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DNA topoisomerase I is essential in *Drosophila melanogaster*

(gene mapping/gene cloning/transposon mutagenesis/genetic complementation/developmental expression)

MAXWELL P. LEE, SHERYL D. BROWN, ALICE CHEN, AND TAO-SHIH HSIEH

Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

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ABSTRACT **Both biochemical and genetic experiments suggest that the type I DNA topoisomerase may participate in DNA replication, recombination, transcription, and other aspects of DNA metabolism. Despite its apparent importance, genetic studies in unicellular organisms including eubacteria and yeasts indicate that topoisomerase I is not essential for viability. We have previously isolated the cDNA clone encoding DNA topoisomerase I from *Drosophila melanogaster*. We report here the cytogenetic mapping of *top1* to the X chromosome at 13C1 and isolation of *top1* genomic DNA. Using *P*-element mutagenesis, we have isolated a mutant deficient in *Drosophila* topoisomerase I functions. Genetic studies of this mutant show that topoisomerase I is essential for the growth and development of the fruit fly, a multicellular organism. The biological functions of topoisomerase I are inferred from our analysis of the regulation of topoisomerase I expression during *Drosophila* development.**

Both type I and II DNA topoisomerases are ubiquitous in nature. These enzymes can reversibly break DNA backbone bonds and thereby alter the higher order structure of DNA and chromosomes. DNA topoisomerases play a critical role in all aspects of DNA metabolism, including replication, transcription, and recombination (1–3). While genetic analysis indicates that type II DNA topoisomerases are essential in both eubacteria and yeast, it appears that type I enzymes are not necessary for the viability of these organisms (4). Mutations in the genes encoding bacterial type I DNA topoisomerases are not lethal (5, 6). However, bacteria carrying a tight mutation such as a deletion of *topA*, a gene encoding the most abundant topoisomerase I in bacterial cells, usually acquire an extragenic suppressor mutation (7–9), suggesting an important role of *topA* in the normal growth of bacteria. Both the budding and fission yeasts can tolerate mutations in the genes encoding type I DNA topoisomerases (10–13), again indicating the nonessentiality of topoisomerase I in these unicellular eukaryotic organisms.

Recent studies reveal several possible functions of DNA topoisomerase I inside yeast cells. Although yeast cells are viable with mutations in *top1*, a gene that encodes the major topoisomerase I activity in yeast, they grow at a slower rate than wild-type cells (12, 13). An apparent biological function of topoisomerase I is to provide a DNA swivel during replication and transcription. Genetic studies of yeast *top2* mutants indicate that DNA topoisomerase II is required to segregate the newly replicated daughter chromosomes (10, 14, 15). Whereas the lethality of temperature-sensitive (ts) $top2^{ts}$ mutants at the nonpermissive temperature occurs during mitosis, $top1/top2^{ts}$ double mutants, when shifted to the restrictive temperature, are arrested immediately at any stage of cell cycle (10, 11, 16). It has been shown that both synthesis of DNA and transcription of rRNA-encoding DNA (rDNA) are greatly diminished in *top1*/*top2* double mutants, but mRNA and tRNA synthesis are affected only 3-fold (16, 17). Based on the above studies, it is suggested that either topoisomerase I or topoisomerase II can serve as DNA swivels to relieve the torsional stress generated during DNA replication or transcription, especially at a highly transcribed locus like rDNA. In supporting the notion that DNA topoisomerase I is involved in DNA replication, it has been demonstrated that DNA topoisomerase I plays a role in the elongation of short nascent DNA chains (18). Involvement of DNA topoisomerase I in transcription is also supported by the reports demonstrating the physical association of *Drosophila* DNA topoisomerase I with actively transcribed regions in the genome (19, 20). Another possible function of DNA topoisomerase I in the process of transcription is associated with the repression of gene expression at the stationary phase of yeast cells (21). It is suggested that topoisomerase I may have an important role to alter the structure of chromatin or transcription machinery during the diauxic shift of yeast cell cycle. DNA topoisomerase I has also been implicated in the recombination pathway of yeast cells. One of the phenotypes associated with the *top1* mutation is the increased mitotic recombination at the rDNA locus (22, 23). This locus is unique in that it contains 200 tandem repeats of a highly transcribed rDNA unit. Therefore, DNA topoisomerase I may suppress recombination among the repetitive DNA sequence to maintain genome stability in the rDNA locus. Another type I DNA topoisomerase, *TOP3*, is identified by a mutation that increases the recombination between repeated δ elements of yeast transposon Ty (13). Sequence analysis of *TOP3* indicated that it is homologous to *Escherichia coli* DNA topoisomerase I. The *top3* mutant is viable, but it also shows a slow growth phenotype (13). Interestingly, the *top1*/*top3* double mutant is viable, but it grows more slowly than either of the single mutants (13).

