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Chapter

Senataxin: A Putative RNA: DNA Helicase Mutated in ALS4—Emerging Mechanisms of Genome Stability in Motor Neurons

Arijit Dutta, Robert Hromas and Patrick Sung

Abstract

Amyotrophic lateral sclerosis type 4 (ALS4) is a rare, autosomal dominant childhood- or adolescent-onset motor neuron disease caused by genetic defects in senataxin (SETX), a putative RNA–DNA helicase. Studies on the yeast SETX ortholog Sen1 revealed its role in small RNA termination pathways. It has been postulated that ALS4-associated neuronal pathologies could stem from defects in RNA metabolism and altered gene expression. Importantly, SETX prevents the accumulation of R-loops, which are potentially pathogenic RNA–DNA hybrids that stem from perturbations in transcription. SETX also interacts with the tumor suppressor BRCA1 that helps promote DNA double-strand break repair by homologous recombination. As such, SETX could contribute toward the removal of harmful R-loops and DSBs in postmitotic neurons. This chapter will visit the plausible mechanistic role of SETX in R-loop removal and DNA break repair that could prevent the activation of apoptotic cell death in neurons and pathological manifestation of ALS4.

Keywords: spinal muscular atrophy, SETX, transcription, R-loop, DNA double-strand break, homologous recombination, nonhomologous DNA end joining

1. Introduction

Amyotrophic lateral sclerosis (ALS) type 4 is a rare form of distal spinal muscular atrophy (SMA) with onset at age 25 years or younger. The disease first manifests itself with weakness of ankles and wrists and gradually paralyzes the limbs due to severe muscle wasting. However, unlike classical ALS, the respiratory and bulbar muscles, sensory abilities, and cognitive functions are largely preserved in ALS4 patients. Hence, ALS4 patients, while having to endure severe disabilities, could expect an otherwise normal life expectancy with proper medical attention.

In 1998, the joint effort of Phillip Chance and David Cornblath described the Mattingly disease, a hereditary peripheral neuropathy as ALS type 4 (ALS4) [1]. The Mattingly disease was first seen among the descendants of a seventeenth-century English colonist, Thomas Mattingly from Maryland. With collaborations of the Mattingly clan, the work of Chance and Cornblath led to the identification of the disease gene locus located at chromosome 9q34 [1]. The causative gene was

identified to be *Senataxin* (SETX), which is a large protein with features that typify RNA–DNA helicases [2]. Three distinct mutations in SETX were found in pedigree analysis of ALS4 patients. Some later studies also reported sporadic mutations in SETX [3, 4], which are summarized in **Table 1**. It should be noted that the other three forms of juvenile ALS (JALS) stem from mutations in different genes, namely, *ALS2* (ALS2), *SPG11* (ALS5), and *SIGMAR1* (ALS16).

Pathological studies of ALS4 have been hampered because of the rarity of the disease, with only about a dozen of diagnosed families around the world. Chen et al. [2] detected degeneration of anterior horn cells in spinal cords and corticospinal tracts in postmortem tissues from two aged individuals of pedigree K7000. Specifically, even though sensory abilities were not significantly affected in these individuals, a significant loss of dorsal root ganglia and posterior columns was detected, along with marked axonal degeneration of motor and sensory roots and peripheral nerves.

In another study, cytosolic mislocalization of the transactive response DNA-binding protein (TDP-43) was observed in spinal cord motor neurons in postmortem tissues from all the ALS4 patients examined [8]. TDP-43 is an RNA metabolism factor and is a well-documented biomarker that forms toxic protein aggregates in multiple neurodegenerative diseases including ALS [9, 10]. Recapitulation of TDP-43 histopathology in motor neurons of mice carrying ALS4 mutations led the authors to imply that dysfunction of SETX converges on TDP-43 pathology causing an ALS-type motor neurodegeneration [8], although the mechanism was not identified.

SETX has also been found mutated in another neurodegenerative disorder termed ataxia with oculomotor apraxia type 2 (AOA2) [11]. However, in this case, disease is caused by missense mutations leading to premature termination of the SETX mRNA transcript. AOA2 patients suffer from progressive cerebellar ataxia with peripheral neuropathy, cerebellar atrophy, and occasional oculomotor apraxia. However, unlike in ALS4, the motor neuron functions are largely preserved in AOA2 patients [12]. It has been suggested that distinct pathologies of AOA2 and ALS4 stem from unique alterations in expression of genes regulated via SETX in neuronal cells [13].

In spite of the seminal discovery of SETX mutations as being the root cause of ALS4, the etiopathogenesis of this disease remains largely unknown. In this

Mutation	Amino acid substitution	Family history	Origin	References
c.8C → T	T3I	Positive	Austria	Chen et al. [2]
c.1166T → C	L389S	Positive Positive	United States Italy	Rabin et al. [5] Chen et al. [2] Avemaria et al. [6]
c.2672T → T	V891A	Positive	Germany	Rudnik-Schoneborn et al. [7]
c.4660T → G	C1554G	Negative	United States	Hirano et al. [3]
c.6085C → G	K2029Q	Negative	United States	Hirano et al. [3]
c.6407G → A	R2136H	Positive	Belgium	Chen et al. 2004 [2]
c.6406C → T	R2136C	Negative	Japan	Saiga T et al., 2012 [4]
c.7640T → C	I2547T	Negative	United States	Hirano et al. [3]

Table 1.
ALS4-associated mutations in SETX.

chapter we will consider the properties of SETX and its role in the maintenance of genomic stability that are likely germane for the health of motor neurons and ALS4 pathology.

2. Senataxin at the crossroads of RNA metabolism and genomic stability

Studies on SETX predate its ALS4 association. SETX is the likely ortholog of a budding yeast protein, splicing endonuclease 1 (Sen1), so named because of its suspected role in the endonucleolytic processing of tRNA during its splicing and maturation [14]. However, because Sen1 lacks endonuclease activity, it likely functions as a non-catalytic effecter of the nucleolytic entity within the splicing machinery [15]. Sen1 possesses sequence motifs characteristic of superfamily 1 (SF1B) nucleic acid helicases [16]. Consistent with this, Sen1 possesses a helicase activity capable of unwinding RNA-DNA hybrids [17–19]. Like other SF1B helicases, Sen1 translocates on nucleic acid strands in the 5′ → 3′ direction [20].

SETX is of low abundance (<500 molecules/cell) predominantly a nuclear protein with some studies reporting its presence in the nucleolus [21, 22]. SETX interacts with RNA polymerase II (pol II) and helps ensure correct termination of transcription and, as such, is important for the processing of noncoding RNAs (ncRNAs) and mRNAs [23, 24]. Importantly, recent studies suggest a role of SETX in maintaining genomic stability across highly transcribed genomic regions via resolution of RNA–DNA hybrids called R-loops, which arise as a consequence of RNA pol II stalling or perturbations of a transcription-coupled process such as mRNA splicing [25]. Moreover, SETX could also clear RNA–DNA hybrids at genomic breaks and promote DNA repair via homologous recombination (HR) [26]. We will explore the various functions of SETX/Sen1 and possible mechanisms by which SETX mutations give rise to ALS4.

