Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6958–6962, November 1984
Biochemistry

Drosophila DNA topoisomerase I is associated with transcriptionally active regions of the genome

(immunofluorescent staining/polytene chromosomes/heat shock loci/nucleolus)

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Communicated by Mary Lou Pardue, July 23, 1984

ABSTRACT **The distribution of DNA topoisomerase I within *Drosophila* polytene chromosomes was observed by immunofluorescent staining with affinity-purified antibodies. The enzyme is preferentially associated with active loci, as shown by prominent staining of puffs. The heat shock loci *87A–87C* are stained after, but not before, heat shock induction. A detailed comparison of the distribution of topoisomerase I with that of RNA polymerase II reveals a similar, although not identical, pattern of association. Topoisomerase I is also found in association with the nucleolus, the site of transcription by RNA polymerase I.**

The polytene chromosomes of the *Drosophila* salivary gland provide an excellent system for investigating the distribution of specific chromosomal proteins. Although highly organized, these giant chromosomes behave like diploid interphase chromatin in many assays of function and fine structure (1–6). They can, however, be easily observed under the light microscope; some substructure, such as transcriptionally active sites, can be recognized. It is possible to localize proteins in these chromosomes and thereby obtain some information as to the biological processes in which the given proteins might be involved. In order to visualize the chromosomal proteins, the method of indirect immunofluorescence was developed several years ago (7, 8). The distribution patterns of a number of chromosomal proteins of unknown function have been determined by this approach (8–11). The method has allowed identification of a subclass of nonhistone chromosomal proteins that are prominently associated with loci that are active or inducible at some time in the salivary glands of the third instar larvae and prepupae (10, 12, 13).

In addition, distribution patterns of proteins of known function have been analyzed—e.g., RNA polymerase II (14–17) and ribonucleoproteins (18). These proteins are preferentially associated with the transcriptionally active regions of the genome. In this paper we report the distribution pattern of DNA topoisomerase I. This enzyme is well characterized at the molecular level, but the range of its biological functions in eukaryotes has yet to be established (for reviews, see refs. 19–25). The ability of eukaryotic topoisomerase I to relax either negatively or positively supercoiled DNA hints that the enzyme might play a role in gene activation, either effecting structural changes in chromatin as it assumes a more "open" conformation (e.g., as detected by the appearance of puffs) and/or facilitating transcription per se (26, 27). There is some evidence in favor of a role of topoisomerase in transcription. For example, topoisomerase has been shown to be associated with ribosomal gene chromatin actively expressing rRNA (28); topoisomerase I is recovered with nucleosomes in a fraction enriched for transcriptionally active genes (29). Complex formation between eukaryotic DNA topoisomerase I and chromosomal high mobility group proteins and histone H1 has also been reported (30). However, there has been no direct evidence as to those eukaryotic cellular functions in which the enzyme might be involved.

We have compared the distribution pattern of DNA topoisomerase I in *Drosophila* polytene chromosomes with the patterns shown by both RNA polymerase II and by an uncharacterized nonhistone chromosomal protein of 130 kDa, which appears at most sites that become active at some time during the third instar. We have also investigated the change in distribution after the induction of the heat shock puffs. The results demonstrate a correlation between the presence of topoisomerase I and transcriptional activity.

MATERIALS AND METHODS

DNA topoisomerase I of *Drosophila melanogaster* Oregon R was prepared as described Javaherian *et al.* (31) or with modifications to be described elsewhere. A protein fraction enriched in the enzyme was run on a 10% $NaDodSO_4$/polyacrylamide gel (32), and the gel band representing the topoisomerase I (135 kDa) was excised, freeze-dried, and ground to a powder. Rabbits were immunized with the powder by the procedure of Tjian *et al.* (33) with minor modifications (7). Three independent preparations of antibodies were used in these studies; all gave essentially the same results.

