code for CPE-mediated translational control. Cell 132:434–448. doi:10.1016/j.cell.2007.12.038

Potireddy S, Midic U, Liang C-G et al (2010) Positive and negative cis-regulatory elements directing postfertilization maternal mRNA translational control in mouse embryos. Am J Physiol Cell Physiol 299:C818–827. doi:10.1152/ajpcell.00166.2010

Prasanth KV, Prasanth SG, Xuan Z et al (2005) Regulating gene expression through RNA nuclear retention. Cell 123:249–263. doi:10.1016/j.cell.2005.08.033

Puoti A, Gallegos M, Zhang B et al (1997) Controls of cell fate and pattern by 3' untranslated regions: the Caenorhabditis elegans sperm/oocyte decision. Cold Spring Harb Symp Quant Biol 62:19–24

Pyronnet S (2000) Phosphorylation of the cap-binding protein eIF4E by the MAPK-activated protein kinase Mnk1. Biochem Pharmacol 60:1237–1243

Racki WJ, Richter JD (2006) CPEB controls oocyte growth and follicle development in the mouse. Development 133:4527–4537. doi:10.1242/dev.02651

Reich A, Klatsky P, Carson S, Wessel G (2011) The transcriptome of a human polar body accurately reflects its sibling oocyte. J Biol Chem 286:40743–40749. doi:10.1074/jbc.M111.289868

Richter JD (2007) CPEB: a life in translation. Trends Biochem Sci 32:279–285. doi:10.1016/j.tibs.2007.04.004

Rinn JL, Kertesz M, Wang JK et al (2007) Functional demarcation of active and silent chromatin domains in human HOX loci by non-coding RNAs. Cell 129:1311–1323. doi:10.1016/j.cell.2007.05.022

Romasko EJ, Amarnath D, Midic U, Latham KE (2013) Association of maternal mRNA and phosphorylated EIF4EBP1 variants with the spindle in mouse oocytes: localized translational control supporting female meiosis in mammals. Genetics 195:349–358. doi:10.1534/genetics.113.154005

Rossant J, Tam PPL (2004) Emerging asymmetry and embryonic patterning in early mouse development. Dev Cell 7:155–164. doi:10.1016/j.devcel.2004.07.012

Sanfins A, Plancha CE, Overstrom EW, Albertini DF (2004) Meiotic spindle morphogenesis in in vivo and in vitro matured mouse oocytes: insights into the relationship between nuclear and cytoplasmic quality. Hum Reprod 19:2889–2899. doi:10.1093/humrep/deh528

Saraogi I, Shan S (2011) Molecular mechanism of co-translational protein targeting by the signal recognition particle. Traffic 12:535–542. doi:10.1111/j.1600-0854.2011.01171.x

Sauer G, Körner R, Hanisch A et al (2005) Proteome analysis of the human mitotic spindle. Mol Cell Proteomics 4:35–43. doi:10.1074/mcp.M400158-MCP200

Scantland S, Grenon J-P, Desrochers M-H et al (2011) Method to isolate polyribosomal mRNA from scarce samples such as mammalian oocytes and early embryos. BMC Dev Biol 11:8. doi:10.1186/1471-213X-11-8

Scheper GC, Proud CG (2002) Does phosphorylation of the cap-binding protein eIF4E play a role in translation initiation? Eur J Biochem 269:5350–5359

Scheper GC, Morrice NA, Kleijn M, Proud CG (2001) The mitogen-activated protein kinase signal-integrating kinase Mnk2 is a eukaryotic initiation factor 4E kinase with high levels of basal activity in mammalian cells. Mol Cell Biol 21:743–754. doi:10.1128/MCB.21.3.743-754.2001

Schier AF (2007) The maternal-zygotic transition: death and birth of RNAs. Science 316:406–407. doi:10.1126/science.1140693

Schlaitz A-L, Thompson J, Wong CCL et al (2013) REEP3/4 ensure endoplasmic reticulum clearance from metaphase chromatin and proper nuclear envelope architecture. Dev Cell 26:315–323. doi:10.1016/j.devcel.2013.06.016

Schuh M, Ellenberg J (2007) Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse oocytes. Cell 130:484–498. doi:10.1016/j.cell.2007.06.025

Schultz RM (2002) The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum Reprod Update 8:323–331

Schultz RM, Wassarman PM (1977) Biochemical studies of mammalian oogenesis: Protein synthesis during oocyte growth and meiotic maturation in the mouse. J Cell Sci 24:167–194

Schultz RM, LaMarca MJ, Wassarman PM (1978) Absolute rates of protein synthesis during meiotic maturation of mammalian oocytes in vitro. Proc Natl Acad Sci U S A 75:4160–4164

Seydoux G, Braun RE (2006) Pathway to totipotency: lessons from germ cells. Cell 127:891–904. doi:10.1016/j.cell.2006.11.016

Shapiro E, Biezuner T, Linnarsson S (2013) Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat Rev Genet 14:618–630. doi:10.1038/nrg3542

Shishova KV, Lavrentyeva EA, Dobrucki JW, Zatsepina OV (2015) Nucleolus-like bodies of fully-grown mouse oocytes contain key nucleolar proteins but are impoverished for rRNA. Dev Biol 397:267–281. doi:10.1016/j.ydbio.2014.11.022

Siemer C, Smiljakovic T, Bhojwani M et al (2009) Analysis of mRNA associated factors during bovine oocyte maturation and early embryonic development. Mol Reprod Dev 76:1208–1219. doi:10.1002/mrd.21096

Slayter HS, Warner JR, Rich A, Hall CE (1963) The visualization of polyribosomal structure. J Mol Biol 7:652–657

Smith WB, Starck SR, Roberts RW, Schuman EM (2005) Dopaminergic stimulation of local protein synthesis enhances surface expression of GluR1 and synaptic transmission in hippocampal neurons. Neuron 45:765–779. doi:10.1016/j.neuron.2005.01.015

Sonenberg N, Gingras AC (1998) The mRNA 5' cap-binding protein eIF4E and control of cell growth. Curr Opin Cell Biol 10:268–275

Sonenberg N, Morgan MA, Merrick WC, Shatkin AJ (1978) A polypeptide in eukaryotic initiation factors that crosslinks specifically to the 5'-terminal cap in mRNA. Proc Natl Acad Sci U S A 75:4843–4847

Stein P, Rozhkov NV, Li F et al (2015) Essential Role for endogenous siRNAs during meiosis in mouse oocytes. PLoS Genet 11:e1005013. doi:10.1371/journal.pgen.1005013

Stitzel ML, Seydoux G (2007) Regulation of the oocyte-to-zygote transition. Science 316:407–408. doi:10.1126/science.1138236

Strome S, Lehmann R (2007) Germ versus soma decisions: lessons from flies and worms. Science 316:392–393. doi:10.1126/science.1140846

Suh N, Blelloch R (2011) Small RNAs in early mammalian development: from gametes to gastrulation. Development 138:1653–1661. doi:10.1242/dev.056234

Suh N, Baehner L, Moltzahn F et al (2010) MicroRNA function is globally suppressed in mouse oocytes and early embryos. Curr Biol 20:271–277. doi:10.1016/j.cub.2009.12.044

Susor A, Ellederova Z, Jelinkova L et al (2007) Proteomic analysis of porcine oocytes during in vitro maturation reveals essential role for the ubiquitin C-terminal hydrolase-L1. Reproduction 134:559–568. doi:10.1530/REP-07-0079

Susor A, Jelínková L, Karabínová P et al (2008) Regulation of cap-dependent translation initiation in the early stage porcine parthenotes. Mol Reprod Dev 75:1716–1725. doi:10.1002/mrd.20913

Susor A, Liskova L, Toralova T et al (2010) Role of ubiquitin C-terminal hydrolase-L1 in antipolyspermy defense of mammalian oocytes. Biol Reprod 82:1151–1161. doi:10.1095/biolreprod.109.081547

Susor A, Jansova D, Cerna R et al (2015) Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway. Nat Commun 6:6078. doi:10.1038/ncomms7078

Swetloff A, Conne B, Huarte J et al (2009) Dcp1-bodies in mouse oocytes. Mol Biol Cell 20:4951–4961. doi:10.1091/mbc.E09-02-0123

Szöllösi MS, Kubiak JZ, Debey P et al (1993) Inhibition of protein kinases by 6-dimethylaminopurine accelerates the transition to interphase in activated mouse oocytes. J Cell Sci 104(Pt 3):861–872

Tay J, Richter JD (2001) Germ cell differentiation and synaptonemal complex formation are disrupted in CPEB knockout mice. Dev Cell 1:201–213

Tomek W, Melo Sterza FA, Kubelka M et al (2002a) Regulation of translation during in vitro maturation of bovine oocytes: the role of MAP kinase, eIF4E (cap binding protein) phosphorylation, and eIF4E-BP1. Biol Reprod 66:1274–1282

Tomek W, Torner H, Kanitz W (2002b) Comparative analysis of protein synthesis, transcription and cytoplasmic polyadenylation of mRNA during maturation of bovine oocytes in vitro. Reprod Domest Anim 37:86–91. doi:10.1046/j.1439-0531.2002.00336.x

Tsai M-C, Manor O, Wan Y et al (2010) Long noncoding RNA as modular scaffold of histone modification complexes. Science 329:689–693. doi:10.1126/science.1192002

Vallée M, Dufort I, Desrosiers S et al (2009) Revealing the bovine embryo transcript profiles during early in vivo embryonic development. Reproduction 138:95–105. doi:10.1530/REP-08-0533

Vassalli JD, Stutz A (1995) Translational control. Awakening dormant mRNAs. Curr Biol 5:476–479

Vautier D, Besombes D, Chassoux D et al (1994) Redistribution of nuclear antigens linked to cell proliferation and RNA processing in mouse oocytes and early embryos. Mol Reprod Dev 38:119–130. doi:10.1002/mrd.1080380202

Vedeler A, Pryme IF, Hesketh JE (1991) Compartmentalization of polysomes into free, cytoskeletal-bound and membrane-bound populations. Biochem Soc Trans 19:1108–1111

VerMilyea MD, Maneck M, Yoshida N et al (2011) Transcriptome asymmetry within mouse zygotes but not between early embryonic sister blastomeres. EMBO J 30:1841–1851. doi:10.1038/emboj.2011.92

Villalba A, Coll O, Gebauer F (2011) Cytoplasmic polyadenylation and translational control. Curr Opin Genet Dev 21:452–457. doi:10.1016/j.gde.2011.04.006

Vinot S, Le T, Maro B, Louvet-Vallée S (2004) Two PAR6 proteins become asymmetrically localized during establishment of polarity in mouse oocytes. Curr Biol 14:520–525. doi:10.1016/j.cub.2004.02.061

Waskiewicz AJ, Flynn A, Proud CG, Cooper JA (1997) Mitogen-activated protein kinases activate the serine/threonine kinases

Mnk1 and Mnk2. EMBO J 16:1909–1920. doi:10.1093/emboj/16.8.1909

Watson AJ, Wiemer KE, Arcellana-Panlilio M, Schultz GA (1992) U2 small nuclear RNA localization and expression during bovine preimplantation development. Mol Reprod Dev 31:231–240. doi:10.1002/mrd.1080310402

Weatheritt RJ, Gibson TJ, Babu MM (2014) Asymmetric mRNA localization contributes to fidelity and sensitivity of spatially localized systems. Nat Struct Mol Biol 21:833–839. doi:10.1038/nsmb.2876

Weston A, Sommerville J (2006) Xp54 and related (DDX6-like) RNA helicases: roles in messenger RNP assembly, translation regulation and RNA degradation. Nucleic Acids Res 34:3082–3094. doi:10.1093/nar/gkl409

Yang L, Duff MO, Graveley BR et al (2011) Genomewide characterization of non-polyadenylated RNAs. Genome Biol 12:R16. doi:10.1186/gb-2011-12-2-r16

Yang C-X, Du Z-Q, Wright EC et al (2012) Small RNA Profile of the Cumulus-Oocyte Complex and Early Embryos in the Pig. Biol Reprod 87(Article 117):1–11. doi:10.1095/biolreprod.111.096669

Yurttas P, Vitale AM, Fitzhenry RJ et al (2008) Role for PADI6 and the cytoplasmic lattices in ribosomal storage in oocytes and translational control in the early mouse embryo. Development 135:2627–2636. doi:10.1242/dev.016329

Zeng F, Schultz RM (2005) RNA transcript profiling during zygotic gene activation in the preimplantation mouse embryo. Dev Biol 283:40–57. doi:10.1016/j.ydbio.2005.03.038

Zheng P, Baibakov B, Wang X, Dean J (2013) PtdIns(3,4,5)P3 is constitutively synthesized and required for spindle translocation during meiosis in mouse oocytes. J Cell Sci 126:715–721. doi:10.1242/jcs.118042

