Akademie věd České republiky

**Teze disertace**
**k získání vědeckého titulu „doktor věd“**
**ve skupině molekulárně-biologických a lékařských věd.**

# “Ribozooming” – initiating, terminating and controlling protein synthesis.

Komise pro obhajoby doktorských disertací v oboru:
molekulární biologie a genetika

**Leoš Shivaya Valášek**

Mikrobiologický ústav AVČR, v.v.i.

Jeřmanice, 30. dubna 2016

## TABLE OF CONTENT

## SUMMARY

Protein synthesis is a fundamental biological mechanism bringing the DNA-encoded genetic information into life by its translation into molecular effectors - proteins. The initiation phase of translation is one of the key points of regulation of gene expression in eukaryotes, playing a role in numerous processes from development to aging. Translation termination is also a subject of translational control via so called programmed stop codon readthrough that increases a variability of the proteome by extending C-termini of the selected proteins, for example upon stress. Indeed, the importance of the study of protein synthesis is increasing with the growing list of genetic diseases caused by mutations that affect mRNA translation. In order to grasp how this regulation is achieved or altered in the latter case, we must first understand the molecular details of all underlying processes of the translational cycle.

My DSc. thesis entitled **"Ribozooming" – initiating, terminating and controlling protein synthesis** is meant to be an adventurous and perhaps also amusing story that will not only provide the reader with our current knowledge on regulation of translation/protein synthesis, but also illustrate how unpredictable the science journey sometimes is and how serendipitous one can be when meeting the right people at the right time. First I discuss recent advances in our comprehension of the molecular basis of particular initiation and termination reactions and provide several examples of their regulation that concern our work in the lab. In the final section I am taking a reader on a historical journey describing our contributions to this field in time and space.

Our contributions start by showing how several initiation factors including the largest initiation factor of all, eIF3, promote and regulate assembly of pre-initiation complexes composed of small ribosomal subunit, initiator Met-tRNA and mRNA, and what steps need to be taken towards reaching the most critical point of the entire initiation process – selection of a proper start of translation; i.e. the beginning of the coding sequence. I hope the reader will appreciate the step-by-step growing mosaic of approaches of yeast genetics, molecular biology, biochemistry, biophysics and later also structural biology that all progressively intertwined to provide us with a complex picture of the entire process that we have at our disposal today. Subsequently, I will illustrate how interconnected the beginning and end of a translational cycle are, as they are both promoted by the initiation factor eIF3. eIF3 rather surprisingly interacts with release/termination factors (eRFs) and modulates the fidelity of their stop codon recognition in yeast as well as in mammals. It also promotes incorporation of near-cognate tRNAs and thus plays a key role in programmed stop codon readthrough. Finally, we will together explore the rules by which near-cognate tRNAs re-decode the stop codon of those mRNAs that are pre-destined to be read through.

## SOUHRN

Syntéza proteinů je základní biologický mechanismus, během kterého je genetická informace „přeložena“ z DNA do proteinů, které fungují v živé buňce jako molekulární efektory. Iniciační fáze tohoto překladu je jedním z klíčových kroků regulace genové exprese u eukaryot. Hraje důležitou roli v mnoha procesech od vývoje po stárnutí. Taktéž terminační fáze překladu je regulovatelná. Jedním příkladem takové regulace je tzv. programované pročítání stop kodónu, které zvyšuje variabilitu proteomu prodloužením C-konců vybraných proteinů, například při stresu. Je neoddiskutovatelným faktem, že s rostoucím seznamem genetických chorob způsobených mutacemi, které negativně ovlivňují právě translaci mRNA, význam hlubšího studia proteosyntézy neustále vzrůstá. Aby bylo možné zjistit, jakým způsobem regulace translace probíhá, popř. jak je v případě nemocí negativně modifikována, musíme nejprve pochopit molekulární podstatu všech základních procesů translačního cyklu.

Má DSc. práce nazvaná **“Ribozooming” – initiating, terminating and controlling protein synthesis** má býti dobrodružným a snad i zábavným příběhem, který čtenáři nejen poskytne přehled o našich současných znalostech týkajících se regulace proteosyntézy (translace), ale rovněž mu má ukázat, jak nepředvídatelná je někdy cesta vědy a jakou roli na této cestě hraje pověstná štěstěna, která nám ve správný čas zcela neočekávaně přivede do cesty správné lidi. Nejprve zde podrobně popisuji nedávný značný pokrok v našem chápání molekulárních principů iniciace a terminace translace. Rovněž uvádím několik příkladů, které úzce souvisí s naší prací, jak jsou tyto dvě klíčové fáze regulované. V závěrečné části pak zvu čtenáře na cestu minulostí, kde obšírně popisuji náš přínos tomuto směru bádání v čase a prostoru.

Kapitola popisující náš přínos začíná tím, že ukážu, jak několik iniciačních faktorů (včetně největšího iniciačního faktoru ze všech, eIF3) stimuluje a reguluje sestavování pre-iniciačních komplexů skládajících se z malé ribozomální podjednotky, iniciátorové Met-tRNA a mRNA. Dále ukážu, co všechno se musí odehrát, aby došlo k dosažení nejkritičtějšího bodu celého procesu iniciace, kterým je beze sporu výběr správného začátku translace – tedy nalezení správného začátku kódující sekvence. Věřím, že čtenář ocení postupné skládání mozaiky výzkumných technik a přístupů z genetiky kvasinek, molekulární biologie, biochemie, biofyziky a později i strukturní biologie, které se v jednom okamžiku vzájemně propojily a poskytly nám současný celistvý obraz procesu iniciace translace. Následně budu ilustrovat, jak jsou začátek a konec translačního cyklu vzájemně propojeny, neboť jsou oba stimulovány iniciačním faktorem eIF3. eIF3 poněkud překvapivě interaguje s terminačními faktory (eRF) a moduluje přesnost, s jakou rozpoznávají stop kodón jak v kvasinkách, tak u savců. eIF3 rovněž podporuje začlenění „*near-cognate*“ tRNA na místo stop kodónu a hraje tak klíčovou roli v programovaném pročítání stop kodónu. Nakonec společně prozkoumáme pravidla, kterými se řídí „*near-cognate*“ tRNA, když dekódují stop kodón jako „*sense*“ kodón u těch mRNA, které jsou určeny k pročítání.

# INTRODUCTION

Regulation of gene expression at the level of transcription and protein synthesis represents the core of molecular biology. The main objective of researchers who have entered this field is to study how genetic instructions encode for biological functions. One of the critical regulatory steps of gene expression occurs during translation initiation, which is in fact the most controlled phase of the whole translation process. Compared to transcriptional regulation, translational control of existing mRNAs allows for more rapid changes in cell's proteome, which gives cells flexibility to adapt to a variable environment, external signals or damage to the cell etc. In addition, translational control can be used to modulate more permanent changes in cell physiology or fate. Thus it is not surprising that the process of translation is also increasingly recognized as an important component in the etiology and maintenance of cancer. There are also numerous examples demonstrating that deregulation of translational control either directly causes various diseases or significantly contributes to their rapid development (for example neurodegenerative conditions, diabetes, etc.).

Translation can be divided into four steps: initiation, elongation, termination and ribosome recycling. As mentioned above, translational regulation is believed to occur primarily during the initiation phase of protein synthesis, as this phase is rate-limiting for most mRNAs. Such regulation may be mediated by altered levels of translational components such as ribosomes and/or some initiation factors (eIFs) – i.e. quantitatively, or by signal transduction pathways that upon various stimuli either change phosphorylation status of the key eIFs or target specific features carried by mRNAs and as such affect protein synthesis qualitatively. Hence, the mechanism of translation initiation; i.e. localization of the authentic start of the coding sequence of each gene in the 5' region of its mRNA – in most cases defined by the first AUG triplet encoding methionine, has been intensively studied for decades in order to elucidate molecular basis of every potential control point (reviewed in (Valášek 2012 - **pub 22**; Hinnebusch 2014)).

Since terminating protein synthesis at the appropriate stop codon is undoubtedly as important as initiating at the proper start codon, similar effort has been invested into understanding how the elongating ribosomes recognize the end of the coding sequence and stop protein synthesis at the right in-frame stop signal. There are altogether three stop codons – UAA, UAG and UGA - lacking its cognate transfer RNA (tRNA), which are decoded by a protein complex of two release factors. These trigger polypeptide release from the peptidyl-tRNA occurring in the P site of the terminating ribosome and thus capture the entire protein synthesis process (reviewed in (Jackson et al. 2012)). There are many examples in all three kingdoms of life describing ribosomes that in some cases purposely bypass the stop codon on specific mRNAs to extend the nascent polypeptide to add a signaling domain or alter the protein's properties. This mechanism is called programmed stop codon readthrough and over the recent decade it has gained a lot of attention due to its potential implication in the medical research. More than 15% of all human genetic

diseases are actually caused by an existence of a premature termination (non-sense) codon (PTC) in the coding region of a functionally essential protein often leading to its deleterious truncation. Hence many labs have started searching for otherwise non-toxic drugs that could specifically prevent termination at these PTCs; in other words that would purposefully increase readthrough at them. However, a successful completion of this task requires identification of all factors involved in programmed readthrough and detailed description of its molecular mechanism. Simply speaking, one has to understand all factors that make sense in non-sense readthrough.

## TRANSLATION INITIATION AND CONTROL IN EUKARYOTES

Translation initiation in eukaryotes is a complex series of reactions leading to the formation of an 80S ribosomal complex that contains initiator methionyl-tRNA (Met-$tRNA_i^{Met}$) base paired to the AUG start codon in the ribosomal P-site. The main initiation pathway in eukaryotes is cap-dependent and is orchestrated by numerous proteins and protein complexes called eukaryotic initiation factors (eIFs). The conventional view of the translation initiation pathway is presented in Fig. 1.

The new cycle of translation initiation starts with the recruitment of the ternary complex (TC) consisting of Met-$tRNA_i^{Met}$ and GTP-bound form of eIF2 to the 40S ribosomal subunit to form the 43S pre-initiation complex (PIC). The binding of TC to the 40S subunit is promoted by eIFs 1, 1A, 5 and the multisubunit eIF3 complex (reviewed in (Valášek 2012; Hinnebusch 2014)). There are two major ways of how eIFs can associate with the ribosomes to form the 43S PIC: i) the “stochastic – prokaryotic-like” pathway where eIFs bind to the small subunit on individual basis; and ii) the “higher order – eukaryotic” pathway, where eIFs 1, 3, 5 and the TC first assemble into a large multifactor complex (MFC) and then bind to the 40S ribosome as a preformed unit (Fig. 1). The MFC was shown to occur in yeast (Asano et al. 2000), plants (Dennis et al. 2009) and also mammals (Sokabe et al. 2011) and studies in yeast showed that it enhances the formation and stability of the 43S PICs (Valášek 2012 - **pub 22**). After binding of these initiation factors, eIFs 1 and 1A serve to stabilize a specific conformation of the 40S head relative to its body that opens the mRNA binding channel for mRNA loading. That requires dissolving the latch formed by helices 18 (h18) and 34 (h34) of 18S rRNA and establishing a new interaction between RPS3 and h16 (Passmore et al. 2007; Hussain et al. 2014; Llacer et al. 2015; Zhang et al. 2015) .

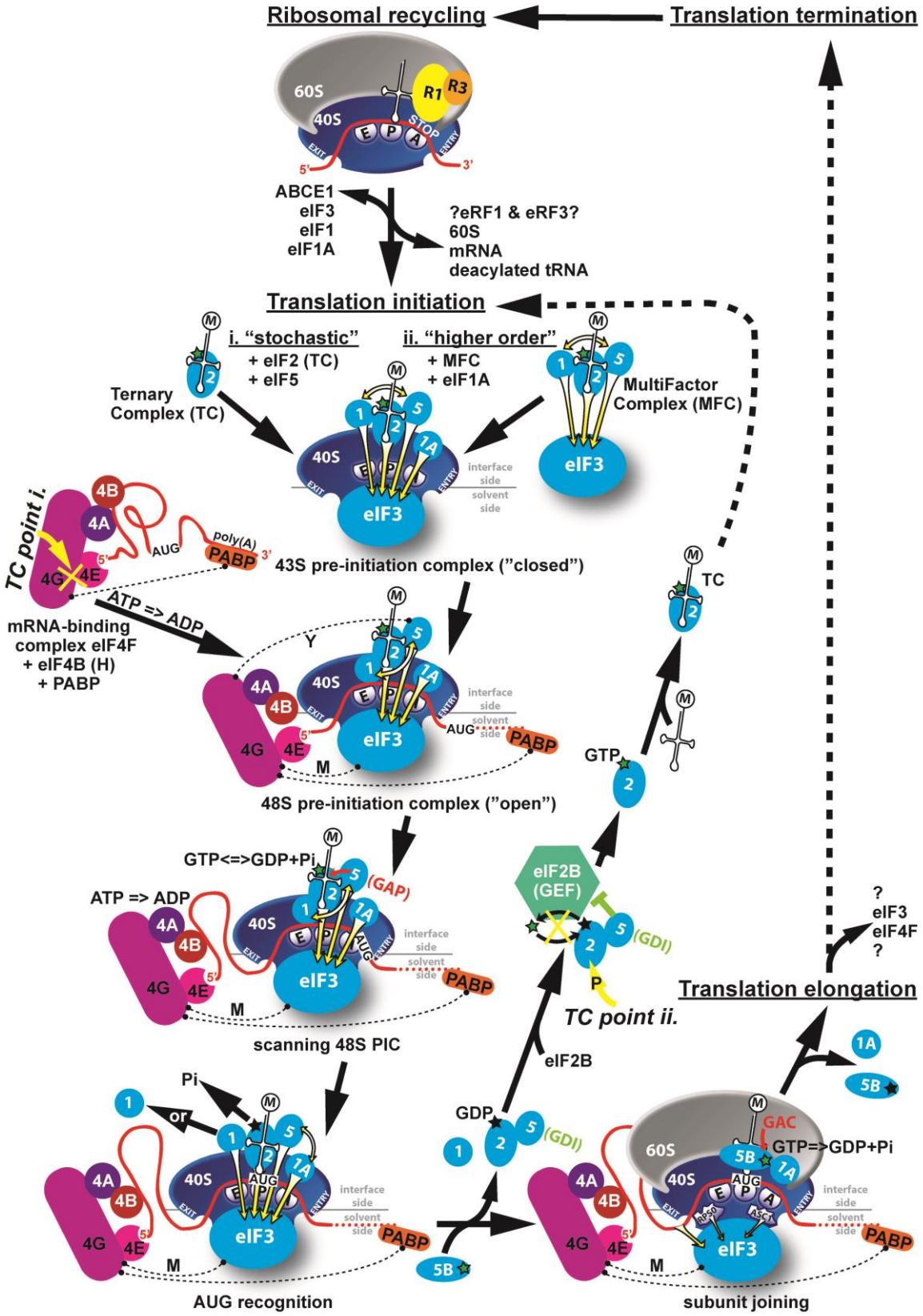


**Figure 1.** Schematic of the canonical translation pathway in eukaryotes with the ribosomal recycling and initiation phases shown in detail. This figure combines findings from both yeast and mammals and indicates potential differences (Valášek 2012 - **pub 22**).

Next step is the loading of mRNA to the 43S PIC. With the help of eIF4F, eIF4B, poly(A)-binding protein (PABP) and eIF3, the 43S PICs activated by eIFs 1 and 1A bind to the capped 5' end of mRNA and form the 48S PIC. The eIF4F complex is comprised of the cap-binding protein eIF4E, scaffolding protein eIF4G, and the DEAD-box RNA helicase eIF4A. The eIF4A component likely unwinds any cap-proximal secondary structures so that the 43S PIC can bind. One consequence of this process is that the mRNA cannot be threaded into the 40S subunit, because eIF4F is bound to the 5′ end, and hence must be loaded laterally into the mRNA channel. In mammals the interaction between the eIF4F•mRNA complex and the 43S PIC is likely bridged by eIF3 which directly binds the eIF4G through three of its subunits (Villa et al. 2013) (Fig. 1 – "M" dashed line). It should also be mentioned that formation of an interaction between the cap-binding protein eIF4E and eIF4G has been shown to serve as one of the two major targets for the general translational control, especially in mammalian cells (Fig. 1 – "TC point i"; see also below).

Once bound near the cap, the resulting 48S PIC scans the mRNA until the AUG start codon in the optimal context is recognized (Kozak 1986). Scanning is accompanied by unwinding secondary structures in an ATP-dependent reaction stimulated by helicases eIF4A, DHX29 and DED1 (reviewed in (Hinnebusch 2014)). The mRNA slides through a tunnel formed by the ribosomal proteins, eIF1A and eIF1, which ensures scanning processivity by keeping the mRNA unstructured and properly oriented for the inspection of the nucleotide sequence in the P site by Met-tRNA$_i^{Met}$. In mammalian reconstituted systems, eIFs 1, 1A, and 3 sufficiently promoted location of the AUG start codon on mRNAs with unstructured 5' UTRs and insertion of even a weak secondary structure in the leader imposed a need for the eIF4F complex (Pestova and Kolupaeva 2002). Yeast genetic data then indicate that in addition to these, eIF5 is also required *in vivo* (Valášek 2012 - **pub 22**; Saini et al. 2014).

During scanning ribosomes have to read, integrate and respond to a variety of signals that orchestrate the AUG recognition (reviewed in (Hinnebusch 2014)). In the open conformation of the 40S ribosome that is induced by eIFs 1 and 1A, as mentioned above, the anticodon of Met-tRNA$_i^{Met}$ is not fully engaged in the ribosomal P-site in order to prevent premature engagement with putative start codons. eIF5 stimulates partial GTP hydrolysis by eIF2 to GDP and Pi, but the Pi is not released from the scanning complex until the anticodon of Met-tRNA$_i^{Met}$ base-pairs with the AUG start codon, which induces dissociation or displacement of eIF1 (Algire et al. 2005; Cheung et al. 2007; Karaskova et al. 2012). The Met-tRNA$_i^{Met}$ is then fully accommodated in the P-site and the 48S PIC switches its conformation to the closed/scanning arrested form (Saini et al. 2010; Saini et al. 2014; Llacer et al. 2015). This irreversible reaction serves as the decisive step stalling the entire machinery at the AUG start codon. Besides the aforementioned factors, there is an increasing number of reports suggesting that also the multisubunit eIF3 contributes

to the regulation of AUG recognition (Valášek et al. 2004 - **pub 10**; Chiu et al. 2010 - **pub 15**; Herrmannová et al. 2012 - **pub 18**; Karaskova et al. 2012 - **pub 21**).

Subsequently, after AUG start codon has been recognized, GTP-bound eIF5B stimulates joining of the 60S ribosomal subunit (Pestova et al. 2000; Fernández et al. 2013). Upon subunit joining, most eIFs are ejected with exception of eIF1A (Unbehaun et al. 2004), and possibly also eIF3 (Szamecz et al. 2008 - **pub 13**; Munzarová et al. 2011 - **pub 17**) and eIF4F (Pöyry et al. 2004). Finally, GTP-hydrolysis on eIF5B stimulated by the GTP-ase activating center of the 60S subunit triggers the release of eIF1A and eIF5B itself producing an elongation-competent 80S ribosome.

For a new round of initiation a pool of separated ribosomal subunits has to be generated from those that have just finished (terminated) translation (reviewed in (Jackson et al. 2012; Valášek 2012 - **pub 22**)) and the ejected eIF2-GDP must be recycled to eIF2-GTP by the guanine nucleotide exchange factor eIF2B in order to form the new ternary complex with Met-tRNA$_i^{Met}$ (Jennings and Pavitt 2010; Jennings et al. 2013). It is important to note that the step of the ternary complex formation is the other of the two major targets of the general translational control (Fig. 1 – “TC point ii").