We are interested in extending the functional studies of DNA topoisomerases to a multicellular organism; to this end, we have isolated the gene coding for DNA topoisomerase I from *Drosophila melanogaster* (24). We report here the chromosomal location of DNA topoisomerase I, isolation of *top1* genomic DNA, isolation and characterization of a mutant deficient in topoisomerase I functions, and determination of the pattern of expression of topoisomerase I protein and RNA at various stages of development.

MATERIALS AND METHODS

***In Situ* Hybridization.** The salivary glands from the late third instar larvae were dissected and squashed on slides as described (25, 26). Biotinylated probes were prepared for top1 cDNA and *P* element DNA. The probes were detected with avidin peroxidase complex (Enzo Biochem).

Cloning and Sequencing. All of the cloning and sequencing experiments were performed as described (24). A radioactive DNA probe made by nick translation of a top1 cDNA was

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Abbreviations: ts, temperature sensitive; rDNA, rRNA-encoding DNA.

used to screen two *Drosophila melanogaster* genomic libraries, one cloned in the λEMBL4 vector and the other constructed in the λGem12 vector (a generous gift of B. Judd). Among the positive recombinant phages isolated from both libraries, two of them with the longest inserts, λgtop1-1 and λgtop1-2, were further analyzed by subcloning and sequencing.

***Drosophila* Strains and Crosses.** Balancer chromosomes and genetic markers of *Drosophila* strains are as described by Lindsley and Zimm (27). Strain 438 was from the Bloomington Stock Center, Bloomington, IN. Flies were grown at 22°C either on standard cornmeal/agar medium or "Instant *Drosophila* Medium" (28). Crosses presented in Tables 1 and 2 were set up on day 0, parents were removed on day 8, and F_1 flies were counted on days 13, 16, and 19.

Mutagenesis. Using the protocol described by Robertson *et al.* (29), we generated the *top1* mutant *77*, by providing a source of genomic transposase in trans to mobilize the *438 P* element:

P (mass) $FM7/Y\ ;\ ry^{506}/ry^{506}$

$\times +/+; P[ry^+ \Delta 2\text{-}3]\ Sb/\text{TM6}$

F_1 (mass) $438P[13C\ ry^+]/Y;\ ry^{506}/ry^{506}$

$\times FM7/+\ ;\ P[ry^+\ \Delta 2\text{-}3]\ Sb/ry^{506}$

F_2 (mass) $FM7/Y;\ ry^{506}/ry^{506}$

$\times FM7/438P[13C\ ry^+];\ P[ry^+\ \Delta 2\text{-}3]\ Sb/ry^{506}$

F_3 (single) $FM7/Y;\ ry^{506}/ry^{506} \times FM7/438^*;\ ry^{506}/ry^{506}$

F_4 score for the absence of 438*/Y.

FM7 is an X chromosome balancer and *TM6* is a third chromosome balancer. We screened for putative *top1* mutants based on three criteria: (*i*) selecting for F_3 ry^- daughters from $FM7/438^*;\ ry^{506}/ry^{506}$, which lack the transposase source $P[ry^+\ \Delta 2\text{-}3]$ and have lost ry^+ at 13C (denoted as *438** here); (*ii*) testing for male lethal phenotype in F_4; (*iii*) DNA changes detected by the polymerase chain reaction using the primers flanking the *P* element insertion site. The expected amplification product from a fly that has undergone precise excision of the *P* element is a 1 kilobase (kb) DNA, whereas products with altered sizes or no amplification products suggest the presence of imprecise excision events. After screening about 100,000 flies, we isolated one putative *top1* mutant, *77*, which was male lethal.

