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RNA Polymerases I and III in development and disease

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Abstract

Ribosomes are macromolecular machines that are globally required for the translation of all proteins in all cells. Ribosome biogenesis, which is essential for cell growth, proliferation and survival, commences with transcription of a variety of RNAs by RNA Polymerases I and III. RNA Polymerase I (Pol I) transcribes ribosomal RNA (rRNA), while RNA Polymerase III (Pol III) transcribes 5S ribosomal RNA and transfer RNAs (tRNA) in addition to a wide variety of small non-coding RNAs. Interestingly, despite their global importance, disruptions in Pol I and Pol III function result in tissue-specific developmental disorders, with craniofacial anomalies and leukodystrophy/neurodegenerative disease being among the most prevalent. Furthermore, pathogenic variants in genes encoding subunits shared between Pol I and Pol III give rise to distinct syndromes depending on whether Pol I or Pol III function is disrupted. In this review, we discuss the global roles of Pol I and III transcription, the consequences of disruptions in Pol I and III transcription, disorders arising from pathogenic variants in Pol I and Pol III subunits, and mechanisms underpinning their tissue-specific phenotypes.

Keywords

RNA Polymerase I; RNA Polymerase III; Treacher Collins syndrome; POLR3-related leukodystrophy; rRNA; ribosome biogenesis

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1. Introduction

The regulation of cell growth, proliferation, survival, and death during development is a carefully coordinated process. Central to this process is the flow of genetic information from DNA to RNA to protein. Three distinct RNA polymerases transcribe all the RNAs necessary in eukaryotic cells. RNA Polymerase II (Pol II) primarily transcribes mRNAs which are translated into proteins, while Pol I and Pol III transcribe non-coding RNAs that are critical for cellular homeostasis, including those for making ribosomes, the macromolecular machines that translate mRNAs into proteins. Despite a global requirement for Pol I and III during development, disruption of genes encoding Pol I or Pol III subunits results in distinct, tissue-specific phenotypes. In this review, we discuss recent advances in our understanding of the function of Pol I and Pol III, how mis-regulation of these polymerases result in disease, and the mechanistic basis for tissue specific regulation of Pol I and III.

2.1 RNA Polymerase I

RNA Polymerase I is a multi-subunit complex that transcribes ribosomal RNA (rRNA) in the nucleolus of the cell. rRNA constitutes an estimated 60% of all transcription in a cell [1] and the production of rRNA is a rate-limiting step in the process of ribosome biogenesis [2]. Pol I is therefore essential for cell growth and survival [3–5]. In humans, Pol I consists of a 10-subunit core complex plus 3 additional subunits [6, 7] (Figure 1A). Five of the subunits present in Pol I, POLR2E, POLR2F, POLR2H, POLR2K, and POLR2L, are components of all three RNA polymerases. Two subunits of Pol I, RPAC1/POLR1C and RPAC2/POLR1D, are shared with Pol III. The remainder, RPA190/POLR1A, RPA135/POLR1B, RPA49/POLR1E, RPA43/POLR1F, RPA34/POLR1G, and RPA12/POLR1H are unique to Pol I and have distinct roles in Pol I transcription. POLR1A contains the active site of Pol I and interacts with POLR1B to form the DNA binding cleft. POLR1E, POLR1G and POLR1H contribute to Pol I passage through nucleosomes. The function of POLR1F is not as well understood but may be important for interactions with Pol I initiation factors [8, 9].

Pol I transcribes rRNA from rDNA, which is present in hundreds of tandem copies in the genome. The rDNA repeats are separated by intergenic spacer regions and a single repeat consists of an upstream control element, a core promoter, the 47S rDNA, and a downstream terminator element [10] (Figure 1B). Due to its highly repetitive nature, rDNA is subject to copy number variation, and the number of active repeats has dual roles in rRNA transcription and genome stability [11]. The number of active rDNA repeats is regulated by their chromatin status [11], and approximately 40% of rDNA repeats are typically silent in mammalian cells [12].

Pol I transcription requires Upstream Binding Transcription Factor (UBTF), Selectivity Factor 1 (SL1; known as TIF-IB in other vertebrates), and Transcription Initiation Factor IA (TIF-IA; RRN3 in yeast). Association of TIF-IA with Pol I is required for basal transcription initiation at the rDNA promoter [13, 14]. Full transcriptional activity depends on UBTF binding to the upstream promoter, which correlates with active rRNA genes [15], through its recruitment of SL1 to the core promoter [16]. Post-translational modifications including phosphorylation and acetylation of initiation complex proteins exhibit regulatory effects on Pol I transcription. These modifications can be activating or inhibiting and occur in

response to several signaling pathways including mTOR, AKT, p53, Rb, CK2, and ERK [17]. Transcription of rRNA is also dynamically regulated during the cell cycle through cyclin-dependent regulation of UBTF and SL1, with the highest levels occurring during S and G2 phases [18]. Additionally, Pol I can exist in an inactive dimer conformation in a manner sensitive to stress such as nutrient deprivation or inhibition of protein synthesis [19–21]. Thus, Pol I and its initiation factors are precisely regulated in response to several cellular events including cell cycle status, nutrient availability, and signaling, which helps to govern the amount of rRNA produced. This is important because rRNA synthesis is a key regulator of ribosome quantity and is integral to increased or decreased protein translation to meet a cell's specific growth, proliferation, or other metabolic needs.

2.2 RNA Polymerase III

RNA polymerase III (Pol III) is a 17-subunit enzymatic complex responsible for the transcription of ubiquitously expressed, small non-coding RNAs (small ncRNA) [22, 23] (Fig. 1C). POLR3A/RPC1 and POLR3B/RPC2 form the active site of the enzyme and are part of the 10-subunit core which also contains two subunits shared with Pol I (POLR1C, POLR1D), and five subunits shared with Pol I and II (POLR2E, POLR2F, POLR2H, POLR2L, POLR2K), and POLR3K/RPC11. The remaining seven subunits of Pol III form subcomplexes important for transcription initiation and termination. POLR3H and CRCP form the stalk complex, important for Pol III initiation, while POLR3C, POLR3F, and POLR3G form a heterotrimer and POLR3D and POLR3E form a heterodimer, both of which are important for transcription initiation and termination [24, 25]. Furthermore, Pol III can exist in two distinct isoforms defined by inclusion of subunit POLR3G or POLR3GL [26].

The function of Pol III can be defined by the roles of its transcripts, perhaps the most well-known of which are the 5S rRNA and transfer RNAs (tRNAs) [22, 23]. Both of these play crucial roles in protein synthesis, highlighting the critical housekeeping role for Pol III in all cells. As the sole rRNA not transcribed by Pol I, 5S rRNA is not only an integral component of the ribosome but thought to play an additional role in ribosome biogenesis through forming the peptidyl transferase center functional domain [27]. Meanwhile, tRNAs, all of which are transcribed by Pol III, are crucial for decoding mRNA transcripts to mediate translation [28]. In addition, Pol III transcribes multiple small ncRNA whose function can be broadly grouped into one or more of the following categories: 1) transcription; 2) RNA processing and/or localization; and 3) translation (Fig. 2). In terms of transcription, Pol III transcribes 7SK RNA, an indirect inhibitor of Pol II transcription, which illustrates a role for Pol III transcripts in the expression of protein-coding genes [29]. Pol III transcripts involved in RNA processing include U6 RNA, RNAse P RNA, and RMRP RNA. U6 RNA forms the active site of the spliceosome and works with Pol II-transcribed spliceosome RNAs to catalyze the removal of introns from pre-mRNA [30]. RNAse P RNA regulates tRNA transcription and facilitates the removal of the 5' leader sequence of pre-tRNA, creating a link between tRNA transcription and processing [31–33]. Meanwhile, RMRP RNA functions in pre-rRNA processing through its role in cleaving the common rRNA precursor produced by Pol I, highlighting an additional role for Pol III in ribosome biosynthesis [33]. Localization of RNA in a cell is assisted by the Pol III transcripts vault RNA and 7SL [22]. Notably, vault RNAs are involved in various

cellular pathways including regulation of mRNA, proliferation, differentiation, apoptosis, and autophagy [34]. However, the contribution of vault RNA to the nuclear pore complex implicates it in nuclear-cytoplasmic transport and, in turn, RNA localization [34]. 7SL is involved in targeting mRNA for secretion by serving as a scaffold for the signal recognition particle, a ribonucleoprotein that will recognize protein-coding transcripts destined for secretion and target them to the endoplasmic reticulum [22, 35].

Beyond these functions, certain Pol III ncRNAs play less canonical roles. Y RNAs are an emerging class of highly conserved, small ncRNAs which bind Ro60, a ring-shaped protein that associates with misfolded non-coding RNAs and pre-5S rRNA. Y RNAs play a perhaps counter-intuitive role by inhibiting Ro60 from binding aberrantly folded RNA, thereby inhibiting an RNA quality control mechanism [36]. Y RNA is also required for DNA replication initiation during the cell cycle [37]. Short Interspersed Element (SINE) retrotransposons are DNA repeat elements found abundantly throughout the human genome, the physiological impact of which are beginning to be elucidated. For example, SINEs such as Alu, most commonly thought of as “genetic parasites”, have been implicated in RNA editing and translation regulation [38]. Finally, the primate specific BC200 RNA, and its rodent counterpart Bc1 are expressed almost exclusively in neurons where they function in regulating local protein translation in dendrites [39]. Overall, the functions of the various small ncRNAs transcribed by Pol III illustrate the crucial and extensive roles for Pol III in all cells.

Pol III transcribes from three distinct promoter types, known as Type 1, Type 2, and Type 3, which vary based on the structure of the promoter elements and variable use of transcription initiation factors [40, 41]. Type 1 promoters utilize transcription initiation factors TFIIIA, TFIIIB, and TFIIIC and exclusively transcribe 5S rRNA. tRNAs and some small ncRNAs are transcribed by Type 2 promoters which utilize TFIIIB and TFIIIC, while other ncRNA transcripts are transcribed by Type 3 promoters. Type 3 promoters are unique among Pol III promoters for their use of upstream promoter elements and their transcriptional machinery, specifically the use of a different form of TFIIIB, which contains a BRF2 subunit instead of BRF1, and a small nuclear RNA activating protein complex (SNAPc) instead of TFIIIC [41]. As the only factor common to all Pol III promoter types, the three subunit TFIIIB complex is an important regulator of Pol III and consists of a TATA-binding protein (TBP), BDP1, and BRF1 or BRF2 [42].

Pol III activity in a cell is under control of the master regulator MAF1, a mechanism conserved from *Saccharomyces cerevisiae* to mammalian cells [43, 44], as well as tumor suppressor and oncogene pathways including p53 and RB1 [45, 46]. MAF1 inhibits Pol III activity in response to nutrient deprived conditions, as Pol III transcription places a large metabolic demand on a cell [47, 48]. In response to environmental and genetic stressors, Maf1 becomes dephosphorylated, triggering its import and accumulation in the nucleus. This enables Maf1 to bind to and allosterically inhibit Pol III [49–51], such that it cannot interact with the basal transcription machinery Brf1-Tbp promoter complex, thereby preventing Pol III transcription at the initiation step [51, 52]. Maf1 also binds elongating Pol III, playing an additional repressive role by preventing re-initiation and local recycling of the complex [51]. MAF1 in humans is thought to be more evolved and play a

more complex role than in yeast. While Maf1 in yeast can function under normal growth conditions, it is predominantly active during stress, such as nutrient deprivation. In contrast, MAF1 in humans has been shown to have a significant role in mediating Pol III-repression in unstressed conditions [44]. Furthermore, in humans, MAF1 represses Pol III function through an additional indirect function, physically binding to and regulating BRF1 as well as the gene that encodes TBP [44], thus acting through both direct and indirect mechanisms. Pol III transcription is further regulated by the tumor suppressor proteins p53 and RB1 [45, 46]. Similar to MAF1, p53 inhibits Pol III transcription at the initiation step, in this case by binding the TBP subunit of TFIIIB and preventing TFIIIB interaction with other transcription factors at the promoter site [46, 53]. Thus, p53 blocks the formation of the basal transcription machinery complex and in turn, halts the recruitment of Pol III to its DNA target sites [53]. Similarly, the RB1 tumor suppressor protein also blocks the formation of the basal transcription machinery complex and prevents Pol III recruitment by binding to BRF1 in TFIIIB [45, 48]. MAF1, p53, and RB1 therefore all function, at least in part, by hindering the activity of TFIIIB, the transcription initiation factor ultimately responsible for Pol III recruitment and thereby blocking transcription initiation [45, 48, 53, 54].

2.3 RNA Polymerases I and III in Ribosome Biogenesis

Ribosome biogenesis, the process of making ribosomes, occurs in the nucleolus, a membrane-less structure within the nucleus [55] that has important regulatory functions in the cell [56] (Figure 3A). This process commences with the transcription of various types of RNA by RNA Pol I and Pol III, which ultimately constitutes about 80% of all nuclear transcription in a cell, and up to 95% of all RNA content [57]. The transcription of 47S pre-rRNA by Pol I is coordinated with rRNA processing, and their subsequent integration with ribosomal proteins [58, 59]. As the 47S rRNA is transcribed, processing, which involves numerous nucleolar proteins and small nucleolar RNAs (snoRNAs), then occurs to remove the Externally Transcribed Spacer (ETS) and Internally Transcribed Spacer (ITS) regions to make the 5.8S, 18S, and 28S rRNAs, which are modified post-transcriptionally and incorporated into ribosomes [60, 61]. These post-transcriptional modifications contribute to the structure of rRNAs with modified bases tending to cluster within functionally important domains in the mature ribosome [62]. Pol III-mediated transcription produces 5S rRNA as well as non-coding RNAs involved in rRNA processing during ribosome biogenesis [63, 64]. The 5S, 5.8S and 28S rRNAs, together with large subunit ribosomal proteins (RPLs) form the large 60S ribosomal subunit, while the 18S rRNA is assembled with small subunit ribosomal proteins (RPSs) to form the small 40S ribosomal subunit (Figure 3A). Once exported to the cytoplasm, 40S and 60S pre-ribosomes undergo further maturation, and associate with an mRNA to form the 80S ribosome, which then functions in protein synthesis.

3. Disorders caused by disruptions in Pol I

Disruptions in any step of ribosome biogenesis can lead to disorders known as ribosomopathies, which display a wide range of tissue-specific phenotypes [65, 66]. Here we highlight two broad categories of developmental disorders, craniofacial and neurodevelopmental, which are considered ribosomopathies due to perturbed Pol I function.

3.1 Craniofacial disorders

The craniofacial complex is an intricate assemblage of primary sense organs, central and peripheral nervous systems, and musculoskeletal components of the head and neck. The blueprint for human craniofacial development is established early in embryogenesis but is sensitive to genetic and environmental perturbation [67]. Craniofacial anomalies account for about one-third of all congenital birth defects and are typically attributed to disruptions in neural crest cell (NCC) development [68–70]. NCCs are a dynamic, multipotent progenitor population that arise at the neural plate border, undergo an epithelial-to-mesenchymal transition (EMT), and migrate throughout the embryo, giving rise to a variety of different cell types and tissues [71, 72]. For example, NCCs generate most of the cartilage and bone in the head and face, and also contribute to tooth development [72, 73].

3.1.1 Treacher Collins syndrome—Treacher Collins syndrome (TCS) is a rare mandibulofacial dysostosis condition that occurs with an estimated incidence of 1:50,000 live births [74]. The primary features of TCS include micrognathia, maxillary and malar hypoplasia, downward slanting palpebral fissures, microtia, and coloboma of the eyelid (Fig. 4A, B). Some individuals also present with cleft palate and conductive hearing loss [75–78]. TCS has been associated with pathogenic variants in four genes to date; *TCOF1* (TCS1; OMIM: 154500), *POLR1B* (TCS4; OMIM: 618939), *POLR1C* (TCS3; OMIM: 248390), and *POLR1D* (TCS2; OMIM: 613717) which can be inherited in an autosomal dominant (*TCOF1*, *POLR1B, POLR1D*) or autosomal recessive (*POLR1C*, *POLR1D*) manner [74, 79–82]. Pathogenic variants in *TCOF1* are responsible for about 80% of TCS cases, whereas variants in *POLR1B, POLR1C*, and *POLR1D* contribute to a relatively small proportion of cases. However, in some instances of TCS, the pathogenic variant remains unknown [75, 76, 83]. *TCOF1* encodes the nucleolar phosphoprotein Treacle, which together with UBF and SL1, forms a complex with Pol I and functions in rDNA transcription and rRNA processing [74, 81, 84–87]. The majority of *TCOF1* variants result in nonsense-mediated mRNA decay or carboxy terminus truncation of the nuclear and nucleolar import motifs in Treacle, which lead to its accumulation in the cytoplasm and inability to promote rDNA transcription. POLR1C and POLR1D are subunits of Pol I and Pol III, which raises the question of whether TCS is both a Pol I and Pol III associated disorder. Modeling of TCS specific *POLR1C* variants indicated they disrupt the localization of POLR1C in the nucleolus and therefore primarily affect Pol I function with little to no effect on Pol III structure or function [6, 88]. However, analysis of a pathogenic *POLR1D* variant in yeast suggests that it can disrupt both Pol I and Pol III [89], though the relative contribution of Pol III to TCS remains to be determined. The recent identification of pathogenic variants in Pol I specific *POLR1B*, however, provide further evidence that TCS is primarily caused by Pol I dysfunction [80, 90].