Antibodies prepared by this procedure were affinity purified following the principles of Olmsted (34). Purified topoisomerase I was run on a 10% $NaDodSO_4$/polyacrylamide gel and transferred electrophoretically (35) to aminophenylthioether paper (36). Subsequent incubation of the paper with 3% bovine serum albumin/0.145 M NaCl/10 mM Tris·HCl, pH 7.6/0.1 mM phenylmethylsulfonyl fluoride was performed to block the remaining binding sites. The precise region of the paper containing the 135-kDa topoisomerase I band was cut out and incubated for 3 hr in 1 ml of serum. The piece of paper was washed in 10 mM Tris·HCl, pH 7.6/0.145 M NaCl for 1 hr and then extracted twice with 200 μl each of 0.2 M glycine·HCl (pH 2.6) for 1.5 min. Subsequent addition of 0.1 vol of 1 M Tris base to the extract raised the pH to ≈7.5. This procedure was repeated three times with the same strip of paper and the same serum. These affinity-purified antibodies were used in the indirect immunofluorescence staining assay developed by Silver and Elgin (7, 10) using acetic acid-fixed polytene chromosomes. The specificity of the antibodies against topoisomerase I was confirmed

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by transferring the proteins from a $NaDodSO_4$/polyacrylamide gel to nitrocellulose paper and incubating the gel replica first with the antibodies and subsequently with ^{125}I-labeled donkey anti-rabbit $F(ab')_2$ fragments or ^{125}I-labeled protein A of *Staphylococcus aureus* (35).

An antiserum (P-215) against the large subunit of *Drosophila* RNA polymerase II was generously supplied by A. Greenleaf (38). *Drosophila* DNA topoisomerase II was kindly provided by T.-s. Hsieh (37). Protein 120/130 was prepared from the nuclear proteins of 6- to 18-hr *Drosophila* embryos and an antiserum prepared as described above.

RESULTS

Antibodies were prepared in a rabbit by using as antigen the ≈135-kDa band of purified *Drosophila* topoisomerase I from a $NaDodSO_4$/polyacrylamide gel. When a crude extract of *Drosophila* nuclear proteins was separated in a $NaDodSO_4$/polyacrylamide gel and the proteins were transferred to nitrocellulose paper, the antiserum generally showed binding to several protein bands of lower molecular mass in addition to reacting with the intact topoisomerase I band. The majority of these bands is thought to be breakdown products of topoisomerase I (31). To eliminate possible ambiguity, antibodies were affinity purified by using the 135-kDa fraction of purified topoisomerase I. The purified antibodies showed a high specificity for purified topoisomerase I. In particular, in a direct test, no reaction of the antibodies with purified RNA polymerase II or DNA topoisomerase II of *Drosophila* was observed (Fig. 1).

Incubation of polytene chromosomes with the affinity-purified antibodies resulted in the staining of a distinct set of loci (Fig. 2). One observes prominent staining at a small subset of loci, with lesser staining at many other positions. The brightest staining appeared over the large puffs such as those that occurred at *68C*, *74EF*, or *75B* (Fig. 3). To further dem-

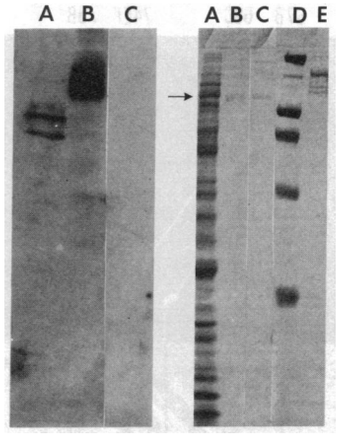

FIG. 1. Selectivity of affinity-purified anti-*Drosophila* topoisomerase I antibodies. Proteins were electrophoresced on 8% $NaDodSO_4$/polyacrylamide gels. (*Right*) The gel was stained with Coomassie blue. Lanes: A, crude fraction containing the lower molecular mass forms of *Drosophila* DNA topoisomerase I; B, purified 135-kDa *Drosophila* topoisomerase I; C, purified *Drosophila* RNA polymerase II; D, protein markers (Bethesda Research Laboratories); E, purified *Drosophila* DNA topoisomerase II. The major bands in the topoisomerase I and polymerase II lanes (B and C) are faint but distinct on the original gel; the position of the topoisomerase I band is marked by an arrow. (*Left*) Proteins from a similar gel were transferred electrophoretically to a nitrocellulose sheet and incubated with the affinity-purified anti-*Drosophila* topoisomerase I antibodies, followed by staining with ^{125}I-labeled protein A. Lanes A–C are as described above. Prominent staining of purified topoisomerase I is observed (lane B), whereas there is no significant staining of RNA polymerase II (lane C) or topoisomerase II (not shown). The major reactive components in the crude nuclear fraction (lane A) are breakdown products of the enzyme.

