MOLECULAR AND CELLULAR BIOLOGY, Dec. 1987, p. 4308–4316
0270-7306/87/124308-09$02.00/0

Vol. 7, No. 12

Microinjection of Anti-Topoisomerase I Immunoglobulin G into Nuclei of *Chironomus tentans* Salivary Gland Cells Leads to Blockage of Transcription Elongation

ENDRE EGYHÁZI[1]* AND EGON DURBAN[2]

Department of Medical Cell Biology, Karolinska Institutet, S-104 01 Stockholm, Sweden,[1] and Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77030[2]

Received 22 June 1987/Accepted 4 September 1987

Purified anti-topoisomerase I immunoglobulin G (IgG) was microinjected into nuclei of *Chironomus tentans* salivary gland cells, and the effect on DNA transcription was investigated. Synthesis of nucleolar preribosomal 38S RNA by RNA polymerase I and of chromosomal Balbiani ring RNA by RNA polymerase II was inhibited by about 80%. The inhibitory action of anti-topoisomerase I IgG could be reversed by the addition of exogenous topoisomerase I. Anti-topoisomerase I IgG had less effect on RNA polymerase II-promoted activity of other less efficiently transcribing heterogeneous nuclear RNA genes. The pattern of inhibition of growing nascent Balbiani ring chains indicated that the transcriptional process was interrupted at the level of chain elongation. The highly decondensed state of active Balbiani ring chromatin, however, remained unaffected after injection of topoisomerase I antibodies. These data are consistent with the interpretation that topoisomerase I is an essential component in the transcriptional process but not in the maintenance of the decondensed state of active chromatin.

Topoisomerases are enzymes that modify the topological structure of DNA by strand breakage and rejoining (for reviews, see references 28 and 29). Type I topoisomerases catalyze unwinding of supercoiled plasmid DNA in steps of single turns and do not require an energy source or divalent cations as cofactors (28, 29).

The function of eucaryotic topoisomerase I is not yet established, although a role in transcriptional regulation is emerging. A topoisomerase I-like protein was observed near the 5′ end and 3′ end of the *Tetrahymena* DNA transcription unit coding for rRNA (16). Furthermore, topoisomerase I has been detected at loci of *Drosophila* polytene chromosomes that contain transcriptionally active genes; importantly, the sites of heat shock genes immunoreacted with affinity-purified topoisomerase I antibody after, but not before, heat shock induction (13). Photo-cross-linking studies showed that topoisomerase I is concentrated in regions of actively transcribed *Drosophila* genes and not on nontranscribed flanking regions (15).

Despite this suggestive evidence that topoisomerase I might be involved in activation of eucaryotic genes, no direct demonstration exists that topoisomerase I has an essential function in the transcription process.

In an attempt to gain information on a possible involvement of topoisomerase I in DNA transcription, we have injected topoisomerase I immunoglobulin G (IgG) into nuclei of living salivary gland cells from *Chironomus tentans* and analyzed its effect on DNA transcription. Analysis of the transcription of the large tissue-specific puffs, Balbiani rings, enabled us to distinguish between possible effects on RNA synthesis and chromatin structure. We show here that the rate of RNA polymerase I, as well as that of RNA polymerase II-promoted transcription, was inhibited after injection of anti-topoisomerase I IgG into the nuclei. The synthesis of nucleolar pre-rRNA and of Balbiani ring RNA was lowered by about 80%. The transcription of non-Balbiani ring heterogeneous nuclear RNA (hnRNA) genes was less affected.

MATERIALS AND METHODS

Polyclonal rabbit anti-topoisomerase I IgG. Rabbits were injected at 1-month intervals with 20 μg of topoisomerase I purified from Novikoff hepatoma cells (3a, 4). Before injection, topoisomerase I was thoroughly mixed with Freund adjuvant (complete in the first injection and incomplete in subsequent boosters). After the fifth injection, antibody titer could be detected by an enzyme-linked immunosorbent assay (21).

IgG fractions were prepared by addition of $(NH_4)_2SO_4$ to 33% saturation (14). Precipitated protein was collected by centrifugation, dissolved in 20 mM Tris hydrochloride (pH 8.0)–28 mM NaCl, and loaded on a DEAE-Affigel blue column (Bio-Rad Laboratories, Richmond, Calif.). The IgG-containing flow-through fractions were pooled and stored at −20°C. IgG fractions of preimmune sera collected from rabbits before injection were prepared by the same procedure.

Cross-reactivity of rat anti-topoisomerase IgG with topoisomerase I in nuclear extract of *Chironomus* salivary gland cells. Nuclei from about 100 glands were isolated (25) and subsequently extracted after resuspension and sonication in 100 μl of 40 mM Tris hydrochloride (pH 7.6)–10 mM $MgCl_2$–50 mM KCl–20 mM $(NH_4)_2SO_4$–2 mM dithiothreitol–2.6% glycerol–100 μg of bovine serum albumin per ml at 4°C for 30 min. After centrifugation, the supernatant was removed and used for the enzyme assay. Topoisomerase I assays were performed in a final volume of 10 μl, including extraction buffer and 2 to 5 μl of nuclear extract, at 30°C for 20 min after addition of supercoiled pBR322 plasmid DNA (Sigma) in the absence of ATP. Camptothecin (lactone form) (NSC 94600) was a gift from the Drug Synthesis and

* Corresponding author.

Chemistry Branch, Division of Cancer Treatment, National Cancer Institute. It was dissolved in dimethyl sulfoxide at 10 mM concentration. Dimethyl sulfoxide had no effect on the nuclear topoisomerase activity. The nuclear extract was treated with camptothecin at 25 to 125 μM.

Gel electrophoresis, immunoblots, and immunofluorescence microscopy. Extracts (0.35 M NaCl) of Novikoff hepatoma chromatin were fractionated with 50 to 85% saturated ammonium sulfate to achieve partial enrichment for topoisomerase I (3a, 4) and then were subjected to polyacrylamide gel electrophoresis. For isolation of *Chironomus* salivary gland proteins, 50 to 75 glands were excised and fixed in 70% ethanol for 30 min at 4°C (5). The cells were then released from the secretion and subsequently extracted with 0.5 ml of sample buffer (22) containing 5% mercaptoethanol by boiling for 3 min. Samples of 50 μl were used for gel electrophoresis.