Zhu K, Yan L, Zhang X et al (2015) Identification of a human subcortical maternal complex. Mol Hum Reprod 21:320–329. doi:10.1093/molehr/gau116

Zuccotti M, Ponce RH, Boiani M et al (2002) The analysis of chromatin organisation allows selection of mouse antral oocytes competent for development to blastocyst. Zygote 10:73–78

Zuccotti M, Merico V, Cecconi S et al (2011) What does it take to make a developmentally competent mammalian egg? Hum Reprod Update 17:525–540. doi:10.1093/humupd/dmr009

CELL CYCLE
2017, VOL. 16, NO. 10, 927–939
http://dx.doi.org/10.1080/15384101.2017.1295178

Taylor & Francis
Taylor & Francis Group

REPORT

OPEN ACCESS

# Regulation of 4E-BP1 activity in the mammalian oocyte

Denisa Jansova[a], Marketa Koncicka[a], Anna Tetkova[a], Renata Cerna[a], Radek Malik [b], Edgar del Llano [a], Michal Kubelka [a], and Andrej Susor[a]

[a]Institute of Animal Physiology and Genetics, ASC, Libechov, Czech Republic; [b]Institute of Molecular Genetics, ASCR, Prague, Czech Republic

**ABSTRACT**

Fully grown mammalian oocytes utilize transcripts synthetized and stored during earlier development. RNA localization followed by a local translation is a mechanism responsible for the regulation of spatial and temporal gene expression. Here we show that the mouse oocyte contains 3 forms of cap-dependent translational repressor expressed on the mRNA level: 4E-BP1, 4E-BP2 and 4E-BP3. However, only 4E-BP1 is present as a protein in oocytes, it becomes inactivated by phosphorylation after nuclear envelope breakdown and as such it promotes cap-dependent translation after NEBD. Phosphorylation of 4E-BP1 can be seen in the oocytes after resumption of meiosis but it is not detected in the surrounding cumulus cells, indicating that 4E-BP1 promotes translation at a specific cell cycle stage. Our immunofluorescence analyses of 4E-BP1 in oocytes during meiosis I showed an even localization of global 4E-BP1, as well as of its 4E-BP1 (Thr37/46) phosphorylated form. On the other hand, 4E-BP1 phosphorylated on Ser65 is localized at the spindle poles, and 4E-BP1 phosphorylated on Thr70 localizes on the spindle. We further show that the main positive regulators of 4E-BP1 phosphorylation after NEBD are mTOR and CDK1 kinases, but not PLK1 kinase. CDK1 exerts its activity toward 4E-BP1 phosphorylation via phosphorylation and activation of mTOR. Moreover, both CDK1 and phosphorylated mTOR co-localize with 4E-BP1 phosphorylated on Thr70 on the spindle at the onset of meiotic resumption. Expression of the dominant negative 4E-BP1 mutant adversely affects translation and results in spindle abnormality. Taken together, our results show that the phosphorylation of 4E-BP1 promotes translation at the onset of meiosis to support the spindle assembly and suggest an important role of CDK1 and mTOR kinases in this process. We also show that the mTOR regulatory pathway is present in human oocytes and is likely to function in a similar way as in mouse oocytes.

**ARTICLE HISTORY**
Received 10 October 2016
Revised 31 January 2017
Accepted 8 February 2017

**KEYWORDS**
4E-BP1; CDK1; cumulus cells; kinase; mTOR; mRNA; meiosis; oocyte; spindle; translation

## Introduction

Translational control of specific mRNAs is a widespread mechanism of gene regulation and contributes to diverse biologic processes in many cell types. During the meiotic division of mammalian oocytes (so called oocyte maturation) protein synthesis plays an important role in controlling the progress of meiosis, since the regulation of gene expression on the level of transcription is ceased. At the onset of the first meiotic division, nuclear envelope breakdown (NEBD; G2/M transition) occurs, chromosomes condense and a bipolar spindle forms from the microtubule organizing centers.[1] During meiosis I, the spindle migrates from the center of the oocyte to the cortex, and the oocyte undergoes an asymmetric division resulting in a large egg competent for fertilization and a relatively small polar body. Proper positioning of the spindle during asymmetric cell division ensures correct partitioning of cellular determinants.[2] How these events are orchestrated in detail remains unclear.

The importance of protein synthesis for meiotic and mitotic progression has been shown previously. Those published results revealed that protein synthesis is not required for NEBD in mouse oocytes, although the formation of the spindle and progression to metaphase II requires active protein synthesis.[3] In contrast, positive regulators of the cap-dependent translational pathway become activated post NEBD and inactivated after fertilization.[4-9]

Regulation of translation occurs mainly at the initiation step, which was shown to be rate limiting for overall protein synthesis.[10] Protein factors that bind to the cap structure at the 5′UTR (untranslated region) and to the 3′UTR-poly(A) sequence of mRNAs have been identified as being essential for this process. Most of the interactions of these proteins are regulated by phosphorylation.[11,12] The best described protein kinase regulating translation initiation is the mTOR/FRAP kinase, the targets of which are the Eukaryotic initiation factor 4E-binding protein 1 (4E-BP1)[13] and the S6 kinase.[14] Hypo-phosphorylated 4E-BP1 binds eIF4E and in such a way inhibits the formation of a translation initiation complex (eIF4F) at the cap structure. EIF4F contains eIF4E (the cap-binding protein), eIF4G1 (the scaffold protein) and eIF4A (an RNA helicase). This complex is probably critical for the translation of mRNAs with extensive secondary structure in their 5′UTR. Upon resumption of meiosis, 4E-BP1

**CONTACT** Andrej Susor susor@iapg.cas.cz Institute of Animal Physiology and Genetics, ASC, Rumburska 89, Libechov, Czech Republic.

Color versions of one or more of the figures in this article can be found online at www.tandfonline.com/kccy.

Supplemental data for this article can be accessed on the publisher's website.

becomes phosphorylated at several sites resulting in its release from eIF4E, allowing eIF4F formation. Phosphorylation at Ser65 and Thr70 modulates the binding of 4E-BP1 to eIF4E directly. Phosphorylation of these sites depends upon 4E-BP1s C-terminal TOR signaling motif that binds Raptor, a component of the mTORC1. Phosphorylation at Thr37/46, which is known to be mediated by mTOR, is required for the modification of Thr70 and Ser65, reflecting the hierarchical phosphorylation of 4E-BP1,[15] and depends upon 4E-BP1s N-terminal RAIP motif.[16] Phosphorylation of Thr37/46 is profoundly inhibited by starving cells of amino acids, which inactivates mTOR signaling.[17] mTORC1 signaling is activated via phosphatidylinositide 3-kinase and protein kinase B (PKB, also termed AKT) and by the Ras/Raf/ERK pathway.[18] AKT plays a substantial role during the progression of meiosis from GV-stage (germinal vesicle – nucleus in the oocytes) to the MI/MII-stage.[19,20] Involvement of the mTOR/4F axis in translational regulation during mitosis might be used as a model case for the meiotic cell. Increased phosphorylation of 4E-BP1 has been detected during the meiotic progression of mammalian oocytes,[4,21,22] and different phosphorylated forms of 4E-BP1 have been shown to co-localize with the meiotic spindle in mouse oocytes.[9,22] Blocking of 4E-BP1 phosphorylation during maturation has also resulted in the irreversible arrest of metaphase I in bovine oocytes,[23] abnormal formation of MII spindles in mouse oocytes[9] or affected asymmetric division.[24]

The aim of this work was to study the metabolic pathways which are involved in 4E-BP1 phosphorylation during in vitro meiotic maturation of mouse oocytes. We discovered that 4E-BP1 becomes phosphorylated in post-NEBD stage oocytes and this phosphorylation remains constant until the MII stage of oocyte maturation and promotes specific translation, which affects spindle assembly. Furthermore, we have uncovered the involvement of different kinases which are potentially involved in the phosphorylation of 4E-BP1.

## Results

### *Only 4E-BP1 is present in the mouse oocyte*

In mammals 3 genes code 4E-BP1, 2 and 3.[25] Our first objective was to determine which form is dominant during mouse oocyte meiotic maturation from the GV to MII stage on the mRNA level. Quantitative RT-PCR analysis showed the presence of all 3 forms of *4e-bps* but with a slightly higher abundance of *4e-bp3*. The global amount of the mRNAs for the 3 different *4e-bps* remained constant throughout meiosis from GV to MII oocytes (Fig. 1A).

Next, we analyzed the presence of all 3 isoforms on the protein level. Our WB analyses showed an absence of 4E-BP2 and 4E-BP3 proteins in the oocytes, which is in the contrary to the results obtained from WB analyses of brain lysate (Fig. 1B and C). However, 4E-BP1 was highly abundant in mouse oocytes with an increased mobility shift post-NEBD (Fig. 1B). Our data showed higher presence of the 4E-BP1 protein in the oocytes than in the brain sample (Fig. 1B and C; Supplementary Fig. 1A). WB also showed that whole population of 4E-BP1 in MII stage oocytes is present as the upper (presumably phosphorylated) band. Treatment of MII oocyte lysate with lambda protein phosphatase (LPP) resulted in the disappearance of the upper band and mobility shift toward lower band, similar pattern to that seen in the GV stage oocytes (Fig. 1D). The experiment in the Fig. 1D shows that mobility shift represents phosphorylation of the 4E-BP1. Moreover, the appearance of mobility shift was confirmed by microinjection of oocytes with RNA coding for 4E-BP1 protein tagged with hemagglutinin (HA). The oocytes were kept in the GV stage or matured for 3 h to NEBD and to MII for 12 h and analyzed by WB, using HA antibody. Our data showed no phosphorylation shift in the GV oocytes, appearance of 2 bands in the NEBD oocytes and whole expressed exogenous HA-4E-BP1 was phosphorylated in the MII stage (Supplementary Fig. 1B).

It is well established that phosphorylation of 4E-BP1 plays an important role in the regulation of cap-dependent translation.[25-30] We thus investigated the localization of 4E-BP1 and its phosphorylated forms (Thr37/46/70 and Ser65) during meiosis I. We analyzed different meiotic stages of maturing oocytes; a germinal vesicle (nucleus is present, prophase I) stage was collected directly post isolation; oocytes underwent NEBD following release from the 3-Isobutyl-1-methylxanthine (IBMX) block, oocytes undergone naturally NEBD within 1 h, a group post-NEBD was collected 3 h post IBMX wash (PIW); a metaphase I (MI) stage was collected 7 h PIW and metaphase II (MII) oocytes were collected 12 h PIW. Cell cycle progression was monitored by timing and by immunocytochemistry (ICC) using DNA staining with DAPI. Pan 4E-BP1 antibody was used to analyze the localization of global 4E-BP1 during GV to MII (Fig. 2A). In GV oocytes global 4E-BP1 was evenly distributed throughout the cytoplasm but with a higher signal visible in the nucleoplasm (Fig. 2A and Supplementary Fig. 3), without staining in the nucleolus (marked by asterisk). In the post-NEBD stages the global 4E-BP1 was also spread evenly with just a slight increase at the spindle. ICC experiments using phospho-specific antibody against the Thr37/46 form showed no signal in the GV and a similar localization was seen as total 4E-BP1 protein in the post-NEBD. Antibody recognizing 4E-BP1 phosphorylated at Ser65 showed an increased fluorescence signal in the vicinity of chromosomes, at the spindle assembly area and later at the spindle poles. The pattern of 4E-BP1 phosphorylation at Thr70 showed significant localization at the newly forming spindle post-NEBD or bipolar spindle at MI and MII, and was also present in the extruded polar body. The phospho-specific antibodies did not show a positive signal in the GV stage, which is in a good agreement with our WB data (Fig. 1B and Supplementary Fig. 1A, B). Moreover, double staining of 4E-BP1 phosphorylated at Ser65 or Thr70 with marker of microtubule organizing centers $\gamma$-tubulin showed significant enrichment of the 4E-BP1(Ser65) signal in the region with stained $\gamma$-tubulin; however, 4E-BP1(Thr70) was distributed along the whole spindle (Fig. 2B).

As 4E-BP1 phosphorylated at the Thr70 was found to be exclusively localized at the forming spindle, we therefore speculated whether this localization was tubulin-dependent. We disrupted the spindle by treatment with 1 $\mu$M Nocodazole (Noco) for 1h post-NEBD. Although the dissolved spindle changed the 4E-BP1 (Thr70) pattern, the fluorescence signal still persisted at the chromosomal area (Fig. 2C).

