Both calf and Drosophila contain a type II casein kinase with similar molecular structure and catalytic activity. Purified calf thymus casein kinase II is composed of three subunits of $M_r = 44,000$ ($\alpha$), $40,000$ ($\alpha'$), and $26,000$ ($\beta$) (Dahmus, M. E. (1981) J. Biol. Chem. 256, 3319–3325), whereas the Drosophila enzyme is composed of two subunits of $M_r = 36,700$ ($\alpha$) and $28,200$ ($\beta$) (Glover, C. V. C., Shelton, E. R., and Brutlag, D. L. (1983) J. Biol. Chem. 258, 3258–3265). The native form of the enzyme is an $\alpha_2\beta_2$ tetramer. Polyclonal antibodies prepared against each enzyme react with both the $\alpha$ and $\beta$ subunits of the homologous enzyme and cross-react with both subunits of the heterologous enzyme. Reaction of polyclonal antibodies with proteins resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis establishes that no significant difference in subunit molecular weight exists between the purified enzymes and the enzyme present in initial cell extracts. Each antibody effectively inhibits the \textit{in vitro} activity of the homologous enzyme and causes a slight inhibition in the activity of the heterologous enzyme. Peptide maps derived from purified subunits indicate that the $\alpha$ and $\beta$ subunits are unique and that there is extensive primary sequence homology between the corresponding subunits of the calf and Drosophila enzyme. Casein kinase II from both sources phosphorylates the same subunits of calf thymus RNA polymerase II and an identical set of proteins in a complex mixture of acid-soluble proteins from Drosophila tissue culture cells. The striking similarity in molecular structure and catalytic activity between the calf and Drosophila enzyme suggests that casein kinase II has been highly conserved in evolution.

Phosphorylation of protein substrates has proven to be extremely common in eukaryotic cells, and it is now well recognized that this modification plays a major role in regulating the activity of both enzymatic and structural proteins (Krebs and Beavo, 1979; Cohen, 1982). A variety of enzymes that catalyze the phosphorylation of protein substrates have been purified to homogeneity and characterized in detail (Flockhart and Corbin, 1982). Although the physiological role of some of these protein kinases (e.g. CAMP-dependent kinase) is well understood, the function of others remains obscure.

Casein kinases are cyclic nucleotide-independent protein kinases that phosphorylate casein and phosphotylin \textit{in vitro} but not histones or protamines (Hathaway and Traugh, 1982). Casein kinases that have been purified to date are of two types. The type I enzyme is composed of a single subunit of $M_r = 30,000–40,000$ and exists in solution as a monomer; the type II enzyme is composed of two dissimilar subunits, $\alpha$ ($M_r = 37,000–44,000$) and $\beta$ ($M_r = 26,000$), and exists in solution as an $\alpha_2\beta_2$ tetramer. Both enzymes appear to be ubiquitous in eukaryotic organisms, and each phosphorylates a broad spectrum of endogenous substrates including translational initiation factors, membrane proteins, proteins associated with mRNP particles, glycogen synthase, and nonhistone nuclear proteins. They are not localized to any particular tissue and can be isolated from a number of subcellular compartments including nuclei, cytosol, membranes, and ribosomes. Although the function of casein kinases is unknown, their broad substrate specificity and wide distribution suggest that they play an important role in the regulation of cellular activity. They are not related to the enzyme that functions in the phosphorylation of casein \textit{in vivo} (Hathaway and Traugh, 1982).

Casein kinase II has been purified from a variety of mammalian and avian species (Hathaway and Traugh, 1982), from Drosophila (Glover et al., 1983), and from yeast (Bell et al., 1977; Rigobello et al., 1982). The enzymes prepared from these various sources are generally found to be remarkably similar in their properties. These properties include a native $M_r \sim 130,000–140,000$, autophosphorylation of the $\beta$ subunit, self-aggregation at low ionic strength, ability to use GTP in place of ATP as nucleotide substrate, and extreme sensitivity to heparin. In spite of the similarities, however, actual homology between any of these enzymes has not yet been demonstrated at the level of primary structure. Furthermore, although the subunit structure is invariably found to be a tetramer consisting of two dissimilar subunits, considerable variation has been observed in the $\alpha$ subunit. The enzyme purified from most mammalian and avian species contains $\alpha$ and $\alpha'$ subunits (Hathaway and Traugh, 1982) which, in the case of the calf thymus enzyme, have been shown to be related in primary structure (Dahmus, 1981a). In contrast, purified casein kinase II from yeast (Rigobello et al., 1982), Drosophila (Glover et al., 1983), and calf brain (Walinder, 1973) appears to contain a single $\alpha$ subunit. The significance of this variation is not completely understood, although proteolysis \textit{in vitro} appears to account for at least some of it (Hathaway and Traugh, 1982).

