



*Review*

## **Role of nucleolar dysfunction in neurodegenerative disorders: a game of genes?**

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**Abstract:** Within the cell nucleus the nucleolus is the site of rRNA transcription and ribosome biogenesis and its activity is clearly essential for a correct cell function, however its specific role in neuronal homeostasis remains mainly unknown. Here we review recent evidence that impaired nucleolar activity is a common mechanism in different neurodegenerative disorders. We focus on the specific causes and consequences of impaired nucleolar activity to better understand the pathogenesis of neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis/frontotemporal dementia (ALS/FTD). In particular, we discuss the genetic and epigenetic factors that might regulate nucleolar function in these diseases. In addition, we describe novel animal models enabling the dissection of the context-specific series of events triggered by nucleolar disruption, also known as nucleolar stress. Finally, we suggest how this novel mechanism could help to identify strategies to treat these still incurable disorders.

**Keywords:** nucleolus; cellular stress; rRNA; neurodegeneration; mouse models; epigenetic regulation

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## 1. Introduction

The nucleolus is not only the cellular site of rRNA gene (rDNA) transcription and ribosomal assembly, it is also a principal sensor and mediator of the cellular stress response [1,2,3]. Indeed, to optimize energy consumption under stress conditions, the nucleolus precisely adjusts its activity enabling cell adaption to potentially harmful conditions [3]. In turn, “nucleolar stress”, defined as the impairment of rDNA transcription and disruption of nucleolar integrity, results in altered turnover of the transcription factor p53 thereby controlling stress response pathways and cell survival [1,4]. Intriguingly, this emerging function of the nucleolus is gaining attention in the last years, in particular in the context of neuronal homeostasis and mechanisms of neurodegeneration [5,6]. Certainly, a major problem in therapy and diagnosis of neurodegenerative disorders is that most of them are sporadic and even for those of known genetic basis the mechanisms of preferential neuronal vulnerability are not completely understood. Hence, the understanding of the multiple factors contributing to the disease state is critical to develop effective therapeutic approaches and reliable biomarkers.

Here we review the role of the nucleolus as a fundamental component of the neurodegenerative process, beyond the well-known impact on ribosome assembly and protein synthesis. We also focus on the genetic and epigenetic factors altering rDNA transcription and nucleolar activity in various neurodegenerative disorders and under cellular stress. Epigenetic factors regulating rRNA genes in response to stress conditions could provide a further, as for now less explored, link to neurodegenerative disorders. Moreover we show that mouse models characterized by inhibition of rRNA synthesis in specific neurons could be valid tools to dissect context-specific nucleolar-dependent signaling pathways.

## 2. Evidence of genetic and epigenetic factors leading to nucleolar stress in neurodegenerative disorders

A causal link between known genetic causes of some neurodegenerative disorders and nucleolar stress emerged very recently for certain forms of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) [7,8]. One of the most common causes of ALS and FTD is the expansion of the hexanucleotide GGGGCC sequence in a noncoding region of the *C9orf72* gene: repeat-associated non-ATG (RAN) translation occurs in these expanded regions [9]. Interestingly, the resulting poly-proline–arginine (PR) and poly-glycine-arginine (GR) peptides localize to the nucleolus [10]. Especially transcription and maturation of rRNA is reduced by these aberrant polypeptide species and by abortive transcripts that form RNA G-quadruplexes, ultimately causing nucleolar stress [7]. This loss of nucleolar integrity is visualized by changes in the distribution of nucleolar proteins, such as nucleolin and nucleophosmin, in B lymphocytes, iPSC-induced motor neurons, and fibroblasts from *C9orf72* hexanucleotide repeat expansion (HRE) patients [7,10]. A summary of the studies showing the link of the nucleolus to *C9orf72* HRE is presented in Table 1 [7,10,11,12]. The results revealed that nucleolar stress is induced by poly-dipeptide, particularly of the GR type, indicating that nucleolar stress may be a primary cause of neurodegeneration [11,12].

