Review

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Mark Steven LeDoux *

Posted Date: 4 April 2024

doi: 10.20944/preprints202404.0322.v1

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 doi:10.20944/preprints202404.0322.v1

Review

Polymerase I as a Target for Treating Neurodegenerative Disorders

Mark S. LeDoux [1,2]

[1] Department of Psychology and College of Health Sciences, University of Memphis, Memphis, TN, 38152, USA; msledoux@memphis.edu or mledoux@veracityneuroscience.com
[2] Veracity Neuroscience LLC, Memphis, TN, 38157, USA

Abstract: Polymerase I (Pol I) is at the epicenter of ribosomal RNA (rRNA) synthesis. Pol I is a target for treatment of cancer. Given the many cellular commonalities between cancer and neurodegeneration (i.e., different faces of the same coin) it seems rational to consider targeting of Pol I or, more generally, rRNA synthesis for treatment of disorders associated with the death of terminally differentiated neurons. Principally, ribosomes synthesize proteins and, accordingly, Pol I can be considered the starting point for protein synthesis. Given that cellular accumulation of abnormal proteins such as α-synuclein and tau is an essential feature of neurodegenerative disorders such as Parkinson disease and fronto-temporal dementia, reduction of protein production is now considered as a viable target for treatment of these and closely related neurodegenerative disorders. Abnormalities in polymerase I activity and rRNA production may also be associated with nuclear and nucleolar stress, DNA damage, and childhood onset neuronal death as is the case for the UBTF E210K neuroregression syndrome. Moreover, restraining the activity of Pol I may be a viable strategy to slow aging. Before starting down the road of Pol I inhibition for treating non-cancerous disorders of the nervous system, many questions must be answered. First, how much Pol I inhibition can neurons tolerate and for how long? Should inhibition of Pol I be continuous or pulsed? Will cells compensate for Pol I inhibition by upregulating the number of active rDNAs? At present, we have no effective and safe disease modulatory treatments for Alzheimer disease, α-synucleinopathies, or tauopathies, and novel therapeutic targets and approaches must be explored.

Keywords: polymerase I; DNA; neurodegeneration; neuroregression; nucleolus; upstream binding transcription factor (UBTF)

1. Introduction

Polymerase I (Pol I) is the rate limiting enzyme in the production of ribosomes. Ribosomes are intricate ribonucleoprotein complexes that translate mRNA into protein. Production of ribosomes utilizes a high percentage of intracellular energy and resources. In non-neuronal eukaryotic cells, well over 50% of active transcription is devoted to rRNA. In the nucleolus, Pol I transcribes a 47S rRNA precursor. Processing of this precursor into 28S, 18S and 5.8S rRNA and pre-assembly of ribosomes also occurs in the nucleolus. Nucleolar assembly of ribosomes requires movement of ribosomal proteins, ribosomal biogenesis proteins, 5S rRNA, and snoRNAs into the nucleolus. Pre-assembled ribosomes are exported out of the nucleolus into the nucleus for further maturation and then into the cytoplasm for final maturation. Abnormalities in ribosomes, the rate of ribosomal biogenesis, and the quality and quantity of protein production plays a role in numerous monogenic disorders, cancers, and, neurodegeneration. As such, upstream control of the rate limiting enzyme Pol I is an attractive therapeutic target for a number of medical disorders.

 doi:10.20944/preprints202404.0322.v1

2. The Nucleolus and Ribosomal DNA (rDNA)

The nucleolus is plurifunctional and harbors one to several hundred active rDNA copies, >100 small nucleolar RNAs required for rRNA processing, and >350 proteins [1]. Many of the proteins in the nucleolus do not end up in mature ribosomes. Numerous nucleolar proteins are involved in DNA repair, cell-cycle control and signal recognition. The telomerase complex is assembled in the nucleolus and telomere and rDNA silencing share factors important in genome stability [2,3]. For example, the action of two important cell-cycle regulators, p53 and MDM2, are regulated by sequestration in the nucleolus. Induction of rRNA synthesis and other nucleolar activities play a role in normal neural processes such as neurite outgrowth and memory consolidation during spatial training [4].

