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Review

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Mechanisms of ribosomopathy and phase separation-related ribosomopathy

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Abstract: Ribosome is an intracellular ribonucleoprotein particle that serves as the site of protein biosynthesis. Ribosomal dysfunction caused by mutations in genes encoding ribosomal proteins (rPs) and ribosomal biogenesis factors (RBFs) can lead to a spectrum of diseases, collectively known as ribosomopathy. Phase separation is a thermodynamic process that produces multiple phases from a homogeneous mixture. The formation of membrane-less organelles and intracellular structures, including ribosomes and nucleoli, cannot occur without the involvement of phase separation. Here, the ribosome structure, biogenesis, and their relationship to ribosomopathy are systematically reviewed. The tissue specificity of ribosomopathy and the role of phase separation in ribosomopathy are particularly discussed, which may offer some clues for understanding the mechanisms of ribosomopathy. Then, some new ideas for the prevention, diagnosis, and treatment of ribosomopathy are provided.

Key words: Ribosome; Phase separation; Ribosomopathy; Tissue specificity

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1 Introduction

The ribosome is an important cellular organelle responsible for the translation of RNA sequences into proteins. In eukaryotes and prokaryotes, ribosomes have similar structures and functions (Filbeck, et al., 2022). For example, ribosomes in eukaryotes and prokaryotes both contain two subunits, catalyze protein synthesis, follow codon and anticodon binding rules, and require complex modifications to perform their functions. Ribosomopathy refers to a group of highly heterogeneous genetic disorders that affect the structure and function of the ribosome. Ribosomopathy can affect different aspects of ribosome function, including ribosome assembly, subunit stability, and accuracy of protein synthesis (Paolini, et al., 2017). These disorders can lead to a variety of developmental and growth abnormalities, including skeletal malformations (Robertson, et al., 2022), craniofacial deformities (Smallwood, et al., 2023), neurological disorders (Bourque, et al., 2018), and anemia (Da Costa L, 2020). Ribosomopathy can be caused by gene mutations that encode ribosomal proteins (rPs), the

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proteins involved in ribosome biogenesis. Phase separation (PS) is a newly prospering field in biological research that is closely related to biological processes such as chromatin organization (Gibson, et al., 2019), gene expression (Cai, et al., 2019), ribosome biogenesis (Banani, et al., 2017), cell signaling (Su, et al., 2021), and synaptic formation (Zeng, et al., 2016), as well as cellular life activities such as cell division (Ong and Torres, 2020) and autophagy (Noda, et al., 2020).

In this review, the ribosome's structure and biogenesis and their relationship to ribosomopathy are systematically reviewed. Moreover, starting from pathogenesis, several ribosomopathies are classified with explicitly linked etiology and clinical features. Simultaneously, some putative ribosomal diseases are described, with the expectation of finding commonalities in these diseases with known ribosomopathies. Finally, the tissue specificity of ribosomopathy and the role of PS in ribosomopathy are discussed. This would provide insights into the underlying mechanisms of ribosomopathy, potentially leading to novel approaches for its prevention, diagnosis, and treatment.

2 Ribosome structure and biogenesis

The eukaryotic ribosome consists of a 40S small subunit (SSU) and a 60S large subunit (LSU). The SSU, comprising 18S rRNA and 33 rPs, is assembled into regions encompassing the head, beak, shoulder, platform, body, and foot (Jobe, et al., 2019; Rabl, et al., 2011). LSU contains 5S, 5.8S, and 28S rRNAs and 47 rPs (Khatter, et al., 2015; Klinge, et al., 2011). Compared with the prokaryotic ribosomes, the eukaryotic ribosomes are more complex in structure, with a large number of eukaryotic-specific proteins in the LSU and SSU (De La Cruz, et al., 2015). These eukaryotic-specific proteins are generally localized at the solute contact surface of the ribosome (Pilla and Bahadur, 2019). The 5' extended sequences (ESs) and 5' variable regions (VRs) in the SSU, together with 16 ESs and 2 VRs in the LSU, are all associated with the presence of eukaryotic-specific proteins (Wilson and Doudna Cate, 2012). Generally, this spatial organization pattern of eukaryotic-specific proteins, while maintaining the relative conservation of the subunits' contact interfaces, gives rise to different physicochemical properties between ribosomes of different species.

Ribosome biogenesis is an overarching description of the process from synthesizing rRNAs and rPs to assembling ribosomal subunits (Bassler and Hurt, 2019). To date, over 200 assembly factors required for ribosome biogenesis, alongside a large number of functionally complexed small nucleolar RNAs (snoRNAs), have been characterized (Nerurkar, et al., 2015; Ojha, et al., 2020; Pena, et al., 2017; Sloan Ke, 2017). These assembly factors and non-coding RNAs (ncRNA) are primarily found in the nucleolus (Nerurkar, et al., 2015). The nucleolus, one of the most important membrane-less intracellular structures, is the starting point and center of ribosome biogenesis (Pederson, 2011). The nucleolus can be divided into three layers from inside to outside based on structural differences: the fibrillar center (FC), dense fibrillar component (DFC), and granular component (GC) (Hernandez-Verdun, et al., 2010). The FC deposits rDNA and RNA polymerase I (RNA pol I), which lack transcriptional activity, whereas active rDNA is transcribed into pre-rRNA catalyzed by RNA pol I at the FC/DFC site (Wu M, 2021). pre-rRNA undergoes post-transcriptional processing, including splicing, base modification, and folding, to become rRNA in the DFC (Smirnov, et al., 2016). In the GC, immature subunits assembled from rRNAs and rPs are eventually transported to the cytoplasm for function (Boisvert, et al., 2007).

The nucleolus exerts multiple roles in ribosome biogenesis and the generation of ribosomopathy. Abnormalities in the structural organization of the nucleolus, including the quantity or quality alterations of trans-acting factors and snoRNAs that play a regulatory role, could affect the normal synthesis of the ribosome and contribute to the development of ribosomopathy (Lafontaine, et al., 2021). In addition, abnormalities in rPs and rRNAs, while affecting ribosome structure or function, may also be accompanied by nucleolus malformation. Therefore, the nucleolus and ribosome are inseparable wholes when exploring ribosome biogenesis and disease generation.

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3 Abnormal ribosome biogenesis and diseases

Ribosomopathies, caused by abnormal ribosome biogenesis, are highly complex in clinical manifestation and pathogenesis. RBFs, rRNA, and rPs are the key elements for proper ribosome formation. Currently, there is no consensus on the classification of ribosomopathies. The challenges in classification are mainly twofold: (1) The concurrence of congenital and acquired ribosomopathies entails a challenge in understanding their pathogenesis; and (2) the intertwining of ribosomopathies caused by abnormal ribosome biogenesis and multi-factorial mechanisms, which are controversial to define in clinical manifestations (Venturi and Montanaro, 2020). For these reasons, starting from pathogenesis, several ribosomopathies are classified with explicitly linked etiology and clinical features (Table 1). Simultaneously, some putative ribosomal diseases are described, with the expectation of finding commonalities in these diseases with known ribosomopathies.

3.1 rDNA: auxiliary mechanism in ribosomopathy

rDNA forms tandem repeat sequences in the nucleolar organizing region (NOR) in a multi-copy pattern (Van Sluis and Mcstay, 2017). Multi-copy of rDNA is a reliable way to meet the high cellular demand for ribosomes, hence avoiding the burden of excessive rDNA transcription rates or high rRNA stability in proliferating cells (Nelson, et al., 2019; Tsekrekou, et al., 2017). Approximately 50% of the rDNAs in the genome are functional enough to meet cellular demand, and this functional redundancy increases tolerance to rDNA mutations (Tsekrekou, et al., 2017).

Under normal conditions, rDNA arranged in a tandem repeat pattern in the eukaryotic genome is prone to rearrangements, whereas subtle mechanisms ensure a relatively constant rDNA copy number (Iida and Kobayashi, 2019). However, large-scale rearrangements of rDNA have been found in several diseases with high oncogenic risk, such as Bloom syndrome and ataxia capillaris (Killen, et al., 2009). Moreover, rDNA copy number variants or rearrangements have also been found in osteosarcoma, AIDS-associated lymphoma, esophageal adenocarcinoma, lung cancer, and colorectal cancer (Xu, et al., 2017). Studies have further demonstrated that the aforementioned rDNA abnormalities do not directly disrupt cell proliferation, rRNAs, or protein synthesis (Xu, et al., 2017). In contrast, they are linked to alterations in the SNP, the copy number of protein-coding genes, cellular hypersensitivity to injury, and the functional status of TP53 (Feng, et al., 2020; Wang and Lemos, 2017). Although these studies do not provide a clear elucidation of the molecular mechanisms, the available data suggest that rDNA abnormality is frequently not a direct factor but rather plays a complementary role in ribosomopathies. Consistent with this idea, no ribosomopathy caused solely by rDNA abnormalities has been identified. However, considering the relevance of ribosomopathies to tumorigenesis, the status of rDNA in tumor patients may be tested to improve patients' prognosis and provide new therapeutic directions.

3.2 RBFs contribute to ribosomopathies through multiple mechanisms

RBFs are biomolecules that regulate ribosome biogenesis, which are mainly localized in the nucleolus and function in multifarious aspects of ribosome maturation, including synthesis, processing, and folding of rRNA and RP, along with ribosome assembly (Kovacevic, et al., 2019). RBFs have become a focus and a challenge in the study of ribosomopathies due to their functional complexity. Based on the functional diversity of RBFs, ribosomopathies with relatively similar main pathogenesis are categorized in this section.

