Molecular Cloning of Casein Kinase II α Subunit from *Dictyostelium discoideum* and Its Expression in the Life Cycle

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A *Dictyostelium discoideum* cDNA encoding an α -type subunit of casein kinase II was isolated, and its cDNA was used to study developmental expression of casein kinase II during the *Dictyostelium* life cycle. The 1.3-kb cDNA insert contained an open reading frame of 337 amino acids (M_r 39,900). The deduced amino acid sequence has high homology with those of casein kinase II α subunits from other species. Genomic Southern blot analysis suggested that there is a single gene encoding casein kinase II α subunit in *D. discoideum*. Northern (RNA) blot analysis showed that the casein kinase II α -subunit gene is expressed constitutively as a 1.9-kb mRNA throughout vegetative growth and multicellular development. Casein kinase purified from normal vegetative cells contained a major protein band of ~36 kDa, which was recognized by antisera raised against rat testis casein kinase II. Comparison of the in vitro transcription/translation product of the α -subunit cDNA clone and the purified 36-kDa protein by partial proteolysis indicated that the isolated cDNA clone encodes the *Dictyostelium* casein kinase II α subunit. No protein corresponding to a β subunit was detected in purified casein kinase. Immunoblot analysis using anti-rat casein kinase II sera showed that the α subunit of casein kinase II is expressed constitutively like its mRNA during the life cycle of *D. discoideum*. Casein kinase II activity measured by using a specific peptide substrate paralleled the level of α subunit detected by immunoblotting during the life cycle, with a maximum variation of ~2-fold. We were unable to obtain disruptants of the casein kinase II α gene, suggesting that there is a single casein kinase II α gene, which is essential for vegetative growth of *D. discoideum*.

Casein kinase II is a ubiquitous protein-serine/threonine kinase in eukaryotic cells that catalyzes the phosphorylation of many protein substrates and has been regarded as a second-messenger-independent protein kinase (reviewed in references 27, 43, 82, and 109). When isolated from most species, this enzyme is composed of an α subunit (37 to 44 kDa) and a β subunit (24 to 28 kDa) and exists as an $\alpha_2\beta_2$ tetramer. The primary amino acid sequences of the α and β subunits of several species, which have been determined by molecular cloning and by direct protein sequence analysis, are well conserved among the species thus far studied (8, 14, 25, 45, 47, 49, 51, 58, 66, 69, 80, 83, 87, 96, 104, 106). The α subunit contains a protein kinase domain that is homologous to the catalytic domain of other protein kinases (42), whereas the β subunit shows no significant homology with other proteins except the *Drosophila melanogaster* steinbock gene products (67). The β subunit is presumed to regulate the catalytic activity (17), although the precise role of this subunit is not clear (59). There is some evidence that the β subunit stabilizes the α subunit, but it is not required for the catalytic activity of the α subunit (32, 40, 108). The β subunit is known to be autophosphorylated; the sites of phosphorylation have been mapped (65), but the physiological role of this phosphorylation is not clear. At least two different α subunits, designated α and α' , encoded by distinct genes have been detected in the budding yeast *Saccharomyces cerevisiae* (14, 87), humans (69, 83), cows (66), and chickens

(80). In yeast cells, disruption of both α -subunit genes causes inviability, whereas disruption of either one of these genes alone has no detectable phenotype. This finding indicates that at least one of the α -type subunits is essential and that the two α -type subunits can complement one another in *S. cerevisiae* (87).

Casein kinase II is detected in both the nucleus and the cytoplasm by fractionation experiments (43, 109). Immunolocalization studies have yielded somewhat conflicting results about the subcellular localization of casein kinase II (30, 60, 112). In one study, all three subunits were found to be predominantly nuclear under all conditions (60), whereas in the another study, the α and β subunits were found to be mainly cytoplasmic in interphase and the α' subunit was nuclear in G_1 and cytoplasmic in S phase (112). In the third study, casein kinase II was found in the nucleus of growing cells but distributed in the nucleus and cytoplasm in quiescent cells (30). A role for casein kinase II in the nucleus is suggested by recent studies showing that a number of the nuclear proteins, such as c-Myc (72), Max (7), c-Myb (71), serum response factor (74, 79), c-Fos (12), c-Jun (64), DNA ligase (90), topoisomerase II (10), the p53 tumor suppressor protein (81), the adenovirus E1A protein (12), the human papillomavirus E7 protein (6, 35), and the simian virus 40 large T antigen (41, 59), are phosphorylated by casein kinase II in vitro at physiological sites and that the DNA-binding activity of some of these nuclear proteins is regulated by phosphorylation by casein kinase II (7, 64, 71, 74, 79). Evidence from microinjection experiments with purified casein kinase II and casein kinase II inhibitor peptides indicates a role for casein kinase II in upregulating the

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activity of serum response factor (36) and downregulating the activity of c-Jun (64) in the intact cell.

Whether casein kinase II activity is regulated in the cell is uncertain. Although it has been reported that some mitogens, including insulin, insulin-like growth factor (56, 101), epidermal growth factor (1, 2, 101), serum (11), and phorbol ester (12), rapidly increase the activity of casein kinase II or a casein kinase II-like enzyme in some mammalian cells, in other cells, such as Swiss 3T3 cells, no increase of casein kinase II activity is detected (4). Because mitogen-induced changes in casein kinase II activity were detected in the absence of de novo protein synthesis, it has been suggested that casein kinase II activity is regulated by posttranslational modification, but there is no direct evidence for this. Moreover, in the case of c-Jun, phosphorylation by casein kinase II at the inhibitory sites appears to occur constitutively, and it has been proposed that the dephosphorylation of these sites that results from mitogen treatment is due to activation of a c-Jun phosphatase rather than inhibition of casein kinase II (64). Thus, casein kinase II may have important roles in the signal transduction system of growth control, but the precise function of this enzyme remains to be clarified.

