Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 8434–8438, September 1992
Plant Biology

Casein kinase II protein kinase is bound to lamina–matrix and phosphorylates lamin-like protein in isolated pea nuclei

(lamin A/nuclear envelope/p34^{cdc2}/*Pisum*)

HAIMIN LI AND STANLEY J. ROUX*

Department of Botany, University of Texas, Austin, TX 78713

Communicated by Eldon H. Newcomb, June 11, 1992

ABSTRACT **A casein kinase II (CK II)-like protein kinase was identified and partially isolated from a purified envelope–matrix fraction of pea (*Pisum sativum* L.) nuclei. When [γ-^{32}P]ATP was directly added to the envelope–matrix preparation, the three most heavily labeled protein bands had molecular masses near 71, 48, and 46 kDa. Protein kinases were removed from the preparation by sequential extraction with Triton X-100, EGTA, 0.3 M NaCl, and a pH 10.5 buffer, but an active kinase still remained bound to the remaining lamina–matrix fraction after these treatments. This kinase had properties resembling CK II kinases previously characterized from animal and plant sources: it preferred casein as an artificial substrate, could use GTP as efficiently as ATP as the phosphoryl donor, was stimulated by spermine, was calcium independent, and had a catalytic subunit of 36 kDa. Some animal and plant CK II kinases have regulatory subunits near 29 kDa, and a lamina–matrix-bound protein of this molecular mass was recognized on immunoblot by anti-*Drosophila* CK II polyclonal antibodies. Also found associated with the envelope–matrix fraction of pea nuclei were p34^{cdc2}-like and Ca^{2+}-dependent protein kinases, but their properties could not account for the protein kinase activity bound to the lamina. The 71-kDa substrate of the CK II-like kinase was lamin A-like, both in its molecular mass and in its cross-reactivity with anti-intermediate filament antibodies. Lamin phosphorylation is considered a crucial early step in the entry of cells into mitosis, so lamina-bound CK II kinases may be important control points for cellular proliferation.**

The nuclear lamina consists of a meshwork of proteins that underlies the nuclear envelope. The lamina is thought to play a role in nuclear stability, chromatin organization, and transcription (1, 2). Nuclear envelope breakdown is a common feature of mitosis and meiosis (3). It involves disassembly of the nuclear membrane (4) and the nuclear lamina (5, 6). Nuclear lamina depolymerization is preceded by and is thought to require an increase in the phosphorylation level of the lamins, a class of intermediate filaments that comprise the predominant proteins in the lamina layer and appear to be localized mainly in this layer (7, 8). The proposed central role of lamin phosphorylation in the initiation of mitosis has generated increased interest in the kinases that control this step. Based on mainly indirect data, several different kinases have been proposed to be involved in lamin phosphorylation, including p34^{cdc2} (9), protein kinase C (10), and S6 kinase II (11).

Dessev *et al.* (12) partially characterized protein kinase activity tightly bound to the lamina-enriched fraction of Ehrlich ascites tumor cell nuclei and suggested that several different kinases might account for this activity, including at least one that was Ca^{2+} and cAMP independent. Among the best characterized Ca^{2+}- and cAMP-independent protein kinases are the casein kinase II (CK II) kinases, which share conserved properties among all eukaryotic organisms examined (13, 14). Members of this family are located in the cell nucleus as well as in the cytosol, and they phosphorylate a broad spectrum of nuclear and cytosolic proteins (13). CK II kinases play a central role in the transition between prophase and metaphase during meiotic cell division of full-grown *Xenopus laevis* oocytes (15). In plant cells a CK II-type kinase has been implicated in the phosphorylation and DNA-binding activity of a trans-acting factor that binds to the promoter of light-regulated genes (16, 17). Although there is evidence that CK II kinases are predominantly nuclear enzymes (18), there is little information on the presence or function of CK II kinases in the nuclear lamina. Here we report that a CK II-like protein kinase is tightly bound to a lamina-enriched fraction of isolated pea nuclei and phosphorylates a lamin-like protein in this fraction.

