The phosphorylation of DNA topoisomerase II in Drosophila Kc tissue culture cells was characterized by in vivo labeling studies and in vitro studies that examined the modification of exogenous enzyme in total homogenates of these embryonic cells. Several lines of evidence identified casein kinase II as the kinase primarily responsible for phosphorylating DNA topoisomerase II. First, the only amino acyl residue modified in the enzyme was serine. Second, partial proteolytic maps of topoisomerase II which had been labeled with \([\gamma-32P]\)phosphate by Drosophila cells in vitro, by cell homogenates in vitro, or by purified casein kinase II were indistinguishable from one another. Third, phosphorylation in cell homogenates was inhibited by \(\mu g/ml\) concentrations of heparin, micromolar concentrations of nonradioactive GTP, or anti-Drosophila casein kinase II antiserum. Fourth, cell homogenates were able to employ \([\gamma-32P]GTP\) as a phosphate donor nearly as well as \([\gamma-32P]ATP\). Although topoisomerase II was phosphorylated in homogenates under conditions that specifically stimulate protein kinase C, calcium/calmodulin-dependent protein kinase, or cAMP-dependent protein kinase, modification was always sensitive to anti-casein kinase II antiserum or heparin. Thus, under a variety of conditions, topoisomerase II appears to be phosphorylated primarily by casein kinase II in the Drosophila embryonic Kc cell system.

DNA topoisomerase II is essential for the viability of eukaryotic cells (1–4) and is involved in many aspects of nucleic acid metabolism (reviewed in Refs. 5–7), including DNA replication (8–12), transcription (12–14), and chromosome segregation (2–4, 15, 16). In addition, the enzyme also appears to play important roles in chromosome structure and condensation (15–22) and in the organization of the nuclear matrix (23, 24).

Despite the importance of topoisomerase II to the eukaryotic cell, very little is understood about its physiological regulation. Enzyme levels and activity rise at least 10-fold during periods of cell proliferation (25–28). Moreover, mitogenic agents, such as concanavalin A (29) and epidermal growth factor (30),1 have been shown to stimulate topoisomerase II activity in mammalian cells. Although the activity of the enzyme remains relatively constant over the cell cycle of proliferating cells (31, 32), levels of the protein increase 1.5- to 2-fold during mitosis (27, 33). In addition, the intracellular location of topoisomerase II redistributes during the process of cell division. During interphase, the enzyme is a major polypeptide component of the nuclear matrix (23). During mitosis, a portion of the enzyme specifically associates with metaphase chromosomes (17–19), while the rest disperses throughout the cytoplasm (19, 23). Unfortunately, virtually nothing is known about the factors that modulate the in vivo activity or intracellular location of topoisomerase II.

Since phosphorylation/dephosphorylation events have long been known to alter the functions of many enzymes (34) and structural proteins (35, 36), including topoisomerase I (37–40), the role of phosphorylation as a physiological regulator of topoisomerase II has been investigated. Indeed, the type II enzyme appears to exist in the eukaryotic cell as a phosphoprotein (41–43). In vitro, topoisomerase II is readily phosphorylated by casein kinase II (42) or protein kinase C (43, 44), and modification by either kinase stimulates enzyme activity by about 3-fold. Topoisomerase II also serves as an in vitro substrate for calcium/calmodulin-dependent protein kinase (44), but considerably higher levels of kinase are required for modification. In contrast, cAMP-dependent protein kinase shows no ability to phosphorylate the enzyme (39, 44). While this last finding indicates that the cellular modification of topoisomerase II must be carried out by specific enzymes, the kinase(s) that phosphorylates the enzyme in vivo, the site(s) of modification, and the mechanism by which phosphorylation stimulates enzyme activity are completely unknown.

Clearly, before the physiological regulation of topoisomerase II can be fully defined, the cellular phosphorylation of the enzyme must be characterized. As a first step toward this end, the in vivo phosphorylation of DNA topoisomerase II in Drosophila Kc cells and the in vitro modification of exogenous Drosophila melanogaster enzyme in total Kc cell homogenates have been examined. On the basis of peptide mapping, enzymological and immunological studies, casein kinase II appears to be the kinase primarily responsible for phosphorylating topoisomerase II in this embryonic cell system.

