

To live or to die: a matter of processing damaged DNA termini in neurons

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NEURODEGENERATION: THE CEREBELLUM IS A COMMON TARGET

The putative role of DNA repair deficiency in the pathogenesis of several neurological disorders has been the subject of intense scientific scrutiny. [Ataxia telangiectasia](#) (AT) is the prototype example of such disorders and yet it is still not completely understood why deficiency of the protein mutated in AT (Ataxia Telangiectasia mutated, ATM) leads to the severe neurological abnormalities seen in AT. Cerebellar degeneration is the most common neurological presentation of AT, which is also a shared feature among at least three distinct hereditary diseases: spinocerebellar ataxia with axonal [neuropathy](#) 1 (SCAN1), ataxia oculomotor apraxia 1 (AOA1) and ataxia oculomotor apraxia 2 (AOA2). A unique feature of the cerebellum is its extended postnatal development. During this phase of growth rapid cell proliferation is expected to generate replication stress leading to more DNA breaks than in other parts of the nervous system. Whether this explains the selective degeneration of the cerebellum in AT, SCAN1, AOA1 and AOA2 is yet to be determined.

Although SCAN1, AOA1 and AOA2 share most of the neurological presentations, they lack the cancer predisposition and immunodeficiency observed in AT. However, there is a fair degree of overlap in the extra-neurological features (Fig 1). For example, whereas AT and AOA2 patients present with high levels of alpha fetoprotein (AFP), SCAN1 and AOA1 patients present with reduced levels of serum albumin (Date et al, 2001; Moreira et al, 2001; Takashima et al, 2002). It is unknown

Defects in the repair of deoxyribonucleic acid (DNA) damage underpin several hereditary neurological diseases in humans. Of the different activities that repair chromosomal DNA breaks, defects in resolving damaged DNA termini are among the most common causes of neuronal cell death. Here, the molecular mechanisms of some of the DNA end processing activities are reviewed and the association with human neurodegenerative disease is discussed.

whether this reflects a consequence of the associated DNA repair defect or a result of a progressive functional decline. AOA2 is associated with defects in senataxin (SETX) – a putative DNA/RNA helicase that has been implicated in the coordination of transcription and DNA repair (Suraweera et al, 2007, 2009). SCAN1 and AOA1 are associated with defects in the two DNA end-processing enzymes – tyrosyl DNA phosphodiesterase 1 (TDP1) and aprataxin (APTX), respectively. Intriguingly, both TDP1 and APTX appear to process DNA breaks that arise due to abortive activities of endogenous enzymes: APTX is a 5'-AMP hydrolase and TDP1 is a 3'-end processing enzyme that repairs topoisomerase 1 (Top1) mediated DNA damage.

REPAIR OF TOPOISOMERASE-MEDIATED DNA DAMAGE AND NEURODEGENERATION: SAME PATHWAY WITH DIFFERENT CLINICAL FEATURES

DNA topoisomerases

The compact and supercoiled nature of the DNA double helix requires [topological modification](#) during important cellular processes, such as transcription, replication and repair. This modification is conducted by DNA topoisomerases and involves transient cleavage of one or both strands of DNA followed by religation of the double helix (Champoux, 2001). This dangerous mode of catalysis involves an intermediate structure in which the topoisomerase is attached either to the 3'-end (Top1), or to the 5'-end (Top2), via a covalent phosphotyrosyl bond. The half-life of these intermediates *in vivo* is normally

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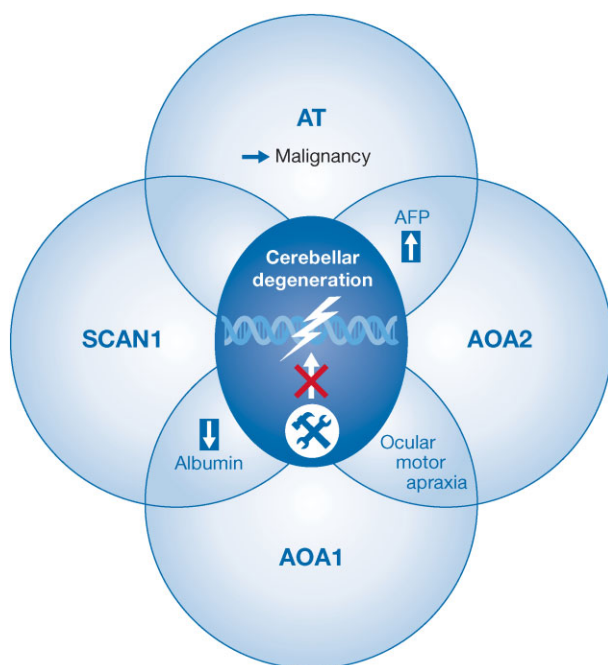


Figure 1. The cerebellum is a common target for disorders with DNA repair defects. Ataxia telangiectasia (AT), Spinocerebellar ataxia with axonal neuropathy 1 (SCAN1), Ataxia oculomotor apraxia 1 (AOA1), and Ataxia oculomotor apraxia 2 (AOA2) are DNA repair-related disorders that share cerebellar degeneration as the most striking clinical feature. In contrast to AT where patients present with immunodeficiency and cancer, symptoms of SCAN1, AOA1 and AOA2 appear to be restricted to the nervous system. It is interesting to note that there is some degree of overlap in the extra-neurological features. Whereas AT and AOA2 patients present with high levels of AFP, SCAN1 and AOA1 present with reduced levels of serum albumin. This may reflect a consequence of the associated DNA repair defect or arise as a result of progressive functional decline.

have been linked to at least three distinct human neurological disorders ranging from mental retardation (Kerzendorfer et al, 2010) and cerebellar degeneration (Takashima et al, 2002) to microcephaly (Shen et al, 2010).