2.1 Biochemical and structural features of SETX

SETX is a large protein of 2677 amino acid residues (303 kDa) and, like yeast Sen1, harbors SF1B-type helicase motifs (**Figure 1A**). It should be noted that even though Sen1 is known to unwind RNA-DNA hybrids [14, 15], such an activity has not yet been demonstrated for SETX. However, ALS4-associated missense mutations (K2029Q, R2136H, and I2547T) are all located in the putative C-terminal helicase domain of SETX (**Figure 1A**). Both SETX and Sen1 have an N-terminal domain that undergoes SUMO modification (**Figure 1A**) and that mediates protein–protein interactions with factors that function in RNA metabolism [27]. SETX likely forms a homodimer via the N-terminal domain, but the hereditary ALS4 mutations do not appear to affect protein dimerization [28].

2.1.1 SETX has a large intrinsically disordered region (IDR)

In silico analysis suggests that there is a large IDR in SETX that spans more than 1000 amino acid residues, a structural feature that is absent in the yeast ortholog Sen1. This putative IDR could confer to SETX the ability to interact with different protein partners, to bind nucleic acids [29]. IDRs in nucleic acid-binding proteins are often subject to post-translational modifications and could undergo phase separation, a molecular phenomenon of rearrangement of molecules in a homogenous solution into distinctly concentrated regions of space called condensates [30–33]. However, unrestrained phase separation causes protein aggregation that is observed with FUS [34] and TDP-43 [35], two extensively studied factors associated with

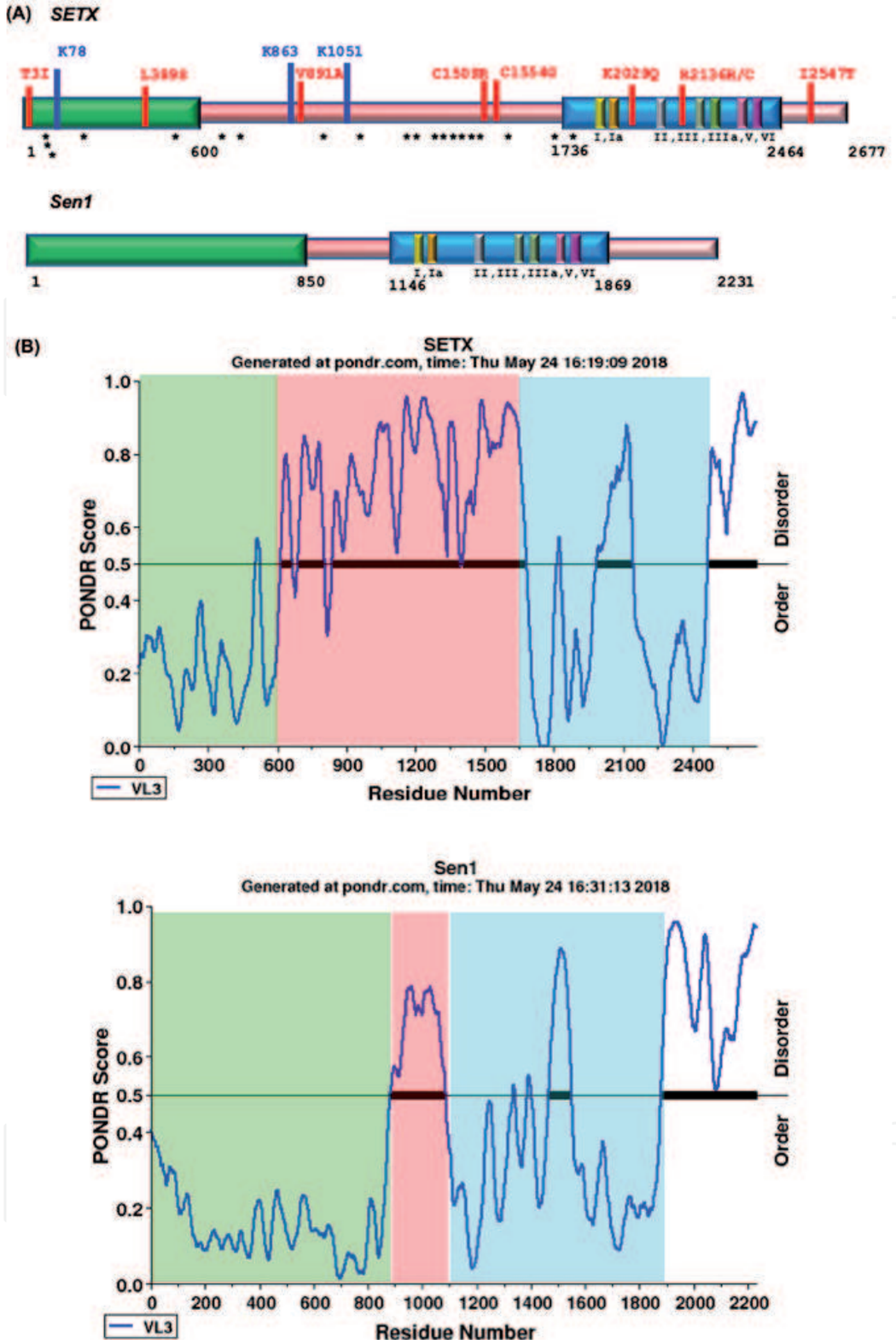

Figure 1.
(A) Schematic diagram of SETX, indicating N-terminal domain (green), central domain (red), and C-terminal helicase domain (blue); conserved motifs are highlighted: Motif I interacts with Mg2+ and NTP, conserved G maintains a flexible loop, motif 1a binds with substrate nucleic acid and transduces energy from the ATP-binding site to the DNA-binding site, motif II binds and hydrolyses ATP, motif III couples ATP hydrolysis with helicase activity, motif V binds substrate nucleic acid, and motif VI couples ATP hydrolysis with helicase activity; ALS4 mutation residues (red) are T3I, L389S, V891A, C1554G, K2029Q, R1236H/C, and I2647T; predicted sumoylation residues (blue) are K78, K863, and K1051 [119]; and cysteine residues predicted by CYSPRED (reliability≥8) to form disulfide bonds (indicated by stars) are C4, C5, C7, C145, C555, C637, C688, C997, C1080, C1123C,1153, C1262, C1277, C1398, C1442, C1509, C1672, C1719, and C2622. (B) Prediction of natural disordered region of SETX (upper panel), and Sen1 (lower panel), with the in silico metapredictor PONDR-VL3 [120]. N-terminal domain (green), central disordered region (red), C-terminal helicase domain (blue).

ALS, which are known to form stress granules in diseased neurons. It could be that SETX, through a phase separation mechanism, forms macromolecular complexes with factors associated with transcription and DNA damage repair. Pathological mutations in SETX could then lead to protein aggregation and loss of protein function in ALS4. This premise awaits experimental testing.

