Drosophila topo III α is required for the maintenance of mitochondrial genome and male germ-line stem cells

Jianhong Wu^a, Liping Feng^b, and Tao-shih Hsieh^{a,1}

^aDepartment of Biochemistry, and ^bDepartment of Obstetrics and Gynecology, Duke University Medical Center, Durham, NC 27710

Communicated by James C. Wang, Harvard University, Cambridge, MA, February 18, 2010 (received for review December 29, 2009)

Topoisomerase III α (topo III α), a member of the conserved Type IA subfamily of topoisomerases, is required for the cell proliferation in mitotic tissues, but has a lesser effect on DNA endoreplication. The *top3 α* gene encodes two forms of protein by utilizing alternative translation initiation sites: one (short form) with the nuclear localization signal only, exclusively localized in the nuclei, and the other (long form), retaining a mitochondrial import sequence at the N-terminus and the nuclear localization sequence at the C-terminus, localized primarily in the mitochondria, though with a small portion in the nuclei. Both forms of topo III α can rescue the viability of null mutants of *top3 α* . No apparent defect is associated with the flies rescued by the long form; short-form-rescued flies (referred to as *M1L*), however, exhibit defects in fertilities. *M1L* females are sterile. They can lay eggs but with mitochondrial DNA (mtDNA) copy number and ATP content decreased by 20- and 2- to 3-fold, respectively, and they fail to hatch. Of the newly eclosed *M1L* males, 33% are completely sterile, whereas the rest have residual fertilities that are quickly lost in 6 days. The fertility loss of *M1L* males is caused by the disruption of the individualization complex and a progressive loss of germ-line stem cells. This study implicates topo III α in the maintenance of mtDNA and male germ-line stem cells, and thus is a causative candidate for genetic disorders associated with mtDNA depletion.

mtDNA depletion | DNA replication | DNA segregation | topoisomerase

Intertwining of DNA duplex provides an elegant means to store and transmit genetic information. However, because of this bihelical structure, the transaction of genetic information leads to DNA entanglement such as supercoiling, knotting, and catenation. DNA topoisomerases are nature's solution for resolving the topological problems associated with DNA metabolism. Based on structure and mechanism, there are two main groups of these enzymes (1, 2). Type I topoisomerases can reversibly cleave one DNA strand at a time, and type II enzymes are able to generate transient double strand breaks and transport another DNA segment through the reversible breaks. There are additional subtypes for each group. Bacterial topo I/III and eukaryotic topo III belong to type IA enzymes, and they work via a mechanism of strand passage through an enzyme-bridged single strand break. There are two isozymes of topo III, α and β , in metazoans: topo III α is essential for viability, and topo III β is not (3, 4). Eucaryotic topo I is classified as type IB enzymes, and it forms an evolutionarily distinct class when compared with type IA enzymes, presumably a descendent of site-specific recombinases in bacteria (5). These enzymes work by generating a strand break where protein is linked to the 3'-phosphoryl end, allowing the free 5' end to undergo a restrained rotation around the noncatalytic strand (6). Type IB can thus serve as an efficient swivel to remove supercoiling accumulated during replication or transcription. Whereas type IA enzymes can also function as a swivel, their strand passage activity can promote reactions as diverse as segregating replicated chromosomes and regulating the formation/resolution of recombination intermediates (7). Members in type

II enzymes are grouped into A and B subtypes, and they are closely related both in structure and mechanism. These enzymes are able to alter DNA supercoiling, but have essential functions in the segregation of daughter chromosomes. Most organisms have at least one or two members of each type of topoisomerases. The presence and function of topoisomerases in organelles like mitochondria and chloroplast, not as thoroughly investigated as their nuclear counterpart, remain to be elucidated.

Mitochondria have a number of critical functions beyond a source for metabolic energy, including processing of key metabolites and the control of programmed cell death (8). Abnormal mitochondria and mitochondrial dysfunction are known to be associated with a number of clinically important human diseases (9). Whereas the nuclear genome encodes all of the functions involved in the intermediate metabolism and biogenesis of mitochondria, the mitochondrial genome encodes part of the mitochondrial protein synthesis machinery, and more importantly, the core peptides for oxidative phosphorylation. Thus, mutations affecting the stable maintenance and transmission of mitochondrial genome can disrupt oxidative phosphorylation and ATP production.