MATERIALS AND METHODS

Nuclei isolation and fractionation experiments were carried out at 4°C except where noted. Nuclei were isolated from the plumules of 7-day-old dark-grown pea (*Pisum sativum* var. Alaska) seedlings by the method of Datta *et al.* (19). A nuclear envelope–matrix (E-M) fraction was prepared from purified pea nuclei by the method of Kaufmann *et al.* (20) with some modification. Briefly, 3–5 × 10^8 nuclei were suspended in 5 ml of buffer A (10 mM Tris·HCl, pH 7.5/0.2 mM $MgCl_2$/1 M sucrose/0.5 mM phenylmethylsulfonyl fluoride/0.5% aprotinin) and incubated with DNase I at 250 μg/ml and boiled RNase A at 250 μg/ml for 1 h at 10°C. The nuclei were centrifuged at 880 × *g* for 20 min, and the nuclease digestion step was repeated one more time. The digested nuclei were then washed with buffer A and resuspended in 5 ml of buffer A. To the suspension was added NaCl (final concentration = 1.6 M) and 2-mercaptoethanol [final concentration = 1% (vol/vol)], and then it was incubated for 15 min with gentle agitation and centrifuged 30 min at 1600 × *g*. The extraction with 1.6 M NaCl was performed as above one more time, except that 2-mercaptoethanol was omitted. The pellet was resuspended in buffer B [10 mM Tris·HCl, pH 7.5/0.2 mM $MgCl_2$/10% (wt/vol) sucrose/0.5 mM phenylmethylsulfonyl fluoride/0.5% aprotinin], and then the above digestion and NaCl extraction steps were repeated once. The resultant preparation was considered to be a purified nuclear E-M fraction of pea nuclei. Note that Kaufmann *et al.* (20) and other authors refer to this fraction simply as nuclear envelopes. However, as discussed below, many "envelope" fractions prepared from plant and animal cells by this method retain in their lumen matrix proteins undigested by the DNase and RNase treatments. Here we are acknowledging the

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CK II, casein kinase II; E-M, envelope–matrix; L-M, lamina–matrix.

*To whom reprint requests should be addressed.

presence of matrix material in pea envelope preparations by referring to them as E-M preparations.

The E-M fraction was used either as freshly prepared or stored in buffer C (30 mM Tris·HCl, pH 7.5/5 mM $MgCl_2$/10 mM dithiothreitol/50% glycerol) at −80°C. In some cases before this fraction was isolated, the nuclei were labeled with ^{32}P by suspending them in kinase reaction buffer (19) plus 100 μM [γ-^{32}P]ATP (850 cpm/pmol) or GTP (350 cpm/pmol), incubating them at room temperature (≈21°C) for 15 min, and then washing them with buffer A. The E-M fraction was then isolated from the labeled nuclei as described above except that 5 mM NaF and 0.2 mM ammonium molybdate were included in buffer A and buffer B.

To prepare a nuclear lamina–matrix (L-M) fraction, the E-M fraction was washed and resuspended in 5 ml of buffer B, to which 0.5 ml of 20% (wt/vol) Triton X-100 (prepared in buffer B) was added. The solution was gently agitated for 15 min on ice and centrifuged at 880 × *g* for 15 min. The pellet from this centrifugation is referred to below as the nuclear L-M preparation. For long-term storage, this preparation was washed two times with buffer B, suspended in buffer B, and stored at −25°C.

To purify lamin proteins, the L-M preparation was resuspended in buffer D (10 mM Tris·HCl, pH 7.5/1 mM EDTA/0.5 mM phenylmethylsulfonyl fluoride/0.5% aprotinin), and then urea was added to a final concentration of 6 M. The solution was incubated at room temperature for 10 min and centrifuged at 12,000 × *g* for 30 min. The supernatant was dialyzed against buffer D containing 0.5 M NaCl overnight and then centrifuged at 12,000 × *g* for 30 min. After the pellet was washed twice with buffer D containing 0.5 M NaCl, it was referred to as the lamin protein preparation.

To extract protein from the L-M, three different extraction media were used: EGTA, EGTA plus 0.3 M NaCl, and Tris base plus 0.3 M NaCl, using the following procedures. The L-M preparation was resuspended in buffer B, to which 2 mM EGTA (pH 7.5) was added. The solution was incubated on ice for 30 min with gentle agitation and centrifuged at 880 × *g* for 15 min. The resultant supernatant is referred to below as the EGTA-extracted protein preparation. The pellet was washed with 10 mM EGTA and 0.3 M NaCl in buffer B. After centrifugation, the supernatant is referred to below as the EGTA/salt-extracted protein preparation. The pellet was washed two times with buffer B and resuspended in a high pH solution (100 mM Tris base, pH 10.5/0.3 M NaCl), incubated on ice for 30 min with gentle agitation, and centrifuged as above. The supernatant obtained is referred to below as the high pH-extracted protein preparation. All the supernatants were concentrated by using an Amicon Centricon-10 concentrator. Protein kinase activity was assayed both in the supernatant and in the pellet fractions after each extraction.

