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Eukaryotic translation initiation factor 3 and its role in plant translation regulation

Eukaryotický translační iniciační faktor 3 a jeho role v rostlinné regulaci translace

Bachelor´s thesis

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Declaration

I hereby declare that I have compiled this thesis independently, using the listed literature and resources only. Content of the thesis or any part of it has not been used to gain any other academic title.

Prohlášení

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

Prague, 7th May 2018

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Abstract

After transcription, mRNA translation is another highly regulated process in gene expression. In plants, translation regulation plays an important role during progamic phase, fertilization and seed development, where synthesized transcripts are stored and selectively translated later in development. Translation regulation is also broadly used in stress responses as a fast and flexible tool to change gene expression; therefore, it plays an essential role in the survival strategy of sessile organisms like plants. Both regulation of the global translational rate as well as selective regulation of specific transcripts modulate the final gene expression response. Most of the regulatory mechanisms are concentrated in the stage of initiation, which is facilitated by several translation initiation factors. Eukaryotic translation initiation factor 3 (eIF3) is the largest and most complex of these factors, consisting of 12 conserved subunits. Its key function in the initiation is to scaffold the formation of the translation initiation complex and in the scanning mechanism accuracy. In past decades, additional eIF3 functions were discovered acting upon the whole translation cycle, including its importance in global and specific translation regulation. The aim of this work is to review eIF3 functions and to discuss current evidence for eIF3-mediated translation regulation in flowering plants.

Key words

gene expression, regulation of gene expression, translation, translation regulation, plants

Abstrakt

Translace mRNA je po transkripci další vysoce regulovanou etapou genové exprese. U rostlin nabývá regulace translace značného významu během progamické fáze, oplození a vývoje semen, kdy jsou s různou mírou selektivity translatovány již skladované molekuly mRNA. Dále se regulace translace uplatňuje díky své rychlosti a flexibilitě i v reakci na stresové situace, což má velký význam pro schopnost přežití rostlin jakožto přisedlých organismů. Přesná modulace genové exprese je výsledkem globální translační regulace stejně jako specifické regulace určitých transkriptů. Většina těchto regulačních mechanismů je soustředěna do iniciační fáze translace, které se účastní celá řada pomocných translačních iniciačních faktorů. Eukaryotický translační iniciační faktor 3 (eIF3) je největším a nejsložitějším translačním iniciačním faktorem, skládajícím se z 12 konzervovaných podjednotek. Jeho role strukturního lešení v iniciační fázi translace je klíčová pro vytvoření translačního iniciačního komplexu a pro přesnost skenovacího mechanismu. V posledních letech byly objeveny další funkce, které rozšířily působnost eIF3 do celého translačního cyklu, a to včetně jeho významu v globální i specifické translační regulaci. Cílem předkládané bakalářské práce je popsat funkce eIF3 a diskutovat poznatky o jeho roli v translační regulaci krytosemenných rostlin.

Klíčová slova

genová exprese, regulace genové exprese, translace, regulace translace, rostliny

List of frequently used abbreviations

ABA	-	abscisic acid
ATP	-	adenosine triphosphate
CTD	-	C-terminal domain
CaMV	-	Cauliflower mosaic virus
eEF	-	eukaryotic translation elongation factor
eIF	-	eukaryotic translation initiation factor
EPP	-	EDTA/puromycin-resistant particle
eRF	-	eukaryotic translation release factor
GTP	-	guanosine triphosphate
IRES	-	internal ribosome entry site
MFC	-	multi-factor complex
MPN	-	Mpr-Pad1-N-terminal domain
MUMP-		microbe associated molecular patterns
NMD	-	nonsense mediated decay
NTD	-	N-terminal domain
ORF	-	open reading frame
PABP	-	poly adenylate binding protein
PCI	-	Proteasome, COP9 signalosome, eIF3 domain
PIC	-	pre-initiation complex
PRR	-	pattern recognition receptor
PTC	-	peptidyl transferase center
S	-	Svedberg unit
SAM	-	shoot apical meristem
TAV	-	transactivator viroplasmin protein
TC	-	ternary complex
UTR	-	untranslated region
YLC	-	yeast-like core

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1 Introduction

Translation is an essential step for gene expression. The process of translation requires not only large amount of cell energy, different RNA molecules, ribosomal proteins but also precise regulation. In plants, expression of some key developmental regulators is specifically controlled at the translational level. Moreover, stress-related transcripts are selectively translated under stress conditions, when translation is globally downregulated. Specific translational regulation comes to importance also during pollen maturation and pollen tube growth, where the complex process of mRNA storage and subsequent selective translation needs precise control. Most of the translational regulation is centered in the translation initiation phase, where several translation initiation factors ensure mRNA association with the ribosome, precise start codon recognition and correct ribosome assembly.

Eukaryotic translation initiation factor 3 (eIF3) is the largest initiation factor, conserved across eukaryotic kingdom and is consisting of 12 subunits in plants. eIF3 forms two structural modules that are joined together; PCI/MPN octamer and yeast-like core (YLC). In initiation process, the octamer module plays a role of scaffold structure that interacts with multiple components of translational machinery, promotes assembly of pre-initiation complex and joins together mRNA and small ribosomal subunit, while the YLC module participates in scanning and stringency of start codon recognition. Recent studies also uncovered many additional species-specific roles for eIF3 in other translational processes and for individual eIF3 subunits in global and specific translation regulation.

Plant eIF3 composition is similar to the mammalian eIF3 complex, and some plant eIF3 subunits were analyzed during the last 20 years using mainly *Arabidopsis thaliana* as a model organism. Plant eIF3 research focused mainly on the subunit eIF3h and described its ability in promoting the translation of specific transcripts that contain inhibitory upstream open reading frames (uORFs) in their 5´ leader. Several other studies pointed to the importance of other eIF3 subunits in various plant developmental stages as well as in abiotic and biotic stress response. However, the knowledge about the exact regulation mechanisms pertained by eIF3 is still mostly unknown.

The aim of this work was to review the current evidence for the translation regulation mediated by individual eIF3 subunits in flowering plants and to suggest future directions in plant eIF3 research. Because of the translation context complexity, preceding the description of eIF3 structure and functions, the process of translation initiation is presented for better understanding and orientation in the topic.

2 Eukaryotic translation

Translation is the last step of the central dogma of molecular biology, where the nucleotide sequence is translated into amino acids by the rules of the genetic code. Translation consumes the majority of the cell energy and is a conserved, multistep and multifactorial process. Excluding translation in organelles, this chapter presents the general process of eukaryotic translation for better orientation in the plant translation regulation.

2.1 Components of the translational machinery

The complex process of translation requires many proteins with structural, enzymatic and regulatory function. Also, three types of RNA play key informational, structural and functional roles in protein synthesis: ribosomal (rRNA), transfer (tRNA) and messenger (mRNA).

rRNAs and ribosomal proteins form ribosomes, massive ribonucleoprotein complexes with catalytic activity ensuring protein synthesis. Across species, the catalytic function is performed by rRNA in the conserved peptidyl transferase center (PTC), while ribosomal proteins have rRNA stabilizing and regulative function (Graifer and Karpova, 2015). Within the eukaryotic cell, ribosomes are localized freely in cytoplasm or associated with endoplasmic reticulum. Eukaryotic cells also contain subpopulations of prokaryotic-like ribosomes in semi-autonomous organelles (mitochondria, plastids). Cytoplasmic 80S ribosome consists of four rRNA chains; 28S, 18S, 5.8S, and 5S, and 70-80 ribosomal proteins, all divided into two subunits: large 60S and small 40S. In the assembled 80S ribosome (Figure 1) there are three channels; polypeptide exit channel, mRNA entry and mRNA exit channel. For the tRNA, three sites are identified; aminoacyl-tRNA binding site (A site), peptidyl-tRNA binding site (P site) and exit site (E site).

tRNA is a short non-coding RNA with a specific cloverleaf-like secondary structure, L-shaped tertiary structure and many base modifications. It plays a major role in deciphering the genetic code during the translation, as it holds the three-nucleotide anticodon on one of its loops and can covalently bind the proper amino acid on the acceptor stem. The binding of amino acid to its specific tRNA (tRNA charging) is performed with high efficiency by aminoacyl-tRNA synthetases.

mRNA carries the sequence information from the nucleus to cytoplasm, where the protein synthesis apparatus is located. From the start of transcription, the elongating nascent transcript undergoes mRNA processing; a series of co- and posttranscriptional modifications, that add 7-methylguanosine cap (5′cap) on its 5′ end, polyadenylate tail (polyA) on 3′end and remove introns (Reviewed by Moore and Proudfoot, 2009). Moreover, transcripts are never fully "naked", but associate with proteins to form the ribonucleoprotein particles (mRNPs). The

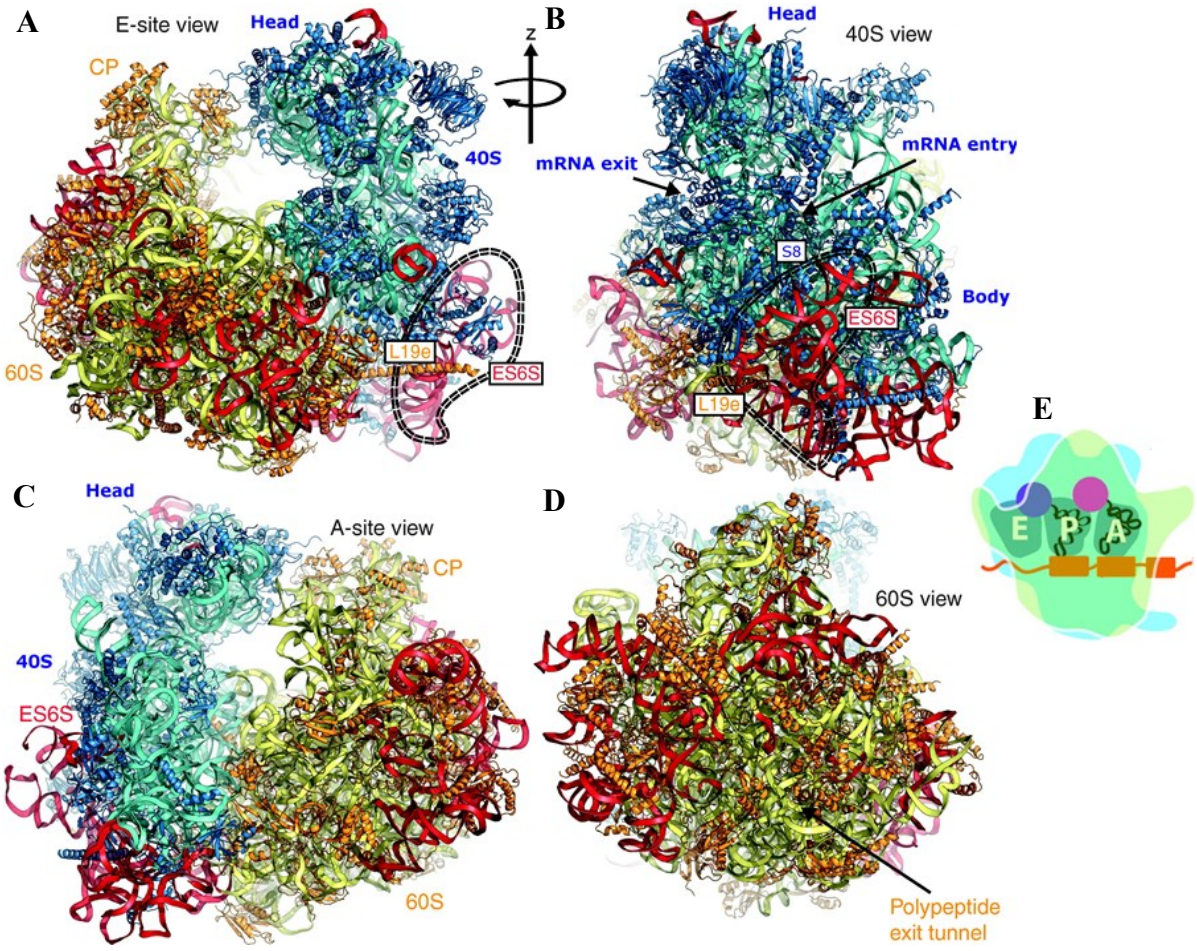

Figure 1: Crystal structure of the S. cerevisiae 80S ribosome. Views from the E site **(A)**, small subunit side **(B)**, A site **(C)** and large subunit side **(D)**. Polypeptide exit tunnel, mRNA entry and exit tunnels are indicated. The large 60S subunit is shown in yellow with orange proteins and the small 40S subunit in cyan with blue proteins. Adapted from (Jenner et al., 2012). Schematic model of three sites for tRNA **(E)**, here shown with mRNA and two aminoacyl-tRNA molecules in P and A site. Modified from (Browning and Bailey-Serres, 2015)

composition of mRNPs is highly dynamic and changes several times during the mRNA life, as it directs the mRNA molecule throughout the RNA processing, nuclear export, translation viability, mRNA storage or the rate of degradation (Singh et al., 2015). For translation, mRNA is activated by several initiation factors and multiple copies of the cytoplasmic Poly(A)-binding proteins (PABPs).

During all of the steps of translation, ribosomes need the support of many other proteins, known as translation factors (Table 1). Initiation factors control the activation of mRNA, its assembly with small ribosomal subunit and initiator tRNA, scanning of mRNA and start codon recognition. Then they are released from the initiation complex to allow for the joining of the 60S to form an elongation-competent 80S ribosome. Elongation factors supply charged tRNAs to the A-site and mediate mRNA translocation within the ribosome. Their functions are fueled by GTP hydrolysis. Release factors are responsible for the proper termination of translation by recognizing the stop codon and promote the separation of ribosomal subunits.

Table 1: Overview of eukaryotic translation factors and their function in canonical translation. Simplified from the list of *Arabidopsis thaliana* translation factors in (Browning and Bailey-Serres, 2015).

Protein		Function
	Initiation factors (eIFs)	
eIF1		PIC formation, scanning, AUG selection, controls eIF5 activity, promotes 40S open conformation
eIF1A		PIC formation, scanning, AUG selection, promotes 40S open conformation
eIF2	subunits α, β, γ	Small GTPase, forms ternary complex with GTP and Met-tRNA$_i^{Met}$
eIF2B		GDP-GTP recycling factor for eIF2
eIF3	12 subunits	Formation of PIC, scanning and AUG recognition, mRNA joining
eIF3j		eIF3 associated factor, promotes eIF3 binding to 40S
eIF4A		ATP-dependent helicase, unwinds secondary structure of mRNA, binds mRNA to 40S
eIF4B		Cofactor of eIF4A
eIF4F	**eIF4E** **eIF4G**	5´cap binding protein Scaffold protein
eIF5		GTPase activating protein for eIF2, Met-tRNA$_i^{Met}$ placement on AUG
eIF5B		Joining of 60S subunit, GTPase
PABP		Binds poly(-A) tail, interacts with eIF4G
	Elongation factors (eEFs)	
eEF1A		small GTPase, binds aminoacyl tRNA and GTP
eEF1B	subunits α, β, γ	Recycling factor of eEF1A
eEF2		tRNA and mRNA translocation
	Release factors (eRFs)	
eRF1		Termination/peptide release
eRF3		Termination/peptide release
ABCE1		Ribosome recycling

2.2 Variability of translational machinery enables specific regulation

2.2.1 Ribosomal proteins build populations of heterogenous ribosomes

In ribosomes, rRNA molecules are enveloped by ribosomal proteins. In plants, there are more than 200 genes for 80 gene families of ribosomal proteins, each family being encoded by two to five paralogs in Arabidopsis (Browning and Bailey-Serres, 2015). In all eukaryotes, most of the ribosomal proteins are found in some ribosomal complex, therefore one cell contains many populations of heterogenous ribosomes (Simsek et al., 2017). Moreover, distinct ribosomes were found to preferentially translate specific subpopulations of mRNA (Shi et al., 2017), increasing their role in selective translation. Plants are no exception from this phenomenon, and show as well some plant specifics in ribosomal biogenesis (Weis et al., 2015).