Repair of DNA topoisomerase-linked DNA breaks

Removal of the covalently linked topoisomerase from DNA is mandatory prior to subsequent repair steps. This could be achieved by a non-specific nucleolytic cleavage of DNA, liberating the topoisomerase and a fragment of DNA (Connelly & Leach, 2004). A number of nucleases have been postulated to possess roles in this mechanism in eukaryotic cells, such as the MRN complex, XPF/ERCC1, Mus81, SLX1/SLX4, CtIP and ARTEMIS (Connelly & Leach, 2004; Eid et al, 2010; Hamilton & Maizels, 2010; Nakamura et al, 2010; Pommier et al, 2006). Cell cycle status and the choice of a particular nuclease can subsequently influence the nature of the DNA break and the mechanism of its repair. None of these nucleases has been conclusively shown to play a part in the repair of Top1-linked DNA **single-strand breaks** (Top1-SSBs). In contrast, these nucleases have been shown to be involved in the repair of Top1-linked DNA **double-strand breaks** (DSBs) (Shrivastav et al, 2008). For this process, cells can either employ a homologous

very short and breaks are sealed rapidly (Wang, 2002). However, collision with the DNA replication machinery, RNA polymerases or the proximity to other types of DNA lesions can inhibit or delay religation, creating potentially deleterious protein-linked DNA breaks. The nature of these breaks depends on the type of the topoisomerase, the biological process involved and on the stage of the cell cycle (Pommier et al, 2006). These protein-linked DNA breaks can interfere with important cellular processes such as transcription, can trigger cell death or, if repaired inaccurately, may lead to mutations. Defects in the pathway of repairing Top1-mediated DNA breaks

Glossary

Ataxia telangiectasia

A heritable neurodegenerative disease that leads to severe disability and increased risk of cancer. Ataxia is a lack of coordination of muscle movements and telangiectasia refers to small dilated blood vessels.

Camptothecin

A cytotoxic chemical derived from plants that binds to Top1 and DNA resulting in a stabilized ternary complex, preventing DNA re-ligation. It therefore causes DNA damage.

Double-strand break

A break or discontinuity in two strands of the DNA double helix.

Homologous recombination

A process in which nucleotide sequences are exchanged between two homologous or identical molecules of DNA, leading to accurate repair of harmful double-strand breaks.

Neuropathy

Damage to nerves of the peripheral nervous system.

Non-homologous end joining

A process in which DNA breaks are directly ligated without the need for a homologous template.

Nucleotide excision repair

A DNA repair process by which cells can prevent unwanted mutations by removing UV-induced DNA damage.

Relative microcephaly

A head size that falls within the normal range but is disproportionately small when compared with the patient's length or weight centiles.

Single-strand break

A discontinuity or a break in only one strand of the DNA double helix.

Somatotropic axis

An intricate network that controls metabolism and comprises of hypothalamic regulatory centres, the anterior pituitary gland, peripheral target organs, receptors and signalling molecules.

Topological modification

Alteration of the superhelical density of the DNA double helix: *i.e.* a change in the number of links and knots in the DNA double helix.

template to repair the break by **homologous recombination** (HR) or directly ligate the two DSB ends during **non-homologous end joining** (NHEJ). It is worth noting that HR is absent in non-cycling cells and thus NHEJ would be the prevailing pathway for repair of DSBs.

Tyrosyl DNA phosphodiesterase 1 (TDP1)

In striking contrast to the nucleolytic cleavage of DNA to remove 'stalled' topoisomerases, cells employ another more accurate and error-free repair mechanism, which involves the hydrolytic cleavage of the covalent phosphotyrosyl bond linking the topoisomerase to DNA termini. The prototype enzyme for such an activity was first reported in yeast and named TDP1 (Yang et al, 1996). The human homologue of the yeast enzyme was subsequently reported and its defect was associated with defects in the repair of topoisomerase 1 DNA breaks (El-Khamisy et al, 2005; Interthal et al, 2005a). The importance of this mode of catalysis is highlighted by the human genetic, neurological disease SCAN1, which is associated with mutations in TDP1 and defects in the repair of Top1-SSBs induced by the Top1 poison **camptothecin** (CPT), hydrogen peroxide or ionizing radiation (El-Khamisy et al, 2005; Interthal et al, 2005a; Katyal et al, 2007; Takashima et al, 2002). Unlike AT and AOA1, SCAN1 patients identified to date possess a later onset (~16 years) but they also present with cerebellar atrophy and peripheral neuropathy. The unique phosphodiesterase activity of TDP1 has also been implicated in the repair of a variety of oxidative 3'-termini, such as 3'-phosphoglycolate (Inamdar et al, 2002; Interthal et al, 2005b; Zhou et al, 2005, 2009). TDP1 also possesses a limited RNA and DNA 3'-nucleosidase activity, removing a single nucleotide from the 3'-hydroxyl terminus of the oligonucleotide (Bahmed et al, 2010; Interthal et al, 2005b). More recently, the corresponding activity that repairs Top2-mediated DNA breaks was identified for the enzyme TTRAP and was subsequently named TDP2 (Ledesma et al, 2009; Zeng et al, 2011). Whether defects in TDP2 lead to neurological disorders in humans or mice, and whether TDP2 contributes to the repair of Top1-breaks remains to be determined.

