Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 3664–3668, June 1986
Biochemistry

Isolation and characterization of the gene encoding *Drosophila* DNA topoisomerase II

(cDNA cloning/gene organization/*in vitro* translation/*in situ* hybridization)

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Communicated by Robert L. Hill, January 21, 1986

ABSTRACT **We have isolated the gene coding for the *Drosophila* type II DNA topoisomerase by immunochemically screening a *Drosophila* cDNA library constructed with a phage λ expression vector, λgt11. The identity of the cloned gene is confirmed by the analysis of an antigenic fusion protein produced in *Escherichia coli* and by the *in vitro* translation of its RNA. The gene is 5.1 kilobases in length, the expected size for a gene encoding topoisomerase II (M_r 170,000), and it is divided into five exons. By *in situ* hybridization to the polytene chromosomes from salivary glands, we have mapped it to chromosome 2L at 37D.**

DNA and chromatin in cells are likely to undergo many structural changes during the cell cycle. DNA topoisomerases are a class of enzymes that can mediate these structural changes in response to various functional needs of the chromosomes (for recent reviews, see refs. 1–4). Topoisomerases catalyze the interconversion of DNA topoisomers by reversibly breaking phosphodiester bonds in DNA. Two types of these enzymes are known, and both are ubiquitous in nature. Type I DNA topoisomerases work by transiently breaking one strand at a time, whereas type II enzymes mediate the passage of a segment of DNA through a reversible double-strand break. In bacteria, three genetic loci encoding topoisomerases are known: *topA* encodes topoisomerase I (ω protein) and *gyrA* and *gyrB* encode gyrase. Topoisomerase III is another type I enzyme in *Escherichia coli* (5), and its genetic locus has yet to be mapped. With the combination of biochemistry and genetics, the functions of DNA topoisomerases in bacteria have been analyzed in detail. They are involved in essentially all aspects of DNA metabolism, including replication, transcription, recombination, repair, and transposition (2–6). In recent years the genes encoding type I and type II topoisomerases have been identified in yeast (7–11). It is clear that *TOP1* (coding for topoisomerase I) is not essential for yeast growth, whereas *TOP2* (coding for topoisomerase II) is an essential gene and is required specifically at the time of mitosis, most likely in the step of segregating intertwined daughter chromosomes (7, 12). There is, however, evidence suggesting that *TOP2* is also involved in other aspects of chromosomal functions during the cell cycle in addition to mitosis. Although the *TOP1* mutation does not alter yeast growth, conditions under which *TOP1* and *TOP2* are rendered inactive result in immediate growth arrest and eventual killing of yeast cells (9–11). Therefore, both types of topoisomerases have complementary roles in modulating the necessary chromatin structure changes during cellular growth. There is also increasing evidence suggesting that the eukaryotic topoisomerase II may play an active role, such as generating DNA supercoils, in the chromatin domains where genes are actively transcribed (see recent reviews in refs. 4 and 13).

We are interested in the function of *Drosophila* DNA topoisomerase II and in establishing a molecular genetic system to rigorously analyze its functions during *Drosophila* development. Using antibody against topoisomerase II, we have screened a *Drosophila* cDNA library for the production of antigenic determinants in *E. coli*. We isolated the gene encoding *Drosophila* topoisomerase II and analyzed its structure. In this paper, we also report the results of cytogenetic mapping of this gene in *Drosophila* chromosomes.

MATERIALS AND METHODS

Construction of cDNA Library and Immunological Screening. Total poly(A)$^+$ RNA was isolated from *Drosophila* embryos. The first- and second-strand cDNA was synthesized according to a published procedure (14, 15). Following the addition of *Eco*RI linkers and digestion with *Eco*RI restriction endonuclease, the cDNAs were inserted in the single *Eco*RI cleavage site of λgt11 expression vector (16). When the packaged cDNA library was plated in the presence of 5-bromo-4-chloro-3-indolyl β-D-galactoside, 95% of the plaques formed colorless plaques because of the inactivation of *lacZ* by inserted cDNA fragments. DNA was isolated from 19 randomly chosen plaques and analyzed by *Eco*RI digestion. Nine of 19 contained inserts, the average size being about 800 base pairs (bp).

Screening of the λgt11 cDNA library was carried out as described (17). Antitopoisomerase II antibodies were preabsorbed with filter-immobilized total proteins from *E. coli* Y1090 lysate. This treatment resulted in removal of most anticoliform antibodies and gave significantly lower background in subsequent screening of plaques.