**EXPERIMENTAL PROCEDURES**

DNA topoisomerase II was purified from the nuclei of frozen Drosophila Kc tissue culture cells as described by Shelton et al. (45). Casein kinase II was prepared from frozen 6- to 18-h-old D. melanogaster embryos as described by Glover et al. (46). Purified catalytic

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1. P. Ackerman, S. Cohen, and N. Osheroff, unpublished results.
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by the addition of cell suspension was homogenized on ice using mers were incubated at pH using bovine serum albumin as a standard. Freshly prepared Kc cell Tris-C1, pH was phosphorylated in a ml of Tris-C1, pH was examined over a dilution range of 1:120 to 1:30. Reactions designed to stimulate protein kinase C included 100 μM CaCl₂ and 33 μg/ml phosphatidylerine; those designed to stimulate calcium/calmodulin-dependent protein kinase included 100 μM CaCl₂ and 6 μM calmodulin; and those designed to stimulate CAMP-dependent protein kinase included 10 μM CAMP (44).

Reaction mixtures were prepared for the identification of phosphorylated amino acids and the generation of partial proteolytic maps by incubating 10% supernatant was collected and used for the immunoprecipitation procedures described below. Purification of in vivo-labeled Topoisomerase II from Nuclear Extracts—Topoisomerase II was purified from the nuclear extracts described above by immunoprecipitation. The procedure of Doderquist and Carpenter (50) was employed. Briefly, nuclear extracts were incubated with IgG volume of rabbit anti-Drosophila topoisomerase II antisera (23, 45), and topoisomerase II was precipitated by the addition of formalin-fixed S. aureus cells (51). Metabolically labeled enzyme was located by autoradiography using Protein kinase C was stimulated by trichloroacetic acid precipitation onto Whatman GF/C filters (46). Phosphorylation was quantitated using a Beckman LS-7500 liquid scintillation counter and ACS scintillant. Identification of Phosphorylated Amino Acid Residues—In vivo- and in vitro-modified enzymes were prepared as described above. Samples were subjected to electrophoresis on 7% polyacrylamide gels as described by Laemmli (51). Bands corresponding to phosphorylated amino acid residues were visualized by autoradiography and excised. Labeled enzyme was extracted by electroatton for 23 h using a Bio-Rad Model 422 microfuge. Alternatively, gel slices were washed twice for 2 h at 23°C in 10% methanol to remove salts and SDS. Phosphorylated topoisomerase II was extracted at 23°C overnight in several changes of 50 mM N-ethylmorpholine acetate, pH 8.2, with continuous rotation. With either procedure, supernatants were pooled, dialyzed overnight against 100 mM ammonium bicarbonate, and concentrated by centrifugation under reduced pressure. Modified topoisomerase II was hydrolyzed in 6 N HCl for 2 h at 110°C under reduced pressure. The hydrolysate was recovered, mixed with phosphorylated amino acid standards (1 mg/ml each of Ser (P), Thr (P), and Tyr (P)), and analyzed by two-dimensional electrophoresis. Reaction samples in proteolysis buffer (Brinkmann) as described by Hunter and Sefton (54). Standards were located by ninhydrin staining and [32P]phosphate was visualized by autoradiography as described above.

Partial Proteolytic Mapping of Phosphorylated Topoisomerase II—Phosphorylated topoisomerase II was isolated as described under “Identification of Phosphorylated Amino Acid Residues.” Enzyme samples were dissolved in proteolysis buffer (125 mM Tris-Cl, pH 6.8, 0.5% SDS, 10% glycerol, 0.001% bromphenol blue) containing bovine serum albumin (0.5 mg/ml) and digested by the method of Cleveland et al. (55). Proteolytic digests were freshly prepared, added immediately to phosphorylated topoisomerase II samples. Final enzyme concentrations were α-chymotrypsin, 67 μg/ml; elastase, 67 μg/ml; S. aureus V8 protease, 10 μg/ml; S. griseus protease, 20 μg/ml; trypsin, 200 μg/ml. Mixtures were incubated at 37°C for 30 min. Following the addition of β-mercaptoethanol and SDS to final concentrations of 10% and 2%, respectively, samples were heated at 95°C for 3 min and subjected to electrophoresis on 11%
polycrylamide gels by the procedure of Laemmli (51). Phosphorylated peptides were visualized by autoradiography as described above.

