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Regulace translace během imunitních reakcí

Translational control in immune response

Bachelor's thesis

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Prohlášení

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V Praze, 22.8.2013 ………………………………………………

Adam Hlaváček

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Table of Contents

Abstract 5

Abstrakt 6

List of abrevations 7

1. Introduction 9

2. Translation initiation 11

3. Translational control 14

4. Translational control in immune cells during immune response 17

4.1. Regulation of translation initiation in T cell activation and anergy 17

4.2. Regulation of translation initiation in dendrocyte activation and survival 20

4.3. Regulation of translation initiation in NK cell activation 22

4.4. Regulation of translation initiation in macrophage activation and apoptosis 24

5. Translational control in non-immune cells during immune response 26

5.1. Regulation of translation initiation in non-infected cells 26

5.2. Regulation of translation initiation in cells infected by viruses and interferon response 27

6. Conclusions 28

7. References 30

Abstract

Immune reaction often requires a prompt modification of gene expression that in turn alters cellular physiology. There are an increasing number of articles supporting a critical role of translational control in this aspect of cellular biology. The aim of this work is to present some of cellular and molecular mechanisms that connect translational control and immune reaction in immune and somatic cells and can be possibly misused by some viruses. Perhaps not surprisingly, many immunologically relevant translational control mechanisms are similar to those acting during the stress response.

Over the years it has been documented that the T cells, dendrocytes, Natural killer cells and macrophages utilize translational control for their immunological activation following stimulation. Combination of general and gene-specific translational control mechanisms enables fast changes in proteome and physiology that are characteristic for immune cell activation. The overall impact of translational control on immune response is further illustrated by the fact that it acts upon each stage of life of immune cells – from their activation, through survival, to a programmed cell death.

Even in some non-immune cells the translational control plays an important role with respect to immunity, as these cells are known to have an ability to influence the overall immune response. For example, fibroblasts and endothelial cells were shown to modify expression of pro-inflammatory proteins in response to cytokines and other stimuli. Also, cells infected by viruses rapidly alter their mode of expression via translational control mechanisms in order to reduce the spread of infection.

Abstrakt

Imunitní reakce často vyžaduje rychlé změny v expresi genů, které mění fyziologii daných buněk. Množství článků dokládajících rozhodující vliv translační kontroly v tomto aspektu biologie buňky roste. Cílem této práce je poukázat na některé buněčné a molekulární mechanismy, které spojují translační kontrolu a imunitní reakce v buňkách imunitního systému, ale také ostatních somatických buňkách a popřípadě mohou být zneužívány některými viry. Řada imunologicky významných kontrolních mechanismů vykazuje podobnost s mechanizmy účastnícími se odpovědi na stress.

V minulých letech bylo doloženo, že T buňky, dendrocyty, přirození zabíječi (z angl. natural killer) a makrofágy využívajítranslační kontrolu jako součásti aktivace imunitních buněk následující po stimulaci. Kombinace obecných a genově-specifických mechanismů translační kontroly umožňuje rychlé změny v proteomu a fyziologii, které jsou charakteristické pro aktivaci imunitních buněk. Celkový účínek translační kontroly na imunitní odpověď je dale dokreslen tím, že se podílí na všech fázích aktivace imunitní buňky – vlastní aktivaci, přežití a smrti buňky.

Také translační kontrola v ne-imunitních buňkách je významná s ohledem na imunitu, protože tyto buňky mají schopnost ovlivňovat celkovou imunitní odpověď. Například fibroblasty a endotelialní buňky mění v závislosti na cytokinech a dalších stimulech expresi prozánětlivých proteinů. Také buňky infikované viry prudce mění způsob exprese skrz translační kontrolu ve snaze zamezit šíření infekce.

List of abrevations

Akt	protein kinase B
Apaf 1	apoptotic protease activating factor 1
APC	antigen presenting cell
ARE	AU rich element
ARE-BP	AU rich element - binding protein
AUG	stat codone (adenin-uracil-adenin)
COX-2	cyclooxygenase 2
DALIS	dendritic cell aggresome-like structure
DAMP	damage associated molecular pattern
DC	dendrocyte
DNA	deoxyribonucleic acid
DRiPs	defective ribosomal products
ds RNA	double strand RNA
eIF	eukaryotic initiation factor
ERPS	glutamyl- prolyl-tRNA synthetase
GAIT	IFN-γ–activated inhibitor of translation
GAPDH	glyceraldehydes-3-phosphate dehydrogenase
GCN 4	general control non repressed
GDP	guanosin diphosphate
GEF	GTP exchange factor
GTP	guanosin triphosphate
Gzmb	Granzyme B
HuR	human antigen R
IAP	inhibitor of apoptosis
IFN	interferon
IL	interleukin
iNOS	inducible nitric oxide synthase
IRE	iron response element
IRES	internal ribosome entry site
IRP	iron response protein
ITAF	IRES trans-acting factor
LPS	lipopolysaccharide
Met-tRNAiMet	methionyl initiator tRNA
MHC	major histocompatibility complex
miRNA	micro RNA
MRE	miRNA response element
mRNA	messenger RNA
mTOR	mammalian target of rapamycin
m7G	7-methylguanosine
NK cell	natural killer cell

PABP	poly(A)-binding protein
PAMP	patogen associated molecular patern
PIC	pre-initiation complex
PI3K	phosphatidylinositide 3-kinase
PKR	protein kinase RNA-activated
poly(A)	polyadenosine
Prf 1	perforin
P site	peptdyl site
RIG	retionic acid-inducible gene
RNA	ribonucleic acid
S	Svedberg, unit of sedimentation coeficient
ss RNA	single strand RNA
TC	ternary complex
TCR	T cell receptor
TIA	T cell inflamatory antigen
TIAR	TIA-related protein
TLR	Toll-like receptor
TNF	tumor necrosis factor
TTP	tristetraprolin
uORF	upstrea open reading frames
UTR	untranslated region
4E-BP	eIF4E-binding protein
40S	eukaryotic small ribosomal subunit
43S	pre-initiation complex
48S	pre-initiation compex with attached mRNA
60S	eukaryotic large ribosomal subunit
80S	assembled ribosome 40S + 60S subunit

1. Introduction

Translation is one of the informational transfers in central dogma of molecular biology. The path of the information flow from genes (encoded in DNA) to proteins is not as straightforward as it might seem from the textbook illustrations. Gene expression can be regulated at many of if steps and sub-steps in terms of both quantity and quality. The degree of this regulation further varies in its scale, impact and effectiveness by which it influences the overall expression profile of the cell. Undoubtedly, transcription of genetic information from DNA to pre-mRNA is the energetically most efficient point of regulation, but on the other hand it takes a relatively long time to put the changes in transcriptome into effect in proteome, which is the ultimate goal. Hence many other regulatory points following those operating on the transcriptional level evolved to speed up the whole process where needed, such as those acting upon RNA splicing, transport of mRNA from nucleus to cytoplasm, mRNA stability and finally on its translatability.

