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Isolation and Sequencing of cDNA Clones Encoding Alpha and Beta Subunits of *Drosophila melanogaster* Casein Kinase II

AMIT SAXENA, RAMESH PADMANABHA, AND CLAIBORNE V. C. GLOVER*

Department of Biochemistry, University of Georgia, Athens, Georgia 30602

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Cloned cDNAs encoding both subunits of *Drosophila melanogaster* casein kinase II have been isolated by immunological screening of λgt11 expression libraries, and the complete amino acid sequence of both polypeptides has been deduced by DNA sequencing. The alpha cDNA contained an open reading frame of 336 amino acid residues, yielding a predicted molecular weight for the alpha polypeptide of 39,833. The alpha sequence contained the expected semi-invariant residues present in the catalytic domain of previously sequenced protein kinases, confirming that it is the catalytic subunit of the enzyme. Pairwise homology comparisons between the alpha sequence and the sequences of a variety of vertebrate protein kinases suggested that casein kinase II is a distantly related member of the protein kinase family. The beta subunit was derived from an open reading frame of 215 amino acid residues and was predicted to have a molecular weight of 24,700. The beta subunit exhibited no extensive homology to other proteins whose sequences are currently known.

Casein kinase II is a cyclic nucleotide-independent, Ca^{2+}- and calmodulin-insensitive protein kinase which is widely distributed among eucaryotic organisms (15). The enzyme phosphorylates a broad spectrum of both nuclear and nonnuclear substrates (1, 5, 11, 15), suggesting that it may function in the regulation or integration of cell metabolism. Casein kinase II from most sources is composed of two dissimilar subunits, alpha (35 to 44 kilodaltons [kDa]) and beta (24 to 29 kDa), which combine to form a native $\alpha_2\beta_2$ tetramer with a molecular weight of 130,000 to 150,000. The enzyme can utilize either ATP or GTP as the nucleoside triphosphate donor and phosphorylates serine or threonine residues in protein substrates. A cluster of acidic residues located immediately C-terminal to the modified residue appears to be important for substrate recognition (15, 25). The alpha polypeptide has been identified as the catalytic subunit by three independent methods (6, 17, 26). The beta subunit becomes phosphorylated when the enzyme is allowed to undergo autophosphorylation, but the function of this subunit and the significance of autophosphorylation are not known.

In an effort to initiate a genetic analysis of casein kinase II, we previously isolated and characterized the enzyme from *Drosophila melanogaster* (14). The purified kinase is composed of a 37-kDa alpha and 28-kDa beta subunit which form a 130,000-dalton $\alpha_2\beta_2$ tetramer. Like its counterpart in other species, the enzyme phosphorylates both serine and threonine residues, utilizes either ATP or GTP, exhibits autophosphorylation of the beta subunit, and is strongly inhibited by heparin, a characteristic feature of casein kinase II (15). In addition, the *Drosophila* enzyme displays a protein substrate specificity which is virtually indistinguishable from that exhibited by the enzyme from calf thymus (7). Peptide mapping and immunological studies demonstrate that the insect and mammalian enzymes are homologous and suggest that casein kinase II has been highly conserved during evolution (7).

Here we report the isolation and sequencing of cDNA clones encoding both subunits of *Drosophila* casein kinase II. The alpha sequence displays the expected homology to the catalytic domain of other protein kinases, confirming its identification as the catalytic subunit. The beta subunit exhibits no extensive homology to other sequenced proteins but does contain possible casein kinase II recognition sites, one or more of which may represent the autophosphorylation site(s) on this subunit.

MATERIALS AND METHODS

Reagents. The M13 sequencing kit, [α-^{35}S]dATP (1,000 Ci/mmol), and ^{125}I-protein A (30 mCi/mg) were obtained from Amersham Corp. Nitrocellulose membranes (BA-85) and DEAE membrane (NA-45) were purchased from Schliecher and Schuell. Restriction enzymes, T4 DNA ligase, and T4 DNA polymerase were purchased from New England BioLabs, and terminal deoxynucleotidyl transferase was from Pharmacia. Bromphenol blue, 4-chloro-1-naphthol, 5-bromo-4-chloro-3-indolyl-β-D-galactoside (X-gal), isopropyl-β-D-thiogalactopyranoside (IPTG), and protein molecular weight standards (myosin, β-galactosidase, phosphorylase B, bovine serum albumin, egg albumin, and carbonic anhydrase) were all obtained from Sigma Chemical Co. Horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (IgG) and sodium dodecyl sulfate (SDS) were purchased from Bio-Rad Laboratories. *Escherichia coli* strains used were Y1089 [*ΔlacU169 proA*$^+$ *Δlon araD139 strA hflA150* (*chr*::Tn*10*) (pMC9)], Y1090 [*ΔlacU169 proA*$^+$ *Δlon araD139 strA supF* (*trpC22*::Tn*10*) (pMC9)] (45), and JM105 (*Δlac-pro thi strA endA sbcB15 hsdR4* F′ *traD36 proAB lacI*^{q}*Z M15*) (43).

λgt11 libraries. The λgt11 genomic library used here was a gift of J. Wang (Harvard). This library was prepared from total genomic DNA of *D. melanogaster* (Oregon R) and consists of 10^7 independent recombinants which have been amplified on *E. coli* Y1088 (G. Pflugfelder and J. Wang, personal communication). The λgt11 cDNA library, a gift of T.-S. Hsieh (Duke), was prepared from total polyadenylated [poly(A)$^+$] RNA isolated from *D. melanogaster* embryos (29).

Antisera. Production and characterization of rabbit antisera against native *Drosophila* casein kinase II and the SDS gel-purified alpha and beta subunits of the *Drosophila* kinase have been described previously (7, 31). Rabbit antiserum

* Corresponding author.

directed against native casein kinase II of calf thymus (7) was a gift of M. Dahmus (U. C. Davis). All sera were used at a working dilution of 1:100 (in 50 mM Tris, 150 mM NaCl, 5 mM EDTA, 3% bovine serum albumin, pH 8.0) and were preadsorbed (M. D. Snyder, D. Sweetser, R. A. Young, and R. W. Davis, Methods Enzymol., in press) to remove anti-*E. coli* antibodies. Affinity-purified alpha- and beta-specific antibodies were also used in some experiments. These were isolated from the native *Drosophila* antiserum by the technique of Olmsted (30) with the SDS-denatured subunits of the *Drosophila* enzyme as the affinity ligand.