**Table 1. Summary of the findings linking the nucleolus to C9ORF72 expansion.**

Reference	Toxic species	Effects on nucleolus	Model system
Haeusler AR et al, Nature, 2014 [7]	RNA G-quadruplexes from C9orf72 hexanucleotide expansions (GGGGCC)n	Dispersion of NCL and NPM from the nucleoli  Impaired processing of 45S pre-rRNA	B lymphocytes, fibroblasts, iPS motor neurons, motor cortex tissue from C9orf72 HRE patients
Kwon I et al, Science, 2014 [10]	PR20/GR20 peptides	PR20 and GR20 binds to nucleoli  Altered rRNA transcription/processing	Human astrocytes
Wen X et al, Neuron, 2014 [11]	PolyPR peptides	Aggregation of polyPR in the nucleoli  Dispersion of nucleolin, larger nucleolar size	Rat primary cortical neurons, human iPSC-derived neurons, spinal cord tissues from C9-ALS and C9-ALS/FTD
Tao Z et al, Human Molecular Genetics, 2015 [12]	PolyGR (and polyPR) Peptides in comparison with other RAN products	Nucleolar swelling, NPM translocation to the nucleus, increased area occupied in the nucleus  Reduced 18S rRNA and 28S rRNA	HEK293 cells, mouse motor neurons NSC-34

Yet important open questions remain: why does it take so long for the disease symptoms to appear? And why is there a selective neuronal vulnerability of motor neurons in ALS and frontal and/or anterior temporal lobes in FTD to the effects of the *C9orf72* mutation? Important clues might come from the observation that different dosages of the 20-dipeptide-long repeats for PR (PR20) have a different effect on rRNA production in cell culture [10]. Paradoxically, low doses lead to an increase of the 45S rRNA precursor (pre-rRNA), while a higher dose of PR20 significantly decreases pre-rRNA levels [10]. It would be definitely instructive to investigate when, and if, these effects are detected at different disease stages.

Interestingly, another recent study focusing on the murine superoxide dismutase 1 carrying glycine to alanine substitution at residue 93 (hSOD1G93A model of ALS) reports a high rate of rDNA transcription in ALS motor neurons [13]. This has been explained as a compensatory response to altered protein homeostasis in this model, in line with what has been observed in response to proteasome inhibitors [13,14]. As for now, nucleolar stress in ALS/FTD is considered the end-point of the pathogenic process in relation to the *C9orf72* mutation, being typical for C9orf72 HRE patients and being absent in non-C9orf72 ALS fibroblasts [7]. A systematic analysis of nucleolar activity and integrity at different stages of the disease and also in different forms of ALS/FTD is missing. However, the results suggest distinct disease phases with important implications for the function of the nucleolus during the course of the disease. Moreover, how rRNA gene activity is

inhibited and whether poly-PR and poly-GR directly interfere with the transcription machinery is still unknown. The answer to these questions is further complicated, as the G-quadruplex structure formed by the hexanucleotide repeats, allows per se the binding to nucleolin [7], a nucleolar protein that regulates rDNA transcription [15]. Interestingly, nucleolin appears dispersed in *C9orf72* ALS tissues, but not in non-ALS and non-*C9orf72* ALS tissues. Whether the abortive RNA transcripts containing 21 GGGGCC-repeats interact with nucleolin and in this way influence rRNA synthesis and processing is an important question to be addressed in the future, in particular for its therapeutic implications.

Intriguingly, a potential role of nucleolin in linking RNA repeat expansion and aberrant protein species to a malfunctioning nucleolus has been reported also in polyglutamine diseases, such Huntington's disease (HD) [8,16,17]. HD is a dominantly inherited neurodegenerative disorder characterized by motor dysfunction and progressive cognitive decline [18]. The disturbances of voluntary movements are ascribed to degeneration of the striatum. The disease is fatal and no treatment is available to halt or slow down its progression [18]. HD is caused by CAG trinucleotide expansion in the mutant Huntingtin (mHtt) gene that partially accounts for the variability in the clinical onset [18]. Hence, a deeper understanding of the mechanisms [19] triggered by mHtt, in particular those that alter transcriptional and translational programs, is necessary to identify disease modifiers and to devise efficient therapeutic strategies halting or slowing down neurodegeneration.