Translation is the last major step in gene expression. It is a process that converts information encoded in sequence of mRNA's nucleotide string into a chain of amino acids in a polypeptide. Translation is one of the fundamental cellular processes, which, from the human health point of view, represents a very important target for various drugs and antibiotics owing to the fact that a long term inhibition of proteosynthesis leads to cellular death.

As such, it is not surprising that regulation of translation is one of the key ways how to regulate protein expression from both qualitative and quantitative point of view. Translation takes place in the cytoplasm, where the messenger RNA (mRNA) is brought to the small 40S ribosomal subunit that is subsequently joined by the large 60S subunit to form the 80S initiation complex. The entire process of translation is divided in four functional steps – initiation, elongation, termination and ribosome recycling. Each of these steps requires specific factors – proteins and protein complexes with vital functions for each and every step.

A growing number of relatively recent studies suggests that translational control significantly impacts cellular immunity. Given the need for a swift reaction on an immunological stimulus, translational control is ideal for desired phenotypical changes accompanying many immunological events. Interestingly, importance of translation control in immunity is not restrained only to leukocytes such as T cells (Grolleau et al. 2002), dendrocytes (DC) (Ceppi et al. 2009), natural killer (NK) cells (Fehniger et al. 2007) and macrophages (Kitamura et al. 2008), all of which, when undergoing activation, exhibit changes in translational profiles. Also translational control in several somatic, non-immune cells can be immunologically relevant. For example, in infected cells

translational control is vital for attenuation of viral replication. In endothelial cells (Kraiss et al. 2003) and also in LPS or TNF-α stimulated fibroblasts (Hao and Baltimore 2009)specific translational changes are observed leading to modulation of immune response in a given microenvironment.

The aim of this text is to present the reader an overview of various immunologically relevant cellular events that are critically influenced by translational control.

2. Translation initiation

Translation initiation in eukaryotic cells relies on complex interactions between the mRNA, methionyl initiator tRNA (Met-tRNA$_i^{Met}$), eukaryotic initiation factors (eIFs) and ribosomal subunits 40S and 60S. The result of these interactions is a fully assembled 80S ribosome with Met-tRNA$_i^{Met}$ bound in its P (peptidyl- tRNA) –site that is with its anticodon base-paired to the start AUG codon of mRNA (Algire et al. 2002). A basic outline of the translation initiation pathway is presented in Figure 1.

Cap-dependent translation initiation in eukaryotes starts with assembly of a ternary complex (TC) composed of Met-tRNA$_i^{Met}$, eIF2 and GTP bound to eIF2. In order to associate with Met-tRNA$_i^{Met}$ effectively, eIF2 has to be in its GTP form (eIF2-GTP) (Levin et al. 1973). eIF2 has an internal GTPase activity, which is activated by eIF5 in later stages of initiation, and leads to conformational changes important for AUG recognition and locking the Met-tRNA$_i^{Met}$ on the AUG start codon (Yamamoto et al. 2005). When eIF5 stimulates the GTPase activity of eIF2, GTP is hydrolyzed to free phosphate and GDP and following the AUG recognition, the eIF2-GDP binary complex leaves the initiating complex. For the new round of initiation, it has to be recycled back to its GTP form by the GTP exchange factor (GEF) eIF2B, which dramatically increases the efficiency of this nucleotide exchange. Importantly, this exchange of GDP to GTP on eIF2 is one of the two key regulatory steps in general translational control as the changes in eIF2-GTP levels have a dramatic impact on the overall translation initiation rates. The eIF2B GEF activity can be inhibited by phosphorylation on the Ser-51 residue of the eIF2-α subunit as this way modified eIF2 complex has a higher binding affinity for eIF2B than a non-phosphorylated form of eIF2. Phosphorylated eIF2 bound to eIF2B inhibits the GDP to GTP exchange and therefore lowers the amount of available eIF2-GTP for formation of the TC, which is the key to the initiation process. (Krishnamoorthy et al. 2001).

Assembly of the 43S pre-initiation complex (PIC) from 40S ribosomal subunit and the TC, as the next step in initiation, is promoted by the eukaryotic initiation factors eIF1, eIF1A, eIF3 and eIF5. Mainly eIF3 acts as a nucleation core for the 43S PIC and later on also for the 48S PIC, containing mRNA (Asano 2000). Mammalian eIF3 is composed of thirteen different subunits and it influences translation on many levels. Mammalian eIF3 contains binding sites for eIF1, eIF2 (Valášek et al. 2002), eIF4G (Siridechadilok et al. 2005), the 40S ribosomal subunit (Fraser et al. 2004) and also for eIF5 that plays an important role in formation of the 43S PIC as it interacts with other eIFs that are present in PIC, namely eIF1, eIF2 and eIF3 (Yamamoto et al. 2005). The interaction of eIF3 with eIF4G seems to be especially important for the 48S PIC assembly as it brings the activated mRNA into contact with the 43S PIC (LeFebvre et al. 2006)

In detail, to recruit the capped mRNA to the 43S PIC, it first has to be activated by a complex of specific mRNA-binding proteins. This complex called eIF4F promotes 43S PIC attachment to mRNA and represents the second major of general regulatory mechanisms. eIF4F is composed of eIF4E, eIF4A and eIF4G. eIF4E binds the cap structure and can be negatively regulated by phosphorylation or by binding of eIF4E-binding proteins (4E-BPs) that inhibit the eIF4F complex formation and thus strongly repress translation (Haghighat et al. 1995). eIF4A has a helicase function that creates a landing pad for the 40S ribosomal subunit on mRNA. eIF4A helicase activity is also needed on the 5' UTRs with a rich secondary structure (Pestova and Kolupaeva 2002). eIF4G serves as a scaffold protein bringing together not only eIF4E and eIF4A, as parts of the eIF4F complex, but also poly(A)-binding protein (PABP) and eIF3 (Hinton et al. 2007). A bond between eIF4G and PABP circularizes mRNA and creates an opportunity for interaction of elements present on 3' UTR with eIFs present on 5' UTR. eIF4G and eIF3 binding then promotes attachment of activated mRNA to the 43S PIC resulting in a formation of the 48S PIC. eIF4G is often a target of cleavage by numerous viruses disrupting cap-dependent translation, because binding domains of eIF4G can be separated and still retain their functions. The N-terminal fragment contains binding site for eIF4E, thereby effectively competes with non-cleaved eIF4G for eIF4E binding and thus inhibits cap-dependent translation. The C-terminal fragment has the ability to bind eIF3 and eIF4A and takes part in the internal ribosome entry site (IRES) mediated translation (Zamora et al. 2002). As aforementioned, the cleavage of eIF4G or dephosphorylation of eIF4E are important regulatory tools in translational control of the cell. Therefore various members of the eIF4 gene families evolved over times with varying binding activities, affinities and functions to further increase range of regulatory tools (reviewed in (Hernández and Vazquez-Pianzola 2005).

