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Expression of the *Drosophila* Type II Topoisomerase Is Developmentally Regulated[†]

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ABSTRACT: The expression of the type II topoisomerase from *Drosophila melanogaster* was studied during development and in tissue culture cells. RNA blot and protein blot analyses using probes specific for *Drosophila* topoisomerase II show that the enzyme is developmentally regulated. Levels of both RNA transcript and protein appear highest during early embryogenesis and pupation, periods which are known to have the highest mitotic activity. Tissue culture analysis using *Drosophila* K_c cells supports these results as levels of topoisomerase II message are higher in rapidly dividing cells than in quiescent cells. Analysis of topoisomerase II levels in early embryos suggests that levels are adequately high for the enzyme to act in DNA replication or segregation at termination of replication. Apparent *in vivo* proteolysis of topoisomerase II is seen throughout the life cycle, in spite of careful precautions. Whether these proteolytic fragments are important *in vivo* is still uncertain.

Topoisomerases are enzymes that are found in both prokaryotes and eukaryotes and are involved in maintaining the topology of DNA [for recent reviews, see Wang (1985) and Vosberg (1985)]. There are two types of topoisomerases, defined by their mechanism of action. Type I topoisomerases make a transient nick in DNA, allowing the broken strand to swivel around the intact strand to change the twist of the DNA. Type II topoisomerases cause a double-strand break in DNA and allow a nearby helix to pass through the broken helix to change the writhe of the DNA. Most type II enzymes require ATP for their action. Both enzymes make a change in the net supercoiling of the DNA. In prokaryotes, topoisomerases regulate the supercoil density of the DNA. In *Escherichia coli*, the type I topoisomerase removes negative supercoils, and the type II topoisomerase (DNA gyrase) induces negative supercoils in DNA. In eukaryotes, no purified supercoiling activity has been identified; however, both type I and type II topoisomerases can remove supercoils. In fact, eukaryotic topoisomerases can remove both negative and positive supercoils.

Whereas the functions of the prokaryotic enzymes are fairly well understood, relatively little is known about the function(s) of the eukaryotic topoisomerases. Double mutants in yeast show that there is an absolute requirement for topoisomerase II. The topoisomerase II requirement appears to be involved

in replication termination (segregation) and medial nuclear division (Goto & Wang, 1985; Holm et al., 1985; DiNardo et al., 1983; Uemura & Yanagida, 1984). A class of drugs known as epipodophylotoxins has been used to study the *in vivo* role of topoisomerase II by trapping topoisomerase II-DNA complexes via a reversible covalent intermediate. Nelson et al. (1986) used teniposide (an epipodophylotoxin) to show that, in mammalian cells, topoisomerase II is associated with nascent DNA fragments near DNA replication forks, and Heck and Earnshaw (1986), using immunofluorescence labeling, showed that the appearance of topoisomerase II exactly parallels the onset of DNA replication in chicken cell lines. In addition to an enzymatic role, topoisomerase II has also been suggested to play a structural role. Berrios et al. (1985) have suggested that topoisomerase II is a component of the nuclear matrix in *Drosophila*. Earnshaw and Heck (1985), Earnshaw et al. (1985), and Gasser et al. (1986) have shown that topoisomerase II acts as a chromosomal scaffold protein both in chicken and in HeLa cells.

Other work has suggested a role for DNA topoisomerases in regulating transcription. Ryoji and Worcel (1984, 1985) described two types of chromatin in *Xenopus* oocytes. These types were called "dynamic" and "static". Dynamic chromatin is found to be torsionally constrained and was shown to be the transcriptionally active form of chromatin. Later, Kmiec and Worcel (1985) found that TFIIIA, the positive transcription factor of the 5S RNA gene, is required to make and maintain dynamic chromatin and confers a gyrase-like activity on the

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endogenous type II topoisomerase.

In prokaryotes, the dynamic superhelical state of the chromosome is maintained by the balance of activities of DNA gyrase (type II topoisomerase) and ω (type I topoisomerase) (Gellert et al., 1983). The DNA gyrase genes have been shown to be autoregulated by a homeostatic mechanism that maintains the superhelical density of the bacterial chromosome (Menzel & Gellert, 1983).