The complexity of mitochondrial genomes varies greatly, with those from fungi and plants having the most complexity. Animal mitochondrial DNA (mtDNA) is a small genome, and typically exists as a circular, covalently closed molecule ranging from 15 to 20 kb (10). The known nuclear genes that are essential for mitochondrial DNA replication include DNA polymerase gamma, polymerase gamma accessory protein, DNA helicase, mitochondrial transcriptional factor A, and RNA polymerase serving as a primase ([11, 12]; reviewed in [13]). Interestingly, whether any DNA topoisomerases have a critical function in mitochondrial DNA replication remains to be elucidated. For DNA replication in kinetoplast, a mitochondrion-like organelle in trypanosome, both topoisomerase II (topo II) and a type IA topoisomerase are known to have essential functions (14, 15). The picture is less clear for animal cells. There exists a dedicated mitochondrial type IB enzyme in mammalian cells (16). However, genetic studies suggest that it is dispensable for mitochondrial DNA maintenance (17). Biochemical analysis using subcellular fractionation provides evidence for the presence of a truncated form of topo II β in bovine heart (18). There is also clear evidence to demonstrate the presence of a mitochondria-targeting form of a type IA enzyme using an alternate translational initiation of *top3 α* gene in human cells (19). However, whether these two enzymes are essential for mitochondrial DNA maintenance remains to be determined.

Author contributions: J.W. and T.-S.H. designed research; J.W., L.F., and T.-S.H. performed research; J.W. and T.-S.H. contributed new reagents/analytic tools; J.W. and T.-S.H. analyzed data; J.W. and T.-S.H. wrote the paper.

The authors declare no conflict of interest.

¹To whom correspondence should be addressed. E-mail: hsieh@biochem.duke.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/1001855107/DCSupplemental.

Fig. 2. The unconserved amino (N) termini of topo III α contain mitochondrial import signals. (A) N-terminal sequences of topo III α of human, mouse, chicken, frog, and fruit fly. The mitochondrial import probabilities were obtained by using the algorithm developed by Claros and Vincens (21). Arrowhead indicates the mitochondrial endopeptidase cleavage sites (19). The second methionines are colored green. (B) The N-termini of proteins encoded by the wildtype and mutant transgenes of *Drosophila*. Methionines are colored green and the mutated amino acid is marked red. For M1L-YFP transgene, the first AUG was mutated to UUG, therefore the translation would start exclusively from the second AUG. (C) Confocal images of live S2 cells transfected with the plasmid DNA containing a transgene of topo III α -YFP, M1L-YFP, or M26L-YFP. Arrow indicates an S2 cell not transfected with transgenic vector. Scale bar, 10 μ m. (D) Confocal images of fixed ovaries of M1L-YFP and M26L-YFP transgenic females. Scale bar, 25 μ m. (E) Subcellular fractionation and immunoblots. Mitochondria fractions (Lanes 1–3) and nuclei fractions (Lanes 4–6) were separated as described in *Materials and Methods*, and were subjected to SDS-PAGE, followed by Western blotting with antibodies against topo III α , lamin (nuclear marker) and cytochrome c (mitochondrial marker). Asterisk indicates the endogenous topo III α and arrow marks YFP fusion products.

With the second AUG was changed to UUG, the translation will be exclusively from the first AUG, giving rise to a protein with a mitochondrial import sequence at its N-terminus and a nuclear

localization signal at its C-terminal portion (Fig. 2B). This protein is predominantly localized in mitochondria, with a detectable fraction in the nuclei (Fig. 2C and D). This result thus suggests that the second AUG in the wildtype *top3 α* is used as an alternative translation initiation codon to produce the major nuclear form of topo III α .

To further confirm the localization patterns of M1L-YFP and M26L-YFP, we isolated nuclei and mitochondria by sucrose gradient centrifugation. Western blotting with topo III α antibody showed that whereas M1L-YFP could be detected in nuclei only (Fig. 2E, Lane 1 vs. Lane 4), M26L-YFP was present in both mitochondria and nuclei, albeit with a lower level in nuclei (Fig. 2E, Lane 2 vs. Lane 5). These biochemical data are consistent with observations in the immunostaining. Altogether, these results demonstrate that the polypeptide between the first and second methionine of *Drosophila* topo III α (Fig. 2B) has a function in mitochondrial import.