Isolation and characterization of cDNA and genomic clones in λgt11. λgt11 phage libraries were screened as described by Snyder et al. (in press). Phage were plated on *E. coli* Y1090 at a density of 50,000 phage per 150-mm plate. Following incubation at 42°C for 3 h, each plate was overlaid with an IPTG-impregnated nitrocellulose filter to induce fusion protein synthesis and simultaneously form a replica on the filter. Filters were then incubated with immune serum followed by horseradish peroxidase-conjugated goat anti-rabbit IgG to visualize antibody positives (Snyder et al., in press). The antiserum against native *Drosophila* casein kinase II (7) was used in the primary screen, as well as in subsequent screens required to plaque purify the initial positives.

Plaque-purified positives were counterscreened with preimmune and subunit-specific sera by a spot assay described by Young and Davis (45). Drops of plaque-purified phage (100 PFU/10-μl drop) were spotted in an array on a freshly prepared lawn of *E. coli* Y1090. Following phage growth, a replica of the array was transferred to IPTG-impregnated nitrocellulose and probed with the desired antiserum followed by ^{125}I-protein A (Snyder et al., in press). The dried membrane was autoradiographed at room temperature on Cronex 4 X-ray film with a Lightning-Plus intensifying screen (Dupont).

Antigenic polypeptides synthesized by individual λgt11 recombinants were analyzed as described by Snyder et al. (in press). Selected positives were first converted to lysogens in *E. coli* Y1089. Each lysogen was grown at 30°C to an A_{600} of 0.5, temperature-induced at 44°C for 15 min to initiate the lytic cycle, and then incubated for 1 h at 37°C in the presence of 10 mM IPTG to induce expression from the *lac* promoter. Total *E. coli* proteins were isolated as described by Snyder et al. (in press) and analyzed by electrophoresis in 8% SDS–polyacrylamide gels (22). Proteins were electrophoretically transferred from the gel to nitrocellulose as described by Burnette (3), except that 0.1% SDS was included in the transfer buffer. The nitrocellulose was then probed with the desired antiserum followed by ^{125}I-protein A and autoradiographed as described above.

DNA sequencing. The *Eco*RI inserts of selected λgt11 recombinants were subcloned (27) in both orientations into the *Eco*RI site of the M13 vector mp18 (43). Approximately 400 ng of *Eco*RI-digested phage DNA and 80 ng of *Eco*RI-cleaved mp18 were ligated and used to transform *E. coli* JM105. Clear plaques were analyzed by direct gel electrophoresis to identify recombinants having an insert of the expected size, and the relative orientation of the inserts was then established via a complementarity test (27). The latter procedure was modified in that hybridization was carried out in 5 mM EDTA (pH 8.0)–0.3 M NaCl–0.6% SDS–0.02% bromphenol blue–12% formamide (44) and agarose gel electrophoresis was carried out in the presence of ethidium bromide (0.5 μg/ml). The latter modification greatly facilitates visualization of positives by increasing the separation of hybridized from nonhybridized molecules.

For sequencing, individual mp18 subclones were subjected to the nested deletion procedure of Dale et al. (8). Overlapping deletions were identified by direct gel electrophoresis and sequenced by the dideoxy chain termination method (35). Sequencing was carried out with the Amersham sequencing kit with [α-^{35}S]dATP as described by the manufacturer. The Microgenie Sequence Analysis Program (32) was used for routine DNA sequence analysis. Homology comparisons among various protein kinases were carried out with a Fortran program written by M. Murata. This program generates alignments via the algorithm of Needleman and Wunsch (28), using as a weighting scheme the 250 PAM mutation data matrix of Dayhoff et al. (9). For each sequence comparison, 200 alignments between random sequences of identical length and composition were carried out to provide statistics for calculating an alignment score. The latter was calculated as the number of standard deviations by which the score obtained for the real sequences differed from the mean score obtained for random sequences.

N-terminal amino acid sequencing of the alpha polypeptide. The alpha subunit of *Drosophila* casein kinase II was purified by electroelution from preparative SDS-polyacrylamide gels exactly as described by Padmanabha and Glover (31). A 300-pmol sample of the eluted polypeptide was concentrated to a volume of 30 μl by rotary evaporation and sequenced by automated Edman degradation on an Applied Biosystems 470A protein sequencer equipped with an on-line Applied Biosystems 120A PTH analyzer. Initial yield was approximately 40 pmol of amino acid derivative per 100 pmol of protein sequenced. Repetitive yield was estimated at 90%, based on the yields obtained for several of the more stable amino acids. Sequencing was carried out at the Molecular Genetics Instrumentation Facility at the University of Georgia.

Genomic Southern analysis. Genomic DNA was isolated from adult *D. melanogaster* (Canton S) by method 1 of Kuner et al. (21). Samples of the DNA were digested with the appropriate restriction enzymes and electrophoresed (7.5 μg/lane) in a 0.8% agarose gel (24). The fragments were transferred to nitrocellulose by the procedure of Southern (38) and hybridized with the ^{32}P-labeled cDNA insert of either Dm95 (alpha) or Dm98 (beta). Insert DNA was isolated by *Eco*RI digestion of phage DNA, followed by preparative agarose gel electrophoresis. The insert fragment was recovered from the gel by the band interception technique with NA45 DEAE membrane (Schleicher and Schuell, application update no. 364) and ^{32}P-labeled by random hexamer priming as described by Feinberg and Vogelstein (12). Hybridization was carried out in 40% formamide at 42°C, and filters were washed twice in 0.1× SSC (15 mM NaCl, 1.5 mM sodium citrate, pH 7.0) at 65°C for 1 h each. The air-dried filters were autoradiographed at −80°C on Cronex 4 X-ray film with a Lightning-Plus intensifying screen (Dupont).

