

# The evolving role of ribosomes in the regulation of protein synthesis

Karolina Gościńska<sup>✉</sup> and Ulrike Topf

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Department of Genetics, Warszawa, Poland

**Maintenance of the cellular homeostasis is firmly linked with protein synthesis. Therefore, it is tightly controlled at multiple levels. An advancement in quantitative techniques, mainly over the last decade, shed new light on the regulation of protein production, which pointed the ribosome as a new player. Ribosomes are macromolecular machines that synthesize polypeptide chains using mRNA as a template. The enormous complexity of ribosomes provides many possibilities of changes in their composition and consecutively in their target specificity. However, it is not clear how this specialization is enforced by the cell and which stimuli provoke that diversity. This review presents an overview of currently available knowledge about ribosome heterogeneity, focusing on changes in protein composition, and their role in the control of translation specificity. Importantly, besides the potential advantage of ribosome-mediated regulation of protein synthesis, its failure can play a crucial role in disease development.**

**Key words:** ribosome, translation regulation, ribosome specialization, heterogeneous ribosomes

**Received:** 05 June, 2020; **revised:** 08 September, 2020; **accepted:** 09 September, 2020; **available on-line:** 27 November, 2020

<sup>✉</sup>e-mail: [kgoscinska@ibb.waw.pl](mailto:kgoscinska@ibb.waw.pl)

**Acknowledgments of Financial Support:** This work was supported by the National Science Centre (Poland) grant 2015/19/B/NZ1/03444

**Abbreviations:** RP, ribosomal proteins; RBPs, ribosome binding proteins

## INTRODUCTION

Protein synthesis (translation) is a fundamental cellular process essential to maintain the integrity of the proteome. Cells use complex molecular machineries called ribosomes that translate genetic information into polypeptide chains. Protein production is inevitably linked to cell growth and proliferation. Imbalance in protein production can be detrimental for the cell, destroying cellular protein homeostasis and even leading to cell death (Hipp *et al.*, 2014; Rothman, 2010; Santra *et al.*, 2019). Consequently, protein synthesis is tightly regulated and immediately adjusts to environmental changes. Modulation of translation is one of the essential cellular mechanisms responding to stress conditions, such as heat shock, nutrient deprivation, and an increase in reactive oxygen species (ROS) production (Crawford & Pavitt, 2019; Harding *et al.*, 2003; Hinnebusch, 2005; Liu & Qian, 2014; Topf *et al.*, 2018; Wrobel *et al.*, 2015). Generally, cells decrease protein synthesis in response to stress to save energy and prevent overproduction of proteins that could overwhelm the capacity of cellular mechanisms protecting from an

accumulation of damaged or unfolded proteins (Grant, 2011; Harding *et al.*, 2003; Mohanraj *et al.*, 2020; Topf *et al.*, 2019).

The ribosome is a large ribonucleoprotein machine responsible for synthesizing proteins in all cells using messenger RNA (mRNA) as a template. Eukaryotic ribosomes are comprised of four ribosomal RNA (rRNA) species and 79 ribosomal proteins (RPs) distributed among two distinct subunits together constituting the monosome (80S, named according to the apparent sedimentation velocity) (Thomson *et al.*, 2013). The small ribosomal subunit (40S) is built of 18S rRNA and 33 RPs, whereas the large ribosomal subunit (60S) consists of three rRNAs (5S, 5.8S, 25S) and 46 RPs.

The biogenesis of ribosomes takes place within the nucleolus, nucleoplasm, and cytoplasm. In the nucleolus, the RNA polymerase I (Pol I) synthesizes 5S, 5.8S, and 18S rRNA in a form of a single 47S transcript, known as pre-rRNA. The last rRNA, 5S, is transcribed in the nucleus by RNA polymerase III (Pol III). Concomitantly, RNA polymerase II transcribes ribosomal protein-coding genes and the arising mRNA is translated in the cytoplasm. Pre-ribosomal subunits, the 40S and 60S, are formed in the nucleolus from processed pre-rRNA, 5S rRNA and ribosomal proteins imported from the cytoplasm. Some of the ribosomal proteins assemble later, after independent export of immature ribosomal subunits to the cytoplasm, including eL24 (L24), eL40 (L40), uL16 (L10) (Fernandez-Pevida *et al.*, 2012; Kruiswijk *et al.*, 1978; Saveanu *et al.*, 2003; Zhou *et al.*, 2019). The size of the mature eukaryotic ribosome is in the range of 3.5 megadaltons (MDa) to 4.0 MDa in higher organisms (Yusupova & Yusupov, 2017). Although intense studies have been conducted on ribosomes for decades, many questions are still pending, for example, do they consist of the same components in every cell or how ribosome heterogeneity changes the translational output? Here, we discuss the latest findings in the regulatory role of the ribosome in translation, specifically focusing on the role of ribosomal proteins and their modifications.

## THE EMERGING CONCEPT OF SPECIALIZED RIBOSOMES

In 1958, George Palade, who discovered ribosomes, proposed a theory of heterogeneous ribosome particles based on electron microscopy observations of differences in their shape and size (Siekevitz & Palade, 1958). In parallel, Francis Crick worked on “one gene-one ribosome-one protein hypothesis”, according to which the ribosome carries information in its RNA for single protein synthesis (Crick, 1958). Nevertheless, this was disproved by Brenner and colleagues who

**Table 1. Overview of alterations in ribosomes that can contribute to the formation of ribosome specialization.**  
Refer to the review text for details and references.

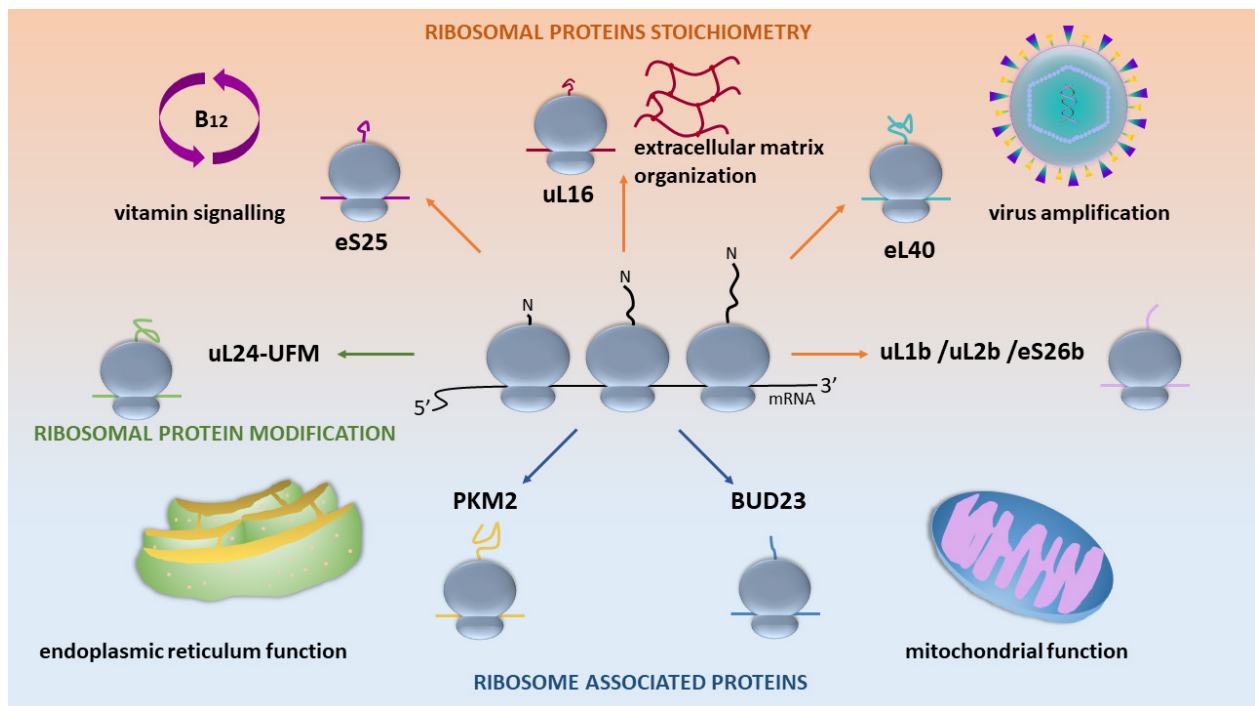
Ribosome alterations	Examples
Ribosomal proteins	
– Paralogs	uL1b, uL2b, eL8, uL18, eL32, uS4, eS26
– Stoichiometry	uL3, uL18, uL1, eL38, eL40, uS7, eS25, eS26
Post-translational modifications (PTMs) of ribosomal proteins	acetylation, methylation, glycosylation, phosphorylation, ubiquitination, oxidation, UFMylation
PTMs of rRNA	Methylation, Pseudouridylation
Ribosome-associated proteins	PKM2, CDK1, BUD23

