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Review

Ribosomopathies: Old Concepts, New Controversies

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Ribosomopathies are a diverse subset of diseases caused by reduced expression of, or mutations in, factors necessary for making ribosomes, the protein translation machinery in the cell. Despite the ubiquitous need for ribosomes in all cell types, ribosomopathies manifest with tissue-specific defects and sometimes increased cancer susceptibility, but few treatments target the underlying cause. By highlighting new research in the field, we review current hypotheses for the basis of this tissue specificity. Based on new work, we broaden our understanding of the role of ribosome biogenesis in diverse tissue types throughout embryonic development. We also pose the question of whether previously described human conditions such as aging can be at least partially attributed to defects in making ribosomes.

Highlights

Ribosomopathies are disorders of making ribosomes that manifest in afflicted humans with tissue-specificity.

Several new ribosomopathies have recently been discovered, helping to reveal the link between ribosome biogenesis and human development.

The specialized ribosomes hypothesis states that the tissue-specific defects of ribosomopathies are due to ribosome heterogeneity caused by changes in ribosomal protein composition or modification, rRNA composition or modification, or accessory protein binding.

The ribosome concentration hypothesis states that the tissue-specific defects of ribosomopathies are due to changes in mRNA translation as a consequence of reduced ribosome number.

Dysregulation of nucleolar activity is associated with aging and neurodegeneration, suggesting that these disorders may perhaps also be ribosomopathies.

Human Diseases of Making Ribosomes – The Ribosomopathies

Ribosomopathies are a diverse subset of largely developmental disorders that result from aberrant ribosome production. Ribosome synthesis is an essential and energy-intensive cellular process that requires the coordination of all three RNA polymerases, ~200 accessory factors, and 80 ribosomal proteins (r-proteins) to process and assemble the mature ribosomal RNAs (rRNAs) [1]. In humans, the bulk of ribosome biogenesis initiates in the nucleolus with transcription of the 47S pre-rRNA from ribosomal DNA (rDNA) loci by RNA polymerase I (RNAPI). Following transcription, the pre-rRNA is processed by a series of endo- and exonucleolytic steps to yield three of the four mature rRNAs (18S, 5.8S, and 28S) [2]. The rRNAs are also modified by box C/D and H/ACA small nucleolar ribonucleoproteins (snoRNPs), and assembled with r-proteins and the fourth rRNA (5S), transcribed by RNA polymerase III from an extranucleolar locus [2]. Together, these steps yield the mature small subunit (SSU, 40S) and the large subunit (LSU, 60S) of the ribosome that come together in the cytoplasm to translate mRNA into proteins.

When it was first discovered that difficulties in making ribosomes could lead to the bone marrow failure syndrome, Diamond–Blackfan anemia (DBA) [3], it was unclear how a defect in the ubiquitous process of making ribosomes could lead to a tissue-specific disorder. Since then, new ribosomopathies have been identified, each with tissue-specific manifestations.

There have been several recent comprehensive reviews on ribosomopathies [4–8], and we therefore discuss only the new and controversial aspects of their pathogenesis. First, we highlight new ribosomopathies based on which step of ribosome biogenesis is affected. Second, we discuss the tissue-specific manifestations of the ribosomopathies, and present a hypothesis defining how defects in the global process of making ribosomes may only affect some tissues. Finally, we pose key open questions and discuss current controversies surrounding ribosomopathies, such as (i) do specialized ribosomes influence the pathogenesis of ribosomopathies? (ii) How can defects in cell growth cause cancer? (iii) Are aging and neurodegenerative diseases also ribosomopathies? Although there is currently no single unifying principle that explains the pathogenesis of all known ribosomopathies, substantial progress has been made in recognizing

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the source of these diseases and in gaining mechanistic insights into their pathogenesis. The hope is that in the future we may see the development of novel therapeutic options.

Highlighting the Molecular Pathology of Ribosomopathies

The diversity of clinical presentations of ribosomopathies makes them difficult to unify. Although all ribosomopathies share defects in ribosome production, not all are caused by defects in the same step in the process. We classify ribosomopathies here by the step at which ribosome production is affected. Based on current knowledge, there are ribosomopathies that result from defects in (i) pre-rRNA transcription and modification, (ii) pre-rRNA processing, and (iii) ribosome assembly (Figure 1). Highlights from the past 5 years are discussed below.

Pre-rRNA Transcription and Modification

Multiple ribosomopathies are caused by proteins implicated in pre-rRNA transcription and modification, including the well-studied mandibulofacial dysostosis Treacher Collins syndrome (TCS). TCS is caused by mutations in treacle (TCOF1) and the RNAPI subunits, POLR1C and POLR1D [9–12]. In depletion and knockout models of TCOF1, a nearly 50% reduction in transcription of the 47S transcript was observed, suggesting a subsequent reduction in overall ribosome numbers [13]. TCOF1 also interacts with a core component of the box C/D snoRNP, NOP56, and studies in animal models reveal an effect of TCOF1 on 2′-*O*-methylation of the pre-18S rRNA [14].