3.2.1 rDNA transcription and ribosomopathy

Treacher‐Collins syndrome (TCS) is an autosomal dominant craniofacial developmental malformation (Mj., 1995), characterized by lid fissures, ear malformations, and craniofacial skeletal dysplasia (Plomp, et al., 2016; Tse, 2016). TCOF1, POLR1B, POLR1C, and POLR1D have been identified as predominant pathogenic genes in TCS (Sanchez, et al., 2020). Mutations in these genes affect rDNA transcription, interfering with

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ribosome synthesis in cranial neural crest cells (CNCCs) and neuroepithelial cells (Levasseur, et al., 2018; Sanchez, et al., 2020).

The vast majority of TCS is caused by pathogenic mutations in TCOF1. TCOF1 encodes Treacle protein, a nucleolus phosphorylation protein with nucleolus localization signals at the C-terminus and a nuclear export signal at the N-terminus, indicating that Treacle protein functions in different subcellular regions (Grzanka and Piekielko-Witkowska, 2021). Treacle protein co-localizes in the nucleolus with the upstream binding factor (UBF), RNA pol I, and Nopp140 in the transcription initiation complex at the rDNA promoter, where Treacle regulates 47S pre-rRNA synthesis (Marszalek-Kruk, et al., 2021). Abnormal Treacle caused by pathogenic mutations in TCOF1 can interfere with rDNA transcription and reduce rRNA levels in cells, resulting in increased neuroepithelial cell-specific apoptosis and decreased neural crest cell proliferation (Dixon J, 2006). Additionally, Treacle can bind to NOP56, a core component of box C/D small nucleolar ribonucleoprotein (snoRNP) particles. Reduced methylation at specific sites of 18S pre-rRNA in TCOF1 heterozygous mutant mice suggests that Treacle can regulate rRNA post-transcriptional processing through Treacle-NOP56 interaction (Gonzales, et al., 2005). POLR1B, POLR1C, and POLR1D are all genes that encode RNA pol subunits (Noack Watt, et al., 2016; Sanchez, et al., 2020). Pathogenic mutations in these genes could also affect RNA pol I functions, contributing to low levels of rRNA and ultimately leading to characteristic malformations of craniofacial tissue (Dauwerse, et al., 2011; Kwong, et al., 2018). To summarize, TCS is mainly triggered by reduced rDNA transcription rather than by significant structural changes in ribosomal components. Thus, low intracellular rRNA levels result in hypoplasia of the first and second branchial arches and congenital craniofacial abnormalities (Levasseur, et al., 2018) (Fig.1).

Abnormal rDNA transcription has likewise been found in other ribosomopathies. For instance, pathogenic mutations of POLR1A in Acrofacial Dysostosis—Cincinnati type (AFDCIN), which encodes for the RNA Pol I component, generate craniofacial deformities through a similar mechanism to that of TCS (Terrazas, et al., 2017; Watt, et al., 2018) (Fig.1). In addition, the reduced rDNA transcription detected in Diamond–Blackfan anemia (DBA) and 5q-syndrome (Le Goff S, 2021), exacerbates erythrocyte dysplasia. However, the primary cause in both disorders is the alteration of ribosomal protein genes (RPGs), and rDNA transcription may occur only as a cofactor in these diseases or as a side effect of nucleolus stress due to decreased rPs synthesis.

Several putative ribosomopathies are potentially associated with rDNA transcription. The Cockayne syndrome A (CSA) protein, a transcription factor for RNA pol I, enhances rDNA transcription and may lead to premature aging and death in children (Koch, et al., 2014). Repression or upregulation of rDNA transcription has also been detected in patients with Aplasia Cutis Congenita (ACC) (Zhu, et al., 2022) and some lung adenocarcinomas (Ohashi, et al., 2020). Unfortunately, studies of these diseases have not yet developed a valid model to explain the connection between rDNA transcription and clinical manifestations.

In summary, TCS stands out from other ribosomopathies caused by abnormal ribosome biogenesis because of its clear connection between the underlying cause and the observed clinical symptoms. The possibility that the clinical features of TCS may be worsened by additional non-ribosomal functions carried out by the increased ribosomal proteins during ribosome assembly raises interesting questions that warrant further exploration. Either way, the correlation between TCS and rDNA transcription leads to abnormalities in ribosome synthesis and function, which in turn affects cell growth and differentiation, ultimately leading to facial and craniofacial deformities. This correlation could play an important role in disease treatment and finding targets for new drugs. An in-depth study of the relationship between TCS and rDNA transcription can help to reveal the molecular mechanism of the occurrence and development of the disease, to provide a theoretical basis for the development of new therapies. Second, drugs or interventions that target abnormal rDNA transcription may become new targets for treating TCS. By regulating the activity or stability of rDNA transcription, it is possible to attempt to correct abnormalities in ribosome synthesis and function, thereby reducing or improving symptoms in patients with TCS. This correlation could be further explored in future studies, and therapeutic strategies targeting rDNA transcription could be attempted to lead to better outcomes

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for patients with TCS.

3.2.2 Post-transcriptional process of rRNA and ribosomopathy

Regulating the post-transcriptional process of rRNA is the main function of RBFs. Post-transcriptional processing is a fundamental regulation of ribosome topology and plays an essential role in the emergence of a variety of ribosomopathies (Taoka, et al., 2018).

RBFs are involved in pre-rRNA splicing. The Cartilage Hair Hypoplasia-Anauxetic dysplasia (CHH-AD) spectrum includes a range of disorders with mild to severe symptoms, the most common of which are defects in skin, bone, blood, and immune system development (Elalaoui, et al., 2016; Matesic and Hagan, 2007; Vakkilainen, et al., 2020). POP1 encodes the core protein of RNase MRP, and RMRP encodes the RNA component of RNase MRP (Makitie, et al., 2001; Mattijssen, et al., 2010). Mutations in POP1 and RMRP can impair RNase MRP assembly and pre-5.8S rRNA splicing, interfering with ribosome biogenesis and resulting in CHH-AD (Barraza-Garcia, et al., 2017) (Fig.1). Abdulhadi-Atwan, et al. (2020) reported three patients with novel POP1 mutations that, inconsistent with the previous studies, do not have a significant accumulation of pre-5.8S rRNA despite reduced RNase MRP levels while exhibiting only mild skeletal dysplasia (Barraza-Garcia, et al., 2017; Thiel, et al., 2007). This POP1 mutation may compensate for the loss of RNase MRP through an unclear mechanism, rescuing the induction of skeletal developmental abnormalities caused by improper rRNA splicing, for which further studies are needed. In patients with North American Indian Childhood Cirrhosis (NAIC), a severe form of cholestasis, mutations in Cirhin and its binding factor NOL11 have been identified (Chagnon P, 2002; Freed, et al., 2012). Both RBFs are involved in pre-18S rRNA splicing (Freed, et al., 2012; Rouin E, 2000; Sondalle and Baserga, 2014) (Fig.1). The precise mechanism by which improper rRNA splicing brings on NAIC is still unknown, and future resolution of this issue may facilitate insight into the mechanisms of ribosomopathies' tissue specificity. Until then, however, the question of why this global and fundamental process of ribosome biogenesis affects only hepatocytes in NAIC will always haunt us.

On the other hand, RBFs are involved in rRNA base modification. Clinical manifestations of Dyskeratosis Congenita (DC/DKC) are mainly related to telomerase function (Jyonouchi, et al., 2011; Vulliamy and Dokal, 2008), but altered rRNA pseudouridylation caused by DKC1, NOP10 (Balogh, et al., 2020), and NHP2 (Vulliamy T, 2008) mutations, as well as rRNA methylation caused by mutations in NPM1 (Nachmani, et al., 2019), all contribute to the pathogenesis of DC (Fig.1). In addition, abnormal expression of rRNA 2'-O-methyltransferase fibrillarin (FBL) can alter rRNA modifications in breast cancer and acute myeloid leukemia (AML) development (Belin, et al., 2009; Hindley, et al., 2021). rRNA base modifications affect its three-dimensional structure, control rRNA folding, ribonucleoprotein assembly, and even ribosomal activity (Decatur and Fournier, 2003). It is still unclear how such subtle structural changes induce disease generation, as accurate intracellular capture of ribosomal component structures remains a great challenge, hindering further studies.

One possible explanation for these diseases is the degradation of rRNAs that are not properly matured in the nucleolus, manifesting as a reduction of ribosomes, which in turn induces ribosomopathies through pathways like nucleolar stress. When there is insufficient quality control of rRNAs, structurally imperfect rRNAs can be incorporated into ribosome assembly to form a subpopulation of incompetent functioning ribosomes, triggering reduced translational fidelity and impaired protein synthesis, causing or exacerbating ribosomopathies. In terms of disease treatment, understanding the molecular pathways involved in ribosome biogenesis and quality control could provide insights into potential therapeutic targets. Developing drugs that enhance ribosomal RNA maturation and promote the formation of functional ribosomes may offer promising avenues for treating ribosomopathies. Furthermore, in the context of disease prevention, early detection of abnormalities in ribosome biogenesis and quality control mechanisms could be crucial. Screening strategies aimed at identifying individuals at risk for ribosomopathies based on genetic markers or biomarkers associated with ribosomal dysfunction could help in implementing preventive measures or interventions to

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mitigate the development of these diseases.