Mouse and zebrafish models have revealed common mechanisms underlying perturbed Pol I function in the pathogenesis of TCS. Mice that are haploinsufficient for *Tcof1* exhibit cranioskeletal anomalies characteristic of TCS in humans [91] (Fig. 4C). *Tcof1* loss-of-function results in diminished rRNA transcription, which induces p53-dependent cell cycle arrest and apoptosis particularly within the neuroepithelium and progenitor NCCs. This leads to the generation of fewer NCCs, which exhibit reduced proliferation, underpinning

hypoplasia of the craniofacial skeleton [91–93]. This mechanism has also been demonstrated in *polr1c* and *polr1d* homozygous mutant zebrafish [94] (Fig. 4D) and in *polr1b* morphant zebrafish [80]. Furthermore, conditional deletion of *Tcof1* and *Polr1c* within NCCs revealed that these genes are required in a cell autonomous manner for NCC survival during early embryo development in mice [92].

3.1.2 Acrofacial dysostosis, Cincinnati type—Pathogenic variants in the largest subunit of Pol I, *POLR1A*, are associated with Acrofacial Dysostosis, Cincinnati Type (AFDCIN; OMIM: 616462) [95], which phenotypically overlaps with TCS. Affected individuals exhibit micrognathia and downward slanting palpebral fissures but can also present with hypoplasia of the zygomatic arches and maxilla. What distinguishes AFDCIN from TCS is the variable presence of limb anomalies [95]. Recent work suggests two AFDCIN variants may affect the active site of Pol I [6], and analysis of the E593Q variant in human cells revealed that mutant POLR1A segregated to the nucleolar periphery into nucleolar caps [96], structures observed in concert with Pol I inhibition and nucleolar stress [97]. AFDCIN may occur as a consequence of *POLR1A* haploinsufficiency, similar to *TCOF1* in TCS, but recent studies suggest that the E593Q variant acts in a dominant negative manner to inhibit rRNA transcription [96].

AFDCIN pathogenesis also mechanistically overlaps with TCS. *polr1a* loss-of-function in zebrafish results in diminished rRNA transcription and ribosome biogenesis, which induces p53-dependent cell cycle arrest and apoptosis particularly within the neuroepithelium and progenitor NCCs. This leads to the generation of fewer NCCs, which exhibit reduced proliferation, underpinning the absence of most of the craniofacial cartilage [95]. Furthermore, conditional deletion of *Polr1a* in NCCs in mice revealed that this gene is required in a cell autonomous manner for NCC survival [92]. Interestingly, *Polr1a* conditional knockout mice and *polr1a* zebrafish exhibit similar but slightly stronger phenotypes than *Polr1c/polr1c* or *polr1d* animals (Fig. 4D), which reflects the more critical requirement for *Polr1a/polr1a* in Pol I as it contains the catalytic site for rRNA transcription.

The similar activation of p53 in the pathogenesis of TCS and AFDCIN is indicative of a conserved underlying molecular mechanism responding to Pol I disruption [92, 94, 95, 98, 99]. Under conditions of normal growth and proliferation, rRNA and ribosomal proteins exist in a stoichiometric ratio conducive to ribosome formation to meet a cell's specific needs. At the same time, Mdm2 (Murine double minute 2), an E3 ligase, binds to and ubiquitinylates p53, targeting it for proteasomal degradation (Figure 3B) [100–102]. However, when rRNA transcription is disrupted, this creates an imbalance between rRNAs and ribosomal proteins, resulting in an excess of free ribosomal proteins [103]. A ribonucleoprotein (RNP) complex of ribosomal proteins RPL11 and RPL5 together with 5S rRNA, called the 5S RNP then binds to Mdm2. The ensuing conformational change in Mdm2 renders it incapable of binding to and ubiquitinylating p53 [104], which leads to the stabilization and accumulation of p53, and consequently cell cycle arrest and apoptosis (Figure 3C). A point mutation in Mdm2 which specifically disrupts binding of the 5S RNP [105] can prevent p53 activation, confirming the importance of the 5S RNP-mediated mechanism in regulating p53 activity in response to disrupted ribosome biogenesis.

Consistent with p53 activation in response to diminished rRNA transcription, there is decreased binding between Mdm2 and p53 in *Tcof1*, *Polr1a*, and *Polr1c* mutant mouse embryonic fibroblasts [92]. Inhibition of *p53* prevents neuroepithelial and NCC death and rescues the craniofacial anomalies and viability of $Tcof1^{+/-}$ mice [98], and improves craniofacial cartilage development in *polr1a*, *polr1c* and *polr1d* mutant zebrafish, but not their viability [94, 106]. This suggests that both p53-dependent and p53-independent pathways act in response to perturbations in rRNA transcription in these models of TCS and AFDCIN.

3.2 Neurodevelopmental disorders

While the majority of pathogenic variants in genes encoding Pol I subunits described to date affect craniofacial development, several studies have indicated a potential role for Pol I in neurodevelopment. In fact, animal models with mutations in *Tcof1, polr1a, polr1c*, and *polr1d* display microcephaly in addition to their cranioskeletal anomalies [94, 95, 107]. Consistent with this neurodevelopmental phenotype, Pol I transcription has previously been shown to be required for the survival of neural precursor cells and cortical neurons [108, 109], as well as influence neurite length and branching in hippocampal neurons [110]. Altogether, these studies indicate that rRNA transcription plays an important role in neurodevelopment and disruptions in Pol I subunit or initiation factor function have the potential to disrupt neurological development and maintenance.

3.2.1 Leukodystrophy—A novel pathogenic variant in *POLR1A* was recently identified in a family with features of neurodegenerative disease [111]. The two affected individuals displayed cerebellar features (i.e. head titubation, truncal ataxia), pyramidal signs (i.e. spasticity) and intellectual disability. One sibling displayed neurodegeneration while the other had seizures. Brain MRI revealed white matter abnormalities consistent with a leukodystrophy, as well as cerebral atrophy, thin corpus callosum and cerebellar atrophy/hypoplasia. In contrast to AFDCIN associated *POLR1A* variants, the *POLR1A* c.2801C>T (p.Ser934Leu) neurodegeneration variant is inherited in an autosomal recessive manner [111]. Nucleolar expression of POLR1A was reduced in patient-derived fibroblasts [111], but how this variant functionally changes rRNA transcription in these cells remains to be determined. The identification of additional individuals with a similar clinical presentation are needed to confirm the typical pattern of inheritance for *POLR1A* associated leukodystrophy and also whether neurodegeneration variants are structurally or functionally distinct from those causing AFDCIN. Furthermore, it will be important in future studies to define the consequences of distinct variants in *POLR1A* to understand the specific role of POLR1A in neurological development.

3.3 Tissue specificity and Pol I transcription

Studies in mice and zebrafish have demonstrated the essential requirement for *Polr1a, Polr1b, Polr1c, Polr1d, and Tcof1* in embryo survival [92, 94, 95, 106, 112, 113]. Although transcription by Pol I is required in all cells, disruptions in Pol I transcription result in distinct, tissue-specific phenotypes. This is confounding given the global importance of rDNA transcription and ribosome biogenesis and implies there must be tissue-specific requirements and/or regulators of rDNA transcription and ribosome biogenesis, or cell and

tissue-specific threshold sensitivities to their disruption. One attractive hypothesis is that different tissues have distinct requirements for Pol I-mediated rRNA transcription. During development, rRNA is differentially expressed in the mouse eye [114] and neuroepithelium [92], giving rise to the idea that the regulation of rRNA transcription may be cell-type specific [115]. Interestingly, rRNA is downregulated during cellular differentiation [116–119] and premature downregulation of rRNA can trigger precocious differentiation [116]. Therefore, the tissue-specificity of TCS and AFDCIN may be due to a particular requirement for rRNA transcription in NCCs. In fact, NCCs display high levels of rRNA compared to other cell types during early craniofacial development [92]. How differential rRNA levels and thus regulation of Pol I activity are achieved is not well understood.

Dynamic and elevated *polr1a, polr1c*, and *polr1d* mRNA expression is evident in the head and central nervous system around 1 day post fertilization during zebrafish embryonic development [94, 95]. Similarly, mouse embryos exhibit broad expression of *Polr1a, Polr1c*, and *Polr1d*, with slightly elevated expression within the neuroepithelium [92], the location of neural and NCC progenitors. *Tcof1* is also broadly expressed with elevated levels in the neuroepithelium and cranial mesenchyme [91, 92]. Furthermore, single-cell RNA sequencing revealed that Pol I genes as well as associated factor *Tcof1* are enriched in NCCs [92]. This suggests that the high levels of rRNA transcription are underpinned by high levels of Pol I subunit expression and therefore Pol I function. Consistent with this idea, higher levels of *Ubtf* expression, which is also required for increased rRNA transcription, are found in NCCs and portions of the central nervous system in *Xenopus* [120] and mouse [121] embryos. The levels of Pol I subunits also tend to correlate with rRNA levels in cancer. Upregulated expression of Pol I subunits correlates with increased rRNA levels in various cancer models [122–124], and are associated with increased tumor size [123] and/or poor prognosis [125, 126]. In addition to the levels of Pol I subunit expression, tissue-specific transcription factors may also regulate rRNA transcription in specific tissues as has been demonstrated in bone development [116, 127] and EMT [128]. Hypoxia may also upregulate Pol I transcription and reprogram 2’-O-methylations [129], and hypoxia is particularly prevalent within the neuroepithelium during embryonic development [130, 131]. Furthermore, changes in the number of active rDNA repeats [132, 133], or regulation of the Pol I transcription initiation machinery in response to the cellular conditions [17, 18] may also contribute to differential regulation of rRNA. These examples add support to the idea that tissue-specific factors may interact with the rDNA promoter to regulate levels of rRNA transcription.

There are still many open questions about how distinct pathogenic variants in Pol I subunits change rRNA levels and its regulation in different tissues, and the relative contributions of the proposed mechanisms described above. Further exploration of the structural and functional consequences of pathogenic variants in Pol I subunits as well as roles for Pol I subunits in a tissue-specific manner in multiple model systems including human cells, yeast, zebrafish, and mouse models will advance our understanding of the tissue-specific nature of Pol I transcription as well as the conserved roles for Pol I throughout evolution.

4. Disorders caused by disruptions in Pol III

Pol III performs fundamental, constitutive functions in all cells. Nonetheless, biallelic pathogenic variants in genes encoding subunits of Pol III (*POLR3A, POLR3B, POLR1C, POLR3K* and *POLR3GL*) have been found to cause a heterogenous group of disorders with overlapping characteristic neurodevelopment features (Table 1).

4.1.1 Hypomyelinating leukodystrophy and neurodevelopmental disorders

A subset of leukodystrophies, inherited white matter disorders of the brain, were the first diseases found to arise from abnormal Pol III function. Biallelic pathogenic variants in *POLR3A* (OMIM 607694) [134] and *POLR3B* (OMIM 614381) [135, 136], encoding the two largest subunits of Pol III and forming its catalytic core, were found to cause five hypomyelinating leukodystrophies: leukodystrophy with oligodontia [137]; ataxia delayed dentition with hypomyelination [138]; 4H syndrome [139]; hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum [140]; and tremor ataxia with central hypomyelination [141]. Because of the overlapping clinical and radiological features, together with the identification of *POLR1C* (OMIM 616494) [88] and *POLR3K* (OMIM 619310) [142] as causative genes, these disorders are now recognized as POLR3-related hypomyelinating leukodystrophy (POLR3-HLD) or 4H (Hypomyelination, Hypodontia and Hypogonadotropic Hypogonadism) leukodystrophy [143]. Variants in *POLR3A*, *POLR3B*, *POLR3K* and *POLR1C* genes include missense, nonsense, small insertions or deletions, exonic or intronic splice site variants and large exonic deletions [88, 134, 135, 143–148].

Individuals with POLR3-HLD display diffuse hypomyelination [149] with sparing of early-myelinating structures (i.e. dentate nucleus, optic radiation, anterolateral nucleus of the thalamus, globus pallidus, and, in some patients, corticospinal tracts at the level of the posterior limb of the internal capsule), with or without thinning of the corpus callosum and cerebellar atrophy [145, 150]. Phenotypically, these individuals present with varying degrees of neurological and non-neurological manifestations. The former consists predominantly of motor signs, including cerebellar (e.g., ataxia, dysmetria, dysarthria), pyramidal (e.g., spasticity) and extrapyramidal (e.g., dystonia) signs with variable cognitive involvement [145, 148, 151, 152]. The later includes abnormal dentition (e.g., hypodontia, delayed dentition, natal teeth), endocrine abnormalities (e.g., hypogonadotropic hypogonadism leading to absent, delayed or arrested puberty, short stature with or without growth hormone deficiency) and ocular abnormalities (commonly myopia) [145, 148, 153–157].

Most patients present in early childhood with motor anomalies and exhibit disease progression over several years. A minority of patients present late in childhood with intellectual disability or cognitive plateauing and then subsequently develop motor anomalies. There is however a wide phenotypic spectrum, including a very severe form presenting in the first few months of life with failure to thrive, prominent hyperkinetic movement disorder, rapid developmental regression and premature death, to an extremely mild form of the disease diagnosed incidentally in adult patients via brain MRI [145, 158, 159]. Interestingly, the severely affected patients all carry a nonsense variant in a compound heterozygous state with a specific *POLR3A* splice site variant that has been previously implicated in a striatal phenotype involving the putamen, caudate nucleus and

red nucleus, but in which affected individuals have normal myelination [160–163]. Biallelic *POLR3B* variants have also been identified in individuals with isolated Hypogonadotropic Hypogonadism [164]. Some patients with variants in *POLR3B* also present with endosteal sclerosis [165].

The pathophysiology of POLR3-HLD remains somewhat enigmatic, though the fact that no patient carrying two null mutations have been reported indicates that the complete lack of POLR3 activity is incompatible with life [134]. POLR3-HLD causing mutations have been shown to hinder the biosynthesis or stability of the Pol III complex, alter the ability of Pol III to interact with DNA and/or lead to less protein expression of the affected subunit suggesting a common hypomorphic outcome [24, 88, 134, 135, 166–168].

POLR3-HLD is not the only POLR3-related disorder. Recently, it was shown that *de novo* pathogenic variants in *POLR3B* cause a completely different phenotype characterized by ataxia, spasticity, and demyelinating sensorimotor peripheral neuropathy, with variable intellectual disability, motor delay and epilepsy, but normal brain myelination [169]. Further, these individuals do not display endocrine dysfunction, growth defects, dental or ocular abnormalities. A recent report of one patient with a previously published *de novo POLR3B* variant [169] displaying isolated demyelinating peripheral neuropathy suggests that this novel POLR3-related disorder is also associated with a spectrum of severity [170]. Protein modelling and affinity purification coupled to mass spectrometry has shown these *de novo POLR3B* variants either cluster in a region of POLR3B that interacts with DNA and/or at a subunit interface, impacting the interaction between POLR3B and either POLR3A, POLR3C, POLR3F, POLR2H, POLR2K, or CRCP. This is distinct from POLR3-HLD where mutations at subunit interfaces are thought disrupt the entire POLR3 complex [166]. Moreover, in fibroblasts cultured from an individual with a *de novo* mutation in *POLR3B*, there was no reduction of POLR3B expression, indicating that the variants have the potential to function in a dominant-negative manner [169].

4.1.2 Wiedemann-Rautenstrauch syndrome

Beyond the critical role Pol III seems to play in the nervous system, mutations in *POLR3A* (OMIM 264090)*, POLR3GL* and, most recently, *POLR3B*, have been shown to underlie some cases of Wiedemann-Rautenstrauch syndrome (WRS) [171–176]. WRS is a rare genetic disorder with heterogeneous clinical features including intrauterine growth restriction, poor postnatal growth, facial dysmorphia, and lipodystrophy [177, 178]. Similar to POLR3D-HLD, dental abnormalities including natal teeth and hypodontia are common in WRS, while myopia and hyperopia are found in a subgroup of patients [173]. Typically, no hypomyelination is observed in WRS, and most individuals display normal motor function, cognition and speech [173]. However, a subset of patients exhibits developmental delay and/or hypotonia, and one individual has been reported with cerebellar signs, muscle weakness and unintelligible speech [171–173]. Short stature is almost universal in WRS, whereas only 61% of individuals with POLR3-HLD are similarly affected [145, 153, 173]. Additionally, while not present in POLR3-HLD, WRS individuals have characteristic facial features including mandibular hypoplasia, triangular face, widened fontanelles and pseudohydrocephalus [173]. WRS-associated variants in genes encoding Pol III subunits

are thought to cause partial loss of Pol III function, with *POLR3A* mutations disrupting biogenesis or stability of the Pol III complex, while at least one *POLR3B* missense mutations lies in the catalytic site and disrupts Pol III-DNA interactions [24, 166, 173, 174]. The reported homozygous *POLR3GL* mutation is a nonsense mutation thought to be degraded via nonsense-mediated mRNA decay and shown to cause an 84% reduction in overall *POLR3GL* mRNA, indicative of a severely hypomorphic state [171].