What could be the source of TDP1-dependent breaks in neurons? One of the most common causes of nicks in DNA is oxidative attack by endogenous reactive oxygen species (ROS), which produces, at least in the case of H₂O₂, more SSBs than DSBs (Bradley & Kohn, 1979). It has been shown that nicks, gaps and discontinuities in DNA can lead to the formation of Top1-breaks (Pourquier et al, 1997). It is therefore plausible to speculate that Top1-breaks arise endogenously in cells and that TDP1 is required to deal with them. In addition, collision of Top1 cleavage complexes with RNA polymerases can also lead to Top1-breaks (Bendixen et al, 1990). Un-repaired DNA breaks with damaged termini have been shown to block progression of RNA polymerases *in vitro* (Bendixen et al, 1990) and thus could provide a possible explanation for neuronal cell death in case of defective TDP1. Another possible explanation for neuronal cell death could be the accumulation of excessive DNA breaks in the absence of TDP1 with subsequent over-activation of poly-(ADP) ribose polymerases and the depletion of neural NAD⁺ and ATP. This may trigger neural cell death by apoptosis. Neural

mitochondria may play role in this mode of cell death via the release of apoptosis-inducing factor (AIF) (see below).

Although TDP1-dependent repair is a housekeeping process and TDP1 exists in a complex with the SSB-repair machinery via interaction with DNA ligase III α (Lig3 α) under unperturbed culture conditions (El-Khamisy et al, 2005), the level of this interaction might still be regulated. Central to this regulation is its post-translational modification by phosphorylation, which was first reported for TDP1 following DNA damage by the Povirk laboratory, Department of Pharmacology and Toxicology, Richmond, USA (Zhou et al, 2005). More recently, a serine residue in the less conserved amino terminus domain of TDP1 (serine 81) was reported to mediate interaction with Lig3 α (Chiang et al, 2010) and the phosphorylation of this serine was mediated by ATM or DNA PK (Das et al, 2009). Mutation of TDP1 serine 81 to a residue that cannot be phosphorylated was found to abrogate interaction with Lig3 α in a yeast-two hybrid analysis. This change has also resulted in a significant reduction of TDP1 interaction with XRCC1/Lig3 α as measured by co-immunoprecipitation, when TDP1 was over-expressed in mammalian cells (Chiang et al, 2010; Das et al, 2009).

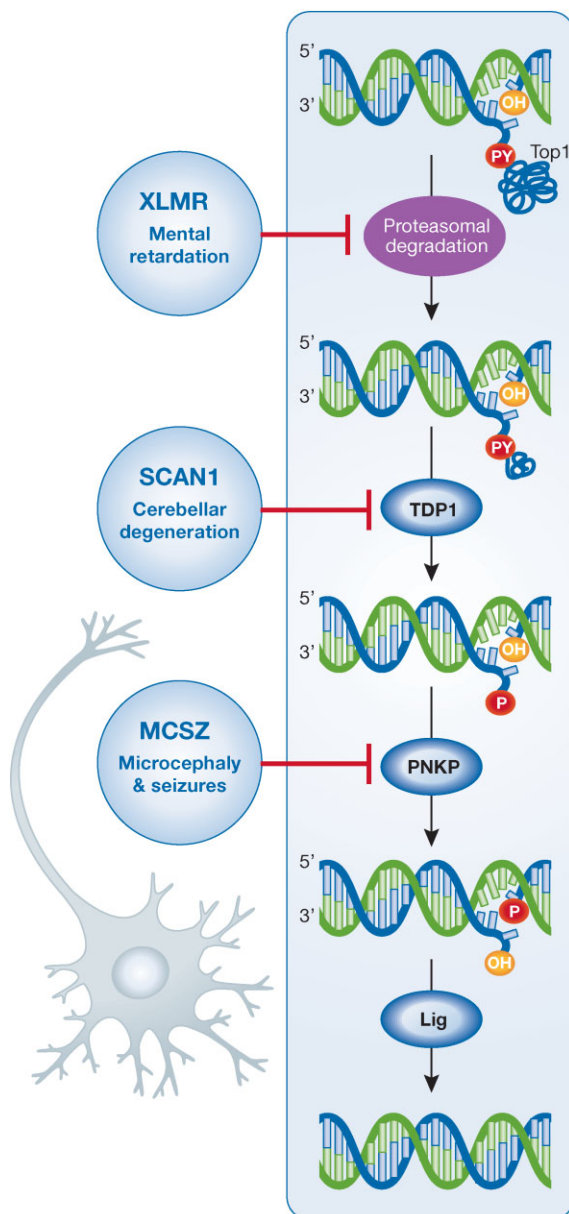
What could be the biological significance of TDP1 phosphorylation and/or interaction with Lig3 α ? Phosphorylation of serine 81 has been shown to promote TDP1 stability following exposure of cells to CPT (Chiang et al, 2010; Das et al, 2009). TDP1 may be chaperoned by Lig3 α , thereby increasing its concentration at sites of damage to ensure completion of repair and to prevent non-specific spurious DNA activities on undamaged DNA. Consistent with this notion, TDP1 phosphorylation at serine 81 facilitates its recruitment to sites of DNA damage (Das et al, 2009). It is not clear whether this reflects phosphorylation of serine 81 only or a subsequent requirement for the interaction with Lig3 α . The latter possibility seems unlikely since Lig3 α -deficient cells lack measurable nuclear DNA repair defects (Chiang and El-Khamisy, unpublished observations). It is thus possible that TDP1/Lig3 α interaction facilitates repair of Top1-SSBs at specific sites, such as clustered DNA lesions. It is worth noting that Lig3 α possesses an N-terminal zinc finger (ZNF) domain homologous to that of PARP1. This ZNF domain is believed to facilitate binding and ligation of DNA breaks located near DNA secondary structures (Cotner-Gohara et al, 2008; Taylor et al, 2000) and thus may be important for targeting TDP1 to closely spaced Top1-SSBs. Alternatively, the TDP1-Lig3 α interaction may channel TDP1 to an alternative NHEJ process to repair Top1-DSBs, for which a role for Lig3 α has been proposed (Audebert et al, 2004). It is also possible that the interaction of TDP1 with Lig3 α is dispensable for nuclear DNA repair but critical for mitochondrial DNA repair, for which both enzymatic activities have been observed (see below).