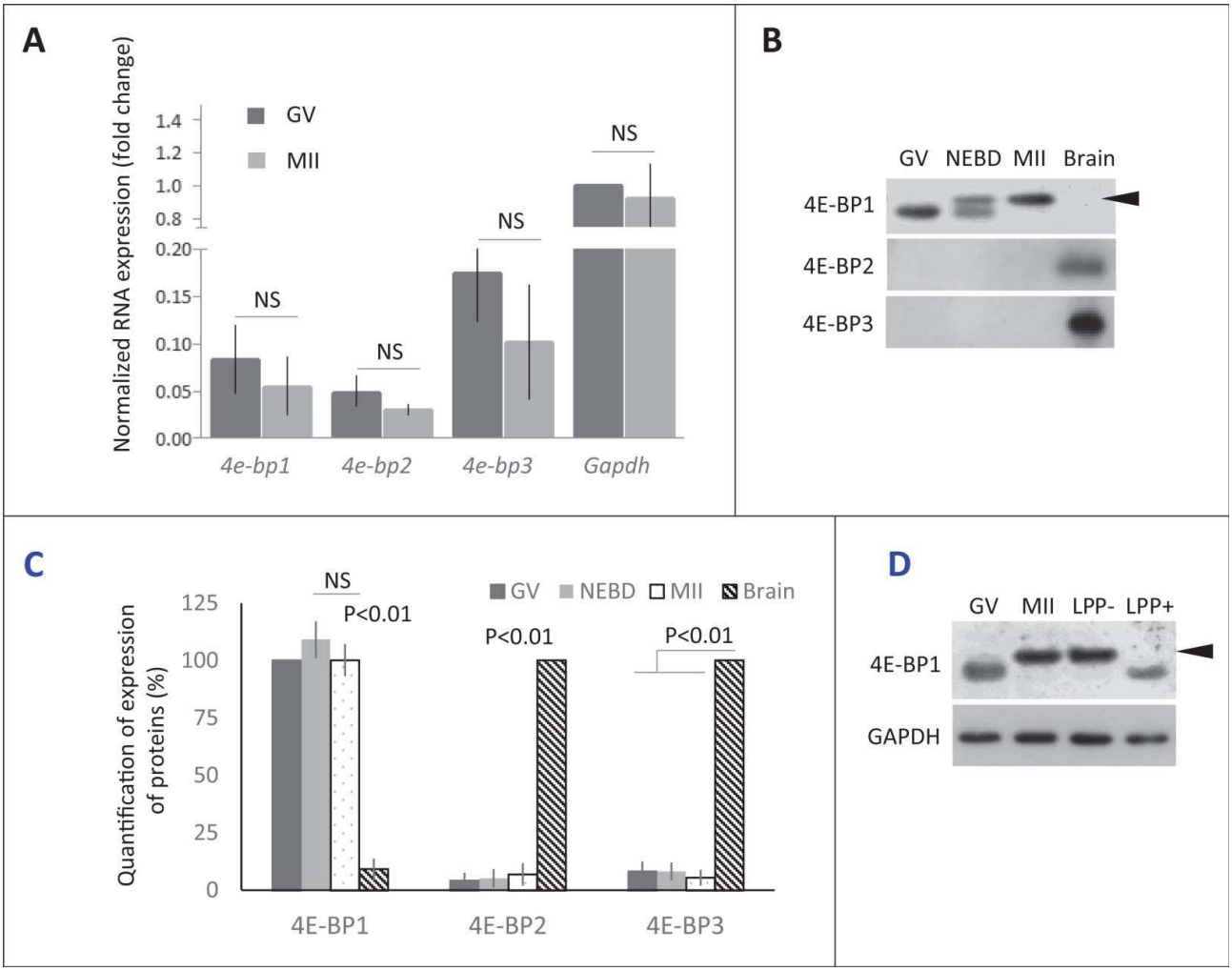



**Figure 1.** Expression of 4E-BP forms in mouse oocytes. (A) Quantitative RT-PCR analysis shows all 3 forms of *4e-bp* mRNA, which are stable during oocyte maturation (NS = non-significant, n ≥ 3). Results were normalized to the relative internal standard *Gapdh* mRNA in GV. (B) Immunoblotting shows presence of only 4E-BP1 form on the protein level. Both 4E-BP2 and 4E-BP3 are absent in the oocytes, although they are present in the brain. Expression of the 4E-BP1 in the brain sample is significantly lower in comparison with oocytes (See Fig. S1A). 4E-BP1 displays visible phosphorylation shift (arrowhead) post NEBD (a typical experiment from at least 3 replicates is shown). (C) Quantification of protein expression of the 4E-BP1–3 in the oocytes during maturation and brain samples. Data are presented as mean ± SD, Student's t-test. (D) Treatment of the lysate from MII oocytes with Lambda Protein Phosphatase (LPP+) suppressed mobility shift of the 4E-BP1 on the WB. Arrowhead points to phospho 4E-BP1 form. See Figure S1A and B.

### *Activity of mTOR is increased in the human oocyte post-NEBD*

As the mouse oocyte is a model organism for the study of human oocytes, we speculated whether mTOR(Ser2448) in human oocytes would be activated similarly as in the mouse oocyte, with a comparable localization pattern. ICC staining of human oocytes in GV, NEBD and MII stages showed that there was no signal for phospho-specific antibody against mTOR (Ser2448) in the GV stage (Supplementary Fig. 3) but increased fluorescence was visible in the NEBD and MII stage. The MII oocyte produced normally formed spindle stained with anti-tubulin antibody with a strong signal for mid-body structure positive for mTOR(Ser2448) (Supplementary Fig. 3).

### *4E-BP1 phosphorylation requires mTOR and CDK1 activity*

The timing of increased phosphorylation of 4E-BP1 positively correlates with increased cap-dependent translation after NEBD in the mouse, porcine and bovine oocyte.[5,9,21] Also, the timing of the increased phosphorylation of mTOR after NEBD.[9,31] suggests a potential role for mTOR in 4E-BP1 phosphorylation during mammalian meiosis.

Previously we have shown that suppression of mTOR activity using 100 nM mTOR inhibitor Rapamycin (Rapa) significantly represses phosphorylation of 4E-BP1, however, it does not prevent the oocytes to reach MII stage.[18] Phosphorylation of 4E-BP1 by CDK1 kinase[32-34] has been also described in other systems, in which it becomes activated at the onset of both mitosis,[34,35] and meiosis.[36] In mammalian oocytes, CDK1 activity is essential for the major morphological events occurring during meiotic maturation (including NEBD, chromosome congression and condensation, formation of the meiotic spindle) and its inhibition in the beginning of maturation results in the complete block of meiosis with oocytes arresting in the GV stage.[37] We therefore investigated the ability of the CDK1 inhibitor 10 $\mu$M Roscovitine (Rosco), as well as 100 nM mTOR inhibitor Rapa, to suppress phosphorylation of 4E-BP1 post

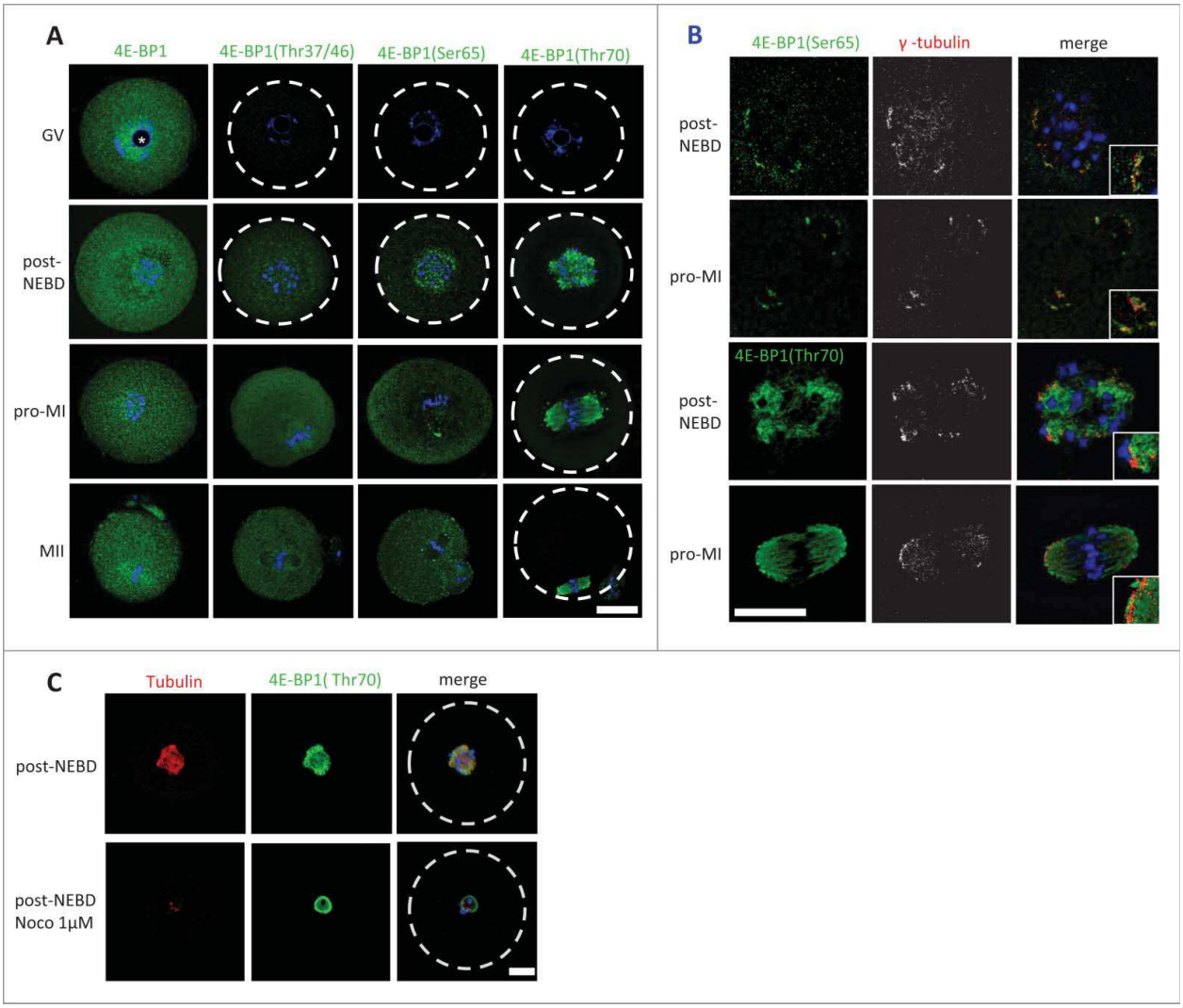



**Figure 2.** Localization of 4E-BP1 and its phosphorylated forms in the oocytes. (A) Confocal images of different meiotic stages GV (germinal vesicle), post-NEBD (3 h post IBMX wash, PIW), pro-MI (7 h PIW) and MII (12 h) stained with phospho-specific antibody (green) and DAPI (blue), white line indicates oocyte edge. Scale bar = 25 $\mu$m. Nucleolus is depicted by asterisk, from at least 3 replicates and n $\geq$ 30. (B) Marker of the microtubule-organizing centers, gamma tubulin (pseudo-colored and red) co-localizes with 4E-BP1 (Ser65) and (Thr70). Scale bar = 25 $\mu$m, n = 10. Enlarged detail in the right bottom corner. (C) Confocal images of control oocytes and oocytes treated with 1 $\mu$M Noco for 1 h in the post-NEBD stage (n $\geq$ 28), tubulin (red), 4E-BP1 (green) and DNA (blue). Scale bar = 20 $\mu$m.

NEBD. Rapa or Rosco were added to the culture media 1h PIW. Similarly to Rapa, the inhibition of CDK1 also showed significant suppression of phosphorylation shift (Fig. 3A). Next, based on its activity described in mitotic cells, we decided to determine whether PLK1 is also involved in the phosphorylation of 4E-BP1.[38] We added 100 nM specific PLK1 inhibitor BI2536[39] to the oocytes 1h PIW. However, no effect of BI2536 on 4E-BP1 phosphorylation was seen after 2h of culture (Fig. 3A and B).

Our study supports other published research[32-34] documenting that CDK1/CYCB1 (MPF) kinase is also involved in 4E-BP1 phosphorylation and in the inactivation of the its suppressor function. Mitosis is commonly thought to be associated with reduced cap-dependent protein translation, however, our previously published results.[4,5,9] show that the main regulators of cap-dependent translation initiation become activated at the onset of meiosis in pig oocytes. Therefore, we elucidated whether MPF had an impact on the activation of mTOR in mouse oocytes. By downregulation of CDK1 using Rosco treatment (added post-NEBD) we found a significant decrease in phosphorylation of mTOR(Ser2448) (Fig. 3C and D). On the other hand, treatment with Okadaic Acid (OA) substantially increased phosphorylation/activation of mTOR in the treated oocytes, when compared with control oocytes (Fig. 3C and D). Our WB data revealed that MPF influenced the activity of mTOR in the mammalian oocyte after the re-initiation of meiosis. We expected a positive correlation between the localization of the kinases and that of the phosphorylated forms of 4E-BP1. The ICC experiments indeed showed that fluorescence for both mTOR(Ser2448) and CDK1 kinases are present at the newly forming spindle or bipolar spindle (Fig. 3E), which was in good agreement with the localization of phosphorylated 4E-BP1 (Fig. 2).