Nucleolar stress, which involves aberrant rRNA expression, has been associated with neurodegenerative disorders including Alzheimer disease and Parkinson disease [5,6] and, more recently, neuroregression [7]. Werner syndrome, characterized by premature aging and early death, is due to recessive LOF mutations in *WRN* which encodes a DNA helicase localized in the nucleolus [8,9]. Rett syndrome, an X-linked neuroregression syndrome due to *MECP2* mutations may also be due, in part, to nucleolar dysfunction [10]. MECP2 is a chromatin-associated protein that binds to methylated CpGs including those associated with rDNA [11]. In preweanling mice, loss of MECP2 results in significantly smaller nucleoli and increased numbers of nucleoli in primary cortical neurons [12]. Overexpression of MECP2 in human 5Y cells results in larger nucleoli compared to the parental cell line [12]. Accordingly, longitudinal study of rRNA expression and nucleolar structure in mouse models may generate novel avenues for exploration of other neurodegenerative disorders, neuroregression syndromes, and aging.

The nucleolus forms around active chromosomal rDNA arrays. Nucleolus number and size is a dynamic process closely tied to cell cycle regulation, rRNA production and environmental cues. Clusters of rDNA arrays (nucleolus organizer regions, NORs) are present on human acrocentric chromosomes 13, 14, 15, 21 and 22. The haploid human and mouse genomes contain around 200 rRNA genes (rDNA) arranged in direct repeats at the NORs. The NORs are subject to highly elevated levels of recombination and have been implicated in a number of genetic diseases including Robertsonian and other chromosomal translocations [13]. Each cluster consists of multiple 45S rDNA repeat units that vary in number among individuals and chromosomes. The rDNA loci are arranged in telomere to centromer orientation. Epigenetic control of rDNA involves a multitude of factors including CpG methylation of rDNA, UBTF binding, and chromatin modifications [14,15]. Some NORs are permanently silenced by CpG methylation (meCpG). Active NORs correspond to visibly decondensed AgNOR loci (stained by silver) secondary constrictions in metaphase spreads, while meCpG silenced rDNA corresponds to condensed NORs [16]. Tissue-specific methylation of rDNA may increase with aging and correlates with a decline in rRNA expression [17].

Acrocentric chromosomes are characterized by a non-central centromere. Each of these chromosomes harbors many copies of rDNA, presumably to support high levels of rRNA and ribosome production particularly during cellular proliferation and periods of increased physiological demand. Using 2546 human genomes from the 1000 Genomes Project, it has been estimated that human rDNA copy number ranges from 61 to 1590 per diploid human genome (range: 61-1590, mean: 315) [18]. Other types of rDNA variation have been explored but incompletely characterized in human brain: 5S rDNA position relative to 28S rDNA (S-type or L-type), single nucleotide coding and non-coding variants, retrotransposons, and structural variants (i.e., inversions) [19]. Moreover, particularly in the context of neurological disease and post-mitotic neurons *in situ*, little is known about rRNA transcription, ribosome production, ribosome stability, mosaicism and ribosome turnover. All of these variables could impact the rate and fidelity of ribosome biogenesis, and response to inhibition of Pol I.

 doi:10.20944/preprints202404.0322.v1

3. Structure and function of Pol I

A total of 13 genes encodes proteins required for formation of the 600 kDa Pol I complex. The active center of Pol I is composed of RPA1 and RPA2. Five Pol I subunits (RPABC1, RPABC2, RPABC3, RPABC4, and RPABC5) are common to all three polymerases (Pol I, Pol II, and Pol III). Pol II transcribes mRNAs, small nuclear RNAs (snRNAs) and microRNAs. Pol III transcribes 5S rRNA and tRNA. RPA1 harbors numerous functional domains (from N-terminal to C-terminal: clamp, active center, pore, funnel, cleft, foot, jaw, expander, jaw, cleft and clamp) [20–22]. RPA2 is a smaller protein with protrusion, lobe, fork, hybrid binding, wall and clamp domains. DNA is loaded into the cleft localized between the clamp and protrusion domains. RPA49 and RPA34 form a heterodimer that contributes to initiation and elongation. Cryo-electron microscopy (Cryo-EM) structural studies suggests that RPA43 functions in termination, open complex formation, elongation and termination [23].

Pol I exhibits a high initiation rate and elongation speed [24]. In yeast, overall initiation rate, and not the number of active rDNA genes, determines rRNA transcription rates [24]. Over 100 Pol I molecules may be simultaneously active on a single rDNA gene. Pol I transcription is regulated by post-translational modification of components of the pre-initiation complex, and changes in the number of active rDNA genes via epigenetic mechanisms.