Here, we describe an analysis of the developmental expression of topoisomerase II in the life cycle of *Drosophila melanogaster* and suggest a correlation with cell division. With the availability of cDNA clones for the topoisomerase gene and polyclonal antibodies against purified topoisomerase II (Heller et al., 1986; personal communication), we have probed the expression of topoisomerase II during *Drosophila* development and in tissue culture cell growth at the level of RNA and protein. We have found that levels of topoisomerase II message and protein are highest during early embryogenesis and pupation, which are developmental stages with increased mitotic activity. Comparison of actively dividing K_c tissue culture cells with quiescent cells shows that levels of topoisomerase II message are higher in the actively dividing cells.

MATERIALS AND METHODS

Drosophila Staging and Collection. All studies were conducted with the Oregon-R strain of *Drosophila melanogaster*. RNA and crude protein extracts were collected from 13 stages in *Drosophila* development. Eight 3-h collections spanning embryogenesis were made on grape media plates and incubated for an appropriate length of time at 25 °C to obtain samples ranging from 0–3 to 21–24 h. Embryos were collected on grape media plates for 6 h and allowed to incubate at 25 °C for 27 h to obtain first-instar larvae. Second- and third-instar larvae, pupae, and adults were collected from corn media cages that were inoculated with dechorionated, 0–6-h-old embryos. Staging for second instar was by collection after 57 h of incubation. Wall crawlers were used for third-instar collections. Pupae were separated from third-instar larvae by flotation in water. Adults were collected after 2 days of growth. All stages were washed in 0.01% Triton and 0.1 M NaCl prior to processing.

Developmental Analysis. Stages during *Drosophila* development were collected, and extracts were prepared at a ratio of 1:5 w/v in 6.4 M guanidine hydrochloride, 0.1 M NaOAc, pH 5.2, 2 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl),¹ pH 7.5, 0.4 mM MgOAc₂, and 0.1% Triton using a Wheaton Dounce. RNA was collected from these extracts by the method of Chirgwin et al. (1979). All RNA samples were analyzed by spectrophotometry (A_{260}/A_{280}) and gel electrophoresis for purity and extent of degradation. RNA samples were heated at 50 °C for 1 h in 5 mM sodium phosphate, pH 6.8, 0.625 M glyoxal, and 50% DMSO. The RNA was then electrophoresed until the bromphenol blue dye front had run out 8 cm in a 1.0% agarose gel using a recirculating buffer system containing 0.04 M Tris-acetate and 0.001 M EDTA (1× TAE). RNA was then transferred to Gene-Screen Plus (New England Nuclear) by electroblotting in a Hoefer TE42 transfer electrophoresis cell at 10 V for 1 h followed by 40 V for an additional 2 h. After

transfer, the filter was washed 15 s in 50 mM NaOH and 30 s in 1× SSC and baked for 1 h at 80 °C in a vacuum oven. The filter was prehybridized at 42 °C for 18 h in 50% formamide, 4× SSC, 1% SDS, 50 mM sodium phosphate, pH 6.5, 5× Denhardt's solution (Denhardt, 1966), 0.5 mg/mL denatured salmon sperm DNA, and 1% glycine and then hybridized for 15 h in 50% formamide, 4× SSC, 1% SDS, 20 mM sodium phosphate, pH 6.5, 1× Denhardt's solution, 0.1 mg/mL denatured salmon sperm DNA, an 2×10^7 cpm/mL probe. The probe for topoisomerase II was a 1.3 kbp cDNA from the 3' region of the gene (Heller, personal communication). The probe for the cytoplasmic actin gene was from a 0.64 kbp clone from the 3' end so that the DNA was unique for the one species of actin (Fyrberg et al., 1983). Both DNA fragments were labeled with [α -³²P]dCTP (Amersham) by the method of Feinberg and Vogelstein (1983). The filter was then washed 3 times in 0.1% SDS and 2× SSC for 5 min at room temperature and 2 times in 0.1% SDS and 0.1× SSC for 15 min at 60 °C. Finally, the filter was wrapped in Saran Wrap and exposed to preflashed Kodak X-Omat film at –80 °C using a single Dupont Cronex Lightning-Plus intensifying screen.

