Cloning and Characterization of the Casein Kinase II α Subunit Gene from the Lymphocyte-Transforming Intracellular Protozoan Parasite *Theileria parva*[†,‡]

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Received January 24, 1992; Revised Manuscript Received April 21, 1992

ABSTRACT: *Theileria parva* is an obligate intracellular protozoan parasite which is the causative agent of East Coast fever, an acute, leukemia-like disease of cattle. The intralymphocytic stage of the parasite induces blastogenesis and clonal expansion of quiescent bovid lymphocytes. Experiments in our laboratory have shown a marked increase of casein kinase II- (CK II-) like activity in *T. parva*-transformed lymphocytes. We have also detected CK II activity in purified *T. parva* schizonts. To explore the significance of this increase, we used a *Drosophila melanogaster* CK II α cDNA probe [Saxena et al. (1987) *Mol. Cell Biol.* 7, 3409-3417] to isolate a *T. parva* genomic clone encoding a CK II catalytic subunit. The clone contains a 1.3-kb open reading frame coding for a predicted protein of 420 amino acids (M_r 50 200). Northern blot analysis revealed a single transcript of 1.65 kb. The deduced *T. parva* CK II catalytic subunit sequence shows, over 321 residues comprising the C-terminus of the molecule, extensive identity with CK II α and α' sequences from both vertebrate and invertebrate organisms. The *T. parva* CK II subunit amino acid sequence displays 68% identity with the *Drosophila* α subunit and 67% with the *Caenorhabditis elegans* α subunit but only 58% and 56% sequence identity with the *Saccharomyces cerevisiae* α and α' subunits, respectively. Comparison of the *T. parva* sequence with higher eukaryotic α and α' sequences reveals that it is most identical with the α subunit. A unique component of the *T. parva* CK II α subunit is a 99 amino acid sequence at the N-terminus, which contains a sequence motif with features characteristic of signal peptides.

Casein kinase II is a serine/threonine protein kinase that is messenger-independent and that has, as its potential physiologic substrates, a diverse and expansive spectrum of proteins involved in metabolism, differentiation, and cellular proliferation [for recent reviews see Edelman et al. (1987), Krebs et al. (1988), and Tuazon and Traugh (1991)]. Casein kinase (CK) II is ubiquitous among eukaryotes and the enzyme or the genes encoding its subunits have been isolated from many sources, including mammalian and avian species (Hathaway & Traugh, 1982; Maridor et al., 1991), *Drosophila* (Glover et al., 1983; Saxena et al., 1987), yeast (Meggio et al., 1986; Padmanabha & Glover, 1987; Chen-Wu et al., 1988; Padmanabha et al., 1990), *Caenorhabditis elegans* (Hu & Rubin, 1990) and *Dictyostelium discoideum* (Renart et al., 1984). The enzyme has been shown to be predominantly cytosolic in distribution but has also been localized to a variety of subcellular organelles, including the nuclear compartment (Hathaway & Traugh, 1983), coated pits (Bar-Zvi & Branton, 1986) and mitochondria (Damuni & Reed, 1988). Furthermore, immunocytochemical studies have shown a marked shift of CK II from the cytoplasm into the nucleus during active proliferation of bovine adrenocortical cells in culture (Filhol et al., 1990). Employing CK II subunit-specific antibodies in immunocytochemical studies, Yu et al. (1991) showed that, in HeLa cells, CK II α and β subunits are predominantly cytoplasmic during interphase but become distributed throughout the cell during mitosis. In contrast, the α' subunit is mostly nuclear in localization during G1 but moves to the cytoplasm during S phase. Although these studies indicate that the localization of CK II is not static but rather depends on the stage of the cell cycle, the report by Krek et al. (1992) suggests that, in primary chick embryo fibroblasts, chicken hepatoma cells, and HeLa cells transfected with cDNAs encoding the subunits of chicken CK II, the enzyme is predominantly localized in the nucleus.

In most higher eukaryotes, the type II casein kinases are heterotetrameric, with possible holoenzyme isoforms of $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, and $\alpha'_2\beta_2$ [for review see Tuazon and Traugh (1991)]. The α subunits (M_r 37 000-44 000) have been shown to be catalytic, while the β subunit (M_r 24 000-28 000), which undergoes autophosphorylation in the presence of ATP or GTP, has a regulatory role (Hathaway et al., 1981; Feige et al., 1983; Cochet & Chambaz, 1983; Meggio & Pinna, 1984; Palen & Traugh, 1991). Until recently the structural relationship between the catalytic subunits of CK II was unclear (Dahmus et al., 1984). However, now with the availability of CK II sequences of these subunits, evidence has been presented showing that, in several species, the α and α' subunits are products of different genes (Chen-Wu et al., 1988; Meisner et al., 1989; Padmanabha & Glover, 1987; Padmanabha et al., 1990; Lozeman et al., 1990; Litchfield et al., 1990; Maridor et al., 1991). However, in *Drosophila* only one form of the catalytic subunit has been reported (Saxena et al., 1987).

Casein kinase II phosphorylates serine or threonine residues in a large number of potential protein substrates, including a variety of enzymes, cell membrane receptors, cytoskeletal proteins, transcription factors, and products of oncogenes [Meggio et al., 1984; see review by Tuazon and Traugh (1991)]. The phosphorylated residues are usually N-terminal to a cluster of acidic amino acid residues. An increasing number of these proteins have been shown to be phosphorylated in vivo at the phosphorylation site motifs specific for CK II (Krebs et al., 1988; Lozeman et al., 1990). Among these are eukaryotic or viral proteins that may be involved in cell

[†] This is ILRAD publication no. 1034.
[‡] The nucleic acid sequence in this paper has been submitted to GenBank under Accession Number M92048.
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0006-2960/92/0431-6193$03.00/0

growth or transformation including SV40 large T antigen (Krebs et al., 1988; Grasser et al., 1988), Myc (Lüscher et al., 1989), DNA topoisomerase II (Ackerman et al., 1988), human papilloma virus E7 protein (Firzlaff et al., 1989), mouse p53 (Meek et al., 1990), and ornithine decarboxylase, an enzyme which is rapidly induced following stimulation of cells with growth-promoting agents (Meggio et al., 1987; Rosenberg-Hasson et al., 1991).

Casein kinase II activity is enhanced within minutes after the addition of insulin to cells propagated in vitro (Sommercorn et al., 1987; Klarlund & Czech, 1988). The enzyme is also induced during cell differentiation and by exposure of cells to growth factors, such as EGF or serum (Sommercorn & Krebs, 1987; Sommercorn et al., 1987; Krebs et al., 1988; Carroll & Marshak, 1989; Ackerman & Osheroff, 1989). High levels of casein kinase II activity have also been reported during early developmental stages of *C. elegans* (Hu & Rubin, 1990) and in early chicken embryos (Maridor et al., 1991). These data suggest that casein kinase II may function as an important element of postreceptor, signal-transducing pathways activated by hormones and by a variety of growth factors.