RESULTS

In Vivo Phosphorylation of Drosophila DNA Topoisomerase II—DNA topoisomerase II has been reported to exist in Drosophila embryonic cells as a phosphoprotein (41, 42). To provide direct evidence for in vivo phosphorylation, nuclear extracts of Drosophila Kc tissue culture cells that had been metabolically labeled with [32P]orthophosphate were immunoprecipitated with rabbit anti-Drosophila topoisomerase II antiserum. Following electrophoresis on a 7% polyacrylamide gel (51), one predominant 32P-labeled protein band was observed in autoradiograms (Fig. 1, lane 2). This band was identified as topoisomerase II on the basis of its comigration with purified Drosophila type II enzyme (compare lanes 1 and 2), its positive reaction with anti-Drosophila topoisomerase II antiserum, and its absence when preimmune serum was employed in the immunoprecipitation procedures (not shown). These experiments provide conclusive evidence that DNA topoisomerase II exists in Drosophila embryonic cells as a phosphoprotein. A recent report draws a similar conclusion with respect to DNA topoisomerase I (39).

Fig. 1. Metabolic labeling of topoisomerase II in Drosophila Kc tissue culture cells. Samples were subjected to electrophoresis on a 7% polyacrylamide gel and phosphorylated proteins were visualized by autoradiography (not shown). The labeled band in the autoradiogram was identified as topoisomerase II on the basis of 1) its complete absence when exogenous enzyme was not included in the assay mixture; 2) its dependence on the concentration of added topoisomerase II; 3) its polypeptide molecular mass of approximately 166,000 daltons (45, 56); and 4) immunoblot analysis (not shown), in which this radioactive band was identified as topoisomerase II by anti-Drosophila topoisomerase II antiserum and its absence when preimmune serum was employed in the immunoprecipitation procedures (not shown). These experiments provide conclusive evidence that DNA topoisomerase II exists in Drosophila embryonic cells as a phosphoprotein. A recent report draws a similar conclusion with respect to DNA topoisomerase I (39).

Phosphorylation of DNA Topoisomerase II by Total Homogenates of Drosophila Kc Cells—In order to further characterize the cellular phosphorylation of the type II enzyme, the ability of total homogenates of Drosophila Kc cells to modify exogenous topoisomerase II was examined. As seen in the polyacrylamide gel of Fig. 2, the enzyme was phosphorylated by these cell homogenates. The labeled band in the autoradiogram was identified as topoisomerase II on the basis of 1) its complete absence when exogenous enzyme was not included in the assay mixture; 2) its dependence on the concentration of

FIG. 2. Phosphorylation of exogenous D. melanogaster DNA topoisomerase II (Topo II) by Drosophila Kc cell homogenates. Reaction mixtures containing 0 (lane 1), 17 (lane 2), or 35 nM (lane 3) topoisomerase II were subjected to electrophoresis on a 7% polyacrylamide gel, and phosphorylated proteins were visualized by autoradiography. Marker proteins are as in Fig. 1 with the following addition: bovine serum albumin, 66 kDa.

Under the exposure conditions employed in Fig. 2, the phosphorylation of endogenous Kc cell topoisomerase II was not visualized. This is due to the fact that levels of the cellular enzyme (~0.1% total protein in Kc cells (45)) were approximately 2 orders of magnitude lower than those of the exogenous enzyme. Upon overexposure of the autoradiogram, bands representing phosphorylated endogenous proteins became visible (not shown).