Extracted proteins were separated by sodium dodecyl sulfate-polyacrylamide (7.5% acrylamide) gel electrophoresis by the method of Laemmli (22). Proteins were subsequently transferred electrophoretically to nitrocellulose paper (27). Nitrocellulose was blocked with 10% goat serum–3% bovine serum albumin, incubated with polyclonal antibody (1:10 dilution in blocking solution), washed with 50 mM Tris hydrochloride (pH 8.0)–150 mM NaCl–0.05% Tween-20, incubated with goat anti-rabbit IgG conjugated to alkaline phosphatase (1:5,000 diluted in blocking solution) (Promega), and finally developed with 5-bromo-4-chloro-3-indolyl phosphate and Nitro Blue Tetrazolium substrate dyes (Promega).

Chromosome IV was prepared from isolated nuclei and used in the indirect immunofluorescence staining assay essentially as previously described (11, 25, 26). The chromosomes were fixed for 0 to 20 s. After staining, the chromosomes were mounted in the presence of *p*-phenylenediamine to retard fading during microscopy (20).

Microinjection. Salivary glands were isolated from fourth-instar larvae of the dipteran *C. tentans* and explanted into modified Cannon medium. Injections of cell nuclei were carried out by means of the micromanipulation technique described by De Fonbrune (3). One salivary gland in a volume of modified Cannon medium (24) was placed on the under side of a cover slip and then inverted and placed on a glass plate with a groove (oil chamber). Liquid paraffin was then added in the groove between glass and cover slip. The microinjection was performed at room temperature and controlled through a Zeiss microscope. A pipette (1.5-μm tip diameter) with enough material to inject all the cells in one gland was introduced into the cell nucleus from below, piercing the cell and nuclear wall by pressing against the cover slip. When the pipette tip was visible inside the nucleus, a volume of liquid was injected by a hand-operated syringe. The injected fluid caused a transient swelling of the nucleus by 10% of the nuclear diameter; thus the injected volume was approximately 30% of the nuclear volume. The visual estimate of the injected volume, even if not quite precise, permits fast work, so that injection of one cell takes less than 1 min. As there are about 36 cells in each gland, one gland could be injected in about 30 min. As cells in a gland are not identical, we injected them in groups of three, alternating between immune and preimmune IgG and buffer alone. New pipettes were used for each solution. The fixed positions of the cells within the gland enabled us to keep track of immune- and preimmune-IgG-injected cells (10). Anti-topoisomerase I IgG and preimmune IgG were solubilized in 20 mM KCl, including 28 mM NaCl and 20 mM Tris hydrochloride (pH 8.0), and injected into the nuclei.

Labeling of RNA, microdissection, and electrophoresis of RNA. The injected gland was then incubated in 3 μl of Cannon medium containing the radioactive RNA precursor, added into the oil chamber. The microinjected gland was incubated with [α-^{32}P]ATP (600 to 700 Ci/mmol; New England Nuclear Research Products, Boston, Mass.) for 20 to 40 min at 18°C. After microinjection and subsequent incubation with the radioactive precursor, the gland was fixed in 70% ethanol for 30 min at 4°C, transferred to a mixture of ethanol and glycerol (1:1) for 60 min at 4°C, and finally placed on the under surface of a cover slip and covered with paraffin. The gland was continuously immersed in a small remaining volume of ethanol-glycerol. Two glass needles connected to the micromanipulator were used for the dissection. The nuclei were removed from the cells, and further subfractionation was carried out. Chromosomes I to III plus nuclear sap were collected, and nucleoli on chromosomes II and III were separated from the chromosome arms. Finally, Balbiani rings 1 and 2 were isolated from chromosome IV (5).

The pooled samples of each nuclear fraction were then transferred by one of the glass needles to a test tube and subsequently digested for 2 min at 20°C in 100 μl of a solution of 20 mM Tris hydrochloride buffer (pH 7.4)–0.5% sodium dodecyl sulfate (Serva, Heidelberg, Federal Republic of Germany)–0.1% proteinase K (Merck, Darmstadt, Federal Republic of Germany). A 20-μg sample of *Escherichia coli* RNA was added as a carrier, and the RNA was precipitated with 2.5 volumes of ethanol and stored at 4°C for about 18 h. The RNA precipitate was then sedimented, dissolved, and subjected to electrophoresis in 1% agarose gel slabs (12). At the end of the run, the gel slab was washed in 5% cold trichloroacetic acid for 1 h and finally with running water overnight. The gel was then dried and exposed to Kodak X-Omat AR film at −70°C with a light-intensifying screen. Dried gels and autoradiograms were scanned in a Shimadzu Dual-Wavelength Chromato-Scanner (model CS/930).

RESULTS

Polyclonal anti-topoisomerase IgG. The polyclonal anti-topoisomerase I IgG was monospecific for topoisomerase I in that it reacted with a single band of 110 kilodaltons (kDa) on immunoblots of nuclear Novikoff hepatoma extracts (Fig. 1). Preincubation of topoisomerase I with polyclonal IgG inhibited topoisomerase I enzymatic activity, whereas preimmune IgG had no effect on enzyme activity (Fig. 2). The polyclonal antibody raised against rat topoisomerase I cross-reacted with *Chironomus* topoisomerase I, as indicated by its reaction with a 130-kDa protein in *Chironomus* salivary gland cell extract (Fig. 3). This molecular mass is similar to that of *Drosophila* topoisomerase I, reported to be around 135 kDa (19). The second immunoreactive band of 50 kDa (Fig. 3, lane 1) is thought to be a topoisomerase I proteolytic product, several of which were also seen in the highly purified Novikoff hepatoma topoisomerase I preparation (Fig. 3, lane 2) with the major 110-kDa moiety. Topoisomerase I is known to be extremely sensitive to proteolytic cleavage in the course of protein extraction.