We have been studying the molecular mechanisms responsible for bovine T lymphocyte transformation induced by *Theileria parva*. *T. parva*, an obligate intracellular protozoan parasite transmitted by a tick vector, causes East Coast fever, an acute and often fatal lymphoproliferative disease of cattle in eastern and central Africa. The mammalian-infective stage of *Theileria* is the sporozoite, which is injected into the host with the saliva during feeding. Although *T. parva* sporozoites can enter a variety of bovine leukocytes engaging a receptor/ligand-like endocytotic process, the parasite appears to survive in, and induce proliferation of, mostly T cells (Fawcett et al., 1982; Baldwin et al., 1988). The sporozoite, which is encapsulated by the host cell plasma membrane during entry, induces rapid dissolution of that membrane soon after entry into the lymphocyte (Fawcett et al., 1982). The dissolution of the host plasma membrane surrounding the sporozoite occurs concurrently with the diminution of the electron-dense material contained in the rhoptries and micronemes, subcellular organelles which have been shown to mediate host cell entry by related parasitic protozoa such as *Plasmodium* spp. Within the lymphocyte, the sporozoite, whose plasma membrane is entirely exposed to the host cell cytoplasm, then differentiates into a multinucleate schizont, which induces clonal expansion and immortalization of *T. parva*-infected cells in vitro as well as in vivo (Baldwin et al., 1988; Emery et al., 1988). Studies on merogony in *T. parva* suggest that the schizont undergoes fission to form merozoites, which bud off from the lymphocyte to enter the circulation where they infect erythrocytes, within which they differentiate into piroplasms. *Theileria*-infected cells have been considered transformed because they exhibit cellular pleiomorphism, have short (16–25 h in vitro) doubling times and surface phenotype alterations involving differentiation antigens, and, when inoculated into athymic mice, form tumorlike masses, which metastasize and infiltrate most organs (Irvin et al., 1975; Baldwin et al., 1988; Conrad et al., 1989). The *T. parva*-transformed lymphocytes are thought to be primarily responsible for the pathology associated with the disease.

In an attempt to determine the molecular mechanisms responsible for the *T. parva*-induced cellular transformation, we have found a marked increase in protein kinase activity in protein preparations from infected cells (ole-MoiYoi et al., 1990). On the basis of substrate specificity and susceptibility to various inhibitors, the enzymes we have found in *T. parva*-infected cells are serine/threonine kinases with the specificities of casein kinase II. We have used *Drosophila* cDNA clones encoding the casein kinase II α or β subunits as probes on Southern blots of both bovine and *T. parva* DNA and have subsequently cloned the gene for an α subunit of the parasite enzyme. In this paper we report the isolation and characterization of the gene for *T. parva* casein kinase II α subunit.

Materials and Methods

Parasites. Infection of cattle with sporozoite stabilates of different stocks of *T. parva* was carried out as previously described by Conrad et al. (1987) and Dolan et al. (1984). A *T. parva* stabilate, isolated at Muguga (TpM: ILRAD isolate 836), Kenya, was used as a source of *T. parva* materials described in this paper.

Cloning of Bovine Lymphocytes and Infection with T. parva. IL 2-dependent bovine cell lines (B657.G6 and A19) had been cloned by limiting dilution from concanavalin A-stimulated bovine lymphocytes as previously described (Brown & Grab, 1985; Morrison et al., 1987); these cell lines belong to the $CD8^+$ subset of T cells (Baldwin et al., 1988). Infection of the IL 2-dependent cell lines with *T. parva* was achieved by incubating the cells with sporozoites of *T. parva* purified from tick salivary gland homogenates (Dobbelaere et al., 1984). The IL 2-dependent lymphocyte clone and the same clone after infection with *T. parva* shall hereafter be referred to as G6 and G6TpM, respectively.

The G6 and the infected G6TpM cell lines were cultured in RPMI 1640 medium containing 25 mM HEPES (Gibco Europe, Paisley, UK) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (Hyclone, Logan, UT), 2 mM L-glutamine, 50 μM 2-mercaptoethanol, and 50 μg of gentamycin/mL. The cells were incubated at 37 °C in an atmosphere of 3–5% CO_2 in air as previously described (Brown & Grab, 1985). At times, G6TpM was also cultured in L-15 medium, which had been supplemented with 10% tryptose phosphate broth and fetal bovine serum, L-glutamine, 2-mercaptoethanol, and gentamycin at the concentrations given above.

Preparation of Lymphocyte Homogenates and Purification of Schizonts. G6 and G6TpM cells, in log-phase growth, were washed several times with phosphate-buffered saline (PBS, pH 7.4) and used for preparation of homogenates for protein kinase assays or for purification of schizonts from *T. parva*-infected lymphocytes. The cells were suspended in cold homogenization buffer (250 mM sucrose, 25 mM Tris-HCl, pH 7.2, 0.01 mM EDTA, 5 mM $MgCl_2$, 120 mM KCl, 1 mM PMSF, and 8 μg of aprotinin/mL) at a concentration of 1×10^8 cells/mL and sonicated at 4 °C for 2 min (0.5-s pulses) at a setting of 230 W with a Branson ultrasonic cell disrupter equipped with a jacket horn. The homogenates were separated into pellet and supernatant by centrifugation at 100000*g* for 30 min at 4 °C. The pellet was recovered and resuspended in a volume of cold homogenization buffet equal to that of the 100000*g* supernatant. Both the pellet and supernatant fractions were analyzed for protein kinase activity on endogenous or exogenous substrates as described below. In both cell lines, most of the enzymatic activity (>90%) was in the 100000*g* pellets. Homogenates of schizonts purified from infected lymphocytes, as described by Sugimoto et al. (1988), were also prepared and used as a source of protein kinase. Briefly, *T. parva*-infected lymphocytes were suspended (4×10^7 cells/mL) in 10 mM HEPES, pH 7.4, 150 mM NaCl, 20 mM KCl, and 1 mM $CaCl_2$ containing 20 μg/mL each of the following protease inhibitors: leupeptin, antipain, chymostatin, and *trans*-epoxysuccinyl-L-leucylamido-(4-guanidino)butane (E-64)

and 2% (w/v) Ficoll 400 (Pharmacia Fine Chemicals, Uppsala, Sweden). To release schizonts, the *T. parva*-infected lymphocytes were lysed by the addition of the cytotoxins aerolysin (10 μg/mL) or Ah-1 hemolysin (23 μg/mL), both purified from *Aeromonas hydrophila*. The cells were incubated at 23 °C for 10 min (aerolysin) or 15 min (Ah-1 hemolysin). Schizont aggregation was inhibited by addition of EDTA to a final concentration of 5 mM. Percoll stock, made in the HEPES–EDTA buffer, was added to the cell lysate to give a final concentration of 64.6% (v/v). The schizonts were then separated from the cellular debris by centrifugation (110000*g*) through a discontinuous Percoll gradient (45–64.6%) for 30 min at 4 °C. Schizonts purified in this way were essentially free from host cell debris and retained their intralymphocytic morphology as assessed by electron microscopical examination. When the analysis of phosphorylation of exogenous substrates was performed, highly diluted schizont homogenates (20–60 ng of protein) were used as a source of enzyme.

For the experiments reported herein, protein kinase assays were performed by incubation, for 10–15 min at 23 °C, of reaction mixtures containing 10 mM $MgCl_2$, 120 mM KCl, 50 mM Tris-HCl (pH 7.2), 1 mM 3-isobutyl-1-methylxanthine (IBMX), 15 μM aprotinin, 40 μM [γ-^{32}P]ATP (specific activity 7500 cpm/pmol) and 20–80 μg of protein as substrate. To this mixture was added 20–60 ng of schizont lysate as enzyme source.

Polyacrylamide Gel Electrophoresis. Samples, each containing 20–60 μg of protein, were incubated in vitro in the protein kinase assays described above and, after incubation, boiled in sodium dodecyl sulfate (SDS) sample buffer for 5 min and run on SDS–polyacrylamide gradient (5–17.5% or 7.5–15%) gels according to Laemmli (1970). The gels were stained with Coomassie blue, destained, and dried. The dried gels were exposed to Fuji RX 100 film for autoradiography, mostly at −80 °C with intensifying screens for varying periods of time. For better resolution of phosphorylated protein bands, it was occasionally necessary to carry out the exposure at 23 °C using Hyperfilm β max (Amersham International plc., Amersham, UK) without intensifying screens.