showed that *Escherichia coli* ribosomes synthesize phage proteins regardless of infection, demonstrating lack of ribosome specificity (Brenner *et al.*, 1961). For decades, ribosomes were thought to be stable and invariable in composition macromolecular machines which translate available messenger RNA into a protein, however, there is increasing evidence showing their regulatory role (reviewed in Dalla Venezia *et al.*, 2019; Dinman, 2016; Genuth & Barna, 2018; Xue & Barna, 2012). In 2002, Mauro and Edelman proposed a ribosome filter hypothesis according to which mRNAs interact differently with ribosomal subunits through changes in their protein composition or rRNA, shedding a light on ribosome control in gene expression (Mauro & Edelman, 2002). Nowadays, the concept of specialized ribosomes in regulation of translation expands and comprises several layers. Heterogeneity in the ribosome can be determined by differential RP stoichiometry, RP modifications (e.g. phosphorylation, glycosylation), rRNA alterations (e.g. methylation, pseudouridylation) or binding of ribosome-associated proteins (RAPs) (Crawford & Pavitt, 2019; Shi & Barna, 2015; Simsek & Barna, 2017; Sloan *et al.*, 2017; Xue & Barna, 2012) (Table 1). This variability increases even more when considering interdependent factors in protein synthesis, mRNA and its diversity, such as internal entry sites (IRES), poly-A sites in the 3' untranslated region (UTR) or nucleotide modifications (Kozak, 2005; Spriggs *et al.*, 2008). Moreover, ribosome specialization can manifest at the level of a subcellular location, as well as dependence on the cell developmental state, cell type, or even tissue specificity (Guimaraes & Zavolan, 2016; Gupta & Warner, 2014; Kondrashov *et al.*, 2011; Marygold *et al.*, 2007; Simsek *et al.*, 2017; Slavov *et al.*, 2015; Wong *et al.*, 2014). Tissue-specific expression was shown for a quarter of human ribosomal proteins (Guimaraes & Zavolan, 2016). Interestingly, changes in ribosomes affect the translation of a subset of mRNAs rather than global protein synthesis. Perhaps this facilitates responses to urgent cellular protein demand or increases the capability to effectively react to environmental changes, e.g. stress conditions. Nonetheless, the etiology of the variability in ribosome composition and further their regulatory activity remains largely unclear.

The emerging field of ribosome heterogeneity is currently rapidly developing and is driven by implementation of specialized techniques to analyse protein synthesis and ribosomes. However, the findings are scattered and their biological impact touches different fields of cell biology. Thus, we focus on a few recently outstanding findings that highlight modulation of ribosomes at the level of ribosomal proteins themselves and the ribosome – associated proteins (Table 1).

## RIBOSOMAL PROTEIN PARALOGS AND DIFFERENTIAL PROTEIN STOICHIOMETRY IN THE RIBOSOME

The existence of ribosomal protein paralogs has been discovered in many organisms, such as: *Arabidopsis thaliana* (Barakat *et al.*, 2001; Falcone Ferreyra *et al.*, 2013), the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Palumbo *et al.*, 2017; Sun *et al.*, 2013), *Drosophila melanogaster* (Mageeny & Ware, 2019; Marygold *et al.*, 2007) and in rodents and human cells (Guimaraes & Zavolan, 2016; Lopes *et al.*, 2010; O'Leary *et al.*, 2013; Sugihara *et al.*, 2010). Among them, the budding yeast appears to be an excellent eukaryotic model for exploring the ribosome heterogeneity. As a result of genome duplication, 59 from 79 RPs of *S.cerevisiae* are present in paralog pairs with high sequence similarity (Parenteau *et al.*, 2015; Wapinski *et al.*, 2010; Wolfe & Shields, 1997). Despite their similarity, in most cases deletion of one ribosomal paralog from the pair results in various phenotypes, suggesting their functional diversification (Lu *et al.*, 2015; Ni & Snyder, 2001; Palumbo *et al.*, 2017; Samir *et al.*, 2018; Segev & Gerst, 2018). To gain further insight, Ghulam *et al.* compared the expression of ribosomal protein paralog pairs (Ghulam *et al.*, 2020). Focusing on RP paralogs with differences in one or more amino acids (37 out of 59 RP paralogs), specifically pairs producing proteins that could be reproducibly distinguished by applied peptide-based mass-spectrometry (23 pairs). In 20 cases, under normal growth conditions, one of the RP paralogs was predominantly produced (major copy), regardless of their A or B nomenclature. A striking difference was observed for uS4 (S9), where the B paralog represents over 80% of total protein expression. Interestingly, exposure to stress, such as hygromycin or NaCl, reduced or reversed the ratio of the generated RP paralogs in most cases, favouring expression of the minor gene copy. Overall genome-wide CHIP-seq study showed no correlation between RNA polymerase II association and the amount of produced ribosomal protein, indicating post-transcriptional regulation of ribosomal protein paralog production (Ghulam *et al.*, 2020). A more in-depth study that used RNA-sequencing and polyribosome association, revealed the importance of RNA abundance in the preferential translation of certain RP paralogs, mainly caused by differences in splicing and 3'end formation (Ghulam *et al.*, 2020). Another study also pointed out the relevance of introns within RPs in control of ribosome function (Parenteau *et al.*, 2011). Altogether this indicates a multilayer regulation of the expression of ribosomal protein paralogs and their distinctive participation in actively translating ribosomes. In response to environmental changes, a subpopulation of the ribosomes that varies in RP paralog occupancy can



**Figure 1. Ribosome heterogeneity regulates translational output.**

The variability in translation machinery composition can be manifested at many levels, including differential ribosomal protein stoichiometry, modification of ribosomal proteins or binding of ribosome associated proteins. The subpools of heterogeneous ribosomes arise in response to cellular protein demand and translate distinct mRNAs. The presence of certain ribosomal proteins in the ribosome results in favoured synthesis of proteins involved in vitamin B12 signalling (eS25 (S25)), extracellular matrix organization (uL16 (L10)) or mitochondrial function (uL1b (L1b), uL2b (L2b), eS26b (S26b)). The large ribosomal subunit protein, eL40 (L40), is required for the translation initiation of vesicular stomatitis virus mRNAs. Also, binding of non-ribosomal proteins increases ribosome specialization and results in production of proteins which are necessary for mitochondrial (via BUD23) or endoplasmic reticulum (via PKM2) functions. Moreover, post-translational modification of the ribosomal protein, uL24 (L24) by UFMylation protects from endoplasmic reticulum stress. These examples demonstrate the importance of ribosome heterogeneity for preserving cellular homeostasis.

provide a specific translation output, perhaps to quickly and specifically adapt to a current cellular need.

Functional diversification of RP paralogs can also affect the production of certain groups of the protein. Segev and Gerst showed the relevance of RP paralogs in the translation of mitochondrial proteins (Segev & Gerst, 2018). The growth of different yeast mutants harbouring deletions of RP paralogs on fermentable (glucose) and non-fermentable (glycerol) carbon sources showed that uL1b (L1b), uL2b (L2b), and eS26b (S26) are necessary under respiratory conditions (Fig. 1). Puromycin-associated nascent chain proteomics (PUNCH-P) technique revealed high downregulation of mitochondrial protein production in these RP mutants. This observation was specific for one RP paralog from the pair, even though they encode an identical protein, such as *uL1a* (L1a) and *uL1b* (L1b). Notwithstanding, how this specificity is conferred remains unknown.

Concurrently, the eL8 (L8) paralog switch in the 80S ribosome was reported in response to shift in the carbon source from glucose to glycerol, in a yeast culture (Samir *et al.*, 2018). eL8b (L8b) supports cell doubling under respiratory conditions in contrast to eL8a (L8a), confirming no interchangeable functions of RPs paralogs. Moreover, isobaric tags for relative and absolute quantitation (iTRAQ) labelling and mass spectrometry-based quantitative proteomics showed an imbalance in single-copy ribosomal protein production upon changes in growth conditions, e.g. uL3 (L3) and uL18 (L5), which in turn refers to the new concept of sub-stoichiometric composition of the ribosome (Samir *et al.*, 2018). Recent advances in quantitative techniques also enabled the

precise and accurate measurement of ribosome component stoichiometry. In contrast to the uniform ribosome dogma, many studies prove the existence of several heterogeneous subpopulations of translation machinery in the cell at the same time (Shi *et al.*, 2017; Slavov *et al.*, 2015). The appearance of such distinction is usually related to physiological conditions or tissue type. Substantial progress on ribosome stoichiometry was done based on research on embryonic stem cells (Kondrashov *et al.*, 2011; Rao *et al.*, 2012; Shi *et al.*, 2017; Slavov *et al.*, 2015). Shi and others (Shi *et al.*, 2017) applied Selected Reaction Monitoring (SRM) to identify compositions of actively translating ribosomes in mouse embryonic stem cells (mESC). Absolute quantification of 15 RPs in the polysome fractions showed four core RPs to be significantly depleted (uS7 (S5), eS25 (S25), uL1 (L10A), and eL38 (L38)). Also, uL1 (L10A) and eS25 (S25) associate with certain sub-pools of transcripts, e.g. encoding extracellular matrix proteins or involved in B12 vitamin signalling, respectively (Fig. 1). Hence, mESC ribosomes lacking one or more of RPs can still actively engage in the protein synthesis.