Among the multiple cellular functions altered by mHtt, recent studies point to a downregulation of rRNA synthesis and disrupted nucleoli in HD due to direct interference of mHtt with the RNA Polymerase I complex [8,17,20,21]. Impaired rDNA transcription has been reported in cellular and mouse models of HD [17,19,20,21]. A recent study showed that nucleolin is sequestered by interaction with expanded CAG RNAs from the rDNA promoter, causing promoter hypermethylation and transcriptional inhibition [16]. In parallel, other epigenetic mechanisms regulating rDNA transcription have been also proposed in HD [19,21,22]. These imply the acetylation and methylation of the upstream binding factor (UBF), a nucleolar transcription factor that is essential for active chromatin architecture of rRNA genes [19,21,22]. UBF acetylation is reduced and rRNA transcription is impaired in cellular and animal models of HD [21]. Moreover, HD-linked UBF methylation increases chromatin condensation, thus reducing nucleolar transcription [22].

While epigenetic mechanisms regulating rDNA transcription in HD have been recently reviewed [23], these are still largely unexplored in PD. Epigenetic alterations as a molecular mechanism of PD have been reported [24,25] and we can now only speculate that similar mechanisms as in HD might be involved in PD.

Impaired rRNA synthesis has been reported in PD brains and in pharmacological rodent models of PD caused by treatment with mitochondrial neurotoxins [26,27,28,29]. However the association of nucleolar stress with known genetic mutations causing PD is not well characterized yet and it is limited to mutations associated with autosomal recessive early onset forms of PD [29,30]. Interestingly, the DJ-1 L166P missense mutation has been shown to alter rRNA biogenesis in a neuroblastoma model of PD and upon proteasome inhibition [30,31], showing that mutant proteins may influence nucleolar activity in a pathological model. More recently, decreased rRNA transcription and induction of nucleolar stress have been demonstrated in a mouse model of PD based on the conditional knock-out of the parkin gene [29]. In this model nucleolar stress is detected in absence of neuronal loss three months after induction of the mutation, while neurodegeneration occurs ten months later [29]. Future studies should address the role played by nucleolar stress in the

degenerative process in these mutants, nevertheless these initial findings suggest that nucleolar stress is an early pathogenic event rather than a consequence of neurodegeneration. Indeed, the increased interaction of PARIS (Parkin Interacting Substrate, ZNF46), one of the Parkin substrates, with RNA Polymerase I subunits could repress rRNA transcription [29].