Generation of *top3 α* Null Mutants. The *top3 α* transgenes with differential targeting to either nucleus or mitochondrion provide a genetic tool to dissect its functions in these subcellular compartments. Our earlier experiments have identified a lethal *top3 α* mutant, *top3 α ¹⁹¹*, demonstrating the essentiality of *top3 α* in *Drosophila* development (4). Because *top3 α ¹⁹¹* is a hypomorphic mutant, we will need to isolate a null mutation that can be used to investigate the distinct nuclear vs. mitochondrial function of topo III α . In the *top3 α* null mutant with the transgenes having specific targeting preference, the only functional *top3 α* would be that from the transgenes, thus allowing us to dissect its function in distinct subcellular compartments. The knock-out mutants were generated through imprecise excision after mobilizing a P-element in the fly strain *top3 α ^{EP2272}* (4). We isolated two imprecise

Fig. 3. The *top3 α* gene and its null mutant *top3 α ⁶* and *top3 α ⁵⁴*. (A) Genomic structure of *top3 α* gene and its mutants *top3 α ⁶* and *top3 α ⁵⁴*. Open triangles represent the remnant inserts derived from P-element and the dashed lines indicate the deleted sequences. Introns are shown as gray bars. (B) Mutants *top3 α ⁶* and *top3 α ⁵⁴* were verified as null mutants by Western blotting. Lysate of ovaries was subjected to SDS-PAGE and the transferred protein bands were probed with antibodies against topo III α , and actin (loading control). Upper bands correspond to topo III α -YFP fusion protein, encoded by the transgene *top3 α -YFP*, and the lower bands to the endogenous topo III α . (C, D) Brains and attached imaginal discs (Arrowheads) were dissected from wandering larvae of control (C) and *top3 α ⁵⁴* mutant (D) visualized with DAPI staining. Salivary gland nuclei (E, F) and fat body nuclei (G, H) of wandering larvae from control (E, G) and *top3 α ⁵⁴* mutants (F, H) were stained with DAPI.

excision alleles that are homozygous lethal, *top3a*⁶ and *top3a*⁵⁴, both of which can be rescued by the genomic transgene of *top3a*, *top3a*-YFP (Fig. 3A and Table S1). Molecular mapping results indicate that at the original P-element insertion site, *top3a*⁶ and *top3a*⁵⁴ have remnant insertions of 638 bp and 331 bp derived from the P-element, respectively. In addition, they also have a deletion of 1540 bp and 2437 bp from nucleotide 203 bp downstream of the first translational initiation codon (Fig. 3A). Western blotting confirmed that *top3a*⁶ and *top3a*⁵⁴ are null mutants (Fig. 3B).

***top3a* Is Required for Cell Proliferation in Mitotic Cells.** Homozygotes of *top3a*⁶ and *top3a*⁵⁴ could develop through third instar stage with a 9 day delay as compared to their heterozygous siblings and died at pupation stage. Because topoisomerases play important roles in DNA metabolism, we examined the tissues active in DNA replication from third instar larvae by DAPI staining. Imaginal discs are the only mitotically active tissues during late larval development. Compared with the brain tissue and imaginal discs of their heterozygous siblings (Fig. 3C), third instar larvae of *top3a*⁵⁴ (or *top3a*⁶) mutants had about normal size brains, but surprisingly, a total absence of any imaginal discs (Fig. 3D). Therefore, *top3a* is required for proliferation of mitotic cells. They did have, however, salivary glands and fat bodies with nuclei size comparable to those of heterozygous siblings (Fig. 3E vs. F and G vs. H). There is endoreplication in these tissues, a specialized DNA replication undergoing multiple rounds of DNA synthesis without cytokinesis, hence giving rise to polyploidy nuclei. Because of the potential presence of low levels of maternally stored topo III α , endoreplication either does not require topo III α or low levels of topo III α would suffice. Interestingly, the origin recognition complex components Orc1 and Orc2 are also essential for mitotic cell division, and the endoreplication was not affected in their null mutants (22).

