Plant Physiol. (1993) 101: 1005–1011

The Major Nucleoside Triphosphatase in Pea (*Pisum sativum* L.) Nuclei and in Rat Liver Nuclei Share Common Epitopes Also Present in Nuclear Lamins[1]

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The major nucleoside triphosphatase (NTPase) activities in mammalian and pea (*Pisum sativum* L.) nuclei are associated with enzymes that are very similar both biochemically and immunochemically. The major NTPase from rat liver nuclei appears to be a 46-kD enzyme that represents the N-terminal portion of lamins A and C, two lamina proteins that apparently arise from the same gene by alternate splicing. Monoclonal antibody (MAb) G2, raised to human lamin C, both immunoprecipitates the major (47 kD) NTPase in pea nuclei and recognizes it in western blot analyses. A polyclonal antibody preparation raised to the 47-kD pea NTPase (pc480) reacts with the same lamin bands that are recognized by MAb G2 in mammalian nuclei. The pc480 antibodies also bind to the same lamin-like bands in pea nuclear envelope-matrix preparations that are recognized by G2 and three other MAbs known to bind to mammalian lamins. In immunofluorescence assays, pc480 and anti-lamin antibodies stain both cytoplasmic and nuclear antigens in plant cells, with slightly enhanced staining along the periphery of the nuclei. These results indicate that the pea and rat liver NTPases are structurally similar and that, in pea nuclei as in rat liver nuclei, the major NTPase is probably derived from a lamin precursor by proteolysis.

Purified pea (*Pisum sativum* L.) nuclei contain an NTPase activity that is photoreversibly controlled by the regulatory pigment, phytochrome; i.e. its activity can be stimulated by red light, and far-red light reverses this effect (Chen and Roux, 1986). Because the stimulation of this NTPase by red light can be blocked by EGTA, Roux (1987) has concluded that this enzyme is probably regulated by Ca^{2+} as well as by phytochrome. Fractionation of pea nuclei reveals that over 50% of their NTPase activity is associated with a 47-kD enzyme, which can be stimulated over 3-fold by Ca^{2+} through the mediation of calmodulin (Chen et al., 1987). Because of its Ca^{2+} sensitivity, this enzyme, which is as yet the only calmodulin-regulated nuclear enzyme that has been purified and characterized, is the most likely candidate for being the nuclear NTPase that is controlled by phytochrome.

As part of our effort to assess the role of Ca^{2+} in mediating phytochrome responses, we are attempting to determine the specific function of the 47-kD NTPase in pea nuclei. Included among several different approaches being utilized to investigate this question is a comparative biochemical one. Often, proteins with critical functions have highly conserved structures that are strikingly similar in different species throughout the animal and plant kingdoms. Here, we report that the 47-kD NTPase from pea nuclei is biochemically and immunologically similar to the major 46-kD NTPase from rat liver NS, which is derived by proteolysis from lamin A/C molecules in the NS (Clawson et al., 1988, 1990), and which is thought to function in the regulation of RNA export from nuclei (Clawson et al., 1984). Our results suggest that there are lamin-like proteins in pea nuclei and that the 47-kD pea NTPase, like the 46-kD rat liver NTPase, may be derived from lamin precursors through the action of NS-associated protease activity.

MATERIALS AND METHODS

Plant Growth

Seedlings of pea (*Pisum sativum* L. cv Alaska) were grown in the dark for 7 d at 22 ± 3°C.

Chemicals

a-ATP was purchased from ICN. SB substrates were obtained from Enzyme Systems Products (Livermore, CT). Phosphatase-labeled second antibodies and the phosphatase substrate system were purchased from Kirkegaard and Perry Labs (Gaithersburg, MD). ECL western blotting detection reagents with horseradish peroxidase-labeled secondary antibodies were purchased from Amersham. All buffers were prepared with water purified by a MilliQ water purification system (Millipore Corp.).

Primary Antibodies

The MAb's used included G2 and G13, both anti-human lamin C (Clawson et al., 1990); C23, an anti-chicken caldesmon directed to an epitope that contains a repeat sequence

[1] This work was supported by grants from the National Science Foundation (DCB-9106245) and the National Aeronautics and Space Administration (NAGW 1519) to S.J.R. and by U.S. Public Health Service grants CA 40145 and CA 21141 to G.A.C.