A wide variety of stimuli and cellular stresses cause eIF2 to be targeted by various protein kinases (for example GCN2 in *Saccharomyces cerevisiae*, and GCN2, PERK, PKR and HRI in mammalian cells). All phosphorylate eIF2 at the same position, serine 51 within α subunit of eIF2 (reviewed in (Jackson et al. 2010)). The resulting phosphorylated eIF2 (eIF2αP) acts as a competitive inhibitor of eIF2B, restricting its exchange activity and reactivation of eIF2 from its GDP to GTP form (Pavitt et al. 1998). This applies a brake, lowering levels of active eIF2 leading to a decrease in general protein synthesis initiation (reviewed in (Jennings and Pavitt 2014)). At the same time certain mRNAs are up-regulated, including specific mRNAs required for the cellular stress response. One well studied class of mRNAs that increase expression following phosphorylation of eIF2 are *GCN4* in yeast and ATF4 in mammalian cells. Both possess short ORFs upstream of the main coding region that normally limit the flow of ribosomes to the main coding AUG. eIF2αP promotes ribosomes to bypass the inhibitory upstream ORF(s) and initiate at the main ORF (reviewed in (Hinnebusch 2005; Baird and Wek 2012; Gunisova and Valasek 2014 - **pub 25**)).

A second extensively used mechanism in eukaryotes to control the rate of translation initiation involves the mRNA 5’ cap recognition process by eIF4F. The interaction between eIF4G and eIF4E in the eIF4F complex is inhibited by members of a family of related proteins, termed eIF4E-binding proteins (4E-BPs) (reviewed in (Pelletier et al. 2015)). The 4E-BPs compete with eIF4G for a shared binding site on eIF4E (Marcotrigiano et al. 1999). Consequently, 4E-BPs inhibit cap-dependent, but not IRES-dependent, translation (IRES stands for Internal Ribosome Entry Site). 4E-BP binding to eIF4E is controlled by phosphorylation. Hypophosphorylated 4E-BPs bind strongly to eIF4E, whereas phosphorylation of 4E-BPs weakens their interaction with eIF4E. A critical kinase, which phosphorylates 4E-BPs, is mTOR (mammalian

target of rapamycin). mTOR is a downstream Ser/Thr kinase in the PI3K/Akt signaling pathway and senses and integrates signals from extracellular stimuli, amino acid availability, and oxygen and energy status of the cells (reviewed in (Dowling et al. 2010)).

## TRANSLATION TERMINATION AND STOP CODON READTHROUGH IN EUKARYOTES

The end of a translational cycle involves another series of steps that culminate in the release of a newly synthesized polypeptide from the translating ribosome (the termination phase), and in the dissolution of the ribosome:tRNA:mRNA complex (the recycling phase) (reviewed in (Jackson et al. 2012)). Termination begins when a stop codon enters the ribosomal A-site, forming a pre-termination complex (pre-TC) (Alkalaeva et al. 2006). In eukaryotes, all three stop codons are decoded by the eukaryotic release factor 1 (eRF1). According to recent models (Shoemaker and Green 2011; Becker et al. 2012; Brown et al. 2015; Matheisl et al. 2015), eRF1 enters the ribosomal A-site in complex with a second release factor, eRF3, in its GTP bound form. Recognition of a stop codon triggers GTPase activity of eRF3, which leads to its dissociation from the complex in its GDP bound form. eRF1 is then free to activate the ribosomal peptidyl transferase center, which hydrolyses the bond between the P-site tRNA and the nascent polypeptide. Importantly, these steps are promoted by RLI1 in an ATP-independent manner. Molecular details of this RLI1 role in termination are not known, nevertheless, the proposed active role of RLI1 in stop codon recognition is consistent with observations that conditional down regulation of RLI1 protein levels increases stop codon read-through in yeast (Khoshnevis et al. 2010a). Based on the most recent structural model, RLI1 binds to the same site on the terminating ribosome as eRF3 (thus their binding is mutually exclusive), and its 4Fe-4S domain interacts with the C-terminal domain of eRF1 to push the conserved GGQ motif in the middle domain of eRF1 to the peptidyl transferase center next to the acceptor stem of the P-site tRNA to trigger polypeptide release (Becker et al. 2012).

Recycling of eRF1-associated post-termination complexes (post-TCs) is also mediated by ABCE1/RLI1, this time, however, in an ATP-dependent manner (Pisarev et al. 2010; Shoemaker and Green 2011). It was hypothesized that RLI1, upon binding and hydrolyzing ATP, switches its conformation into a closed state, and the mechanochemical work generated by this switch splits post-TCs into free 60S subunits and deacylated tRNA- and mRNA-bound 40S subunits (40S-post-TC) (Becker et al. 2012). Recently, it was proposed that RLI1/ABCE1 not only recycles terminating ribosomes but also controls translation reinitiation in 3' UTRs *in vivo* (Young et al. 2015). Finally, Pisarev *et al.* showed that the release of tRNA and mRNA from the 40S-post-TCs is *in vitro* ensured by the *bona fide* initiation factors eIF1, eIF1A and eIF3 (Pisarev et al. 2007; Pisarev et al. 2010). eIF3, and in particular its j subunit (HCR1 in yeast), were suggested to play the key role in mRNA

dissociation. We showed that yeast eIF3 and HCR1 control termination and stop codon readthrough (Beznosková et al. 2013 - **pub 23**).

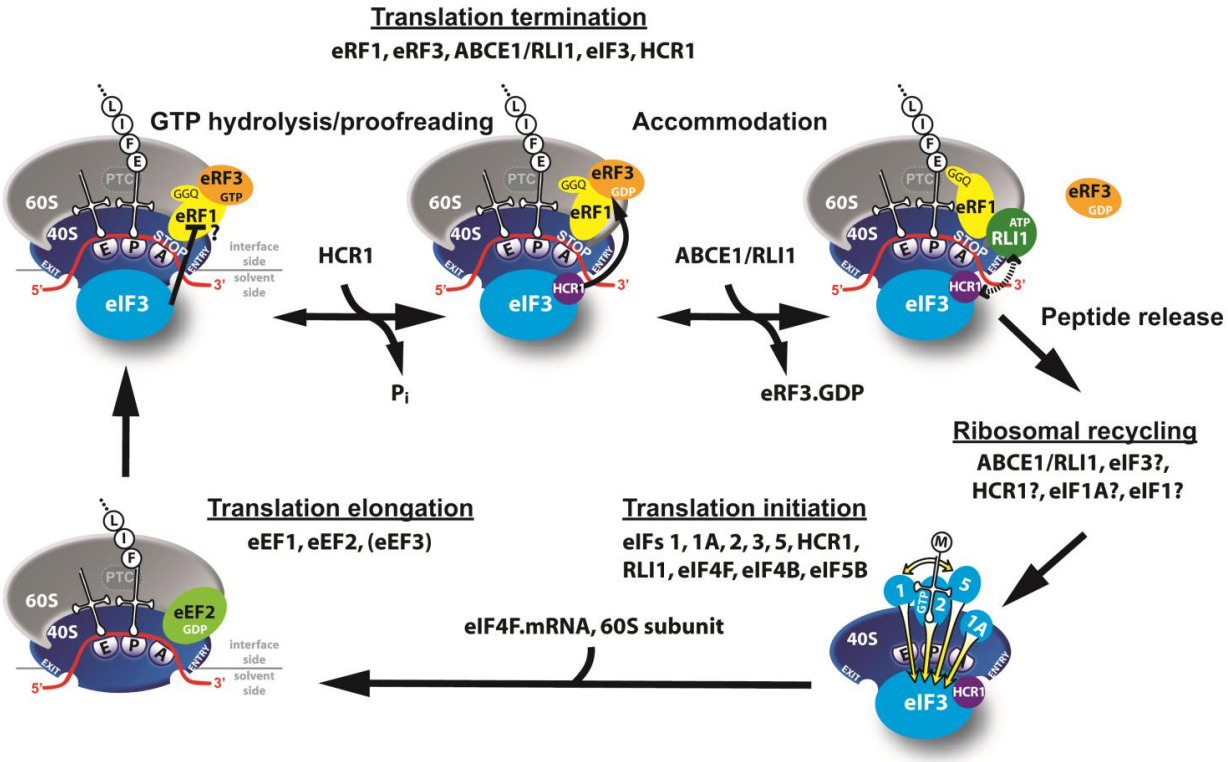


**Figure 2.** Schematic of the translation termination and recycling pathways in eukaryotes with the proposed roles of initiation factors eIF3 and HCR1 in them (Beznosková et al. 2013 - **pub 23**).

In some specific cases, not all stop codons signal the proper end of translation, which can thus continue beyond to the next stop codon. Generally speaking, translation termination can be viewed as a competition between stop codon recognition by release factors and stop codon decoding by near-cognate tRNAs. This competition differs genome-wide in its efficiency. The efficiency can be influenced by the identity of the stop codon (Robinson and Cooley 1997; Chao et al. 2003; Napthine et al. 2012), the nucleotide context of the stop codon (Bonetti et al. 1995; McCaughan et al. 1995; Cassan and Rousset 2001), the identity of the last two amino acids incorporated into the polypeptide chain (Janzen et al. 2002), the identity of the P-site tRNA (Mottagui-Tabar et al. 1998), cellular levels and the identity of near-cognate/suppressor tRNAs (Beznoskova et al. 2015 - **pub 27**; Beznoskova et al. 2016 - **pub 28**), and the presence of stimulatory elements downstream from the stop codon (Skuzeski et al. 1991; Namy et al. 2001; Harrell et al. 2002). All these features increase the odds of the stop codon being decoded by a near-cognate, natural suppressor tRNA rather than by eRF1, resulting in the process termed stop codon readthrough. This allows production of C-terminally extended polypeptides with new or at least modified biological roles compared to their shorter, original versions. The mechanism whereby near-cognate tRNAs outcompete conventional

stop codon recognition by eRF1 has not been known until recently (Beznoskova et al. 2016 - **pub 28**), nor has been known which protein factors might be functionally important for stop codon readthrough (Beznoskova et al. 2015 - **pub 27**).

In recent years several groups have proposed that the stop codon readthrough mechanism is specifically regulated by cis-acting RNA elements downstream of the first stop codon that may exist to generate proteome diversity in response to changing environmental conditions. The rapidly growing list of cellular genes under the control of this "programmed stop codon readthrough" mechanism, the typical, long standing example of which is from the tobacco mosaic virus (TMV) genome (Skuzeski et al. 1991), strongly suggests that programmed stop codon readthrough is an important contributor to general translational control in all kingdoms of life (for review; see (Namy et al. 2004; Dreher and Miller 2006; von der Haar and Tuite 2007; Bidou et al. 2010). A recent ribosome profiling study detected many readthrough events occurring at biologically relevant levels in budding yeast, fruit fly, and human data sets, suggesting that this mechanism is highly conserved (Dunn et al. 2013).

## AUTHOR'S CONTRIBUTION TO THE FIELD SET IN THE HISTORICAL PERSPECTIVE

The story to be told begins in 1994, when I was unexpectedly contacted by Dr. Jiří Hašek from the Institute of Microbiology CAS, v.v.i. in Prague. My dream after graduating from the Charles University was to find an interesting PhD position somewhere in the US or quit science and find a job in a private sector. Jiří offered me to join the peroxisomal group of Prof. Helmut Ruis in the Vienna Biocentrum (nowadays Max F. Perutz Laboratories) associated with the University of Vienna, whom he closely collaborated with, as a new PhD student. I was very pleased by his interest but, considering my dream, not that much excited about the place. Nevertheless, we agreed on going there together for two days to find out how it feels. I was truly amazed by a brand new institute at Dr. Bohr-Gasse 9 street and the people there and all possibilities that this serendipity was offering to me. The topic of my thesis was supposed to be a characterization of a newly identified budding yeast protein of an unknown function, initially designated as MAP110 but later renamed to RPG1 (for Required for Passage through G1 phase) (Kovarik et al. 1998 **- pub 1**). This protein was selected in a phage display assay carried out by Dr. Pavel Kovarik because it cross-reacted with antibody raised against mammalian Microtubule Associated Protein 2 (MAP2). The idea was that a new yeast microtubule associated protein was discovered that could have an interesting impact on cytoskeleton dynamics. It took three long years of hard work on my PhD thesis - having nothing in my hands that would make sense - to realize that this protein, which may have some connection to the cytoskeleton after all (Kovarik et al. 1998 **- pub 1**; Hašek et al. 2000), is actually and primarily the largest subunit of the budding yeast translation initiation factor 3 (eIF3).

This out of the blue realization dates back to spring of 1997. The group around Dr. William (Bill) C. Merrick from the Case Western Reserve University published an

article in J. Biol. Chem., where they described identification of cDNA clones for the large subunit of eukaryotic translation initiation factor 3 (eIF3) and deposited their sequences to the NCBI databases (Johnson et al. 1997). Indeed, I was carrying out regular blast searches with the RPG1 sequence hoping to find its homolog(s) (preferably with known biological roles) for all those years. But only after this report had been released my blast search finally retrieved something. This "something" was a huge surprise to all of us; at the same time, it was a true break point of my PhD work and undoubtedly the turning point of my entire career. Just a note out of curiosity, the blast searches at those days were not on-line taking seconds to retrieve the scoring matches; the sequences had to be sent via email to the NCBI staff and it took about a week or two to receive a response from them.

Never mind, an extensive (and relieving at the same time) literature search began in order to find out what is known about the eIF3 complex, what functions it performs etc. I quickly learned that there were at least 10 proteins called initiation factors implicated in initiation of protein synthesis at that time, among which eIF3 represented the largest and the most complex one (Merrick and Hershey 1996). eIF3 was thought to be involved in the dissociation of the 80S ribosome into 40S and 60S subunits, to bind to 40S subunits and thereby to act as an anti-association factor. Furthermore, eIF3 was believed to stabilize the Met-tRNA$_i$·eIF2·GTP ternary complex by binding to the 40S subunit and to promote recruitment of mRNA to 40S and 80S ribosomes (Benne and Hershey 1978; Trachsel and Staehelin 1979). eIF3 was shown to bind to eIF4F via the eIF4G subunit (Lamphear et al. 1995) and to eIF4B (Methot et al. 1994). As mentioned above, eIF4F is a protein complex composed of three subunits, eIF4G, eIF4A and eIF4E, which is responsible for binding of the capped end of mRNA, melting of mRNA secondary structure and binding to the 43S preinitiation complex. The Johnson's 1997 "breakthrough" paper even suggested that eIF3 might be the major factor orchestrating the accurate positioning of mRNA for binding to the 40S subunit and subsequent recognition of the initiation AUG codon (Johnson et al. 1997). At the end of their discussion they claimed that: "The timing of all translation initiation events as well as their coordination are only poorly understood. Thus, a characterization of not yet described proteins involved in that process can bring more light to our understanding of the mechanism of the initiation step of protein synthesis." This was very encouraging with respect to my situation – having an unknown protein waiting to be characterized genetically and biochemically with a clear homology to the largest subunit (p180) of also poorly characterized human eIF3 and with the end of my PhD endeavor rapidly approaching.

I also learned that the group of John W.B. Hershey from the UC Davis had been very actively involved in characterization of both budding yeast and mammalian eIF3. They revealed that mammalian initiation factor 3 is composed of at least ten non-identical subunits (Asano et al. 1997); later it was shown that the total number is 13 (reviewed in (Hinnebusch 2006; Valášek 2012 - **pub 22**)). On the other hand, yeast eIF3 was predicted to comprise eight subunits of apparent molecular masses ranging from 16 to 135 kDa (Naranda et al. 1994). At that time, only four yeast homologues of mammalian eIF3 subunits had been identified. These were PRT1,

NIP1, TIF34, and TIF35, having homologs in the human eIF3 subunits p110, p116, p36 and p44, respectively. Yeast eIF3 also supposedly contained two previously described proteins, GCD10 (Garcia-Barrio et al. 1995) and SUI1 (Naranda et al. 1996), but corresponding homologues were not found in mammalian eIF3. Later it was shown that the latter two proteins were falsely classified as eIF3 subunits and that the total number of budding yeast subunits was six (Hinnebusch 2006; Valášek 2012 - **pub 22**). The Johnson's 1997 paper also stated that: "The *S. cerevisiae* genome encodes one additional protein, p110, homologous to the human eIF3 subunit p180, which has not been identified and characterized yet (Johnson et al. 1997). It was clear to me that either Bill's or John's leading translation group – or both – is after identification and characterization of our yeast RPG1 homologue of mammalian p180 and that there was not much time left to "publish and not perish".

Being inspired by several papers published by these two groups in the past, I promptly carried out a series of custom-tailored experiments and in co-operation with Hans Trachsel's lab from the University of Bern; i.e. another leading translation group where I spent 6 weeks in early 1998 working on RPG1, we quickly demonstrated that the essential *S. cerevisiae* gene *RPG1* encoding a polypeptide with a calculated molecular weight of approximately 110 kDa is indeed the functional homologue of the mammalian p180 protein; i.e. the largest subunit of yeast eIF3 (Valášek et al. 1998 - **pub 2**). Coincidentally, during these 6 weeks in Switzerland John paid a visit to his "old buddy" Hans in Bern where he talked about eIF3 and all subunits that remained to be characterized including yeast p110. It was intense to experience this kind of competition at this stage of my career. Luckily we made it in time and a year later (in 1999) the John's group also succeeded with a similar story confirming and extending our findings (Vornlocher et al. 1999). However, in the same year when we published our RPG1 story (1998), another big player joined the eIF3 game – Dr. Alan Hinnebusch from NIH. His group showed in two excellent papers that all five yeast proteins homologous to human eIF3 subunits are components of a stable heteromeric complex *in vivo* that may comprise the conserved core of yeast eIF3 (Asano et al. 1998; Phan et al. 1998). They also demonstrated that the NIP1 subunit of eIF3 interacts with eIF5 (the GTPase activating protein (GAP) for the Met-$tRNA_i$·eIF2·GTP ternary complex) and with SUI1 (nowadays eIF1); interestingly, both eIF5 and SUI1/eIF1 have been implicated in accurate recognition of the AUG start codon (Huang et al. 1997). Hence they proposed that eIF5 and eIF1 may be recruited to the 40S ribosomes through physical interactions with the NIP1 subunit of eIF3 – and they were correct. Since these two traditional translation groups (Alan's and John's) designated p110 as TIF32 (for Translation Initiation Factor 32), we later gave up on the RPG1 name and started using TIF32 too.

In the remaining time of my PhD in Vienna I also managed to identify and characterize the sixth and last *Saccharomyces cerevisiae* homolog of human eIF3, the HCR1 subunit homologous to p35, which I isolated as a High Copy suppressor of a temperature-sensitive mutation in RPG1 in 1997 (Valášek et al. 1999 - **pub 3**). This paper in principle captured the overall effort to characterize the composition of yeast eIF3 as it also captured my PhD thesis. It was also my first paper where I feature as

a corresponding author. All these sudden, successive and successful events were like a fuel for my scientific engine that almost conked out in early 1997. I once again started dreaming about the US – this time as a post-doctoral fellow in the field of translation. I contacted 12 "translational" PIs, whose articles excited me the most during my extensive literature search, by faxing them my CV and a motivation letter on one July Sunday afternoon. In the following months I received 7 offers to come, including the one from John Hershey from California. In late summer of 1998 I participated at the Yeast Genetics and Molecular Biology meeting held by the University of Maryland where I met Alan Hinnebusch (another serendipity), who was unintentionally left out of my list of 12 PIs. We thoroughly talked over my "RPG1" poster and I was very much impressed by his depth and interest but also by his modesty. Towards the end of our discussion I indeed expressed my interest to continue with my research in a well-established gene expression lab in the US. He replied: "I will see what I can do.", and left me for a beer break. Two months later I was offered a post-doctoral position (the NIH Visiting Fellow Award) from Alan, with three pages long email describing three potential projects I could choose from in an astonishing detail. With 8 offers to choose from I talked to a very much respected scientist at the Vienna Biocentrum, Dr. Gustav Ammerer. Gustav went silently through my list and told me without hesitation: "Go to Hinnebusch." So, in May 1999 I joined Alan's lab - without hesitation.