3.2.3 Ribosome assembly and ribosomopathy

Another important function of RBFs is regulating ribosome assembly. In line with this, a malfunctioning ribosome assembly process has the potential to cause multifarious ribosomopathies, amplifying the susceptibility to oncogenesis (Warren, 2018).

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive disorder characterized by hypoplasia of the pancreas, hematopoietic system, and skeleton (Burroughs, et al., 2009), with a small proportion of patients also having myelodysplasia (MDS) and acute myeloid leukemia (AML) (Dror, 2005). SBDS, EFL1, DNAJC2, and SRP54, as the SDS pathogenic genes, are all involved in ribosome biogenesis (Bezzerri and Cipolli, 2019) (Fig.2). Among them, SBDS, SRP54, and EFL1 are responsible for removing eIF6 from the pre-60S large subunit, completing the maturation and assembly of the ribosomal large subunit, thus forming a substantial contribution to SDS (Weis, et al., 2015). DNAJC21 stabilizes assembled 80S ribosome (Bezzerri and Cipolli, 2019). Hence, SDS is classified as a ribosome assembly-related ribosomopathy based on the importance of these genes in LSU assembly. Furthermore, SBDS is involved in regulating biological processes such as spindle stabilization, cellular stress, and apoptosis, which are more like side effects of ribosome assembly disorders (Ambekar, et al., 2010; Orelio, et al., 2009). Intriguingly, unlike DBA, SDS patients tend to exhibit impaired granulopoiesis (Xia, et al., 2018), while red blood cell lineage development is less affected. Tissue specificity is an important manifestation of ribosomopathies, and it is worth investigating how different abnormalities in ribosome biogenesis affect bone marrow hematopoiesis and result in inconsistent clinical phenotypes.

Bowen-Conradi syndrome (BCS) is caused by abnormal ribosome assembly factor EMG1, with the main clinical manifestations being developmental delay, and facial and osteoarticular dysplasia (Hunter Ag, 1979; Lowry, et al., 2003; Wurm, et al., 2010). EMG1 binds to rPs1, participates in ribosome assembly, and functions as a methyltransferase required for eukaryotic 18S rRNA site-specific N1 pseudouridine modification (Thomas, et al., 2011; Wurm, et al., 2010) (Fig.1). Mutations in EMG1 disrupt its nucleolus localization, causing aggregation and degradation of abnormal EMG1 in the nucleus (Warda, et al., 2016). Meyer et al. (Meyer, et al., 2011) found that EMG1 mutations had no effect on its methyltransferase activity, implying a more important role of defects in ribosomal small subunit assembly in BCS. Therefore, BCS is classified as a ribosome assembly-related ribosomopathy.

Although the current research has made significant strides in unraveling the complexities of ribosomopathies, further investigations are still needed to fully elucidate the underlying mechanisms and identify novel therapeutic strategies. Continued efforts in this field, including more comprehensive studies and translational research, will be essential in advancing our understanding of ribosomopathies and developing effective treatments for these diseases. Incorporating these points into the manuscript would undoubtedly enhance its appeal and significance in the scientific community. Now, there are many controversies and gaps in the research of ribosome assembly-related diseases. Further research on the pathogenic genes such as SBDS, EFL1, DNAJC2, SRP54, EMG1, etc., will be conducive to the precise interpretation of the influence pathway of ribosome assembly abnormalities on ribosomal diseases, and the development of possible therapeutic means based on this.

3.3 Ribosomal proteins and ribosomopathy

The eukaryotic ribosomes contain approximately 80 rPs, the most diverse component of the ribosome, which are encoded by ribosomal protein genes (RPGs). Many RPGs exist in a single-copy pattern (Dharia, et al., 2014), implying a more pronounced influence of these genes on ribosomal biogenesis processes compared to rDNA.

Bone marrow is one of the main affected tissues in ribosomopathies. RPGs mutations and deletions can change ribosomal physicochemical properties, resulting in Diamond-Blackfan anemia (DBA), 5q- syndrome

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(Boultwood, et al., 2010; Ear, et al., 2016; Ebert, et al., 2008), T-cell acute lymphoblastic leukemia (T-ALL) (De Keersmaecker, et al., 2013), chronic lymphocytic leukemia (CLL) (Ljungstrom, et al., 2016), AML and a variety of other hematologic disorders (Fig.2). Among these diseases, DBA has received more attention, providing additional guidance in understanding this group of diseases. DBA was the first ribosomopathy to be described (Dorn, et al., 2021), and the dominating manifestation of this disease is impaired red lineage development (Flygare and Karlsson, 2007).

A large number of RPGs mutations have been detected in DBA patients. A subset of RPGs, including RPS19 (Mirabello, et al., 2017; Morimoto, et al., 2007), RPS17 (Farrar, et al., 2011), RPL5, and RPL11 (Cao P, 2021; Li, et al., 2020), is more clearly associated with DBA. Studies on RPS19 have revealed the role of ribosomal protein structure in DBA development. Malformation of RPS19 can impact the hydrophobic core or helix structure and reduce protein structural stability. RPS19 mutations can also interfere with hydrogen bond generation, weaken the tightness of protein folding, increase spatial site resistance, or alter surface electrostatic properties to disrupt multivalent interactions with rRNA (An, et al., 2021). In general, these mutations impede the assembly process of rPs into ribosomes and affect the formation of mature, fully functional ribosomal subunits. Additionally, RPL5 and RPL11 bind to MDM2 to inhibit the ubiquitinated degradation of p53 (Tong, et al., 2020) (Fig.2). Mutations in RPGs may cause an imbalance in the number of rPs and rRNAs. The escalation of free rPs in the nucleus, followed by p53 enrichment, would result in cell cycle arrest and augmented cell apoptosis. Unfortunately, more pathogenic genes, particularly RPS26-29 (Gripp, et al., 2014; Mirabello, et al., 2014), RPL15 (Wlodarski, et al., 2018), RPL18, and RPL35 (Mirabello, et al., 2017), only have a few case reports and/or relevant studies, and the understanding of these genes is still stagnant in its infancy.

RPGs mutations have also been detected in isolated congenital asplenia (ICA) (Bolze, et al., 2013), autism (Klauck, et al., 2006), microcephaly (Bourque, et al., 2018; Brooks, et al., 2014), and a subset of familial colorectal cancer type X (FCCX) (Nieminen, et al., 2014). Although it is unclear how RPGs mutations affect the clinical manifestations of these diseases, several hypotheses have attempted to explain these phenomena. For instance, the specialized ribosome and ribosome concentration hypotheses, which will be discussed in the followed section about tissue specificity of ribosomopathy.

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4 Tissue specificity of ribosomopathy

Tissue specificity is the most attractive feature of ribosomopathy. Synthesis disorders of the ribosome, this fundamental organelle, often display symptoms in specific tissues (Table 2). Commonly affected tissues and organs include the neural crest, bone marrow, and liver (Farley-Barnes, et al., 2019). Similar tissue development defects can arise from ribosomal disorders brought on by different mechanisms. For instance, the craniofacial malformations in TCS (Marszalek-Kruk, et al., 2021), AFDCIN (Watt, et al., 2018), and DBA (Dorn, et al., 2021), either as the bone marrow failure and anemia exhibited by patients with SDS (Burroughs, et al., 2009), DBA, and 5q syndrome (Ganapathi and Shimamura, 2008), indicating a complex network of mechanisms behind the tissue specificity of ribosomopathies.

4.1 Specialized ribosome and ribosome concentration - qualitative and quantitative hypotheses of tissue specificity

The notion of ribosomal heterogeneity has sparked widespread speculation since it’s first proposed six decades ago. It is only in the last decade or so that technological advances have allowed us to approach this theory. This heterogeneity stems from rPs homologous paralog substitutions (Gay, et al., 2022), discordant expression levels of RPGS (Slavov N, 2015), RPGs or rDNA mutations (Panda, et al., 2020; Xue and Barna, 2012), and differences in modifications of ribosomal components, manifested as subtle differences in

composition or structure between ribosome subpopulations. Hitherto, solid and credible evidence has confirmed the existence of ribosomal heterogeneity.

This heterogeneity lays the foundation of another relatively vague derived concept, the (functional) specialized ribosome. Although there is no consensus on the definition, researchers have generalized similarly to the conception of specialized ribosome (Ferretti Mb, 2019; Genuth and Barna, 2018; Norris, et al., 2021), so we simplified this concept to a subgroup of ribosomes that are involved in global protein synthesis, regulate translation processes, and output results. Several studies have provided more plausible evidence for the existence of specialized ribosomes. Shigeoka, et al. (2019) found in Xenopus embryonic neuronal cells that rPs synthesized in axons participate in ribosome assembly in a nucleolus-independent manner, and that blocking this process interferes with axon formation, implying a more refined function acquired by these ribosomes.RPL38 is a eukaryotic-specific ribosomal protein located close to the extended fragment ES27 (Kondrashov, et al., 2011). Large-scale changes in gene expression occur in RPL38-deficient mouse embryos, showing a significant reduction of the Hox gene expression. These changes are associated with anomalies in axial skeletal and neural tube patterns, as evidenced by previous studies (Gopanenko, et al., 2021; Kondrashov, et al., 2011).