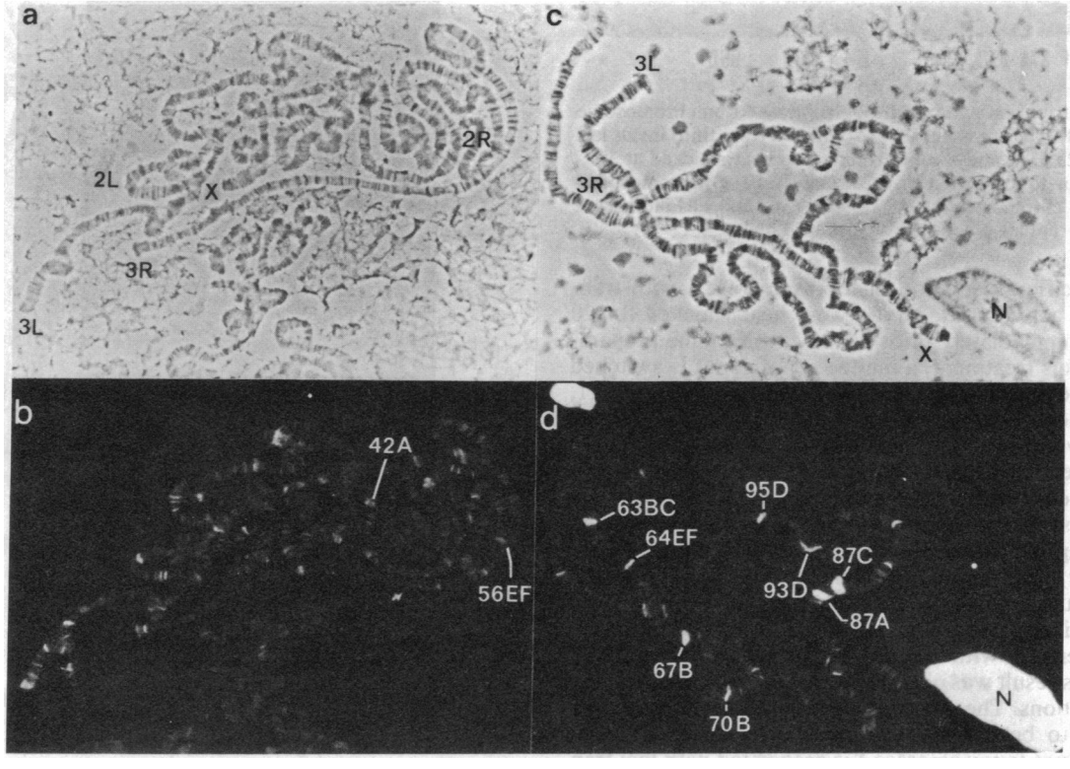

FIG. 2. Distribution pattern of topoisomerase I on polytene chromosomes. Topoisomerase I was visualized by the method of indirect immunofluorescence. Chromosomes were obtained from third instar larvae grown at 25°C (*a* and *b*) and from larvae heat shocked at 37°C for 20 min prior to dissection (*c* and *d*). (*a* and *c*) Phase-contrast images; (*b* and *d*) fluorescent images. N, nucleolus; X, 2L, 2R, 3L, and 3R, chromosome arms; specific loci as indicated.

