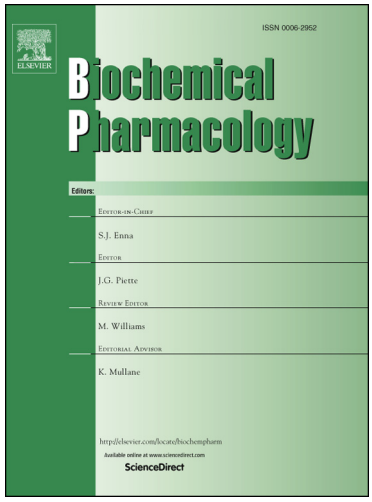
Journal Pre-proofs



Commentary

Ribosomal heterogeneity – a new inroad for pharmacological innovation

Dianne Ford

PII: S0006-2952(20)30102-7
DOI: https://doi.org/10.1016/j.bcp.2020.113874
Reference: BCP 113874

To appear in: *Biochemical Pharmacology*

Received Date: 5 January 2020
Accepted Date: 20 February 2020

Please cite this article as: D. Ford, Ribosomal heterogeneity – a new inroad for pharmacological innovation, *Biochemical Pharmacology* (2020), doi: https://doi.org/10.1016/j.bcp.2020.113874

Ribosomal heterogeneity – a new inroad for pharmacological innovation

Author details:

Dianne Ford
Northumberland Building, Northumbria University, Northumberland Road, Newcastle upon Tyne, NE1 8ST, United Kingdom
Tel: 0191 215 6100
Email: Dianne.ford@northumbria.ac.uk

Abstract

The paradigm of ribosome usage in protein translation has shifted from a stance proposed as scientists began to unpick the genetic code that each mRNA was partnered by its own, unique ribosome to a rapid reversal of this view that ribosomes are completely interchangeable and simply recruited to mRNAs from a completely homogenous cellular pool. Evidence that the ribosomal proteome, ribosomal gene transcriptome and ribosome protein and RNA modifications differ between cells and tissues points to the fact that ribosomes are heterogeneous in their composition and have a degree of specialisation in their function. It has also been posited that the tissue-specificity of ribosome diseases provides an indication of functional ribosome heterogeneity, but there are substantial caveats to this interpretation. Only now have proteomic technologies developed to a level enabling accurate stoichiometric comparison of the abundance of specific ribosomal proteins in actively translating ribosomes and to measure protein in non-denatured ribosomes. This poises the field for the provocation that ribosome heterogeneity offers a novel and powerful inroad for the pharmacological targeting of disease. Such ribosome-targeted treatments may extend beyond specific ribosomopathies through strategies such as targeting features of ribosomes that are unique to diseased cells, particularly cancer cells, or to activated immune cells, as well as augmenting the action of other drugs through weakening the production of new proteins in target tissues. We may also be able to

harness the potential power in ribosome diversity and specialism to better tune synthetic biology for the production of pharmaceutical proteins.

Key words: ribosomopathy, ribosomal protein, drug design, rRNA modification

1. Discovery of the ribosome

The ribosome was first described by George Palade in the 1950s, who observed dense intracellular particles using electron microscopy (EM) [1]. Palade's initial terminology 'microsome' caused confusion among biologists because the microsome fraction of the cell separated physically includes protein and lipid. The protein and lipid component was viewed as contaminant of the particulate matter considered to be the microsome fraction by some biologists, whereas others considered the protein and lipid to be the microsomal fraction and the particulate matter the contaminant. The suggested term 'ribonucleoprotein particles of the microsome fraction' was, rightly, considered cumbersome, hence adoption of the term ribosome. Palade, along with Albert Claude and Christian de Duve, was awarded the Nobel Prize in 1974 for discovery of the ribosome.

2. Structure of the ribosome

At the simplest level of description, the ribosome is a complex of RNA and protein - a ribonucleoprotein. The size of the ribosome components is measured in Svedberg units (S), a measure of the rate of sedimentation during centrifugation. In both bacterial and eukaryotic cells, the ribosome (total 60S in bacteria and 80S in eukaryotes) has a smaller and larger subunit. The smaller subunit (30S in bacteria, 40S in eukaryotes) contains single ribosomal RNA (rRNA) chain (16S and 18S in bacterial and eukaryotes, respectively). There are two RNA chains in the 50S bacterial large subunit (23S and 5S) and three in the 60S eukaryotic large subunit (28S, 5.8S and 5S). Ribosomal RNA is transcribed by RNA polymerase I. The

bacterial small subunit typically contains ~22 proteins and the eukaryotic small subunit ~32 proteins; 15 of these are common to both. The bacterial large subunit typically contains ~32 proteins and the eukaryotic large subunit contains ~45 proteins, of which 18 are common to both. The ribosomal protein nomenclature system initially evolved organically, leading to some complexity and potential confusion. Most proteins are numbered sequentially for each of the small and large subunit with a prefix of S denoting a protein of the small ribosomal subunit and L a protein of the large subunit. A recommended system for universal nomenclature is to also use the prefix b (e.g. bS1; bL9), e (e.g. eS1; eL6) or u (e.g. uS2; uL1) to denote proteins that are exclusively bacterial, exclusively eukaryotic or universal, respectively [2]. This system is used throughout this article. The protein and rRNA makeup of the bacterial and eukaryotic ribosome is summarised in Figure 1. Since the turn of the millennium, X-ray crystallography has revealed the atomic structure of the ribosome at increasing resolution. Published structures include some with the ribosome in complex with the mRNA being translated and with the tRNAs responsible for assembly of their amino acid cargoes (reviewed in [3]).

3. Early history of the concept of ribosome heterogeneity

Palade suggested the ribosome could be a heterogeneous particle based on his early observation of apparent size and shape difference between the dense particulate matter he observed by EM. Francis Crick was an enthusiastic proponent of a ‘one gene-one ribosome-one protein hypothesis’, which encompassed the concept of there being a bespoke ribosome for each protein – ribosome heterogeneity at its most extreme [4]. However, in 1963, only three years after Crick

proposed this model, the field underwent a complete volte-face. Landmark experiments by Brenner and co-workers demonstrated that *E. coli* cells used ribosomes already present in the cell before phage infection to translate the phage proteins [5], leading to the view that ribosomes are completed non-specialised and simply synthesise the protein determined by the message present. The view that the ribosome is a largely passive element of the cellular gene-decoding machinery that simply responds non-discriminately to translate whichever mRNAs are transcribed prevailed over the several decades that followed and indeed is still the way ribosomes and their function are preened in most modern textbooks.

4. A re-evaluation of the position on ribosome heterogeneity

4.1. Tissue-specific presence of ribosomal elements

Throughout the 1980s and 1990s reports of differential expression of specific ribosomal proteins or in the rRNA content under different conditions or in different cell types in a variety of model organisms began to emerge. For example, a GUS (β-glucuronidase) reporter sequence fused to the promoter sequence of each of 2 paralogues of the uL16 ribosomal protein in *Arabidopsis* revealed differential expression in proliferating versus non-proliferating tissue [6] and changes in the expression and modification of specific ribosomal proteins were observed the slime mould *Dictyostelium discoideum* during the transition from unicellular growth to the multicellular fruiting body [7].

A substantial body of experimental observations now points indirectly to the likelihood that the cell alters ribosome synthesis, and the specific ratios of the ribosomal components, under a variety of conditions. A bioinformatic analysis of the burgeoning body of RNAseq data focused on ribosomal protein genes found that transcripts for 80%–90% of ribosomal protein genes were present in a stoichiometry that spanned a range of less than threefold and with little tissue specificity [8]. Nonetheless, this reveals either that not all ribosomes contain all protein components in equimolar amounts or that there is regulated translation and/or differential turnover of ribosomal proteins. Moreover, the analysis revealed that several of seven annotated ribosomal protein subsidiary genes appeared to be transcribed in a tissue-specific manner, including *RPL10L* in testis and *RPL3L* in muscle. Also, work from this author's laboratory probing the role of a zinc-sensitive transcription factor ZNF658 through determining the response of the transcriptome to its knockdown revealed that of 77 genes up-regulated 29 were ribosomal proteins or annotated as ribosomal protein pseudogenes [9]. It is possible that these are, in fact, functional genes repressed by ZNF658 when the zinc supply is adequate and expressed to alter ribosome composition and thus mRNA preference adaptively to conditions of zinc restriction.

Other evidence demonstrates more-directly that the ribosomal population of the cell differs in its makeup in a way that is both dynamic and cell-specific and that the cell invokes ribosomal protein paralogs as an element of likely functional variability. A body of work reveals that different cell and tissue types have characteristic ribosomal protein transcript profiles. For example, analysis of the heterogeneity of expression of 90 ribosomal protein genes, including 19 paralogs, in a panel of human tissues,

primary cells, and tumours revealed that approximately one quarter of human ribosomal proteins are expressed in a tissue-specific manner [10]. Ribosomal protein genes in yeast cells (*Saccharomyces cerevisiae*) exist in pairs created by gene duplication; it was shown recently that one member is most active under normal growth conditions and the other under conditions of stress, leading to modification of ribosome composition in response to changes in growth conditions [11]. Other work presenting evidence of differential use of the ribosomal protein paralogs for likely (though still unproven) functional consequence includes studies on the switching from use in skeletal muscle of predominantly RPuL3 to RPuL3L in response to an anabolic stimulus [12] . Mechanisms underlying the switching between use of ribosomal protein paralogs are potentially diverse; currently we have knowledge of only isolated specific examples such as for the switch between RPeL22L1 and RPeL22, which is via RPeL22 destabilising the mRNA for RPeL22L1 through a direct binding interaction [13].

Speculatively, detailed work in polarised cells, such the intestinal epithelial cell (enterocyte) and neurone, may reveal variation in ribosome composition at the subcellular level. Polarised distribution of mRNA, presumably to achieve translation into the corresponding proteins at their sites of function, is observed in polarised cells, as well-established for the enterocyte [14, 15]. Localised mRNA translation to protein would be further honed by a corresponding polarised distribution of variant ribosomes matched to translation of the polarised mRNAs.