topo III α Is Required for the Mitochondrial Genome Maintenance and Fertilities of Flies. To explore the biological function of topo III α in mitochondrion or nucleus, we introduced the transgenes with a preference in organelle-targeting, M1L-YFP or M26L-YFP, into *top3a* null background. In such a background, transgenes were the only functional topo III α . Both M1L-YFP and M26L-YFP could fully rescue the viability of null mutant *top3a*⁵⁴ and *top3a*⁶ (Table S1). M26L-YFP produces a protein with both mitochondrial and nuclear import sequence. Despite the greatly diminished amount of M26L-YFP imported into the nuclei (Fig. 2C–E), it is sufficient to sustain the essential functions for viability and fertility. However, M1L-YFP produces topo III α without a mitochondrial import sequence and is exclusively localized in the

nuclei (Fig. 2C–E). M1L-YFP rescued flies (referred to as *M1L*) exhibit fertility defects in both sexes. *M1L* females are completely sterile. Compared with the heterozygous siblings, *M1L* females have no significant difference in egg yields during a 12 day period, but their eggs appear to be smaller as a whole, and 17% shorter in length (Fig. 4A–C). They are also prone to collapse, indicative of defects in vitellogenesis (23, 24). The remaining intact embryos could not hatch, but could develop to different stages up to gastrulation. There is significant DNA fragmentation, suggesting severe defects in nuclear DNA replication. To examine the effect on mtDNA content, we examined whether there was a change in mtDNA copy number in the 0–1 hr *M1L* embryos by quantitative PCR. The mtDNA copy number was decreased by 20-fold as a result of the removal of mitochondrial import signal of topo III α (Fig. 4D), suggesting that topo III α has a critical function in mitochondrial genome maintenance. Because mitochondria are the major source for cellular energy, we made measurements for ATP content in *M1L* embryos. ATP content of *M1L* was 2- to 3-fold lower than those of their heterozygous siblings (Fig. 4D). Interestingly, no apparent change in the level of cytochrome c, a nucleus-encoded protein, was detected in these embryos (Fig. 4D). These defects in mtDNA copy number and ATP content are not limited to embryos. Both ovaries and testes of *M1L* also have a lower ATP level (Fig. S1) and a decreased mtDNA content (Fig. S2) relative to their heterozygous siblings.

The fertility of *M1L* males is also severely impaired. Their fertility decreases drastically as the flies age. In the newly eclosed *M1L* males, 33% of them were completely sterile, and the rest of them had a fertility <10% of their heterozygous siblings (Fig. 4E). To analyze the dependence of fertility on the fly age, we conducted sperm depletion experiments in a period of 12 days. *M1L* males completely lost fertility after 6 days, whereas heterozygous siblings maintained a steady level of fertility throughout 12 days (Fig. 4E).

Individualization Complexes of *M1L* Testes Are Disrupted During Spermatogenesis. There are two major aspects in the fertility defects of *M1L* males: a severe defect for the newly eclosed males and its rapid deterioration over time. We first examined the spermatogenesis of newly eclosed males. The final stage of spermatogenesis requires the resolution of a spermatid cyst into separate gametes by packaging each of 64 elongated spermatids in its own plasma membrane, a process referred to as individualization (20). Individualization complex (IC) is an actin-rich structure that is assembled at the nuclear end of the cyst and can be clearly visualized by phalloidin-TRITC staining (Fig. S3A, B, and Insets). In control testes, the ICs showed distinct triangular shape, and they moved in a coordinated fashion (Fig. S3A and Inset). In

Fig. 4. The topo III α is required for mtDNA genome maintenance and fertilities of flies. (A, B) Eggs laid by *M1L* females, which are shorter than their heterozygous siblings, cannot hatch. (C) Embryo length of 20 eggs for each genotype was measured with Qcapture Pro version 5.1 (Qimaging) and analyzed with Student's *t* test. (D) 0–1 hr embryos laid by *M1L* females have lower mtDNA copy number and ATP content, although cytochrome c level is not affected. (E) Residual fertility of *M1L* males is lost after 6 day sperm depletion.

MIL testes, the ICs were not aligned as in the control, but were scattered along the testes (Fig. S3 B and Inset), suggesting that they did not move in synchrony. The triangular shape of ICs was not affected, implicating that it could be the movement, not the formation of the ICs that had been impaired.

