Mapping Topoisomerase Sites in Mitochondrial DNA with a Poisonous Mitochondrial Topoisomerase I (Top1mt)*

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Background: Top1mt is the mtDNA untwisting enzyme. It is present and conserved in vertebrates.

Results: Using a mutated toxic enzyme, we determined Top1mt sites and mtDNA damage.

Conclusion: Top1mt accumulates in the regulatory region of mtDNA. Stable Top1mt cleavage complexes rapidly deplete mtDNA.

Significance: This is the first map of Top1mt sites across the entire mitochondrial genome and a new setup to elicit mtDNA damage.

Mitochondrial topoisomerase I (Top1mt) is a type IB topoisomerase present in vertebrates and exclusively targeted to mitochondria. Top1mt relaxes mitochondrial DNA (mtDNA) supercoiling by introducing transient cleavage complexes wherein the broken DNA strand swivels around the intact strand. Top1mt cleavage complexes (Top1mtcc) can be stabilized in vitro by camptothecin (CPT). However, CPT does not trap Top1mtcc efficiently in cells and is highly cytotoxic due to nuclear Top1 targeting. To map Top1mtcc on mtDNA in vivo and to overcome the limitations of CPT, we designed two substitutions (T546A and N550H) in Top1mt to stabilize Top1mtcc. We refer to the double-mutant enzyme as Top1mt*. Using retroviral transduction and ChIP-on-chip assays with Top1mt* in Top1mt knock-out murine embryonic fibroblasts, we demonstrate that Top1mt* forms high levels of cleavage complexes preferentially in the noncoding regulatory region of mtDNA, accumulating especially at the heavy strand replication origin O1H, in the ribosomal genes (12S and 16S) and at the light strand replication origin O1L. Expression of Top1mt* also caused rapid mtDNA depletion without affecting mitochondria mass, suggesting the existence of specific mitochondrial pathways for the removal of damaged mtDNA.

Type IB topoisomerases (Top1 and Top1mt) relax DNA superhelical tension by allowing the swiveling of one DNA strand around the other, which rapidly dissipates DNA supercoiling (1–3). To execute these reactions, Top1 and Top1mt introduce DNA single-strand breaks through their catalytic tyrosine residues, which remain transiently covalently bound to the 3’-end of the nicks. These catalytic intermediates are referred to as Top1 cleavage complexes (Top1cc). They are normally transient as the 5’-hydroxyl DNA ends quickly religate the nicks by attacking the 3’-tyrosyl-DNA bonds. Camptothecin (CPT) or its clinical derivatives topotecan and irinotecan, as well as the noncamptothecin indenoisoquinolines, trap Top1cc by binding at the Top1-DNA interface with high selectivity, thereby inhibiting the DNA religation step (4–7). In S phase, collisions between Top1cc and the replication machinery produce DNA double-strand breaks and eventually cell death, which accounts for the fact that nuclear Top1 is the target of anticancer drugs (1, 8).

Studies in yeast have shown that, even in the absence of CPT, substitution of conserved residues in the active site of Top1 alter the catalytic cycle of the enzyme. Substitution of Thr722 with Ala (T722A) produces a CPT-mimetic effect, enhancing the stability of Top1cc by reducing DNA religation (inhibiting the reverse reaction) (9), whereas mutation of Asn726 to His (N726H) increases the Top1-DNA binding rate (forward reaction) (10). Combination of both mutations in a single polypeptide converts Top1 to a potent cytotoxin by enhancing its DNA cleavage and reducing its DNA religation rate at the same time (11).

Sequences of Top1mt and Top1 are highly similar except for their N-terminal domains, which contain mitochondrial and nuclear targeting sequences, respectively (3, 12). Top1mt and Top1 are most likely paralogs that originated from nuclear targeting sequences, respectively (3, 12). Top1mt and Top1 are likely to remove topological stress from mitochondrial DNA (mtDNA). mtDNA is a circular multicopy genome that encodes 13 essential subunits of the mitochondrial respiratory chain, two ribosomal RNAs (12S and 16S) and 22 tRNAs (14, 15) (see Fig. 2B). Genetic information is tightly packed in mtDNA as both strands, denoted as heavy (H) and light (L), are actively transcribed. The only noncoding region (NCR) of substantial size is the mtDNA regulatory region, containing the promoters for mtDNA transcription (HSP1, HSP2, and LSP), the origin of H-strand replication (O1H), and several conserved sequences with regulatory functions (for reviews on mtDNA transcription and replication, see Refs. 15–17 and schemes in Fig. 2). mtDNA
replication is frequently aborted approximately 600 base pairs downstream from OH (18). The nascent DNA chain, called 7S DNA, displaces the parental H-strand, generating a triple-stranded displacement loop (named the D-loop) (see Fig. 2C). The function of the D-loop is not fully understood.