2.1.2 SETX structure could be regulated via disulfide bonding

Multiple neurodegenerative diseases including ALS have been classified among protein misfolding disorders, with disruption of protein disulfide isomerases (PDIs) causing aggregation of superoxide dismutase (SOD1) and TDP-43 in ALS neurons [36]. PDIs are a family of proteins that catalyze formation of disulfide bonds and proper folding of proteins, particularly especially those that harbor an IDR [37]. SETX has 31 cysteine residues in its IDR, and in silico analysis of this region using two independent neural network based predictors, CYSPRED [38] and DIpro [39], revealed that at least 14 cysteine residues in the SETX IDR could engage in disulfide bonding (**Figure 1A**), which is expected to be catalyzed by a PDI. This notion is supported by a proteomic analysis where PDIA6 was detected as a component of the SETX-interactome (**Figure 2**), [40]. We also note that amino acid residue C1554, expected to engage in disulfide linkage with C1509 (**Figure 1A**), is mutated in a sporadic case of ALS4 [3]. Testing of SETX regulation via redox homeostasis [41, 42] merits the effort of ALS researchers.

2.2 Involvement of SETX in RNA metabolism

2.2.1 Role in transcription termination

The role of SETX in regulation of coding and noncoding transcripts is highly conserved. RNA-seq analysis showed that SETX mutations that cause either ALS4 or AOA2 induce unique changes in gene expression patterns [13]. Studies in yeast have shown that Sen1 interacts directly with RNA pol II and is an integral component of the transcription termination machinery consisting of two other

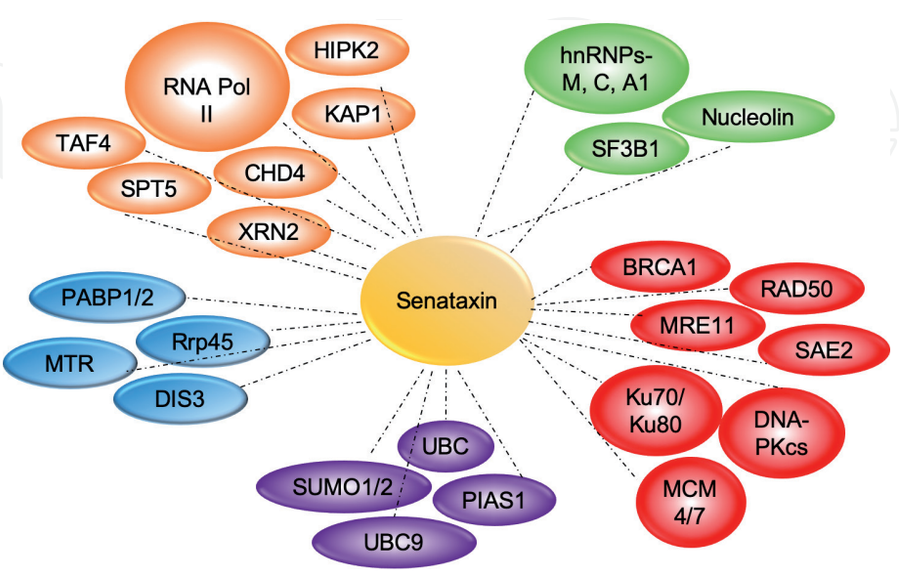

Figure 2.
SETX interactome: DNA repair factors (red), sumoylation and ubiquitination-associated factors (purple), RNA exosome factors (blue), RNAP II and transcription-associated factors (orange), splicing factor and RNA-binding proteins (green), adapted from [40].

factors (Nrd1 and Nab3) that regulate the generation of small nuclear RNAs (snRNAs) and small nucleolar RNAs (snoRNAs) [43–45]. During aberrant RNA pol II pausing, the Nrd1-Nab3-Sen1 (NNS) complex is recruited via direct interaction of Sen1 and Nrd1 with the C-terminal domain (CTD) of RNA pol II [46, 47]. Moreover, Pcf11, a component of the cleavage and polyadenylation complex (CPAC), facilitates RNA pol II CTD Ser2 phosphorylation and handoff of Sen1 from the NNS complex to RNA pol II [48]. NNS complex also captures polyadenylated RNAs and channels them to the RNA exosome complex for degradation, which we will discuss further in Section 2.2.2 [49]. Sen1 is also necessary for recruitment of Rat1/Xrn2, a 5′ → 3′ exoribonuclease at G-rich RNA pol II pause sites for degradation of the nascent transcripts and to prevent the accumulation of pathogenic R-loops [18, 50, 51]. Human lymphoblastoid and fibroblast cells with loss of both SETX or XRN2 result in increased R-loops and DNA double-strand breaks (DSBs) at transcriptional pause sites and hypersensitivity of cells to replications of stress and DNA damage induced by ionizing radiation, ultraviolet light, and oxidative stress [52, 53], which will be discussed further in Section 2.3.3. Thus, defects in pathways of RNA metabolism can lead to the induction of DNA damage.

2.2.2 Role in RNA surveillance machinery

SETX interacts with the RNA exosome, a highly conserved multiprotein ribonuclease complex that processes or degrades a diverse spectrum of RNAs in cells [54]. The exosome removes improperly processed coding and noncoding RNAs (ncRNAs) in the nucleus and regulates mRNA turnover in the cytoplasm. Other critical functions of the exosome include generation of mature ribosomal RNAs, processing of ncRNAs into snRNAs and snoRNAs, and turnover of tRNAs. The human RNA exosome is a ten-subunit complex with a central six-subunit core that constitutes a channel (EXOSC4–9), a three-subunit cap (EXOSC1–3) and a ribonuclease (EXOSC11) subunit located at the bottom of the channel. The nuclear form of the exosome also harbors a riboexonuclease subunit, EXOSC10 [54, 55]. The current model posits that a RNA strand enters the exosome through the cap and is threaded through the channel to be fed to the ribonuclease module for nucleolytic processing [55].

Importantly, SETX interacts with EXOSC9, and complex formation requires SUMOylation of the N-terminal domain of SETX [27]. It has been inferred that SETX-exosome interaction reflects a vital mechanistic axis for resolving co-transcriptional R-loops and preventing genomic instability at heavily transcribed regions in neurons. It might also be surmised that the RNA exosome is recruited via SETX at R-loops to help resolve these pathogenic structures via degradation of the RNA moiety.

Interestingly, the RNA exosome has also been linked to spinal SMA-type neuropathies. Familial missense mutations in EXOSC3 [56] and EXOSC8 [57] are linked to an infantile neuronal disorder, pontocerebellar hypoplasia type 1 (PCH1), that is marked by cerebellar atrophy and progressive microcephaly along with developmental defects and degeneration of spinal motor neurons. Hereditary mutations in EXOSC10 also cause similar neurological defects [58]. Taken together, the available evidence points to a critical role of the exosome in the avoidance of motor neuropathies. Given the interaction noted for SETX and exosome, it might be contemplated that an R-loop removal defect in spinal motor neuronal precursor and differentiated cells could represent the underlying basis for PCH1.

2.3 SETX: a guardian of genomic stability across highly transcribed genomic landscapes

2.3.1 Transcription-coupled (TC) repair

Nucleotide excision repair (NER) is a conserved DNA repair pathway that removes bulky DNA lesions such as those induced by ultraviolet light. When the RNA polymerase II ensemble is obstructed by such a bulky lesion, Cockayne syndrome B (CSB) protein, which interacts with RNA pol II, mediates the recruitment of NER factors such as the Cockayne syndrome A (CSA)-E3-ubiquitin ligase complex [59] and the endonucleases ERCC1-XPF and XPG to mediate the removal of the DNA lesion. In yeast cells, Sen1 has been shown to play a direct role in TC-NER via interaction with Rad2, the yeast XPG ortholog [47]. Whether SETX also functions in TC-NER in humans remains an open question. However, in the absence of SETX, the TC-NER endonucleases XPF and XPG generate DSBs at R-loops, leading to the activation of DNA damage response pathways and repair via HR or the alternate DSB repair pathway of nonhomologous DNA end joining (NHEJ) [60].