4.2. Variation in ribosome interacting proteins

The contribution of differences in protein content to variations in ribosomal structure extend beyond differences in ribosomal protein content to differences in composition with respect to other interacting proteins, of which a recent study revealed several hundred [16]. Significantly, this work uncovered the fact that the enzyme pyruvate kinase, in a non-canonical role, was associated preferentially with the ribosomes of the ER compared with cytosolic ribosomes in mouse embryonic stem cells. This highlights again the likelihood that ribosomal heterogeneity at the sub-cellular level may be important functionally. It also points to the ribosome interacting proteins as are a potential site to target pharmacologically to achieve a desired specific functional intervention.

4.3. Variation in ribosome protein post-translational modification

Protein posttranslational modification comprises a further level of potential variation between ribosomes in their protein composition that contributes to heterogeneity and has potential effects on function. As a principle, it has been known for many years that the core ribosomal proteins undergo posttranslational modification. Phosphorylation and ubiquitination have been studied most extensively (reviewed in [17]. Indeed, phosphorylation of RPeS6 is so well-established as a downstream effect of activation of the mTORC pathways and also of physiological or pharmacological neurone activation in mice that it is used experimentally as robust marker of both processes [18, 19]. Nonetheless, the functional effects of this reproducible response are less evident. The phenotype of RPeS6 phosphorylation-deficient mice is subtle and tissue-specific, including smaller pancreatic beta cells and impaired glucose homeostasis [20, 21].

The finding that the ribosomal protein RPL26 is the major target for the protein modification UFMylation provides compelling evidence for a posttranslational modification of a ribosomal protein that has functional effects. UFMylation comprises addition of an 85-amino acid modifier, ubiquitin fold modifier 1 (UFM1), to lysine residues on target proteins, similar to ubiquitination. Until recently, although known to be essential for brain and hematopoietic development [22, 23] the biological function and protein targets of this modification were unknown. UFMylome analysis in human (K562 erythroleukemia) cells engineered to lack either UFMylation or de-UFMylation revealed RPL26 as the principal target of this modification. The work uncovered a dynamic cycle of UFMylation and de-UFMylation of RPL26 by enzymes at the cytoplasmic surface of the endoplasmic reticulum in close proximity to the SEC61 translocon, indicating that RPL26 UFMylation is a specific functional ribosomal modification that plays a role in protein biogenesis in the early secretory pathway [24].

Abnormality in ribosomal protein post-translational modification has been uncovered as the basis for some human diseases. A striking example is the intimate relationship between phosphorylation of RPuS19 and Parkinson's disease [25]. The kinase LRRK2, which phosphorylates RPuS17, RPuS19, and RPeS27, is mutated in familial and sporadic forms of the disease. In *Drosophila*, mutation of the substrate site on RPuS19 rescues the toxicity of mutant LRRK2 whereas incorporation of the phosphomimetic form of RPuS19 causes neurotoxicity, providing evidence for a causal relationship. Also, human post-mortem brain samples from Parkinson's disease patients with the LRRK2 mutation revealed hyperphosphorylation of

RPuS19. Together, this body of evidence supports strongly the premise that RPuS19 phosphorylation has substantial functional effects.

Although there are myriad unequivocal strands of evidence that ribosomal protein posttranslational modification is observed and that it at least can be functional in some instances, there remains a question concerning whether or not these modifications can be bespoke to specific, individual ribosomes to bestow heterogeneity and functional diversity either within or between cells.

4.4. Variation in ribosomal RNA sequence and post-transcriptional modification

We should also explore the potential for ribosomes to be divergent at the level of the rRNA, as well as in terms of ribosomal protein composition. Should this emerge as a level of variance with impactful functional relevance then gene-therapy approaches that target ribosomal RNA diversity may be another avenue of therapeutic intervention through exploiting ribosome heterogeneity to develop.

The four eukaryotic rRNAs are encoded by multiple copies of ribosomal DNA (rDNA) on different chromosomes [26-28]. The 18 and 5.8 and 28S rRNAs are transcribed as a 45S rRNA precursor that undergoes post-transcriptional processing to yield the mature rRNAs. Mapping of rRNA sequence data to these different rDNAs indicates that many rRNA alleles are expressed in a tissue-specific manner [29-31]. Intriguingly, the binding sequence for the zinc-regulated transcription factor ZNF658, which, as mentioned above, may have a role in the transcription of specific ribosomal

protein variants, occurs in multiple copies in the 45S rRNA precursor 5' to the start of the mature 18S rRNA and is also present 5' to each of the mature 5.8 and 28S rRNA sequences [9]. Though unproven, it is possible that these ZTREs provide sites through which the cell modifies 45S rRNA processing in response to zinc availability. Variation in these ZNF658 biding sequences (ZTREs) between the multiple rDNAs may, speculatively, play a role alongside refinement of the ribosomal protein complement produced in adapting the cellular ribosome makeup to respond to changes in the zinc supply, which requires a tightly regulated homeostatic response across the phyla [32]. An understanding of switching between rRNA genes and of its likely functional importance is particularly well advanced with regard to zebrafish development. Sequencing the ribosomal transcriptome from eggs, embryos and adult tissue has revealed that for the 5S rRNA [33] then the 18, 5.8 and 28S products of the common 45S precursor [34] a switch from maternal sequences to somatic sequences, mapping to different rRNA genes. Although demonstration of functional importance is still rudimentary, in silico work suggests that expansion segments in 18S rRNA that appear to be involved directly in ribosome–mRNA interactions may preferentially interact with specific mRNA genes.

In addition to selective rDNA transcription in specific tissues, and potentially under other different conditions, variation at the level of rRNA occurs also through post-transcriptional modification. Approximately 2% (over 200 sites in humans) of rRNA nucleotides are modified. The most common modification is 2'-O-methyation of the sugar (reviewed in [35]) but pseudouridylation, ribosylation, base methylation and acetylation is also observed [17]. The view generally held is that rRNA chemical modifications stabilise secondary and tertiary structures, which is a plausible

mechanism through which functional effect may be achieved. Modifications at some sites are sub-stoichiometric, commensurate with, though not sufficient for, them being a layer of functional ribosomal specialisation (reviewed in [36]). For example, of 112 sites modified by pseudouridylation in yeast, 18 were modified on fewer than 85% of ribosomes [37]. In human HeLa and HCT116 cells, approximately one- third of 2'-O-methylation sites were fractionally, rather than fully, modified. Importantly, there were distinct differences between the two cell lines at some sites, adding to the evidence for ribosome specialisation specifically at the level of rRNA post-translational modification [38].

4.5. Do observed differences in ribosome structure have functional effects?

The most obvious manner in which ribosome specialisation is likely to play out functionally is through it being a mechanism to target ribosomes specifically to the translation of specific mRNAs or affect the efficiency with which specific mRNAs are translated. However, there are important caveats to observations that are commensurate with the view that ribosomes are heterogeneous in a manner that targets ribosomes of specific composition to the translation of specific transcripts. For example, while variation in ribosome composition may be observed unequivocally, specificity of interaction between ribosomes of specific composition and particular mRNAs has not been demonstrated directly. A recent analysis that raises pertinent questions about functional relevance highlights this current challenge to the field [39]. For example, could variation in the composition of microsomes be merely reflective of a level of tolerance in the process of ribosome quality control? Some studies report very similar effects on mRNA translation after

knockdown of diverse ribosomal proteins, which the authors proffer may simply be a manifestation of a general defect in ribosome function as may be the result of overall reduced ribosome numbers, which, in turn, could affect differentially subclasses of mRNAs. The authors recommend controls to validate functional diversity, including gain-of-function assays and showing that selective effects on translation of specific mRNAs can be induced under physiological conditions.

4.6. New inroads through technological advances

Despite longstanding challenges, however, the field now stands poised for rigorous evaluation of the penetration of functional ribosome heterogeneity because proteomic technologies have now developed to a level enabling accurate stoichiometric comparison of the abundance of specific ribosomal proteins in in actively translating ribosomes. Such an analysis was achieved for 15 ribosomal proteins in mouse embryonic stem cells using the technique of selected reaction monitoring (SRM) mass spectrometry [40]. The technique determines absolute abundance of a specific protein by comparison of the signal strength for known peptides with the signal strength for a spiked heavy isotope standard of the same peptides. Nine of the 15 ribosomal proteins (five of the large subunit and four of the small subunit) were present in quantities that did not vary significantly, interpreted to reveal ribosomal components that were core to translating all mRNAs in this cell type. Two proteins (of the large subunit) were present at levels only slightly lower than these but four of the ribosomal proteins measured (two in each of the large and small ribosome subunits) were present at only 60%–70% of this level, indicating their incorporation in only a subset of ribosomes of likely selective function. Two of these

non-stoichiometric ribosomal proteins (one on each subunit; RPSeS25 and RPuL1) were selected for analysis of the mRNA populations to which ribosomes incorporating them bind on the basis that they occupy a position in the ribosome that flanks the mRNA exit tunnel. This analysis, comprising mRNA footprinting and RNAseq before and after CRISPR/Cas-mediated knockdown and affinity purification of mRNAs bound to FLAG-tagged immunoprecipitates, revealed that they are incorporated in ribosomes that bind selectively to subsets of mRNAs that differ for each of the two individual ribosomal proteins. Some RNA footprints were enriched and others depleted after knockdown, revealing both preferential and disfavoured binding interactions. Interestingly, there was also a tendency for opposite enrichment or depletion of sub-pools of mRNAs related by opposite function. For example, footprints for ribosomes containing RPuL1 were enriched in genes promoting growth or implicated in cancer metastasis but depleted in transcripts functioning in the stress response and cell death. Arguably, this work provides compelling direct evidence that ribosomes differencing in composition with respect to their protein content are responsible for the translation of a subset of transcripts with specific cellular functions. A finding of particular potential significance to potentially exploiting ribosome heterogeneity as a therapeutic target was that RPeS25-containing ribosomes were significantly over-represented among the transcripts of all components of the vitamin B12 pathway (transport, cellular uptake and utilisation). This finding hints at the possibility that ribosome heterogeneity may operate on the components of entire metabolic pathways, akin to operons in bacterial cells, and thus be a particularly powerful lever through which to intervene in a given pathway.