In addition to TCS, two newly described ribosomopathies also result from defects in RNAPI transcription of rDNA. First, compound heterozygous mutations in *TAF1A*, encoding an RNAPI-associated factor, were reported in two sisters who presented with end-stage dilated

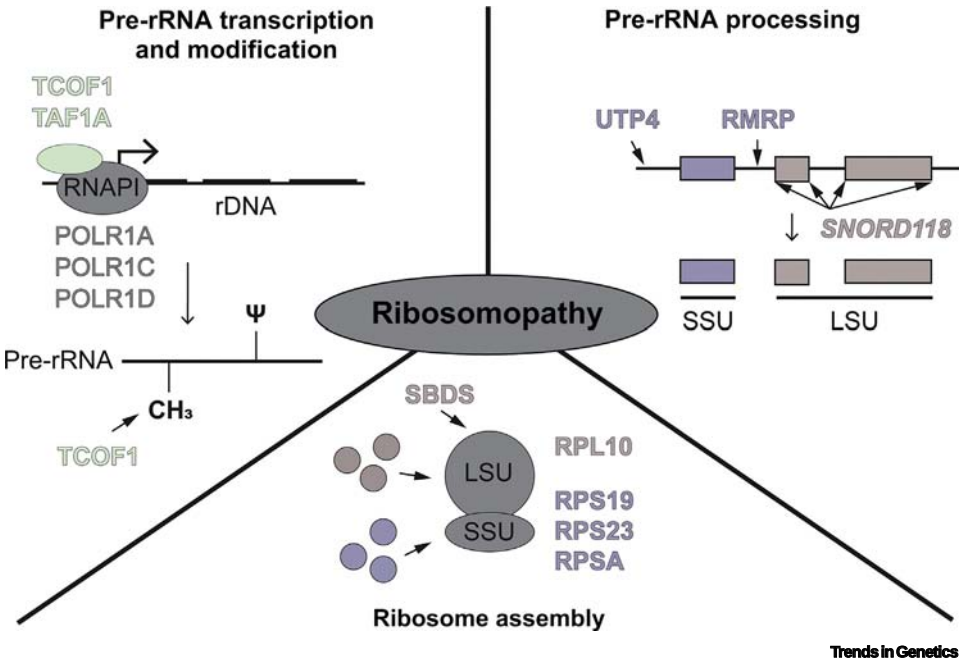

Figure 1. How Ribosomopathies Affect Ribosome Production. Ribosomopathies can affect: (top left) pre-ribosomal RNA (pre-rRNA) transcription and modification including pseudouridylation (ψ) and 2′-*O*-methylation (CH_3), (top right) pre-rRNA processing, and (bottom) ribosome assembly. Proteins named in each section are highlighted in this review, including RNA polymerase I (RNAPI) subunits (grey), RNAPI-associated factors (green), small subunit (SSU) components and factors (purple), and large subunit (LSU) components and factors (red).

cardiomyopathy. The cardiac phenotype was recapitulated in a zebrafish *taf1a* knockout model [15]. However, biochemical follow-up was not performed [15]. Second, mutations in the gene encoding the largest RNAPI subunit, *POLR1A*, cause acrofacial dysostosis, Cincinnati type, resulting in craniofacial abnormalities in both humans and zebrafish [16]. In this case levels of pre-rRNA were determined by qPCR, which revealed a significant reduction in pre-rRNA levels in *polr1a* mutants, again suggesting an associated reduction in overall ribosome numbers and a role for ribosome biogenesis in the pathogenesis of this disease.

Pre-rRNA Processing

Ribosomopathies can also arise from defects in processing of the 47S pre-rRNA into mature rRNAs. Such disorders include North American Indian childhood cirrhosis caused by a mutation in the gene encoding the SSU processome component *UTP4* (Cirhin) [17,18] and cartilage hair hypoplasia (CHH) caused by mutations in *RMRP*, which encodes the RNA component of the MRP endoribonuclease complex [19]. In addition, a new disorder caused by mutations in the U8 snoRNA has recently been described. Although typically involved in rRNA modification, a small number of snoRNPs are required for pre-rRNA cleavage events. Among these is the U8 snoRNP, that is required for maturation of the 5.8S and 28S LSU rRNAs, likely by guiding proper pre-rRNA folding and recruiting the necessary cleavage factors [20,21]. Mutations in the U8 snoRNA (transcribed from the *SNORD118* locus) were originally described in 40 patients who presented with leukoencephalopathy with calcifications and cysts (LCC or Labrune syndrome [22]) in the brain. Since then there have been several reports of additional patients with LCC who have mutations in the U8 snoRNA [23–26], providing additional support that mutations in U8 are the root cause of LCC pathogenesis. Biochemical experiments support an underlying defect in the function of the mutated U8 snoRNAs [22], but these mutations have yet to be introduced into an animal model for direct verification of their centrality.