2.3.2 Resolution of R-loops

R-loops are RNA–DNA hybrid structures with a displaced single DNA strand and are generated upon reannealing of a nascent transcript with the sense strand [61]. R-loops are transiently formed in many regions of the genome, including those transcribed by RNA pol I, II, and III [62]. R-loops are abundant at promoters of RNA pol II-transcribed genes [63–65], at sequences that are prone to forming G-quadruplex or hairpin structures in the non-template DNA strand [66]. Perturbations of transcription-coupled processes, such as mRNA splicing, also result in R-loop formation [61, 67]. R-loops are detected in the genome via DNA–RNA immunoprecipitation (DRIP) [68] and immunofluorescence assays with the monoclonal antibody S9.6, which has high affinity for RNA–DNA hybrids [69]. Typically, to ensure that the signal detected is specific for RNA–DNA hybrids, one would include the expression of RNase H in cells (to digest the hybrids) or pretreat samples for sequencing with this enzyme.

Depending upon their location and size, R-loops could impart beneficial or harmful effects. R-loops could extend from a few hundred base pairs to kilo base pairs in size. In immunoglobulin (Ig) class switch regions, R-loops serve an important role in Ig class switch recombination by promoting the induction of DNA breaks via the action of activation-induced cytidine deaminase (AID) and base excision repair factors [70]. R-loops also prime DNA replication in the mitochondrial genome [71]. In human fibroblast cells, R-loops can influence the expression of over 1200 genes by facilitating transcription via suppression of DNA methylation [72] and recruitment or eviction of chromatin remodeling complexes [73]. On the contrary, R-loops could interfere with transcription at certain genomic loci like rDNA [62, 74]. The major threat from unscheduled R-loops is the generation of lethal DSBs because of head-on collisions with the DNA replication machinery [75, 76].

Because of the potential harm that R-loops could cause, their levels are tightly regulated via a variety of mechanisms. In this regard, RNAseH1/2 provides a major means for R-loop clearance via ribonucleolytic cleavage of RNA strand [77, 78]. While topoisomerase I prevents R-loop formation by reducing negative supercoiling behind the elongating RNA pol II, TRanscription EXport (TREX) complex factors (THOC1–7, UAP56) and serine−/arginine-rich splicing factor 1 (SRSF1) suppress R-loops by removing the nascent mRNA [79]. RNA biogenesis factors like Trf4/Air2/Mtr4p polyadenylation

(TRAMP) complex and, as discussed earlier, the RNA exosome complex also participate in the regulation of R-loop formation or removal [80]. Moreover, R-loops can be dissociated via the nucleic acid unwinding activity of Sen1/SETX [23, 50, 81] and other helicase proteins such as Pif1 [82], DEAH box protein 9 (DHX9) [83], and Fanconi Anemia Complementation factor M (FANCM) [84]. It seems reasonable to postulate that each of the above-named factors operates at specific genomic milieu.

Studies so forth have suggested that SETX is involved in resolving R-loops at paused transcription sites [50] via forming a physiological complex with the tumor suppressor protein BRCA1 to prevent R-loop-associated DNA damage [25]. This warrants further investigation on SETX-BRCA1 axis to reveal the molecular mechanisms of R-loop resolution.

2.3.3 DNA double-strand break repair and replication fork stability

Occurrence of DSBs and their repair in terminally differentiated neurons were reported in the early 1970s [85, 86]. Recent studies have provided evidence that ALS is associated with a defect in DSB repair [87–89]. DSBs are generated in neurons via endogenous oxidative stress [90, 91] or a topoisomerase IIβ-dependent mechanism that is essential for expression of early genes regulating vital neuronal functions [92]. A recent study showed that neuronal cells from SMA express only low levels of SETX and DNA-PKcs, a highly conserved NHEJ factor. As a result, SMA neurons display higher levels of R-loops that culminate DSB formation, and cellular toxicity [93]. Importantly, these phenotypic manifestations can be corrected by the overexpression of SETX. These observations aptly underscore the genome protective role of SETX in neurons.

Importantly, SETX colocalizes at DSBs with various factors that function in the DNA damage response and repair factors, including γH2AX, 53BP1, and BRCA1, and it forms a co-immunoprecipitable complex with DNA-PKcs, MRE11, RAD50 [24, 25, 53, 94]. AOA2-pateint derived lymphoblastoid cell lines lacking SETX are sensitive toward topoisomerase I inhibitor, camptothecin, DNA crosslinking agent mitomycin C and hydrogen peroxide [53], again indicative of a role of SETX in the DNA damage response. Moreover, *Setx*−/− mice displays a defect in germ cell maturation due to defective meiotic recombination with unrepaired DSBs [81]. Interestingly, recent findings revealed that nascent transcripts or small ncRNAs could accumulate at DSBs via diverse mechanisms, and affect DSB repair via distinct pathways of HR or NHEJ [95–103]. This was further evidenced by the studies demonstrating that both excess removal and impaired clearance of RNA–DNA hybrids result in defective DSB repair [100], suggesting the role of RNA in DSB repair via processes that are yet to be characterized. In this context, SETX has been implicated in enhancing HR-mediated DSB repair that is catalyzed by the recombinase RAD51, via resolving RNA–DNA hybrids at DSBs [26]. Further biochemical investigations are required to dissect the mechanistic underpinnings of this process.

It should be noted that cycling cells face an incessant threat of genomic instability via replication-transcription collisions, wherein the replication and transcription machineries could engage in head-on collisions [104]. This could directly lead to formation of DSBs [76]. Importantly, SETX associates with DNA replication forks and promotes their progression across RNA pol II transcribed regions, a function that appears to be independent of its transcription termination role [105].

2.3.4 Telomere maintenance

Telomeres, which comprise repetitive DNA sequences, cap the ends of each chromosome, and their attrition leads to cellular senescence. Telomerase reverse

transcriptase (TERT), the catalytic subunit of telomerase that maintains the normal length of telomeres, is present at a low level in most differentiated cells including neurons [106]. TERT levels appear to be significantly lower in the spinal cord tissues of ALS patients than healthy individuals [107]. Ex vivo studies have suggested that while telomere damage induces neuronal cell death [108], the activation of telomerase can enhance neuronal cell viability [109, 110]. Interestingly, a novel compound that enhances telomerase activity in neurons also appears to ameliorate the symptoms of ALS [109].

Importantly, SETX is present at telomeres, and AOA2 lymphocytes and lymphoblasts showed reduced telomere length along with higher sensitivity toward oxidative stress and DNA-damaging agents [111]. Another study has implicated SETX in the maintenance of telomeres in *Myotis* bats [112]. These observations should constitute the basis for investigating the mechanistic role of SETX in telomere maintenance in motor neurons and other cell types.