A further unanswered question concerning ribosome heterogeneity is to what extent the potential enormous level of diversity that arises from the known number of

ribosomal protein variants, possible differences in ribosome protein stoichiometry, rDNA variants and rRNA and ribosomal protein modification all in combination is actually exploited by the cell. Although the body of data on all of these features demonstrates that ribosomes in different cells in the same organism can differ *per se*, it is not sufficiently granular to reveal what extent of the capacity for individual ribosomes to differ from each other is actually used. We can address this question only by observing ribosomes on an individual basis. One approach that offers promise and has been used to observe the assembly and stoichiometry of individual ribosomal proteins in ribosomal particles of bacterial, plant and human origin is native mass spectrometry. Testing the capacity of this technique to reveal details of the human 40S subunit resolved a minor population of particles lacking either the S25 or S10 protein and also allowed detection of bound viral RNA fragments [41]. Cryo-electron microscopy also offers promise to observe the composition of individual ribosomes. The technique has been used to visualise the structure of the human and *Drosophila* ribosome [42] but, until a very recent report (made available in pre-print form, pending validation by peer-review, in January 2020 [43]) had not been applied to observing ribosome heterogeneity. A challenge to overcome was to achieve adequate resolution of individual structural differences using a technique that averages data from many complexes into an individual structure. In this recent study, the level of purification achieved by sucrose-gradient centrifugation was adequate to generate a dataset with average resolution of 3.5 Å from *Drosophila* testis and 3.0 Å from ovary, which allowed the generation of atomic models for 80S ribosomal complexes from both tissues. Analysis of the same ribosomal preparations by quantitative mass spectrometry revealed that ribosomal protein

paralog switching made the biggest contribution to ribosome heterogeneity, and mapping the switching paralogs onto the ribosome structures revealed locations at the surface, suggesting that switching could alter the ribosome surface and hence enable different proteins to regulate translation. A further level of purification of ribosomal sub-populations, for example by affinity purification based on use of antibodies specific to particular ribosomal protein variants, may also prove effective in yielding samples suitable for visualisation of differences at high resolution.

5. Ribosomopathies and associated indications of ribosome heterogeneity

Towards the end of the millennium came a discovery that supports directly the provocation that ribosomes may be a feature we can target for therapeutic benefit and that revealed ribosomal disorders can have effects that are tissue specific. The underlying cause of the disease Diamond-Blackfan Anaemia, a deficiency in the production of erythroid precursors in the bone marrow, was found to be abnormality in the gene encoding ribosomal protein eS19 [44]. The disease is now linked to mutations in over 15 different ribosomal proteins (reviewed in [45]). Mutations in ribosomal protein genes in other diseases, which also have tissue specificity, have since been revealed. These include asplenia in humans associated with loss of RPuS2 [46] and a form of hair loss inherited through a mutation of RPeL21 [47]. A recent meta-analysis of genome-wide association studies (GWAS) of atrial fibrillation that uncovered associations with a missense and a splice donor variant of RPL3L [48]. The researchers sourced RNA from the cardiac aorta of 167 people of whom two were found to carry the splice donor variant. In both individuals, and in contrast to the other 165 people in the sample, an alternative isoform of RPL3L was present.

It remains to be established if the variant affects ribosome function and if this has any effect on cardiac function. Other diseases caused by abnormalities in ribosome structure that presumably have functional effects include dyskeratosis congenita, in which pathogenic mutations result in abnormal patterns of rRNA pseudouridylation that promote tumorigenesis through impaired translation of p53 and p27 tumour suppressors and of the antiapoptotic factors Bcl-xL and XIAP [49-51].

The number of disorders now shown or identified as likely to have at the root a ribosomal dysfunction has expanded as RNAi-based screens have uncovered a plethora of ribosome biogenesis factors that encompasses many disease-associated proteins and biomarkers (reviewed in [52]). Table 1 presents a list of diseases in which ribosome abnormality has a role and in which tissue-specific symptoms are manifest.

These discoveries expand the range of potential therapeutic targets for the treatment of not only ribosomopathies but also other diseases in which intervention targeted to ribosomal function may be effective. Thus these discoveries highlight further the importance of first developing a robust knowledge base of the extent to which the cell exploits the potential capacity for ribosome heterogeneity and of the specific relationship between cell types and specific ribosome variants, as well as of ribosome variant functionality.

Tissue-specific effects of ribosomal abnormalities are also observed in model organisms. For example, the various different Drosophila minute phenotypes of small bristles and delayed development mapped to numerous different ribosomal

protein loci have distinctive features, one of which is a specific effect on wing growth associated with point mutations in *RpL38* and *RpL5* [81]. Mice carrying mutations in the *Rpl38* gene, characterised phenotypically by skeletal patterning defects, showed perturbed translation of a subset of homeobox mRNAs identified by polysome profiling whilst global protein synthesis and Hox mRNA levels were unchanged. This phenotype was specific to the *Rpl38* gene and not apparent in mice carrying mutations in any of five other ribosomal protein genes, demonstrating selectivity the relationship between RPeL38 and the affected mRNAs. Also, expression of RPeL38 was found to be highly enriched in the embryonic tissues affected by the mutation, which is commensurate with there being a tissue-specific function of RPeL38 at these sites [82]. Male infertility in RPLP1 mutant mice is a second example of a ribosomal protein abnormality in mice that manifests in a tissue-specific manner [83].

It has been posited that the different underlying causes of ribosomopathies and their different tissue-specific phenotypes provides evidence that ribosome heterogeneity exists and has functional consequence. However, judicious attention to many caveats is required to avoid over-interpretation. Consider, for example, Diamond Blackfan Anaemia. Researchers have argued that the bone-marrow-specific effects of the causative ribosomal protein mutations reveals that ribosomes that include these proteins have specific roles in haematopoiesis. This argument can be challenged, however, by the proposal that an effect of a mutation on ribosome activity and overall rate of translation that is global and non-specific may nonetheless be manifest in a cell-specific manner. Most obviously, a rapid rate of cell division may make certain cells, including the haematopoietic cells of the bone marrow, more susceptible than others (akin to the manner in which some chemotherapies, though

generic in action, target the rapidly dividing cancer cells). However, some observations provide a counter-argument to such a proposal. For example, not every ribosomal protein mutation that decreases the rate of protein synthesis is manifest as Diamond Blackfan Anaemia. Mice lacking RPeL29, for example, exhibit lower rates of protein synthesis without the symptoms of Diamond Blackfan Anaemia, but are small and have fragile bones [84]. Another way a given ribosomal protein mutation could manifest as tissue-specific symptoms that could, incautiously, be interpreted as evidence for tissue-specific ribosome complements would be if specific ribosomal proteins common to all ribosomes bind only to specific mRNAs. In this instance, manifestation of any symptoms of ribosomal protein mutation or deficiency would depend purely on whether or not a specific transcript is expressed in the tissue in question. Another model proposed to account for tissue-specific effects of abnormality in the fundamental and ubiquitous process of ribosome function or assembly that need not invoke the concept of functional ribosome heterogeneity pivots on stabilisation of the tumour suppressor p53 via an extra-ribosomal function of the 5S RNP. Non-ribosome-associated 5S RNP, resulting from defective ribosome assembly, binds to and inhibits the E3 ubiquitin ligase HDM2, which removes p53 through targeting the protein for proteasomal degradation [85]. As a result, cellular p53 levels rise [86-88]. In this model, tissue specificity is based on differences in the level of p53 activation in different cell types [89, 90]. Consistent with this model being the explanation for tissue-specific effects at least in some instances of ribosomopathy is that many such specific effects appear to be p53 dependent. For example, symptoms of Treacher Collins syndrome and 5q syndrome can be rescued by inhibiting p53 function [91, 92].

6. Ribosome heterogeneity as a potential therapeutic inroad

Current exploitation of the ribosome as a druggable target includes the treatment of Duchenne muscular dystrophy with ataluren, which is effective in the by virtue of its action to promote read-through by the ribosome of premature stop codons in the transcript of the mutated dystrophin gene [93]. There is also burgeoning interest in the potential use of RNA polymerase I inhibitors in cancer therapy (reviewed in [94]). A number of possible avenues for the development of more-refined therapeutic approaches that target the ribosome open up if ribosome heterogeneity proves a way that distinguishes cells within different tissues, cells in different states of development, activation or disease and/or that substantially alters the efficiency with which specific mRNAs are translated.

6.1. The hypothetical immunoribosome and the immune response as a target for ribosome-based therapy

The provocation that a highly specialised ribosome, termed the immunoribosome, exists is particularly intriguing [95]. The model proposed accounts for the rapidity of viral-antigen presentation from stable viral proteins. The proposal is that this as yet hypothetical specialised ribosome is responsible for the production of peptides presented by MHC Class I molecules on activated T-cells that are, in the main, particularity transient components of proteins that, when produced in the accurate and functional form are, nonetheless, rapidly degraded (RPDs – rapidly degraded polypeptides). The researchers propose that these peptides, named defective ribosomal products (DRiPs), are produced from tumour cells or intracellular

pathogens as polypeptides that fail to achieve native structure due to errors in their synthesis. The immunoribosomal population of the cell is a hypothetical subset of ribosomes dedicated to the production of DRiPs for antigen processing. Advances in proteomic techniques for granular study of ribosome heterogeneity and preferential mRNA translation may provide an opportunity to identify immunoribosomes, if a real phenomenon, and in turn to ultimately harness the potential of these putative specialist ribosomes for therapeutic applications that could include cancer immunotherapy and T-cell-targeted immunosuppression.