Upon formation of the 48S PIC, the ribosome can start its search for usually the first AUG codon in the process called scanning, which is greatly influenced by eIF1 and eIF1A. eIF1 binding to the 40S subunit stabilizes the "open" conformation, that favors scanning, but is inadequate for start codon selection in most cases. Only the AUG codon with a suitable nucleotide context can be selected as a start codon and promote a conformational change of the ribosome from the scanning-conducive, "open" to scanning-incompetent, "closed" conformation (Pestova and Kolupaeva 2002). eIF5 promoting the GTPase activity of eIF2 also stimulates eIF1 dissociation from the P site after AUG start codon recognition; both of these actions further stabilize the "closed" conformation. Upon AUG recognition, most of the eIFs dissociate from the 48S PIC, which is subsequently joined by the large ribosomal subunit 60S to form the 80S initiation complex ready for proteosynthesis (reviewed in (Valášek 2012)).

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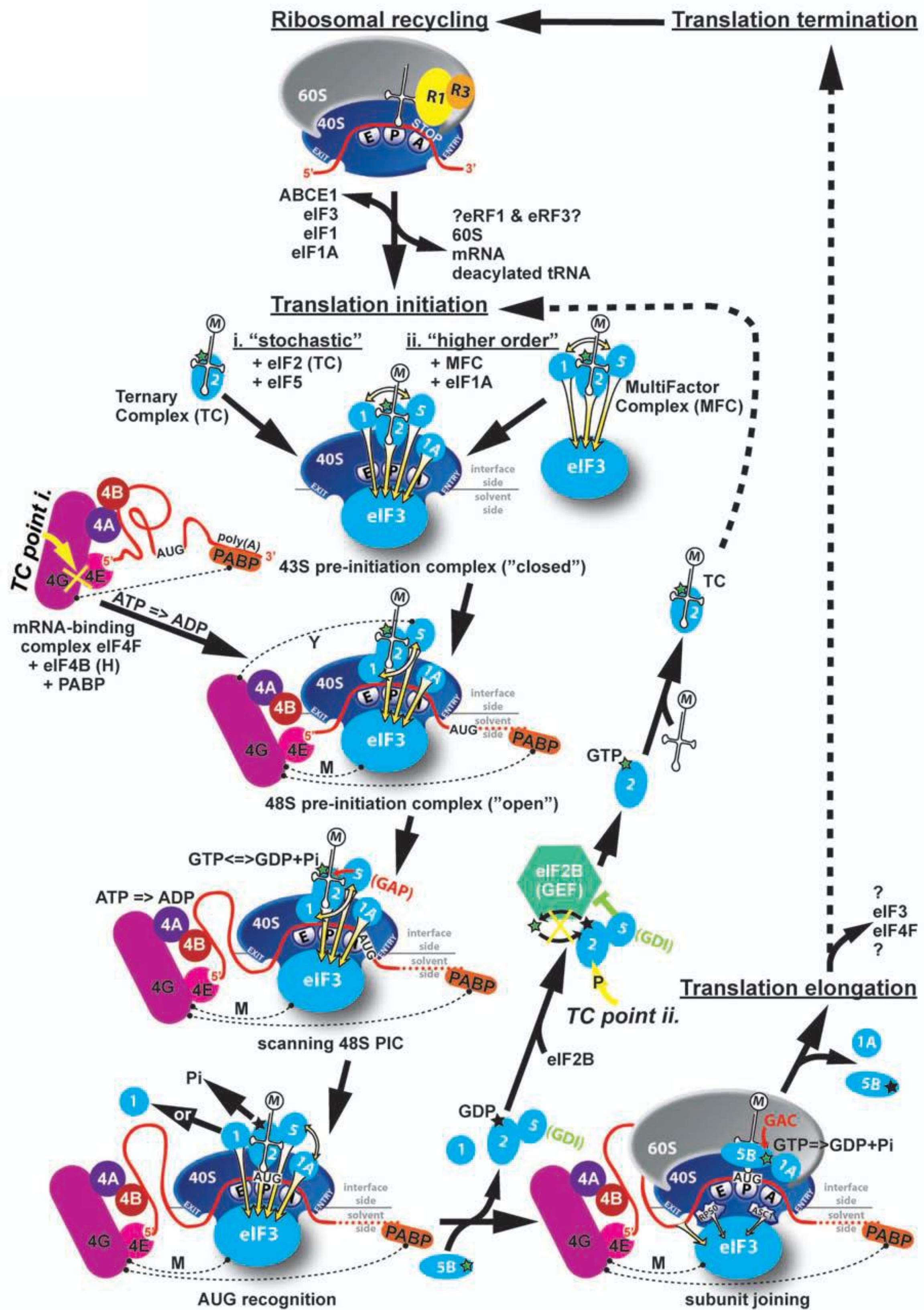

Figure 1: Schematic of eukaryotic cap-dependent translation initiation. (Valášek 2012)

3. Translational control

Each protein in the cell is synthesized in the process of translation. If we consider the turn-over time of proteins and constant need to replenish them, it makes translation one of the energetically most demanding processes in the cell. Thus, the translation has to be strictly regulated in order to enable the cell to react to internal and external stimuli. Translational control can be general or gene-specific. The general control is mostly governed by ribosome and active eIFs availability in cytosol (see above). The gene-specific control is mediated by various cis- and trans-acting elements occurring in the 5’ and 3’ untranslated regions (UTR) of mRNAs (Sonenberg and Hinnebusch 2009).

The most abundant and probably the most crucial form of general translational control is the universal down-regulation of proteosynthesis during cellular stresses such as for example hypoxia, amino acid starvation, unfolded protein response etc. (Holcik and Sonenberg 2005), the mechanistic details of which are described above. Less abundant but still very critical control mechanisms operate only on certain groups of mRNAs, leaving the translatability of the others unaffected. For example, the internal ribosomal entry site (IRES) dependent translation is specific for mRNAs coding for pro- and anti- apoptotic genes and genes connected to stress response (Nevins et al. 2003). According to an IRES database (*http://iresite.org*), there is up to 115 cellular mRNAs with the IRES sequence (Mokrejs et al. 2006). It is noteworthy that in spite of their relatively small number, a reasonable fraction of those cellular mRNAs that do contain an IRES takes part in stress response to heat shock, amino acid starvation, and participate in regulation of stress survival and apoptosis. Highly structured 5’ UTR and certain motifs containing 3’ UTR are specific for mRNAs coding proliferative factors, transcriptional factors, oncogenes and immunologically active proteins (interleukins etc.) (Kozak, 1991). The translation control via modification of eukaryotic initiation factors (eIFs) and their mutual interactions and interactions with mRNAs is also an efficient mean of regulation and as such it represents one of the main topics of this text.