The *Chironomus* topoisomerase I activity and its cross-reactivity with anti-topoisomerase I IgG from Novikoff hepatoma were also examined in nuclear extracts derived from salivary gland cells. The rationale for this experiment was that topoisomerase I activity can be distinguished from that of topoisomerase II in a crude extract. This is because

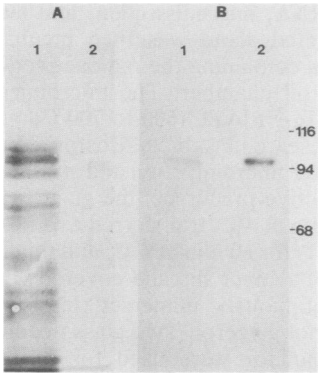


FIG. 1. Specificity of rabbit polyclonal topoisomerase I antibodies. Samples of 2 μg of purified topoisomerase I (lane 2) and 50 μg of a 50 to 85% ammonium sulfate-saturated fraction of a 0.35 M NaCl extract of Novikoff hepatoma chromatin (the first step in the purification procedure of topoisomerase I) (lane 1) were electrophoresed in 7.5% polyacrylamide-sodium dodecyl sulfate gels and transferred to nitrocellulose paper. (A) Nitrocellulose strip stained with amido black; (B) nitrocellulose strip incubated with rabbit polyclonal anti-topoisomerase I IgG, followed by incubation with ^{125}I-protein A and autoradiography.

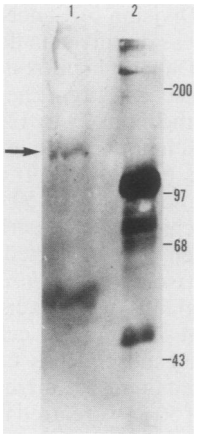


FIG. 3. Cross-reactivity of rat polyclonal anti-topoisomerase I IgG with *Chironomus* topoisomerase I. A 50-μl sample of extract of *Chironomus* salivary gland proteins was electrophoresed on a 7.5% polyacrylamide-sodium dodecyl sulfate gel (lane 1). Lane 2, Topoisomerase I (2 μg), purified from Novikoff hepatoma. Proteins were transferred to nitrocellulose paper and immunoreacted with polyclonal anti-topoisomerase I IgG. Immunoreactive proteins were visualized by incubation with goat anti-rabbit IgG conjugated to alkaline phosphatase, followed by incubation with substrate dyes (see Materials and Methods). Arrow points to the cross-reacting 130-kDa *Chironomus* protein, a tentative topoisomerase I.

the activity of topoisomerase I is ATP independent and selectively inhibited by the alkaloid camptothecin, whereas topoisomerase II is ATP dependent and resistant to camptothecin (17, 18). The relaxation of supercoiled pBR322 plasmid DNA after incubation with *Chironomus* nuclear extract is shown in Fig. 4A. The DNA relaxation after incubation in the absence of nuclear extract and with purified rat topoisomerase I is shown for comparison. As seen, the nuclear extract was capable of relaxing the supercoiled DNA essentially completely under our assay conditions (Fig. 4A). The introduction of 125 μM camptothecin or of 2 μl of anti-topoisomerase I IgG into the enzyme assay inhibited topoisomerase relaxation activity, as evidenced by appearance of the supercoiled form I pBR322 DNA (Fig. 4B). The relaxation activity of nuclear topoisomerase was lowered by more than 90% and by about 65%, respectively, by camptothecin and anti-topoisomerase I IgG. The addition of preimmune IgG to the relaxation assay did not seem to affect nuclear topoisomerase activity since the supercoiled form I pBR322 DNA was completely abolished (Fig. 4B). Thus the results in Fig. 4 indicate that the rat Novikoff hepatoma anti-topoisomerase I IgG is able to inhibit the relaxation activity of *Chironomus* topoisomerase I.

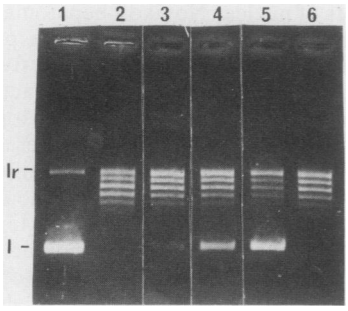


FIG. 2. Inhibition of topoisomerase I by anti-topoisomerase I IgG. Topoisomerase I (1.5 ng) was preincubated for 60 min on ice with various amounts of DEAE-Affigel-Blue-purified IgG, followed by addition of 0.1 μg of supercoiled pBR322 plasmid DNA and incubation at 30°C for 20 min. DNA relaxation was evaluated by agarose gel electrophoresis (4, 23). Lane 1, pBR322 DNA (I, supercoiled form; Ir, relaxed form); lane 2, relaxation in the presence of 1.5 ng of topoisomerase I and 2 μg of preimmune IgG; lanes 3 through 5, relaxation of DNA in the presence of 0.2, 0.6, and 2 μg of anti-topoisomerase I IgG, respectively; lane 6, relaxation in the presence of 1.5 ng of topoisomerase I (no antibodies).

The cross-reaction of anti-topoisomerase I IgG with endogenous topoisomerase I associated with transcriptionally active chromosome regions received further support from indirect immunofluorescence staining of isolated chromosome IV (Fig. 5). As seen, the staining with the anti-topoisomerase IgG was largely located in the Balbiani ring 1 and 2 regions (Fig. 5B). The condensed band regions were only slightly stained. The size of the puffs appeared to be correlated with the intensity of staining. It should be pointed out that Balbiani rings 1 and 2 on chromosome IV represent only about 0.5% of chromosome IV DNA (30) but manufacture more than 90% of chromosome IV RNA (7). Incubation of isolated chromosome IV with preimmune sera yielded a weak and unspecific staining over the whole chromosome (Fig. 5A).

Effects of microinjected anti-topoisomerase I IgG on transcription of rRNA and hnRNA genes. We have previously described DNA transcription in chromosomes, Balbiani rings, and nucleoli of *Chironomus* salivary gland cells. The synthesis of chromosomal hnRNA in the 16S to 100S range has been found to be sensitive to α-amanitin (9) as well as to 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (8) and is therefore considered to represent RNA polymerase II-based transcription. The nucleolar synthesis of preribosomal 38S RNA and its processing has also been established (24). For labeling of RNA synthesis, we have taken advantage of the fact that the plasma membrane of *Chironomus* salivary gland cells permits the penetration of ATP into the nucleus in an undegraded form and thus that labeled ATP can be used as a