Tissue Culture Analysis. *Drosophila* K_c cells were grown in Eschalier's D₂₂ media (Eschalier & Ohanessian, 1969) to densities of 4×10^6 , 8×10^6 , and 12×10^6 cells/mL as determined by counting on a hemacytometer. The first two concentrations reflect logarithmic growth (doubling time = 20–24 h, and the last reflects stationary growth (saturation of culture). Cells were collected by a low-speed centrifugation from 500-mL, spinner-flask cultures and processed for RNA according to the method described above. RNA from the K_c cells was poly(A)⁺ selected on an oligo(dT) (Collaborative Research) column by loading samples in 0.5 M NaCl and eluting the poly(A)⁺ RNA in quartz, doubly distilled, DEPC-treated water. The poly(A)⁺ RNA was ethanol precipitated and redissolved in DEPC-treated water. Appropriate amounts of poly(A)⁺ RNA (as determined by spectrophotometry) were heated at 50 °C for 60 min in 5 mM sodium phosphate, pH 6.8, 0.7 M glyoxal, and 50% DMSO. The RNA was electrophoresed until the bromphenol blue dye front had run out 8 cm on a 0.8% agarose gel in a recirculating buffer containing 10 mM sodium phosphate, pH 6.8. The poly(A)⁺ RNA was then capillary blotted to aminothiophenol (ATP) paper in 0.2 M NaOAc, pH 4.0. ATP paper was prepared by the method of Wahl et al. (1979) and activated just before use in 1.2 M HCl and 0.027% NaNO₂. The filter was prehybridized, hybridized using the topoisomerase II cDNA as a probe, and washed as described above. After exposure to preflashed Kodak X-Omat film, the filter was stripped of probe by incubating at 65 °C for 60 min in 98% formamide. The filter was then reprobed under identical conditions using the cytoplasmic actin cDNA fragment. The data for both the developmental RNA and tissue culture RNA analyses were scanned by densitometry using a Quick Scan Jr. (Helena Laboratory) densitometer.

Protein Analysis. Crude extracts of all the *Drosophila* stages were made by using a 7-mL Wheaton Dounce 5× with a loose pestle and 5× with a tight pestle in a 1:2 mass:volume ratio of 10% glycerol, 5% 2-mercaptoethanol, 3% SDS, 0.125 M Tris-HCl, pH 7.2, and 5 M urea (buffer A). Extracts were immediately frozen in liquid nitrogen and stored at –80 °C until ready for use. The crude extracts were thawed on ice and centrifuged for 2 min in an Eppendorf centrifuge. Aliquots of the supernatant were removed and extracted 3 times in an equal volume of chloroform and methanol (3:1 v/v), being careful to leave the interface intact. Samples were dried down

¹ Abbreviations: DEPC, diethyl pyrocarbonate; DMSO, dimethyl sulfoxide; EDTA, disodium ethylenediaminetetraacetate; kbp, kilobase pair(s); SDS, sodium dodecyl sulfate; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; 1× SSC, 0.15 M NaCl and 0.015 M sodium citrate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; PMSF, phenylmethanesulfonyl fluoride; kDa, kilodalton(s); bp, base pair(s).

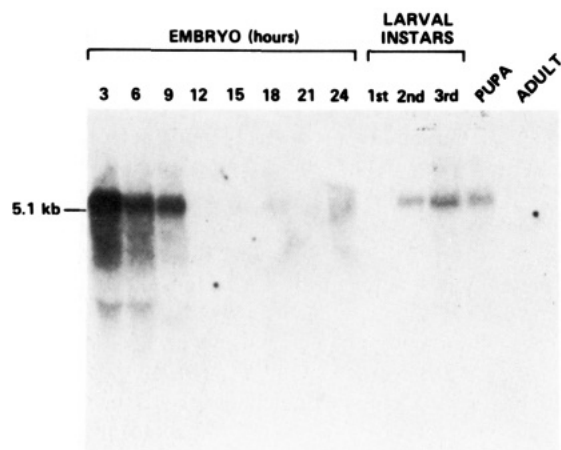


FIGURE 1: Changes in levels of *Drosophila* topoisomerase II message during development. Each lane contains 50 μ g of total RNA. RNA was extracted, purified, run out on a glyoxal gel, transferred to Gene-Screen Plus, and probed as described under Materials and Methods. Lanes marked 3, 6, 9, 12, 15, 18, 21, and 24 represent time spans of 0–3, 3–6, 6–9, 9–12, 12–15, 15–18, 18–21, and 21–24 h during embryogenesis, respectively.