2.2.2 Sequence and structure determine translation of individual mRNAs

Some mRNAs contain special features that influence their lifespan or translation efficiency (Figure 2). These additional features are mostly found in 5´untranslated region (5´UTR), less commonly in 3´untranslated region (3´UTR) or in main ORF (mORF). Recognition of transcript specificity is dependent mostly on sequence and ability to form secondary structures. Upstream

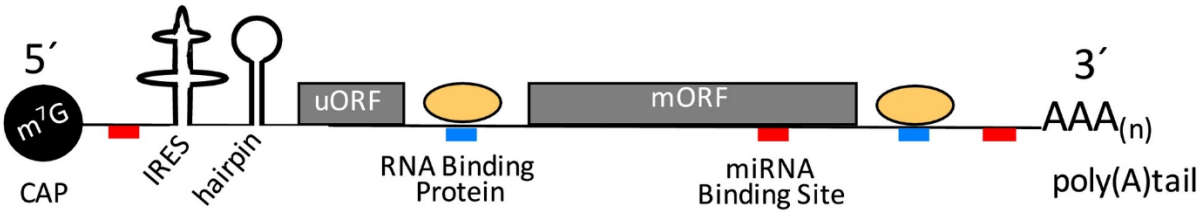

Figure 2: Structural features of mRNA that influence translation. The 5′ Cap and the poly(A) tail enhance translation. Internal ribosomal entry sites (IRESs) promote cap-independent translation; hairpins and upstream open reading frames (uORFs) generally reduce translation of the main ORF (mORF). Light orange ovals represent RNA-binding proteins that recognize specific sequences in the transcript, marked as blue bands, and can either inhibit or enhance translation. Red bands represent small RNA binding sites and enhance mRNA degradation and specific gene silencing. Adapted and modified from (Merchante et al., 2017).

open reading frames (uORFs) have inhibitory effect on translation of the major ORF. Another well-known regulatory mechanism used is sequence-specific translation repression mediated by small RNAs (Reviewed by Islam et al., 2018 and Liu et al., 2018). Hairpin secondary structures in mRNA are found to be a barrier in scanning process and can be surpassed by additional helicases or rarely by ribosome shunting. More complicated structures mediate IRES-like initiation or might be recognizable by classical or additional RNA binding proteins (Merchante et al., 2017).

2.2.3 Plant-specific initiation factors operate on different mRNA pools

Similarly to ribosomal proteins, plants frequently encode a translation factor by more than one gene. Moreover, two structurally distinct eIF4F complexes are found in plants to provide mRNA activation; canonical eukaryotic eIF4F and plant specific eIF4isoF (Figure 3). eIFisoF is comprised of a plant specific cap binding protein eIF4isoE and a scaffold protein eIF4isoG. However, mixed complexes of eukaryotic and plant specific factors could be formed, too (Mayberry et al., 2011). Recent studies suggest that both complexes are differentially expressed and operate on different mRNA pools (Mayberry et al., 2009; Martínez-Silva et al., 2012; Gallie, 2016). The plant eIF4B is non-conserved and may have alternative functions (Browning and Bailey-Serres, 2015). Plants also possess large variety of PABPs (Belostotsky, 2003).

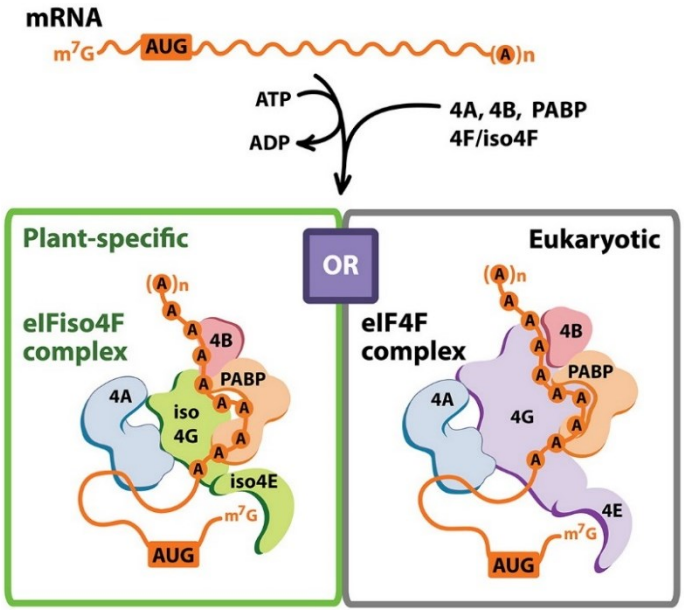

Figure 3: Plant options of mRNA activation. After export from the nucleus, mRNA is activated by cap-binding complex eIF4F or plant-specific eIFiso4F at the 5′ end, PABP at the 3′ end and additional factors eIF4A and eIF4B before loading to 43S PIC. Adapted from (Browning and Bailey-Serres, 2015)

2.3 The translation cycle

2.3.1 Initiation phase

Initiation is the opening step of translation (Figure 4), accomplished by the scanning mechanism in eukaryotes (Reviewed by Hinnebusch, 2014; 2017). Initiation starts with formation of the Ternary Complex (TC) from eIF2-GTP and initiator Met-tRNA$_i^{Met}$. The TC is then bound to free 40S ribosomal subunit along with initiation factors eIF1, eIF1A, eIF3 and eIF5 to form the 43S pre-initiation complex (43S PCI). In this assembly, eIF3 plays a role of a scaffold that joins other proteins together. In addition, eIF1, eIF2, eIF3 and eIF5 could pre-assemble, independently on 40S, forming the so called multi-factor complex (MFC). Free cytoplasmic MFC was observed in yeast (Asano et al., 2000), plants (Dennis et al., 2009), and in mammals (Sokabe et al., 2012). In the 43S PIC, Met-tRNA$_i^{Met}$ is placed almost precisely in the future P-site of the ribosome with anticodon CAU complementary to start codon AUG.

Activated mRNA joins 43S PIC along with associated proteins; eIF4E, eIF4A, eIF4B, PABP and eIF4G. 43S PIC and mRNA are joined together via the eIF3-eIF4G bond to form 48S pre-initiation complex (48S PIC) in the open conformation (Villa et al., 2013). The open 48S PIC starts scanning the 5´UTR until the AUG codon is reached in Kozak consensus

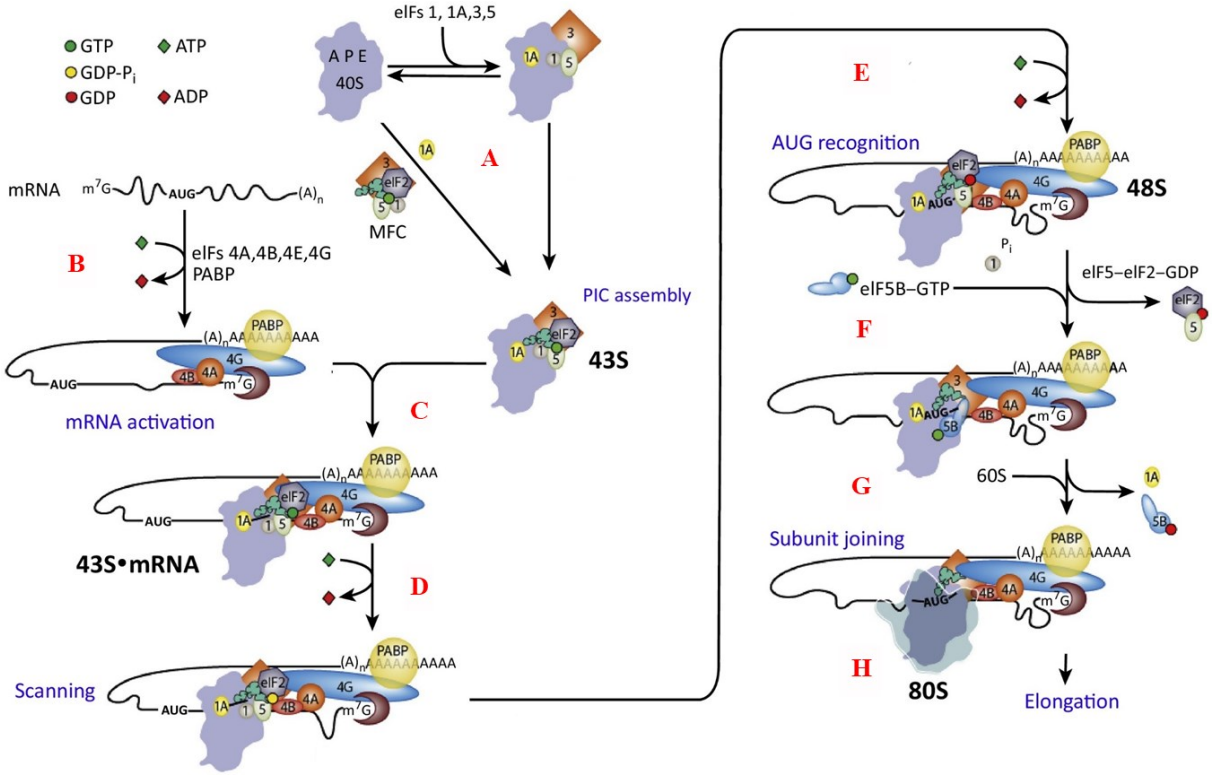

Figure 4: Model of the Scanning Mechanism of Eukaryotic Translation Initiation. Here shown as a series of steps. Starting with 43S PIC assembly **(A)** and mRNA activation **(B)**, followed by mRNA acquisition to form 43S-mRNA PIC **(C)**. GTP hydrolysis on eIF2 starts subsequent scanning of the mRNA **(D)**, accompanied with ATP dependent unwinding of mRNA by eIF4A **(E)**. AUG recognition form the closed 48S that triggers the release of eIF1, Pi, and eIF5-eIF2-GDP to, while eIF5B-GTP binds to the complex **(F)** and promotes joining of the 60S subunit to the PIC, with the release of residual initiation factors **(G)**. Formed 80S IC is then ready for the first round of elongation **(H)**. Adapted and modified from (Hinnebusch, 2017)

sequence, a favorable context of surrounding nucleotides (Kozak, 1987). Scanning is first driven by hydrolysis of GTP on eIF2, a process promoted by eIF5, where the phosphate remains bound to the eIF2 through the scanning process (Algire et al., 2005). Then, eIF4A unwinds the 5´UTR using the ATP hydrolysis as an energy source. Start codon in Kozak sequence consensus is recognized by the codon-anticodon pairing with Met-tRNA$_i^{Met}$ with the support of eIFs. The following release of eIF1 opens the way for the phosphate to dissociate from eIF2 (Algire et al., 2005). The phosphate release is followed by the dissociation of eIF1A, eIF2-GDP, eIF3 and eIF5. PIC is then in the closed conformation, ready to bind with the 60S large ribosomal subunit, with Met-tRNA$_i^{Met}$ anticodon interacting with start codon in the future P-site. The 60S subunit is joined with the help of eIF5B, in a process fueled by GTP hydrolysis, resulting in the 80S initiation complex (80S IC), ready for the first round of elongation.

2.3.2 Elongation, termination and ribosome recycling

In one elongation cycle, eEF1A-GTP delivers charged tRNA to the A-site, accompanied by GTP hydrolysis. After the ribosome catalyzes new peptide bonding in the PTC, eEF2 promotes tRNA translocation within the ribosome sites and allows mRNA to move by one codon, a process powered by another GTP hydrolysis. The GDP in the eEF1A is exchanged for GTP by eEF1B, while eEF2 requires no recycling factor for recharging. As the ribosome completes the elongation, one tRNA molecule binds stepwise to the codon in the A-site, moves to the P-site, holding the growing peptidyl chain and leaves through the E-site after another elongation round.

The elongation cycle is repeated, until a stop codon (UAA, UGA, UAG) is reached. In most organisms, there is no tRNA with proper anticodon. Instead, eRF1 recognizes all three stop codons in the A-site and binds eRF3. eRF3-mediated GTP hydrolysis releases the polypeptide from the ribosome by the hydrolysis of tRNA-eRF3 bond within P-site (Brown et al., 2015).

Ribosome recycling starts after the polypeptide chain has been released and 80S ribosome is still bound to mRNA. In eukaryotes, there is no homolog of prokaryotic ribosome recycling factor (RRF). Instead, eRF1 remains bound to the 80S after termination and may have a role in ribosome recycling (Pisarev et al., 2007). The dissociation of the 80S ribosome to 40S and 60S subunits is in eukaryotes promoted by highly conserved ATP-binding cassete E (ABCE1) (Pisarev et al., 2010). Elongation, termination and ribosome recycling are discussed in detail in Dever and Green, 2012.

2.3.3 Non-canonical translation events

There are several exceptions, where general rules of translation cycle are challenged. To be treated independently of global translation and its regulation, viruses developed several mechanisms to do so, and as was mentioned in previous chapter, some cell transcripts also

possess specific features enabling them to fine-tune their own translation. In *Leaky scanning*, the start codon is surpassed during the mRNA scanning, when AUG is in weak surrounding sequence (Kozak, 2002). To the contrary, during *non-AUG initiation* ribosomes initiate translation on codon other than AUG (Hsu et al., 2016). Viral internal ribosome entry sites (IRES) are complex structures in their 5´UTR that bind 40S, initiation factors, and place viral RNA in ribosome with AUG directly in P-site. Some cell transcripts also possess IRES-like structures to promote *Cap-independent initiation* (Yamamoto et al., 2017). Other mRNA secondary structures, inhibitory hairpins in 5´UTR, can be bypassed by the scanning complex by the so-called *ribosome shunting*, a mechanism performed by some viruses as well (Pooggin and Ryabova, 2018). *Reinitiation* occurs on post-termination ribosomes, where mRNA remains bound to 40S and fresh TC and eIFs are loaded to form a new 43S PIC that is able to scan and reinitiate (Skabkin et al., 2013). During *Stop codon readthrough*, successful recognition of stop codon is decreased and ribosomes continue in elongation cycles (Schueren and Thoms, 2016).

3 Eukaryotic initiation factor 3

In the previous chapter, eukaryotic translation initiation factor 3 (eIF3) was shown as a part of the initiation machinery. This chapter provides a more detailed description of structural and functional features of this protein complex. In most studies, yeast and mammalian eIF3 was examined, but much could be applied on other organisms, because eIF3 is conserved in all eukaryotes, comprised of as much as 12 subunits. First was isolated from rabbit reticulocytes in the 1970´s (Benne and Hershey, 1976; reviewed by Cate, 2017) and from then, several eIF3 functions were discovered, with some of them not even related to the translation initiation.

3.1 Composition differences in organisms

eIF3 is likely to be present in every eukaryotic cell. Individual subunits are conserved in their structure, protein domains and at least the basal functions (Table 2). In the beginning of the 21st century, a unified letter nomenclature for eIF3 subunits was proposed instead of molecular weight (Browning et al., 2001; Burks et al., 2001). The most diverged eIF3 complex that has been discovered and intensively studied is in *Saccharomyces cerevisiae*, consisting of just 5 subunits (-3a, -3b, -3c, -3i, and -3g) with molecular mass of ~360 kDa (Asano et al., 1998; Phan et al., 1998; Khoshnevis et al., 2012), suggesting for the essential subunits needed for eIF3 functions in translation initiation. eIF3j is also found in *S.c.* and is necessary for eIF3 binding to 40S, but is only loosely attached to eIF3 (Valášek et al., 2001; Elantak et al., 2010) and not present in purified yeast eIF3 complex, considered as an eIF3 associated factor (Asano et al., 1998; Khoshnevis et al., 2012). In mammals, homologs of all 5 yeast eIF3 subunits are found

Table 2: Table of eIF3 subunit composition in different organisms. Not including eIF3 associated protein eIF3j. Modified from (Valášek et al., 2017), with respect to (Li et al., 2016) and (Li et al., 2017).

Subunit	Domains	Organism					
		S. cerevisiae	*S. pombe*	*T. brucei*	*H. sapiens*	*A. thaliana*	*O. sativa*
eIF3a	PCI, Spectrin	**x**	**x**	**x**	**x**	**x**	**x**
eIF3b	WD40, RRM	**x**	**x**	**x**	**x**	**x**	**x**
eIF3c	PCI	**x**	**x**	**x**	**x**	**x**	**x**
eIF3d	5´cap binding?		**x**	**x**	**x**	**x**	**x**
eIF3e	PCI		**x**	**x**	**x**	**x**	**x**
eIF3f	MPN		**x**	**x**	**x**	**x**	**x**
eIF3g	RRM, Zn finger	**x**	**x**	**x**	**x**	**x**	**x**
eIF3h	MPN		**x**	**x**	**x**	**x**	**x**
eIF3i	WD40	**x**	**x**	**x**	**x**	**x**	**x**
eIF3k	PCI			**x**	**x**	**x**	**x**
eIF3l	PCI			**x**	**x**	**x**	**x**
eIF3m	PCI		**x**		**x**	**x**	**x**

in the purified complex, together with 7 additional subunits -3d, -3e, -3f, -3h, -3k, -3l and -3m (Zhou et al., 2008; Smith et al., 2013). In mammals and *Neurospora crassa*, eIF3-associated protein eIF3j shows similar functions as its *S.c.* ortholog (Fraser et al., 2004; Smith et al., 2013). Plant eIF3 composition is similar to the mammalian model (Burks et al., 2001), suggesting for a high conservation of the 12 subunits and that the yeast eIF3 is likely reduced. In *Excavata,* eIF3 composition is also similar to the mammalian model, although subunits differ in sequence, keeping conserved only their domains, and for some species from this group, there is a lack of evidence for some subunits (Rezende et al., 2014; Han et al., 2015; Meleppattu et al., 2015). In Bacteria and Archaea, there is no homology for eIF3 subunits, except of the homology with the subunit eIF3i found in Archaea (Benelli and Londei, 2011; Rezende et al., 2014), corresponding with the fact that neither Bacteria nor Archaea initiate translation by the scanning mechanism.