Ribosome Assembly

Finally, there are also several ribosomopathies that result from mutations in large and small subunit r-proteins and assembly factors that affect the number or proportion of functional ribosomes. Examples of these include DBA and Shwachman–Diamond syndrome (SDS). In DBA, mutations in one of 18 r-proteins that result in haploinsufficiency have been implicated in causing the disease. Although often ribosome composition remains unaffected, overall ribosome levels are reduced [27]. In SDS, on the other hand, disease is caused by mutations in *SBDS* in a majority of patients, which leads to a failure in the cytoplasmic maturation of the LSU and prevents LSU and SSU joining, reducing the overall number of translationally competent ribosomes [4].

Mutations in the r-protein gene, *RPL10* (*uL16*), were first discovered to be associated with autism [28,29]. More recently, however, mutations in *RPL10* (K78E) have also been shown to cause X-linked microcephaly, intellectual disability, and seizures [30,31]. When the latter was modeled in zebrafish, Rpl10 depletion caused a reduction in head size and increased apoptosis as assayed by TUNEL (terminal deoxynucleotidyl transferase dUTP nick end labeling) staining – defects that could be rescued by expression of the human mRNA and autism-associated mutations, but not with mRNA that carried the K78E mutation, highlighting the importance of studying these orthologous mutations in animal models [30].

In addition, two autosomal dominant ribosomopathies were recently described that are likely caused by r-protein haploinsufficiency. Two unrelated children presented with overlapping symptomatology including brachycephaly, trichomegaly, and developmental delay (BTDD). Whole-exome sequencing revealed amino acid changes in RPS23 (uS12) [32]. Each child

bears a variant of *RPS23* (R67K or F120I) in one *RPS23* allele. When tested for function in *Saccharomyces cerevisiae*, the orthologous R67K mutation resulted in slower growth, smaller colony size, and decreased translational fidelity, indicating that the mutation is deleterious. Studies in patient fibroblasts also revealed reduced translational fidelity. Likewise, mutations in *RPSA* (*uS2*) cause isolated congenital asplenia (ICA) [33,34]. Knockdown of *Rpsa* in *Xenopus tropicalis* revealed impaired spleen development and pre-rRNA processing defects, and the ICA-causing mutation was unable to rescue either defect [35]. Interestingly, however, the heterozygous null mouse ($Rpsa^{+/-}$) did not demonstrate ICA [33]. The plethora of these newly identified ribosomopathies, with more likely to come, will continue to spark insight into the role of ribosome biogenesis in embryonic development and disease.

However, it is important to note that, although most of the ribosomopathies discussed here may be traced to a defect in a particular aspect of ribosome biogenesis, the disease process may also include additional defective steps in making ribosomes.

Furthermore, it is possible that the ribosome biogenesis factors implicated may also influence extraribosomal cellular functions [36,37]. The potential contributions of these alternative functions to disease pathogenesis remain to be elucidated. Thus, to define ribosomopathies based solely on the step at which ribosome biogenesis is affected, although convenient for this discussion, may not fully encapsulate the complexity of ribosomopathies as we currently understand them. Further studies, particularly in animal models, will be necessary to gain a more comprehensive understanding of how failure in ribosome production contributes to the natural history of each condition.

Hints at the Basis for the Tissue Specificity of Ribosomopathies

The ribosomopathies manifest as diverse disorders, each with a tissue-specific clinical presentation. Despite the requirement for ribosome function in all cell types, disruptions in the process of making ribosomes often affect the development of specific tissues. We attempt here to synthesize the expansive list of affected tissues by dividing them into ribosomopathies that affect the tissues derived from the neural crest, and ribosomopathies that affect non-neural crest derived tissues. The mechanisms dictating how a specific mutation in a protein required for this ubiquitous process can affect only particular tissues is an active area of investigation.

Ribosomopathies Derived from Hindered Development of Neural Crest Cells

It has been known for some time that there is a strong link between neural crest development and ribosome biogenesis [38]. Neural crest cells in the developing embryo are multipotent migratory cells that differentiate into numerous cell and tissue types including the teeth, peripheral nervous system and glia, heart, pigment cells of the skin, and head skeletal structures. Multiple ribosomopathies present with defective tissues derived from neural crest cells during embryogenesis (Figure 2). For example, heart defects can be found in multiple ribosomopathies including DBA [39], the RPL10 ribosomopathy [31], SDS [40], and non-ischemic dilated cardiomyopathy caused by *TAF1A* mutations [15]. In addition, skin and pigmentation defects can be found in aplasia cutis congenita [41], dyskeratosis congenita [42–45], DBA (mouse model) [46], alopecia, neurologic defects, and endocrinopathy (ANE) syndrome [47], a ribosomopathy arising from mutations in *DNAJC21* similar to SDS [48,49], and the Rps23/uS12 ribosomopathy [32].