RNA (Northern) Blots and Immunoblots (Western Blots). Samples were collected from each of the following stages of the *Drosophila* life cycle: embryos with postoviposition ages of 0–2 h, 2–6 h, 6–12 h, and 12–20 h, first instar larva, second instar larva, third instar larva, pupa, and adult. To isolate total protein, ≈0.1 g of each frozen, pulverized sample was homogenized in 0.35 ml of 50 mM Tris·HCl (pH 8.0) containing 50 mM NaCl, 5 mM EDTA, 0.1 mM dithiothreitol, 0.5% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, and 2 μg of leupeptin and 2 μg of pepstatin per ml. The protein concentration of the supernatant after pelleting was determined by Coomassie dye binding assay. An equal volume of lysed sample and SDS sample buffer was mixed together, boiled for 2 min, and pelleted. Each protein sample (≈100 μg) was run on an SDS/7% polyacrylamide gel (30), and immunoblot analysis was performed as described (31). Total RNA was isolated by homogenizing ≈1 g of each frozen, pulverized sample in 10–15 ml of extraction buffer consisting of an equal-volume mixture of phenol and a buffer containing 10 mM Tris·HCl (pH 8.0), 20 mM EDTA, and 1% SDS. After centrifugation, the aqueous phase was extracted twice with phenol and once with a mixture of phenol/chloroform/isoamyl alcohol, 25:25:1 (vol/vol). This nucleic acid mixture, containing mostly RNA, was precipitated with ethanol, and concentrations were determined by absorbance of 260 nm. Approximately 5 μg of total RNA from each sample was fractionated by electrophoresis on a 1% agarose gel containing formaldehyde and analyzed by blot hybridization (32).

RESULTS

Chromosomal Location of *Drosophila top1* Gene. To identify the genetic locus for DNA topoisomerase I in *Drosophila*, we cloned the cDNA sequence coding for this enzyme. Both homology of the predicted amino acid sequence and biochemical data confirmed the identity of the cloned sequence (24). Using the top1 cDNA clone as a molecular probe, we mapped the *top1* gene to cytological position 13C1 on the X chromosome (Fig. 1). There was no known mutant at that cytological locus, which appeared to affect DNA topoisomerase I function. However, de Cicco and Spradling had generated a fly strain that carries a single transposable *P* element insertion at this cytological location (33). The strain is referred to as *438* here. *In situ* hybridization experiments using the *438* fly confirmed cytogenetic colocalization of a single *P* element and the *top1* gene at the chromosome band 13C1 (data not shown).

Isolation of *top1* Genomic DNA and Molecular Mapping of the *P* Element. To find out whether the *P* element in *438* flies is located in the *top1* gene at the molecular level, we proceeded to isolate and characterize the genomic DNA containing the *top1* gene. Using top1 cDNA probe, we obtained several overlapping genomic DNA clones covering about a 20-kb region (Fig. 2). Restriction mapping and nucleotide sequence analysis indicate that the *top1* gene consists of eight exons and seven introns. We initially mapped the *P* element in *438* flies by genomic Southern blots, and the restriction digestion data suggested that the *top1* locus in *438* harbored an insertional DNA element (data not shown). We have subsequently designed primers and utilized polymerase chain reaction to amplify the junction sequence. Sequence analysis of the junction revealed that the *P* element insertion created an 8-bp duplication underlined in the following sequence: 5′-ATATAATGTGAACGCAAA-3′. The first A corresponds to nucleotide 4799 of the longer cDNA, which is 5132 base pairs (bp) in length excluding poly(A) sequences (24). Therefore, in *438* flies, the *P* element insertion is mapped in the untranslated region of *top1* at a position