The fertility defect of *MIL* males is also reflected in their testes structure. As a whole, 3 day old *MIL* testes appeared to be 50% smaller in diameter than the control (Fig. S3 C and D, and quantified in Fig. S3E). Half of the testes had no motile sperm observed in the sperm storage organ seminal vesicles, and the rest had rare. The spermatid nuclei in the same cyst of a normal testis can elongate and condense to form a needle-shaped nuclear bundle (20) (Fig. S3 C and Inset). However, in the *MIL* testis, the condensed nuclei were scattered throughout the testis tube (Fig. S3 D and Inset).

topo III α Is Required for the Maintenance of Male Germ-Line Stem Cells (GSC). *MIL* males, though having residual fertilities, became completely sterile over a period of 6 days, while the fertilities of their heterozygous siblings remained unchanged (Fig. 4E). To pinpoint the cellular cause, we examined the testes from the males either newly eclosed or with an age of 6 day by triple immunostaining for Vasa, a marker of germ-line cells (25, 26), Fasciclin III, a marker of hub cells (27, 28), and DNA. The hub cells provide a niche necessary for the self-renewal of GSC. For the freshly eclosed males, control testes all had apparent Fasciclin in the hub cells (Fig. 5 A and Inset) and different stages of germ-line cells, which were laid out throughout the testes (Fig. 5 A and D). In contrast, 25% of *MIL* testes had no apparent Fasciclin signal and very few germ-line cells scattered along the testes (Fig. 5 B and D). At the age of 6 days, whereas the control testes had the same staining pattern as the newly eclosed (Fig. 5D), the *MIL* testes exhibited apparent deterioration in spermatogenesis. Fifty-six percent of the testes showed a pattern of few scattered germ-line cells and no Fasciclin signal for hub cells (Fig. 5 B and D). Furthermore, 31% of them were completely devoid of germ-line cells (Fig. 5 C and D). We did not detect cell death at the apical tip of testes from two-day old males by Tunel Assay, suggesting that GSC loss is a result of defects in cell proliferation. Altogether these data demonstrate that the functions of topo III α in mitochondria are indispensable for the maintenance of male GSC.

Discussion

We have demonstrated that *Drosophila* *top3 α* is essential for cell proliferation, but not so stringently required for endoreplication, a specialized form of DNA replication with cells undergoing multiple round of DNA replication without cytokinesis. *top3 α* encodes two forms of topo III α by using alternative translational

initiation codons. One (long form) with a mitochondrial import sequence at the amino terminus and a nuclear localization signal at the carboxyl terminus of the protein, is predominantly localized in the mitochondria, with a detectable fraction in the nuclei. The other one (short form) without the mitochondrial import sequence, is present exclusively in the nuclei (Fig. 2). Both forms of topo III α can fully rescue the viabilities of *top3 α* null mutants, suggesting the topo III α 's functions in nuclei are essential for viabilities. No apparent defect in fertility was observed for flies harboring the long form of topo III α . However, both males and females retaining only the short form of topo III α exhibit fertility defects, implying that topo III α 's functions in mitochondria are indispensable for the fertility of flies. The females are completely sterile, and the males have reduced fertility with the residual fertility lost over a period of 6 days due to disruption of ICs and the loss of germ-line stem cells. Moreover, deprivation of topo III α in mitochondria led to the loss of mtDNA, implying topo III α is required for the mitochondrial genome maintenance. The work presented in this paper thus clearly demonstrates the essential function of a topoisomerase in the metazoan mitochondria.