5’ UTR can contain various cis- acting elements that contribute to both general and specific translational control. These elements are: (i) the 7-methylguanosine (m7G) cap structure, (ii) primary sequence context of the start codon, (iii) upstream open reading frames (uORFs), (iv) specific secondary structures, (v) internal ribosomal entry sites (IRES) and others. (i) The m7G cap structure is present in all nuclear eukaryotic mRNAs and is essential for scanning dependent translation. (ii) A primary sequence context of the start codon affects the efficiency with which the 48S PIC selects a given AUG codon, and thus can influence usage of alternative, non-canonical start sites (Kozak, 1986). (iii) Upstream open reading frames (uORFs) generally inhibit translation of most mRNAs,

however, in some special cases - like in the case of yeast GCN4 – they can be a part of a very sophisticated gene control mechanism (Abastado et al. 1991). (iv) Secondary structures and their stability and position can obstruct 43S interaction with mRNA via steric hindrance, if present close to cap structure. Secondary structures in 5' UTR can also bind regulatory proteins. Well known example of this phenomenon is iron response element (IRE) in 5' UTR of ferritin mRNA and iron response proteins (IRPs) that bind this element and inhibit translation (reviewed in (Rouault 2006)). (V)Other example of very specific secondary structures and their role in 5' UTR are the internal ribosomal entry sites (IRES), which are responsible for cap-independent translation. IRES is a sequence in the 5'UTR of mRNA preceding the coding sequence in a similar way as the Shine-Dalgarno motif in prokaryotic mRNAs. It facilitates assembly of the translation machinery at a position close to or directly at the initiation codon that is independent on cap-mediated translation initiation (Chen and Sarnow 1995). The last but not least important attribute of 5' UTR is simply its length. It has to be sufficient to support ribosome loading as the translation becomes more efficient with increasing length of 5' UTR. In majority of higher eukaryotic mRNAs the 5' UTR length varies from 20 to 60 nucleotides, but 5' UTRs as long as 450 nucleotides are not exceptional (Connor and Lyles 2002).

3' UTRs also harbor many structures important for translational control. The most important feature is the polyadenosine (poly(A)) tail at the very 3' end of mRNA. Length of poly(A) tail influences a half-life of mRNA and through its interaction with poly(A) binding protein (PABP) also translation efficiency. There are also other means of translational control operating at 3' UTR. Micro RNAs (miRNAs) are the recently discovered one the most pronounced small non-coding RNAs, which interfere with translation and destabilize transcripts by binding to partially complementary sequence in the 3' UTR called miRNA response elements (MRE). An immunologically important miRNA is for instance the miR-155, which is upregulated by LPS or dsRNA stimulation. miR-155 silences translation of several pro-inflammatory transcripts (Mazumder et al. 2010) and enhances translation of others, for instance TNF-α (Piccinini and Midwood 2012), suggesting a context dependent function.

3' UTRs contain also various AU-rich elements (ARE). These cis-acting elements attract AU rich element binding proteins (ARE-BPs) that can alter the mRNA half-life through interactions with nucleases. And since the mRNA is circularized during translation they can also interact with eIFs at 5' UTR and affect the translational rates (Nakagawa 2008). The most prominent ARE-BP is human antigen R (HuR). HuR has mostly beneficial effect on mRNA stability, but studies on its role in translational control seem ambiguous, suggesting that HuR's role in translation is influenced by context of other ARE-BPs and cis- and trans- acting elements present on the mRNA (Katsanou et al. 2005) (Sureban et al. 2007).

To conclude this part, as recently demonstrated, post-transcriptional and translational control are responsible for a significantly larger portion of phenotypical changes then anticipated – in mouse embryonic fibroblasts only about 40% of variation in protein levels can be explained by variation in transcriptome (Schwanhäusser et al. 2011). The main reasons for utilization of translational control as the tool of regulation of gene expression are its immediacy, accuracy, efficiency and redundancy. Translational control affects cellular phenotype in matter of minutes. General translational down-regulation or increased translation from pre-synthesized mRNA that was previously translationally silenced saves time in comparison to de novo synthesis of mRNA (Fehniger et al. 2007). Translation control can also achieve much more precise control and fine tuning at the late stage of gene expression than transcriptional control at its beginning. It can act in combination with transcriptional control to ensure that expression of toxic or proliferation stimulating proteins is strictly regulated on multiple levels and that they are expressed only when needed (Brant-Zawadzki et al. 2007).

4. Translational control in immune cells during immune response

Immune system takes part in maintaining the integrity and homeostasis by reacting on external (bacterial, viral and many others) or internal (cells with pathological phenotype) agents unfamiliar to the organism. In these reactions there is often a need for swift change in cell's proteome. This change is attainable by several means also on the post-transcriptional level of gene expression, most notably during translation initiation, in particular by modifications of eukaryotic initiation factors. The well-studied examples of this type of regulation are rapid changes in translational rates and modes of operation occurring during activation of dendrocytes (DC), T cells, macrophages and NK cells.

4.1. Regulation of translation initiation in T cell activation and anergy

T cells can be divided into many subgroups according to their function and structure. The very basic division is based on presence of surface molecules CD4 and CD8. CD8+ cells are called cytotoxic T cells, CD4+ are called T helper cells and are divided into functional subgroups: T_h1 and T_h2, T_h17 and T_{reg} (O'Brien and Zhong 2012).

In order to gain its function, T cell has to go through activation. During this process the naive T cell is transformed and gains specific functions. T cell activation is a good example of physiological changes exerted by specific translation control of various transcripts. Translational control is used as a very efficient and specific tool for fast cellular differentiation in this case.

Stimulation of a T cell receptor (TCR) by the right antigen and stimulation by proliferative lymphokines triggers chain of biochemical changes in a T cell. TCR stimulation activates signaling cascade PI3K/AKT/mTOR and leads to increase in cytoplasmatic concentration of calcium cations and diacylglycerol (DAG) (Gorentla et al. 2011). Mammalian target of rapamycin (mTOR) kinase integrates signals from many pathways and according to them modulates translational rates by phosphorylation of 4E-BP1 (as an eIF4E inhibitor) and S6K. Phosphorylation of 4E-BPs releases eIF4E to promote recruitment of ribosome machinery in protein translation (Richter and Sonenberg 2005). Activated S6K can then phosphorylate other targets like S6 small ribosomal protein (Jefferies et al. 1997), eukaryotic elongation factor 2 (Wang et al. 2001) or eIF4B (reviewed in (Gingras et al. 2001)), yet the exact mechanism how it contributes to translational regulation remains unclear. These changes occur in the matter of tens of minutes, between 15 and 30 minutes, after stimulation and they markedly enhance translational rates. TCR stimulation influences cellular amounts of an array of proteins impacting translational control, which are made more available and abundant in the cytoplasm.