Agnostic to the existence of the immunoribosome, the immune response generally may be a process in which exploiting ribosome heterogeneity for therapeutic benefit offers particular opportunities. It would seem likely, though still an idea to investigate, that the rapidly dividing cells of the activated immune system employ particular ribosome variants to achieve rapid translation transcripts pivotal in their expansion.

6.2. Ribosome-associated cancers and the hypothetical cancer ribosome

A number of cancers are associated with ribosomal gene abnormality. Some features of these cancers, while not providing direct evidence for the involvement of ribosomes that are not all uniform and interchangeable in function, are nonetheless consistent with ribosomes being a heterogeneous population of organelles that differ between cells. Incidences of gene copy-number changes in cancer are generally much lower than incidences of point mutations. However, this trend is reversed in the case of ribosomal protein gene mutations associated with cancer; abnormality in

ribosomal protein genes in cancer is more often through copy-number changes than point mutations [96]. This situation may reflect a requirement for tight control over the relative stoichiometry of production of specific ribosomal proteins, which would seem commensurate with normal cell function and controlled cell proliferation requiring a ribosome complement of very specific composition as would be likely in the case of functional ribosome heterogeneity. Thus, although not direct evidence for the phenomenon, this observation made in cancer is arguablly supportive of the principle.

Multiagent combination therapy cocktails are required to overcome the low efficacy of single-agent cancer therapies due to resistance development and to treat cancer with high efficacy and low toxicity. The size and complexity of the ribosome makes it a good target for such approaches, some of which, such as use of RNA polymerase I inhibitors, are based on 'starving' cancer cells of ribosomes to prevent translation. However, ribosome heterogeneity created by the genetic abnormalities that underlie ribosome-related cancers is arguably the most promising new therapeutic target for ribosome-based cancer therapy. The hypothetical 'onco-ribosome' may be an attractive drug target. This could be considered a form of abnormal ribosome heterogeneity or of 'uncontrolled ribosome heterogeneity'. High-resolution structures of human ribosomes bound to various antibiotics and, in future, of mutant ribosomes open the drug discovery field to finding new ribosomal inhibitors that interact specifically with defective ribosomes, which could be through the design of small molecules that bind specifically to 'onco-ribosomes'. Repurposed prokaryotic antibiotics that target the ribosome, identified by screening for interaction with

abnormal human cancer ribosomes, may also prove effective new therapeutic agents [96].

6.3. Other avenues for ribosome-targeted therapies

In the same way that distinguishing features of the ribosomes of cancer cells or activated immune cells may provide therapeutic targets, so might we ultimately be able harness ribosome heterogeneity by co-administering a ribosome-targeted drug to manipulate the susceptibility of specific tissues to other drugs to improve specificity and reduce side effects. An assault on cell-type-specific ribosomes through targeting their distinctive components to weaken the ability of cells to produce new proteins due to compromised mRNA translation may achieve this. At the most-refined level of drug targeting, intracellular rRNA heterogeneity could potentially be exploited to target and prevent translation of specific transcripts, such as proteins responsible for disease symptoms, that are translated by ribosomes with druggable distinctive components. The discovery that ribosomes containing RPS25/eS25 preferentially translate mRNAs encoding all stages of the vitamin B12 pathway [40] tempts speculation that the paradigm may apply to other cellular pathways also. This remains to be proven. However, should this apply to pathways that are therapeutic targets for particular diseases then targeting drug treatment to heterogenous features of ribosomes may be particularly efficacious, akin to the strategy of Systems Pharmacology.

A description of ribosome assembly and the state of current knowledge about the process is beyond the scope of this article, but is the subject of a comprehensive

recent review [52]. Differences in individual components of the assembly machinery responsible for the production of variant ribosomes offer another potential target to deliver therapy targeted to specific cells or intracellular ribosome subpopulations.

The therapeutic potential of ribosome heterogeneity may extend, either through targeted manipulation of ribosome activity in the intracellular environment or in cell-free protein synthesis systems, to synthesis of therapeutic peptides such as antibodies, insulin and tissue plasminogen activator. Creation of bespoke ribosomes optimised for the translation of the corresponding mRNAs in engineered cells or cell-free systems may add to the synthetic biology toolkit and improve the process of producing recombinant proteins therapeutics.

Figure 2 presents the range possible approaches to exploiting intercellular and intracellular ribosome heterogeneity for therapeutic application discussed above.

7. Conclusions and Future Directions

A large body of data now demonstrates that cells in the same organism can differ in their complement of ribosomal components, including differences in ribosomal protein and/or RNA content and modification. Thus, the principle that ribosome heterogeneity exits on an intercellular basis is probably unequivocal. However, major questions remain concerning ribosome heterogeneity on an intracellular level and, more importantly, concerning if differences in ribosome composition either between or within cells have any functional consequences.

The advancement of proteomic techniques to a point where the ribosomal protein composition of individual ribosomes can be determined now poises the field at a nexus to determine how much of the capacity for individual ribosome heterogeneity is exploited. However, determining directly that ribosomes of specific composition differ in their function, for example with respect to relative efficiency with which they translate specific mRNAs, will be an ongoing challenge. One way forward may be to harness future improved understanding of how the process of ribosome assembly introduces such heterogenous features and recapitulate this ex-vivo to build synthetic ribosomes to test in cell-free systems. We may also be able to exploit such approaches for therapeutic benefit in the future through customisation of synthetic ribosomes to optimise the synthesis of peptide-based therapeutics. Detailed knowledge of how the process of ribosome assembly introduces heterogeneity may also reveal druggable targets to affect differentially affect the synthesis of specific ribosome variants for therapeutic benefit in vivo.

As advances in structural biology reveal greater ribosome structural detail, rational design of drugs to target specific ribosome variants may become feasible. Putative variants that may be particularly high-priority drug targets include the proposed immunoribosomes of activated T-cells, for the purpose of cancer immunotherapy and T-cell-targeted immunosuppression, and the proposed oncoribosome, to target cancer cells.

In summary, ribosome heterogeneity stands poised to potentially become a major new target for therapeutic intervention. However, advances in understanding the

basic principles of ribosome heterogeneity and the assembly processes responsible for creating this heterogeneity are required to harness this potential.

Figure legends

Figure 1. Potential variations in ribosome composition. Ribosomal RNA (rRNA) is depicted in blue (large subunit) or green (small subunit). Proteins (except where variant) are depicted in yellow. Alternative colouring (or, for rRNA shading pattern) indicates variation. The tables show the composition of the bacterial and eukaryotic ribosome.

Figure 2. Potential approaches to exploiting features of ribosome heterogeneity for therapeutic benefit. Coloured arrows point to potential drug target sites (shown in the same colour) on ribosomes that differ between cells or ribosomes with cells. A flat arrowhead (and colour difference) indicates the drug does not interact with the site indicated. A round arrowhead indicates blocking a process. An effective therapeutic strike is shown as disordered ribosome structure and fragmentation of the cell membrane.

Statement on conflict of interest

The author has no conflict of interest to declare.

References

[1] G.E. Palade, A small particulate component of the cytoplasm, J Biophys Biochem Cytol 1(1) (1955) 59-68.

[2] N. Ban, R. Beckmann, J.H. Cate, J.D. Dinman, F. Dragon, S.R. Ellis, D.L. Lafontaine, L. Lindahl, A. Liljas, J.M. Lipton, M.A. McAlear, P.B. Moore, H.F. Noller, J. Ortega, V.G. Panse, V. Ramakrishnan, C.M. Spahn, T.A. Steitz, M. Tchorzewski, D. Tollervey, A.J. Warren, J.R. Williamson, D. Wilson, A. Yonath, M. Yusupov, A new system for naming ribosomal proteins, Curr Opin Struct Biol 24 (2014) 165-9.

[3] A. Jobe, Z. Liu, C. Gutierrez-Vargas, J. Frank, New Insights into Ribosome Structure and Function, Cold Spring Harb Perspect Biol 11(1) (2019).

[4] F.H. Crick, On protein synthesis, Symp Soc Exp Biol 12 (1958) 138-63.

[5] S. Brenner, F. Jacob, M. Meselson, An unstable intermediate carrying information from genes to ribosomes for protein synthesis, Nature 190 (1961) 576-581.

[6] M.E. Williams, I.M. Sussex, Developmental regulation of ribosomal protein L16 genes in Arabidopsis thaliana, Plant J 8(1) (1995) 65-76.

[7] S. Ramagopal, Induction of cell-specific ribosomal proteins in aggregation-competent nonmorphogenetic Dictyostelium discoideum, Biochem Cell Biol 68(11) (1990) 1281-7.

[8] V. Gupta, J.R. Warner, Ribosome-omics of the human ribosome, RNA 20(7) (2014) 1004-13.

[9] O.A. Ogo, J. Tyson, S.J. Cockell, A. Howard, R.A. Valentine, D. Ford, The zinc finger protein ZNF658 regulates the transcription of genes involved in zinc homeostasis and affects ribosome biogenesis through the zinc transcriptional regulatory element, Mol Cell Biol 35(6) (2015) 977-87.

[10] J.C. Guimaraes, M. Zavolan, Patterns of ribosomal protein expression specify normal and malignant human cells, Genome Biol 17(1) (2016) 236.

[11] M.M. Ghulam, M. Catala, S. Abou Elela, Differential expression of duplicated ribosomal protein genes modifies ribosome composition in response to stress, Nucleic Acids Res (2019).

[12] T. Chaillou, T.J. Kirby, J.J. McCarthy, Ribosome biogenesis: emerging evidence for a central role in the regulation of skeletal muscle mass, J Cell Physiol 229(11) (2014) 1584-94.