The cellular slime mold *Dictyostelium discoideum* is an excellent system for the study of signal transduction (reviewed in references 24, 34, 37, 52, 54, and 110). The cells grow vegetatively as individual amoebae, which, upon starvation, initiate a developmental program to form a multicellular organism. Aggregates of approximately 10^5 cells develop over a 25-h period to form mature fruiting bodies consisting of two major cell types, spores and stalk cells (24, 34, 68). Mutants affecting signal transduction processes for several developmental stages have been isolated, and their analysis has led to an understanding of the various processes controlling signal transduction and cellular differentiation. Signaling pathways mediating cyclic nucleotide formation, inositol phospholipid hydrolysis, and other mechanisms are known to be involved in development of *D. discoideum* (34, 54, 110). Many of these signal pathways use protein phosphorylation, and it is reasonable to assume that a number of protein kinases are required for cellular differentiation. In fact, several protein kinases have been purified or partially purified from *D. discoideum* and shown to have properties similar to but, in some cases, distinct from those of mammalian enzymes. For example, cyclic AMP (cAMP)-dependent protein kinase has been purified from *D. discoideum* (15, 20–22, 63, 73), and clones for the regulatory (R) (84) and catalytic (C) (9, 77) subunits of this protein kinase have been isolated. Myosin heavy-chain kinases (18, 92), myosin light-chain kinase (105), protein kinase C (53, 70), and casein kinases (86, 93) have also been purified or partially purified from *D. discoideum*.

The *Dictyostelium* cAMP-dependent protein kinase has been shown to be a dimer consisting of one R and one C subunit, whereas in other organisms, this enzyme is generally found as a tetramer of R_2C_2 (27). Cells carrying a disruption of the C subunit are impaired in cAMP-mediated activation of adenyl cyclase and thus cannot aggregate, but can still be attracted to an exogenous cAMP signal. Mutants lacking active R subunit or overexpressing C subunit develop precociously and show aberrant morphogenesis (5, 78).

In addition, *D. discoideum* has the advantage that one can use genetic techniques such as gene disruption and antisense inactivation to analyze gene function (23, 61, 85). In this paper, we report the cloning of the *Dictyostelium* casein

kinase II catalytic α subunit and analysis of its expression in the life cycle.

MATERIALS AND METHODS

Growth and development of *D. discoideum*. *D. discoideum* KAX-3 was grown axenically in HL-5 medium and developed as described previously (33, 75, 76). For the developmental time course of protein expression, cells were plated on 15-cm-diameter agar plates (10^9 per plate), starved, and harvested from the plates at various times by washing with ice-cold 12 mM sodium phosphate buffer at pH 6.1 followed by centrifugation.

Design of PCR primers. Fully degenerate oligonucleotide primers deduced from the amino acid sequences of casein kinase II α subunits, which are completely conserved among three different species, *Drosophila melanogaster* (96), *S. cerevisiae* (14), and humans (83), were synthesized and used as primers for polymerase chain reaction (PCR). The oligonucleotides contained nondegenerate 5' ends; the sense-strand oligonucleotide contained a *Bam*HI restriction site with four additional bases (GTTT) on its 5' end, while the antisense-strand oligonucleotides had a *Sal*I restriction site with four additional bases (GAAA) on their 5' ends. The following sense-strand primer corresponds to *Drosophila* amino acid residues 20 to 25 (Glu-Tyr-Trp-Asp-Tyr-Glu):

5'-GTTTGGATCC GAA TAC TGG GAC TAC GAA-3'
 G T T T G

The two antisense strand primers correspond to *Drosophila* amino acid residues 159 to 164 (Asn-Val-Met-Ile-Asp-His) and 228 to 233 (Glu-Pro-Phe-Phe-His-Gly). Their sequences are as follows:

5'-GAAAGTCGAC GTG GTC GAT CAT NAC GTT-3'
 A A A A
 T
5'-GAAAGTCGAC NCC ATG AAA AAA NGG CTC-3'
 G G G T

cDNA cloning and sequencing. A cDNA library constructed in Lambda ZAP II by using a Uni-ZAP XR cloning kit (Stratagene, La Jolla, Calif.) prepared from *D. discoideum* at the 4-h stage of its development, kindly donated by Peter K. Howard (University of California, San Diego), was used as a source of DNA templates for PCR and to isolate full-length cDNA clones for the α subunit of casein kinase II. Phage DNA was prepared from the library by precipitation with polyethylene glycol and NaCl (95). The cDNA templates (~ 0.4 μ g) and oligonucleotides corresponding to *Drosophila* Glu-20 to Glu-25 and Glu-228 to Gly-233 as sense and antisense primers, respectively, were used for PCR. The reaction was carried out under conditions described previously (97), with 30 cycles of denaturation (1.5 min at 94°C), annealing (2 min at 37°C), and elongation (2 min at 72°C). After amplification, the reaction mixture was diluted 2,000-fold and used for reamplification with oligonucleotides corresponding to *Drosophila* Glu-20 to Glu-25 and Asn-159 to His-164 as sense and antisense primers, respectively, and a DNA fragment of the expected size on agarose gel electrophoresis was obtained. The reamplified fragment was recovered from the agarose gel, digested with *Bam*HI and *Sal*I, and directionally cloned into pSP73 (Promega, Madison, Wis.). The cloned fragment was sequenced from both ends on double-stranded plasmid DNA by using modified T7 DNA polymerase (Sequenase; U.S. Biochemical, Cleve-

land, Ohio) as recommended by the manufacturer. The cloned fragment containing the α subunit of casein kinase II was excised from the plasmid and ^{32}P labeled by the random oligonucleotide primer method (29). *Escherichia coli* XL1-Blue was infected with the cDNA library (5×10^6 PFU/15-cm-diameter plate; nine plates), and duplicate replicas of the plaques were made onto nylon membranes. The prehybridization, hybridization, and washes were carried out by standard methods (95). Positive phages were isolated. The cloned cDNA in pBluescript SK was excised and circularized according to Stratagene's protocol, and the sizes of inserts were analyzed. The clone with the longest insert, designated $\alpha 4$, was used for further study. Clone $\alpha 4$ was sequenced from both ends on double-stranded DNA as described above, using nested sets of deletion mutants from both directions generated by exonuclease III digestion (double-stranded nested deletion kit; Pharmacia LKB, Piscataway, N. J.). The gaps in the sequences among the exonuclease III deletion clones were covered by using synthetic oligonucleotide primers (17-mer) based on the sequences obtained from the deletion mutants. Clone $\alpha 4$ was sequenced in both directions multiple times. Intelligenetics software was used for data analysis.