Analysis of Antigenic Fusion Proteins. *E. coli* Y1089 and its lysogen containing λ3c1 were grown in LB medium supplemented with 50 μg of ampicillin per ml at 32°C until cell density reached 5 × 10^6. The cells were induced by shifting the temperature to 42°C and the addition of 10 mM isopropyl β-D-thiogalactoside (IPTG). After an induction period of 15 min, the cells were incubated at 38°C for another 30 min and then harvested by centrifugation. The proteins in cell pellets were separated by NaDodSO$_4$/PAGE (18). The immunological activity of these proteins was analyzed by transferring them electrophoretically to a sheet of nitrocellulose membrane (19) and detecting the antigenic species by means of ^{125}I-labeled protein A or peroxidase-conjugated second antibody.

***In Vitro* Translation and Immunoprecipitation.** Templates for *in vitro* translation were prepared by *in vitro* transcription of cloned topoisomerase genes using phage SP6 or T7 RNA polymerase (19). Sense-strand template was transcribed with phage T7 RNA polymerase from the cDNA clone pGFc1, which had been linearized in the 3′ polylinker with *Xba* I.

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Abbreviations: kb, kilobase(s); IPTG, isopropyl β-D-thiogalactoside; bp, base pair(s).

Reaction conditions were 40 mM Tris·HCl (pH 7.8), 6 mM $MgCl_2$, 20 mM dithiothreitol, 2 mM spermidine, 0.5 mM (four) ribonucleoside triphosphates, 20 μg of DNA per ml, 1200 units of RNasin (Promega Biotec, Madison, WI) per ml, 1000 units of T7 RNA polymerase (United States Biochemical, Cleveland, OH) per ml, and 100 μCi of [α-^{32}P]ATP per ml (1 Ci = 37 GBq) at 37°C for 2 hr. Anti-sense RNA was prepared by cleaving pGFc1 in the 5′ noncoding region with *Mlu* I. Transcription of this template with purified SP6 RNA polymerase (Promega Biotec) was performed according to manufacturer's recommendations. Messenger-sense RNA was also transcribed with purified SP6 polymerase from the genomic clone pPX5.6, which had been cleaved in the 3′ polylinker with *Xba* I. The size of these templates was determined on formaldehyde/agarose gels (20).

Template RNAs were translated in nuclease-treated rabbit reticulocyte lysates (Promega Biotec) under conditions recommended by the manufacturer. The final concentration of template was 10 μg/ml. Proteins were labeled with [^{35}S]methionine and visualized by autoradiography of $NaDodSO_4$/polyacrylamide gels. For immunoprecipitations, 5 μl of the translation reaction was added to 100 μl of binding buffer containing 25 mM Hepes (pH 7.6), 0.1 mM EDTA, 100 mM KCl, 1 mM phenylmethylsulfonyl fluoride, 2% Triton X-100, and 0.1% $NaDodSO_4$. Antitopoisomerase antibody was added to a final concentration of 68 μg/ml. After 5 hr of mixing at room temperature, 20 μl of protein A-Sepharose (Pharmacia) was added. After 5 additional hr of mixing at room temperature, the protein A-Sepharose-bound material was collected by centrifugation, and pellets were washed five times with 0.5 ml of binding buffer. Bound material was eluted by boiling 3 min in gel sample buffer (2% $NaDodSO_4$/100 mM dithiothreitol/50 mM Tris·HCl, pH 6.8/10% glycerol/0.01% bromophenol blue), and samples were loaded directly onto $NaDodSO_4$/polyacrylamide gels.

S1 Nuclease Digestion. Poly(A)$^+$ embryo RNA (3.8 μg) (or yeast tRNA for controls) was precipitated by ethanol along with 0.1 μg of pPX5.6 that had been digested with *Cla* I or *Bgl* II. The precipitate was resuspended in 15 μl of 80% formamide/40 mM Pipes, pH 6.4/0.4 M NaCl/1 mM EDTA. Digestion by S1 nuclease was carried out as described (21, 22) with some modification. After digestion the samples were fractionated in a 1.2% alkaline agarose gel, transferred to a nylon membrane filter, and hybridized with various labeled probes.