in a SpeedVac (Savant) and redissolved in SDS-PAGE loading buffer as described by Laemmli (1970). In an alternative procedure, crude extracts were made in 10% glycerol, 5% 2-mercaptoethanol, 0.05% SDS, 62.5 mM Tris-HCl, pH 6.8, 5 M urea, 10 mM $\text{Na}_2\text{S}_2\text{O}_5$, and 1 mM phenylmethanesulfonyl fluoride (PMSF) (buffer B) so that protein concentrations could be determined. Samples were extracted and dried down as in the previous procedure and redissolved in buffer B. Aliquots were removed and precipitated in 5 volumes of acetone for 15 min at -20°C , spun for 10 min in an Eppendorf centrifuge, and redissolved in loading buffer. Samples were heated at 90°C for 2 min and electrophoresed along with prestained protein molecular weight standards (Bethesda Research Laboratories) on 10% acrylamide gels (Studier, 1973) at 10 mA for the stacking gel and 30 mA for the resolving gel for a distance of 12 cm. The protein was then transferred to nitrocellulose (Schleicher & Schuell) for 12 h by electroblotting in a Hoefer apparatus in 20 mM Trizma, 150 mM glycine, and 20% methanol (Burnette, 1981). The protein blots were probed with a 1:1000 dilution of a rabbit anti-topoisomerase II polyclonal antiserum [995; the antiserum was made against the purified topoisomerase II by Heller et al. (1986)]. The filter was then probed with ^{125}I -labeled protein A (ICN) and autoradiographed. Analysis with pre-immune serum (1:1000 titer) was identical with the procedure above.

RESULTS

***Drosophila* Topoisomerase II Gene Is Temporally Modulated.** Topoisomerase II temporal regulation was studied at the level of transcription. RNA was collected from various stages of *Drosophila* development, subjected to polyacrylamide gel electrophoresis under denaturing conditions, and transferred to Gene-Screen Plus for analysis. The RNA was probed with a cDNA clone (coding for the 3' end of the gene) from a λ gt10 library which was labeled to high specific activity by the random hexamer annealing reaction (see Materials and Methods). The developmental profile of the topoisomerase II message is shown in Figure 1.

A band is seen with an apparent length of 5.1 kbp in agreement with previous results (Heller, personal communication). It also appears to be the only transcript made as no other bands are apparent. A cDNA from the 5' end of the

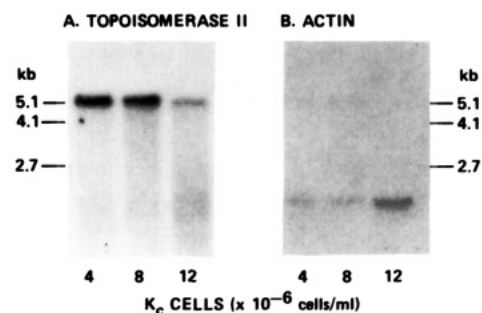


FIGURE 2: Variations in levels of topoisomerase II and actin message in *Drosophila* K_c tissue culture cells. Each lane contains 10 μ g of poly(A)⁺-selected RNA. RNA was extracted, purified, poly(A)⁺-selected, run out on a glyoxal gel, transferred to ATP paper, and probed as described under Materials and Methods.

topoisomerase II gene was also used as a probe; there is no partial transcription, as the same length RNA was detected (data not shown).

The developmental profile of topoisomerase II shows striking fluctuations in the levels of message. Early embryos contain a very high level of topoisomerase II message. This level of message rapidly decreases about 100-fold at mid-embryogenesis and begins increasing in amount during late embryogenesis to about one-tenth the level of early embryogenesis. No message was detected in first-instar larvae, but an increase in signal comparable to late embryogenesis was observed throughout second instar and third instar with levels plateauing in the pupal stage. No topoisomerase II message was detected in adults. The high levels of RNA in early embryos are presumed to be due to the presence of maternal transcripts. The levels of message for topoisomerase II appear to correlate with cell division, with levels of transcript being highest during periods when cell division is the most frequent. The levels of message in the later stages are more difficult to interpret as cell division becomes tissue specific and is highly asynchronous for the organism.