Why do lower eukaryotes such as yeast not need the N-terminus domain of TDP1? It is possible that higher eukaryotes require more efficient mechanisms for repairing endogenous Top1-breaks to keep their steady-state level below a certain threshold. This may be particularly important in non-dividing cells where cell death may be dictated by a much lower threshold of DNA breaks within a specific cellular compartment. It is also

possible that the evolutionary addition of the N-terminus domain of TDP1 reflects the need for better orchestration of repair processes due to bigger genome size and consequently the extent of breaks arising *per* cell. It would be interesting to determine the relative proportion of Top1-linked DNA breaks arising endogenously in lower eukaryotes to that in human.

What is the relevance of TDP1 serine 81 to SCAN1? The known SCAN1 patients possess a homozygous mutation (H493R) within the active site of TDP1 that results in a ~3-fold reduction of expression, presumably due to instability of the mutant protein. It is not known whether this instability is due to improper folding of the mutant TDP1^{H493R} protein. It is also not known whether TDP1^{H493R} can be efficiently phosphorylated at S81 *in vivo* and whether this affects its steady-state level at endogenous levels of DNA damage. Cell free assays utilizing

anti-TDP1 immunoprecipitates revealed no detectable TDP1 activity from SCAN1 lymphoblastoid cells (LCLs) and experiments utilizing recombinant TDP1^{H493R} protein or whole cell extracts from SCAN1 cells revealed 25-fold or 100-fold reduction of enzymatic activity, respectively (El-Khamisy et al, 2005; Interthal et al, 2005a). It is worth noting that the mutant TDP1^{H493R} remains bound to the 3'-terminus after releasing Top1, but the relative proportion of Top1-linked breaks *versus* TDP1-linked breaks in SCAN1 is not fully resolved (Hirano et al, 2007; Interthal et al, 2005a). Importantly, the relative impact of TDP1-linked breaks *versus* Top1-linked breaks on neurological function is not determined. Accumulation of the latter has been associated with age-dependent reduction of cerebellar size in *Tdp1*^{-/-} mice (Katyal et al, 2007). Investigation of the impact of TDP1-linked breaks on neurological function awaits the generation of *Tdp1*^{H493R} knock-in mice.



Polynucleotide kinase phosphatase (PNKP)

One of the most common forms of damaged DNA termini induced by ROS is 3'-phosphate, which is a major substrate for polynucleotide kinase phosphatase (PNKP, Chen et al, 1991). Importantly, the product of TDP1 activity is 3'-phosphate and 5'-hydroxyl termini, both of which require the 3'-phosphatase and 5'-kinase activities of PNKP to create ligatable termini (Fig 2). Mutations in *PNKP* were recently identified in several pedigrees of Arabic and European origin that possess an autosomal recessive disorder, which features microcephaly, infantile-onset seizures, and developmental delay denoted MCSZ (Shen et al, 2010). Similar to SCAN1, MCSZ patients had no reported evidence for increased cancer predisposition or immunodeficiency. However, in an intriguing contrast to SCAN1, MCSZ patients lack ataxia 'lack of muscle coordination' and cerebellar degeneration. Western blotting and RT-PCR analyses of cells from the affected individuals suggest a correlation between the severity of clinical symptoms and expression/activity of PNKP (Shen et al, 2010). Consistent with a role for PNKP in the repair of Top1-mediated breaks, A549 cells depleted for PNKP (El-Khamisy et al, 2005) or LCLs from MCSZ patients (Shen et al, 2010) accumulate higher levels of CPT-induced DNA breaks, compared to control cells. More importantly, LCLs from MCSZ patients repair hydrogen peroxide-induced damage at a much lower rate than normal cells (Shen et al, 2010). It is worth noting that through its fork head associated (FHA) domain, PNKP

Figure 2. Repair of topoisomerase-mediated DNA damage and neurodegeneration: same pathway with different clinical features. Stalled DNA topoisomerase 1 (Top1) is subjected to proteasomal degradation to form a smaller peptide that is then acted upon by TDP1. TDP1 cleaves the phosphodiester bond between Top1 peptide and DNA, creating 3'-phosphate and 5'-hydroxyl termini. In preparation for ligation, PNKP restores conventional 3'-hydroxyl and 5'-phosphate termini, followed by sealing the nick by a DNA ligase. Mutations in *CUL4B* (involved in proteasomal degradation) underlie defects in the degradation step of DNA topoisomerase 1 repair, causing mental retardation and motor neuron impairment in XLMR. Defects in the downstream step catalysed by TDP1 causes cerebellar degeneration and peripheral neuropathy typified by patients with SCAN1. Mutations in *PNKP* underlie microcephaly and seizures observed in MCSZ.

interacts with XRCC1 or XRCC4, components of the single- and double-strand break repair machinery (Chappell et al, 2002; Jilani et al, 1999; Karimi-Busheri et al, 1999; Whitehouse et al, 2001).