***In Situ* Hybridization.** Late third instar larval salivary glands were squashed on slides that had been treated with Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin). Biotinylated probes were prepared by nick-translation with biotin-11-dUTP (ref. 37; Bethesda Research Laboratories) and were purified by isopropanol precipitation. Each slide was hybridized with 100 ng of probe in 20 μl of a buffer containing 0.3 M NaCl, 30 mM sodium citrate, 10 mM sodium phosphate (pH 7.0), 50% formamide, 10% dextran sulfate, 0.5 mg of salmon sperm DNA per ml, and 0.5 mg of yeast tRNA per ml. Hybridizations were performed overnight at 37°C in a humid chamber. Slides were washed and the probe was detected with avidin-peroxidase complex (Enzo Detek-I hrp) according to the manufacturer's instructions. Slides were stained with Giemsa and photographed by phase-contrast on a Zeiss photomicroscope III with Panatomic-X film.

RESULTS

Isolation of the Gene Encoding DNA Topoisomerase II from *Drosophila*. We first constructed a *Drosophila* cDNA library using the expression vector system of λgt11 (16, 17). With monospecific antibodies against topoisomerase II (23), we screened the library for the production of antigenic determinants in *E. coli*. Of 5 × 10^6 plaques screened, five independent recombinant phages reproducibly yielded positive immunochemical signals throughout various plaque purification cycles and with different preparations of monospecific antibodies. All five recombinant phages contain inserts with sizes between 1.3 and 1.5 kilobases (kb). Southern blot hybridization analysis and restriction endonuclease digestion experiments indicated that the insert sequences are homologous to each other.

To further characterize these recombinant phages, the production of their fusion proteins was analyzed. We first lysogenized *E. coli* Y1089 with these phages. From these lysogens the phages were thermally induced, and expression of the cloned sequence under the control of *lac* promoter was activated by the addition of IPTG (16). The proteins from these cells were analyzed by $NaDodSO_4$/PAGE (17) and immunoblots (18, 24). Fig. 1 shows the results with one of the topoisomerase II-clone candidates, λ3c1. With induction by temperature and IPTG, *E. coli* cells lysogenic for λ3c1 produced a series of proteins that are antigenic toward antitopoisomerase antibodies (Fig. 1, lanes 4 and 12). The M_r of the largest of these antigenic polypeptides is 167,000, consistent with it being a fusion between β-galactosidase (M_r 114,000) and a polypeptide of M_r 53,000 produced from the 1.45-kb insert in λ3c1.

The smaller, induced, antigenic polypeptides are likely generated from the larger one by partial proteolysis, since their yields increase during prolonged storage of these protein extracts. We have also isolated the fraction of antibodies that can bind specifically to these fusion proteins and demonstrated that these affinity-purified antibodies can also bind specifically with *Drosophila* topoisomerase II (data not shown). Therefore, λ3c1 and its homologous recombinant phages likely contain an inserted segment of DNA sequence encoding part of topoisomerase II in *Drosophila*.

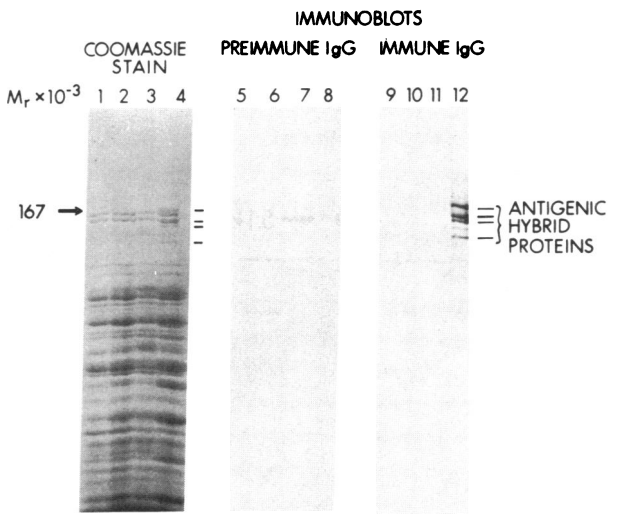

FIG. 1. Induction of the synthesis of antigenic fusion proteins in λ3c1 lysogen. Total proteins from *E. coli* Y1089 and its λ3c1 lysogen were isolated and analyzed by $NaDodSO_4$/PAGE. An identical triplicate of the gel either was stained with Coomassie blue (lanes 1–4) or transferred to a sheet of nitrocellulose membrane and subsequently treated with antitopoisomerase II antibodies (lanes 9–12) or preimmune antibodies (lanes 5–8). The immunoblots were processed to detect the antigenic polypeptides. Lanes 1, 5, and 9 and lanes 2, 6, and 10 show the samples from Y1089 before and after induction, respectively. Lanes 3, 7, and 11 and lanes 4, 8, and 12 show the samples from the lysogen before and after the identical induction conditions. The bars beside lanes 4 and 12 indicate the major antigenic polypeptides.