RESULTS

Immunological screening of λgt11 expression libraries. To isolate recombinant clones encoding the subunits of *Drosophila* casein kinase II, we screened both a cDNA and a genomic λgt11 expression library with a rabbit antiserum raised against native *Drosophila* casein kinase II. This serum reacts with both the alpha and beta subunits of the kinase (7) and should thus be capable of identifying recombinant clones encoding either subunit. Eighty-one positives (designated Dm10 through Dm90) were identified in a primary screen of

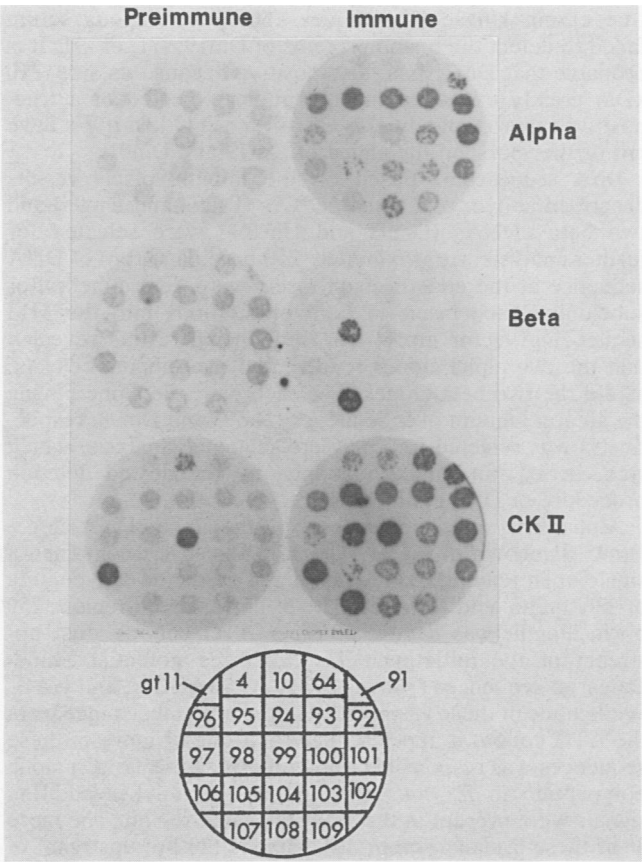

FIG. 1. Immunological reactivity of selected λgt11 recombinants. Six lawns of *E. coli* Y1090 were spotted with an identical array of 21 plaque-purified λgt11 recombinants. Following phage growth, a replica of the array was transferred to IPTG-impregnated nitrocellulose and probed with the desired antiserum, followed by ^{125}I-protein A. An autoradiogram of the filter is shown. Alpha, Antiserum against the SDS gel-purified alpha subunit. Beta, Antiserum against the SDS gel-purified beta subunit. CK II, Antiserum against native casein kinase II (originally used to isolate the recombinants and reactive with both subunits). Immune sera are shown on the right; preimmune sera from the corresponding rabbits are on the left. Numbers in the key at the bottom refer to the individual recombinants. A negative control, consisting of the λgt11 vector without an insert, was included in the array (gt11). Clone 4 contains a yeast DNA insert and is unrelated to the experiments described here.

3×10^6 recombinant phage from the genomic library. Of these, 49 rescreened and were plaque-purified. Nineteen positives (designated Dm91 through Dm109) were identified in a primary screen of 6×10^5 recombinant phage from the cDNA library. All of these positives rescreened and were plaque-purified.

A spot test described by Young and Davis (45) was used to counterscreen each positive with preimmune serum (to eliminate one class of false-positives) and with subunit-specific antisera (to further confirm true-positives and also to distinguish alpha from beta recombinants). The subunit-specific sera were raised against the SDS gel-purified alpha and beta polypeptides and exhibit a high degree of specificity for the respective subunit (31). Spot tests for two of the genomic and all 19 of the cDNA positives are shown in Fig. 1. As expected, all of the recombinants gave positive signals with the antibody originally used in their isolation (CKII, immune). The intensity of these signals varied over a considerable range and reflected the intensity observed during the original screening. Three clones (Dm10, Dm99, and Dm106) could be readily identified as false-positives by their strong reaction with preimmune serum from the same rabbit (CKII, preimmune). At least one polypeptide displaying strong reactivity with both preimmune and immune serum from this rabbit can, in fact, be readily detected on Western blots of whole *Drosophila* extracts (7). Of the remaining 18 clones, 15 reacted with the alpha-specific serum, two (Dm98 and Dm107) with the beta-specific serum, and one (Dm64) with neither.

Western blotting. To characterize further the remaining positive clones, each was first converted to a lysogen in *E. coli* Y1089. Lysogens were grown and temperature- and IPTG-induced as described in Materials and Methods. Total proteins from each lysogen were then isolated and analyzed by SDS gel electrophoresis. Immunologically reactive polypeptides were detected by Western blotting and immunological staining (Fig. 2). When probed with the antiserum originally used in their isolation (Fig. 2A), approximately 50% of the recombinants (e.g., Dm98) were found to synthesize an antigenically reactive polypeptide of high molecular weight. This was as expected, since λgt11 is designed to produce an IPTG-inducible translational fusion between the amino-terminal 112 kDa of β-galactosidase and the polypeptide encoded by the foreign DNA insert (Snyder et al., in press). However, a large proportion of the clones (e.g., Dm65) synthesized a low-molecular-weight antigen of 37 kDa. This polypeptide exactly comigrated with the *Drosophila* alpha subunit (Fig. 2, lane CK) and was only observed in clones which reacted with alpha-specific antiserum (Fig. 1 and data not shown). These results strongly suggest that the alpha polypeptide is synthesized intact in these clones and hence that these recombinants represent bona fide alpha positives. The results also predict that a fortuitous *E. coli* ribosome-binding site should be found upstream of the alpha coding region (see below) and, since several clones which produce the 37-kDa antigen were genomic, that the alpha coding region contains no introns.

Two cDNA clones (Dm92 and Dm108) produced both the 37-kDa antigen and a small amount of a high-molecular-weight fusion protein. Although we have no definitive explanation for this, both clones produced a relatively large amount of truncated β-galactosidase (as detected by staining with Coomassie blue, data not shown), so it is possible that a low level of translational readthrough or perhaps frameshifting leads to production of the fusion material. All of the immunologically reactive polypeptides detected in Fig. 2A were nonreactive with preimmune serum (Fig. 2C), and all were under IPTG control (data not shown). Finally, two of the recombinants shown in Fig. 2A (Dm59 and Dm90) produced no antigenically detectable polypeptide. The most plausible explanation for this is that the epitope(s) recognized in the plaque assay is denatured during SDS gel electrophoresis.