Fig. 3. Identity of Phosphorylated Amino Acyl Residues—The amino acyl residue of Drosophila topoisomerase II that was phosphorylated by Kc cells in vivo (Fig. 3, left panel) or by cell homogenates in vitro (right panel) was identified by partial acid hydrolysis and two-dimensional electrophoresis. As previously shown for the casein kinase II-catalyzed reaction (42), only serine was modified. No phosphothreonine or

3 Purified casein kinase II incorporates a maximum of 2 to 3 phosphate groups per homodimer of topoisomerase II (42) (see Table III). Reaction times (i.e. 10 min) that yielded a submaximal ~1:1 modification of the enzyme were employed for the cell homogenate experiments described below in order to optimize the effects of added kinase inhibitors and stimulators. Higher levels of topoisomerase II phosphorylation were observed at longer reaction times.
Phosphorylation of DNA Topoisomerase II

In Vivo

In Vitro

FIG. 3. Identification of the amino acyl residue phosphorylated in vivo by Drosophila Kc cells (left panel) and in vitro by Kc cell homogenates (right panel). Phosphorylated topoisomerase II was subjected to partial acid hydrolysis, mixed with phosphorylated amino acid standards (Ser(P), Thr(P), and Tyr(P)), and analyzed by two-dimensional thin-layer electrophoresis at pH 1.9 and 3.5. Autoradiograms of the thin-layer plates are shown. Dashed circles indicate the positions of amino acid standards. P₁ designates the location of inorganic phosphate.

Phosphotyrosine was observed, even upon overexposure of the autoradiograms.

Partial Proteolytic Maps of Phosphorylated Topoisomerase II—Partial proteolytic maps of modified topoisomerase II were generated by the procedure of Cleveland et al. (55) in order to compare sites of phosphorylation labeled in vivo with those labeled in homogenate- and casein kinase II-catalyzed reactions. Five proteases with different cleavage specificities were employed for these experiments, including chymotrypsin (specific for aromatic amino acid residues), elastase (specific for neutral aliphatic residues), S. aureus V8 protease (specific for glutamic acid residues), S. griseus protease (nonspecific), and trypsin (specific for lysyl and arginyl residues). For each of the above proteases, unique banding patterns of phosphorylated peptides were observed following SDS-polyacrylamide gel electrophoresis and autoradiography. In all cases, corresponding peptide maps of in vivo-, homogenate-, and casein kinase II-phosphorylated topoisomerase II were identical to one another. Maps generated with chymotrypsin, elastase, or S. aureus V8 protease are shown in Fig. 4 (upper panel). Maps produced with S. griseus protease contained 7 major phosphorylated bands ranging in size from ~20 to ~3 kDa and those produced with trypsin contained 14 major phosphorylated bands ranging in size from ~120 to ~5 kDa (not shown).

A 15-fold overexposure of the low molecular mass portion of the partial proteolytic maps (Fig. 4, lower panel) revealed labeled peptides with masses in the 2.5- to 3-kDa range. This limits the site of phosphorylation in topoisomerase II to a region no larger than 20 to 30 amino acyl residues and makes it likely that the kinase primarily responsible for modifying the enzyme in both Kc cells and cell homogenates is casein kinase II. As described below, several additional experiments were carried out in order to confirm this identification. All of the following work was designed to characterize directly the enzymological nature of the kinase that is responsible for phosphorylating topoisomerase II in Kc cell homogenates.

Inhibition of Homogenate-catalyzed Phosphorylation by Heparin—The modification of topoisomerase II by Kc cell homogenates was strongly inhibited by the inclusion of heparin in assay mixtures (Fig. 5). As determined from a semilog plot of [heparin] versus percent phosphorylation (not shown), 50% inhibition occurred at a heparin concentration of approximately 3 μg/ml.