Top1mt is not essential for murine development. Yet, Top1mt KO mouse embryonic fibroblasts (MEFs) show altered oxidative phosphorylation, increased glycolysis and lipid oxidation, defective mitochondrial potential, and increased mitophagy in liver tissues (19). The mtDNA defects of Top1mt KO mice are also exhibited by the hypersensitivity of those mice to the cardiototoxicity of doxorubicin (20) and their altered mtDNA supercoiling (21). Here we set out to map Top1mt binding sites in vivo in the whole mtDNA genome. Previous mapping of Top1mt sites trapped by CPT has been done by ligation-mediated PCR and limited to the mitochondrial NCR (22). It revealed that treatment of isolated mitochondria with high CPT concentrations preferentially trapped Top1mt at a cluster of sites downstream to the end of 7S DNA (22). However, using CPT has limitations because of the limited permeability of mitochondria to noncationic molecules and because CPT is readily inactivated at alkaline pH (8, 23). Furthermore, CPT traps Top1 at selective sequences that are dictated by the drug stacking with the CPT traps Top1 at selective sequences that are dictated by the 

**EXPERIMENTAL PROCEDURES**

**Cell Culture, Cloning, and Expression of Mutated of Top1mt**—Wild type (WT) and Top1mt knock-out (KO) MEFs were grown as described (19). The coding sequence of mouse Top1mt (NM_028404.2) was cloned in the retroviral vector pFB-Neo (Agilent, Santa Clara, CA) through link-mediated PCR, generating pFB-Top1mt-Neo. Residues Thr546 and Asn550 of Top1mt were mutated to Ala and His, respectively, using overlap-extension PCR (25), generating pFB-Top1mt*-Neo. MMLV-based viruses were generated co-transfecting pFB-Top1mt-Neo or pFB-Top1mt*-Neo with the two packaging vectors pVPack-GP and pVPack-VSV-G Neo (Agilent) in 293T cells. pFB-Neo was co-transfected with the packaging vectors to produce empty viruses utilized as control for transduction. Supernatants were diluted in equal volume of dilution buffer (50 mM Tris–HCl, pH 8.0, 0.5% Nonidet P-40, 10 mM MgCl₂, 5 mM DTT) with protease inhibitors and incubated overnight at 4 °C on a rotating platform with 5 μg/ml anti-FLAG antibody (F1804; Sigma). The following day, 150 μl/ml A/G-Plus agarose beads (Santa Cruz Biotechnology) were added. After incubation for 2 h at 4 °C on a rotating platform, the beads were washed three times with wash buffer, three times with wash buffer + 200 mM NaCl, three times with LiCl buffer, and twice with Tris-EDTA (TE) (according to the Agilent protocol). The beads were resuspended in 300 μl of TE, 0.5% SDS and transferred in a new tube. At this point the saved 50-μl no-IP lysates were thawed and diluted in 300 μl of TE, 0.5% SDS and transferred in a new tube. For each probe on the array, the no-IP signal was subtracted from the IP signal (log2 values). Positive values indicate mtDNA regions where the IP signal is higher than its corresponding no-IP (Top1mt-enriched regions).

**Recombinant Top1mt Expression**—For the production of recombinant Top1mt, the mature form of human Top1mt (without mitochondrial targeting sequence, amino acids 25–601 (3)) was cloned in the baculovirus Destination Vector pDest-605 and expressed using Bac-to-bac Expression System (Invitrogen) according to the manufacturer’s procedure. For production of Top1mt*, human mature Top1mt was mutated (T554A and N558H) and expressed as described previously (19). The primary antibody used were: anti-Top1mt (19) and Anti-OxPhos Complex kit (457999; Invitrogen).