The best studied neural crest-derived ribosomopathies affect development of the face. Craniofacial development is affected in several ribosomopathies including TCS (reviewed in [50]), DBA [51], and acrofacial dysostosis, Cincinnati type [16] (Table 1). Further linking development of the face and

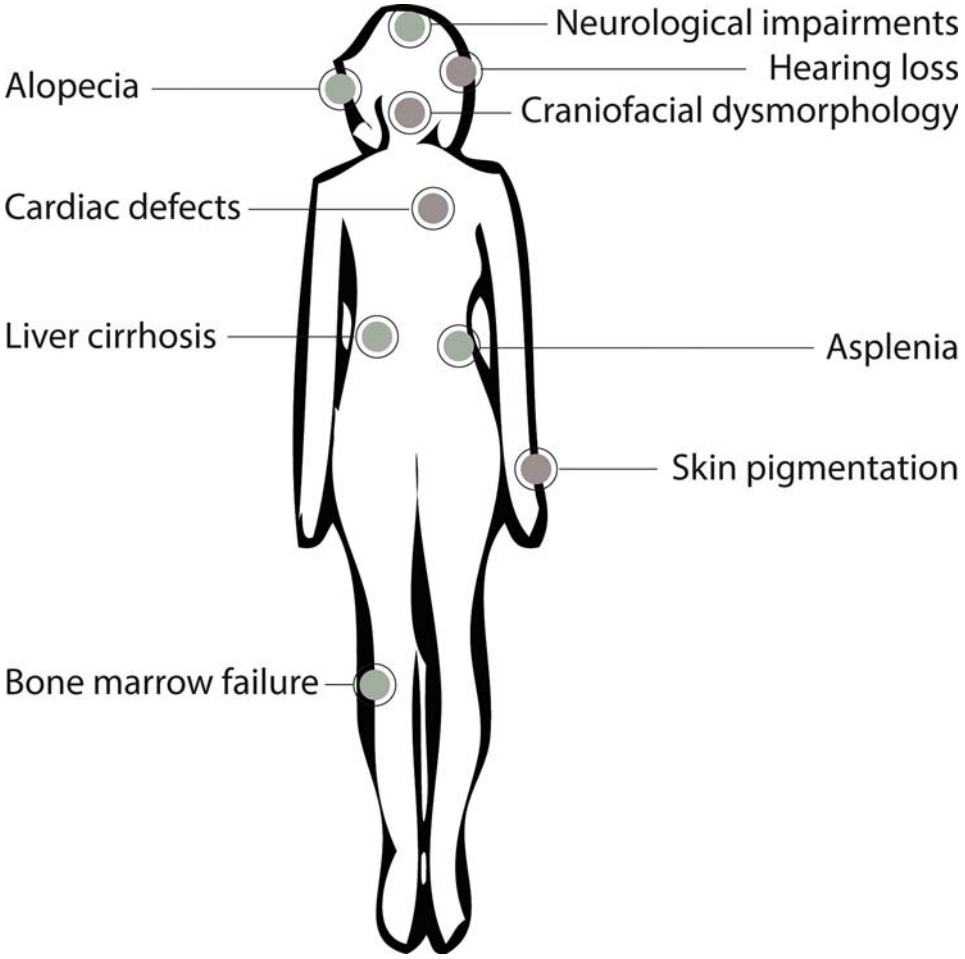

Figure 2. The Clinical Manifestations of Ribosomopathies. The diverse pathologies associated with ribosomopathies are depicted. These pathologies arise from neural crest cell (red) and non-neural crest cell lineages (green).

ribosomopathies, several ribosome biogenesis factors that have not been associated with human disease cause craniofacial defects in model organisms (Table 1). In addition, ear development is influenced by neural crest cells [52], and multiple ribosomopathies manifest in hearing loss. Hearing loss is usually seen in ribosomopathies that affect the development of the face and head as a whole, such as TCS [53], the RPS23/uS12 ribosomopathy [32], the RPS10 ribosomopathy [30], DBA [54], a ribosomopathy resulting from mutations in *DNAJC21* [48], and acrofacial dysostosis, Cincinnati type [16]. Therefore, aberrant ribosome biogenesis often results in defects manifested through neural crest-derived tissues.

p53-Mediated Nucleolar Stress Response

Some, but not all, of the disease manifestations of ribosomopathies have been attributed to the increased sensitivity of the affected neural crest progenitor cells to the stabilization of the proapoptotic factor p53. This occurs in response to dysfunctional ribosome biogenesis during a window of development [7]. Supporting this idea, the clinical manifestations of several ribosomopathies can be rescued by codepletion of p53 [55–59]. However, not all phenotypes can be rescued this way, suggesting that not all of the signs and symptoms associated with ribosomopathies are mediated by p53 [4,56,60–64]. In addition, other stress response pathways

Table 1. Genes with Roles in Both Ribosome Biogenesis and Craniofacial Development