Correlation of Topoisomerase II Regulation with Cell Division. RNA was collected from three points in the growth cycle of *Drosophila* K_c (Echalier & Ohanessian, 1969) tissue culture cells. Two time points represented logarithmic growth of the culture, in which the cells divided about every 20–24 h. The last time point represented a saturated culture in which cell division had decreased dramatically (no increase in cell density was observed over a prolonged time period). The RNA from these cultures was poly(A)⁺ selected, subjected to electrophoresis, and transferred to aminothiophenol (ATP) paper. The same probes were used as described in the previous section. Results are shown in Figure 2.

The level of message for topoisomerase II remains constant for the log growth phase but drops more than 2-fold when the culture is saturated. On the basis of the number of cells that were required to load 15 μ g of poly(A)⁺ RNA on a gel for each sample, the calculated change in the amount of message per cell also stays constant when the two time points are compared during log growth but drops at least 10-fold when the culture becomes saturated. This change is reflected in a drop from approximately 10–20 transcripts/cell to about 1 transcript/cell.

Comparison of Topoisomerase II Message Levels with Cytoplasmic Actin Message. Although equal amounts of RNA as determined by spectrophotometric analysis [whether it was total RNA or poly(A)⁺-selected RNA] were electrophoresed, the changes in levels of topoisomerase II message might still be due to problems such as total RNA degradation. A probe for the cytoplasmic actin gene whose developmental

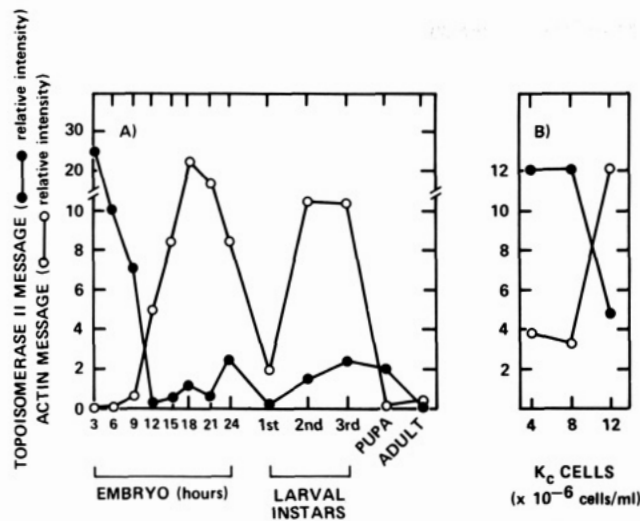


FIGURE 3: Comparison of levels of message for topoisomerase II and actin during development and in tissue culture growth. Panel A is a plot of densitometric scans of levels of topoisomerase II and actin during development. Panel B represents the same type of scans for message levels in K_c tissue culture cells. Relative intensities of the two analyses were normalized to the same values for convenience and do not reflect absolute differences. Although this figure represents a scan only of data shown in this paper, two other separate RNA collections analyzed for both topoisomerase II and actin showed the same profile except that one of the profiles shows a shift in the region near late embryogenesis and first-instar larvae, due to variations in staging.

profile has already been reported (Fyrberg et al., 1983) was used to compare message levels to those for topoisomerase II. Identical experiments were done as reported above to standardize the changes in topoisomerase II message levels to changes in the actin message levels. The comparison is shown in graphic form in Figure 3. These graphs are based on densitometry analysis of Figures 1 and 2 and developmental Northern blots probed with the actin DNA. Care was taken to scan film that was exposed at the linear range of its capacity. The profile for actin message, while not identical with published data, is qualitatively identical. Separate collections of RNA analyzed for actin message also give some variance in signal. While no direct comparison can be made between the levels of message for actin and topoisomerase II, relative values can be illustrated. (On the basis of the length of time needed to detect a signal for both messages and length of homology, the actin message is at least 10-fold more abundant.) There are strong differences in message levels for actin and topoisomerase II. This is especially evident in early embryos, where the highest levels of topoisomerase II message and the lowest levels of actin message are seen during development. This would argue that the changes in the levels of message for topoisomerase II are real and specific for topoisomerase II. Comparison of RNA for actin and topoisomerase II in tissue culture cells shows an inverse relationship in RNA level, verifying that the decrease in message for topoisomerase II in saturated cultures is not just due to general RNA degradation.