Effected proteins are for example eIF2B, which serves as GEF for eIF2-GDP and thereby enables increase in the initiation rates (Kleijn and Proud 2002), or HuR (Fan and Steitz 1998), which stabilizes transcripts with AREs in their 3' UTR. These changes permit the rise in the translational rates for the mRNAs present in the cell and this fast increase in translation represents a fundamental step in the T cell activation process that precedes other changes in their physiology, such as transcriptional changes.

The latter change in the activated T-cell translational rates varies for various mRNA species; some transcripts are more sensitive to these changes than the others. This is determined by the fact that not only the general translational control is involved, but also a specific translational control utilizing the sequential motives present in the mRNA's 5' and 3' UTRs contribute to the overall regulation. In the following study on the T-cells activation (Garcia-Sanz et al. 1998) it was observed that 12,6% of mRNAs were translationally regulated. Despite a general increase in translation efficiency after the T cell activation, ~4,7% of these mRNA species were translationally repressed. This nicely demonstrates the delicate specificity of the entire process, as the increased translational rate in general is simply not a warranty of a more effective translation of all transcripts.

One of the translationally upregulated genes is TNF-α. This signaling molecule participates in pro-inflammatory reaction by activation of transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-ƘB) and mitogen-activated protein kinase (MAPK). The 3' UTR of TNF-α mRNA contains AU-rich elements (AREs) that can control the transport of the TNF-α mRNA from the nucleus to the cytoplasm (Dumitru et al. 2000), destabilize the message (Kontoyiannis et al. 1999), and inhibit its translation (Han et al. 1990). The regulation conferred by the AREs is mediated through proteins that bind to them - the ARE binding proteins (ARE-BPs). In resting T cells, ARE-BPs that participate in shortening of poly(A) tail prevail, but in activated T cells the ratio of stabilizing and destabilizing ARE-BPs shifts towards ARE-BPs with protective function (Buxadé et al. 2005).

One of the ARE-BPs with a protective function is HuR, which is also up regulated during the T cell activation HuR protects mRNA from ARE-dependent degradation. During the T cell activation, HuR is phosphorylated and migrates from karyoplasm to cytoplasm. This consequently increases the half-life of mRNAs containing AREs. The share of these transcripts is substantial, up to 11% of mRNA species in cell contains ARE and among them are major cytokines and lymphokines. You can find examples of ARE containing cytokines in Table 1. From 950 mRNA species with AREs tracked during THP1 cell activation, 40 were stabilized (reviewed in (Nakagawa 2008)).

Another protein specifically regulated on translational level is the interleukin 2 receptor (IL-2R) subunit α. IL-2 signaling induces expression of cyclines necessary for transition from G0 to G1 and

from G1 to S phase. Stimulated T cells produce IL-2 in autocrine manner to induce their own activation and proliferation. The IL-2Rα mRNA contains upstream open reading frames (uORFs) in its 5’UTR. These uORFs decrease translational rates of IL-2Rα mRNAs (Weinberg and Swain 1990) when the amount of active eIF is high, as in the case of activated T cells. This decreases expression of receptors for IL-2 on the surface of activated T cells and balances effect of increased expression of IL-2 by the T cell, effectively modulating the strength of proliferative signals effecting T cell. IL-2 does not only play a vital role in T cell activation and proliferation, but also in the induction of T cell anergy. During the establishing of anergy there is drop in the level of IL-2 protein, but the level of its mRNA stays stable. The number of ribosomes bound to IL-2 mRNA declines from polysomes in activated T cell, to one or none in anergyc cell. This event is IL-2 specific, house-keeping genes are translated in same extend as in active T cells. Additionally, sucrose gradients showed that IL-2 mRNA is able to bind pre-initiation complex, but there is only one ribosome bound on the mRNA (Garcia-Sanz and Lenig 1996). These results suggest translation initiation as the regulatory point; the exact mechanism of control however remains elusive.

Th1 group	
INF-γ	U**AUUUA**UU**AUUUA**ACAUU**AUUUA**UAU
IL-2	U**AUUUA**UUUAAU**AUUUA**AAUUUUAU**AUUUA**UU
IL12	GUUUGUUU**AUUUAUUUAUUUA**UUUUUGCAU
Th2 group	
IL-4	AUAUUUUUA**AUUUAU**GAGUUUUUGAUAGCUUUAUUUUUA
IL-5	AUUUGGUAAAUUAGU**AUUUA**UUUAAUGUUAU
IL-10	**AUUUAUUA**CC UCUGAUACCU CAACCCCCAUUUCU**AUUUAUUUA**CUGAGCU
Th17 group	
IL-17A	CUUGGGAAUUUUAUU**AUUUA**AAAGGUAAAACCUGU**AUUUA**UUUG
	AGCU**AUUUA**AGGAUCUAUUUAUGUUUAAGUAUUUAG
IL-23A	UGGGGACAGUUUGGGGAGGAUU**AUUUA**UUGU**AUUUA**UAUUUGAAUUA
	UGUACUUUUUUCAAUAAAGUCUUAUUUUGUGGCUAAAAAAA
IL-6	ACACU**AUUUA**AUUAUUUUUA**AUUUA**UUAAU**AUUUA**AAUAUGUG
	AAGCUGAGUUA**AUUUA**UGUAAGUCAU**AUUUA**UAUUU
Treg group	
IL-10	UCAACCCCCAUUUCU**AUUUAUUUA**CUGAGCUUCUCUGUGAACG**AUUUA**G
	AAAGAAGCCCAAUAUUAUAAUUUUUUUCAAU**AUUUA**UUAUUUCACCUGUUUUU
IL-2	U**AUUUAUUUA**AU**AUUUA**AAUUUUAU**AUUUA**UU
Other immunologically important cytokines	
IL-1β	U**AUUUAUUUAUUUA**UUUGUUUGUUUGUUUUAUU
IL-8	U**AUUUA**UU**AUUUA**UGU**AUUUAUUUA**A
TNF-α	AUU**AUUUA**UU**AUUUAUUUA**UU**AUUUAUUUAUUUA**
IL-13	A**AUUUA**UUGUUUUUCCUCGU**AUUUA**AAUAUUAAAUAUGUU
COX-2	UAUUA**AUUUA**AUUAUUUAAUAAU**AUUUA**UUAAAA

Table 1: Cytokines containing ARE in 3′ UTR of mRNAs (Nakagawa 2008)

4.2. Regulation of translation initiation in dendrocyte activation and survival

Dendrocytes (DC) belong among antigen presenting cells (APC). Their function is to activate antigen specific immune response through interaction with T cells. Dendritic cells recognize general pathogen associated molecular patterns (PAMPs) due to their receptors from toll-like receptor (TLR) and retinoic acid-inducible gene (RIG) family. The signals transferred by these receptors cause DC maturation and critically affect presentation of peptide fragments by major histocompatibility complexes (MHC) (reviewed in (Lee and Kim 2007).