3.2 Structure and assembly

Size, complexity and structural flexibility are barriers in solving the complete eIF3 structure. In recent years, major progress pushed the boundaries of knowledge further, as the structure of eIF3 in different contexts of PICs has been solved in yeast (Erzberger et al., 2014; Korostelev, 2014; Aylett et al., 2015) and in mammals (Hashem et al., 2013a; Querol-Audi et al., 2013; des Georges et al., 2015; Simonetti et al., 2016; Eliseev et al., 2018), together with the biochemical data of proposed eIF3 subunit assembly, composition and mutual interactions (Zhou et al., 2005; Masutani et al., 2007; Zhou et al., 2008; Sun et al., 2011; Herrmannová et al., 2012; Khoshnevis et al., 2012; Wagner et al., 2014; 2016).

Mammalian eIF3 can be divided into two modules (Figure 5C), first module being an octamer formed from eIF3a, -3c, -3e, -3f. -3h, -3k, -3l and -3m subunits. Assembly of this octamer is enabled by a scaffold structure of 6 subunits (-3a, -3c, -3e, -3k, -3l and -3m)

containing a PCI (for Proteasome, CSN, eIF3) domain and 2 subunits (-3h and -3f) containing a MPN (for Mpr1-Pad1-N-terminal) domain. This PCI/MPN octamer has a five-lobed structure (Siridechadilok et al., 2005; Querol-Audi et al., 2013), stabilized by two interaction hubs; the PCI domain-containing subunits form a structure known as β-sheet arc, plus every subunit of the octamer joins with one α-helix, forming the helical bundle (des Georges et al., 2015). This structure is shared between otherwise functionally unrelated protein complexes (Figure 5); CSN (COP9 signalosome) and 26S proteasome lid (Pick et al., 2009; Enchev et al., 2010; Ellisdon and Stewart, 2012). The second module consists of eIF3 subunits -3b, -3i and -3g, structurally similar to the yeast orthologs. Both modules are connected via -3a/-3b interaction. In the most reduced *S.c.* complex, -3a PCI domain binds with the second PCI domain of eIF3c to form one subcomplex. The second subcomplex consist of the mutual interaction between eIF3g and eIF3i. Both subcomplexes are bridged by eIF3b, binding both -3g and -3i via its CTD, while its NTD binds with eIF3a. Associated protein eIF3j binds loosely to the spectrin domain of eIF3a and to RNA recognition motif (RRM) of eIF3b (Valášek et al., 2001; 2003).

The assembly of the 12 subunit eIF3 was proposed by Wagner and co-workers in 2016. In this model, the -3a binds -3b, forming the nucleation core, then -3i and -3g join the -3b to form a subcomplex of -3a, -3b, -3g and -3i, containing four out of five budding yeast eIF3 subunits, therefore called yeast-like core (YLC) (Figure 5C), which alone is still able to bind to 40S and recruit activated mRNA *in vivo* (Wagner et al., 2014). Assembly of other PCI/MPN core subunits proceeds, starting with -3c joining -3a to form the "head", followed by -3m, -3f and -3h to form the "left leg". Then, the "right arm" -3e joins, followed by the "right leg" -3l and -3k to complete the octamer. eIF3d is a non-octameric subunit, which is bound to -3e. Although in this model, the proposed human nucleation core is the -3a/-3b dimer and the YLC is considered a key prerequisite for octamer assembly, the whole human octamer alone was reconstituted *in vitro* and subunits -3b, -3d and -3i completed the whole complex when added later on. In this study, the dimer of -3a/-3c was proposed as the main nucleation core (Sun et al., 2011). From these slightly different evidences it might be suggested that both modules can be formed independently, therefore variable eIF3 subcomplexes might be present *in* vivo, still capable of at least the basal functions. Moreover, such subcomplexes were observed in some aforementioned experiments as well as in recent reconstitution of *Neurospora crassa* eIF3 (Smith et al., 2016). In this study, minimal subcomplex containing -3a, -3b, -3c, -3f, -3g, -3i, and -3m subunit was proposed as sufficient for survival (Smith et al., 2016). As suggested on these little differences between the essential subunits needed, the probability of species-specific functions for eIF3 subunits is apparent.

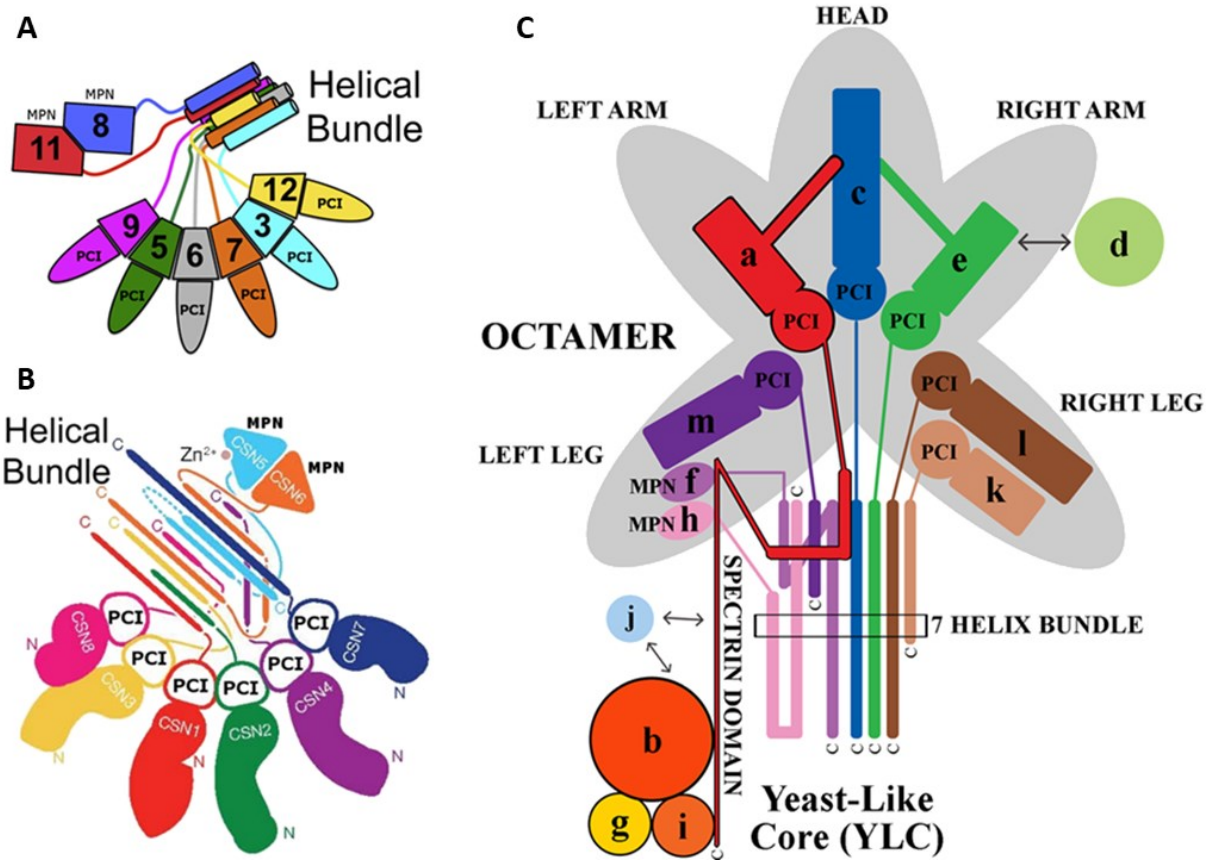

Figure 5: Structure comparison of the 26S proteasome lid, COP9 signalosome and eIF3 protein complexes that share PCI/MPN core and helical bundle interactions. Cartoon model of the yeast 26 S proteasome lid **(A),** modified from (Estrin et al., 2013). Cartoon model of human COP9 signalosome **(B)**, modified from (Lingaraju et al., 2014). Cartoon model of human eIF3 **(C)**, modified from (Wagner et al., 2016).

3.3 eIF3 in translation initiation

eIF3 alone is able to bind to 40S and in the case of some initiation factor missing in PIC, eIF3 prevents the 40S and 60S association (Kolupaeva et al., 2005). After binding to 40S, eIF3 enhances TC, eIF1, eIF1A, eIF5 loading on the 40S (Valášek, 2012). Moreover, eIF3 can bind eIF1, eIF1A, eIF2 and eIF5 without the presence of 40S, forming the multifactor complex (MFC) that loads the Met-tRNA$_i^{Met}$ on the 40S (Sokabe et al., 2012). In the MFC assembly, the eIF3c provides several interactions with other factors (Karásková et al., 2012; Obayashi et al., 2017). In assembled 43S PIC, eIF3 encircles the 40S in a clamp-like fashion. In *S.c.*, eIF3c and -3a bind to the solvent side of 40S and eIF3b, -3g and -3i are placed near mRNA entry channel (Aylett et al., 2015). eIF3g interacts with two entry channel ribosomal proteins, Rps3 and Rps20 (Cuchalová et al., 2010), and associated eIF3j interacts with two other ribosomal proteins, Rps2 and Rps23, in entry channel (Elantak et al., 2010). The position of the 12 subunit mammalian eIF3 on 40S ribosome seems to be analogous, the PCI/MPN octamer is placed on the solvent side of the 40S with eIF3a NTD reaching the mRNA exit channel, and YLC placed on the intersubunit side near the mRNA entry channel (des Georges et al., 2015), interacting

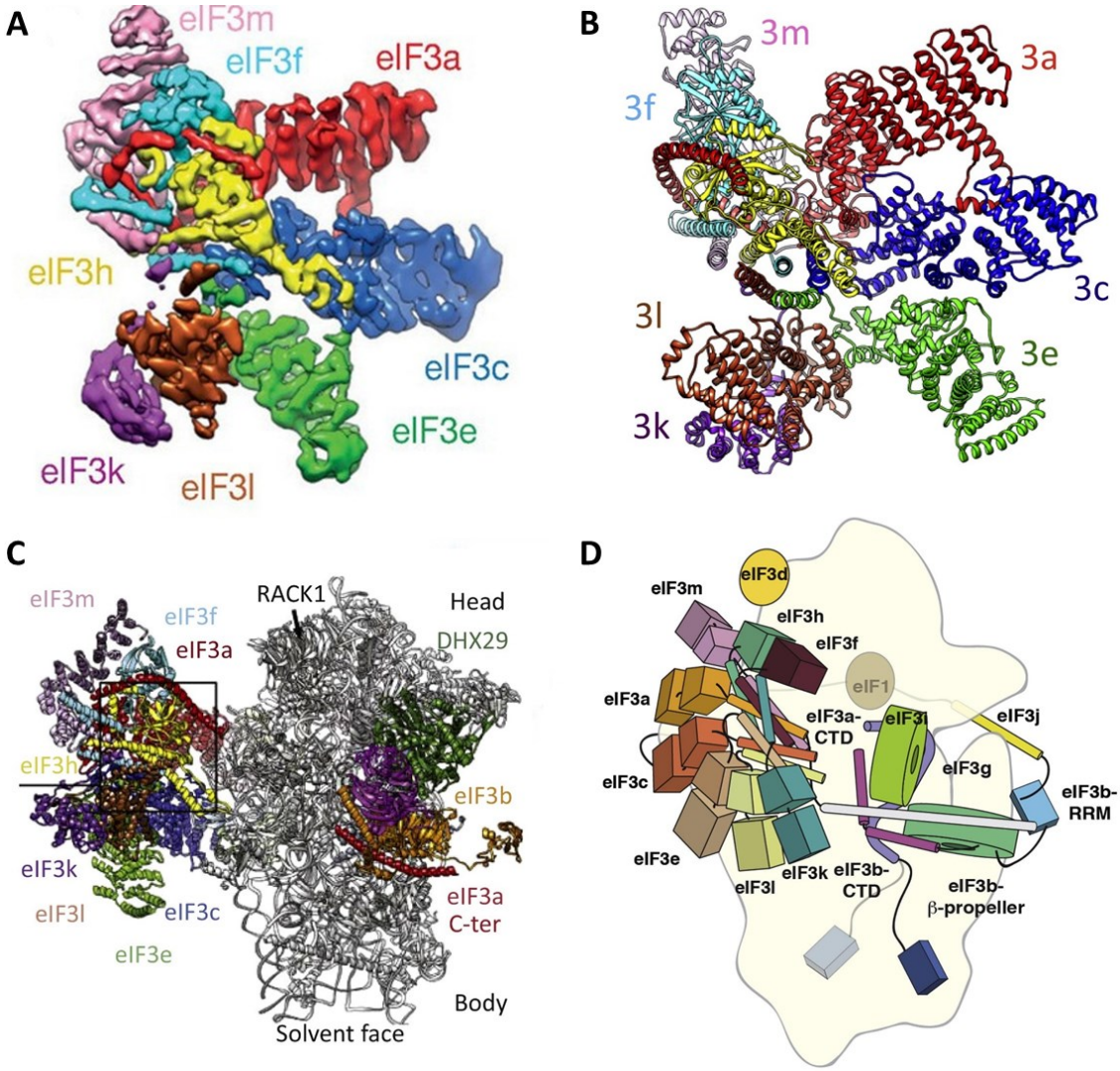

Figure 6: eIF3 structure position in the context of mammalian 43S PIC. Cryo-EM reconstruction of the octamer reconstituted from mammalian eIF3 **(A)**, Adapted from (des Georges et al., 2015) Polyalanine-level model of the eIF3 PCI/MPN octamer m43S PIC, showing the seven-helix bundle formed by subunits -3h, -3c, -3e, -3l, -3f, and -3k **(B)**, adapted from (Hinnebusch, 2017). Location of eIF3 subunits in the mammalian 43S PIC, with the PCI/MPN octamer bound the mRNA exit channel and peripheral domains bound near the mRNA entry channel **(C)**, adapted from (Hinnebusch, 2017), originally from (des Georges et al., 2015). Model of mammalian 40S-eIF1-eIF3 complex. Here, the problematic placement of flexible YLC is modelled accordingly to the yeast eIF3 **(D)**, adapted from (Erzberger et al., 2014).

with the 40S by a 9-bladed β-propeller structure in eIF3b (Liu et al., 2014). After the 43S PIC is formed, eIF3 is necessary for loading the activated mRNA to the 43S PIC (Mitchell et al., 2010). The recruitment of mRNA to PIC is enabled by the direct binding of the eIF4G to a surface made from three eIF3 subunits; -3c, -3d, -3e (Villa et al., 2013). eIF3a interacts directly with mRNA in the exit channel (Pisarev et al., 2008), thus the mRNA stabilization on 40S is enhanced by eIF3 on both mRNA entry and exit channel, presumably by extending the interactions of PIC and mRNA by few nucleotides (Aitken et al., 2016). The processivity of scanning and stringent AUG recognition are also enhanced by eIF3, probably by stabilizing the conformation on the mRNA and factors involved in scanning (Cuchalová et al., 2010; Elantak et al., 2010; Chiu et al., 2010; Karásková et al., 2012; Aitken et al., 2016; Obayashi et al., 2017;

Valášek et al., 2017). Analyzed by two recent structural studies, the YLC module undergoes rearrangement on 40S during mRNA binding, as it relocates from the solvent exposed site to the intersubunit surface and stabilizes the interaction of the scanning complex (Simonetti et al., 2016). Upon AUG recognition, YLC is relocated back to the solvent exposed site, probably via a conformational change of eIF2 (Eliseev et al., 2018).

3.4 eIF3 beyond initiation

Translation reinitiation occurs mostly after short uORFs in the presence of eIF3, as it was shown that eIF3 remains bound to the elongating ribosomes for a short time during first couple of elongation cycles on uORFs and subsequent reinitiation (Mohammad et al., 2017). In *S.c.*, eIF3a promotes reinitiation after recognizing the secondary structure on the uORF of GCN4 transcript (Szamecz et al., 2008; Gunišová et al., 2018), while eIF3h promotes reinitiation in mammals (Hronová et al., 2017) and in plants, in a process which will be described later on.

In yeast, eIF3 participates in stop codon readthrough, as suggested by eIF3 interacting with eRF1 *in vivo*, eIF3 presence in terminating complexes, and decreased rate of stop codon readthrough in systems with mutated eIF3 (Beznosková et al., 2013). The mechanism proposed was that eIF3, by binding to eRF1, interferes with eRF1 ability of decoding the third nucleotide in stop codon, thus enabling a near-cognate tRNA binding to stop codon and the continuation of elongation (Beznosková et al., 2015; Dabrowski et al., 2015). The eIF3j, to the contrary, decreased the rate of stop codon readthrough (Beznosková et al., 2013).