Other studies focus on sub-stoichiometry in ribosome composition as an effect of dynamic conditions, like cellular stress. In yeast, high pH or high salt concentrations lead to the production of eS26 (S26)-depleted ribosomes (Ferretti *et al.*, 2017). Indeed, transcriptome examination revealed that eS26 (S26)-deficient ribosomes preferentially translate mRNAs implicated in the stress response. Conversely, eS26 (S26)-containing ribosomes occupy transcripts related to the translation process recognizing the Kozak sequence elements. Thus, this work illustrates



the enhancement of well-known transcriptional stress response at the level of translation which is mediated by the ribosome heterogeneity.

Finally, some RPs exhibit more specialized functions. By studying vesicular stomatitis virus (VSV) protein synthesis upon host shutoff, Lee and others (Lee *et al.*, 2013) discovered that the 60S ribosomal subunit protein, eL40 (L40) is needed for translation initiation, in particular, 80S formation on viral mRNAs (Fig. 1) (Fernandez-Pevida *et al.*, 2012). Deep sequencing of polysome associated-mRNA in a yeast model showed eL40 (L40)-dependent transcript selectivity, mainly involved in the stress response. Surprisingly, the depletion of eL40 (L40) did not influence the bulk of mRNA – polysome associations (93%), confirming the remarkable specialization of eL40 (L40)-bound ribosomes.

## RIBOSOMAL PROTEIN MODIFICATIONS

Ribosome's mode of action can be also changed by RPs' modifications. Such protein modifications include acetylation, methylation, glycosylation, phosphorylation and ubiquitination (Filipovska & Rackham, 2013; Simsek & Barna, 2017; Xue & Barna, 2012). Recently, thiol oxidation and UFMylation are getting increased attention (Shcherbik & Pestov, 2019; Simsek *et al.*, 2017; Topf *et al.*, 2018; Wang *et al.*, 2020). One of important mechanisms controlling protein function is reversible oxidation and reduction of its cysteine (Cys) residues. An advanced quantitative redox proteomics technique, oxidative isotope-coded affinity tags (OxiCAT), revealed several RPs from both ribosome subunits to be ROS sensitive in different organisms (Leichert *et al.*, 2008; Menger *et al.*, 2015; Topf *et al.*, 2018). The SILAC-iodoTMT method, which allows to simultaneously monitor the redox state and protein expression level, also confirms RPs to be one of the most significant groups of proteins affected by the hydrogen peroxide treatment (Vajrychova *et al.*, 2019). Topf *et al.* proposed a concept that RPs can serve as redox sensors upon oxidative stress conditions (Topf *et al.*, 2018). Dysfunction of mitochondrial protein import results in increased ROS production and rapid attenuation of global protein synthesis. On the other hand, inhibition of protein production caused by exogenous addition of hydrogen peroxide can be reversed by removing the source of stress, suggesting quick translational reprogramming. OxiCAT analysis upon mitochondrial stress and exogenous hydrogen peroxide treatment identified overlapping ROS-sensitive ribosomal proteins. Importantly, global translation upon deletions of these proteins was less affected under oxidative stress. Altogether, this renders RPs potential thiol-based redox switches, which might effectively respond to environmental changes. Such a molecular mechanism could be beneficial for the cell, allowing for immediate protein synthesis regulation, without energy-consuming *de novo* ribosome assembly. Nevertheless, it is still not clear how oxidation of certain RPs influences the work of translation machinery and whether the main outcome of this event is attenuation of the process or rather its reprogramming. Interestingly, other redoxome studies in yeast and also in human cells show that some of the RPs are oxidized under normal growth conditions and play a role in functional pathways (Go *et al.*, 2011; Le Moan *et al.*, 2006). This supports the importance of reversible changes in ribosomal protein redox state in preserving cellular homeostasis.

UFMylation is one of the latest identified ribosomal protein modifications. Ubiquitin-fold modifier 1 (UFM1)

is an ubiquitin-like small protein identified in metazoans, plants, and mammals, but not in yeast (Wei & Xu, 2016). It conjugates to lysine residues on substrates via a specific cascade of enzymatic processes that involve ligases and proteases (Wei & Xu, 2016). The role of UFM1 is expanding, showing mostly its significance in the unfolded stress response (UPR) in the endoplasmic reticulum (ER) and haematopoiesis (Cai *et al.*, 2016; Lemaire *et al.*, 2011; Tatsumi *et al.*, 2011; Wang *et al.*, 2020; Zhang & Xu, 2016). An intricate study of Walczak *et al.*, involving UFMylome and MS analysis, revealed that the primary target of UFM1 posttranslational modification is the large ribosomal subunit protein, uL24 (L26), and particularly its C-terminal lysines which are localized next to the ribosome's peptide exit tunnel (Walczak *et al.*, 2019). UFMylated uL24 (L26) was found to be enriched at the ER membrane-bound ribosomes and polysomes, which correlates with the fact that the enzyme complexes catalysing UFMylation and de-UFMylation bind to the ER cytoplasmic surface. Moreover, disruptions of uL24 (L26) UFMylation result in ER stress (Fig. 1). Exploring the function of UFMylation of uL24 (L26) in ER-associated ribosomes, Wang *et al.* uncovered its implication in the degradation of stalled nascent chains (Wang *et al.*, 2020). Ribosome UFMylation triggers degradation of ER translocation-arrested proteins directing them to the lysosome, in contrast to previously described ribosome-associated quality control (RQC) mechanism, which targets nascent chains for proteasomal degradation (Brandman & Hegde, 2016). Thus, modifications of ribosomal proteins can occur at any level of protein production and can have a prominent impact not only on translation regulation but also on the entailed cellular processes.

## THE ACTIVITY OF RIBOSOME-ASSOCIATED PROTEINS

Binding of non-canonical proteins to the ribosome can also contribute to the increase in the diversity of the translational output. A ribo-interactome study performed in mammalian cells revealed hundreds of proteins associating with ribosomes that belong to different functional groups, including protein- and RNA-modifying enzymes, RNA binding proteins, but also proteins involved in the energy metabolism, redox homeostasis and cell cycle (Simsek *et al.*, 2017). Interestingly, a subcellular pool of translating ribosomes interacting with the muscle pyruvate kinase 2 (PKM2) was identified. PKM2 converts phosphoenolpyruvate (PEP) and adenosine diphosphate (ADP) to pyruvate and adenosine triphosphate (ATP) in the last step of glycolysis. This metabolic enzyme appeared to act as a translation activator regardless of its catalytic activity. To find direct PKM2 and RNA interactions, three techniques were combined: ultraviolet (UV) crosslinking, immunoprecipitation (IP), and high-throughput sequencing, together known as the iCLIP analysis. This comprehensive study uncovered that PKM2 interacts with 18S rRNA and 28S rRNA in the proximity to the aminoacyl site (A-site) on the ribosome, where charged t-RNA molecules bind during protein synthesis. Additionally, PKM2 mainly targets mRNAs translated by ER-associated ribosomes, encoding components of the ER itself and cellular membrane proteins (Fig. 1). Potentially, it might couple cell metabolism and proliferation. Notably, other iCLIP studies also revealed metabolic enzymes that can interact with RNA, giving a chance to expand the current hypothesis (Baltz *et al.*, 2012; Castello *et al.*, 2012; Liu *et al.*, 2019). Overall,

this data shows that ribosome associated proteins could boost translational selectivity of spatially localized mRNAs.