We have undertaken a detailed comparison of casein kinase II from calf thymus and Drosophila. These two sources were
chosen because of the relatively large phylogenetic distance separating the two organisms and because of the differences reported in the subunit structure of their corresponding enzymes. We report here that casein kinases II from calf and *Drosophila* are related at the level of primary structure, as determined by immunological cross-reactivity and by two-dimensional peptide mapping, that the subunit structure of the enzyme in rapidly prepared whole cell extracts resembles that of the purified proteins, and that the two enzymes have indistinguishable protein substrate specificities. These results imply that casein kinase II has been highly conserved both structurally and functionally during evolution.

**Experimental Procedures**

**Materials**

Unlabeled ATP was obtained from P-L Biochemicals; [γ-32P]ATP (3000 Ci/mmol) was from Amersham. Carrier-free Na<sup>226</sup>I was purchased from New England Nuclear.

Casein, obtained from Nutritional Biochemicals, was treated as described by Reimann et al. (1971). Marker proteins, phosphorylase b, albumin, ovalbumin, carboxy anhydrase, trypsin inhibitor, and α-lactalbumin were purchased from Pharmacia. Trypsin (t-1-tosylamido-2-phenylethyl chloromethyl ketone) was obtained from Worthington. Thermolysin was obtained from Boehringer Mannheim.

Iodogen (1,3,4,6-tetrachloro-3a,6a-diphenylglycoluril) was purchased from Pierce Chemical Co. TLC cellulose sheets, layer thickness of 0.1 mm, were obtained from EM Reagents. Phenylmethylsulfonyl fluoride was obtained from Sigma.

**Methods**

**Protein Kinase Assay**—The standard reaction mixture contained 50 mM Tris-HCl, pH 7.9, 100 mM NaCl, 10 mM MgCl<sub>2</sub>, 0.25 mM EDTA, 0.25 mM dithiothreitol, 5% glycerol, 0.025% Triton X-100, 1 μM [γ-32P]ATP (1 cpm/reaction), 1 mg/ml casein, and enzyme in a final volume of 28 μl. Serum and enzyme samples were diluted in 20 mM Tris-HCl, pH 7.9, 0.5 mM EDTA, 200 mM NaCl, 0.5 mM dithiothreitol, 10% glycerol, and 0.05% Triton X-100 and preincubated at 25 °C for 30 min in a final volume of 14 μl. Salts, nucleotide, and protein substrate were added and the incubation continued for 15 min at 25 °C. Reactions were stopped by the addition of 10 μl of 1 mM ATP and 1 μl of 20% trichloroacetic acid. Precipitates were collected on nitrocellulose filters (Millipore HAWP 02400). The filters were washed three times with 10-ml aliquots of 3% trichloroacetic acid, dried, and counted in 2,5-diphenyloxazole toluene scintillant.

**Gel Electrophoresis**—Electrophoresis in 10 or 12% polyacrylamide-SDS<sup>1</sup> gels was as described by Laemmli (1970). Acid-urea polyacrylamide gels (12% acrylamide, 0.08% N,N'-methylene bisacrylamide containing 6 M urea and 5% acetic acid) were made as described by Panyim and Chalkley (1969) and run as previously described (Glover, 1982).

**Preparation of Polyclonal Antibodies**—Antibodies to calf thymus casein kinase II were prepared as previously described (Dahmus, 1981c). Antibodies to *Drosophila* casein kinase II (isolated from 6-18 h embryos as described (Glover et al., 1983)) were prepared as follows. A female New Zealand White rabbit was injected intramuscularly at approximately six sites in the back with 800 μg of purified enzyme emulsified with an equal volume of complete Freund's adjuvant. The rabbit was boosted at 4- to 8-week intervals (five boosts total) with 250 μg of enzyme emulsified with an equal volume of incomplete Freund's adjuvant. Serum was collected at 1-week intervals beginning 1 week after the first boost and stored in aliquots at -80 °C. A maximum titer was reached after the third boost (50% inhibition of enzyme activity at a 1:100 serum dilution) and maintained thereafter.