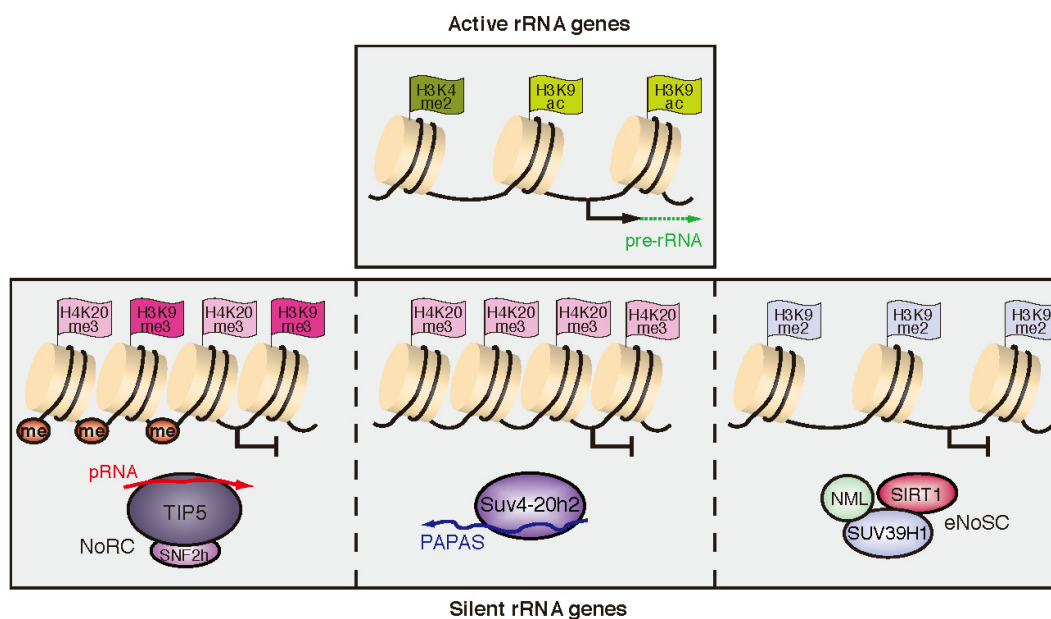
Moreover, it would be interesting to assess whether nucleolar activity is lower in dopaminergic (DA) neurons of the substantia nigra pars compacta (SNpc), preferentially lost in PD, than in DA neurons of the ventral tegmental area (VTA). Two recent studies investigate the effects of a partial unilateral intrastriatal lesion by 6-hydroxydopamine (6-OHDA) on nucleolar volume in dopaminergic cells [26,27]. Although the number of DA neurons is most severely reduced within the SNpc, nucleolar volume was equally decreased also in less vulnerable DA neurons within the VTA. This observation is quite interesting because it dissociates the neurotoxic effect of the 6-OHDA lesions from the morphological impact of nucleolar structure and activity: the nucleolus being equally affected nevertheless mediates different context-dependent [26,27]. The data are in line with the fact that for example genetic mutations have a stronger impact in specific neuronal sub-populations. Clearly the open question is to identify the factors accounting for the differential vulnerability.

While specific mutant proteins and mRNAs interfere with the rRNA transcriptional machinery in PD, in polyglutamine diseases and in C9orf72 ALS/FTD, similar mechanisms have not been shown in Alzheimer's disease (AD). While for these other diseases a systematic analysis of changes in the nucleolar structure changes and in the chromatin status of the rDNA locus is still missing, a very detailed and accurate description of nucleolar volume and changes in rDNA promoter methylation states has been known since many years in cortical and hippocampal tissues from AD patients [32,33]. In particular, nucleolar hypertrophy was observed in asymptomatic AD in contrast with the reduced nucleolar volume typical of mild cognitive impairment and manifest AD. In fact, 28S rRNA was significantly reduced in AD prefrontal cortex [34].

Interestingly the initial increase of nucleolar size and activity has been proposed as a compensatory mechanism preventing progression to dementia [32]. Although testing this possibility will require additional functional studies in model organisms, a more recent study of mild cognitive impairment (MCI)/AD-associated methylation status of the rDNA promoter supports that nucleolar activity is silenced in MCI and correlate with AD pathology [33]. The triggers of these methylation changes are not known as well as the role of these changes on the disease course, but potential mechanisms will be discussed in the next paragraph.

### **3. Epigenetic regulation of rRNA genes and its possible link to neurodegeneration**

As discussed above, nucleolar stress in neurodegenerative disorders can impinge on the RNA polymerase I transcription machinery, thereby impairing rRNA gene transcription. In addition, nucleolar stress might also affect the intricate epigenetic regulation of rRNA genes. Several epigenetic processes that either promote or antagonize transcription operate on rDNA and contribute to the fine-tuning of rRNA synthesis in response to developmental programs and external signals. So far, three major different epigenetic mechanisms of rDNA silencing are known that might become aberrant in neurodegenerative settings and thus might enhance the nucleolar functional decline (Figure 1). These mechanisms include silencing of rDNA repeats by promoter hypermethylation, quiescence- and aging-induced heterochromatin formation at rRNA genes and epigenetic regulation of rRNA genes in response to the cellular energy supply.