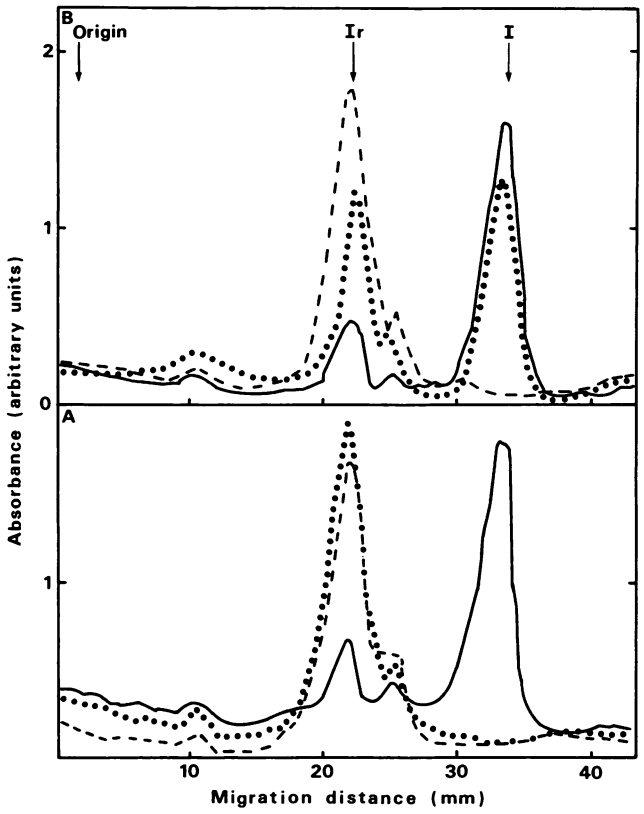


FIG. 4. Topoisomerase activity in *Chironomus* nuclear extract in the presence of camptothecin and of anti-topoisomerase I from rat Novikoff hepatoma. Nuclear extract was preincubated for 10 min at 20°C with 125 μM camptothecin or with 2 μl of anti-topoisomerase I IgG (1.5 mg/ml), followed by the addition of 0.1 μg of supercoiled pBR322 plasmid DNA and incubation at 30°C for 20 min. Topoisomerase I relaxation activity was also assayed in the presence of preimmune IgG and in the absence of any topoisomerase effector. (A) Solid line, DNA incubated in the absence of nuclear extract; dotted line, DNA incubated with nuclear extract; dashed line, DNA incubated with purified Novikoff hepatoma topoisomerase I without nuclear extract. (B) Solid line, DNA incubated with nuclear extract plus camptothecin; dotted line, DNA incubated with nuclear extract plus anti-topoisomerase I IgG; dashed line, DNA incubated with nuclear extract plus preimmune IgG. For other information, see the legend to Fig. 2 and Materials and Methods.

direct precursor for RNA synthesis (12). The electrophoretic pattern of ^{32}P-labeled hnRNA, derived from the nonnucleolar portion of the nucleus, shows the usual heterogenous distribution of label in the 16 to 100S range of the pattern (Fig. 6).

The electrophoretic pattern of normal nucleolar RNA is dominated by the label in preribosomal 38S RNA (Fig. 6A). The labeling of 38S RNA in cells injected with the anti-topoisomerase I IgG was reduced by more than 80% compared with nucleolar RNA from control cells. In contrast, the injection of purified IgG from a nonimmunized rabbit did not block the synthesis of 38S RNA. Thus the interference with the transcription of DNA coding for rRNA is a specific feature of the anti-topoisomerase I IgG. Figure 6B shows the electrophoretic distribution of hnRNA. As seen, anti-topoisomerase I IgG lowered hnRNA synthesis to a smaller extent than the labeling of rRNA, whereas the addition of IgG from nonimmunized animals did not interfere with hnRNA synthesis. The labeling of hnRNA derived from cells injected with anti-topoisomerase I IgG decreased by about 50% in comparison with that of hnRNA obtained after injection of nonimmune IgG.

The effect of anti-topoisomerase I IgG injected after 5-fold and 25-fold dilutions of the original stock solution of the antibodies (1.5 mg/ml) is shown in Fig. 7. Nonimmune IgG was used for injection of control cells. The introduction of 0.3 and 0.06 mg of antibodies per ml into the salivary gland nuclei diminished the labeling of nucleolar 38S RNA by about 75 and 70%, respectively (Fig. 7A). The incorporation of ^{32}P into hnRNA was diminished after injection of 0.3 and 0.06 mg of anti-topoisomerase I IgG per ml by about 50 and 43%, respectively. Thus the anti-topoisomerase IgG solution retained its ability to interfere with DNA transcription even at substantially lowered antibody concentrations. A 25-fold dilution of antibody solution resulted in less than a 10% drop in inhibitory efficiency. On the other hand, a significant portion of DNA transcription escaped inhibition even at the highest antibody concentration. Notably, the RNA polymerase I-mediated DNA transcription seemed more vulnerable to anti-topoisomerase I IgG than did transcription based on RNA polymerase II, and the differential inhibitory effect of anti-topoisomerase I IgG on the synthesis of nucleolar pre-rRNA (about 80%) and chromosomal hnRNA (about 50%) persisted even after the addition of diluted IgG.

Inhibitory action of anti-topoisomerase I IgG on transcription of rRNA genes, reversed by addition of topoisomerase I. We also examined the question of whether the inhibitory activity of microinjected topoisomerase IgG on transcription of DNA coding for rRNA could be modified by preincubation of the antibody with its homologous antigen before injection into gland nuclei. Equal volumes of anti-topoisomerase I IgG and rat Novikoff hepatoma topoisomerase I were combined, and the mixture was preincubated. The antigen-antibody mixture was then microinjected into one third of the nuclei of one gland, while the remaining two thirds cells in the gland were injected either with topoisomerase I or with medium only (Fig. 8). The analysis of ^{32}P incorporation into nucleolar RNA with [α-^{32}P]ATP revealed that coinjection of topoisomerase I with anti-topoisomerase I IgG was able to reverse the inhibitory effect of the antibody on rRNA labeling. The incorporation of ^{32}P into pre-rRNA

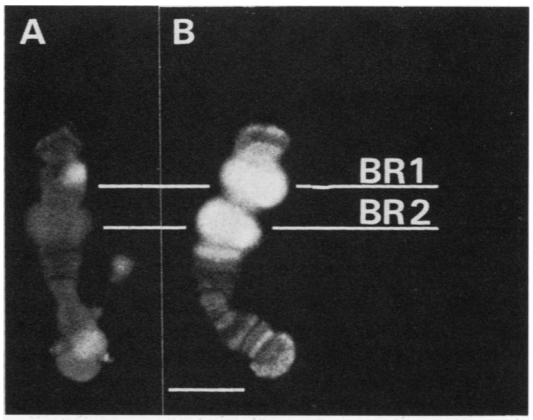


FIG. 5. Fluorescence distribution on isolated chromosome IV after indirect immunostaining with anti-topoisomerase I IgG (B) and preimmune IgG (A) and with fluorescein isothiocyanate-labeled swine anti-rabbit IgG. The chromosomes were immunostained without fixation. Bar, 14 μm.

