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DNA topoisomerases in mtDNA maintenance and ageing☆

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ABSTRACT

DNA topoisomerases pass DNA strands through each other, a function essential for all DNA metabolic processes that create supercoils or entanglements of DNA. Topoisomerases play an ambivalent role in nuclear genome maintenance: Deficiency compromises gene transcription, replication and chromosome segregation, while the inherent DNA-cleavage activity of the enzymes endangers DNA integrity. Indeed, many DNA-damaging agents act through enhancing topoisomerase DNA cleavage. Mitochondrial DNA (mtDNA) clearly requires topoisomerase activity for transcription and replication, because it is a closed, double-stranded DNA molecule. Three topoisomerases have so far been found in mammalian mitochondria (I, IIβ, IIIα), but their precise role in mtDNA metabolism, mitochondrial maintenance and respiratory function remains mostly unclear. It is a reasonable surmise that these enzymes exhibit similar ambiguity with respect to genome maintenance and gene transcription as their nuclear counterparts. Here, we review what is known about the physiological roles of mitochondrial topoisomerases and draft three scenarios of how these enzymes possibly contribute to ageing-related mtDNA attrition and respiratory chain dysfunction. These scenarios are: mtDNA attrition by exogenously stimulated topoisomerase DNA cleavage, unbalancing of mitochondrial and nuclear transcription by direct effects on mitochondrial transcription, and contributions to enhanced mtDNA entanglement and recombination.

1. Physiological functions of mitochondrial topoisomerases

Human mtDNA is a closed, double-stranded DNA circle (Iborra et al., 2004; Lawrence et al., 1996), although more complex topological structures are found in certain post-mitotic tissues of aged humans (Pohjoismaki and Goffart, 2011). A recent survey of the topology of mammalian mtDNA has revealed more than 25 distinct topological forms of mtDNA also including DNA/RNA hybrids and variety of single stranded DNA molecules (Kolesar et al., 2013). Strand separation during transcription and replication of the closed double stranded mtDNA form creates topological stress that interferes with these processes unless that stress is released in a timely fashion. The only enzymes capable of performing this task are topoisomerases (Wang, 2002). Three topoisomerases have been found in mammalian mitochondria, two enzymes catalysing the transient cleavage and ligation of single stranded DNA (mitochondrial topoisomerases I and IIIα) and one enzyme catalysing cleavage and ligation of both strands of the DNA double helix (mitochondrial topoisomerase IIβ). All three enzymes are encoded in the nuclear genome. Currently, very little is known about the role of these enzymes in mtDNA metabolism and maintenance. However, it appears that their biological functions differ markedly from those of the corresponding enzymes in the cell nucleus.

Mitochondrial topoisomerase I (TOP1MT) is the only known mitochondrial topoisomerase encoded by a separate gene. Splitting of the TOP1 gene into nuclear and mitochondrial paralogs is highly conserved in vertebrates (Zhang et al., 2001, 2004, 2007) and reflects a functional specialisation: Nuclear TOP1 is incompatible with mtDNA transcription/replication, while TOP1MT is unable to interact with nuclear chromosomes (Dalla Rosa et al., 2009). TOP1MT catalyses the transient cleavage and ligation of one strand of the DNA double helix, thereby providing the major activity for relaxation of mtDNA supercoils. TOP1MT DNA-cleavage activity is clustered in an mtDNA region downstream of the displacement loop (D-loop) (Zhang and Pommier, 2008) that contains an additional DNA strand (7S DNA). 7S DNA is either a prerequisite or a side product of mtDNA replication (Falkenberg et al., 2007). Depletion of 7S DNA upon inhibition of TOP1MT (Zhang and Pommier, 2008) suggests an involvement of the enzyme in D-loop maintenance or replication. However, an essential role of TOP1MT in mtDNA maintenance is unlikely, since *TOP1MT*$^{-/-}$ mice express mtDNA-encoded proteins (Douarre et al., 2012). In the cell nucleus, TOP1 is an essential cofactor promoting rRNA- and mRNA transcription (Christensen et al., 2002a; Kretzschmar et al., 1993; Zhang et al., 1988). In mitochondria, TOP1MT is not essential for mtDNA-transcription. On the contrary, it acts as a negative regulator of mtDNA transcription.