On the other hand, a more direct interplay between cell proliferation and protein synthesis provides cyclin-dependent kinase 1 (CDK1), lately shown as a translational activator (Haneke *et al.*, 2020). CDK1 is a conserved, central kinase regulating cell cycle, which was identified among other kinases and phosphatases in the siRNA screen upon the formation of stress granules in human cells (Haneke *et al.*, 2020). Although it was already reported as a protein involved in protein synthesis control during mitosis (Shuda *et al.*, 2015), for the first time its role goes beyond the cell cycle control. By synchronizing or arresting certain phases of cell cycle progression, the authors were able to show a reduction in global protein synthesis upon pharmacological inhibition or knockdown of CDK1 in the HeLa cells, as well as in the primary mouse embryonic fibroblasts (MEFs) (Shuda *et al.*, 2015). Many molecular mechanisms contribute to this effect which is downstream of CDK1, such as phosphorylation of eIF2 $\alpha$  or S6K1 signalling (Haneke *et al.*, 2020). It is also important to mention that CDK1 co-sediments with the polysome fraction, which was confirmed using a mass spectrometry approach (Simsek *et al.*, 2017). The known substrate of CDK1 is the large ribosomal subunit protein, uL11 (L12), which undergoes phosphorylation upon its activation. Interestingly, uL11 (L12) phosphorylation, in turn, enhances the mitotic protein synthesis program (Imami *et al.*, 2018). Finally, ribosome footprinting (Ribo-Seq) revealed that CDK1 boosts 5'TOP mRNA translation, including RPs. Thus, CDK1 serves as a hub connecting the control of protein synthesis and regulation of cell cycle progression.

Further, several studies implicate a role of ribosome heterogeneity in production of mitochondrial proteins (Crawford & Pavitt, 2019; Segev & Gerst, 2018). Recently, Baxter and others (Baxter *et al.*, 2020) identified the ribosome associated protein, BUD23, belonging to this group. BUD23 methyltransferase plays a dual, independent role in the formation of the translation apparatus. It is involved in the processing of pre-18S rRNA and adding of methyl mark on its key guanosine residue located within the E site, where deacylated tRNA exits the ribosome and peptidyl-tRNA site (P-site) of the 40S ribosomal subunit. Loss of BUD23 does not significantly affect the global protein production but causes an imbalance in translation of specific mRNA. Translational efficiency (TE) of certain transcripts was calculated as a proportion between 'heavy' polysome fractions, representing more than three ribosomes loaded on one mRNA, and 'light' (less than three). Analysis of combined TE and RNA-seq results showed that BUD23-ribosomes preferentially bind to mRNA transcripts with modest 5'UTR GC content. Surprisingly, depletion of BUD23 impairs the synthesis of nuclear-encoded mitochondrial proteins, especially those comprising complex I, IV, and V of the electron transport chain (Fig. 1). In turn, this greatly diminishes generation of mitochondrial ATP. Ribosomes with associated BUD23 were shown to be necessary for maintaining mitochondrial function, both *in vitro* and *in vivo*. In genetically modified mice models, loss of BUD23 has led to embryo-lethality, whereas BUD23 loss restricted to mitochondrial-dependent cells, cardiomyocytes, caused cardiomyopathy and pre-mature death of animals. These findings exemplify the high importance of extrinsic proteins modifying ribosomes to regulate protein synthesis and its impact on organismal physiology.

## RIBOSOME MODIFICATION-RELATED DISEASES

Ribosomopathies are inherited diseases originating from dysfunction of ribosomes caused by mutations in ribosomal genes or rRNA, which give a wide spectrum of clinical phenotypes. However, this definition broadens as a consequence of heterologous ribosomes discoveries. In 1999, the Diamond-Blackfan Anemia (DBA), a bone marrow failure syndrome, was identified as the first disease caused by ribosomal protein mutation, in particular eS19 (S19), thereby supporting the concept of ribosome heterogeneity (Draptchinskaia *et al.*, 1999). The list of DBA related RPs mutations was extended and now involves RPs from both ribosomal subunits, e.g. eS17 (S17), uL18 (L5), and additionally, changes in rRNA were reported (Farrar *et al.*, 2011; Gazda *et al.*, 2008; Lezzerini *et al.*, 2020; Quarello *et al.*, 2016). Ribosomopathies in most cases manifest themselves as hematopoietic deficiencies, and perhaps this is connected to the high diversity in RPs' expression observed in the primary hematopoietic cells. An intriguing phenomenon in ribosomopathies is the transition from hypo- to hyper-proliferation phenotypes, introduced by William Dameshek in 1967 (Dameshek, 1967). Patients who suffer from diseases characterized by a diminished proliferation potential, such as anaemia, with time are at a higher risk of developing hyper-proliferative diseases, such as cancer (Dameshek, 1967; De Keersmaecker, Sulima, Dinman, 2015).

Protein synthesis also plays a crucial role in cancer progression. Cancer cells are characterized by a high proliferative potential and therefore increased mRNA translation demand. Mutations of RPs are linked to several human cancer types, including e.g. breast cancer and T-cell acute lymphoblastic leukaemia (De Keersmaecker *et al.*, 2015; Ferreira *et al.*, 2014; Kampen *et al.*, 2019; Rao *et al.*, 2012). Nevertheless, the so-called oncogenic ribosomes are also characterized by changes in protein composition. One of the newly discovered cancer-related proteins involved in translational reprogramming is the FK506-binding protein 10 (FKBP10) (Ramadori *et al.*, 2020). It is specifically expressed in lung cancer cells and its presence is negatively correlated with the patients' survival. FKBP10 is an ER chaperone with peptidyl-prolyl-cis-trans-isomerase (PPIase) activity (Chen *et al.*, 2017). Depletion of FKBP10 in A549 cells reduces global protein synthesis by half. Polysome profiling, followed by western blot analysis, detected FKBP10 in the monosome (the 80S) and in a light fraction of polysomes. Further, knockdown of FKBP10 in A549 cells and *in vivo* in lung tumours in mice models showed accumulation of FKBP10 in the monosome fraction together with a decrease in the polysomes, suggesting its implication in translation elongation. Importantly, upon these conditions ribosome occupancy specifically increases at the proline codons. These results indicate a mechanism relying on the ribosome binding protein, FKBP10, adapted by cancer cells to support their growth by an increase in the protein synthesis. Hence, this could be a promising target for new anticancer therapy (Liang *et al.*, 2019).

Yet, emerging issues are neurodevelopmental syndromes caused by mutations in the ribosomal machinery's components or association of trans-acting proteins, e.g. intellectual retardation and schizophrenia (Zhou *et al.*, 2018). Several studies report mutations in the uL16 (L10) gene which gives different phenotypes depending on localization of the mutation (Brooks *et al.*, 2014). Mutation at the N-terminus of the uL16 (L10) protein results in inter alia microcephaly, whereas at C-terminus

correlates with autism (Brooks *et al.*, 2014). Knockdown of uL16 (L10) in zebrafish gives a microcephaly-like phenotype and results in a decrease in general protein synthesis in the brain, which could not be rescued by mutated uL16 (L10) identified in patients (Brooks *et al.*, 2014). This proves the role of uL16 (L10) in symptoms developed by affected individuals, but the exact mechanism remains unknown. Similarly, protein synthesis dysregulation was observed in patients with developmental delay carrying a mutation in a small ribosomal protein, uS12 (S23) (Paolini *et al.*, 2017). Corresponding mutations in yeast show impairment in accuracy of mRNA translation, while global protein synthesis remains unchanged, most likely meaning that mutated uS12 (S23) is an integral part of translational machinery (Paolini *et al.*, 2017). Noteworthy, fibroblasts from affected patients display high sensitivity to oxidative stress (Paolini *et al.*, 2017). In conclusion, changes in ribosomes are the cause of many distinct pathological phenotypes implicated in a wide spectrum of diseases, thus making them an attractive treatment targets, as well as potential disease prognosis markers.

## CONCLUSIONS AND FURTHER PERSPECTIVES

Protein synthesis is a crucial biological process in which deregulation has a tremendous impact on the cellular or even organismal fitness. The discovery of ribosome heterogeneity is a milestone in understanding of gene expression. Advancement in molecular and analytical techniques, such as mass spectrometry, paves the way towards deciphering ribosome functions in the control of protein production (Genuth & Barna, 2018; Samir *et al.*, 2018; Simsek *et al.*, 2017; Topf *et al.*, 2018). Multiple high-throughput analyses allow for identification of a vast number of alterations in the translation machinery, such as diversity in protein composition, changes in rRNA structure, or association of extrinsic proteins. The ribosome is an intricate ribonucleoprotein complex and for this reason it can also be a source of countless modifications, which in turn renders its study challenging. Generation of specialized ribosome sub-populations is usually linked to development, the status of the cell, or environmental conditions, such as stress. Pieces of evidence also suggest ribosome differentiation according to cell type or tissue specificity (Guimaraes & Zavolan, 2016; Kondrashov *et al.*, 2011; Wong *et al.*, 2014). A seemingly subtle change, a single variation in the translation machinery can modulate translational output or even leads to a phenotypic effect in the whole organism. Likewise, such diversity also increases the risk of dysfunctional ribosomes' formation or production of redundant proteins, which can disturb cell homeostasis. In the context of diseases, the finding of specialized ribosomes results in a more detailed understanding of certain molecular mechanisms and in consequence the pathophysiology of human diseases (Tahmasebi *et al.*, 2018). Importantly, this opens new perspectives for targeted therapies. As already mentioned, a single modification in the translation machinery can cause a wide range of symptoms, proving its enormous impact on protein synthesis and further organismal health and vitality. Altogether, occurrence of ribosome heterogeneity opens many questions. Among them are the existence of signals for the generation of new ribosome subpopulations, what determines their localization, or if their appearance changes with aging? Currently, available knowledge regarding ribosome heterogeneity and its regulatory role in gene expression

superficially touches different fields and is lacking consistency. There is a need for more systematic studies and identification of functional consequences to further understand its implication in physiology and pathophysiology.