Most cellular kinases are totally insensitive to concentrations of heparin which are lower than 10 μg/ml (59, 60). In contrast, casein kinase II is strongly inhibited by heparin concentrations in the 0.1 to 1 μg/ml range (46, 59, 60). Although this range is somewhat lower than that observed for

FIG. 4. Partial proteolytic maps of phosphorylated topoisomerase II. Phosphorylation was catalyzed in vitro by Kc cell homogenate (Kc) (lanes 1, 4, and 7), in vitro by purified casein kinase II (CKII) (lanes 2, 5, and 8), or in vivo by metabolic labeling of Kc cells (In Vivo) (lanes 3, 6, and 9). Phosphorylated topoisomerase II was digested with chymotrypsin (lanes 1–3), elastase (lanes 4–6), or S. aureus V8 protease (lanes 7–9). Digests were subjected to electrophoresis on an 11% polyacrylamide gel. An autoradiogram of the gel is shown. Marker proteins are as in Fig. 2 with the following additions: soybean trypsin inhibitor, 20; myoglobin fragment IV, 14.4; fragment III, 8.2; fragment II, 6.2; fragment I, 2.5. A 15-fold overexposure of the low molecular mass region of the peptide maps is shown in the lower panel.

FIG. 5. Inhibition of Kc cell homogenate-catalyzed phosphorylation of topoisomerase II by heparin. Reaction mixtures containing 0, 2, 4, 8, or 20 μg/ml heparin (lanes 1–5) were subjected to electrophoresis on a 7% polyacrylamide gel. An autoradiogram of the gel is shown. Marker proteins are as in Fig. 1.
the homogenate-catalyzed activity, the heparin inhibition of casein kinase II can be overcome by the presence of basic proteins, such as histones (not shown), which are present in high concentrations in the embryonic Kc cell line (49).

**Inhibition of Phosphorylation by Nonradioactive GTP**—The ability of nonradioactive GTP to inhibit the homogenate-catalyzed modification of topoisomerase II (when [γ-32P]ATP was used as a phosphate donor) is shown in Fig. 6. As determined from a semilog plot (not shown), 50% inhibition was estimated to occur at 17 μM GTP. While most kinases require millimolar levels of GTP for inhibition, the Ki value of GTP for purified casein kinase II is 9 μM (61).

**Utilization of [γ-32P]GTP as a Phosphate Donor by Kc Cell Homogenates**—A distinguishing characteristic of type II casein kinases is their ability to employ GTP as a phosphate donor (Kₗ = 66 μM) nearly as effectively as they do ATP (Kₗ = 17 μM) (60, 61). Therefore, the ability of homogenates to utilize [γ-32P]GTP was examined. As seen in Fig. 7 (lane 4), homogenates could incorporate [32P]phosphate into Drosophila topoisomerase II using [γ-32P]GTP as a phosphate donor. By normalizing levels of phosphorylation obtained with homogenates to those found with purified casein kinase II (i.e. homogenate-catalyzed phosphorylation/casein kinase II-catalyzed phosphorylation), it was possible to determine the relative abilities of cell homogenates to employ [γ-32P]GTP and [γ-32P]ATP as phosphate donors (Fig. 7). Under the conditions employed, the relative utilization of radioactive GTP by cell homogenates (39%) (compare lanes 3 and 4) was remarkably similar to their relative utilization of radioactive ATP (41%) (compare lanes 1 and 2).

To ensure that the [γ-32P]GTP that was included in assay mixtures was not converted to [γ-32P]ATP by the action of nucleoside diphosphate kinase or other nucleotide salvage enzymes (62), the fate of labeled GTP was followed over the course of a phosphorylation reaction. As determined by thin layer chromatography (58), less than 0.5% of the nucleoside triphosphate was hydrolyzed to GDP and, at most, less than 0.2% was converted to labeled ATP. Thus, the observed modification of topoisomerase II by cell homogenates must result from the direct utilization of GTP as a phosphate donor.