4.1.3 Endosteal Hyperostosis

Recently, exome sequencing identified *POLR3GL* mutations in three individuals with endosteal hyperostosis, oligodontia, growth impairment, dysmorphic facial features and in two of those individuals, delayed puberty (OMIM 619234) [179]. Other than delays in motor development which were seen in all individuals, neurological impairment was minimal, and no ocular abnormalities were present. One individual exhibited a thin corpus callosum, without hypomyelination. The variants in *POLR3GL* reported to date are all splice site variants, found in either a homozygous or compound heterozygous state, and cause skipping of exon 2, which is involved in translation initiation, or exon 5, that is part of the domain thought to mediate interactions between POLR3GL and POLR3C [180]. RNA-sequencing of blood revealed an absence of full length *POLR3GL* RNA [179]. Since POLR3GL is the sole POLR3 subunit with an isoform, and POLR3G, has been shown to compensate for POLR3GL *in vivo*, this perhaps explains how the lack of wild-type POLR3GL does not affect viability [181].

4.1.4 Disorders associated with Pol III co-factors and transcripts

Biallelic variants in genes encoding proteins that interact with Pol III have been shown to cause a phenotype reminiscent of POLR3-related disorders. These include variants in *BRF1*, a subunit of the transcription factor TFIIIB, which is involved in recruiting Pol III to its DNA targets [182]. Hypomorphic biallelic pathogenic variants in *BRF1* alter Pol III recruitment and transcription, and affected individuals present with cerebellar hypoplasia and thin corpus callosum, as well as non-neurological features such as dysmorphic facial features, dental abnormalities, and short stature [182]. Conditional deletion of *Brf1* in mice results in perturbed 5S rRNA and tRNA transcription, diminished 80S ribosome production and loss of translation [183]. These studies suggest that *Brf1* may be required in a dynamic spatiotemporal manner. Overall, this cerebellar-facial-dental syndrome further implicates Pol III hypofunction in this specific subset of tissues.

Mutations in certain Pol III transcripts cause disorders with partially overlapping phenotypic features. For example, hypomorphic mutations in *RMRP* RNA, the Pol III transcript involved in processing the common rRNA precursor produced by Pol I, is known to underlie various forms of skeletal dysplasia, including cartilage-hair hypoplasia (CHH), anauxetic dysplasia (AD) and kyphomelic dysplasia (KD) [184, 185]. These three disorders share abnormalities in connective tissues, which manifest as short stature, while individuals with AD also exhibit mild intellectual disability and abnormal dentition, and patients with KD display mild facial dysmorphism [185]. Each disorder demonstrates a critical role for Pol III transcription in connective tissues, dentition, and neurodevelopment.

4.2 Tissue specific regulation of Pol III and Pol III transcripts

How certain tissues like the cerebral white matter are particularly vulnerable to reduced Pol III function remains intriguing. Spared early myelinated structures suggests myelination arrest during development [145]. There are two major pathophysiological hypotheses, the first being that reduced Pol III transcription leads to insufficient tRNA synthesis during a critical timepoint in development, such as during the high-protein demand of central nervous system myelination, and the second being that specific Pol III-transcribed ncRNAs are required for myelination [54, 88, 186]. Moreover, the pathogenesis of tissue-specific phenotypes as a result of disruption in Pol III regulatory factors suggest that there are tissue-specific responses to changes in Pol III transcription which may be relevant to multiple Pol III-related disorders.

4.2.1 Tissue-specific regulation of Pol III transcription—Tissue-specific regulation of Pol III may be dependent upon Pol III initiation factors as well as negative regulators. Pol III is regulated indirectly via its basal transcription factors TFIIIB and TFIIIC, both at the level of their expression and phosphorylation-mediated ability to interact, and through proposed epigenetic mechanisms that modulate Pol III interaction with its target genes [187, 188]. TFIIIB and TFIIIC also influence Pol III-mediated transcription by regulating tRNA levels in response to growth conditions or signaling pathways [57, 187]. Furthermore, activity of negative regulator Maf1 [48] is modulated in response to nutrients and stress signaling pathways [189]. Null or conditional mutations of *Maf1* in mice result in tissue-specific phenotypes related to formation of the mesoderm lineage, adipogenesis, and bone density, all of which are associated with increased Pol III activity [190–192].

Recent studies have suggested additional Pol III regulation mechanisms such as the SUMOU-biquitin-Cdc48 segregase pathway, which was identified in *S. cerevisiae* as targeting Pol III for proteasomal degradation [193]. Interestingly, this mechanism seems to preferentially target defective Pol III by recognizing defects in transcription initiation and/or elongation that cause Pol III to stall on the chromatin [193]. The prerequisite that the Pol III complex be formed and perhaps chromatin-bound likely limits the relevance of this pathway in many disease-causing *POLR3* mutations that elicit defects in Pol III complex biogenesis or DNA interaction [24, 166]. However, certain POLR3-HLD causing mutations, which result in a growth defect in yeast can be rescued by disrupting this cascade [193]. Overall, the regulation of Pol III is quite complex, which is not surprising considering the critical role of Pol III in all cells and its association with multiple diseases.

4.2.2 Tissue-specific expression of Pol III and Pol III transcripts—Pol III transcribes multiple ncRNAs that likely contribute to the tissue-specificity of phenotypes arising from Pol III disruption. Similar to Pol I, the expression levels of these RNA transcripts as well as that of Pol III subunits may be cell type specific. In zebrafish, the expression of *polr3b* dynamically changes during development [194], similar to that of *polr1c* and *polr1d* [94]. *polr3b* expression is initially ubiquitous before becoming enriched in the central nervous system and developing gut [194]. *Polr3g* and *Polr3gl* have overlapping but distinct expression patterns in *Xenopus* embryos, with *Polr3gl* being more highly expressed in the branchial arches, neural tube and somites [195] where it may influence

craniofacial, central nervous system, skeletal, or muscle development. *Polr3g* is also highly expressed in pluripotent stem cells in mice and this particular isoform may be important for early embryonic development [196]. Furthermore, upregulated Pol III gene expression has been observed in cancer [197] and liver regeneration [198]. While these studies indicate roles for Pol III in different tissues, and increased expression levels of some Pol III subunits within the central nervous system, there is not always a clear correlation between expression level and the tissue type affected in human diseases. Therefore, a comprehensive examination of Pol III expression during development is needed to determine whether Pol III activity correlates with the levels of Pol III subunit expression or if the regulation of Pol III transcripts and their tissue-specific effects are due to other factors.

Tissue-specific expression of Pol III transcripts including 5S rRNA, tRNAs, and other non-coding RNAs has been demonstrated in multiple contexts. The 5S rRNA exists as a maternal/oocyte variant and somatic variant in *Xenopus* [199] and zebrafish [200], although the functional consequences of expressing one over another remain unclear. The primate-specific cytoplasmic RNA, BC200, is highly expressed in the brain [201] and a recent study identified changes in BC200 in association with POLR3-HLD, suggesting that this lncRNA may be important in its pathogenesis [186]. Pathogenic variants in lncRNA *RMRP* which is highly expressed in hypertrophic chondrocytes [202] and involved in cell cycle regulation [203], are associated with Cartilage-Hair Hypoplasia (CHH) [204]. RMRP is the source of two small RNAs which regulate the expression of target genes including those involved in hematopoiesis, growth, and bone development [205].

Variants in tRNA sequences, tRNA modification, and tRNA maturation also have the potential to lead to changes in translation which may contribute to tissue-specific phenotypes associated with human disorders [206]. tRNAs are expressed at different levels across tissues in humans [207], with one tRNA specifically expressed in the central nervous system [208]. Furthermore, tRNAs are intricately linked to neurological disorders. Biallelic pathogenic variants in genes coding for various aminoacyl tRNA synthetases including *EPRS1*, *DARS1* and *RARS1*, have been shown to cause hypomyelinating leukodystrophies [209–211]. Pathogenic variants in genes encoding proteins involved in the processing and maturation of tRNAs (i.e. *CLP1, TSEN*) are also associated with various neurological disorders including neurodegeneration and pontocerebellar hypoplasia [28], which further demonstrates a particular neurodevelopmental sensitivity to altered tRNA biogenesis. The tRNA pool may also be spatiotemporally regulated during development [212] as tRNAs are differentially expressed during proliferation and differentiation in cancer cell lines [213]. The response to changes in the tRNA pool may also be cell type specific due to the activity of tRNA-derived fragments or codon composition of mRNAs expressed within a tissue type. tRNA-derived fragments, which can be produced from immature or mature tRNAs, have regulatory functions and roles in cell proliferation [214] and may act as microRNAs, bind to RNA-binding proteins, or function in ribosome biogenesis [214, 215]. Further, if changes in the tRNA pool are induced by Pol III dysfunction, this could have a stronger effect on some mRNAs versus others due to their codon composition. Recent work has demonstrated that codon composition correlates with mRNA stability and “optimal” codons correlate with tRNAs that are highly expressed [216, 217]. This mechanism is used in the maternal-to-zygotic transition in zebrafish and *Drosophila* [216], and may also be important

in the response to stress [218] or cell cycle state [219]. However, while mRNA decay is important during neural development for cell fate decisions, codon optimality may not be the primary driver [220, 221]. Further studies are necessary to understand the cell type specific regulation of tRNA-derived fragments or the tRNA pool during development, which represents a new area of research in which Pol III-derived transcripts may regulate ribosome biogenesis and translation.

5. Discussion

5.1 Tissue-specific roles for ribosome biogenesis

The distinctive phenotypes of disorders in Pol I and Pol III suggest that there are tissue-specific requirements and functions for the corresponding subunits, their regulation, and/or the RNAs transcribed by Pol I and III in ribosome biogenesis. While Pol I-related disorders are clearly defined as ribosomopathies, Pol III-related disorders are not referred to as ribosomopathies because of the diverse array of genes transcribed by Pol III that function outside of ribosome biogenesis. However, Pol III-mediated transcription of the 5S rRNA is an essential component of the ribosome as well as the response to ribosomal stress. In addition, the Pol III transcript *RMRP* is involved in processing rRNAs transcribed by Pol I, and U6 snRNA is modified within the nucleolus before its assembly into the spliceosome where it has catalytic function to remove introns from mRNAs, including those encoding ribosomal proteins. Therefore, Pol III transcription is required at multiple steps of ribosome biogenesis and its activity is coordinated with Pol I by many of the same pathways including mTOR [222], c-Myc, RB, and p53 [57] to regulate the synthesis of ribosomes and proteins.

The tissue-specific nature of phenotypes arising from disruptions in ribosome biogenesis have led to two broad hypotheses to explain this phenomenon. First, that ribosome concentration varies across tissues, and that deficiencies in ribosome biogenesis affect translation globally. The tissue-specificity in this model arises from the idea that mRNAs which initiate translation poorly will be more sensitive to a reduction in ribosome concentration or function [223]. Second, that ribosomes are heterogeneous with different compositions in different tissues, and that these “specialized” ribosomes translate specific subsets of mRNAs [224]. This model arose in part, from the observations that ribosomal proteins are tissue-specifically expressed and that subsets of genes were specifically downregulated [225]. However, given that no changes in cap-dependent or IRES-dependent translation were observed, perhaps ribosome concentration is key [223]. Studies in mouse embryonic stem cells have revealed differences in the stoichiometry of ribosomal proteins in polysomes suggesting heterogeneity within this cell type [226]; however, no differences in ribosome composition were observed in the mouse brain [227] or human hematopoietic cells despite evidence for tissue-specific transcript changes [228]. This suggests that we do not fully understand the degree to which “specialized” ribosomes may be present in individual tissues or the sensitivity of different mRNA transcripts in these tissues to differences in ribosome concentration. Alternatively, some ribosome biogenesis associated proteins may have non-ribosomal functions [229]. For example, in addition to rRNA transcription and processing [230] *Tcof1*/Treacle plays a critical role in the DNA damage response during embryogenesis particularly within the neuroepithelium [130, 131, 231–233].

Emerging evidence suggests that distinct rRNA variants in different tissues may impact spatiotemporal specificity. rRNA is a catalytic component of the ribosome and therefore changes in rRNA sequence have the potential to affect translation depending on the location and structural consequences of the variant. 47S and 5S rDNA variants have been detected in several species including bacteria [234], *Xenopus* [199], zebrafish [200, 235], mice [236, 237], and humans [237], and each variant is tissue-specifically expressed, which could potentially result in "specialized" ribosomes. Analysis of rRNA variants in mice and humans showed that they map to the functional center of the ribosome and may also lead to changes in rRNA modifications [237]. Recent studies found expression of specific rRNA variants under hypoxic conditions [129] and in long term memory [238]. Together these results indicate that both environmental conditions and intrinsic tissue-specific factors could influence the expression of rRNA variants. Further work is needed to understand the functional consequences of rRNA variants and how the expression of these variants and the amount of rRNA produced may contribute to tissue-specific consequences of disruptions in Pol I transcription.

5.2 Ribosomal Stress as a consequence of disruptions in Pol I and III transcription

Nucleolar integrity is tightly linked to rRNA transcription and ribosome biogenesis, and one of the consequences of disruptions in ribosome biogenesis is nucleolar stress or perturbed nucleolar structure. This triggers the nucleolar surveillance pathway or ribosomal stress response, which results in activation of p53 [102, 239, 240]. However, defects in ribosome biogenesis do not necessarily always disrupt nucleolar integrity [119, 241, 242]. We therefore use ribosomal stress to refer to disruptions in ribosome biogenesis, and note that disruption in nucleolar morphology, or nucleolar stress may or may not be present. Either way, activation of the ribosomal protein (RP)-Mdm2-p53 cascade leads to p53 stabilization, cell cycle arrest and apoptosis.

A recent high-throughput screen identified RPL5 and RPL11, which comprise part of the 5S RNP as the only RPs necessary for the ribosomal stress response [243], suggesting that this is the primary mechanism by which p53 becomes stabilized in response to ribosomal stress. P53 activation has been observed in both Pol I and Pol III mutant models [92, 94, 95, 194, 244, 245], but it is important to consider the underlying causes of p53 activation in addition to its downstream consequences. p53 has the potential to regulate both Pol I and Pol III transcription, distinct from its known roles in cell cycle arrest and apoptosis. P53 interacts with SL1 at the Pol I promoter to inhibit rRNA transcription [246], and p53 can specifically inactivate TFIIIB to inhibit Pol III-mediated transcription [53, 247]. Further, in the context of ribosome biogenesis, nucleolar stress is variably present and not always dependent upon p53 [109]. P53 can be activated in the absence of a disruption in nucleolar morphology, simply through an imbalance in rRNAs to ribosomal proteins [103]. Together, these observations suggest that activation of p53 can occur through nucleolar or ribosomal stress, but that blocking p53 does not prevent nucleolar stress or its p53-independent consequences.

The degree to which the RP-Mdm2-p53 pathway is triggered is dependent upon the rate of ribosome biogenesis [248] and this would suggest that tissues with relatively high rates

of rRNA transcription would be more likely to trigger robust p53 activation than cells maintaining a basal level of transcription. In mouse embryos, inhibition of Pol I with BMH-21 specifically increased neuroepithelial apoptosis, consistent with its relatively high rate of rRNA transcription, which was demonstrated by quantitative analysis of pre-rRNA [92]. Recent work in mouse embryonic fibroblasts demonstrated that the RP-Mdm2-p53 response is activated upon *Polr1a, Polr1c, and Tcof1* loss-of-function [92]. Importantly, the levels of Mdm2, Rpl5, and Rpl11 protein were unchanged between controls and mutants, while in contrast p53 protein increased in the mutants. Furthermore, previous studies in *Tcof1*$^{+/-}$ mutants showed that p53 protein levels increased in mutant embryos, but that p53 mRNA levels remained unchanged [91, 98]. Together, these studies mechanistically demonstrate that the post-translational stabilization of p53, and not activation of *p53* mRNA, contrary to one recent hypothesis [233], dictates the cellular response to disruptions in rRNA transcription.

The induction of p53 in the ribosomal stress response is highly dependent upon the 5S RNP [105, 243] and mutations causing a disruption in Pol I transcription of rRNAs, as well as mutations in ribosomal proteins can trigger 5S RNP binding to Mdm2 [91, 92, 94, 106, 239, 241]. Disruptions in Pol III reducing 5S rRNA, in the context of the 5S RNP, would therefore be expected to result in reduced binding of the 5S RNP to Mdm2, thus preventing p53 activation. However, the 5S rRNA also binds Mdm4. Knockdown of 5S rRNA reduces protein levels of Mdm4, but not mRNA levels or p53 protein levels, and consequently p21 expression increases, which results in growth arrest [249]. Therefore, in the context of POLR3-related pathogenic variants which downregulate 5S rRNA, these, hypothetically, may initially result in p53 activation in an Mdm4-dependent manner, but this has not been experimentally demonstrated. P53 activation has however been observed in Pol III mutant animal models [194, 244] as well as human fibroblasts [250]. Activation of p53 in zebrafish *rpc9*$^{-/-}$ mutants contributes to cell death, but has no effect on proliferation, and inhibition of *p53* rescues cell death [245]. In the future, it will also be important to consider the degree to which p53-independent effects on cellular proliferation and survival occur due to Pol I or Pol III disruption.