Similarly, enrichment or deletion of RPS25, RPS26, and RPL10A would confer ribosome high selectivity towards a subset of the translated mRNAs, which are involved in cellular stress response, cell cycle, metabolism, and developmental regulation (Cheng, et al., 2019; Ferretti, et al., 2017; Shi, et al., 2017). All these evidences suggest that beyond the transcriptional level, regulation at the translational level affecting the ribosome is also an important aspect of gene expression, which links ribosomal functional specialization to tissue-specific defects. When ribosomal affinity alters the expression of tissue-specific genes, it may cause developmental defects in the corresponding embryo tissues. However, related studies have been hampered by following obstacles: (1) The manipulation of specific rPs through mutagenesis or knockdown resulted in the alteration of ribosome concentration to varying extents, which in turn complicated the interpretation of various effects. RPS19 haploinsufficient can reduce translation of the hematopoietic transcription factor GATA1, but RPS19 was not found to interact with clear cis-elements on mRNA (Xue, et al., 2015), suggesting that GATA1 translation require a high ribosome level. (2) The emphasis on rPs stoichiometry overshadows ribosomal function tests. Hence, ribosomes in assembly or degradation may be identified as specialized ribosomes due to their ordered, step-wise assemblage or degradation (Bassler and Hurt, 2019; Ferretti Mb, 2019). (3) Although translation viability assays are conducted, it is not assured that the origin, composition, and purity of the ribosomes used are reliable. Addressing these challenges may further refine ribosome function and explain the tissue specificity of ribosomopathies from a qualitative standpoint, but this is destined to require a great deal of effort.

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Compared to the specialized ribosome hypothesis, the ribosome concentration hypothesis concerns quantitative factors of ribosomes. Multiple copies and high transcriptional levels of rDNA, according to this hypothesis, limit the extent of their influence (Xu, et al., 2017), while RBFs and rPs appear to be the key factors in tissue specificity. Both RBFs and rPs can obstruct the formation of sufficient mature ribosomes through complex mechanisms, which are described above. Several reasons may explain why insufficient ribosomes, the ubiquitous and essential organelle for life activity, frequently affect specific tissues: (1) Affected tissues in ribosomopathies have high metabolic or proliferative rates and require large numbers of ribosomes to perform functions, such as the proliferation of neural crest, bone marrow, and germ cell, along with pancreas secretion (Dutt, et al., 2011; Levasseur, et al., 2018; Noack Watt, et al., 2016). Inadequate ribosomes disrupt the normal function of these tissues, resulting in clinical manifestations. (2) High levels of ribosomes are required for the expression of a single gene or group of genes, in contrast to the overall high demand for ribosomes in specific cell types. When ribosomes are in short supply, genes that highly express for a long time or express explosively at specific developmental stages would undergo reduced expression or silencing, producing tissue specific developmental defects (Morimoto, et al., 2007). Consistent with this idea, mice with RPL5 intron ENU mutations exhibit impaired erythroid maturation at embryonic day 12.5, with

relieving symptoms by embryonic day 14 (Yu, et al., 2021). Moreover, this condition is associated with the ribosome sensitivity of specific mRNAs (Mills and Green, 2017). Genes with high ribosome sensitivity may be more vulnerable to abnormal ribosome concentration, thus, the specialized ribosome and ribosome concentration hypotheses seem to be in tandem.

In conclusion, the specialized ribosome and ribosome concentration hypotheses both explain part of the tissue specificity of ribosomopathies. Effects that alter ribosome properties or quantity are not mutually exclusive and co-exist in ribosomopathies. Hence, neither theory can fully elucidate the phenomenon of tissue specificity. For the tissue specificity of ribosomopathies, it may be necessary to move away from the limitations of a particular hypothesis. The way forward for research would involve changes in ribosome properties and numbers being assessed in an integrated manner, as well as connecting abnormalities in ribosome biogenesis to downstream effects that directly affect the phenotype (e.g., activation of p53).

4.2 p53-dependent and -independent pathways induce developmental defects in specific tissues

The nucleolus is responsible for rDNA transcription, cell growth, and metabolism (Boulon, et al., 2010). Nucleolar stress, also known as ribosomal stress, can be induced by nucleolus structure or function perturbation (Pfister, 2019). rPs mutations, blocked rDNA transcription, abnormal rRNA processing, and other ribosomal pathogenic factors are triggers of the nucleolar stress response (Kang, et al., 2021). Nucleolar stress has the potential to elicit downstream effects through p53-dependent and -independent pathways, contributing to tissue specific clinical phenotypes.

4.2.1 Imbalance between rRNA and rPs may activate p53-dependent pathways

The evolutionarily conserved TP53 gene encodes TP53, a protein that extensively involved incellular stress response (Hernandez Borrero and El-Deiry, 2021; Levine, 2020). TP53 functions as a hub in the cellular life cycle, connecting stress signals to downstream target genes and effectors (Liebl and Hofmann, 2021), subsequently initiating biological effects such as repairment of DNA, cell cycle arrest, cellular senescence, apoptosis, and autophagy (Boisvert and Lamond, 2010; Engeland, 2018; Liu, et al., 2019). This response to ribosome biogenesis defects occurs through mechanisms including altered p53 post-translational modifications, protein interactions, and increased p53 translation (Kang, et al., 2021), which play a crucial role in a variety of ribosomopathies (Narla and Ebert, 2010).

Tcof1+/- mice is the first TCS model (Dixon J, 2006), in which loss of p53 function is able to reduce apoptosis in neuroepithelial and neural crest cells and rescue craniofacial deformities (Achilleos and Trainor, 2015). However, ribosomal biogenesis defects are not rescued (Jones, et al., 2008), indicating a direct role for p53 in the craniofacial tissue defects in TCS. Tcof1 mutations lead to deficient Treacle synthesis and consequently reduced rRNA levels in the nucleolus (Sakai and Trainor, 2009). Firstly, the decline in rRNA transcription during ribosome assembly prevents the formation of sufficient mature ribosomes to meet the exuberant demand for ribosomes in neuroepithelial and neural crest cells (Jones, et al., 2008). Secondly, the relative excess of rPs contributes to an increase in non-ribosomal rPs. MDM2 is an E3 ubiquitin ligase that promotes p53 ubiquitination-dependent proteasomal hydrolysis (Wu and Prives, 2018). rPs that are not involved in ribosome assemblies, such as RPL5 and RPL11, can bind to MDM2 and inhibit p53 ubiquitination, resulting in downstream effects like p53 accumulation and activation in response to nucleolar stress (Turi, et al., 2018) (Fig.1). In a recent study, Falcon, et al. (2022) utilized a series of animal experiments to elucidate possible mechanisms of craniofacial tissue specific malformations.

AFDCIN, as previously described, has similar pathogenesis and clinical presentation to TCS. The mice with double heterozygous of Tcof1 and RNA Pol I subunits encoding gene Polr1a have a more severe defect in NCC formation compared to Tcof1+/- mice. In addition, the loss of RNA Pol I catalytic core POLR1A causes the most severe symptoms in mice embryos compared to other single gene deletion mice, highlighting the susceptibility of NCC and its derivatives to RNA Pol I dysfunction, along with the possible connection between RNA Pol I and congenital defects. This may be attributed to the high activity of Pol I/Ⅱ in neural

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progenitor cells and NCC. When RNA Pol I malfunctions, RPGs expression is not significantly affected, as RPGs are transcribed by the relatively normal functioning Pol Ⅱ. To meet the high demand for ribosomes in the NCC, the rate of rPs synthesis far exceeds that of rRNAs, which ultimately induces NCC apoptosis through p53-dependent pathways.

Similarly, Chakraborty et al. (Chakraborty, et al., 2009) found craniofacial and neurological defects, together with elevated expression of p53-related genes in RPL11 knock-out zebrafish. After excluding interference brought by the binding of other rPs to MDM2, RPL11 knockdown could perform cellular surveillance functions by modulating p53-related checkpoint response that senses the biogenetic integrity of ribosomes, rather than altering p53 ubiquitination levels. Besides, following RPL5 knockdown in Xenopus laevis embryos, p53 activates and represses the activity of the proto-oncogene BCL2 (responsible for apoptosis inhibition), triggering a craniofacial and hematological phenotype similar to that of DBA (Schreiner, et al., 2022). The sensitivity of p53 to bidirectional changes in ribosomal protein concentration suggests a pivotal role in the regulation of ribosomal protein content homeostasis, regardless of whether free RPL5/11 is increased or decreased. This homeostatic phenomenon may not directly monitor the expression levels of ribosomal components, but rather limit the relative content between rRNA and rPs. Consistent with this idea, Donati, et al. (2011) proposed that downregulation of rRNA synthesis was able to activate p53, whereas simultaneous downregulate synthesis of rRNA and rPs did not cause p53 functional stabilization.

Furthermore, the tissue specific enrichment of p53 and abnormal red lineage development caused by RPS14 and RPS19 deletions (Dutt, et al., 2011), as well as the activation of p53 following mutations or insufficient numbers of more rPs (e.g. RPS6, RPS8, RPS18, RPS20, RPL22, RPL35, etc.) (Amsterdam A, 2004; Anderson, et al., 2007; Mcgowan, et al., 2008; Panic, et al., 2006), indicate that p53 operates as a more general mechanism in the generation of tissue specific developmental defects.