TLR 4 stimulation by LPS stimulates signaling cascade PI3K/AKT/mTOR leading to phosphorylation of many intracellular proteins. These include: mTOR's target 4E-BP1 and S6K as

described in above in chapter 4.1. and depicted in Figure 2. All these changes lead to increase in the translational rates of cap-dependent transcripts; in the first four hours after activation the translational rates get elevated by ~3-4 times (Lelouard et al. 2007). This increase in translational rates is substantial for the antigen presenting function of mature dendrocytes. Extensive boost of translation leads to formation of stress-like response**.** Many of newly synthesized polypeptides form defective ribosomal products (DRiPs), which are unfolded, ubiquitinilated and subsequently degraded in proteasome, products of degradation are then presented by MHCs on cellular surface. DRiPs aggregate into dendritic cell aggresome-like structure (DALIS). In DALIS are these polypeptide fragments protected from cleavage and are stored for later processing by proteasome and MHC. DALIS might function as a storage units and as loading controllers for presentation of antigens by MHC (Lelouard et al. 2002). The existence of many unfolded proteins can lead to activation of unfolded protein response (UPR) by PKR-like endoplasmatic reticulum kinase (PERK) and other stress activated kinases. PERK activation leads to phosphorylation of eIF2-α subunit, which in turn leads to decrease in cap dependent translation (Schröder and Kaufman 2005). Prolonged UPR stimulates apoptosis.

Approximately 8 hours after stimulation by LPS, the mode of translation shifts from the cap-dependent form prevalent in early stage of maturation to IRES mediated translation in the later stage. This change is caused by proteasomal cleavage of eIF4GI; interestingly, the resulting fragments slightly vary from fragments generated by viral proteases or pro-apoptotic cleavage by caspases. These fragments still possess their binding activity, so they can interfere with the cap binding activity of intact eIF4GI and other fragments can function as factors for IRES (Lelouard et al. 2007). The level of eIF4GI rises in this stage of maturation as its translation is IRES dependent. Level of DAP5, member of eIF4G family, rises as well, since it is also translated in the IRES-dependent manner. Cleavage of those eIFs is important, because fragments generated by this cleavage work as factors for IRES and without them, there would not be any IRES mediated stress response (Holcik and Sonenberg 2005). Genes translated in this later phase of dendrocyte maturation are mostly genes connected to stress reaction and survival, like the inhibitor of apoptosis (IAP) etc. (Warnakulasuriyarachchi et al. 2004).

Based on these findings, two stages can be distinguished in the dendrocyte maturation. First stage is characterized by increased translation of cap-dependent transcripts that code for proteins essential for activation of the dendrocyte's APC function. The second one is characterized by decrease in scanning mediated translation and an increase in the IRES-mediated translation. This change is required for survival of activated dendrocyte, because long-lasting high levels of unfolded DRiPs accumulated in DALIS and act as a strong pro-apoptotic signal that has to be overcome by increased expression of anti-apoptotic proteins, which are expressed in the IRES-dependent manner.

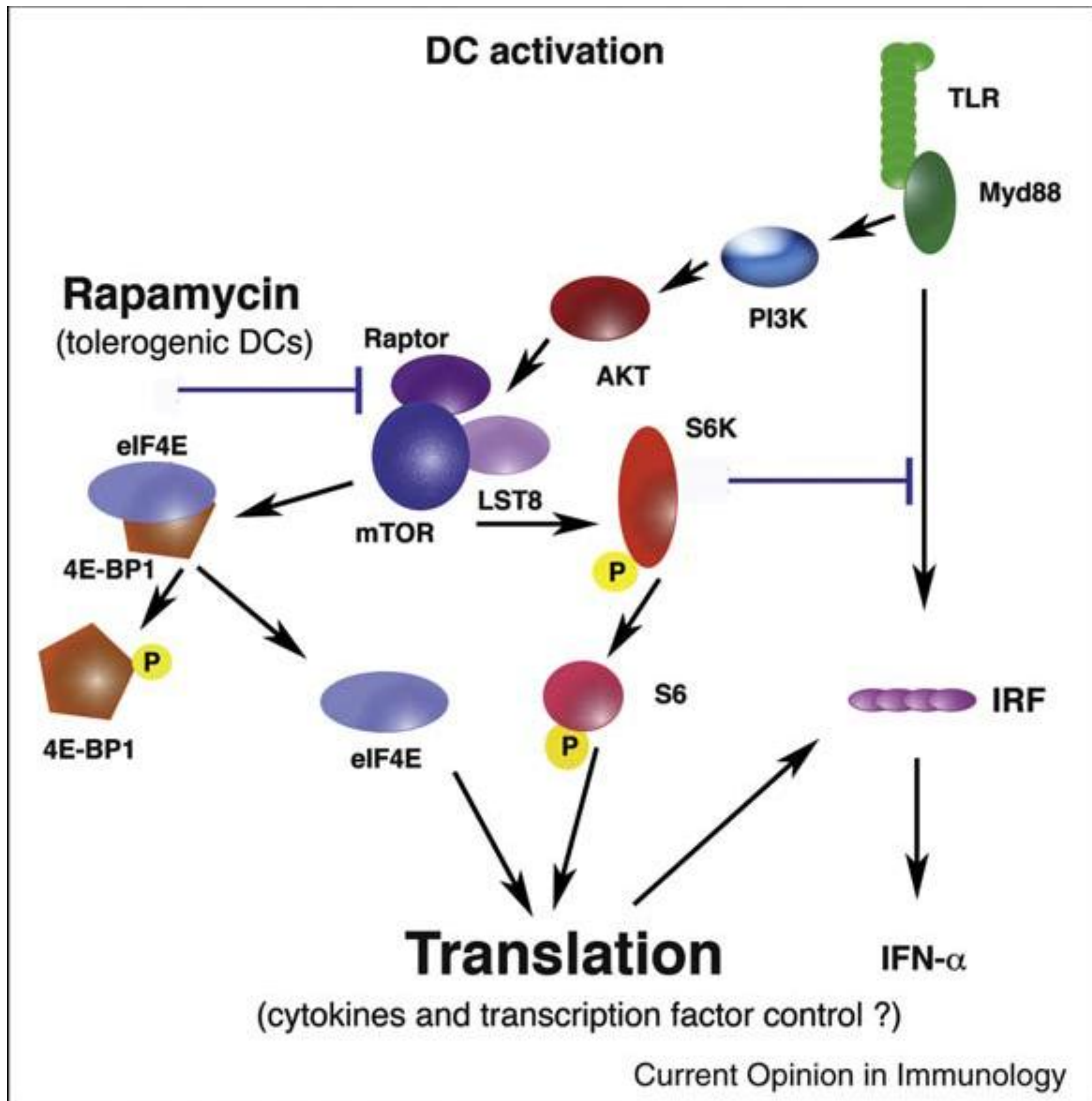

Figure 2: Dendrocyte signaling pathway influencing translation control. (Pierre 2009)

4.3. Regulation of translation initiation in NK cell activation

Natural killer (NK) cells are a potent part of innate immunity. They act as cytotoxic cells, but they functionally differ from the CD8+ T cells. Whereas T cells can be activated only by cells with MHC that bind to their T cell receptor, NK cells can be activated by any cell expressing pathogen associated molecular patterns or damage associated molecular patterns (DAMPs) on its surface. NK cells target mostly cells infected by viruses and also tumorogenic cells. Other significant function of

NK cells is production of immunoregulatory cytokines such as IL-10, TNF-α and most importantly INF-γ (reviewed in (Zucchini et al. 2008)).