5.3 Pol I and III – Pathogenic variants in one gene can lead to different syndromes; one syndrome may be caused by pathogenic variants in different genes

Pathogenic variants in genes encoding Pol I and Pol III subunits give rise to a variety of distinct disorders with some overlap in the specific tissues involved. For example, craniofacial and/or dental anomalies are associated with pathogenic variants in *POLR1A, POLR1B, POLR1C, POLR1D, POLR3A*, *POLR3B, POLR3GL*, and *POLR3K*. Similarly, central nervous system white matter involvement is associated with pathogenic variants in *POLR1A, POLR1C, POLR3A, POLR3B*, and *POLR3K*. Despite these commonalities, there are also important differences between disorders. Furthermore, there is considerable phenotypic variability within each disorder.

The ability to distinguish disorders arising from pathogenic variants in the same gene is best exemplified by biallelic pathogenic variants in *POLR1C*, which were first associated with TCS [79] and then associated with POLR3-HLD [88]. Molecular analysis showed that

POLR1C variants associated with POLR3-HLD specifically affected the assembly of Pol III and the ability of Pol III, but not Pol I, to bind to its target promoters [88]. While it was originally suggested that POLR3-HLD associated variants in *POLR1C* affect Pol III while sparing Pol I activity, a more recent analysis identified individuals diagnosed with both POLR3-HLD and craniofacial abnormalities, including 1 individual with a typical TCS phenotype [88, 148]. Recent structural analysis of different variants has also supported the initial classification of these distinct syndromes according to their disruption of either Pol I or Pol III [6]. However, work in yeast models has raised the possibility that Pol III dysfunction may contribute to TCS [89]. While *POLR1C* pathogenic variants tend to preferentially affect Pol I or Pol III function, several open questions remain regarding the tissue-specificity of the phenotypes, and the relative contribution of Pol I or Pol III transcription in these tissues. For example, pathogenic variants in *POLR1A* are associated with AFDCIN [95] and a leukodystrophy [111] which have distinct, non-overlapping clinical manifestations. Furthermore, distinct pathogenic variants in *POLR3A* are associated with POLR3-HLD [145], the mild and severe striatal forms of POLR3-HLD [158], WRS [173], or susceptibility to varicella-zoster virus infection [251]. Interestingly, both Pol I and Pol III-mediated transcription are disrupted in fibroblast cells from one WRS patient [250], which displayed reduced rRNA transcription and disrupted nucleolar integrity, despite location of a pathogenic variant within Pol III. Whether pathogenic variants in Pol I subunits have a reciprocal effect on Pol III transcription remains to be determined. Together, this raises many questions regarding the origins of the variability and tissue-specificity of Pol I or Pol III-related disorders and their molecular underpinnings. Current hypotheses, which are combinatorial, include variant-specific effects, tissue-specific expression of Pol I and III transcripts, tissue-specific interactions with initiation factors, genetic background, epigenetic modifications, and environmental influences.

5.4 Future perspectives

Altogether, there are several mechanisms by which Pol I and III transcription are regulated throughout development and while our understanding of these processes is improving, multiple questions remain to be addressed. First, how Pol I or III subunits and their transcripts are differentially expressed during embryogenesis and whether these levels can be mechanistically tied to tissue-specificity. Second, how phenotypic variability arising from pathogenic variants in the same gene occurs, and whether environmental stressors are important. Third, how Pol I and Pol III are coordinately regulated, if at all, in Pol I and III-related disorders and if this confers additional phenotypic variation. Fourth, how, mechanistically, the integration of cellular signaling pathways gives rise to tissue-specific phenotypes associated with Pol I and Pol III function. Resolving these questions has the potential to uncover novel regulators of development and new targets for the treatment of Pol I and III transcription associated disorders.

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Abbreviations:

4H	Hypomyelination, Hypodontia and Hypogonadotropic Hypogonadism
AFDCIN	Acrofacial dysostosis, Cincinnati type
BRF1	Brf1 Transcription Initiation Factor IIIB subunit
DARS1	Aspartyl-tRNA-synthetase 1
EPRS1	Glutamyl-Prolyl-tRNA synthetase 1
ETS	Externally Transcribed Spacer
ITS	Internally Transcribed Spacer
Mdm2	Murine double minute 2
mRNA	messenger RNA
ncRNA	non-coding RNA
Pol I	RNA Polymerase I
Pol III	RNA Polymerase III
POLR3-HLD	RNA polymerase III-related hypomyelinating leukodystrophy
RARS1	Arginyl-tRNA-synthetase 1
RMRP	Mitochondrial RNA-processing endoribonuclease
RNP	ribonucleoprotein
RPL	large subunit ribosomal protein
RPS	small subunit ribosomal protein
rRNA	ribosomal RNA
SL1	Selectivity Factor 1
SNAPc	small nuclear RNA activating protein complex
snoRNA	small nucleolar RNA
TCS	Treacher Collins syndrome
TFIIIB	Transcription Factor for polymerase III B
TIF-IA	Transcription Initiation Factor IA

tRNA	transfer RNA
UBTF	Upstream Binding Transcription Factor
WRS	Wiedemann-Rautenstrauch syndrome

References

[1]. Warner JR. The economics of ribosome biosynthesis in yeast. Trends in Biochemical Sciences 1999;24:437–40. [PubMed: 10542411]

[2]. Laferté A, Favry E, Sentenac A, Riva M, Carles C, Chédin S. The transcriptional activity of RNA polymerase I is a key determinant for the level of all ribosome components. Genes & Development 2006;20:2030–40. [PubMed: 16882981]

[3]. Nogi Y, Vu L, Nomura M. An approach for isolation of mutants defective in 35S ribosomal RNA synthesis in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences 1991;88:7026.

[4]. Dequard-Chablat M, Riva M, Carles C, Sentenac A. RPC19, the gene for a subunit common to yeast RNA polymerases A (I) and C (III). Journal of Biological Chemistry 1991;266:15300–7. [PubMed: 1869554]

[5]. Mann C, Buhler J-M, Treich I, Sentenac A. RPC40, a unique gene for a subunit shared between yeast RNA polymerases A and C. Cell 1987;48:627–37. [PubMed: 3815519]

[6]. Misiaszek AD, Girbig M, Grotsch H, Baudin F, Murciano B, Lafita A, et al. Cryo-EM structures of human RNA polymerase I. Nature structural & molecular biology 2021;28:997–1008.

[7]. Zhao D, Liu W, Chen K, Wu Z, Yang H, Xu Y. Structure of the human RNA polymerase I elongation complex. Cell Discovery 2021;7:97. [PubMed: 34671025]

[8]. Merkl PE, Pilsl M, Fremter T, Schwank K, Engel C, Längst G, et al. RNA polymerase I (Pol I) passage through nucleosomes depends on Pol I subunits binding its lobe structure. Journal of Biological Chemistry 2020;295:4782–95. [PubMed: 32060094]

[9]. Beckouët F, Mariotte-Labarre S, Peyroche G, Nogi Y, Thuriaux P. Rpa43 and its partners in the yeast RNA polymerase I transcription complex. FEBS Letters 2011;585:3355–9. [PubMed: 21983101]

[10]. Goodfellow SJ, Zomerdijk JC. Basic mechanisms in RNA polymerase I transcription of the ribosomal RNA genes. Subcell Biochem 2013;61:211–36. [PubMed: 23150253]

[11]. Ide S, Miyazaki T, Maki H, Kobayashi T. Abundance of Ribosomal RNA Gene Copies Maintains Genome Integrity. Science 2010;327:693–6. [PubMed: 20133573]

[12]. Santoro R, Grummt I. Molecular Mechanisms Mediating Methylation-Dependent Silencing of Ribosomal Gene Transcription. Molecular Cell 2001;8:719–25. [PubMed: 11583633]

[13]. Pilsl M, Crucifix C, Papai G, Krupp F, Steinbauer R, Griesenbeck J, et al. Structure of the initiation-competent RNA polymerase I and its implication for transcription. Nature Communications 2016;7:12126.

[14]. Blattner C, Jennebach S, Herzog F, Mayer A, Cheung ACM, Witte G, et al. Molecular basis of Rrn3-regulated RNA polymerase I initiation and cell growth. Genes & Development 2011;25:2093–105. [PubMed: 21940764]

[15]. Sanij E, Poortinga G, Sharkey K, Hung S, Holloway TP, Quin J, et al. UBF levels determine the number of active ribosomal RNA genes in mammals. Journal of Cell Biology 2008;183:1259–74. [PubMed: 19103806]

[16]. Tuan JC, Zhai W, Comai L. Recruitment of TATA-binding protein-TAFI complex SL1 to the human ribosomal DNA promoter is mediated by the carboxy-terminal activation domain of upstream binding factor (UBF) and is regulated by UBF phosphorylation. Mol Cell Biol 1999;19:2872–9. [PubMed: 10082553]

[17]. Hein N, Hannan KM, George AJ, Sanij E, Hannan RD. The nucleolus: an emerging target for cancer therapy. Trends in Molecular Medicine 2013;19:643–54. [PubMed: 23953479]

[18]. Grummt I Wisely chosen paths – regulation of rRNA synthesis. The FEBS Journal 2010;277:4626–39. [PubMed: 20977666]

[19]. Torreira E, Louro JA, Pazos I, González-Polo N, Gil-Carton D, Duran AG, et al. The dynamic assembly of distinct RNA polymerase I complexes modulates rDNA transcription. eLife 2017;6:e20832. [PubMed: 28262097]

[20]. Fernández-Tornero C RNA polymerase I activation and hibernation: unique mechanisms for unique genes. Transcription 2018;9:248–54. [PubMed: 29372670]

[21]. Heiss FB, Daiß JL, Becker P, Engel C. Conserved strategies of RNA polymerase I hibernation and activation. Nature Communications 2021;12:758.

[22]. Dieci G, Fiorino G, Castelnuovo M, Teichmann M, Pagano A. The expanding RNA polymerase III transcriptome. Trends Genet 2007;23:614–22. [PubMed: 17977614]

[23]. White RJ. Transcription by RNA polymerase III: more complex than we thought. Nat Rev Genet 2011;12:459–63. [PubMed: 21540878]

[24]. Ramsay EP, Abascal-Palacios G, Daiss JL, King H, Gouge J, Pilsl M, et al. Structure of human RNA polymerase III. Nat Commun 2020;11:6409. [PubMed: 33335104]

[25]. Li L, Yu Z, Zhao D, Ren Y, Hou H, Xu Y. Structure of human RNA polymerase III elongation complex. Cell Research 2021;31:791–800. [PubMed: 33674783]

[26]. Haurie V, Durrieu-Gaillard S, Dumay-Odelot H, Da Silva D, Rey C, Prochazkova M, et al. Two isoforms of human RNA polymerase III with specific functions in cell growth and transformation. Proceedings of the National Academy of Sciences 2010;107:4176.

[27]. Huang S, Aleksashin NA, Loveland AB, Klepacki D, Reier K, Kefi A, et al. Ribosome engineering reveals the importance of 5S rRNA autonomy for ribosome assembly. Nat Commun 2020;11:2900. [PubMed: 32518240]

[28]. Schaffer AE, Pinkard O, Coller JM. tRNA Metabolism and Neurodevelopmental Disorders. Annu Rev Genomics Hum Genet 2019;20:359–87. [PubMed: 31082281]

[29]. Egloff S, Studniarek C, Kiss T. 7SK small nuclear RNA, a multifunctional transcriptional regulatory RNA with gene-specific features. Transcription 2018;9:95–101. [PubMed: 28820318]

[30]. Didychuk AL, Butcher SE, Brow DA. The life of U6 small nuclear RNA, from cradle to grave. RNA 2018;24:437–60. [PubMed: 29367453]

[31]. Jarrous N, Reiner R. Human RNase P: a tRNA-processing enzyme and transcription factor. Nucleic Acids Res 2007;35:3519–24. [PubMed: 17483522]

[32]. Guerrier-Takada C, Gardiner K, Marsh T, Pace N, Altman S. The RNA moiety of ribonuclease P is the catalytic subunit of the enzyme. Cell 1983;35:849–57. [PubMed: 6197186]

[33]. Esakova O, Krasilnikov AS. Of proteins and RNA: the RNase P/MRP family. RNA 2010;16:1725–47. [PubMed: 20627997]

[34]. Hahne JC, Lampis A, Valeri N. Vault RNAs: hidden gems in RNA and protein regulation. Cell Mol Life Sci 2021;78:1487–99. [PubMed: 33063126]

[35]. Nagai K, Oubridge C, Kuglstatter A, Menichelli E, Isel C, Jovine L. Structure, function and evolution of the signal recognition particle. EMBO J 2003;22:3479–85. [PubMed: 12853463]

[36]. Boccitto M, Wolin SL. Ro60 and Y RNAs: structure, functions, and roles in autoimmunity. Crit Rev Biochem Mol Biol 2019;54:133–52. [PubMed: 31084369]

[37]. Christov CP, Trivier E, Krude T. Noncoding human Y RNAs are overexpressed in tumours and required for cell proliferation. Br J Cancer 2008;98:981–8. [PubMed: 18283318]

[38]. Hasler J, Strub K. Alu elements as regulators of gene expression. Nucleic Acids Res 2006;34:5491–7. [PubMed: 17020921]

[39]. Sosinska P, Mikula-Pietrasik J, Ksiazek K. The double-edged sword of long non-coding RNA: The role of human brain-specific BC200 RNA in translational control, neurodegenerative diseases, and cancer. Mutat Res Rev Mutat Res 2015;766:58–67. [PubMed: 26596549]

[40]. Canella D, Praz V, Reina JH, Cousin P, Hernandez N. Defining the RNA polymerase III transcriptome: Genome-wide localization of the RNA polymerase III transcription machinery in human cells. Genome Res 2010;20:710–21. [PubMed: 20413673]

[41]. Schramm L, Hernandez N. Recruitment of RNA polymerase III to its target promoters. Genes & Development 2002;16:2593–620. [PubMed: 12381659]

[42]. Geiduschek EP, Kassavetis GA. The RNA polymerase III transcription apparatus11Edited by P. E. Wright. Journal of Molecular Biology 2001;310:1–26. [PubMed: 11419933]

[43]. Pluta K, Lefebvre O, Martin NC, Smagowicz WJ, Stanford DR, Ellis SR, et al. Maf1p, a negative effector of RNA polymerase III in Saccharomyces cerevisiae. Mol Cell Biol 2001;21:5031–40. [PubMed: 11438659]

[44]. Goodfellow SJ, Graham EL, Kantidakis T, Marshall L, Coppins BA, Oficjalska-Pham D, et al. Regulation of RNA polymerase III transcription by Maf1 in mammalian cells. J Mol Biol 2008;378:481–91. [PubMed: 18377933]

[45]. Gjidoda A, Henry RW. RNA polymerase III repression by the retinoblastoma tumor suppressor protein. Biochim Biophys Acta 2013;1829:385–92. [PubMed: 23063750]

[46]. Stein T, Crighton D, Warnock LJ, Milner J, White RJ. Several regions of p53 are involved in repression of RNA polymerase III transcription. Oncogene 2002;21:5540–7. [PubMed: 12165852]

[47]. Willis IM. Maf1 phenotypes and cell physiology. Biochim Biophys Acta Gene Regul Mech 2018;1861:330–7. [PubMed: 29248739]

[48]. Upadhya R, Lee J, Willis IM. Maf1 is an essential mediator of diverse signals that repress RNA polymerase III transcription. Mol Cell 2002;10:1489–94. [PubMed: 12504022]

[49]. Oficjalska-Pham D, Harismendy O, Smagowicz WJ, Gonzalez de Peredo A, Boguta M, Sentenac A, et al. General repression of RNA polymerase III transcription is triggered by protein phosphatase type 2A-mediated dephosphorylation of Maf1. Mol Cell 2006;22:623–32. [PubMed: 16762835]

[50]. Roberts DN, Wilson B, Huff JT, Stewart AJ, Cairns BR. Dephosphorylation and genome-wide association of Maf1 with Pol III-transcribed genes during repression. Mol Cell 2006;22:633–44. [PubMed: 16762836]

[51]. Vannini A, Ringel R, Kusser AG, Berninghausen O, Kassavetis GA, Cramer P. Molecular basis of RNA polymerase III transcription repression by Maf1. Cell 2010;143:59–70. [PubMed: 20887893]

[52]. Vorlander MK, Baudin F, Moir RD, Wetzel R, Hagen WJH, Willis IM, et al. Structural basis for RNA polymerase III transcription repression by Maf1. Nature structural & molecular biology 2020;27:229–32.