Protein kinase activity was assayed by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into casein (a mixture of α- and β-casein from Sigma) or into the synthetic decapeptide RRREEETEEE, purchased from Peninsula Laboratories. The assay was carried out at room temperature as described by Roskoski (21). In some cases the reaction mixture was boiled in SDS/PAGE sample buffer and subjected to SDS/PAGE (22); then the gel was stained, destained, dried, and autoradiographed using Kodak AR film, as described by Li *et al.* (23). All autoradiographs were developed overnight using an intensifying screen.

To assay the autophosphorylation of E-M and L-M, these preparations were suspended in reaction buffer (25 mM Tris·HCl, pH 8.0/10 mM $MgCl_2$/5 mM NaF/0.2 mM ammonium molybdate/100 μM [γ-^{32}P]ATP) and incubated at room temperature for 15 min. The reaction mixture was brought to 6 M urea/2.5% SDS/50 mM iodoacetamide, added to SDS/PAGE sample buffer, briefly heated to 80°C, and then subjected to SDS/PAGE (10% gel) and autoradiography as above. In some experiments lamins were purified from labeled L-M and then were solubilized in 6 M urea/2.5% SDS/50 mM iodoacetamide and analyzed by SDS/PAGE and autoradiography.

The procedure for detecting protein kinase activity on gels after SDS/PAGE was carried out as described (23), except that both histone III-S at 1 mg/ml and casein at 1 mg/ml were included in the separating gel before polymerization.

Western blot analyses were carried out as described by Li *et al.* (23). The antibodies used for these analyses included anti-*Drosophila* CK II kinase polyclonal antibody (24), kindly provided by Claiborne Glover III (University of Georgia); anti-human $p34^{cdc2}$ monoclonal antibody directed to the PSTAIR hexapeptide sequence, purchased from Upstate Biotechnology (Lake Placid, NY); and anti-human intermediate filament monoclonal antibody (25), obtained from cultured hybridoma cells (ATCC no. TIB 131) purchased from the American Type Culture Collection. These antibodies, all raised to animal antigens, were used to test the antigenic similarity of pea L-M proteins to animal L-M proteins. For those blot analyses testing the anti-intermediate filament antibodies, Tween 20 was used instead of nonfat dry milk as the blocking agent, as described by La Claire (26).

For transmission electron microscopy, nuclear E-M preparations were resuspended in CM buffer (50 mM sodium cacodylate, pH 7.4/5 mM $MgCl_2$), and glutaraldehyde was added to 1%. After 20 min, the E-M preparation was sedimented at 880 × *g* for 10 min, resuspended in CM buffer with 1% glutaraldehyde, washed four times with 1% glutaraldehyde in CM buffer, suspended in 2% osmium tetroxide, and left at room temperature for 2 h. The samples were subsequently washed in distilled water and stained for 12 h at 4°C in 1% uranyl acetate, which was prepared in maleate buffer (50 mM, pH 5.6). The tissue was then washed in maleate buffer, dehydrated in a graded ethanol series, transferred to absolute acetone, and embedded in Spurr's low viscosity resin (27). Thin sections were cut by using a diamond knife, poststained with 5% uranyl acetate and lead citrate (28), and examined in a Hitachi HU11E electron microscope.

To negatively stain the lamin protein preparation, samples purified as described above were resuspended and gently agitated in three drops of H_2O; then about 40 μl of the suspension was placed on a 300-mesh Formvar-coated copper grid. After removing excess water from the suspension by touching it to filter paper, the specimen (now attached to the Formvar) was washed with five drops of water, three drops of 0.25% Triton X-100, and again with five drops of water and then stained by one drop of 1.5% uranyl acetate with addition of bacitracin (0.1 mg/ml) as a spreading agent. Stained specimens were examined and photographed in a Philips 420 electron microscope.

RESULTS

As described by Datta *et al.* (19) and by Li *et al.* (23), the nuclear preparation used as the starting material for the E-M purification was highly pure and essentially free of plastid, mitochondrial, and bacterial contamination. By electron microscopic analysis, the envelopes in the E-M preparation were more electron transparent than the nuclei from which they were derived, but otherwise the two structures were remarkably similar in appearance, both in their diameter (5–6 μm) and in their content of nucleolar-like structures (compare Fig. 1*A* with Fig. 1*B*). However, histochemically, the E-M preparations did not stain positive for DNA or RNA, and by chemical analysis they contained <0.5% of the original nuclear content of RNA and DNA (data not shown). Negatively stained preparations of purified lamin protein were highly enriched in filamentous structures that ranged in diameter from 6 to 12 nm (Fig. 1*C*).