In the absence of topo III α in mitochondria, though the *MIL* flies can survive to adulthood, they have a 4- to 15-fold decrease in mtDNA copy number, and a lowered ATP content by 2- to 3-fold in ovaries or testes, indicating that topo III α plays a key role in mtDNA maintenance and function. *Drosophila* mtDNA is a circular molecule of about 20 kb. One expects its maintenance would require at least one member from each type of topoisomerases. Among all the topoisomerases present in *Drosophila*, topo III α (4) and topo III β (29) (Type IA), topo I (30) (Type IB), and topo II (31) (Type II), only topo III α has been shown to be apparently imported into both the nuclei and the mitochondria. Although it has been reported that human nuclear genome encodes a mitochondria-specific Type 1B topoisomerase, Top1mt (16), which is not essential for viability (32), no such enzyme exists in *Drosophila* based on the genome sequence. The topo II immunostaining of the testes, a powerful tool to examine the mitochondrial localization of proteins, revealed that the sole Type II topoisomerase in *Drosophila* is exclusively localized in the nuclei, implying that Type II topoisomerase might be dispensable for the segregation of mtDNA. Mitochondrial import analysis predicts that topo I and topo II in human, *Drosophila*, and yeast have import probabilities lower than 5% (Table S2). Whereas topo III α is required for mtDNA maintenance and function in *Drosophila*, there is no report on the effect on mtDNA in yeast without *top3*. An interesting question is how mtDNA segregation is achieved if topo III α or topo III is the only topoisomerase in mitochondria in *Drosophila* or yeast. Several lines of experiments demonstrated that mtDNA replicates itself via a strand displacement mechanism (33). Specifically, replication of the leading

Fig. 5. The functions of topo III α in mitochondria are required for male germ-line stem cell maintenance. Testes dissected from the control (A) and *MIL* (B and C) males after 6 day sperm depletion (SI Materials and Methods) were fixed and stained with antibody against Vasa (marker for germ-line cells), Fasciclin III (marker for hub cells, the niche of germ-line stem cells), and DAPI. Insets (A–C) highlight the tips, pointed by the arrows, of testes with higher magnification. Arrowhead in (A) indicates the hub cells marked by Fasciclin III. The Vasa/Fasciclin III immunostaining patterns fell into three types: Type I (Panel A), with distinct Fasciclin III and Vasa signals, Type II (Panel B), without apparent Fasciclin III, but with germ-line cells scattered throughout the testis tube and Type III (Panel C), no germ-line cells present. (D) Quantification of three types of Fasciclin III/Vasa pattern for testes of males newly eclosed and after 6 day sperm depletion. Scale bars, 50 μ m.

strand initiates at the origin O_H . Once the leading strand synthesis proceeds for two-thirds of the genome, the synthesis of the complementary strand initiates at the origin O_L of the displaced strand. The daughter molecule containing the strand initiated at O_L lags in completion and segregates before DNA is fully replicated (34). With this model of DNA replication, it is plausible that segregation of daughter chromosomes with extensive single-stranded regions can be accomplished by a type IA topoisomerase alone, without the participation of a Type II topoisomerase. Interestingly, earlier biochemical experiments showed that *Escherichia coli* topo III can efficiently decatenate the replicated plasmid DNA provided there exists single stranded regions in the daughter molecules (35).

The embryos laid by *MIL* females can develop to some extent with DNA fragmented, and die at the stage of early embryogenesis. The lethality could be caused by the defects in mtDNA content and decreased ATP level. Reduction of mitochondrial ATP production by 60% can impair G1-S phase transition in adult flies (36). Furthermore, the loss and mutations of mtDNA could trigger additional pathways that are deleterious to cells. Veatch et al. (37) recently reported that loss of mtDNA resulted in nuclear genome instability by inhibiting the production of proteins containing iron-sulfur clusters. Although mtDNA content is decreased by 4- to 15-fold, the *MIL* flies can survive to adults; however, their embryos cannot with a 20-fold decrease in mtDNA copy number. These results indicate that there may be a threshold of mtDNA copy number required for mitochondria's cellular functions. Reduction of mtDNA copy number by <70% is defined as mtDNA depletion, which will lead to human diseases (38). Many clinical cases with instability in mtDNA failed to reach

a molecular diagnosis (39), and thus the causative agent for mtDNA depletion remains unknown. Absence of the mitochondria-targeting sequence in topo III α leads to a decrease of mtDNA copy number by at least 75%, suggesting that mistargeting topo III α can be a source of human disorders associated with mtDNA depletion. We have generated valuable tools to further dissect the mechanisms underlying the potential genetic disorder in mtDNA depletion due to topo III α deprivation in mitochondria.

Materials and Methods

See *SI Materials and Methods* for a detailed description of materials and methods.