[53]. Crighton D, Woiwode A, Zhang C, Mandavia N, Morton JP, Warnock LJ, et al. p53 represses RNA polymerase III transcription by targeting TBP and inhibiting promoter occupancy by TFIIIB. EMBO J 2003;22:2810–20. [PubMed: 12773395]

[54]. Lata E, Choquet K, Sagliocco F, Brais B, Bernard G, Teichmann M. RNA Polymerase III Subunit Mutations in Genetic Diseases. Front Mol Biosci 2021;8:696438. [PubMed: 34395528]

[55]. Lafontaine DLJ, Riback JA, Bascetin R, Brangwynne CP. The nucleolus as a multiphase liquid condensate. Nature Reviews Molecular Cell Biology 2021;22:165–82. [PubMed: 32873929]

[56]. Moss T, Stefanovsky VY. At the Center of Eukaryotic Life. Cell 2002;109:545–8. [PubMed: 12062097]

[57]. White RJ. RNA polymerases I and III, non-coding RNAs and cancer. Trends in Genetics 2008;24:622–9. [PubMed: 18980784]

[58]. Wittekind M, Kolb JM, Dodd J, Yamagishi M, Mémet S, Buhler JM, et al. Conditional expression of RPA190, the gene encoding the largest subunit of yeast RNA polymerase I: effects of decreased rRNA synthesis on ribosomal protein synthesis. Molecular and Cellular Biology 1990;10:2049–59. [PubMed: 2183018]

[59]. Schneider DA, Michel A, Sikes ML, Vu L, Dodd JA, Salgia S, et al. Transcription Elongation by RNA Polymerase I Is Linked to Efficient rRNA Processing and Ribosome Assembly. Molecular Cell 2007;26:217–29. [PubMed: 17466624]

[60]. Turowski TW, Tollervey D. Cotranscriptional events in eukaryotic ribosome synthesis. WIREs RNA 2015;6:129–39. [PubMed: 25176256]

[61]. Stenström L, Mahdessian D, Gnann C, Cesnik AJ, Ouyang W, Leonetti MD, et al. Mapping the nucleolar proteome reveals a spatiotemporal organization related to intrinsic protein disorder. Molecular Systems Biology 2020;16:e9469. [PubMed: 32744794]

[62]. Sloan KE, Warda AS, Sharma S, Entian KD, Lafontaine DLJ, Bohnsack MT. Tuning the ribosome: The influence of rRNA modification on eukaryotic ribosome biogenesis and function. RNA Biol 2017;14:1138–52. [PubMed: 27911188]

[63]. Ciganda M, Williams N. Eukaryotic 5S rRNA biogenesis. Wiley Interdisciplinary Reviews: RNA 2011;2:523–33. [PubMed: 21957041]

[64]. Lindahl L, Bommankanti A, Li X, Hayden L, Jones A, Khan M, et al. RNase MRP is required for entry of 35S precursor rRNA into the canonical processing pathway. RNA 2009;15:1407–16. [PubMed: 19465684]

[65]. Yelick PC, Trainor PA. Ribosomopathies: Global process, tissue specific defects. Rare Diseases 2015;3:e1025185. [PubMed: 26442198]

[66]. Trainor PA, Merrill AE. Ribosome biogenesis in skeletal development and the pathogenesis of skeletal disorders. Biochim Biophys Acta 2014;1842:769–78. [PubMed: 24252615]

[67]. Fitriasari S, Trainor PA. Diabetes, Oxidative Stress, and DNA Damage Modulate Cranial Neural Crest Cell Development and the Phenotype Variability of Craniofacial Disorders. Frontiers in cell and developmental biology 2021;9:644410. [PubMed: 34095113]

[68]. Watt KEN, Trainor PA. Chapter 17 - Neurocristopathies: The Etiology and Pathogenesis of Disorders Arising from Defects in Neural Crest Cell Development. In: Trainor PA, editor. Neural Crest Cells. Boston: Academic Press; 2014. p. 361–94.

[69]. Crane JF, Trainor PA. Neural crest stem and progenitor cells. Annual review of cell and developmental biology 2006;22:267–86.

[70]. Trainor PA. Craniofacial birth defects: The role of neural crest cells in the etiology and pathogenesis of Treacher Collins syndrome and the potential for prevention. Am J Med Genet A 2010;152A:2984–94. [PubMed: 20734335]

[71]. Gammill LS, Bronner-Fraser M. Neural crest specification: migrating into genomics. Nature Reviews Neuroscience 2003;4:795–805. [PubMed: 14523379]

[72]. Bhatt S, Diaz R, Trainor PA. Signals and switches in Mammalian neural crest cell differentiation. Cold Spring Harb Perspect Biol 2013;5.

[73]. Santagati F, Rijli FM. Cranial neural crest and the building of the vertebrate head. Nature Reviews Neuroscience 2003;4:806–18. [PubMed: 14523380]

[74]. The Treacher Collins Syndrome Collaborative G, Dixon J, Edwards SJ, Gladwin AJ, Dixon MJ, Loftus SK, et al. Positional cloning of a gene involved in the pathogenesis of Treacher Collins syndrome. Nat Genet 1996;12:130–6. [PubMed: 8563749]

[75]. Vincent M, Geneviève D, Ostertag A, Marlin S, Lacombe D, Martin-Coignard D, et al. Treacher Collins syndrome: a clinical and molecular study based on a large series of patients. Genetics in Medicine 2016;18:49–56. [PubMed: 25790162]

[76]. Altug Teber Ö, Gillessen-Kaesbach G, Fischer S, Böhringer S, Albrecht B, Albert A, et al. Genotyping in 46 patients with tentative diagnosis of Treacher Collins syndrome revealed unexpected phenotypic variation. European Journal of Human Genetics 2004;12:879–90. [PubMed: 15340364]

[77]. Li C, Mernagh J, Bourgeois J. Novel craniofacial and extracraniofacial findings in a case of Treacher Collins syndrome with a pathogenic mutation and a missense variant in the TCOF1 gene. Clinical Dysmorphology 2009;18.

[78]. Giampietro PF, Armstrong L, Stoddard A, Blank RD, Livingston J, Raggio CL, et al. Whole exome sequencing identifies a POLRID mutation segregating in a father and two daughters with findings of Klippel–Feil and Treacher Collins syndromes. American Journal of Medical Genetics Part A 2015;167:95–102.

[79]. Dauwerse JG, Dixon J, Seland S, Ruivenkamp CAL, van Haeringen A, Hoefsloot LH, et al. Mutations in genes encoding subunits of RNA polymerases I and III cause Treacher Collins syndrome. Nat Genet 2011;43:20–2. [PubMed: 21131976]

[80]. Sanchez E, Laplace-Builhé B, Mau-Them FT, Richard E, Goldenberg A, Toler TL, et al. POLR1B and neural crest cell anomalies in Treacher Collins syndrome type 4. Genetics in Medicine 2020;22:547–56. [PubMed: 31649276]

[81]. Wise CA, Chiang LC, Paznekas WA, Sharma M, Musy MM, Ashley JA, et al. TCOF1 gene encodes a putative nucleolar phosphoprotein that exhibits mutations in Treacher Collins Syndrome throughout its coding region. Proceedings of the National Academy of Sciences 1997;94:3110–5.

[82]. Schaefer E, Collet C, Genevieve D, Vincent M, Lohmann DR, Sanchez E, et al. Autosomal recessive POLR1D mutation with decrease of TCOF1 mRNA is responsible for Treacher Collins syndrome. Genet Med 2014;16:720–4. [PubMed: 24603435]

[83]. Cesaretti C, Gentilin B, Bianchi V, Melloni G, Bonaguro M, Rossi C, et al. Occurrence of complete arhinia in two siblings with a clinical picture of Treacher Collins syndrome negative for TCOF1, POLR1D and POLR1C mutations. Clinical dysmorphology 2011;20:229–31 10.1097/MCD.0b013e3283491725. [PubMed: 21772136]

[84]. Gonzales B, Henning D, So RB, Dixon J, Dixon MJ, Valdez BC. The Treacher Collins syndrome (TCOF1) gene product is involved in pre-rRNA methylation. Human Molecular Genetics 2005;14:2035–43. [PubMed: 15930015]

[85]. Valdez BC, Henning D, So RB, Dixon J, Dixon MJ. The Treacher Collins syndrome (TCOF1) gene product is involved in ribosomal DNA gene transcription by interacting with upstream binding factor. Proceedings of the National Academy of Sciences of the United States of America 2004;101:10709–14. [PubMed: 15249688]

[86]. Gladwin AJ, Dixon J, Loftus SK, Edwards S, Wasmuth JJ, Hennekam RCM, et al. Treacher Collins Syndrome may Result from Insertions, Deletions or Splicing Mutations, Which Introduce a Termination Codon into the Gene. Human Molecular Genetics 1996;5:1533–8. [PubMed: 8894686]

[87]. Edwards SJ, Gladwin AJ, Dixon MJ. The Mutational Spectrum in Treacher Collins Syndrome Reveals a Predominance of Mutations That Create a Premature-Termination Codon. American Journal of Medical Genetics 1997;60:515–24.

[88]. Thiffault I, Wolf NI, Forget D, Guerrero K, Tran LT, Choquet K, et al. Recessive mutations in POLR1C cause a leukodystrophy by impairing biogenesis of RNA polymerase III. Nature Communications 2015;6:7623.

[89]. Walker-Kopp N, Jackobel AJ, Pannafino GN, Morocho PA, Xu X, Knutson BA. Treacher Collins syndrome mutations in Saccharomyces cerevisiae destabilize RNA polymerase I and III complex integrity. Human Molecular Genetics 2017;26:4290–300. [PubMed: 28973381]

[90]. Enomoto Y, Tsurusaki Y, Tominaga M, Kobayashi S, Inoue M, Fujita K, et al. A Recurrent Variant in POLR1B, c.3007C<T; p.Arg1003Cys, Associated with Atresia of the External Canal and Microtia in Treacher Collins Syndrome Type 4. Molecular Syndromology 2021;12:127–32. [PubMed: 34012383]

[91]. Dixon J, Jones NC, Sandell LL, Jayasinghe SM, Crane J, Rey J-P, et al. Tcof1/Treacle Is Required for Neural Crest Cell Formation and Proliferation Deficiencies That Cause Craniofacial Abnormalities. Proceedings of the National Academy of Sciences of the United States of America 2006;103:13403–8. [PubMed: 16938878]

[92]. Falcon KT, Watt KEN, Dash S, Achilleos A, Zhao R, Sakai D, et al. Dynamic regulation and requirement for ribosomal RNA transcription during mammalian development. bioRxiv 2021:2021.09.22.461379.

[93]. Sakai D, Trainor PA. Treacher Collins syndrome: unmasking the role of Tcof1/treacle. The international journal of biochemistry & cell biology 2009;41:1229–32. [PubMed: 19027870]

[94]. Noack Watt KE, Achilleos A, Neben CL, Merrill AE, Trainor PA. The Roles of RNA Polymerase I and III Subunits Polr1c and Polr1d in Craniofacial Development and in Zebrafish Models of Treacher Collins Syndrome. PLOS Genetics 2016;12:e1006187. [PubMed: 27448281]

[95]. Weaver KN, Watt Kristin EN, Hufnagel Robert B, Navajas Acedo J, Linscott Luke L, Sund Kristen L, et al. Acrofacial Dysostosis, Cincinnati Type, a Mandibulofacial Dysostosis Syndrome with Limb Anomalies, Is Caused by POLR1A Dysfunction. The American Journal of Human Genetics 2015;96:765–74. [PubMed: 25913037]

[96]. Ide S, Imai R, Ochi H, Maeshima K. Transcriptional suppression of ribosomal DNA with phase separation. Science Advances 2020;6:eabb5953. [PubMed: 33055158]

[97]. van Sluis M, McStay B. A localized nucleolar DNA damage response facilitates recruitment of the homology-directed repair machinery independent of cell cycle stage. Genes & Development 2015;29:1151–63. [PubMed: 26019174]

[98]. Jones NC, Lynn ML, Gaudenz K, Sakai D, Aoto K, Rey JP, et al. Prevention of the neurocristopathy Treacher Collins syndrome through inhibition of p53 function. Nat Med 2008;14:125–33. [PubMed: 18246078]

[99]. Panoutsopoulos AA, De Crescenzo AH, Lee A, Lu AM, Ross AP, Borodinsky LN, et al. Pak1ip1 Loss-of-Function Leads to Cell Cycle Arrest, Loss of Neural Crest Cells, and Craniofacial Abnormalities. Frontiers in cell and developmental biology 2020;8:510063. [PubMed: 32984348]

[100]. Haupt Y, Maya R, Kazaz A, Oren M. Mdm2 promotes the rapid degradation of p53. Nature 1997;387:296–9. [PubMed: 9153395]

[101]. Kubbutat MHG, Jones SN, Vousden KH. Regulation of p53 stability by Mdm2. Nature 1997;387:299–303. [PubMed: 9153396]

[102]. Pestov DG, Strezoska Ž, Lau LF. Evidence of p53-Dependent Cross-Talk between Ribosome Biogenesis and the Cell Cycle: Effects of Nucleolar Protein Bop1 on G1/S Transition. Molecular and Cellular Biology 2001;21:4246–55. [PubMed: 11390653]

[103]. Donati G, Bertoni S, Brighenti E, Vici M, Treré D, Volarevic S, et al. The balance between rRNA and ribosomal protein synthesis up- and downregulates the tumour suppressor p53 in mammalian cells. Oncogene 2011;30:3274–88. [PubMed: 21399665]

[104]. Donati G, Peddigari S, Mercer CA, Thomas G. 5S ribosomal RNA is an essential component of a nascent ribosomal precursor complex that regulates the Hdm2-p53 checkpoint. Cell Rep 2013;4:87–98. [PubMed: 23831031]

[105]. Jaako P, Debnath S, Olsson K, Zhang Y, Flygare J, Lindstrom MS, et al. Disruption of the 5S RNP-Mdm2 interaction significantly improves the erythroid defect in a mouse model for Diamond-Blackfan anemia. Leukemia 2015.

[106]. Watt KEN, Neben CL, Hall S, Merrill AE, Trainor PA. tp53-dependent and independent signaling underlies the pathogenesis and possible prevention of Acrofacial Dysostosis–Cincinnati type. Human Molecular Genetics 2018;27:2628–43. [PubMed: 29750247]

[107]. Sakai D, Dixon J, Dixon MJ, Trainor PA. Mammalian neurogenesis requires Treacle-Plk1 for precise control of spindle orientation, mitotic progression, and maintenance of neural progenitor cells. PLoS Genet 2012;8:e1002566. [PubMed: 22479190]

[108]. Parlato R, Kreiner G, Erdmann G, Rieker C, Stotz S, Savenkova E, et al. Activation of an Endogenous Suicide Response after Perturbation of rRNA Synthesis Leads to Neurodegeneration in Mice. The Journal of Neuroscience 2008;28:12759. [PubMed: 19036968]

[109]. Kalita K, Makonchuk D, Gomes C, Zheng J-J, Hetman M. Inhibition of nucleolar transcription as a trigger for neuronal apoptosis. Journal of Neurochemistry 2008;105:2286–99. [PubMed: 18315559]

[110]. Gomes C, Smith SC, Youssef MN, Zheng J-J, Hagg T, Hetman M. RNA Polymerase 1-driven Transcription as a Mediator of BDNF-induced Neurite Outgrowth*. Journal of Biological Chemistry 2011;286:4357–63. [PubMed: 21098478]

[111]. Kara B, Köroğlu Ç, Peltonen K, Steinberg RC, Maraş Genç H, Hölttä-Vuori M, et al. Severe neurodegenerative disease in brothers with homozygous mutation in POLR1A. European Journal of Human Genetics 2017;25:315–23. [PubMed: 28051070]

[112]. Chen H, Li Z, Haruna K, Li Z, Li Z, Semba K, et al. Early pre-implantation lethality in mice carrying truncated mutation in the RNA polymerase 1–2 gene. Biochemical and Biophysical Research Communications 2008;365:636–42. [PubMed: 18023416]

[113]. Miao X, Sun T, Golan M, Mager J, Cui W. Loss of POLR1D results in embryonic lethality prior to blastocyst formation in mice. Mol Reprod Dev 2020;87:1152–8. [PubMed: 33022126]

[114]. Qian J, Lavker RM, Tseng H. Mapping ribosomal RNA transcription activity in the mouse eye. Developmental Dynamics 2006;235:1984–93. [PubMed: 16673408]

[115]. Tseng H Cell-type-specific regulation of RNA polymerase I transcription: a new frontier. BioEssays 2006;28:719–25. [PubMed: 16850402]

[116]. Neben CL, Lay FD, Mao X, Tuzon CT, Merrill AE. Ribosome biogenesis is dynamically regulated during osteoblast differentiation. Gene 2017;612:29–35. [PubMed: 27847259]