Reduced cap-dependent protein translation is believed to be connected with mitosis. However, Heesom et al.[32] and Huda et al.[34] have demonstrated that cap-dependent translation is generally sustained during mitosis and 4E-BP1 becomes phosphorylated after entry to mitosis. Thus we isolated cumulus cells (CCs) from GV and MII oocyte-

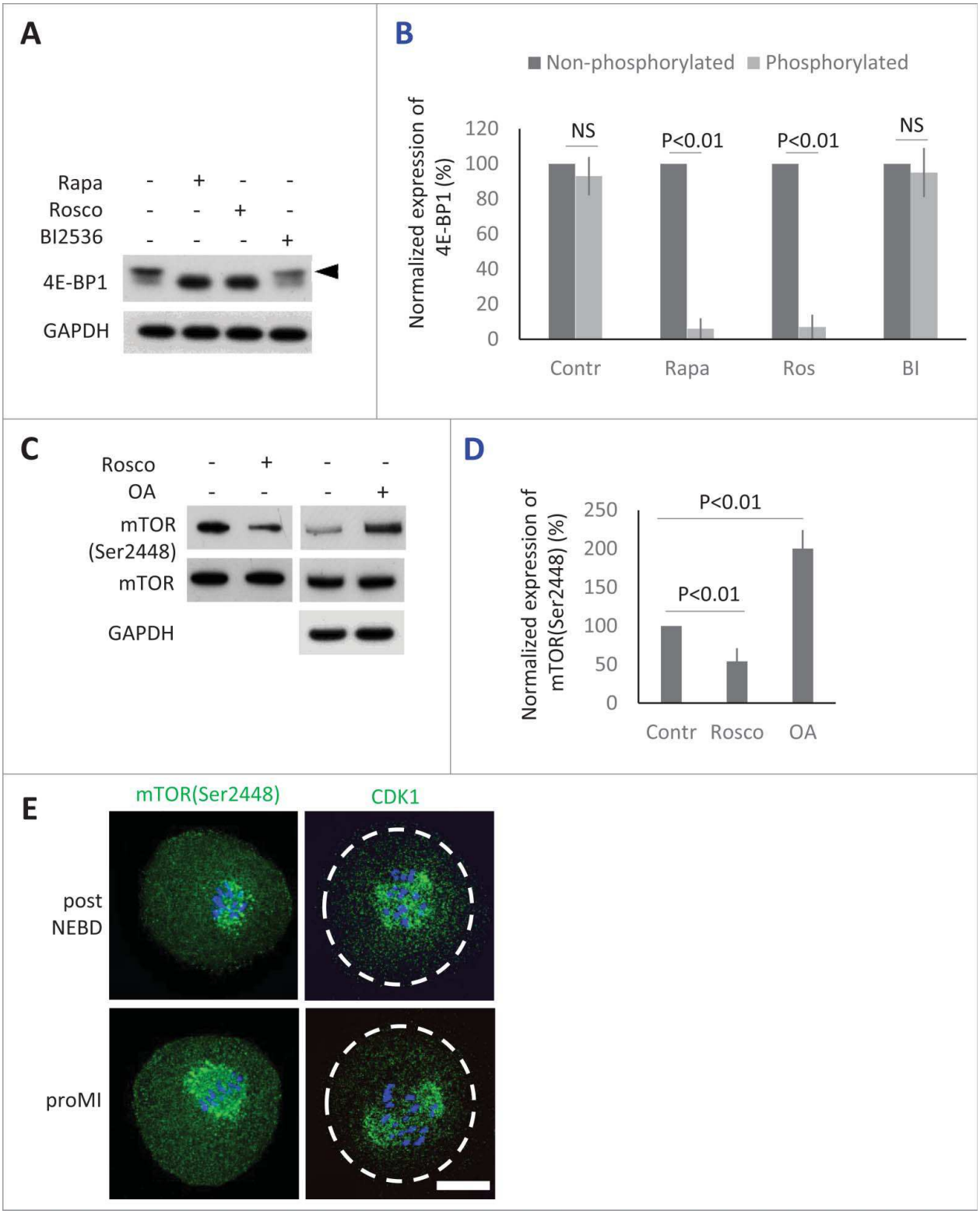



**Figure 3.** Protein kinases phosphorylating 4E-BP1 in the oocytes. (A) Detection of 4E-BP1 by immunoblotting in the oocytes treated with specific inhibitors Rapa (100 nM), Rosco (10 $\mu$M), or BI2536 (100 nM) post-NEBD. Arrowhead marks the presence of upper band (phosphorylation shift) of 4E-BP1 in the oocytes treated for 2 h post-NEBD, GAPDH was used as a loading control, a typical experiment from at least 3 replicates is shown. (B) Quantification of non-phosphorylated and phosphorylated form of 4E-BP1 in the post NEBD oocytes. Data are presented as mean ± SD, Student's t-test, NS = non-significant. (C) CDK1 effect on mTOR phosphorylation in the oocytes treated by Rosco (10 $\mu$M) or OA (1 $\mu$M). Immunoblot was probed with mTOR(Ser2448) and control (mTOR and GAPDH) antibodies. Twenty oocytes were used per sample. (D) Presence of mTOR(Ser2448) normalized to the mTOR in the Rosco or OA treated oocytes. Data are presented as mean ± SD, Student's t-test. (E) Localization of mTOR (Ser2448) and CDK1 in the post NEBD and pro-MI stage oocytes, n ≥ 30, phospho-specific antibody (green) and DNA (blue). Scale bar = 20 $\mu$m.

cumulus complexes to investigate the phosphorylated status of 4E-BP1 in other cells that are naturally present in the G0 or G1 stage[40] of the cell cycle. WB data from CCs lysates revealed that 4E-BP1 was not phosphorylated in the CCs isolated either from GV or from MII CCs (Supplementary Fig. 4A). Oocytes in GV and MII stages were used as a

control of the protein mobility shift. Accordingly, in the ICC experiments there was no 4E-BP1 phosphorylation signal observed for Thr37/46/70 and Ser65 in the CCs, although 4E-BP1 was present in this cell type (Supplementary Fig. 4B).

Altogether, our results suggest that upon exit from prophase the activity of CDK1/CYCB1 (MPF) is required for the phosphorylation of 4E-BP1, most likely via activation of mTOR.

### *Expression of a dominant negative 4E-BP1 mutant promotes aberrant spindle formation*

4E-BP1 phosphorylation releases eIF4E binding to permit translation initiation; the overall increase in phospho-4E-BP1 in the cytoplasm may facilitate maternal mRNA translational recruitment in the cytoplasm. To down-regulate phosphorylation of 4E-BP1, we expressed RNA coding for 4E-BP1 with all 4 phospho-sites mutated - Thr37/46/70 and Ser65 (4E-BP1–4Ala; Fig. 4A). Microinjection (Fig. 4B) of the *in vitro* transcribed (IVT) RNA coding for 4E-BP1-wild type (4E-BP1-Wt) or 4E-BP1–4Ala showed that the whole population of endogenous and exogenous 4E-BP1-Wt was phosphorylated in the MII oocytes (Fig. 4C; also see Fig. 1A and D), however, in MII oocytes microinjected with *4E-BP1–4Ala* RNA 2 not phosphorylated bands were present (depicted by arrowhead) and upper band of phosphorylated endogenous 4E-BP1 (Fig. 4C). Moreover, ICC detection with 4E-BP1 antibody in the microinjected oocytes showed significant increase of the intensity of the 4E-BP1 protein level for both injected constructs in comparison with no injected group (Supplementary Fig. 5; mean value ± 29 % in the 4E-BP1-Wt and mean value ± 23 % in the 4E-BP1–4Ala, $P<0.001$ Student's *t*-test). Microinjected oocytes with *4E-BP1–4Ala* RNA extruded a polar body, however, ICC analysis showed significant increase in aberrant spindles accompanied by the absence of chromosome alignment to the

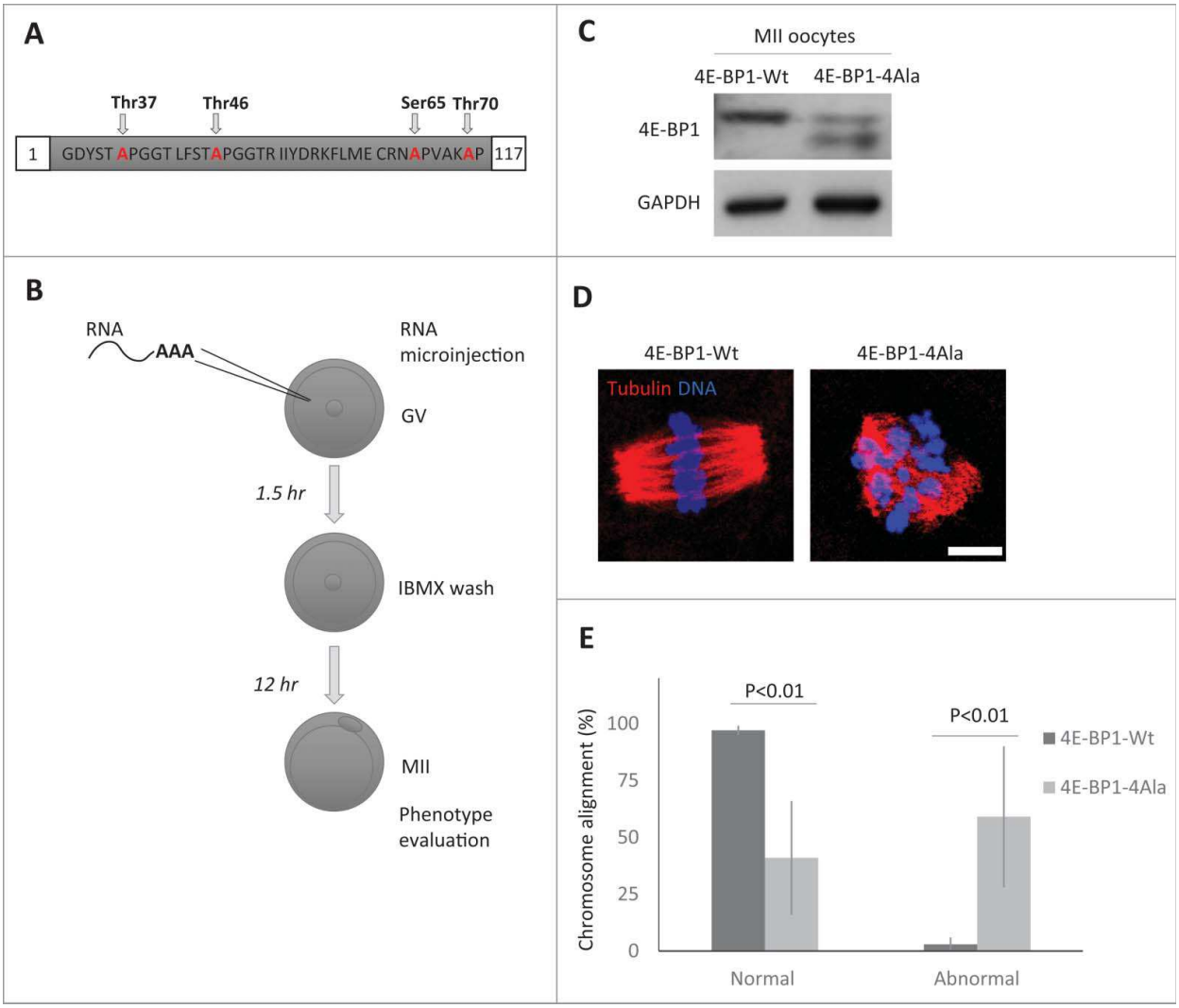



**Figure 4.** Down-regulation of 4E-BP1 phosphorylation in oocytes results in defects in the MII spindle assembly. (A) Scheme of dominant negative mutant construct of 4E-BP1–4Ala used for in vitro transcription. (B) Scheme of experimental procedure to express 4E-BP1 RNA constructs in the oocyte. (C) Immunoblotting evaluation of expression of microinjected non-phosphorylable form (marked by arrowhead) of 4E-BP1 in the matured MII oocytes n = 2. GAPDH was used as a loading control. See Figure S5. (D) Confocal images of MII spindles of oocytes microinjected with 4E-BP1-Wt or dominant negative mutant 4E-BP1–4Ala, Tubulin (red) and DNA (blue). Scale bar = 10 $\mu$m. (E) Quantification of chromosome alignment in the metaphase plate, MII oocytes expressing 4E-BP1-Wt or 4E-BP1-Ala RNA. Data are presented as mean ± SD, Student's t-test, n ≥ 25.