3. Conclusion

Defects in RNA metabolism factors have been associated with multiple motor neuron diseases including ALS and SMA [113, 114]. Despite having distinctive pathological manifestations and clinical onsets, such motor neuropathies could stem from related underlying mechanisms pertaining to RNA homeostasis. In this chapter, we reviewed how defective RNA metabolic pathways could ensue genomic instability, an emerging mechanism in neurodegenerative diseases. Co-transcriptional R-loops are crucial for regulating gene expression in both dividing and postmitotic neurons; however, when not timely resolved, it will lead to genomic instability via generation of DNA strand breaks. While replication-transcription conflicts are the major source of R-loop-induced DSBs in dividing cells that need repair via HR or NHEJ, how R-loops trigger DSBs in postmitotic neurons remains to be investigated. Moreover, small RNAs at DSBs could impede repair and must be cleared nucleolytically or via unwinding of RNA–DNA hybrids. Genetic and cell-based studies of human SETX, together with biochemical characterization of its yeast ortholog Sen1, have suggested that SETX protects genomic stability via resolution of R-loops, assisting replication fork progression across transcribing genomic regions and promoting HR at DSBs. In the light of recent findings on implication of genomic instability in neurodegenerative diseases, as reviewed in [115–118], in-depth studies are required to precisely delineate role of SETX in ALS4. Thus, it is apposite to test if ALS4 gain-of-function mutations affect SETX activities pertaining to DSB repair.

Treatment of ALS4 or other JALS is currently limited to physical and occupational therapies to promote mobility and independence. While an FDA-approved glutamate inhibitor drug, riluzole, that slows down symptoms and prolongs survival is used clinically to treat ALS, there are currently no specific treatment for juvenile ALS diseases. Further studies are obligatory to recognize SETX as a therapeutic target for treatment of ALS4.

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Conflict of interest

The authors declare no conflict of interest.

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References

[1] Chance PF, Rabin BA, Ryan SG, Ding Y, Scavina M, Crain B, et al. Linkage of the gene for an autosomal dominant form of juvenile amyotrophic lateral sclerosis to chromosome 9q34. American Journal of Human Genetics. 1998;**62**:633-640

[2] Chen YZ, Bennett CL, Huynh HM, Blair IP, Puls I, Irobi J, et al. DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). American Journal of Human Genetics. 2004;**74**:1128-1135

[3] Hirano M, Quinzii CM, Mitsumoto H, Hays AP, Roberts JK, Richard P, et al. Senataxin mutations and amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis. 2011;**12**:223-227

[4] Saiga T, Tateishi T, Torii T, Kawamura N, Nagara Y, Shigeto H, et al. Inflammatory radiculoneuropathy in an ALS4 patient with a novel SETX mutation. Journal of Neurology, Neurosurgery, and Psychiatry. 2012;**83**:763-764

[5] Rabin BA, Griffin JW, Crain BJ, Scavina M, Chance PF, Cornblath DR. Autosomal dominant juvenile amyotrophic lateral sclerosis. Brain. 1999;**122**(**Pt 8**):1539-1550

[6] Avemaria F, Lunetta C, Tarlarini C, Mosca L, Maestri E, Marocchi A, et al. Mutation in the senataxin gene found in a patient affected by familial ALS with juvenile onset and slow progression. Amyotrophic Lateral Sclerosis. 2011;**12**:228-230

[7] Rudnik-Schoneborn S, Arning L, Epplen JT, Zerres K. SETX gene mutation in a family diagnosed autosomal dominant proximal spinal muscular atrophy. Neuromuscular Disorders. 2012;**22**:258-262

[8] Bennett CL, Dastidar SG, Ling SC, Malik B, Ashe T, Wadhwa M, et al. Senataxin mutations elicit motor neuron degeneration phenotypes and yield TDP-43 mislocalization in ALS4 mice and human patients. Acta Neuropathologica. 2018;**136**:425-443

[9] Gao J, Wang L, Huntley ML, Perry G, Wang X. Pathomechanisms of TDP-43 in neurodegeneration. Journal of Neurochemistry. 2018;**146**:7-20

[10] Cohen TJ, Lee VM, Trojanowski JQ. TDP-43 functions and pathogenic mechanisms implicated in TDP-43 proteinopathies. Trends in Molecular Medicine. 2011;**17**:659-667

[11] Moreira MC, Klur S, Watanabe M, Nemeth AH, Le Ber I, Moniz JC, et al. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. Nature Genetics. 2004;**36**:225-227

[12] Anheim M, Monga B, Fleury M, Charles P, Barbot C, Salih M, et al. Ataxia with oculomotor apraxia type 2: Clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. Brain. 2009;**132**:2688-2698

[13] Fogel BL, Cho E, Wahnich A, Gao F, Becherel OJ, Wang X, et al. Mutation of senataxin alters disease-specific transcriptional networks in patients with ataxia with oculomotor apraxia type 2. Human Molecular Genetics. 2014;**23**:4758-4769

[14] Winey M, Culbertson MR. Mutations affecting the tRNA-splicing endonuclease activity of Saccharomyces cerevisiae. Genetics. 1988;**118**:609-617

[15] DeMarini DJ, Winey M, Ursic D, Webb F, Culbertson MR. SEN1, a positive effector of tRNA-splicing endonuclease in *Saccharomyces cerevisiae*.

Molecular and Cellular Biology. 1992;**12**:2154-2164

[16] Kim HD, Choe J, Seo YS. The sen1(+) gene of Schizosaccharomyces pombe, a homologue of budding yeast SEN1, encodes an RNA and DNA helicase. Biochemistry. 1999;**38**:14697-14710

[17] Martin-Tumasz S, Brow DA. Saccharomyces cerevisiae Sen1 helicase domain exhibits 5′- to 3'-helicase activity with a preference for translocation on DNA rather than RNA. The Journal of Biological Chemistry. 2015;**290**:22880-22889

[18] Han Z, Libri D, Porrua O. Biochemical characterization of the helicase Sen1 provides new insights into the mechanisms of non-coding transcription termination. Nucleic Acids Research. 2017;**45**:1355-1370

[19] Leonaite B, Han Z, Basquin J, Bonneau F, Libri D, Porrua O, et al. Sen1 has unique structural features grafted on the architecture of the Upf1-like helicase family. The EMBO Journal. 2017;**36**:1590-1604

[20] Buttner K, Nehring S, Hopfner KP. Structural basis for DNA duplex separation by a superfamily-2 helicase. Nature Structural & Molecular Biology. 2007;**14**:647-652

[21] Chen YZ, Hashemi SH, Anderson SK, Huang Y, Moreira MC, Lynch DR, et al. Senataxin, the yeast Sen1p orthologue: Characterization of a unique protein in which recessive mutations cause ataxia and dominant mutations cause motor neuron disease. Neurobiology of Disease. 2006;**23**:97-108

[22] Beck M, Schmidt A, Malmstroem J, Claassen M, Ori A, Szymborska A, et al. The quantitative proteome of a human cell line. Molecular Systems Biology. 2011;**7**:549

[23] Suraweera A, Lim Y, Woods R, Birrell GW, Nasim T, Becherel OJ, et al. Functional role for senataxin, defective in ataxia oculomotor apraxia type 2, in transcriptional regulation. Human Molecular Genetics. 2009;**18**:3384-3396