DNA Preparation. DNA was prepared from the G6 and G6TpM lymphocyte clones as well as from *T. parva* piroplasms according to Conrad et al. (1987). Briefly, purified piroplasms or lymphoblastoid cell pellets (final concentration 5×10^7 cells/mL) were suspended in 1× TNE (25 mM Tris-HCl, 5 mM EDTA, and 100 mM NaCl, pH 8.0), and SDS and boiled RNase A were added to the cell suspensions to give final concentrations of 0.5% and 100 μg/mL, respectively. The cell lysates were mixed gently and incubated at 37 °C for 1–2 h. Proteinase K (BRL, Gaithersburg, MD) was added to a final concentration of 100 μg/mL and the preparations were incubated for 3 h at 50 °C. Further extraction of DNA was performed as described by Sambrook et al. (1989).

Genomic Library Screening. A library of *T. parva* (Muguga) piroplasm sheared genomic DNA was constructed in λgt11 as previously described (Sambrook et al., 1989; Conrad et al., 1987). The library, which yielded 1.1×10^6 independent recombinant phage clones from 0.1 μg of *T. parva* DNA, was plated out at 5×10^3 plaque-forming units (pfu) per 150-mm plate and screened using a cDNA clone of *D. melanogaster* CK II α subunit (Dm 95, a gift from Dr. Claiborne V. C. Glover; Saxena et al., 1987). After incubation, duplicate filters (Schleicher & Schüell, Keene, NH) were prepared, the DNA was denatured, and the filters were neutralized and baked at 80 °C for 2 h. The *Drosophila* CK II α cDNA probe was labeled by random priming according to Feinberg and Vogelstein (1983) to a specific activity of about 1×10^9 cpm/μg of DNA and used to probe the duplicate filters in 4× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 10× Denhardt's solution, 0.1% SDS, and 0.1% sodium orthophosphate at 50 °C for 16 h. After hybridization, the filters were washed twice in 4× SSC/0.1% SDS at 50 °C for 1 h and once in 2× SSC/0.1% SDS at 50 °C for 1 h. The filters were then dried and exposed for 16 h to Fuji RX 100 film (Fuji Photo Film Co., Japan) with intensifying screens at −80 °C. One positive clone (λg*Tp*MCK2) was identified and was plaque-purified by two additional rounds of screening using the conditions described above. This clone contained a 4.5-kb insert of *T. parva* DNA.

Subcloning and DNA Sequencing. The 4.5-kb genomic DNA fragment of *T. parva* in the clone λg*Tp*MCK2 was subcloned into pUC19 and a partial restriction map was generated. Subsequently, a 1.4-kb *Eco*RI/*Hpa*II fragment and a 0.9-kb *Hpa*II fragment were also subcloned into pUC19. Nested deletions were generated by digestion of the DNA inserts with Bal 31 exonuclease. The inserts were transferred into M13mp18/19 and all of the sequencing was performed using the dideoxy chain-termination method (Yanisch-Perron et al., 1985; Sanger et al., 1977), predominantly using Sequenase (U.S. Biochemicals, Cleveland, OH) and [α-^{35}S]dATP according to the manufacturer's instructions. The sequencing gels were read using a sonic digitizer interfaced to a microcomputer (Science Accessories Corp.). Each base was sequenced at least twice on each of the strands. The sequence data were analyzed by DNASIS/PROSIS (Hitachi Software Engineering, Brisbane, CA) on an IBM PS/2 Model 80 computer. Amino acid sequence comparisons were performed using the Clustal software program (Higgins & Sharp, 1988).

Southern Blot Analysis. DNA prepared from uninfected or *T. parva*-infected lymphocytes or from piroplasms was digested with various restriction enzymes and fractionated on 0.8% agarose gels, which were subsequently processed as described by Sambrook et al. (1989). DNA fragments were transferred onto Hybond N$^+$ nylon filters (Amersham International) and hybridized with either ^{32}P-labeled Dm 95 *Drosophila* cDNA (Saxena et al., 1987) or with an *Eco*O109I/*Xho*I fragment of *T. parva* DNA from the λg*Tp*MCK2 clone. The 888-bp *Eco*O109I/*Xho*I fragment contained most of the open reading frame of the *T. parva* CK II gene. In addition to the low and intermediate stringency washing conditions described above, Southern blot filters hybridized with the latter probe were additionally washed in 0.1× SSC/0.5% SDS at 65 °C for 1 h.

RNA Isolation and Northern Blot Analysis. Total RNA was prepared as described by Han et al. (1987) from the G6 lymphoblasts in log-phase growth, the from G6TpM cell line, and from *T. parva* piroplasms. Poly(A)-enriched RNA was isolated by oligo(dT)–cellulose (Type 3) chromatography according to the manufacturer's instructions (Collaborative Research Inc., Bedford, MA). Poly(A)-rich RNA was denatured, fractionated on 1.4% agarose–formaldehyde gels, and transferred onto Hybond N$^+$ nylon membranes (Amersham). Antisense ^{32}P-labeled RNA transcripts were synthesized in vitro using a 1.5-kb *T. parva* CK II α cDNA cloned into pBluescript SK (Stratagene, La Jolla, CA). Hybridization was performed for 16 h at 60 °C according to the instructions provided by the supplier of T7 RNA polymerase (Stratagene). After hybridization, the filters were washed twice, each time for 30 min, in 0.5× SSC/0.5% SDS at 65 °C. Autoradiography was performed at −80 °C as described above.

Construction and Screening of a cDNA Library. Double-stranded cDNA was made from poly(A)-enriched RNA (5 μg), prepared from *T. parva*-infected bovine lymphocytes, according to the instructions provided by the manufacturer (BRL cDNA Synthesis System). The first strand was primed with oligo(dT) and the second strand was made with RNase H and DNA polymerase I. The double-stranded cDNA was methylated on *Eco*RI sites and blunt-end ligated to *Eco*RI linkers (New England Biolabs, Beverly, MA) using T4 DNA ligase (Amersham). The *Eco*RI linkers were removed by digestion with *Eco*RI and separated from the cDNA by size fractionation on a 5–20% potassium acetate gradient in a Beckman SW50.1 rotor for 3 h at 49000 rpm at 22 °C (Aruffo & Seed, 1987). The cDNA with sizes greater than 0.8 kb was mixed with λgt11 arms (Promega, Madison, WI) and ligated using T4 DNA ligase. The library had about 2×10^6 independent recombinants. This library was screened using the ^{32}P-labeled *Eco*O109I/*Xho*I fragment as probe, employing the high-stringency washing conditions described above. Several cDNA clones were isolated and the clone with the largest insert (1.5 kb) was further characterized.

Results

Identification of Casein Kinase II Activity in T. parva Schizonts. Comparison of kinase activity of 100000*g* particulate or supernatant preparations from the IL 2-dependent G6 clone and those from the G6TpM cell line revealed that the infected cell line had an 11–18-fold increase in kinase activity per microgram of protein (ole-MoiYoi et al., 1990). In order to understand the significance of this finding, it was essential to determine the contribution of the intralymphocytic schizont to the total protein kinase activity detected in the *T. parva*-infected cells. For these studies, we employed homogenates prepared from schizonts purified from infected lymphocytes as an enzyme source to phosphorylate heat-inactivated 100000*g* pellet preparations from the infected lymphocytes as well as a variety of commercially-available kinase substrates. These experiments revealed that *T. parva* schizonts had a kinase activity that was capable of phosphorylating caseins, phosvitin, and glycogen synthase but that failed to phosphorylate, to any extent, histones 2A, III-S, or V-S, protamine, or kemptide (data not shown). Figure 1 depicts an autoradiogram of an SDS–polyacrylamide gradient (7.5–15%) gel in which substrates were resolved after phosphorylation with the schizont-associated kinase. The phosphorylation of mixed casein or phosvitin was essentially abolished by 750 ng of heparin/mL (Figure 1, compare lanes 2 and 3 with lanes 5 and 6, respectively), whereas that of glycogen synthase was less affected (Figure 1, compare lane 4 with lane 7). There were only a few faint bands (M_r 86000 and 14000–17000) visible in the schizont lysate itself (Figure 1, lane 8). There were no detectable protein bands in the M_r range of bovine CK II subunits. The schizont lysate nevertheless phosphorylated the heat-inactivated (94 °C, 10 min) 100000*g* pellet proteins, which by themselves had no residual kinase activity, to give patterns (Figure 1, lane 1) similar to those seen when uninactivated endogenous enzymatic activity was used to phosphorylate endogenous lymphocyte (G6TpM) substrates (ole-MoiYoi et al., manuscript in preparation). The enzyme in the schizont preparations was activated by spermine and polylysine but not by cyclic nucleotides (data not shown).