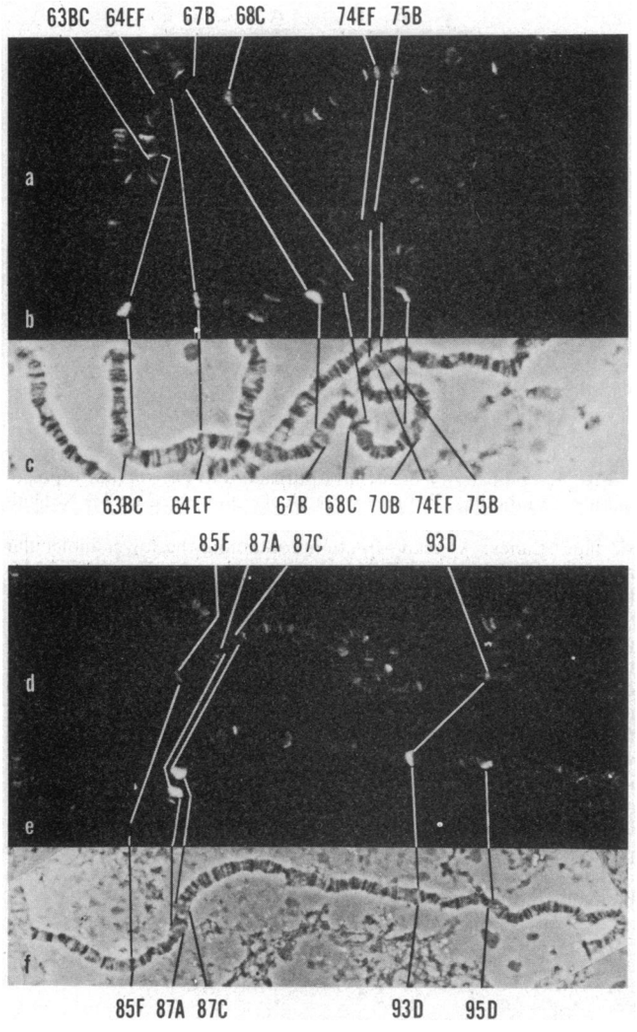

FIG. 3. Distribution pattern of topoisomerase I on chromosome arms 3L and 3R. Chromosomes were obtained from third instar larvae grown at 25°C (*a* and *d*) and from larvae heat shocked at 37°C for 20 min prior to dissection (*b*, *c*, *e*, and *f*). (*a–c*) Chromosomal arm 3L; (*d–f*) chromosomal arm 3R. (*a*, *b*, *d*, and *e*) Fluorescent images; (*c* and *f*) phase-contrast images of *b* and *e*.

onstrate specific association of topoisomerase I with transcriptionally active sites, larvae were heat shocked for 20 min at 37°C immediately prior to the chromosome preparation. Upon this treatment, a limited set of genes is switched on and can be visualized as the so-called heat shock puffs (1, 39, 40), while the expression of the developmentally active genes is severely reduced (41, 42). After heat shock, the newly induced puffs (*63BC*, *64EF*, *67B*, *70B*, *87A*, *87C*, *93D*, *95D*) exhibited a strong fluorescence, while a reduced fluorescence was observed in many previously active loci—e.g., *68C*, *85F* (Fig. 3). Notice that in the case of *68C*, the puff had not yet fully regressed, but only minor amounts of topoisomerase I could be detected.

Another site of high transcriptional activity, the nucleolus, reacted intensely with the antibodies to topoisomerase I (Fig. 2). This result was obtained under both normal and heat shock conditions. The rRNA genes, located in the nucleolus, are shown to be transcribed under both conditions (43). Whether or not topoisomerase I is associated with loci transcribed by RNA polymerase III is more difficult to assess. Locus *42A* of the second chromosome was stained by using antibodies against topoisomerase I (Fig. 2). This locus contains a cluster of four tRNA genes (44). Other tRNA gene sites such as *61D*, *62A*, or *84AB* are too small to be readily analyzed with this technique. Staining with anti-topoisomerase I (Fig. 2), but not with anti-RNA polymerase II (data not shown), was observed at locus *56EF*, the site of the 5S RNA genes (45).

A comparative analysis of the distribution patterns of topoisomerase I, RNA polymerase II, and a nuclear protein of 130 kDa (protein 120/130) was carried out by using chromosomes from an early puffing stage (1–2), a stage when only a few loci are puffed (40, 46). To be certain to obtain chromosome squashes at the same developmental stage, one salivary gland was divided, several slides were prepared, and the separate parts were stained independently by using the affinity-purified antibodies against topoisomerase I, a specific antiserum against RNA polymerase II, and the antiserum against protein 120/130. Appreciable staining for topoisomerase I and RNA polymerase II was found only at a few sites, as demonstrated for the third chromosome arm 3L (Fig. 4). Loci *63C*, *68C*, and *71EF* were stained with comparable intensities by using antibodies against either enzyme. Loci *63A* and *67B* showed more pronounced staining with anti-RNA polymerase II antiserum, whereas locus *71CD* showed more pronounced staining with anti-topoisomerase I antibodies. These differences were observed consistently for all chromosomes derived from the same squash, with only minor differences in the relative staining intensities. The sites that were stained prominently by only one of the two antisera in several cases could also be detected with the other antiserum in other chromosome squashes of slightly different developmental stages. Protein 120/130 was found in more loci; the pattern observed was similar to that reported for the Band 2- and ρ-antigens, which are generally observed in those loci in the salivary gland that are active at some time during the third larval instar or prepupal stages (10, 12, 13).