We speculate that any disruption in rPs content homeostasis could potentially act as a direct or indirect signal for p53 activation. Unfortunately, this necessitates time-consuming experiments to validate and is destined for an uncertain future. In addition, RPL26 can bind to p53 mRNA and induce elevated p53 translation following DNA damage, resulting in p53-mediated growth arrest and apoptosis (Chen, et al., 2012; Chen, et al., 2017). If more rPs that function by analogous mechanisms can be identified, it will undoubtedly be beneficial to construct a clearer web of relationships between p53 and ribosomopathies' tissue specificity, thus deepening our understanding of this peculiar phenomenon.

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4.2.2 Checkpoints for multiple mechanisms may exist in the p53-dependent pathways

Abnormalities in some nucleolar proteins can disturb tissue specific patterns in ways that are not yet fully understood. Nucleolar protein FBL, nucleolin (NCL), and NOL11 are all abundantly expressed in NCC and its derived craniofacial tissues, which are all involved in the post-transcriptional processing of pre-rRNA (Freed, et al., 2012; Guillen-Chable, et al., 2020; Jia, et al., 2017). Knockdown of these proteins stimulates ocular and/or craniofacial developmental defects (Dash and Trainor, 2022; Delhermite, et al., 2022; Griffin, et al., 2015). WDR43 determines the subcellular localization of nucleoproteins including Treacle protein, MPP10, and NCL. In addition, the DNA helicase DDX21 is responsible for regulating RNA Pol I/II activity and coordinating the synthesis of rRNA and rPs (Calo, et al., 2018). Malfunction or dislocation of WDR43 and DDX21 disrupts branchial arch development, while DDX21-deficient embryos also exhibit red lineage deficiency (Calo, et al., 2018; Zhao, et al., 2014). Moreover, URB2, a nucleolar protein responsible for LSU biogenesis and its deficiency significantly reduces hematopoietic stem cells and T cells in the thymus (Cai, et al., 2018). Similar to Tcof1+/- mice, blocking p53 rescues the phenotype of these mutants, but not the ribosomal biogenesis defects, implying that these nucleolar protein abnormalities eventually converge on the p53-dependent pathways and downstream effects aroused by p53 activation play a crucial role in tissue-specific phenotypes.

It is still unclear how p53 activates in these circumstances, and we speculate that there are several approaches: (1) These nucleolar proteins may be directly implicated in the p53 metabolic process and

p53-dependent pathways. It has been reported that p53 can bind to FBL and inhibits its expression, but it is unknown whether there is a feedback mechanism from FBL to p53 (Marcel, et al., 2013). (2) Malfunction of nucleolar proteins may interfere with ribosome biogenesis and indirectly activates a p53-dependent surveillance mechanism in an aforementioned manner.

4.2.3 The p53-independent pathways as powerful complement to the tissue-specificity of ribosomopathies

Although p53 plays a crucial role in the tissue specificity of ribosomopathy, this does not negate the significance that p53-independent pathways impact the development of particular tissue abnormalities. Inhibiting p53 function prevents neuroepithelial cell apoptosis in polr1a knock-out zebrafish embryos, but defects in neural crest cell proliferation and craniofacial cartilage development persist (Watt, et al., 2018). Similarly, p53 knockdown restore the physiological function of the pancreas to some extent in a zebrafish model of SDS (Tourlakis, et al., 2015), but the various phenotypes of SDS are not completely rescued (Provost, et al., 2012). These two studies suggest the existence of potential p53-independent pathways in AFDCIN and SDS. However, the exact pathways responsible for the phenotype specific to the tissue remain unclear. Because previously identified p53-independent nucleolar stress response modalities, such as E2F1 downregulation and p27kip1 upregulation, have not been correlated with recognized tissue specific developmental defects (Holmberg Olausson, et al., 2012; Zhou, et al., 2015b). Additionally, RPL5 and RPL11 also bind to p73 and compete for the binding site of MDM2 (Zhou, et al., 2015a). Even in cells where p53 is silenced, increased ribosomal-free rPs promotes p73-mediated apoptosis (Zhou, et al., 2015a). The extent to which p73 contributes to the ribosomopathies' phenotype has not been explored, but p73 could serve as a bypass for p53, eliminating cells with defects in ribosome biogenesis.

The investigation into the tissue-specificity of ribosomopathies remains incomplete; however, it has been confirmed that both p53-dependent and -independent pathways are co-exist in such circumstances. More in-depth studies of the relationship between these two pathways and tissue specific developmental defects will facilitate the selection of a more rational and accurate comprehensive treatment plan for patients, improving the outcome for patients with related ribosomopathies.

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5 Phase separation and ribosomopathy

Biomolecular condensates, or condensates, arise from phase separation (PS) and constitute the basis for numerous intracellular membraneless structures (e.g., nucleoli, stress granules, transcription factories, centrioles, etc.) (Tsang B, 2020; Zhang, et al., 2020). Thermodynamically, the PS of biomolecules is dose-dependent. When the concentration of structural components in solution reaches a critical value, the whole system undergoes PS to reduce the free energy, forming coexisting dilute solution phase and biomolecule-rich condensate phase (Lyon, et al., 2021). From a molecular biology perspective, the formation of biomolecular condensates is structure-dependent. PS is triggered by multivalent interactions, and these weak intermolecular interactions include electrostatic interactions, hydrogen bonding, hydrophobic bonding, and stacking of aromatic amino acid residues (Banani, et al., 2017; Gabryelczyk, et al., 2019). Therefore, biomolecules with structures that have a proneness to form multivalent interactions are potential PS constituents. The physicochemical characteristics, subcellular placement, and biological roles of distinct intracellular membraneless structures are are determined by the concentration and arrangement of their major components. Hence, the theory of biomolecular liquid-liquid PS offers a valuable addition to the conventional cellular structure hypothesis.

The components within the nucleolus play a non-negligible role in the formation of ribosomes and the generation of ribosomopathies. Previous findings showed that a subset of disease-associated variants in disordered regions in HMGB1 alter PS, cause mispartitioning into the nucleolus and altering rRNA biogenesis,

followed by disrupting nucleolar function and causing brachyphalangy, polydactyly and tibial aplasia syndrome, a rare complex malformation syndrome. These data identify the cause of a rare complex syndrome and suggest that a large number of genetic variants may dysregulate nucleoli and other biomolecular condensates in humans (Mensah, et al., 2023). In-depth studies of intracellular PS, particularly those associated with nucleolus and ribosome biogenesis, would be beneficial to explore different aspects of ribosomopathies' etiology and pathology.

5.1 Multifunctional PS condensates

Biomolecular condensates participate in processes including chromatin organization, gene expression, ribosome biogenesis, cell signaling, and synapse formation (Banani, et al., 2017; Chen, et al., 2020; Hiraoka, 2020; Rieder, et al., 2012), as well as in cellular activities including cell division (Spannl, et al., 2019) and autophagy (Fujioka and Noda, 2021; Noda, et al., 2020). The multiple functions of condensates are dependent on their properties: (1) Condensates enhance the velocity of biochemical reactions through multiple strategies. Enrichment of specific structural components through multivalent interactions provides suitable sites for biochemical reactions and promotes reaction rate (Fujioka and Noda, 2021; Shin and Brangwynne, 2017). This also enables a series of successive biochemical reactions to take place at adjacent sites, reducing the time and energy consumption of intracellular transportation. In addition, the orderly open structure of condensates allows efficient transport of substrates and products, controlling reaction homeostasis (Wang, et al., 2021). (2) Condensates improve biochemical reaction specificity. High concentrations of specific components in condensates enhance reaction specificity by excluding extraneous factors and attenuating competitive inhibition (Shin and Brangwynne, 2017). Meanwhile, condensates can reinforce the site-specificity of the reaction through combination with specific membrane components or assembly at specific sites (Spannl, et al., 2019; Zhao and Zhang, 2020).

5.2 Abnormal PS can interfere with the proper organization of rDNA

Gibson, et al. (2019) discovered that recombinant chromatin forms droplets after spontaneous PS and hypothesized that chromatin also exhibits liquid-like properties in vivo. In disagreement with this conclusion, a series of controlled experiments demonstrated that liquid-like chromatin condensates are produced only under specific conditions. Chromatin under physiological conditions prefers to compress into solid or gel-like structures, while chromatin-binding proteins undergo liquid-liquid PS around such scaffolds (Strickfaden, et al., 2020). The surface tension that generates from condensates of chromatin-binding proteins can both pull distal chromatin fragments together and nudge the surrounding high-density chromatin background apart, creating space for membraneless compartments (Shin, et al., 2018). This mechanical influence of droplets alters the spatial layout of chromatin and plays a role in the formation of intracellular membraneless structures (Welsh, et al., 2018). Through multivalent interactions, these condensates also function as molecular filters, further solubilizing needed DNA, RNA, and proteins, simultaneously excluding unnecessary components (Nott, et al., 2016). With the concentration of these functionally interconnected biomolecules, precisely organized and functionally diverse membraneless structures are formed in the nucleus (Bhat, et al., 2021).