Isolation of Nuclei and Mitochondria. Dissected ovaries were homogenized in buffer containing 5 mM Hepes (pH 7.9), 0.3 M sucrose, 2 mM EDTA, 5 mM KCl. After differential centrifugation, both crude nuclei and mitochondria pellets were resuspended and purified by a linear sucrose gradient, and analyzed by Western blotting.

ATP Assay. Ovaries and testes were dissected, ground, and boiled in Buffer D (5 mM Tris, pH 8.0, 0.5 mM EDTA). ATP assay was performed with ATP Bioluminescent Assay Kit (Sigma) using a Beckman LS6500 scintillation counter.

Quantitative PCR. 0–1 hr embryos (dechorionated), ovaries of 3 day old females or testes were prepared for quantitative PCR using the primers listed in *SI Text*.

ACKNOWLEDGMENTS. We thank Larry Lee and Shane Chen for technical assistance. We also thank Dr. Jody Plank for providing topo III α antigen, and Lored Asllani for assistance in ATP measurement. This study was supported by National Institutes of Health Grant GM29006.

- Schoeffler AJ, Berger JM (2008) DNA topoisomerases: Harnessing and constraining energy to govern chromosome topology. *Q Rev Biophys* 41(1):41–101.
- Wang JC (2002) Cellular roles of DNA topoisomerases: A molecular perspective. *Nat Rev Mol Cell Biol* 3(6):430–440.
- Li W, Wang JC (1998) Mammalian DNA topoisomerase III α is essential in early embryogenesis. *Proc Natl Acad Sci USA* 95(3):1010–1013.
- Plank JL, Chu SH, Pohlhaus JR, Wilson-Sali T, Hsieh TS (2005) *Drosophila melanogaster* topoisomerase III α preferentially relaxes a positively or negatively supercoiled bubble substrate and is essential during development. *J Biol Chem* 280(5):3564–3573.
- Krogh BO, Shuman S (2002) A poxvirus-like type IB topoisomerase family in bacteria. *Proc Natl Acad Sci USA* 99(4):1853–1858.
- Stewart L, Redinbo MR, Qiu X, Hol WG, Champoux JJ (1998) A model for the mechanism of human topoisomerase I. *Science* 279(5356):1534–1541.
- Plank J, Hsieh TS (2009) Helicase-appended topoisomerases: new insight into the mechanism of directional strand transfer. *J Biol Chem* 284(45):30737–30741.
- Wang C, Youle RJ (2009) The role of mitochondria in apoptosis. *Annu Rev Genet* 43:95–118.
- Wallace DC, Fan W (2009) The pathophysiology of mitochondrial disease as modeled in the mouse. *Genes Dev* 23(15):1714–1736.
- Boore JL (1999) Animal mitochondrial genomes. *Nucleic Acids Res* 27(8):1767–1780.
- Greenleaf AL, Kelly JL, Lehman IR (1986) Yeast RPO41 gene product is required for transcription and maintenance of the mitochondrial genome. *Proc Natl Acad Sci USA* 83(10):3391–3394.
- Larsson NG, et al. (1998) Mitochondrial transcription factor A is necessary for mtDNA maintenance and embryogenesis in mice. *Nat Genet* 18(3):231–236.
- Copeland WC (2008) Inherited mitochondrial diseases of DNA replication. *Annu Rev Med* 59:131–146.
- Lindsay ME, Gluenz E, Gull K, Englund PT (2008) A new function of *Trypanosoma brucei* mitochondrial topoisomerase II is to maintain kinetoplast DNA network topology. *Mol Microbiol* 70(6):1465–1476.
- Scocca JR, Shapiro TA (2008) A mitochondrial topoisomerase IA essential for late theta structure resolution in African trypanosomes. *Mol Microbiol* 67(4):820–829.
- Zhang H, et al. (2001) Human mitochondrial topoisomerase I. *Proc Natl Acad Sci USA* 98(19):10608–10613.
- Zhang H, Pommier Y (2008) Mitochondrial topoisomerase I sites in the regulatory D-loop region of mitochondrial DNA. *Biochemistry* 47(43):11196–11203.
- Low RL, Orton S, Friedman DB (2003) A truncated form of DNA topoisomerase II β associates with the mtDNA genome in mammalian mitochondria. *Eur J Biochem* 270(20):4173–4186.