Resting murine NK cells are far less efficient in cytotoxicity against tumor cells than activated NK cells. Main effectors of cytotoxic function of NK cell are perforins and granyzmes. Study on resting murine NK cells show clues for post-transcriptional and translational control of perforin (Prf 1) and granzyme B (Gzmb). While mRNA levels of Prf and Gzmb, between resting and activated cells vary only slightly, their protein levels increase up to 4-fold. Activation is achieved by IL-15 or IL-2 stimulation, 6 hours after stimulation levels of Prf and Gzmb markedly increased and after 24 hours 86% of NK cells were activated. This noticeable boost in Gzmb and Prf1 protein production and only a small change in mRNA abundance suggests that activation is caused by the release of a yet to be described block in mRNA translation (Fehniger et al. 2007).

NK and T cells are main producers of INF-γ. They produce it after stimulation by infected cells or by cytokines IL-2 or IL-12. As many other cytokines, IFN-γ mRNA has ARE sequences in its 3'UTR that bind trans-acting ARE-binding proteins. Tristetraprolin (TTP) is a significant player in IFN-γ degradation, other ARE-binding proteins such as HuR, T cell inflammatory antigen (TIA) or TIA-related protein (TIAR) affect mRNA stability, half-life and translational activity (Piecyk et al. 2000). IFN-γ has also one very interesting and unusual regulatory element in its 5' UTR; it contains a pseudoknot and this dsRNA structure attracts and activates PKR. Surprisingly, PKR phosphorylation of eIF2-α does not affect general translation but is strongly localized and utilized for the IFN-γ mRNA specific translational down-regulation (Ben-Asouli et al. 2002).

INF-γ can evoke specific translational regulation in stimulated cells. IFN-γ signalization induces assembly of IFN-γ–activated inhibitor of translation (GAIT) complex. Human GAIT components are: ribosomal protein L13a, glutamyl- prolyl-tRNA synthetase (ERPS), NS1-associated protein-1 and glyceraldehydes-3-phosphate dehydrogenase (GAPDH). IFN-γ stimulation evokes phosphorylation of ribosomal protein L13a, which then dissociates from 60S subunit (Mazumder et al. 2003), and phosphorylation of ERPS, which dissociates from multisynthetase complex (Sampath et al. 2004). GAIT complex binds to specific region in the 3' UTR, but it also interacts with eIF4G. GAIT interaction with eIF4G interferes with interaction of eIF3 and eIF4G effectively interfering with formation of 48 S PIC at the 5' UTR (Kapasi et al. 2007). Experiments on monocytes indicate existence of range of mRNAs with GAIT element in their 3' UTR. These translationally controlled mRNAs code for cytokines, cytokine receptors and other immunologically active proteins. (Vyas et al. 2009).

TNF-α produced by NK cells stimulates proliferation and function of B cells, dendrocytes, macrophages and T cells. TNF-α expression is strongly influenced on post-transcriptional and

translational level by ARE-BPs. TTP and HuR are opponents in maintaining the stability of TNF-α mRNA. TTP bound on ARE decreases mRNA stability, while HuR stabilizes transcript and prolongs its half-life. TIA-1 acts as translational silencer. Repression is especially strong in resting cells and is reduced in activated cells (Piecyk et al. 2000).

NK cells are also major producers of anti-inflammatory cytokine IL-10 in later stages of infection. IL-10 mRNA contains cis- acting regulatory element in the 3' UTR, elements contain AUUUA sequence and are related to ARE structurally and functionally. They modulate half-life of IL-10 mRNA. (Maroof et al. 2008). IL-10 anti-inflammatory function is mainly given by inhibition of p38 MAPK. p38 MAPK stimulated by pro-inflammatory stimuli increases translation of pro-inflamatory mRNAs through phosphorylation of ARE-BP (Mavropoulos et al. 2005).

4.4. Regulation of translation initiation in macrophage activation and apoptosis

Macrophages are one of the first cell types that react on the bacterial infection, they can be found at the site of inflammation in first stages of response. They are very sensitive to LPS activating signal and activate quickly.

Study conducted on macrophage-like J774.1 cells stimulated by LPS examined extend of post-transcriptional and translational control to macrophage activation. Cells were observed 1, 2 and 4 hours after LPS stimulation and more than 500 mRNAs are under the influence of translational control, exactly 115 mRNAs are up- and 418 are down regulated. Up-regulated genes are enriched by genes active in immune response, immune reaction and apoptosis, which is fairy expectable. Among the up-regulated genes belong TNF-α, IL-1β, IL-8 and cyclooxygenase 2 (COX-2). Surprising and noteworthy is the composition of the down-regulated group. Down-regulated genes were enriched by genes active in H^+ transport, ATP biosynthesis and ATP transport. Among the down-regulated genes belong the cytochrome c oxidase, proton transporting ATP synthase, mitochondrial F_o complex and other elements of mitochondrial respiratory chain complexes I, IV and V (Kitamura et al. 2008). This result implies that LPS stimulation of immune cells affects even the translation control of non-inflammatory proteins. Decreased pool of respiratory active proteins causes decrease in ATP pool and eventually energy depletion. Since macrophages can inflict harm to tissues if they are activated extensively, their activity has to be regulated. Energy depletion caused by respiratory genes down-regulation inflicts cellular death and so the same LPS stimulation that leads to macrophage activation insures its death.

LPS stimulation increases expression of COX-2 and inducible nitric oxide synthase (iNOS) in macrophage (Chen et al. 1999). LPS stimulation activates p38 MAPK, which leads to phosphorylation of ARE-BPs and thereby stabilizates COX-2 mRNA containing ARE sequences (Mavropoulos et al. 2005) (see Table 1.) Without LPS stimulation the mRNAs for COX-2 and iNOS are very unstable and have short half-lives that highly reduce translation from these mRNAs.