Cloning of a T. parva CK II Gene. The preliminary experiments described above suggested that *T. parva* schizonts had a casein kinase II-like enzyme. We therefore used the *Drosophila* CK II α or β cDNAs (Dm 95 and Dm 98, respectively; Saxena et al., 1987) to probe Southern blots of

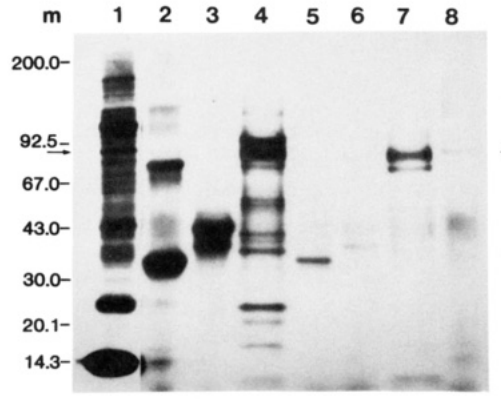
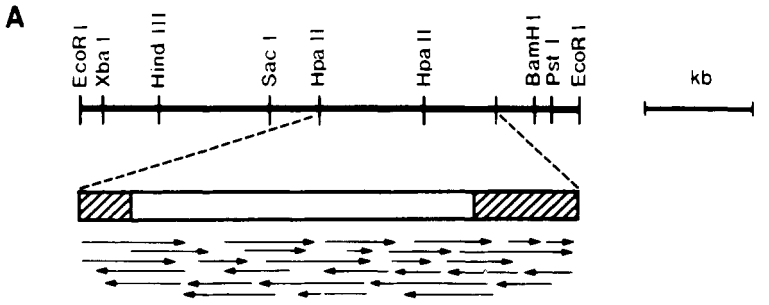


FIGURE 1: Inhibition by heparin of protein phosphorylation by a *T. parva* schizont-associated CK II. A homogenate of a highly purified schizont preparation (lane 8) was used to phosphorylate 20 μg each of mixed casein, phosvitin, and glycogen synthase (lanes 2, 3, and 4, respectively). For each substrate, a reaction mixture containing heparin (750 ng/mL) was processed in parallel (depicted in lanes 5–7). Phosphorylation of heat-inactivated (94 °C, 10 min) 100000*g* protein pellet from *T. parva*-infected lymphocytes, which had no detectable residual kinase activity, was also used as substrate for the schizont-associated kinase (lane 1). The arrow depicts a protein band (M_r 86000) present in both the schizont and lymphocyte pellet preparations. Molecular masses of the markers (m) are in kilodaltons.

DNA prepared from the uninfected IL 2-dependent G6 lymphocyte clone, G6TpM, and *T. parva* piroplasm DNA. The *Drosophila* CK II β cDNA hybridized to bovine DNA, but not to *Theileria* DNA under intermediate stringency (2× SSC, 50 °C) washing conditions. In contrast, the *Drosophila* CK II α cDNA probe showed no hybridization to bovine DNA at washings of intermediate stringency but did hybridize to fragments of approximately 15 kb and 10 kb of *Eco*RI- and *Hin*dIII-digested *T. parva* DNA, respectively (data not shown; however, see below).

When the *Drosophila* CK II α cDNA probe was used to screen a library of *T. parva* piroplasm genomic DNA in λgt11, one positive clone, containing a 4.5-kb insert, was identified when the filters were washed at the same stringency as for Southern blots. This clone, λg*Tp*MCK2, was plaque-purified and used for preparation of DNA. A 1.4-kb *Eco*RI/*Hpa*II fragment and a 0.9-kb *Hpa*II fragment were then subcloned for sequencing. Bal 31 nuclease digestion time points of these two fragments were generated for cloning into M13mp18/19 for sequencing. A partial restriction map of the insert cloned in λg*Tp*MCK2 is shown in Figure 2A.

Properties of the CK II Gene Sequence of T. parva. The stop codon map for the three reading frames of the cloned sequence in the forward direction revealed an open reading frame (reading frame 1) with an in-frame TGA stop codon 25 nucleotides upstream of a potential ATG start codon (Figure 2B). The open reading frame (ORF) starts from nucleotide 50 and extends to a TAA termination codon ending at nucleotide 1336. Within the 5′ part of the *T. parva* CK II α gene encoding the 99 amino acid sequence unique to this organism, there were three potential ATG initiation codons starting at nucleotides 74, 290, and 299. We have not found any typical consensus sequences for translation initiation within the expected distance from any of these ATG initiation codons. The ORF encodes a protein of 420 amino acids with a calculated M_r of 50200. The translated region of this gene is 64.7% AT, whereas the 3′ untranslated region is 80.4% AT. However, the short 5′ untranslated region is only slightly more AT-rich (65.3%) than the coding region. There was a potential polyadenylation signal (AATAAA) starting at nucleotide 1368. Interestingly, sequence analysis of two cDNA clones



B

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1     CCGGGTAAGAGATTCTTGACAGAATCCACTGTAATTTTAAGGCTATGATGGCAAGAAATAATTTTAAATCGT ATG ATA TAT AGA
                                                                              *   M   I   Y   R   4

85    TAT AAA TTT TAT AAA TAT ATA ATA TAC CTG TTT GCC ATC ATT ATC CAC AAA TCC TAC TGT ATA GAA TTT CAT AAA TAT ATT AAT
      Y   K   F   Y   K   Y   I   I   Y   L   F   A   I   I   I   H   K   S   Y   C   I   E   F   H   K   Y   I   N  32

169   CAA TAC AAT TTA TCA TTT GTA AAG CCA ATA AAT AAT AAA GAT ATT ATT TAT AGG TTA AAA AGA GAT TAT TTA CAG TTA AAT AAG
      Q   Y   N   L   S   F   V   K   P   I   N   N   K   D   I   I   Y   R   L   K   R   D   Y   L   Q   L   N   K  60

253   GAA GAA AAT GGT ATA TTC AAT CAG CCA ATA CTA AAT ATG GAT CAG ATG GAC TTG GTT GAT ACC GAT AAA AGC AGG AAG TAT CCT
      E   E   N   G   I   F   N   Q   P   I   L   N   M   D   Q   M   D   L   V   D   T   D   K   S   R   K   Y   P  88

337   GCA AAA GAC CTT AAA ATT ATA TTA CCA AAA ACA TAC GCA GAT GTA AAT AGT AAA AAG GGA CCT GAG TAT TGG GAT TAC GAG AAT
      A   K   D   L   K   I   I   L   P   K   T   Y   A   D   V   N   S   K   K   G   P   E   Y   W   D   Y   E   N 116

421   ATA ACA CTA AAG TGG AAT GTA CCT GAC TCA TAC GAG ATA GTC AGG AAG ATC GGA AGA GGG AAG TTC AGT GAA GTT TTT GAG GGA
      I   T   L   K   W   N   V   P   D   S   Y   E   I   V   R   K   I   G   R   G   K   F   S   E   V   F   E   G 144