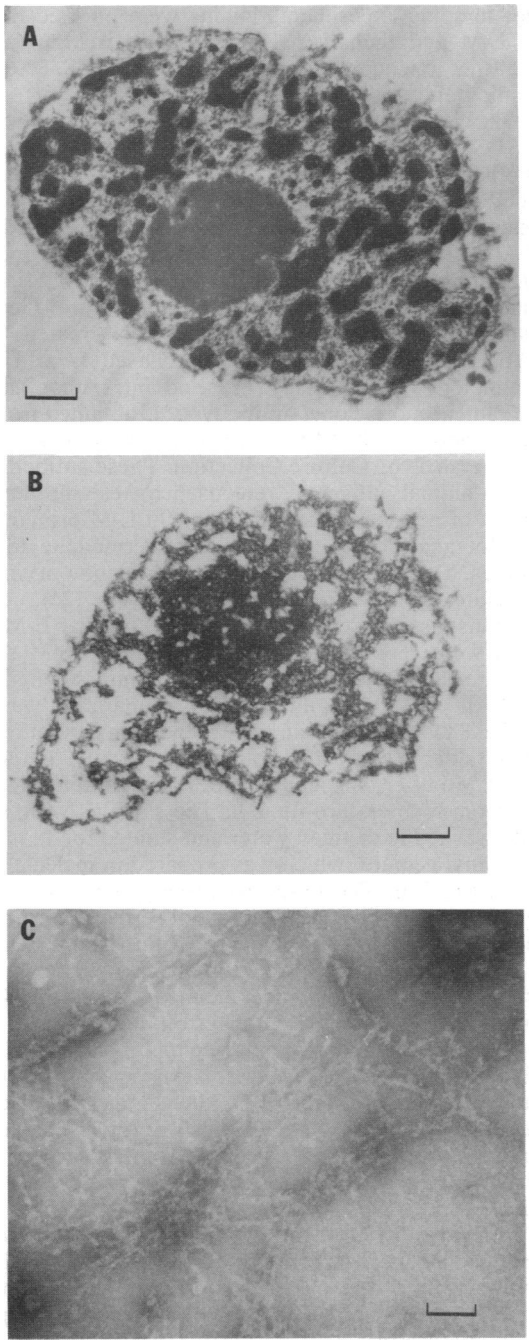

FIG. 1. Transmission electron microscopy of purified pea nuclei (*A*), purified E-M fraction (*B*), and negatively stained pea lamin preparation (*C*). (*A* and *B*, bar = 1.0 μm; *C*, bar = 0.1 μm.)

When the E-M, L-M, and lamin preparations were assayed by SDS/PAGE and Coomassie blue staining, many protein bands were apparent (Fig. 2, lanes A and B). The three major stained bands in the lamin preparation had apparent molecular masses of 71, 67, and 60 kDa (Fig. 2, lane C), similar to those of the lamins in animal nuclei (8). Anti-human intermediate filament antibody recognized the 71- and 60-kDa protein bands (Fig. 2, lane E).

Both the pea nuclear E-M and L-M preparations contain high levels of protein kinase activity and strongly prefer casein over histone as an artificial substrate (Table 1). When [γ-^{32}P]ATP was directly added to the preparations, the same four protein bands were the most heavily labeled in the E-M (Fig. 3*A*) and in the L-M (Fig. 3*B*) preparations. Their molecular masses were 92 kDa, 71 kDa, and an incompletely

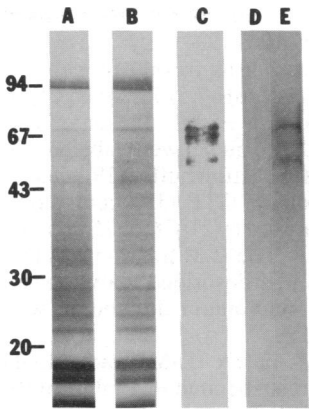

FIG. 2. Coomassie blue stain (lanes A–C) and immunoblots (lanes D and E) of the protein bands separated on SDS/10% PAGE gels from nuclear E-M preparation (lane A), L-M preparations (lanes B, D, and E), and lamin preparations of proteins (lane C). The primary antibodies used for the immunoblots of the lamina proteins were nonimmune serum (lane D) and the anti-mouse intermediate filament monoclonal antibody TIB 131 (lane E).

resolved doublet near 48 and 46 kDa. When lamin proteins were purified from L-M that had been labeled with ^{32}P, the same labeled bands copurified with the lamins, although in this preparation only the 46-kD band of the doublet appears to be present (Fig. 3*C*). The kinase activity that is associated with the E-M and tightly bound to the L-M can use GTP as efficiently as ATP as the phosphate donor (Fig. 3 *A–C*, lanes 1 and 2).