Abbreviations: AMPK, AMP-dependent protein kinase; AMPKα2, alpha-subunit of AMP-dependent protein kinase; MEFs, Murine embryonic fibroblasts; mtDNA, mitochondrial DNA; mTOR, Mammalian target of rapamycin; ROS, Reactive oxygen species; TFAM, Mitochondrial transcription factor A; TOP1, DNA topoisomerase I; TOP1MT, Mitochondrial topoisomerase I; TOP2, DNA topoisomerase II; TOP2A, topoisomerase IIα; TOP2B, DNA topoisomerase IIβ; TOP3A, DNA topoisomerase IIIα.

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This function is not linked to the mito-biogenic nuclear programme, indicating a direct, intra-mitochondrial regulation mechanism (Sobek et al., 2014). The negative impact of TOP1MT on mtDNA transcription involves a direct interaction with mitochondrial RNA polymerase and probably the removal of supercoils from mtDNA, as it is dependent on catalytic activity (Sobek et al., 2014). DNA supercoiling is a known requirement for transcription initiation in bacteria and yeast (Drolet, 2006; Schultz et al., 1992). mtDNA harbours a certain base line level of supercoiling (Kolesar et al., 2013), which is most probably created in the course of compaction by mitochondrial transcription factor A (TFAM) (Kaufman et al., 2007; Pohjoismaki et al., 2006). TFAM-mediated mtDNA compaction and bending is required for the assembly of transcription complexes (Hallberg and Larsson, 2011). TOP1MT possibly counteracts mitochondrial transcription initiation by reducing supercoiling below the level that permits optimal transcription. TOP1MT is up regulated in cellular stress responses (Goto et al., 2006) and cancer cells (Zoppoli et al., 2011) suggesting an involvement in stress-adaptation of respiratory capacity and the Warburg effect. However, this seems not the only function: *TOP1MT*$^{-/-}$ murine embryonic fibroblasts (MEFs) exhibit a complex pattern of mitochondrial dysfunction including hyper-fusion and constitutive retrograde activation of the nuclear mito-biogenic programme(Douarre et al., 2012). The mitochondrial dysfunctions of TOP1MT$^{-/-}$ MEFs cannot be abolished by expressing TOP1MT (Sobek et al., 2014) indicating an essential and constitutive role of the enzyme in mtDNA maintenance during development, which is possibly related to the principal ability of TOP1 to process RNA/DNA hybrids, suppress R-loops (Tuduri et al., 2009) and stabilise circular mini-chromosomes (Trigueros and Roca, 2001).

Mitochondrial topoisomerases IIIα (TOP3A) is created by alternative translation initiation of a common transcript coding for nuclear and mitochondrial representatives of the enzyme (Wang et al., 2002). Currently, it is not known how alternative translation of TOP3A is regulated to control the relative amounts of nuclear and mitochondrial isoforms made from the common transcript. In the nucleus TOP3A acts on single stranded DNA transiently disengaged from the double helix (DNA solenoids) and plays an essential role in nuclear transcription by resolving DNA solenoids formed upon strand separation (Wang, 2002). TOP3A seems to be a sufficient topoisomerase co-factor of mtDNA transcription, inasmuch as it is capable of upholding base line levels of mitochondrial transcripts in the absence of TOP1MT and TOP2B (Sobek et al., 2014). TOP3A deficiency causes a depletion of mtDNA in male germ-line stem cells of *Drosophila* (Wu et al., 2010) pointing to an essential role in mtDNA maintenance, which is possibly linked to its presumed interaction with Twinkle helicase (see: section 2.3). However, currently it is not clear which functional deficit(s) cause this phenotype and whether it is due to deficiency of nuclear or mitochondrial products of the TOP3A gene. Moreover, the relevance of this finding with respect to mammalian mtDNA maintenance is questionable, since the machinery supporting these functions is very different in insects.