Southern and Northern (RNA) blot hybridization analysis. The ^{32}P -labeled insert of clone $\alpha 4$ was used as a probe. Genomic DNA was isolated as previously described (85); the DNA (1 μg per lane) was digested with restriction enzymes, resolved on a 0.7% agarose gel, and transferred to a nylon membrane; and the membrane was prehybridized (95). For hybridization under stringent conditions, the membrane was hybridized at 37°C for 32 h in a solution containing 50% formamide, $3\times$ SSC (1 $\times$ SSC is 150 mM NaCl plus 15 mM sodium citrate, pH 7.0), 10 mM EDTA, 60 mM sodium phosphate (pH 7.2), 4 $\times$ Denhardt solution (1 $\times$ Denhardt solution is 0.02% polyvinylpyrrolidone, 0.02% Ficoll, and 0.02% bovine serum albumin), 0.6% sodium dodecyl sulfate (SDS), 50 μg of denatured salmon sperm DNA per ml, and the ^{32}P -labeled probe. The membrane was washed in $0.1\times$ SSC–0.1% SDS at 37°C for 2 h and then at 55°C for 1 h. Less stringent hybridization was carried out in a solution containing 40% instead of 50% formamide; the membrane was washed in $0.2\times$ SSC–0.1% SDS at 37°C for 2 h and then at 50°C for 1 h. The membrane was exposed to Kodak XAR X-ray film for autoradiography. For Northern blot analysis, total RNA was isolated at each time point of development and hybridization analysis was performed as described previously (75, 76, 85), using 6 μg of the total RNA per lane.

Protein purification. *Dictyostelium* casein kinase II was purified from vegetative cells by phosphocellulose column chromatography as described previously (100) and further purified by hydroxyapatite and Mono Q column chromatographies. During the purification procedures, casein kinase activity was monitored by using $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (ICN, Costa Mesa, Calif.) and casein (Fisher, Pittsburgh, Pa.) as a substrate essentially as described previously (44). Where indicated, casein kinase II activity was measured by using a specific substrate peptide, Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (Peninsula, Belmont, Calif.), as a phosphate acceptor essentially as described previously (101). The following procedures were carried out at 0 to 4°C . The cells grown in liquid medium (10^{10} cells, 10 g [wet weight]) were collected by centrifugation and washed with 12 mM sodium phosphate (pH 6.1). The cells were homogenized by sonication with a Heat System sonicator (six periods of 15 s) in 50 mM Tris-HCl (pH 7.5)–30% sucrose–40 mM sodium pyrophosphate–2 mM EDTA–2 mM ethyleneglycol bis(β -amino-

ethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA)–0.1 mM *N*-tosyl-L-lysine chloromethyl ketone–1 mM benzamidine hydrochloride–10 mM 2-mercaptoethanol–1 mM phenylmethylsulfonyl fluoride and centrifuged for 15 min at $25,000 \times g$. The supernatant was diluted with the same volume of ice-cold water and then centrifuged for 60 min at $100,000 \times g$ as described previously (102). The final supernatant was applied to a phosphocellulose column (1.6 by 5 cm; P11; Whatman, Maidstone, England) equilibrated with 50 mM Tris-HCl (pH 7.5)–1 mM EDTA–10 mM EGTA–10 mM 2-mercaptoethanol–0.1 M NaCl. The column was washed with the same buffer containing 0.3 M NaCl, and the enzyme was eluted by application of a 50-ml linear concentration gradient of NaCl (0.35 to 1.0 M). The casein kinase activity was recovered and applied to a hydroxyapatite column (0.9 by 1.9 cm; HTP; Bio-Rad, Richmond, Calif.) equilibrated with 15 mM potassium phosphate (pH 6.9)–1 mM EDTA–1 mM EGTA–10 mM 2-mercaptoethanol. The column was washed, and the enzyme was eluted with 3 ml of 400 mM potassium phosphate (pH 6.9)–1 mM EDTA–1 mM EGTA–10 mM 2-mercaptoethanol. The eluate was dialyzed against 20 mM Tris-HCl (pH 7.5)–1 mM EDTA–1 mM EGTA–10 mM 2-mercaptoethanol–0.1 M NaCl and applied to a Mono Q column (HR5/5; Pharmacia LKB) connected to an FPLC system (Pharmacia LKB) and equilibrated with the same buffer. After washing, the enzyme was eluted with a 20-ml linear concentration gradient of NaCl (0.1 to 0.57 M). The fraction with the highest enzyme activity was used for subsequent experiments. For the developmental time course experiment, *Dictyostelium* casein kinase II was purified partially from the cell extract by phosphocellulose column chromatography as described above in the presence of the additional protease inhibitor leupeptin (10 $\mu\text{g}/\text{ml}$) and used for immunoblot analysis. Cells at various stages (10^9 cells at each stage) were homogenized by sonication in homogenization buffer containing leupeptin as described above, and supernatants were collected by two successive centrifugations. Each of the cell extracts from the various time points (35 mg of protein) was applied to a P11 phosphocellulose column (0.6 by 0.9 cm); the columns were washed with the buffer described above containing 0.3 M NaCl and eluted with 0.9 ml of buffer containing 0.8 M NaCl in the presence of leupeptin. Twenty-five microliters of each of these eluates (containing 21 to 24 μg of protein) was used for immunoblot analysis.

Preparation of antisera. Antisera against casein kinase II were raised by using purified enzyme from rat testis as the antigen. Rat testis casein kinase II was first separated from casein kinase I by phosphocellulose column chromatography and further purified by hydroxyapatite and Mono Q column chromatographies as described above. Briefly, frozen rat testes (80 g [wet weight]) were homogenized in 50 mM Tris-HCl (pH 7.5)–1 mM EDTA–10 mM 2-mercaptoethanol–1 mM phenylmethylsulfonyl fluoride. The homogenate was centrifuged for 60 min at $100,000 \times g$, and the supernatant was applied to a P11 phosphocellulose column (2 by 10 cm) equilibrated with 50 mM Tris-HCl (pH 7.5)–1 mM EDTA–10 mM 2-mercaptoethanol–0.1 M NaCl. After a wash with buffer containing 0.3 M NaCl, the enzyme was eluted by a 150-ml linear concentration gradient of NaCl (0.35 to 1.0 M). Two major peaks of casein kinase activity, casein kinases I and II, appeared. The casein kinase II (the activity eluting second) was recovered and applied to an HTP hydroxyapatite column (0.9 by 9.5 cm) equilibrated with 15 mM potassium phosphate (pH 6.9)–1 mM EDTA–10 mM 2-mercaptoethanol. The column was washed, and casein

mRNA origin. The clone containing the longest insert, $\alpha 4$, was used for further analysis.