## REFERENCES

- Baltz AG, Munschauer M, Schwanhauser B, Vasile A, Murakawa Y, Schueler M, Youngs N, Penfold-Brown D, Drew K, Milek M, *et al* (2012) The mRNA-bound proteome and its global occupancy profile on protein-coding transcripts. *Mol Cell* **46**: 674–690. <https://doi.org/10.1016/j.molcel.2012.05.021>
- Barakat A, Szick-Miranda K, Chang IF, Guyot R, Blanc G, Cooke R, Delseny M, Bailey-Serres J (2001) The organization of cytoplasmic ribosomal protein genes in the Arabidopsis genome. *Plant Physiol* **127**: 398–415
- Baxter M, Voronkov M, Poolman T, Galli G, Pinali C, Goosey L, Knight A, Krakowiak K, Maidstone R, Iqbal M, Zi M, Prehar S, Cartwright EJ, Gibbs J, Matthews LC, Adamson AD, Humphreys NE, Rebelo-Guioimar P, Minczuk M, Bechtold DA, Loudon A, Ray D (2020) Cardiac mitochondrial function depends on BUD23 mediated ribosome programming. *Elife* **9**. <https://doi.org/10.7554/eLife.50705>
- Ben-Sahra I, Manning BD (2017) mTORC1 signaling and the metabolic control of cell growth. *Curr Opin Cell Biol* **45**: 72–82. <https://doi.org/10.1016/j.ccb.2017.02.012>
- Brandman O, Hegde RS (2016) Ribosome-associated protein quality control. *Nat Struct Mol Biol* **23**: 7–15. <https://doi.org/10.1038/nstruc.2016.017>
- Brenner S, Jacob F, Meselson M (1961) An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **190**: 576–581. <https://doi.org/10.1038/190576a0>
- Brooks SS, Wall AL, Golzio C, Reid DW, Kondyles A, Willer JR, Botti C, Nicchitta CV, Katsanis N, Davis EE (2014) A novel ribosomopathy caused by dysfunction of RPL10 disrupts neurodevelopment and causes X-linked microcephaly in humans. *Genetics* **198**: 723–733. <https://doi.org/10.1534/genetics.114.168211>
- Cai YF, Singh N, Li HL (2016) Essential role of Ufm1 conjugation in the hematopoietic system. *Exp Hematol* **44**: 442–446. <https://doi.org/10.1016/j.exphem.2016.03.007>
- Castello A, Fischer B, Eichelbaum K, Horos R, Beckmann BM, Strein C, Davey NE, Humphreys DT, Preiss T, Steinmetz LM, Krijgsvelde J, Hentze MW (2012) Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. *Cell* **149**: 1393–1406. <https://doi.org/10.1016/j.cell.2012.04.031>
- Chen Y, Terajima M, Banerjee P, Guo H, Liu X, Yu J, Yamauchi M, Kurie JM (2017) FKBP65-dependent peptidyl-prolyl isomerase activity potentiates the lysyl hydroxylase 2-driven collagen cross-link switch. *Sci Rep* **7**: 46021. <https://doi.org/10.1038/srep46021>
- Costa-Mattioli M, Walter P (2020) The integrated stress response: From mechanism to disease. *Science* **368**(6489). <https://doi.org/10.1126/science.aat5314>
- Crawford RA, Pavitt GD (2019) Translational regulation in response to stress in *Saccharomyces cerevisiae*. *Yeast* **36**: 5–21. <https://doi.org/10.1002/yea.3349>
- Crick FH (1958) On protein synthesis. *Symp Soc Exp Biol* **12**: 138–163
- Dalla Venezia N, Vincent A, Marcel V, Catez F, Diaz J. J (2019) Emerging role of eukaryote ribosomes in translational control. *Int J Mol Sci* **20**: <https://doi.org/10.3390/ijms20051226>
- Dameshek W (1967) Riddle – What do aplastic anemia paroxysmal nocturnal hemoglobinuria (Pnh) and hypoplastic leukemia have in common. *Blood* **30**: 251–254
- De Keersmaecker K, Sulima SO, Dinman JD (2015) Ribosomopathies and the paradox of cellular hypo- to hyperproliferation. *Blood* **125**: 1377–1382. <https://doi.org/10.1182/blood-2014-10-569616>
- Dever TE, Dinman JD, Green R (2018) Translation elongation and recoding in eukaryotes. *Cold Spring Harb Perspect Biol* **10**: <https://doi.org/10.1101/cshperspect.a032649>
- Dinman JD (2016) Pathways to specialized ribosomes: the brussels lecture. *J Mol Biol* **428**(10 Pt B): 2186–2194. <https://doi.org/10.1016/j.jmb.2015.12.021>
- Donnelly N, Gorman AM, Gupta S, Samali A (2013) The eIF2 alpha kinases: their structures and functions. *Cell Mol Life Sci* **70**: 3493–3511. <https://doi.org/10.1007/s00018-012-1252-6>
- Draptchinskaia N, Gustavsson P, Andersson B, Pettersson M, Willig T. N, Dianzani I, Ball S, Tchernia G, Klar J, Mattsson H, Tentler D, Mohandas N, Carlsson B, Dahl N (1999) The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia. *Nature Genet* **21**: 169–175
- Falcone Ferreyra ML, Casadevall R, Luciani MD, Pezza A, Casati P (2013) New evidence for differential roles of 110 ribosomal proteins from Arabidopsis. *Plant Physiol* **163**: 378–391. <https://doi.org/10.1104/pp.113.223222>