[117]. Zhang Q, Shalaby NA, Buszczak M. Changes in rRNA transcription influence proliferation and cell fate within a stem cell lineage. Science 2014;343:298–301. [PubMed: 24436420]

[118]. Hayashi Y, Kuroda T, Kishimoto H, Wang C, Iwama A, Kimura K. Downregulation of rRNA transcription triggers cell differentiation. PLoS One 2014;9:e98586. [PubMed: 24879416]

[119]. Slomnicki LP, Pietrzak M, Vashishta A, Jones J, Lynch N, Elliot S, et al. Requirement of Neuronal Ribosome Synthesis for Growth and Maintenance of the Dendritic Tree. J Biol Chem 2016;291:5721–39. [PubMed: 26757818]

[120]. Delhermite J, Tafforeau L, Sharma S, Marchand V, Wacheul L, Lattuca R, et al. Systematic mapping of rRNA 2'-O methylation during frog development and involvement of the methyltransferase Fibrillarin in eye and craniofacial development in Xenopus laevis. PLOS Genetics 2022;18:e1010012. [PubMed: 35041640]

[121]. Gray Paul A, Fu H, Luo P, Zhao Q, Yu J, Ferrari A, et al. Mouse Brain Organization Revealed Through Direct Genome-Scale TF Expression Analysis. Science 2004;306:2255–7. [PubMed: 15618518]

[122]. Morral C, Stanisavljevic J, Hernando-Momblona X, Mereu E, Álvarez-Varela A, Cortina C, et al. Zonation of Ribosomal DNA Transcription Defines a Stem Cell Hierarchy in Colorectal Cancer. Cell Stem Cell 2020;26:845–61.e12. [PubMed: 32396863]

[123]. Wang X, Wu Z, Qin W, Sun T, Lu S, Li Y, et al. Long non-coding RNA ZFAS1 promotes colorectal cancer tumorigenesis and development through DDX21-POLR1B regulatory axis. Aging 2020;12:22656–87. [PubMed: 33202381]

[124]. Camps J, Pitt JJ, Emons G, Hummon AB, Case CM, Grade M, et al. Genetic Amplification of the NOTCH Modulator LNX2 Upregulates the WNT/β-Catenin Pathway in Colorectal Cancer. Cancer Research 2013;73:2003–13. [PubMed: 23319804]

[125]. Wang M, Niu W, Hu R, Wang Y, Liu Y, Liu L, et al. POLR1D promotes colorectal cancer progression and predicts poor prognosis of patients. Mol Carcinog 2019;58:735–48. [PubMed: 30582221]

[126]. Tian Y, Sun F, Zhong Y, Huang W, Wang G, Liu C, et al. Expression and Clinical Significance of POLR1D in Colorectal Cancer. Oncology 2020;98:138–45. [PubMed: 31722331]

[127]. Young DW, Hassan MQ, Pratap J, Galindo M, Zaidi SK, Lee SH, et al. Mitotic occupancy and lineage-specific transcriptional control of rRNA genes by Runx2. Nature 2007;445:442–6. [PubMed: 17251981]

[128]. Prakash V, Carson BB, Feenstra JM, Dass RA, Sekyrova P, Hoshino A, et al. Ribosome biogenesis during cell cycle arrest fuels EMT in development and disease. Nature Communications 2019;10:2110.

[129]. Metge BJ, Kammerud SC, Pruitt HC, Shevde LA, Samant RS. Hypoxia re-programs 2′-O-Me modifications on ribosomal RNA. iScience 2021;24:102010. [PubMed: 33490918]

[130]. Sakai D, Dixon J, Achilleos A, Dixon M, Trainor PA. Prevention of Treacher Collins syndrome craniofacial anomalies in mouse models via maternal antioxidant supplementation. Nature Communications 2016;7:10328.

[131]. Sakai D, Trainor PA. Face off against ROS: Tcof1/Treacle safeguards neuroepithelial cells and progenitor neural crest cells from oxidative stress during craniofacial development. Development, growth & differentiation 2016;58:577–85.

[132]. Xu B, Li H, Perry JM, Singh VP, Unruh J, Yu Z, et al. Ribosomal DNA copy number loss and sequence variation in cancer. PLOS Genetics 2017;13:e1006771. [PubMed: 28640831]

[133]. Lopez FB, Fort A, Tadini L, Probst AV, McHale M, Friel J, et al. Gene dosage compensation of rRNA transcript levels in Arabidopsis thaliana lines with reduced ribosomal gene copy number. The Plant Cell 2021;33:1135–50. [PubMed: 33793816]

[134]. Bernard G, Chouery E, Putorti ML, Tetreault M, Takanohashi A, Carosso G, et al. Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. Am J Hum Genet 2011;89:415–23. [PubMed: 21855841]

[135]. Tetreault M, Choquet K, Orcesi S, Tonduti D, Balottin U, Teichmann M, et al. Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. Am J Hum Genet 2011;89:652–5. [PubMed: 22036172]

[136]. Saitsu H, Osaka H, Sasaki M, Takanashi J, Hamada K, Yamashita A, et al. Mutations in POLR3A and POLR3B encoding RNA Polymerase III subunits cause an autosomal-recessive hypomyelinating leukoencephalopathy. Am J Hum Genet 2011;89:644–51. [PubMed: 22036171]

[137]. Atrouni S, Daraze A, Tamraz J, Cassia A, Caillaud C, Megarbane A. Leukodystrophy associated with oligodontia in a large inbred family: fortuitous association or new entity? Am J Med Genet A 2003;118A:76–81. [PubMed: 12605447]

[138]. Wolf NI, Harting I, Innes AM, Patzer S, Zeitler P, Schneider A, et al. Ataxia, delayed dentition and hypomyelination: a novel leukoencephalopathy. Neuropediatrics 2007;38:64–70. [PubMed: 17712733]

[139]. Timmons M, Tsokos M, Asab MA, Seminara SB, Zirzow GC, Kaneski CR, et al. Peripheral and central hypomyelination with hypogonadotropic hypogonadism and hypodontia. Neurology 2006;67:2066–9. [PubMed: 17159124]

[140]. Sasaki M, Takanashi J, Tada H, Sakuma H, Furushima W, Sato N. Diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum. Brain Dev 2009;31:582–7. [PubMed: 18851904]

[141]. Bernard G, Thiffault I, Tetreault M, Putorti ML, Bouchard I, Sylvain M, et al. Tremor-ataxia with central hypomyelination (TACH) leukodystrophy maps to chromosome 10q22.3–10q23.31. Neurogenetics 2010;11:457–64. [PubMed: 20640464]

[142]. Dorboz I, Dumay-Odelot H, Boussaid K, Bouyacoub Y, Barreau P, Samaan S, et al. Mutation in POLR3K causes hypomyelinating leukodystrophy and abnormal ribosomal RNA regulation. Neurol Genet 2018;4:e289. [PubMed: 30584594]

[143]. Bernard G, Vanderver A. POLR3-Related Leukodystrophy. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Mirzaa G, et al., editors. GeneReviews((R)). Seattle (WA)2012.

[144]. Daoud H, Tetreault M, Gibson W, Guerrero K, Cohen A, Gburek-Augustat J, et al. Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. J Med Genet 2013;50:194–7. [PubMed: 23355746]

[145]. Wolf NI, Vanderver A, van Spaendonk RM, Schiffmann R, Brais B, Bugiani M, et al. Clinical spectrum of 4H leukodystrophy caused by POLR3A and POLR3B mutations. Neurology 2014;83:1898–905. [PubMed: 25339210]

[146]. Gutierrez M, Thiffault I, Guerrero K, Martos-Moreno GA, Tran LT, Benko W, et al. Large exonic deletions in POLR3B gene cause POLR3-related leukodystrophy. Orphanet J Rare Dis 2015;10:69. [PubMed: 26045207]

[147]. La Piana R, Cayami FK, Tran LT, Guerrero K, van Spaendonk R, Ounap K, et al. Diffuse hypomyelination is not obligate for POLR3-related disorders. Neurology 2016;86:1622–6. [PubMed: 27029625]

[148]. Gauquelin L, Cayami FK, Sztriha L, Yoon G, Tran LT, Guerrero K, et al. Clinical spectrum of POLR3-related leukodystrophy caused by biallelic POLR1C pathogenic variants. Neurol Genet 2019;5:e369. [PubMed: 32042905]

[149]. Steenweg ME, Vanderver A, Blaser S, Bizzi A, de Koning TJ, Mancini GM, et al. Magnetic resonance imaging pattern recognition in hypomyelinating disorders. Brain 2010;133:2971–82. [PubMed: 20881161]

[150]. La Piana R, Tonduti D, Gordish Dressman H, Schmidt JL, Murnick J, Brais B, et al. Brain magnetic resonance imaging (MRI) pattern recognition in Pol III-related leukodystrophies. J Child Neurol 2014;29:214–20. [PubMed: 24105487]

[151]. Al Yazidi G, Tran LT, Guerrero K, Vanderver A, Schiffmann R, Wolf NI, et al. Dystonia in RNA Polymerase III-Related Leukodystrophy. Mov Disord Clin Pract 2019;6:155–9. [PubMed: 30838315]

[152]. Osterman B, Sylvain M, Chouinard S, Bernard G. Tremor-ataxia with central hypomyelination (TACH): dystonia as a new clinical feature. Mov Disord 2012;27:1829–30. [PubMed: 23208740]

[153]. Pelletier F, Perrier S, Cayami FK, Mirchi A, Saikali S, Tran LT, et al. Endocrine and Growth Abnormalities in 4H Leukodystrophy Caused by Variants in POLR3A, POLR3B, and POLR1C. J Clin Endocrinol Metab 2021;106:e660–e74. [PubMed: 33005949]

[154]. Wolff A, Koch MJ, Benzinger S, van Waes H, Wolf NI, Boltshauser E, et al. Rare dental peculiarities associated with the hypomyelinating leukoencephalopathy 4H syndrome/ADDH. Pediatr Dent 2010;32:386–92. [PubMed: 21070704]

[155]. Billington E, Bernard G, Gibson W, Corenblum B. Endocrine Aspects of 4H Leukodystrophy: A Case Report and Review of the Literature. Case Rep Endocrinol 2015;2015:314594. [PubMed: 26113998]

[156]. Potic A, Brais B, Choquet K, Schiffmann R, Bernard G. 4H syndrome with late-onset growth hormone deficiency caused by POLR3A mutations. Arch Neurol 2012;69:920–3. [PubMed: 22451160]

[157]. Potic A, Popovic V, Ostojic J, Pekic S, Kozic D, Guerrero K, et al. Neurogenic bladder and neuroendocrine abnormalities in Pol III-related leukodystrophy. BMC Neurol 2015;15:22. [PubMed: 25868523]

[158]. Perrier S, Gauquelin L, Fallet-Bianco C, Dishop MK, Michell-Robinson MA, Tran LT, et al. Expanding the phenotypic and molecular spectrum of RNA polymerase III-related leukodystrophy. Neurol Genet 2020;6:e425. [PubMed: 32582862]

[159]. DeGasperis SM, Bernard G, Wolf NI, Miller E, Pohl D. 4H leukodystrophy: Mild clinical phenotype and comorbidity with multiple sclerosis. Neurol Genet 2020;6:e409. [PubMed: 32337336]

[160]. Azmanov DN, Siira SJ, Chamova T, Kaprelyan A, Guergueltcheva V, Shearwood AJ, et al. Transcriptome-wide effects of a POLR3A gene mutation in patients with an unusual phenotype of striatal involvement. Hum Mol Genet 2016;25:4302–14. [PubMed: 27506977]

[161]. Hiraide T, Kubota K, Kono Y, Watanabe S, Matsubayashi T, Nakashima M, et al. POLR3A variants in striatal involvement without diffuse hypomyelination. Brain Dev 2020;42:363–8. [PubMed: 31932101]

[162]. Harting I, Al-Saady M, Krageloh-Mann I, Bley A, Hempel M, Bierhals T, et al. POLR3A variants with striatal involvement and extrapyramidal movement disorder. Neurogenetics 2020;21:121–33. [PubMed: 31940116]

[163]. Wu S, Bai Z, Dong X, Yang D, Chen H, Hua J, et al. Novel mutations of the POLR3A gene caused POLR3-related leukodystrophy in a Chinese family: a case report. BMC Pediatr 2019;19:289. [PubMed: 31438894]

[164]. Richards MR, Plummer L, Chan YM, Lippincott MF, Quinton R, Kumanov P, et al. Phenotypic spectrum of POLR3B mutations: isolated hypogonadotropic hypogonadism without neurological or dental anomalies. J Med Genet 2017;54:19–25. [PubMed: 27512013]

[165]. Ghoumid J, Petit F, Boute-Benejean O, Frenois F, Cartigny M, Vanlerberghe C, et al. Cerebellar hypoplasia with endosteal sclerosis is a POLR3-related disorder. Eur J Hum Genet 2017;25:1011–4. [PubMed: 28589944]

[166]. Girbig M, Misiaszek AD, Vorlander MK, Lafita A, Grotsch H, Baudin F, et al. Cryo-EM structures of human RNA polymerase III in its unbound and transcribing states. Nature structural & molecular biology 2021;28:210–9.

[167]. Choquet K, Pinard M, Yang S, Moir RD, Poitras C, Dicaire MJ, et al. The leukodystrophy mutation Polr3b R103H causes homozygote mouse embryonic lethality and impairs RNA polymerase III biogenesis. Mol Brain 2019;12:59. [PubMed: 31221184]

[168]. Choquet K, Yang S, Moir RD, Forget D, Lariviere R, Bouchard A, et al. Absence of neurological abnormalities in mice homozygous for the Polr3a G672E hypomyelinating leukodystrophy mutation. Mol Brain 2017;10:13. [PubMed: 28407788]

[169]. Djordjevic D, Pinard M, Gauthier MS, Smith-Hicks C, Hoffman TL, Wolf NI, et al. De novo variants in POLR3B cause ataxia, spasticity, and demyelinating neuropathy. Am J Hum Genet 2021;108:186–93. [PubMed: 33417887]

[170]. Xue YY, Cheng HL, Dong HL, Yin HM, Yuan Y, Meng LC, et al. A de novo variant of POLR3B causes demyelinating Charcot-Marie-Tooth disease in a Chinese patient: a case report. BMC Neurol 2021;21:402. [PubMed: 34666706]

[171]. Beauregard-Lacroix E, Salian S, Kim H, Ehresmann S, D'Amours G, Gauthier J, et al. A variant of neonatal progeroid syndrome, or Wiedemann-Rautenstrauch syndrome, is associated with a nonsense variant in POLR3GL. Eur J Hum Genet 2020;28:461–8. [PubMed: 31695177]

[172]. Paolacci S, Li Y, Agolini E, Bellacchio E, Arboleda-Bustos CE, Carrero D, et al. Specific combinations of biallelic POLR3A variants cause Wiedemann-Rautenstrauch syndrome. J Med Genet 2018;55:837–46. [PubMed: 30323018]

[173]. Wambach JA, Wegner DJ, Patni N, Kircher M, Willing MC, Baldridge D, et al. Bi-allelic POLR3A Loss-of-Function Variants Cause Autosomal-Recessive Wiedemann-Rautenstrauch Syndrome. Am J Hum Genet 2018;103:968–75. [PubMed: 30414627]

[174]. Wu SW, Li L, Feng F, Wang L, Kong YY, Liu XW, et al. Whole-exome sequencing reveals POLR3B variants associated with progeria-related Wiedemann-Rautenstrauch syndrome. Ital J Pediatr 2021;47:160. [PubMed: 34289880]

[175]. Jay AM, Conway RL, Thiffault I, Saunders C, Farrow E, Adams J, et al. Neonatal progeriod syndrome associated with biallelic truncating variants in POLR3A. Am J Med Genet A 2016;170:3343–6. [PubMed: 27612211]

[176]. Perrier S, Gauquelin L, Wambach JA, Bernard G. Distinguishing severe phenotypes associated with pathogenic variants in POLR3A. Am J Med Genet A 2021.

[177]. Rautenstrauch T, Snigula F. Progeria: a cell culture study and clinical report of familial incidence. Eur J Pediatr 1977;124:101–11. [PubMed: 319005]

[178]. Wiedemann HR. An unidentified neonatal progeroid syndrome: follow-up report. Eur J Pediatr 1979;130:65–70. [PubMed: 569581]

[179]. Terhal PA, Vlaar JM, Middelkamp S, Nievelstein RAJ, Nikkels PGJ, Ross J, et al. Biallelic variants in POLR3GL cause endosteal hyperostosis and oligodontia. Eur J Hum Genet 2020;28:31–9. [PubMed: 31089205]

[180]. Boissier F, Dumay-Odelot H, Teichmann M, Fribourg S. Structural analysis of human RPC32beta-RPC62 complex. J Struct Biol 2015;192:313–9. [PubMed: 26394183]

[181]. Wang X, Gerber A, Chen W-Y, Roeder RG. Functions of paralogous RNA polymerase III subunits POLR3G and POLR3GL in mouse development. Proceedings of the National Academy of Sciences 2020;117:15702.