**Figure 1. Different ways of epigenetic rRNA gene silencing.** The scheme depicts changes in key epigenetic features upon silencing of the rDNA promoter by the three different chromatin-modifying complexes, i.e. NoRC/pRNA, Suv4-20h2/PAPAS and eNoSC. NoRC is recruited by the long non-coding RNA pRNA and induces DNA methylation (me) and heterochromatic histone modifications like H3K9 trimethylation (H3K9me3) and H4K20me3. The long non-coding RNA PAPAS originates from rDNA transcription in antisense orientation and guides the histone methyltransferase Suv4-20h2 to rDNA, thereby triggering H4K20 trimethylation and chromatin compaction. eNoSC silences rDNA by SIRT1-dependent deacetylation of H3K9 and SUV39H1-mediated H3K9 dimethylation. For further explanation please see the main text.

In somatic tissues, rDNA repeats exist in two epigenetic states, a transcription-permissive, euchromatic state and a silent state characterized by heterochromatic features [35]. The silent rDNA fraction is maintained even in cycling cells with high ribosome production and the key player, which mediates heterochromatin formation at rDNA, is the Nucleolar Remodeling Complex NoRC. NoRC consists of the remodeling factor SNF2h and TIP5 (also known as BAZ2A in humans) [36], the large subunit that interacts with histone deacetylases and DNA and histone methyltransferases. Moreover, TIP5 binds also to a long non-coding RNA (lncRNA) that originates upstream of the rRNA gene promoter [37]. This promoter-associated RNA (pRNA) recruits NoRC and its associated epigenetic factors to rDNA and thus orchestrates promoter methylation, heterochromatin formation and transcriptional silencing. Interestingly, the balance between active and silent rDNA copies is disturbed in AD, as there is a significant and robust hypermethylation of the rDNA promoter in AD patients [33]. The increased rDNA silencing in AD is in line with a decline in nucleolar activity. The question remains how rDNA hypermethylation is triggered. One possible scenario would be that forced expression of TIP5 in AD would lead to elevated NoRC activity. Consistently, ectopic expression of TIP5 in cell culture systems has been shown to cause rDNA hypermethylation and silencing [38]. However, a more recent study found that overexpression of TIP5 in cancer cells

sustains proliferation and rRNA synthesis by aberrant silencing of protein-coding genes [39], indicating that elevated levels of endogenous TIP5 could paradoxically also promote nucleolar activity. Further investigation of TIP5 expression and NoRC function in a neurodegenerative context will help to elucidate its role in the pathological process.

Another explanation for rDNA hypermethylation in AD would be that pRNA is up-regulated and increases the recruitment of NoRC to rDNA promoters. Indeed, several lncRNA have been shown to be dysregulated in neurological disorders [40]. The level of pRNA might be increased if methylation-dependent silencing is restricted to the main rDNA promoter, while the upstream promoter is still competent for transcription. In addition, the stability of pRNA might be increased. Interestingly, pRNA is degraded by the exosome, an evolutionary conserved RNA surveillance machinery [41] and exosome mutations have been recently found in the context of motor neuron degeneration [42]. Thus, impaired exosome function might be a common feature of neurodegenerative processes, causing elevation of pRNA levels, which in turn triggers epigenetic silencing of rRNA genes.

While NoRC and pRNA keep a constant fraction of rRNA genes repressed, the activity of the transcription-permissive rRNA genes is tightly regulated according to the developmental and metabolic state of cells. For instance, rRNA synthesis is shutdown when cell proliferation ceases, either due to growth-factor depletion or due to terminal differentiation. Under these conditions rDNA antisense transcripts termed 'PAPAS' (promoter and pre-rRNA antisense) are up-regulated and induce heterochromatin formation [43]. The PAPAS lncRNA interacts with the histone methyltransferase Suv4-20h2 and thereby directs trimethylation of histone H4 at lysine 20 (H4K20me3) to rDNA. This heterochromatic mark leads to chromatin compaction and renders the rDNA promoter inaccessible for the transcription machinery. Interestingly, lncRNA-mediated induction of H4K20me3 is not only restricted to rDNA but occurs globally in postmitotic cells [43]. Moreover, H4K20me3 is also up-regulated upon cellular senescence and organismal aging [44,45,46], providing a possible link to age-related neurological disorders. Given that PAPAS levels increase in aged brains, the repressive effect on rRNA synthesis might promote nucleolar stress. Similar to pRNA, PAPAS is also targeted by the exosome [47] and might be aberrantly stabilized in neurodegenerative settings that are linked to compromised exosome function.