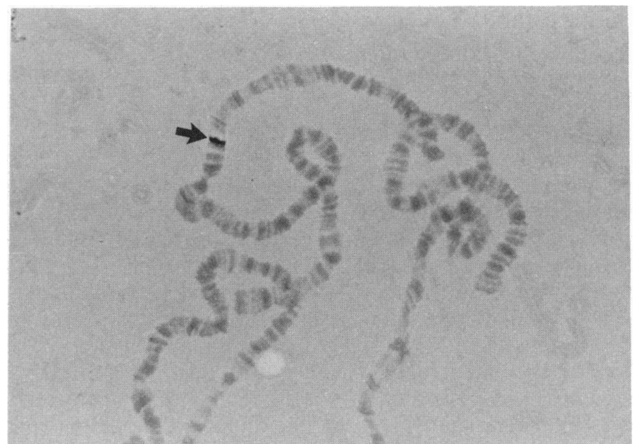

FIG. 1. *In situ* hybridization of the top1 cDNA probe to the salivary gland polytene chromosomes. The *top1* gene is mapped to 13C1 on the X chromosome as indicated by the arrow.

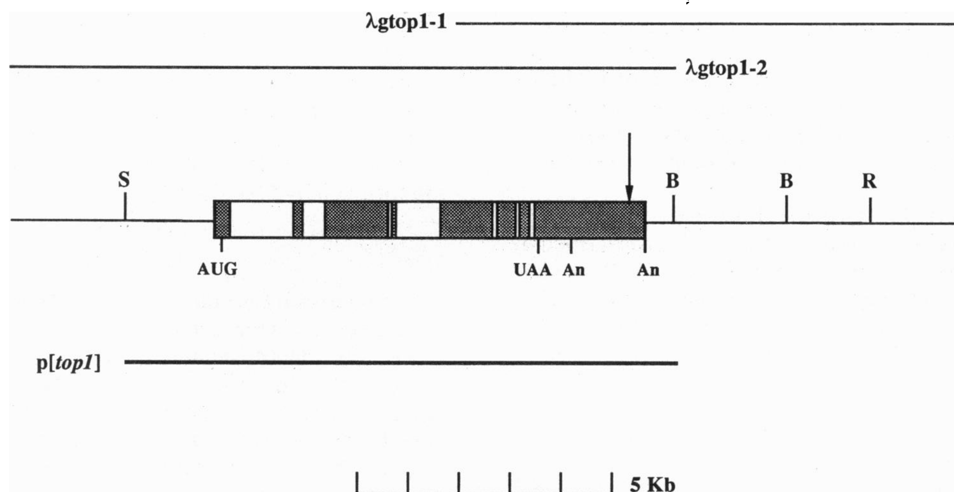

FIG. 2. A physical map showing the structure of the *Drosophila top1* gene. λgtop1-1 and λgtop1-2 are the two isolated genomic clones. They overlap and cover a 20-kb segment of genomic DNA containing the *Drosophila top1* gene. Some of the mapped restriction sites are shown as S (*Spe* I), B (*Bam*HI), and R (*Eco*RI). The nucleotide sequence between *Spe* I and the proximal *Bam*HI has been determined and analyzed. The stippled and open boxes represent the exons and introns, respectively. The structures of cDNAs have been described (24). Two poly(A) sites of the two isolated cDNAs are indicated by "An." The protein coding region is marked by the initiation codon AUG and the termination codon UAA. The *P* element insertion site at the *top1* locus of *438* is marked with an arrow. p[*top1*] contains a 10-kb *Spe* I–*Bam*HI fragment subcloned into *Xba* I/*Bam*HI sites of the CaSpeR vector.

of 1634 bp downstream from the TAA termination codon (Fig. 2).

Isolation of a *top1* Mutant. Both male and homozygous female *438* flies were viable and fertile. To generate flies with lesions in the *top1* locus and with the possible phenotypes of lethality or sterility, we carried out genetic crosses to remobilize the resident *P* element in the X chromosome of the fly *438*. Imprecise excision events occasionally occur during *P* element transposition and thus result in deletion or insertion mutations at the *P* element locus (34, 35). Using this mutagenesis method, we isolated a putative *top1* mutant, *77*, which exhibited a male lethal phenotype (see *Materials and Methods*). We were not able to recover any male fly carrying this *77* X chromosome, and the *77* X chromosome was passed from the mother to the daughter flies. *77* had an altered *top1* locus in comparison with the X chromosomes from either wild type or the *438* fly. The initial molecular mapping experiment indicated that the *top1* mutant *77* contained a 3-kb insertion in the 3′ untranslated region of *top1*. The context of DNA sequence surrounding the insertion site in *77* mutant X chromosome is different from that in the mutant *438* chromosome, which may be responsible for the difference of phenotypes observed for these two strains. However, further experiments will be needed to examine whether any sequence changes also exist in other regions of *top1*.