[24] Yuce O, West SC. Senataxin, defective in the neurodegenerative disorder ataxia with oculomotor apraxia 2, lies at the interface of transcription and the DNA damage response. Molecular and Cellular Biology. 2013;**33**:406-417

[25] Hatchi E, Skourti-Stathaki K, Ventz S, Pinello L, Yen A, Kamieniarz-Gdula K, et al. BRCA1 recruitment to transcriptional pause sites is required for R-loop-driven DNA damage repair. Molecular Cell. 2015;**57**:636-647

[26] Cohen S, Puget N, Lin YL, Clouaire T, Aguirrebengoa M, Rocher V, et al. Senataxin resolves RNA: DNA hybrids forming at DNA double-strand breaks to prevent translocations. Nature Communications. 2018;**9**:533

[27] Richard P, Feng S, Manley JL. A SUMO-dependent interaction between Senataxin and the exosome, disrupted in the neurodegenerative disease AOA2, targets the exosome to sites of transcription-induced DNA damage. Genes & Development. 2013;**27**:2227-2232

[28] Bennett CL, Chen Y, Vignali M, Lo RS, Mason AG, Unal A, et al. Protein interaction analysis of senataxin and the ALS4 L389S mutant yields insights into senataxin post-translational modification and uncovers mutant-specific binding with a brain cytoplasmic RNA-encoded peptide. PLoS One. 2013;**8**:e78837

[29] Oldfield CJ, Dunker AK. Intrinsically disordered proteins and intrinsically disordered protein regions.

Annual Review of Biochemistry. 2014;**83**:553-584

[30] Posey AE, Holehouse AS, Pappu RV. Phase separation of intrinsically disordered proteins. Methods in Enzymology. 2018;**611**:1-30

[31] Hahn S. Phase separation, protein disorder, and enhancer function. Cell. 2018;**175**:1723-1725

[32] Elbaum-Garfinkle S. Matter over mind: Liquid phase separation and neurodegeneration. The Journal of Biological Chemistry. 2019;**294**:7160-7168

[33] Boeynaems S, Alberti S, Fawzi NL, Mittag T, Polymenidou M, Rousseau F, et al. Protein phase separation: A new phase in cell biology. Trends in Cell Biology. 2018;**28**:420-435

[34] Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, et al. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. Cell. 2015;**162**:1066-1077

[35] Mann JR, Gleixner AM, Mauna JC, Gomes E, DeChellis-Marks MR, Needham PG, et al. RNA binding antagonizes neurotoxic phase transitions of TDP-43. Neuron. 2019;**102**:321-338

[36] Andreu CI, Woehlbier U, Torres M, Hetz C. Protein disulfide isomerases in neurodegeneration: From disease mechanisms to biomedical applications. FEBS Letters. 2012;**586**:2826-2834

[37] Hatahet F, Ruddock LW. Protein disulfide isomerase: A critical evaluation of its function in disulfide bond formation. Antioxidants & Redox Signaling. 2009;**11**:2807-2850

[38] Fariselli P, Riccobelli P, Casadio R. Role of evolutionary information in predicting the disulfide-bonding state of cysteine in proteins. Proteins. 1999;**36**:340-346

[39] Cheng J, Saigo H, Baldi P. Large-scale prediction of disulphide bridges using kernel methods, two-dimensional recursive neural networks, and weighted graph matching. Proteins. 2006;**62**:617-629

[40] Groh M, Albulescu LO, Cristini A, Gromak N. Senataxin: genome guardian at the interface of transcription and neurodegeneration. Journal of Molecular Biology. 2017;**429**:3181-3195

[41] Paul BD, Sbodio JI, Snyder SH. Cysteine metabolism in neuronal redox homeostasis. Trends in Pharmacological Sciences. 2018;**39**:513-524

[42] Franco R, Vargas MR. Redox biology in neurological function, dysfunction, and aging. Antioxidants & Redox Signaling. 2018;**28**:1583-1586

[43] Steinmetz EJ, Brow DA. Repression of gene expression by an exogenous sequence element acting in concert with a heterogeneous nuclear ribonucleoprotein-like protein, Nrd1, and the putative helicase Sen1. Molecular and Cellular Biology. 1996;**16**:6993-7003

[44] Steinmetz EJ, Conrad NK, Brow DA, Corden JL. RNA-binding protein Nrd1 directs poly(A)-independent 3′-end formation of RNA polymerase II transcripts. Nature. 2001;**413**:327-331

[45] Steinmetz EJ, Warren CL, Kuehner JN, Panbehi B, Ansari AZ, Brow DA. Genome-wide distribution of yeast RNA polymerase II and its control by Sen1 helicase. Molecular Cell. 2006;**24**:735-746

[46] Chinchilla K, Rodriguez-Molina JB, Ursic D, Finkel JS, Ansari AZ, Culbertson MR. Interactions of Sen1, Nrd1, and Nab3 with multiple phosphorylated forms of the Rpb1

C-terminal domain in Saccharomyces cerevisiae. Eukaryotic Cell. 2012;**11**:417-429

[47] Ursic D, Chinchilla K, Finkel JS, Culbertson MR. Multiple protein/protein and protein/RNA interactions suggest roles for yeast DNA/RNA helicase Sen1p in transcription, transcription-coupled DNA repair and RNA processing. Nucleic Acids Research. 2004;**32**:2441-2452

[48] Grzechnik P, Gdula MR, Proudfoot NJ. Pcf11 orchestrates transcription termination pathways in yeast. Genes & Development. 2015;**29**:849-861

[49] Arndt KM, Reines D. Termination of transcription of short noncoding RNAs by RNA polymerase II. Annual Review of Biochemistry. 2015;**84**:381-404

[50] Skourti-Stathaki K, Proudfoot NJ, Gromak N. Human senataxin resolves RNA/DNA hybrids formed at transcriptional pause sites to promote Xrn2-dependent termination. Molecular Cell. 2011;**42**:794-805

[51] Wagschal A, Rousset E, Basavarajaiah P, Contreras X, Harwig A, Laurent-Chabalier S, et al. Microprocessor, Setx, Xrn2, and Rrp6 co-operate to induce premature termination of transcription by RNAPII. Cell. 2012;**150**:1147-1157

[52] Morales JC, Richard P, Patidar PL, Motea EA, Dang TT, Manley JL, et al. XRN2 links transcription termination to DNA damage and replication stress. PLoS Genetics. 2016;**12**:e1006107

[53] Suraweera A, Becherel OJ, Chen P, Rundle N, Woods R, Nakamura J, et al. Senataxin, defective in ataxia oculomotor apraxia type 2, is involved in the defense against oxidative DNA damage. The Journal of Cell Biology. 2007;**177**:969-979

[54] Morton DJ, Kuiper EG, Jones SK, Leung SW, Corbett AH, Fasken MB. The RNA exosome and RNA exosome-linked disease. RNA. 2018;**24**:127-142

[55] Kilchert C, Wittmann S, Vasiljeva L. The regulation and functions of the nuclear RNA exosome complex. Nature Reviews. Molecular Cell Biology. 2016;**17**:227-239

[56] Wan J, Yourshaw M, Mamsa H, Rudnik-Schoneborn S, Menezes MP, Hong JE, et al. Mutations in the RNA exosome component gene EXOSC3 cause pontocerebellar hypoplasia and spinal motor neuron degeneration. Nature Genetics. 2012;**44**:704-708