As a further immunological test for true casein kinase II positives, selected recombinants were probed with a heterologous antiserum (a gift of M. Dahmus) prepared against native casein kinase II of calf thymus. This antiserum strongly crossreacts with both subunits of *Drosophila* casein kinase II (7) (Fig. 2B, lane CK). As shown in Fig. 2B, this serum efficiently recognized the 37-kDa polypeptide in every clone which produced it, as well as the fusion proteins produced by Dm92, -98, -105, -107, and -108. This result further supports the identification of these recombinants as

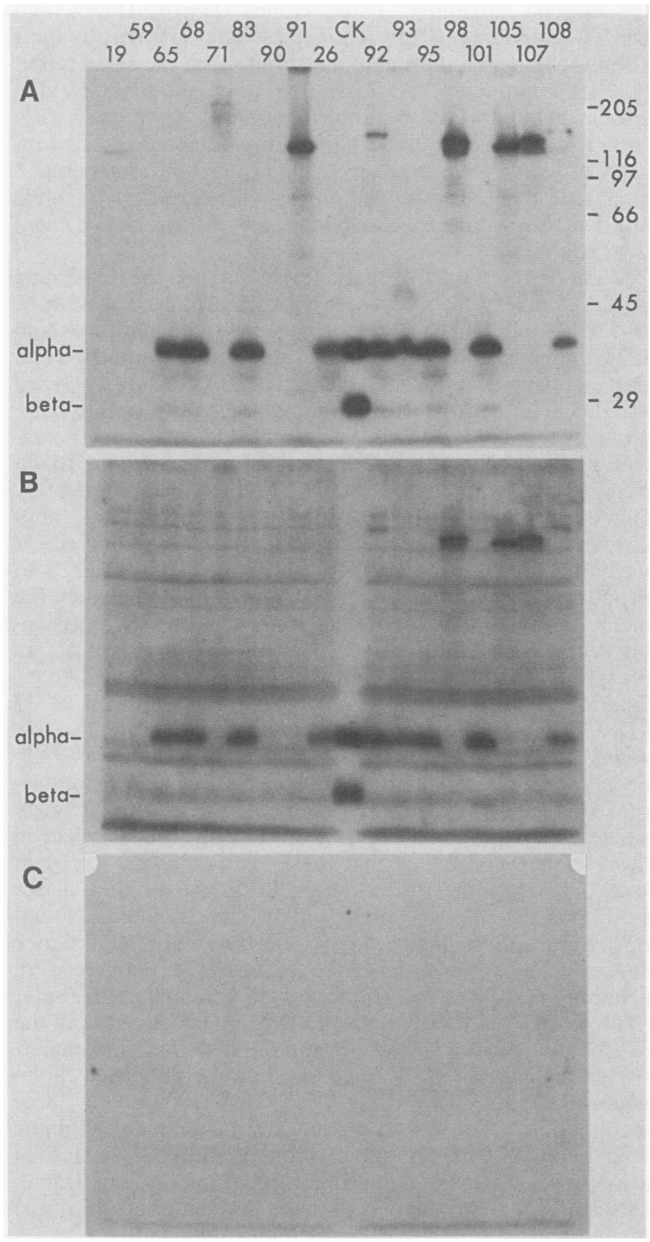

FIG. 2. Immunological reactivity of fusion proteins synthesized by selected λgt11 recombinants. Total *E. coli* proteins were isolated from induced lysogens and subjected to electrophoresis in three identical 8% polyacrylamide–SDS gels. Each gel was transferred to a nitrocellulose filter, and each filter was then incubated with a different serum, followed by ^{125}I-labeled protein A. An autoradiogram of the filters is shown. (A) Antiserum against native casein kinase II of *Drosophila*. (B) Antiserum against native casein kinase II of calf thymus. (C) Preimmune serum from the same rabbit as in panel A. The numbers at the top refer to individual λgt11 recombinants. The mobilities of size standards are indicated at the right: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), egg albumin (45 kDa), and carbonic anhydrase (29 kDa). The lane labeled CK was loaded with 100 ng of homogeneous *Drosophila* casein kinase II, purified as described by Glover et al. (14). The alpha and beta subunits are indicated at the left in panels A and B.

true casein kinase II positives. The heterologous serum failed to detect the fusion proteins of Dm19, -71, or -91. It is probable that Dm71 is a false-positive because its size (210 kDa) greatly exceeded the maximum expected of a true-positive (approximately 112 + 37 kDa = 149 kDa). We have not further pursued the status of Dm19 and Dm91.

DNA sequencing. Based on the immunological results described above, two alpha cDNAs (Dm92 and Dm95) and two beta cDNAs (Dm98 and Dm107) were selected for further analysis. Approximately 250 base pairs (bp) of DNA sequence at the ends of each insert were determined after subcloning each insert in both orientations into the M13 sequencing vector mp18. The sequencing results revealed that the two alpha clones represented overlapping cDNAs, as did the two beta clones. For each pair, the clone having the greater amount of 5′ sequence (Dm95 and Dm98, respectively) was selected for complete sequence analysis, which was carried out on both strands by the nested deletion procedure of Dale et al. (8).

Alpha. The complete sequence of the alpha cDNA clone, Dm95, is shown in Fig. 3. The 1,483-bp insert contained a single open reading frame encoding a predicted polypeptide of 336 amino acid residues. The 5′ untranslated region, 259 bp in length, was devoid of other ATG codons. Just upstream of the initiating ATG lay three potential Shine-Dalgarno sequences (36), AAGGAGG, AAGGA, and GAG. While none of these was located at the optimal distance from the ATG codon, it appears likely that one or more of these sequences was responsible for synthesis of the 37-kDa alpha polypeptide in *E. coli*. Two AATAAA poly(A) addition signals were present in the 3′ untranslated region. The more 3′ of these lay an appropriate distance (20 bp) upstream of the terminal poly(A) stretch, suggesting that it is used in vivo.

We have previously reported an N-terminal amino acid sequence for the alpha subunit of *Drosophila* casein kinase II (31). This sequence was determined by automated Edman degradation of the intact enzyme, an approach made possible by the fact that the beta subunit is N-terminally blocked (Padmanabha and Glover, unpublished observation). We have recently confirmed that this amino acid sequence derives from the alpha subunit by sequencing the SDS gel-purified alpha subunit (see Materials and Methods). The 45 residues identified during these protein-sequencing experiments corresponded to those predicted from the cDNA sequence (Fig. 3, underlined amino acids), confirming that Dm95 encodes the alpha subunit of casein kinase II. The protein sequencing results also demonstrated that the initiating methionine residue is removed in vivo. The newly exposed N-terminal residue, threonine (Fig. 3), is a stabilizing amino acid according to the N-end rule of Bachmaier et al. (2). The mature alpha polypeptide was predicted to have a molecular mass of 39,833 daltons, 8% above the 37,000-dalton estimate obtained by SDS gel electrophoresis (14).