Figure 1 shows the nucleotide sequence of clone $\alpha 4$ (1,305 bp) together with the deduced amino acid sequence. $\alpha 4$ contains an open reading frame of 1,014 bp initiated at ATG (nucleotides 101 to 103) and terminated at TAA (nucleotides 1112 to 1114), which encodes a protein of 337 amino acids with a calculated molecular weight of 39,900, preceded by 100 bp of 5' untranslated sequence and followed by 191 bp of 3' untranslated sequence. In the 5' untranslated sequence, an in-frame TAA stop codon is found at nucleotides 86 to 88, and three stop codons for the other two reading frames are at nucleotides 60 to 62, 63 to 65, and 91 to 93, indicating that the ATG codon at nucleotides 101 to 103 is the physiological initiation codon. The 5' and 3' untranslated sequences are AT rich, as found in other *Dictyostelium* genes (55), but no poly(A) tract is found at the end of the 3' untranslated sequence. Nucleotides 188 to 622 correspond to the sequence of the PCR-amplified fragment. Nucleotides 188 to 205, 605 to 662, and 812 to 829 correspond to the PCR primers, showing a perfect match to the sequences of the primers except for nucleotides 197 and 814. The deduced amino acid sequence shares extensive homology with the catalytic subunit of casein kinase II isolated from other species (14, 25, 47, 66, 69, 80, 83, 87, 96) and has all of the hallmark protein kinase motifs (42). Figure 2 shows the alignment of the deduced amino acid sequences of catalytic subunits of casein kinase II from various species.

Analysis of the casein kinase II α -subunit gene. To estimate the copy number, genomic mapping was carried out by using the ^{32}P -labeled whole insert of $\alpha 4$. Figure 3A shows the result of Southern blot analysis under stringent conditions (see Materials and Methods). When the genomic DNA was digested with *EcoRI*, which does not cleave within the probe sequence, a single hybridizing fragment of 5.9 kb was detected. There is a single restriction site for *ClaI* (nucleotide 646) within $\alpha 4$ DNA. Two fragments, 4.1 and 1.8 kb, were observed when the DNA was digested with *ClaI* and *EcoRI*, consistent with a single *ClaI* cleavage site in the 5.9-kb *EcoRI* fragment. The $\alpha 4$ cDNA has two sites for *HindIII* (nucleotides 204 and 452) and for *NcoI* (nucleotides 829 and 963). When the DNA was digested with *HindIII* and *EcoRI*, one major fragment of 4.3 kb and two faint fragments of 0.9 and 0.7 kb were observed. These two fragments are seen more clearly under less stringent hybridization conditions (Fig. 3B and C, lanes 3). In the case of digestion with *NcoI* and *EcoRI*, two major bands of 3.5 and 2.2 kb were detected (a theoretical 0.2-kb small fragment was not observed). Thus, all of the DNA fragments detected under the stringent conditions could be generated from a single gene. The restriction digestion map suggests the existence of some intron sequences in the coding region of the gene, especially between the *HindIII* restriction sites. Further analysis of the structure of the casein kinase II α -subunit gene is necessary to determine the exact number and positioning of the introns. Even under the less stringent conditions, only rather faint additional fragments were detected in addition to the fragments mentioned above (Fig. 3B and C). These results suggest that there is probably only a single gene for casein kinase II α subunit in *D. discoideum*.

Analysis of casein kinase II α -subunit mRNA expression. Expression of casein kinase II α -subunit mRNA was analyzed by hybridizing total RNA prepared from different developmental stages of *D. discoideum*, using the insert of $\alpha 4$ as a probe. One major 1.9-kb mRNA was expressed constitutively during the *Dictyostelium* life cycle, and no

obvious change in the level of expression of this mRNA was observed (Fig. 4). This mRNA is larger than that of the insert of $\alpha 4$ (1,305 bp). $\alpha 4$ seems to lack some of the 3' untranslated sequence, as this cDNA clone has no poly(A) tract. It is also possible that $\alpha 4$ lacks some of the 5' untranslated sequence.

Purification of casein kinase from *D. discoideum*. We purified casein kinase from vegetative cells, measuring protein kinase activity by using casein as a protein substrate. After three steps of column chromatography, one major and one minor protein band of ~36 and ~39 kDa, respectively, were stained with silver in the purified enzyme preparation (Fig. 5A). Purified rat testis casein kinase II analyzed in parallel contains an α subunit (39 kDa), an α' subunit (doublet of 37 and 36 kDa), and a β subunit (26 kDa). The major protein band from *D. discoideum* is slightly smaller than that of mammalian casein kinase II α subunits (Fig. 5A). The 36-kDa protein band was detected upon immunoblotting by antisera raised against rat testis casein kinase II (Fig. 5B), indicating that this 36-kDa protein band is immunologically related to the mammalian casein kinase II α subunits (the 39-kDa band was not detected by immunoblotting, and its origin is unknown). However, no protein band of the size of the β subunit of casein kinase II (24 to 28 kDa) was detected by either silver staining or immunoblot analysis.

To study the relationship of the 36-kDa protein and the product of the $\alpha 4$ cDNA clone, in vitro transcription/translation of the $\alpha 4$ cDNA was carried out. The ^{35}S -labeled $\alpha 4$ in vitro translation product comigrated with the 36-kDa protein (Fig. 6 and data not shown). The patterns of partial proteolysis products generated from the in vitro translation product and the 36-kDa protein after limited digestion with *S. aureus* V8 protease were essentially identical, if the bands of V8 protease itself are excluded (Fig. 6). These structural similarities indicate that the 36-kDa protein band purified from the vegetative cells is most likely encoded by the gene from which the $\alpha 4$ cDNA clone was derived and is the α subunit of *Dictyostelium* casein kinase II.

Expression of casein kinase II during development. Casein kinase II activity could not be measured accurately in whole cell lysates, and to determine whether casein kinase II activity changed during development, we had to partially purify the enzyme. For this purpose, casein kinase II was partially purified by phosphocellulose column chromatography from cell extracts prepared at several time points during development. The immunoblot signals obtained with the anti-rat casein kinase II antiserum described above were not strong enough for us to measure the levels of the 36-kDa casein kinase II α band in whole cell lysates; therefore, the partially purified casein kinase II preparations were also used to determine the amount of the 36-kDa protein at different times during development by immunoblotting (Fig. 7). As a control, the α' and β subunits of the rat enzyme and purified *Dictyostelium* casein kinase II were analyzed in parallel (Fig. 7, lanes 1 and 2). To normalize the different time points, cell extracts containing the same amounts of protein were applied to each column, and the same volumes of the eluates were used for immunoblot analysis and assay of enzymatic activity. The protein staining of the comparable SDS-polyacrylamide gel also showed very similar profiles of protein staining from each time point.