[13] M.N. O'Leary, K.H. Schreiber, Y. Zhang, A.C. Duc, S. Rao, J.S. Hale, E.C. Academia, S.R. Shah, J.F. Morton, C.A. Holstein, D.B. Martin, M. Kaeberlein, W.C. Ladiges, P.J. Fink, V.L. Mackay, D.L. Wiest, B.K. Kennedy, The ribosomal protein Rpl22 controls ribosome composition by directly repressing expression of its own paralog, Rpl22l1, PLoS Genet 9(8) (2013) e1003708.

[14] A.E. Moor, M. Golan, E.E. Massasa, D. Lemze, T. Weizman, R. Shenhav, S. Baydatch, O. Mizrahi, R. Winkler, O. Golani, N. Stern-Ginossar, S. Itzkovitz, Global mRNA polarization regulates translation efficiency in the intestinal epithelium, Science 357(6357) (2017) 1299-1303.

[15] C.M. Pedder, D. Ford, J.E. Hesketh, Targeting of transcripts encoding membrane proteins in polarized epithelia: RNA-protein binding studies of the SGLT1 3'-UTR, Biochem Soc Trans 36(Pt 3) (2008) 525-7.

[16] D. Simsek, G.C. Tiu, R.A. Flynn, G.W. Byeon, K. Leppek, A.F. Xu, H.Y. Chang, M. Barna, The Mammalian Ribo-interactome Reveals Ribosome Functional Diversity and Heterogeneity, Cell 169(6) (2017) 1051-1065 e18.

[17] D. Simsek, M. Barna, An emerging role for the ribosome as a nexus for post-translational modifications, Curr Opin Cell Biol 45 (2017) 92-101.

[18] O. Meyuhas, Ribosomal Protein S6 Phosphorylation: Four Decades of Research, Int Rev Cell Mol Biol 320 (2015) 41-73.

[19] Z.A. Knight, K. Tan, K. Birsoy, S. Schmidt, J.L. Garrison, R.W. Wysocki, A. Emiliano, M.I. Ekstrand, J.M. Friedman, Molecular profiling of activated neurons by phosphorylated ribosome capture, Cell 151(5) (2012) 1126-37.

[20] I. Ruvinsky, N. Sharon, T. Lerer, H. Cohen, M. Stolovich-Rain, T. Nir, Y. Dor, P. Zisman, O. Meyuhas, Ribosomal protein S6 phosphorylation is a determinant of cell size and glucose homeostasis, Genes Dev 19(18) (2005) 2199-211.

[21] I. Ruvinsky, M. Katz, A. Dreazen, Y. Gielchinsky, A. Saada, N. Freedman, E. Mishani, G. Zimmerman, J. Kasir, O. Meyuhas, Mice deficient in ribosomal protein S6 phosphorylation suffer from muscle weakness that reflects a growth defect and energy deficit, PLoS One 4(5) (2009) e5618.

[22] Y. Cai, N. Singh, H. Li, Essential role of Ufm1 conjugation in the hematopoietic system, Exp Hematol 44(6) (2016) 442-6.

[23] E.M.C. Hamilton, E. Bertini, L. Kalaydjieva, B. Morar, D. Dojcakova, J. Liu, A. Vanderver, J. Curiel, C.M. Persoon, D. Diodato, L. Pinelli, N.L. van der Meij, B. Plecko, S. Blaser, N.I. Wolf, Q. Waisfisz, T.E.M. Abbink, M.S. van der Knaap, H.A.B.C.R.G. Recessive, UFM1 founder mutation in the Roma population causes recessive variant of H-ABC, Neurology 89(17) (2017) 1821-1828.

[24] C.P. Walczak, D.E. Leto, L. Zhang, C. Riepe, R.Y. Muller, P.A. DaRosa, N.T. Ingolia, J.E. Elias, R.R. Kopito, Ribosomal protein RPL26 is the principal target of UFMylation, Proc Natl Acad Sci U S A 116(4) (2019) 1299-1308.

[25] I. Martin, J.W. Kim, B.D. Lee, H.C. Kang, J.C. Xu, H. Jia, J. Stankowski, M.S. Kim, J. Zhong, M. Kumar, S.A. Andrabi, Y. Xiong, D.W. Dickson, Z.K. Wszolek, A. Pandey, T.M. Dawson, V.L. Dawson, Ribosomal protein s15 phosphorylation

mediates LRRK2 neurodegeneration in Parkinson's disease, Cell 157(2) (2014) 472-485.

[26] A.S. Henderson, D. Warburton, K.C. Atwood, Location of ribosomal DNA in the human chromosome complement, Proc Natl Acad Sci U S A 69(11) (1972) 3394-8.

[27] R.D. Little, D.C. Braaten, Genomic organization of human 5 S rDNA and sequence of one tandem repeat, Genomics 4(3) (1989) 376-83.

[28] D.M. Stults, M.W. Killen, H.H. Pierce, A.J. Pierce, Genomic architecture and inheritance of human ribosomal RNA gene clusters, Genome Res 18(1) (2008) 13-8.

[29] N. Arnheim, E.M. Southern, Heterogeneity of the ribosomal genes in mice and men, Cell 11(2) (1977) 363-70.

[30] M.M. Parks, C.M. Kurylo, R.A. Dass, L. Bojmar, D. Lyden, C.T. Vincent, S.C. Blanchard, Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression, Sci Adv 4(2) (2018) eaao0665.

[31] H. Tseng, W. Chou, J. Wang, X. Zhang, S. Zhang, R.M. Schultz, Mouse ribosomal RNA genes contain multiple differentially regulated variants, PLoS One 3(3) (2008) e1843.

[32] K.J. Waldron, J.C. Rutherford, D. Ford, N.J. Robinson, Metalloproteins and metal sensing, Nature 460(7257) (2009) 823-30.

[33] M.D. Locati, J.F. Pagano, W.A. Ensink, M. van Olst, S. van Leeuwen, U. Nehrdich, K. Zhu, H.P. Spaink, G. Girard, H. Rauwerda, M.J. Jonker, R.J. Dekker, T.M. Breit, Linking maternal and somatic 5S rRNA types with different sequence-specific non-LTR retrotransposons, RNA 23(4) (2017) 446-456.

[34] M.D. Locati, J.F.B. Pagano, G. Girard, W.A. Ensink, M. van Olst, S. van Leeuwen, U. Nehrdich, H.P. Spaink, H. Rauwerda, M.J. Jonker, R.J. Dekker, T.M.

Breit, Expression of distinct maternal and somatic 5.8S, 18S, and 28S rRNA types during zebrafish development, RNA 23(8) (2017) 1188-1199.

[35] I.A. Roundtree, M.E. Evans, T. Pan, C. He, Dynamic RNA Modifications in Gene Expression Regulation, Cell 169(7) (2017) 1187-1200.

[36] K.E. Sloan, A.S. Warda, S. Sharma, K.D. Entian, D.L.J. Lafontaine, M.T. Bohnsack, Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function, RNA Biol 14(9) (2017) 1138-1152.

[37] M. Taoka, Y. Nobe, Y. Yamaki, Y. Yamauchi, H. Ishikawa, N. Takahashi, H. Nakayama, T. Isobe, The complete chemical structure of Saccharomyces cerevisiae rRNA: partial pseudouridylation of U2345 in 25S rRNA by snoRNA snR9, Nucleic Acids Res 44(18) (2016) 8951-8961.

[38] N. Krogh, M.D. Jansson, S.J. Hafner, D. Tehler, U. Birkedal, M. Christensen-Dalsgaard, A.H. Lund, H. Nielsen, Profiling of 2'-O-Me in human rRNA reveals a subset of fractionally modified positions and provides evidence for ribosome heterogeneity, Nucleic Acids Res 44(16) (2016) 7884-95.

[39] M.B. Ferretti, K. Karbstein, Does functional specialization of ribosomes really exist?, RNA 25(5) (2019) 521-538.

[40] Z. Shi, K. Fujii, K.M. Kovary, N.R. Genuth, H.L. Rost, M.N. Teruel, M. Barna, Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide, Mol Cell 67(1) (2017) 71-83 e7.

[41] M. van de Waterbeemd, S. Tamara, K.L. Fort, E. Damoc, V. Franc, P. Bieri, M. Itten, A. Makarov, N. Ban, A.J.R. Heck, Dissecting ribosomal particles throughout the kingdoms of life using advanced hybrid mass spectrometry methods, Nat Commun 9(1) (2018) 2493.

[42] A.M. Anger, J.P. Armache, O. Berninghausen, M. Habeck, M. Subklewe, D.N. Wilson, R. Beckmann, Structures of the human and Drosophila 80S ribosome, Nature 497(7447) (2013) 80-5.

[43] T. Hopes, M. Agapiou, K. Norris, C.G.P. McCarthy, M.J. O'Connell, J. Fontana, J.L. Aspden, (2020).

[44] N. Draptchinskaia, P. Gustavsson, B. Andersson, M. Pettersson, T.N. Willig, I. Dianzani, S. Ball, G. Tchernia, J. Klar, H. Matsson, D. Tentler, N. Mohandas, B. Carlsson, N. Dahl, The gene encoding ribosomal protein S19 is mutated in Diamond-Blackfan anaemia, Nat Genet 21(2) (1999) 169-75.

[45] N. Danilova, H.T. Gazda, Ribosomopathies: how a common root can cause a tree of pathologies, Dis Model Mech 8(9) (2015) 1013-26.

[46] A. Bolze, N. Mahlaoui, M. Byun, B. Turner, N. Trede, S.R. Ellis, A. Abhyankar, Y. Itan, E. Patin, S. Brebner, P. Sackstein, A. Puel, C. Picard, L. Abel, L. Quintana-Murci, S.N. Faust, A.P. Williams, R. Baretto, M. Duddridge, U. Kini, A.J. Pollard, C. Gaud, P. Frange, D. Orbach, J.F. Emile, J.L. Stephan, R. Sorensen, A. Plebani, L. Hammarstrom, M.E. Conley, L. Selleri, J.L. Casanova, Ribosomal protein SA haploinsufficiency in humans with isolated congenital asplenia, Science 340(6135) (2013) 976-8.