**Protein Transfer**—Protein was transferred from SDS-polyacrylamide gels to either DPT paper (Figs. 3 and 4) or nitrocellulose (Fig. 5). DPT paper was prepared as described by Seed (1982) using Whatman 540 paper. Proteins were transferred and reacted with

<sup>1</sup>The abbreviations used are: SDS, sodium dodecyl sulfate; DPT, diazophenylthioether paper.

**Results**

**Subunit Composition of Purified Enzymes**—Calf thymus casein kinase II is composed of three subunits, α, α', and β, having Mr = 44,000, 40,000, and 26,000, respectively (Dahmus, 1981a). Purified *Drosophila* casein kinase II is composed of two subunits, α and β, having Mr = 36,700 and 28,200, respectively (Glover et al., 1983). A comparison of the electrophoretic mobility of calf thymus and *Drosophila* casein kinase II subunits in an SDS-polyacrylamide gel is shown in Fig. 1. The mobility of all of the subunits is consistent with previous molecular weight assignments, confirming that the *Drosophila* α subunit has a molecular weight lower than that of the α' subunit of calf thymus casein kinase II. The *Drosophila* β subunit has a mobility slightly less than that of the corresponding calf thymus subunit and is somewhat more diffuse.

**Immunological Cross-reactivity between Calf Thymus and Drosophila Casein Kinase II**—Polyclonal antibodies were prepared against calf and *Drosophila* casein kinase II by immu-

![Fig. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of calf thymus and Drosophila casein kinase II.](image)

Electrophoresis was carried out in a 10% polyacrylamide gel according to the procedure of Laemmli (1970) and stained with Coomassie blue. *Lane 1*, 2.5 μg of calf thymus casein kinase II; *lane 2*, 2.5 μg of *Drosophila* casein kinase II; *lane 3*, 2.5 μg each of calf thymus and *Drosophila* kinase; *lane 4*, molecular weight markers: phosphorylase b (97,000), albumin (67,000), ovalbumin (45,000), carbonic anhydrase (30,000), and trypsin inhibitor (20,100). The mobility of the α, α', and β subunits of calf thymus casein kinase II (subscript B for bovine) is indicated at the left. The mobility of the α and β subunits of *Drosophila* casein kinase II is indicated at the right. The molecular mass (in kilodaltons) of standard proteins is also indicated at the right.
nization of rabbits with purified native protein. The effect of increasing concentrations of immune serum on the activity of calf thymus and Drosophila casein kinase II is shown in Fig. 2. Each serum is an effective inhibitor of its homologous casein kinase in a standard reaction. There is a slight but reproducible inhibition of enzymatic activity with the heterologous sera.

The subunits of calf thymus and Drosophila casein kinase II were resolved by polyacrylamide gel electrophoresis in the presence of SDS and transferred to DPT paper as described under “Methods.” The transfers were reacted with serum and reactive subunits were visualized by subsequent treatment with 125I-protein A and autoradiography. The results presented in Fig. 3, B–D (lane 1) show that antibody prepared against calf thymus casein kinase II reacts with the α, α', and β subunits of the calf enzyme. Although the sera from three different rabbits all react with each subunit, quantitative differences in reactivity are apparent. Similarly, antibodies prepared against Drosophila casein kinase II react with both the α and β subunits of Drosophila casein kinase II (Fig. 3E, lane 2). Antibody prepared against calf thymus casein kinase II cross-reacts with both the α and β subunit of Drosophila casein kinase II (Fig. 3, B–D, lane 2). Quantitative differences in the reactivity of serum from different rabbits is again apparent. Antibodies prepared against Drosophila casein kinase II cross-react with comparable intensity with the β subunit of the calf enzyme and to a lesser extent with the α and α' subunits (Fig. 3E, lane 1). None of the sera cross-reacts with any of the standard proteins used (Fig. 3, lane 2). These results indicate a high degree of conservation in the determinants recognized by these antibodies.

**Polypeptide Composition of Casein Kinase II in Vivo**—The data of Fig. 1 confirm that the calf and Drosophila enzymes have significantly different molecular structures with respect to the α subunit. This result raises the question of whether or not the molecular structure of the purified enzymes is an accurate reflection of their in vivo structure, particularly since limited proteolysis has been reported to give rise to the α' subunit of casein kinase II in rabbit reticulocytes (Hathaway and Traugh, 1982). This question was addressed by using the casein kinase II-specific antisera to visualize the enzyme subunits in crude fractions and initial homogenates.