The project that I picked was to characterize a potential role of the NIP1 subunit of eIF3; i.e. the subunit connecting eIF3 to eIF1 and eIF5, in regulation of the AUG start codon recognition. However, since I brought my RPG1, well, TIF32 and HCR1 projects with me, I also continued working on them. In less than two years since the onset of my post-doc we reported that: 1) a subcomplex of three eIF3 subunits (TIF32, NIP1 and PRT1) binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and Met-$tRNA_i^{Met}$ (Phan et al. 2001 - **pub 4**); 2) the sequentially related eIF3 subunits TIF32 and HCR1 interact with an RNA recognition motif in PRT1 and are required for the eIF3 integrity and ribosome binding (Valášek et al. 2001b - **pub 7**); and also that 3) HCR1/eIF3j has a dual function in translation initiation and in processing of 20S pre-rRNA during ribosome biogenesis (Valášek et al. 2001a - **pub 6**). Alan's group also revealed that eIF1, eIF2, eIF3, eIF5, and initiator $tRNA^{Met}$ form a so called multifactor complex (MFC) (Fig. 3) that is an important translation initiation intermediate *in vivo* (Asano et al. 2000). The interaction between eIF3 and eIF2 in the MFC was thought to be mediated by eIF5. These budding yeast results were meanwhile confirmed and extended in other organisms such as fission yeast and mammals by labs of Drs. Tayana Pestova, Gerhard Wagner and Umadas Maitra (see for example (Das et al. 1997; Pestova et al. 1998; Bandyopadhyay and Maitra 1999; Fletcher et al. 1999; Das and Maitra 2000; Pestova and Kolupaeva 2002; Majumdar et al. 2003).

Next we wished to 1) obtain a detailed subunit-subunit interaction map of yeast eIF3, 2) to elucidate the roles of individual subunits in the initiation process, 3) to map the eIF3 binding site on the 40S ribosomal subunit and 4) to identify the mutual intermolecular bridges between eIF3 and the 40S subunit. This effort yielded

several key publications that fulfilled our initial plan. We even discovered the long suspected but never shown direct contact between eIF2 and eIF3, and the first "sausage" model of the yeast eIF3 3D "structure" in the context of the entire MFC was born (Fig. 3). We also proposed where eIF3 and its associated factors bind on the small subunit (Fig. 4) (Asano et al. 2001; Valášek et al. 2002 - **pub 7**; Valášek et al. 2003 - **pub 8**; Nielsen et al. 2004 - **pub 9**; Jivotovskaya et al. 2006 - **pub 11**; Nielsen et al. 2006 - **pub 12**).

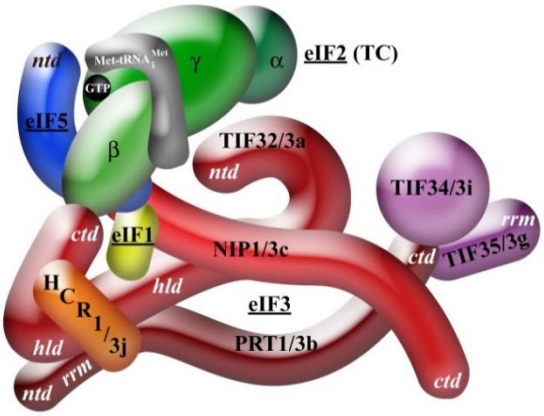


**Figure 3.** Schematic of eIF3 in the MFC (Valášek et al. 2002 - **pub 7**).

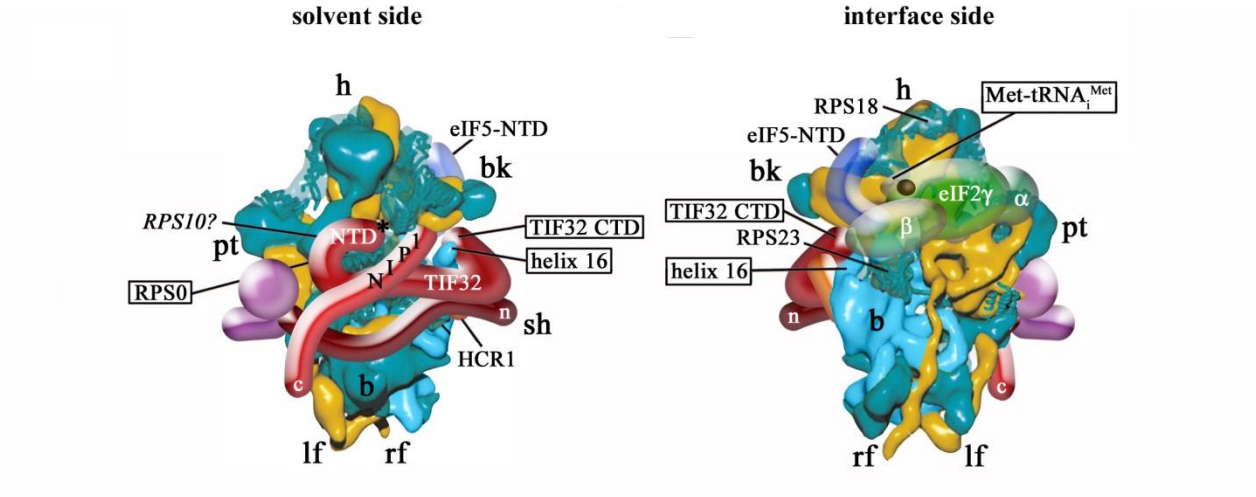


**Figure 4.** Schematic of the MFC constituents bound to the 40S ribosome (Valášek et al. 2003 - **pub 8**).

When this was done, it was time to resuscitate my initial project; i.e. to characterize a potential role of the NIP1 subunit of eIF3 in regulation of the AUG start codon recognition. However, after four years of exciting but at the same time very hard work in Alan's lab I felt pretty worn down and decided to leave his lab, thinking of transferring this project with already promising results to somebody else. A totally changed atmosphere in the US following the September 11$^{th}$ events in 2001 also strongly contributed to my/our decision. My NIH contract was set to be terminated in June 2003 and I and my wife could not wait to spend two months travelling across the US national parks before coming back home. With practically zero scientific history in the Czech Republic, however, there were not many options for me, if any, to continue with my career in science as a PI back in 2003. Hence, once again, I started thinking about quitting and going to the private sector. The priority of both of us was to return home no matter what. But here again the serendipity took the stage.

With the value of the American dollar – earned with great effort in Alan's lab – going down the drain post the aforementioned events, a good friend of mine convinced me to stay one more year and "regain" some of its value by smart investment into real estate. Alan was very surprised but immediately agreed to my delight. He only told me that my bench had already been given to somebody else – luckily to my collaborator and best friend Klaus Nielsen – and as such we would have to share it, if I wished to stay one more year. Klaus also immediately agreed, so I could restart my post-doc soon after our arrival from the amazing 2-months long trip across the USA. This was critical and serendipitous at the same time for my career, because a year later, in 2004, The Czech Academy of Sciences launched a new program – Fellowship of Jan E. Purkyně – for successful researchers working abroad who wished to return back to the Czech Republic. This fellowship was associated with a guaranteed position at the one of the CAS institutes (provided that there was such a position available) and a decent salary. Hence returning home in 2004 was a lot easier with respect to staying in basic science compared to a year before. But back to my last post-doc year, I rushed to show that interactions of the eIF3 subunit NIP1 with eIF1 and eIF5 promote preinitiation complex assembly and mainly regulate start codon selection by a molecular mechanism that had yet to be described in detail (Valášek et al. 2004 - **pub 10**). This once and for all completed my scientific endeavor in Alan's lab.

Following this paper, other members of Alan's lab and also the groups of Drs. Tatyana Pestova and John Lorsch have gone a long way to demonstrate that dissociation of eIF1 from the 40S ribosomal subunit is the key step in start codon selection *in vivo* and that the N- and C-terminal residues of eIF1A have opposing effects on its fidelity. In particular, they showed that eIF1 and eIF1A induce an open/scanning-conducive conformation of the 40S ribosome and that regulatory elements in the eIF1A terminal tails control the fidelity of start codon selection by modulating Met-$tRNA_i^{Met}$ binding to the ribosome. In the "open" scanning complex it occurs in the so-called $P^{out}$ state, which refers to a loosely bound mode of the $tRNA_i^{Met}$ binding to the ribosomal P-site allowing successive inspection of incoming nucleotides. Upon AUG selection it shifts to the $P^{in}$ state referring to a stable mode of binding, which prevents further scanning of the ribosome that adopts the closed/scanning-arrested conformation. All these intricate changes are under delicate control of mainly eIFs 1 and 1A. Finally, they demonstrated that the free Pi release from eIF2, not GTP hydrolysis *per se*, is the step controlled by start-site selection during eukaryotic translation initiation and that eIF5 promotes the accuracy of start codon recognition by regulating the Pi release and conformational transitions (open to closed) of the preinitiation complex (Lomakin et al. 2003; Unbehaun et al. 2004; Algire et al. 2005; Fekete et al. 2005; Maag et al. 2005; Lomakin et al. 2006; Pisarev et al. 2006; Fekete et al. 2007; Cheung et al. 2007; Passmore et al. 2007; Yu et al. 2009; Saini et al. 2010; Saini et al. 2014). All these mostly genetic, biochemical and biophysical results were recently confirmed by structural studies coming from the lab of the Nobel laureate Dr. Venki Ramakrishnan (Hussain et al. 2014; Llacer et al. 2015). It will never stop enrapturing me how these guys could predict all these

intricate reactions in such a detail using only indirect tools (Fig. 5) (reviewed in (Hinnebusch 2014)).

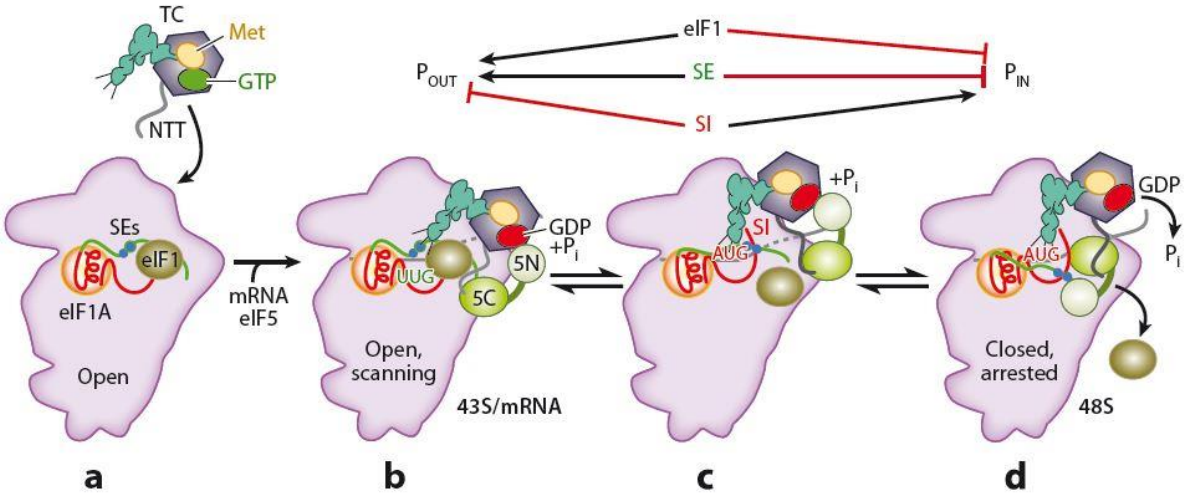


**Figure 5.** Model of structural rearrangements in the preinitiation complex (PIC) accompanying start-codon recognition. (*a*) Binding of eukaryotic initiation factors (eIFs) eIF1 and eIF1A to the 40S subunit evokes an open conformation conducive to rapid ternary complex (TC) binding, which forms the 43S PIC. The N-terminal tail (NTT) of eIF2β is shown as a wavy line attached to the TC. (*b*) The 43S subunit scans the messenger RNA (mRNA) 5' untranslated region; the anticodon stem loop (ASL) of methionyl initiator transfer RNA (Met-tRNAi) is not fully engaged with the P site ($P^{out}$ state) but can sample triplets for complementarity to the anticodon as they enter the P site. The GAP domain in eIF5-NTD (N-terminal domain, abbreviated 5N) stimulates GTP hydrolysis to produce GDP-Pi (phosphate), but release of Pi is impeded. The unstructured NTT of eIF2β interacts with eIF1 to stabilize this open conformation of the PIC. (*c*) Base-pairing between the ASL and the AUG codon promotes movement of the tRNA from the $P^{out}$ state to the $P^{in}$ state, displacing eIF1 from its location near the P site to a new 40S binding site that overlaps with the eIF5-CTD (C-terminal domain, abbreviated 5C) binding site. This movement of eIF1 eliminates its interaction with eIF2β-NTT, and the latter interacts tightly with eIF5-CTD instead. (*d* ) eIF1 dissociates from the 40S subunit to stabilize the closed, scanning-incompatible conformation of the 40S subunit. Ejection of eIF1 allows eIF5-NTD to dissociate from the G domain of eIF2γ and bind to the 40S subunit at a location that overlaps the eIF1 binding site, facilitating a functional interaction with the eIF1A C-terminal tail (CTT) that triggers release of free Pi from eIF2-GDP-Pi and blocks re-association of eIF1 with the 40S subunit (Hinnebusch 2014).

During the last couple of months in Alan's lab I contacted my old "Viennese" friend Dr. Jiří Hašek with a simple request: "Would there be an available position for me at the Institute of Microbiology CAS (IM CAS), so that I could apply for the Fellowship of Jan E. Purkyně?" The answer was Yes, so I applied, received this award and started as a research scientist in Jiři's lab in June 2004; i.e. two weeks

after our arrival from the US. Hence, taking a retrospective look from the eagle's perspective, we did not stay in the US one more year to capitalize on our savings (in fact, we did not make any investments at all at the end of the day) but to hold on for a moment until something (like the JEP fellowship) appeared to pave my way to start performing science on the home ground.

The first year of my first Czech employment was spent mostly with writing international grants. And it paid off because in the other half of 2005 I received NIH Global Health Research Initiative Program Award, Wellcome Trust International Senior Research Fellowship and Howard Hughes Medical Institute International Research Scholar award – all with great funding (mainly from the Trust) and all for 5 years. I started hiring, submitted a request with the IM CAS to have my own Laboratory of Regulation of Gene Expression established and above all, we started working hard to fulfill our goals (see below). In the fall of 2005 Dr. Miroslav Flieger did a huge, never-to-be-forgotten favor to my group by surrendering a nice, spacious lab at the ground floor of building L (it has remained our leading lab-ship ever since). In June 2006 the lab consisted of 7 people excluding myself and was officially brought to life by the IM CAS director, Prof. B. Říhová.

Our initial major goals were:

• How does eIF3 promote the assembly of the 43S preinitiation complex; i.e. binding of the multifactor complex (MFC) containing the Met-$tRNA_i^{Met}$-eIF2-GTP ternary complex to the 40S ribosome?

• What are the critical contact points between eIF3 and other components of the MFC and the 40S ribosome?

• Where do these factors bind on the small ribosome?

• What role does eIF3 play in regulation of the post-assembly events such as scanning, stringent selection of the start AUG codon, and GTP-hydrolysis?

We first made our own existence known to the world by an unexpected finding that came along as a lovely surprise while we were working on the first two aims. Bela Szamecz, my first post-doc showed that the interaction between the N-terminal domain of the largest subunit of eIF3, TIF32, and a small ribosomal protein RPS0 is not only an important intermolecular bridge between eIF3 and the 40S ribosome but also critically promotes so called translation reinitiation (REI) (Szamecz et al. 2008 - **pub 13**).

REI is a gene-specific regulatory mechanism exploiting the presence of short upstream uORFs in mRNA leaders (i.e. 5' untranslated regions – 5' UTRs) of various genes. The molecular key to this potentially abundant regulation (Calvo et al. 2009; Hood et al. 2009; Zhou et al. 2010) is the ability of some of these short uORFs (in yeast up to 5 codons in length (Vilela et al. 1998; Rajkowitsch et al. 2004; Szamecz et al. 2008 - **pub 13**), in plants up to 16 (von Arnim et al. 2014) and in mammals up to 30 codons (Kozak 2005)) to retain 40S ribosomal subunits on the same mRNA molecule even after they have been translated and the large 60S subunit has been recycled by the ribosome recycling factors (reviewed in (Jackson et al. 2012; Valášek 2012 - **pub 22**)). Such post-termination 40S subunits are then able to resume

scanning downstream and upon acquisition of the new ternary complex (TC), composed of Met-tRNA$_i^{Met}$ and eukaryotic initiation factor eIF2 in its GTP form, they are capable to recognize the AUG start codon of a next ORF and reinitiate translation thereon.

Generally speaking, short uORFs in principle impose a functional barrier for sufficient expression of a downstream main ORF. This repressive effect of uORFs can be, however, alleviated under specific conditions such as various types of stress in order to boost expression of some regulatory uORF-containing mRNAs that help the cell to cope with the sudden environmental changes. It has been shown that the efficiency of REI depends on four main factors: i) time required for uORF translation, which is determined by the relative length of uORF and the translation elongation rate; ii) its 5' and 3' flanking sequences, which contain specific *cis*-acting features with poorly understood molecular roles; iii) translation initiation factors (eIFs) involved in the primary initiation event such as the eIF3 and eIF4F complexes, which are believed to remain associated with the ribosome throughout the short elongation as well as termination and recycling phases; and iv) its distance to the next open reading frame, which determines the likelihood of acquisition of the new TC by the post-termination 40S ribosome that has resumed scanning (Kozak 1987; Dever et al. 1992; Pöyry et al. 2004; Szamecz et al. 2008 - **pub 13**).

Translational control of one of the yeast most influential stress-related transcription factors, GCN4, represents undoubtedly the best studied model of eukaryotic translation REI. The *GCN4* mRNA containing altogether four short uORFs has been studied in great detail for several decades and found to be very sensitive to the TC levels that are changing in response to different nutrient conditions and to rely mainly on the first REI-permissive uORF1 and the last REI-non-permissive uORF4 (reviewed in (Hinnebusch 2005) and recently revised in (Gunisova and Valasek 2014 - **pub 25**) – see below). Briefly, the first of the four uORFs is efficiently translated under both nutritional replete and deplete conditions and after its translation the post-termination 40S subunit remains attached to the mRNA and resumes scanning downstream for REI at the next AUG (Fig. 6; both panels).

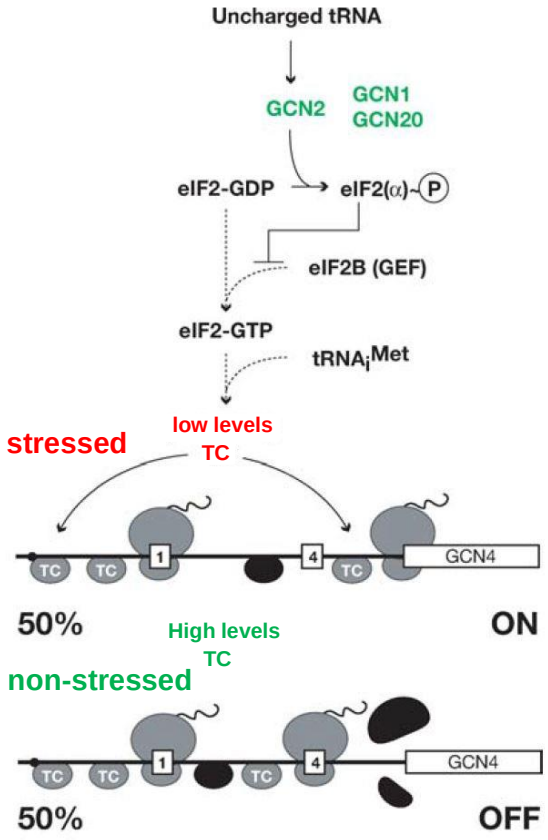


**Figure 6.** *GCN4* translational control *via* reinitiation (REI) (Hinnebusch 2005).

In non-stressed cells, where the TC levels are high, nearly all of the ribosomes that translated REI-permissive uORF1 and resumed scanning can rebind the TC before reaching one of the last two distant uORFs (uORFs 3 and 4), neither of which supports efficient REI. As a result, ribosomes terminating on one of these two uORFs undergo the full ribosomal recycling step, which prevents them from reaching and translating the main *GCN4* ORF (Fig 6; lower panel).

Under starvation conditions, the GCN2 kinase phosphorylates eIF2, which suspends formation of new TCs in the cytoplasm. Consequently, post-termination 40S ribosomes traveling from the uORF1 stop codon downstream will require more time to rebind the TC to be able to recognize the next AUG start codon. This will allow a large proportion of them to bypass uORF3 and uORF4 and reacquire the TC downstream of uORF4 but still upstream of the *GCN4* start codon (Fig. 6; upper panel). Thus, whereas global protein synthesis is significantly down-regulated under nutrient deplete conditions, protein expression of the GCN4 transcriptional activator is concurrently induced.