This model may explain the formation of compartments in the nucleus (e.g., nucleoli) to some extent. The assembly of the nucleolus starts at the rDNA transcriptionally active site formed by liquid-liquid PS (Shin, et al., 2018). UBF which has low sequence-specificity and extensively binding to the rDNA promotes transcription by repressing nucleosome reorganization in this scope (Moss, et al., 2019). Inside the UBF condensates, transcriptionally active rDNA forms discrete chromatin loops that function as the core of nucleolus assembly and disassembly (Maiser, et al., 2020). RNA Pol I catalyzes pre-47S rRNA synthesis. The cross-linking of nascent rRNA to rDNA promotes their intercalation with ribonucleoproteins and chromatin-binding proteins, contributing to the formation of mixed droplets (Lawrimore, et al., 2021) (Fig.3B). Several studies have been conducted to construct the link between PS and nucleolus assembly. When RNA-DNA cross-linked fragments are digested through overexpression of endogenous nucleases, the mutual

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solubility between ribonucleoproteins and chromatin-binding proteins decreases, followed by the disintegration of the hierarchically assembled nucleolus structure, suggesting that normal rRNA synthesis facilitates nucleolus assembly (Lawrimore, et al., 2021).

In the transcriptionally active nucleolus, condensates of Pol I confine rDNA to the FC region. Nevertheless, in a patient with a Pol I mutation and craniofacial malformations, the mutated Pol I seizes the rDNA motif and binds tightly, causing the normal Pol I to separate from rDNA and dissolve into the liquid structure derived from the FC region (Ide S, 2020). This suggests that both Pol I and newly synthesized rRNA play a role in regulating PS during nucleolar assembly (Fig.3A).

The evidence above reminds us of Pol I mutations in several ribosomal disorders, including TCS and AFDCIN. These mutations cause Pol I functional imbalance and abnormal PS of nucleolus components. Possible mechanisms in these circumstances include: (1) Self-association of components that should be miscible, such as rDNA, Pol I, and chromatin binding proteins, leading to excessive condensation and nucleolus dissociation. For instance, mutations in the Pol I core subunit POLR1A lead to affinity changes of Pol I for rDNA (Falcon, et al., 2022). Either reduced Pol I affinity leading to rDNA compaction or increased affinity leading to normal Pol I over condensed may contribute to the pathogenesis of AFDCIN. In addition, Treacle recruits SL1, required for Pol I transcription, to the rDNA promoter and assists UBF in replacing nucleosomes (Grzanka and Piekielko-Witkowska, 2021). Treacle insufficiency induced by the TCOF1 missense mutations reshapes the rDNA condensation and accounts for nucleolus dissociation by allowing chromatin to exhibit more solid properties (Grzanka and Piekielko-Witkowska, 2021); (2) the decreased inherent organization of the nucleolus triggers a weakening of the molecular filter, which could result in the intermingling of extraneous elements that disrupt the proper functioning of the nucleolus. Misfolded disordered proteins have been found to enter the granular component under stress without affecting the rRNA processing capacity of the nucleolus (Frottin F, 2019). However, there is a paucity of knowledge on the molecular filter function of the nucleolus. However, the existence of this phenomenon makes searching for unconventional components that mix into the nucleolus a direction to explore the mechanisms of ribosomopathies' generation.

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5.3 Abnormal PS within the nucleolus may impede ribosome synthesis orderliness

Components relevant to rDNA organization and transcription are clustered in the FC region at the center of the nucleolus, encapsulated by the peripheral DFC and GC. These two layers are replete of RBFs with intrinsic disorder regions (IDR), including NOPP140, NOP10, NHP2, NPM1, and FBL, all responsible for rRNA processing (Uversky, 2017).

Among the above RNA-binding proteins (RBFs), NPM1 and FBL have garnered the most focus. Feric et al. (2016) determined the physical properties of the nucleolus in vitro and in vivo, demonstrating that the nucleolus is an immiscible multiphase droplet composed of nucleic acids and proteins. The distinct structural features of the primary constituents in each stratum dictate the multiphase configuration. FBL, predominantly present in the dense fibrillar component (DFC), harbors a methyltransferase domain (MD) and a low-complexity sequence GAR domain (Shubina, et al., 2020). By self-conjugation PS, the GAR domain forms the DFC, whereas the MD binds nascent pre-47S rRNA and directs it from the FC/DFC interface, where transcription occurs, to the DFC and undergoes post-transcriptional processing (Yao, et al., 2019). In addition, NPM1, localized in the GC, has an oligomeric domain (OD) at its N-terminus that promotes the formation of NPM1 pentamer, a disordered ribonuclease active domain in the middle, and a C-terminal domain responsible for RNA binding (Box, et al., 2016; Mitrea, et al., 2016). Through multivalent interactions, the disorder regions of NPM1 and FBL alone achieve complete miscibility (Lafontaine, et al., 2021), whereas the presence of the RNA-binding and oligomeric domains drives the separation of GC from DFC (Feric, et al., 2016). Surface tension is responsible for the formation of such subcompartments within the nucleolus (Guillen-Chable, et al., 2020). Structural domains that are not miscible increase the number of intermolecular free sites and thus the entropy of the system (Pyo, et al., 2022). This discrepancy amplifies the difference in

the multivalent interactions between homologous molecules, enabling GC with lower surface tension to encapsulate DFC (Feric, et al., 2016). Consequently, the sequentially arranged nucleolus compartment functions as a "production line," ensuring the complicated rRNA processing and modification procedures are performed efficiently (Fig.3C).

In summary, the cooperation of protein factors is necessary for rRNA synthesis within the nucleolus. The negative impact of components such as UBF, Pol I, SL1, and Treacle on PS is reflected by normal nucleolus structure disassembly and rDNA transcription declination. Meanwhile, NPM1 and FBL affect rRNA post-transcriptional processing. In addition, some rPs (e.g., RPL5, RPL27A) can interact multivalently with rDNA and NPM1 after transfer to the nucleolus, modulating nucleolus assembly and morphology, which may explain the down-regulation of rDNA transcription in DBA (Matsumori, et al., 2022).

These factors could cause downstream effects through pathways, as described previously. For instance, the self-association of components that should be miscible can lead to excessive condensation and nucleolus dissociation. The decreased inherent organization of the nucleolus triggers a weakening of the molecular filter and may also result in the intermingling of extraneous elements that disrupt the proper functioning of the nucleolus. Thus, the phenomenon of aberrant PS disrupts multiple aspects of ribosomal genesis, leading to ribosomopathies. The investigation of the intricate network of PS structures within the nucleolus still faces numerous difficulties. For instance, it remains unclear that how the cell allows these components to both maintain a certain degree of orderly condensation throughout time and not to form irreversible cytotoxic condensates (Hondele, et al., 2020).

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6 Conclusion

Ribosome is an intracellular ribonucleoprotein particle that serves as the site of protein biosynthesis. Ribosomal dysfunction caused by mutations in genes encoding rPs and RBFs can lead to a spectrum of diseases collectively known as ribosomopathy. The definition of ribosomopathy covers highly heterogeneous disorders. Classification based on pathogenesis and screening for several categories of diseases related to rDNA transcription, rRNA post-transcriptional processing, ribosome assembly, and rPs would facilitate the exploration of common or distinct pathways in the development of different ribosomopathies. Unlike classification based on the site of disease initiation or inheritance pattern, this classification can better guide the prevention and early diagnosis of ribosomopathies, reducing the difficulty in treatment. However, classification by pathogenesis also has drawbacks. Firstly, ribosomopathies are often caused by a combination of multiple ribosomal biogenesis-related mechanisms, and it is difficult to measure the extent of their contribution, for example, understanding the regulatory role of RPL5 in rDNA transcription and ribosome assembly. Secondly, this classification does not demarcate the boundary between abnormal ribosome biogenesis and multifactorial pathogenesis in diseases like DC. Thus, there is still a long way to go to achieve an exhaustive, consensus classification of ribosomopathies.

Tissue specificity is a typical feature of ribosomopathies, focussing on abnormal ribosome biogenesis in specific tissues. This may be because affected tissues in ribosomopathies tend to have high metabolic or proliferative rates and require large numbers of ribosomes to fulfill their functions, such as the neural crest, bone marrow, pancreas, and germ cells. Ribosome deficiency disrupts the normal physiologic function of these tissues, leading to the corresponding clinical manifestations. In the neural crest, bone marrow, pancreas, and germ cells, the high expression of functionally relevant individual genes or groups of genes requires abundant ribosomes. Insufficient ribosome availability can lead to decreased expression or silencing of genes that are consistently highly expressed or exhibit intense activity during particular developmental phases. This can result in tissue developmental abnormalities by triggering downstream effects, like apoptosis, autophagy, and cell cycle interruption, through checkpoint activation. Moreover, irregular ribosome quantities are linked to the sensitivity of certain mRNAs to ribosomes, with genes displaying high ribosome sensitivity being more

vulnerable to deviations in ribosome levels. In contrast to ribosome quantity abnormalities, ribosome quality abnormalities also play a role in the tissue-specificity of ribosomopathies. When the quality control of rRNAs and rPs is insufficient, structurally imperfect rRNAs and rPs may be incorporated into the ribosome assembly, forming a subpopulation of dysfunctional ribosomes, triggering reduced translational fidelity and impaired protein synthesis, leading to ribosomal disease or exacerbating pre-existing-symptoms. The tissue-specificity of ribosomopathies reflects the special demand for ribosome quantity and quality in affected tissues. Thus, an in-depth study of the relationship between abnormalities in ribosome quantity and quality and tissue-specificity in ribosomopathies is of great significance in unraveling the disease mechanism and finding therapeutic strategies.