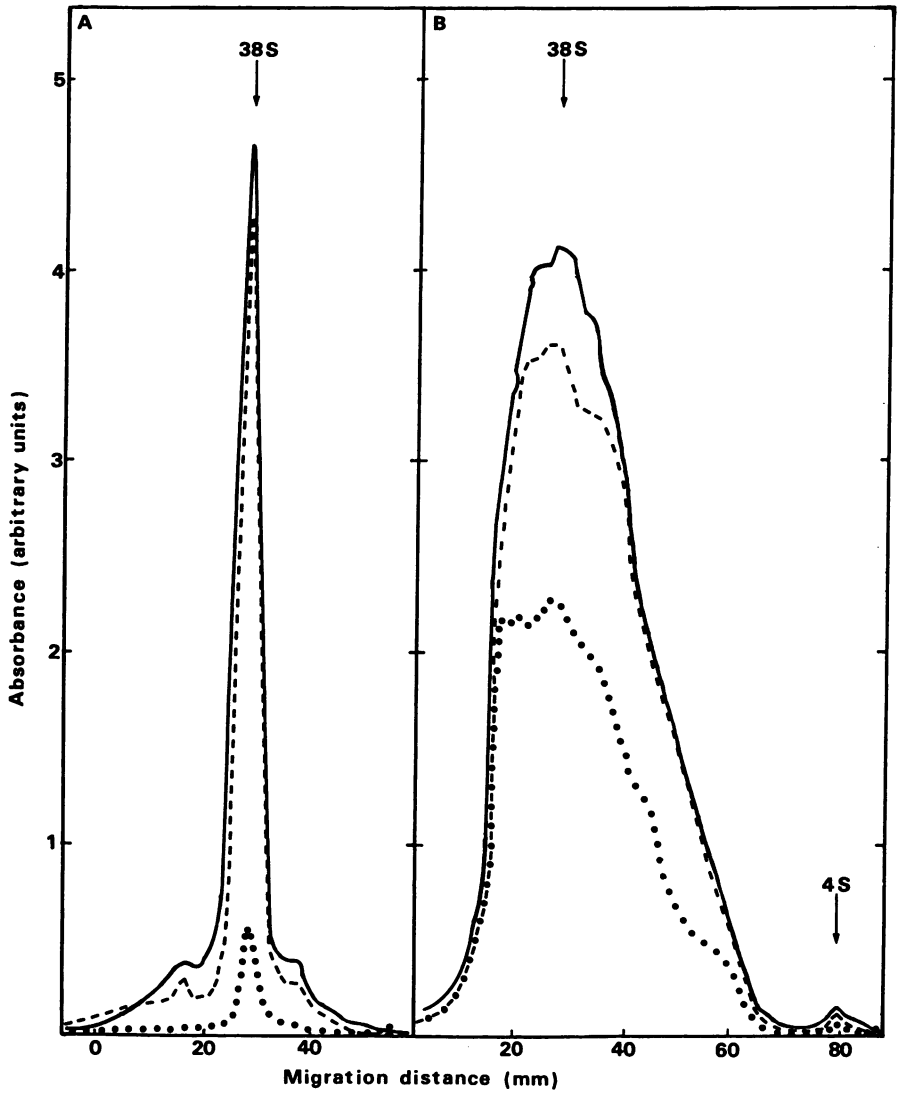


FIG. 6. Electrophoretic analysis of nucleolar pre-rRNA (A) and chromosomal hnRNA (B) labeled for 30 min after microinjection of anti-topoisomerase I IgG (dotted line), nonimmune IgG (dashed line), or medium (containing 20 mM KCl, 28 mM NaCl, and 20 mM Tris hydrochloride buffer) (solid line). Before being labeled with [α-^{32}P]ATP at 3 μM concentration, the injected gland was preincubated for 10 min in the absence of the isotopic precursor. About one third of the cells in a gland were injected with medium, whereas the other two thirds were injected with anti-topoisomerase I IgG (1.5 mg/ml) or nonimmune IgG (1.5 mg/ml). After labeling, the gland was fixed, chromosomes and nucleoli were dissected out, and the RNA was extracted and separated in a 1% agarose gel. For other data, see Materials and Methods.

derived from the antigen-antibody-treated cells was not significantly different from that obtained by analysis of control cells (Fig. 8). The microinjection of topoisomerase I alone stimulated the incorporation of ^{32}P into the nucleolar pre-rRNA by about 40%. In another similar experiment, the concentration of anti-topoisomerase I IgG in the preincubation mixture was increased fivefold (1.5 mg/ml) and the concentration of topoisomerase I was decreased twofold (0.025 mg/ml), and a volume of this new antigen-antibody solution was subsequently injected into nuclei. The outcome of this experiment showed that topoisomerase I, when added at a 10-fold lower antibody/antigen ratio, could no longer inactivate the coinjected antibody and could not abolish its inhibitory action on DNA transcription (data not shown). The rate of nucleolar pre-rRNA synthesis was found to be reduced by about 70%, which implied that the inhibitory action becomes similar to that obtained when anti-topoisomerase IgG was injected in the absence of topoisomerase I (Fig. 7).

Anti-topoisomerase IgG probably inhibits the rate of Balbiani ring transcription elongation. Owing to the complex nature of in vivo expression of hnRNA and rRNA and to the use of preincubation and relatively long labeling time, events reflecting initiation and elongation in the transcriptional process could not be distinguished. The main obstacle to the identification of specific transcriptional steps is the fact that the transcriptional and posttranscriptional events are superimposed in the course of the expression of most transcriptional units in living cells. This problem could be circumvented by the use of Balbiani ring transcription to elucidate the sequence of transcriptional events which are spacially separated in the nucleus. The electrophoretic pattern of Balbiani ring 1 and 2 RNA displays an asymmetrically distributed radioactivity peak which reflects simultaneous