Silent rDNA exists in a closed heterochromatin state whereas active rDNA exists in the euchromatin state. Epigenetic states are controlled by cycle-cell, environmental factors and total rDNA copy number [25,26]. The epigenetics of rDNA regulation in terminally-differentiated neurons is difficult to study and poorly understood. Moreover, study of rDNA in the brains of animal models such as mice may be a poor surrogate for the aged adult human brain.

4. Pol I Interactions

The synthesis of rRNA by Pol I is critically dependent on transcription and termination factors, particularly Upstream Binding Transcription Factor (UBTF). UBTF binds to the Pol I Upstream Control Element (UCE, -200 to -107) and core element (-45 to +20). UBTF is a multi-HMGB (High Mobility Group B)-box architectural DNA binding protein essential for rRNA transcription by Pol I and for ribosome biogenesis in the nucleolus [15,27]. UBTF exists as two isoforms, UBTF1 (CCDS42346.1) and UBTF2 (CCDS1140.1), that are expressed at similar levels in differentiated cell types. These replace histone chromatin across active copies of the several hundred rDNA genes present in the genome, the UBTF1/2 ratio corresponding closely with the fraction of active rRNA genes.

UBTF contains a dimerization domain, and 6 tandem HMGB boxes, the first 3 of which bind in the minor DNA groove and induce bending [28–30]. UBTF replaces histone chromatin across active rRNA genes, inducing a 16kbp long Nucleosome-Free Region (NFR) [31]. It is also found genome-wide at GC rich NFRs adjacent to nucleosomes containing the H2A.Z histone variant. These NFRs also lie immediately upstream of RNA polymerase 2 (Pol II) transcribed mRNA genes implicated in chromatin formation and cell cycle progression that have been suggested to be regulated by UBTF [32].

UBTF1 and UBTF2 are present across rDNA and at NFRs genome-wide as hetero- and homo-dimers, but only UBTF1 can cooperate with the TBP-complex SL1 to form the preinitiation complex. Thus, UBTF1 is essential for rDNA transcription, but UBTF1 and UBTF2 are equivalent for NFR formation and potentially for Pol II regulation. Knock-down of UBTF has been shown to cause genome instability [32]. This may be related to the essential role this factor plays in rDNA transcription and/or its ability to replace nucleosomes and stabilize NFRs. Defects in ribosome biogenesis lead to nucleolar stress, the stabilization of p53, cell cycle arrest and apoptosis [33–36], and loss of UBTF has been shown to have these effects [32,37]. Loss or mutation of UBTF could affect the formation of both rDNA-specific and genome-wide NFRs, possibly leaving the underlying DNA poorly protected and explaining the enhanced damage that has been observed. Extrapolating from yeast studies of the ortholog Hmo1, it is likely that UBTF-dependent NFRs are setup during genome replication [38]. Loss of UBTF induces enhanced H2A.Z acetylation, a marker of H2A.Z histone

 doi:10.20944/preprints202404.0322.v1

turnover, adjacent to associated NFRs indicating increased chromatin instability at these sites [15]. Thus, mutations in UBTF could not only cause genome instability by inducing nucleolar stress, but also by inducing NFR instability.

Pol I recruitment to rDNA requires a Pre-Initiation Complex (PIC) consisting of UBTF and SL1. UBTF binds to the SL1 complex composed of the TATA-binding protein (TBP) and four TBP-associated factors (TAF1A, TAF1B, TAF1C, TAF1D). RRN3 associates with Pol I and enables interaction with the PIC. Once Pol I clears the promoter, the PIC remains bound and ready to recruit another Pol I molecule. Transcription termination factor 1 (TTF-1) binds to a consensus terminator element downstream of the 3′ end of pre-rRNA and mediates termination of pre-rRNA synthesis. In theory, drugs that target interactions of Pol 1 with rDNA or its transcription factors could alter production of rRNA.

5. Pol I Inhibitors

The molecular mechanisms by which putative "Pol I inhibitors" operate remain poorly understood. Possible mechanisms include inhibition of elongation, prevention of promoter escape during initiation, and activation of a DNA damage response [39–41]. Drug classes include DNA intercalators, G4-stabilizers, TOP2 (Topoisomerase 2) inhibitors, DNA crosslinkers, and TOP1 (Topoisomerase 1) inhibitors [42]. Numerous Pol I inhibitors have been reported in the literature but only a few have made it to clinical trials or regulatory approval [42].