Gene name	Function in ribosome biogenesis	Defects in craniofacial development	Name of human disease[a]	Refs
DDX11	rDNA transcription	Long faces, narrow eyes, low mouths	Warsaw breakage syndrome (WABS)	[126,135,136]
DDX21	rDNA binding, transcription of r-proteins	Hypoplasia of mandible/zygomatic complex	N/a; may be linked to Treacher Collins syndrome	[57]
ESF1	Pre-rRNA processing	Jaw malformations, microcephaly	N/a	[137]
NOL11	rDNA transcription and pre-rRNA processing	Microcephaly, reduced size of pharyngeal cartilages	N/a	[58]
PAK1IP1	Pre-rRNA processing	Midline facial cleft	N/a	[138,139]
POLR1A	rDNA transcription	Range of mandibulofacial dystoses including downslanting palpebral fissures, eyelid clefts, and micrognathia	Acrofacial dystosis, Cincinnati type	[16]
RPL38/eL38	Large subunit ribosomal protein	Midline facial cleft, cleft palate	N/a	[78]
RPS19/eS19, *RPL5/uL18*, *RPL11/uL5*, *RPL35a/eL33*, *RPS26/eS26*, *RPS24/eS24*, *RPS17/eS17*, *RPS7/eS7*, *RPS10/eS10*, *RPL19/eL19*, *RPL26/uL24*, *RPS29/uS14*, *RPL31/eL31*, *RPS28/eS28*, *RPS20/uS10*, *RPL15/eL15*, *RPL17/uL22*, *GATA1*, *TSR2*	Mainly ribosomal proteins	Cleft lip, cleft palate, flat nasal bridge, hypertelorism	Diamond–Blackfan anemia	[51]
TCOF1, *POLR1C*, *POLR1D*	rDNA transcription	Hypoplasia of mandible/zygomatic complex, some dental anomalies, cleft palate	Treacher–Collins syndrome	[13,140,141]
WDR43	rDNA transcription	Reduced size of pharyngeal cartilages, hydrocephaly	Linked to Miller, McKusick, and Malvaux (3-M) syndrome	[59]

[a]N.a., not applicable.

in various affected and unaffected tissues have yet to be fully explored [65]. In the future, it would be pertinent to examine the sensitivity of various cell types to p53 throughout development so as to untangle some of the questions surrounding the cell type specificity of ribosomopathies.

Ribosomopathies Derived from Hindered Development of Non-Neural Crest Cells

Some ribosomopathies affect tissues that are not derived from the neural crest (Figure 2). For example, bone marrow failure and anemia are present in SDS (also characterized by neutropenia [66]), DBA [51], 5q– syndrome [67], dyskeratosis congenita [68,69], and a newly described ribosomopathy arising from mutations in *DNAJC21* [49]. In addition, hair development is affected in ANE syndrome [47], CHH [19], the RPS23/uS12 ribosomopathy [32], and a hair-loss disorder (hereditary hypotrichosis simplex) caused by mutations in *RPL21* (*eL21)* [70]. Because both the bone marrow and hair develop from continuously dividing cells, it is possible that these tissues have an increased reliance on making ribosomes for their growth. However, neither North American Indian childhood cirrhosis, which affects liver function [71], nor ICA, which affects spleen development [33], fit this model. Furthermore, ribosomopathies often present with neurodevelopmental defects such as microcephaly and intellectual disability [22,25,30–32,47,72]. Rescue experiments via co-knockout of *Tp53* in animal models have succeeded for some phenotypes in a subset of these disorders, suggesting that some non-neural crest derived cell types also

have an increased dependence on p53 [61–64]. Additional studies of animal models of these developmental disorders will therefore be necessary to parse out the mechanisms of these tissue-specific clinical manifestations.

Do Specialized Ribosomes Influence the Pathogenesis of Ribosomopathies?

One explanation for how ribosomopathies can manifest in tissue-specific defects is that different tissues comprise ribosomes with distinct compositions that endow them with 'specialized' functions in translating specific mRNAs. The specialized ribosome hypothesis is as old as the discovery of ribosomes themselves; Francis Crick postulated that each individual gene had a single ribosome responsible for its translation (often referred to as the one gene–one ribosome–one protein hypothesis) [73]. Ribosome heterogeneity would be predicted to occur through a multitude of mechanisms including changes in rRNA sequence, core r-protein composition, rRNA or protein modifications, or binding of accessory proteins (Figure 3; reviewed in [74]). These subtle changes could thus lead to altered translation of specific mRNAs required for the proper development of some cell types but not others.

Despite enthusiasm for finding specialized ribosomes, their existence remains controversial. This is because a high standard must be applied to demonstrate not only that there is variation in ribosome composition but also that such variation results in functional differences in protein synthesis. Evidence supporting the specialized ribosome hypothesis has been thoroughly reviewed elsewhere [74–76], and we therefore only highlight a few of the most controversial and recent developments in the field.