- Farrar JE, Vlachos A, Atsidaftos E, Carlson-Donohoe H, Markello TC, Arceci RJ, Ellis SR, Lipton JM, Bodine DM (2011) Ribosomal protein gene deletions in Diamond-Blackfan anemia. *Blood* **118**: 6943–6951. <https://doi.org/10.1182/blood-2011-08-375170>
- Fernandez-Pevida A, Rodriguez-Galan O, Diaz-Quintana A, Kressler D, de la Cruz J (2012) Yeast ribosomal protein L40 assembles late into precursor 60 S ribosomes and is required for their cytoplasmic maturation. *J Biol Chem* **287**. <https://doi.org/10.1074/jbc.M112.400564>
- Ferreira AM, Tuominen I, van Dijk-Bos K, Sanjabi B, van der Sluis T, van der Zee AG, Hollema H, Zazula M, Sijmons RH, Aaltonen LA, Westers H, Hofstra RM (2014) High frequency of RPL22 mutations in microsatellite – unstable colorectal and endometrial tumors. *Hum Mutat* **35**: 1442–1445. <https://doi.org/10.1002/humu.22686>
- Ferretti MB, Ghalei H, Ward EA, Potts EL, Karbstein K (2017) Rps26 directs mRNA-specific translation by recognition of Kozak sequence elements. *Nat Struct Mol Biol* **24**: 700–707. <https://doi.org/10.1038/nsmb.3442>
- Filipovska A, Rackham O (2013) Specialization from synthesis: How ribosome diversity can customize protein function. *FEBS Letters* **587**: 1189–1197. <https://doi.org/10.1016/j.febslet.2013.02.032>
- Gazda HT, Sheen MR, Vlachos A, Choemsel V, O'Donohue MF, Schneider H, Darras N, Hasman C, Sieff CA, Newburger PE, Ball SE, Niewiadomska E, Matysiak M, Zaucha JM, Glader B, Niemeyer C, Meerpoel JJ, Atsidaftos E, Lipton JM, Gleizes PE, Beggs AH (2008) Ribosomal protein L5 and L11 mutations are associated with cleft palate and abnormal thumbs in Diamond-Blackfan anemia patients. *Am J Hum Genet* **83**: 769–780. <https://doi.org/10.1016/j.ajhg.2008.11.004>
- Genuth NR, Barna M (2018) The discovery of ribosome heterogeneity and its implications for gene regulation and organismal life. *Mol Cell* **71**: 364–374. <https://doi.org/10.1016/j.molcel.2018.07.018>
- Ghulam MM, Catala M, Abou Elela S (2020) Differential expression of duplicated ribosomal protein genes modifies ribosome composition in response to stress. *Nucleic Acids Res* **48**: 1954–1968. <https://doi.org/10.1093/nar/gkz1183>
- Go YM, Duong DM, Peng J, Jones DP (2011) Protein cysteines map to functional networks according to steady-state level of oxidation. *J Proteomics Bioinform* **4**: 196–209. <https://doi.org/10.4172/jpb.1000190>
- Gold VA, Chroschicki P, Bragoszewski P, Chacinska A (2017) Visualization of cytosolic ribosomes on the surface of mitochondria by electron cryo-tomography. *EMBO Rep* **18**: 1786–1800. <https://doi.org/10.15252/embr.201744261>
- Grant CM (2011) Regulation of translation by hydrogen peroxide. *Antioxid Redox Signal* **15**: 191–203. <https://doi.org/10.1089/ars.2010.3699>
- Guimaraes JC, Zavolan M (2016) Patterns of ribosomal protein expression specify normal and malignant human cells. *Genome Biol* **17**: 236. <https://doi.org/10.1186/s13059-016-1104-z>
- Gupta V, Warner JR (2014) Ribosome-omics of the human ribosome. *RNA* **20**: 1004–1013. <https://doi.org/10.1261/ma.043653.113>
- Haneke K, Schott J, Lindner D, Hollensen AK, Damgaard C, K, Mongis C, Knop M, Palm W, Ruggieri A, Stoeklin G (2020) CDK1 couples proliferation with protein synthesis. *J Cell Biol* **219**: <https://doi.org/10.1083/jcb.201906147>
- Harding HP, Zhang Y, Zeng H, Novoa I, Lu PD, Calfon M, Sadri N, Yun C, Popko B, Paules R, Stojdl DF, Bell JC, Hettmann T, Leiden JM, Ron D (2003) An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. *Mol Cell* **11**: 619–633. [https://doi.org/10.1016/s1097-2765\(03\)00105-9](https://doi.org/10.1016/s1097-2765(03)00105-9)
- Hinnebusch AG (2005) Translational regulation of GCN4 and the general amino acid control of yeast. *Annu Rev Microbiol* **59**: 407–450. <https://doi.org/10.1146/annurev.micro.59.031805.133833>
- Hipp MS, Park SH, Hartl FU (2014) Proteostasis impairment in protein-misfolding and -aggregation diseases. *Trends Cell Biol* **24**: 506–514. <https://doi.org/10.1016/j.tcb.2014.05.003>
- Holz MK, Ballif BA, Gygi SP, Blenis J (2005) mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. *Cell* **123**: 569–580. <https://doi.org/10.1016/j.cell.2005.10.024>
- Imami K, Milek M, Bogdanow B, Yasuda T, Kastelic N, Zaubner H, Ishihama Y, Landthaler M, Selbach M (2018) Phosphorylation of the ribosomal protein RPL12/uL11 affects translation during mitosis. *Mol Cell* **72**: 84–98 e89. <https://doi.org/10.1016/j.molcel.2018.08.019>
- Jackson RJ, Hellen CU, Pestova TV (2010) The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* **11**: 113–127. <https://doi.org/10.1038/nrm2838>
- Kampen KR, Sulima SO, Verbelen B, Girardi T, Vereecke S, Rinaldi G, Verbeeck J, Op de Beeck J, Uytendaele A, Meijerink JPP, et al (2019) The ribosomal RPL10 R98S mutation drives IRES-dependent BCL-2 translation in T-ALL. *Leukemia* **33**: 319–332. <https://doi.org/10.1038/s41375-018-0176-z>
- Klinge S, Woolford JL (2019) Ribosome assembly coming into focus. *Nat Rev Mol Cell Biol* **20**: 116–131. <https://doi.org/10.1038/s41580-018-0078-y>
- Kondrashov N, Pusic A, Stumpf CR, Shimizu K, Hsieh AC, Ishijima J, Shiroishi T, Barna M (2011) Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. *Cell* **145**: 383–397. <https://doi.org/10.1016/j.cell.2011.03.028>
- Kozak M (2005) Regulation of translation *via* mRNA structure in prokaryotes and eukaryotes. *Gene* **361**: 13–37. <https://doi.org/10.1016/j.gene.2005.06.037>
- Kruiswijk T, Planta RJ, Krop JM (1978) The course of the assembly of ribosomal subunits in yeast. *Biochim Biophys Acta* **517**: 378–389. [https://doi.org/10.1016/0005-2787\(78\)90204-6](https://doi.org/10.1016/0005-2787(78)90204-6)
- Kwan T, Thompson SR (2019) Noncanonical translation initiation in eukaryotes. *Cold Spring Harb Perspect Biol* **11**: <https://doi.org/10.1101/cshperspect.a032672>
- Le Moan N, Clement G, Le Maout S, Tacnet F, Toledano MB (2006) The *Saccharomyces cerevisiae* proteome of oxidized protein thiols – contrasted functions for the thioredoxin and glutathione pathways. *J Biol Chem* **281**: 10420–10430. <https://doi.org/10.1074/jbc.M513346200>
- Leichert LI, Gehrke F, Gudiseva HV, Blackwell T, Ilbert M, Walker AK, Strahler JR, Andrews PC, Jakob U (2008) Quantifying changes in the thiol redox proteome upon oxidative stress *in vivo*. *Proc Natl Acad Sci U S A* **105**: 8197–8202. <https://doi.org/10.1073/pnas.0707723105>
- Lemaire K, Moura RF, Granvik M, Igoillo-Esteve M, Hohmeier HE, Hendrickx N, Newgard CB, Waelkens E, Cnop M, Schuit F (2011) Ubiquitin fold modifier 1 (UFM1) and its target UFBP1 protect pancreatic beta cells from ER stress-induced apoptosis. *PLoS One* **6**: e18517. <https://doi.org/10.1371/journal.pone.0018517>
- Lezzerini M, Penzo M, O'donohue MF, Vieira CMD, Saby M, Elfrink HL, Diets IJ, Hesse AM, Coute Y, Gastou M, Nin-Velez A, Nikkels PGJ, Olson AN, Zonneveld-Huijssoon E, Jongmans MCJ, Zhang G, van Weeghel M, Houtkooper RH, Wolodarski MW, Kuiper RP, Bierings MB, van der Werff Ten Bosch J, Leblanc T, Montanaro L, Dinman JD, Da Costa L, Gleizes PE (2020) Ribosomal protein gene RPL9 variants can differentially impair ribosome function and cellular metabolism. *Nucleic Acids Res* **48**: 770–787. <https://doi.org/10.1093/nar/gkz1042>
- Liang L, Zhao K, Zhu JH, Chen G, Qin XG, Chen JQ (2019) Comprehensive evaluation of FKBP10 expression and its prognostic potential in gastric cancer. *Oncol Rep* **42**: 615–628. <https://doi.org/10.3892/or.2019.7195>
- Liu B, Qian SB (2014) Translational reprogramming in cellular stress response. *Wiley Interdiscip Rev RNA* **5**: 301–315. <https://doi.org/10.1002/wrna.1212>
- Liu LC, Li T, Song G, He QX, Yin YF, Lu JYY, Bi XJ, Wang KL, Luo S, Chen YS, Yang Y, Sun BF, Yang YG, Wu J, Zhu H, Shen X (2019) Insight into novel RNA-binding activities *via* large-scale analysis of lncRNA-bound proteome and IDH1-bound transcriptome. *Nucleic Acids Res* **47**: 2244–2262. <https://doi.org/10.1093/nar/gkz032>
- Lopes AM, Miguel RN, Sargent CA, Ellis PJ, Amorim A, Affara NA (2010) The human RPS4 paralogue on Yq11.223 encodes a structurally conserved ribosomal protein and is preferentially expressed during spermatogenesis. *Bmc Mol Biol* **11**. <https://doi.org/10.1186/1471-2199-11-33>
- Lu H, Yao XW, Whiteway M, Xiong J, Liao ZB, Jiang YY, Cao YY (2015) Loss of RPS41 but not its paralog RPS42 results in altered growth filamentation and transcriptome changes in *Candida albicans*. *Fungal Genet Biol* **80**: 31–42. <https://doi.org/10.1016/j.fgb.2015.03.012>
- Mageeey CM, Ware VC (2019) Specialized eRPL22 paralogue-specific ribosomes regulate specific mRNA translation in spermatogenesis in *Drosophila melanogaster*. *Mol Biol Cell* **30**: 2240–2253. <https://doi.org/10.1091/mbc.E19-02-0086>
- Magnuson B, Ekim B, Fingar DC (2012) Regulation and function of ribosomal protein S6 kinase (S6K) within mTOR signalling networks. *Biochem J* **441**: 1–21. <https://doi.org/10.1042/Bj20110892>
- Mahamid J, Pfeffer S, Schaffer M, Villa E, Danev R, Cuellar LK, Forster F, Hyman AA, Plitzko JM, Baumeister W (2016) Visualizing the molecular sociology at the HeLa cell nuclear periphery. *Science* **351**: 969–972. <https://doi.org/10.1126/science.1248857>
- Marygold SJ, Roote J, Reuter G, Lambertsson A, Ashburner M, Millburn GH, Harrison PM, Yu Z, Kenmochi N, Kaufman TC, Leevers SJ, Cook KR (2007) The ribosomal protein genes and Minuto loci of *Drosophila melanogaster*. *Genome Biol* **8**: R216. <https://doi.org/10.1186/gb-2007-8-10-r216>
- Mauro VP, Edelman GM (2002) The ribosome filter hypothesis. *Proc Natl Acad Sci U S A* **99**: 12031–12036. <https://doi.org/10.1073/pnas.192442499>
- Menger KE, James AM, Cocheme HM, Harbour ME, Chouchani ET, Ding SJ, Fearnley IM, Partridge L, Murphy MP (2015) Fasting but not aging dramatically alters the redox status of cysteine residues on proteins in *Drosophila melanogaster*. *Cell Reports* **13**: 1285–1285. <https://doi.org/10.1016/j.celrep.2015.10.048>