The exceptionally high REI potential of uORF1 has been ascribed in the past to 1) its 5' sequences (Grant et al. 1995), 2) the first 10 nt immediately following the uORF1 stop codon (Grant and Hinnebusch 1994), and 3) the third coding triplet of uORF1 in combination with its 3' UTR (Grant and Hinnebusch 1994). Bela revealed that the 5' sequences of uORF1 contain *cis*–acting elements that functionally interact with the N-terminal domain (NTD) of the TIF32 subunit of eIF3 and that this interaction is critically required for stabilization of post-termination 40S subunits on the uORF1 stop codon. Without formation of this interaction, the small subunit is also recycled, cannot resume scanning and thus the expression of *GCN4* remains off even when the cells are starved (Szamecz et al. 2008 - **pub 13**). This unexpected discovery initiated a brand new path of our research.

In the following years Vanda Munzarová in my lab identified and characterized four discernible REI-promoting *cis*–acting elements (RPEs i. – iv.), all of which together make up the so called 5´ enhancer (Fig. 7) (Munzarová et al. 2011 - **pub 17**). Genetic epistatic experiments revealed that two of these RPEs, RPE i. and RPE iv., operate in synergy and in the TIF32-NTD dependent manner, whereas RPEs ii. and iii. contribute by a different, yet to be elucidated mechanism. Likewise, two separate regions within the TIF32-NTD were described and implicated in promoting REI in concert with RPEs i. and iv. (they were called Boxes 6 and 17 and each of them is composed of 10 aa residues). A combination of computational and biochemical approaches revealed the 2D structure of the entire 5´ enhancer. The two key features of it are a 9 bp-long bulged stem and a double-circle hairpin representing the RPEs ii. and iv., respectively. In addition, we also showed that the TIF32-NTD interacts with the small ribosomal protein RPS0A (Valášek et al. 2003 - **pub 8**; Kouba et al. 2012a - **pub 20**), occurring virtually at the 40S mRNA exit channel (Aylett et al. 2015), where the 5' sequences of uORF1 also occur during termination on uORF1, suggesting that the a/TIF32-Boxes 6 and 17 directly interact with uORF1 RPEs i. and iv. (we are currently preparing a manuscript clearly showing that this is indeed the case *in vivo*). Collectively we proposed that the specific

secondary structures have to fold progressively while the ribosome scans through them prior uORF1 translation in order to form fully active REI enhancer upon uORF1 termination that is capable of binding to the TIF32-NTD. Strikingly, the structural motif similar to *GCN4* RPE iv. was also identified upstream of the REI-permissive uORF in the mRNA leader of yet another yeast transcriptional activator YAP1 (Munzarová et al. 2011 - **pub 17**). The fact that it likewise operated in the a/TIF32-NTD-dependent manner suggested that at least in yeasts the underlying mechanism of REI on short uORFs might be conserved.

Subsequently, Slávka Gunišová from my lab made a rather striking discovery by showing that the solitary uORF2 of *GCN4* is nearly as REI-permissive as uORF1, and not REI-non-permissive as was believed before. The similarly high efficiency of REI promoted by uORF2 was found to stem from the same *modus operandi* shared by uORF1 and uORF2 (Gunisova and Valasek 2014 - **pub 25**). In particular, we revealed that the REI competence of uORF2 strictly relies on: a) the structured, eIF3-independent RPE ii. of uORF1, which thus represents a common REI-promoting element for both of these uORFs, and b) a specific, 10 bp-long element designated as RPE v., which occurs in the vicinity of the 40S mRNA exit channel of the 80S ribosome terminating on uORF2 and, not surprisingly, operates in the TIF32-NTD-dependent manner. Thus, together with *GCN4* uORF1 and *YAP1* uORF, the *GCN4* uORF2 was the third short uORF that promotes REI by *cis*-acting elements upstream of its coding region, some of which functionally interact with the TIF32 subunit of eIF3.

These findings allowed us to markedly revise the long standing model of the *GCN4* translational control that has served as a textbook example of REI (compare Figs. 6 and 7). We proposed that the second REI-permissive uORF, uORF2 with ~80-90% of the uORF1 REI activity, serves as a backup of uORF1 to capture all ribosomes that leaky scanned the uORF1 AUG (Gunisova and Valasek 2014 - **pub 25**), especially during stress conditions that seem to increase the frequency of leaky scanning in general (Lee et al. 2009; Raveh-Amit et al. 2009; Palam et al. 2011; Sundaram and Grant 2014). This ensures that the maximum capacity of this intriguing regulatory system is met. Similarly, two consecutive uORFs with minimal or no REI-promoting potential occurring further downstream also prevent “leakiness” of this system but during nutrient replete conditions (Gunisova and Valasek 2014 - **pub 25**). Hence the tightness of *GCN4* translational control is ensured by a fail-safe mechanism that effectively prevents or triggers *GCN4* expression under nutrient replete or deplete conditions, respectively (Fig. 7).

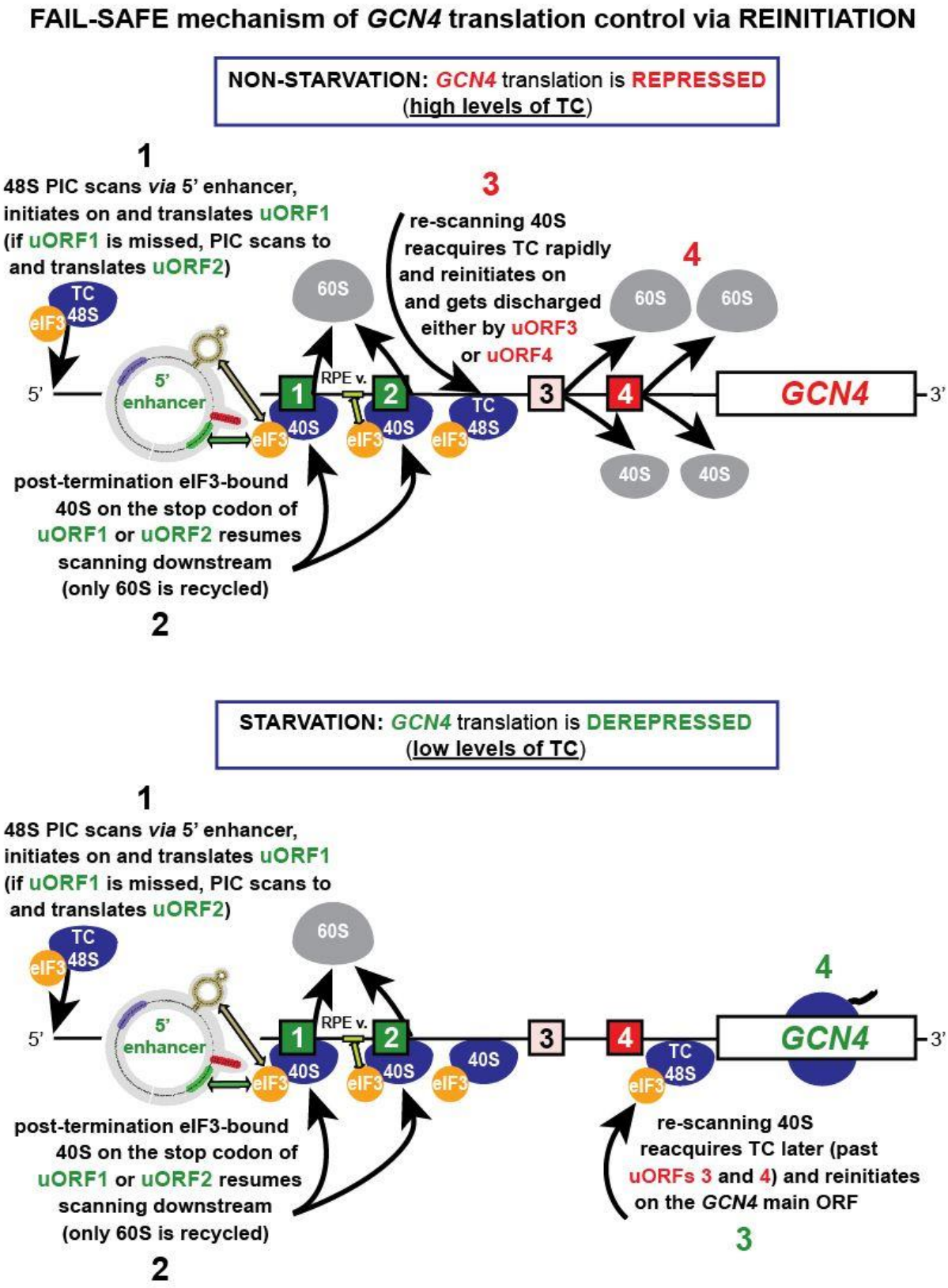


**Figure 7.** Revised fail-safe mechanism of *GCN4* translational control *via* reinitiation (Gunisova and Valasek 2014 - **pub 25**).

In our most recent article (Gunisova et al. 2016 - **pub 29**), we wished to investigate the molecular role of the first 10 nt immediately following the uORF1 stop codon that were shown to be critically required for efficient REI after uORF1 (Grant and Hinnebusch 1994) as well as the contributions of individual triplets of all uORFs. Our systematic analysis of all potential *cis*-determinants that either promote or inhibit reinitiation on *GCN4* mRNA revealed the attributes of individual uORFs that are summarized in Fig. 8.

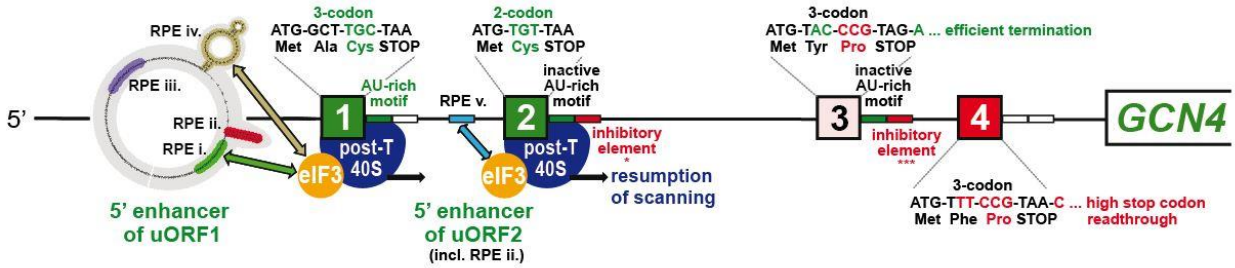


**Figure 8.** Summary of all *cis*-determinants that either promote or inhibit reinitiation on *GCN4* after translation of its four short uORFs (Gunisova et al. 2016 - **pub 29**).

The 3' sequences of uORFs 1 - 3, in particular the first 12 nt immediately following their stop codons, contain a conserved $AU_{1-2}A/UUAU_2$ motif that promotes REI independently of other REI-promoting elements but only when situated at the defined distance from the *GCN4* AUG start codon, in principle corresponding to the position of uORF1. Hence, despite carrying this autonomous motif in their 3' sequences, uORF2 and uORF3 do not utilize it. Intriguingly, the 3' sequences of specifically these two uORFs in addition contain inhibitory elements that immediately follow the AU-rich motif and decrease the REI potential of these two uORFs. Furthermore, Slávka also revealed that the authentic length of both REI-permissive uORFs has to be maintained for their optimal activity and that the last coding triplet can most probably tolerate a wide range of codons with the exception of the REI-inhibiting proline CCG triplet. Indeed, specifically this Pro triplet occurs as the last triplet in uORF3 and uORF4 and, in fact, features in all ultimate uORFs in the *GCN4* mRNA leaders across yeast species. Finally, we showed that the ~4-fold difference between the REI potential of modestly-REI-permissive uORF3 and REI-non-permissive uORF4 does not lie in the supposedly inhibitory 3' sequence of uORF4, as suggested before (Gunisova and Valasek 2014 - **pub 25**), but is manifested through the specific effects of the sequence composition of their second codon and of the identity of their stop codon tetranucleotide, which together impact the efficiency of stop codon recognition in a positive (uORF3) or negative (uORF4) way. In other words, we demonstrated for the first time that there is a direct negative correlation between the efficiency of reinitiation and efficiency of translation termination. Collectively this comprehensive approach highlighted an intriguing complexity of this delicate regulatory system that depends on several REI-promoting as well as inhibiting features that mutually fine tune their often autonomous effects on the overall efficiency of REI on *GCN4* mRNA in order to keep it as low as possible during

non-starvation conditions or as high as possible during starvation/stress conditions (Gunisova et al. 2016 - **pub 29**).

As mentioned above, we are currently preparing a manuscript that will provide strong *in vivo* evidence for a long-standing hypothesis that some critical initiation factors needed for reinitiation (like eIF3) are preserved on the 80S ribosome post the subunit joining step and remain 80S-bound for a few elongation cycles to promote resumption of scanning of the post-termination 40S subunits. We are also characterizing a molecular mechanism of translational control of human functional orthologue of yeast *GCN4*, the ATF4 transcriptional activator (Vattem and Wek 2004). Preliminary results suggest that there will be more differences than similarities.

In the meantime, the main stream of our laboratory research aimed at answering the aforementioned four major goals; in short, where eIF3 and its associated eIFs bind on the ribosome and what is their role. We first solved the structure of the RRM domain of PRT1 bound to HCR1 in collaboration with the NMR specialist Dr. Peter Lukavsky from MRC LMB in Cambridge and Susan Wagner from my lab demonstrated that HCR1 closely cooperates with the PRT1-RRM and eIF1A on the ribosome to ensure proper formation of the scanning-arrested conformation required for stringent AUG recognition (ElAntak et al. 2010 - **pub 14**). We also mapped the HCR1 position on the ribosome to lie at the mRNA entry channel, which was in good accord with the earlier position-mapping for mammalian eIF3j by Dr. Doudna's group (Fraser et al. 2007). Next Lucka Cuchalová mapped the position of the TIF35 subunit of eIF3 by revealing its interactions with RPS3 and RPS20, which are located near the ribosomal mRNA entry channel (Cuchalová et al. 2010 - **pub 16**). In this paper we also implicated TIF35 in ensuring the processivity of scanning through stable secondary structures and showed that it is required for resumption of scanning for downstream reinitiation by post-termination 40S ribosomes, like the TIF32-NTD mentioned above. Finally, we implicated the TIF34 subunit of eIF3 in stimulation of the linear scanning. Subsequently, we showed that the extreme CTD of TIF32 binds to RPS2 and RPS3, both situated near the mRNA entry channel, and directly stabilizes the 43S subunit-mRNA interaction. We also found that the TIF32-CTD directly interacts with the aforementioned PRT1-RRM–HCR1 partial assembly and that this trimeric "submodule" of eIF3 regulates the transition between scanning-conducive and scanning-arrested (initiation-competent) conformations of the PIC, described above (Chiu et al. 2010 - **pub 15**). The follow-up study, again in close collaboration with Peter, reported a 2.2 Å resolution crystal structure of the complex between the seven-bladed β-propeller TIF34 and a C-terminal α-helix of PRT1. Functional analysis of critical residues mediating this contact carried out by Anička Herrmannová revealed that the C-terminus of PRT1 orchestrates co-operative recruitment of TIF34 and TIF35 to the 40S subunit for a stable and proper assembly of 48S pre-initiation complexes necessary for stringent AUG recognition on mRNAs (Herrmannová et al. 2012 - **pub 18**).

Two of my PhD students meanwhile focused on the opposite termini of the NIP1 subunit of eIF3. Tomáš Kouba showed that the extreme C-terminus directly interacts with the small ribosomal protein RACK1/ASC1, which is a part of the 40S head, and, consistently, that deletion of ASC1 impaired eIF3 association with the 40S ribosome. The extreme C-terminus is preceded by a well-defined PCI domain that we computer-modelled and showed that it binds to RNA. It was the first ever evidence implicating this typical protein-protein binding domain in mediating also the protein-RNA interaction. Our mutational analysis of this domain entitled us to propose that the NIP1 C-terminal region forms an important intermolecular bridge between eIF3 and the 40S head region by contacting RACK1/ASC1 and most probably also 18S rRNA, which promotes assembly of translation preinitiation complexes (Kouba et al. 2012b - **pub 19**). Martina Karásková took over my first and last project in Alan's lab and wished to understand the molecular role of the N-terminal domain (NTD) of NIP1, which mediates eIF3 binding to eIF1 and eIF5, in ensuring high fidelity of AUG recognition in great detail. She demonstrated that eIF5 binds to the extreme NIP1-NTD (residues 1-45) and that impairing this interaction predominantly affects the 43S PIC formation. She also revealed that eIF1 interacts with the region (60-137) that immediately follows, and altering this contact deregulates AUG recognition. Together our data indicated that binding of eIF1 to the NIP1-NTD is equally important for its initial recruitment to PICs and for its proper functioning in selecting the translational start site (Karaskova et al. 2012 - **pub 21**). All these findings of ours and others were summarized in my "Ribozoomin" review from 2012 (Fig. 9), where I also proposed a model suggesting where all eIFs are binding on the 40S ribosome and what their role is (Fig. 10) (Valášek 2012-**pub 22**).

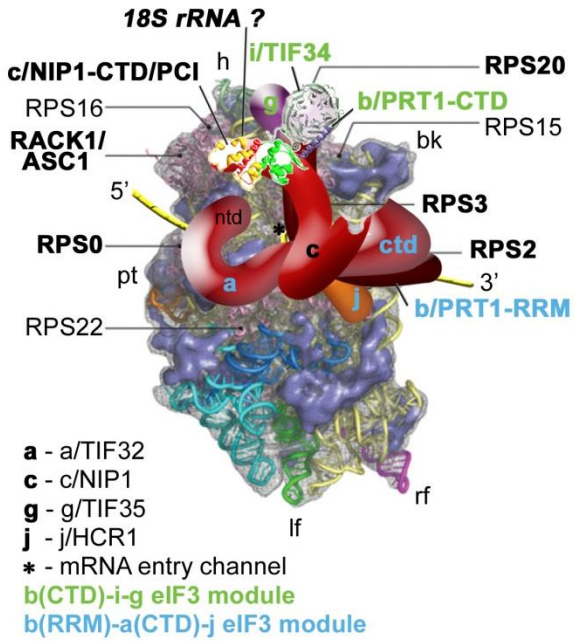


**Figure 9.** Model of the hypothetical location of eIF3 on the *S. cerevisiae* small ribosomal subunit (Kouba et al. 2012b - **pub 29**). The Cryo-EM reconstruction of the 40S subunit is shown from the solvent side with ribosomal RNA represented as tubes. Ribosomal proteins, with known homologs and placement, are shown as pink cartoons and labeled. The position of RACK1/ASC1 is highlighted in bold. The mRNA entry channel is designated by an asterisk. Hypothetical location of *S. cerevisiae* eIF3 on the back side of the 40S subunit is based on the data of several studies including the interactions between NIP1-CTD and ASC1 (and potentially also with 18S rRNA), RPS0 and TIF32-NTD, RPS2 and HCR1, RPS2 and 3 and TIF32-CTD, helices 16-18 of 18S rRNA and TIF32-CTD, and RPS3 and 20 and g/TIF35. The yellow lines represent mRNA.