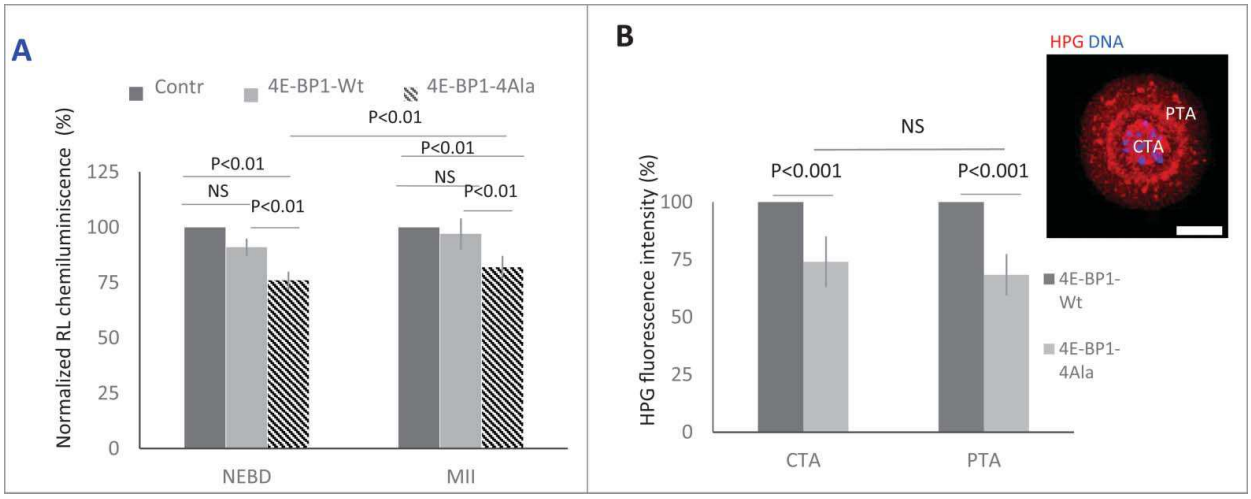



**Figure 5.** 4E-BP1 effects on protein synthesis in the oocytes. (A) Renilla luciferase reporter carrying 5′UTR TOP motive of *Eef2* co-injected with *4E-BP1-Wt* or *4E-BP1-Ala* RNA. In the control no *4E-BP1* RNA was used and the IRES motive Firefly Luciferase was used as a loading control. Chemiluminescence was measured in the post NEBD stage (mean value ± 6 and 4 %, Student's t-test, NS = non-significant) and MII stage oocytes (mean values ± SD, Student's t-test). Data are presented as mean ± SD, n ≥ 10 replicates. (B) Measurement of *in situ* translation intensity in the chromosomal area (CTA, mean value ± SD, Student's t-test, NS = non-significant) and perispindular translational area (PTA, mean value ± SD, Student's t-test) in the post NEBD oocytes, HPG (red) and DNA (blue). Data are presented as mean ± SD, n ≥ 21. Scale bar = 20 $\mu$m.

metaphase plate in 59% of the 4E-BP1–4Ala injected oocytes (mean value ± 31%; $P<0.01$, Student's *t*-test), whereas only 3% of the 4E-BP1-Wt injected oocytes produced them (mean value ± 2 %; $P < 0.01$, Student's *t*-test, Fig. 4E).

It is accepted that 4E-BP1 is a key player in cap-dependent translation[27] which predominantly utilizes mRNA with TOP motif.[41] To further investigate this, we examined the expression of a dominant negative mutant of 4E-BP1[41] and its influence on the translation of the *Renilla* Luciferase (RL) reporter with canonical TOP motive of the *Eef2*[41]. We performed microinjection of IVT RNA coding 4E-BP1-Wt or 4E-BP1–4Ala with RL reporter RNA and *Firefly* Luciferase (FL) with IRES motif as a microinjection loading control. Analysis of chemiluminescence showed a significant decrease of RL expression in the oocytes expressing 4E-BP1–4Ala in comparison with the control injected with RL and FL (Fig. 5A). Decrease of RL expression was non-significant in 4E-BP1-Wt RNA injected (mean value ± 6%, $P > 0.05$, Student's *t*-test) in the post-NEBD stage in comparison with 24% significant decrease in the *4E-BP1–4Ala* RNA injected groups (mean value ± 4%, $P<0.01$, Student's *t*-test). RL expression in the MII oocytes showed significant (18%) decrease in the oocytes injected with 4E-BP1–4Ala RNA in comparison with the control group (mean value ± 5%, $P < 0.01$, Student's *t*-test). Moreover, we analyzed in situ translation (Fig. 5B) in the 2 distinct areas of the oocyte after expression of 4E-BP1-Wt or 4E-BP1–4Ala, one at the area of the newly forming spindle (Chromosomal Translational Area; CTA) and the second at the Perispindular Translational Area (PTA). We detected a significant decrease of translation at the CTA (26 %, mean ± 11 %; $P < 0.01$, Student's *t*-test) and PTA (32 %, mean ± 9 %; $P < 0.01$, Student's *t*-test), however, without significant differences between CTA and PTA ($P > 0.05$; Fig. 5B).

## Discussion

Here we present an analysis of regulation of 4E-BP1 phosphorylation during meiotic division of the mammalian oocyte, a cell that naturally undergoes NEBD, then enters prometaphase and resumes meiosis further by asymmetric cytokinesis creating a fertilizable egg and a polar body. The progress of oocytes through cell cycle is highly synchronized, with rapid inactivation/phosphorylation of 4E-BP1, which suggests that cap-dependent translation is highly active in this cell type and stage.

In accordance with Mayer et al.[23] we were not able to detect 4E-BP2 and 4E-BP3 proteins suggesting that 4E-BP1 is the only form of eIF4E-binding protein present in mouse and bovine oocytes. However, mRNAs coding all 3 isoforms are present and stable in mouse oocytes during maturation indicating their role post-fertilization during early embryonic development, or alternatively, they might be translated to substitute 4E-BP1 in case of an insufficiency of the 4E-BP1 form.[42]

Here we show that the main effector kinases of 4E-BP1 phosphorylation are mTOR and CDK1, which become highly active after the resumption of meiosis both in mouse, human and, also bovine oocytes (mTOR,[9,23] and MPF[36]), which is similar to mitosis.[43] It has been reported that also PLK1 promotes phosphorylation of 4E-BP1 in mitotic cells,[44] however, inhibition of PLK1 in mammalian oocytes did not show any effect on 4E-BP1 phosphorylation in our model system. Inhibition of mTOR or CDK1, on the other hand, strongly affects 4E-BP1 phosphorylation in a very similar manner. These findings suggest the existence of a different mechanism of 4E-BP1 phosphorylation in the meiotic cell. We further show that inhibition of CDK1 kinase activity results in inhibition of mTOR phosphorylation on the site activation (Ser2448), suggesting that CDK1 exerts its effect on 4E-BP1 phosphorylation via activation of mTOR, although we cannot exclude the possibility that CDK1 phosphorylates 4E-BP1 directly. So, in accordance with Heesom et al.[32] we show that the main regulator of 4E-BP1 phosphorylation in mouse oocytes is mTOR, on the other hand, CDK1 activity is in our system required for the full mTOR activation rather than for direct 4E-BP1 phosphorylation. It is known that mTOR is phosphorylated and activated in mitotic cells by AKT,[45] however, according to our results, it

seems that during mammalian meiosis this pathway is not sufficient for full mTOR activation, which is likely to be mediated by CDK1.

An increase in 4E-BP1 phosphorylation has been previously seen in porcine, bovine and mouse oocytes,[4,8,9,21,22] however, only recently the localization of the differently phosphorylated forms of 4E-BP1 has been described in mouse oocytes.[38] The nature and role of Ser65 and Thr70 phosphorylation for spindle localization is unclear at the present time, although it should be noted that cap-dependent translation becomes elevated at the onset of meiosis and is inactivated later when it exits meiosis (fertilization).[6] Romasko et al.[22] show that 4E-BP1(Ser112) has similar localization as 4E-BP1(Ser65) in our study. Regulation of 4E-BP1 phosphorylation at the spindle is likely to be temporally and mechanistically distinct from its regulation in the rest of the oocyte. The dynamic spatial and temporal pattern of localization of phosphorylated 4E-BP1 that forms at the spindle is indicative of a novel mechanism promoting localized protein production related to transcripts localized at the spindle. Depolymerization of the newly forming spindle by Noco treatment changed the 4E-BP1(Thr70) pattern, however, phosphorylation still persisted at the chromosomal area. This suggests the existence of a mechanism, which maintains phosphorylation at this position, most likely involving Lamin A/C and/or endoplasmic reticulum structures surrounding the spindle assembly area. Such a mechanism would promote the accumulation of specific proteins by microtubule-independent machinery, involving some sort of semipermeable membrane[46] formed from microfilaments,[47,48] ER,[49-51] LMN[9] and possibly other constituents.

A number of studies,[22,52-57] have reported the enrichment of specific mRNAs at the spindle, which may contribute to the local proteome. Beside the enrichment of global translation at the oocyte spindle,[9] Romansko et al.[22] has also shown that *Mis18a* mRNA coding MIS18 Kinetochore Protein A is localized at the oocyte spindle, which is required for metaphase alignment and proper chromosome segregation.[58] Another example of localized translation has been documented by Bomar et al.[52] who identified the localization of *Akap95* (A kinase-anchoring protein) mRNA at the MII spindle without protein expression at this stage, but the mRNA was then translated after fertilization and the protein was present in the female pronucleus causing an unequal distribution between maternal and paternal nuclei in the zygote. Local transcriptome coupled with its translation suggests the role of translational machineries, where mTOR, CDK1 and 4E-BP1 are key players, the mechanism that is used by meiotic and mitotic cells of various species. However, differences between the cell types suggest there are distinct modes of regulation.

There are various factors involved in spindle formation. Apart from the specific transport of mRNA to the spindle, a population of RNA might already be present in the nucleus,[9,56,59,60] which indicates a significant contribution of the local transcriptome to the formation of spindle directly post-NEBD. In accordance with this, 4E-BP1 is enriched in the nucleus in its non-phosphorylated state. 4E-BP1 in the nucleus might by bound to the 5′UTR of mRNAs, where it probably functions as a translational repressor. Consequently, after its hyperphosphorylation following NEBD, it becomes inactivated and in such a way promotes the translation of specific mRNAs at the newly forming spindle. These results suggest that the function of mRNA retention in the nucleus may be to sustain translational repression, and that their subsequent translation can be regulated in a spatiotemporally restricted manner in response to cell cycle events.

We propose that meiotic phosphorylation of 4E-BP1 on Ser65 and Thr70 by mTOR acts to stimulate cap-dependent translation as the oocyte proceeds though meiosis (particularly after NEBD) and that specific localization of the key cap-dependent translation regulatory factors,[22,61] is essential for the translation of specific mRNAs at the spindle area to ensure errorless meiotic progression. We identify the 2 kinases mTOR and CDK1 involved in the inactivation of the 4E-BP1 at the spindle where all the important regulators are present. Using PLK1 inhibitor BI2536 we show that PLK1 kinase is not involved in 4E-BP1 phosphorylation in mouse oocytes and also, that CDK1 exerts its influence via the phosphorylation (and as such further activation) of mTOR, which as a result is likely to phosphorylate Ser65 and Thr70 of 4E-BP1. However, we cannot exclude the possibility that CDK1 phosphorylates at least one of these sites directly, as was previously reported by Heesom et al.[32] and Shuda et al.[34] Since the effect of CDK1 inhibition on the level of 4E-BP1 phosphorylation is less pronounced in later stages of meiosis (data not shown) it is tempting to speculate that the increased activation of mTOR mediated by CDK1 might be temporally and possibly also spatially restricted to the most critical process during early meiosis, i.e. formation of the meiotic spindle. Such hypothesis is supported also by the data obtained by us and other studies[38] showing the increased presence of 4E-BP1 phosphorylated forms at the spindle and in the chromosomal area. It is also interesting to note that CDK1 has been shown to directly phosphorylate the key mTOR binding partner Raptor during mitosis.[62] This reinforces our conclusions and those from other studies suggesting that mTOR activity is highly regulated by cell cycle progression. A number of other proteins involved in the regulation of translation have also been described previously. Papst[63] reported that Ribosomal Protein S6 Kinase is a substrate for CDK1/CYCB1 in mitosis and Elongation factor-1[64] in the *Xenopus* oocytes during meiotic cell division is a physiologic substrate of CDK1/CYCB1 in mitosis.