Another role for eIF3 was discovered in Nonsense-Mediated mRNA decay (NMD). NMD is a transcript degradation pathway that occurs when a premature termination codon (PTC) is recognized on mRNA during the first round of elongation (Celik et al., 2017; Gupta and Li, 2018). UPF1, a key NMD factor, binds to eIF3 after mRNA is marked for NMD and probably inhibits the translation initiation on such transcripts (Isken et al., 2008; Flury et al., 2014). Moreover, eIF3e was shown as the eIF3 subunit needed for NMD pathway initiation (Morris et al., 2007), while NMD inhibitory effects were proposed for eIF3g, -3f and –3h, (Choe et al., 2012; Peixeiro et al., 2012; Pereira et al., 2015).

Under stress conditions, eIF4F activity is inhibited and eIF3 was shown to bind the 5´cap itself via eIF3d and -3l (Kumar et al., 2016; Lee et al., 2016). Moreover, eIF3 binds to transcripts encoding cell growth, differentiation or apoptosis proteins (Lee et al., 2015), and mRNAs containing N6-methyladenosine (m^6A) in their 5′ UTR (Meyer et al., 2015). IRES-like containing transcripts also need eIF3 for efficient translation initiation (Sun et al., 2013; Hashem et al., 2013b). DHX29 helicase interacts with the RRM motif of eIF3b and CTD of

eIF3a to cooperate in scanning on the complicated secondary structures in 5´UTRs (Pisareva and Pisarev, 2016). *In vitro*, eIF3 splits the post-terminated 80S ribosomes to individual subunits, suggesting eIF3 can act as a recycling factor. Presence of other initiation factors increased the ability of eIF3-dependent ribosome recycling. It is possible, that recycling factor ABCE1 and eIF3 cooperate in recycling, as both factors directly interact (Pisarev et al., 2007).

4 Plant translation regulation

4.1 Regulation of translation in response to environment changes and stress

Protein synthesis is the most ATP consuming cell process (Buttgereit and Brand, 1995), so logically when cell energy resources decrease, so does translation. Plants are sessile autotrophic organisms, and their energy state depends mostly on photosynthesis. Environmental changes that affect photosynthesis have therefore an impact on translation as well. A decrease in translation is observed typically when there is lack of light during nights, or in the immediate light to dark shift. Global decrease in translation is also observed during abiotic stress responses, such as sucrose starvation, water deficiency, high salt condition, oxygen deprivation, ROS, heat and cold stress or in response to abscidic acid (ABA) (Reviewed by Merchante et al., 2017). During microbial biotic stress, specific recognition of microbe associated molecular patterns (MUMPs) by pattern recognition receptors (PRRs) triggers reprogramming of the translation that is not correlating with the simultaneous changes in the transcription (Xu et al., 2017). In case of viral infections, viruses modulate the translation for their own multiplication by commonly using non-canonical modifications (Echevarría-Zomeño et al., 2013).

Several protein kinases regulate translation through phosphorylation, doing so after perceiving signals of changed cell state and environment change. Many initiation factors and ribosomal proteins are phosphorylated with a different impact on their function (Reviewed by Browning and Bailey-Serres, 2015). One of the classic examples of global downregulation is the phosphorylation of eIF2α. In plants, eIF2α is phosphorylated by GCN2 in response to amino acid starvation, UV exposure or cold treatment. The phosphorylation increases eIF2 affinity for binding its recycling factor eIF2B, thus impairing the formation of TC, leading to inhibition of the initiation (Lageix et al., 2008).

The general downregulation of translation, however, doesn´t affect some transcripts that are able to bypass the repression and are efficiently translated. For example, during nights, polysomal occupancy is low, but transcripts of ribosomal protein and mitochondrial respiratory chain are translated with high efficiency (Missra et al., 2015). Also, facilitating proper abiotic stress response requires translation of specific stress-responding proteins even though global

translation is repressed. Such translational specificity is also seen for some key development regulators and is generally a result of the great variability in translation machinery (briefly described in 2.2), that enables almost individual regulation for some special transcripts.

4.2 mRNA storage during plant reproduction and development

In plant life cycle, two generations are altering, haploid gametophyte and diploid sporophyte. In flowering plants, gametophytic generation is reduced to just a couple of cells. Translation regulation occurs in all developmental stages, being very interesting during male gametophyte development. In the tissues of anther loculi, diploid microsporocyte undergoes meiosis, resulting in four haploid microspores. Microspores then undergo two cell divisions; asymmetric division forms the vegetative and the generative cell, and subsequent division of the generative cell into two sperm cells (Figure 7). After pollination, pollen is activated, and pollen tube bursts out of the pollen grain. The pollen tube elongates through the stigma, style and ovary to the embryo sac, where one gamete fuses with the egg cell and the other gamete fuse with the central cell, a process known as double fertilization (Reviewed by Hafidh et al., 2016).

During early microgametogenesis, some mRNAs are stored in large ribonuclear particles, known as EPPs (Honys et al., 2000). After pollination, mRNA translation rapidly increases, as the stored mRNAs in EPPs are quickly released and translated. Moreover, some EPPs carry transcripts needed later on during the tip growth. These EPPs are transported to the tip of the pollen tube and stored mRNAs are continuously activated (Honys et al., 2000; Honys et al., 2009). Similar regulation of specific mRNA storage is found in plant embryo and endosperm during seed maturation, and their rapid translation during seed germination (Rajjou et al., 2004; Nakabayashi et al., 2005; Galland et al., 2014). EPPs contain some components of the translational machinery; ribosomal proteins, rRNA, translation factors and other accessory proteins. EPPs in Tobacco pollen contain several proteins that could regulate the specific translation; eIF4E, eIF4isoE, eIF4A, PABPs, translation elongation factors and also eIF3a and

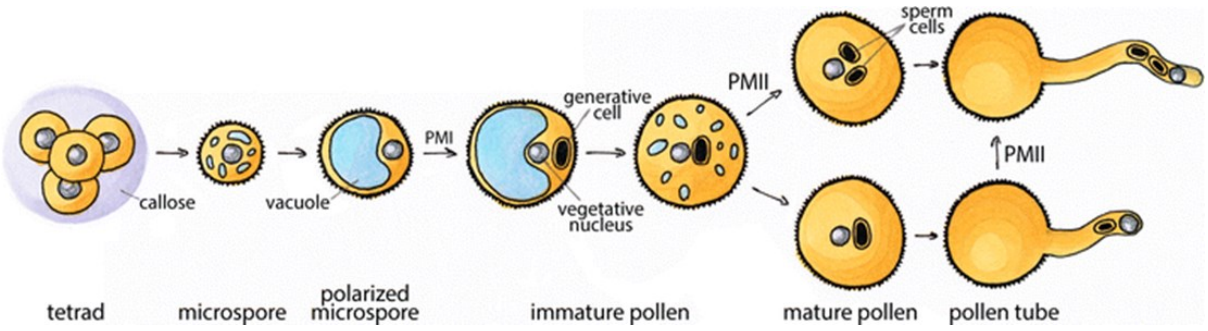

Figure 7: Diagram describing pollen development. After meiosis, tetrad of microspores is covered by callose, from which are later released. Released microspores undergo asymmetric division, pollen mitosis I **(**PMI**)**, to form generative and vegetative cell. The generative cell is engulfed in the cytoplasm of vegetative cell and divides once more in pollen mitosis II **(**PMII**)** into two sperm cells - male gametes. Adapted from (Hafidh et al., 2016)

eIF3b (Honys et al., 2009). Whether all these factors are found in EPPs just to be at hand when mRNA is activated or are participating in the regulation and control of pollen specific translation is yet unknown.

5 eIF3-mediated translation regulation in flowering plants

As most of the eIF3 structure and functions discoveries were done in yeast and mammals, we can only confirm via biochemical analyses that plant eIF3 is a complex of 12 subunits in *Arabidopsis thaliana, Triticum aestivum* and *Oryza sativa* (Browning et al., 2001; Burks et al., 2001; Li et al., 2016). Similar composition and domain conservation as the mammalian eIF3, suggest also structural similarities. However, additional functions of individual subunits might have diverged in plants. Even though plant eIF3 knowledge is still limited, existing studies of individual subunits have presented some of the regulative functions of plant eIF3 subunits. Most of plant eIF3 studies are concentrating on *Arabidopsis thaliana*, a model dicot for plant basic research. Arabidopsis genome has 21 coding sequences for eIF3 subunits, including two genes for eIF3j. Five subunits are encoded by one gene; eIF3a, -3e, -3f, -3h, -3k, and seven subunits are encoded by two genes; eIF3b, -3c, -3d, -3g, -3i, -3l, -3m (Browning and Bailey-Serres, 2015). This chapter gathers the existing studies on the individual plant eIF3 subunits, including the associated protein eIF3j and suggests possibilities for future plant eIF3 research. Where the knowledge is limited, suggestions are based on eIF3 subunit studies from other eukaryotes.

5.1 Expression profiles of individual eIF3 subunits share common pattern

eIF3 is undoubtedly an essential factor for translation and therefore it comes to no surprise that its expression correlates with global translation rate in plant tissues. The available mRNA expression profiles from Microarray data sets via ePlant browser (Winter et al., 2007; http://bar.utoronto.ca/eplant/), RNA sequencing via Araport (Krishnakumar et al., 2015; https://www.araport.org) and from promoter-reporter constructs in referred studies confirm this fact and locate highest eIF3 expression in rapidly proliferating and growing tissues like the shoot apical meristem (SAM), root apical meristem, leaf primordia, flowers, female reproductive tissues and during pollen, seed and embryo development. At the subcellular level, eIF3 is localized mainly in the cytoplasm. As all the eIF3 subunits share common expression profile, only the exceptions are mentioned in the following individual eIF3 subunit sections.

5.2 eIF3a is an essential eIF3 subunit

eIF3a is encoded by a single gene in Arabidopsis, and there is no evidence of any plant eIF3a mutant phenotype, as it is probably an essential gene like it is in mammals and yeast (Valášek et al., 2017 ref. to Luo et al., 2014). ATEIF3A was recognized in several studies as an

interacting partner with histone deacetylase GCN5 (Servet et al., 2008), as substrate of phosphorylation in the light to dark shift response (Boex-Fontvieille et al., 2013), as a ubiquitin-conjugate protein (Kim et al., 2013), and as part of the EPP particle in tobacco (Honys et al., 2009). Another interaction was discovered with plant reinitiation supporting protein (RISP), that increase reinitiation on *Cauliflower mosaic virus* (CaMV) mRNA (Thiébeauld et al., 2009). The implementation of eIF3 in CaMV translation is discussed in the eIF3g subunit section (5.8). One older study presented *Oryza sativa OSEIF3A* as a gene induced by auxin (Li et al., 2003).

eIF3a is the first octamer subunit in eIF3 assembly and the described modifications might impact the assembly of the eIF3 complex. Whether plant eIF3a is a gene regulated by auxin, and what impact has the phosphorylation or ubiquitin modification would require further research.

5.3 eIF3b is an essential eIF3 subunit with an impact on development

eIF3b is encoded in Arabidopsis by two genes, *ATEIF3B1* and *ATEIF3B2*. *ATEIF3B1* expression is higher in all tissues when compared to the *ATEIF3B2* expression levels. Homozygous *ateif3b2* insertion plants have delayed development but are viable (Roy, 2010; Linhart, 2017). Mutant *ateif3b1* plants were obtained only as heterozygotes, thus *ATEIF3B1* is an essential gene in plants. Heterozygous *ateif3b1* plants had higher frequency of aborted embryos than wild type and could not be fully complemented by *ATEIF3B2*. Neither of the single eIF3b mutants affected pollen development or fertilization (Linhart, 2017). Both Arabidopsis eIF3b subunits were found as a substrate of phosphorylation in the light to dark shift response (Boex-Fontvieille et al., 2013), and as part of the EPP particle in tobacco (Honys et al., 2009).

The plant insertion mutant lines confirmed that eIF3b is essential for eIF3 function, and that the two Arabidopsis paralogs are not redundant and might play distinct roles in plant development, as suggested from the *ateif3b2* delayed development phenotype. What is the impact of phosphorylation on eIF3b protein function is not known.

5.4 eIF3c is phosphorylated by CK2 and promotes the plant MFC assembly

In Arabidopsis, eIF3c is encoded by two genes, *ATEIF3C1* and *ATEIF3C2*. Expression of *ATEIF3C1* shows the common eIF3 expression pattern, whereas *ATEIF3C2* gene is expressed only during pollen development and in the endosperm. The heterozygous *ateif3c1* insertion mutant had defects in embryo development, leading to higher rate of aborted seeds (Roy, 2010).

Arabidopsis and wheat eIF3c was shown to be phosphorylated on multiple sites by CK2 kinase (Dennis et al., 2009), an essential and highly conserved serine/threonine kinase that is

involved in cell cycle, cell proliferation and apoptosis (Nuñez de Villavicencio-Diaz et al., 2017). In plants, CK2 affects plant development and is under control of plant hormones (Mulekar and Huq, 2014; Vilela et al., 2015). Amongst other substrates, plant CK2 phosphorylates initiation factors eIF2 and eIF5 and already mentioned eIF3c, which altogether increases the interaction affinity among these factors and thus the tendency to form free MFC in cytoplasm (Dennis et al., 2009) (Figure 8). Another interaction discovered was with RISP, a protein that increases reinitiation on CaMV mRNA (Thiébeauld et al., 2009). The implementation of eIF3 in CaMV translation is discussed in the eIF3g subunit section (5.8).

eIF3c was shown as essential for the octamer stability in mammals (Wagner et al., 2016). In plants, *ATEIF3C1* is dispensable during male gametophyte development, probably complemented by *ATEIF3C2*. If the low expression of *ATEIF3C2* in the mutant *ateif3c1* embryo suffice for proper eIF3 complex function is not known. The higher levels of *ATEIF3C2* found in endosperm and pollen could cause distinct subpopulations of eIF3 complexes with different roles. Also, CK2 could phosphorylate both ATEIF3C differently. Thus, plant eIF3c shows some interesting features that might bring novel findings in future research of plant development regulation.

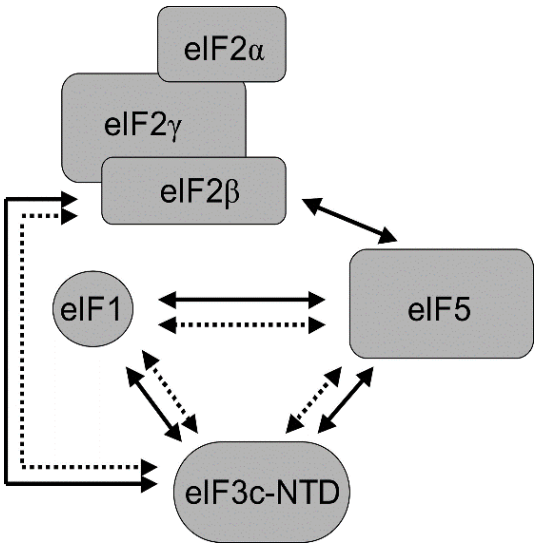

Figure 8: Model for in vitro plant multifactor complex interactions and the influence of CK2. Solid lines indicate interactions within plant MFC. Dotted lines are interactions that are enhanced by CK2 phosphorylation in vitro. Adapted from (Dennis et al., 2009)

5.5 eIF3d

Plant eIF3d lacks PCI or MPN domain but is bound to eIF3e in the octamer. There are two genes encoding eIF3d in Arabidopsis, *ATEIF3D1* and *ATEIF3D2*. Single insertion mutants had no phenotype, but in *ateif3d1*, the insertion is in the 3´UTR and its expression was not affected. Both genes show similar expression patterns, peaking during male microgametogenesis. In mammals, eIF3d was recently identified as a cap binding protein, that enables eIF3 to bind cell-proliferative transcripts and loading them on 40S, independently of eIF4F (Lee et al., 2015; Lee et al., 2016). In plants, eIF4isoE is one of the transcript specific cap binding proteins, as is eIF3d in mammals. Thus, plant eIF3d might have lost this function in the evolution without affecting the plant fitness.

5.6 eIF3e regulates global translation and is controlled by CSN

eIF3e is another octamer subunit encoded by a single gene in Arabidopsis. The *ateif3e* insertion mutant impaired pollen germination but did not affect pollen grain development or pollen tube elongation of the very rarely germinating mutant pollen grains. Also the fitness of female gametophyte was decreased (Roy, 2010; Roy et al., 2011; Linhart, 2017). Interestingly, *ATEIF3E* overexpression leads to retardation defects during seedling, vegetative and floral development. Therefore, loss of function in *ateif3e* insertion mutant as well as its overexpression leads to phenotypic defects. ATEIF3E was proposed be an essential regulator of translation in plants (Yahalom et al., 2008; Roy, 2010). In contrast to eIF3h, eIF3e affects the rate of translation initiation on global level rather than on specific transcripts. Normally, ATEIF3E protein is a part of the eIF3 complex and contributes in the binding of eIF4G to the PIC, but it was shown that excess of free ATEIF3E inhibits translation rate *in vitro*, probably by impairing the ability of entire eIF3 complex in binding to 40S (Paz-Aviram et al., 2008; Yahalom et al., 2008). To maintain the desired correct protein level in the cell, the strict homeostasis of ATEIF3E is controlled by COP9 signalosome (CSN), a nuclear protein complex that shares the PCI/MPN architecture with eIF3 and 26S proteasome lid and regulates activity of ubiquitin ligases, thus controlling specific protein degradation (Stratmann and Gusmaroli, 2012; Jin et al., 2018). There are multiple described interactions between all three PCI/MPN complexes *in vitro* (Karniol et al., 1998; Yahalom et al., 2001; Pick et al., 2009). However, ATEIF3E was not only shown to interact with CSN7 subunit but was also localized in nucleus in photosynthetically active tissues (Yahalom et al., 2001). Based on similarities between *ateif3e* and *csn* mutants, overexpression phenotypes and *in vitro* analyses, ATEIF3E is believed to be a substrate for degradation in proteasome in a CSN dependent way (Yahalom et al., 2008).