Both serine/threonine and tyrosine protein kinases contain a homologous catalytic domain (20). One version of a protein kinase consensus sequence is shown in Fig. 4. The alpha subunit of casein kinase II exhibited a good fit to this consensus sequence, confirming earlier evidence (6, 17, 26) that it is the catalytic subunit of the enzyme. To quantitate the degree of homology between casein kinase II and other protein kinases, pairwise comparisons were made between the core catalytic domain of the alpha subunit (residues 42 to 229 of the mature protein) and the corresponding region of a variety of vertebrate serine/threonine and tyrosine protein kinases (Table 1). Although the catalytic domain of casein

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TTTTTTTTACTAAGACTGCAAAACTGTGAGGAACATACCCGGCATACACTGTGGATATCATACGAGACGGAGAAATCTTTTAAAATACTTTGGCAAGCCAGCTTATAACAATCAATCTTC  120

AAAAATTGGATTGTTGTACAATAATAATATTGTTAGGACCACCAACCGACTGAGAACGGAATCAGCTCACATAAACTTACTGCTACAACCCGAAAAAATTTTAACAAGGAGGCAGAAAGG  240

AACGTTTTGTTGAGAAAAATGACACTTCCTAGTGCGGCTCGCGTGTACACAGATGTCAATGCGCACAAACCGGATGAATATTGGGACTATGAAAATTATGTGGTTGATTGGGGCAATCAA  360
                    M  T  L  P  S  A  A  R  V  Y  T  D  V  N  A  H  K  P  D  E  Y  W  D  Y  E  N  Y  V  V  D  W  G  N  Q    34

GACGATTATCAGTTGGTCCGTAAATTAGGCCGTGGAAAGTATTCTGAGGTCTTCGAGGCCATTAATATTACGACCACGGAAAAGTGCGTTGTTAAAATTCTGAAACCTGTTAAAAAAAAG  480
D  D  Y  Q  L  V  R  K  L  G  R  G  K  Y  S  E  V  F  E  A  I  N  I  T  T  T  E  K  C  V  V  K  I  L  K  P  V  K  K  K    74

AAGATAAAGCGTGAAATCAAAATTTTGGAGAACTTGCGTGGAGGAACTAATATAATAACACTTTTAGCCGTTGTCAAGGACCCAGTTTCTCGAACACCAGCGTTGATTTTTGAGCACGTC  600
K  I  K  R  E  I  K  I  L  E  N  L  R  G  G  T  N  I  I  T  L  L  A  V  V  K  D  P  V  S  R  T  P  A  L  I  F  E  H  V   114

AACAACACAGATTTCAAGCAACTTTACCAAACATTAACTGATTATGAGATTCGTTACTACTTGTTTGAGCTTCTTAAGGCACTTGACTACTGCCACAGCATGGGAATAATGCATCGTGAT  720
N  N  T  D  F  K  Q  L  Y  Q  T  L  T  D  Y  E  I  R  Y  Y  L  F  E  L  L  K  A  L  D  Y  C  H  S  M  G  I  M  H  R  D   154

GTAAAGCCCCACAATGTTATGATAGATCACGAAAATCGAAAATTGCGCCTTATAGATTGGGGACTTGCCGAATTTTACCATCCTGGTCAAGAATATAATGTTCGTGTGGCTTCGAGATAC  840
V  K  P  H  N  V  M  I  D  H  E  N  R  K  L  R  L  I  D  W  G  L  A  E  F  Y  H  P  G  Q  E  Y  N  V  R  V  A  S  R  Y   194

TTTAAAGGTCCCGAATTACTGGTAGATTACCAGATGTATGATTACTCACTGGATATGTGGTCACTAGGTTGTATGTTGGCGTCGATGATATTCCGAAAAGAGCCATTTTTCCACGGACAT  960
F  K  G  P  E  L  L  V  D  Y  Q  M  Y  D  Y  S  L  D  M  W  S  L  G  C  M  L  A  S  M  I  F  R  K  E  P  F  F  H  G  H   234

GATAACTATGATCAATTGGTACGCATTGCCAAGGTGCTGGGCACCGAAGAACTCTACGCATATTTGGATAAATACAATATTGACCTCGATCCAAGATTTCACGACATTCTACAGCGTCAC 1080
D  N  Y  D  Q  L  V  R  I  A  K  V  L  G  T  E  E  L  Y  A  Y  L  D  K  Y  N  I  D  L  D  P  R  F  H  D  I  L  Q  R  H   274

TCACGAAAGCGATGGGAAAGATTTGTCCATTCTGACAACCAACATCTAGTTTCTCCTGAAGCACTAGACTTTCTTGATAAACTTTTACGCTATGATCACGTTGATCGACTCACAGCTCGC 1200
S  R  K  R  W  E  R  F  V  H  S  D  N  Q  H  L  V  S  P  E  A  L  D  F  L  D  K  L  L  R  Y  D  H  V  D  R  L  T  A  R   314

GAAGCAATGGCCCATCCATATTTCTTACCTATTGTCAATGGTCAAATGAATCCCAATAATCAGCAATAAGAAGTTTTTTCATTTTGATGAATACTGTAATTCGAGTTTGGGATAGAAGCC 1320
E  A  M  A  H  P  Y  F  L  P  I  V  N  G  Q  M  N  P  N  N  Q  Q  *                                                        336

ATTTAACAATATGATAATTATGCAAAAAAAAAAATATAAAATCCGAAGAAAAATAAAATAACTTAAGCTACTGTCTTAAAAATGACTTCAACTGTTCGTTAAGGGATTAAACAGAGAAATC 1440