[57] Boczonadi V, Muller JS, Pyle A, Munkley J, Dor T, Quartararo J, et al. EXOSC8 mutations alter mRNA metabolism and cause hypomyelination with spinal muscular atrophy and cerebellar hypoplasia. Nature Communications. 2014;**5**:4287

[58] Burns DT, Donkervoort S, Muller JS, Knierim E, Bharucha-Goebel D, Faqeih EA, et al. Variants in EXOSC9 disrupt the RNA exosome and result in cerebellar atrophy with spinal motor neuronopathy. American Journal of Human Genetics. 2018;**102**:858-873

[59] Fousteri M, Mullenders LH. Transcription-coupled nucleotide excision repair in mammalian cells: Molecular mechanisms and biological effects. Cell Research. 2008;**18**:73-84

[60] Sollier J, Stork CT, Garcia-Rubio ML, Paulsen RD, Aguilera A, Cimprich KA. Transcription-coupled nucleotide excision repair factors promote R-loop-induced genome instability. Molecular Cell. 2014;**56**:777-785

[61] Crossley MP, Bocek M, Cimprich KA. R-loops as cellular regulators and genomic threats. Molecular Cell. 2019;**73**:398-411

[62] El Hage A, French SL, Beyer AL, Tollervey D. Loss of topoisomerase I leads to R-loop-mediated transcriptional blocks during ribosomal RNA synthesis. Genes & Development. 2010;**24**:1546-1558

[63] Chen L, Chen JY, Zhang X, Gu Y, Xiao R, Shao C, et al. R-ChIP using inactive RNase H reveals dynamic coupling of R-loops with transcriptional pausing at gene promoters. Molecular Cell. 2017;**68**:745-757

[64] Ginno PA, Lott PL, Christensen HC, Korf I, Chedin F. R-loop formation is a distinctive characteristic of unmethylated human CpG island promoters. Molecular Cell. 2012;**45**:814-825

[65] Dumelie JG, Jaffrey SR. Defining the location of promoter-associated R-loops at near-nucleotide resolution using bisDRIP-seq. Elife. 2017;**6**:e28306

[66] Skourti-Stathaki K, Proudfoot NJ. A double-edged sword: R loops as threats to genome integrity and powerful regulators of gene expression. Genes & Development. 2014;**28**:1384-1396

[67] Li X, Manley JL. Inactivation of the SR protein splicing factor ASF/SF2 results in genomic instability. Cell. 2005;**122**:365-378

[68] Sanz LA, Chedin F. High-resolution, strand-specific R-loop mapping via S9.6-based DNA-RNA immunoprecipitation and high-throughput sequencing. Nature Protocols. 2019;**14**:1734-1755

[69] Boguslawski SJ, Smith DE, Michalak MA, Mickelson KE, Yehle CO, Patterson WL, et al. Characterization of monoclonal antibody to DNA.RNA and its application to immunodetection of hybrids. Journal of Immunological Methods. 1986;**89**:123-130

[70] Roy D, Yu K, Lieber MR. Mechanism of R-loop formation at immunoglobulin class switch sequences. Molecular and Cellular Biology. 2008;**28**:50-60

[71] Lee DY, Clayton DA. Initiation of mitochondrial DNA replication by transcription and R-loop processing. The Journal of Biological Chemistry. 1998;**273**:30614-30621

[72] Grunseich C, Wang IX, Watts JA, Burdick JT, Guber RD, Zhu Z, et al. Senataxin mutation reveals how R-loops promote transcription by blocking DNA methylation at gene promoters. Molecular Cell. 2018;**69**:426-437

[73] Chen PB, Chen HV, Acharya D, Rando OJ, Fazzio TG. R loops regulate promoter-proximal chromatin architecture and cellular differentiation. Nature Structural & Molecular Biology. 2015;**22**:999-1007

[74] Belotserkovskii BP, Soo Shin JH, Hanawalt PC. Strong transcription blockage mediated by R-loop formation within a G-rich homopurine-homopyrimidine sequence localized in the vicinity of the promoter. Nucleic Acids Research. 2017;**45**:6589-6599

[75] Gan W, Guan Z, Liu J, Gui T, Shen K, Manley JL, et al. R-loop-mediated genomic instability is caused by impairment of replication fork progression. Genes & Development. 2011;**25**:2041-2056

[76] Hamperl S, Bocek MJ, Saldivar JC, Swigut T, Cimprich KA. Transcription-replication conflict orientation modulates R-loop levels and activates distinct DNA damage responses. Cell. 2017;**170**:774-786

[77] El Hage A, Webb S, Kerr A, Tollervey D. Genome-wide distribution of RNA-DNA hybrids identifies RNase H targets in tRNA genes, retrotransposons and mitochondria. PLoS Genetics. 2014;**10**:e1004716

[78] Wahba L, Amon JD, Koshland D, Vuica-Ross M. RNase H and multiple

RNA biogenesis factors cooperate to prevent RNA:DNA hybrids from generating genome instability. Molecular Cell. 2011;**44**:978-988

[79] Santos-Pereira JM, Aguilera A. R loops: New modulators of genome dynamics and function. Nature Reviews. Genetics. 2015;**16**:583-597

[80] Laffleur B, Basu U. Biology of RNA surveillance in development and disease. Trends in Cell Biology. 2019;**29**:428-445

[81] Becherel OJ, Yeo AJ, Stellati A, Heng EY, Luff J, Suraweera AM, et al. Senataxin plays an essential role with DNA damage response proteins in meiotic recombination and gene silencing. PLoS Genetics. 2013;**9**:e1003435

[82] Tran PLT, Pohl TJ, Chen CF, Chan A, Pott S, Zakian VA. PIF1 family DNA helicases suppress R-loop mediated genome instability at tRNA genes. Nature Communications. 2017;**8**:15025

[83] Cristini A, Groh M, Kristiansen MS, Gromak N. RNA/DNA hybrid Interactome identifies DXH9 as a molecular player in transcriptional termination and R-loop-associated DNA damage. Cell Reports. 2018;**23**:1891-1905

[84] Schwab RA, Nieminuszczy J, Shah F, Langton J, Lopez Martinez D, Liang CC, et al. The fanconi anemia pathway maintains genome stability by coordinating replication and transcription. Molecular Cell. 2015;**60**:351-361

[85] Price GB, Modak SP, Makinodan T. Age-associated changes in the DNA of mouse tissue. Science. 1971;**171**:917-920

[86] Wheeler KT, Lett JT. Formation and rejoining of DNA strand breaks in irradiated neurons: in vivo. Radiation Research. 1972;**52**:59-67

[87] Mitra J, Guerrero EN, Hegde PM, Liachko NF, Wang H, Vasquez V, et al. Motor neuron disease-associated loss of nuclear TDP-43 is linked to DNA double-strand break repair defects. Proceedings of the National Academy of Sciences of the United States of America. 2019;**116**:4696-4705

[88] Welty S, Teng Y, Liang Z, Zhao W, Sanders LH, Greenamyre JT, et al. RAD52 is required for RNA-templated recombination repair in post-mitotic neurons. The Journal of Biological Chemistry. 2018;**293**:1353-1362