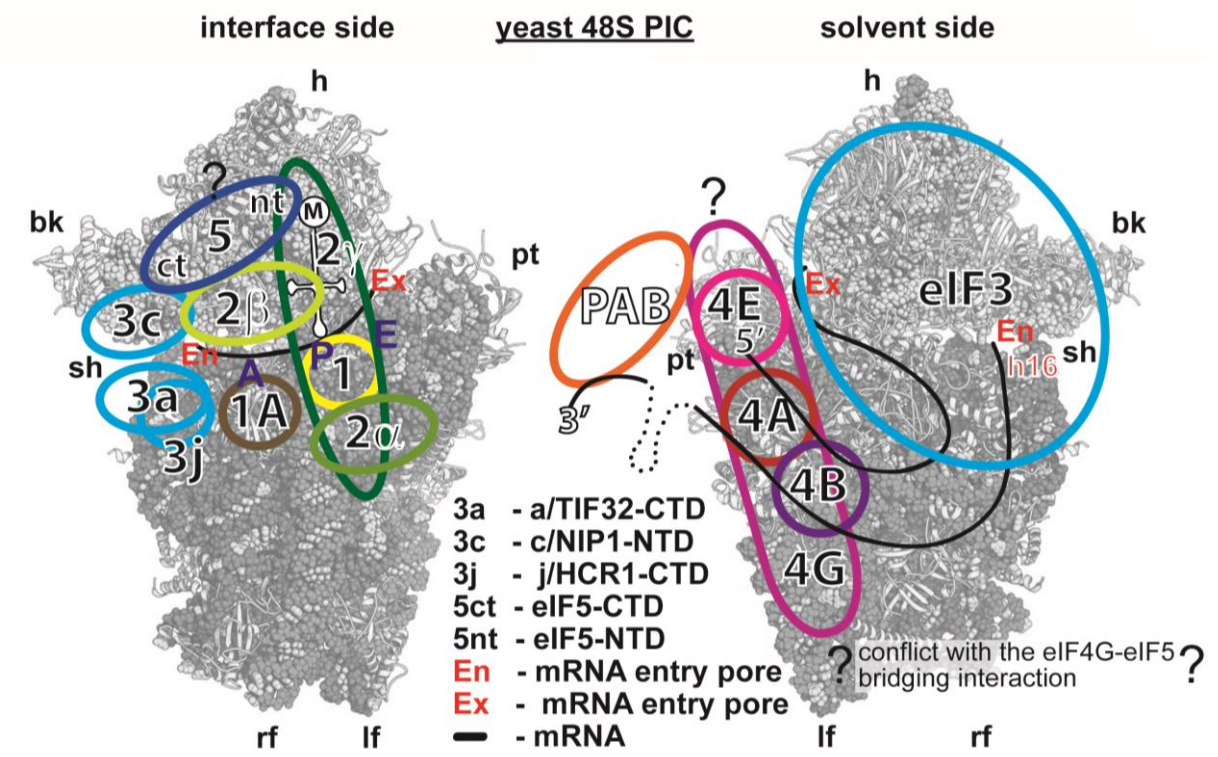


**Figure 10.** Hypothetical summary model of the structural arrangement of the yeast 48 PIC. Interface and solvent-exposed views of the tertiary structure of the 40S showing the 18S rRNA as spheres and the proteins as gray cartoons. Positions of the individual eIFs is schematically depicted as color-coded ovals based on studies referenced throughout this text. Positions with the question marks were not determined experimentally, not even proposed by structural modeling, and thus represent only the author's best estimate (Valášek 2012 **- pub 22**).

Two years later we published our last yeast story on this topic until now, this time in collaboration with the structural group of Dr. Ralf Ficner from the University in Gottingen. They solved a crystal structure of the PCI domain of the TIF32 subunit of eIF3 at 2.65-Å resolution and Slávka Gunišová and Vlaďka Vlčková from my lab showed that it is required for integrity of the eIF3 core and, similarly to the NIP1-PCI, is capable of RNA binding. We also performed its mutational analysis and identified a 10-Ala-substitution (Box37) that severely reduced amounts of model mRNAs in the 43-48S PICs *in vivo* as the major, if not the only, detectable defect. The putative RNA-binding surface was found to be defined by positively charged areas containing two Box37 residues, R363 and K364. Their substitutions with alanines severely impaired the mRNA recruitment step *in vivo*, which suggested that the TIF32-PCI represents one of the key domains ensuring stable and efficient mRNA delivery to the PICs (Khoshnevis et al. 2014 - **pub 24**). In the same year the Ralf's group managed to solve also the structure of the unusual nine-bladed beta-propeller of the PRT1-CTD and demonstrate that it interacts with the 40S ribosomal subunit via RPS9 (Liu et al. 2014). With these two studies done we presented our final, so far, model of the eIF3 arrangement on the ribosome (Fig. 11), because soon after these achievements

several long-awaited Cryo-EM structures, unfortunately still not in a very high resolution, of yeast 43S PICs were presented by Nenad Ban's and Venki Ramakrishnan's groups (Erzberger et al. 2014; Aylett et al. 2015; Llacer et al. 2015) (Fig. 12). To our delight and satisfaction, they all nicely agreed with our models that we had been putting together step-by-step using yeast genetics and biochemistry for over ten years beginning in the Alan's lab.

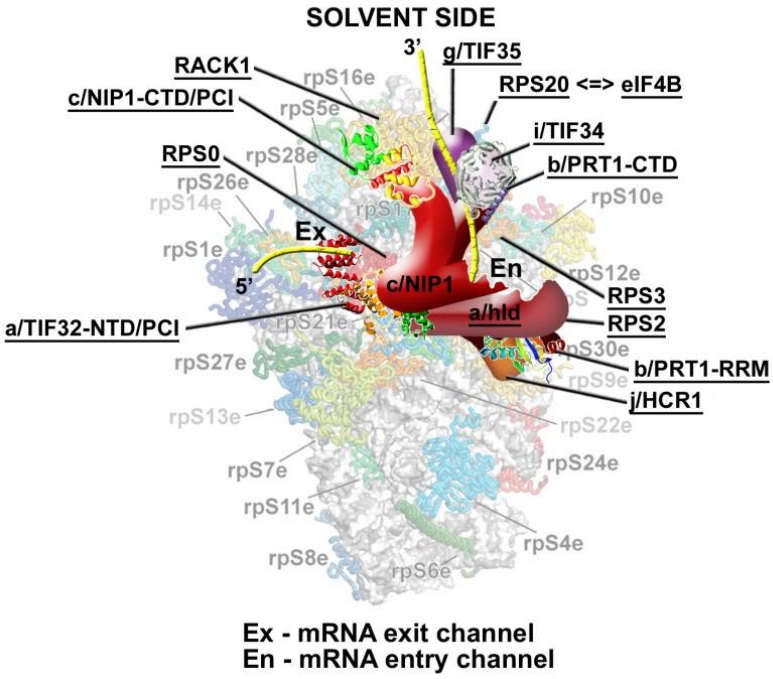


**Figure 11.** A revised model of eIF3 on the 40S ribosome spanning the mRNA exit and entry channels (Khoshnevis et al. 2014 - **pub 24**). The crystal structure of the 40S subunit is shown from the solvent side with ribosomal proteins shown as cartoons in individual colors; rRNA is shown as gray surface. A hypothetical location of *S. cerevisiae* eIF3 on the back side of the 40S subunit is based on the published interactions between RACK1 and the NIP1-CTD/PCI (Kouba et al. 2012b - **pub 19**), RPS0 and the TIF32-NTD/PCI (Valášek et al. 2003 - **pub 8**; Szamecz et al. 2008 - **pub 13;** Kouba et al. 2012a - **pub 20**); RPS2 and HCR1 (ElAntak et al. 2010 - **pub 14**); RPS2 and 3 and the TIF32-CTD (Chiu et al. 2010 - **pub 15**); helices 16-18 of 18S rRNA and the TIF32-CTD (Valášek et al. 2003 - **pub 8**); and RPS3 and 20 and TIF35 (Cuchalová et al. 2010 - **pub 16**). The extreme N-terminal and C-terminal domains of c/NIP1 and a/TIF32, respectively, are predicted to interact with the interface side of the 40S subunit (Valášek et al. 2003 - **pub 8**), as hinted. The interaction between RPS20 and eIF4B is indicated by a double-headed arrow (Walker et al. 2013). Positions of all eIF3 subunits as well as RACK1, RPS0, 2, 3 and 20 are highlighted in bold. Schematic representations of TIF34 bound to the PRT1-CTD, the PRT1-RRM, the TIF32-NTD/PCI, and the NIP1-CTD/PCI were replaced with the X-ray structures (Khoshnevis et al. 2010b - **pub 24**; Herrmannová et al. 2012 - **pub 18**) or the 3D structural model (Kouba et al. 2012b - **pub 19**), respectively. The yellow lines represent mRNA.

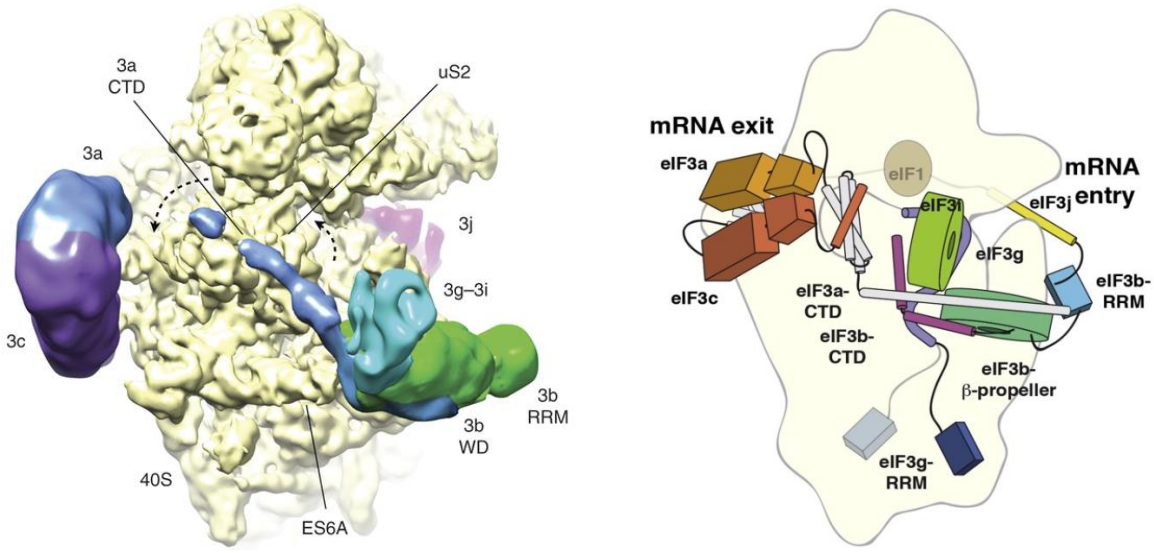


**Figure 12.** Cryo-EM structure of the budding yeast eIF1–eIF1A–eIF3–eIF3j initiation complex bound to the 40S (left panel). Model of the consensus positions of various eIF3 elements in the yeast 40S·eIF1·eIF3 complexes (right panel) (Erzberger et al. 2014; Aylett et al. 2015).

We will resume working on this interesting topic; i.e. on functional characterization of individual eIF3 subunits and their domains, as soon as close-to-atomic resolution crystal structures of these complexes will be available. Besides other labs, we too are working on this important and rather "secretive" goal in collaboration with Dr. Marat Yusupov from the Inst. de Génétique et de Biologie Moléculaire et Cellulaire in France.

While we were working on characterization of subunit composition and structure of budding yeast eIF3, several groups invested a lot of effort into the same goal but with mammalian eIF3. The mammalian eIF3 complex has a molecular mass ~ 800 kDa that accounts for about two thirds of the mass of the 40S ribosomal subunit (~1200 kDa). Human eIF3 contains altogether 13 subunits (eIF3s-eIF3m) in contrast to 6 subunits in yeast; only eIF3a, eIF3b, eIF3c, eIF3g, eIF3i and eIF3j have yeast homologues as introduced above. Many interactions are believed to be conserved in both organisms suggesting that they share a common functional core (reviewed in (Hinnebusch 2006; Valášek 2012 - **pub 22**)), however, comprehensive experimental evidence especially for human eIF3 is still rather poor. Initial mass spectrometric analysis of human eIF3 suggested that human eIF3 comprises three modules. Module i contains all but one yeast eIF3 core subunits (a, b, g, i), module ii comprises eIF3c, d, e, k and l, and module iii contains subunits eIF3f, h, and m (Zhou et al. 2008). Later it was proposed that the structural core of human eIF3 is formed by the so called PCI/MPN octameric complex composed of 8 subunits (a, c, e, f, k, l, m and n) (Fig. 13 – left panel) to which other 5 subunits are more flexibly linked (Fig. 13 – right panel) (Hashem et al. 2013; Querol-Audi et al. 2013; des Georges et al. 2015).

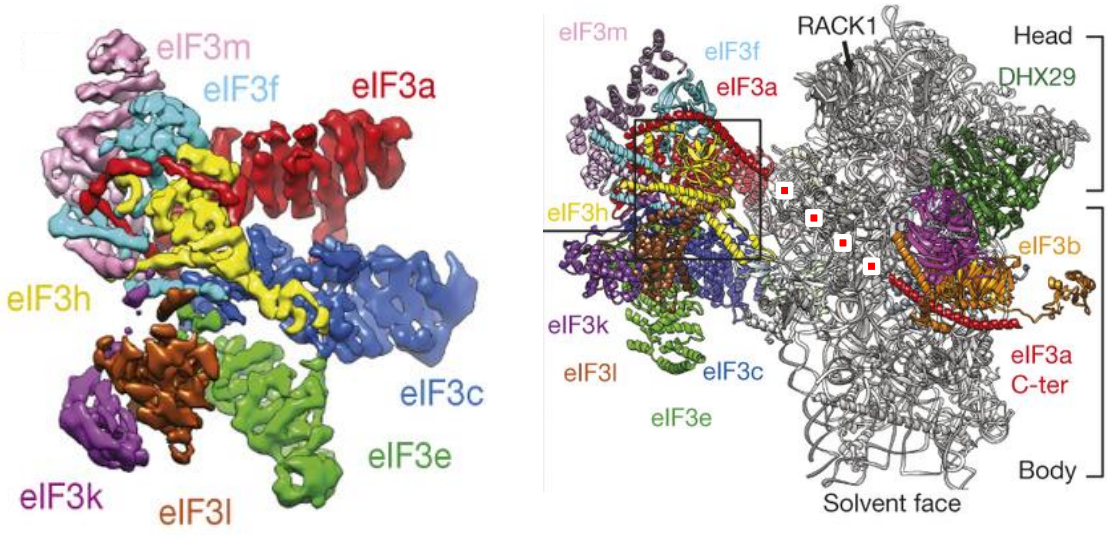


**Figure 13.** Structural of the core of human eIF3 formed by the so called PCI/MPN octamer (left panel) and contacts of the PCI/MPN eIF3 core and of the remaining subunits with the 40S subunit in the 43S complex (right panel) (des Georges et al. 2015)

Several years ago we have also turned our attention to human eIF3 and our recent biochemical work and a follow-up study that will be submitted shortly suggested that at least two stable eIF3 subcomplexes may exist *in vivo*: the PCI/MPN octamer and the "yeast eIF3-like" a-b-g-i formation - both of them share the eIF3a subunit (Fig. 14) (Wagner et al. 2014 - **pub 26**). Importantly, in this published study we developed several novel *in vivo* assays to monitor the integrity of the 13-subunit human eIF3 complex, defects in assembly of 43S PICs, efficiency of mRNA recruitment, and post-assembly events such as AUG recognition. We knocked down expression of the PCI domain-containing eIF3c and eIF3a subunits and of eIF3j in human cells and analyzed the functional consequences. Whereas eIF3j downregulation had barely any effect and eIF3a knockdown disintegrated the entire eIF3 complex, eIF3c knockdown produced a separate assembly of the "yeast eIF3-like" a-b-g-I (Fig. 14), which preserved high 40S-binding affinity, an ability to promote mRNA recruitment to 40S subunits and displayed defects in AUG recognition. Both eIF3c and eIF3a knockdowns also severely reduced protein but not mRNA levels of many other eIF3 subunits and indeed shut off translation. Based on these results we proposed that eIF3a and eIF3c control abundance and assembly of the entire eIF3 and thus represent its crucial scaffolding elements critically required for formation of PICs (Wagner et al. 2014 - **pub 26**). Interestingly, three of the four "yeast eIF3-like" subcomplex subunits (a, b, g) plus eIF3d were recently implicated in controlling expression of a special set of mRNAs and the authors suggested that binding of eIF3 to these mRNAs could be targeted to control carcinogenesis (Lee et al. 2015).

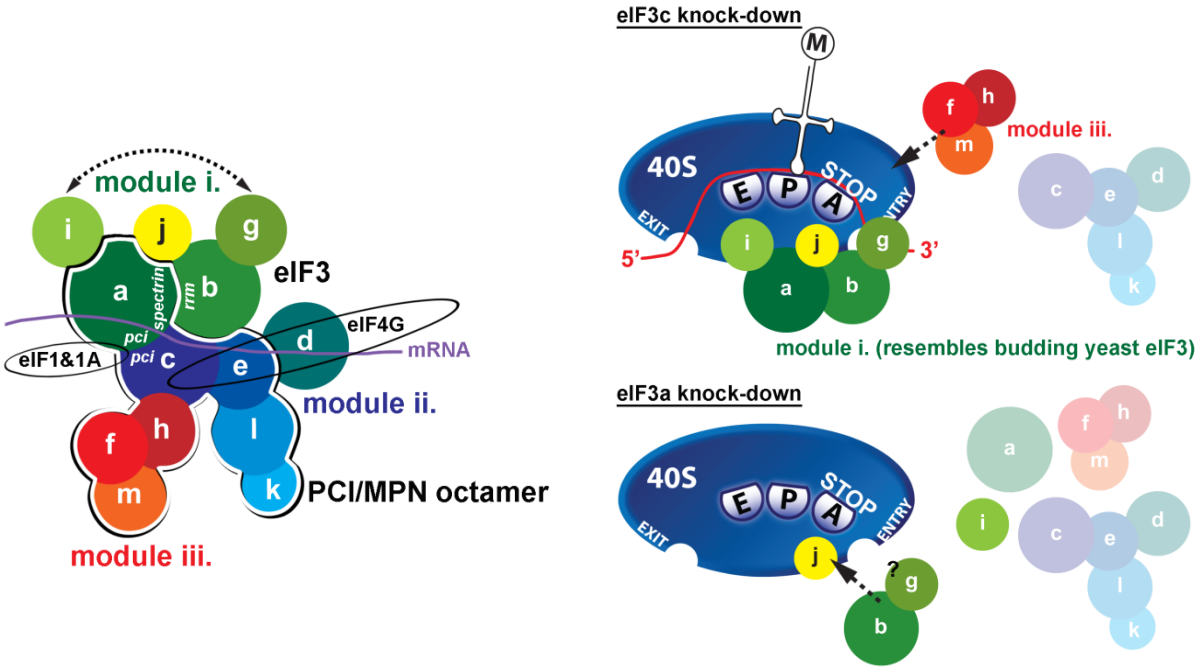


**Figure 14.** A schematic model of human eIF3 and its binding partners; individual eIF3 modules as well as eIF3 subunits forming the PCI/MPN octamer are color-coded (left panel). Schematics illustrating the effects of eIF3c and eIF3a knock-downs on integrity of the human eIF3 holocomplex and its ability to stimulate formation of the 48S PICs (right panel) (Wagner et al. 2014 - **pub 26**).

Earlier work by the Pestova's group implicated several eukaryotic initiation factors like eIF3 in ribosomal recycling *in vitro* (Pisarev et al. 2007). As mentioned above, recycling of eRF1-associated post-termination complexes (post-TCs) is mainly ensured by the ATPase ABCE1/RLI1, which splits post-TCs into free 60S subunits and deacylated tRNA- and mRNA-bound 40S subunits (40S-post-TC) in an ATP-dependent manner (Pisarev et al. 2010; Shoemaker and Green 2011; Becker et al. 2012). The release of tRNA and mRNA from the 40S-post-TCs can be *in vitro* mediated by the *bona fide* initiation factors eIF1, eIF1A and eIF3 (Pisarev et al. 2007; Pisarev et al. 2010). eIF3, and in particular its j subunit (HCR1 in yeast), were suggested to play the key role in mRNA dissociation.