**ChIP-on-chip**—ChIP-on-chip experiments were performed according to the Agilent mammalian ChIP-on-chip protocol version 10.2. Briefly, five 50% confluent T175-cm² flasks of Top1mt KO MEFs were transduced with Top1mt*-FLAG or empty vector as control (see Fig. 1). Twenty four hours after transduction, cells were treated with formaldehyde (1.1% final concentration) for 10 min at room temperature, and reactions were stopped by adding 125 mM glycine for 5 min. Cells were then washed with PBS, scraped, and lysed in 2 ml of lysis buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, 0.5% Nonidet P-40, 10 mM MgCl₂, 5 mM DTT) with protease inhibitors and incubated on ice for 30 min. Lysates were sonicated seven times for 30 s at 30% power to obtain chromatin fragments of approximately 200 base pairs and centrifuged at 14,000 rpm for 10 min at 4 °C. 50 μl of lysates were saved frozen at this point for “no-IP” control. Supernatants were diluted in equal volume of dilution buffer (50 mM Tris–HCl, pH 8.0, 0.5% Nonidet P-40, 5 mM DTT) with protease inhibitors and incubated overnight at 4 °C on a rotating platform with 5 μg/ml anti-FLAG antibody (F1804; Sigma). The following day, 150 μl/ml A/G-Plus agarose beads (Santa Cruz Biotechnology) were added. After incubation for 2 h at 4 °C on a rotating platform, the beads were washed three times with wash buffer, three times with wash buffer + 200 mM NaCl, three times with LiCl buffer, and twice with Tris-EDTA buffer. 20 mg/ml proteinase K were added to all samples, followed by incubation for 6 h at 65 °C with continuous shaking. After centrifugation (14,000 rpm, 1 min) the chromatin in the supernatants was extracted with an equal volume of phenol–chloroform–isoamyl alcohol, and ethanol-precipitated with 20 mg/ml glycogen. DNA pellets were resuspended in 65 μl of 10 mM Tris, pH 8.0. Both IP and no-IP DNA samples were amplified, labeled, and hybridized according to the Agilent protocol. For each probe on the array, the no-IP signal was subtracted from the IP signal (log2 values). Positive values indicate mtDNA regions where the IP signal is higher than its corresponding no-IP (Top1mt-enriched regions).

**Detection of Top1mt Cleavage Complexes (ICE Bioassay)**—ICE bioassays were performed as described (26). Membranes were incubated overnight at 4 °C with anti-Top1mt antibody (19) and 1 h at room temperature with anti-mouse HRP-linked secondary antibody (GE Healthcare). Signals were detected using ECL Plus Western Blotting Detection Reagents (GE Healthcare). Western blotting was performed as described previously (19). The primary antibody used were: anti-Top1mt (19) and Anti-OxPhos Complex kit (457999; Invitrogen).

**In Vivo Mapping of Top1mt Binding Sites**—In Vivo Mapping of Top1mt Binding Sites was previously (19). The primary antibody used were: anti-Top1mt (19) and Anti-OxPhos Complex kit (457999; Invitrogen).
DNA, 10 mM Tris-HCl, pH 8.5, 50 mM KCl, 0.1 mM EDTA, 5 mM MgCl$_2$, 15 µg/ml BSA, 1 mM DTT, and 10% dimethyl sulfoxide or 10 µM CPT. 70 nM human recombinant Top1mt or Top1mt* was added to reaction mixtures. Following incubation at 25 °C for 1 h, reactions were stopped by adding 0.5% SDS (final concentration). Reaction products were separated in 20% denaturing PAGE and imaged by a PhosphorImager. Oligonucleotide sequences used for Top1mt* biochemical mapping and quantification of mitochondrial DNA were designed to map Top1mt binding sites in the mitochondrial genome. To do so, we designed a tiling array for the mouse mtDNA including 3260 probes, where 55 of 60 nucleotides of each probe overlapped with the next one (Fig. 2A, left). We performed ChIP in Top1mt KO MEFs expressing Top1mt* and hybridized the DNA fragments covalently bound to Top1mt* on the array (Fig. 2A, right and bottom panels).