Temporal Expression of Topoisomerase II. The levels of RNA for topoisomerase II during the development of *Drosophila* do not necessarily represent a true picture of the expression of the protein, mainly because the translation efficiency and the turnover rate for the protein are not known. We also looked at the levels of protein during development in order to address this issue.

The same stages in development were used to make crude homogenates for protein analysis. The homogenates were

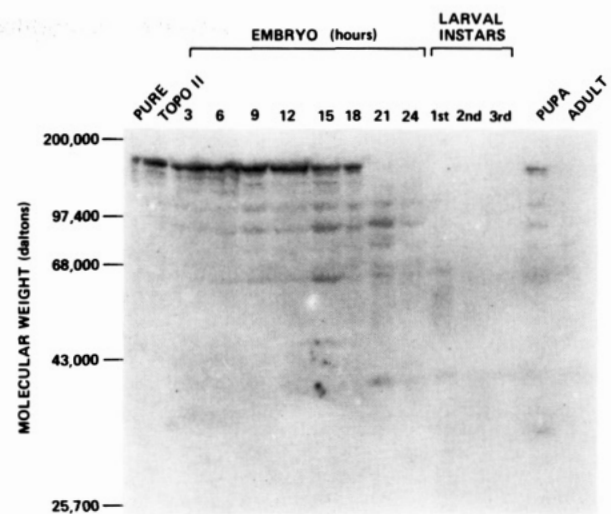


FIGURE 4: Changes in levels of topoisomerase II protein during *Drosophila* development. Lane 1 contains 0.5 μ g of purified topoisomerase II from 6–18-h-old embryos. Lanes marked 3, 6, 9, 12, 15, 18, 21, and 24 represent time spans of 0–3, 3–6, 6–9, 9–12, 12–15, 15–18, 18–21, and 21–24 h in age during embryogenesis, respectively. Molecular weight markers are myosin, phosphorylase B, bovine serum albumin, ovalbumin, and α -chymotrypsinogen, going from largest to smallest.

extracted with organic solvents to remove lipids. Samples were then subjected to SDS-PAGE in which equal weights of extracts from each stage were loaded. The samples were then transferred to nitrocellulose and probed with a polyclonal antiserum directed against purified topoisomerase II. The results of such an experiment are shown in Figure 4. The first lane represents purified topoisomerase II, and the remaining lanes represent all the stages of *Drosophila* development. A major band running in the same position as topoisomerase II (about 167 kDa) is seen in early and mid-embryogenesis. Purified topoisomerase II also contains three minor bands that run just below the main band, and this pattern persists throughout development. The most striking features are the disappearance of topoisomerase II in late embryogenesis and the high levels of topoisomerase II in pupae. Upon longer exposure, faint bands can be picked up in the larval stages also running at about 167 kDa. A number of controls were performed to verify that the decrease in signal was not simply an artifact. Extracts run on SDS-PAGE were also stained for total protein by using Coomassie blue stain to show that a decrease in topoisomerase II signal was not simply due to total protein degradation. The topoisomerase II analysis was repeated by electrophoresing equal amounts of protein per sample (by the Bio-Rad Microassay) using SDS-PAGE in order to compare the changes in topoisomerase II signal to the analysis shown in Figure 4. The results gave a similar pattern to that shown in Figure 4 (data not shown). Aliquots were removed during each step of the processing for 0–3-h embryos and first-instar larvae (the latter stage was especially difficult to process due to the high level of lipids that interfered with the analysis), measured for protein concentration, and run out on a gel in order to show that there is no change in topoisomerase II levels due to the processing of the homogenates. After transfer and probing with the antiserum, the levels of topoisomerase II were shown to remain constant throughout the processing of these two samples.