Since the implication of eIF3 in the recycling process was deduced only from experiments carried out with 11-codon long model mRNA in mammalian *in vitro* reconstituted systems, we decided to investigate whether or not eIF3 also plays a direct role in translation termination and/or ribosomal recycling in the living cell. Thanks to an extensive research by Petra Beznosková and Lucie Cuchalová, we showed that the five-subunit core of eIF3 and its loosely associated subunit – HCR1 – control translation termination and stop codon readthrough in yeast, although in the opposite manner. A substantial proportion of eIF3, HCR1 and eukaryotic release factor 3 (eRF3) but not eIF5 (a well-defined "initiation-specific" binding partner of eIF3, as described above) specifically co-sedimented with 80S couples isolated from

RNase-treated heavy polysomes in an eRF1-dependent manner, indicating the presence of eIF3 and HCR1 on terminating ribosomes. eIF3 and HCR1 also occurred in ribosome- and RNA-free complexes with both eRFs and the recycling factor ABCE1/RLI1. Several eIF3 mutations reduced rates of stop codon readthrough and genetically interacted with mutant eRFs. In contrast, slow growing deletion of *hcr1* increased readthrough and accumulated eRF3 in heavy polysomes in a manner suppressible by overexpressed ABCE1/RLI1. Based on these and other findings we proposed that 1) in the pre-TC, eIF3 interacts with the N domain of eRF1 *via* its two small g/TIF35 and i/TI34 subunits and modulates, perhaps inhibits, its stop codon recognition activity during the proofreading step; 2) upon stop codon recognition the GTP molecule on eRF3 is hydrolyzed and HCR1 promotes eRF3·GDP ejection from the post-termination complexes to allow binding of its interacting partner ABCE1/RLI1 (Fig. 2). Furthermore, the fact that high dosage of ABCE1/RLI1 fully suppressed the slow growth phenotype of *hcr1Δ* as well as its termination but not initiation defects strongly indicated that the termination function of HCR1 is more critical for optimal proliferation than its function in translation initiation. Together our work characterized novel, unexpected roles of eIF3 and HCR1 in stop codon recognition and thus defined a communication bridge between the initiation and termination/recycling phases of translation (Beznosková et al. 2013 - **pub 23**).

In the follow-up study, we wished to reveal molecular details of the eIF3 role in the programmed stop codon readthrough. Programmed readthrough is a post-transcription regulatory mechanism specifically increasing proteome diversity by creating a pool of C-terminally extended proteins. During this process, the stop codon is decoded as a sense codon by a near-cognate (nc) tRNA, which programs the ribosome to continue elongation. The efficiency of competition for the stop codon between release factors (eRFs) and near-cognate tRNAs is largely dependent on its nucleotide context. Petra Beznosková showed that eIF3 really critically promotes programmed readthrough on all three stop codons (Beznoskova et al. 2015 - **pub 27**). In order to do so, eIF3 must associate with pre-termination complexes where it interferes with the eRF1 ability to recognize the third/wobble position of any programmed stop codon leading to the rejection of the eRF1-eRF3.GTP complex from pre-TCs. At the same time eIF3 promotes incorporation of nc-tRNAs with a mismatch at the same position and thus represents one of the key players in programmed stop codon readthrough (Fig. 15). For UGA there are two nc-tRNAs with the mismatch at the third/wobble position, Trp- and Cys-tRNAs. The third and last nc-tRNA with the wobble mismatch, Tyr-tRNA, is shared by the UAA and UAG stop codons. Incorporation of all of these three nc-tRNAs is promoted by eIF3 and, in fact, we provided evidence that efficient readthrough at UGA is enabled exclusively by the former two nc-tRNAs with the mismatch at the third/wobble position. This does not seem to apply to the other two stop codons, however, because other studies based on different approaches that were mentioned above showed that in addition to Tyr-tRNA, UAA and UAG can be also recoded by Gln- and Lys-tRNAs; i.e. nc-tRNAs with the mismatch at the first position (Blanchet et al. 2014; Roy et al. 2015). The UGA stop codon has two nc-tRNAs with the first position mismatch, Gly- and Arg-tRNA,

the latter of which was also suggested to insert at the A-site with low frequencies by these authors; however, it did not do so in our hands. Importantly, Petra also showed that the eIF3 role in programmed readthrough is conserved between yeast and humans.

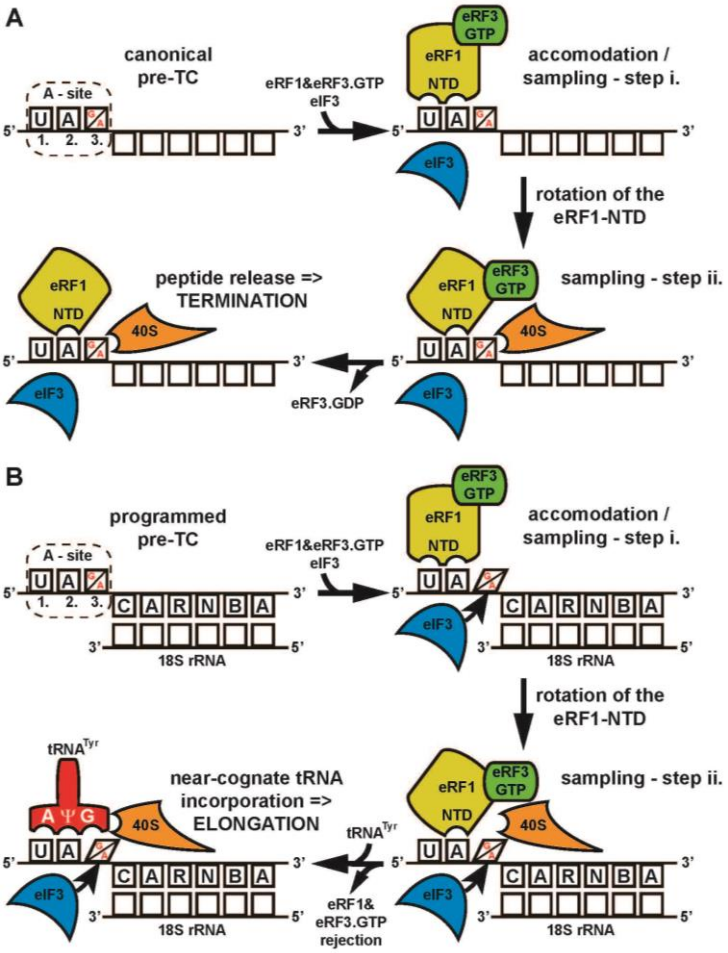


**Figure 15.** Translation initiation factor eIF3 promotes programmed stop codon readthrough. (**A**) Canonical termination; stop codon in the termination favorable context appears in the A-site (only UAG and UAA stop codons are indicated for illustration purposes; UGA works by the same mechanism), eRF1 in complex with eRF3.GTP binds to it and samples the codon in a two-step process including conformational re-arrangements of the eRF1-NTD. During the second step the ribosome by itself co-participates in this accommodation phase that ultimately leads to GTP hydrolysis on eRF3, polypeptide release and ribosomal recycling (see text for further details). (**B**) Programmed stop codon readthrough; stop codon occurs in the unfavorable termination context bearing specific consensus sequences like CAR-NBA in its 3' UTR – in this particular case proposed to base-pair with 18S rRNA. The eIF3 presence in the pre-TC (perhaps in co-operation with these sequences) alters decoding property of the nucleotide at the 3$^{rd}$ stop codon position. This prevents its proper decoding during the second sampling step and subsequently, after the eRF1-eRF3.GTP complex rejection, allows incorporation of near-cognate tRNAs with the mismatch at the 3$^{rd}$ position to read through the stop codon and continue with elongation (Beznoskova et al. 2015 - **pub 27**).

In our most recent study, we specifically focused on the leakiest stop codon of all three (UGA) that, when featuring as a premature termination codon (PTC; see below), responds rather unpredictably to various types of anti-PTC treatment (Linde and Kerem 2008; Lee and Dougherty 2012). Our genetic experiments suggested that the key determinant of the highest readthrough levels displayed specifically by the

UGA-C tetranucleotide (Bonetti et al. 1995; Beznoskova et al. 2015 - **pub 27**) is the impaired ability of eRF1 to unambiguously recognize the stop codon when it is followed by the cytosine base (Beznoskova et al. 2016 - **pub 28**). Importantly, Petra Beznosková also revealed that this "cytosine-specific termination effect" has a general character as it was manifested also on the UAA and UAG stop codons. In addition, we also showed that the identity of the +4 base determines the preference of nc-tRNAs for a given UGA-N tetranucleotide. In particular, that UGA-A and UGA-G tetranucleotides are preferentially read through by tryptophan and cysteine nc-tRNAs, respectively (Beznoskova et al. 2016 - **pub 28**). The preferences for UAA-N and UAG-N decoding have also been recently identified by Petra, who is just completing our new story on this topic.

I would like to finish this thesis by mentioning our hope that our insightful understanding of the readthrough process might actually help to develop a better nonsense mutation suppression strategy. Premature termination codons (PTCs) logically result in premature termination and may involve a synthesis of truncated, abnormal proteins potentially toxic to cells through dominant negative or gain-of-function effects (Davies et al. 2007). As such premature termination constitutes the molecular basis of many genetic diseases, including cystic fibrosis. Nonsense suppression therapy encompasses approaches aimed at suppressing translation termination at in-frame premature termination codons to restore deficient protein function. That can be accomplished by applying chemical compounds (low molecular weight drugs) or by applying other approaches to either enhance premature termination codon readthrough, for example via suppressor tRNA, or to inhibit the nonsense-mediated mRNA decay (summarized in (Keeling et al. 2014)). Among the readthrough inducing drugs, the group of aminoglycoside antibiotics are the most important representatives; unfortunately their administration is very often associated with adverse, toxic effects. We believe that the new observations we made in (Beznoskova et al. 2015; Beznoskova et al. 2016; Gunisova et al. 2016) open a brand new avenue of the PTC-oriented research because they strongly indicate that the degree of readthrough at any of the three stop codons is specifically determined by 1) the identity of the +4 base and 2) the cellular level of nc-tRNA (from now on called the readthrough inducing tRNA - rti-tRNA) that is specific for a given stop codon tetranucleotide; i.e. specifically incorporates at the A-site occupied by this tetranucleotide compared to other existing nc-tRNAs. This means that depending on a character of a given problematic PTC, one could first make a judgment what rti-tRNA is specific for it and then attempt to use it in high dosage, perhaps in combination with a lower than usual dosage of some of the aforementioned drugs, as a specific treatment agent(s). Hence the exploration of the application of naturally occurring nc-tRNAs as the readthrough inducing agents and their crosstalk with readthrough inducing drugs is one of our primary research interests at the moment. End of story as of April 10, 2016.

## CONCLUSIONS

We have:

- isolated and characterized the largest subunit of translation initiation factor eIF3 – eIF3a/TIF32/RPG1 (pub 1,2,5)
- isolated and characterized the loosely associated subunit of translation initiation factor eIF3 – eIF3j/HCR1 (pub 3,5,6)
- mapped a subunit composition of yeast eIF3 and its subunit-subunit interactions as well as its interactions with other eIFs (eIF1, 2 and 5) together occurring in the so called Multifactor complex (pub 4,5,7,8,11)
- characterized the roles of eIF3 and its associated eIFs in assembly of pre-initiation complexes composed of the 40S ribosome, Met-tRNA$_i^{Met}$ and mRNA and in the stringent selection of the proper AUG start site (pub 5,7,8,9,10,11,12,14,15,16,18,19,21,24)
- in collaboration with foreign labs, solved NMR or x-ray structures of several eIF3 subunits or its critical domains and investigated the function of their critical, conserved residues in translation (pub 14,18,24)
- discovered and characterized the importance and molecular role of eIF3 in translation reinitiation (pub 13,17,20)
- identified and characterized the molecular role of cis-acting sequences flanking short uORFs, some of which interact with eIF3, in the GCN4 mRNA leader in translation reinitiation; based on these results we revised a long-standing, text book model of translation reinitiation (pub 13,17,25,29)
- most of these results were summarized in my “Ribozoomin” review (pub 22)
- characterized modularity (subunit subassemblies/modules) and basic molecular functions of human eIF3 in living cells (pub 26)
- discovered and characterized the surprising role of eIF3 and HCR1 in translation termination and programmed stop codon readthrough (pub 23,27)
- described new decoding rules that near-cognate tRNAs observed during programmed stop codon readthrough (pub 28)

## REFERENCES


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**: 251-262.

Alkalaeva EZ, Pisarev AV, Frolova LY, Kisselev LL, Pestova TV. 2006. In vitro reconstitution of eukaryotic translation reveals cooperativity between release factors eRF1 and eRF3. *Cell* **125**: 1125-1136.

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**: 2534-2546.

Asano K, Kinzy TG, Merrick WC, Hershey JWB. 1997. Conservation and diversity of eukaryotic translation initiation factor eIF3. *J Biol Chem* **272**: 1101-1109.

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.

Asano K, Shalev A, Phan L, Nielsen K, Clayton J, Valášek L, Donahue TF, Hinnebusch AG. 2001. Multiple roles for the carboxyl terminal domain of eIF5 in translation initiation complex assembly and GTPase activation. *EMBO J* **20**: 2326-2337.

Aylett CH, 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**: 269-271.

Baird TD, Wek RC. 2012. Eukaryotic Initiation Factor 2 Phosphorylation and Translational Control in Metabolism. *Advances in Nutrition: An International Review Journal* **3**: 307-321.

Bandyopadhyay A, Maitra U. 1999. Cloning and characterization of the p42 subunit of mammalian translation initiation factor 3 (eIF3): demonstration that eIF3 interacts with eIF5 in mammalian cells. *Nucleic Acids Research* **27**: 1331-1337.

Becker T, Franckenberg S, Wickles S, Shoemaker CJ, Anger AM, Armache JP, Sieber H, Ungewickell C, Berninghausen O, Daberkow I et al. 2012. Structural basis of highly conserved ribosome recycling in eukaryotes and archaea. *Nature* **482**: 501-506.

Benne R, Hershey JWB. 1978. The mechanism of action of protein synthesis initiation factors from rabbit reticulocytes. *J Biol Chem* **253**: 3078-3087.

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.

Beznoskova P, Gunisova S, Valasek LS. 2016. Rules of UGA-N decoding by near-cognate tRNAs and analysis of readthrough on short uORFs in yeast. *RNA* **22**: 456-466.

Beznoskova P, Wagner S, Jansen ME, von der Haar T, Valasek LS. 2015. Translation initiation factor eIF3 promotes programmed stop codon readthrough. *Nucleic Acids Res* **43**: 5099-5111.

Bidou L, Rousset JP, Namy O. 2010. Translational errors: from yeast to new therapeutic targets. *FEMS Yeast Res* **10**: 1070-1082.

Blanchet S, Cornu D, Argentini M, Namy O. 2014. New insights into the incorporation of natural suppressor tRNAs at stop codons in Saccharomyces cerevisiae. *Nucleic Acids Res* **42**: 10061-10072.

Bonetti B, Fu L, Moon J, Bedwell DM. 1995. The efficiency of translation termination is determined by a synergistic interplay between upstream and downstream sequences in Saccharomyces cerevisiae. *J Mol Biol* **251**: 334-345.

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

Calvo SE, Pagliarini DJ, Mootha VK. 2009. Upstream open reading frames cause widespread reduction of protein expression and are polymorphic among humans. *PNAS* **106**: 7507-7512.

Cassan M, Rousset JP. 2001. UAG readthrough in mammalian cells: effect of upstream and downstream stop codon contexts reveal different signals. *BMC Mol Biol* **2**: 3.

Cuchalová L, Kouba T, Herrmannová A, Danyi 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**: 4671-4686.

Das S, Maiti T, Das K, Maitra U. 1997. Specific interaction of eukaryotic translation initiation factor 5 (eIF5) with the b-subunit of eIF2. *J Biol Chem* **272**: 31712-31718.

Das S, Maitra U. 2000. Mutational analysis of mammalian translation initiation factor 5 (eIF5): role of interaction between the beta subunit of eIF2 and eIF5 in eIF5 function in vitro and in vivo. *Mol Cell Biol* **20**: 3942-3950.

Davies JC, Alton EW, Bush A. 2007. Cystic fibrosis. *BMJ* **335**: 1255-1259.

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**: 20615-20628.

des Georges A, Dhote V, Kuhn L, Hellen CU, Pestova TV, Frank J, Hashem Y. 2015. Structure of mammalian eIF3 in the context of the 43S preinitiation complex. *Nature* **525**: 491-495.

Dever TE, Feng L, Wek RC, Cigan AM, Donahue TD, Hinnebusch AG. 1992. Phosphorylation of initiation factor 2a by protein kinase GCN2 mediates gene-specific translational control of *GCN4* in yeast. *Cell* **68**: 585-596.

Dowling RJO, Topisirovic I, Fonseca BD, Sonenberg N. 2010. Dissecting the role of mTOR: Lessons from mTOR inhibitors. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics* **1804**: 433-439.

Dreher TW, Miller WA. 2006. Translational control in positive strand RNA plant viruses. *Virology* **344**: 185-197.

Dunn JG, Foo CK, Belletier NG, Gavis ER, Weissman JS. 2013. Ribosome profiling reveals pervasive and regulated stop codon readthrough in Drosophila melanogaster. *Elife* **2**: e01179.

ElAntak L, Wagner S, Herrmannová A, Karásková M, Rutkai E, Lukavsky PJ, Valášek L. 2010. The indispensable N-terminal half of eIF3j co-operates with its structurally conserved binding partner eIF3b-RRM and eIF1A in stringent AUG selection. *J Mol Biol* **396**: 1097-1116.

Erzberger JP, Stengel F, Pellarin R, Zhang S, Schaefer T, Aylett CH, Cimermancic P, Boehringer D, Sali A, Aebersold R et al. 2014. Molecular Architecture of the 40SeIF1eIF3 Translation Initiation Complex. *Cell* **158**: 1123-1135.

Fekete CA, Applefield DJ, Blakely SA, Shirokikh N, Pestova T, Lorsch JR, Hinnebusch AG. 2005. The eIF1A C-terminal domain promotes initiation

complex assembly, scanning and AUG selection in vivo. *Embo J* **24**: 3588-3601.

Fekete CA, Mitchell SF, Cherkasova VA, Applefield D, Algire MA, Maag D, Saini AK, Lorsch JR, Hinnebusch AG. 2007. N- and C-terminal residues of eIF1A have opposing effects on the fidelity of start codon selection. *EMBO J* **26**: 1602–1614.

Fernández IS, Bai X-C, Hussain T, Kelley AC, Lorsch JR, Ramakrishnan V, Scheres SHW. 2013. Molecular Architecture of a Eukaryotic Translational Initiation Complex. *Science* **342**.

Fletcher CM, Pestova TV, Hellen CUT, Wagner G. 1999. Structure and interactions of the translation initiation factor eIF1. *EMBO Journal* **18**: 2631-2639.

Fraser CS, Berry KE, Hershey JW, Doudna JA. 2007. 3j is located in the decoding center of the human 40S ribosomal subunit. *Mol Cell* **26**: 811-819.

Garcia-Barrio MT, Naranda T, Cuesta R, Hinnebusch AG, Hershey JWB, Tamame M. 1995. GCD10, a translational repressor of *GCN4*, is the RNA-binding subunit of eukaryotic translation initiation factor-3. *Genes Dev* **9**: 1781-1796.

Grant CM, Hinnebusch AG. 1994. Effect of sequence context at stop codons on efficiency of reinitiation in *GCN4* translational control. *Mol Cell Biol* **14**: 606-618.

Grant CM, Miller PF, Hinnebusch AG. 1995. Sequences 5' of the first upstream open reading frame in *GCN4* mRNA are required for efficient translational reinitiation. *Nuc Acids Res* **23**: 3980-3988.

Gunisova S, Beznoskova P, Mohammad MP, Vlckova V, Valasek LS. 2016. In-depth analysis of cis-determinants that either promote or inhibit reinitiation on GCN4 mRNA after translation of its four short uORFs. *RNA* **22**: 542-558.

Gunisova S, Valasek LS. 2014. Fail-safe mechanism of GCN4 translational control-uORF2 promotes reinitiation by analogous mechanism to uORF1 and thus secures its key role in GCN4 expression. *Nucleic Acids Res* **42**: 5880-5893.

Harrell L, Melcher U, Atkins JF. 2002. Predominance of six different hexanucleotide recoding signals 3' of read-through stop codons. *Nucleic Acids Res* **30**: 2011-2017.