Structure of the *TOP2* Gene and Its Transcript. Purified *Drosophila* topoisomerase II is a homodimer of polypeptides with size of M_r 170,000 (23, 25). Using λ3c1 as a hybridization probe, the size of *TOP2* mRNA was determined to be 5.1 kb (Fig. 2), very close to the size predicted from polypeptide length. The cloned insert in λ3c1 therefore represents only a portion (about 30%) at the 3′ end of *TOP2* cDNA (Fig. 3).

We isolated several recombinant λEMBL4 phages (26) containing overlapping *Drosophila* genomic DNA sequences that are homologous to the λ3c1 insert. Some of these genomic clones, λ102, λ106, and λ107, are shown in Fig. 3. Using various restriction fragments generated from these genomic clones as hybridization probes, the coding region for *TOP2* RNA was mapped between the restriction sites of *Bgl* II and *Eco*RI (from map coordinates −1 to 6.6 in Fig. 3). This region represents a length of ≈7 kb, which is significantly larger than the size of *TOP2* mRNA, thus suggesting the presence of intervening sequences in the *TOP2* gene. To further characterize the gene structure we proceeded to isolate full-length cDNA from our λgt11-Dm cDNA library. We screened about 3×10^6 plaques with a radioactively labeled probe generated from a *Bam*HI/*Eco*RI restriction fragment (from map coordinates 1.5 to 3.3 in Fig. 3) using the plaque hybridization procedure (27). Six recombinant phages were recovered and all of them contain an apparently identical insert, 5.1 kb in length, corresponding to the size of *TOP2* mRNA. The size of the inserts in these recombinant phages suggests that they contain most, if not all, of the cDNA sequence from *TOP2*. One of them, λFc1 (Fig. 3), was further characterized. It has a tract of dA/dT at the end of the insert and shares the same sequence as the λ3c1 sequence. By comparing the genomic DNA sequence in this region with that of cloned cDNA using restriction enzyme digestion and nucleotide sequence analysis (data not shown), we concluded that *TOP2* RNA is encoded by five exons (Fig. 3). The largest intron is about 1 kb in length, whereas the other three introns are <100 bp in length.

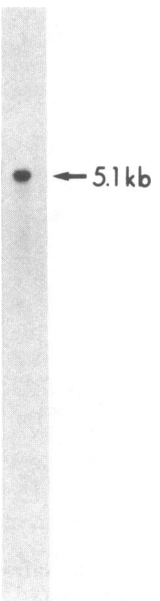

FIG. 2. RNA transfer blot hybridization of the topoisomerase II transcript. Total poly(A)$^+$ RNA from *Drosophila* was fractionated in a 1% agarose gel containing formaldehyde, electrophoretically transferred to a nylon membrane, and probed by radioactively labeled pGFc1, a plasmid DNA containing the insert from λFc1. The molecular weight of *TOP2* mRNA was determined through comparison with RNA size markers generated by transcribing cloned DNA sequences with phage SP6 RNA polymerase.

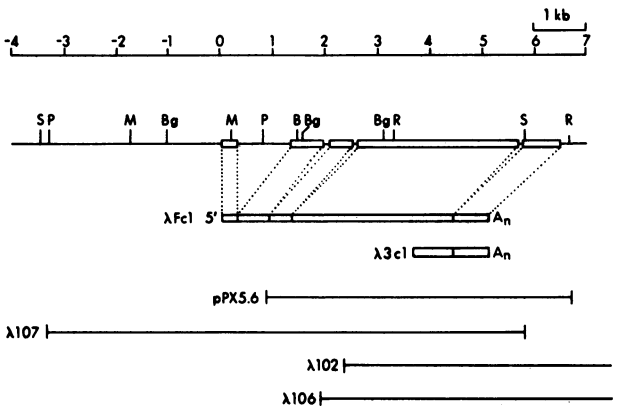

FIG. 3. Restriction map of cDNA and genomic DNA clones containing *Drosophila TOP2*. The following restriction sites were mapped: *Sal* I (S), *Pst* I (P), *Mlu* I (M), *Bgl* II (Bg), *Bam*HI (B), and *Eco*RI (R). λFc1 and λ3c1 are cDNA clones and λ102, λ106, and λ107 are from genomic clones. pPX5.6 was constructed from λ102 and λ107. The bars and lines beside these clones indicate the span of the cloned region, whereas the open end of the line indicates the end is mapped to a region beyond the map shown here. The boxes denote the exons of *TOP2*. A_n stands for the poly(A) end, determined from nucleotide sequence analysis, in cDNA clones.