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Abbreviations: a-ATP, azido-[^{32}P]ATP; NS, nuclear scaffolding; NTPase, nucleoside triphosphatase; MAb, monoclonal antibody; SB, S-benzyl.

similar to one found in nuclear lamin A (Lin et al., 1991), kindly provided as a gift by Dr. J. Lin (University of Iowa); and TIB 131, obtained from cultured hybridoma cells (American Type Culture Collection No. TIB 131). TIB 131 recognizes an epitope common to all intermediate filaments (Pruss et al., 1981). All of the MAb's were used without purification, and all of them were in ascites fluid except TIB 131, which was used as a culture supernatant. Polyclonal anti-pea NTPase antibodies (pc480) were raised in guinea pigs to an NTPase preparation that was purified first through the HPLC DEAE anion-exchange column chromatography step (Chen et al., 1987), then on SDS-PAGE. The purified 47-kD NTPase band was excised from the gel after SDS-PAGE and sent to Pocono Farms (Canadensis, PA) to be used for inoculation. The pc480 immune serum was affinity-purified on protein A-agarose before use.

Nuclei, NS, and Nuclear Envelope-Matrix Preparations

Nuclei were isolated from the plumules of 7-d-old dark-grown pea seedlings by the method of Datta et al. (1985). A nuclear envelope-matrix fraction was prepared from purified pea nuclei by the method of Li and Roux (1992). Rat liver NS was prepared as described in Clawson et al. (1990).

Photolabeling and Immunoprecipitation of NTPases from Pea Nuclear Protein Preparation

Pea nuclear NTPase was purified through the DEAE column chromatography step as described by Chen et al. (1987). For photolabeling experiments, 5 μg of pea NTPase was photolabeled with a-ATP, specific activities of 6.4 to 9.5 Ci/mmol, at concentrations of 0.1 to 1.0 μM for 5 min at room temperature in buffer A (50 mM Tris-HCl, pH 7.6, 25 mM KCl, 5 mM $MgCl_2$), as described by Clawson et al. (1990). Photolabeled peptides from the NTPase preparation were then solubilized in 0.5% SDS with heating. The SDS was diluted with a 4-fold excess of Triton X-100, and the sample was incubated with 50 μg of antibody or preimmune serum, or with buffer (no-primary-antibody control) for 12 h at 4°C. The samples were centrifuged for 5 min at maximum speed in a Fisher microcentrifuge before adding the protein A-Sepharose. This centrifugation does not pellet immune complexes, but can remove any lamin complexes that may have spontaneously formed insoluble filaments during the incubation. This precautionary centrifugation did not pellet any labeled components. After incubation with protein A-Sepharose for 30 min at room temperature, the immune complexes were pelleted by centrifugation at 2000*g* for 5 min and rinsed extensively with buffer A. Bound complexes were dissociated by boiling in SDS-PAGE sample buffer and resolved by electrophoresis in 12% gels (Clawson et al., 1990). The gels were dried and autoradiography was performed with X-Omat-AR film (Kodak) at −80°C with intensifying screens.

Immunoblotting

Discontinuous SDS-PAGE was performed according to Laemmli (1970) using minislab gels containing 9% acrylamide. The electrophoretic transfer of the separated proteins to nitrocellulose, and the blocking and immunostaining of the blot (using alkaline phosphatase-labeled second antibody) were as described by Guo et al. (1990). In Figure 3, the immunostaining was carried out by the ECL western blotting detection reagents following instructions of the manufacturer (Amersham).

Assays of Nuclear Protease Activity

Colorimetric protease assays were conducted as previously described (Clawson et al., 1990) using SB substrates obtained from Enzyme Systems Products (Livermore, CT). These substrates included $AAPF_{SB}$, Y_{SB}, $FGAL_{SB}$, and VLK_{SB}. The SB substrates were added at 0.25 mM, dithiodipyridine was added to 1 mM, and reactions were for 5.5 h at 37°C. Standard assays contained 0.5 μg of DEAE-purified pea NTPase preparation or 25 μg of rat liver NS protein. For K_m determinations, $AAPF_{SB}$ was tested at 0.05, 0.1, 0.25, 0.5, 0.75, and 1 mM.

Isolation and Immunoblot Analysis of Proteins from Mammalian Nuclei

Nuclei were gradient purified from pig liver by the same procedure used to purify pea nuclei (Chen and Roux, 1986). After the nuclei were stripped of their envelopes by treatment with EDTA and Triton X-100 (Chen et al. 1987), they were treated with 0.5 M NaCl for 20 min, then centrifuged in a Fisher microcentrifuge at 13,600*g* for 5 min. The pellet (about 5 μg of total protein) was used as the sample analyzed in Figure 2A. A partially purified preparation of NS proteins from rat liver nuclei (about 2 μg) was used as the sample analyzed in Figure 2B.