Aliquots containing equal units of calf thymus casein kinase II, at various stages of purification, were denatured in the presence of SDS and resolved by polyacrylamide gel electrophoresis. Proteins were transferred to DPT paper and reacted with antibody and 125I-protein A as described under “Methods.” Fig. 4 shows that the molecular composition of the enzyme in the initial cell extract (lane 1) is identical to that of the purified enzyme (lane 9). The α' subunit is present in all stages and the αα' ratio does not appear to change significantly during the course of purification. Identical results were obtained when calf thymus tissue was homogenized directly in sample application buffer containing SDS and β-mercaptoethanol, denatured at 85 °C, and loaded onto the polyacrylamide gel within minutes after cell disruption.

In a similar set of experiments, antibodies prepared against Drosophila casein kinase II were reacted with whole cell extracts from either intact embryos or exponentially growing tissue culture cells (Fig. 5). The mobility of the α and β subunits in extracts from both whole embryos (lane 1) and tissue culture cells (lane 2) is identical to that of the purified enzyme (lane 3). The αβ ratio is also the same in the whole cell homogenates as in the purified enzyme, and no bands are
sensing the relationship between calf and Drosophila casein kinase II, the α and β subunits were analyzed by peptide mapping. Subunits were purified by SDS-polyacrylamide gel electrophoresis, eluted from the gel, and labeled with 125I. Electrophoresis of the 125I-labeled subunits established that the subunits were intact and free of contamination. The iodinated subunits were digested with trypsin or thermolysin and the peptides were resolved by electrophoresis in the first dimension and by chromatography in the second dimension. Resolution of the thermolytic peptides for the various subunits is shown in Fig. 6. The map of the calf thymus α′ subunit closely resembles that of the α subunit and is not included in this figure. For each enzyme, the map of the α subunit is distinct from that of the β subunit indicating that the α and β subunits are not related in primary sequence.

There is, however, extensive similarity between the peptide maps of the α subunits from the two sources (Fig. 6, A and C). The map in B represents a 1:1 mixture of peptides from the calf and Drosophila α subunit and confirms that these subunits give rise to numerous peptides of identical mobility. Similarly, the thermolytic peptide map of the calf thymus β subunit is strikingly similar to the β subunit of Drosophila casein kinase II (Fig. 6, D and F). The composite map (Fig. 6E) confirms that peptides derived from the two sources have identical mobilities. The tryptic peptides (data not shown) were analyzed in the same way and also support the conclusion that there is extensive primary sequence homology between corresponding subunits in the calf and Drosophila enzymes.

**Substrate Specificity of Calf Thymus and Drosophila Casein Kinase II**—Reactions containing either calf thymus casein kinase II, Drosophila casein kinase II, or the catalytic subunit of bovine brain cAMP-dependent protein kinase were incubated with acid-soluble proteins from Drosophila Kc tissue culture cells in the presence of [γ-32P]ATP. The 32P-labeled proteins were then analyzed by acid-urea-polyacrylamide gel electrophoresis. The results presented in Fig. 7 show that the apparent in the region corresponding to proteins of \( M_r = 40,000-44,000 \). The prominent band in lane 1 of Fig. 5B, with \( M_r = 47,000 \), corresponds to yolk protein. Some nonspecific reaction with yolk protein as well as with other unidentified peptides is observed for both immune and preimmune serum.

**Analysis of Tryptic and Thermolytic Peptides of Calf and Drosophila Casein Kinase II**—As an alternate means of analyzing the relationship between calf and Drosophila casein kinase II, the α and β subunits were analyzed by peptide mapping. Subunits were purified by SDS-polyacrylamide gel electrophoresis, eluted from the gel, and labeled with 125I. Electrophoresis of the 125I-labeled subunits established that the subunits were intact and free of contamination. The iodinated subunits were digested with trypsin or thermolysin and the peptides were resolved by electrophoresis in the first dimension and by chromatography in the second dimension. Resolution of the thermolytic peptides for the various subunits is shown in Fig. 6. The map of the calf thymus α′ subunit closely resembles that of the α subunit and is not included in this figure. For each enzyme, the map of the α subunit is distinct from that of the β subunit indicating that the α and β subunits are not related in primary sequence. There is, however, extensive similarity between the peptide maps of the α subunits from the two sources (Fig. 6, A and C). The map in B represents a 1:1 mixture of peptides from the calf and Drosophila α subunit and confirms that these subunits give rise to numerous peptides of identical mobility. Similarly, the thermolytic peptide map of the calf thymus β subunit is strikingly similar to the β subunit of Drosophila casein kinase II (Fig. 6, D and F). The composite map (Fig. 6E) confirms that peptides derived from the two sources have identical mobilities. The tryptic peptides (data not shown) were analyzed in the same way and also support the conclusion that there is extensive primary sequence homology between corresponding subunits in the calf and Drosophila enzymes.