The intron–exon structure was further confirmed by S1 nuclease digestion analysis (28) of the *TOP2* message. Plasmid pPX5.6 DNA, which contains all but the first exon of the *TOP2* transcript (from the *Pst* I site at 0.8 to *Eco*RI at 6.8, Fig. 3), was cut with *Cla* I or *Bgl* II (Fig. 4*a*). This unlabeled DNA was hybridized to *Drosophila* embryo poly(A)$^+$ RNA, digested with S1 nuclease, and fractionated by alkaline agarose gel electrophoresis. The DNA was transferred to a nylon membrane and sequentially probed with various labeled DNAs.

Fig. 4*a* shows the DNA fragments used for S1 nuclease protection as well as the labeled probes used to detect the protected sequences on the blot in this analysis. Fig. 4*b* shows the autoradiographic result for each of these probes. For A, B, and C, only the protection of *Cla* I fragments is shown; for probe D, *Cla* I and *Bgl* II are shown. Probe A is the full-length cDNA, which detects bands at 3100, 600, and 400 bp protected by message in lane 1; no bands are seen in lane 2 (no message). The intensity of the 600-bp band suggests that it is an unresolved doublet. Only the 3100-bp exon is detected with probe B; probe C detects this exon as well as a 600-bp band, which must be derived from sequences between the *Sal* I site and the external *Eco*RI site. Although probe D (a *Bgl* II fragment from coordinates 1.7 to 3.0) does not cover the *Sal* I to *Eco*RI region, it hybridizes to a 600-bp exon, which must, then, be different from the one detected with probe C. In addition, probe D hybridizes to the 400-bp and 3100-bp exons. Lanes 3 and 4 for probe D are S1 protection experiments using *Bgl* II fragments. A 1300-bp band caused by renaturation of an unlabeled protection fragment is seen in the control and message lanes. A broad band of about 400 bp is protected by message in lane 3. This results from the clipping of one of the 600-bp exons and the 3100-bp exon by *Bgl* II. Probe D detects only the intact 400-bp exon and the 350- to 400-bp segments cut by *Bgl* II from the exons flanking it.

From this information, we can unambiguously map the order and location of all of the exons of pPX5.6. The 5′ end of the gene was mapped by S1 nuclease protection and primer extension techniques; it is mapped within 20 nucleotides from the 5′ end of the λFc1 insert (data not shown). All splice sites predicted by S1 nuclease have been confirmed by DNA sequencing. Since *Cla* I cuts 100 bp upstream of the poly(A) addition site, the size of the last exon is 700 bp, instead of the