Although the ultimate outcome of TDP1 deficiency is similar to PNKP deficiency, resulting in neuronal cell loss, the cause of this cell loss is likely to be fundamentally different, causing cerebellar degeneration in SCAN1 and microcephaly in MCSZ (Shen et al, 2010). Microcephaly results from proliferation defects or cell loss during neurogenesis. This could result from DNA damage interfering with replication or during the differentiation of neuron progenitors. Mouse models for *LIG4*, *ATR* and *BRCA2* point at profound apoptosis during neuronal development, resulting in microcephaly (Barnes et al, 1998; Frappart et al, 2007; Murga et al, 2009). Whether PNKP deficiency in mice will also result in microcephaly is yet to be tested.

Cullin 4B

Cullin 4B is an E3-ubiquitin ligase that is encoded by the *CUL4B* gene and controls a variety of DNA repair processes. Mutations in *CUL4B* have been recently associated with mental and growth retardation, **relative microcephaly** and motor neuron impairment; a syndrome called X-linked mental retardation (XLMR) (Cabezas et al, 2000; Tarpey et al, 2007; Zou et al, 2007, 2009). In contrast to SCAN1, *Cul4B*-mutated patients do not present with cerebellar atrophy, however they develop gait ataxia with increasing age (Cabezas et al, 2000; Tarpey et al, 2007; Zou et al, 2007, 2009). LCLs from *CUL4B*-patients and siRNA knockdown of *CUL4B* in A549 cells exhibit CPT sensitivity (Kerzendorfer et al, 2010), pointing to a role for Cullin 4B in the repair of Top1-mediated DNA damage. Consistent with these observations, Top1 degradation following CPT damage was significantly impaired in *CUL4B*-mutated LCLs compared to wild-type cells. Since cellular and cell-free assays using recombinant proteins have shown that proteasomal degradation of Top1 to a small peptide is a prerequisite for TDP1 action (El-Khamisy et al, 2007; Interthal et al, 2001), the increased sensitivity of *CUL4B*-mutated LCLs to CPT points to a role for Cullin 4B upstream of TDP1 (Fig 2). This notion was reinforced by the work of Kerzendorfer et al, who showed increased levels of CPT-induced DNA breaks in *CUL4B*-mutated LCLs compared to wild-type cells (Kerzendorfer et al, 2010). The authors employed a modified version of the alkaline comet assay to measure Top1-cleavage complexes and thus events upstream of TDP1. It is likely that the impaired degradation of Top1 results in accumulation of protein-linked breaks that will interfere with transcription, causing the specific neuronal involvement in *CUL4B*-mutated patients. Whether *CUL4B* deficiency in mice will also result in growth and mental retardation is yet to be tested.

REPAIR OF ABORTIVE LIGATION PRODUCTS AND NEURODEGENERATION

Aprataxin

Aprataxin (APTX) is mutated in AOA1 which is an autosomal recessive spinocerebellar ataxia that shares the neurological

features of AT but lacks the non-neurological features such as immunodeficiency and cancer predisposition. AOA1 accounts for approximately 10% of all autosomal recessive cerebellar ataxias (Le Ber et al, 2003). AOA1 patients present with cerebellar atrophy, ataxia and oculomotor apraxia (limited eye movement after instruction), hypoalbuminaemia, hypercholesterolaemia and involuntary muscle movements (Date et al, 2001; Moreira et al, 2001).

Aprataxin is a member of the histidine triad (HIT) domain superfamily of nucleotide hydrolases/transferases. It possesses a divergent FHA domain at the N-terminus, similar to PNKP. The FHA domain of APTX mediates its interaction with casein kinase 2 (CK2) phosphorylated XRCC1 or XRCC4 (Clements et al, 2004; Iles et al, 2007; Koch et al, 2004). In addition, APTX has been shown to associate with the nucleolar proteins nucleolin, nucleophosmin and upstream binding factor (UBF1, Becherel et al, 2006; Gueven et al, 2004). This may reflect a requirement for APTX at sites of high transcriptional demand. It would be interesting to examine the effect of APTX deletion on gene expression under conditions of high transcriptional activity or elevated oxidative stress.

Consistent with a requirement for APTX activity for neurological function, most of the mutations identified to date in AOA1 are located within or in very close proximity to the HIT domain. The impact of these mutations on the function of APTX seems to correlate well with clinical features (Seidle et al, 2005; Tranchant et al, 2003). Consistent with possessing a HIT domain, APTX can release AMP from AMP-lysine, albeit with low catalytic activity (Kijas et al, 2006). APTX has also been reported to process 3'-phosphate and 3'-phosphoglycolate termini, but again with low catalytic activity (Takahashi et al, 2007). APTX activity is much higher for AMP linked to 5'-DNA termini (Ahel et al, 2006; Rass et al, 2007a), raising the possibility that DNA lesions with 5'-AMP termini are the physiological substrate for APTX in cells. These structures are normal intermediates of DNA ligation and the prediction is that an incomplete ligation cycle may lead to the formation of DNA lesions with 5'-AMP, for which APTX is needed to remove the AMP and re-set the ligase (Rass et al, 2007b). Incomplete 'premature' ligation is likely to happen if the ligase binds to DNA and transfers the AMP to the 5'-terminus before 3'-end processing has occurred. This notion has been supported by observations in mouse astrocytes (El-Khamisy et al, 2009) and in yeast (Daley et al, 2010), where co-deletion of APTX together with enzymes that process 3'-damaged termini had synergistic defect in the ability of cells to repair oxidative DNA damage.