Table 1: Oligonucleotide sequences used for Top1mt* biochemical mapping

| Peak | Strand | Sequence | 5'→3'
|------|--------|----------|
| 15335 (R) | L | gtgtcTgtaaaccctgaanTgataacctcttttccttcttgaag |
| 15420 (C) | L | tctttgagaagagagatTcatttctctctctcagtaagacc |
| 15690 (D) | L | tccctattttgcttatTaatcttctctactctctctctctcgataag |
| 16060 (E) | L | attactggtgTgtacgtaaatTTgataaactaatcatccatcaccat |

Quantification of Mitochondrial Mass—Mitochondrial mass was measured by nonyl acridine orange staining (Invitrogen). Cells (from one T25-cm$^2$ flask) were treated with 50 nM nonyl acridine orange for 30 min at 37 °C and then trypsinized and washed in phosphate-buffered saline (PBS). Cells were resuspended in 1 ml of prewarmed Hanks’ balanced salt solution buffer and immediately analyzed by flow cytometry with a FACScan flow cytometer (BD Biosciences).

RESULTS

Generation of the CPT-mimetic Top1mt—Our rationale for these experiments is that substitutions of Thr$^{722}$ (T722A) and Asn$^{726}$ (N726H) in yeast Top1 alter its cleavage/religation equilibrium, increasing the stability of Top1cc and enhancing the Top1-DNA binding rate (9–11). Because the active sites of yeast Top1 and Top1mt are well conserved (Fig. 1A), we hypothesized that the corresponding mutations in Top1mt* would generate an enzyme prone to trapping mtDNA. Thus, we mutated Thr$^{446}$ and Asn$^{550}$ in mouse Top1mt (T546A and N550H) and transduced native or mutated Top1mt constructs (Top1mt and Top1mt*, respectively) in Top1mt KO MEFs (Fig. 1B). Top1mt and Top1mt* polypeptides were readily detected at comparable expression levels 1 day after transfection (Fig. 1C). To determine whether Top1mt* formed the expected high levels of topoisomerase-mtDNA complexes, we compared Top1mtcc in cells expressing Top1mt* versus Top1mt (Fig. 1D). We also used high concentrations of topotecan (TPT), the clinical water-soluble camptothecin derivative (8), which enters mitochondria (28) to trap Top1mtcc. Unlike wild type Top1mt, which required the presence of CPT to form cleavage complexes, Top1mt*cc were readily detectable in the absence of drug (Fig. 1D). To demonstrate the specificity of the Top1mtcc detection, we also treated Top1mt KO cells transfected with empty vector with CPT. Lack of Top1mt signal confirmed the selectivity of our Top1mt antibody, as TPT, under these conditions induces high levels of cleavage complexes with nuclear Top1 (29). These results show that Top1mt* bearing the two substitutions T546A and N550H produces elevated levels of Top1mt*cc in the absence of any drugs.

Mapping Top1mt Cleavage Complexes—Having established that Top1mt* behaved as a poisoning topoisomerase, we used it to map Top1mt binding sites in the mitochondrial genome. To do so, we designed a tiling array for the mouse mtDNA including 3260 probes, where 55 of 60 nucleotides of each probe overlapped with the next one (Fig. 2A, left). We performed ChIP in Top1mt KO MEFs expressing Top1mt* and hybridized the DNA fragments covalently bound to Top1mt* on the array (Fig. 2A, right and bottom panels). Fig. 2B summarizes the distribution of Top1mt* sites over the entire mitochondrial genome (shown linearized instead of circular to simplify the presentation). Top1mt* sites were prominently enriched in the NCR, as well as at multiples sites within the ribosomal genes (125 and 165S). To ensure that the signals observed were Top1mt-specific, we performed the same ChIP-on-chip protocol in Top1mt KO MEFs transfected with empty vector (supplemental Fig. 1). Because these cells did not express Top1mt, all sequences immunoprecipitated in these samples can be considered nonspecific. IP signals from Top1mt KO cells gave completely different readouts in the negative control, particularly in the NCR, eliminating the possibility that the peaks from Top1mt* mapping results could be false positive peaks.

An expanded view of the Top1mt* sites in the NCR is shown in Fig. 2C. The NCR (approximately 880 base pairs long) contains the promoters for mitochondrial transcription (black arrowheads) and the O$_{H}$ (15). Transcription of mtDNA starts from three different promoters (HSP1, HSP2, and LSP) and proceeds bidirectionally (Fig. 2C, bottom scheme). In a circular genome such as mtDNA, bidirectional transcription leads to accumulation of negative supercoils (i.e. underwound DNA) behind the transcription machinery and positive supercoils ahead of it (30). The high density of Top1mt* sites in the NCR and ribosomal mtDNA genes, which are highly transcribed, could reflect the need for Top1mt to resolve topological tensions related to mtDNA transcription. Indeed, Top1 is involved in the removal of negative supercoils during transcription elongation in the nucleus, avoiding R-loop formation behind the RNA polymerase (8, 31–35). Accordingly, cells lacking Top1 mtDNA accumulate highly negative supercoiled mtDNA (21), consistent with a role of Top1mt in removing transcription-associated supercoiling in the mitochondrial genome.