PS is a thermodynamic process that produces multiple phases from a homogeneous mixture. The phenomenon of biomolecule PS sheds some light on the possible causes of several ribosomopathies. Abnormal proteins synthesized by mutations in ribosomal disease-causing genes can cause an imbalance in Pol I function and abnormal PS of the nucleolus, leading to self-coalescence of otherwise miscible nucleolus components (e.g., rDNA, Pol I, and chromatin-binding proteins) and triggering nucleolus segregation. This reduction in intrinsic nucleolus components leads to a weakening of the molecular filter effect, which may result in the admixture of extraneous elements that interfere with the normal function of the kernel, thereby disrupting the normal function of the nucleolus. For now, little is known about the molecular filtering function of the nucleolus, but the existence of this phenomenon makes the search for unconventional components mixed into the nucleolus a direction for exploring the mechanism of ribosomal disease production. In addition, because different cells do not express the same genes, even mutations in genes that are widely expressed in most tissues may result in tissue-specific impairment of ribosomal function and corresponding pathological manifestations by binding to other tissue-specific proteins to form tissue-specific nucleolar three-dimensional structures or PS agglutinates. Studies of ribosomopathies need to delve into these phase-separating and tissue-specific effects to reveal deeper mechanisms of disease development. To explore the tissue-specificity of ribosomopathies and the related PS phenomena, in the future, it may be necessary to analyze gene expression using bulkRNA-seq and scRNA-seq, determine intracellular protein abundance using Label-free quantitative proteomics, monitor intracellular translation in real-time using ribosome-profiling, use co-inmunoprecipitation and ChIP-qPCR to study the differences in biomolecule interactions, and use in vitro droplet formation and fluorescence bleaching recovery experiments to explore the phenomenon of in vivo and in vitro PS.

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Data availability statement

This article is a review and does not involve the generation of new primary data. The information presented in this review is based on previously published research articles, books, reports, and other relevant sources. As such, the original datasets used in the individual studies cited are not directly available from the authors of this review. However, the references provided in this article can be used to locate and access the original data, if needed. We encourage readers to consult the original sources for further details on data availability and methodology. In cases where publicly available datasets or repositories were utilized in the cited studies, the corresponding references have been included in this review. Readers are advised to follow the data availability statements provided in the original studies to access and utilize these datasets. It is important to note that the authors of this review have not conducted any new experiments or analyses using the original datasets. The purpose of this review is to summarize and interpret the existing research in a given field. Therefore, data availability for this review article itself is not applicable.

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Author contributions

All authors contributed to the study conception and design. The first draft of the manuscript was written by Zhiyuan Pan

and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethics guidelines

Zhiyuan Pan, Guofen Lin, Hao Liu, Guozhi Li, Xiaoyi Zhang and Jiewen Dai declare that they have no conflict of interest. This is an review article. This article does not contain any studies with human or animal subjects performed by any of the authors. The Shanghai Jiao Tong university Research Ethics Committee has confirmed that no ethical approval is required. The datasets generated during and/or analysed during the current study are available in the PubMed repository, https://pubmed.ncbi.nlm.nih.gov.

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Figure Legends

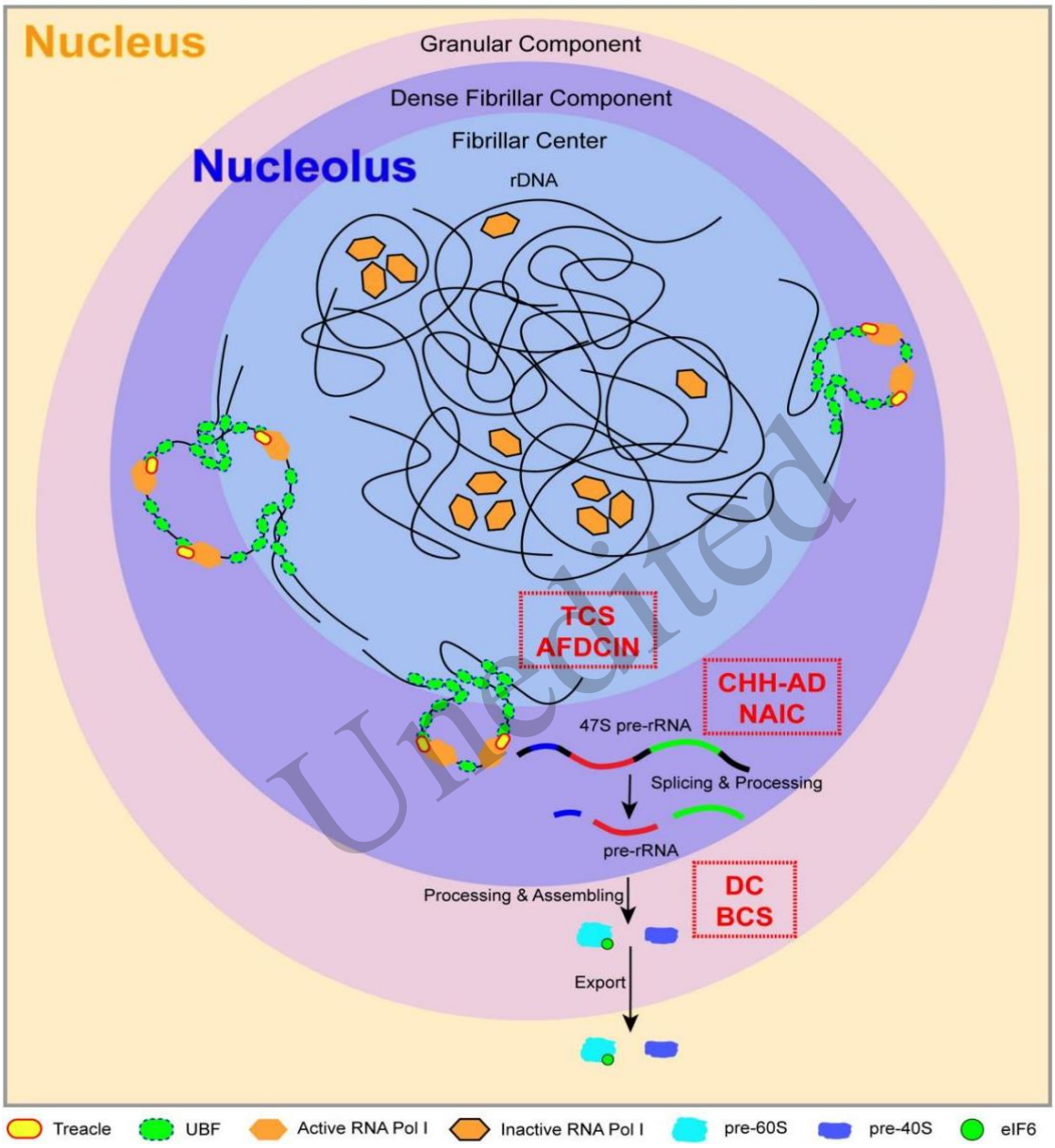

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Fig 1. Intra-nucleolar ribosome biogenesis process and associated ribosomopathies. The red dashed frames highlight the abnormal steps in ribosome biogenesis factors that give rise to several ribosomopathies: Treacher–Collins syndrome (TCS), Acrofacial Dysostosis—Cincinnati type (AFDCIN), Cartilage Hair Hypoplasia—Anauxetic dysplasia spectrum (CHH-AD), North American Indian Childhood Cirrhosis (NAIC), Dyskeratosis Congenita (DC/DKC), and Bowen–Conradi syndrome (BCS). The legend in the lower left corner identifies important ribosomal biogenesis factors and other components.