[89] Walker C, El-Khamisy SF. Perturbed autophagy and DNA repair converge to promote neurodegeneration in amyotrophic lateral sclerosis and dementia. Brain. 2018;**141**:1247-1262

[90] Pollari E, Goldsteins G, Bart G, Koistinaho J, Giniatullin R. The role of oxidative stress in degeneration of the neuromuscular junction in amyotrophic lateral sclerosis. Frontiers in Cellular Neuroscience. 2014;**8**:131

[91] Wang X, Michaelis EK. Selective neuronal vulnerability to oxidative stress in the brain. Frontiers in Aging Neuroscience. 2010;**2**:12

[92] Madabhushi R, Gao F, Pfenning AR, Pan L, Yamakawa S, Seo J, et al. Activity-induced DNA breaks govern the expression of neuronal early-response genes. Cell. 2015;**161**:1592-1605

[93] Kannan A, Bhatia K, Branzei D, Gangwani L. Combined deficiency of Senataxin and DNA-PKcs causes DNA damage accumulation and neurodegeneration in spinal muscular atrophy. Nucleic Acids Research. 2018;**46**:8326-8346

[94] Roda RH, Rinaldi C, Singh R, Schindler AB, Blackstone C. Ataxia with

oculomotor apraxia type 2 fibroblasts exhibit increased susceptibility to oxidative DNA damage. Journal of Clinical Neuroscience. 2014;**21**:1627-1631

[95] Storici F, Bebenek K, Kunkel TA, Gordenin DA, Resnick MA. RNA-templated DNA repair. Nature. 2007;**447**:338-341

[96] Keskin H, Shen Y, Huang F, Patel M, Yang T, Ashley K, et al. Transcript-RNA-templated DNA recombination and repair. Nature. 2014;**515**:436-439

[97] Wei W, Ba Z, Gao M, Wu Y, Ma Y, Amiard S, et al. A role for small RNAs in DNA double-strand break repair. Cell. 2012;**149**:101-112

[98] Wang Q, Goldstein M. Small RNAs recruit chromatin-modifying enzymes MMSET and Tip60 to reconfigure damaged DNA upon double-Strand break and facilitate repair. Cancer Research. 2016;**76**:1904-1915

[99] Gao M, Wei W, Li MM, Wu YS, Ba Z, Jin KX, et al. Ago2 facilitates Rad51 recruitment and DNA double-strand break repair by homologous recombination. Cell Research. 2014;**24**:532-541

[100] Ohle C, Tesorero R, Schermann G, Dobrev N, Sinning I, Fischer T. Transient RNA-DNA hybrids are required for efficient double-strand break repair. Cell. 2016;**167**:1001-1013

[101] Chakraborty A, Tapryal N, Venkova T, Horikoshi N, Pandita RK, Sarker AH, et al. Classical non-homologous end-joining pathway utilizes nascent RNA for error-free double-strand break repair of transcribed genes. Nature Communications. 2016;**7**:13049

[102] Francia S, Michelini F, Saxena A, Tang D, de Hoon M, Anelli V, et al. Site-specific DICER and DROSHA RNA products control the DNA-damage response. Nature. 2012;**488**:231-235

[103] Francia S, Cabrini M, Matti V, Oldani A, d'Adda di Fagagna F. DICER, DROSHA and DNA damage response RNAs are necessary for the secondary recruitment of DNA damage response factors. Journal of Cell Science. 2016;**129**:1468-1476

[104] Hamperl S, Cimprich KA. Conflict resolution in the genome: How transcription and replication make it work. Cell. 2016;**167**:1455-1467

[105] Alzu A, Bermejo R, Begnis M, Lucca C, Piccini D, Carotenuto W, et al. Senataxin associates with replication forks to protect fork integrity across RNA-polymerase-II-transcribed genes. Cell. 2012;**151**:835-846

[106] Eitan E, Hutchison ER, Mattson MP. Telomere shortening in neurological disorders: An abundance of unanswered questions. Trends in Neurosciences. 2014;**37**:256-263

[107] De Felice B, Annunziata A, Fiorentino G, Manfellotto F, D'Alessandro R, Marino R, et al. Telomerase expression in amyotrophic lateral sclerosis (ALS) patients. Journal of Human Genetics. 2014;**59**:555-561

[108] Cheng A, Shin-ya K, Wan R, Tang SC, Miura T, Tang H, et al. Telomere protection mechanisms change during neurogenesis and neuronal maturation: Newly generated neurons are hypersensitive to telomere and DNA damage. The Journal of Neuroscience. 2007;**27**:3722-3733

[109] Eitan E, Tichon A, Gazit A, Gitler D, Slavin S, Priel E. Novel telomerase-increasing compound in mouse brain delays the onset of amyotrophic lateral sclerosis. EMBO Molecular Medicine. 2012;**4**:313-329

[110] Zhu H, Fu W, Mattson MP. The catalytic subunit of telomerase protects neurons against amyloid beta-peptide-induced apoptosis. Journal of Neurochemistry. 2000;**75**:117-124

[111] De Amicis A, Piane M, Ferrari F, Fanciulli M, Delia D, Chessa L. Role of senataxin in DNA damage and telomeric stability. DNA Repair (Amst). 2011;**10**:199-209

[112] Foley NM, Hughes GM, Huang Z, Clarke M, Jebb D, Whelan CV, et al. Growing old, yet staying young: The role of telomeres in bats' exceptional longevity. Science Advances. 2018;**4**:eaao0926

[113] Grohmann K, Schuelke M, Diers A, Hoffmann K, Lucke B, Adams C, et al. Mutations in the gene encoding immunoglobulin mu-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. Nature Genetics. 2001;**29**:75-77

[114] Gama-Carvalho M, L Garcia-Vaquero M, R Pinto F, Besse F, Weis J, Voigt A, Schulz JB, De Las Rivas J. Linking amyotrophic lateral sclerosis and spinal muscular atrophy through RNA-transcriptome homeostasis: A genomics perspective. Journal of Neurochemistry. 2017;**141**:12-30

[115] Madabhushi R, Pan L, Tsai LH. DNA damage and its links to neurodegeneration. Neuron. 2014;**83**:266-282

[116] Konopka A, Atkin JD. The emerging role of DNA damage in the pathogenesis of the C9orf72 repeat expansion in amyotrophic lateral sclerosis. International Journal of Molecular Sciences. 2018;**19**:E3137

[117] Penndorf D, Witte OW, Kretz A. DNA plasticity and damage in amyotrophic lateral sclerosis. Neural Regeneration Research. 2018;**13**:173-180

[118] Wang H, Hegde ML. New mechanisms of DNA repair defects in fused in sarcoma-associated neurodegeneration: Stage set for DNA repair-based therapeutics? Journal of Experimental Neuroscience. 2019;**13**:1179069519856358

[119] Yeo AJ, Becherel OJ, Luff JE, Graham ME, Richard D, Lavin MF. Senataxin controls meiotic silencing through ATR activation and chromatin remodeling. Cell Discovery. 2015;**1**:15025

[120] Xue B, Dunbrack RL, Williams RW, Dunker AK, Uversky VN. PONDR-FIT: A meta-predictor of intrinsically disordered amino acids. Biochimica et Biophysica Acta. 2010;**1804**:996-1010