The coincidence of Top1mt sites with mtDNA replication origins for both the heavy and light strand (O$_{H}$ (Fig. 2, B and C) and O$_{L}$ (Fig. 2B)) suggests a potential role for Top1mt in mtDNA replication. As shown in Fig. 2C, H-strand DNA synthesis starts at O$_{H}$ and can stop prematurely producing the so-called 7S DNA. 7S DNA has a short half-life and is continu-
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The very strong Top1mt sites at the RNA-DNA switch site (site 16060 in Fig. 2C) and over on a broad area downstream could reflect Top1mt involvement in adjusting mtDNA topology in association with replication initiation. Similarly, a cluster of strong Top1mt sites was found at the OL region (Fig. 2B). In addition, a cluster of strong Top1mtcc sites was detected at the end of the D-loop (centered on sites 15335 and 15420), matching the strong site detected previously by ligation-mediated PCR (22) and the termination-associated sequence element. Hence, it appears that Top1mt* cleavage sites are concentrated in two regions corresponding to the 3′- and 5′-end of 7S DNA (18, 22, 37), indicating that Top1mt acts at the edge of the D-loop region. This finding might be related to the fact that Top1 cleavage is driven not only by DNA sequence but also by secondary structure of the template (38).

To compare the sites generated by Top1mt* with those induced by Top1cc-targeted drugs, we performed the same ChIP-on-chip experiments in cells expressing WT Top1mt and treated with TPT (supplemental Fig. 2). Interestingly, TPT-induced sites were less concentrated in the NCR and spread over the entire mitochondrial genome. The different distributions between TPT induced and Top1mt* sites are likely due to the drug sequence bias in trapping Top1 (24) that redistributes the enzyme from its “natural” positions. Together, our mapping results support the implication of Top1mt in mtDNA transcription and replication.

Strand-specific Sequencing of the Top1mt* Cleavage Sites in the NCR

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FIGURE 1. T546A and N550H mutations trap Top1mt. A, alignment of the C-terminal region of yeast Top1 with corresponding regions of mouse and human Top1mt polypeptides. Thr722 and Asn726 of yeast Top1 are conserved in Top1mt. B, experimental approach. Thr546 and Asn550 of mouse Top1mt were mutated to Ala and His, respectively. Native (Top1mt) and mutated (Top1mt*) enzymes were expressed in Top1mt KO MEFs using a lentiviral expression system. Top1mt KO MEFs transduced with empty virus (EV) were used as control. C, Western blot showing Top1mt expression in control cells (EV) and cells expressing Top1mt and Top1mt* 24 h after transduction. D, formation of Top1mtcc in MEFs transduced with empty virus, Top1mt, or Top1mt* in the presence or absence of TPT. Total cellular DNA was extracted and 650 ng was blotted to PVDF membrane using a slot-blot vacuum system. Top1mt-mtDNA complexes were visualized by Western blotting using Top1mt-specific antibody.
(±20 bases from the peak) (Table 1 and Fig. 3, oligonucleotides B–E). End labeling of the H- or L-strand allowed the detection of Top1mt cleavage sites on both DNA strands (Fig. 3). Consistent with the in vivo data (Figs. 1C and 2B and supplemental Fig. S2), Top1mt* cleaved DNA much more efficiently than Top1mt in the absence of CPT (Fig. 1C). Moreover, Top1mt sites induced by CPT were often at different positions from those produced by Top1mt* (compare third and fourth lanes). The sequence-selective trapping of Top1cc by CPT likely accounts for these differences (24).

For the two strong sites at the edges of the 7S DNA region (positions 15420 and 16060) (see Fig. 2C), Top1mt cleaved...
mainly the L-strand sequence (Fig. 3, C and E). On the contrary, around positions 15335 and 15690 Top1mt primarily cleaved the H-strand (Fig. 3, B and D). The consensus sequence for nuclear Top1 cleavage has been defined as −4 (AT), −3 (GAC), −2 (AT), −1 (T), and +1 (GA) (24, 39). The analysis of Top1mt cleavage sites mapped in Fig. 3 revealed high similarity between Top1mt and Top1 biochemical sites (see Table 1), indicating that, in addition to DNA structure, the DNA sequence itself play an important role in determining the trapping of Top1mt*. Indeed, the sites in oligonucleotides were also present in cells (compare Figs. 2 and 3).