Rescue of Lethal Phenotype of *77* by an Ectopic *top1* Copy. To test whether the lethality of strain 77 was due to a lesion in the *top1* gene, we attempted to rescue the lethal phenotype of *77* with an ectopic copy of the cloned wild-type *top1* gene in transgenic fly strains. A 10-kb genomic DNA fragment between *Spe* I and *Bam*HI sites, containing the entire *top1* transcribed region plus sequence 1.3 kb upstream and 0.5 kb downstream (Fig. 2), was subcloned into the CaSpeR *P* element vector (36). This 10-kb fragment likely contained no gene other than *top1* since our initial sequence analysis of this region revealed no significant open reading frame except for that of topoisomerase I (data not shown). Furthermore, developmental Northern hybridization experiments using this 10-kb genomic DNA as probe yielded identical patterns to those shown in Fig. 3, which were obtained with the top1 cDNA as probes. We microinjected the *P* element vector containing this cloned *top1* genomic sequence into *Drosophila* embryos using the established transformation procedure (38, 39) and screened for transformants carrying the ectopic *top1* gene on the second chromosome. Genetic crosses were carried out to test whether the cloned *top1* on the second chromosome could rescue the lethal phenotype of *77* (Table 1). We observed all of the progeny from the cross shown in Table 1, except for those with the genotype *77*/Y; *Sco*/*CyO*, again demonstrating the lethality of *77*. In a marked contrast, flies of *77*/Y; p[*top1*]/*Sco* or *CyO* were recovered, thus establishing that an ectopic copy of *top1* could rescue the lethality of *77*. Since we obtained flies of *77*/Y; p[*top1*]/*CyO*, we could further test whether *77*/*77* females were viable. It was clear from the cross shown in Table 2 that *77*/*77*; *Sco*/*CyO* flies were not viable, while *77*/*77*; p[*top1*]/*Sco* or *CyO* flies survived. Therefore, these genetic crosses have demonstrated that the mutation of *77* is due to a lesion in the *top1* gene and that topoisomerase I is essential in both male and female *Drosophila*.

Developmental Expression of DNA Topoisomerase I. The possible essential functions of DNA topoisomerase I were investigated by examining the regulation of DNA topoisomerase I expression during *Drosophila* development (Fig. 3). A substantial amount of topoisomerase I protein was present in the 0–2 h embryos, and the expression was maximal in 6- to 12-h embryos (Fig. 3 *A* and *C*). Northern blot analysis indicated that there were two populations of top1 mRNA—one with a size of 5.2 kb and another that was heterogeneous with an average size of 4.0 kb (Fig. 3*B*). At least some of the 4.0-kb messages were generated by using a polyadenylylation site upstream to that of the 5.2-kb RNA (ref. 24; see also Fig. 2). The 5.2-kb transcript followed the profile of topoisomerase I protein in that it reached the maximal level in 6- to 12-h embryos and was very low in the third instar larval stage (Fig. 3 *B* and *C*). In contrast to the protein expression, the 5.2-kb message increased more significantly during the pupal/adult stage. The expression of the 4.0-kb transcript was mostly restricted to early embryos and adult (Fig. 3 *B* and *C*). The abundance of the top1 protein and message in the first 2 h of embryonic development suggested that they were maternally derived (40, 41). Further analysis of *top1* expression in adult tissues indicated that both the protein and RNA expression were concentrated in the ovaries, confirming the contribution of the maternal expression and storage (data not shown). The maximal expression of *top1*, which was, however, in the 6- to