Immunolocalization

Sectioned Materials

Pea plumule tissue was fixed in 3% formaldehyde in 70 mM Na-phosphate buffer, pH 7.4, for 20 min at room temperature. During fixation, the samples were sliced and evacuated to promote penetration of the fixative. Samples were washed in buffer, dehydrated in ethanol, cleared in a graded series of ethanol and xylene ending in 100% xylene, then embedded in paraffin wax by standard procedures. Cut sections (approximately 5–7 μm thick) were attached to glass slides, deparaffinized, hydrated, treated with blocking buffer (Na phosphate, pH 7.5, 0.1 M NaCl, 3% nonfat dry milk [Carnation]) for 1 h, then incubated overnight at 4°C with pc480 antibodies, protein A purified preimmune serum, G13, or TIB 131 antibodies. All antibodies were used at a 1:20 dilution except G13, which was used at a 1:100 dilution. After several washes with blocking buffer, the samples were incubated with rabbit anti-guinea pig or anti-mouse immunoglobulin G tetramethylrhodamine isothionate conjugate secondary antibody (Sigma) at a 1:75 dilution for 1 h and washed several times. Stained samples were mounted in glycerol and analyzed on a Zeiss microscope equipped for epifluorescence, with the filter system appropriate for rhodamine fluorescence.

Isolated Cell Preparations

Plumules were fixed in 2% formaldehyde buffered in 0.1 M Na phosphate, then washed briefly in distilled water. Cells were gently teased apart from the tissue by making small cuts with a scalpel in a droplet of distilled water on slides that had been treated with poly-L-Lys. Often, broken cells release intact nuclei during this process. These cells and nuclei released by them were then treated with blocker (2% BSA in PBS) and stained by the same sequence of primary antibody, wash, secondary antibody as described above.

RESULTS

Nuclei and Nuclear Envelope-Matrix Preparations

The pea nuclear preparation used as the starting material for these experiments was examined by electron microscopic analysis and found to be highly pure and essentially free of plastid, mitochondrial, and bacterial contamination (Datta et al., 1985; see also Li et al., 1991; Li and Roux, 1992). The nuclear envelope-matrix preparation used was essentially identical to that characterized by Li and Roux (1992) and had the following properties. The envelope-matrix vesicles contained the double-nuclear membrane and were enriched with intermediate filaments characteristic of the lamina layer described in animal nuclei and also present in plant nuclear matrices (Bevan et al., 1991; Frederick et al., 1992; Li and Roux, 1992; McNulty and Saunders, 1992). By histochemical analysis, the preparation did not stain positive for DNA or RNA. However, the envelopes retained in their lumen matrix proteins undigested by the DNase and RNase treatments.

Immunoprecipitation of a-ATP-Labeled Peptides from a Partially Purified Preparation of Pea NTPase

The MAb G2 immunoprecipitates from rat NS preparations a 46-kD protein photolabeled with a-ATP (Clawson et al., 1990). Similarly, G2 also immunoprecipitates an a-ATP-labeled 47-kD protein from a preparation of pea NTPase purified through the DEAE chromatography step (Chen et al., 1987) under conditions where a no-primary-antibody control did not contain labeled protein (Fig. 1A). The labeled material at the top of lane 1 in Figure 1A represents protein that did not enter the running gel. The photolabeled 47-kD protein can also be immunoprecipitated by pc480, a polyclonal antibody raised in guinea pigs to the 47-kD pea NTPase (lane 2 of Fig. 1B), and preimmune controls show approximately 5-fold less label associated with the protein A pellet than obtained with G2 and pc480 (lane 3 of Fig. 1B). The autoradiogram in Figure 1B was overdeveloped to demonstrate the bands near 29 and 19 kD, so the top of lanes 1 and 2 is obscured by label trapped at the top of the gel, but a band near 60 kD (the molecular mass of lamin C) can be detected above the background in both lanes. The levels of 29- and 19-kD immunoreactive peptides vary from preparation to preparation and may reflect different levels of proteolysis of the 47-kD NTPase, although their appearance is not affected by a cocktail of protease inhibitors including leupeptin, aprotinin, benzamidine, PMSF, and pepstatin (Clawson et al., 1988) (data not shown).