Rice *OSEIF3E* has similar expression pattern as the Arabidopsis ortholog, and shows a mRNA increase during cold stress and in ABA response. RNAi knockdown plants were mannitol sensitive and had slower and dwarf development, defects in pollen maturation, reduced reproductive organ size and lower seed biomass (Wang et al., 2016). OSEIF3E forms homodimers and interacts with inhibitors of cyclin dependent kinases *in vitro* (Wang et al., 2016), suggesting an unknown role in cell cycle regulation and possible self-regulation, considering that homodimers might impair the inhibition effect of the OSEIF3E monomer.

In summary, eIF3e is normally a part of eIF3 complex, while its monomer negatively regulates global translation and is under control of CSN in Arabidopsis. Mutants studies suggest its role in multiple developmental stages, but the influenced stages differ between species.

5.7 eIF3f is needed for pollen development and embryogenesis

In plants, eIF3f characterized in *Arabidopsis thaliana* and *Oryza sativa* is encoded by a single gene in both species (Roy, 2010; Xia et al., 2010; Li et al., 2016). In a knockdown insertion mutant, *ateif3f*, pollen grains were unable to germinate, although microsporogenesis and microgametogenesis was unaffected. The embryo development of the homozygous mutant, obtained by pollen rescue, showed higher rates of abortion at different developmental stages. Some of abnormal embryos formed seeds and even seedlings. These seedlings were malformed in stem shape, root system, lacked chlorophyll, and were sensitive to sugar (Xia et al., 2010), similarly to the *eIF3h* mutant (Kim et al., 2004). Xia et al., 2010 further analyzed the transcriptome of homozygous seedlings to identify 3100 genes with significantly altered expression. The number of upregulated transcripts in *ateif3f* was almost as high as the number of downregulated transcripts. The phenotype could be at least partially explained by downregulation of genes important for sugar response (*ASN1, ProDH2*), normal pollen tube growth and embryo development (*CSLA7*), plastid differentiation and chlorophyll synthesis (*SCO1, NAP7, CAO*) (Xia et al., 2010). Unfortunately, authors in the study concede lack of plant material from homozygous seedlings to perform any protein analyses (Xia et al., 2010). However, some presumption could be assumed from the similarity with eIF3h. Sugar response genes *ASN1* and *ProDH2* are regulated by transcription factor ATBZIP11 (Hanson et al., 2008), its specific translation is described in eIF3h subunit section (5.9). Transcript levels of *ASN1* and *ProDH2* genes were downregulated in *ateif3f*, while *ATBZIP11* transcript level was normal (Xia et al., 2010). It could be assumed that in the *ateif3f* knockdown, most eIF3 complexes lack ATEIF3F, thus disrupting translation of *ATBZIP11* but not affecting its mRNA level. Therefore, genes regulated by *ATBZIP11* would be lowered, which was the case of *ASN1* and *ProDH2*.

In rice, expression of *OSEIF3F* was increased in anthers, pollen and seeds development. RNAi knockdown of *OSEIF3F* caused no abnormalities in vegetative growth, but reduced seed production and caused male sterility by decreasing pollen viability. In contrast to the reduced pollen germination observed in Arabidopsis *ateif3f*, rice *oseif3f* pollen grains were arrested at various stages of microgametogenesis (Li et al., 2016). Surprisingly, *OSEIF3F* expression showed novel localization in endoplasmic reticulum, leaving another eIF3-question mark for future research (Li et al., 2016).

Taken together, plant eIF3f affects translation rate of specific transcripts that are transcription regulators for many other genes. Function of *ATEIF3F* seems to be similar to *ATEIF3H*, but not identical, as eIF3f comes to importance in different developmental processes, such as pollen maturation and seed development in rice or pollen germination in Arabidopsis.

5.8 eIF3g regulates translation in both abiotic and biotic stress conditions

Arabidopsis eIF3g is encoded by two paralog genes, *ATEIF3G1* and *ATEIF3G2*. In single gene knockout insertion mutants, no phenotype has been observed for both mutated genes (Roy, 2010). No evidence was found about the double mutant characterization. Even though expression of *ATEIF3G1* is consistently several times higher than *ATEIF3G2*, both genes might be functionally redundant, because eIF3g is considered essential for basal function in initiation.

The *Triticum aestivum TAEIF3G* has been shown to play an important role in abiotic stress response and tolerance. *TAEIF3G* expression was highly increased during drought, cold, high osmolarity and high salinity stress and induced by brassinosteroids and salicylic acid (Singh et al., 2007; Singh et al., 2013). *TAEIF3G* overexpression in Arabidopsis increased the tolerance to drought, osmotic and salinity stress, increased concentration of soluble proteins and stress hormone abscisic acid, provided better stability of PSII under stress conditions and decreased levels of oxidative membrane damage stress (Singh et al., 2013).

Plant eIF3g is a target of some viral proteins that regulate translation. *Cauliflower mosaic virus* (CaMV) is a circular dsDNA plant pararetrovirus, that produce capped polycistronic RNA containing six protein coding ORFs. To translate all six proteins, ribosomes need to reinitiate after long ORFs (Schepetilnikov and Ryabova, 2014; Schoelz et al., 2016). The product of ORF VI, transactivator viroplasmin protein (TAV), controls reinitiation after long uORF (Park et al., 2001) by recruiting eIF3 through interactions with eIF3g and plant specific reinitiation supporting protein (RISP) to polysomes (Park et al., 2001; Ryabova et al., 2004; Thiébeauld et al., 2009). After termination, one TAV binds 60S, a second TAV binds eIF3 bound to 40S and exchanges TC to reinitiate on next ORF. In a *Papaya ringspot virus* (PRSV) study in *Carica papaya*, *CPEIF3G* mRNA was increased 2-4.5 times during PRSV infection (Gao et al., 2015). Taken with the *TAEIF3G* increase during abiotic stress, plant eIF3g seems to be involved in both abiotic and biotic stress responses. Moreover, viral nuclear inclusion protein (Nia-Pro) binds to *CPEIF3G*, and probably decrease its contribution in stress response (Gao et al., 2015).

There is no evidence about the exact role of plant eIF3g during stress responses. What is known is that stress resistance transcripts are extensively translated in the presence of high eIF3g protein levels. Similar increase of stress tolerance was found by just increasing global translation rate or when other initiation factors were overexpressed (Wang et al., 2012; Sun and Hong, 2013), facts that decrease the eIF3g specificity in the regulation of stress response genes. Further research is needed to reveal the inducing mechanism of stress tolerance by eIF3g, which could also have an impact on developing crop plants tolerant to abiotic stress or viral infections.

5.9 eIF3h promotes reinitiation on uORF-containing transcripts

eIF3h is encoded by a single gene in Arabidopsis, *ATEIF3H,* and is found as a stable part of plant eIF3 complex, but dispensable for eIF3 general function. However, pleiotropic growth defects in postembryonic growth, female fertility, sugar sensitivity and pollen fitness are found in insertion mutant *ateif3h*, leading to seedling lethality (Kim et al., 2004; Roy et al., 2011). Some of these growth defects also altered auxin response. To surpass the lethality, addition of sucrose partially rescued the seedlings, enabling the plants to reproduce (Kim et al., 2004).

Behind this severe phenotype is the ATEIF3H ability to stimulate the translation efficiency of specific transcripts containing one or more inhibitory uORFs in their 5´ leader sequences, by promoting reinitiation on major ORF after termination event on short uORF. In Arabidopsis, over 30 % of mRNA possess at least one uORF, with uAUG in favorable Kozak context in nearly half of them (Kim et al., 2007). A plant model for eIF3h translation regulation was formulated for one uORFs containing gene discovered to be translationally dependent on eIF3h; *ATB2* gene encodes the Arabidopsis basic leucine zipper protein 11 (ATBZIP11), which is a sugar sensitive transcription factor that activates genes involved in amino acid and sugar metabolism (Hanson et al., 2008; Ma et al., 2011). The mRNA has a long 5´ leader that contains five uAUGs in four uORF and some of the neighboring uORFs are partially overlapping (Figure 9A). uORF1 has an uAUG in weak context and is mostly skipped by leaky scanning. In the case that initiation on uORF1 occurs, the long distance between uORF1 and mORF makes consequent reinitiation highly unlikely. When uORF1 is skipped, uORF2a/2b is translated from strong uAUGs. Translation of uORF2 makes initiation on uORF3 and uORF4 impossible because their start codons overlap with uORF2. After uORF2, ribosome reinitiate on mORF and ATBZIP11 protein is produced (Roy et al., 2010). In *ateif3h* mutant, reinitiation events decrease after uORF2, as well as polysomal occupancy on *ATBZIP11* transcript, thus downregulation of ATBZIP11 protein level is observed. The uORF2 encodes an inhibitory 28 residues peptide that, in presence of sucrose, stalls the ribosome during its translation and efficiently inhibits *ATBZIP11* translation (Rahmani et al., 2009; Canfield et al., 2017). Stalled ribosomes are found to be more associated with coding uORFs (Hou et al., 2016), which suggest that this regulatory mechanism is used more widely.

In over 250 genes translationally downregulated in *ateif3h*, gene classes of transcriptional regulators and protein modifying enzymes are enriched (Kim et al., 2007; Roy et al., 2010; Tiruneh et al., 2013). Many bZIP family genes are involved in low or high sugar responses and possess uORFs (Wiese et al., 2005), which could explain, why *eIF3h* seedlings were sensitive to various sugar levels (Kim et al., 2004). The eIF3h ability to perform reinitiation after uORFs

is also needed in maintaining the correct shoot apical meristem (SAM) size and functionality**.** SAM is the primary stem cell niche organized into three layers and several zones of different stem cells populations and its size is controlled by a *CLAVATA3-WUSHEL* autoregulatory negative feedback loop (Figure 9B) (Soyars et al., 2016). CLAVATA3 (CLV3) is a short extracellular peptide expressed in the central zone and recognized by receptor-like kinase CLAVATA (CLV1) in underlying cell layers of the so called organizing center. CLV1 signaling represses transcription of *WUSCHEL* (*WUS*), a transcription factor that is delivered through plasmodesma back to central zone, where it activates the *CLV3* expression and ensure the size of stem cell population (Aichinger et al., 2012; Somssich et al., 2016; Soyars et al., 2016). In this case, it is the receptor CLV1, which is restrained on the translational level. There are four uORFs in the *CLV1* 5´ leader and translation of main ORF is highly dependent on the presence of eIF3h. With reinitiation events inhibited in *ateif3h* mutant, the CLV1 protein level is downregulated, which disrupts the inhibition of *WUS* in the negative feedback loop. In *eIF3h*, SAM is indeed enlarged, quiescent and has a different dome-like shape than SAM in wild type (Zhou et al., 2014). Analogically, the translation rate of the auxin response factors (*ARFs*) class of transcription factors with diverse uORFs in 5´ leader is downregulated in *ateIF3h* mutant (Figure 10) (Zhou et al., 2010), as well as the adaxializing transcription factor that cooperate in leaf polarity, *ASSYMETRIC LEAVES1* (*AS1*) (Figure 9B), that possess three inhibitory uORFs and its mutant has similar defects in leaf morphology as in *ateIF3h* mutant (Zhou et al., 2014). Selective translation of uORF containing transcripts is also proposed to be one of important regulative mechanism during seed germination (Basbouss-Serhal et al., 2015).

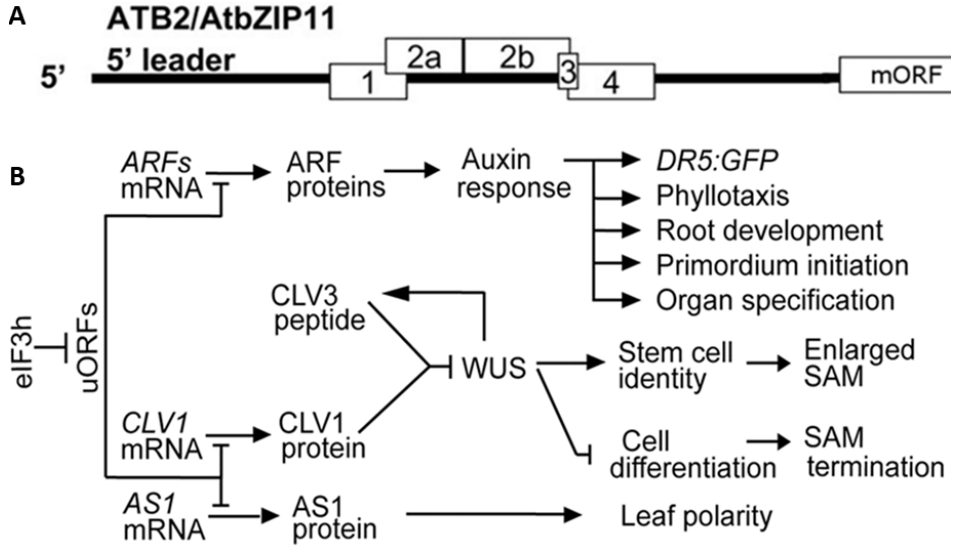

Figure 9: Plant eIF3h increases translation of development regulators by promoting reinitiation after uORFs. Diagram of the 5´ leader of AtbZIP11 showing four uORFs in 5'UTR **(A)**, adapted and modified from (Kim et al., 2004). A concept map for the role of eIF3h in Arabidopsis SAM maintenance and auxin response **(B)**. By overcoming the translational repression of uORFs, eIF3h promotes the translation of ARFs, CLV1 and AS1 and plays an important role in SAM maintenance and organogenesis. Adapted from (Zhou et al., 2014)

Ensuring control over developmental genes, ATEIF3H function is controlled by target of rapamycin kinase (TOR), which integrates cytoplasmic auxin, stress, nutrient and energy signals (Schepetilnikov and Ryabova, 2018). Stimulated TOR binds to initiation complexes or polysomes and activates kinase S6K1, that phosphorylates EIF3H to increase its ability to promote reinitiation (Figure 10) (Schepetilnikov et al., 2011; Schepetilnikov et al., 2013).

Taken together, plant eIF3h does not change global translational levels, but its presence is essential to overcome the inhibitory effects of uORFs in 5´ leaders on specific genes that regulate metabolism, stem cell maintenance and organogenesis. Its activity is promoted indirectly by TOR kinase, a cytoplasmic node of signal transduction. Similar phenotypes and involvement in uORF regulated translation were found in some mutants of ribosomal proteins, RPL4A, RPL4D, RPL5A, RPL24B (Nishimura et al., 2005; Zhou et al., 2010; Rosado et al., 2012; Tiruneh et al., 2013), suggesting cooperation between large ribosomal subunits and eIF3 in reinitiation and complex cooperation of signal transduction, initiation factors, composition of ribosome and structural features in mRNA in translation regulation of specific genes.