When purified, intact nuclei were incubated with [γ-^{32}P]ATP and GTP, the E-M isolated from these nuclei, in contrast to E-M directly reacted with labeled ATP or GTP, showed no labeled lamin-like band near 71 kDa. The only band clearly phosphorylated had an apparent molecular mass near 92 kDa (Fig. 3*D*). This phosphorylated protein was still significantly labeled in the E-M preparation (Fig. 3*A*) but was relatively diminished in the L-M (Fig. 3*B*) and lamin (Fig. 3*C*) preparations.

Certain treatments allowed various kinase activities to be extracted from the L-M preparation. The EGTA-extracted protein preparation had no detectable protein kinase activity when either histone or casein was tested as an artificial substrate (data not shown). Both the EGTA/salt-extracted protein preparation and the high pH-extracted protein preparation had significant activity, as did the remaining L-M preparation. When tested on an activity gel, all of them appeared to have a 36-kDa catalytic subunit (Fig. 4*A*). The EGTA/salt-extracted and the L-M-bound fractions contained a 29-kDa protein that could be recognized by anti-*Drosophila* CK II polyclonal antibodies, which have been shown to bind to the 29-kDa regulatory subunit of CK II kinases (24) (Fig. 4*B*).

When a monoclonal antibody directed to the PSTAIR sequence of p34^{cdc2} protein kinase was used to test the various L-M fractions in a Western blot analysis, the results indicated that this kinase is present in pea lamina and that some of it could be washed out both by the EGTA and the EGTA/salt extraction treatments (Fig. 4). The same 34-kDa band recognized by the anti-p34 antibody also bound to the anti-*Drosophila* CK II antibody, probably because both share similar sequences (29). However, the anti-PSTAIR antibody did not recognize the 29-kDa putative CK II subunit protein.

Table 1. Comparison of the protein kinase activity of the LM-bound CK II kinase using different artificial substrates and ATP and GTP as phosphoryl donors

Substrate	Specific activity, pmol·min^{-1}·mg^{-1}	
	ATP	GTP
Casein	185	173
Histone	87	80
CK II substrate peptide (RRREEETEEE)	75	72

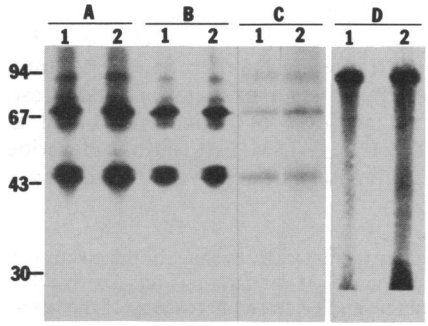

FIG. 3. Autoradiograph of SDS/PAGE pattern of ^{32}P-labeled proteins from nuclear E-M fraction (*A*), nuclear L-M fraction (*B*), nuclear lamin (*C*), and nuclear E-M fraction isolated from ^{32}P-labeled pea nuclei (*D*). Lane 1, labeled by [γ-^{32}P]ATP; lane 2, labeled by [γ-^{32}P]GTP.

When casein was used as the artificial substrate, the EGTA/salt- and high pH-extracted protein preparations and the kinase activity that remained bound to the L-M after the extractions all had properties similar to the kinase activity in the lamina before the washes (Fig. 5). All of these preparations could also label the decapeptide RRREEETEEE, considered specific for CK II kinases (14, 30) (Table 1). They all could use ATP and GTP as phosphoryl donors (Fig. 5, lanes 1 and 2), and all were stimulated by spermine (Fig. 5, lanes 3). None of them appeared to be inhibited significantly by heparin (Fig. 5, lanes 4).

