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# Synergistic decrease of DNA single-strand break repair rates in mouse neural cells lacking both Tdp1 and aprataxin

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ABSTRACT

Ataxia oculomotor apraxia-1 (AOA1) is an autosomal recessive neurodegenerative disease that results from mutations of aprataxin (*APTX*). APTX associates with the DNA single- and double-strand break repair machinery and is able to remove AMP from 5′-termini at DNA strand breaks *in vitro*. However, attempts to establish a DNA strand break repair defect in APTX-defective cells have proved conflicting and unclear. We reasoned that this may reflect that DNA strand breaks with 5′-AMP represent only a minor subset of breaks induced in cells, and/or the availability of alternative mechanisms for removing AMP from 5′-termini. Here, we have attempted to increase the dependency of chromosomal single- and double-strand break repair on aprataxin activity by slowing the rate of repair of 3′-termini in aprataxin-defective neural cells, thereby increasing the likelihood that the 5′-termini at such breaks become adenylated and/or block alternative repair mechanisms. To do this, we generated a mouse model in which APTX is deleted together with tyrosyl DNA phosphodiesterase (TDP1), an enzyme that repairs 3′-termini at a subset of single-strand breaks (SSBs), including those with 3′-topoisomerase-1 (Top1) peptide. Notably, the global rate of repair of oxidative and alkylation-induced SSBs was significantly slower in $Tdp1^{-/-}/Aptx^{-/-}$ double knockout quiescent mouse astrocytes compared with $Tdp1^{-/-}$ or $Aptx^{-/-}$ single knockouts. In contrast, camptothecin-induced Top1-SSBs accumulated to similar levels in $Tdp1^{-/-}$ and $Tdp1^{-/-}/Aptx^{-/-}$ double knockout astrocytes. Finally, we failed to identify a measurable defect in double-strand break repair in $Tdp1^{-/-}$, $Aptx^{-/-}$ or $Tdp1^{-/-}/Aptx^{-/-}$ astrocytes. These data provide direct evidence for a requirement for aprataxin during chromosomal single-strand break repair in primary neural cells lacking Tdp1.


## 1. Introduction

Defects in the appropriate response to DNA damage have been associated with several neurological disorders [1]. The most common form of DNA damage is that produced in one strand of the double helix, resulting in DNA single-strand breaks (SSBs). One of the most frequent sources of SSBs is oxidative attack of endogenous reactive oxygen species (ROS) on DNA [2]. In addition, SSBs can also arise during the enzymatic activities of DNA topoisomerase-1 (Top1) or DNA ligase. The abortive activity of the latter is associated with one of the most common ataxias, ataxia oculomotor apraxia-1 (AOA1). This recessive disease resembles the archetypal DNA damage-associated syndrome, ataxia telangiectasia (A-T), in neurological phenotype, but lacks the extra-neurological features of A-T such as immunodeficiency and cancer predisposition [3–5]. The gene that is mutated in AOA1, *APTX*, encodes the protein aprataxin (APTX). Based on sequence alignments, it has been shown that aprataxin belongs to the histidine triad superfamily of nucleotide hydrolases/transferases [6]. Consistent with this, aprataxin can remove adenosine monophosphate (AMP) from various AMP-linked substrates *in vitro* [7]. However, the most robust substrate so far reported for aprataxin is adenylated DNA, in which AMP-linked to the 5′-terminus of single- or double-stranded DNA breaks, suggesting that this may be the physiological substrate for this enzyme [8].

Adenylated DNA breaks are normal intermediates of DNA ligation reactions, and are usually very transient, because DNA ligases rapidly catalyse nucleophilic attack of the 5′-AMP by the 3′-hydroxyl terminus, releasing AMP and sealing the nick. However, in cases of DNA damage most 3′-termini lack a hydroxyl group and so require repair by 3′-end processing enzymes before DNA ligation