The intensity of the 36-kDa *Dictyostelium* α -subunit bands differed up to twofold between time points, but the α subunit was clearly expressed constitutively throughout development. Table 1 shows the casein kinase II activity of samples at the same time points, which was measured by using a casein kinase II-specific peptide substrate (101). Casein

D. disc.	MNHSSKKKNRILRNKARIYCDVNLHKKKEYWNYEALN-VKWET-QDDYEIIRKLGRGKYSEVFEGA	65
Zea mays	MS...V.A...VLR...D...T-.Q.GE-...VV..V.....I	52
Yeast CKA1	MKCRVWSE..V.TNI.KQRTE...D..NTV-ID.S.NTK...EN.V.....Q.V	58
Yeast CKA2	MPLPP.TLNQKSNRVYSV..V.KNACEER.Q...D..QGVITD.GKIS-N...N.I.....S.R	68
C. elegans	MPPIPSR..V.AE..PSR.R...D..HM-IE.G-QI...QLV.....F	56
Drosophila	MT.PSA..V.T...A...D...D..NYV-.D.G-N...QLV.....AI	55
Human α	MSGPVPSR..V.T...T.R.R...D..SHV-.E.G-N...QLV.....AI	57
Human α'	MPGPAAGSR..V.AE..SLRSR...D..HV-PS.G-N...QLV.....AI	58
Chicken α	MSGPVPSR..V.T...T.R.R...D..SHV-.E.G-N...QLV.....AI	57
Chicken α'	MPGPAAGSR..V.AE..SLRSR...D..HV-PS.G-N...QLV.....AI	58
D. disc.	NIKNNEKCVIKVLKPKVKKKIKREIKILQNLG-----	97
Zea mays	.VN....I..I.....	84
Yeast CKA1	KLDSKVKI...M.....TD.SNEKVPPTTLFPQKDQYYTNQKEDVLKFIIRPYIFDQ	125
Yeast CKA2	C.V..Q.....M...Y..L...T...T.....	100
C. elegans	KMSTD..V.V.I.....E..R-----	88
Drosophila	..TTT...V.I.....E..R-----	87
Human α	..T...V.V.I.....E..R-----	89
Human α'	..T...RV.V.I.....V..E..R-----	90
Chicken α	..T...V.V.I.....E..R-----	89
Chicken α'	..T...RV.V.I.....V..E..R-----	90
D. disc.	---GGPNIIITLYDVVRDPQSKTSPSLIFEYINNTDFKHLSPTLTDFDVRYYIRELLHALDFCHSNGIM	161
Zea mays	---...VK.L.I...QH.....V.....V.Y.....Y.I...Y..K...Y...Q...	148
Yeast CKA1	PHN.HA...H.F.IIK..I...A.V...VD.V..RI.Y.K...LEI.F.MF...K...Y...M...	192
Yeast CKA2	---...VVG...I.Q.AD..I.A...E.K.V..RT.Y..FKLP.IQ..FTQ..I...Y...M...	164
C. elegans	---..T...L...K..I.R..A...HV..S...Q.YQ..S.Y.I...LY...K...Y...Q...	152
Drosophila	---..T...LA..K..V.R..A...HV...Q.YQ...YEI...LF...K...Y...M...	151
Human α	---..T...A.I.K..V.R..A.V..HV...Q.YQ...Y.I.F.MY.I.K...Y...M...	153
Human α'	---..T...K.I.T.K..V...A.V...Q.YQI...I.F.MY.I.K...Y...K...	154
Chicken α	---..T...A.I.K..V.R..A.V..HV...Q.YQ...Y.I.F.MY.I.K...Y...M...	153
Chicken α'	---..T...N.I.T.K..V...A.V...Q.YQI...I.F.MY.I.K...Y...K...	154
D. disc.	HRDVKPSNVMDHQRKLYLIDWGLAEFYHFNQDYNVRVASRPYKGPPELLVDMEDYDYSLDMWSLGC	228
Zea mays	H.....EL...R.....GKE.....YF.....LQ.....	215
Yeast CKA1	H.....KNK..R.....VNME.....FF.....YRM.....L..F.T	259
Yeast CKA2	Q.....PTE..R.....GV.....YH.....NLNQ.....L..V..	231
C. elegans	H.....AE..E.R.....R.....YF.....YQC.....	219
Drosophila	H.....EN..R.....G.E.....YF.....YQM.....	218
Human α	H.....EH..R.....G.E.....YF.....YQM.....	220
Human α'	H.....QK..R.....A.E.....YF.....YQM.....	221
Chicken α	H.....EH..R.....G.E.....YF.....YQM.....	220
Chicken α'	H.....K...R.....A.E.....YF.....YQM.....	221
D. disc.	MFAGMLFQKDPFFHGHNDIDQLVKIVKILGTEEFYAYLDKYGIVVDHTILSIIGK-HPKKPWSRFIT	294
Zea mays	...I.R.E...Y...H...A.V...DGLNV..N..R.EL.PQLEALV.R-.SR...LK.MN	281
Yeast CKA1	.L.S.I.KRE...TS.T...V..TSD.EK..L..E.TLPREFYD--MDQYIR..H..N	324
Yeast CKA2	.L.AIV.K.E...K.SS.P...ATV...K.LLG..G...LHLPSEY-DN.MRDFT..S.THLL	297
C. elegans	.L.S.I.R.E...Y...R.A.V...D.L.E.IAR.H.DL.PRFND.L.R-.SR.R.E..H	285
Drosophila	.L.S.I.R.E...Y...R.A.V...L...N.DL.PRFND.LQR-.SR.R.E..VH	284
Human α	.L.S.I.R.E...Y...R.A.V...DL.D.I...N.EL.PRFND.L.R-.SR.R.E..VH	286
Human α'	.L.S.I.RRE...Q..Y...R.A.V...L.G..K..H.DL.PHFND.L.Q-.SR.R.EN..H	287
Chicken α	.L.S.I.R.E...Y...R.A.V...DL.D.I...N.EL.PRFND.L.R-.SR.R.E..VH	286
Chicken α'	.L.S.I.RRE...Q..Y...R.A.V...DL.D.L.G..K..H.EL.PHFND.L.Q-.SR.R.EN..H	287
D. disc.	KENQH LAV-PEAIDFLEKLLRYDPAERLTREAMEHPYFKPLSH	337
Zea mays	AD...VS-.....D...HQ...AL...T...QVRAAENSRTA	332
Yeast CKA1	DG.K..SGND.I..LIDN...HQ...AK...G..W.A.IREQIEK	372
Yeast CKA2	S.TK-...-VV.LIDN...HQ...AK...D.KF.KTKFE	339
C. elegans	A....-..T...L...D...H...AQ...G.E..R.VVEAHARANGTEQADGGASNSASSQ	351
Drosophila	SD....-..S...L...D...HVD..A...A...L.IVNGQMNPNNQ	336
Human α	S....-..S...L...D...HQS...A...YTVVKDQARMGSSSMPGGSTPVSSANMM	352
Human α'	S...R...-..S...L...D...HQQ...AK...Y.VVKEQSQPCADNAVLSGLTAAR	350
Chicken α	S....-..S...L...D...HQS...A...Y.IVKDQARMGSSSMPGGSTPVSSASMM	352
Chicken α'	S...R...-..S...VL.L.D...HQQ...AK...Y.VVKEQSQPCADNAVLSGLTTAR	350
C. elegans	SSDAKIDGA	360
Human α	SGISSVPTSPPLGPLAGSPVIAANPLGMPVPAAGAQQ	391
Chicken α	SGISSVPTSPPLGPLAGSPVISATTTLGMPVPAAGAQQ	391