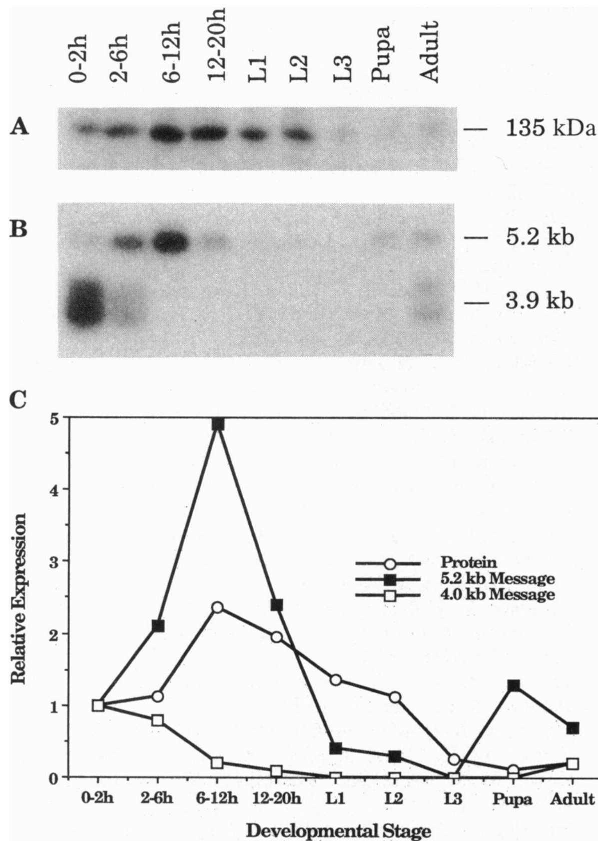

FIG. 3. Developmental expression profile of *Drosophila* topl protein and RNA. Immunoblot analysis (*A*) and Northern blot analysis (*B*) during *Drosophila* development. Marked on the right are the positions of the 135-kDa topoisomerase I and topl RNA size markers 5.2 kb and 3.9 kb long made by *in vitro* transcription of cloned segments of the topl cDNAs. (*C*) Graphic representation of the relative expression of topoisomerase I protein and RNA during *Drosophila* development. The developmental profile of the *topl* gene expression at the protein and RNA levels is quantitated by densitometric scanning of the autoradiograms shown in Fig. 3 *A* and *B*. Northern blot analysis was performed by using ^{32}P-labeled probes made from both the cloned 5.2-kb topl cDNA and *Drosophila ras* DNA. Hybridization to *ras* message was used to normalize the total amount of RNA (37). The relative expression of topoisomerase I protein (open circles), topl 5.2-kb message (closed squares), and topl 4.0-kb message (open squares) is plotted versus each developmental stage, and it is normalized to the 0- to 2-h embryo stage. The topl 4.0-kb message profile includes the quantitation of message populations ranging from 3.8 to 4.2 kb.

12-h embryos, was apparently of zygotic origin. This period also correlated with the maximal DNA synthesis activity during the *Drosophila* development (40, 41), suggesting a critical role for DNA topoisomerase I in embryonic DNA replication. The expression of topoisomerase I has been shown to increase in proliferating mammalian cells, and it is possible that there is also a correlation between topoisomerase I levels and DNA synthesis in mammalian cells (42–45).

DISCUSSION

We have demonstrated here that DNA topoisomerase I is essential for development of a multicellular organism, *Drosophila melanogaster*. By contrast, DNA topoisomerase I is not essential for eubacteria and yeast. Both the lethal phenotype of *77* and its rescue by p[*topl*] are absolute (Tables 1 and 2). The developmental expression profile suggests that DNA topoisomerase I is likely required during several stages of fly development (Fig. 3)—in particular, during the 6- to 12-h postfertilization embryo stage, when rapid DNA synthesis takes place (40). Several genes essential for the process of DNA replication have similar developmental expression profiles. These include *Drosophila* topoisomerase II (46, 47) and DNA polymerase α (48), for which the expression is also maximal in the embryos with an age of around 6 h.

An important biological function for DNA topoisomerase I is likely to provide a swivel at the fork of DNA replication (49, 50). Based on the genetic analyses of yeast and biochemical studies of the mammalian *in vitro* DNA replication system, DNA topoisomerase I is implicated in the process of DNA synthesis (18, 51); however, this function may be substituted by topoisomerase II. From the mechanism of action for these two different classes of topoisomerases, type II enzyme is not expected to serve a DNA swivel function as efficiently and directly as the type I enzyme. Even in the presence of topoisomerase II, an absence of topoisomerase I activity results in a delay in the elongation of newly synthesized DNA fragments in the yeast cell (18). It is therefore plausible that in a multicellular organism, an absence of adequate topoisomerase I activity can lead to a disruption in the coordination of DNA replication and cell cycling (52). This disruption could ultimately result in cell death and account for the essentiality of topoisomerase I in a multicellular organism.