DISCUSSION

Dessev *et al.* (12) were the first to describe a protein kinase that was tightly bound to rat liver nuclear lamina. They characterized this kinase as a serine/threonine kinase that was cAMP independent, but they were unable to dissociate it from the lamina without denaturing it. The L-M-bound protein kinase in pea nuclei resembles the one characterized by Dessev *et al.* (12) in that it is able to phosphorylate threonine (in a synthetic peptide) and is cAMP independent. However, we have been able to characterize the pea kinase further and show that it is a CK II kinase by several standard criteria: it prefers casein to histone as an artificial substrate (Table 1); it can phosphorylate the decapeptide RRREEE-

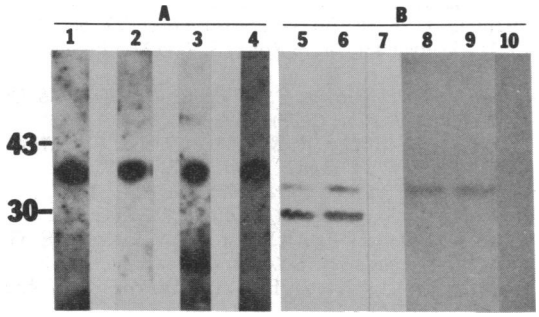

FIG. 4. (*A*) Detection of casein kinase activity in gels after SDS/PAGE and sample renaturation. (*B*) Immunoblots of protein samples, using the following as the first antibody: anti-*Drosophila* CK II antibody (lanes 5 and 6), rabbit nonimmune serum (lane 7), anti-p34^{cdc2} monoclonal antibody (lanes 8 and 9), and mouse nonimmune serum (lane 10). The protein samples separated on SDS/PAGE were nuclear L-M fraction before any extraction steps (lane 1; 30 μg of protein loaded), fraction extracted from lamina by 10 mM EGTA plus 0.3 M NaCl (lanes 2, 5, 7, 8, 10; 4 μg of protein loaded per lane), fraction extracted from L-M by pH 10 buffer (lane 3; 3 μg of protein loaded), and protein remaining associated with L-M after all extraction steps (lanes 4, 6, and 9; 25 μg of protein loaded per lane).

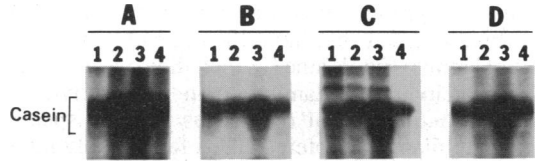

FIG. 5. Level of casein phosphorylating activity of CK II kinase present in various fractions of L-M proteins. The activity was detected by autoradiography of labeled casein substrate after 15 μg of the casein (per lane) was electrophoresed on SDS/PAGE gels. The L-M fractions tested for activity were L-M ($\approx$4 μg) before any extraction steps (*A*), fraction extracted from L-M by 10 mM EGTA plus 0.3 M NaCl ($\approx$0.5 μg) (*B*), fraction extracted by pH 10 buffer ($\approx$0.3 μg) (*C*), and protein still associated with L-M after all extraction steps ($\approx$3 μg) (*D*). Lane 1, labeled with [γ-^{32}P]GTP; lanes 2, 3, and 4, labeled with [γ-^{32}P]ATP; lane 3, 2 mM spermine present during labeling; lane 4, heparin at 10 μg/ml present during labeling. The amounts in parentheses in the legend represent the approximate amount (in μg of total protein) of each fraction used as the CK II kinase source for reaction with the 15 μg of casein per assay.

TEEE, considered specific for CK II kinases (30); it can use GTP as efficiently as ATP as the phosphoryl donor; and it is stimulated by spermine. It also has a catalytic subunit of 36 kDa, similar to that observed for many other CK II kinases.

Most CK II kinases in animals and plants are heteromeric and have a noncatalytic (β) subunit between 25 and 29 kDa. For example, Li and Roux (14) recently purified a CK II kinase from pea nuclei that has a regulatory subunit of 29 kDa. This kinase resembles the L-M-associated one described here in that it has a catalytic subunit of 36 kDa, but it differs in that it is associated with and easily solubilized from a chromatin-enriched fraction of the pea nuclei. Only the α (36 kDa) subunit of the chromatin-associated CK II kinase reacts with the anti-*Drosophila* CK II antibody (14), so it differs antigenically from the lamina-bound CK II kinase described here. Whether these two differently localized CK II kinases are two forms of the same heteromeric protein remains to be seen. Nonetheless, because a known pea CK II kinase has a 29-kDa subunit, the fact that the pea L-M fraction contains a tightly bound 29-kDa peptide recognized by an anti-*Drosophila* CK II antibody (24) is consistent with the conclusion that there is a CK II kinase in the L-M.

The one property of the L-M-bound kinase that is not characteristic of CK II kinases is that it is insensitive to heparin. However, this may or may not reflect an intrinsic property, because its tight associations with lamins (in the lamina) or with other proteins (when solubilized) may have biased these results.