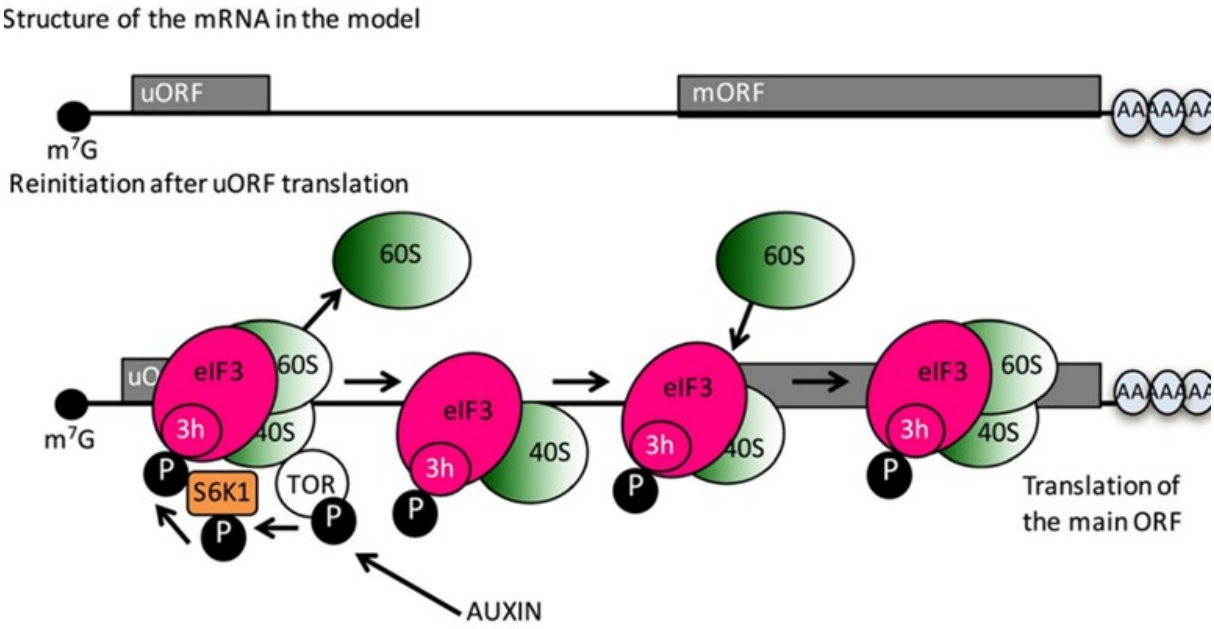

Figure 10: Auxin induced activation of eIF3h mediated reinitiation after uORFs. In response to auxin, TOR is phosphorylated and recruited to polysomes, which triggers the phosphorylation of eIF3h through S6K. After the ribosome subunits disassemble at the uORF stop, the phosphorylation on eIF3h allows 40S to recruit new 60S at the AUG of the followed. Adapted and modified from (Merchante et al., 2017).

5.10 eIF3i is activated by brassinosteroids

eIF3i is encoded by two duplicate genes in Arabidopsis, *ATEIF3I1* and *ATEIF3I2*, that form direct tandem repeat on chromosome two, with 517 bp between them. Expression estimated from RNA sequencing experiments shows very low expression of *ATEIF3I2*, while *ATEIF3I1* expression follows the common eIF3 pattern. Arabidopsis *ateif3i* RNAi lines were probably

knockdowns of both genes and lead to severe developmental defects; seeds exhibited delayed germination, seedlings had dwarf phenotype and died before growing first true leaves. For some seedlings that survived, developmental malformations in leaf morphology, apical dominance and aberrant flower development were observed (Jiang and Clouse, 2001; Roy, 2010). It was also shown that eIF3i transcription is regulated by brassinosteroids. Adding brassinosteroids increased the level of the *Phaseolus vulgaris PVEIF3I* mRNA in bean, tobacco cells and Arabidopsis seedlings (Jiang and Clouse, 2001). Moreover, EIF3I was phosphorylated *in vitro* by BRASSINOSTEROID-INSENSITIVE 1 (BRI1) receptor serine/threonine kinase (Ehsan et al., 2005), suggesting brassinosteroids control eIF3i activity also post-translationally.

In mammals, eIF3i is phosphorylated by transforming growth factor beta (TGF-β) receptor kinase and modulates TGF-β response (Choy and Derynck, 1998). Supporting the role of eIF3i in translation regulation on proliferative genes, eIF3i dysregulation was shown in various types of cancer (Ali et al., 2017). In plants, at*eif3i* mutants also showed developmental defects, but did not disrupt translation efficiency of *ATBZIP11* (Roy, 2010). However, no study identified how brassinosteroids or phosphorylation affects the function of eIF3i within the eIF3 complex or in interacting with ribosomal proteins to modulate the initiation process and thus global or specific translation.

5.11 eIF3j

The eIF3j associated protein has two genes in Arabidopsis, *ATEIF3J1* and *ATEIF3J2*, and is not found as part of eIF3 complex in the rice analysis (Li et al., 2016). Expression of *ATEIF3J2* is slightly higher in all tissues when compared to *ATEIFJ1*. In other eukaryotes, eIF3j has stabilizing role in PIC assembly and promotes 40S-mRNA dissociation during ribosome recycling (Pisarev et al., 2007). Mammalian eIF3j is phosphorylated by CK2 protein kinase, a modification needed for attachment to eIF3 and normal translation efficiency. Phosphorylation of plant eIF3j is not known, as well as any other regulatory function.

5.12 eIF3k

eIF3k is encoded by a single gene in Arabidopsis. The insertion mutant of *ateif3k* had no phenotype and the translational efficiency of *ATBZIP11* was not compromised (Roy, 2010; Tiruneh et al., 2013). ATEIF3K was identified as a ubiquitin-conjugate (Kim et al., 2013). In other eukaryotes eIF3k is also non-essential, and shows interactions with cell cycle associated proteins (Shen et al., 2004; Lin et al., 2008; Huang et al., 2012). In *Caenorhabditis elegans*, eIF3k with eIF3l induce apoptosis and loss of function mutants lead to extended lifespan and higher ER stress resistance (Huang et al., 2012; Cattie et al., 2016).

5.13 eIF3l

There are two eIF3l genes are in Arabidopsis genome, *ATEIF3L1* and *ATEIF3L2*, and expression of *ATEIF3L2* is overall stronger than *ATEIF3L1*. ATEIF3L1 was identified as a ubiquitin-conjugate (Kim et al., 2013), but since no plant mutant characterization was done for eIF3l, it is hard to make suggestions on its function.

5.14 eIF3m

Two genes, *ATEIF3M1* and *ATEIF3M2*, encode the eIF3m subunit in Arabidopsis. Single and double mutants lacked any phenotype and the double mutant had no effect on *ATBZIP11* expression (Roy, 2010). The *ATEIF3M1* transcript levels are higher than the *ATEIF3M2* levels. In fission yeast, eIF3m is essential for protein synthesis and polysome loading (Zhou et al., 2005). In mice eIF3m is essential for developing embryos and organ size control (Zeng et al., 2013) and is detected as dysregulated in various human tumors (Gomes-Duarte et al., 2018).

6 Summary

In contrast with the well-known complex regulation of transcription, knowledge is still restricted for the translation regulation mechanisms. Recent advanced methodologies have just outlined the complexity of translation regulation, where transcript sequence and structure possess many regulatory features, heterogeneous ribosomes are occupying different mRNA populations and the initiation phase of translation is performed by the initiation factors that are controlled by various signaling pathways. Across the eukaryotic kingdom, eIF3 is mostly studied in yeast and mammals, where individual subunits show essential regulatory roles that change the translation efficiency and the overall translatome in the organism and their mutations cause developmental dysfunctions and various types of tumors (Gomes-Duarte et al., 2018).

In plants, a similar role for the eIF3 subunits participating in posttranscriptional regulation is undoubtedly confirmed, but still little is known about the exact mechanisms. Several plant eIF3 subunits were proven to regulate translation, affecting vegetative development as well as the gametophytic generation and stress response. On the global level, eIF3a and eIF3b are essential, needed for the eIF3 complex assembly and normal function. Monomeric eIF3e is a negative regulator of the initiation phase, while phosphorylation on eIF3c promotes assembly of the initiation MFC. Increased levels of eIF3g during various stresses enhance the translation of stress response genes. On the level of specific regulation, eIF3h increases the rate of reinitiation after short uORFs, which leads to increased gene expression of specific development regulators. eIF3f plays presumably a similar role as eIF3h, but in different developmental stages. This is also supported by the fact that eIF3h and eIF3f both contain the

MPN domain and are part of the same “left leg” lobe in the PCI/MPN octamer module. Still, further analysis of the affected proteins in the *ateif3f* mutant is needed for confirmation. eIF3i showed impact on development by being under control of brassinosteroids. That suggest a possible involvement in translation regulation, supported by the similarity to the mammalian eIF3i role of selective translational regulation on transcript of proliferative genes in mammals TGF-β response.

For the best characterized plant subunit eIF3h, a detailed structural characterization of reinitiation in plants might bring out answers and/or new questions about the exact mechanism, or at least provide new plant eIF3 structural data. For the less well understood subunits, recent studies hint connections to plant hormonal regulation or cell cycle. Post-translational modifications of some subunits also indicate that their function might be a part of some regulation mechanisms as well. Further analysis of the character of eIF3-hormonal communication and impact of those modifications is needed. For some plant subunits, very little is known. The first step for more information for them is the missing characterization of knockout double mutant for both paralog genes.

An interesting field for future research is the characterization of eIF3 subcomplexes, as they were found *in vivo* and able to perform at least partially some eIF3 functions. Whether they are just yet unassembled complexes or they have non-identical functions in the translation or its regulation is yet to be known. In Arabidopsis, this functional modulation might be the case for non-redundant eIF3b paralogs and different expression profiles of eIF3c paralogs in male gametophyte. Moreover, presence of eIF3a and eIF3b in tobacco pollen EPPs, both being first subunits for the eIF3 assembly, suggest the actual need for eIF3 complex after pollen activation.

This work described eIF3 structure and function in the initiation phase of translation, using the well-known mammalian eIF3 model, which is similar to the plant eIF3 complex. With the understanding of eIF3 in the translational context, discussion of the current knowledge about eIF3 role in translation regulation of flowering plants ensued. The interesting, but limited information about plant eIF3 lead to suggestions on future plant eIF3 research that could bring not just novel findings in the emerging field of posttranscriptional regulation of gene expression, but also improve applied research especially in the field of stress tolerance of crop plants.

7 References

Aichinger E, Kornet N, Friedrich T, Laux T (2012) Plant stem cell niches. Annu Rev Plant Biol **63**: 615–36

Aitken CE, Beznosková P, Vlčkova V, Chiu W-L, Zhou F, Valášek LS, Hinnebusch AG, Lorsch JR (2016) Eukaryotic translation initiation factor 3 plays distinct roles at the mRNA entry and exit channels of the ribosomal preinitiation complex. Elife. doi: 10.7554/eLife.20934

Algire MA, Maag D, Lorsch JR (2005) Pi release from eIF2, not GTP hydrolysis, is the step controlled by start-site selection during eukaryotic translation initiation. Mol Cell **20**: 251–62

Ali MU, Ur Rahman MS, Jia Z, Jiang C (2017) Eukaryotic translation initiation factors and cancer. Tumour Biol **39**: 1010428317709805

Asano K, Clayton J, Shalev A, Hinnebusch AG (2000) A multifactor complex of eukaryotic initiation factors, eIF1, eIF2, eIF3, eIF5, and initiator tRNA(Met) is an important translation initiation intermediate in vivo. Genes Dev **14**: 2534–46

Asano K, Phan L, Anderson J, Hinnebusch AG (1998) Complex Formation by All Five Homologues of Mammalian Translation Initiation Factor 3 Subunits from Yeast Saccharomyces cerevisiae. J Biol Chem **273**: 18573–18585

Aylett CHS, Boehringer D, Erzberger JP, Schaefer T, Ban N (2015) Structure of a yeast 40S-eIF1-eIF1A-eIF3-eIF3j initiation complex. Nat Struct Mol Biol **22**: 269–71

Basbouss-Serhal I, Soubigou-Taconnat L, Bailly C, Leymarie J (2015) Germination Potential of Dormant and Nondormant Arabidopsis Seeds Is Driven by Distinct Recruitment of Messenger RNAs to Polysomes. Plant Physiol **168**: 1049–65

Belostotsky DA (2003) Unexpected complexity of poly(A)-binding protein gene families in flowering plants: three conserved lineages that are at least 200 million years old and possible auto- and cross-regulation. Genetics **163**: 311–9

Benelli D, Londei P (2011) Translation initiation in Archaea: conserved and domain-specific features. Biochem Soc Trans **39**: 89–93

Benne R, Hershey JW (1976) Purification and characterization of initiation factor IF-E3 from rabbit reticulocytes. Proc Natl Acad Sci U S A **73**: 3005–9

Beznosková P, Cuchalová L, Wagner S, Shoemaker CJ, Gunišová S, von der Haar T, Valášek LS (2013) Translation initiation factors eIF3 and HCR1 control translation termination and stop codon read-through in yeast cells. PLoS Genet **9**: e1003962

Beznosková P, Wagner S, Jansen ME, von der Haar T, Valášek LS (2015) Translation initiation factor eIF3 promotes programmed stop codon readthrough. Nucleic Acids Res **43**: 5099–111

Boex-Fontvieille E, Daventure M, Jossier M, Zivy M, Hodges M, Tcherkez G (2013) Photosynthetic Control of Arabidopsis Leaf Cytoplasmic Translation Initiation by Protein Phosphorylation. PLoS One **8**: e70692

Brown A, Shao S, Murray J, Hegde RS, Ramakrishnan V (2015) Structural basis for stop codon recognition in eukaryotes. Nature **524**: 493–496

Browning KS, Bailey-Serres J (2015) Mechanism of cytoplasmic mRNA translation. Arab B **13**: e0176

Browning KS, Gallie DR, Hershey JWB, Hinnebusch AG, Maitra U, Merrick WC, Norbury C (2001) Unified nomenclature for the subunits of eukaryotic initiation factor 3. Trends Biochem Sci **26**: 284

Burks EA, Bezerra PP, Le H, Gallie DR, Browning KS (2001) Plant initiation factor 3 subunit composition resembles mammalian initiation factor 3 and has a novel subunit. J Biol Chem **276**: 2122–31

Buttgereit F, Brand MD (1995) A hierarchy of ATP-consuming processes in mammalian cells. Biochem J **312 (Pt 1**: 163–7

Canfield SG, Stebbins MJ, Morales BS, Asai SW, Vatine GD, Svendsen CN, Palecek SP, Shusta E V (2017) An isogenic blood-brain barrier model comprising brain endothelial cells, astrocytes, and neurons derived from human induced pluripotent stem cells. J Neurochem **140**: 874–888

Cate JHD (2017) Human eIF3: from 'blobology' to biological insight. Philos Trans R Soc Lond B Biol Sci **372**: 20160176

Cattie DJ, Richardson CE, Reddy KC, Ness-Cohn EM, Droste R, Thompson MK, Gilbert W V., Kim DH (2016) Mutations in Nonessential eIF3k and eIF3l Genes Confer Lifespan Extension and Enhanced Resistance to ER Stress in Caenorhabditis elegans. PLoS Genet **12**: e1006326

Celik A, He F, Jacobson A (2017) NMD monitors translational fidelity 24/7. Curr Genet **63**: 1007–1010

Cuchalová L, Kouba T, Herrmannová A, Dányi I, Chiu W-L, Valášek L (2010) The RNA recognition motif of eukaryotic translation initiation factor 3g (eIF3g) is required for resumption of scanning of posttermination ribosomes for reinitiation on GCN4 and together with eIF3i stimulates linear scanning. Mol Cell Biol **30**: 4671–86

Dabrowski M, Bukowy-Bieryllo Z, Zietkiewicz E (2015) Translational readthrough potential of natural termination codons in eucaryotes--The impact of RNA sequence. RNA Biol **12**: 950–8

Dennis MD, Person MD, Browning KS (2009) Phosphorylation of plant translation initiation factors by CK2 enhances the in vitro interaction of multifactor complex components. J Biol Chem **284**:

20615–28

Dever TE, Green R (2012) The elongation, termination, and recycling phases of translation in eukaryotes. Cold Spring Harb Perspect Biol **4**: a013706

Ehsan H, Ray WK, Phinney B, Wang X, Huber SC, Clouse SD (2005) Interaction of Arabidopsis BRASSINOSTEROID-INSENSITIVE 1 receptor kinase with a homolog of mammalian TGF-beta receptor interacting protein. Plant J **43**: 251–61

Echevarría-Zomeño S, Yángüez E, Fernández-Bautista N, Castro-Sanz AB, Ferrando A, Castellano MM (2013) Regulation of Translation Initiation under Biotic and Abiotic Stresses. Int J Mol Sci **14**: 4670–83

Elantak L, Wagner S, Herrmannová A, Karásková M, Rutkai E, Lukavsky PJ, Valášek L (2010) The indispensable N-terminal half of eIF3j/HCR1 cooperates with its structurally conserved binding partner eIF3b/PRT1-RRM and with eIF1A in stringent AUG selection. J Mol Biol **396**: 1097–116

Eliseev B, Yeramala L, Leitner A, Karuppasamy M, Raimondeau E, Huard K, Alkalaeva E, Aebersold R, Schaffitzel C (2018) Structure of a human cap-dependent 48S translation pre-initiation complex. Nucleic Acids Res **46**: 2678–2689

Ellisdon AM, Stewart M (2012) Structural biology of the PCI-protein fold. Bioarchitecture **2**: 118–23

Enchev RI, Schreiber A, Beuron F, Morris EP (2010) Structural insights into the COP9 signalosome and its common architecture with the 26S proteasome lid and eIF3. Structure **18**: 518–27

Erzberger JP, Stengel F, Pellarin R, Zhang S, Schaefer T, Aylett CHS, Cimermančič P, Boehringer D, Sali A, Aebersold R, et al (2014) Molecular architecture of the 40S·eIF1·eIF3 translation initiation complex. Cell **158**: 1123–1135

Estrin E, Lopez-Blanco JR, Chacón P, Martin A (2013) Formation of an intricate helical bundle dictates the assembly of the 26S proteasome lid. Structure **21**: 1624–35