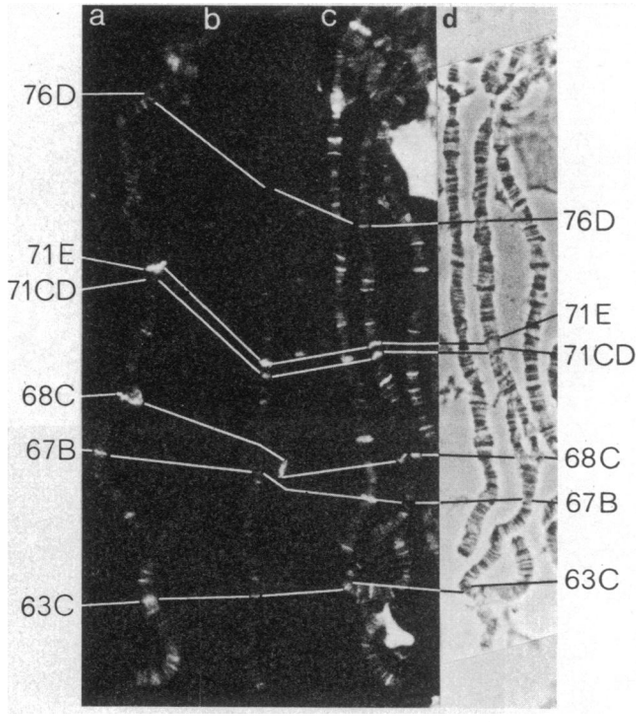

FIG. 4. Comparison of the distribution pattern of RNA polymerase II, topoisomerase I, and protein 120/130 on polytene chromosome 3L. A salivary gland of a third instar larva at puffing stage 1–2 was divided into several parts and chromosome squashes were prepared. Squashes were incubated with anti-RNA polymerase II antiserum (*a*), affinity-purified anti-topoisomerase I antibodies (*b*), and anti-protein 120/130 antiserum (*c*). (*a–c*) Fluorescence images; (*d*) phase-contrast image of *c*.

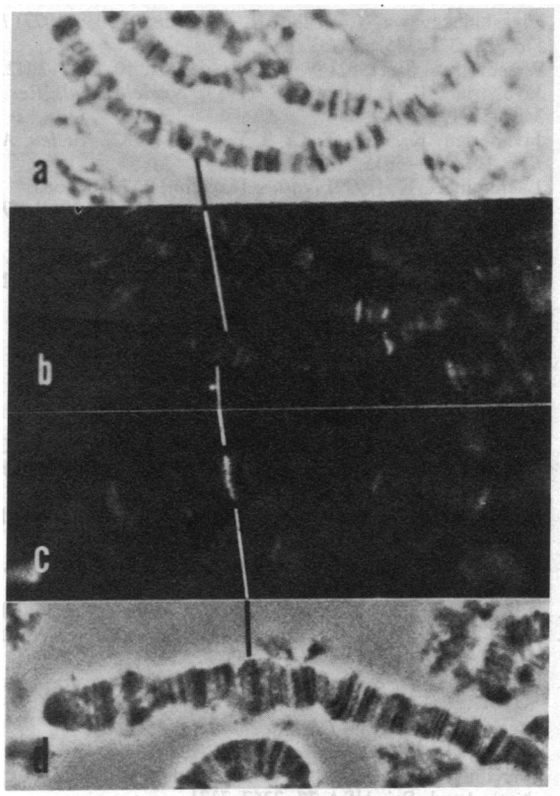

FIG. 5. Comparison of the occurrence of topoisomerase I in chromosomal position 3C11-12 of *D. melanogaster* strain BER-1 and Oregon R. Squashes from BER-1 (*a* and *b*) or Oregon R (*c* and *d*) were incubated with affinity-purified anti-topoisomerase I antibodies. (*b* and *c*) Fluorescence images: (*a* and *d*) phase-contrast images of *b* and *c*, respectively.

We have also used a well-characterized mutant of *Drosophila* to examine the nature of the correlation between the presence of topoisomerase I and transcription. The *Sgs-4* locus at position 3C11-12 of the X chromosome codes for one of the glue proteins used to attach the puparium to a surface (47, 48). The gene is normally expressed at high levels during puffing stage 1–2. In the BER-1 strain of *Drosophila*, this gene carries a deletion of ≈100 base pairs at a position ≈400–500 base pairs upstream from the mRNA initiation site. No transcript of the glue gene can be detected (49) nor are any other transcripts detected from the 16- to 19-kilobase region of this locus during third instar in BER-1 larvae (refs. 48 and 50; J. C. Eissenberg, personal communication). An analysis of the chromatin structure of this locus has shown that in the BER-1 larvae one fails to see the development of DNase I hypersensitive sites 5′ to the gene which are characteristic of the active state as observed in Oregon R larvae (51). In contrast to results obtained with Oregon R larvae, almost no RNA polymerase II can be detected by using immunofluorescence staining at this position in BER-1 larvae of the same developmental stage (52). Similarly, little staining was observed by using antibodies to topoisomerase I (Fig. 5). This result indicates that the localization of topoisomerase I as detected here is closely correlated with the actual transcription event in the process of gene activation.