**Substrate Specificity of Calf Thymus and Drosophila Casein Kinase II**—Reactions containing either calf thymus casein kinase II, Drosophila casein kinase II, or the catalytic subunit of bovine brain cAMP-dependent protein kinase were incubated with acid-soluble proteins from Drosophila Kc tissue culture cells in the presence of [γ-32P]ATP. The 32P-labeled proteins were then analyzed by acid-urea-polyacrylamide gel electrophoresis. The results presented in Fig. 7 show that the 

**Fig. 4. Reaction of antibody prepared against calf thymus casein kinase II with partially purified enzyme.** Samples of partially purified casein kinase II were resolved by SDS-polyacrylamide gel electrophoresis on a 12% slab gel and electrophoretically transferred to DPT paper. The paper was reacted with a 1:50 dilution of calf thymus casein kinase II antisera and 125I-protein A as described under "Methods." Lanes 1–7 contain aliquots of casein kinases I and II at various stages of purification (Dahmus, 1981a). Loadings in lanes 1–7 were adjusted so that each lane contained approximately 2 units of casein kinase II except lane 1 which contained 1 unit and lane 6 which contained 2 units of casein kinase I. Lane 1, whole cell extract, 105 µg of protein; lane 2, ammonium sulfate precipitate, 120 µg; lane 3, protamine sulfate supernatant, 72 µg; lane 4, DEAE-cellulose flow-through, 26 µg; lane 5, phosphocellulose P1, 14 µg; lane 6, casein kinase I phosphocellulose P11, 3 µg; lane 7, casein kinase II phosphocellulose P11, 0.6 µg; lane 8, purified casein kinase I, 2 µg; lane 9, purified casein kinase II, 2 µg. The mobility of the α, α′, and β subunits of calf thymus casein kinase II is indicated at the right.

**Fig. 5. Reaction of antibody prepared against Drosophila casein kinase II with Drosophila cell extracts.** Three identical sets of protein samples were analyzed in parallel by SDS gel electrophoresis in a 12% polyacrylamide gel. One set (A) was stained with Coomassie blue. The remaining two sets were electrophoretically transferred to nitrocellulose paper and reacted with either a 1:50 dilution of antiserum prepared against Drosophila casein kinase II (B) or a 1:50 dilution of preimmune serum from the same rabbit (C). Bound antibodies were visualized by reaction with 125I-protein A and autoradiography (Burnette, 1981). All lanes contained approximately the same amount of casein kinase II activity. Lane 1, whole Drosophila proteins from intact embryos, 50 µg/lane; lane 2, whole Drosophila proteins from exponentially growing tissue culture cells (line Kc), 30 µg/lane; lane 3, purified Drosophila casein kinase II, 0.05 µg/lane. For whole protein samples, the fresh tissue was either lysed (Kc cells) or homogenized (embryos) directly in sample application buffer (Laemmli, 1970) heated to 100°C for 5 min, frozen, and stored at -20°C until used. The mobility and molecular mass (in kilodaltons) of standard proteins electrophoresed in the same gel are indicated at the left. The mobility of the α and β subunits of Drosophila casein kinase II is indicated at the right.

**Fig. 6. Analysis of thermolytic peptides from calf thymus and Drosophila casein kinase II.** [125I]-labeled subunits were prepared and digested with thermolysin as described under "Methods." Samples were subjected to electrophoresis toward the cathode at pH 4.4. Chromatography in the second dimension was as described under "Methods." The origin is at the lower right of each map. A–C are peptide maps of the α subunit. A, calf thymus α subunit (2.4 × 10^6 cpm); B, a 1:1 mixture of calf thymus and Drosophila α subunits (4.8 × 10^6 cpm); C, Drosophila α subunit 2.4 × 10^6 cpm). D–F are peptide maps of the β subunit. D, calf thymus β subunit (2.5 × 10^6 cpm); E, a 1:1 mixture of calf thymus and Drosophila β subunits (2.5 × 10^6 cpm); F, Drosophila β subunit (2.5 × 10^6 cpm). Peptides common to a specific subunit are numbered. Autoradiograms were exposed for 2 days for A–C and 4, 6, and 8 days for D–F, respectively.
DISCUSSION