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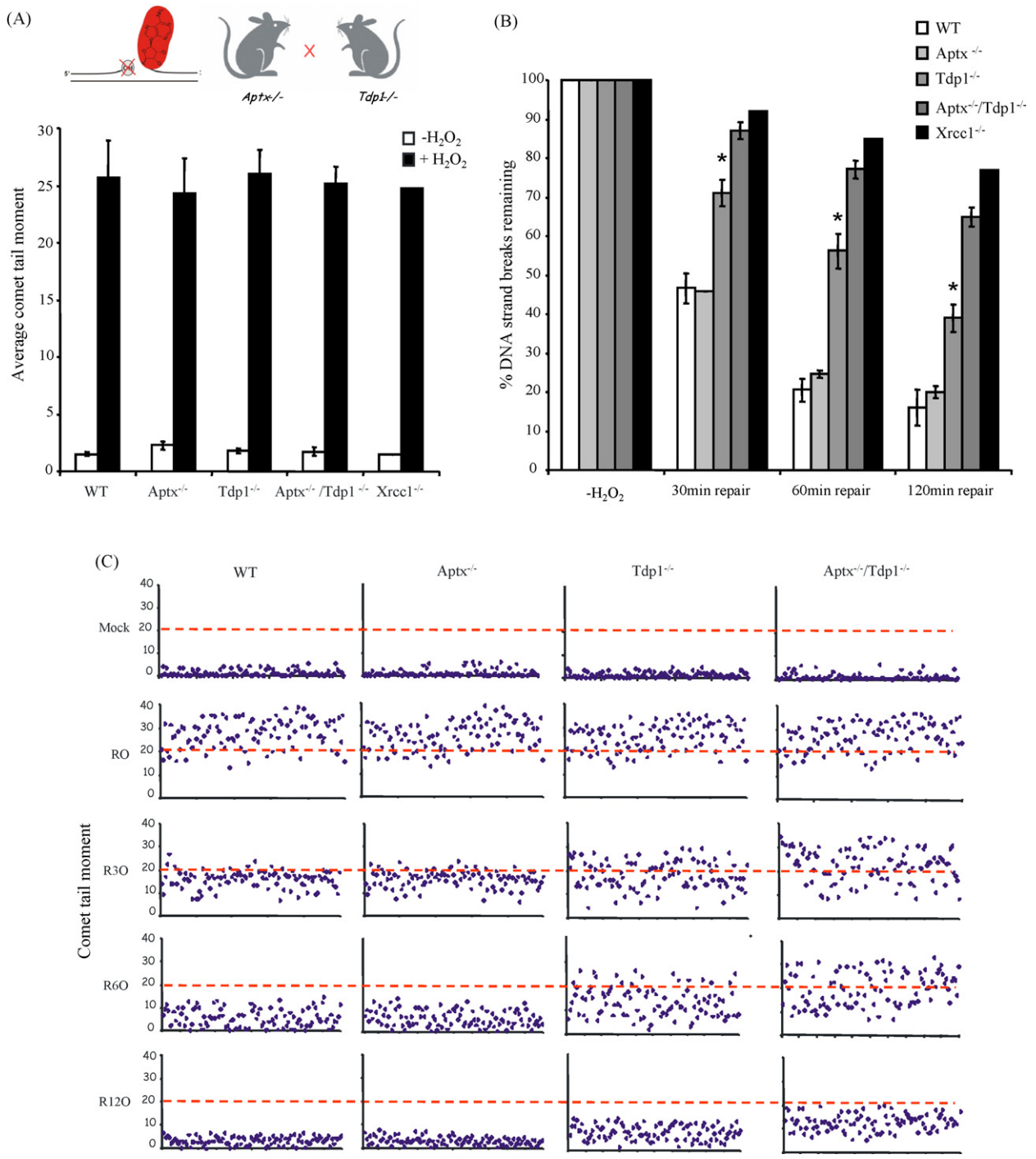


**Fig. 1.** Deletion of *Tdp1* in *Aptx*$^{-/-}$ neural cells uncovers a requirement for aprataxin during repair of "oxidative" DNA strand breaks. (A) Primary quiescent cortical astrocytes from wild-type, *Aptx*$^{-/-}$, *Tdp1*$^{-/-}$, and *Tdp1*$^{-/-}$/*Aptx*$^{-/-}$ littermates were mock treated ($-H_2O_2$) or treated ($+H_2O_2$) with 75 μM $H_2O_2$ and levels of DNA strand breakage were quantified by alkaline comet assays. *Inset*: Cartoon depicting the approach employed here to increase the sub-fraction of 5′-AMP breaks, by slowing the rate of repair of 3′-termini at SSBs via co-deletion of Tdp1 in aprataxin-defective neural cells. (B) Following treatment with $H_2O_2$ as indicated above, cells were incubated for the indicated repair periods in drug-free medium and mean fraction of DNA strand breaks remaining at the indicated time points were quantified by alkaline comet assays. Mean tail moments were quantified for 100 cells/sample/experiment and data are the average for three independent experiments (±S.E.M.). Asterisks denote statistically significant ($P < 0.05$; *t*-test) differences between *Tdp1*$^{-/-}$ and *Tdp1*$^{-/-}$/*Aptx*$^{-/-}$ histograms at the indicated time points (C) Representative scatter plots from one of the experiments included in (B), showing comet tail moments of 100 individual cells per sample at the time points indicated.

can occur. Failure to do so may result in premature adenylation of the 5′-terminus and persistence of 5′-AMP, which is subsequently removed by aprataxin [9]. Aprataxin may thus reverse premature adenylation reactions at DNA breaks in a reaction that involves a covalent AMP-APTX intermediate [10].

SSBs can also arise from the abortive activity of DNA topoisomerase-1, which results in SSBs whereby Top1 is covalently attached to the 3′-terminus. Top1 peptides are removed from 3′-termini by TDP1, mutation of which is responsible for the neurodegenerative disease, spinocerebellar ataxia with axonal neuropathy-1 (SCAN1) [11]. SCAN1 patients and Tdp1, knock-out mice exhibit late onset cerebellar degeneration, pointing at an important role for TDP1 in non-dividing neural cells [12]. Top1 associated DNA breaks can arise by collision of cleavage