After fertilization when the nuclear envelope is reformed again at the end of meiosis, phosphorylation of 4E-BP1 disappears.[5,9] This indicates a specific/exclusive role of this pathway in meiotic maturation, which is also supported by our findings showing that no phosphorylated 4E-BP1 is present in the CCs, naturally occurring in the G0 or G1 stage. We might conclude that phosphorylation of 4E-BP1 follows exit from prophase of the cell cycle. It has been reported previously that overall protein synthesis becomes reduced during meiosis.[4,9,65] However, studies in synchronized HeLa cells have shown that this inhibition ceases by late telophase[66] and that overall protein synthesis increases rapidly as cells enter G1-phase.[67]

Here we show that the presence of a non-phosphorylated 4E-BP1 population in an oocyte that progresses through meiosis results in aberrant morphology of the metaphase II spindle that is most likely the result of impaired translation of a subset of RNAs. Previously, we have described the effect of mTOR/4F

pathway downregulation on in situ translation at the chromosomal area.[9] Our current finding shows that a non-phosphorylated mutant does not display significant differences in the level of translation between the chromosomal and perispindular areas. This might be explained by the fact that exogenous 4E-BP1, which is loaded to the cytoplasm in the form of RNA, and its consequent 4E-BP1 protein, lacks endogenous localization in this large cell and so influences both translational areas within the cell. On the other hand, the expression of a mutant in the cytoplasm which is unable to be phosphorylated leads to downregulation of translation in the cytoplasm and at the chromosomal area.

4E-BP1 null mice are viable and fertile.[42] However, we have observed aberrant spindle formation in the MII oocytes expressing a non-phosphorylatable 4E-BP1 form, which might suggest that the role of 4E-BP1 is rather in the fine tuning of meiotic progression. Regulation of 4E-BP1 in the oocyte might be affected by cell stress or by the age of the female. Moreover, insulin stimulates the mTOR signaling pathway[68] and insulin signaling promotes the production of high-quality oocytes.[69] Consistently, oocytes from diabetic mice display spindle abnormalities, which can be reversed by pancreatic islet transplantation.[70] Our findings showing localization of phosphorylated/inactivated 4E-BP1 at the spindle also suggest the existence of a mechanism that links maternal age and environmental exposures to diminished oocyte quality arising from defective spindle formation and function. We show that mTOR becomes also activated post NEBD in the human oocyte, with strong signal at midbody in the MII oocyte, suggesting its similar role in the human oocyte meiosis in specific translational regulation, as it plays in the mouse oocyte. Here, mTOR pathway might contribute to the age related chromosome segregation errors in the woman oocytes, similarly as it has been documented in the mouse model,[9] as well as in mammalian and yeast cells.[71] Lapasset et al.[7] showed that the treatment with Rapa resulted in the prevention of extrusion of second polar body in starfish oocytes. They present the absence of eIF4E dissociation from 4E-BP in the presence of Rapa without the effect on translation of Cyclin B1 or Mos. Taken together, mTOR involvement is indispensable for inactivation of translational repressor 4E-BP1, which prevents the synthesis of essential proteins necessary for a correct completion of the meiotic and mitotic divisions. In addition to translational initiation factors, Ribosomal protein S3 (RPS3) is present at the mitotic[72] or newly forming meiotic spindle.[73] RPS3 knockdown causes arrest in mitotic metaphase,[72] which resembles the effect of mTOR inhibition[23] in the bovine oocyte. The influence of known effector kinases in the inactivation of the translational repressor 4E-BP1 might be essential for the temporal and spatial translation of specific mRNAs at the spindle area to ensure errorless meiotic progression.

In this study we propose that localized translational regulation at the oocyte spindle regulated though an mTOR/CDK1 pathway might represent a mechanism which links spindle formation and function with the temporal and spatial regulation of the local transcriptome in the particular subcellular areas, which affects oocyte quality. There is still much to learn about the dynamics of distribution of mRNA and translational regulatory components, as well as how exactly these are regulated in the different cellular compartments. Further elucidation of the relationship between cytoskeletal elements and translation machinery may help to explain the logistics of translational control of spindle assembly and chromosome segregation.

## Material and methods

### Oocytes isolation and maturation

Mouse ovaries were obtained from CD1 mice at least 6 weeks old which were stimulated to by intraperitoneal injection of 5 UI of pregnant mare serum gonadotropin (PMSG; Folligon, Merck Animal Health) 46 h before collection. GV oocytes were isolated into transfer medium Tetkova et al.[74] supplemented with 100 $\mu$M of 3-isobutyl-1-methylxanthine (IBMX) used to prevent spontaneous resumption of meiosis. Selected oocytes were stripped of the cumulus cells and cultured in M16 medium (Millipore) without IBMX at 37°C, 5% $CO_2$. After 70 min post IBMX wash (PIW) at least 90% of oocytes underwent nuclear envelope breakdown (NEBD, resumption of meiosis; G2/M transition) and oocytes arrested in the GV were discarded. Pro-metaphase I (pro-MI) and metaphase I (MI) stage oocytes were collected after post IBMX wash at 3 h (post-NEBD), 7 h (pro-MI) and 12 h (MII). All animal work was conducted according to Act No 246/1992 on the protection of animals against cruelty. Human oocytes, not used in human reproduction, were obtained from the Obstetrics and Gynecology Clinic of the General University Hospital in Prague. The project was accredited (#30/12) by the Ethical Committee of the General Hospital, Prague.

### Oocyte treatments

Mouse oocytes were treated with 100nM BI2536 for 2 hours post NEBD (Axon Medchen), 1 $\mu$M Nocodazole for 1 h (M1404, Sigma-Aldrich), 100 nM Rapamycin (#9904, CST) or 10 $\mu$M Roscovitine (R7772, Sigma-Aldrich); 1 $\mu$M Okadaic acid (OA, CAS 459616, Millipore) for 2 h after NEBD. For nascent protein synthesis specific stage NEBD-2 h, oocytes were cultured in methionine-free medium (Gibco) supplemented with 1% dialyzed fetal bovine serum (10,000MW; Sigma) and 50 mM L-homopropargylglycine (HPG) for 30 min. HPG was detected by using a Click-iT Cell Reaction Kit (Life Technologies). *In situ* translation detection showed increased incorporation of HPG in the chromosomal area (CTA) and perispindular area (PTA[9])

### RNA isolation and quantitative RT-PCR

RNA was extracted with RNeasy Plus Micro kit (Qiagen) according to manufacturer's instructions. Genomic DNA was depleted using guide columns. $H_2O$ for qRT-PCR was used for RNA elution in amount of 25 $\mu$L for 25 oocytes. Samples were stored at −80°C until expression analysis. mRNA equivalent for 1 oocyte was amplified by a One-step RT-PCR kit (Qiagen) with real-time detection using SybrGreenI fluorescent dye on a Rotor Gene 3000 instrument (Corbett Research, Australia). The qRT-PCR reactions were prepared in duplicates in one

run. Reaction conditions were: reverse transcription at 50°C for 30 min, initial activation at 95°C for 15 min, cycling: denaturation at 95°C for 20 sec, annealing at a temperature specific for each set of primers (see Table S1) for 20 sec, extension at 7°C for 30 sec. Products were verified by melting analysis and gel electrophoresis on 1.2% agarose gel with ethidium bromide staining. The relative concentration of templates in different samples was determined using comparative analysis software (Corbett Research). The results for individual target genes were normalized according to the relative internal standard GAPDH. The data are presented from at least 3 biologic replicates. The significant differences between GV and MII were evaluated using *t*-test (PrismaGraph5).

### Immunocytochemistry

Mouse and human oocytes were fixed for 20 min in 4% PFA in phosphate saline buffer (PBS). Oocytes were permeabilized for 10 min in 0.2% Triton X-100 in PBS, then washed with PVA/PBS. Oocytes were incubated with primary antibodies at 4°C overnight. We are using human 4E-BP1 nomenclature to unify the text discussing human and mouse systems. The human 4E-BP1 sequence of amino acid numbers is greater by one. The following antibodies were used in 1:100 dilution: rabbit anti-4E-BP1 (#9452, CST), rabbit anti-phospho-4E-BP1(Thr70) (#13396, CST), rabbit anti-phospho-4E-BP1(T37/46) (#9459, CST), rabbit anti-phospho-4E-BP1(Ser65) (#9451, CST), rabbit anti-CDK1 (#9112, CST), mouse anti-tubulin (#T6793, Sigma) and $\gamma$-tubulin (#T6557, Sigma), rabbit anti-phospho-mTOR(Ser2448, #2971, CST) and mouse anti-LMNA/C (SAB4200236, Sigma Aldrich). After washing in PBS, detection of the primary antibodies was performed by cultivation of the oocytes with relevant Alexa Fluor 488, 594 or 647 conjugates (diluted 1: 250) for 1 h at room temperature. Oocytes were then washed 2 times for 15 min in PVA/PBS and mounted using Vectashield Mounting Medium with DAPI (H-1200, Vector Laboratories). Samples were visualized using a Leica SP5 inverted confocal microscope (Leica Microsystems) in 16 bit depth. Images were assembled in LEICA LasAFX (Leica Microsystems) software and equatorial sections were quantified by Image J software (http://rsbweb.nih.gov/ij/).

### Western blot

Oocytes were lysed with 6 $\mu$l of Millipore $H_2O$ and 2, 5 $\mu$l of 4x lithium dodecyl sulfate, sample buffer NP 0007 and 1 $\mu$l reduction buffer NP 0004 (Novex, Thermo Fisher Scientific) and boiled at 100°C for 5 min. If not stated otherwise, sample of 50 oocytes per sample was used. To detect phosphorylation shift, oocytes were dissolved in the 20 $\mu$l of the 1x NEBuffer with 800 U of LPP enzyme (P0753, New England BioLabs) and incubated overnight at 30°C, LPP was omitted in the control sample (LPP-). Lysates were separated using a 4–12% gradient polyacrylamide gel SDS (NP323BOX, Life Technologies) page and transferred to an immobilon P membrane (PVDF; Millipore) using semidry blotting system (Biometra GmbH). Membranes were blocked for 1 h, in 1–5% skimmed milk dissolved in Tween-Tris-buffer saline (TTBS, pH 7,4) according to antibody (list of primary antibodies and dilutions is below). After 3 cycles for 10 min washing in TTBS, membranes were incubated at 4°C overnight in 1% milk/TTBS with the following primary antibodies: GAPDH (rabbit, G9545, Sigma-Aldrich) and Tubulin (mouse, T6793, Sigma-Aldrich) antibodies were diluted 1:30 000 and 4E-BP1 (rabbit, 9452, CST), 4E-BP1(T69) (rabbit, 9455S, CST), 4E-BP1(T36/45) (rabbit, 9459, CST), 4E-BP1 (S64) (rabbit, 9451S, CST), anti HA (rabbit, 3724, CST) antibodies were diluted 1:500; mTOR(Ser2448) (rabbit, 2971S, CST), mTOR (rabbit, 2972, CST) antibodies were diluted 1:8 000 and 1:2 000 respectively. After 3 cycles of 10 min washing in TTBS the membrane was incubated for 1 h with secondary antibody Peroxidase Anti-Rabbit Donkey (711–035–152, Jackson immunoresearch) or Peroxidase Anti-mouse Donkey (715–035–151, Jackson immunoresearch) in 1:7.500 dilution in 1% milk/TTBS 1 h at room temperature. Immunodetected proteins were visualized by ECL (Amersham, GE Healthcare life science), films were scanned using a GS-800 calibrated densitometer (Bio-Rad) and quantified using Image J software (http://rsbweb.nih.gov/ij/).

### Microinjection

GV stage mouse oocytes were microinjected in transfer medium with IMBX on an inverted microscope Leica DMI 6000B with Transferman NK2 and Femtojet (Eppendorf). Oocytes were injected with *in vitro* transcribed RNA (mMessage, Ambion) from mutant plasmid pCW57.1–4E-BP1–4Ala[41] and pCMV3-N-HA-4E-BP1 (generous gift of professor Nahum Sonenberg, McGill University, Montreal, Canada; Gingras et al.[27]). Approximately 5 pl of RNA solutions of 4E-BP1-Ala or 4E-BP1-Wt diluted in RNAse free water, to concentration 50 ng/$\mu$l were microinjected into oocytes.

### Dual-luciferase assay

Oocytes were injected in the presence of IBMX with 50 ng/$\mu$l of IVT RNA (mMessage, Ambion) from *Renilla Luciferase* constructs (*Eef2*–5′UTR - RL; #38235; Addgene) with combination of injection amount control *Firefly Luciferase* (FL; #18964; Addgene[75]) and RNA for 4E-BP1-Wt or 4E-BP1–4Ala in the presence of IBMX. Oocytes were cultured for 5 h without IBMX. At least 5 oocytes were lysed in 5 $\mu$l of Passive Lysis Buffer and stored at −80°C until measurement of chemiluminescence by Dual-Luciferase Assay System (Promega) according to the manufacturer's instructions. Signal intensities were measured using a Glomax Luminometer (Promega). Activity of RL was normalized to the FL luciferase.