The background of the autoradiogram was highly variable, depending on how the extracts were made and how they were stored. However, all bands except for those around 167 kDa and just below and the one around 40 kDa also appeared when the extracts were probed with the preimmune serum. The

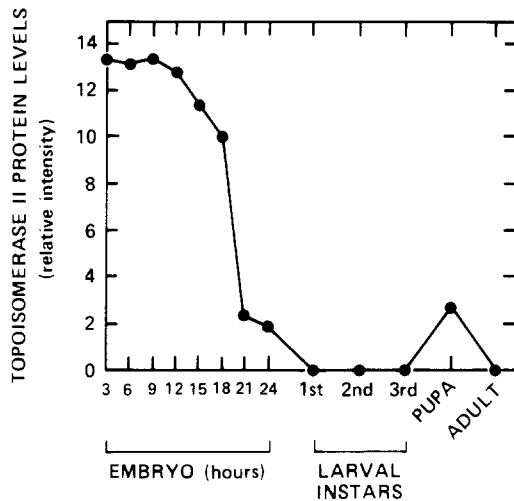


FIGURE 5: Plot of densitometric scan from protein blot analysis.

presence of these smaller molecular weight bands has also been seen by others, but whether they are *in vivo* fragments or artifacts of the extraction procedure is yet to be established (Shelton et al., 1983; Sander & Hsieh, 1983; Heller et al., 1986).

The autoradiogram in Figure 4 was scanned by densitometry to analyze the levels of topoisomerase II throughout development. The ratio between the major band at 167 kDa and the lower three bands varied in each preparation [as reported earlier by Heller et al. (1986)], so all four bands were scanned to get a true reflection of the amount of topoisomerase II at each stage. The results are shown in Figure 5. Topoisomerase II levels remain constant in early embryogenesis but drop about 6-fold during mid-embryogenesis. The signal continues to drop but at a slower rate during late embryogenesis. There are virtually no detectable levels of topoisomerase II in the larval or adult stages; however, there is a fairly strong signal in the pupal stage.

The data from the scan of Figure 4 were used to compare the topoisomerase II signals from the extracts to the signal for the purified topoisomerase II in the autoradiogram and then to calculate the amount of topoisomerase II found per individual organism for each stage during development. This is shown in Table I. Calculations were based on data from Chapter 4 in Robertson (1978). The level of topoisomerase II can be estimated by comparing the average wet weight of an individual to the wet weight of the extract electrophoresed by SDS-PAGE for each stage during development (for Figure 4; 20 mg wet weight was loaded). Also included in the table is the ratio of base pairs of DNA to topoisomerase II. The molar ratio in early embryogenesis is about 25 000 base pairs of DNA per one topoisomerase II enzyme. This ratio becomes extremely large in the larval stages, implying that topoisomerase II is needed only rarely along chromosomes or perhaps some other mechanism can replace the function of topoisomerase II. The levels of topoisomerase are difficult to interpret in the larval stage because while some tissues may be actively dividing, most are not replicating at all.

The expression of topoisomerase II protein parallels the levels of topoisomerase II message throughout development. Both message and protein are abundant in early embryos. Both decrease dramatically during mid-embryogenesis. The increase of message in late embryogenesis may explain the slower rate of decline of the protein during the same period. Message levels increase during the late larval stages and may explain the presence of protein in the pupae. Although it is surprising that no protein is seen in late larvae concomitant

Table I: Levels of Topoisomerase II during Development

stage	signal relative to pure topo II	wt of total topo II (ng)	molecules of topo II/individual ($\times 10^8$)	bp of DNA/individual ($\times 10^{13}$)	kbp of DNA/topo II
0-3 h	1.42	710	12.7	2.9	23
3-6 h	1.41	705	12.7	3.4	27
6-9 h	1.44	720	12.9	4.2	32
9-12 h	1.37	685	12.3	5.1	41
12-15 h	1.22	610	11.0	5.9	54
15-18 h	1.08	540	9.70	6.7	69
18-21 h	0.26	130	2.34	7.5	320
21-24 h	0.20	100	1.80	8.4	467
first instar			<0.20	12.2	>6 100
second instar			<0.20	24.8	>12 400
third instar			<0.20	80.8	>40 400
pupa	0.40	200	233	~82	35
adult			<0.20	~82	>41 000

to the increase in message, this might be explained by a delay in the processing of the RNA to make the protein.