Topoisomerase IIβ (TOP2B) is the only known type II topoisomerase present in mitochondria, which is able to catalyse cleavage, passage and ligation of both strands of the DNA double helix. In the cell nucleus, TOP2B plays an essential role in transcriptional activation (Haffner et al., 2010; Ju et al., 2006) or repression (Tiwari et al., 2012) of certain gene loci, while chromosome condensation and segregation is exclusively supported by the other isoform topoisomerase IIα (TOP2A) (Grue et al., 1998; Linka et al., 2007). Mammalian mitochondria contain a proteolytic derivative of TOP2B, which lacks the entire N-terminal domain of the enzyme (Low et al., 2003). The N-terminal domain is the portion most divergent between TOP2A and TOP2B. It is responsible for recruiting TOP2B and TOP2A to isoform-specific tasks (Linka et al., 2007) but is dispensable for the enzymes' basic catalytic activities (Jensen et al., 1996). Therefore, the N-terminally truncated, mitochondrial form of TOP2B is expected to be fully active in terms of cleavage, passage and ligation of DNA double strands, and could in principle be the enzyme separating intertwined mtDNA molecules that are inadvertently formed during replication (Wang, 2002). However this seems not to be the case, since the degree of mtDNA-intertwining as well as the rate of mtDNA-transcription are normal in *TOP2B*$^{-/-}$ MEFs. Consequently, there must exist another enzyme activity in mitochondria that catalyses the separation of intertwined mtDNA molecules (see also section 2.3). We recently observed that TOP2B is significantly up-regulated in *TOP1MT*$^{-/-}$ MEFs and capable of maintaining increased levels of mtDNA transcription in the absence of TOP1MT (Sobek et al., 2014), which possibly indicates a redundant role in the removal of negative DNA supercoils that are generated during mtDNA transcription and normally removed by TOP1MT.

2. Possible contributions of topoisomerases to age-related alterations in mtDNA homeostasis

2.1. mtDNA attrition by chronic exogenous "poisoning" of mitochondrial TOP1 and TOP2

Many compounds induce breaks in the DNA backbone through interference with the cleavage/ligation reaction of TOP1 and/or TOP2. Such compounds attenuate the ligation step of topoisomerases and thereby prolong the half-life of a catalytic intermediate consisting of a DNA break with topoisomerase covalently attached to the free 3′- or 5′-phosphate of the break. Such topoisomerase-linked DNA breaks are not readily recognised as DNA damage and consequently are less efficiently repaired than other DNA breaks (Deweese and Osheroff, 2009a; Pommier et al., 2006; Soubeyrand et al., 2010). Substances inducing DNA breaks by inhibiting the ligation step of TOP1 or TOP2 are termed topoisomerase poisons because they turn the entire topoisomerase complement of a cell into a DNA-damaging agent. Topoisomerase poisons encompass a large and heterogeneous group of chemical compounds. Many widely prescribed anti-cancer drugs act by poisoning mammalian TOP2. These include the podophyllotoxins etoposid (VP16) and teniposide (VM26), the anthracyclins doxo- and daunorubicine, the phenathracene Mitoxantrone and the aminoacridine m-Amsacrine (Liu, 1989; Nitiss, 2009; Pommier et al., 2010). TOP2 is also poisoned by various natural alkaloids produced by fungi or plants. Typical examples are the plant alkaloid Lycobetain (Barthelmes et al., 2001) and the fungal toxin Alternariol, which is a frequent carcinogenic food contaminant (Fehr et al., 2009). TOP2 poisoning is also an established carcinogenic mechanism of 1,4-benzoquinone metabolites derived from industrial waste products containing benzene (Lindsey et al., 2005). Interestingly the widely prescribed analgesic drug Acetaminophen is likewise metabolised to a 1,4-benzoquinone derivative that effectively poisons mammalian TOP2 and possibly triggers liver carcinogenesis in humans (Bender et al., 2004). Moreover, certain bioflavonoids that are constituents of normal human diet exhibit a strong TOP2-poisoning activity. Most notably, the isoflavone Genistein contained in soybeans is a strong poison of TOP2B (Bandele and Osheroff, 2007). Habitual consummation of soy-based dietary supplements or food fortified with isoflavones in purified form is assumed to raise serum levels of Genistein up to 10 μM (Nielsen and Williamson, 2007), a concentration that effectively poisons TOP2B in cultured human cells (Kalfalah et al., 2011). It is assumed that Genistein consumed at high doses during pregnancy plays a role in triggering infant leukaemia (Ross, 2000; Ross and Kasum, 2002). Poisoning of TOP1 is the established mechanism of a group of widely prescribed anti-cancer drugs derived from the plant alkaloid camptothecin (Nitiss, 2009; Pommier et al., 2010). TOP1-poisoning is also a side effect of the Dihydropyridine Dexniguldipine, a drug prescribed as a calcium antagonist (Straub et al., 1997). The isoflavone Quercetin contained in nuts and coffee is an effective TOP1-poison (Boege et al., 1996). Genistein is highly enriched in certain food supplements, but it is not known whether its

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