The molecular structure of purified calf thymus casein kinase II appears to be an accurate reflection of the structure of this enzyme in vivo. This is indicated by the fact that cell extracts prepared in the presence of protease inhibitors and denatured within minutes of cell disruption contain both α and α' subunits in the same molar ratio as the purified enzyme. Therefore, in spite of the fact that α and α' are related in primary sequence, α' does not appear to arise from α by proteolytic processing during enzyme purification. This is also supported by the observation that HeLa cells, lysed in Laemmli sample application buffer and analyzed by similar procedures, contain both α and α' (data not shown). Two possibilities that cannot be distinguished at this time are that there are two distinct but related α genes, one for α and one for α', or that α and α' are related by a physiologically relevant processing step either at the level of transcription or translation. In either case, one might expect the molar ratio of α:α' to be dependent on the tissue type, developmental stage, and physiological state. This is consistent with the variability in casein kinase II structure observed in various tissues of different organisms (Walinder, 1973; Dahmus and Natzle, 1977; Thornburg and Lindell, 1977; Hathaway and Traugh, 1979; Dahmus, 1981a; Rigobello et al., 1982; Huang et al., 1982; Ahmad et al., 1982; Glover et al., 1983).

Hathaway and Traugh (1982) have reported that casein kinase II from rabbit reticulocytes contains both α and α' subunits when purified in the absence of protease inhibitors but only an α subunit when purified in the presence of inhibitors. Furthermore, they have observed complete conversion of α into a α' in vitro during prolonged storage of the enzyme at 4 °C. These results indicate that only one α subunit is expressed in rabbit reticulocytes and that the presence of α' is an artifact for this particular tissue. At first glance, it appears surprising that a polypeptide of the same molecular weight should be a legitimate subunit of casein kinase II in the case of calf thymus but an artifact produced in vitro in the case of rabbit reticulocytes. However, if the α polypeptide is physiologically processed to α' in vitro in at least some tissue, then it is possible that small amounts of the responsible protease are present in reticulocytes and effect the same cleavage in vitro during purification or long term storage of the enzyme. Alternatively, if there are two genes or two different mRNAs derived from a single gene, then perhaps α and α' differ by an additional small domain (perhaps encoded by a separate exon) which is sensitive to attack in vitro by nonspecific proteases.

Purified Drosophila casein kinase II consists of a single α and β subunit. The α subunit is unusually small, suggesting that it may correspond to the α' subunit of calf casein kinase II. The immunological transfer experiments indicate that the subunit composition of the purified enzyme is an accurate reflection of the structure of the enzyme in vivo in both embryos and tissue culture cells. In particular, there is no indication of heterogeneity to subunit α. The results suggest that there is only a single α gene expressed in Drosophila and that the product is not processed physiologically. While it is possible that a second α subunit exists but is not expressed in 6–18-h embryos (or in tissue culture cells), this seems unlikely in view of the number of tissue types present at this stage. A more complete analysis of the relationship between the α and α' subunits of calf thymus and the α subunit of Drosophila casein kinase II will require primary sequence analysis of these subunits as well as a characterization of the genes that code for these subunits.

The results presented here establish that Drosophila casein
kinase II is homologous to casein kinase II from mammalian sources. Antibodies prepared against the calf and *Drosophila* enzyme show extensive cross-reactivity in protein transfers. Furthermore, a slight but reproducible inhibition of enzymatic activity was observed when antibody was reacted with the heterologous enzyme. The enzymes from the two sources also show extensive structural similarities. Native casein kinase II from calf thymus and *Drosophila* have $M_r = 132,000-140,000$ and 130,000, respectively and are composed of polypeptides of approximately the same size (Dahmus, 1981a; Glover et al., 1983). Peptide maps derived from purified subunits establish that homologous subunits from the two enzymes share extensive primary sequence homology. The catalytic activity of the two enzymes is also comparable in that each enzyme can utilize either ATP or GTP as nucleotide substrate and is inhibited by heparin (Dahmus, 1981a; Glover et al., 1983). The protein substrate specificity of calf thymus and *Drosophila* casein kinase II also appears to be identical. This is true even when the enzymes are challenged with complex mixtures of potential substrates. Such striking similarities in structure and function imply a high degree of conservation of casein kinase II since the divergence of these organisms.

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