Finally, synthesis of rRNA is the biggest metabolic burden in cells and is therefore efficiently switched off when the intracellular energy supply is exhausted. In energy-deprived cells epigenetic rDNA silencing is mediated by a ternary protein complex termed eNoSC (energy-dependent nucleolar silencing complex) [48]. eNoSC consists of the NAD<sup>+</sup>-dependent protein deacetylase SIRT1, the histone H3K9 methyltransferase SUV39H1 and the nucleolar protein nucleomethylin (NML). Reduced intracellular availability of energy leads to an increase of the NAD<sup>+</sup>/NADH ratio, which activates eNoSC to deacetylate H3K9 by SIRT1 and to dimethylate H3K9 by SUV39H1 at rDNA. Thereby, the heterochromatic H3K9me2 mark is elevated at rDNA and transcription is impaired. The down-regulation of pre-rRNA synthesis restores the energy balance and protects cells from apoptosis [48]. This function of SIRT1 in the eNoSC complex is in line with its well-established role in promoting cell survival and longevity, which also holds true for the nervous system [49,50]. However, there is also growing realization that under certain conditions SIRT1 activity can worsen neurodegeneration. In this regard it is noteworthy that SIRT1 inhibition protects neurons in rats against oxidative damage [51] and that the specific SIRT1 inhibitor Selisistat (EX-527) ameliorates HD pathology in cell and mouse models and is currently tested in phase I

clinical trials for HD treatment [52,53]. Thus, one might envision that the adverse nature of SIRT1 in neurons might also be in part attributed to its function in eNoSC and the inhibitory effect on nucleolar activity. Apparently, this hypothesis needs further experimental investigation to uncover if, and under which circumstances, SIRT1/eNoSC-dependent rDNA silencing can sustain neurodegeneration.

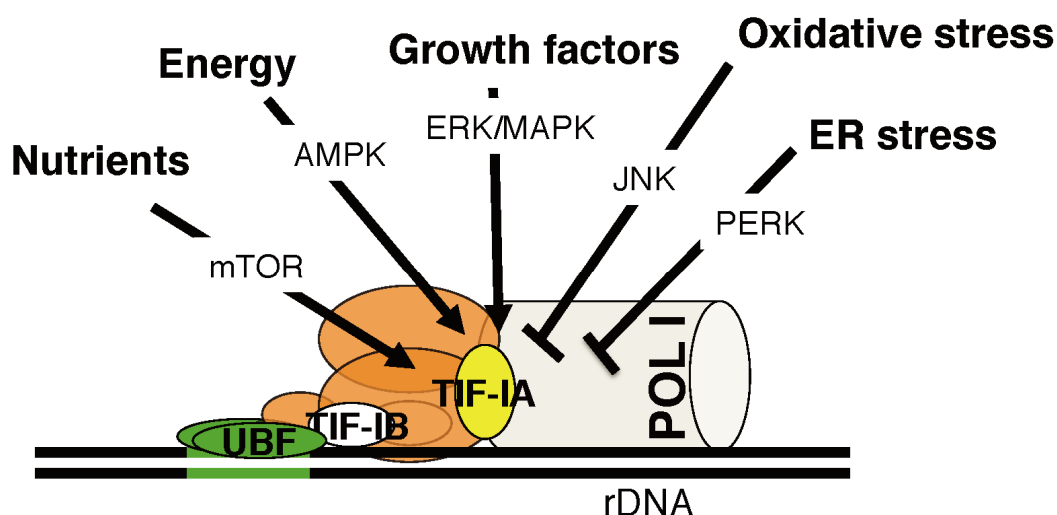
Taken together, aberrant epigenetic silencing of rRNA genes represents a likely mechanism that contributes to nucleolar impairment and stress in degenerative pathologies of the nervous system. Hypermethylation of the rDNA promoter in AD represents a first example in this direction and it will be interesting to further assess if and how deregulation of the three rDNA silencing machineries, NoRC/pRNA, Suv4-20h2/PAPAS and eNoSC, is causally linked to different neurodegenerative disorders. A deeper understanding of the epigenetic factors that elicit nucleolar stress will provide novel therapeutic targets and strategies to intervene in the age- and injury-caused decline of brain performance.