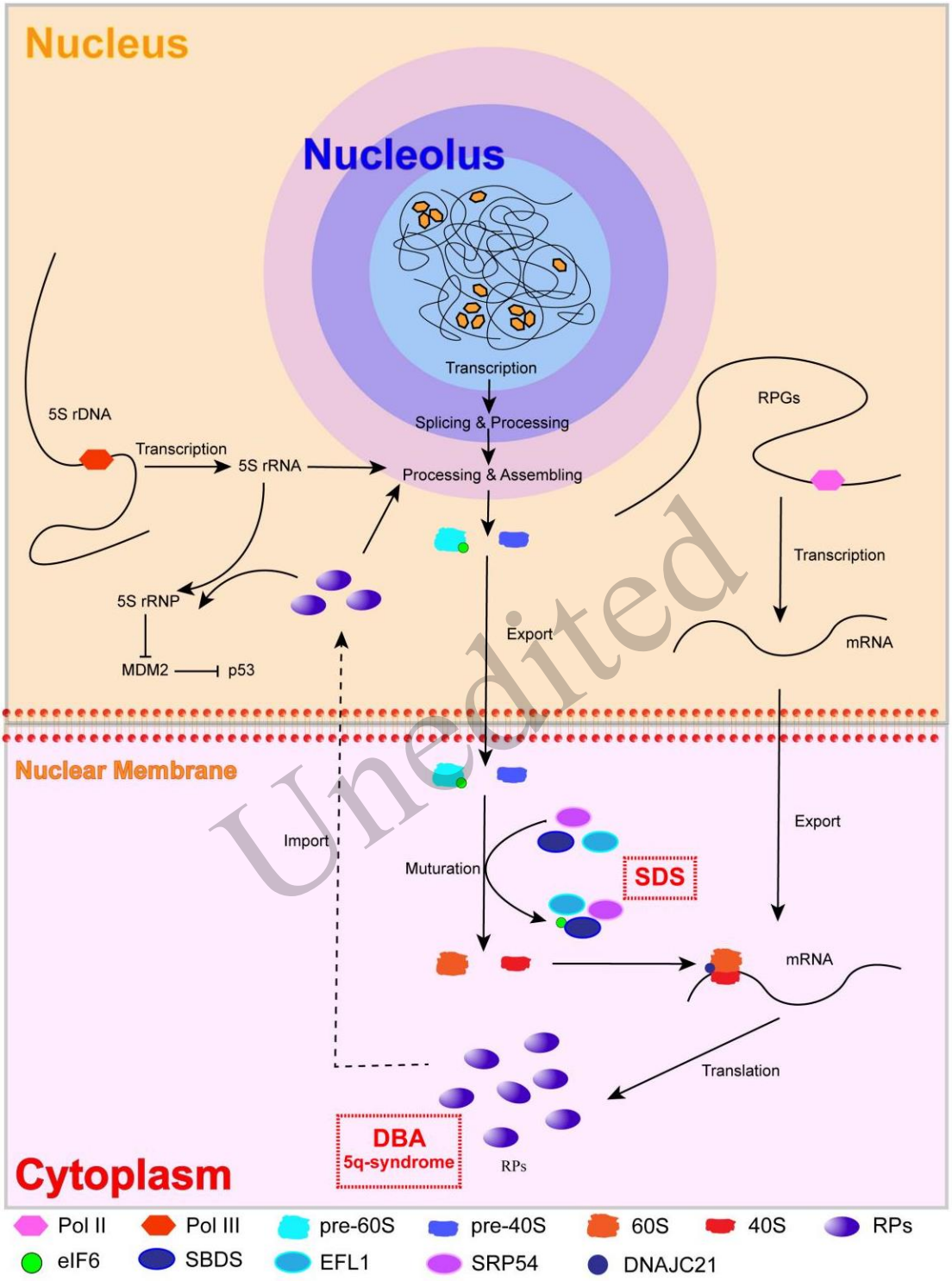

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Fig 2. Extra-nucleolar ribosome biogenesis process and associated ribosomopathies. This diagram simplifies ribosome biogenesis in the nucleolus. The red dashed frames highlight additional typical ribosomopathies: Shwachman–Diamond syndrome (SDS), Diamond–Blackfan anemia (DBA), and 5q-syndrome. The legend in the lower left corner identifies important ribosomal biogenesis factors and other components.

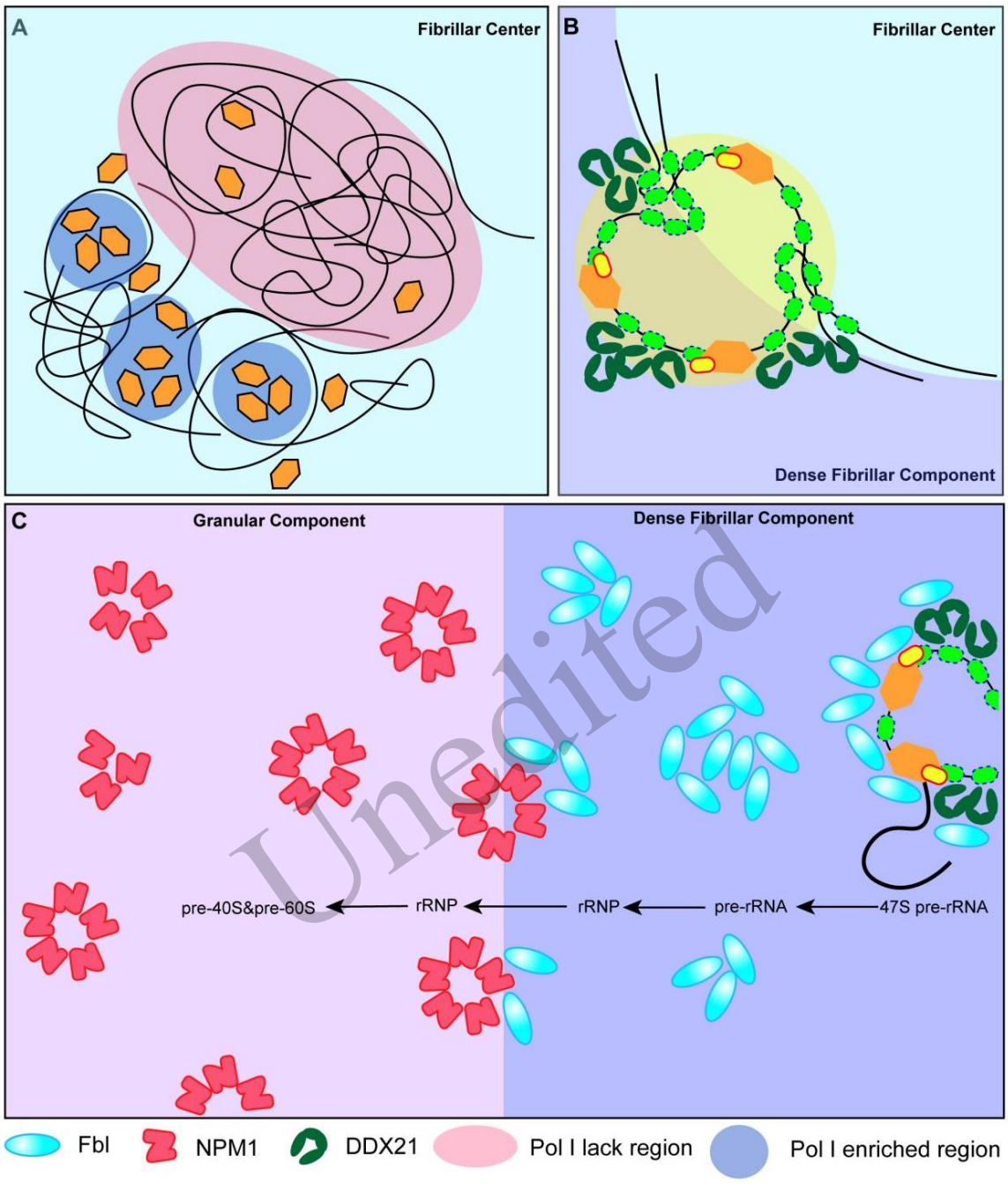

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Fig 3. Phase separation in the nucleolar organization and ribosome biogenesis. (A) Inactive Pol l in fibrillar center restrict transcriptionally inactive rDNA to specific regions. (B) The cross-linking of nascent rRNA to rDNA motifs promotes their intercalation with ribonucleoproteins and chromatin-binding proteins, contributing to the formation of mixed droplets. (C) Through multivalent interactions, DFC with higher surface tension is encapsulated by GC, functioning as a "production line" to enable efficient rRNA processing and modification.

Table 1. Mechanisms of ribosomopathies.

Classification	Disease	Gene	Function in ribosome biogenesis
rDNA transcriptional-associated ribosomopathy	Treacher-Collins syndrome	*TCOF1*	rDNA transcription, 18S pre-rRNA methylation, SSU maturation and modification
		POLR1B, POLR1C, POLR1D	Encoding RNA pol subunit, rDNA transcription
	AFDCIN	*POLR1A*	Encoding RNA pol subunit, rDNA transcription
rRNA post-transcriptional processing- associated ribosomopathy	CHH-AD	*POP1, RMRP*	Constitute RNase MRP, pre-5.8S rRNA splicing
	NAIC	*CIRHIN, NOL11*	pre-18S rRNA splicing
	Dyskeratosis Congenita	*Dyskerin, NOP10, NHP2*	rRNA pseudouridylation
		NPM1	rRNA methylation
Ribosome assembly- associated ribosomopathy	Shwachman-Diamond syndrome	*SBDS, SRP54, EFL1*	Assembly and maturation of LSU
		DNAJC2	Stabilization of assembled 80S ribosome
	Bowen-Conradi syndrome	*EMG1*	Binding RPS19, SSU assembly, 18S rRNA pseudouridylation
Ribosomal protein-associated ribosomopathy	Diamond-Blackfan anemia	*RPS19, RPS17, RPS26-29, RPL5, RPL11, RPL15, RPL18, RPL35, etc.*	Ribosomal proteins
	5q-syndrome	*RPS14*	Ribosomal protein

AFDCIN=Acrofacial Dysostosis—Cincinnati type; CHH-AD=Cartilage Hair Hypoplasia-Anauxetic dysplasia; NAIC=North American Indian Childhood Cirrhosis

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Table 2. Main affected areas of ribosomopathies.

Classification	Disease	Affected tissues, organs, and systems							
		Neural crest	**Bone Marrow**	**Hepatic**	**Pancreas**	**Immune System**	**Central Nervous System**	**Locomotor System**	**Reproductive System**
rDNA transcriptional-associated ribosomopathy	Treacher-Collins syndrome	√							
	AFDCIN	√						√	
rRNA post-transcriptional processing- associated ribosomopathy	CHH-AD		√			√		√	
	NAIC			√		√			
	Dyskeratosis Congenita	√	√			√			
Ribosome assembly-associated ribosomopathy	Shwachman-Diamond syndrome		√		√	√		√	
	Bowen-Conradi syndrome	√					√	√	
Ribosomal protein-associated ribosomopathy	DBA	√	√					√	√
	Diamond-Blackfan anemia		√						

AFDCIN=Acrofacial Dysostosis—Cincinnati type; CHH-AD=Cartilage Hair Hypoplasia-Anauxetic dysplasia; NAIC=North American Indian Childhood Cirrhosis

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