- Wang Y, Lyu YL, Wang JC (2002) Dual localization of human DNA topoisomerase III α to mitochondria and nucleus. *Proc Natl Acad Sci USA* 99(19):12114–12119.
- Fuller MT (1993) Spermatogenesis. *The Development of Drosophila melanogaster*, eds M Bate and AM Arias (Cold Spring Harbor Lab Press, Cold Spring Harbor, New York), Vol 1, pp 71–147.
- Claros MG, Vincens P (1996) Computational method to predict mitochondrially imported proteins and their targeting sequences. *Eur J Biochem* 241(3):779–786.
- Park SY, Asano M (2008) The origin recognition complex is dispensable for endoreplication in *Drosophila*. *Proc Natl Acad Sci USA* 105(34):12343–12348.
- Spradling AC (1993) Developmental genetics of oogenesis. *The Development of Drosophila melanogaster*, eds M Bate and AM Arias (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), Vol 1, pp 1–70.
- Yan YL, Postlethwait JH (1990) Vitellogenesis in *Drosophila*: Sequestration of a yolk polypeptide/invertase fusion protein into developing oocytes. *Dev Biol* 140(2):281–290.
- Hay B, Jan LY, Jan YN (1988) A protein component of *Drosophila* polar granules is encoded by vasa and has extensive sequence similarity to ATP-dependent helicases. *Cell* 55(4):577–587.
- Lasko PF, Ashburner M (1990) Posterior localization of vasa protein correlates with, but is not sufficient for, pole cell development. *Genes Dev* 4(6):905–921.
- Brower DL, Smith RJ, Wilcox M (1981) Differentiation within the gonads of *Drosophila* revealed by immunofluorescence. *J Embryol Exp Morphol* 63:233–242.
- Gonczy P, Viswanathan S, DiNardo S (1992) Probing spermatogenesis in *Drosophila* with P-element enhancer detectors. *Development* 114(1):89–98.
- Wilson-Sali T, Hsieh TS (2002) Generation of double-stranded breaks in hypernegatively supercoiled DNA by *Drosophila* topoisomerase II β , a type IA enzyme. *J Biol Chem* 277(30):26865–26871.
- Lee MP, Brown SD, Chen A, Hsieh TS (1993) DNA topoisomerase I is essential in *Drosophila melanogaster*. *Proc Natl Acad Sci USA* 90(14):6656–6660.
- Wyckoff E, Hsieh TS (1988) Functional expression of a *Drosophila* gene in yeast: Genetic complementation of DNA topoisomerase II. *Proc Natl Acad Sci USA* 85(17):6272–6276.
- Zhang H, Meng LH, Pommier Y (2007) Mitochondrial topoisomerases and alternative splicing of the human TOP1mt gene. *Biochimie* 89(4):474–481.
- Brown TA, Cecconi C, Tkachuk AN, Bustamante C, Clayton DA (2005) Replication of mitochondrial DNA occurs by strand displacement with alternative light-strand origins, not via a strand-coupled mechanism. *Genes Dev* 19(20):2466–2476.
- Shadel GS, Clayton DA (1997) Mitochondrial DNA maintenance in vertebrates. *Annu Rev Biochem* 66:409–435.
- DiGate RJ, Marians KJ (1988) Identification of a potent decatenating enzyme from *Escherichia coli*. *J Biol Chem* 263(26):13366–13373.
- Mandal S, Guptan P, Owusu-Ansah E, Banerjee U (2005) Mitochondrial regulation of cell cycle progression during development as revealed by the tenured mutation in *Drosophila*. *Dev Cell* 9(6):843–854.
- Veatch JR, McMurray MA, Nelson ZW, Gottschling DE (2009) Mitochondrial dysfunction leads to nuclear genome instability via an iron-sulfur cluster defect. *Cell* 137(7):1247–1258.
- Rotig A, Poulton J (2009) Genetic causes of mitochondrial DNA depletion in humans. *Biochim Biophys Acta* 1792(12):1103–1108.
- Hudson G, et al. (2006) POLG1, C10ORF2, and ANT1 mutations are uncommon in sporadic progressive external ophthalmoplegia with multiple mitochondrial DNA deletions. *Neurology* 66(9):1439–1441.