[47] C. Zhou, D. Zang, Y. Jin, H. Wu, Z. Liu, J. Du, J. Zhang, Mutation in ribosomal protein L21 underlies hereditary hypotrichosis simplex, Hum Mutat 32(7) (2011) 710-4.

[48] R.B. Thorolfsdottir, G. Sveinbjornsson, P. Sulem, J.B. Nielsen, S. Jonsson, G.H. Halldorsson, P. Melsted, E.V. Ivarsdottir, O.B. Davidsson, R.P. Kristjansson, G. Thorleifsson, A. Helgadottir, S. Gretarsdottir, G. Norddahl, S. Rajamani, B. Torfason, A.S. Valgardsson, J.T. Sverrisson, V. Tragante, O.L. Holmen, F.W. Asselbergs, D.M.

Roden, D. Darbar, T.R. Pedersen, M.S. Sabatine, C.J. Willer, M.L. Lochen, B.V. Halldorsson, I. Jonsdottir, K. Hveem, D.O. Arnar, U. Thorsteinsdottir, D.F. Gudbjartsson, H. Holm, K. Stefansson, Coding variants in RPL3L and MYZAP increase risk of atrial fibrillation, Commun Biol 1 (2018) 68.

[49] A. Yoon, G. Peng, Y. Brandenburger, O. Zollo, W. Xu, E. Rego, D. Ruggero, Impaired control of IRES-mediated translation in X-linked dyskeratosis congenita, Science 312(5775) (2006) 902-6.

[50] C. Bellodi, N. Kopmar, D. Ruggero, Deregulation of oncogene-induced senescence and p53 translational control in X-linked dyskeratosis congenita, EMBO J 29(11) (2010) 1865-76.

[51] C. Bellodi, O. Krasnykh, N. Haynes, M. Theodoropoulou, G. Peng, L. Montanaro, D. Ruggero, Loss of function of the tumor suppressor DKC1 perturbs p27 translation control and contributes to pituitary tumorigenesis, Cancer Res 70(14) (2010) 6026-35.

[52] K.E. Bohnsack, M.T. Bohnsack, Uncovering the assembly pathway of human ribosomes and its emerging links to disease, EMBO J 38(13) (2019) e100278.

[53] I. Boria, E. Garelli, H.T. Gazda, A. Aspesi, P. Quarello, E. Pavesi, D. Ferrante, J.J. Meerpohl, M. Kartal, L. Da Costa, A. Proust, T. Leblanc, M. Simansour, N. Dahl, A.S. Frojmark, D. Pospisilova, R. Cmejla, A.H. Beggs, M.R. Sheen, M. Landowski, C.M. Buros, C.M. Clinton, L.J. Dobson, A. Vlachos, E. Atsidaftos, J.M. Lipton, S.R. Ellis, U. Ramenghi, I. Dianzani, The ribosomal basis of Diamond-Blackfan Anemia: mutation and database update, Hum Mutat 31(12) (2010) 1269-79.

[54] S.R. Ellis, P.E. Gleizes, Diamond Blackfan anemia: ribosomal proteins going rogue, Semin Hematol 48(2) (2011) 89-96.

[55] B.L. Ebert, J. Pretz, J. Bosco, C.Y. Chang, P. Tamayo, N. Galili, A. Raza, D.E. Root, E. Attar, S.R. Ellis, T.R. Golub, Identification of RPS14 as a 5q- syndrome gene by RNA interference screen, Nature 451(7176) (2008) 335-9.

[56] N.A. Paolini, M. Attwood, S.B. Sondalle, C. Vieira, A.M. van Adrichem, F.M. di Summa, M.F. O'Donohue, P.E. Gleizes, S. Rachuri, J.W. Briggs, R. Fischer, P.J. Ratcliffe, M.W. Wlodarski, R.H. Houtkooper, M. von Lindern, T.W. Kuijpers, J.D. Dinman, S.J. Baserga, M.E. Cockman, A.W. MacInnes, A Ribosomopathy Reveals Decoding Defective Ribosomes Driving Human Dysmorphism, Am J Hum Genet 100(3) (2017) 506-522.

[57] B.C. Valdez, D. Henning, R.B. So, J. Dixon, M.J. Dixon, The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor, Proc Natl Acad Sci U S A 101(29) (2004) 10709-14.

[58] J.G. Dauwerse, J. Dixon, S. Seland, C.A. Ruivenkamp, A. van Haeringen, L.H. Hoefsloot, D.J. Peters, A.C. Boers, C. Daumer-Haas, R. Maiwald, C. Zweier, B. Kerr, A.M. Cobo, J.F. Toral, A.J. Hoogeboom, D.R. Lohmann, U. Hehr, M.J. Dixon, M.H. Breuning, D. Wieczorek, Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome, Nat Genet 43(1) (2011) 20-2.

[59] M. Vincent, D. Genevieve, A. Ostertag, S. Marlin, D. Lacombe, D. Martin-Coignard, C. Coubes, A. David, S. Lyonnet, C. Vilain, A. Dieux-Coeslier, S. Manouvrier, B. Isidor, M.L. Jacquemont, S. Julia, V. Layet, S. Naudion, S. Odent, L. Pasquier, S. Pelras, N. Philip, G. Pierquin, F. Prieur, N. Aboussair, T. Attie-Bitach, G. Baujat, P. Blanchet, C. Blanchet, H. Dollfus, B. Doray, E. Schaefer, P. Edery, F. Giuliano, A. Goldenberg, C. Goizet, A. Guichet, C. Herlin, L. Lambert, B. Leheup, J. Martinovic, S. Mercier, C. Mignot, M.L. Moutard, M.J. Perez, L. Pinson, J.

Puechberty, M. Willems, H. Randrianaivo, K. Szakszon, A. Toutain, A. Verloes, J. Vigneron, E. Sanchez, P. Sarda, J.L. Laplanche, C. Collet, Treacher Collins syndrome: a clinical and molecular study based on a large series of patients, Genet Med 18(1) (2016) 49-56.

[60] J. Rainger, H. Bengani, L. Campbell, E. Anderson, K. Sokhi, W. Lam, A. Riess, M. Ansari, S. Smithson, M. Lees, C. Mercer, K. McKenzie, T. Lengfeld, B. Gener Querol, P. Branney, S. McKay, H. Morrison, B. Medina, M. Robertson, J. Kohlhase, C. Gordon, J. Kirk, D. Wieczorek, D.R. Fitzpatrick, Miller (Genee-Wiedemann) syndrome represents a clinically and biochemically distinct subgroup of postaxial acrofacial dysostosis associated with partial deficiency of DHODH, Hum Mol Genet 21(18) (2012) 3969-83.

[61] H. Vega, Q. Waisfisz, M. Gordillo, N. Sakai, I. Yanagihara, M. Yamada, D. van Gosliga, H. Kayserili, C. Xu, K. Ozono, E.W. Jabs, K. Inui, H. Joenje, Roberts syndrome is caused by mutations in ESCO2, a human homolog of yeast ECO1 that is essential for the establishment of sister chromatid cohesion, Nat Genet 37(5) (2005) 468-70.

[62] C. Selmi, C.A. Feghali-Bostwick, A. Lleo, S.A. Lombardi, M. De Santis, F. Cavaciocchi, L. Zammataro, M.M. Mitchell, J.M. Lasalle, T. Medsger, Jr., M.E. Gershwin, X chromosome gene methylation in peripheral lymphocytes from monozygotic twins discordant for scleroderma, Clin Exp Immunol 169(3) (2012) 253-62.

[63] N.S. Heiss, S.W. Knight, T.J. Vulliamy, S.M. Klauck, S. Wiemann, P.J. Mason, A. Poustka, I. Dokal, X-linked dyskeratosis congenita is caused by mutations in a highly conserved gene with putative nucleolar functions, Nat Genet 19(1) (1998) 32-8.

[64] S.W. Knight, T.J. Vulliamy, B. Morgan, K. Devriendt, P.J. Mason, I. Dokal, Identification of novel DKC1 mutations in patients with dyskeratosis congenita: implications for pathophysiology and diagnosis, Hum Genet 108(4) (2001) 299-303.

[65] T.J. Vulliamy, S.W. Knight, P.J. Mason, I. Dokal, Very short telomeres in the peripheral blood of patients with X-linked and autosomal dyskeratosis congenita, Blood Cells Mol Dis 27(2) (2001) 353-7.

[66] T. Vulliamy, R. Beswick, M. Kirwan, A. Marrone, M. Digweed, A. Walne, I. Dokal, Mutations in the telomerase component NHP2 cause the premature ageing syndrome dyskeratosis congenita, Proc Natl Acad Sci U S A 105(23) (2008) 8073-8.

[67] J. Armistead, S. Khatkar, B. Meyer, B.L. Mark, N. Patel, G. Coghlan, R.E. Lamont, S. Liu, J. Wiechert, P.A. Cattini, P. Koetter, K. Wrogemann, C.R. Greenberg, K.D. Entian, T. Zelinski, B. Triggs-Raine, Mutation of a gene essential for ribosome biogenesis, EMG1, causes Bowen-Conradi syndrome, Am J Hum Genet 84(6) (2009) 728-39.

[68] M. Ridanpaa, H. van Eenennaam, K. Pelin, R. Chadwick, C. Johnson, B. Yuan, W. vanVenrooij, G. Pruijn, R. Salmela, S. Rockas, O. Makitie, I. Kaitila, A. de la Chapelle, Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia, Cell 104(2) (2001) 195-203.

[69] E.F. Freed, S.J. Baserga, The C-terminus of Utp4, mutated in childhood cirrhosis, is essential for ribosome biogenesis, Nucleic Acids Res 38(14) (2010) 4798-806.

