Senataxin protects the genome
Implications for neurodegeneration and other abnormalities

Martin F. Lavin,1,2,* Abrey J. Yeo1,3 and Olivier J. Becherel1,4
1Queensland Institute of Medical Research; Radiation Biology and Oncology; Brisbane, QLD, Australia; 2University of Queensland Centre for Clinical Research; Herston, QLD, Australia; 3School of Medicine; University of Queensland; Herston, QLD, Australia; 4School of Chemistry & Molecular Biosciences; University of Queensland; St. Lucia, QLD, Australia

Ataxia oculomotor apraxia type 2 (AOA2) is a rare autosomal recessive disorder characterized by cerebellar atrophy, peripheral neuropathy, loss of Purkinje cells and elevated α-fetoprotein. AOA2 is caused by mutations in the SETX gene that codes for the high molecular weight protein senataxin. Mutations in this gene also cause dominant neurodegenerative disorders. Similar to that observed for other autosomal recessive ataxias, this protein protects the integrity of the genome against oxidative and other forms of DNA damage to reduce the risk of neurodegeneration. Senataxin functions in transcription termination and RNA splicing and it has been shown to resolve RNA/DNA hybrids (R-loops) that arise at transcription pause sites or when transcription is blocked. Recent data suggest that this protein functions at the interface between transcription and DNA replication to minimise the risk of collision and maintain genome stability.

Our recent data using SETX gene-disrupted mice revealed that male mice were defective in spermatogenesis and were infertile. DNA double strand-breaks persisted throughout meiosis and crossing-over failed in SETX mutant mice. These changes can be explained by the accumulation of R-loops, which interfere with Holiday junctions and crossing-over. We also showed that senataxin was localized to the XY body in pachytene cells and was involved in transcriptional silencing of these chromosomes. While the defect in meiotic recombination was striking in these animals, there was no evidence of neurodegeneration as observed in AOA2 patients. We discuss here potentially different roles for senataxin in proliferating and post-mitotic cells.

Autosomal recessive cerebellar ataxias are a class of progressive neurodegenerative disorders that result from cerebellar atrophy and spinal tract dysfunction.1 One of these, ataxia oculomotor apraxia type 2 (AOA2) is characterized by progressive cerebellar atrophy and peripheral neuropathy, oculomotor apraxia and elevated α-fetoprotein serum levels, with an onset between 10–20 y of age.3,4 Brain MRI reveals diffuse cerebellar atrophy and electroneuromyography confirms the peripheral neuropathy.4 The major clinical features of this disorder are shown in Table 1. In a post-mortem AOA2 case, Criscuolo et al.5 observed reduced brain size and cerebellar atrophy which was most evident at the level of the vermis and anterior lobe; the cerebellar cortex had marked loss of Purkinje cells and brainstem and spinal cord were slightly reduced. Thus, as with other autosomal recessive ataxias, pathology in the cerebellum features strongly. However, unlike that for the related disorder ataxia telangiectasia (A-T), there is no evidence of increased cancer susceptibility in AOA2.

The gene mutated in AOA2 was initially mapped to chromosome 9q34 and subsequently identified as SETX.6 SETX is predicted to code for a 2,667 amino acid protein (senataxin) that contains a highly conserved C-terminal seven-motif domain found in the superfamily 1 of
DNA/RNA helicases and an N-terminal domain important for protein-protein interaction. Generally speaking, mutations in a single gene, such as SETX, gives rise to one syndrome, which, of course, may show heterogeneity depending on the nature and localization of the mutations. In the case of SETX, up to 4 different syndromes are associated with mutations in this gene (Table 2). Juvenile amyotrophic lateral sclerosis (ALS4) is a form of juvenile ALS characterized by distal muscle weakness and atrophy, normal sensation and pyramidal tract signs. The ALS4 locus maps to chromosome 9q34. Chen et al. detected missense mutations in a single allele in the SETX gene (which maps to this locus) which segregated with the disease. Subsequent studies have detected SETX mutations in additional ALS4 patients. Heterozygous SETX gene mutations were also detected in patients with autosomal dominant proximal spinal muscular atrophy. These patients showed proximal and distal muscular atrophy and pareses. While there was overlap with ALS4, this appeared to be a discrete entity. A dominant SETX mutation, causing a cerebellar phenotype termed tremor-ataxia syndrome, has been described for a mother and daughter. These patients showed cerebellar atrophy, oculomotor defects and tremor but no evidence of peripheral neuropathy or pyramidal signs. In short, mutations in SETX can give rise to both dominant and recessive disorders with some overlap in features. A greater insight into the function of senataxin and the proteins it interacts with will help to resolve the quandary of several distinct disorders from mutations in a single gene.