[182]. Borck G, Hog F, Dentici ML, Tan PL, Sowada N, Medeira A, et al. BRF1 mutations alter RNA polymerase III-dependent transcription and cause neurodevelopmental anomalies. Genome Res 2015;25:155–66. [PubMed: 25561519]

[183]. Liko D, Mitchell L, Campbell KJ, Ridgway RA, Jones C, Dudek K, et al. Brf1 loss and not overexpression disrupts tissues homeostasis in the intestine, liver and pancreas. Cell Death & Differentiation 2019;26:2535–50. [PubMed: 30858608]

[184]. Welting TJ, Mattijssen S, Peters FM, van Doorn NL, Dekkers L, van Venrooij WJ, et al. Cartilage-hair hypoplasia-associated mutations in the RNase MRP P3 domain affect RNA folding and ribonucleoprotein assembly. Biochim Biophys Acta 2008;1783:455–66. [PubMed: 18164267]

[185]. Martin AN, Li Y. RNase MRP RNA and human genetic diseases. Cell Res 2007;17:219–26. [PubMed: 17189938]

[186]. Choquet K, Forget D, Meloche E, Dicaire MJ, Bernard G, Vanderver A, et al. Leukodystrophy-associated POLR3A mutations down-regulate the RNA polymerase III transcript and important regulatory RNA BC200. J Biol Chem 2019;294:7445–59. [PubMed: 30898877]

[187]. Graczyk D, Ciesla M, Boguta M. Regulation of tRNA synthesis by the general transcription factors of RNA polymerase III - TFIIIB and TFIIIC, and by the MAF1 protein. Biochim Biophys Acta Gene Regul Mech 2018;1861:320–9. [PubMed: 29378333]

[188]. Bhargava P Epigenetic regulation of transcription by RNA polymerase III. Biochim Biophys Acta 2013;1829:1015–25. [PubMed: 23732820]

[189]. Moir RD, Willis IM. Regulation of pol III transcription by nutrient and stress signaling pathways. Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms 2013;1829:361–75. [PubMed: 23165150]

[190]. Bonhoure N, Byrnes A, Moir RD, Hodroj W, Preitner F, Praz V, et al. Loss of the RNA polymerase III repressor MAF1 confers obesity resistance. Genes & Development 2015;29:934–47. [PubMed: 25934505]

[191]. Chen CY, Lanz RB, Walkey CJ, Chang WH, Lu W, Johnson DL. Maf1 and Repression of RNA Polymerase III-Mediated Transcription Drive Adipocyte Differentiation. Cell Rep 2018;24:1852–64. [PubMed: 30110641]

[192]. Busschers E, Ahmad N, Sun L, Iben J, Walkey CJ, Rusin A, et al. Maf1, a repressor of RNA polymerase III-dependent transcription, regulates bone mass. bioRxiv 2021:2021.11.09.467887.

[193]. Wang Z, Wu C, Aslanian A, Yates JR 3rd, Hunter T. Defective RNA polymerase III is negatively regulated by the SUMO-Ubiquitin-Cdc48 pathway. Elife 2018;7.

[194]. Yee NS, Gong W, Huang Y, Lorent K, Dolan AC, Maraia RJ, et al. Mutation of RNA Pol III Subunit rpc2/polr3b Leads to Deficiency of Subunit Rpc11 and Disrupts Zebrafish Digestive Development. PLOS Biology 2007;5:e312. [PubMed: 18044988]

[195]. McQueen C, Hughes GL, Pownall ME. Skeletal muscle differentiation drives a dramatic downregulation of RNA polymerase III activity and differential expression of Polr3g isoforms. Developmental Biology 2019;454:74–84. [PubMed: 31173763]

[196]. Lund RJ, Rahkonen N, Malonzo M, Kauko L, Emani MR, Kivinen V, et al. RNA Polymerase III Subunit POLR3G Regulates Specific Subsets of PolyA+ and SmallRNA Transcriptomes and Splicing in Human Pluripotent Stem Cells. Stem Cell Reports 2017;8:1442–54. [PubMed: 28494942]

[197]. White RJ. RNA polymerase III transcription and cancer. Oncogene 2004;23:3208–16. [PubMed: 15094770]

[198]. Yeganeh M, Praz V, Carmeli C, Villeneuve D, Rib L, Guex N, et al. Differential regulation of RNA polymerase III genes during liver regeneration. Nucleic Acids Res 2019;47:1786–96. [PubMed: 30597109]

[199]. Wolffe AP, Brown DD. Developmental regulation of two 5S ribosomal RNA genes. Science 1988;241:1626–32. [PubMed: 3420414]

[200]. Locati MD, Pagano JFB, Ensink WA, van Olst M, van Leeuwen S, Nehrdich U, et al. Linking maternal and somatic 5S rRNA types with different sequence-specific non-LTR retrotransposons. RNA 2017;23:446–56. [PubMed: 28003516]

[201]. Tiedge H, Chen W, Brosius J. Primary structure, neural-specific expression, and dendritic location of human BC200 RNA. The Journal of Neuroscience 1993;13:2382. [PubMed: 7684772]

[202]. Steinbusch MMF, Caron MMJ, Surtel DAM, Friedrich F, Lausch E, Pruijn GJM, et al. Expression of RMRP RNA is regulated in chondrocyte hypertrophy and determines chondrogenic differentiation. Scientific Reports 2017;7:6440. [PubMed: 28743979]

[203]. Vakkilainen S, Skoog T, Einarsdottir E, Middleton A, Pekkinen M, Öhman T, et al. The human long non-coding RNA gene RMRP has pleiotropic effects and regulates cell-cycle progression at G2. Scientific Reports 2019;9:13758. [PubMed: 31551465]

[204]. Ridanpää M, van Eenennaam H, Pelin K, Chadwick R, Johnson C, Yuan B, et al. Mutations in the RNA component of RNase MRP cause a pleiotropic human disease, cartilage-hair hypoplasia. Cell 2001;104:195–203. [PubMed: 11207361]

[205]. Rogler LE, Kosmyna B, Moskowitz D, Bebawee R, Rahimzadeh J, Kutchko K, et al. Small RNAs derived from lncRNA RNase MRP have gene-silencing activity relevant to human cartilage-hair hypoplasia. Hum Mol Genet 2014;23:368–82. [PubMed: 24009312]

[206]. Lant JT, Berg MD, Heinemann IU, Brandl CJ, O'Donoghue P. Pathways to disease from natural variations in human cytoplasmic tRNAs. J Biol Chem 2019;294:5294–308. [PubMed: 30643023]

[207]. Dittmar KA, Goodenbour JM, Pan T. Tissue-Specific Differences in Human Transfer RNA Expression. PLOS Genetics 2006;2:e221. [PubMed: 17194224]

[208]. Ishimura R, Nagy G, Dotu I, Zhou H, Yang XL, Schimmel P, et al. RNA function. Ribosome stalling induced by mutation of a CNS-specific tRNA causes neurodegeneration. Science 2014;345:455–9. [PubMed: 25061210]

[209]. Mendes MI, Gutierrez Salazar M, Guerrero K, Thiffault I, Salomons GS, Gauquelin L, et al. Bi-allelic Mutations in EPRS, Encoding the Glutamyl-Prolyl-Aminoacyl-tRNA Synthetase, Cause a Hypomyelinating Leukodystrophy. Am J Hum Genet 2018;102:676–84. [PubMed: 29576217]

[210]. Nafisinia M, Sobreira N, Riley L, Gold W, Uhlenberg B, Weiss C, et al. Mutations in RARS cause a hypomyelination disorder akin to Pelizaeus-Merzbacher disease. Eur J Hum Genet 2017;25:1134–41. [PubMed: 28905880]

[211]. Taft RJ, Vanderver A, Leventer RJ, Damiani SA, Simons C, Grimmond SM, et al. Mutations in DARS cause hypomyelination with brain stem and spinal cord involvement and leg spasticity. Am J Hum Genet 2013;92:774–80. [PubMed: 23643384]

[212]. Sagi D, Rak R, Gingold H, Adir I, Maayan G, Dahan O, et al. Tissue- and Time-Specific Expression of Otherwise Identical tRNA Genes. PLOS Genetics 2016;12:e1006264. [PubMed: 27560950]

[213]. Gingold H, Tehler D, Christoffersen Nanna R, Nielsen Morten M, Asmar F, Kooistra Susanne M, et al. A Dual Program for Translation Regulation in Cellular Proliferation and Differentiation. Cell 2014;158:1281–92. [PubMed: 25215487]

[214]. Lee YS, Shibata Y, Malhotra A, Dutta A. A novel class of small RNAs: tRNA-derived RNA fragments (tRFs). Genes & Development 2009;23:2639–49. [PubMed: 19933153]

[215]. Kim HK, Xu J, Chu K, Park H, Jang H, Li P, et al. A tRNA-Derived Small RNA Regulates Ribosomal Protein S28 Protein Levels after Translation Initiation in Humans and Mice. Cell Reports 2019;29:3816–24.e4. [PubMed: 31851915]

[216]. Bazzini AA, Del Viso F, Moreno-Mateos MA, Johnstone TG, Vejnar CE, Qin Y, et al. Codon identity regulates mRNA stability and translation efficiency during the maternal-to-zygotic transition. EMBO J 2016;35:2087–103. [PubMed: 27436874]

[217]. Wu Q, Medina SG, Kushawah G, DeVore ML, Castellano LA, Hand JM, et al. Translation affects mRNA stability in a codon-dependent manner in human cells. Elife 2019;8.

[218]. Torrent M, Chalancon G, de Groot NS, Wuster A, Madan Babu M. Cells alter their tRNA abundance to selectively regulate protein synthesis during stress conditions. Sci Signal 2018;11.

[219]. Guimaraes JC, Mittal N, Gnann A, Jedlinski D, Riba A, Buczak K, et al. A rare codon-based translational program of cell proliferation. Genome Biology 2020;21:44. [PubMed: 32102681]

[220]. Burow DA, Umeh-Garcia MC, True MB, Bakhaj CD, Ardell DH, Cleary MD. Dynamic regulation of mRNA decay during neural development. Neural Dev 2015;10:11. [PubMed: 25896902]

[221]. Burow DA, Martin S, Quail JF, Alhusaini N, Coller J, Cleary MD. Attenuated Codon Optimality Contributes to Neural-Specific mRNA Decay in Drosophila. Cell Rep 2018;24:1704–12. [PubMed: 30110627]

[222]. Mayer C, Grummt I. Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. Oncogene 2006;25:6384–91. [PubMed: 17041624]

[223]. Mills EW, Green R. Ribosomopathies: There's strength in numbers. Science 2017;358.

[224]. Xue S, Barna M. Specialized ribosomes: a new frontier in gene regulation and organismal biology. Nat Rev Mol Cell Biol 2012;13:355–69. [PubMed: 22617470]

[225]. Kondrashov N, Pusic A, Stumpf CR, Shimizu K, Hsieh AC, Ishijima J, et al. Ribosome-mediated specificity in Hox mRNA translation and vertebrate tissue patterning. Cell 2011;145:383–97. [PubMed: 21529712]

[226]. Shi Z, Fujii K, Kovary KM, Genuth NR, Röst HL, Teruel MN, et al. Heterogeneous Ribosomes Preferentially Translate Distinct Subpools of mRNAs Genome-wide. Mol Cell 2017;67:71–83 e7. [PubMed: 28625553]

[227]. Amirbeigiarab S, Kiani P, Velazquez Sanchez A, Krisp C, Kazantsev A, Fester L, et al. Invariable stoichiometry of ribosomal proteins in mouse brain tissues with aging. Proceedings of the National Academy of Sciences 2019;116:22567–72.

[228]. Khajuria RK, Munschauer M, Ulirsch JC, Fiorini C, Ludwig LS, McFarland SK, et al. Ribosome Levels Selectively Regulate Translation and Lineage Commitment in Human Hematopoiesis. Cell 2018;173:90–103 e19. [PubMed: 29551269]

[229]. Warner JR, McIntosh KB. How common are extraribosomal functions of ribosomal proteins? Mol Cell 2009;34:3–11. [PubMed: 19362532]

[230]. Hayano T, Yanagida M, Yamauchi Y, Shinkawa T, Isobe T, Takahashi N. Proteomic analysis of human Nop56p-associated pre-ribosomal ribonucleoprotein complexes. Possible link between Nop56p and the nucleolar protein treacle responsible for Treacher Collins syndrome. J Biol Chem 2003;278:34309–19. [PubMed: 12777385]

[231]. Ciccia A, Huang J-W, Izhar L, Sowa ME, Harper JW, Elledge SJ. Treacher Collins syndrome TCOF1 protein cooperates with NBS1 in the DNA damage response. Proceedings of the National Academy of Sciences 2014;111:18631–6.

[232]. de Peralta MSP, Mouguelar VS, Sdrigotti MA, Ishiy FAA, Fanganiello RD, Passos-Bueno MR, et al. Cnbp ameliorates Treacher Collins Syndrome craniofacial anomalies through a

pathway that involves redox-responsive genes. Cell Death & Disease 2016;7:e2397–e. [PubMed: 27711076]

[233]. Calo E, Gu B, Bowen ME, Aryan F, Zalc A, Liang J, et al. Tissue-selective effects of nucleolar stress and rDNA damage in developmental disorders. Nature 2018;554:112–7. [PubMed: 29364875]

[234]. Song W, Joo M, Yeom JH, Shin E, Lee M, Choi HK, et al. Divergent rRNAs as regulators of gene expression at the ribosome level. Nat Microbiol 2019;4:515–26. [PubMed: 30718849]

[235]. Locati MD, Pagano JFB, Girard G, Ensink WA, van Olst M, van Leeuwen S, et al. Expression of distinct maternal and somatic 5.8S, 18S, and 28S rRNA types during zebrafish development. RNA 2017;23:1188–99. [PubMed: 28500251]

[236]. Tseng H, Chou W, Wang J, Zhang X, Zhang S, Schultz RM. Mouse Ribosomal RNA Genes Contain Multiple Differentially Regulated Variants. PLOS ONE 2008;3:e1843. [PubMed: 18365001]

[237]. Parks MM, Kurylo CM, Dass RA, Bojmar L, Lyden D, Vincent CT, et al. Variant ribosomal RNA alleles are conserved and exhibit tissue-specific expression. Science Advances 2018;4:eaao0665. [PubMed: 29503865]

[238]. Allen KD, Regier MJ, Hsieh C, Tsokas P, Barnard M, Phatarpekar S, et al. Learning-induced ribosomal RNA is required for memory consolidation in mice-Evidence of differentially expressed rRNA variants in learning and memory. PLoS One 2018;13:e0203374. [PubMed: 30281601]

[239]. Yuan X, Zhou Y, Casanova E, Chai M, Kiss E, Gröne H-J, et al. Genetic Inactivation of the Transcription Factor TIF-IA Leads to Nucleolar Disruption, Cell Cycle Arrest, and p53-Mediated Apoptosis. Molecular Cell 2005;19:77–87. [PubMed: 15989966]

[240]. Rubbi CP, Milner J. Disruption of the nucleolus mediates stabilization of p53 in response to DNA damage and other stresses. EMBO J 2003;22:6068–77. [PubMed: 14609953]

[241]. Fumagalli S, Di Cara A, Neb-Gulati A, Natt F, Schwemberger S, Hall J, et al. Absence of nucleolar disruption after impairment of 40S ribosome biogenesis reveals an rpL11-translation-dependent mechanism of p53 induction. Nat Cell Biol 2009;11:501–8. [PubMed: 19287375]

[242]. Dutt S, Narla A, Lin K, Mullally A, Abayasekara N, Megerdichian C, et al. Haploinsufficiency for ribosomal protein genes causes selective activation of p53 in human erythroid progenitor cells. Blood 2011;117:2567–76. [PubMed: 21068437]

[243]. Hannan KM, Soo P, Wong MS, Lee JK, Hein N, Evers M, et al. Nuclear stabilisation of p53 requires a functional nucleolar surveillance pathway. bioRxiv 2021:2021.01.21.427535.