- Mohanraj K, Nowicka U, Chacinska A (2020) Mitochondrial control of cellular protein homeostasis. *Biochem J* **477**: 3033–3054. <https://doi.org/10.1042/BCJ20190654>
- Morita M, Gravel SP, Hulea L, Larsson O, Pollak M, St-Pierre J, Topisirovic I (2015) mTOR coordinates protein synthesis mitochondrial activity and proliferation. *Cell Cycle* **14**: 473–480. <https://doi.org/10.4161/15384101.2014.991572>
- Ni L, Snyder M (2001) A genomic study of the bipolar bud site selection pattern in *Saccharomyces cerevisiae*. *Mol Biol Cell* **12**: 2147–2170. <https://doi.org/DOI 10.1091/mbc.12.7.2147>
- O'Leary MN, Schreiber KH, Zhang Y, Duc ACE, Rao SY, Hale JS, Academia EC, Shah SR, Morton JF, Holstein CA, Martin DB, Kaeberlein M, Ladiges WC, Fink PJ, Mackay VL, Wiest DL, Kennedy BK (2013) The ribosomal protein Rpl22 controls ribosome composition by directly repressing expression of its own paralogs Rpl22L1. *PLoS Genet* **9**: <https://doi.org/ARTNe100370810.1371/journal.pgen.1003708>
- Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM (2016) The integrated stress response. *EMBO Rep* **17**: 1374–1395. <https://doi.org/10.15252/embr.201642195>
- Palumbo RJ, Fuchs G, Lutz S, Curcio MJ (2017) Paralogs-specific functions of RPL7A and RPL7B mediated by ribosomal protein or snoRNA Dosage in *Saccharomyces cerevisiae*. *G3 (Bethesda)* **7**: 591–606. <https://doi.org/10.1534/g3.116.035931>
- Paolini NA, Attwood M, Sondalle SB, Vieira C, van Adrichem AM, di Summa FM, O'Donohue MF, Gleizes PE, Rachuri S, Briggs JW, et al (2017) A ribosomopathy reveals decoding defective ribosomes driving human dysmorphism. *Am J Hum Genet* **100**: 506–522. <https://doi.org/10.1016/j.ajhg.2017.01.034>
- Parenteau J, Durand M, Morin G, Gagnon J, Lucier JF, Wellinger RJ, Chabot B, Abou Elela S (2011) Introns within ribosomal protein genes regulate the production and function of yeast ribosomes. *Cell* **147**: 320–331. <https://doi.org/10.1016/j.cell.2011.08.044>
- Parenteau J, Lavoie M, Catala M, Malik-Ghulam M, Gagnon J, Abou Elela S (2015) Preservation of gene duplication increases the regulatory spectrum of ribosomal protein genes and enhances growth under stress. *Cell Rep* **13**: 2516–2526. <https://doi.org/10.1016/j.celrep.2015.11.033>
- Pavitt GD (2018) Regulation of translation initiation factor eIF2B at the hub of the integrated stress response. *Wiley Interdisciplinary Reviews-Rna* **9**: <https://doi.org/ARTNe149110.1002/wrna.1491>
- Pena C, Hurt E, Panse VG (2017) Eukaryotic ribosome assembly transport and quality control. *Nat Struct Mol Biol* **24**: 689–699. <https://doi.org/10.1038/nsmb.3454>
- Quarello P, Garelli E, Carando A, Mancini C, Foglia L, Botto C, Farruggia P, De Keersmaecker K, Aspesi A, Ellis SR, Dianzani I, Ramenghi U (2016) Ribosomal RNA analysis in the diagnosis of Diamond-Blackfan Anaemia. *Br J Haematol* **172**: 782–785. <https://doi.org/10.1111/bjh.13880>
- Ramadori G, Ioris RM, Villanyi Z, Firnkes R, Panasenko OO, Allen G, Konstantinidou G, Aras E, Brenachot X, Biscotti T, Charollais A, Luchetti M, Bezrukov F, Santinelli A, Samad M, Baldi P, Collart MA, Coppari R (2020) FKBP10 regulates protein translation to sustain lung cancer growth. *Cell Rep* **30**: 3851–3863 e3856. <https://doi.org/10.1016/j.celrep.2020.02.082>
- Rao SY, Lee SY, Gutierrez A, Perrigoue J, Thapa RJ, Tu ZG, Jeffers JR, Rhodes M, Anderson S, Oravecz T, Hunger SP, Timakhov RA, Zhang R, Balachandran S, Zambetti GP, Testa JR, Look AT, Wiest DL (2012) Inactivation of ribosomal protein L22 promotes transformation by induction of the stemness factor Lin28B. *Blood* **120**: 3764–3773. <https://doi.org/10.1182/blood-2012-03-415349>
- Reid DW, Nicchitta CV (2015) Diversity and selectivity in mRNA translation on the endoplasmic reticulum. *Nat Rev Mol Cell Biol* **16**: 221–231. <https://doi.org/10.1038/nrm3958>
- Rothman S (2010) How is the balance between protein synthesis and degradation achieved? *Theor Biol Med Model* **7**: <https://doi.org/10.1186/1742-4682-7-25>
- Roux PP, Topisirovic I (2018) Signaling pathways involved in the regulation of mRNA translation. *Mol Cell Biol* **38**: <https://doi.org/10.1128/MCB.00070-18>
- Samir P, Browne CM, Rahul Sun M, Shen BX, Li W, Frank J, Link AJ (2018) Identification of changing ribosome protein compositions using mass spectrometry. *Proteomics* **18**: <https://doi.org/ARTN 180021710.1002/pmic.201800217>
- Santra M, Dill KA, de Graff AMR (2019) Proteostasis collapse is a driver of cell aging and death. *Proc Natl Acad Sci U S A* **116**: 22173–22178. <https://doi.org/10.1073/pnas.1906592116>
- Saveanu C, Namane A, Gleizes PE, Lebreton A, Rousselle JC, Noaillac-Depyre J, Gas N, Jacquier A, Fromont-Racine M (2003) Sequential protein association with nascent 60S ribosomal particles. *Mol Cell Biol* **23**: 4449–4460. <https://doi.org/10.1128/mcb.23.13.4449-4460.2003>
- Saxton RA, Sabatini DM (2017) mTOR Signaling in growth metabolism and disease. *Cell* **169**: 361–371. <https://doi.org/10.1016/j.cell.2017.03.035>
- Segev N, Gerst JE (2018) Specialized ribosomes and specific ribosomal protein paralogs control translation of mitochondrial proteins (vol 217 pg 117 2018) *J Cell Biol* **217**: 117–126. <https://doi.org/10.1083/jcb.201706059>
- Shcherbik N, Pestov DG (2019) The impact of oxidative stress on ribosomes: from injury to regulation. *Cells* **8**: <https://doi.org/10.3390/cells8111379>
- Shi Z, Barna M (2015) Translating the genome in time and space: specialized ribosomes RNA regulons and RNA-binding proteins. *Annu Rev Cell Dev Biol* **31**: 31–54. <https://doi.org/10.1146/annurev-cell-bio-100814-125346>
- Shi Z, Fujii K, Kovary KM, Genuth NR, Rost HL, Teruel MN, Barna M (2017) Heterogeneous ribosomes preferentially translate distinct subpools of mRNAs genome-wide. *Mol Cell* **67**: 71–83 e77. <https://doi.org/10.1016/j.molcel.2017.05.021>
- Shuda M, Velasquez C, Cheng E, Cordek DG, Kwun HJ, Chang Y, Moore PS (2015) CDK1 substitutes for mTOR kinase to activate mitotic cap-dependent protein translation. *Proc Natl Acad Sci U S A* **112**: 5875–5882. <https://doi.org/10.1073/pnas.1505787112>
- Siekevitz P, Palade GE (1958) Heterogeneity of pancreatic ribosome-nucleoprotein particles. *Fed Proc* **17**: 311–311
- Simsek D, Barna M (2017) An emerging role for the ribosome as a nexus for post-translational modifications. *Curr Opin Cell Biol* **45**: 92–101. <https://doi.org/10.1016/j.celb.2017.02.010>
- Simsek D, Tiu GC, Flynn RA, Byeon GW, Leppik K, Xu AF, Chang HY, Barna M (2017) The mammalian ribosome-interactome reveals ribosome functional diversity and heterogeneity. *Cell* **169**: 1051–1065 e1018. <https://doi.org/10.1016/j.cell.2017.05.022>
- Slavov N, Semrau S, Airoidi E, Budnik B, van Oudenaarden A (2015) Differential stoichiometry among core ribosomal proteins. *Cell Reports* **13**: 865–873. <https://doi.org/10.1016/j.celrep.2015.09.056>
- Sloan KE, Warda AS, Sharma S, Entian KD, Lafontaine DLJ, Bohnsack MT (2017) Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. *RNA Biol* **14**: 1138–1152. <https://doi.org/10.1080/15476286.2016.1259781>
- Spriggs KA, Stoneley M, Bushell M, Willis AE (2008) Re-programming of translation following cell stress allows IRES-mediated translation to predominate. *Biol Cell* **100**: 27–38. <https://doi.org/10.1042/BC20070098>
- Sriram A, Bohlen J, Teleman AA (2018) Translation acrobatics: how cancer cells exploit alternate modes of translational initiation. *EMBO Rep* **19**: <https://doi.org/10.15252/embr.201845947>
- Sugihara Y, Honda H, Iida T, Morinaga T, Hino S, Okajima T, Matsuda T, Nadano D (2010) Proteomic analysis of rodent ribosomes revealed heterogeneity including ribosomal proteins L10-like L22-like 1 and L39-like. *J Proteome Res* **9**: 1351–1366. <https://doi.org/10.1021/pr9008964>
- Sun L, Yang XW, Chen FF, Li RP, Li XS, Liu ZX, Gu YY, Gong XY, Liu ZH, Wei H, et al (2013) Paralogous ribosomal protein L32-1 and L32-2 in fission yeast may function distinctively in cellular proliferation and quiescence by changing the ratio of Rpl32 paralogs. *PLoS One* **8**: <https://doi.org/10.1371/journal.pone.0060689>
- Tahmasebi S, Khoutorsky A, Mathews MB, Sonenberg N (2018) Translation deregulation in human disease. *Nat Rev Mol Cell Biol* **19**: 791–807. <https://doi.org/10.1038/s41580-018-0034-x>
- Tatsumi K, Yamamoto-Mukai H, Shimizu R, Waguri S, Sou YS, Sakamoto A, Taya C, Shitara H, Hara T, Chung CH, Tanaka K, Yamamoto M, Komatsu M (2011) The Ufm1-activating enzyme Uba5 is indispensable for erythroid differentiation in mice. *Nat Commun* **2**: <https://doi.org/10.1038/ncomms1182>
- Thomson E, Ferreira-Cerca S, Hurt E (2013) Eukaryotic ribosome biogenesis at a glance. *J Cell Sci* **126**: 4815–4821. <https://doi.org/10.1242/jcs.111948>
- Thoreen CC (2017) The molecular basis of mTORC1-regulated translation. *Biochem Soc Trans* **45**: 213–221. <https://doi.org/10.1042/BST20160072>
- Topf U, Suppanz I, Samluk L, Wrobel L, Boser A, Sakowska P, Knapp B, Pietrzyk MK, Chacinska A, Warscheid B (2018) Quantitative proteomics identifies redox switches for global translation modulation by mitochondrially produced reactive oxygen species. *Nat Commun* **9**: 324. <https://doi.org/10.1038/s41467-017-02694-8>
- Topf U, Uszczynska-Ratajczak B, Chacinska A (2019) Mitochondrial stress-dependent regulation of cellular protein synthesis. *J Cell Sci* **132**: <https://doi.org/jcs22625810.1242/jcs.226258>
- Vajrychova M, Salovska B, Pimkova K, Fabrik I, Tambor V, Kondelova A, Bartek J, Hodny Z (2019) Quantification of cellular protein and redox imbalance using SILAC-iodoTMT methodology. *Redox Biol* **24**: <https://doi.org/10.1016/j.redox.2019.101227>
- Walczak CP, Leto DE, Zhang L, Riepe C, Muller RY, DaRosa PA, Ingolia NT, Elias JE, Kopito RR (2019) Ribosomal protein RPL26 is the principal target of UFMylation. *Proc Natl Acad Sci U S A* **116**: 1299–1308. <https://doi.org/10.1073/pnas.1816202116>
- Wang L, Xu Y, Rogers H, Saidi L, Noguchi CT, Li H, Yewdell JW, Guydosh NR, Ye Y (2020) UFMylation of RPL26 links translocation-associated quality control to endoplasmic reticulum protein ho-