FIG. 2. Comparison of the amino acid sequences of casein kinase II catalytic subunits from various species. Gaps (—) have been introduced for optimal alignment; dots show identities with the *D. discoideum* α subunit (this report). Shown are sequences for *D. discoideum* (D. disc.), *Zea mays* (25), *S. cerevisiae* CKA1 (14), *S. cerevisiae* CKA2 (87), *C. elegans* (47), *D. melanogaster* (96), human α (69, 83) and α' (69), and chicken α (80) and α' (80).

kinase II activity was detected throughout the life cycle. In this experiment, the activity was highest in vegetative cells, fell immediately after starvation, increased at 10 to 15 h, and then decreased again at 20 to 25 h. The changes in enzyme activity are consistent with the levels of the α subunit detected by immunoblot analysis. Given the difficulties inherent in ensuring that the partial purification procedure is quantitative, we do not believe that the twofold changes in

the level of casein kinase II α subunit and activity are likely to be physiologically significant.

Interestingly, 5 μ M heparin, which is reported to be a potent inhibitor for mammalian casein kinase II (27, 43, 44), showed almost no inhibitory effect on either purified (data not shown) or partially purified (Table 1) *Dictyostelium* casein kinase II activity yet inhibited the rat testis casein kinase II activity potently, as reported by others (data not shown).

FIG. 3. Genomic Southern blot analysis of casein kinase II α subunit. Total *Dictyostelium* genomic DNA was digested with the indicated restriction enzymes, electrophoresed, transferred to a nylon membrane, and hybridized with the ^{32}P -labeled cDNA insert of $\alpha 4$ under stringent conditions (A), less stringent conditions (B), and longer exposure of the less stringent conditions (C) as described in Materials and Methods. The membrane was exposed to X-ray film with an intensifying screen for 15 (A and B) or 60 (C) h at -70°C . The positions of molecular weight markers are indicated in kilobase pairs.

A band of ~ 27 kDa was detected in the partially purified enzyme preparation from vegetative cells (Fig. 7, lane 3). This band was also detected during development, and although its abundance did not change significantly, its abundance relative to that of the α -subunit band seemed to vary during development (Fig. 7, lanes 4 to 8). This band is slightly larger than that of mammalian casein kinase II β subunit, and it is possible that this 27-kDa band is the β subunit of *Dictyostelium* casein kinase II, which is absent from purified preparations because of its loss during the purification procedures (Fig. 5).

Genetic analysis of casein kinase II α function. We have attempted to determine the function of the *Dictyostelium* casein kinase II α gene by either under- or overexpressing casein kinase II α . To decrease casein kinase II α expression, attempts were made both to disrupt the casein kinase II α gene and to reduce its expression by using an antisense expression vector. The possible effect of overexpression of casein kinase II α was also tested. In all cases, the constructs were examined carefully to ensure that the vector had the correct structure. To attempt to create a null casein kinase II α mutant, the *Thy-1* selectable marker (26) (thymine prototrophy) was cloned into the *HindIII* site of the 652-bp *ClaI-XhoI* casein kinase II α fragment, replacing the 247-bp *HindII* fragment. The clone was digested with *ClaI* and *XhoI*, extracted with phenol, and transformed in duplicate or triplicate into the *thy-1* auxotroph strain, JH10 (26, 61, 77). In each experiment a control with the *Thy-1* gene alone was

carried out, and *Thy* $^+$ clones were obtained at the expected frequency. Three independent attempts to obtain *Thy* $^+$ clones with the casein kinase II α construct were unsuccessful. Although a few *Thy* $^+$ clones were obtained in the first two experiments and a larger number were obtained in the third, when these clones were examined by Southern analysis, none of them was found to be disrupted in the casein kinase II α gene as determined by Southern analysis (data not shown).

Antisense RNA expression was achieved by cloning the 652-bp *ClaI-XhoI* casein kinase II α fragment containing the entire coding region downstream from the actin 15 (*Act15*) promoter in the antisense/sense expression vector BS18 (61), which gives a high level of expression both in the vegetative growth state and in early development. Clonal isolates were examined for the antisense and endogenous sense RNAs, which could be distinguished on the basis of size. For only one clone was there a detectable level of antisense RNA, and this was accompanied by a small ($\sim 30\%$) reduction in casein kinase II α mRNA. Casein kinase II α protein levels were not checked, but successful antisense RNA expression in *D. discoideum* generally leads to decreased protein expression by reducing mRNA levels (19, 46, 57, 61, 94, 99, 103). No growth or developmental phenotype was observed in any of the antisense clones.

Finally, overexpression of casein kinase II α was achieved by cloning the complete coding region downstream from the *Act15* promoter, using the natural casein kinase II α ATG for translation initiation in the BS18 vector. Several clones were

FIG. 4. Northern blot analysis of casein kinase II α subunit during development. RNA was isolated at the indicated times during development, electrophoresed, transferred to a nylon membrane, and hybridized with the ^{32}P -labeled cDNA insert of $\alpha 4$ as described in Materials and Methods. The membrane was exposed to X-ray film with an intensifying screen for 15 h at -70°C . Numbers indicate the time in hours after starvation. The positions of 18S and 28S rRNA markers are indicated.