Why does APTX deficiency lead to neuronal cell death? Studies on cultured mammalian cells revealed little impact of the loss of APTX alone on cellular DNA repair capacity, with variations in the extent of this impact depending on the experimental read out adopted and the type of cells employed (Becherel et al, 2006; Clements et al, 2004; Gueven et al, 2004, 2007; Harris et al, 2009; Mosesso et al, 2005; Reynolds et al, 2009). This likely reflects the low frequency of formation of APTX-dependent lesions and suggests that neuronal cell death recurs due to stochastic accumulation of these lesions over years. Un-repaired 5'-AMP breaks could interfere with cellular transcription and may affect global gene expression. Consistent

with this, a recent study by the Lavin laboratory (Radiation Biology and Oncology Laboratory, Queensland, Australia) showed reduced expression of PARP-1, apurinic endonuclease 1 (APE1) and OGG1 in AOA1 cells (Harris et al, 2009). Based on the assumption that a DNA ligase dissociates 'falls off' after transferring the AMP to the 5'-DNA terminus, *in vitro* studies with 5'-AMP synthetic substrates revealed no inhibitory effect of the 5'-AMP on the repair of the 3'-terminus by PNKP or on the addition of one nucleotide by DNA polymerase β (POL β) (Reynolds et al, 2009). It is worth noting that these experiments uncouple two steps of the ligation cycle that may not be uncoupled *in vivo* and thus whether this will also be true in the context of a full ligation cycle in cells is yet to be tested. It has also been proposed that APTX loss is associated with defects in DNA ligation, which could be overcome by addition of non-adenylated DNA ligase (Reynolds et al, 2009), raising the intriguing possibility that cellular levels of ATP may determine the frequency of 5'-AMP lesions. If this is true then identification of small molecules that promote de-adenylation of DNA ligase could potentially reverse the abortive DNA ligase step that creates 5'-AMP breaks and thus circumvent the requirement for APTX. Since the most likely endogenous cause of 5'-AMP breaks in cells lacking APTX is ROS, it would be interesting to study the role of APTX under conditions of elevated oxidative stress. If it is true that oxidative stress is an important aetiological factor for the neuropathology, will antioxidants be effective at preventing cell loss? The use of animal models will be critical in resolving at least some of these issues and will further our understanding of the associated neuropathology.

Cycling cells possess mechanisms that could deal with APTX-dependent breaks that are absent in non-cycling cells. For example, the use of a homologous template for the repair of DNA damage during HR or the use of replicative DNA polymerases to extend the 3'-terminus, creating a 5'-AMP flap that could be clipped off by FEN1, circumventing the requirement for APTX. Evidence for the latter possibility was supported by studies in mammalian cells and in yeast (Daley et al, 2010; Reynolds et al, 2009). However, the reason that APTX deficiency specifically affects the cerebellum is far more challenging and is yet to be explained.

Mitochondrial dysfunction and neurological disorders

Mitochondria are tiny, lozenge-shaped, semi-autonomously reproductive organelles within eukaryotic cells that carry their own genetic material, the mitochondrial genome (mtDNA). Mitochondria contain between 2 and 10 copies of mtDNA and the most conservative estimate suggests that each eukaryotic cell contains more than 100 mitochondria (Elson & Lightowlers, 2006; Holt et al, 2007). This can vary considerably according to the energy demands of a given tissue. For example, neuronal cells have many thousands of mitochondria. The expression of two tissue-specific mitochondrial proteins Err α and Gabpa/b appear to control the number of mitochondria in a given tissue (Thorburn, 2004). mtDNA is very susceptible to DNA damage due to its unique nature and location within the cell. The mitochondria produce most of the cell's ROS and mtDNA is located on the inner membrane close to the sites of ROS

production, rendering mtDNA particularly vulnerable to DNA damage. In addition, mtDNA lacks protective histones and is almost exclusively transcribed (contains very few non-coding areas). This is in marked contrast to nuclear DNA, where coding genes are interrupted by introns and less than 2% is expressed. It is thus critical to maintain the genomic stability of mtDNA, as it controls many functions important for cell viability, such as energy production, production of ROS and regulation of apoptosis. For example, it is estimated that mitochondria generate ~65 kg of ATP every day (Lane, 2006). Association of human disease and mitochondrial dysfunction is well established (Bishop et al, 2010; Calvo & Mootha, 2010; Westly, 2010). The association between defects in mtDNA and human disease was first reported in 1988 (Holt et al, 1988). Not surprisingly, defects in the repair of breaks in mtDNA are expected to elicit greater impact on tissues with high energy demands, such as the brain (Lightowlers, 1992). Indeed, mitochondrial dysfunction has been associated with a variety of human neurological disorders, such as Alzheimers disease (Bonilla et al, 1999; Cortopassi & Arnhem, 1990; Gredilla et al, 2010), Parkinsons (Barroso et al, 1993; Orth & Schapira, 2002; Sherer et al, 2002), Huntington (Panov et al, 2002), Friedreich's ataxia (Tan et al, 2001), and Amyotrophic lateral sclerosis (Wong et al, 1995). Detailed coverage of the relevance of mitochondrial organization and repair to human disease was recently reviewed (Boesch et al, 2010).