**Inhibition of Topoisomerase II Phosphorylation by Anti-casein Kinase II Antiserum**—The ability of anti-Drosophila casein kinase II antisem to inhibit Kc cell homogenate-catalyzed modification of topoisomerase II was examined. The antisem employed reacts specifically with casein kinase II in Drosophila Kc cell homogenates and inhibits the activity of the purified kinase by approximately 50% at a dilution of 1:150 (53). Despite the fact that preimmune serum appeared to stimulate homogenate catalyzed reactions by about 15%, anti-casein kinase II antisem strongly inhibited the phosphorylation of topoisomerase II (Table I). 50% inhibition was observed at an antisem dilution of approximately 1:120.

**Phosphorylation of Topoisomerase II under Conditions That Stimulate Protein Kinase Activities**—While the enzymological and immunological studies presented above strongly suggest that topoisomerase II is modified by casein kinase II in Kc cell homogenates, they do not rule out possible phosphorylation of the enzyme by other cellular kinases. Therefore, phosphorylation assays were carried out under conditions specifically designed to stimulate protein kinase C or calcium/
dependent protein kinase, both of which have been shown to modify Drosophila topoisomerase II in vitro (44) or cAMP-dependent protein kinase, which shows no ability to modify the enzyme in vitro (39, 44) (see Table III).

Typical results are shown in Table II. While the extent of modification in the presence of calcium/calmodulin was similar to that found under assay conditions lacking these compounds, the inclusion of calcium/phosphatidylserine (which stimulates protein kinase C) or cAMP increased topoisomerase II phosphorylation by approximately 50%. However, even in the presence of these specific kinase inducers, addition of anti-casein kinase II antiserum or heparin strongly inhibited phosphorylation (Table II). Thus, phosphorylation by casein kinase II still appeared to be the event of importance.

Several hypotheses are consistent with the above finding. First, topoisomerase II may become a more suitable substrate for protein kinase C or cAMP-dependent protein kinase only after it is first phosphorylated by casein kinase II. Several examples of such synergistic phosphorylation have been reported (63-67), and, in all cases, the initial and critical phosphorylation event was mediated by casein kinase II. Second, topoisomerase II may be phosphorylated exclusively by casein kinase II, but the kinase's activity is stimulated by a direct interaction (i.e. phosphorylation) with another activated protein kinase. In this regard, it should be noted that casein kinase II exists in the cell as a phosphoprotein (60). Third, topoisomerase II may be phosphorylated exclusively by casein kinase II, but the kinase's activity is stimulated as an indirect result of protein kinase C or cAMP-dependent protein kinase activation. Such an explanation requires a cascade or second messenger mechanism.

Studies were carried out in order to test the first two hypotheses. Experiments described below employed the catalytic subunit of CAMP-dependent protein kinase, since this kinase shows no ability to modify topoisomerase II in vitro (39, 44) (see Table III). The CAMP-dependent enzyme preparation used was highly active as judged by its ability to modify histone or Kemptide substrates (not shown).

The first experiment examined the possibility of synergistic phosphorylation. To this end, topoisomerase II was phosphorylated for 10 min with purified casein kinase II. The kinase was then inhibited by the addition of either 250 μM GTP or a combination of 250 μM GTP and 10 μg/ml heparin. Purified catalytic subunit of CAMP-dependent protein kinase was added to the reaction mixture to determine whether casein kinase II-modified topoisomerase II became a substrate for the CAMP-dependent enzyme. As seen in Fig. 8, no synergistic phosphorylation was observed.

The second experiment examined the possibility that casein kinase II was stimulated by the presence of the CAMP-dependent enzyme. Accordingly, casein kinase II was incubated with the catalytic subunit of CAMP-dependent protein kinase prior to the addition of topoisomerase II. As seen in Table III, the time course for the phosphorylation of topoisomerase II by this kinase mixture was similar to that generated by casein kinase II alone. This result precludes a stimulatory interaction between the catalytic subunit of CAMP-dependent protein kinase and casein kinase II. Thus, it appears likely that the addition of CAMP to cell homogenates increases levels of casein kinase II-mediated topoisomerase II phosphorylation by an indirect process.