GAATAAATTCCTCACAGTATAACATCTAAAAAAAAAAAAAAAA  1483
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FIG. 3. Nucleotide sequence of a cDNA clone encoding the alpha subunit of *Drosophila* casein kinase II. The sequence of the coding strand of the cDNA insert of Dm95 is shown, oriented in the 5′ to 3′ direction. Two potential poly(A) addition signals (AATAAA) are underlined. The deduced amino acid sequence of the longest open reading frame is shown below the sequence, with the one-letter abbreviations. The asterisk indicates the stop codon (TAA). Underlined amino acids were identified during automated Edman degradation of the intact enzyme (the beta subunit is blocked; see text) and/or the SDS gel-purified alpha subunit. No residue was identified at cycles 40 and 44, both of which correspond to arginines in the predicted sequence.

kinase II was clearly related to that of the other enzymes (alignment score, 8 to 12), casein kinase II appeared to be a rather distantly related member of the family. In particular, it appeared to be as distantly related to the other serine/threonine kinases as are the tyrosine kinases. Most of the homology observed between casein kinase II and the other kinases examined was, in fact, primarily a function of the conserved residues depicted in Fig. 4. Even there, casein kinase II was distinguished by a number of conservative replacements at positions which are universally conserved in other protein kinases (e.g., DWG in place of DFG, Fig. 4).

Beta. The sequence of cDNA clone Dm98, encoding the beta subunit of the kinase, is shown in Fig. 5. The 964-bp sequence contained an open reading frame encoding a predicted polypeptide of 215 amino acid residues. The 3′ untranslated region, 294 nucleotides in length, terminated in an oligo(A) stretch which was not preceded at the expected distance by a canonical AATAAA poly(A) addition signal. Partial sequencing of the second beta cDNA clone, Dm107, revealed the presence of two AATAAA poly(A) addition signals located approximately 570 and 720 bp downstream of the translation stop codon. The more 3′ of these signals was located at the expected distance upstream of the oligo(A) tail in Dm107, suggesting that it is used in vivo. It is quite possible, therefore, that Dm98 was primed from an internal A-rich sequence in a longer mRNA.

Although no protein sequencing data are currently available for the *Drosophila* beta subunit, the complete amino acid sequence of the beta subunit of calf lung casein kinase II has recently been determined (40a). This sequence, which contains 209 amino acid residues, was aligned in a gap-free manner with the predicted *Drosophila* sequence (deduced from Dm98), and the aligned sequences were 86% identical. This result confirmed that Dm98 encodes the beta subunit of

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CK II Alpha:  ···--[G•G••S•V]----(12)---[V•K]----(86)---[RDV•••N]--(17)--
Consensus:    ···--[G•G••G•V]--(11-18)--[A•K]--(89-99)--[RDL•••N]--(16)--

CK II Alpha:  --[DWG]----(21)---[GPE]--(12)--[D•W••G]---(8)---[R••P]-- ···
Consensus:    --[DFG]--(19-24)--[APE]--(11)--[D•W••G]--(8-9)--[G••P]-- ···
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FIG. 4. Homology of casein kinase II (CK II) with a protein kinase consensus sequence. The lower line (consensus) depicts invariant or nearly invariant residues which are present in both serine/threonine and tyrosine protein kinases (based on the sequence alignments of Hunter and Cooper [18] and Knopf et al. [19]). The upper line displays the corresponding residues of the alpha subunit of *Drosophila* casein kinase II (based on an alignment obtained by visual inspection). A dot within a boxed region indicates a variable amino acid, and numbers in parentheses indicated the range of spacings observed between the various conserved blocks. The region shown extends from residues 49 to 253 of cyclic AMP-dependent protein kinase (37) and from residues 43 to 228 (not counting the initiating methionine) of casein kinase II (Fig. 3).

TABLE 1. Sequence homology among various protein kinases[a]

Sequence	% Identical amino acid residues \ Alignment score						
	CKII	cA	cG	PKC-1	PhK-γ	MLCK	c-*src*
CKII		9.0	9.0	9.7	9.0	11.9	8.6
cA	25		26.7	27.4	16.4	13.9	11.2
cG	28	53		22.6	16.1	15.6	8.8
PKC-1	30	49	42		15.6	15.3	12.3
PhK-γ	28	38	34	35		14.2	9.4
MLCK	28	31	31	31	37		12.1
c-*src*	26	28	29	30	24	34	

[a] For each comparison, only the region corresponding to the consensus sequence of Fig. 4 plus one additional residue at either end was analyzed. This region, which is approximately 200 amino acids in length, corresponds to residues 48 to 254 of cyclic AMP-dependent protein kinase. Alignments were generated and alignment scores calculated as described in Materials and Methods. Alignment scores are expressed in standard deviation units, i.e., a value of 9.0 indicates an alignment which is 9 standard deviations away from the mean obtained for 200 alignments between random sequences of identical length and composition. CKII, *Drosophila* casein kinase II; cA, cyclic AMP-dependent protein kinase (37); cG, cyclic GMP-dependent protein kinase (41); PKC-1, protein kinase C-1 (19); PhK-γ, phosphorylase kinase, gamma subunit (34); MLCK, myosin light-chain kinase (40); c-*src*, chicken c-*src* tyrosine kinase (39).

casein kinase II. Since the *Drosophila* sequence was 3 amino acids longer than the calf sequence at the amino terminus, the data also make it very likely that Dm98 contains a full-length protein-coding region. The polypeptide encoded by Dm98 was predicted to have a molecular mass of 24,700 daltons (assuming that the initiating methionine residue is removed and neglecting the N-terminal blocking group, the structure of which is unknown). This value is 12% below the 28,000-dalton estimate obtained by SDS gel electrophoresis of the intact beta subunit (14).

A search of the National Biomedical Research Foundation protein data base for sequences related to the beta subunit revealed no statistically significant homologies. The amino acid composition and predicted secondary structure of the polypeptide were not unusual and yielded little information about the possible function(s) of this polypeptide. Since the beta subunit is subject to autophosphorylation, i.e., is a substrate for the catalytic subunit, the beta sequence was examined for the presence of typical casein kinase II recognition sites (a serine or threonine residue followed by a series of acidic residues). The three best candidates present in the sequence were SEE, SDMTE, and SDIPGE (the D and E residues are acidic), the locations of which are indicated in Fig. 5.