ActD inhibits Pol I, and, at higher dosages, Pol II transcript elongation [43]. ActD reduces production of rRNA [44]. ActD is used to treat a variety of malignant tumors including Wilms tumor, Ewing sarcoma, testicular cancer, and trophoblastic neoplasms. Typically, ActD is administered intravenously every 2 - 3 wks. ActD shows poor CNS penetration.

BMH-21 is a planar heterocyclic small molecule DNA intercalator that binds strongly to GC-rich DNA sequences, ultimately inhibiting Pol I, blocking transcription and disrupting nucleolar structure [45]. BMH-21 penetrates the CNS and has been used in murine preclinical studies of spinal cord injury [46], but is not being used in clinical studies at present due to deleterious off-target effects.

CX5461 is an orally bioavailable small molecule that selectively inhibits Pol I-driven transcription relative to Pol II-driven transcription (~ 200-fold in human cell lines) [47–49]. CX5461 was claimed to inhibit Pol I via disruption of the SL1-rDNA complex but the Moss lab has now shown that it actually blocks initiation (manuscript in preparation). CX5461 shows limited CNS penetration. CX5461 is currently in Phase I/II clinical testing (NCT02719977) for solid malignancies.

Orally-bioavailable, improved 2nd-generation Pol I inhibitors that are orally-bioavailable are in early phase clinical trials. PMR-116 is one such example. PMR-116 induces phosphorylation and accumulation of p53 and does not activate CHK2 [50]. PMR-116 is currently in Phase I dose escalation trial in patient with solid tumors (ACTRN12620001146987). PMR-116 has shown efficacy in MYC-driven cancer models. More specifically, these small molecules have been tested in preclinical models of metastatic breast cancer [51].

6. Human Mutations with Direct Effects on the Pol I Enzymatic Complex

Most cases of Treacher Collins Syndrome (TCS) are caused by autosomal mutations, typically *de novo*, of *TCOF1* which encodes treacher (TCS1) [52]. TCS can also be caused by mutations in *POLR1B* (TCS4), *POLR1C* (TCS3) and *POLR1D* (TCS2). Reported mutations in TSC3 are autosomal recessive. TSC2 may be dominant or recessive. TSC4 is dominant. Since *POLR1C* and *POLR1D* are shared with Poly III, the effects of mutations in *POLR1C* and *POLR1D* cannot be ascribed to Pol I dysfunction in isolation (Table 1). TCS is characterized by severe craniofacial structural abnormalities that arise *in utero*. Phenotypic features may include malformed auricles, malar and mandibular hypoplasia, conductive hearing impairment, down slanting palpebral fissures, absence of the lower eyelashes, and, rarely, cleft palate [53]. In general, patients with TCS1 show no intellectual disability. Some patients with TCS2 may show delays in motor and speech development. TCS penetrance may be incomplete and subtle phenotypes have been reported. Many TCS patients require major reconstructive facial surgery.

 doi:10.20944/preprints202404.0322.v1

Autosomal dominant mutations in *POLR1A* cause Acrofacial Dysostosis, Cincinnati Type (AFDCIN)[54]. AFDCIN shares some features with TCS but, in general, is more severe and affected individuals may also manifest short stature, microcephaly, bowed forearms, radial aplasia, heart defects, and neurological dysfunction (developmental delay, epilepsy, infantile spasms, and hypotonia)[55].

Ostensibly autosomal recessive hypomyelinating leukodystrophy (HLD) has been linked to autosomal recessive mutations in *POLR1A* (HLD27) and *POLR1C* (HLD11) (Table 1). HLD may be a misnomer since careful analysis of clinical descriptions indicates that the hypomyelination reported in patients with deleterious *POLR1A* variants is secondary to neuronal loss since affected individuals show clear magnetic resonance imaging (MRI) evidence of global neurodegeneration with reductions in both gray and white matter volumes. Patients show neurodevelopmental abnormalities with later appearance of neuroregression [56,57]. Reported neurological findings include intellectual disability, ataxia, dystonia, oculomotor abnormalities, seizures and spasticity. Similar findings have been reported in individuals with homozygous or compound heterozygous *POLR1C* mutations which would impact both Pol I and Pol III [55,58]. Some patients with *POLR1C* mutations also have dental abnormalities.