Currently, the understanding of nuclear-mitochondrial interactions is limited and roles for mtDNA repair proteins remain contentious. For example, mtDNA has no capacity for independent replication and relies on nuclear-encoded DNA polymerase gamma. In addition, several components of the DNA repair machinery possess mitochondrial versions that are also encoded by nuclear genes (Maynard et al, 2010; Roberts et al, 2008; Weissman et al, 2007). For example, Lig3 α , APE1, APE2 and Cockayne syndrome B (CSB) have been shown to localize to the mitochondria (Aamann et al, 2010; Chattopadhyay et al, 2006). Based on estimates of the number of mitochondrial genes residing in the nuclear genome, around a tenth of the population may be carrying genetic disorders that could affect mitochondrial function (Thorburn, 2004). In this context, mitochondrial DNA repair defects could contribute to the neurological decline in some of the diseases discussed above. For example, an APTX version with a putative mitochondrial targeting sequence has been reported (Caldecott, 2008). Whether this specific isoform targets APTX to mitochondria and, more importantly, whether it exists in cells and thus could contribute, at least in part, to the neurological decline in AOA1 is not clear. Whether the role of APTX in the mitochondria, if any, is due to its DNA related functions or due to novel RNA processing activities is not tested. Defects of mtRNA maturation have been recently associated with an autosomal recessive spastic ataxia with optic atrophy, highlighting the importance of RNA integrity for mitochondrial function (Crosby et al, 2010).

TDP1 activity was recently identified in the kinetoplastid parasite *Leishmania donovani* (Banerjee et al, 2010), in mitochondria of MCF-7 human carcinoma cells (Das et al,

2010), and in the mitochondria of murine neurons (our unpublished observations). Since mtDNA is subject to oxidative DNA damage, some of which may be substrates for TDP1 activity, and because the mitochondria possess a specific mtTop1 isoform that can form Top1-DNA covalent adducts in mtDNA (Zhang & Pommier, 2008), it is sensible for the mitochondria to possess TDP1 activity to deal with endogenous levels of DNA breaks. These observations suggest that nuclear-encoded TDP1 is translocated to the mitochondria. However, we and others have failed to detect a mitochondrial targeting sequence in TDP1, suggesting the presence of novel importers or a specific mitochondrial isoform. It is possible that a TDP1 activity was encoded by mtDNA and has moved from the mitochondria to the nucleus during evolution. A more efficient way for repairing DNA damage in mitochondria is likely to be through moving a pool of nuclear repair proteins rather than inducing the expression of mtDNA genes encoding for the repair proteins. Tracking down genes that have shifted to the nucleus may elucidate important connections to human disease. The physiological importance of TDP1 in the mitochondria was recently suggested by a polymerase chain reaction-based assay to measure mtDNA repair in *Tdp1*^{-/-} mouse embryonic fibroblasts (Das et al, 2010). The results suggest a slower rate of repair of hydrogen peroxide-induced DNA damage in mtDNA in *Tdp1*^{-/-} cells compared to wild-type control cells. Whether mtDNA of SCAN1 or *Tdp1*^{-/-} neuronal cells accumulate damage, mutations or deletions with age is not clear.

DNA repair and physiological aging

Why is a rodent old at ~3 years and a human old at ~80 years? This difference reflects the rapidity of the aging process, which is a syndrome of changes that are irreversible, progressive, and multi-faceted. Damage-associated aging can occur in all biomolecules including DNA, proteins and lipids, affecting different cells and organs. Aging changes are often associated with increased mortality rates. It is thus important to identify aging changes, which are not associated with a specific aging-related disorder, and as such could be used as biomarkers of normal 'physiological' aging. These biomarkers would be better predictors of the increased likelihood of mortality. There are several proposed aging biomarkers but, as yet, none has been validated and widely accepted.

Aging is regulated by the extent of stochastic damage that accumulates over time and the rate at which this damage accumulates (Kirkwood & Holliday, 1979). The latter is dictated by the efficiency of genetic pathways that control longevity (Holzenberger et al, 2003; Kurosu et al, 2005). Mitochondrial function is a key player in these mechanisms. This was first suggested in *Drosophila* (Miquel et al, 1983) and subsequently in mammals where age-dependent accumulation of mutations within mtDNA was reported (Lee et al, 1999; Michikawa et al, 1999). DNA deletions have also been shown to cause premature aging in mitochondrial mutator mice (Vermulst et al, 2008). The link between DNA damage/repair and aging is also rapidly emerging (Fig 3) and was first highlighted by the premature aging-like syndromes that are associated with defects in DNA repair, such as defects in **nucleotide excision repair** (NER,

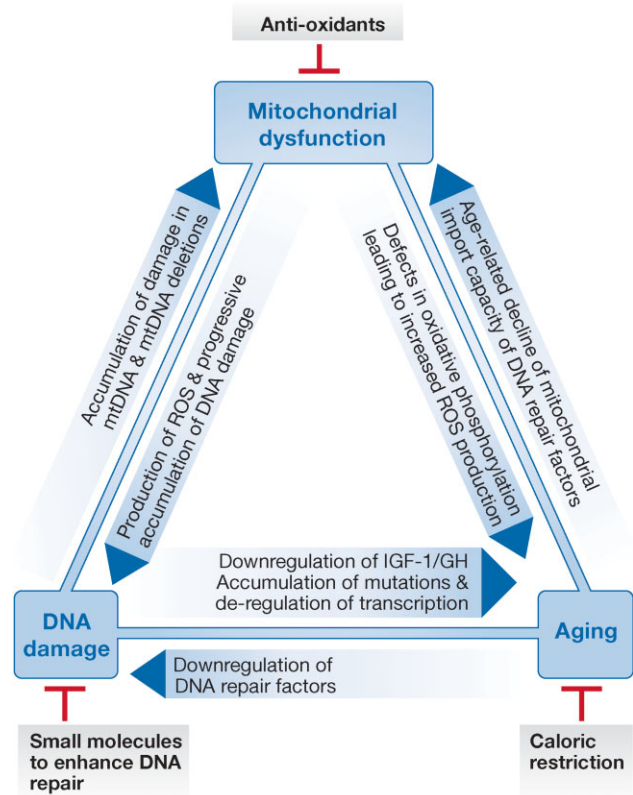


Figure 3. The mitochondrial dysfunction, DNA damage, and aging triangle.