### Statistical analysis

Experiments were repeated at least 3 times unless stated. Mean and SD values were calculated using MS Excel, statistical significance of the differences between the groups was tested using Student's *t*-test (PrismaGraph5) and $P<0.05$ was considered as statistically significant.

## Abbreviations

| | |
|---|---|
| Akap95 | Kinase (PRKA) anchor protein 8 |
| CCs | Cumulus cells |

| | |
|---|---|
| CDK1 | Cyclin dependent kinase 1 |
| CTA | Chromosomal translational area |
| CYCB1 | Cyclin B1 |
| DAPI | 4', 6-diamidino-2-phenylindole |
| ER | Endoplasmic reticulum |
| eIF4E | Eukaryotic initiation factor 4E |
| eIF4G1 | Eukaryotic initiation factor 4G1 |
| eIF4A | Eukaryotic initiation factor 4A |
| FL | Firefly Luciferase |
| FRAP kinase | FKBP-12-rapamycin-associated protein |
| G1-phase | Gap 1 phase |
| GV | Germinal vesical stage |
| HPG | L-homopropargylglycine |
| HA | Hemagglutinin |
| IBMX | 3-Isobutyl-1-methylxanthine |
| IRES | Internal ribosome entry site |
| IVT | *In vitro* transcribed |
| LMN | Lamin A/C |
| Mis18a | MIS18 kinetochore protein A |
| mTOR | Mammalian target of rapamycin |
| MI | Metaphase of first meiotic maturation |
| MII | Metaphase of second meiotic maturation |
| MPF | Maturation promoting factor |
| Noco | Nocodazole |
| NEBD | Nuclear envelope breakdown |
| OA | Okadaic acid |
| PTA | Perispindular translational area |
| PVA | Polyvinyl alcohol |
| PIW | Post IBMX wash |
| PLK1 | Polo-like kinase1 |
| PKB/ AKT | Protein kinase B/ serine/threonine-specific protein kinase |
| PBE | Polar body extrusion. |
| Ras/Raf/ERK pathway | Mitogen-activated protein kinases pathway |
| Rosco | Roscovitine |
| RL | Renilla Luciferase |
| Rapa | Rapamycin |
| S6 kinase | Ribosomal s6 kinase |
| TOP | Terminal oligopyrimidine motif |
| 3′UTR | Three prime untranslated region |
| 4E-BP1 | Eukaryotic translation initiation factor 4E-binding protein 1 |
| 4E-BP2 | Eukaryotic translation initiation factor 4E-binding protein 2 |
| 4E-BP3 | Eukaryotic translation initiation factor 4E-binding protein 3 |
| 5′UTR | Five prime untranslated region |

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Acknowledgments

We thank Jaroslava Supolikova and Marketa Hancova for their exceptional assistance with experiments and Nahum Sonenberg and his laboratory for kindly providing the pCMV3-N-HA- 4E-BP1 plasmid.

## Funding

This work was supported by GACR13–12291S, GACR15–22765S, EXCELLENCE CZ.02.1.01/0.0/0.0/15_003/0000460 OP RDE and Institutional Research Concept RVO67985904.

## ORCID

Radek Malik http://orcid.org/0000-0002-6783-1146
Michal Kubelka http://orcid.org/0000-0002-2264-5884

## References

[1] Schuh M, Ellenberg J. Self-organization of MTOCs replaces centrosome function during acentrosomal spindle assembly in live mouse Oocytes. Cell 2007; 130:484-98
[2] Kusch J, Liakopoulos D, Barral Y. Spindle asymmetry: a compass for the cell. Trends Cell Biol 2003; 13:562-9
[3] Hashimoto N, Kishimoto T. Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. Dev Biol 1988; 126:242-52; PMID:3350209
[4] Ellederova Z, Kovarova H, Melo-Sterza F, Livingstone M, Tomek W, Kubelka M. Suppression of translation during in vitro maturation of pig oocytes despite enhanced formation of cap-binding protein complex eIF4F and 4E-BP1 hyperphosphorylation. Mol Reprod Dev 2006; 73:68-76; PMID:16211600
[5] Ellederová Z, Cais O, Susor A, Uhlírová K, Kovárová H, Jelínková L, Tomek W, Kubelka M. ERK1/2 map kinase metabolic pathway is responsible for phosphorylation of translation initiation factor eIF4E during in vitro maturation of pig oocytes. Mol Reprod Dev 2008; 75:309-17; PMID:17290414
[6] Susor A, Jelínková L, Karabínová P, Torner H, Tomek W, Kovárová H, Kubelka M. Regulation of cap-dependent translation initiation in the early stage porcine parthenotes. Mol Reprod Dev 2008; 75:1716-25; PMID:18386287
[7] Lapasset L, Pradet-Balade B, Vergé V, Lozano J-C, Oulhen N, Cormier P, Peaucellier G. Cyclin B synthesis and rapamycin-sensitive regulation of protein synthesis during starfish oocyte meiotic divisions. Mol Reprod Dev 2008; 75:1617-26; PMID:18361417
[8] Tomek W, Torner H, Kanitz W. Comparative analysis of protein synthesis, transcription and cytoplasmic polyadenylation of mRNA during maturation of bovine oocytes in vitro. Reprod Domest Anim Zuchthyg 2002; 37:86-91
[9] Susor A, Jansova D, Cerna R, Danylevska A, Anger M, Toralova T, Malik R, Supolikova J, Cook MS, Oh JS, et al. Temporal and spatial regulation of translation in the mammalian oocyte via the mTOR-eIF4F pathway. Nat Commun 2015; 6:6078; PMID:25629602
[10] Jackson RJ, Hellen CUT, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. Nat Rev Mol Cell Biol 2010; 11:113-27; PMID:20094052
[11] Gingras AC, Gygi SP, Raught B, Polakiewicz RD, Abraham RT, Hoekstra MF, Aebersold R, Sonenberg N. Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. Genes Dev 1999; 13:1422-37; PMID:10364159
[12] Gingras AC, Raught B, Sonenberg N. Regulation of translation initiation by FRAP/mTOR. Genes Dev 2001; 15:807-26; PMID:11297505
[13] Raught B, Gingras AC, Sonenberg N. The target of rapamycin (TOR) proteins. Proc Natl Acad Sci U S A 2001; 98:7037-44; PMID:11416184
[14] Tavares MR, Pavan ICB, Amaral CL, Meneguello L, Luchessi AD, Simabuco FM. The S6K protein family in health and disease. Life Sci 2015; 131:1-10; PMID:25818187
[15] Gingras AC, Raught B, Gygi SP, Niedzwiecka A, Miron M, Burley SK, Polakiewicz RD, Wyslouch-Cieszynska A, Aebersold R, Sonenberg N. Hierarchical phosphorylation of the translation inhibitor 4E-BP1. Genes Dev 2001; 15:2852-64; PMID:11691836
[16] Tee AR, Proud CG. Caspase cleavage of initiation factor 4E-binding protein 1 yields a dominant inhibitor of cap-dependent translation and reveals a novel regulatory motif. Mol Cell Biol

2002; 22:1674-83; PMID:11865047; http://dx.doi.org/10.1128/MCB.22.6.1674-1683.2002

[17] Wang X, Beugnet A, Murakami M, Yamanaka S, Proud CG. Distinct signaling events downstream of mTOR cooperate to mediate the effects of amino acids and insulin on initiation factor 4E-binding proteins. Mol Cell Biol 2005; 25:2558-72; PMID:15767663; http://dx.doi.org/10.1128/MCB.25.7.2558-2572.2005

[18] Corradetti MN, Guan K-L. Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? Oncogene 2006; 25:6347-60; PMID:17041621; http://dx.doi.org/10.1038/sj.onc.1209885

[19] Kalous J, Kubelka M, Solc P, Susor A, Motlík J. AKT (protein kinase B) is implicated in meiotic maturation of porcine oocytes. Reprod Camb Engl 2009; 138:645-54

[20] Tomek W, Smiljakovic T. Activation of Akt (protein kinase B) stimulates metaphase I to metaphase II transition in bovine oocytes. Reprod Camb Engl 2005; 130:423-30

[21] Tomek W, Melo Sterza FA, Kubelka M, Wollenhaupt K, Torner H, Anger M, Kanitz W. Regulation of translation during in vitro maturation of bovine oocytes: the role of MAP kinase, eIF4E (cap binding protein) phosphorylation, and eIF4E-BP1. Biol Reprod 2002; 66:1274-82; PMID:11967187; http://dx.doi.org/10.1095/biolreprod66.5.1274

[22] Romasko EJ, Amarnath D, Midic U, Latham KE. Association of maternal mRNA and phosphorylated EIF4EBP1 variants with the spindle in mouse oocytes: localized translational control supporting female meiosis in mammals. Genetics 2013; 195:349-58; PMID:23852387; http://dx.doi.org/10.1534/genetics.113.154005

[23] Mayer S, Wrenzycki C, Tomek W. Inactivation of mTor arrests bovine oocytes in the metaphase-I stage, despite reversible inhibition of 4E-BP1 phosphorylation. Mol Reprod Dev 2014; 81:363-75; PMID:24459013; http://dx.doi.org/10.1002/mrd.22305

[24] Lee S-E, Sun S-C, Choi H-Y, Uhm S-J, Kim N-H. mTOR is required for asymmetric division through small GTPases in mouse oocytes. Mol Reprod Dev 2012; 79:356-66; PMID:22407942; http://dx.doi.org/10.1002/mrd.22035

[25] Poulin F, Gingras A-C, Olsen H, Chevalier S, Sonenberg N. 4E-BP3, a New Member of the Eukaryotic Initiation Factor 4E-binding Protein Family. J Biol Chem 1998; 273:14002-7; PMID:9593750; http://dx.doi.org/10.1074/jbc.273.22.14002

[26] Pause A, Belsham GJ, Gingras AC, Donzé O, Lin TA, Lawrence JC, Sonenberg N. Insulin-dependent stimulation of protein synthesis by phosphorylation of a regulator of 5′-cap function. Nature 1994; 371:762-7; PMID:7935836; http://dx.doi.org/10.1038/371762a0

[27] Gingras AC, Raught B, Sonenberg N. eIF4 initiation factors: effectors of mRNA recruitment to ribosomes and regulators of translation. Annu Rev Biochem 1999; 68:913-63; PMID:10872469; http://dx.doi.org/10.1146/annurev.biochem.68.1.913

[28] Mader S, Lee H, Pause A, Sonenberg N. The translation initiation factor eIF-4E binds to a common motif shared by the translation factor eIF-4 gamma and the translational repressors 4E-binding proteins. Mol Cell Biol 1995; 15:4990-7; PMID:7651417; http://dx.doi.org/10.1128/MCB.15.9.4990

[29] Tsukiyama-Kohara K, Vidal SM, Gingras AC, Glover TW, Hanash SM, Heng H, Sonenberg N. Tissue distribution, genomic structure, and chromosome mapping of mouse and human eukaryotic initiation factor 4E-binding proteins 1 and 2. Genomics 1996; 38:353-63; PMID:8975712; http://dx.doi.org/10.1006/geno.1996.0638

[30] Fadden P, Haystead TA, Lawrence JC. Identification of phosphorylation sites in the translational regulator, PHAS-I, that are controlled by insulin and rapamycin in rat adipocytes. J Biol Chem 1997; 272:10240-7; PMID:9092573; http://dx.doi.org/10.1074/jbc.272.15.10240

[31] Kogasaka Y, Hoshino Y, Hiradate Y, Tanemura K, Sato E. Distribution and association of mTOR with its cofactors, raptor and rictor, in cumulus cells and oocytes during meiotic maturation in mice. Mol Reprod Dev 2013; 80:334-48; PMID:23440873; http://dx.doi.org/10.1002/mrd.22166

[32] Heesom KJ, Gampel A, Mellor H, Denton RM. Cell cycle-dependent phosphorylation of the translational repressor eIF-4E binding protein-1 (4E-BP1). Curr Biol 2001; 11:1374-9; PMID:11553333; http://dx.doi.org/10.1016/S0960-9822(01)00422-5

[33] Greenberg VL, Zimmer SG. Paclitaxel induces the phosphorylation of the eukaryotic translation initiation factor 4E-binding protein 1 through a Cdk1-dependent mechanism. Oncogene 2005; 24:4851-60; PMID:15897904; http://dx.doi.org/10.1038/sj.onc.1208624