Senataxin shares extensive homology with the yeast *Saccharomyces cerevisiae* splicing endonuclease 1 protein (Sen1p), which possesses helicase activity, and is involved in the processing of tRNA, rRNA, small nuclear and small nucleolar RNA. Sen1p also interacts with Rad2, which is required for DNA repair, suggesting that the protein may be involved in protecting the genome. We demonstrated that this might also be the case for senataxin by showing that AOA2 patient cells display sensitivity to DNA damaging agents such as H₂O₂, camptothecin and mitomycin C and the cells had elevated levels of oxidative DNA damage. In support of a role for senataxin in the DNA damage response, it has also been demonstrated that telomere length is constitutively reduced in AOA2 lymphocytes and the rate of telomere shortening by DNA damage is increased in these cells. Interaction of Sen1p with Rnt1p (an endoribonuclease required for RNA maturation) suggested that Sen1p is also involved in RNA processing and transcription. We provided evidence for a similar role in human cells by identifying novel senataxin-interacting proteins, the majority of which are involved in transcription and RNA processing, including RNA polymerase II. Binding of RNA polymerase II to candidate genes was significantly reduced in senataxin deficient cells and this was accompanied by decreased transcription of these genes, suggesting a role for senataxin in the regulation/modulation of transcription. RNA polymerase II-dependent transcription termination was defective in cells depleted of senataxin in keeping with the observed interaction of senataxin with poly(A) binding proteins 1 and 2.

### Table 1. Ataxia Oculomotor Apraxia Type 2 (AOA2): Clinical Features

| Disorder                          | Age of onset (yr) | Major Clinical Phenotype                      | Gene/Protein | Inheritance | References |
|-----------------------------------|-------------------|------------------------------------------------|--------------|-------------|------------|
| Ataxia oculomotor apraxia type 2 (AOA2) | 10–20             | Cerebellar ataxia with peripheral neuropathy | Setx/senataxin | Recessive | 1–5        |
| Tremor ataxia syndrome (TAS)      | 3, 13*            | Cerebellar ataxia without peripheral neuropathy | Setx/senataxin | Dominant  | 11         |
| Juvenile Amyotrophic lateral sclerosis (ALS4) | 14**               | Limb weakness and severe muscle wasting | Setx/senataxin | Dominant | 9          |
| Autosomal dominant proximal spinal muscular atrophy (ADSMA) | 10–20             | Muscular atrophy and weakness | Setx/senataxin | Dominant | 10         |

*Age of onset for daughter and mother. **Average age of onset.
efficiency of specific mRNAs and alternate splice-site selection of both endogenous genes and artificial minigenes were altered in senataxin-depleted cells. A role for senataxin in transcription elongation and termination is further supported by a report showing that cells with senataxin knockdown displayed an increase in RNA read-through and Pol II density downstream of the Poly (A) site and also exhibited increased levels of R-loop formation. R-loops are RNA/DNA hybrids that form over transcription pause sites by interaction with a ssDNA template behind an elongating Pol II complex (Fig. 1). These structures are potentially harmful and can cause genomic instability if left unresolved. The yeast ortholog of senataxin Sen1p, has also been shown to protect its heavily transcribed genome from R-loop-mediated DNA damage. More recently, Hazelbaker et al. showed that kinetic competition between elongating RNA pol II and Sen1p helicase likely explains the temporal and spatial window for early Pol II termination. Loss of Sen1p results in transient R-loop accumulation, giving rise to transcription-associated recombination and genome instability. More insight into this was provided recently by Alzu et al. who showed that Sen1p associates with DNA replication forks to protect their integrity across RNA Pol II – transcribed genes. Thus, Sen1p plays an important role in coordinating replication with transcription to protect the genome. Support for a similar role for the human ortholog, senataxin, was provided recently by Yuce-Petronczki and West who showed that senataxin localized to distinct nuclear foci in S/G2 phase cells and that the number of these foci increased in response to impaired replication. These data suggest that senataxin localizes to collision sites between the transcription apparatus and components of the replisome.