505   ATA AAT ACA GTA ACG AAG GAT AAA TGC GTA ATA AAA ATC CTA AAG CCT GTA AAG AAG AAG AAA ATC AAG AGA GAG ATT AAG ATT
      I   N   T   V   T   K   D   K   C   V   I   K   I   L   K   P   V   K   K   K   K   I   K   R   E   I   K   I 172

589   CTA CAG AAC CTA AGA GGA GGT CCA AAT ATA ATA AAG TTA CTC GAT ATT GTA AAA GAC CCT CAA AGC AGA ACA CCC TCG CTA ATT
      L   Q   N   L   R   G   G   P   N   I   I   K   L   L   D   I   V   K   D   P   Q   S   R   T   P   S   L   I 200

673   TTT GAA CAT GTA AAT AAT ACA GAT TTC AAA ACC TTG TAC CCA ACC CTA AGT ATA CAG GAC ATA AAG TAC TAT ATT TAC CAG TTA
      F   E   H   V   N   N   T   D   F   K   T   L   Y   P   T   L   S   I   Q   D   I   K   Y   Y   I   Y   Q   L 228

757   TTG AAG GCC ATG AAT TAC TGT CAC AGT CAA GGC ATT ATG CAC AGA GAT ATA AAA CCG CAC AAC GTT ATG ATA GAT CAT GAA AAG
      L   K   A   M   N   Y   C   H   S   Q   G   I   M   H   R   D   I   K   P   H   N   V   M   I   D   H   E   K 256

841   AAA ATA CTA AGG CTA ATT GAC TGG GGA TTA GCT GAG TTT TAC CAC CCA GAA CAA GAA TAT AGT GTT AGG GTT GCA ACA AGG TAC
      K   I   L   R   L   I   D   W   G   L   A   E   F   Y   H   P   E   Q   E   Y   S   V   R   V   A   T   R   Y 284

925   TAC AAG GGT CCG GAA TTG CTG GTT GAC ATG AGG TAC TAC GAC TAC AGC CTC GAT ATA TGG AGC ATA GGG TGT ATG CTA GCG GGT
      Y   K   G   P   E   L   L   V   D   M   R   Y   Y   D   Y   S   L   D   I   W   S   I   G   C   M   L   A   G 312

1009  ATC ATT TTC AAG AAG GAG CCA TTT TTC TAT GGT CAT GAT AAC TAT GAT CAG CTG GTG AAA ATA GCC AAG GTA CTA GGA ACA GAA
      I   I   F   K   K   E   P   F   F   Y   G   H   D   N   Y   D   Q   L   V   K   I   A   K   V   L   G   T   E 340

1093  GAT TTA CAC CGA TAC TTT GAG AAA TAC GGA CTT AAA TTC GCA CCT GCA TAC CAG GAA ATT TTA GGA AAT CAC TCC AAG AAA CCC
      D   L   H   R   Y   F   E   K   Y   G   L   K   F   A   P   A   Y   Q   E   I   L   G   N   H   S   K   K   P 368

1177  TGG ACC AAG TTC GTA CAC CAT GAA AAT CAA CAC CTG GTA TCA CCT GAG GTC ATG GAC CTG CTG GAT CGC ATG TTA GTT TAT GAC
      W   T   K   F   V   H   H   E   N   Q   H   L   V   S   P   E   V   M   D   L   L   D   R   M   L   V   Y   D 396

1261  CAT ACC AAG AGA ATC ACA CCG CTC GAG GCA ATG GAA CAC CCT TTC TTT AAC GAA ATC AAA AAC AAC TCA GTT TAA TTCTGAATTTA
      H   T   K   R   I   T   P   L   E   A   M   E   H   P   F   F   N   E   I   K   N   N   S   V  **          420

1347  ACGACAGTTTAATGTATTCTAATAAATTATATTTATTATTCTAGTTTTAAATTAATAAAACACATTTTTGAGAATATAATTTAATAAATTATGAAATTTGTGAGTTAAAAT

1460  GTCTTTCACGGGGAATGTGTTTTTATTCAGAAAAGTGTGGCGGAATGAAATGTGTTTATCCAAAACAAATTAATTTATTTTTTAATTAAATTAAATAAAAACA
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FIGURE 2: Sequence of the *T. parva* CK II α subunit gene. (A) Schematic representation and restriction map of the 4.5-kb *T. parva* piroplasm genomic DNA fragment cloned in the recombinant phage λg*Tp*MCK2. The hatched boxes represent the noncoding regions (UTR) of the gene. The open reading frame is shown by the open box. The sequencing strategy is depicted by the arrows under the diagram. Each base was sequenced over at least twice on each strand. (B) Nucleotide and deduced amino acid sequence (single-letter code) of the only major open reading frame within the sequenced fragment of the λg*Tp*MCK2 clone. Numbers at the left and right margins indicate positions of nucleotides and amino acids, respectively. A portion of the putative signal sequence (amino acid residues 9–26) is underlined. When the deduced sequence was analyzed using the weight-matrix scanning algorithm described by von Heijne (1986), a part of the underlined sequence was identified as a potential signal peptide. Potential N-linked glycosylation sites are boxed. The "transcript-instability" sequence motifs in the 3′ UTR are overlined. The two stop codons discussed in the text are shown as * (5′) and ** (3′).

coding for the *T. parva* CK II α subunit revealed that identity between the genomic sequence and the cDNA sequences ended at nucleotide 1374, one nucleotide beyond the potential poly(A) addition signal. This was immediately followed by a poly(A) tail. Such short (3–6 nt) nucleotide sequences between potential polyadenylation signals and the poly(A) tails have been