Table 1. Polymerase I (Pol I).

Subunit Protein	**Gene Symbol**	**Shared with Pol II**	**Shared with Pol III**	**OMIM Associated Disorders**
RPA1	*POLR1A*	No	No	AD: Acrofacial dysostosis, Cincinnati type AR: Leukodystrophy, hypomyelinating, 27
RPA2	*POLR1B*	No	No	AD: Treacher Collins syndrome 4
RPAC1	*POLR1C*	No	Yes	AR: Leukodystrophy, hypomyelinating, 11 AR: Treacher Collins syndrome 3
RPAC2	*POLR1D*	No	Yes	AD/AR: Treacher Collins syndrome 2
RPA49	*POLR1E*	No	No	None
RPA43	*POLR1F*	No	No	None
RPA34	*POLR1G*	No	No	None
RPA12	*POLR1H*	No	No	None
RPABC1	*POLR2E*	Yes	Yes	None
RPABC2	*POLR2F*	Yes	Yes	None
RPABC3	*POLR2H*	Yes	Yes	None
RPABC4	*POLR2K*	Yes	Yes	None
RPABC5	*POLR2L*	Yes	Yes	None

AD, autosomal dominant. AR, autosomal recessive.

Table 2. Pol I Regulatory Factors.

Protein	**Gene Symbol(s)**	**Function**	**OMIM Associated Disorders**
UBTF	*UBTF*	recruitment of Pol I to rDNA, determining a specialized non-nucleosomal chromatin structure on active rDNA, cooperating with other	UBTF E210K neuroregression syndrome (AKA – neurodegeneration, childhood-onset, with brain atrophy [CONDA], hematological malignancies

 doi:10.20944/preprints202404.0322.v1

		components of the pre-initiation complex at the Pol I promoter	
SL1 complex (TBP, TAF1A, TAF1B, TAF1C, TAF1D)	*TBP*, *TAF1A*, *TAF1B*, *TAF1C*, *TAF1D*	essential component of the pre-initiation complex, interacts with UBTF and Pol I	*TBP* (AD: spinocerebellar ataxia 17) TAF1A – none TAF1B – none TAF1C – none TAF1D - none
RRN3	*RRN3*	mediates interaction of Pol I with UBTF and SL1	None
TTF1	*TTF1*	terminates Pol I transcription	None
TCOF1 (Treacle protein)	*TCOF1*	Pol 1 rDNA promotor recognition and recruitment of UBTF	AD: Treacher Collins syndrome 1

AD, autosomal dominant.

In aggregate, analysis of mutations that directly affect Pol I suggests that (1) Pol 1 inhibition should be avoided during early development, (2) some degree of Pol 1 inhibition may be tolerated after the early prenatal period of life, and (3) partial Pol I inhibition may be associated with few adverse neurological effects in adults.

7. Human Mutations with Indirect Effects on Pol I

The UBTF E201K neuroregression syndrome is a recurrent de novo dominant mutation most commonly associated with neurodegeneration beginning at 2.5 years of age [59,60]. In some patients, neuroregression is superimposed on mild developmental delay. Other *UBTF* variants have been reported in patients with similar clinical syndromes [61,62]. The term CONDBA (childhood-onset neurodegeneration with brain atrophy) has been used as a more general term for all pathogenic UBTF variants because MRI imaging in affected subjects shows progressive loss of brain volume [60]. Affected individuals become non-ambulatory and aphasic by their early teen years. Neurological features include ataxia, dystonia, intellectual impairment, dysarthria, and dysphagia. Seizures and Parkinsonism have been reported in some cases[63,64]. Overall, there is substantial clinical and neuroimaging overlap among patients with *UBTF*, *POLR1A* and *POLR1C* mutations.

The TATA box-binding protein (TBP), which contributes to the SL1 complex, has been linked to spinocerebellar ataxia type 17 (SCA17). SCA17 is also known as Huntington Disease-Like 4 (HDL4). Most commonly, SCA17 is associated with CAG/CAA repeat expansions in *TBP* [65]. Some cases are digenic due to intermediate expansions in *TBP* coupled with mutations in *STUB1*. SCA17 typically presents during adult life with variably progressive combinations of cerebellar ataxia, cognitive decline, seizures, chorea, and dystonia. *TBP* is a general transcription factor required for Pol I, polymerase II (Pol II), and Polymerase III (Pol III). To my knowledge, the quantitative effects of TBP repeat expansions on Pol I activity and synthesis of rRNA are not known, and acquisition of this data would be one starting point for targeted therapeutics.