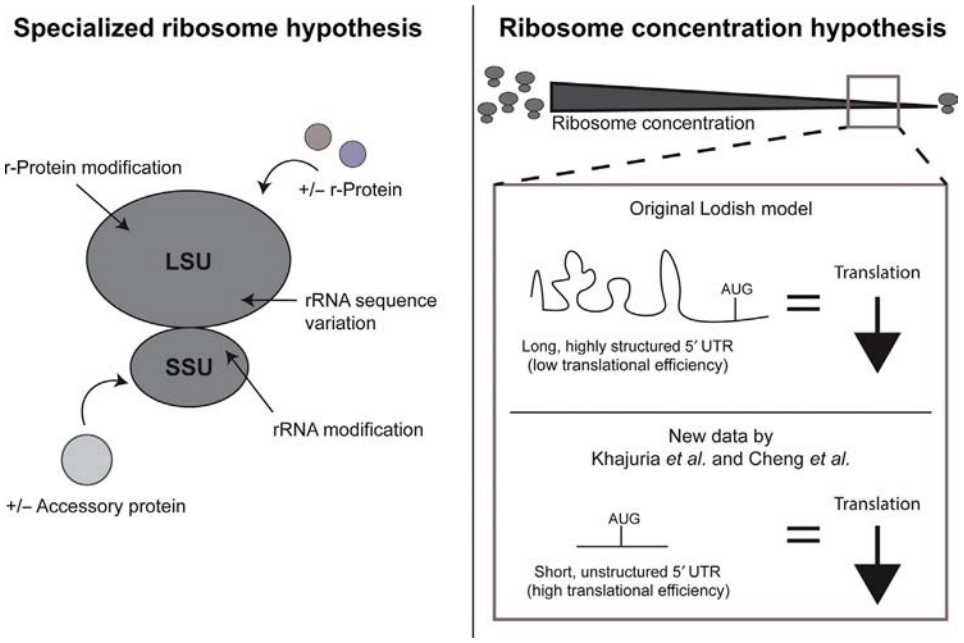

Figure 3. Two Hypotheses for the Tissue-Specific Effects of Ribosomopathies. (Left) The specialized ribosome hypothesis states that modification or changes in rRNA sequence, core ribosomal protein (r-protein) composition, or accessory proteins in either the large subunit (LSU) or small subunit (SSU) of the ribosome can result in heterogeneous ribosomes with differential translation abilities in diverse tissues. (Right) The ribosome concentration hypothesis states that decreased ribosome concentration results in decreased protein levels for specific mRNAs. At low ribosome concentrations, the original Lodish model [81] proposed that mRNAs with long, highly structured 5′-untranslated regions (5′-UTRs) would be most affected by changes in ribosome concentration (above), whereas new data from Khajuria *et al.* [27] and Cheng *et al.* [84] propose the opposite (below).

Ribosomal Proteins as the Basis for Ribosome Heterogeneity

Because the ribosome is composed of rRNAs and r-proteins, one place to search for specialization is in the r-protein composition of the ribosomes themselves. Seminal work in support of the specialized ribosome hypothesis comes from investigating the role of RPL38 (eL38) in murine development [77,78]. It was previously shown that the skeletal patterning defects observed in tail-short (*Ts*) mice are due to mutations in RPL38 that affect its role in the translation of specific *Hox* gene mRNAs [77,78]. However, ribosomes lacking RPL38 in different tissues were not directly measured [77,78]. Another recent example in support of specialized ribosomes examined the role of RPL10A (uL1) in binding to various mRNAs [79]. Although the authors did find that ribosomes containing RPL10A bound a different set of mRNAs than those without [79], these studies utilized tagged r-proteins, which can affect their function (reviewed in [74]). Especially notable for these experiments is that control experiments employed a different tag altogether (HA-RPL22/eL22) than the experimental group (FLAG-RPL10A) [79]. Finally, recent work in yeast has shown that differential mRNA translation occurs in ribosomes with and without Rps26 (eS26), an essential r-protein that is mutated in DBA [80]. Because Rps26 is essential, it is puzzling that ribosomes could be made at all when Rps26 was depleted. Perhaps its essential role is not in ribosome assembly but only in ribosome function. Clearly, ribosomes appear to be more heterogeneous than was previously thought.

That there are specialized ribosomes is both an old and new concept brought recently to attention by new technological developments. Although we embrace these studies that have been on the vanguard of discovery, we caution that finding specialized ribosomes requires additional supporting evidence, and special care must be taken to include stringent controls. One problem with examining a heterogeneous ribosome population is that it may represent failed synthesis intermediates or defective ribosomes on their way to being degraded. Current methods aggregate results because they rely on pools of cells (e.g., sucrose gradient sedimentation) and therefore have limited resolution. Additional orthogonal approaches such as single-cell mass spectrometry and cryo-electron microscopy may help to validate recent discoveries. Nevertheless, to answer the question of whether there are specialized ribosomes, it will certainly require a multipronged approach that combines these new techniques with the old.