Flury V, Restuccia U, Bachi A, Mühlemann O (2014) Characterization of phosphorylation- and RNA-dependent UPF1 interactors by quantitative proteomics. J Proteome Res **13**: 3038–53

Fraser CS, Lee JY, Mayeur GL, Bushell M, Doudna JA, Hershey JWB (2004) The j-subunit of human translation initiation factor eIF3 is required for the stable binding of eIF3 and its subcomplexes to 40 S ribosomal subunits in vitro. J Biol Chem **279**: 8946–56

Galland M, Huguet R, Arc E, Cueff G, Job D, Rajjou L (2014) Dynamic proteomics emphasizes the importance of selective mRNA translation and protein turnover during Arabidopsis seed germination. Mol Cell Proteomics **13**: 252–68

Gallie DR (2016) Eukaryotic Initiation Factor eIFiso4G1 and eIFiso4G2 Are Isoforms Exhibiting Distinct Functional Differences in Supporting Translation in Arabidopsis. J Biol Chem **291**: 1501–13

Gao L, Tuo D, Shen W, Yan P, Li X, Zhou P (2015) NIa-Pro of Papaya ringspot virus interacts with Carica papaya eukaryotic translation initiation factor 3 subunit G (CpeIF3G). Virus Genes **50**: 97–103

des Georges A, Dhote V, Kuhn L, Hellen CUTT, Pestova T V., Frank J, Hashem Y (2015) Structure of mammalian eIF3 in the context of the 43S preinitiation complex. Nature **525**: 491–5

Gomes-Duarte A, Lacerda R, Menezes J, Romão L (2018) eIF3: a factor for human health and disease. RNA Biol **15**: 26–34

Graifer D, Karpova G (2015) Roles of ribosomal proteins in the functioning of translational machinery of eukaryotes. Biochimie **109**: 1–17

Gunišová S, Hronová V, Mohammad MP, Hinnebusch AG, Valášek LS (2018) Please do not recycle! Translation reinitiation in microbes and higher eukaryotes. FEMS Microbiol Rev **42**: 165–192

Gupta P, Li Y-R (2018) Upf proteins: highly conserved factors involved in nonsense mRNA mediated decay. Mol Biol Rep **45**: 39–55

Hafidh S, Fíla J, Honys D (2016) Male gametophyte development and function in angiosperms: a general concept. Plant Reprod **29**: 31–51

Han H, Kong C, Dong H, Zhu S, Zhao Q, Zhai Q, Liang S, Li S, Yang S, Huang B (2015) Molecular characterization and functional analysis of subunit 7 of eukaryotic initiation factor 3 from Eimeria tenella. Exp Parasitol **154**: 118–26

Hanson J, Hanssen M, Wiese A, Hendriks MMWB, Smeekens S (2008) The sucrose regulated transcription factor bZIP11 affects amino acid metabolism by regulating the expression of ASPARAGINE SYNTHETASE1 and PROLINE DEHYDROGENASE2. Plant J **53**: 935–49

Hashem Y, des Georges A, Dhote V, Langlois R, Liao HY, Grassucci RA, Hellen CUTT, Pestova T V., Frank J (2013a) Structure of the mammalian ribosomal 43S preinitiation complex bound to the scanning factor DHX29. Cell **153**: 1108–19

Hashem Y, des Georges A, Dhote V, Langlois R, Liao HY, Grassucci RA, Pestova T V, Hellen CUT, Frank J (2013b) Hepatitis-C-virus-like internal ribosome entry sites displace eIF3 to gain access to the 40S subunit. Nature **503**: 539–43

Herrmannová A, Daujotyte D, Yang J-C, Cuchalová L, Gorrec F, Wagner S, Dányi I, Lukavsky PJ, Valášek LS (2012) Structural analysis of an eIF3 subcomplex reveals conserved interactions required for a stable and proper translation pre-initiation complex assembly. Nucleic Acids Res **40**: 2294–311

Hinnebusch AG (2014) The scanning mechanism of eukaryotic translation initiation. Annu Rev Biochem **83**: 779–812

Hinnebusch AG (2017) Structural Insights into the Mechanism of Scanning and Start Codon Recognition in Eukaryotic Translation Initiation. Trends Biochem Sci **42**: 589–611

Honys D, Combe JP, Twell D, Capková V (2000) The translationally repressed pollen-specific ntp303 mRNA is stored in non-polysomal mRNPs during pollen maturation. Sex Plant Reprod **13**: 135–144

Honys D, Rěnák D, Feciková J, Jedelský PL, Nebesárová J, Dobrev P, Capková V (2009) Cytoskeleton-associated large RNP complexes in tobacco male gametophyte (EPPs) are associated with ribosomes and are involved in protein synthesis, processing, and localization. J Proteome Res **8**: 2015–31

Hou C, Lee W, Chou H-C, Chen A-P, Chou S, Chen H (2016) Global Analysis of Truncated RNA Ends Reveals New Insights into Ribosome Stalling in Plants. Plant Cell **28**: 2398–2416

Hronová V, Mohammad MP, Wagner S, Pánek J, Gunišová S, Zeman J, Poncová K, Valášek LS (2017) Does eIF3 promote reinitiation after translation of short upstream ORFs also in mammalian cells? RNA Biol **14**: 1660–1667

Hsu PY, Calviello L, Wu H-YL, Li F-W, Rothfels CJ, Ohler U, Benfey PN (2016) Super-resolution ribosome profiling reveals unannotated translation events in Arabidopsis. Proc Natl Acad Sci U S A **113**: E7126–E7135

Huang C-Y, Chen J-Y, Wu S-C, Tan C-H, Tzeng R-Y, Lu P-J, Wu Y-F, Chen R-H, Wu Y-C (2012) C. elegans EIF-3.K promotes programmed cell death through CED-3 caspase. PLoS One **7**: e36584

Chiu W-L, Wagner S, Herrmannová A, Burela L, Zhang F, Saini AK, Valášek L, Hinnebusch AG (2010) The C-terminal region of eukaryotic translation initiation factor 3a (eIF3a) promotes mRNA recruitment, scanning, and, together with eIF3j and the eIF3b RNA recognition motif, selection of AUG start codons. Mol Cell Biol **30**: 4415–34

Choe J, Oh N, Park S, Lee YK, Song O-K, Locker N, Chi S-G, Kim YK (2012) Translation initiation on mRNAs bound by nuclear cap-binding protein complex CBP80/20 requires interaction between CBP80/20-dependent translation initiation factor and eukaryotic translation initiation factor 3g. J Biol Chem **287**: 18500–9

Choy L, Derynck R (1998) The Type II Transforming Growth Factor (TGF)-β Receptor-interacting Protein TRIP-1 Acts as a Modulator of the TGF-β Response. J Biol Chem **273**: 31455–31462

Isken O, Kim YK, Hosoda N, Mayeur GL, Hershey JWB, Maquat LE (2008) Upf1 phosphorylation triggers translational repression during nonsense-mediated mRNA decay. Cell **133**: 314–27

Islam W, Noman A, Qasim M, Wang L (2018) Plant Responses to Pathogen Attack: Small RNAs in Focus. Int J Mol Sci **19**: 515

Jenner L, Melnikov S, Garreau de Loubresse N, Ben-Shem A, Iskakova M, Urzhumtsev A, Meskauskas A, Dinman J, Yusupova G, Yusupov M (2012) Crystal structure of the 80S yeast ribosome. Curr Opin Struct Biol **22**: 759–67

Jiang J, Clouse SD (2001) Expression of a plant gene with sequence similarity to animal TGF-beta receptor interacting protein is regulated by brassinosteroids and required for normal plant development. Plant J **26**: 35–45

Jin D, Wu M, Li B, Bücker B, Keil P, Zhang S, Li J, Kang D, Liu J, Dong J, et al (2018) The COP9 Signalosome regulates seed germination by facilitating protein degradation of RGL2 and ABI5. PLoS Genet **14**: e1007237

Karásková M, Gunišová S, Herrmannová A, Wagner S, Munzarová V, Valášek LS (2012) Functional characterization of the role of the N-terminal domain of the c/Nip1 subunit of eukaryotic initiation factor 3 (eIF3) in AUG recognition. J Biol Chem **287**: 28420–34

Karniol B, Yahalom A, Kwok S, Tsuge T, Matsui M, Deng XW, Chamovitz DA (1998) The Arabidopsis homologue of an eIF3 complex subunit associates with the COP9 complex. FEBS Lett **439**: 173–9

Khoshnevis S, Hauer F, Milón P, Stark H, Ficner R (2012) Novel insights into the architecture and protein interaction network of yeast eIF3. RNA **18**: 2306–19

Kim B-H, Cai X, Vaughn JN, von Arnim AG (2007) On the functions of the h subunit of eukaryotic initiation factor 3 in late stages of translation initiation. Genome Biol **8**: R60

Kim D-Y, Scalf M, Smith LM, Vierstra RD (2013) Advanced proteomic analyses yield a deep catalog of ubiquitylation targets in Arabidopsis. Plant Cell **25**: 1523–40

Kim T-H, Kim B-H, Yahalom A, Chamovitz D a, von Arnim AG (2004) Translational regulation via 5' mRNA leader sequences revealed by mutational analysis of the Arabidopsis translation initiation factor subunit eIF3h. Plant Cell **16**: 3341–56

Kolupaeva VG, Unbehaun A, Lomakin IB, Hellen CUT, Pestova T V (2005) Binding of eukaryotic initiation factor 3 to ribosomal 40S subunits and its role in ribosomal dissociation and anti-association. RNA **11**: 470–86

Korostelev AA (2014) A deeper look into translation initiation. Cell **159**: 475–6

Kozak M (1987) An analysis of 5'-noncoding sequences from 699 vertebrate messenger RNAs. Nucleic Acids Res **15**: 8125–48

Kozak M (2002) Pushing the limits of the scanning mechanism for initiation of translation. Gene **299**: 1–34

Krishnakumar V, Hanlon MR, Contrino S, Ferlanti ES, Karamycheva S, Kim M, Rosen BD, Cheng C-Y, Moreira W, Mock SA, et al (2015) Araport: the Arabidopsis information portal. Nucleic Acids

Res **43**: D1003-9

Kumar P, Hellen CUT, Pestova T V. (2016) Toward the mechanism of eIF4F-mediated ribosomal attachment to mammalian capped mRNAs. Genes Dev **30**: 1573–88

Lageix S, Lanet E, Pouch-Pélissier M-N, Espagnol M-C, Robaglia C, Deragon J-M, Pélissier T (2008) Arabidopsis eIF2alpha kinase GCN2 is essential for growth in stress conditions and is activated by wounding. BMC Plant Biol **8**: 134

Lee AS, Kranzusch PJ, Doudna JA, Cate JHD (2016) eIF3d is an mRNA cap-binding protein that is required for specialized translation initiation. Nature **536**: 96–9

Lee ASYY, Kranzusch PJ, Cate JHDD (2015) eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. Nature **522**: 111–4

Li H, Shen SS, Xu ZH, Xue HW (2003) [Isolation and expression pattern analysis of rice auxin-induced eIF3a coding gene]. Shi Yan Sheng Wu Xue Bao **36**: 54–60

Li K, Zhou S, Guo Q, Chen X, Lai D, Lun Z, Guo X (2017) The eIF3 complex of Trypanosoma brucei: composition conservation does not imply the conservation of structural assembly and subunits function. RNA **23**: 333–345

Li Q, Deng Z, Gong C, Wang T (2016) The Rice Eukaryotic Translation Initiation Factor 3 Subunit f (OseIF3f) Is Involved in Microgametogenesis. Front Plant Sci **7**: 532

Lin Y-M, Chen Y-R, Lin J-R, Wang W-J, Inoko A, Inagaki M, Wu Y-C, Chen R-H (2008) eIF3k regulates apoptosis in epithelial cells by releasing caspase 3 from keratin-containing inclusions. J Cell Sci **121**: 2382–93

Lingaraju GM, Bunker RD, Cavadini S, Hess D, Hassiepen U, Renatus M, Fischer ES, Thomä NH (2014) Crystal structure of the human COP9 signalosome. Nature **512**: 161–5

Linhart F (2017) Characterization of eukaryotic translation initiation factor 3 subunits (eIF3) in A . thaliana male gametophyte. Prague. Master thesis. Dep. Exp. Plant Biol. Charles Univ. Fac. Sci.

Liu H, Yu H, Tang G, Huang T (2018) Small but powerful: function of microRNAs in plant development. Plant Cell Rep **37**: 515–528

Liu Y, Neumann P, Kuhle B, Monecke T, Schell S, Chari A, Ficner R (2014) Translation initiation factor eIF3b contains a nine-bladed β-propeller and interacts with the 40S ribosomal subunit. Structure **22**: 923–30

Luo H, Lin Y, Gao F, Zhang C-T, Zhang R (2014) DEG 10, an update of the database of essential genes that includes both protein-coding genes and noncoding genomic elements. Nucleic Acids Res **42**: D574-80

Ma J, Hanssen M, Lundgren K, Hernández L, Delatte T, Ehlert A, Liu C-M, Schluepmann H, Dröge-Laser W, Moritz T, et al (2011) The sucrose-regulated Arabidopsis transcription factor bZIP11 reprograms metabolism and regulates trehalose metabolism. New Phytol **191**: 733–45

Martínez-Silva AV, Aguirre-Martínez C, Flores-Tinoco CE, Alejandri-Ramírez ND, Dinkova TD (2012) Translation initiation factor AteIF(iso)4E is involved in selective mRNA translation in Arabidopsis thaliana seedlings. PLoS One **7**: e31606

Masutani M, Sonenberg N, Yokoyama S, Imataka H (2007) Reconstitution reveals the functional core of mammalian eIF3. EMBO J **26**: 3373–83

Mayberry LK, Allen ML, Dennis MD, Browning KS (2009) Evidence for Variation in the Optimal Translation Initiation Complex: Plant eIF4B, eIF4F, and eIF(iso)4F Differentially Promote Translation of mRNAs. Plant Physiol **150**: 1844–1854

Mayberry LK, Allen ML, Nitka KR, Campbell L, Murphy PA, Browning KS (2011) Plant cap-binding complexes eukaryotic initiation factors eIF4F and eIFISO4F: molecular specificity of subunit binding. J Biol Chem **286**: 42566–74

Meleppattu S, Kamus-Elimeleh D, Zinoviev A, Cohen-Mor S, Orr I, Shapira M (2015) The eIF3 complex of Leishmania-subunit composition and mode of recruitment to different cap-binding complexes. Nucleic Acids Res **43**: 6222–35

Merchante C, Stepanova AN, Alonso JM (2017) Translation regulation in plants: an interesting past, an exciting present and a promising future. Plant J **90**: 628–653

Meyer KD, Patil DP, Zhou J, Zinoviev A, Skabkin MA, Elemento O, Pestova T V., Qian S, Jaffrey SR (2015) 5' UTR m(6)A Promotes Cap-Independent Translation. Cell **163**: 999–1010

Missra A, Ernest B, Lohoff T, Jia Q, Satterlee J, Ke K, von Arnim AG (2015) The Circadian Clock Modulates Global Daily Cycles of mRNA Ribosome Loading. Plant Cell **27**: 2582–99

Mitchell SF, Walker SE, Algire MA, Park E-H, Hinnebusch AG, Lorsch JR (2010) The 5'-7-methylguanosine cap on eukaryotic mRNAs serves both to stimulate canonical translation initiation and to block an alternative pathway. Mol Cell **39**: 950–62

Mohammad MP, Munzarová Pondelícková V, Zeman J, Gunišová S, Valášek LS (2017) In vivo evidence that eIF3 stays bound to ribosomes elongating and terminating on short upstream ORFs to promote reinitiation. Nucleic Acids Res **45**: 2658–2674

Moore MJ, Proudfoot NJ (2009) Pre-mRNA processing reaches back to transcription and ahead to translation. Cell **136**: 688–700

Morris C, Wittmann J, Jäck HM, Jalinot P (2007) Human INT6/eIF3e is required for nonsense-mediated mRNA decay. EMBO Rep **8**: 596–602

Mulekar JJ, Huq E (2014) Expanding roles of protein

kinase CK2 in regulating plant growth and development. J Exp Bot **65**: 2883–93

Nakabayashi K, Okamoto M, Koshiba T, Kamiya Y, Nambara E (2005) Genome-wide profiling of stored mRNA in Arabidopsis thaliana seed germination: epigenetic and genetic regulation of transcription in seed. Plant J **41**: 697–709

Nishimura T, Wada T, Yamamoto KT, Okada K (2005) The Arabidopsis STV1 protein, responsible for translation reinitiation, is required for auxin-mediated gynoecium patterning. Plant Cell **17**: 2940–53

Nuñez de Villavicencio-Diaz T, Rabalski AJ, Litchfield DW (2017) Protein Kinase CK2: Intricate Relationships within Regulatory Cellular Networks. Pharmaceuticals (Basel) **10**: 27