[70] E.F. Freed, J.L. Prieto, K.L. McCann, B. McStay, S.J. Baserga, NOL11, implicated in the pathogenesis of North American Indian childhood cirrhosis, is required for pre-rRNA transcription and processing, PLoS Genet 8(8) (2012) e1002892.

[71] G.R. Boocock, J.A. Morrison, M. Popovic, N. Richards, L. Ellis, P.R. Durie, J.M. Rommens, Mutations in SBDS are associated with Shwachman-Diamond syndrome, Nat Genet 33(1) (2003) 97-101.

[72] S. Tan, L. Kermasson, A. Hoslin, P. Jaako, A. Faille, A. Acevedo-Arozena, E. Lengline, D. Ranta, M. Poiree, O. Fenneteau, H. Ducou le Pointe, S. Fumagalli, B. Beaupain, P. Nitschke, C. Bole-Feysot, J.P. de Villartay, C. Bellanne-Chantelot, J. Donadieu, C. Kannengiesser, A.J. Warren, P. Revy, EFL1 mutations impair eIF6 release to cause Shwachman-Diamond syndrome, Blood 134(3) (2019) 277-290.

[73] S. Dhanraj, A. Matveev, H. Li, S. Lauhasurayotin, L. Jardine, M. Cada, B. Zlateska, C.S. Tailor, J. Zhou, R. Mendoza-Londono, A. Vincent, P.R. Durie, S.W. Scherer, J.M. Rommens, E. Heon, Y. Dror, Biallelic mutations in DNAJC21 cause Shwachman-Diamond syndrome, Blood 129(11) (2017) 1557-1562.

[74] R. Carapito, M. Konantz, C. Paillard, Z. Miao, A. Pichot, M.S. Leduc, Y. Yang, K.L. Bergstrom, D.H. Mahoney, D.L. Shardy, G. Alsaleh, L. Naegely, A. Kolmer, N. Paul, A. Hanauer, V. Rolli, J.S. Muller, E. Alghisi, L. Sauteur, C. Macquin, A. Morlon, C.S. Sancho, P. Amati-Bonneau, V. Procaccio, A.L. Mosca-Boidron, N. Marle, N. Osmani, O. Lefebvre, J.G. Goetz, S. Unal, N.A. Akarsu, M. Radosavljevic, M.P. Chenard, F. Rialland, A. Grain, M.C. Bene, M. Eveillard, M. Vincent, J. Guy, L. Faivre, C. Thauvin-Robinet, J. Thevenon, K. Myers, M.D. Fleming, A. Shimamura, E. Bottollier-Lemallaz, E. Westhof, C. Lengerke, B. Isidor, S. Bahram, Mutations in signal recognition particle SRP54 cause syndromic neutropenia with Shwachman-Diamond-like features, J Clin Invest 127(11) (2017) 4090-4103.

[75] J. Nousbeck, R. Spiegel, A. Ishida-Yamamoto, M. Indelman, A. Shani-Adir, N. Adir, E. Lipkin, S. Bercovici, D. Geiger, M.A. van Steensel, P.M. Steijlen, R. Bergman, A. Bindereif, M. Choder, S. Shalev, E. Sprecher, Alopecia, neurological

defects, and endocrinopathy syndrome caused by decreased expression of RBM28, a nucleolar protein associated with ribosome biogenesis, Am J Hum Genet 82(5) (2008) 1114-21.

[76] R. Spiegel, S.A. Shalev, A. Adawi, E. Sprecher, Y. Tenenbaum-Rakover, ANE syndrome caused by mutated RBM28 gene: a novel etiology of combined pituitary hormone deficiency, Eur J Endocrinol 162(6) (2010) 1021-5.

[77] A.G. Marneros, BMS1 is mutated in aplasia cutis congenita, PLoS Genet 9(6) (2013) e1003573.

[78] E.M. Jenkinson, M.P. Rodero, P.R. Kasher, C. Uggenti, A. Oojageer, L.C. Goosey, Y. Rose, C.J. Kershaw, J.E. Urquhart, S.G. Williams, S.S. Bhaskar, J. O'Sullivan, G.M. Baerlocher, M. Haubitz, G. Aubert, K.W. Baranano, A.J. Barnicoat, R. Battini, A. Berger, E.M. Blair, J.E. Brunstrom-Hernandez, J.A. Buckard, D.M. Cassiman, R. Caumes, D.M. Cordelli, L.M. De Waele, A.J. Fay, P. Ferreira, N.A. Fletcher, A.E. Fryer, H. Goel, C.A. Hemingway, M. Henneke, I. Hughes, R.J. Jefferson, R. Kumar, L. Lagae, P.G. Landrieu, C.M. Lourenco, T.J. Malpas, S.G. Mehta, I. Metz, S. Naidu, K. Ounap, A. Panzer, P. Prabhakar, G. Quaghebeur, R. Schiffmann, E.H. Sherr, K.R. Sinnathuray, C. Soh, H.S. Stewart, J. Stone, H. Van Esch, C.E. Van Mol, A. Vanderver, E.L. Wakeling, A. Whitney, G.D. Pavitt, S. Griffiths-Jones, G.I. Rice, P. Revy, M.S. van der Knaap, J.H. Livingston, R.T. O'Keefe, Y.J. Crow, Mutations in SNORD118 cause the cerebral microangiopathy leukoencephalopathy with calcifications and cysts, Nat Genet 48(10) (2016) 1185-92.

[79] H. Tummala, A.J. Walne, M. Williams, N. Bockett, L. Collopy, S. Cardoso, A. Ellison, R. Wynn, T. Leblanc, J. Fitzgibbon, D.P. Kelsell, D.A. van Heel, E. Payne, V. Plagnol, I. Dokal, T. Vulliamy, DNAJC21 Mutations Link a Cancer-Prone Bone

Marrow Failure Syndrome to Corruption in 60S Ribosome Subunit Maturation, Am J Hum Genet 99(1) (2016) 115-24.

[80] G. Zanni, V.M. Kalscheuer, A. Friedrich, S. Barresi, P. Alfieri, M. Di Capua, S.A. Haas, G. Piccini, T. Karl, S.M. Klauck, E. Bellacchio, F. Emma, M. Cappa, E. Bertini, L. Breitenbach-Koller, A Novel Mutation in RPL10 (Ribosomal Protein L10) Causes X-Linked Intellectual Disability, Cerebellar Hypoplasia, and Spondylo-Epiphyseal Dysplasia, Hum Mutat 36(12) (2015) 1155-8.

[81] S.J. Marygold, C.M. Coelho, S.J. Leevers, Genetic analysis of RpL38 and RpL5, two minute genes located in the centric heterochromatin of chromosome 2 of Drosophila melanogaster, Genetics 169(2) (2005) 683-95.

[82] N. Kondrashov, A. Pusic, C.R. Stumpf, K. Shimizu, A.C. Hsieh, J. Ishijima, T. Shiroishi, M. Barna, Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning, Cell 145(3) (2011) 383-397.

[83] L. Perucho, A. Artero-Castro, S. Guerrero, S. Ramon y Cajal, L.L. ME, Z.Q. Wang, RPLP1, a crucial ribosomal protein for embryonic development of the nervous system, PLoS One 9(6) (2014) e99956.

[84] L.G. Sloofman, K. Verdelis, L. Spevak, M. Zayzafoon, M. Yamauchi, L.M. Opdenaker, M.C. Farach-Carson, A.L. Boskey, C.B. Kirn-Safran, Effect of HIP/ribosomal protein L29 deficiency on mineral properties of murine bones and teeth, Bone 47(1) (2010) 93-101.

[85] X. Wu, J.H. Bayle, D. Olson, A.J. Levine, The p53-mdm-2 autoregulatory feedback loop, Genes Dev 7(7A) (1993) 1126-32.

[86] S. Bursac, M.C. Brdovcak, M. Pfannkuchen, I. Orsolic, L. Golomb, Y. Zhu, C. Katz, L. Daftuar, K. Grabusic, I. Vukelic, V. Filic, M. Oren, C. Prives, S. Volarevic, Mutual protection of ribosomal proteins L5 and L11 from degradation is essential for

p53 activation upon ribosomal biogenesis stress, Proc Natl Acad Sci U S A 109(50) (2012) 20467-72.

[87] G. Donati, S. Peddigari, C.A. Mercer, G. Thomas, 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint, Cell Rep 4(1) (2013) 87-98.

[88] K.E. Sloan, M.T. Bohnsack, N.J. Watkins, The 5S RNP couples p53 homeostasis to ribosome biogenesis and nucleolar stress, Cell Rep 5(1) (2013) 237-47.

[89] E.W. Mills, R. Green, Ribosomopathies: There's strength in numbers, Science 358(6363) (2017).

[90] E. Emmott, M. Jovanovic, N. Slavov, Ribosome Stoichiometry: From Form to Function, Trends Biochem Sci 44(2) (2019) 95-109.

[91] N.C. Jones, M.L. Lynn, K. Gaudenz, D. Sakai, K. Aoto, J.P. Rey, E.F. Glynn, L. Ellington, C. Du, J. Dixon, M.J. Dixon, P.A. Trainor, Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function, Nat Med 14(2) (2008) 125-33.