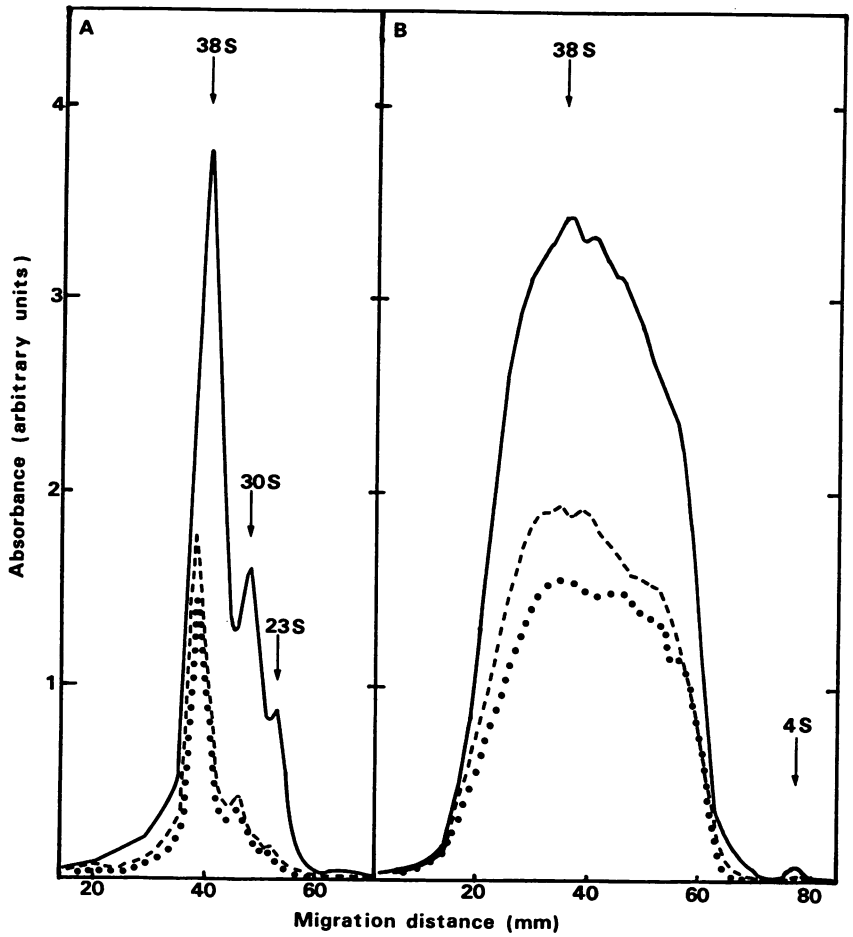
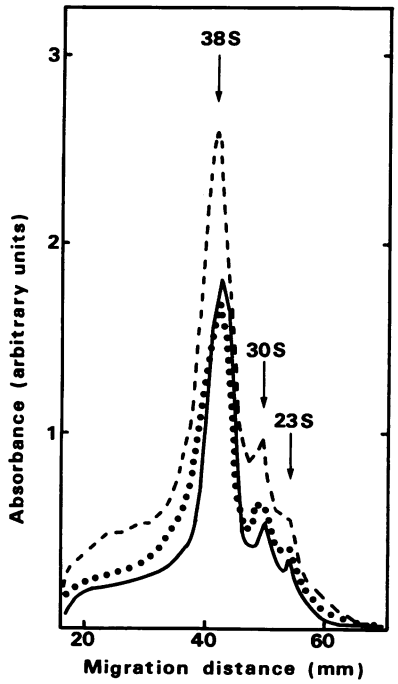


FIG. 7. Electrophoretic analysis of nucleolar pre-rRNA (A) and chromosomal hnRNA (B) labeled for 30 min after microinjection of 0.3 (dotted line) or 0.06 (dashed line) mg of anti-topoisomerase I IgG per ml or of 0.3 mg of nonimmune IgG (solid line). For other data, see the legend to Fig. 1 and Materials and Methods.



growth of multiple RNA chains from one or a few closely related transcription unit(s) (2, 7). The giant finished transcripts (75S RNA) leave the gene loci soon after completion. Thus, growing nascent RNA chains and the finished product can be separated after injection of the antibody.

To examine whether the inhibitory effect of the anti-topoisomerase I IgG on the transcription of Balbiani ring 1 and 2 genes was similar to that observed on total hnRNA synthesis, a gland was injected with anti-topoisomerase I IgG at 1.5 and 0.6 mg/ml. The gland was then incubated for 10 min in the absence of isotopic precursor, followed by 40 min of labeling with [α-^{32}P]ATP (Fig. 9A). A comparison between the electrophoretic profiles of Balbiani ring 1 and 2 RNAs from cells treated with the control IgG and from anti-topoisomerase I IgG-injected cells revealed a substan-

FIG. 8. Electrophoretic analysis of nucleolar pre-rRNA labeled for 45 min after microinjection of anti-topoisomerase I mixed with topoisomerase I (dotted line), of rat topoisomerase I (dashed line), or of medium alone (solid line). Equal volumes of 0.3-mg/ml anti-topoisomerase I IgG and 0.05-mg/ml topoisomerase I were combined, and the mixture was preincubated for 10 to 15 min. The antigen-antibody mixture was then microinjected into one third of the nuclei of one gland. The remaining cells in the gland were injected with either topoisomerase I or medium only. For other data, see Materials and Methods.