8. Targeting Neurodegeneration, Neuroregression, and Aging

Numerous neurodegenerative disorders are due, at least in part, to the accumulation of proteins in the central nervous system (CNS). Protein accumulation may predominate in certain cell types of the CNS with little or no obvious cellular pathology in extra-neural tissues. In Parkinson disease, α-synuclein encoded by *SNCA*, accumulates in neurons, typically with early involvement of monoaminergic neurons of the locus coeruleus and substantia nigra, pars compacta. Prior to the onset of motor dysfunction, the majority of patients destined to develop Parkinson disease manifest one or more non-motor features such as hyposmia, constipation, or REM sleep behavior disorder. Moreover, deleterious variants in LRRK2 and GBA increase the risk of developing Parkinson disease. As such,

 doi:10.20944/preprints202404.0322.v1

there are both clinical and genetic markers that can be used to predict risk of developing Parkinson disease and permit early intervention with disease modulating therapeutics prior to penultimate clinical decline. Nucleolar dysfunction involving p53 and mTOR signaling may contribute to the pathobiology of Parkinson disease [66]. Another α-synucleinopathy, multiple system atrophy, is characterized by deposition of α-synuclein in oligodendroglia.

Tauopathies are a collection of neurodegenerative diseases with both specific and overlapping clinical features that are histopathologically characterized by abnormal accumulation and aggregation of tau within neurons, glia, or both. Tau, encoded by *MAPT*, is a microtubule associated protein. Alternative splicing of the *MAPT* transcript generates 6 CNS isoforms with different numbers of N-terminal inserts and either 3 or 4 repeats (3R or 4R) in the repeat domain. Tauopathies are often divided into primary and secondary forms. Primary forms include frontotemporal lobar degeneration, corticobasal degeneration, and progressive supranuclear palsy. Secondary forms include Alzheimer disease (AD) and chronic traumatic encephalopathy. At the microscopic pathological level, these disorders differ in the relative involvement of neurons, astrocytes and oligodendroglia. At the molecular level, they differ in relative accumulation of 3R and 4R tau. Tau is found in both the nucleus and cytoplasm. In the nucleolus, tau may be involved in silencing rDNA [67].

The spinocerebellar ataxias (SCAs) are a diverse group of inherited disorders mainly characterized by cerebellar atrophy and ataxia on clinical examination. To date, more than 50 autosomal dominant SCAs have been reported in the medical literature and many of the more prevalent SCAs are trinucleotide repeat disorders. SCA1, SCA2, SCA3, SCA6, SCA7, SCA12, and SCA17 are due to CAG repeat expansions. Another movement/neurodegenerative disorder, Huntington disease (HD) is also caused by CAG repeat expansions. Expanded polyQ proteins from insoluble cellular aggregates [68]. In addition, some ataxia-associated polyQ proteins localize to the nucleolus and may impact nucleolar function [69].

AD pathology is characterized by aggregation of amyloid beta (Aβ) proteins into extracellular plaques and tau into intraneuronal neurofibrillary tangles. AD pathology is commonly seen in postmortem brains of aged individuals without clinical evidence of overt cognitive impairment. Other pathologies, particularly limbic-predominant age-related (TDP-43) encephalopathy neuropathological changes (LATE-NC) are commonly found in brains from aged individuals and may contribute to minor deterioration in motor, sensory and cognitive abilities commonly seen as part of the so-called normal aging process [70]. Repeat expansions of *C9orf72* are the most common known genetic cause of amyotrophic lateral sclerosis (ALS). In both C9orf72-associated and sporadic ALS, nucleolar stress appears to be upstream of pathological disease hallmarks, specifically TDP-43 mislocalization and antisense RNA foci [71].

UBTF-associated neuroregression is a potential target for treatment with Pol I inhibition. UBTF E210K shows increased binding to the rDNA promoter [59]. UBTF E210K fibroblasts show increased expression of pre-rRNA and 18S rRNA, nucleolar abnormalities, markedly increased numbers of DNA double-strand breaks (DSBs), defective cell-cycle progression, and apoptosis [60]. *Ubtf*$^{-/-}$ is early embryonic lethal in mice[72] and transgenic expression of human UBTF1 E210K in Drosophila neurons is also lethal [60].