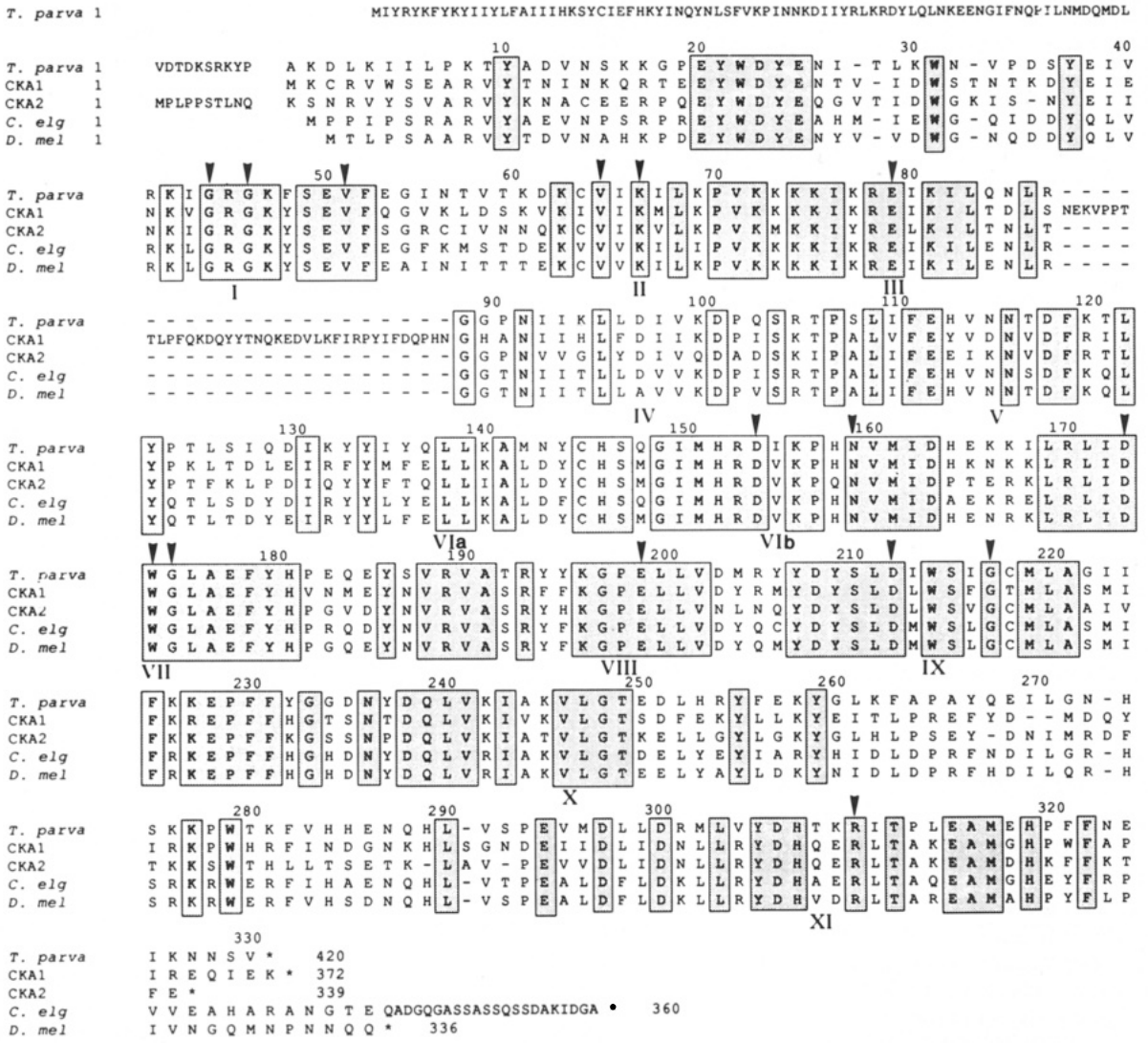

FIGURE 3: Comparison of the *T. parva* CK II α sequence with casein kinase II catalytic subunits from other invertebrates. The deduced amino acid sequence of *T. parva* CK-II α is aligned with other CK II sequences from *S. cerevisiae* α (CKA1; Cheu-Wu et al., 1988), *S. cerevisiae* α' (CKA2; Padmanabha et al., 1990), *C. elegans* (*C. elg*, Hu & Rubin, 1990), *D. melanogaster* (*D. mel*.; Saxena et al., 1987). The numbering is according to that of *Drosophila* CK II α subunit (Saxena et al., 1987; Padmanabha et al., 1990). Arrowheads indicate residues present in virtually all kinases (Hanks & Quinn, 1991). The boxed residues are those present in all CK II catalytic subunits compared here. The total length, in amino acid residues, of each CK II catalytic subunit is given at the end of each sequence.

seen in other genes of *T. parva* (R. Bishop, personal communication). There were two sequences, starting at positions 1343 and 1355 (ATTTA and TTTA, respectively) in the 3′ untranslated region, which have been reported to confer instability to mRNA (Shaw & Kamen, 1986). All three of these sequence motifs were present in the cDNA clones with sequence identity spanning nucleotides 1138–1374.

Within the *T. parva* CK II α gene were two domains (nucleotides 800–900 and 1001–1100) within which the gene had greater than 71% identity with the gene for the *Drosophila* CK II α subunit (Saxena et al., 1987). Contained within the latter domain was a 37-bp region for which the two sequences are nearly 90% identical. When the *Drosophila* CK II cDNA α gene was used to probe *Hpa*II-digested λg*Tp*MCK2 DNA, we had noted that the enzyme cut the insert fragments of about 1.4 and 0.9 kb that hybridized to the heterologous probe, giving signals of equal intensity (data not shown). The *Hpa*II cutting site, which lies between these two conserved nucleotide sequences at nucleotide 935, is consistent with those observations. Interestingly, this *Hpa*II site is within the part of the gene encoding the conserved KGPELLV sequence motif, which is present in all CK II α subunits reported to date.

The *T. parva* CK II α gene appears to have no introns and potentially encodes a protein larger than most other CK II α subunits described in other species. For confirmation of transcription of this gene, we obtained sequence information from a cDNA clone. This clone had an insert of 1.5 kb and revealed complete identity with the consensus genomic sequence from base 53 to 1374.

Comparison of Deduced Amino Acid Sequences of CK II α Subunits from Invertebrates. The amino acid sequence deduced from the *T. parva* casein kinase II α subunit sequence has a high degree of identity with the cell division control (*cdc*) kinases, as has been reported for CK II α sequences from other organisms (Lorincz & Reed, 1984; Takio et al., 1987; Saxena et al., 1987; Chen-Wu et al., 1988). Pairwise comparisons between the *T. parva* CK II α sequence, with identity generally beginning after the conserved tyrosine (Y^{37} in the *D. melanogaster* CK II α sequence; Figure 3), with the *cdc2* human analogue, *cdc28*, and *cdc2* gives identities of 31%, 30%, and 29%, respectively. The alignment of the predicted *T. parva* CK II α subunit sequence with those from other invertebrates (*S. cerevisiae*, *C. elegans*, and *D. melanogaster*) is shown in Figure 3. As has been reported for other catalytic subunits

of CK II, the *T. parva* sequence has Ser instead of Gly in the sequence motif $G^{44}XGXXSXV$ in catalytic subdomain I, Val instead of Ala in the $A^{64}XK$ sequence in catalytic subdomain II, $DW^{175}G$ instead of $DF^{175}G$ in subdomain VII, and $G^{195}PE$ instead of $A^{195}PE$ in subdomain VIII. The last two replacements appear to be unique to the catalytic subunits of casein kinase II (Padmanabha et al., 1990).

The boxed residues shown in Figure 3 are the amino acids that are present in all the five catalytic subunits compared here. In general these CK II α subunits from lower eukaryotes do not differ substantially from a wider comparison in an alignment which included CK II catalytic subunits from mammalian sources (Padmanabha et al., 1990). Of the residues shown to be conserved in all casein kinase II catalytic subunits reported by Padmanabha et al. (1990), there are 17 amino acids which are different in the *T. parva* sequence reported here. Among the 17 differences, however, only one (V^{304}/R) represents a radical replacement. The *T. parva* CK II subunit displays 68% sequence identity with the *Drosophila* α subunit, 67% with the *C. elegans* α subunit, 58% with the *S. cerevisiae* α′ subunit, and 56% with the *S. cerevisiae* α subunit. Among mammalian CK II catalytic subunits, the predicted *T. parva* sequence has about 68% sequence identity with the human, rat, and chicken α subunits (Lozeman et al., 1990; Meisner et al., 1989; Maridor et al., 1991), while identity with the α′ subunits from these species was less, ranging from 65% to 66%. Casein kinase II catalytic subunits have thus been remarkably conserved across wide evolutionary distances.

Padmanabha et al. (1990) pointed out three domains of interest in the casein kinase II subfamily. These include the ARVY and EYWDYE sequence motifs which lie N-terminal to the kinase catalytic subdomain I (Hanks & Quinn, 1991) and the KPVKKKKIKR sequence within catalytic subdomain II. The latter two are completely conserved in *T. parva* CK II α sequence. However, the *T. parva* sequence PKTY, replacing ARVY, contains three conservative replacements, thus leaving tyrosine as the only invariant residue in this sequence motif. With the exception of the *S. cerevisiae* CK II α subunit (*CKA1*), potential N-linked glycosylation sites (NXS/T) were present in all the CK II sequences compared. Among the CK II catalytic subunit sequences available, the *T. parva* subunit has the highest number (5) of potential N-linked glycosylation sites. Only one of these (NNT/S; subdomain V) is shared with the other sequences, including those from mammalian sources. With this one exception, these sites fall within sequence stretches for which the *T. parva* CK II catalytic subunit has no homology with all the other α or α′ subunits.