All of the investigations above have described a role for senataxin in preventing collision between DNA replication forks and ongoing transcription to preserve genome integrity in proliferating cells. However, the major phenotype in AOA2 patients is progressive neurodegeneration in post-mitotic tissue. Under those conditions, ongoing transcription will not encounter DNA replication forks. What then is the role of senataxin in the brain? Vantaggiato et al. provided evidence that senataxin plays a role in neuritogenesis and cytoprotection during neuronal differentiation which is mediated by fibroblast growth factor 8. However, since this is a progressive disease, it is unlikely that the role of senataxin is restricted to development. To address this further, we disrupted the SETX gene in a mouse model for AOA2, which was the subject matter of our recent publication. Unfortunately, we did not observe a neurodegenerative phenotype in the Setx−/− mice and there was no evidence of more subtle behavioral differences in these mice, which limited investigation into the nature of the defect in the brains of these mice. This was not altogether surprising since knock-out of several of the genes causing autosomal recessive ataxias in humans fails to re-capitate the phenotype in mouse...
subsequently showed that senataxin had an essential role in spermatogenesis in mice and in its absence these cells failed to progress past the pachytene stage of prophase 1 of meiosis. The DNA double-strand breaks (DSB) introduced by Spo11 in readiness for meiotic recombination were inefficiently repaired on autosomes, resulting in a failure to complete crossing-over. During the process of crossing-over, autosomes remain transcriptionally active, so it was possible that in the absence of senataxin, R-loops would accumulate in the vicinity of unrepaired DNA DSB leading to collapse of Holiday junctions and inhibition of the crossing-over step. Indeed this was the case since we detected elevated levels of R-loops in both spermatocyte spreads and testes sections (Fig. 2). Wild-type mice showed a very much reduced level of signal. So in the case of spermatocyte differentiation, the R-loops that accumulate in the absence of senataxin appear to collide with Holiday junctions rather than with advancing replication forks. We also screened for the presence of R-loops in the brains of Setx mutant mice but failed to detect these structures by immunofluorescence (unpublished data). This was not altogether surprising since neither DNA replication nor DNA recombination is taking place in this tissue. It is possible that persistence of DNA damage in post-mitotic cells might lead to the accumulation of these structures, which in turn could contribute to the neurodegenerative changes in AOA2 patients. However, we and others have provided evidence for a broader role for senataxin in transcription and other cellular processes. Senataxin plays an important role in transcription termination to prevent RNA readthrough, which may or may not be related to R-loop resolution. The presence of significant readthrough of mRNA may lead to inefficient or aberrant protein synthesis and consequently cell toxicity. Senataxin has also been shown to play a role in the regulation of splicing and deficiency of the SR splicing factor ASF/SF2 leads to R-loop accumulation and genome instability. This in turn may interfere with the fidelity of the transcriptome in AOA2 cerebellum.

AOA2 is just one of several neurodegenerative disorders characterized by models. However, we observed that Setx male mice were infertile and fertility was reduced in females. While there is no information on male fertility in AOA2, there are a few reports of hypogonadism in females. Criscuolo et al. reported two patients who entered menopause in early adulthood which was also observed in a separate study. Ovarian failure has also been observed in a patient with AOA2 and another patient had a diagnosis of polycystic ovarian syndrome. We subsequently showed that senataxin had an essential role in spermatogenesis in mice and in its absence these cells failed to progress past the pachytene stage of prophase 1 of meiosis. The DNA double-strand breaks (DSB) introduced by Spo11 in readiness for meiotic recombination were inefficiently repaired on autosomes, resulting in a failure to complete crossing-over. During the process of crossing-over, autosomes remain transcriptionally active, so it was possible that in the absence of senataxin, R-loops would accumulate in the vicinity of unrepaired DNA DSB leading to collapse of Holiday junctions and inhibition of the crossing-over step. Indeed this was the case since we detected elevated levels of R-loops in both spermatocyte spreads and testes sections (Fig. 2). Wild-type mice showed a very much reduced level of signal. So in the case of spermatocyte differentiation, the R-loops that accumulate in the absence of senataxin appear to collide with Holiday junctions rather than with advancing replication forks. We also screened for the presence of R-loops in the brains of Setx mutant mice but failed to detect these structures by immunofluorescence (unpublished data). This was not altogether surprising since neither DNA replication nor DNA recombination is taking place in this tissue. It is possible that persistence of DNA damage in post-mitotic cells might lead to the accumulation of these structures, which in turn could contribute to the neurodegenerative changes in AOA2 patients. However, we and others have provided evidence for a broader role for senataxin in transcription and other cellular processes. Senataxin plays an important role in transcription termination to prevent RNA readthrough, which may or may not be related to R-loop resolution. The presence of significant readthrough of mRNA may lead to inefficient or aberrant protein synthesis and consequently cell toxicity. Senataxin has also been shown to play a role in the regulation of splicing and deficiency of the SR splicing factor ASF/SF2 leads to R-loop accumulation and genome instability. This in turn may interfere with the fidelity of the transcriptome in AOA2 cerebellum.
defects in RNA metabolism that impact either gene transcription, pre-mRNA splicing, ribonucleoprotein complex formation, mRNA transport, RNA translation or RNA degradation.28 One form of the motor neuron disease, amyotrophic lateral sclerosis (ALS) is caused by defects in TDP-43 and FUS/TLS, both of which contain RNA-binding motifs29 and spinal muscular atrophy (SMA) is caused by deletion or mutation in survival of motor neuron 1 (SMN1). Profound loss of splicing or mutation in survival of motor neuron 1 (SMN1). Profound loss of splicing-deletion or mutation in survival of motor muscular atrophy (SMA) is caused by genetic and clinical study. Brain 2004; 127:759-67; PMID:14756755; http://dx.doi.org/10.1093/brain/awh080

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