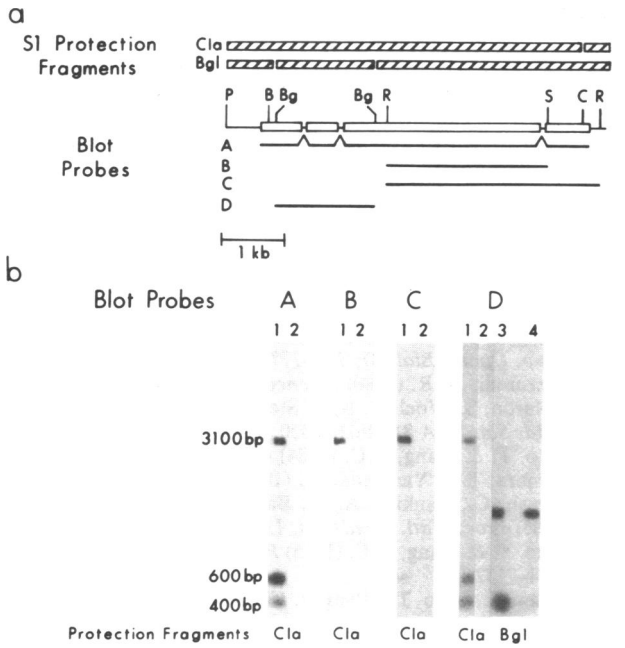

FIG. 4. S1 nuclease map of the *TOP2* transcript. (*a*) Schematic diagrams showing the physical locations of various probes used. "S1 Protection Fragments" indicate the unlabeled DNA used in the S1 nuclease reactions. Blot probes A–D are the labeled sequences used to detect the protected fragments on the blot. Probe A, full-length cDNA; probe B, internal *Eco*RI to *Sal* I; probe C, internal *Eco*RI to external *Eco*RI; probe D, *Bgl* II to *Bgl* II. (*b*) Autoradiographs of S1 digestion blots probed with the fragments shown in *a*. Unlabeled *Cla* I- or *Bgl* II-digested pPX5.6 was hybridized to RNA, S1 nuclease digested, run on an alkaline agarose gel, and blotted. The same blot was then probed with labeled DNA, autoradiographed, stripped, and reprobed. Odd-numbered lanes contain poly(A)$^+$ RNA; even-numbered lanes are controls (tRNA). Lanes 1 and 2, *Cla* I fragment protection; lanes 3 and 4, *Bgl* II fragment protection.

600 bp indicated by protection of *Cla* I fragments from S1 digestion. The length of the first exon, determined from sequence data, is about 230 bp. Therefore, the approximate sizes of the exons in 5′ to 3′ order are 230, 600, 400, 3100, and 700 bp. These total 5000 bp, in good agreement with our RNA transfer blot hybridization result and the size of the full-length cDNA.

***In Vitro* Translation of *TOP2* RNA.** To further confirm the identity of our cloned *Drosophila TOP2* gene and to establish an *in vitro* expression system for future analysis of this gene, we examined *in vitro* translation of *TOP2* RNA. The cDNA insert in λFc1 was isolated and cloned in a plasmid vector so the sense and anti-sense RNAs are transcribed from phage T7 and phage SP6 promoters, respectively. Sense and antisense RNAs were transcribed with purified T7 or SP6 RNA polymerase and translated in a rabbit reticulocyte lysate. In control experiments with no exogenous RNA (Fig. 5, lane 2), a single polypeptide with M_r 50,000 was synthesized. Translation of the sense RNA (Fig. 5, lane 1) gave polypeptides of sizes ranging up to M_r 170,000, the same size as *Drosophila* type II topoisomerase and the size expected from translation of a 5.1-kb RNA. Premature termination or internal initiation probably accounts for the synthesis of the smaller peptides. The M_r 170,000 polypeptide and a few smaller ones were recognized by antitopoisomerase II antibody and can be purified by immunoprecipitation (Fig. 5, lane 7). The immunoprecipitation of these labeled polypeptides was abolished by adding an excess of purified *Drosophila* topoisomerase II, and they were not precipitated by preimmune antibody using the same immunoprecipitation procedure (data not shown). It is also interesting to note that, using an RNA generated from

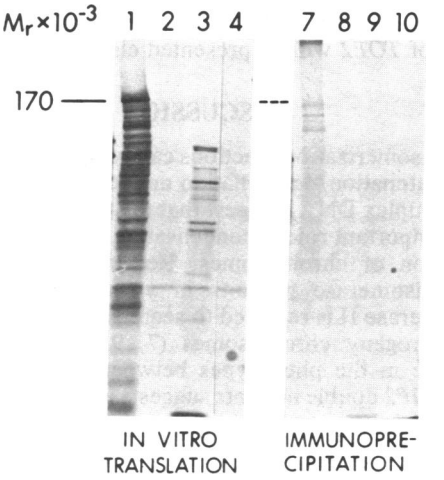

FIG. 5. *In vitro* translation of high molecular weight products and their specific immunoprecipitation by antitopoisomerase antibodies. The [^{35}S]methionine-labeled products of *in vitro* translation in rabbit reticulocyte lysates were analyzed by autoradiography of NaDodSO$_4$/7.5% polyacrylamide gels. RNA templates in the translation reactions: lanes 1 and 7, RNA transcribed in the message-sense direction from the cDNA clone pGFc1; lanes 2 and 8, RNA transcribed in the reverse-sense direction from pGFc1; lanes 3 and 9, RNA transcribed in the message-sense direction from genomic clone pPX5.6; and lanes 4 and 10, no template. Lanes 1–4 are the complete products of the translation reaction; lanes 7–10 are protein products immunoprecipitated by antitopoisomerase antibodies and protein A-Sepharose.

the insert of pPX5.6 by SP6 RNA polymerase, the translation products are much smaller than those made from sense *TOP2* RNA (Fig. 5, lane 3). This is in accord with the presence of introns in this DNA sequence.