Alternative View: the Ribosome Concentration Hypothesis

A second hypothesis put forth to explain the tissue-specificity of ribosomopathies is the ribosome concentration hypothesis (Figure 3). Like the specialized ribosome hypothesis, it was originally proposed several years ago by Lodish [81] but was recently repopularized by Mills and Green [6]. The ribosome concentration hypothesis postulates that the number of available cytoplasmic ribosomes per unit volume results in mRNAs that are translated differently based on structural features such as 5′-untranslated region (5′-UTR) length or structural elements, open reading frame (ORF) length, and Kozak context. Linking back to the problem of tissue specificity of ribosomopathies, the expression of specific proteins may be sensitive to decreased ribosome concentration in that particular cell type.

Although the ribosome concentration hypothesis might at first glance seem more straightforward than the specialized ribosome hypothesis, experimental evidence supporting this theory has also been hard to generate. It has been difficult to directly measure ribosome concentration on a per cell basis, and most studies have only inferred reduced ribosome number.

The often-studied examples in support of the ribosome concentration hypothesis have focused on the protein GATA1, a lineage-determining hematopoietic transcription factor that is implicated in the pathogenesis of DBA. Two recent papers with overlapping groups of authors argue that

decreased ribosome concentration leads to reductions in GATA1 protein levels, resulting in the erythropoiesis-specific defects observed in patients with DBA [27,82]. However, the mechanistic explanation for how reductions in GATA1 protein levels occur is inconsistent between the two studies, making it difficult to reconcile the underlying mechanism. In the first, it is because GATA1 has a highly structured, long 5′ UTR; whereas in the second, it is because GATA1 has a short, unstructured 5′-UTR [27,82]. This discrepancy may partially arise from methodological differences: older studies utilized the 5′ RACE technique (rapid amplification of 5′ cDNA ends) [82], whereas more recent studies utilize CAGE (cap analysis gene expression) [27] to define the GATA1 5′-UTR. The GATA1 NCBI reference sequence, NM_002049.3, reveals a 5′-UTR of 91 nt, which supports a 5′-UTR for GATA1 shorter than the average length of 210.2 nt for human 5′-UTRs [83]. Additional new ribosome profiling results in yeast support the idea that more efficiently translated mRNAs are more affected by reduced ribosome concentration [84]. By contrast, the original mathematical modeling by Lodish [81] and echoed by more recent reviews [6,74], postulated that poorly translated mRNAs (such as GATA1 if its 5'-UTR is long and highly structured) would be dependent on the cell having a high available ribosome concentration, whereas well-translated mRNAs would not have such dependency. These apparently contradictory results emphasize our current lack of understanding of how the structural elements of mRNAs affect their translation. In addition, examination of the molecular basis of ribosomopathies should continue to probe the effects of compensatory processes such as ribosome recycling [6].

It is important to note that these two hypotheses – specialized ribosomes and ribosome concentration – are not mutually exclusive and both may contribute to the clinical manifestations observed in patients with ribosomopathies. In addition, the role of p53 stabilization in tissue specificity cannot be overlooked. It is possible that either or both of the proposed mechanisms could result in reduced translation of a protein that leads to p53 stabilization and apoptosis, ultimately causing the tissue-specific defects. Thus, it is likely that no single mechanism will be able to fully explain all the signs and symptoms of these disorders, which are complicated not only by their tissue specificity but also by their developmental timing.

How Can Defects in Cell Growth Cause Cancer?

Several ribosomopathies – disorders of hypoproliferation – often coincide with a predisposition to cancer, a disease of hyperproliferation. This juxtaposition is referred to as Dameshek's riddle [85]. Although cancer predisposition has not been studied for every ribosomopathy, this paradox has been studied in the context of SDS that is caused by mutations in *SBDS* [86,87]. The neutropenia and bone marrow abnormalities characteristic of SDS often progress to myelodysplastic syndromes (MDS) and acute myelogenous leukemia (AML) [88,89]. Reinforcing this link to cancer, SBDS physically interacts with the r-protein RPL10 (uL16) that is mutated in pediatric T cell leukemia (T-ALL [90,91]). Perhaps the hyperproliferation results as a consequence of cellular compensation for dysfunctional ribosome biogenesis. In support, some cells compensate for *SBDS* mutations by deleting the *EIF6* gene [del(20)q] to upregulate protein synthesis [92,93]. This has been associated with a lower risk of developing MDS and AML, although more longitudinal studies with larger patient cohorts will be necessary to confirm this [94–96]. By contrast, SDS patients often also acquire *TP53* mutations [97]. It is possible that cells sense disruptions in making ribosomes by upregulating p53 levels, and cancer results when the cells acquire additional mutations to bypass this effect. Further insight into Dameshek's riddle may be found in studies showing that niche-derived inflammatory signaling may facilitate or even drive malignant progression in MDS in an effort to compensate for perturbations in hematopoietic stem cells [5,98,99]. In the future, we hope to be able to evaluate the natural progression of all of the compensatory mutations in patients with diverse ribosomopathies.