Although the buffered 1.6 M NaCl solutions used to prepare the nuclear E-M fraction could not free the pea CK II kinase from its bound state, a buffer containing only 0.3 M NaCl plus 2 mM EGTA did dissociate significant levels of this kinase from the L-M. Presumably this effect is due to the calcium-chelating activity of EGTA, which would lower the calcium concentration from its contaminant level in the buffer of near 10 μM to below 0.1 μM. If so, then one can speculate that a submicromolar Ca^{2+} environment permits the conversion of some fraction of the tightly bound population of lamina CK II kinase to a form that can be dissociated with 0.3 M salt.

One of the proteins in the L-M that is phosphorylated by the CK II-like kinase is a 71-kDa protein that can be extracted by a procedure standardly used to purify lamins. This procedure takes advantage of a characteristic intermediate filament property of being soluble in 6 M urea but then becoming insoluble when it is dialyzed out of the urea solution into one not containing urea (11). A similar procedure was used to reconstitute intermediate filaments extracted from carrot suspension culture cells (31). Using this method, we purified

from pea L-M proteins that assemble into intermediate filaments (Fig. 1*C*) and that have the characteristic molecular masses of lamins (Fig. 2, lane C), including one at 71 kDa. The 71-kDa protein is also lamin-like in its reactivity with a monoclonal antibody that recognizes a broad spectrum of intermediate filament proteins. Thus far the only intermediate filament protein near 71 kDa reported to be in nuclei is lamin A in vertebrate cells or its close relatives in invertebrates (6). Thus not only is there a CK II-like kinase tightly bound to the L-M fraction, but this kinase phosphorylates one of the main structural proteins of the lamina. Lamin-like proteins have been described in plant nuclei but are not well characterized. To our knowledge, this is the first report of a specific L-M-associated kinase phosphorylating a lamin-like protein in plant nuclei.

Because lamin phosphorylation is a critical event in the early stages of mitosis and meiosis, there is strong interest in identifying the kinases involved in this step. In animal cells there is better documentation for $p34^{cdc2}$ and S6 kinases phosphorylating lamins than there is for CK II kinases performing this function. However, there is evidence that a combination of kinases may participate in lamin phosphorylation. When Lüscher *et al.* (9) compared the lamin B2 phosphorylation pattern generated by $p34^{cdc2}$ *in vitro* with that which occurred *in vivo* during mitosis, they concluded that another kinase in addition to $p34^{cdc2}$ was probably involved in producing the *in vivo* pattern. If multiple kinases are involved in lamin phosphorylation in pea nuclei, our results indicate that a lamina-bound CK II kinase may be one of them.

Interestingly, the 46- to 48-kDa doublet that is labeled when [γ-^{32}P]GTP is incubated with pea L-M has nearly the same molecular mass as a nuclear envelope-associated 47-kDa NTPase in peas that is known to cross-react with anti-lamin antibodies (32). These antibodies also cross-react with a 46-kDa NTPase from rat liver nuclei, which is postulated to represent the N-terminal portion of lamins A and C (33). It is possible, then, that one of the substrate proteins near 47 kDa in the pea L-M fraction may itself be lamin-related.

There are at least two other protein kinases in the pea E-M fraction, a $p34^{cdc2}$-like kinase (Fig. 4) and a Ca^{2+}-dependent one (unpublished results). However the former cannot use GTP as a phosphoryl donor, and the latter requires Ca^{2+} for its activity. These two kinases, then, cannot account for the ^{32}P-labeling of proteins in pea E-M, because these substrates can be phosphorylated by a kinase that uses GTP as well as ATP and is Ca^{2+} independent. The p34 kinase released from L-M by EGTA was in an inactive state, which is not surprising given that this kinase is a key enzyme in cell division regulation and is active primarily during the earliest stages of mitosis or meiosis (34), when it phosphorylates lamins and promotes the breakdown of the nuclear envelope (35). The EGTA-solubilized p34-like kinase is not inactive due to denaturation, because we can restore its kinase activity by incubating it with an L-M-bound protein phosphatase (unpublished results).

All *in vitro* experiments are necessarily inconclusive in predicting native associations. Thus the failure of the lamin-like protein to be phosphorylated in intact nuclei could be interpreted to mean that the CK II-catalyzed phosphorylation pattern observed in purified L-M preparations is strictly an *in vitro* phenomenon. On the other hand, these results could mean that the intact nucleus, unlike the L-M, may contain factors that inhibit lamin phosphorylation and thus help maintain the nucleus in interphase. Because isolated nuclear envelopes have been used successfully to study such native functions as RNA export and protein import through nuclear pores (36), there is an expectation that these structures maintain a high level of native, functional associations. Whether the CK II–L-M associations described here are native will ultimately be resolved by immunocytochemical methods.