A Novel N-Terminal Extension on T. parva CK II. The *T. parva* CK II catalytic subunit has a unique N-terminal 99 amino acid sequence. Visual inspection of this part of the sequence revealed a putative signal peptide (residues 1–26). We then employed the probability weight-matrix algorithm described by von Heijne (1986) to identify putative signal sequences and their probable peptidase cleavage sites within the whole of the deduced *T. parva* CK II α sequence. There were five frames that had marginally high Si values (von Heijne, 1986), two of which were within the N-terminal sequence unique to *T. parva*. These sequences consisted of a hydrophobic stretch of amino acids, the h-region (residues 10–19), bounded on the N-terminal side by a group of amino acids with a net positive charge, the n-region (residues 1–9), and on the C-terminal end by a stretch of residues, the c-region (residues 20–26), which are more polar than the hydrophobic core.

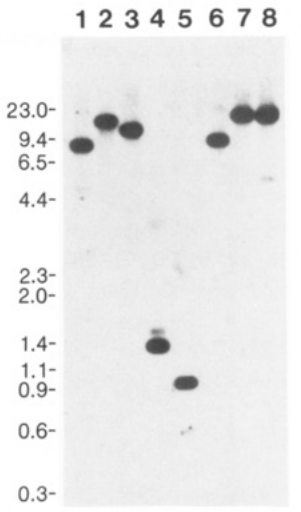

FIGURE 4: Genomic Southern blot analysis of *T. parva* piroplasm DNA using the *Eco*O109I/*Xho*I probe. Total *T. parva* genomic DNA was digested with each of various restriction enzymes, and the fragments were resolved on an (0.8%) agarose gel and blotted onto a nylon filter. The enzymes included *Bam*HI (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), *Ase*I (lane 4), *Eco*O109I/*Xho*I (lane 5), *Kpn*I (lane 6), *Pst*I (lane 7), and *Stu*I (lane 8). The conditions for hybridization and washing are as described under Materials and Methods. Autoradiography was overnight at −70 °C with intensifying screens. The DNA fragment length markers are given in kilobases.

Southern Blot Analysis of the T. parva CK II Gene. Genomic DNA from *T. parva* piroplasms was digested to completion with various restriction enzymes. An *Eco*O109I/*Xho*I fragment (residues 395–1282, which is contained entirely within the *T. parva* CK II α open reading frame) was radiolabeled and used to probe Southern blots of these digests (Figure 4). The restriction enzymes, which have no sites within the sequenced *T. parva* CK II gene, generated DNA fragments larger than the gene [*Bam*HI (8.0 kb), *Eco*RI (15.0 kb), *Hin*dIII (10 kb), *Kpn*I (8.3 kb), *Pst*I (15 kb), and *Stu*I (15 kb)]. The *Eco*RI and *Hin*dIII fragments, which hybridized to the labeled *Eco*O109I/*Xho*I probe, are the same lengths as those seen earlier when *T. parva* digests were probed with the *Drosophila* CK II α subunit probe. Enzymes with restriction sites within the CK II α gene generated fragments of expected lengths. For example, *Ase*I, with cutting sites at nucleotides 164, 1398, and 1527, generated one large strongly hybridizing fragment of about 1.25 kb (Figure 4, lane 4). The other fragment showed no hybridization because it was outside the sequence complementary to the probe. Use of restriction endonucleases having two or more sites within the gene, including *Taq*I, *Hpa*II, *Sac*I, and *Nci*I (data not shown), revealed, in each case, the expected number of fragments hybridizing to the *Eco*O109I/*Xho*I probe. Although these data are not completely conclusive, they suggest that *T. parva* has a single copy of the CK II α gene. Figure 4 also shows that in addition to the main band, all of the other restriction enzymes, perhaps with the exception of *Eco*RI (Figure 4, lane 2) and *Pst*I (Figure 4, lane 7), generated a weakly hybridizing band, which is best seen in the *Ase*I digest (Figure 4, lane 4) where it appears as a satellite above the main band.

T. parva CK II Gene Transcripts. Transcripts derived from the *T. parva* CK II gene were analyzed by northern blot hybridization of poly(A)-enriched RNA prepared from the IL 2-dependent G6 clone, the G6TpM *T. parva*-infected cell line, and *T. parva* piroplasms isolated from blood of infected cattle. An ethidum bromide-stained gel (Figure 5, lanes 1–3) and an autoradiogram of the northern blot are shown. The *T. parva* CK II α Riboprobe showed no hybridization to RNA

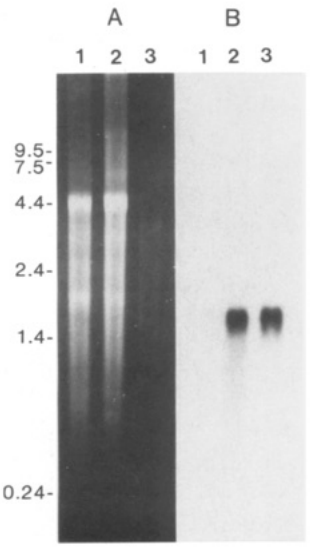

FIGURE 5: Northern blot analysis of bovine and *T. parva* poly(A)-enriched RNA using, as a probe, the 888-bp *Eco*O109I/*Xho*I fragment of the *T. parva* CK II α gene, which was labeled with ^{32}P. Poly(A)-enriched RNA was prepared from the IL 2-dependent G6 T-lymphocyte clone, from the same clone (G6TpM) after it had been infected with *T. parva*, and from *T. parva* piroplasms. The poly(A)-enriched RNAs were resolved in an agarose–formaldehyde gel and blotted onto a filter. The ethidium bromide-stained gel is shown in panel A. Lanes 1 and 2 contained 10 μg each of poly(A) RNA from the uninfected IL 2-dependent G6 parental cell line and the G6TpM cell line infected with *T. parva* (Muguga), respectively. Poly(A)-rich RNA (1 μg) from piroplasms of *T. parva* is shown in lane 3. The filter was hybridized with a ^{32}P-labeled antisense Riboprobe synthesized in vitro using a 1.5-kb *T. parva* CK II α cDNA as described under Materials and Methods. The amounts of *T. parva* poly(A)-enriched RNA used in this experiment were estimated by assuming that 10% of the poly(A)-rich RNA isolated from infected lymphocytes is of theilerial origin as estimated by Rot analysis (Gerhards et al., 1989). The filter was washed with 0.5× SSC/0.1% SDS for 1 h and exposed to X-ray film at −80 °C for 1 day. The size markers are the RNA ladder from BRL.

from the uninfected G6 cell line. In contrast, the CK II α probe hybridized with RNA from the uninfected G6 cell line. In contrast, the CK II α probe hybridized with RNA from the G6TpM T-cell clone infected with *T. parva* as well as to piroplasm RNA, giving bands of equal intensity at 1.65 kb. There was therefore no detectable length heterogeneity in the *T. parva* CK II α transcripts, as has been reported for higher eukaryotes (Meisner et al., 1989; Litchfield et al., 1990; Maridor et al., 1991).

Discussion

In this study, we describe the cloning and characterization of a gene for the casein kinase II α subunit of the intracellular, bovid lymphocyte-transforming protozoan parasite *Theileria parva*. The deduced amino acid sequence for the *T. parva* enzyme has the amino acid residues which are conserved in virtually all protein kinases, as well as those specific for the serine/threonine kinase family (Padmanabha et al., 1990; Hanks & Quinn, 1991). With the exception of the ARVY sequence motif located near the N-terminus of all the other casein kinase II α or α' subunits, the *T. parva* enzyme has essentially all the structural features considered unique to the catalytic subunits of casein kinase II (Saxena et al., 1987; Chen-Wu et al., 1988; Padmanabha et al., 1990). These include the EYWDYE, the basic sequence motif KPVKKKKIKR, DWG, and GPE. The sequence differences between CK II and other kinases, even though some involve conservative replacements when compared to those of other kinase subfamilies, may be sufficient to confer the functional characteristics unique to CK II, such as the capacity to utilize GTP, as well as ATP, as a phosphoryl group donor and its susceptibility to modulation by a variety of effector molecules, including its exquisite sensitivity to inhibition by heparin and other glycosaminoglycans (Feige et al., 1980; Hathaway et al., 1980).