**DISCUSSION**

Casein kinase II appears to be the enzyme primarily responsible for phosphorylating DNA topoisomerase II in *Drosophila*.
were modified in cells or in cell homogenates. Second, peptide lysyltopo-sylata topoisomerase I1 (generated with five different proteolytic enzymes) were indistinguishable from one another. Third, homogenate-catalyzed phosphorylation was strongly inhibited by μg/ml concentrations of heparin or micromolar concentrations of nonradioactive CTP. Sensitivity to these compounds are hallmark characteristics of type II casein kinases (46, 59-61). Fourth, homogenate could employ [γ-32P] GTP as a phosphate donor nearly as effectively as it could [γ-32P]ATP. Once again, this enzymological trait is unique to casein kinase II (46, 60). Fifth, homogenate-catalyzed reactions were specifically inhibited by antiserum directed against Drosophila casein kinase II. Sixth, even under reaction conditions that specifically stimulate protein kinase C, calcium/calfemulin-dependent protein kinase, or cAMP-dependent protein kinase, homogenate-catalyzed reactions were inhibited by anti-casein kinase II antiserum or heparin.

Two experimental models were used to examine topoisomerase II-kinase interactions in the present study: metabolic labeling of topoisomerase II in intact Drosophila Kc cells and in vitro phosphorylation of exogenous enzyme in total homogenates of these embryonic cells. By coupling these two models, it was possible to characterize sites of phosphorylation in topoisomerase II as well as the enzymological and immunological properties of the modification reaction. This dualistic approach was favored over experiments designed to characterize only the modification of endogenous topoisomerase II in intact cells, because the metabolic approach relies totally on the identification of phosphorylation sites or the correlation between levels of phosphorylation and the presence of kinase effectors. When defining interactions between kinase and protein substrates, it is very important to couple enzymological studies with mapping experiments, because it is possible that more than one kinase can phosphorylate the same site on a given protein. As an example, serine 40 of tyrosine hydroxylase is modified by at least four different protein kinases: casein kinase I1 (42), protein kinase C, calcium/calmodulin-dependent protein kinase, or cAMP-dependent protein kinase, homogenate-catalyzed reactions were inhibited by anti-casein kinase II antiserum or heparin.

Although DNA topoisomerase II is primarily a nuclear enzyme in interphase cells, total cell homogenates rather than isolated nuclei were employed for the in vitro portion of the present work. The decision to use total cell homogenates was made for two reasons. First, during mitosis, the nuclear envelope dissolves (35) and topoisomerase II is exposed to the cytoplasm (19, 23). Second, in vitro phosphorylation of Dro-sophila topoisomerase II has been demonstrated with three different protein kinases: casein kinase I1 (42), protein kinase C (43, 44), and calcium/calmodulin-dependent protein kinase (44). In the cell, casein kinase II and the calcium/calmodulin-dependent enzyme are located in both the nucleus and the cytoplasm (60, 69, 70), while protein kinase C is predominantly cytoplasmic (71, 72). Thus, the use of total cell homogenates provided a system that was not biased toward or against any of the above kinases.

Drosophila topoisomerase II exists in the cell as a phospho-protein (41-43) (Fig. 1), but the physiological consequences of the modification are as yet unknown. Considering that 1) the activity of topoiso-merase II increases markedly during the transition from cell quiescence to proliferation (25-30), 2) the enzyme's intracellular location changes during the course of the cell cycle (17-19, 23), and 3) phosphorylation/dephosphorylation events commonly affect both the enzymatic activities (34, 37-40) and cellular locations (35, 36) of many protein systems, it is highly probable that phosphorylation plays a role in regulating at least some of the in vivo functions of topoisomerase II. This conclusion is strongly supported by the fact that serine phosphorylation stimulates the activity of the Drosophila enzyme in vitro (42-44).

At the present time, virtually nothing is known about the biological events that control the cellular modification of topoisomerase II. Clearly, identifying the kinase that mediates the in vitro phosphorylation of the enzyme represents an important step toward elucidating these events. On the basis of the results presented above, it appears likely that casein kinase II is the enzyme that is primarily responsible for phosphorylating DNA topoisomerase II in the embryonic Drosophila Kc cell line.

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