DISCUSSION

The results from analysis of message and protein for the *Drosophila* topoisomerase II show elevated levels during early embryogenesis (probably due to maternal message) and during pupation. These stages in *Drosophila* development are also noted for increased mitotic activity which immediately suggests a role for topoisomerase II in cell division. As mentioned earlier, double mutations in yeast identify topoisomerase II as being especially important in termination of DNA replication, presumably aiding in segregation of chromosomes. There is also evidence that topoisomerase II is actively involved at replication forks. Initially, circumstantial evidence for this activity was reported, suggesting that type II topoisomerases are associated with the nuclear matrix, the structure that supports DNA replication (Pardoll et al., 1980; Noguchi et al., 1983; Berrios et al., 1985). Further, Heller et al. (1986) have shown that, in immunofluorescence studies using a polyclonal antiserum directed against topoisomerase II, the enzyme is evenly distributed in polytene chromosomes from the salivary glands of *Drosophila* but absent from puff regions. This would imply a general role for topoisomerase II, such as in DNA replication. More recently, topoisomerase II has been shown to be correlated directly with DNA replication (Heck & Earnshaw, 1986) and also has been shown to be associated with nascent DNA and DNA replication forks (Nelson et al., 1986).

To further support that topoisomerase II is indeed involved in cell division, analysis of topoisomerase II message in *Drosophila* K_c cells was done. Levels of topoisomerase II message are highest in cultures undergoing logarithmic growth, where cells are dividing every 20-24 h when compared to saturated cultures, when cells are quiescent. Quantification of message levels per cell give approximate values of 10-20 transcripts in log phase growth and about 1 transcript in stationary phase growth.

Protein analysis was also useful because we were able to quantify levels of topoisomerase II throughout *Drosophila* development (see Table I). As noted above, protein levels were highest in early embryos. We calculated an average of 1.5×10^9 molecules of topoisomerase II per embryo which agrees closely with that predicted from the purification of topoisomerase II (Shelton et al., 1983). They reported a yield of 0.44 mg of topoisomerase II from 100 g of 6-18-h-old embryos at a yield of about 6%. This would predict a value of about 3×10^9 molecules of topoisomerase II per embryo. Further

calculations predict that there is approximately 1 topoisomerase II molecule per 25 000 base pairs of DNA, or 1 topoisomerase II molecule per 6 replication forks. This level is well within the limit of topoisomerase II activity as the in vitro turnover rate for the enzyme is relaxation of about 200 supercoils min^{-1} (molecule of topoisomerase II) $^{-1}$ at a ratio of 1 topoisomerase II/15 molecules of DNA. This would allow for the relaxation of approximately 500 supercoils per replication fork per minute which is the amount needed as a replication fork induces a maximum of 260 supercoils per minute (Kriegstein & Hogness, 1974). The amount of topoisomerase II decreases rapidly in late embryogenesis, concomitant to a decrease in the rate of DNA synthesis, and remains at very low levels until pupation, where the enzyme returns to a level almost comparable to embryogenesis at 1 topoisomerase II molecule per 35 000 base pairs of DNA.

Proteolysis of topoisomerase II has always been reported in its purification and in vivo analysis. Extreme precautions were taken to eliminate proteolysis in vitro. However, the characteristic pattern of proteolysis persisted, characterized by a set of three bands just below the major band at 167 kDa and a band at about 40 kDa. Whether these are found in vivo is uncertain yet. The three bands below the major polypeptide at 167 kDa were assigned molecular weights of 151K, 141K, and 132K relative to a reported major band at 170K (Sander & Hsieh, 1983). They were also shown to be related both biochemically and immunologically [see also Heller et al. (1986)]. When extracts were left at room temperature, we saw a reciprocal relationship in intensity of the major band with the minor bands just below the 40-kDa band as proteolysis occurred, indicating discrete labile sites in the protein. It will be interesting to test these fragments for ATP activity and DNA binding activity to determine their relatedness to the enzyme.

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