[244]. Kieckhaefer JE, Lukovac S, Ye DZ, Lee D, Beetler DJ, Pack M, et al. The RNA Polymerase III Subunit Polr3b Is Required for the Maintenance of Small Intestinal Crypts in Mice. Cellular and Molecular Gastroenterology and Hepatology 2016;2:783–95. [PubMed: 28090567]

[245]. Wei Y, Xu J, Zhang W, Wen Z, Liu F. RNA polymerase III component Rpc9 regulates hematopoietic stem and progenitor cell maintenance in zebrafish. Development 2016;143:2103–10. [PubMed: 27151951]

[246]. Zhai W, Comai L. Repression of RNA polymerase I transcription by the tumor suppressor p53. Molecular and cellular biology 2000;20:5930–8. [PubMed: 10913176]

[247]. Cairns CA, White RJ. p53 is a general repressor of RNA polymerase III transcription. The EMBO Journal 1998;17:3112–23. [PubMed: 9606193]

[248]. Scala F, Brighenti E, Govoni M, Imbrogno E, Fornari F, Treré D, et al. Direct relationship between the level of p53 stabilization induced by rRNA synthesis-inhibiting drugs and the cell ribosome biogenesis rate. Oncogene 2016;35:977–89. [PubMed: 25961931]

[249]. Li M, Gu W. A Critical Role for Noncoding 5S rRNA in Regulating Mdmx Stability. Molecular Cell 2011;43:1023–32. [PubMed: 21925390]

[250]. Báez-Becerra CT, Valencia-Rincón E, Velásquez-Méndez K, Ramírez-Suárez NJ, Guevara C, Sandoval-Hernandez A, et al. Nucleolar disruption, activation of P53 and premature senescence in POLR3A-mutated Wiedemann-Rautenstrauch syndrome fibroblasts. Mechanisms of Ageing and Development 2020;192:111360. [PubMed: 32976914]

[251]. Ogunjimi B, Zhang SY, Sørensen KB, Skipper KA, Carter-Timofte M, Kerner G, et al. Inborn errors in RNA polymerase III underlie severe varicella zoster virus infections. J Clin Invest 2017;127:3543–56. [PubMed: 28783042]

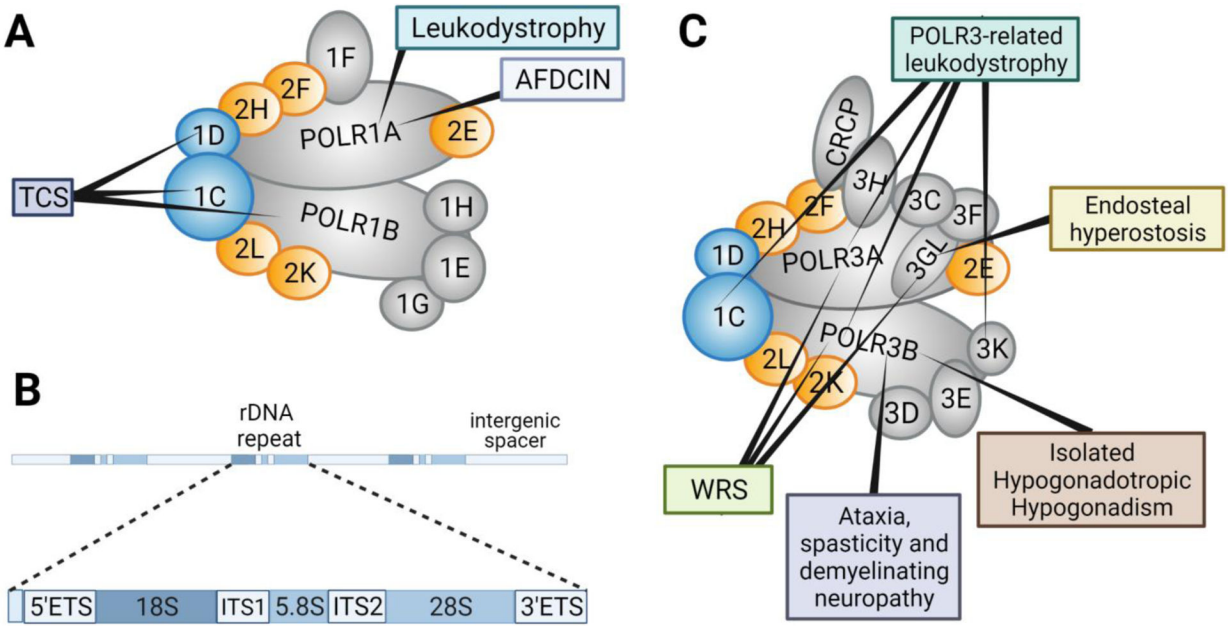

Figure 1.

RNA Polymerase I subunits, rDNA repeat, and RNA Polymerase III subunits. A) Schematic representation of Pol I in humans. Shared Pol I and III subunits POLR1C and POLR1D are in blue and subunits shared across Pols I, II, and III are in orange. B) Structure of the rDNA repeat. The promoter elements are denoted in light blue. The 47S transcript consists of the 5' Externally Transcribed Spacer (ETS), 18S rRNA, Internally Transcribed Spacer (ITS)1, 5.8S rRNA, ITS2, 28S rRNA, and the 3'ETS. These rDNA repeats are separated by intergenic spacers (white). C) Schematic representation of Pol III subunits in humans. Pol III isoform with subunit POLR3GL is represented, but it should be noted that an alternative form of Pol III exists with subunit POLR3G (not shown). Shared subunits are indicated as in A). Disorders associated with Pol I and III subunits are indicated in boxes.

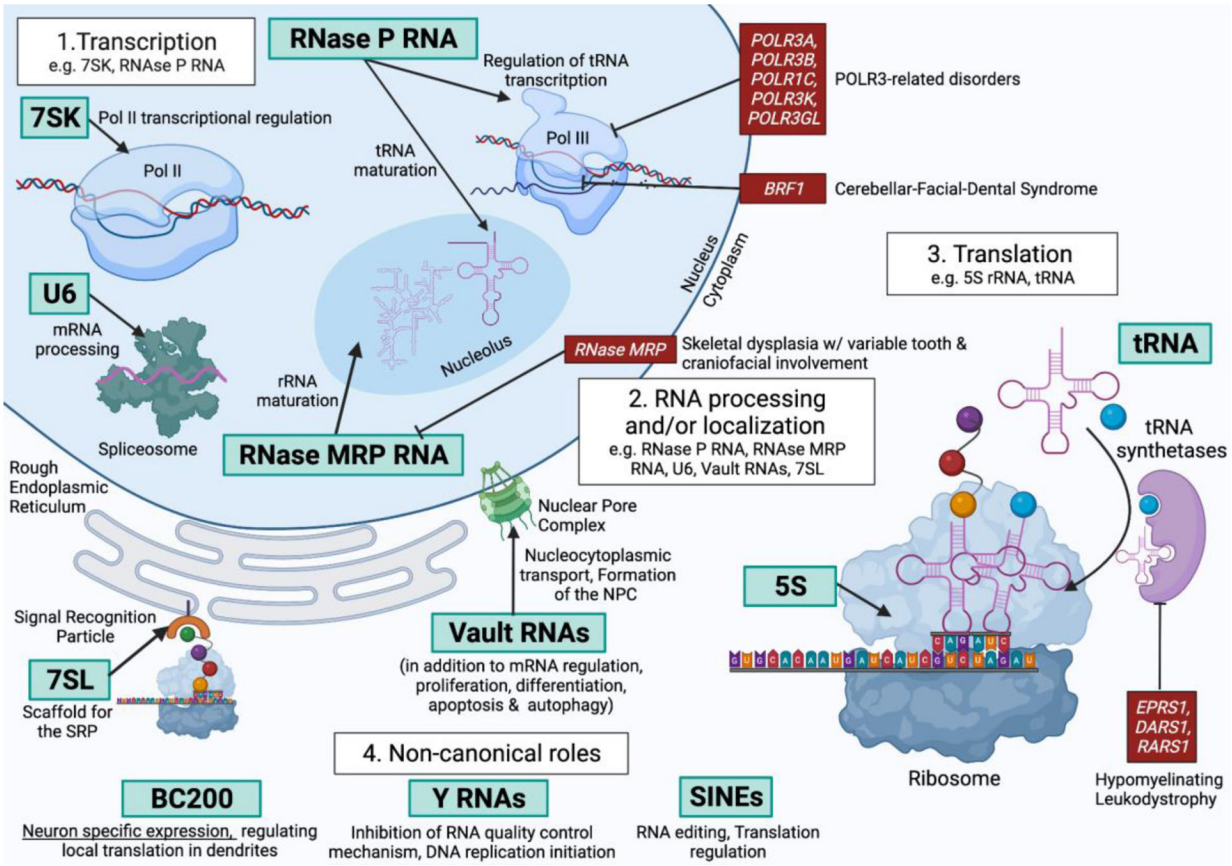

Figure 2.

Broad grouping of classic Pol III transcripts and their functions. Classic RNA polymerase III transcripts and their roles in transcription, RNA processing and/or localization and translation. Abbreviations: Pol II, RNA polymerase II; Pol III, RNA polymerase III ; tRNA, transfer RNA ; rRNA, ribosomal RNA; mRNA, messenger RNA; BRF1, Transcription Factor IIIB ; SRP, signal recognition particle; NPC, nuclear pore complex, EPRS1, Glutamyl-Prolyl-tRNA synthetase 1; DARS1, Aspartyl-tRNA-synthetase 1; RARS1, Arginyl-tRNA-synthetase 1.

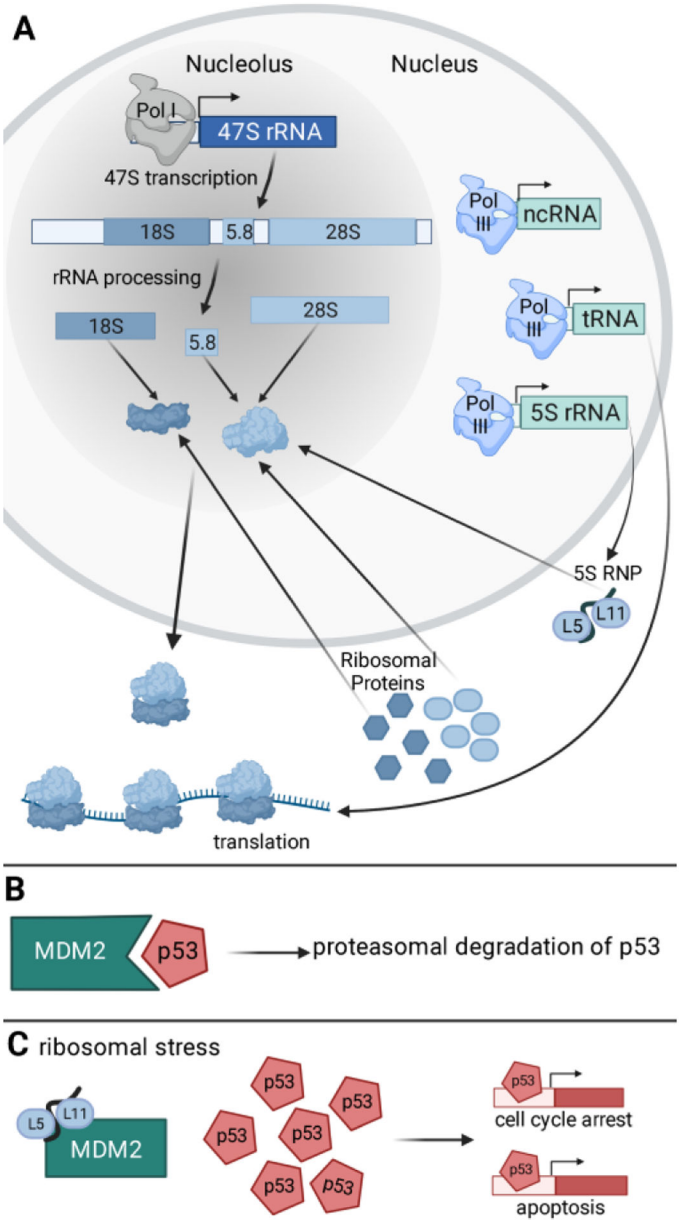

Figure 3.

Ribosome biogenesis occurs in the nucleolus. A) The 47S rRNA is transcribed by Pol I, and is then processed, modified, and incorporated with ribosomal proteins to form the mature ribosome, which functions in translation. Pol III transcribes the 5S rRNA, which associates with Rpl5 and Rpl11 and is incorporated into the 60S ribosomal subunit. Pol III also transcribes non-coding RNAs involved in rRNA processing and tRNAs important for translation. B) Under conditions of normal ribosome biogenesis and cell growth, MDM2 binds to p53 targeting it for proteasomal degradation. C) Under conditions of ribosomal stress, the 5S RNP which includes RPL5 and RPL11 binds MDM2, causing it to undergo a conformational change such that it can no longer bind to p53. This results in p53 accumulation and activation of target genes in cell cycle arrest and apoptosis.

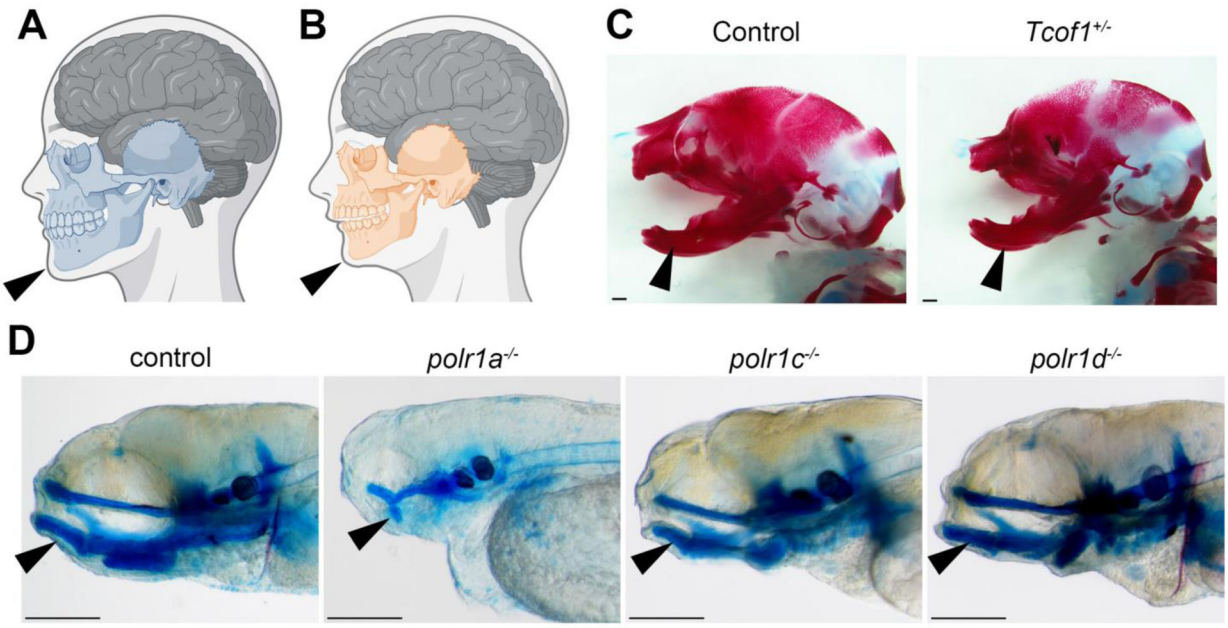

Figure 4.

Models of craniofacial disorders arising from disruption in Pol I transcription. Hypoplasia of NCC derived skeletal elements is present in AFDCIN and TCS (B) compared to unaffected individuals (A). Cartilage and bone staining reveals hypoplasia of skeletal elements in mouse models of TCS (C) and zebrafish models of AFDCIN and TCS (D). Arrowheads indicate areas of mandibular hypoplasia.

Table 1:

Overview of common clinical findings in diseases arising from variants in genes encoding Pol III subunits.

	POLR3-related leukodystrophy	**Ataxia, spasticity and demyelinating neuropathy**	**Wiedemann-Rautenstrauch syndrome**	**Endosteal hyperostosis**	**Isolated Hypogonadotropic Hypogonadism**
Pol III subunit affected	*POLR3A*, *POLR3B*, *POLR1C*, *POLR3K*	*POLR3B*	*POLR3A*, *POLR3B*, *POLR3GL*	*POLR3GL*	*POLR3B*
Inheritance	Autosomal recessive	*De novo*	Autosomal recessive	Autosomal recessive	Autosomal recessive
Neurological features	Cerebellar (ataxia, dysmetria, dysarthria), Pyramidal (spasticity), Extrapyramidal (dystonia) signs, Cognitive involvement	Pyramidal (spasticity), Cerebellar signs (ataxia, dysmetria, dysarthria) Sensorimotor demyelinating peripheral neuropathy Developmental delay, Epilepsy	Not common – Hypotonia and/or Developmental delay in some individuals	Motor delay, Hypotonia, Mild intellectual disability	-
Myelin	Diffuse brain hypomyelination	Peripheral nerves demyelination	-	-	-
Teeth	Hypodontia, Oligodontia, Delayed dentition, Natal teeth, etc.	-	Oligodontia, Natal teeth	Oligodontia	-
Endocrine	Hypogonadotropic hypogonadism (absent, delayed or arrested puberty), growth hormone deficiency, others	-	-	Hypogonadotropic Hypogonadism	Hypogonadotropic Hypogonadism
Growth	Short stature	-	Short stature	Short stature	-
Eyes	Myopia	-	Myopia, Hyperopia or normal vision	Hyperopia	-
Bone	Osteosclerosis (rare), Endosteal Sclerosis in some patients with biallelic *POLR3B* variants	-	Congenital fractures	Axial endosteal hyperostosis	-
Adipose tissue	-	-	Lipodystrophy, Abnormal distribution of fat tissue	-	-
Craniofacial bones	Not common - reported in some patients with biallelic *POLR1C* variants	-	Characteristic facial features ex. triangular face, widened fontanelles, mandibular hypoplasia	Dysmorphic facial features	-