In an attempt to determine whether the gene that we have cloned encoded the α or the α' subunit of CK II, we performed a protein sequence comparison between the *T. parva* catalytic subunit and the CK II α and α' sequences from human (Lozman et al., 1990) and chicken (Maridor et al., 1991). This comparison revealed that, among the positions for which the α and α' sequences differed, there were 18 residues for which the *T. parva* catalytic subunit was identical with either the α or the α' sequences from the other two species. Among these residues, the *T. parva* sequence was most identical with the α (13 of 18 residues) rather than the α' subunit. The *T. parva* gene that we have cloned is, therefore, likely to be that encoding the CK II α subunit of the enzyme.

A most intriguing feature of the *T. parva* CK II α subunit is the presence of a deduced N-terminal 99 amino acid peptide. Within this region of the molecule is a sequence, residues 1–26 of the *T. parva* CK II α sequence, which has features characteristic of presecretory signal peptides (von Heijne, 1986; Gierasch, 1989). These features include the presence of the three signal sequence regions reported by von Heijne (1986): an n-region, which carries a net positive charge, an h-region, consisting of a hydrophobic core, and a c-region, with one or more polar residues. In addition to this putative signal peptide, there are five potential N-linked glycosylation sites in the predicted sequence of the *T. parva* CK II α subunit, four of which are not present in any of the other CK II catalytic subunits. If these sites are at all glycosylated, it would be an additional indicator that the *T. parva* CK II is either secreted or destined for the plasma membrane. We have not found reports in the literature of a CK II with such structural features. However, other serine/threonine kinases have been detected in a variety of mammalian plasma membrane preparations and in intact cells including transformed 3T3 cells, adipocytes, HeLa cells, macrophages, mouse lymphoma cells, and myoblasts (Mastro & Rozengurt, 1976; Remold-O'Donnell, 1978; Kubler et al., 1989; Chen & Lo, 1991). Such ectoprotein kinases phosphorylate endogenous cell membrane proteins as well as those present in the extracellular milieu (Chen & Lo, 1991).

It has been suggested that the highly basic sequence motif (KPVKKKKIKR) may mediate interaction of the α subunit with the highly acidic domains of the β subunit as well as with those of protein substrates, most of which are acidic, and with a variety of effector molecules [Chen-Wu et al., 1988; Lozeman et al., 1990; see review by Tuazon and Traugh (1991)]. It has been noted by Lozeman et al. (1990) that the KPVKKKKIKR sequence also has the features characteristic of nuclear localization sequence (NLS) motifs (Kalderon et al., 1984; Lanford & Butel, 1984; Richardson et al., 1986; Dang & Lee, 1989). NLS motifs have been shown to be present in about a dozen nuclear proteins phosphorylated by CK II and are located at an average distance of about 10–30 amino acid residues N-terminal to putative CK II phosphorylation sites (Rihs et al., 1991). Rihs and Peters (1989) have shown that the NLS motif of SV40 large T antigen was sufficient to induce nuclear accumulation of recombinant fusion proteins containing large T antigen. It is of considerable interest that Rihs et al. (1991) have recently shown that, whereas the specificity of nuclear targeting of proteins is de-

termined by the NLS motif, the rate of transport of the SV40 large T antigen was a function of phosphorylation of the CK II site in the vicinity of the NLS. It has recently been proposed that the dynamics of cytoplasmic/nuclear translocation of molecules capable of modulating cellular replication represents an important level of regulation of cellular growth and differentiation (Rihs et al., 1991). In this context, the possible role of CK II as a transducer of growth signals to the nucleus might be effected through the alteration of the rate of nuclear protein transport, a process which would clearly influence gene expression and cell growth. That the CK II α' subunit is present in the nucleus and, like the CK II α subunit, shows a change in distribution depending on the stage of the cell cycle, raises questions regarding differences in the functions of the catalytic subunits of CK II. Chen-Wu et al. (1988) and Padmanabha et al. (1990) showed that disruption of either the α or the α' subunit genes of *S. cerevisiae* casein kinase II was not lethal and concluded that the catalytic subunits are sufficiently similar to allow each subunit to compensate for the absent functions of the other. However, haploid yeast cells with mutations of both catalytic subunits were growth-arrested and nonviable. Padmanabha et al. (1990) could rescue such yeast cells with double mutations of the catalytic subunits by complementation with the *Drosophila* CK II α subunit; these observations provide functional evidence reflecting the remarkable degree of primary structural conservation of CK II over great evolutionary distances.

In addition to identifying the gene encoding the *T. parva* CK II α subunit, we have also detected casein kinase II activity in lysates of schizonts purified from *T. parva*-infected bovine lymphocytes. However, we have not shown that the casein kinase II gene encodes the enzyme whose activity we have detected. It is known that CK II aggregates to form insoluble, linear filaments in buffers of low ionic strength (Glover, 1986). Although the buffers used to purify schizonts were of sufficiently high ionic strength (Sugimoto et al., 1988) to prevent such precipitation and subsequent copurification of bovine CK II with the schizonts, this possibility has not been entirely ruled out.

In conclusion, the structure of the α subunit of casein kinase II from *T. parva* adds information to recent observations showing that CK II is extremely well conserved among highly divergent species. Differences in molecular masses between the α and the α' subunits of CK II are attributable to C-terminal extensions in the case of vertebrates (Lozeman et al., 1990; Meisner et al., 1989; Maridor et al., 1991) or an internal insertion in the case of yeast (Chen-Wu et al., 1988; Padmanabha et al., 1990). The CK II α subunit of *T. parva*, reported here, has a large N-terminal extension.

Evidence has been presented by others to show that CK II is activated upon hormonal or growth factor stimulation of various cell types (Sommercorn & Krebs, 1987; Sommercorn et al., 1987; Klarlund & Czech, 1988; Krebs et al., 1988; Carroll & Marshak, 1988; Ackerman & Osheroff, 1989). Additionally, increased CK II activity has been observed in rapidly proliferating cells such as human leukemic and virally-transformed cell lines [reviewed by Pinna (1990) and Tuazon and Traugh (1991)]. In this study, we have not established a connection between the *T. parva* CK II α and *Theileria*-induced lymphocyte transformation. However, *T. parva* schizonts are completely exposed to the host cell's cytoplasm. They may therefore induce cellular transformation by releasing effector molecules into the lymphocyte, which then activate pathways for cellular proliferation. Such molecules may also be exposed on the schizont's plasma membrane. In either of these cases, an essential feature of such parasite molecules would be their capacity to effectively interact with, and modulate, host substrates, which implies interesting structural as well as functional conservation of the molecules from both species. The *T. parva* CK II α subunit has a high degree of identity with the catalytic subunits of CK II from higher eukaryotes and has structural features which accord with those of molecules targeted for translocation to the nucleus as well as for secretion.

Acknowledgments

We are grateful to Dr. Claiborne V. C. Glover (University of Georgia, Athens, GA) for *Drosophila* cDNA clones and for his interest, helpful discussions, and provision of manuscripts prior to publication, to Dr. Michael E. and Grace K. Dahmus (University of California, Davis, CA) and to Drs. Daniel R. Marshak and Il Je Yu (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY) for their helpful discussions and for provision of copies of manuscripts prior to publication. We acknowledge Dr. Paul T. Englund and our colleagues at ILRAD, R. P. Bishop, W. R. Fish, J. D. Lonsdale-Eccles, N. B. Murphy, and D. Zilberstein, for critical comments on the manuscript and Mrs. L. W. Thairo for typing the manuscript.

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