[34] Shuda M, Velásquez C, Cheng E, Cordek DG, Kwun HJ, Chang Y, Moore PS. CDK1 substitutes for mTOR kinase to activate mitotic cap-dependent protein translation. Proc Natl Acad Sci U S A 2015; 112:5875-82; PMID:25883264; http://dx.doi.org/10.1073/pnas.1505787112

[35] Velásquez C, Cheng E, Shuda M, Lee-Oesterreich PJ, Pogge von Strandmann L, Gritsenko MA, Jacobs JM, Moore PS, Chang Y. Mitotic protein kinase CDK1 phosphorylation of mRNA translation regulator 4E-BP1 Ser83 may contribute to cell transformation. Proc Natl Acad Sci U S A 2016; 113:8466-71; PMID:27402756; http://dx.doi.org/10.1073/pnas.1607768113

[36] Hampl A, Eppig JJ. Analysis of the mechanism(s) of metaphase I arrest in maturing mouse oocytes. Development 1995; 121:925-33; PMID:7743936

[37] Wang X, Swain JE, Bollen M, Liu X-T, Ohl DA, Smith GD. Endogenous regulators of protein phosphatase-1 during mouse oocyte development and meiosis. Reproduction 2004; 128:493-502; PMID:15509695; http://dx.doi.org/10.1530/rep.1.00173

[38] Shang ZF, Yu L, Li B, Tu WZ, Wang Y, Liu XD, Guan H, Huang B, Rang WQ, Zhou PK. 4E-BP1 participates in maintaining spindle integrity and genomic stability via interacting with PLK1. Cell Cycle 2012; 11:3463-71; PMID:22918237; http://dx.doi.org/10.4161/cc.21770

[39] Lénárt P, Petronczki M, Steegmaier M, Di Fiore B, Lipp JJ, Hoffmann M, Rettig WJ, Kraut N, Peters J-M. The small-molecule inhibitor BI 2536 reveals novel insights into mitotic roles of polo-like kinase 1. Curr Biol CB 2007; 17:304-15; PMID:17291761; http://dx.doi.org/10.1016/j.cub.2006.12.046

[40] Katska L, Bochenek M, Kania G, Ryńska B, Smorag Z. Flow cytometric cell cycle analysis of somatic cells primary cultures established for bovine cloning. Theriogenology 2002; 58:1733-44; PMID:12472143; http://dx.doi.org/10.1016/S0093-691X(02)01043-9

[41] Thoreen CC, Chantranupong L, Keys HR, Wang T, Gray NS, Sabatini DM. A unifying model for mTORC1-mediated regulation of mRNA translation. Nature 2012; 485:109-13; PMID:22552098; http://dx.doi.org/10.1038/nature11083

[42] Tsukiyama-Kohara K, Poulin F, Kohara M, DeMaria CT, Cheng A, Wu Z, Gingras AC, Katsume A, Elchebly M, Spiegelman BM, et al. Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. Nat Med 2001; 7:1128-32; PMID:11590436; http://dx.doi.org/10.1038/nm1001-1128

[43] Vazquez-Martin A, Oliveras-Ferraros C, Bernadó L, López-Bonet E, Menendez JA. The serine 2481-autophosphorylated form of mammalian Target Of Rapamycin (mTOR) is localized to midzone and midbody in dividing cancer cells. Biochem Biophys Res Commun 2009; 380:638-43; PMID:19285014; http://dx.doi.org/10.1016/j.bbrc.2009.01.153

[44] He Z, Wu J, Dang H, Lin H, Zheng H, Zhong D. Polo-like kinase 1 contributes to the tumorigenicity of BEL-7402 hepatoma cells via regulation of Survivin expression. Cancer Lett 2011; 303:92-8; PMID:21330050; http://dx.doi.org/10.1016/j.canlet.2011.01.007

[45] Makker A, Goel MM, Mahdi AA. PI3K/PTEN/Akt and TSC/mTOR signaling pathways, ovarian dysfunction, and infertility: an update. J Mol Endocrinol 2014; 53:R103-118; PMID:25312969; http://dx.doi.org/10.1530/JME-14-0220

[46] Schweizer N, Pawar N, Weiss M, Maiato H. An organelle-exclusion envelope assists mitosis and underlies distinct molecular crowding in the spindle region. J Cell Biol 2015; 210:695-704; PMID:26304726; http://dx.doi.org/10.1083/jcb.201506107

[47] Yu Y, Dumollard R, Rossbach A, Lai FA, Swann K. Redistribution of mitochondria leads to bursts of ATP production during spontaneous mouse oocyte maturation. J Cell Physiol 2010; 224:672-80; PMID:20578238; http://dx.doi.org/10.1002/jcp.22171

[48] Yi K, Rubinstein B, Unruh JR, Guo F, Slaughter BD, Li R. Sequential actin-based pushing forces drive meiosis I chromosome migration

and symmetry breaking in oocytes. J Cell Biol 2013; 200:567-76; PMID:23439682; http://dx.doi.org/10.1083/jcb.201211068

[49] FitzHarris G, Marangos P, Carroll J. Changes in endoplasmic reticulum structure during mouse oocyte maturation are controlled by the cytoskeleton and cytoplasmic dynein. Dev Biol 2007; 305:133-44; PMID:17368610; http://dx.doi.org/10.1016/j.ydbio.2007.02.006

[50] Dalton CM, Carroll J. Biased inheritance of mitochondria during asymmetric cell division in the mouse oocyte. J Cell Sci 2013; 126:2955-64; PMID:23659999; http://dx.doi.org/10.1242/jcs.128744

[51] Schlaitz A-L, Thompson J, Wong CCL, Yates JR, Heald R. REEP3/4 ensure endoplasmic reticulum clearance from metaphase chromatin and proper nuclear envelope architecture. Dev Cell 2013; 26:315-23; PMID:23911198; http://dx.doi.org/10.1016/j.devcel.2013.06.016

[52] Bomar J, Moreira P, Balise JJ, Collas P. Differential regulation of maternal and paternal chromosome condensation in mitotic zygotes. J Cell Sci 2002; 115:2931-40; PMID:12082153

[53] Blower MD, Feric E, Weis K, Heald R. Genome-wide analysis demonstrates conserved localization of messenger RNAs to mitotic microtubules. J Cell Biol 2007; 179:1365-73; PMID:18166649; http://dx.doi.org/10.1083/jcb.200705163

[54] Eliscovich C, Peset I, Vernos I, Méndez R. Spindle-localized CPE-mediated translation controls meiotic chromosome segregation. Nat Cell Biol 2008; 10:858-65; PMID:18536713; http://dx.doi.org/10.1038/ncb1746

[55] Mili S, Macara IG. RNA localization and polarity: from A(PC) to Z (BP). Trends Cell Biol 2009; 19:156-64; PMID:19251418; http://dx.doi.org/10.1016/j.tcb.2009.02.001

[56] Lécuyer E, Yoshida H, Parthasarathy N, Alm C, Babak T, Cerovina T, Hughes TR, Tomancak P, Krause HM. Global Analysis of mRNA localization reveals a prominent role in organizing cellular architecture and function. Cell 2007; 131:174-87; PMID:17923096; http://dx.doi.org/10.1016/j.cell.2007.08.003

[57] Bolton EM, Tuzova AV, Walsh AL, Lynch T, Perry AS. Noncoding RNAs in prostate cancer: the long and the short of it. Clin Cancer Res Off J Am Assoc Cancer Res 2014; 20:35-43; http://dx.doi.org/10.1158/1078-0432.CCR-13-1989

[58] Fujita Y, Hayashi T, Kiyomitsu T, Toyoda Y, Kokubu A, Obuse C, Yanagida M. Priming of centromere for CENP-A recruitment by human hMis18alpha, hMis18beta, and M18BP1. Dev Cell 2007; 12:17-30; PMID:17199038; http://dx.doi.org/10.1016/j.devcel.2006.11.002

[59] Jambor H, Surendranath V, Kalinka AT, Mejstrik P, Saalfeld S, Tomancak P. Systematic imaging reveals features and changing localization of mRNAs in Drosophila development. Elife 2015; 4:e05003; PMID:25838129; http://dx.doi.org/10.1080/15384101.2017.1295178

[60] Susor A, Jansova D, Anger M, Kubelka M. Translation in the mammalian oocyte in space and time. Cell Tissue Res 2016; 363:69-84; PMID:26340983; http://dx.doi.org/10.1007/s00441-015-2269-6

[61] Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell 2007; 12:9-22; PMID:17613433; http://dx.doi.org/10.1016/j.ccr.2007.05.008

[62] Gwinn DM, Asara JM, Shaw RJ. Raptor is phosphorylated by cdc2 during mitosis. PloS One 2010; 5:e9197; PMID:20169205; http://dx.doi.org/10.1371/journal.pone.0009197

[63] Papst PJ, Sugiyama H, Nagasawa M, Lucas JJ, Maller JL, Terada N. Cdc2-Cyclin B Phosphorylates p70 S6 Kinase on Ser411at Mitosis. J Biol Chem 1998; 273:15077-84; PMID:9614117; http://dx.doi.org/10.1074/jbc.273.24.15077

[64] Bellé R, Minella O, Cormier P, Morales J, Poulhe R, Mulner-Lorillon O. Phosphorylation of elongation factor-1 (EF-1) by cdc2 kinase [Internet]. In: Meijer L, Guidet S, Tung HYL, editors. Progress in Cell Cycle Research. Boston, MA: Springer US; 1995 [cited 2016 Aug 2]. 265-70. Available from: http://link.springer.com/10.1007/978-1-4615-1809-9_21

[65] Schultz RM, LaMarca MJ, Wassarman PM. Absolute rates of protein synthesis during meiotic maturation of mammalian oocytes in vitro. Proc Natl Acad Sci 1978; 75:4160-4; PMID:279905; http://dx.doi.org/10.1073/pnas.75.9.4160

[66] King DW, Barnhisel ML. Synthesis of RNA in mammalian cells during mitosis and interphase. J Cell Biol 1967; 33:265-72; PMID:6039370; http://dx.doi.org/10.1083/jcb.33.2.265

[67] Pyronnet S, Pradayrol L, Sonenberg N. A cell cycle-dependent internal ribosome entry site. Mol Cell 2000; 5:607-16; PMID:10882097; http://dx.doi.org/10.1016/S1097-2765(00)80240-3

[68] Vander Haar E, Lee S-I, Bandhakavi S, Griffin TJ, Kim D-H. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol 2007; 9:316-23; PMID:17277771; http://dx.doi.org/10.1038/ncb1547

[69] Wang Q, Ratchford AM, Chi MM-Y, Schoeller E, Frolova A, Schedl T, Moley KH. Maternal diabetes causes mitochondrial dysfunction and meiotic defects in murine oocytes. Mol Endocrinol 2009; 23:1603-12; PMID:19574447; http://dx.doi.org/10.1210/me.2009-0033

[70] Chen J, Chen S, Chen Y, Zhang C, Wang J, Zhang W, Liu G, Zhao B, Chen Y. Circulating endothelial progenitor cells and cellular membrane microparticles in db/db diabetic mouse: possible implications in cerebral ischemic damage. Am J Physiol Endocrinol Metab 2011; 301:E62-71; PMID:21505143; http://dx.doi.org/10.1152/ajpendo.00026.2011

[71] Bonatti S, Simili M, Galli A, Bagnato P, Pigullo S, Schiestl RH, Abbondandolo A. Inhibition of the Mr 70,000 S6 kinase pathway by rapamycin results in chromosome malsegregation in yeast and mammalian cells. Chromosoma 1998; 107:498-506; PMID:9914383; http://dx.doi.org/10.1007/s004120050335

[72] Jang C-Y, Kim HD, Zhang X, Chang J-S, Kim J. Ribosomal protein S3 localizes on the mitotic spindle and functions as a microtubule associated protein in mitosis. Biochem Biophys Res Commun 2012; 429:57-62; PMID:23131551; http://dx.doi.org/10.1016/j.bbrc.2012.10.093

[73] Susor A, Kubelka M. Translational regulation in the mammalian oocyte. In: Oocytes - Maternal information and functions. Results and problems in cell differentiation. Boston, MA: Springer US; 2017.

[74] Tetkova A, Hancova M. Mouse Oocyte Isolation, Cultivation and RNA Microinjection —BIO-PROTOCOL; 6:e1729; https://dx.doi.org/10.21769/BioProtoc.1729

[75] Safran M, Kim WY, O'Connell F, Flippin L, Günzler V, Horner JW, Depinho RA, Kaelin WG. Mouse model for noninvasive imaging of HIF prolyl hydroxylase activity: assessment of an oral agent that stimulates erythropoietin production. Proc Natl Acad Sci U S A 2006; 103:105-10; PMID:16373502; http://dx.doi.org/10.1073/pnas.0509459103