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Review

The evolving role of ribosomes in the regulation of protein synthesis

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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

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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) synthesises 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* (Mageeney & 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

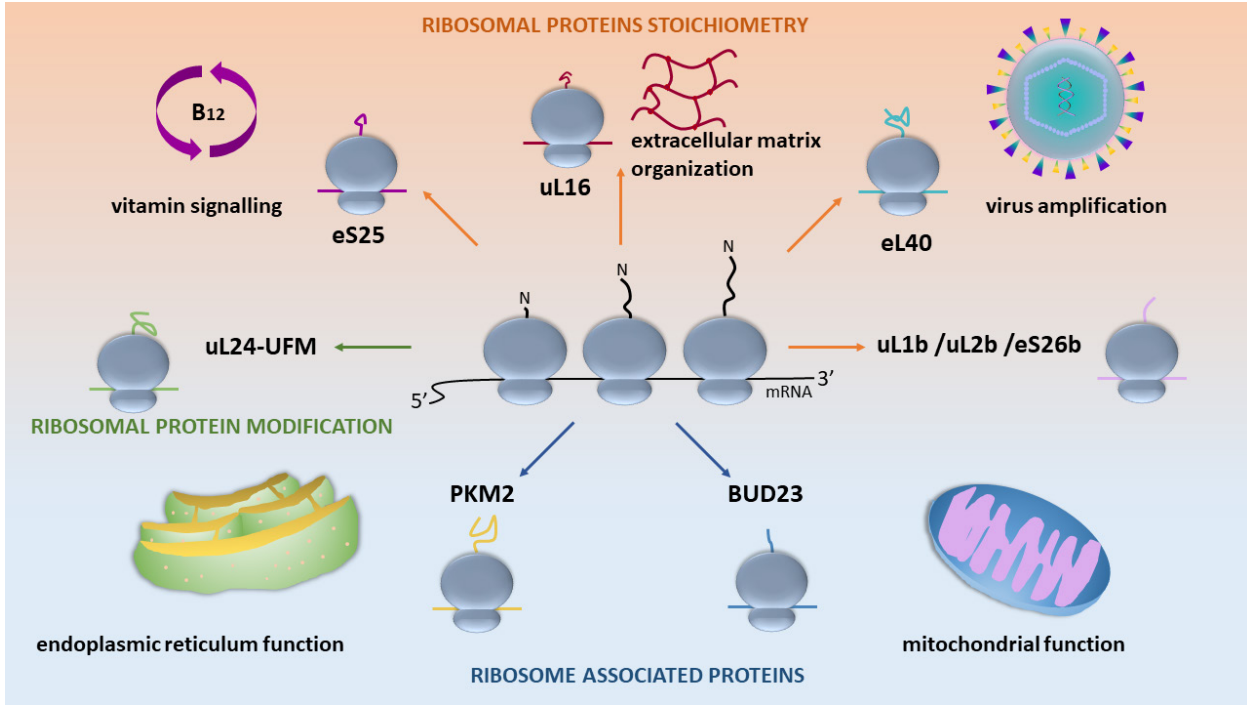

Figure 1. Ribosome heterogeneity regulates translational output.
The variability in translation machinery composition can be manifested at many levels, including differential ribosomal protein stoichiometry, modification of ribosomal proteins or binding of ribosome associated proteins. The subpools of 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, translatome 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)-depended 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 eIF2α or S6K1 signalling (Haneke *et al*., 2020). It is also important to mention that CDK1 cosediments 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.

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