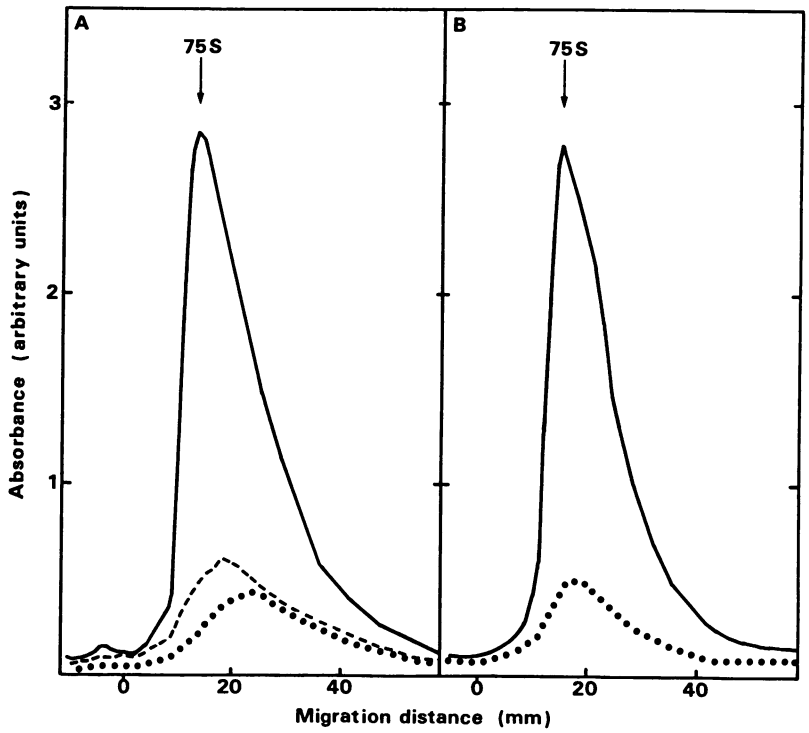


FIG. 9. Electrophoretic analysis of Balbiani ring 1 and 2 RNA after injection of anti-topoisomerase I IgG and nonimmune serum. (A) One third of the cells in a gland were injected with 1.5 mg of nonimmune serum per ml (solid line), and the other two thirds were injected with 1.5 (dotted line) or 0.03 (dashed line) mg of anti-topoisomerase I antibody per ml. The gland was preincubated for 10 min before the addition of [α-^{32}P]ATP and subsequent incubation for 40 min. (B) One third of the cells in another gland were injected with 0.03 mg of anti-topoisomerase I antibody per ml (dashed line), and another third of the cells were injected with the same amount of nonimmune serum (solid line). After injection, the gland was labeled for 20 min in the presence of [α-^{32}P]ATP. For other information, see the legend to Fig. 1 and Materials and Methods.

tial drop in RNA labeling in the latter cells (Fig. 9A). The distribution of the anti-topoisomerase I-resistant Balbiani ring 1 and 2 RNA retained its asymmetric appearance, and thus the pattern indicated that small RNA chains and large chains are inhibited to a similar extent. The degrees of Balbiani ring RNA inhibition with 1.5 and 0.06 mg of antibody per ml—80 and 72%, respectively—are almost identical with the inhibitory effect exerted on synthesis of pre-rRNA.

Figure 9B shows the effect of injected anti-topoisomerase I IgG on Balbiani ring transcription without preincubation of the salivary gland and with a shortened (20-min) labeling time. This modification of the experimental design made the system better suited to distinguish between effect(s) on the initiation and the elongation steps in the Balbiani ring 1 and 2 transcription. Even if the antibody and the radioactive RNA precursor were almost simultaneously added to the cells, the labeling in the low-molecular-weight range and that in the 75S RNA range were interrupted to a similar extent (Fig. 9B). This sequence of inhibitory events is distinctly different from that of the initiation inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole, which under similar experimental conditions causes a preferential inhibition of labeling of short RNA chains, making the normally asymmetric Balbiani ring 1 and 2 RNA pattern gradually symmetric (7). Rather, the action of anti-topoisomerase I IgG resembles that of the elongation inhibitor α-amanitin, which soon after addition to the cells suppresses hnRNA labeling uniformly in the whole molecular size spectrum of hnRNA (6). The result in Fig. 9, of course, does not exclude the possibility that transcription initiation and elongation are simultaneously inhibited.

The highly decondensed structural appearance of the Balbiani ring chromatin system makes it useful for studying the effect of anti-topoisomerase I IgG on the structure of active chromatin. Light microscopic inspection of Balbiani rings on chromosome IV, used in the experiment described in Fig. 9, could not reveal any significant modification in the decondensed state of giant puffs after the antibody regimen. This lack of effect on structural appearance was observed despite an about 80% reduction of the transcriptional activity.

DISCUSSION

Microinjection of anti-topoisomerase I IgG into nuclei of *Chironomus* salivary gland cells appears to interfere with nuclear DNA transcription, consistent with the interpretation that genes transcribed at the highest efficiency are interrupted preferentially irrespective of whether the transcribing RNA polymerase is of type I or II. In salivary glands, these genes include the ubiquitously expressed nucleolar rRNA genes as well as Balbiani ring genes, which are expressed in a tissue-specific manner. It is presently unclear why low-activity genes are less vulnerable to treatment with anti-topoisomerase I IgG. One possibility is that the antibodies are less accessible to topoisomerase I molecules associated with only partially decondensed hnRNA

chromatin than they are to topoisomerase I molecules that are located on highly unfolded and extended chromatin fibers in the nucleolus and Balbiani rings. The experimental results also suggest that DNA transcription per se is dependent on continuous topoisomerase I activity rather than on events involved in maintaining transcribing genes in an activated (decondensed) state.

Giant polytene nuclei with easily recognizable chromosome morphology permit a controlled performance of microinjection under a phase microscope. The standard medium for injection, 20 mM KCl, was supplemented with anti-topoisomerase I IgG or with preimmune IgG including 28 mM NaCl and 20 mM Tris hydrochloride (pH 8.0). The introduction of exogenous substances into cell nuclei always raises the question about artifactual effects on chromatin structure and nuclear metabolism. The appearance and transcription of the highly expanded giant Balbiani ring puffs are sensitive indicators of disturbances in chromosome function. Visual inspection of salivary gland nuclei after injection of salt medium with or without antibody, or with other control proteins like casein and actin (10), did not reveal any significant alteration in the morphology of polytene chromosomes and of the Balbiani rings. Likewise, no effect of the salt medium on DNA transcription was detected with or without control proteins. Furthermore, injection of topoisomerase I stimulated the transcriptional activity, and the inhibitory action of anti-topoisomerase I IgG on nuclear DNA transcription could be reversed by coinjection of antibody with topoisomerase I. Thus, our data taken together strongly suggest that inhibition of transcriptional activity of RNA polymerase I-based rRNA genes and of RNA polymerase II-based Balbiani rings by microinjected purified anti-topoisomerase I IgG reflects interference with the function of chromatin-associated endogenous topoisomerase I due to antigen-antibody cross-reaction (Fig. 3 through 5). The suppression of topoisomerase I activity appears to stop or highly reduce the rate of ongoing relaxation of supercoiled transcribing DNA sequences, thereby hampering RNA polymerase movement along the DNA helix.

An interaction between topoisomerase I and the transcriptional machinery is also consistent with previous reports demonstrating the association of topoisomerase I with transcriptionally active genes on *Drosophila* polytene chromosomes (13) and with actively transcribed DNA sequences of *Drosophila* cells (15). In addition, topoisomerase I and topoisomerase II were found to be required for rRNA synthesis in a yeast double deletion mutant (*top1 top2* [1]). Our own results focus on the functional role of topoisomerase I and are a first direct demonstration that topoisomerase I is necessary for nuclear DNA transcription.