5. Translational control in non-immune cells during immune response

Immune response is a complex process that is executed not only by activities of immune cells, but also by modifications in physiology of non-immune cells. Immune cells form only a fraction of total somatic cells. Other somatic cells form microenvironment for immune response and are possible targets of intracellular pathogens. Immune cells and other somatic cells constantly interact; some of these interactions are affected by translational control in interacting cells.

5.1. Regulation of translation initiation in non-infected cells

Tumor necrosis factor α (TNF-α) secreted by immune cells on site of inflammation stimulates expression of 180 various genes in fibroblasts. RNA stability of mRNAs encoded by these genes and their role in pro-inflammatory reaction are interconnected. In addition, the degree of mRNA stability determines to which of the three groups these genes classify. The group I mRNAs are rich in AREs (10-4) and are very unstable. They code for oncogenes and other potentially dangerous products that have to be strictly regulated, their pool increases and then decreases early after stimulation. The group II genes contain few AREs (4-2) and are more stable than the group I genes. They code for proteins signaling to immune cells and also increase early after stimulation, but their half-life is longer. The group III mRNAs contain one or none ARE and are very stable. This group codes for products that are essential for tissue remodeling in a long term inflammation. The group III mRNAs require longer stimulation for their expression, but their half-life is very long. It is thus conceivable that time sequence of events in stimulated fibroblast is given by difference in mRNA stability (Hao and Baltimore 2009). Although mRNA stabilization is post-transcriptional control mechanism, it can affect translation as well. Some of the mRNAs have very short half-lifes effectively decreasing translation rates and blocking their translation without stabilizing ARE-BPs.

On surface of the TNF-α stimulated endothelial cells is expressed E-selectin, an adhesive protein recognized by immune cells. Levels of expressed E-selectin are in negative correlation with fluid flow effecting endothelial cell. Increasing fluid flow specifically decreases protein levels of E-selectin, but E-selectin mRNA levels do not change. Thus either the fluid flow regulates accessibility of E-selectin mRNA to proteosynthetic apparatus or, more likely, its expression is under yet-to-be-elucidated translation control (Kraiss et al. 2003).

5.2. Regulation of translation initiation in cells infected by viruses and interferon response

Translational and transcriptional apparatuses are hijacked in viral infection and serve to multiplication of virions. Viruses adapted many elegant mechanisms to subdue and misuse cellular resources avoiding, at the same time, their detection by the immune system. From my point of view are the most interesting viral taxons that specifically alter the mode of translation and use it to their own benefit to maximize the gain from one infected cell. In the following sections I will talk about anti-viral defense mechanisms adapted by cells

Viral infection induces expression of interferon α (INF-α) and interferon β (INF-β) in infected cells. INF-α and INF-β induced response is the first line of defense, before immune cells can respond to infection in a massive manner. INFs are secreted in paracrine and autocrine manner and stimulate antiviral response that affects cellular growth and metabolism. Two pathways in this response directly modify activity of translation initiation factors and one modifies translated mRNA(reviewed in (Samuel 2001)).

The first pathway of antiviral response begins with positive stimulation of protein kinase RNA-activated (PKR). After INF stimulation protein kinase PKR associates with ribosomes and is activated by presence of foreign dsRNA or ssRNA. PKR phosphorylates many substrates, one of them is eIF2 where phosphorylation occurs on the Ser-51 residue of its α subunit. The modified p-eIF2 has a higher affinity for eIF2B, which leads to inhibition of the eIF2B GEF activity and therefore lowers the amount of the TC (for details see above). This leads to a general translational shut off (Balachandran et al. 2000).

The second pathway of antiviral response begins with a strong increase in p56 protein levels after INF stimulation. The p56 binds to the “e” subunit of eIF3 and prevents the ternary complex stabilization on the ribosome that is normally ensured by eIF3, resulting in inhibition of proteosyntesis and cell growth. The p56 pathway is exclusively triggered by INF and thus differs from the PKR activation that also required stimulus by foreign dsRNA (Guo et al. 2000) (Hui et al. 2003).

The third pathway of antiviral response is executed by adenosine deaminase acting on RNA (ADAR) that convertes adenosine to inosine. Action of ADAR is ds RNA dependent and and it can lead to codon change resulting in a modification in function of coded protein (Wagner et al. 1989).

6. Conclusions

Information presented in this work clearly implicates translational control in modulating immune response to various stimuli. Translational control is immediate, accurate, efficient and also redundant with other regulatory mechanisms of gene expression, and as such ideally suits for rapid changes of expression that often accompany immune reactions. Surely, translation control is not as powerful regulatory mechanism as transcriptional control, yet it still is a very potent tool enabling rather precise control of protein abundance. The subtle changes that it allows are often essential for functions of various immune cells, because they increase variability in web of their mutual interactions via proteins that they produce at a given time.

On the examples of T cells, dendrocytes, NK cells and macrophages we can see that immune cells robustly modify their translational control as a part of response on activating stimuli. Immune cells utilize both general and specific translation control for their activation. General translation control is governed by abundance of ribosomes and active initiation factors. Specific translation control is governed by cis- and trans- acting elements present in the 5' and 3' UTRs. Combination of these mechanisms enables quick and rather specific change in proteome and cellular physiology, which is characteristic for immune cells and their activation. Immune cells are usually pre-armed with translationally silenced mRNAs of proteins important for function of activated cell, because neo-synthesis of mRNA would be time-inefficient and presence of active proteins would be too dangerous because of their potentially pathologic effect.

Translational control is related to each phase of activation of the presented immune cells. T cell activation relies on increase of cap-dependent translation in early stages. NK cell activation is dependent on release of specific translational silencing granzyme B and perforin mRNAs. Dendrocyte survival and antigen presentation function depends on shift from cap-dependent to IRES mediated translation and thereby on formation of stress-like survival response. Macrophage cellular death is induced by a decrease in translation of a specific mRNA subpopulation encoding genes involved in oxidative respiration; this decrease is induced by the same stimulus that activates macrophage function.

Translational control in non-immune cells can also play an important role in the course of immune response. Fibroblasts stimulated by inflammatory cytokines differentially alter stability of three specific groups of mRNAs, which induce pro-inflammatory response and remodeling of microenvironment. Endothelial cells exposed to higher fluid flow translationally attenuate expression of E-selectin and thereby decrease chance for immune cells to attach to them. Cells infected by

viruses and stimulated by INF-α or INF-β undergo rapid alterations on the translational level resulting in a decrease of cap dependent translation and a relative increase in translation of IRES dependent transcripts. A relatively large fraction of cellular mRNAs with IRES perform role in control of stress survival and apoptosis.

To conclude, translational control is a widely used mechanism employed by various types of cells during response to various immunological stimuli. Undoubtedly, more and more examples of translationally regulated mRNAs encoding immunologically active proteins will be discovered in the future research.

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