DISCUSSION

Analysis of the distribution pattern of DNA topoisomerase I in the polytene chromosomes of *Drosophila* by using affinity-purified antibodies indicates a significant concentration of the enzyme at loci known to be transcriptionally active, including the nucleolus. This correlation with transcription is best demonstrated by examination of the heat shock puffs. The major heat shock loci *87A* and *87C* are not stained at all using chromosomes from control larvae but stain brightly in chromosomes from heat-shocked animals in which the gene is being transcribed at a high rate. The correlation can be confirmed by analysis of mutant loci. Staining with anti-topoisomerase I at locus *3C11–12* is severely reduced in BER-1 larvae (in which no transcription is detected) compared to that seen in Oregon R larvae (in which that gene is active).

It should be noted that in a detailed comparison of the distribution patterns of topoisomerase I and RNA polymerase II, one sees many similarities but does not see coincidence. Several factors may contribute to the differences observed. Comparisons are made by using fragments of one salivary gland to ensure that all cells are at the same developmental stage. Nonetheless, different cells may have slightly different patterns of transcription. In this assay it is the presence, rather than the activity of the enzyme, that is being detected; the timing of association/dissociation of various components of the transcription complex may vary. Lastly, topoisomerase I might be associated with loci transcribed by RNA polymerase III, which would not be stained by the antiserum against RNA polymerase II used here.

In considering the process of gene activation and transcription, two major roles have been suggested for topoisomerase I. First, the enzyme might be involved in necessary alterations of chromatin structure; second, it might be required for efficient transcription of the chromatin template *per se*. Recent evidence suggests that the chromatin structure at the 5′ end of active and/or activatable genes may reflect supercoiling of the DNA (53, 54). Studies of the extrachromosomal genes for rRNA (rDNA) of *Tetrahymena* have indicated the presence of a nuclease activity with analogies to topoisomerase I in the immediate neighborhood of the 5′ DNase I hypersensitive sites (55). In addition to such localized effects, several studies indicate that transcriptional activation requires a broad change in the configuration of the chromatin template to a form that is more sensitive to nucleases (56–61). Changes in the linking numbers of yeast plasmids that are related to the expression of genes residing on the plasmids have also been observed (62).

It might be supposed that structural changes of the type noted above would require relatively little topoisomerase I, with only one or a few molecules associated with each gene. However, the cellular concentration of topoisomerase I would appear to be in excess of such requirements. Liu and Miller (63) have estimated that there is one molecule of topoisomerase I for every 10 nucleosomes in the calf thymus nucleus; if the enzyme is localized in active regions, as seen here, the concentration will be even greater, perhaps one molecule per nucleosome. Although it is not possible to quantitate the staining reaction in the present study, the visual impression is that the staining of puffs is comparable to that obtained with anti-RNA polymerase II, suggesting the presence of many molecules per haploid genome at these loci. Indeed, the enzyme appears to be present in the *Drosophila* nucleus at a level roughly equivalent to that of RNA polymerase II (31). These considerations suggest that the bulk of the topoisomerase I observed in the present study plays a role in the transcriptional process itself. It should be possible to gain further insight into the roles of topoisomerase I by a similar immunocytological analysis of well-characterized mutants of *Drosophila*, in which a specific failure of gene activation and/or transcription can be attributed to a specific change in the DNA sequences at the locus. Such experiments can now be designed by utilizing *in vitro* alteration of a DNA sequence followed by P-factor-mediated transformation of *Drosophila* (64).

We thank Sue Abmayr for technical assistance, A. Greenleaf (Duke University) both for antibodies against *Drosophila* RNA

polymerase II and for a sample of the purified enzyme, and T.-s. Hsieh (Duke University) for a sample of DNA topoisomerase II. This work was supported by grants from the National Science Foundation, the National Institutes of Health, and the American Cancer Society (to S.C.R.E.) and by grants from the National Institutes of Health and the American Cancer Society (to J.C.W.). G.F. and G.P. were supported by fellowships from the Deutsche Forschungsgemeinschaft.

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