ACKNOWLEDGMENTS

This investigation was supported by the Swedish Cancer Society, by the Karolinska Institutet (Reservationsanslaget) (to E.E.), and by the Robert A. Welch Foundation (to E.D.).

LITERATURE CITED

1. **Brill, S. J., S. DiNardo, K. Voelkel-Meiman, and R. Sternglanz.** 1987. Need for DNA topoisomerase activity as a swivel for DNA replication and for transcription of ribosomal RNA. Nature (London) **326**:414–416.
2. **Daneholt, B.** 1972. Giant RNA transcript in a Balbiani ring. Nature (London) New Biol. **240**:229–232.
3. **De Fonbrune, P.** 1949. Technique de micromanipulation. Monographies de l'Institut Pasteur. Masson, Paris.

3a. **Durbane, E., J. S. Mills, D. Roll, and H. Busch.** 1983. Phosphorylation of purified Novikoff hepatoma topoisomerase I. Biochem. Biophys. Res. Commun. **111**:897–905.

4. **Durban, E., D. Roll, G. Beckner, and H. Busch.** 1981. Purification and characterization of a nuclear DNA-binding phosphoprotein in fetal and tumor tissues. Cancer Res. **41**:537–545.
5. **Edström, J.-E.** 1964. Microextraction and micro electrophoresis for determination and analysis of nucleic acids in isolated cellular units, p. 417–447. *In* D. M. Presscott (ed.), Methods in cell physiology, vol. 1. Academic Press, Inc., New York.
6. **Egyházi, E.** 1974. A tentative initiation inhibitor of chromosomal heterogeneous RNA synthesis. J. Mol. Biol. **84**:173–183.
7. **Egyházi, E.** 1975. Inhibition of Balbiani ring RNA synthesis at the initiation level. Proc. Natl. Acad. Sci. USA **72**:947–950.
8. **Egyházi, E., B. Daneholt, J.-E. Edström, B. Lambert, and U. Ringborg.** 1970. Differential inhibitory effect of a substituted benzimidazole on RNA labeling in polytene chromosomes. J. Cell Biol. **47**:516–520.
9. **Egyházi, E., B. D'Monte, and J.-E. Edström.** 1972. Effects of α-amanitin on *in vitro* labeling of RNA from defined nuclear components in salivary gland cells from *Chironomus tentans*. J. Cell Biol. **53**:523–531.
10. **Egyházi, E., and A. Pigon.** 1986. Selective repression of RNA polymerase II by microinjected phosvitin. Chromosoma **94**:329–336.
11. **Egyházi, E., A. Pigon, A. Ossoinak, M. Holst, and U. Tayip.** 1984. Phosphorylation of some non-histone proteins in active genes is blocked by the transcription inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB). J. Cell Biol. **98**:954–962.
12. **Egyházi, E., A. Pigon, and L. Rydlander.** 1983. 5,6-Dichlororibofuranosylbenzimidazole inhibits the rate of transcription initiation in intact *Chironomus* cells. Eur. J. Biochem. **122**:445–451.
13. **Fleischmann, G., G. Pflugfelder, E. K. Steiner, K. Javaherian, G. C. Howard, J. C. Wang, and S. C. R. Elgin.** 1984. Drosophila DNA topoisomerase I is associated with transcriptionally active regions of the genome. Proc. Natl. Acad. Sci. USA **81**:6958–6962.
14. **Garvey, J. S., N. E. Cremer, and D. H. Sussdorf.** 1977. Methods in immunology, 3rd ed. W. A. Benjamin, Inc., Reading, Mass.
15. **Gilmour, D. S., G. Pflugfelder, J. C. Wang, and J. T. Lis.** 1986. Topoisomerase I interacts with transcribed regions in Drosophila cells. Cell **44**:401–407.
16. **Gocke, E., B. J. Bonven, and O. Westergaard.** 1983. A site and strand specific nuclease activity with analogies to topoisomerase I frames the rRNA gene of Tetrahymena. Nucleic Acids Res. **11**:7661–7678.
17. **Hsiang, Y.-H., R. Hertzberg, S. Hecht, and L. F. Liu.** 1985. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J. Biol. Chem. **260**:14873–14878.
18. **Hsieh, T.-S., and D. Brutlag.** 1980. ATP-dependent DNA topoisomerase from D. melanogaster reversibly catenates duplex DNA rings. Cell **21**:115–125.
19. **Javaherian, K., Y.-C. Tse, and J. Vega.** 1982. Drosophila topoisomerase I: isolation, purification and characterization. Nucleic Acids Res. **10**:6945–6955.
20. **Johnson, G. D., and G. M. de C Nougueira Aranjo.** 1981. A simple method of reducing the fading of immunofluorescence during microscopy. J. Immunol. Methods **43**:349–350.
21. **Kelsey, D. E., R. K. Busch, and H. Busch.** 1981. An enzyme immunoassay for the detection of human tumor nucleolar antigens. Cancer Lett. **12**:295–305.
22. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
23. **Liu, L. F.** 1983. HeLa topoisomerase I. Methods Enzymol. **100**:133–137.
24. **Ringborg, U., and L. Rydlander.** 1971. Nucleolar derived ribonucleic acid in chromosomes, nuclear sap and cytoplasm of *Chironomus tentans* salivary gland cells. J. Cell Biol. **51**:355–368.
25. **Sass, H.** 1980. Features of in vitro puffing and RNA synthesis in

polytene chromosomes of *Chironomus*. Chromosoma **78:**33–78.
26. **Sass, H.** 1982. RNA polymerase B in polytene chromosomes: immunofluorescent and autoradiographic analysis during stimulated and repressed RNA synthesis. Cell **28:**269–278.
27. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of protein from polyacrylamide to nitrocellulose sheets: procedures and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
28. **Vosberg, H.-P.** 1985. DNA topoisomerase: enzymes that control DNA conformation. Curr. Top. Microbiol. Immunol. **114:** 19–102.
29. **Wang, J. C.** 1985. DNA topoisomerase. Annu. Rev. Biochem. **54:**665–697.
30. **Wieslander, L.** 1979. Number and structure of Balbiani ring 75 S RNA transcription in *Chironomus tentans*. J. Mol. Biol. **134:** 347–367.