#### **4. Consequences of nucleolar stress and their link to neurodegenerative disorders in mouse models**

To dissect the cellular alterations and molecular mechanisms triggered by nucleolar stress in specific neuronal contexts, we devised a simple and versatile strategy to inhibit rDNA transcription in a controlled fashion. This is based on the conditional ablation of the TIF-IA gene, encoding an evolutionary conserved transcription factor essential for the recruitment of RNA polymerase I to rRNA gene promoters [54,55]. Interestingly, TIF-IA activity is finely tuned by reversible phosphorylation in response to growth factors, nutrients and stress [56,57,58,59] (Figure 2). Based on the conditional knockout approach using the Cre-loxP system in mice we developed a convenient strategy to mimic nucleolar stress and to investigate selective responses virtually in any cell-type [20,28,60,61,62,63]. Similar to TIF-IA-depleted embryonic fibroblasts [64], dividing embryonic neural progenitors lacking TIF-IA are rapidly lost by p53-dependent apoptosis [62]. Interestingly, in vivo this leads to anencephaly that can be partially rescued when p53 is also conditionally ablated in these cells ([62] and R. Parlato, unpublished observations). On the contrary, hippocampal neurons lacking TIF-IA are progressively lost despite p53 increased level. Moreover, death is limited to a subset of neurons, suggesting for the first time specific compensatory mechanisms triggered by impaired nucleolar function [60,62]. Surprisingly, mutant mice lacking TIF-IA in dopaminergic neurons mimic the main behavioral and cellular features of parkinsonism, including mitochondrial dysfunction, increased oxidative damage and progressive but selective neurodegeneration of dopaminergic SNpc neurons, while dopaminergic VTA neurons are less vulnerable [28]. It is still not clear why VTA neurons are less vulnerable, despite induction of nucleolar stress and increased levels of p53 in both regions [28]. Nevertheless, conditional ablation of p53 in dopaminergic neurons under nucleolar stress delays neuronal loss suggesting that p53 may trigger apoptosis in these neurons [28]. Notably, nucleolar stress triggers down-regulation of the mammalian target of rapamycin (mTOR) pathway [28], a central regulator of cellular growth and protein synthesis, revealing that nucleolar stress orchestrates homeostatic responses in neurons. To further investigate the early response to nucleolar stress at the molecular and cellular level, more recently we have shown that decreased mTOR activity upon induction of nucleolar stress in medium spiny neurons of the striatum, mostly affected in HD, triggers activation of autophagy [20]. This represents an early neuroprotective response accounting



for the late striatal degeneration and in fact impaired autophagy accelerates neuronal death. In contrast to dopaminergic neurons, conditional loss of p53 together with nucleolar stress in striatal neurons accelerates neurodegeneration, suggesting that p53 increase may be initially neuroprotective. By comparing mRNAs differentially expressed at different stages in controls and mutants we could identify molecular changes triggered by nucleolar stress common to those reported in HD, including a set of genes up-regulated only before neuronal death takes place [20]. Among these, we noticed PTEN, a known p53 target and a regulator of mTOR. In line with a model in which p53 increase leads to PTEN up-regulation and mTOR down-regulation with consequent activation of autophagy, we showed that the conditional loss of PTEN in striatal neurons under nucleolar stress accelerates death [20,65]. This mechanism is context-specific, because the conditional loss of PTEN in dopaminergic neurons under nucleolar stress results in improved motor deficits [63]. Interestingly, increased PTEN mRNA has been reported also in a mouse model of HD [66], supporting similar pathomechanisms and encouraging further investigation of nucleolar stress in HD. Moreover, the mTOR pathway is dysregulated in HD [67]. Based on a recent study, decreased mTOR activity is indeed observed in HD patients and in mouse models of HD prior to the onset of neurological symptoms [67]. However, the role of mTOR is controversial, because previous studies indicated that mTORC1 down-regulation is protective in HD [68].