FIG. 5. Protein staining and immunoblot analysis of casein kinase II. Casein kinase II was purified from rat testis and from *D. discoideum*; after SDS-gel electrophoresis, gels were stained by silver or used for immunoblot analysis as described in Materials and Methods. The membrane for immunoblot analysis was exposed to X-ray film with an intensifying screen for 70 h at -70°C . (A) Protein staining; (B) immunoblot. Lanes: 1 and 3, rat enzyme (0.3 and 0.03 μg , respectively); 2 and 4, *Dictyostelium* enzyme (0.3 μg). The positions of α , α' , and β subunits of rat enzyme are indicated. The positions of molecular weight markers are indicated in kilodaltons.

examined and found to have mRNA derived from the *Act15*/casein kinase II α fusion, but no obvious growth or developmental phenotype was seen.

DISCUSSION

A cDNA clone encoding casein kinase II α subunit was isolated by PCR from a cDNA library prepared from *D. discoideum* at the 4-h stage of development. No other distinct α -subunit clone was obtained by PCR or during the screening of the cDNA library. When a cDNA library made from vegetative cells was used as a template, only the same clone was amplified by PCR. Even using a degenerate oligonucleotide from another conserved region (Asp-154 to Val-160 of *D. melanogaster*) as a sense primer with the antisense primer corresponding to Glu-228 to Gly-233, we isolated a single α -subunit fragment, which was identical to the equivalent sequence in $\alpha 4$. All of the major DNA fragments detected by genomic Southern blot analysis with the $\alpha 4$ cDNA clone can be accounted for by a single α -subunit gene. Since two α -subunit genes have been found in other organisms, we tested whether there might be a second α -subunit gene in *D. discoideum* by using low-stringency Southern analysis. However, only faint DNA fragments were observed under less stringent conditions, and Northern blot analysis showed a single major mRNA expressed constitutively during growth and development. Moreover, immunoblot analysis using antiserum against mammalian casein kinase II detected a single protein band. Thus, it is most probable that *D. discoideum* has a single casein kinase II α -subunit gene. Nevertheless, because two closely related but distinct genes are present in *S. cerevisiae* (*CKA1* and *CKA2*) (14, 87), mammals (α and α') (66, 69, 83), and birds (α and α') (80), we cannot absolutely rule out the possibility that another casein kinase II α -subunit gene, which is distantly related to the clone that we have isolated, is expressed at some stages in *D. discoideum*. However, if

there is a second casein kinase II α -subunit gene, it is significantly less closely related to the gene that we have cloned than the two casein kinase II α -subunit genes are to each other in other species. Moreover, our genetic analysis indicates that the casein kinase II α gene that we have cloned is an essential gene, and therefore if there is another casein kinase II α gene, it cannot be functionally redundant.

Genetic evidence has been obtained for an essential role for casein kinase II in other organisms. Disruption of the two catalytic subunit genes is lethal in yeast cells, and casein kinase II is therefore essential for viability (14, 87). It has also been reported that the β subunit of casein kinase II can complement partially the sensitivity to UV light of a xeroderma pigmentosum cell line, suggesting the possibility that one cellular response to DNA damage is modulated by protein phosphorylation through casein kinase II (106).

Our genetic analysis of casein kinase II α function in *D. discoideum* does not show unequivocally that casein kinase II α is an essential gene, because the results are negative. However, since *D. discoideum* is a haploid organism, the failure to obtain disruptants with use of the same vector that has been successfully used to generate disruptants in other genes, including a protein kinase gene (61, 77), suggests that casein kinase II α is an essential gene. It has proved impossible to disrupt a number of other *Dictyostelium* genes, and in these cases there is good reason to believe that these are essential genes. Likewise, the fact that all of the antisense clones that we obtained expressed nearly wild-type levels of casein kinase II α mRNA indicates that continued

FIG. 6. Peptide mapping analysis of purified protein from *D. discoideum* and the in vitro transcription/translation product. One-dimensional peptide maps of purified protein from vegetative cells and the ^{35}S -labeled in vitro transcription/translation product were prepared by using different amounts of *S. aureus* V8 protease as described in Materials and Methods. The gel of the in vitro translation product was treated with diphenyloxazole, dried, and exposed to X-ray film with an intensifying screen for 12 days at -70°C . (A) Silver-stained maps of the purified protein; (B) fluorographed maps of the in vitro translation product. Lanes: 1, no V8 protease; 2, 50 ng of V8 protease; 3, 250 ng of V8 protease; 4, 1,250 ng of V8 protease. Arrowheads indicate the positions of V8 protease bands. The positions of molecular weight markers are indicated in kilodaltons.

FIG. 7. Immunoblot analysis of casein kinase II during *Dictyostelium* development. Casein kinase II was partially purified from the cell extracts prepared at each time point of development, and expression of the enzyme was investigated by immunoblot analysis using antisera raised against mammalian casein kinase II as described in Materials and Methods. The membrane was exposed to X-ray film with an intensifying screen for 24 h at -70°C . Lanes: 1, purified rat enzyme (0.03 μg); 2, purified *Dictyostelium* enzyme (0.3 μg); 3 to 8, partially purified *Dictyostelium* enzyme after starvation for 0, 5, 10, 15, 20, and 25 h, respectively. Arrowheads indicate the positions of *Dictyostelium* casein kinase II α subunit and the 27-kDa protein described in text. The positions of molecular weight markers are indicated in kilodaltons.

expression of casein kinase II α is required for outgrowth of clones. In the absence of a phenotype, the overexpression experiments are not easy to interpret. Given that casein kinase II α mRNA was overexpressed, we assume that the level of casein kinase II α protein was increased. If this is the case, then the simplest interpretation is that overexpression of casein kinase II α has no phenotype, because the protein is already present in excess over the amount required. However, it is possible that casein kinase II α expression was not increased because of the lack of overexpression of a putative casein kinase II β subunit, which might lead to instability of casein kinase II α or to a limited level of some other factor required for casein kinase II α activity.

The deduced amino acid sequence of *Dictyostelium* casein kinase II α has strong homology with sequences of both

types of casein kinase II α subunit from other species (Fig. 2). Certain amino acid residues of the *Dictyostelium* α subunit (e.g., His-26, Ile-90, Thr-104, His-244, Asp-267, Arg-291, and Phe-308) are conserved among α clones rather than α' clones of other species; however, other residues (e.g., Ala-36, Lys-115, Ile-124, Phe-139, and Gln-175) are conserved among α' rather than α subunits. Thus, it is possible that the single *Dictyostelium* α subunit is a hybrid of the two types of α subunit found in other organisms.