Hashem Y, des Georges A, Dhote V, Langlois R, Liao HY, Grassucci RA, Hellen CU, Pestova TV, Frank J. 2013. Structure of the Mammalian Ribosomal 43S Preinitiation Complex Bound to the Scanning Factor DHX29. *Cell* **153**: 1108-1119.

Hašek J, Kovarik P, Valášek L, Malinská K, Schneider J, Kohlwein SD, Ruis H. 2000. Rpg1p, the subunit of the Saccharomyces cerevisiae eIF3 core complex, is a microtubule-interacting protein. *Cell Motil Cytoskeleton* **45**: 235-246.

Herrmannová A, Daujotyte D, Yang JC, Cuchalová L, Gorrec F, Wagner S, Danyi 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**: 2294-2311.

Hinnebusch AG. 2005. Translational regulation of GCN4 and the general amino acid control of yeast. *Annu Rev Microbiol* **59**: 407-450.

-. 2006. eIF3: a versatile scaffold for translation initiation complexes. *Trends Biochem Sci* **31**: 553-562.

-. 2014. The scanning mechanism of eukaryotic translation initiation. *Annu Rev Biochem* **83**: 779-812.

Hood HM, Neafsey DE, Galagan J, Sachs MS. 2009. Evolutionary Roles of Upstream Open Reading Frames in Mediating Gene Regulation in Fungi. *Annual Review of Microbiology* **63**: 385-409.

Huang H, Yoon H, Hannig EM, Donahue TF. 1997. GTP hydrolysis controls stringent selection of the AUG start codon during translation initiation in *Saccharomyces cerevisiae*. *Genes Dev* **11**: 2396-2413.

Hussain T, Llacer JL, Fernandez IS, Munoz A, Martin-Marcos P, Savva CG, Lorsch JR, Hinnebusch AG, Ramakrishnan V. 2014. Structural changes enable start codon recognition by the eukaryotic translation initiation complex. *Cell* **159**: 597-607.

Chao AT, Dierick HA, Addy TM, Bejsovec A. 2003. Mutations in eukaryotic release factors 1 and 3 act as general nonsense suppressors in Drosophila. *Genetics* **165**: 601-612.

Cheung YN, Maag D, Mitchell SF, Fekete CA, Algire MA, Takacs JE, Shirokikh N, Pestova T, Lorsch JR, Hinnebusch AG. 2007. Dissociation of eIF1 from the 40S ribosomal subunit is a key step in start codon selection in vivo. *Genes Dev* **21**: 1217-1230.

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**: 4415-4434.

Jackson RJ, Hellen CU, Pestova TV. 2012. Termination and post-termination events in eukaryotic translation. *Adv Protein Chem Struct Biol* **86**: 45-93.

Jackson RJ, Hellen CUT, Pestova TV. 2010. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* **11**: 113-127.

Janzen DM, Frolova L, Geballe AP. 2002. Inhibition of translation termination mediated by an interaction of eukaryotic release factor 1 with a nascent peptidyl-tRNA. *Mol Cell Biol* **22**: 8562-8570.

Jennings MD, Pavitt GD. 2010. eIF5 has GDI activity necessary for translational control by eIF2 phosphorylation. *Nature* **465**: 378-381.

-. 2014. A new function and complexity for protein translation initiation factor eIF2B. *Cell Cycle* **13**: 2660-2665.

Jennings MD, Zhou Y, Mohammad-Qureshi SS, Bennett D, Pavitt GD. 2013. eIF2B promotes eIF5 dissociation from eIF2•GDP to facilitate guanine nucleotide exchange for translation initiation. *Genes & Development* **27**: 2696-2707.

Jivotovskaya A, Valášek L, Hinnebusch AG, Nielsen KH. 2006. Eukaryotic translation initiation factor 3 (eIF3) and eIF2 can promote mRNA binding to 40S subunits independently of eIF4G in yeast. *Mol Cell Biol* **26**: 1355-1372.

Johnson KR, Merrick WC, Zoll WL, Zhu Y. 1997. Identification of cDNA clones for the large subunit of eukaryotic translation initiation factor 3. *J Biol Chem* **272**: 7106-7113.

Karaskova M, Gunisova S, Herrmannova A, Wagner S, Munzarova V, Valasek 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**: 28420-28434.

Keeling KM, Xue X, Gunn G, Bedwell DM. 2014. Therapeutics based on stop codon readthrough. *Annu Rev Genomics Hum Genet* **15**: 371-394.

Khoshnevis S, Gross T, Rotte C, Baierlein C, Ficner R, Krebber H. 2010a. The iron-sulphur protein RNase L inhibitor functions in translation termination. *EMBO Rep* **11**: 214-219.

Khoshnevis S, Gunišová S, Vlčková V, Kouba T, Neumann P, Beznosková P, Ficner R, Valášek LS. 2014. Structural integrity of the PCI domain of eIF3a/TIF32 is required for mRNA recruitment to the 43S pre-initiation complexes. *Nucleic Acids Research* **42**: 4123-4139.

Khoshnevis S, Neumann P, Ficner R. 2010b. Crystal structure of the RNA recognition motif of yeast translation initiation factor eIF3b reveals differences to human eIF3b. *PLoS One* **5**.

Kouba T, Danyi I, Gunišová S, Munzarová V, Vlčková V, Cuchalová L, Neueder A, Milkereit P, Valášek LS. 2012a. Small Ribosomal Protein RPS0 Stimulates Translation Initiation by Mediating 40S-binding of eIF3 via its Direct Contact with the eIF3a/TIF32 Subunit. *PLoS One* **7**: e40464.

Kouba T, Rutkai E, Karasková M, Valášek LS. 2012b. The eIF3c/NIP1 PCI domain interacts with RNA and RACK1/ASC1 and promotes assembly of the pre-initiation complexes. *Nucleic Acids Research* **40**: 2683-2699.

Kovarik P, Hašek J, Valášek L, Ruis H. 1998. RPG1: an essential gene of saccharomyces cerevisiae encoding a 110-kDa protein required for passage through the G1 phase. *Curr Genet* **33**: 100-109.

Kozak M. 1986. Point mutations define a sequence flanking the AUG initiator codon that modulates translation by eukaryotic ribosomes. *Cell* **44**: 283-292.

-. 1987. Effects of intercistronic length on the efficiency of reinitiation by eucaryotic ribosomes. *Mol Cell Biol* **7**: 3438-3445.

-. 2005. Regulation of translation via mRNA structure in prokaryotes and eukaryotes. *Gene* **361**: 13-37.

Lamphear BJ, Kirchweger R, Skern T, Rhoads RE. 1995. Mapping of functional domains in eukaryotic protein synthesis initiation factor 4G (eIF4G) with picornaviral proteases. *J Biol Chem* **270**: 21975-21983.

Lee AS, Kranzusch PJ, Cate JH. 2015. eIF3 targets cell-proliferation messenger RNAs for translational activation or repression. *Nature* **522**: 111-114.

Lee HL, Dougherty JP. 2012. Pharmaceutical therapies to recode nonsense mutations in inherited diseases. *Pharmacol Ther* **136**: 227-266.

Lee YY, Cevallos RC, Jan E. 2009. An upstream open reading frame regulates translation of GADD34 during cellular stresses that induce eIF2alpha phosphorylation. *J Biol Chem* **284**: 6661-6673.

Linde L, Kerem B. 2008. Introducing sense into nonsense in treatments of human genetic diseases. *Trends Genet* **24**: 552-563.

Liu Y, Neumann P, Kuhle B, Monecke T, Schell S, Chari A, Ficner R. 2014. Translation initiation factor eIF3b contains a nine-bladed beta-propeller and interacts with the 40S ribosomal subunit. *Structure* **22**: 923-930.

Llacer JL, Hussain T, Marler L, Aitken CE, Thakur A, Lorsch JR, Hinnebusch AG, Ramakrishnan V. 2015. Conformational Differences between Open and Closed States of the Eukaryotic Translation Initiation Complex. *Mol Cell* **59**: 399-412.

Lomakin IB, Kolupaeva VG, Marintchev A, Wagner G, Pestova TV. 2003. Position of eukaryotic initiation factor eIF1 on the 40S ribosomal subunit determined by directed hydroxyl radical probing. *Genes & Dev* **17**: 2786-2797.

Lomakin IB, Shirokikh NE, Yusupov MM, Hellen CU, Pestova TV. 2006. The fidelity of translation initiation: reciprocal activities of eIF1, IF3 and YciH. *Embo J* **25**: 196-210.

Maag D, Fekete CA, Gryczynski Z, Lorsch JR. 2005. A Conformational Change in the Eukaryotic Translation Preinitiation Complex and Release of eIF1 Signal Recognition of the Start Codon. *Mol Cell* **17**: 265-275.

Majumdar R, Bandyopadhyay A, Maitra U. 2003. Mammalian Translation Initiation Factor eIF1 Functions with eIF1A and eIF3 in the Formation of a Stable 40 S Preinitiation Complex. *J Biol Chem* **278**: 6580-6587.

Marcotrigiano J, Gingras AC, Sonenberg N, Burley SK. 1999. Cap-dependent translation initiation in eukaryotes is regulated by a molecular mimic of eIF4G. *Molecular Cell* **3**: 707-716.

Matheisl S, Berninghausen O, Becker T, Beckmann R. 2015. Structure of a human translation termination complex. *Nucleic Acids Res* **43**: 8615-8626.

McCaughan KK, Brown CM, Dalphin ME, Berry MJ, Tate WP. 1995. Translational termination efficiency in mammals is influenced by the base following the stop codon. *Proc Natl Acad Sci U S A* **92**: 5431-5435.

Merrick WC, Hershey JWB. 1996. The pathway and mechanism of eukaryotic protein synthesis. In *Translational control*, (ed. JWB Hershey, MB Matthews, N Sonenberg), pp. 31-69. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.

Methot N, Pause A, Hershey J, Sonenberg N. 1994. The translation initiation factor eIF-4B contains an RNA-binding region that is distinct and independent from its ribonucleoprotein consensus sequence. *Mol Cell Biol* **14**: 2307-2316.

Mottagui-Tabar S, Tuite MF, Isaksson LA. 1998. The influence of 5' codon context on translation termination in Saccharomyces cerevisiae. *Eur J Biochem* **257**: 249-254.

Munzarová V, Pánek J, Gunišová S, Dányi I, Szamecz B, Valášek LS. 2011. Translation Reinitiation Relies on the Interaction between eIF3a/TIF32 and Progressively Folded cis-Acting mRNA Elements Preceding Short uORFs. *PLoS Genet* **7**: e1002137.

Namy O, Hatin I, Rousset JP. 2001. Impact of the six nucleotides downstream of the stop codon on translation termination. *EMBO Rep* **2**: 787-793.

Namy O, Rousset JP, Napthine S, Brierley I. 2004. Reprogrammed genetic decoding in cellular gene expression. *Mol Cell* **13**: 157-168.

Napthine S, Yek C, Powell ML, Brown TD, Brierley I. 2012. Characterization of the stop codon readthrough signal of Colorado tick fever virus segment 9 RNA. *RNA* **18**: 241-252.

Naranda T, MacMillan SE, Donahue TF, Hershey JW. 1996. SUI1/p16 is required for the activity of eukaryotic translation initiation factor 3 in Saccharomyces cerevisiae. *Mol Cell Biol* **16**: 2307-2313.

Naranda T, MacMillan SE, Hershey JWB. 1994. Purified yeast translational initiation factor eIF-3 is an RNA-binding protein complex that contains the PRT1 protein. *J Biol Chem* **269**: 32286-32292.

Nielsen KH, Szamecz B, Valasek LJ, A., Shin BS, Hinnebusch AG. 2004. Functions of eIF3 downstream of 48S assembly impact AUG recognition and GCN4 translational control. *EMBO J* **23**: 1166-1177.

Nielsen KH, Valášek L, Sykes C, Jivotovskaya A, Hinnebusch AG. 2006. Interaction of the RNP1 motif in PRT1 with HCR1 promotes 40S binding of eukaryotic initiation factor 3 in yeast. *Mol Cell Biol* **26**: 2984-2998.

Palam LR, Baird TD, Wek RC. 2011. Phosphorylation of eIF2 facilitates ribosomal bypass of an inhibitory upstream ORF to enhance CHOP translation. *J Biol Chem* **286**: 10939-10949.

Passmore LA, Schmeing TM, Maag D, Applefield DJ, Acker MG, Algire MA, Lorsch JR, Ramakrishnan V. 2007. The eukaryotic translation initiation factors eIF1 and eIF1A induce an open conformation of the 40S ribosome. *Mol Cell* **26**: 41-50.

Pavitt GD, Ramaiah KVA, Kimball SR, Hinnebusch AG. 1998. eIF2 independently binds two distinct eIF2B subcomplexes that catalyze and regulate guanine-nucleotide exchange. *Genes Dev* **12**: 514-526.

Pelletier J, Graff J, Ruggero D, Sonenberg N. 2015. Targeting the eIF4F translation initiation complex: a critical nexus for cancer development. *Cancer Res* **75**: 250-263.

Pestova TV, Borukhov SI, Hellen CUT. 1998. Eukaryotic ribosomes require initiation factors 1 and 1A to locate initiation codons. *Nature* **394**: 854-859.

Pestova TV, Kolupaeva VG. 2002. The roles of individual eukaryotic translation initiation factors in ribosomal scanning and initiation codon selection. *Genes Dev* **16**: 2906-2922.

Pestova TV, Lomakin IB, Lee JH, Choi SK, Dever TE, Hellen CUT. 2000. The joining of ribosomal subunits in eukaryotes requires eIF5B. *Nature* **403**: 332-335.

Phan L, Schoenfeld LW, Valášek L, Nielsen KH, Hinnebusch AG. 2001. A subcomplex of three eIF3 subunits binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and $tRNA_i^{Met}$. *EMBO J* **20**: 2954-2965.

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**: 4935-4946.

Pisarev AV, Hellen CUT, Pestova TV. 2007. Recycling of Eukaryotic Posttermination Ribosomal Complexes. *Cell* **131**: 286–299.

Pisarev AV, Kolupaeva VG, Pisareva VP, Merrick WC, Hellen CU, Pestova TV. 2006. Specific functional interactions of nucleotides at key -3 and +4 positions flanking the initiation codon with components of the mammalian 48S translation initiation complex. *Genes Dev* **20**: 624-636.

Pisarev AV, Skabkin MA, Pisareva VP, Skabkina OV, Rakotondrafara AM, Hentze MW, Hellen CUT, Pestova TV. 2010. The Role of ABCE1 in Eukaryotic Posttermination Ribosomal Recycling. *Mol Cell* **37**: 196-210.

Pöyry TA, Kaminski A, Jackson RJ. 2004. What determines whether mammalian ribosomes resume scanning after translation of a short upstream open reading frame? *Genes Dev* **18**: 62-75.

Querol-Audi J, Sun C, Vogan JM, Smith MD, Gu Y, Cate JH, Nogales E. 2013. Architecture of human translation initiation factor 3. *Structure* **21**: 920-928.

Rajkowitsch L, Vilela C, Berthelot K, Ramirez CV, McCarthy JE. 2004. Reinitiation and recycling are distinct processes occurring downstream of translation termination in yeast. *J Mol Biol* **335**: 71-85.

Raveh-Amit H, Maissel A, Poller J, Marom L, Elroy-Stein O, Shapira M, Livneh E. 2009. Translational control of protein kinase Ceta by two upstream open reading frames. *Mol Cell Biol* **29**: 6140-6148.

Robinson DN, Cooley L. 1997. Examination of the function of two kelch proteins generated by stop codon suppression. *Development* **124**: 1405-1417.

Roy B, Leszyk JD, Mangus DA, Jacobson A. 2015. Nonsense suppression by near-cognate tRNAs employs alternative base pairing at codon positions 1 and 3. *Proc Natl Acad Sci U S A* **112**: 3038-3043.

Saini AK, Nanda JS, Lorsch JR, Hinnebusch AG. 2010. Regulatory elements in eIF1A control the fidelity of start codon selection by modulating tRNAiMet binding to the ribosome. *Genes Dev* **24**: 97-110.

Saini AK, Nanda JS, Martin-Marcos P, Dong J, Zhang F, Bhardwaj M, Lorsch JR, Hinnebusch AG. 2014. Eukaryotic translation initiation factor eIF5 promotes the accuracy of start codon recognition by regulating Pi release and conformational transitions of the preinitiation complex. *Nucleic Acids Research* **42**: 9623-9640.

Shoemaker CJ, Green R. 2011. Kinetic analysis reveals the ordered coupling of translation termination and ribosome recycling in yeast. *Proc Natl Acad Sci U S A* **108**: E1392-1398.

Skuzeski JM, Nichols LM, Gesteland RF, Atkins JF. 1991. The signal for a leaky UAG stop codon in several plant viruses includes the two downstream codons. *J Mol Biol* **218**: 365-373.

Sokabe M, Fraser CS, Hershey JWB. 2011. The human translation initiation multi-factor complex promotes methionyl-tRNAi binding to the 40S ribosomal subunit. *Nucleic Acids Research* **40**: 905-913.

Sundaram A, Grant CM. 2014. A single inhibitory upstream open reading frame (uORF) is sufficient to regulate Candida albicans GCN4 translation in response to amino acid starvation conditions. *RNA* **20**: 559-567.

Szamecz B, Rutkai E, Cuchalova L, Munzarova V, Herrmannova 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**: 2414-2425.

Trachsel H, Staehelin T. 1979. Initiation of mammalian protein synthesis: The multiple functions of initiation factor eIF-3. *Biochim Biophys Acta* **565**: 305-14.

Unbehaun A, Borukhov SI, Hellen CU, Pestova TV. 2004. Release of initiation factors from 48S complexes during ribosomal subunit joining and the link between establishment of codon-anticodon base-pairing and hydrolysis of eIF2-bound GTP. *Genes Dev* **18**: 3078-3093.

Valášek L, Hašek J, Nielsen KH, Hinnebusch AG. 2001a. Dual function of eIF3j/Hcr1p in processing 20 S Pre-rRNA and translation initiation. *J Biol Chem* **276**: 43351-43360.

Valášek L, Hašek J, Trachsel H, Imre EM, Ruis H. 1999. The *Saccharomyces cerevisiae HCRI* gene encoding a homologue of the p35 subunit of human translation eukaryotic initiation factor 3 (eIF3) is a high copy suppressor of a temperature-sensitive mutation in the Rpg1p subunit of yeast eIF3. *J Biol Chem* **274**: 27567-27572.

Valášek L, Mathew A, Shin BS, Nielsen KH, Szamecz B, Hinnebusch AG. 2003. The Yeast eIF3 Subunits TIF32/a and NIP1/c and eIF5 Make Critical Connections with the 40S Ribosome in vivo. *Genes Dev* **17**: 786-799.

Valášek L, Nielsen KH, Hinnebusch AG. 2002. Direct eIF2-eIF3 contact in the multifactor complex is important for translation initiation in vivo. *EMBO J* **21**: 5886-5898.

Valášek L, Nielsen KH, Zhang F, Fekete CA, Hinnebusch AG. 2004. Interactions of Eukaryotic Translation Initiation Factor 3 (eIF3) Subunit NIP1/c with eIF1 and

eIF5 Promote Preinitiation Complex Assembly and Regulate Start Codon Selection. *Mol Cell Biol* **24**: 9437-9455.

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

Valášek L, Trachsel H, Hašek J, Ruis H. 1998. Rpg1, the *Saccharomyces cerevisiae* homologue of the largest subunit of mammlian translation initiation factor 3, is required for translational activity. *J Biol Chem* **273**: 21253-21260.

Valášek LS. 2012. 'Ribozoomin' – Translation Initiation from the Perspective of the Ribosome-bound Eukaryotic Initiation Factors (eIFs). *Curr Protein Pept Sci* **13**: 305-330.

Vattem KM, Wek RC. 2004. Reinitiation involving upstream ORFs regulates ATF4 mRNA translation in mammalian cells. *Proc Natl Acad Sci USA* **101**:11269-74.

Vilela C, Linz B, Rodrigues-Pousada C, McCarthy JE. 1998. The yeast transcription factor genes YAP1 and YAP2 are subject to differential control at the levels of both translation and mRNA stability. *Nucleic Acids Res* **26**: 1150-1159.