Genomic Southern analysis. To estimate the copy number of the alpha and beta genes, the inserts of Dm95 (alpha) and Dm98 (beta) were labeled with ^{32}P and used to probe restriction digests of total *Drosophila* genomic DNA under high-stringency conditions (0.1× SSC, 65°C). When the alpha probe was hybridized to genomic DNA cleaved with restriction enzymes which do not cut the probe itself (Fig. 6, lanes 1, 2, and 3), one major and at least one minor band were observed in each digest. Since the protein-coding region appeared to contain no introns (see above), the existence of the minor band(s) suggests the possibility of a second, related gene copy. However, we cannot yet rule out the possibility of an intron (containing the necessary restriction sites) in either the 5′ or 3′ untranslated region of Dm95.

Genomic Southern analysis carried out with the beta probe yielded two strongly hybridizing bands for *Eco*RI (Fig. 6, lane 4) and three for *Pst*I (lane 5). However, all of these bands can be explained as deriving from a single locus: preliminary genomic cloning revealed the presence of an *Eco*RI site in an intron interrupting the beta coding region, and the cDNA probe itself contained two *Pst*I sites (which were separated by an intron in the genomic DNA). The results thus suggest the presence of a single copy of the beta subunit gene in the *Drosophila* genome.

DISCUSSION

This paper describes the isolation and sequencing of cDNA clones encoding both subunits of *Drosophila* casein kinase II. Based on the DNA sequencing results, the complete amino acid sequence of both subunits is presented. The identification of these cDNAs is based on (i) their immunological reactivity with antisera directed against native casein kinase II, against the SDS gel-purified alpha and beta subunits, and against calf casein kinase II, (ii) the absence of immunological reactivity with each of the corresponding preimmune sera, (iii) immunological reactivity with affinity-

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CAAAATAATCCCCTTAATCAAAATGAGCAGCTCCGAGGAAGTCTCCTGGGTCACCTGGTTCTGTGGACTTCGTGGCAATGAGTTCTTCTGCGAGGTGGATGAGGACTACATACAGGATAA  120
                    M  S  S  S  E  E  V  S  W  V  T  W  F  C  G  L  R  G  N  E  F  F  C  E  V  D  E  D  Y  I  Q  D  K   33

ATTCAATTTAACTGGTTTAAATGAGCAGGTACCCAACTATCGGCAAGCGTTGGACATGATCTTGGACTTGGAACCGGAGGACGAGCTCGAGGACAATCCACTGCAGTCCGACATGACCGA  240
  F  N  L  T  G  L  N  E  Q  V  P  N  Y  R  Q  A  L  D  M  I  L  D  L  E  P  E  D  E  L  E  D  N  P  L  Q  S  D  M  T  E   73

GCAGGCCGCCGAGATGCTCTACGGCCTCATACACGCCAGATATATACTAACAAATCGCGGCATCGCTCAAATGATCGAGAAATATCAAACTGGCGATTTCGGACATTGTCCACGTGTCTA  360
  Q  A  A  E  M  L  Y  G  L  I  H  A  R  Y  I  L  T  N  R  G  I  A  Q  M  I  E  K  Y  Q  T  G  D  F  G  H  C  P  R  V  Y  113

CTGTGAAAGTCAGCCCATGCTGCCATTGGGTCTGTCGGACATCCCCGGCGAGGCAATGGTTAAGACCTATTGCCCCAAGTGCATTGACGTGTACACACCAAAATCGTCGCGTCACCACCA  480
  C  E  S  Q  P  M  L  P  L  G  L  S  D  I  P  G  E  A  M  V  K  T  Y  C  P  K  C  I  D  V  Y  T  P  K  S  S  R  H  H  H  153

TACCGATGGCGCCTATTTCGGCACTGGATTTCCACACATGCTCTTCATGGTGCATCCCGAATATCGTCCCAAGCGTCCTACTAATCAGTTTGTTCCAAGGCTATATGGATTTAAAATACA  600
  T  D  G  A  Y  F  G  T  G  F  P  H  M  L  F  M  V  H  P  E  Y  R  P  K  R  P  T  N  Q  F  V  P  R  L  Y  G  F  K  I  H  193

CAGCTTAGCTTATCAAATTCAGCTGCAGGCAGCAGCCAATTTTAAAATGCCACTACGAGCGAAAAACTAATAAAATAAATACACCACCAACAACAACAAATACTACAACACAAACACATA  720
  S  L  A  Y  Q  I  Q  L  Q  A  A  A  N  F  K  M  P  L  R  A  K  N  *                                                      215

CGCACGAATACAACAACAAACAATCCATTTAACTGCATGTAAACGAAACAAAAACATAAATTGAAATGATAGTTTACAACGGGAAGACCGATAGACAGCAACACACAACAACAACAACAA  840

CAGCCACACTGAGAGACGGAGAGACAATTGTATTGAGGTTTTTAAACGCTGGGCGCAAAGTTTCATTTATATAAATATAAAAACTAAAAAAAACAACACAAAAAACAAAAAAAAAAAAAA  960

AAAA  964
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FIG. 5. Nucleotide sequence of a cDNA clone encoding the beta subunit of *Drosophila* casein kinase II. The sequence of the coding strand of the cDNA insert of Dm98 is shown, oriented in the 5′ to 3′ direction. The deduced amino acid sequence of the largest open reading frame is given below the sequence. The asterisk indicates the stop codon (TAA). Three possible autophosphorylation sites are indicated by a heavy underline (to indicate the serine residue), followed by light underlines (to indicate the associated acidic residues; see text).

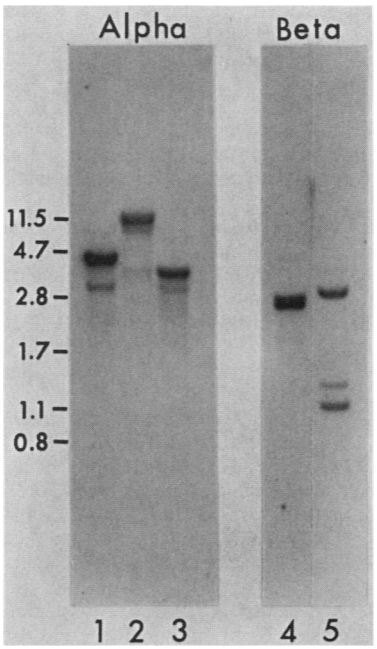

FIG. 6. Genomic Southern analysis. Total *Drosophila* genomic DNA was digested with various restriction enzymes, electrophoresed in a 0.8% agarose gel, transferred to nitrocellulose, and hybridized with the ^{32}P-labeled cDNA insert of either Dm95 (alpha probe, left) or Dm98 (beta probe, right). Lanes: 1, *Eco*RI; 2, *Sst*I plus *Kpn*I; 3, *Pst*I plus *Bam*HI; 4, *Eco*RI; 5, *Pst*I. The mobility and size (in kilobases) of marker restriction fragments are shown at the left.

purified alpha- and beta-specific antibodies (see Materials and Methods; data not shown), (iv) acceptable agreement between the predicted and observed molecular weights, and (v) the presence of very strong homology with the alpha and beta subunits of calf casein kinase II (see below). The correct identification of the alpha cDNA is further supported by (vi) its perfect agreement with the 40-amino-acid N-terminal sequence determined for the *Drosophila* alpha polypeptide by protein sequencing (Fig. 3) and (vii) by the presence of the expected homology with the protein kinase consensus sequence (Fig. 4).