Obayashi E, Luna RE, Nagata T, Martin-Marcos P, Hiraishi H, Singh CR, Erzberger JP, Zhang F, Arthanari H, Morris J, et al (2017) Molecular Landscape of the Ribosome Pre-initiation Complex during mRNA Scanning: Structural Role for eIF3c and Its Control by eIF5. Cell Rep **18**: 2651–2663

Park HS, Himmelbach A, Browning KS, Hohn T, Ryabova LA (2001) A plant viral 'reinitiation' factor interacts with the host translational machinery. Cell **106**: 723–33

Paz-Aviram T, Yahalom A, Chamovitz DA (2008) Arabidopsis eIF3e interacts with subunits of the ribosome, Cop9 signalosome and proteasome. Plant Signal Behav **3**: 409–11

Peixeiro I, Inácio Â, Barbosa C, Silva AL, Liebhaber SA, Romão L (2012) Interaction of PABPC1 with the translation initiation complex is critical to the NMD resistance of AUG-proximal nonsense mutations. Nucleic Acids Res **40**: 1160–73

Pereira FJC, Teixeira A, Kong J, Barbosa C, Silva AL, Marques-Ramos A, Liebhaber SA, Romão L (2015) Resistance of mRNAs with AUG-proximal nonsense mutations to nonsense-mediated decay reflects variables of mRNA structure and translational activity. Nucleic Acids Res **43**: 6528–44

Phan L, Zhang X, Asano K, Anderson J, Vornlocher HP, Greenberg JR, Qin J, Hinnebusch AG (1998) Identification of a translation initiation factor 3 (eIF3) core complex, conserved in yeast and mammals, that interacts with eIF5. Mol Cell Biol **18**: 4935–46

Pick E, Hofmann K, Glickman MH (2009) PCI complexes: Beyond the proteasome, CSN, and eIF3 Troika. Mol Cell **35**: 260–4

Pisarev A V., Hellen CUT, Pestova T V. (2007) Recycling of eukaryotic posttermination ribosomal complexes. Cell **131**: 286–99

Pisarev A V, Kolupaeva VG, Yusupov MM, Hellen CUT, Pestova T V (2008) Ribosomal position and contacts of mRNA in eukaryotic translation initiation complexes. EMBO J **27**: 1609–21

Pisarev A V, Skabkin MA, Pisareva VP, Skabkina O V, Rakotondrafara AM, Hentze MW, Hellen CUT, Pestova T V (2010) The role of ABCE1 in eukaryotic posttermination ribosomal recycling. Mol Cell **37**: 196–210

Pisareva VP, Pisarev A V (2016) DHX29 and eIF3 cooperate in ribosomal scanning on structured mRNAs during translation initiation. RNA **22**: 1859–1870

Pooggin MM, Ryabova LA (2018) Ribosome Shunting, Polycistronic Translation, and Evasion of Antiviral Defenses in Plant Pararetroviruses and Beyond. Front Microbiol **9**: 644

Querol-Audi J, Sun C, Vogan JM, Smith MD, Gu Y, Cate JHD, Nogales E (2013) Architecture of human translation initiation factor 3. Structure **21**: 920–8

Rahmani F, Hummel M, Schuurmans J, Wiese-Klinkenberg A, Smeekens S, Hanson J (2009) Sucrose control of translation mediated by an upstream open reading frame-encoded peptide. Plant Physiol **150**: 1356–67

Rajjou L, Gallardo K, Debeaujon I, Vandekerckhove J, Job C, Job D (2004) The effect of alpha-amanitin on the Arabidopsis seed proteome highlights the distinct roles of stored and neosynthesized mRNAs during germination. Plant Physiol **134**: 1598–613

Rezende AM, Assis LA, Nunes EC, da Costa Lima TD, Marchini FK, Freire ER, Reis CRS, de Melo Neto OP (2014) The translation initiation complex eIF3 in trypanosomatids and other pathogenic excavates--identification of conserved and divergent features based on orthologue analysis. BMC Genomics **15**: 1175

Rosado A, Li R, van de Ven W, Hsu E, Raikhel N V (2012) Arabidopsis ribosomal proteins control developmental programs through translational regulation of auxin response factors. Proc Natl Acad Sci U S A **109**: 19537–44

Roy B (2010) Translational Regulation in Arabidopsis thaliana : Genetic and Functional Characterization of Eukaryotic Initiation Factor 3. PhD diss., University of Tennessee, 2010.

Roy B, Copenhaver GP, von Arnim AG (2011) Fluorescence-tagged transgenic lines reveal genetic defects in pollen growth--application to the eIF3 complex. PLoS One **6**: e17640

Roy B, Vaughn JN, Kim B-H, Zhou F, Gilchrist MA, Von Arnim AG (2010) The h subunit of eIF3 promotes reinitiation competence during translation of mRNAs harboring upstream open reading frames. RNA **16**: 748–61

Ryabova L, Park H-S, Hohn T (2004) Control of translation reinitiation on the cauliflower mosaic virus (CaMV) polycistronic RNA. Biochem Soc Trans **32**: 592–6

Servet C, Benhamed M, Latrasse D, Kim W, Delarue M, Zhou D-X (2008) Characterization of a phosphatase 2C protein as an interacting partner

of the histone acetyltransferase GCN5 in Arabidopsis. Biochim Biophys Acta **1779**: 376–82

Shen X, Yang Y, Liu W, Sun M, Jiang J, Zong H, Gu J (2004) Identification of the p28 subunit of eukaryotic initiation factor 3(eIF3k) as a new interaction partner of cyclin D3. FEBS Lett **573**: 139–46

Shi Z, Fujii K, Kovary KM, Genuth NR, Röst HL, Teruel MN, Barna M (2017) Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide. Mol Cell **67**: 71–83.e7

Schepetilnikov M, Dimitrova M, Mancera-Martínez E, Geldreich A, Keller M, Ryabova LA (2013) TOR and S6K1 promote translation reinitiation of uORF-containing mRNAs via phosphorylation of eIF3h. EMBO J **32**: 1087–102

Schepetilnikov M, Kobayashi K, Geldreich A, Caranta C, Robaglia C, Keller M, Ryabova LA (2011) Viral factor TAV recruits TOR/S6K1 signalling to activate reinitiation after long ORF translation. EMBO J **30**: 1343–56

Schepetilnikov M, Ryabova L (2014) Cauliflower mosaic virus (CaMV) upregulates translation reinitiation of its pregenomic polycistronic 35S RNA via interaction with the cell's translation machinery. Plant Virus–Host Interact. Elsevier, pp 325–343

Schepetilnikov M, Ryabova LA (2018) Recent Discoveries on the Role of TOR (Target of Rapamycin) Signaling in Translation in Plants. Plant Physiol **176**: 1095–1105

Schoelz JE, Angel CA, Nelson RS, Leisner SM (2016) A model for intracellular movement of Cauliflower mosaic virus: the concept of the mobile virion factory. J Exp Bot **67**: 2039–48

Schueren F, Thoms S (2016) Functional Translational Readthrough: A Systems Biology Perspective. PLoS Genet **12**: e1006196

Simonetti A, Brito Querido J, Myasnikov AG, Mancera-Martinez E, Renaud A, Kuhn L, Hashem Y (2016) eIF3 Peripheral Subunits Rearrangement after mRNA Binding and Start-Codon Recognition. Mol Cell **63**: 206–217

Simsek D, Tiu GC, Flynn RA, Byeon GW, Leppek K, Xu AF, Chang HY, Barna M (2017) The Mammalian Ribo-interactome Reveals Ribosome Functional Diversity and Heterogeneity. Cell **169**: 1051–1065.e18

Singh B, Chauhan H, Khurana JP, Khurana P, Singh P (2013) Evidence for the role of wheat eukaryotic translation initiation factor 3 subunit g (TaeIF3g) in abiotic stress tolerance. Gene **532**: 177–85

Singh G, Jain M, Kulshreshtha R, Khurana JP, Kumar S, Singh P (2007) Expression analysis of genes encoding translation initiation factor 3 subunit g (TaeIF3g) and vesicle-associated membrane protein-associated protein (TaVAP) in drought tolerant and susceptible cultivars of wheat. Plant Sci **173**: 660–669

Singh G, Pratt G, Yeo GW, Moore MJ (2015) The Clothes Make the mRNA: Past and Present Trends in mRNP Fashion. Annu Rev Biochem **84**: 325–54

Siridechadilok B, Fraser CS, Hall RJ, Doudna JA, Nogales E (2005) Structural roles for human translation factor eIF3 in initiation of protein synthesis. Science **310**: 1513–5

Skabkin MA, Skabkina O V, Hellen CUT, Pestova T V (2013) Reinitiation and other unconventional posttermination events during eukaryotic translation. Mol Cell **51**: 249–64

Smith MD, Arake-Tacca L, Nitido A, Montabana E, Park A, Cate JH (2016) Assembly of eIF3 Mediated by Mutually Dependent Subunit Insertion. Structure **24**: 886–96

Smith MD, Gu Y, Querol-Audí J, Vogan JM, Nitido A, Cate JHD (2013) Human-like eukaryotic translation initiation factor 3 from Neurospora crassa. PLoS One **8**: e78715

Sokabe M, Fraser CS, Hershey JWB (2012) The human translation initiation multi-factor complex promotes methionyl-tRNAi binding to the 40S ribosomal subunit. Nucleic Acids Res **40**: 905–13

Somssich M, Je B Il, Simon R, Jackson D (2016) CLAVATA-WUSCHEL signaling in the shoot meristem. Development **143**: 3238–48

Soyars CL, James SR, Nimchuk ZL (2016) Ready, aim, shoot: stem cell regulation of the shoot apical meristem. Curr Opin Plant Biol **29**: 163–8

Stratmann JW, Gusmaroli G (2012) Many jobs for one good cop - the COP9 signalosome guards development and defense. Plant Sci **185–186**: 50–64

Sun C, Querol-Audí J, Mortimer SA, Arias-Palomo E, Doudna JA, Nogales E, Cate JHD (2013) Two RNA-binding motifs in eIF3 direct HCV IRES-dependent translation. Nucleic Acids Res **41**: 7512–7521

Sun C, Todorovic A, Querol-Audí J, Bai Y, Villa N, Snyder M, Ashchyan J, Lewis CS, Hartland A, Gradia S, et al (2011) Functional reconstitution of human eukaryotic translation initiation factor 3 (eIF3). Proc Natl Acad Sci U S A **108**: 20473–8

Sun Y-L, Hong S-K (2013) Sensitivity of translation initiation factor eIF1 as a molecular target of salt toxicity to sodic-alkaline stress in the halophytic grass Leymus chinensis. Biochem Genet **51**: 101–18

Szamecz B, Rutkai E, Cuchalová L, Munzarová V, Herrmannová A, Nielsen KH, Burela L, Hinnebusch AG, Valášek L (2008) eIF3a cooperates with sequences 5' of uORF1 to promote resumption of scanning by post-termination ribosomes for reinitiation on GCN4 mRNA. Genes Dev **22**: 2414–25

Thiébeauld O, Schepetilnikov M, Park H-S, Geldreich A, Kobayashi K, Keller M, Hohn T,

Ryabova L a (2009) A new plant protein interacts with eIF3 and 60S to enhance virus-activated translation re-initiation. EMBO J **28**: 3171–84

Tiruneh BS, Kim B-H, Gallie DR, Roy B, von Arnim AG (2013) The global translation profile in a ribosomal protein mutant resembles that of an eIF3 mutant. BMC Biol **11**: 123

Valášek L, Mathew AA, Shin B-S, Nielsen KH, Szamecz B, Hinnebusch AG (2003) The yeast eIF3 subunits TIF32/a, NIP1/c, and eIF5 make critical connections with the 40S ribosome in vivo. Genes Dev **17**: 786–99

Valášek L, Phan L, Schoenfeld LW, Valásková V, Hinnebusch AG (2001) Related eIF3 subunits TIF32 and HCR1 interact with an RNA recognition motif in PRT1 required for eIF3 integrity and ribosome binding. EMBO J **20**: 891–904

Valášek LS (2012) 'Ribozoomin'--translation initiation from the perspective of the ribosome-bound eukaryotic initiation factors (eIFs). Curr Protein Pept Sci **13**: 305–30

Valášek LS, Zeman J, Wagner S, Beznosková P, Pavlíková Z, Mohammad MP, Hronová V, Herrmannová A, Hashem Y, Gunišová S (2017) Embraced by eIF3: structural and functional insights into the roles of eIF3 across the translation cycle. Nucleic Acids Res **45**: 10948–10968

Vilela B, Pagès M, Riera M (2015) Emerging roles of protein kinase CK2 in abscisic acid signaling. Front Plant Sci **6**: 966

Villa N, Do A, Hershey JWB, Fraser CS (2013) Human eukaryotic initiation factor 4G (eIF4G) protein binds to eIF3c, -d, and -e to promote mRNA recruitment to the ribosome. J Biol Chem **288**: 32932–40

Wagner S, Herrmannová A, Malík R, Peclinovská L, Valášek LS (2014) Functional and biochemical characterization of human eukaryotic translation initiation factor 3 in living cells. Mol Cell Biol **34**: 3041–52

Wagner S, Herrmannová A, Šikrová D, Valášek LS (2016) Human eIF3b and eIF3a serve as the nucleation core for the assembly of eIF3 into two interconnected modules: the yeast-like core and the octamer. Nucleic Acids Res **44**: 10772–10788

Wang L, Xu C, Wang C, Wang Y (2012) Characterization of a eukaryotic translation initiation factor 5A homolog from Tamarix androssowii involved in plant abiotic stress tolerance. BMC Plant Biol **12**: 118

Wang W, Xu M, Liu X, Tu J (2016) The Rice Eukaryotic Translation Initiation Factor 3 Subunit e (OseIF3e) Influences Organ Size and Pollen Maturation. Front Plant Sci **7**: 1399

Weis BL, Kovacevic J, Missbach S, Schleiff E (2015) Plant-Specific Features of Ribosome Biogenesis. Trends Plant Sci **20**: 729–740

Wiese A, Elzinga N, Wobbes B, Smeekens S (2005) Sucrose-induced translational repression of plant bZIP-type transcription factors. Biochem Soc Trans **33**: 272–5

Winter D, Vinegar B, Nahal H, Ammar R, Wilson G V., Provart NJ (2007) An 'Electronic Fluorescent Pictograph' browser for exploring and analyzing large-scale biological data sets. PLoS One **2**: e718

Xia C, Wang Y-J, Li W-Q, Chen Y-R, Deng Y, Zhang X-Q, Chen L-Q, Ye D (2010) The Arabidopsis eukaryotic translation initiation factor 3, subunit F (AteIF3f), is required for pollen germination and embryogenesis. Plant J **63**: 189–202

Xu G, Greene GH, Yoo H, Liu L, Marqués J, Motley J, Dong X (2017) Global translational reprogramming is a fundamental layer of immune regulation in plants. Nature **545**: 487–490

Yahalom A, Kim T-H, Roy B, Singer R, von Arnim AG, Chamovitz DA (2008) Arabidopsis eIF3e is regulated by the COP9 signalosome and has an impact on development and protein translation. Plant J **53**: 300–11

Yahalom A, Kim TH, Winter E, Karniol B, Von Arnim AG, Chamovitz DA (2001) Arabidopsis eIF3e (INT-6) associates with both eIF3c and the COP9 signalosome subunit CSN7. J Biol Chem **276**: 334–340

Yamamoto H, Unbehaun A, Spahn CMT (2017) Ribosomal Chamber Music: Toward an Understanding of IRES Mechanisms. Trends Biochem Sci **42**: 655–668

Zeng L, Wan Y, Li D, Wu J, Shao M, Chen J, Hui L, Ji H, Zhu X (2013) The m subunit of murine translation initiation factor eIF3 maintains the integrity of the eIF3 complex and is required for embryonic development, homeostasis, and organ size control. J Biol Chem **288**: 30087–93

Zhou C, Arslan F, Wee S, Krishnan S, Ivanov AR, Oliva A, Leatherwood J, Wolf DA (2005) PCI proteins eIF3e and eIF3m define distinct translation initiation factor 3 complexes. BMC Biol **3**: 14

Zhou F, Roy B, von Arnim AG (2010) Translation reinitiation and development are compromised in similar ways by mutations in translation initiation factor eIF3h and the ribosomal protein RPL24. BMC Plant Biol **10**: 193

Zhou F, Roy B, Dunlap JR, Enganti R, von Arnim AG (2014) Translational control of Arabidopsis meristem stability and organogenesis by the eukaryotic translation factor eIF3h. PLoS One **9**: e95396

Zhou M, Sandercock AM, Fraser CS, Ridlova G, Stephens E, Schenauer MR, Yokoi-Fong T, Barsky D, Leary JA, Hershey JW, et al (2008) Mass spectrometry reveals modularity and a complete subunit interaction map of the eukaryotic translation factor eIF3. Proc Natl Acad Sci U S A **105**: 18139–44