In addition to its zygotic function, topoisomerase I may also have maternal functions based on the developmental expression data. The maternally stored gene products could be critically needed for the rapid-paced DNA replication in the syncytial embryos. Furthermore, using the same mutagenesis scheme described here, we have isolated several female sterile mutants, and the embryonic development of the eggs from one of the homozygous female sterile mutants is arrested at the blastodermal stage (data not shown), a period with active DNA synthesis and little transcriptional activity (40, 41). It is interesting to note that a significant

Table 1. Rescue of the male lethal phenotype of *77* by p[*topl*]

F_1 genotype	Fly numbers	%
FM7/Y ; *Sco*/*CyO*	242	4.7
FM7/Y ; p[*topl*]/*Sco* or *CyO*	527	10.3
77/Y ; *Sco*/*CyO*	0	0
77/Y ; p[*topl*]/*Sco* or *CyO*	1060	20.6
FM7/w$^-$; *Sco*/*CyO*	452	8.8
FM7/w$^-$; p[*topl*]/*Sco* or *CyO*	886	17.3
77/w$^-$; *Sco*/*CyO*	497	9.7
77/w$^-$; p[*topl*]/*Sco* or *CyO*	1092	21.3
Exceptional flies*	378	7.4
Total	5134	100.1

The F_0 cross was ♂ w^-/Y ; p[*topl*]/*CyO* × ♀ *FM7*/*77* ; *Sco*/*CyO*. *CyO*/*CyO* is lethal and it is not included here. p[*topl*] indicates an ectopic *topl* gene integrated into the second chromosome.
*The exceptional flies were the progeny with aneuploidy of sex chromosomes. They were generated presumably through the nondisjunction of *FM7*/*77* chromosomes in F_0 parental female flies.

Table 2. Rescue of female lethal phenotype of *77* by p[*topl*]

F_1	Fly numbers	%
FM7/Y ; *Sco*/*CyO*	223	4.3
FM7/Y ; p[*topl*]/*Sco* or *CyO*	576	11.1
77/Y ; *Sco*/*CyO*	0	0
77/Y ; p[*topl*]/*Sco* or *CyO*	1472	28.3
FM7/*77* ; *Sco*/*CyO*	501	9.6
FM7/*77* ; p[*topl*]/*Sco* or *CyO*	1236	23.7
77/*77* ; *Sco*/*CyO*	0	0
77/*77* ; p[*topl*]/*Sco* or *CyO*	1198	23.0
Total	5206	100.0

The F_0 cross was ♂ *77*/Y ; p[*topl*]/*CyO* × ♀ *FM7*/*77* ; *Sco*/*CyO*.

fraction of the *Drosophila* female sterile mutations actually correspond to maternal hypomorphs of essential genes (53).

During genetic experiments in testing the lethality of the *top1* mutant *77*, we noticed that female flies carrying one *77* X chromosome always produced significant number of progenies with aneuploidy of sex chromosomes (Table 1). They were generated presumably through the nondisjunction of *FM7/77* chromosomes in parental female flies. The background level for sex chromosome nondysjunction is about 0.1% (54). However, the presence of a nonexchange autosome, like *CyO* here, may elevate the X chromosome nondysjunction rate (55). Alternatively, it is also plausible that the presence of a *top1* mutant chromosome like *77* may have an effect on the chromosome pairing and disjoining during meiosis. Further experiments will be necessary to elucidate the genetic basis of the enhanced nondysjunction rate observed here.

The *top1* mutant, which will be named $top1^{77}$, is the initial DNA topoisomerase mutant identified and characterized in a multicellular eucaryote. Additional *top1* mutants can be isolated by using $top1^{77}$, since heterozygotes of new *top1* mutants and $top1^{77}$ could be either lethal or with impaired viability or fertility. Further characterization of $top1^{77}$ and other *top1* mutants will elucidate the critical functions of DNA topoisomerase I in eucaryotic cells.

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