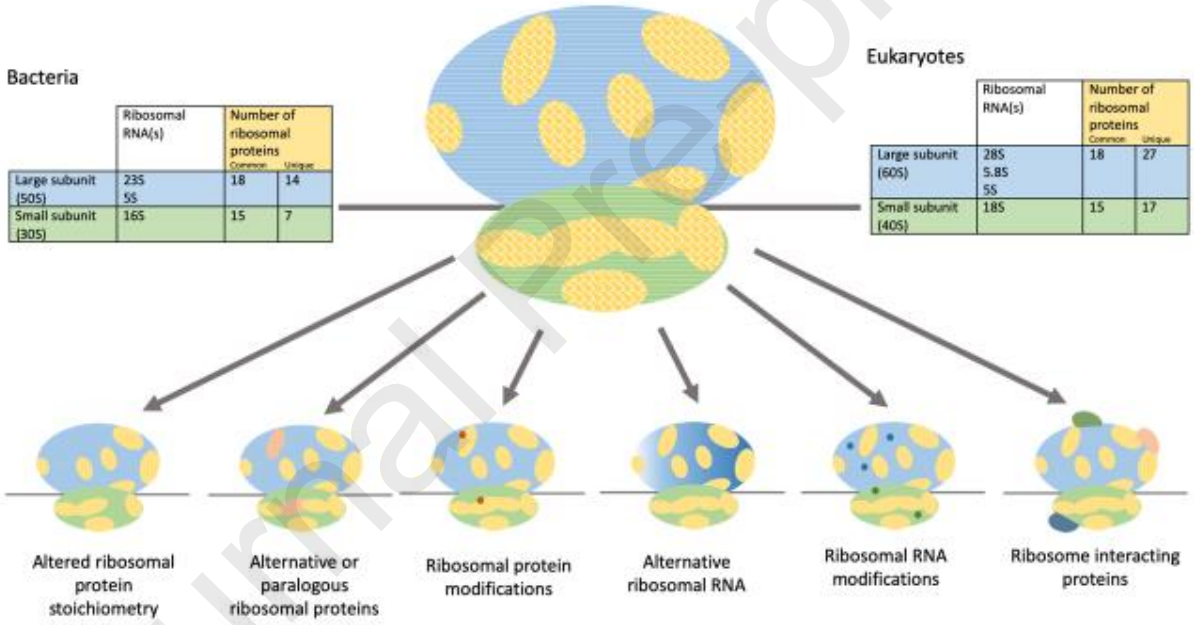
[92] J.L. Barlow, L.F. Drynan, N.L. Trim, W.N. Erber, A.J. Warren, A.N. McKenzie, New insights into 5q- syndrome as a ribosomopathy, Cell Cycle 9(21) (2010) 4286-93.

[93] M. Haas, V. Vlcek, P. Balabanov, T. Salmonson, S. Bakchine, G. Markey, M. Weise, G. Schlosser-Weber, H. Brohmann, C.P. Yerro, M.R. Mendizabal, V. Stoyanova-Beninska, H.L. Hillege, European Medicines Agency review of ataluren for the treatment of ambulant patients aged 5 years and older with Duchenne muscular dystrophy resulting from a nonsense mutation in the dystrophin gene, Neuromuscul Disord 25(1) (2015) 5-13.

[94] R. Ferreira, J.S. Schneekloth, Jr., K.I. Panov, K.M. Hannan, R.D. Hannan, Targeting the RNA Polymerase I Transcription for Cancer Therapy Comes of Age, Cells 9(2) (2020).

[95] J.W. Yewdell, C.V. Nicchitta, The DRiP hypothesis decennial: support, controversy, refinement and extension, Trends Immunol 27(8) (2006) 368-73.

[96] S.O. Sulima, I.J.F. Hofman, K. De Keersmaecker, J.D. Dinman, How Ribosomes Translate Cancer, Cancer Discov 7(10) (2017) 1069-1087.



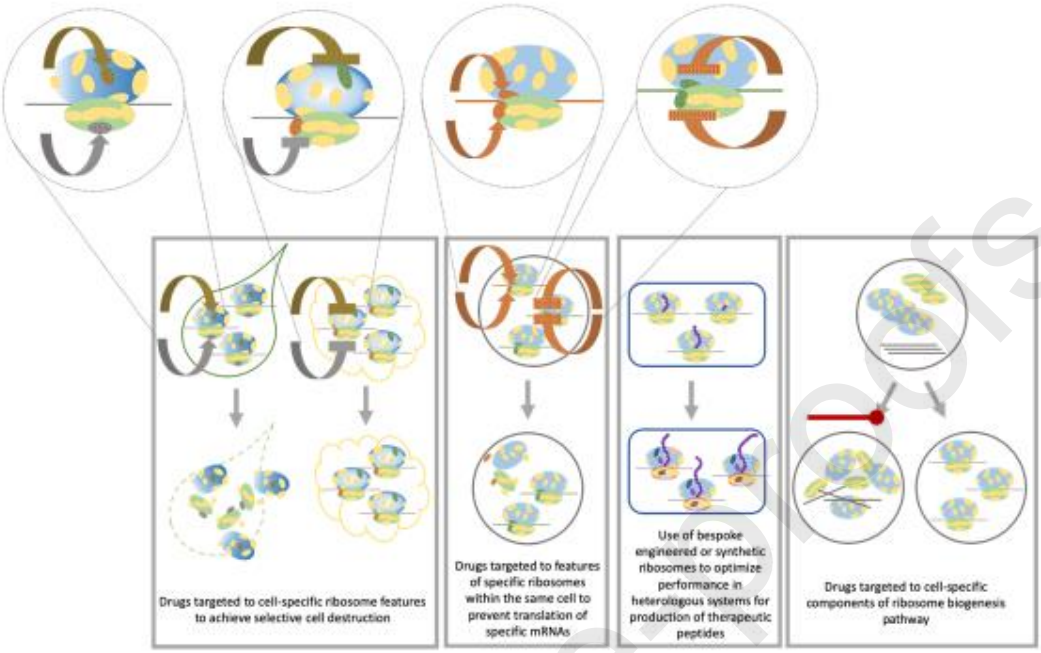
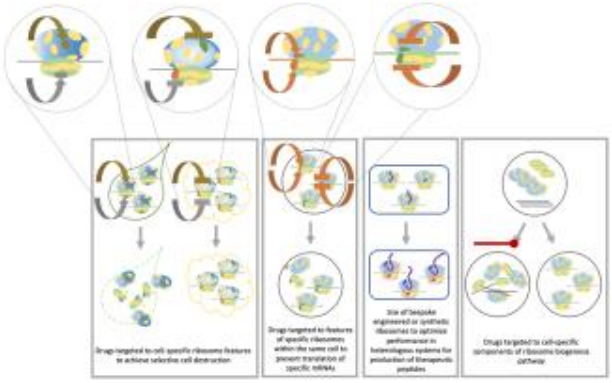


Table 1. Ribosomopathies: diseases caused by defects in ribosomal proteins or in components of the ribosome biogenesis pathway. Adapted from [52].

Disease	OMIM	Affected gene(s)*	Clinical features
Diamond-Blackfan anaemia [53, 54]	105650	*RPL5, RPL11, RPL27, RPL35A, RPS7, RPS10, RPS17, RPS19, RPS24, RPS26, RPS27, RPS28, GATA1, TSR2*	anaemia, cataracts, cleft palate, glaucoma, hypertelorism, malformed or absent thumbs, microcephaly, micrognathia, ptosis, short, webbed neck, strabismus
5q-myelodysplastic Syndrome [55]	153550	*RPS14*	anaemia, dysmegakaryopoiesis, thrombocytosis
isolated congenital asplenia [46]	271400	*RPSA*	absence of spleen, immunodeficiency
RPS23-related Ribosomopathy [56]	617412	*RPS23*	autism spectrum disorder, epicanthic folds in eyes, extra front teeth, facial asymmetry, foetal finger pads, hearing loss, high palate, intellectual disability, low back hairline, microcephaly, simian palmar creases,
Treacher Collins syndrome [57-59]	154500, 613717, 248390	*TCOF1 POLR1C POLR1D*	cleft palate, craniofacial defects, hearing loss, micrognathia,microtia, midface hypoplasia
postaxial acrofacial dysostosis (POADS) [60]	263750	*DHODH*	craniofacial defects, postaxial limb deformities
Roberts syndrome [61]	268300	*ESCO2*	craniofacial defects, limb malformations, prenatal growth retardation
Scleroderma [62]	181750	*UTP14A*	hardened/thickened skin, muscle weakness, ulcers/sores, swollen joints, fingers or toes,
dyskeratosis congenita [63-66]	305000	*DKC1 TERC TERT NOP10 NHP2 TINF2*	bone marrow failure, immunodeficiency, mucocutaneous abnormalities, pulmonary fibrosis
Bowen-Conradi syndrome [67]	211180	*EMG1*	camptodactyly, growth retardation, microcephaly, facial deformities, joint abnormalities, micrognathia, psychomotor delay, rockerbottom feet
cartilage-hair hypoplasia [68]	250250	*RMRP*	Bone deformities, hair growth abnormalities, short stature
North American Indian childhood cirrhosis [69, 70]	604901	*UTP4 NOL11*	biliary cirrhosis, portal hypertension
Shwachman–Diamond Syndrome [71-74]	260400	*SBDS EFL1 DNAJC21 SRP54*	bone marrow dysfunction, exocrine pancreatic insufficiency, leukaemia, skeletal abnormalities
alopecia, neurological and endocrinopathy syndrome (ANE) [75, 76]	612079	*RBM28*	alopecia, endocrinopathy, neurological defects
aplasia cutis congenital [77]	107600	*BMS1*	skin (especially scalp) defects
leukoencephalopathy, intercranial calcifications and cysts [78]	614561	*SNORD118*	leukoencephalopathy, intercranial calcifications and cysts
cancer-prone bone marrow failure syndrome [79]	617052	*DNAJC21*	Acute myeloid leukaemia, bone marrow failure, decreased bone density, microcephaly, short stature
X-linked intellectual disability, cerebellar hypoplasia and	300847	*RPL10*	cerebellar hypoplasia , intellectual disability, spondyloepiphyseal dysplasia

spondyloepiphyseal dysplasia [80]	

*The prefix RP denotes ribosomal protein genes. Other genes listed are involved in ribosome biogenesis. The prefix POL denotes RNA polymerase genes.



The idea for the article was developed solely by the named author, Dianne Ford, who was also responsible for the preparation of all text, tables and figures.