**Top1mt* Expression Leads to mtDNA Elimination**—Having established that Top1mt* efficiently produced Top1mt*cc independently of drug both in cells and in biochemical assays, we investigated the fate of mtDNA after expression of the poisonous Top1mt*. To do so, we transduced Top1mt KO MEFs with double mutant Top1mt* and followed mtDNA copy number for 4 consecutive days (Fig. 4A). Top1mt* expression led to a decrease of mtDNA, reaching approximately 50% of control after 3 days. Unlike Top1mt*, Top1mt expression did not affect mtDNA copy number. Because the mitochondrial genome encodes for 13 essential subunits of the mitochondrial respiratory chain, to test the consequences of Top1mt*-induced mtDNA loss, we measured the steady-state levels of OXPHOS subunits after Top1mt* transduction (Fig. 4B). Three days after Top1mt* transduction, when mtDNA was depleted by 50%, we observed decreases in NDUFA9 and COX4 (complex I and IV subunits, respectively) (Fig. 4B). SDHA (subunits of complex II) was not affected by mtDNA depletion because complex II is entirely nuclear coded (17).
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Previous reports showed that accumulation of nuclear Top1cc after pharmacological Top1 trapping leads to DNA lesions, which have to be repaired or will trigger apoptosis. Because mtDNA is partially depleted after Top1mt* expression, our results suggest that trapped Top1mt*cc are not effectively repaired in mitochondria and damaged mtDNA is eliminated. To test whether damaged mtDNA was degraded by mitochondrial autophagy (mitophagy) (40), we examined mitochondrial mass after Top1mt* transduction (Fig. 4C). Mitochondrial mass was not altered, indicating that Top1mt*-induced cleavage complexes activate an alternative mechanism of quality control for the degradation of highly damaged mtDNA.

DISCUSSION

Here we engineered a mutated Top1mt (Top1mt*) prone to trapping on mtDNA to determine Top1mt catalytic sites in the mitochondrial genome of mammalian cells. Using MEFs from Top1mt KO mice (19), we demonstrate that Top1mt acts preferentially in the regulatory NCR of the mitochondrial genome, with highest occupancy at the promoters and ribosomal RNA coding region. Top1mt sites also appeared concentrated at the beginning and the end of the D-loop region and at the OL. Biochemical mapping of Top1mt*cc within the four major cleavage sites identified in vivo showed a consensus sequence for Top1mt cleavage very similar to the one reported previously for Top1 (39). These data indicate that the DNA sequence plays a role in determining Top1mt* trapping. However, the strong accumulation of Top1mt* in the NCR indicates that other factors contribute to its trapping. The strong topological stress generated in the NCR is likely to be the driving force for Top1mt* accumulation. These results suggest a role for Top1mt in relieving mtDNA supercoiling generated by bidirectional transcription and replication and indicate that both DNA sequence and topology are important in determining Top1mt trapping.

Loss of mtDNA impairs the production of essential subunits of the mitochondrial respiratory chain, compromising the oxidative phosphorylation (OXPHOS) (17). The threshold of mtDNA depletion necessary to cause pathogenic mitochondrial dysfunctions varies from 50 to 80%, depending on cellular type (41, 42). Here we show that high levels of Top1mt*cc deplete mtDNA by 50% and this partial depletion is sufficient to cause a marked decrease in the steady-state levels of OXPHOS subunits. This limited mtDNA depletion could reflect the fact that only a fraction of mtDNA is targeted by Top1mt*. This would be consistent with our recent study showing that Top1mt associates only with the transcriptionally active fraction of mitochondrial nucleoids (43).

Top1cc, which are normally very transient (44, 45), are readily trapped on oxidized DNA bases and at DNA nicks, generating stable Top1-DNA intermediates (46). Considering the high oxidative stress present in mitochondria, the formation of persistent and potentially irreversible Top1mtcc could be a frequent natural event. Even though multiple mtDNA repair pathways are present in mitochondria (47), repair mechanisms are likely not able to counteract high levels of Top1mt*cc, and damaged mtDNA is degraded. These findings provide novel insights and new molecular tools for the field of mtDNA repair.

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