We especially thank Dr. K. Seshan for the electron microscopy of isolated nuclei and E-M preparations and Dr. K. Kudlicka for the negative staining and electron microscopy of the lamin preparation. We also thank Prof. D. Brown (Cell Research Institute) and R. M. Brown, Jr. and G. Cole (Department of Botany) for the use of their facilities. This work was supported by National Science Foundation Grant DCB-9106245.

1. Newport, J. W. & Forbes, D. J. (1987) *Annu. Rev. Biochem.* **56**, 535–565.
2. Nigg, E. A. (1988) *Int. Rev. Cytol.* **110**, 27–92.
3. Masui, Y. & Markert, C. L. (1971) *J. Exp. Zool.* **177**, 129–146.
4. Chai, L. S., Weinfeld, H. & Sandberg, A. A. (1974) *J. Natl. Cancer Inst.* **53**, 1033–1048.
5. Gerace, L. & Blobel, G. (1980) *Cell* **19**, 277–287.
6. Gerace, L. (1986) *Trends Biochem. Sci.* **11**, 443–446.
7. Miake-Lye, R. & Kirschner, M. W. (1985) *Cell* **41**, 165–175.
8. Suprynowicz, F. A. & Gerace, L. (1986) *J. Cell Biol.* **103**, 2073–2081.
9. Lüscher, B., Brizuela, L., Beach, D. & Eisenman, R. N. (1991) *EMBO J.* **10**, 865–875.
10. Fields, A. P., Pettit, G. R. & May, W. S. (1988) *J. Biol. Chem.* **263**, 8253–8260.
11. Ward, G. E. & Kirschner, M. W. (1990) *Cell* **61**, 561–577.
12. Dessev, G., Iovcheva, C., Tasheva, B. & Goldman, R. (1988) *Proc. Natl. Acad. Sci. USA* **87**, 7080–7084.
13. Pinna, L. A. (1990) *Biochim. Biophys. Acta* **1054**, 267–284.
14. Li, H. & Roux, S. J. (1992) *Plant Physiol.* **99**, 686–692.
15. Mulner-Lorillon, O., Marot, J., Cayla, X., Pouhle, R. & Belle, R. (1988) *Eur. J. Biochem.* **171**, 107–117.
16. Datta, N. & Cashmore, A. R. (1989) *Plant Cell* **1**, 1069–1077.
17. Klimczak, L. J., Schindler, U. & Cashmore, A. R. (1992) *Plant Cell* **4**, 87–98.
18. Krek, W., Maridor, G. & Nigg, E. A. (1992) *J. Cell Biol.* **116**, 43–55.
19. Datta, N., Chen, Y.-R. & Roux, S. J. (1985) *Biochem. Biophys. Res. Commun.* **128**, 1403–1408.
20. Kaufmann, S. K., Gibson, W. & Shaper, J. H. (1983) *J. Biol. Chem.* **258**, 2710–2719.
21. Roskoski, R. J. (1983) *Methods Enzymol.* **99**, 3–4.
22. Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685.
23. Li, H., Dauwalder, M. & Roux, S. J. (1991) *Plant Physiol.* **96**, 720–727.
24. Dahmus, G. K., Glover, C. V. C., Brutlag, D. L. & Dahmus, M. E. (1984) *J. Biol. Chem.* **259**, 9001–9006.
25. Pruss, R. M., Mirsky, R. & Raff, M. (1981) *Cell* **27**, 419–428.
26. La Claire, J. W., II (1989) *Planta* **177**, 47–57.
27. Spurr, A. R. (1969) *J. Ultrastruct. Res.* **26**, 31–43.
28. Reynolds, E. S. (1963) *J. Cell Biol.* **17**, 208–222.
29. Takio, K., Kuenzel, E. A., Walsh, K. A. & Krebs, E. G. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 4851–4855.
30. Kuenzel, E. A. & Krebs, E. G. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 737–741.
31. Hargreaves, A. J., Goodbody, K. C. & Lloyd, C. W. (1989) *Biochem. J.* **261**, 679–682.
32. Roux, S. J. (1992) *Photochem. Photobiol.*, in press.
33. Clawson, G. A., Lackey, A. & Tokes, Z. A. (1988) *Exp. Cell Res.* **176**, 180–186.
34. Nurse, P. (1990) *Nature (London)* **334**, 503–508.
35. Dessev, G. & Goldman, R. (1988) *Dev. Biol.* **130**, 543–550.
36. Nigg, E., Baeuerle, P. & Luhrmann, R. (1991) *Cell* **66**, 15–22.