Chromosomal Location of the *TOP2* Gene. Determining the cytogenetic location of the *TOP2* gene is the first step toward our goal in using a molecular genetic approach to analyze the functions of DNA topoisomerase II. To this end, we prepared a biotinylated probe from p3c1, a plasmid DNA containing the insert sequence from λ3c1. This probe was used for *in situ* hybridization to polytene chromosomes from salivary glands (29). It is mapped to the wild-type (Oregon-R, P2) chromosome 2L at position 37D (Fig. 6). Using various fly strains carrying different deletions in this region, the *TOP2* location was mapped to a cytogenetic region between 37D2 and 37D6

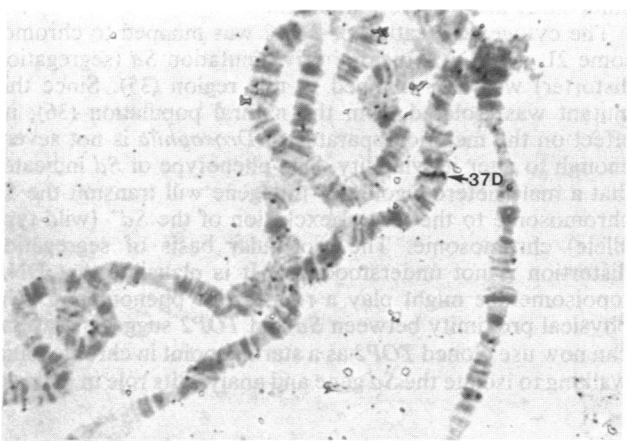

FIG. 6. *In situ* hybridization of the *TOP2* probe to polytene chromosomes. Biotinylated p3c1 DNA was hybridized to third instar larval salivary glands and detected with avidin-peroxidase. The arrow indicates the site of p3c1 hybridization, 37D.

(unpublished data). A detailed account of the cytogenetic location of *TOP2* will be presented elsewhere.

DISCUSSION

The topoisomerization reactions catalyzed by topoisomerase II, like catenation/decatenation and knotting/unknotting of circular duplex DNA, suggest that this class of enzymes may play an important role in condensation, decondensation, and segregation of chromosomes. Recent genetic studies on the topoisomerase mutants in yeast demonstrated that topoisomerase II is required to segregate topologically interlocked progeny chromosomes (7, 9, 12). However, the difference in the phenotypes between *TOP2* mutants and *TOP1/TOP2* double mutants suggests additional topoisomerase II functions in modulating chromosome structures (9–11). Other lines of evidence also implicate a functional role for topoisomerase II in controlling chromosome structure. Topoisomerase II is a major component in the nuclear matrix of *Drosophila* cells (30) and in the scaffold of chicken chromosomes (31). Topoisomerase II in the mitotic chromosomes is concentrated at the bases of radial loop domains (32). The sequence specificity of the topoisomerase II cleavage reaction in DNA and chromatin (33, 34) also suggests preferential location of this enzyme in certain domains of eukaryotic chromosomes.

To establish a genetic system with which we can thoroughly analyze the functions of topoisomerase II during the development of *Drosophila*, we isolated and characterized the gene encoding *Drosophila* topoisomerase II. We confirmed the identity of the cloned gene by the following two types of analysis. One is the immunochemical analysis on the expression in *E. coli* of the fusion protein carrying the carboxyl-terminal portion of topoisomerase II. The other indicates that *in vitro* translation of *TOP2* RNA resulted in the production of polypeptides with sizes up to that of purified topoisomerase II and these high molecular weight polypeptides could be specifically precipitated by antitopoisomerase antibody. Furthermore, the size of *TOP2* mRNA was measured to be 5.1 kb, consistent with the expected size of an RNA coding for topoisomerase II (M_r 170,000). We have isolated various overlapping genomic DNA clones containing *TOP2* and full-length cDNA clones. Detailed restriction digestion analysis of these cloned DNAs and their nucleotide sequence revealed that *TOP2* is encoded by five exons. This result was further confirmed by S1 nuclease protection experiments. The largest intron in this gene is about 1 kb in length and located near the 5′ end of the gene, whereas the other three are <100 bp in length.

The cytogenic location of *TOP2* was mapped to chromosome 2L at 37D. A meiotic drive mutation *Sd* (segregation distorter) was also mapped to this region (35). Since this mutant was isolated from the natural population (36), its effect on the meiotic apparatus of *Drosophila* is not severe enough to alter its viability. The phenotype of *Sd* indicates that a male heterozygous for this gene will transmit the *Sd* chromosome to the virtual exclusion of the Sd^+ (wild-type allele) chromosome. The molecular basis of segregation distortion is not understood yet. It is plausible that DNA topoisomerase might play a role in this phenomenon. The physical proximity between *Sd* and *TOP2* suggests that one can now use cloned *TOP2* as a starting point in chromosomal walking to isolate the *Sd* gene and analyze its role in meiosis.