Accumulation of mutations with age has been extensively studied in mice and humans as one cause of 'physiological' aging. DNA damage can also cause downregulation of genes that are normally associated with the extension of life span, such as IGF-1 and GH, contributing to the aging process. On the other hand, aging leads to reduced expression of DNA repair factors, leading to increased accumulation of DNA damage. Aging also may cause reduced trafficking of DNA repair factors to the mitochondria, contributing to mitochondrial dysfunction. The latter results in defects in the oxidative phosphorylation leading to increased production of ROS and consequent accumulation of DNA damage in mtDNA. This vicious cycle can potentially be broken by counteracting any of its components. For example, enhancing DNA repair by the use of small molecule based approaches or gene therapy may reduce the extent of DNA damage. Anti-oxidants and caloric restriction may reduce the extent of mitochondrial dysfunction and delay the symptoms of aging.

Hasty et al, 2003). The inability to repair bulky DNA lesions from the transcribed strand of DNA through transcription-coupled repair is associated with the progeroid symptoms observed in Cockayne syndrome (CS) and trichothiodystrophy (TTD, Lehmann, 2003; Niedernhofer et al, 2006). Prolonged treatment of mice with DNA damaging agents leads to downregulation of genes that are normally associated with the extension of life span, such as insulin-like growth factor 1 (IGF-1) and growth hormone (GH, Niedernhofer et al, 2006). It is possible that attenuation of the **somatotropic axis** is an indirect consequence of tissue damage induced by the accumulation of DNA damage. There is evidence that accumulation of DNA damage can lead to age-dependent changes in DNA repair

(Moriwaki et al, 1996; Gorbunova et al, 2007). Alternatively, it could represent a direct response to specific types of DNA lesions. Evidence for the latter is supported by the observation that accumulation of transcription-blocking lesions directly causes attenuation of the somatotrophic axis in aging animals (Garinis et al, 2009). Since a defect in the repair of Top1-associated breaks leads to stalled transcription, it is intriguing to speculate that efficiency of this repair process correlates with the aging process. This could include the proteasomal degradation of Top1, the removal of the remaining Top1 peptide by TDP1, or the restoration of conventional 3'-hydroxyl and 5'-phosphate termini by PNKP. Mouse models lacking these components will be an invaluable resource to test this hypothesis.

CONCLUSIONS

The unique structure of the nervous system requires the interplay of distinct but vigilant DNA repair processes to deal with different forms of DNA damage. Defects in these processes have been broadly linked to aging, neurodegeneration and mitochondrial dysfunction. More recently, accumulating evidence suggests that specific defects in processing damaged DNA termini underlie neurological decline. In addition to the examples discussed above, oxidative stress and the relative availability of 5'-end processing activities have also been implicated in the expansion of triplet repeats in Huntington disease (Goula et al, 2009; Kovtun et al, 2007). Further studies are clearly needed to address many unresolved questions. For example, what is the threshold of DNA breaks that trigger cell death in neural cells and how is this compared to other cells?

Pending issues

The ultimate aim for understanding causes of neurodegeneration is to exploit ways to prevent or delay 'pathological' and 'physiological' related neurological demise. To achieve these goals there are still many challenging questions. For example:

What is the nature of age-related changes in DNA repair?

Is mitochondrial dysfunction a cause or a consequence of neural cell dysfunction and aging?

What is the extent of tissue specificity of nuclear-encoded mitochondrial proteins? Does aging reduce DNA repair capacity of eukaryotic cells?

Can we detect accumulation of the proposed lesions with damaged DNA termini *in vivo*?

If so, can we exploit novel molecule inhibitors to prevent their accumulation and delay neurological decline?

If oxidative stress is a major cause of endogenous DNA damage in the nervous system, what would be the outcome of modulating endogenous anti-oxidant mechanisms in DNA repair deficient systems?

Addressing these challenges will enhance strategies to alleviate or delay neurological decline. For example, small molecule-based approaches, pharmacotherapies, RNA interference, viral vector-mediated gene therapy, stem cell therapies and immunotherapy. There is a great hope for future progress in these areas.

How do unrepaired breaks lead to neuronal dysfunction? What is the relative contribution of mitochondrial and nuclear DNA repair in the extent of neurological dysfunction? It is worth noting that brain specific deletion of the 'nuclear' scaffold protein XRCC1 leads to cerebellar defects in mice (Lee et al, 2009). Do mitochondria require similar scaffolding factors? What is the relative proportion of physiological levels of nuclear and mitochondrial Top1-linked breaks? Do mitochondria possess their own version of TDP1, TDP2 and PNK? Why isn't human DNA 'programmed' to re-grow neural cells as it ages? Mouse model systems will be invaluable resources to shed light on some of these crucial issues.

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