Nucleolar stress due to excessive, unnecessary or aberrant Pol I activity and ribosomal synthesis has been also been associated with normal aging and premature aging syndromes such as progeria. Disturbed ribosomal biogenesis and enlarged nucleoli is seen in patients with Hutchinson-Gilford Progeria Syndrome and fibroblasts from aged humans [73]. With aging, the accumulation of somatic mutations in nuclear (nDNA) and mitochondrial DNA (mtDNA) leads to the production of mutant proteins of little or no functional value leading to a deterioration of multiple cellular processes and the cellular burden of eliminating unwanted proteins. Similarly, accumulation of mutations in rDNA would lead to production of aberrant rRNA and ribosomal pathology. Intrinsic and extrinsic triggers of nucleolar stress also lead to a cascade of secondary effects including damage to non-nucleolar nDNA. It should also be emphasized that ribosome biogenesis, both normal and aberrant, is energy demanding, which for disorders such as Parkinson disease that have been linked to mitochondrial

 doi:10.20944/preprints202404.0322.v1

dysfunction, is particularly undesirable. In this context, it is important to note recent work showing that reducing the metabolic burden of rRNA synthesis promotes longevity in *C. elegans* [25].

Gene therapy, particularly antisense oligonucleotides, has been employed in clinical trials of neurodegenerative disorders. Complications of delivery strategies and blood barrier penetration have been limiting. For instance, a trial of tominersen in HD, an antisense oligonucleotide delivered via intrathecal injection, was stopped due to lack of efficacy [74]. Despite technological improvements many critical issues remain, particularly in the context of long-term delivery, and these include antibody formation, inflammatory response, reduction of wild-type protein, penetration of deep brain tissues, and cost. Small molecule enzyme inhibitors that cross the blood brain barrier such as rasagiline (monoamine oxidase type B) for Parkinson disease and donepezil (acetylcholinesterase) for Alzheimer disease have proven to be efficacious and well tolerated by patients for years to decades. The same could apply to Pol I inhibition.

9. Unanswered Questions

The ideal Pol I inhibitor to treat aging and neurodegenerative/neuroregression disorders of the CNS should cross the blood-brain barrier, have no effect on Pol II or Pol III, and have no deleterious off target effects or toxicity. More specifically, is it possible to effectively target post-mitotic neurons when Pol I is critical for survival of extra-neural tissues such as the rapidly dividing cells of the hematopoietic system. Preliminary studies can be done in cells, ideally cultured neurons, or human induced pluripotent stem cells (hiPSCs). Next level studies can use worms (*C. elegans*) and flies (*Drosophila*). Mice are the most practical mammalian system but live less than two years. Mice have often proved to be poor models for late-onset human neurodegenerative disorders and aging. Ultimately, human studies will be required for validation. As always, Phase I and II studies in humans must focus on safety. Efficacy studies (Phase III) will likely require prolonged interventions of at least one year. Shorter studies of disease modifying therapeutics in Parkinson disease and Huntington disease have failed to reach predefined clinical endpoints [75,76].

Implementation of Pol I inhibition for treatment of neurodegeneration, neuroregression, and aging will require answers to several key questions. First, would chronic or intermittent inhibition of Pol I lead to compensatory increases in active rDNA or trigger nucleolar stress? How will Pol I inhibition alter the kinetics of target (e.g., α-synuclein) and off-target protein production and clearance? What are the downstream effects of Pol I inhibition that may be largely independent of protein production? How would chronic, mild inhibition of Pol I affect other aspects of nucleolar function? How would the pharmacokinetics and pharmacodynamics of an orally-available Pol I inhibitor inform dosing strategies? Dosing strategies (twice daily, daily, weekly, monthly, or other) may be also dictated by specific disease state. Effective and inexpensive disease modifying drugs to slow progression of neurodegeneration, neuroregression and aging could transform healthcare and partial Pol I inhibition is one pharmacological approach that warrants evaluation.

Author Contributions: Conceptualization, writing—original draft preparation, writing—review and editing, M.S.L. M.S.L. read and agreed to the published version of the manuscript.

Funding: National Institutes of Health of the United States (NIH) grant number R56NS123059 to M.S.L.
Conflicts of Interest: The authors declare no conflict of interest.

 doi:10.20944/preprints202404.0322.v1

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