**Figure 2. TIF-IA activity senses changes in the extracellular environment.** Schematic representation showing various extracellular stimuli that regulate TIF-IA activity and RNA polymerase I recruitment to the rDNA promoter, in either a positive or negative way. Specific kinases regulates TIF-IA phosphorylation pattern in response to permissive growth conditions like presence of nutrients, energy resources and growth factors or negative conditions like oxidative stress and endoplasmic reticulum (ER) stress.

In summary, loss of TIF-IA mimics nucleolar stress and may lead to down-regulation of the mTOR pathway in dopaminergic and dopaminoceptive neurons. In both cases decreased mTOR activity on the long run might account for neuronal atrophy and degeneration. Nevertheless the causes and consequences of mTOR impairment may be different in the two cases as indicated by the

observations that in dopaminergic neurons, up-regulation of mTOR by PTEN ablation is in part beneficial, while in dopaminoceptive neurons up-regulation of mTOR by PTEN ablation accelerates neurodegeneration [28,63]. Intriguingly, hippocampal neurons lacking TIF-IA show rather an increase of mTOR activity, but the whole impact on neuronal survival requires further investigation [60]. As for now, these results revealed a tight link between nucleolar activity and the mTOR pathway by context-specific factors that could play an important role in different diseases and in the regulation of the homeostasis of this crucial pathway.

Before the development of the TIF-IA mutant mice the effect of impaired rRNA biogenesis was essentially evaluated in cell cultures. Under these conditions perturbed rRNA synthesis certainly leads to rapid cell death [64]. In mice, we could now dissect the specific sequence of events triggered by nucleolar stress in diverse neuronal contexts [28,62,63], revealing that also neuroprotective responses are induced by nucleolar stress [20,60]. Intriguingly, the conditional inducible knockout of UBF in mice and in MEFs produced a different phenotype from the conditional TIF-IA knockout, which can be addressed to the fact that ablation of the UBF gene did not lead to enhanced p53 and activation of stress pathways [69]. Moreover UBF knock-out mutant did not continued to develop beyond the morula stage, while TIF-IA knock-out mice reach E8.5 [64]. These results suggest that inhibition of rRNA transcription by TIF-IA depletion was a serendipitous approach to induce nucleolar stress. Probably, and this is still to be tested, the “particular nature” of TIF-IA as a central integrator of nucleolar stress signals, make its conditional ablation such a versatile tool to mimic very closely a condition of cellular stress.

## 5. Conclusions and open questions

The exciting concept that nucleolar function plays a principal role in neurodegenerative disorders is rapidly advancing. Nevertheless, impaired nucleolar activity is mainly considered as an ending point of the degenerative process. Based on the evidence reviewed here, we hope we have contributed to revisit this traditional view and to motivate future research. For example, the systematic characterization of nucleolar activity and integrity by nucleolar morphology and localization of nucleolar proteins in different neurodegenerative disorders could be critical to monitor different disease stages. Although mutant RNAs and proteins may directly impair the synthesis and processing of rRNA and thus leading to neuronal death, it will be important to investigate when nucleolar stress is a cause or consequence of neuronal impairment. Epigenetic activation of nucleolar transcription could represent a strategy to develop neuroprotective treatments in different neurodegenerative disorders. Finally based on the lesson from the “TIF-IA models”, showing that nucleolar stress triggers homeostatic responses at the molecular, cellular and physiological level, novel therapeutic targets could be developed to halt or slow down neurodegeneration.

In conclusion, a better understanding of the link between nucleolar stress and well-established landmarks of neurodegenerative disorders, such accumulation of protein aggregates, altered mitochondria function and proteasome impairment, will be instrumental to explain causes and mechanisms of neurodegenerative disorders and to identify disease modifiers and treatment strategies.

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## Conflict of interest

The Authors declare no conflict of interest.

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