We thank Mike Murphy for excellent technical assistance and for his tireless effort in starting up our *in situ* hybridization experiments. We are grateful to Gerda Vergara for her assistance in photography and Mary Jo Outlaw for typing this manuscript. This work was supported by a National Institutes of Health predoctoral training grant to J.M.N. and by a grant to T.-s.H. from the National Institutes of Health (GM29006).

1. Cozzarelli, N. R. (1980) *Cell* **22**, 327–328.
2. Gellert, M. (1981) *Annu. Rev. Biochem.* **50**, 879–910.
3. Liu, L. F. (1983) *CRC Crit. Rev. Biochem.* **15**, 1–24.
4. Wang, J. C. (1985) *Annu. Rev. Biochem.* **54**, 665–697.
5. Dean, F., Krasnow, M. A., Otter, R., Matzuk, M. M., Spengler, S. G. & Cozzarelli, N. R. (1983) *Cold Spring Harbor Symp. Quant. Biol.* **47**, 769–777.
6. Cozzarelli, N. R. (1980) *Science* **207**, 953–960.
7. DiNardo, S., Voelkel, K. & Sternglanz, R. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 2616–2620.
8. Goto, T. & Wang, J. C. (1984) *Cell* **36**, 1073–1080.
9. Uemura, T. & Yanagida, M. (1984) *EMBO J.* **3**, 1737–1744.
10. Thrash, C., Bankier, A. T., Barrell, B. G. & Sternglanz, R. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4374–4378.
11. Goto, T. & Wang, J. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7178–7182.
12. Holm, C., Goto, T., Wang, J. C. & Botstein, D. (1985) *Cell* **41**, 553–563.
13. Weintraub, H. (1985) *Cell* **42**, 705–711.
14. Okayama, H. & Berg, P. (1982) *Mol. Cell. Biol.* **2**, 161–170.
15. Gubler, U. & Hoffman, B. J. (1983) *Gene* **25**, 263–269.
16. Young, R. A. & Davis, R. W. (1983) *Science* **222**, 778–782.
17. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1194–1198.
18. Laemmli, U. K. (1970) *Nature (London) New Biol.* **227**, 680–685.
19. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
20. Melton, D. A., Krieg, P. H., Rebagliati, M. R., Maniatis, T., Zinn, K. & Green, M. R. (1984) *Nucleic Acids Res.* **12**, 7035–7056.
21. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 202–203.
22. Favaloro, J., Freisman, R. & Kamen, R. (1980) *Methods Enzymol.* **65**, 718–749.
23. Sander, M. & Hsieh, T. (1983) *J. Biol. Chem.* **258**, 8421–8428.
24. Renart, J., Reiser, J. & Stark, G. R. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
25. Shelton, E. R., Osheroff, N. & Brutlag, D. (1983) *J. Biol. Chem.* **258**, 9530–9535.
26. Frischauf, A.-M., Lehrach, H., Poustka, A. & Murray, N. (1983) *J. Mol. Biol.* **170**, 827–842.
27. Benton, W. D. & Davis, R. W. (1977) *Science* **196**, 180–182.
28. Berk, A. J. & Sharp, P. A. (1977) *Cell* **12**, 721–732.
29. Pardue, M. L. & Gall, J. (1975) *Methods Cell Biol.* **10**, 1–17.
30. Berrios, M., Osheroff, N. & Fisher, P. A. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4142–4146.
31. Earnshaw, W. C., Halligan, B., Cooke, C. A., Heck, N. M. S. & Liu, L. F. (1985) *J. Cell Biol.* **100**, 1706–1715.
32. Earnshaw, W. C. & Heck, M. S. (1985) *J. Cell Biol.* **100**, 1716–1725.
33. Udvardy, A., Schedl, P., Sander, M. & Hsieh, T. (1985) *Cell* **40**, 933–941.
34. Yang, L., Rowe, T. C., Nelson, E. M. & Liu, L. F. (1985) *Cell* **41**, 127–132.
35. Brittnacher, J. G. & Ganetzky, B. (1983) *Genetics* **103**, 659–673.
36. Sandler, L., Hiraizumi, Y. & Sandler, I. (1959) *Genetics* **44**, 223–250.
37. Langer, P. R., Waldrop, A. A. & Ward, D. C. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6633–6637.