Are Aging and Neurodegenerative Diseases also Ribosomopathies?

Recent studies on premature aging diseases, models of longevity, and neurodegeneration continue to support a connection between aging and nucleolar morphology/activity. This connection is consistent with the longstanding proposal that rDNA instability underlies aging phenotypes [100–104]. Hutchinson–Gilford progeria syndrome (HGPS), that is caused by mutated lamin A/C, results in increased nucleolar function through increased rDNA transcription and translation [105]. By contrast, both Werner syndrome (WS) and Cockayne syndrome (CS), canonical disorders of DNA repair [106,107], result in decreased nucleolar activity through reduced rDNA transcription [108]. Furthermore, WS has been shown to disrupt nuclear pores and lamin B1 [109], and lamin B2 has been shown to regulate nucleolar morphology and function [110], suggesting a broader link between the nuclear membrane, the nucleolus, and aging.

Intriguingly, neurodegenerative diseases share similar nucleolar phenotypes to WS and CS. This has been reviewed in greater detail previously [111–116]. In brief, observations in Alzheimer's disease (AD) support reduced nucleolar size, activity, and translation [117,118]. Likewise, in Parkinson's disease (PD) nucleolar disruption has been observed [114], and when the RNAPI transcription factor RRN3 (TIF-1A) is depleted specifically in adult mouse dopaminergic neurons, p53-dependent apoptosis and PD-like symptoms are observed, supporting a link between nucleolar stress and neurodegeneration [119]. However, recent work studying children heterozygous for a gain-of-function mutation in the gene encoding the RNAPI transcription factor UBTF (E210K) also have neurodegeneration, suggesting that our understanding of the association between nucleolar function and neurodegeneration remains incomplete [120].

Finally, it has recently been discerned that small nucleolar size predicts a longer lifespan not only in *C. elegans* but also in fly, mouse, and human [121]. Despite contrasting with the above observations on neurodegeneration, reduced nucleolar size and activity as a hallmark of longevity is consistent with longstanding research on mTOR (mechanistic/mammalian target of rapamycin) and the use of mTOR inhibitors to treat age-related diseases [122]. Further confounding is a new study that identified increased CpG methylation in the rDNA of aged mice (a pattern conserved in canid models and humans) [123]. This would suggest decreased rDNA transcription and greater genome stability in aged individuals, which is contrary to much of the existing literature. Perhaps increased methylation is a compensatory mechanism to counteract cellular processes that are no longer maintaining genome stability. Furthermore, defects in ribosome recycling have also recently been implicated in aging and neurodegeneration [124,125]. Thus, additional studies will be necessary to gain a more comprehensive understanding of the role of ribosome biogenesis in aging and neurodegenerative disease.

Concluding Remarks and Future Perspectives

Can we classify old diseases as new ribosomopathies? (see Outstanding Questions). As we continue to understand more about embryonic development and its relationship to making ribosomes, we will be compelled to re-examine old disorders for new links to ribosome biogenesis. Along these lines, recent research indicates that the microcephaly, small forehead, elongated face, clinodactyly of the fifth fingers, and intellectual disability seen in the cohesinopathy Warsaw breakage syndrome (WABS) may be due in part to a newfound role for the protein DDX11 in RNAPI transcription [126]. Two proteins in another bone marrow failure syndrome, Fanconi anemia (FA), have recently been found to have a role outside of DNA repair in RNAPI transcription, suggesting that there may be a dual pathogenesis for FA [127,128]. In addition, recent results have shown differential expression of r-proteins in response to ethanol exposure, highlighting a potential link between the craniofacial defects observed in fetal alcohol syndrome and making ribosomes [129]. Discovery-based approaches for factors that influence human

Outstanding Questions

In diseases of ribosome biogenesis, why are some tissues affected more often than others?

Are there old disorders that we can now classify as new ribosomopathies?

How do the ribosomopathies result in tissue-specific defects – through specialized ribosomes, reduced ribosome concentration, both, or neither?

How might ribosome composition and number change during development and within different tissues? How is this altered in patients with ribosomopathies?

How does the composition and structure of an mRNA influence its translation? How does this change upon reduced numbers of available ribosomes?

What are the mechanisms through which ribosomopathies result in increased cancer susceptibility? Is this mechanism the same for all ribosomopathies?

What is the relationship between longevity and the nucleolus? Can aging and neurodegenerative diseases be classified as ribosomopathies?

ribosome biogenesis [130–134] may be crucial in identifying these new links. Moving forward, by defining the mechanisms underlying the pathogenesis of ribosomopathies, we hope to eventually be able to alleviate the human suffering that they cause.

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