We were unable to isolate a cDNA clone for a casein kinase II β subunit from *D. discoideum*. PCR was carried out as for the α subunit, with several different combinations of oligonucleotide primers deduced from amino acid residues conserved among the *Drosophila* (96), bovine (104), and human (45, 51) β subunits, but no clone having homology with β subunits from other species was obtained. A cAMP-independent protein kinase has been purified from *D. discoideum* by monitoring its activity with casein as a substrate protein (93). The protein kinase activity resides in a single protein of ~ 38 kDa and has enzymatic properties similar to those of mammalian casein kinase II. Thus, this protein kinase has been regarded as casein kinase II in *D. discoideum*, although it apparently consists of only a single type of subunit. Another difference is that the 38-kDa protein is autophosphorylated when the purified enzyme preparation is incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (93), whereas autophosphorylation of the α subunit is reported to be much less than that of the β subunit in other species (1, 27, 30, 43) unless polylysine is present (88).

The casein kinase II preparation purified in our study also contained one major protein band of 36 kDa corresponding to an α subunit almost identical in size to the reported 38-kDa protein, which was phosphorylated when incubated with $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (data not shown). No β subunit was detected by protein staining or immunoblot analysis in our purified enzyme preparation (Fig. 5), but a ~ 27 -kDa band slightly larger than the rat casein kinase II β subunit was detected by anti-rat casein kinase II antibodies in the partially purified enzyme preparation obtained from the cells at various stages of development (Fig. 7). During the procedures for large-scale purification from vegetative cells, the 27-kDa band was detected by immunoblotting in the enzyme fractions eluted from the phosphocellulose column by a linear salt concentration gradient, but the ratios of the 27-kDa band and the α subunit differed in each fraction (data not shown). Recovery of the 27-kDa band was much lower than that of the α subunit at the hydroxyapatite column step, and no 27-kDa band was detected after the final step of purification on a Mono Q column. Thus, it is possible that this band is a β subunit-like protein that is lost during the purification procedures. This finding suggests either that the association of the α and β subunits is not as strong as that in the enzyme from other species or else that the β subunit is more susceptible to proteases than is the *Dictyostelium* α subunit despite the presence of several protease inhibitors. In this regard, it is interesting that two forms of casein kinase II, one of which apparently contains a free α subunit, have been purified from maize (25).

The exact role of the β subunit in casein kinase II function is not known. In vitro studies with recombinant subunits suggest that the β subunit stabilizes the α subunit, mediates interaction with the basic compounds such as polyamines that stimulate casein kinase activity, and may lower the K_m for substrates (31, 32, 40, 108). However, it is clear that the β subunit is not absolutely required for α -subunit enzymatic activity, and so far there has been no genetic test to

TABLE 1. Casein kinase II activity during the development of *D. discoideum*

Time after starvation (h)	Casein kinase II activity (cpm) ^a	
	-Heparin	+Heparin (5 μM)
0	8,000	7,240
5	2,970	2,680
10	3,950	3,900
15	5,220	4,700
20	2,860	2,670
25	2,470	1,990

^a Measured by using a specific peptide substrate (71) and expressed as ^{32}P radioactivity incorporated into peptide per 10 min per 10 μl of enzyme fraction. Background radioactivity without added enzyme fraction (400 cpm) has been subtracted.

determine whether β -subunit genes are essential. Given the suggestive evidence for a β subunit-like protein in *D. discoideum*, renewed cDNA cloning efforts are in order to determine the basis for the unusual properties of this β subunit. It would also be worth testing whether the *Dictyostelium* α subunit interacts more strongly with a vertebrate β subunit.

Dictyostelium casein kinase II α subunit purified from vegetative cells and casein kinase II partially purified from the cells of various stages showed high enzyme activity in the presence of 5 μ M heparin, which is a potent inhibitor for casein kinase II (27, 43, 44) (Table 1 and data not shown). The rat testis enzyme was completely inhibited under the same conditions. Our results are at variance with those of a previous study (93), in which the heparin sensitivity of what appears to be the same *Dictyostelium* enzyme was found to be close to that of mammalian casein kinase II. We currently have no good explanation for this discrepancy, although it should be noted that certain basic peptides have the ability to reverse heparin inhibition of casein kinase II (13). It seems unlikely that our preparation of *Dictyostelium* casein kinase II is resistant to heparin because it lacks the β subunit, since free recombinant α subunit is reported to be equally sensitive to heparin inhibition (48, 108). Moreover, the basic region in the α subunit implicated by mutagenesis in heparin sensitivity (residues 82 to 88) is conserved in the *Dictyostelium* α subunit (48). Analysis of the properties of recombinant *Dictyostelium* casein kinase α may resolve this issue.

The expression of casein kinase II has been studied in several species. A transient increase of casein kinase II activity was found in developing mouse embryos (98) and fetal rat liver (89). The enzyme activity and the expression of its α subunit are high in *Caenorhabditis elegans* embryos and decrease in subsequent larval stages (47), and expression of the α , α' , and β subunits is high in early chicken embryos and decreases during embryonic development (80). In *D. discoideum*, developmental and, in some cases, cell-type-specific gene expression has been found for genes such as those for the regulatory subunit of cAMP-dependent protein kinase, cAMP receptor, G proteins, and Ras during development (for reviews, see references 24, 34, 39, and 91). In contrast, the Northern blot and immunoblot analyses in our study indicated that the α subunit and possibly the β subunit are expressed constitutively during the *Dictyostelium* life cycle. The partially purified enzyme preparations from the various stages of development showed casein kinase II activity consistent with the protein data. The level of α -subunit mRNA was very constant during development. Moreover, there was no large change in the abundance of the casein kinase II α protein or in casein kinase II activity during *Dictyostelium* development. However, it is possible that its activity toward physiological substrate proteins is regulated by some unknown mechanism, such as phosphorylation or subcellular localization. Regulation of casein kinase II activity by phosphorylation/dephosphorylation has been obtained in vitro (3) and suggested by results obtained in vivo (1), but it is still not clear how this enzyme is regulated in vivo. The regulation of casein kinase II activity in *D. discoideum* and the nature of its substrates need further study.

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ADDENDUM IN PROOF

By using different column chromatography steps, it is possible to obtain a preparation of *Dictyostelium* casein kinase II that retains a low level of what appears to be a β subunit and is stimulated by spermine, which is characteristic of the β subunit (O. Filhol and C. Cochet, personal communication).

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