Immunological and peptide mapping studies (7, 31) have previously indicated that casein kinase II has been highly conserved over large evolutionary distances. As stated in the Results, the beta subunit of *Drosophila* can be aligned with that of calf thymus without the introduction of gaps into either sequence, and the two sequences are 86% identical. A similar degree of homology appears to apply to the alpha subunit as well: of the 65 amino acids currently published for the calf alpha subunit (40a), 63 are identical to the corresponding residues of *Drosophila* alpha, and again no gaps are required to align the two sequences. In addition, the 42-kDa alpha subunit of yeast casein kinase II (31) has recently been cloned and sequenced in this laboratory and is 64% identical to the *Drosophila* sequence (Chen, Padmanabha, and Glover, unpublished).

Based on the limited data available, the unit evolutionary period (i.e., time required to accumulate 1% sequence divergence [42]) of both subunits of casein kinase II appears to be in the vicinity of 30 million years. This value, which exceeds that of cytochrome *c* (42), implies that the evolution of both polypeptides is subject to stringent functional constraints (10). Among such constraints are presumably the large number of protein-protein interactions required for proper casein kinase II function. The alpha and beta subunits must interact heterotypically and possibly also homotypically to form the native $\alpha_2\beta_2$ tetramer, and the tetramers must then interact with a large collection of diverse substrates. In addition, since *Drosophila* casein kinase II forms filaments in vitro under physiological conditions (13), it is also possible that tetramers must interact with one another. Binding sites for low-molecular-weight ligands (e.g., polyamines, which have been shown to stimulate enzyme activity in vitro [5, 16]) may impose additional functional constraints on the evolution of the enzyme.

Although several possible sites of autophosphorylation were identified in the sequence of the beta subunit (Fig. 5), only one of these (SEE) represents a site previously identified in other substrates (see, for example, reference 15). The other two sequences contain rather widely spaced acidic residues and do not correspond to sites which have so far been characterized in either natural proteins or artificial peptides (15, 25). Certainly, none of the three sites exhibits the dramatic acidity typical of known sites in physiological substrates (e.g., SESEDEED in the R_{II} subunit of cyclic AMP-dependent protein kinase [4]). One possibility is that the tertiary structure of the molecule creates a more acidic site from nonadjacent parts of the sequence. Highly acidic stretches, unassociated with serine or threonine residues, do in fact occur in the N-terminal region of the molecule (between the first two potential sites; see Fig. 5). An alternative possibility is that the autophosphorylation site does not resemble a typical phosphorylation site in a protein substrate. Efforts are under way to localize biochemically the autophosphorylation site(s) of the *Drosophila* enzyme.

Whole-genome Southern analysis suggests that the beta gene is single copy. This conclusion is consistent with the results of Takio et al. (40a), who observed no microheterogeneity during protein sequencing of the calf beta subunit. Southern analysis of the alpha gene, in contrast, suggests (though it does not yet prove) the possibility of a second, related alpha gene. This would be consistent with the fact that both *S. cerevisiae* (31) and probably calf (40a) contain more than one alpha gene. However, we think it unlikely that the alpha subunit we obtain from 6- to 18-h-old embryos (14) contains more than one alpha species, since no microheterogeneity was observed during protein sequencing of the N-terminal 45 residues. One possibility is that *Drosophila* contains a second alpha gene which is expressed only in later stages of development.

The sequence comparisons shown in Table 1 indicate that casein kinase II is distantly related to a variety of well-characterized protein kinases, including a number of serine/threonine kinases. While the data in Table 1 may be biased to some extent by the fact that the casein kinase II sequence is dipteran while the other sequences are avian or mammalian, the extremely high evolutionary conservation of casein kinase II (see above) argues strongly that most of the sequence difference is between kinases rather than between species. The data thus imply that casein kinase II is a distantly related member of the protein kinase family.

A possible clue to the function of casein kinase II has recently been provided by Takio et al. (40a), who noted a relatively high level of homology between the alpha subunit of calf casein kinase II and the Ser/Thr protein kinase encoded by the yeast cell division cycle gene *CDC28* (23, 33). This homology is particularly striking in the region C-terminal to the protein kinase consensus depicted in Fig. 4

(K. Takio and E. Krebs, personal communication), an intriguing result since this region is, in general, poorly conserved except between related kinases (e.g., between cyclic AMP- and cyclic GMP-dependent protein kinase). Comparison of the *Drosophila* alpha sequence with *CDC28* reveals a similar pattern of homology. In the region corresponding to Fig. 4, the homology between casein kinase II and *CDC28* was marginally better (33% identity; alignment score, 12.5) than that between casein kinase II and a variety of other kinases (Table 1), but in the C-terminal region the casein kinase II-*CDC28* homology was dramatically better. Here, the two sequences were 39% identical (alignment score, 8.8), with the extreme C-terminus displaying a stretch of 16 of 26 identical residues (62%). In contrast, other protein kinases exhibit little if any meaningful homology to casein kinase II over this region (the catalytic subunit of cyclic AMP-dependent protein kinase, for example, exhibits 17% identity, a value which is not statistically significant [alignment score, 1.2]). If the sequence homology between casein kinase II and *CDC28* reflects any underlying similarity of function, this observation could eventually shed light on the physiological role of both enzymes.

Isolation of the casein kinase II genes of *Drosophila*, an organism suitable for both classic and molecular genetic studies, should facilitate the development of a genetic system for the analysis of casein kinase II function in vivo.

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