Villa N, Do A, Hershey JW, Fraser CS. 2013. Human eukaryotic initiation factor 4G (eIF4G) binds to eIF3c, -d, and -e to promote mRNA recruitment to the ribosome. *J Biol Chem* **288**: 32932-32940.

von Arnim AG, Jia Q, Vaughn JN. 2014. Regulation of plant translation by upstream open reading frames. *Plant Sci* **214**: 1-12.

von der Haar T, Tuite MF. 2007. Regulated translational bypass of stop codons in yeast. *Trends Microbiol* **15**: 78-86.

Vornlocher HP, Hanachi P, Ribeiro S, Hershey JWB. 1999. a 110-kilodalton subunit of translation initiation factor eIF3 and an associated 135-kilodalton protein are encoded by the *Saccharomyces cerevisiae TIF32* and *TIF31* genes. *Journal of Biological Chemistry* **274**: 16802-16812.

Wagner S, Herrmannova A, Malik R, Peclinovska L, Valasek LS. 2014. Functional and Biochemical Characterization of Human Eukaryotic Translation Initiation Factor 3 in Living Cells. *Mol Cell Biol* **34**: 3041-3052.

Walker SE, Zhou F, Mitchell SF, Larson VS, Valasek L, Hinnebusch AG, Lorsch JR. 2013. Yeast eIF4B binds to the head of the 40S ribosomal subunit and promotes mRNA recruitment through its N-terminal and internal repeat domains. *RNA* **19**: 191-207.

Young DJ, Guydosh NR, Zhang F, Hinnebusch AG, Green R. 2015. Rli1/ABCE1 Recycles Terminating Ribosomes and Controls Translation Reinitiation in 3'UTRs In Vivo. *Cell* **162**: 872-884.

Yu Y, Marintchev A, Kolupaeva VG, Unbehaun A, Veryasova T, Lai S-C, Hong P, Wagner G, Hellen CUT, Pestova TV. 2009. Position of eukaryotic translation initiation factor eIF1A on the 40S ribosomal subunit mapped by directed hydroxyl radical probing. *Nucleic Acids Research* **37**: 5167-5182.

Zhang F, Saini AK, Shin BS, Nanda J, Hinnebusch AG. 2015. Conformational changes in the P site and mRNA entry channel evoked by AUG recognition in yeast translation preinitiation complexes. *Nucleic Acids Res* **43**: 2293-2312.

Zhou D, Pallam LR, Jiang L, Narasimhan J, Staschke KA, Wek RC. 2008. Phosphorylation of eIF2 directs ATF5 translational control in response to diverse stress conditions. *J Biol Chem* **283**: 7064-7073.

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.

## LIST OF THE DSc. THESIS PUBLICATIONS

[# indicates co-first authors; * indicates corresponding author(s)]

Kovarik, P.[#], Hašek, J.[#], **Valášek, L**.[#], and Ruis, H., (1998) *RPG1*: an essential gene of *Saccharomyces cerevisiae* encoding a 110 kDa protein required for passage through the G1 phase. *Curr. Genetics*, **33**, 100-109.
IF1998=1.864; Cit. total = 26

**Valášek, L**., Trachsel, H., Hašek, J., and Ruis, H., (1998) Rpg1, the *Saccharomyces cerevisiae* homologue of the largest subunit of mammalian translation initiation factor 3, is required for translational activity. *J. Biol. Chem.,* **273**, 21253-21260.
IF1998=7.199; Cit. total = 31

**Valášek, L**.*, Hašek, J., Trachsel, H., Imre, E.I., and Ruis, H. (1999) The Saccharomyces cerevisiae HCR1 gene encoding a homologue of the p35 subunit of human translation initiation factor 3 (eIF3) is a high copy suppressor of a temperature-sensitive mutation in the Rpg1p subunit of yeast eIF3. *J. Biol. Chem.,* **274**, 27567-27572.
IF1999=7.666; Cit. total = 33

Phan, L., Shoenfeld, L.W., **Valášek, L**., Nielsen, K., Olsen, D., and Hinnebusch, A.G. (2001) A subcomplex of three eIF3 subunits binds eIF1 and eIF5 and stimulates ribosome binding of mRNA and tRNA(i)Met. *EMBO J.,* **20**, 2954-65.
IF2001=12.459; Cit. total = 75

**Valášek, L**., Phan, L., Schoenfeld, L.W., Valášková, V., and Hinnebusch, A.G. (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.**
IF2001=7.258; Cit. total = 62

**Valášek, L**., Hašek, J., Nielsen, K.H., and Hinnebusch, A.G. (2001) Dual function of eIF3j/Hcr1p in processing 20S pre-rRNA and translation initiation. *J. Biol. Chem.,* **276**, 43351-43360.
IF2001=6.144; Cit. total = 41

**Valášek, L**., Nielsen, K.H., and Hinnebusch, A.G. (2002) Direct eIF2-eIF3 Contact in the Multifactor Complex is Important for Translation Initiation in vivo. *EMBO J.,* **21**, 5886-98.
IF2002=10.698; Cit. total = 87

**Valášek, L**., Mathew, A.A., Shin, B-S., Nielsen, K.H., Szamecz, B., and Hinnebusch, A.G. (2003) The Yeast eIF3 Subunits TIF32/a and NIP1/c and eIF5 Make Critical Connections with the 40S Ribosome in vivo. *Genes & Dev* **14,** 2534-2546.
IF2003=17.013; Cit. total = 104

Nielsen, K.H., Szamecz, B., **Valášek, L.,** Jivotovskaya, A., Shin, B-S., and Hinnebusch, G.H. (2004) Functions of eIF3 downstream of 48S assembly impact AUG recognition and GCN4 translational control. *EMBO J.*, **23**, 1166-77.
IF2004=10.492; Cit. total = 72

**Valášek, L**.*, Nielsen, K.H., Zhang, F., Hamilton, A.C., and Hinnebusch, A.G.* (2004) Interactions of eIF3 subunit NIP1/c with eIF1 and eIF5 promote pre-initiation complex assembly and regulate start codon selection. *Mol. Cell Biol.*, **24**, 9437-9455.
IF2004=7.822; Cit. total = 92

Jivotovskaya, A.V., **Valášek, L**., Hinnebusch, A.G., and Nielsen, K.H. (2006) Eukaryotic translation initiation factor 3 (eIF3) and eIF2 can promote mRNA binding to 40S subunits independently of eIF4G in yeast. *Mol. Cell Biol.*, **26**, 1355-72.
IF2006=6.773; Cit. total = 66

Nielsen, K.H., **Valášek, L**., Sykes, C., Jivotovskaya, A.V., and Hinnebusch, A.G. (2006) Interaction of the RNP1 motif in PRT1 with HCR1 promotes 40S binding of eukaryotic initiation factor 3 in yeast. *Mol. Cell Biol.*, **26**, 2984-98.
IF2006=6.773; Cit. total = 45

Szamecz, B., Rutkai, E., Cuchalová, L., Munzarová, V., Herrmannová, A., Nielsen, K.H., Burela, L., Hinnebusch, A.G., and **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**, 2414-2425.
IF2008=13.623; Cit. total = 56

ElAntak, L.[#], Wagner, S.[#], Herrmannová, A.[#], Janošková, M., Rutkai, E., Lukavsky, P.J.*, and **Valášek, L.*** (2010) The indispensable N-terminal half of eIF3j co-operates with its structurally conserved binding partner eIF3b-RRM in stringent AUG selection. *J. Mol. Biol.*, **396**, 1097-116.
IF2010=4.008; Cit. total = 36

Chiu, W.-L., Wagner, S., Herrmannová, A., Burela, L., Zhang, F., Saini, A.K., **Valášek, L.***, and Hinnebusch, A.G.* (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**, 4415-4434.
IF2010=6.188; Cit. total = 36

Cuchalová, L., Kouba, T., Herrmannová, A., Danyi, I., Chiu, W.-l. and **Valášek, L.*** (2010) The RNA Recognition Motif of Eukaryotic Translation Initiation Factor 3g (eIF3g) Is Required for Resumption of Scanning of Post-termination Ribosomes for Reinitiation on GCN4 and Together with eIF3i Stimulates Linear Scanning. *Mol Cell Biol*, **30**, 4671-4686.
IF2010=6.188; Cit. total = 36

Munzarová, V., Pánek, J., Gunišová, S., Danyi, I., Szamecz, B. and **Valášek, L.S.*** (2011) Translation Reinitiation Relies on the Interaction Between eIF3a/TIF32 and Progressively Folded cis-acting mRNA Elements Preceding Short uORFs. *PLoS Genet.*, **7**, e1002137.
IF2011=8.694; Cit. total = 26

Herrmannová, A.[#], Daujotytė, D.[#], Yang, J-C., Cuchalová, L., Gorrec, F., Wagner, S., Dányi, I., Lukavsky, P.J.*, and **Valášek, L.S.*** (2012) Structural Analysis of an eIF3 Subcomplex Reveals Conserved Interactions Required for a Stable and Proper Translation Pre-Initiation Complex Assembly. *Nucleic Acid Res.*, **40**(5**)**, 2294-311. Epub 2011 Nov 15.
IF2012=8.278; Cit. total = 27

Kouba, T., Rutkai, E., Karasková, M., and **Valášek, L.S.*** (2012) The eIF3c/NIP1 PCI Domain Interacts with RNA and RACK1/ASC1 and Promotes Assembly of Translation Pre-initiation Complexes. *Nucleic Acid Res.*, **40**(6), 2683-99. Epub 2011 Nov 28.
IF2012=8.278; Cit. total = 24

Kouba, T., Danyi, I, Gunišová, S., Munzarová, V., Vlčková, V., Cuchalová, L., Neueder, A., Milkereit, P., and **Valášek, L.S.*** (2012) Small Ribosomal Protein RPS0 Stimulates Translation Initiation by Mediating 40S-binding of eIF3 *via* its Direct Contact with the eIF3a/TIF32 Subunit. *PLoS One*, **7**, e40464. Epub 2012 Jul 5.
IF2012=3.730; Cit. total = 14

Karasková, M.[#], Gunišová, S.[#], Herrmannová, A.[#], Wagner, S., Munzarová, V., and **Valášek, L.S.*** (2012) Functional Characterization of the Role of the N-terminal Domain of the c/Nip1 Subunit of eIF3 in AUG recognition. *J Biol Chem,* **287**, 28420-28434.
IF2012=4.651; Cit. total = 9

**Valášek, L.S.*** (2012) "Ribozoomin" – Translation Initiation from the Perspective of the ribosome-bound Eukaryotic Initiation Factors (eIFs). *Curr Protein Pept Sci.*, **13**, 305-330.
F2012=2.326; Cit. total = 36

Beznosková, P.[#], Cuchalová, L. [#], Wagner, S., Shoemaker, C.J., Gunišová, S., Von der Haar, T. and **Valášek, L.S.*** (2013) Translation initiation factors eIF3 and HCR1 control translation termination and stop codon read-through in yeast cells. *PLoS Genet.,* **9**, e1003962
IF2013 = 8.167; Cit. total = 13

Khoshnevis, S.[#], Gunišová, S.[#], Vlčková, V.[#], Kouba, T., Neumann, P., Beznosková, P., Ficner, R.* and **Valášek, L.S.*** (2014) Structural integrity of the PCI domain of eIF3a/TIF32 is required for mRNA recruitment to the 43S pre-initiation complexes. *Nucleic Acid Res.*, **42**(6):4123-39. Epub 2014 Jan 13.
IF2014 = 9.112; Cit. total = 8

Gunišová, S. and **Valášek, L.S.*** (2014) Fail-safe Mechanism of *GCN4* Translational Control – uORF2 Promotes Reinitiation by Analogous Mechanism to uORF1 and thus Secures its Key Role in *GCN4* expression. *Nucleic Acid Res.*, **42**(9), 5880-93. Epub 2014 Mar 12.
IF2014 = 9.112; Cit. total = 0

Wagner, S., Herrmannová, A., Peclinovská, L., Malík, R. and **Valášek, L.S.*** (2014) Functional and biochemical characterization of human eIF3 in living cells. *Mol. Cell Biol.*, **34**, 3041-52.
IF2014 = 4.777; Cit. total = 8

Beznosková, P., Wagner, S., Jansen, M.E., von der Haar, T. and **Valášek, L.S.*** (2015) Translation initiation factors eIF3 promotes programmed stop codon readthrough. *Nucleic Acid Res.*, **43**(10), 5099-111. Epub 2015 April 29.
IF2014 = 9.112; Cit. total = 3

Beznosková, P., Gunišová, S. and **Valášek, L.S.*** (2016) Rules of UGA-N decoding by near-cognate tRNAs and analysis of readthrough on short uORFs in yeast. *RNA*, **22**(3), 456-66. Epub 2016 Jan 12.
IF2014 = 4.936; Cit. total = 1

Gunišová, S.*, Beznosková, P., Mohammad, M.P., Vlčková, V. and **Valášek, L.S.*** (2016) In-depth analysis of *cis*-determinants that either promote or inhibit reinitiation on *GCN4* mRNA after translation of its four short uORFs. *RNA*, **22**(4), 542-58. Epub 2016 Jan 28.
IF2014 = 4.936; Cit. total = 0

## LIST OF OTHER PUBLICATIONS BY THE AUTHOR

[# indicates co-first authors; * indicates corresponding author(s)]

Vondrejs, V., **Valášek, L**. (1994) Comparison of the effect of zymocine K1 on protoplasts and cells of selected yeast species. *Folia Microbiol.,* **39**, 363.
IF1994=0.502; Cit. total = 0

Vondrejs, V., **Valášek, L**. (1994) Comparison of different variants of the rhodamine test in terms of sensitivity of detection of the effect of zymocins in different strains of *Saccharomyces cerevisiae*. *Folia Microbiol.,* **39**, 362.
IF1994=0.502; Cit. total = 0

**Valášek, L.**, Vondrejs, V., Bartůněk, M., and Janderová, B. (1996) Exploitation of Rhodamine B in the Killer Toxin Research. Fluorescence Microscopy and Fluorescence Probes, 163 – 167, Plenum Press, New York, 1996.

Vondrejs, V., Janderova, B, **Valášek, L**. (1996) Yeast killer toxin K1 and its exploitation in genetic manipulations. *Folia Microbiol.,* **41**, 379-93.
IF1996=0.518; Cit. total = 5

Sabelli, P.A., Burgess, S.R., **Valášek, L**., and Shewry, P.R. (1998) Molecular cloning and characterisation of a maize cDNA for a homologue of the large subunit of the eukaryotic initiation factor 3 (eIF3). *Mol Gen Genet.,* **261**, 820-830.
IF1998=2.766; Cit. total = 5

Hašek, J., Kovarik, P., **Valášek, L**., Malínská, K., Schneider J., Kohlwein S.D., and Ruis, H., (2000) Rpg1p, the subunit of the Saccharomyces cerevisiae eIF3 core complex, is a microtubule-interacting protein. *Cell Motil. Cytoskel.,* **45**, 235-46.
IF2000=3.029; Cit. total = 23

Asano K., Phan, L., **Valášek, L**., Schoenfeld, L.W., Shalev, A., Clayton, J., Nielsen, K., Donahue, T.F., and Hinnebusch A.G. A Multifactor Complex of eIF1, eIF2, eIF3, eIF5, and $tRNA_i^{Met}$ Promotes Initiation Complex Assembly and Couples GTP Hydrolysis to AUG Recognition (2001) *Cold Spring Harbor Symposia on Quantitative Bilology*, Vol. **LXVI**, 403-415, CSHL Press
Cit. total = 20

Asano, K., Shalev, A., Phan, L., Nielsen, K., Clayton, J., **Valášek, L**., Donahue, T. F., and Hinnebusch, A.G. (2001) Multiple roles for the C-terminal domain of eIF5 in translation initiation complex assembly and GTPase activation. *EMBO J.,* **20**, 2326-37.
IF2001=12.459; Cit. total = 77

Shalev, A., **Valášek, L**., Pise-Masison, C.A., Radonovich, M., Phan, L., Clayton, J., Brady, J.N., Hinnebusch, A.G, and Asano, K. (2001) *Saccharomyces cerevisiae* protein PCI8 and human protein eIF3e/Int-6 interact with eIF3 core complex by binding to cognate eIF3b subunits. *J Biol Chem*., 276, 34948-57.
IF2001=7.258; Cit. total = 34

Hinnebusch, A.G., Asano, K., Olsen, D.S., Phan, L., Nielsen, K.H., and **Valášek, L**. (2004) Study of Translational Control of Eukaryotic Gene Expression Using Yeast. *Ann N Y Acad Sci.,* **1038**, 60-74.
IF2004=1.789; Cit. total = 18

Hašek, J., Peřinka, L., and **Valášek, L**., (2005) Specification of the monoclonal antibody PK1 reactivity in Chinese hamster ovary cells. *Folia Biol.,* **51**, 50-1.
IF2005=0.719; Cit. total = 0

**Valášek, L.***, Szamecz, B., Hinnebusch, A.G., and Nielsen, K.H.* (2007) In vivo stabilization of pre-initiation complexes by formaldehyde cross-linking. *Methods Enzymol*., Vol **429**, 163-183
IF2007=2.122; Cit. total = 29

Nielsen, K.H.*, and **Valášek, L.*** (2007) In vivo deletion analysis of the architecture of a multiprotein complex of translation initiation factors. *Methods Enzymol*., Vol **431**, 15-32
IF2007=2.122; Cit. total = 10

Groušl, T., Ivanov, P., Frýdlová, I., Vašicová, P., Janda, F., Vojtová, J., Malínská, K., Malcová,I., Nováková, L., Janošková, D., **Valášek, L.**, and Hašek, J. (2009) Robust heat shock induces eIF2alpha-phosphorylation-independent assembly of stress granules containing eIF3 and 40S ribosomal subunits in budding yeast S. cerevisiae. *J. Cell Sci*., **122**, 2078-88.
IF2009=6.144; Cit. total = 84

Nemoto, N., Singh, C.R., Udagawa, T., Wang, S., Thorson, E., Winter, Z., Ohira, T., Ii, M., **Valášek, L.**, Brown, S.J., and Asano K. (2010) Yeast 18 S rRNA is directly involved in the ribosomal response to stringent AUG selection during translation initiation. *J Biol Chem*., **285**, 32200-12.
IF2010=5.328; Cit. total = 6

Mašek, T., **Valášek, L**., and Pospíšek M. (2011) Polysome analysis and RNA purification from sucrose gradients. *Methods Mol Biol*., **703**, 293-309.
Cit. total = 23

Novotný, I., Podolská, K., Blažíková, M, **Valášek, L.S.**, Svoboda, P., Staněk, D. (2012) Nuclear LSm8 affects number of cytoplasmic processing bodies via controlling cellular distribution of Like-Sm proteins. *Mol Biol Cell*, **23**(19), 3776-85.
IF2012=5.372; Cit. total = 6

Pospíšek, M. and **Valášek, L.*** (2013) Polysome profile analysis--yeast. *Methods Enzymol*., **530**, 173-181.
IF2013=2.194; Cit. total = 1

Walker, S.E., Zhou, F., Mitchell, S.F., Larson, V.S., **Valášek, L.**, Hinnebusch, A.G., and Lorsch, J.R. (2013) Yeast eIF4B binds to the head of the 40S ribosomal subunit and promotes mRNA recruitment through its N-terminal and internal repeat domains. *RNA*, **19**(2), 191-207.
IF2013=4.622; Cit. total = 12

Pánek, J.*, Kolář, M., Vohradský, J. and **Valášek, L.S.** (2013) An evolutionary conserved pattern of 18S rRNA sequence complementarity to mRNA 5' UTRs and its implications for eukaryotic gene translation regulation. *Nucleic Acid Res.*, **41**(16), 7625-7634. Epub 2013 Jun 26.
IF2013 = 8.808; Cit. total = 3

von der Haar, T. and **Valášek, L.S.** (2014) mRNA Translation: Fungal Variations on a Eukaryotic Theme, Fungal RNA Biology, Springer International Publishing, Switzerland, 2014

Pánek, J.*, Kolář, M., Herrmannová, A., and **Valášek, L.S.*** (2016) A systematic analysis of the rRNA–3'UTR sequence complementarity reveals a mechanism facilitating ribosomal recycling. *RNA*, in press
IF2014 = 4.936; Cit. total = 0