- meostasis. *Cell Res* **30**: 5–20. <https://doi.org/10.1038/s41422-019-0236-6>
- Wapinski I, Pfiffner J, French C, Socha A, Thompson DA, Regev A (2010) Gene duplication and the evolution of ribosomal protein gene regulation in yeast. *Proc Natl Acad Sci U S A* **107**: 5505–5510. <https://doi.org/10.1073/pnas.0911905107>
- Wei Y, Xu X (2016) UFMylation: A unique & fashionable modification for life. *Genomics Proteomics Bioinformatics* **14**: 140–146. <https://doi.org/10.1016/j.gpb.2016.04.001>
- Wek RC, Jiang HY, Anthony TG (2006) Coping with stress: eIF2 kinases and translational control. *Biochem Soc Trans* **34**(Pt 1): 7–11. <https://doi.org/10.1042/BST20060007>
- Wolfe KH, Shields DC (1997) Molecular evidence for an ancient duplication of the entire yeast genome. *Nature* **387**: 708–713. <https://doi.org/10.1038/42711>
- Wong QWL, Li J, Ng SR, Lim SG, Yang H, Vardy LA (2014) RPL39L is an example of a recently evolved ribosomal protein paralog that shows highly specific tissue expression patterns and is upregulated in ESCs and HCC tumors. *RNA Biol* **11**: 33–41. <https://doi.org/10.4161/rna.27427>
- Wrobel L, Topf U, Bragoszewski P, Wiese S, Sztolsztener ME, Oeljeklaus S, Varabyova A, Lirski M, Chroscicki P, Mroczek S, Januszewicz E, Dziembowski A, Koblovska M, Warscheid B, Chacinska A (2015) Mistargeted mitochondrial proteins activate a proteostatic response in the cytosol. *Nature* **524**: 485–488. <https://doi.org/10.1038/nature14951>
- Xue S, Barna M (2012) Specialized ribosomes: a new frontier in gene regulation and organismal biology. *Nat Rev Mol Cell Biol* **13**: 355–369. <https://doi.org/10.1038/nrm3359>
- Yusupova G, Yusupov M (2017) Crystal structure of eukaryotic ribosome and its complexes with inhibitors. *Phil Trans Royal Soc B – Biol Sci* **372**. <https://doi.org/10.1098/rstb.2016.0184>
- Zhang Y, Xu H (2016) Translational regulation of mitochondrial biogenesis. *Biochem Soc Trans* **44**: 1717–1724. <https://doi.org/10.1042/BST20160071C>
- Zhou Y, Dong F, Lanz TA, Reinhart V, Li M, Liu L, Zou J, Xi HS, Mao Y (2018) Interactome analysis reveals ZNF804A a schizophrenia risk gene as a novel component of protein translational machinery critical for embryonic neurodevelopment. *Mol Psychiatry* **23**: 952–962. <https://doi.org/10.1038/mp.2017.166>
- Zhou Y, Musalgaonkar S, Johnson AW, Taylor DW (2019) Tightly-orchestrated rearrangements govern catalytic center assembly of the ribosome. *Nat Commun* **10**. <https://doi.org/10.1038/s41467-019-08880-0>