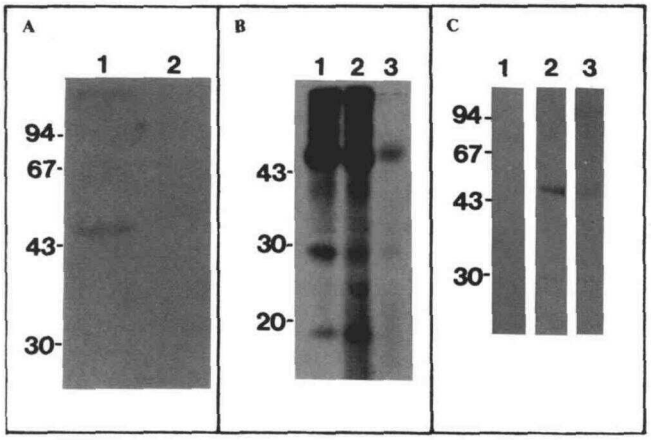

Figure 1. Anti-lamin antibody immunoprecipitates (A and B) and immunostains (C) the pea nuclear NTPase. A and B, Autoradiograms of immunoprecipitated proteins from two different preparations of DEAE-purified pea NTPase that had been photolabeled with a-ATP. A, Photolabeled pea nuclear protein immunoprecipitated with MAb G2 (lane 1). Lane 2 shows no-first-antibody control. B, Photolabeled pea nuclear peptides immunoprecipitated with MAb G2 (lane 1), pc480 (lane 2), or preimmune immunoglobulin G (lane 3). The same amount of NTPase preparation (about 5 μg) was used for each photolabeling/immunoprecipitation experiment. C, Western blot of DEAE-purified pea NTPase reacted with lane 1, preimmune serum; lane 2, pc480; lane 3, MAb G2. A total of about 6 μg of protein of the NTPase preparation was loaded onto a 2-cm wide lane of a minigel, resolved by SDS-PAGE, and electroblotted onto nitrocellulose. The nitrocellulose was divided into three equal strips, which were probed with different first antibodies as noted. The immunostain was developed chromogenically with phosphatase-labeled second antibodies.

Western Blot Analysis of DEAE-Purified NTPase from Pea Nuclei

After its electrophoresis on an SDS polyacrylamide gel and transfer by electroblot to nitrocellulose, a pea NTPase preparation purified through the DEAE column chromatography step (Chen et al., 1987) had two immunostainable bands, one at 47 kD and one at 29 kD. These bands bound both pc480 and G2 (Fig. 1C). Neither band was immunostained with preimmune sera (Fig. 1C). When crude preparations of nuclear proteins were assayed by pc480 in a western blot analysis, the same two bands at 47 and 29 kD were the only ones immunostained (data not shown). In addition to the bands at 47 and 29 kD, G2 (but not pc480) weakly stains bands near 56 and 92 kD (Fig. 1C).

Western Blot Analysis of Proteins Extracted from Mammalian Nuclei

Both pc480 and G2 immunostain three proteins from pig liver NS that migrate at the same molecular mass as lamins A (72 kD), B (68 kD), and C (60 kD) under conditions in which preimmune serum shows little or no staining (Fig. 2A). The pc480 antibodies also faintly stain a band near 46 kD. The immunostaining pattern differs significantly from the protein-staining pattern of pig nuclei. When proteins from rat NS are analyzed, both pc480 and G13 immunostain

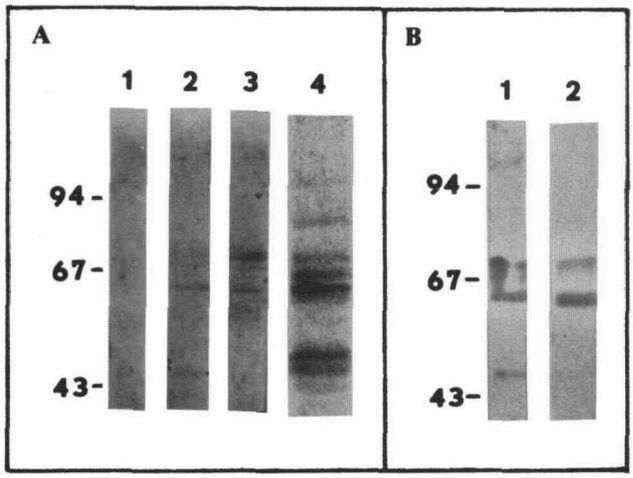

Figure 2. A, Lanes 1–3, Western blot analysis of pig NS proteins remaining with the NS after its extraction with 0.5 M NaCl. Primary antibodies used for the immunostain were: lane 1, preimmune serum; lane 2, pc480; lane 3, G2. Lane 4 is the Coomassie blue staining pattern of pig NS proteins used for the immunoblot analyses shown in lanes 1–3. B, Western blot analysis of partially purified rat liver NS proteins. Primary antibodies used for the immunostain were: lane 1, pc480; lane 2, G13. Staining with preimmune serum for pc480 gave the same results as shown in panel A, lane 1.

proteins near 72, 68, and 60 kD; and pc480 but not G13 strongly stains a band near 46 kD (Fig. 2B).

Western Blot Analysis of Proteins Associated with Purified Pea Nuclear Envelope-Matrix Fraction

Highly purified pea nuclear envelope-matrix preparations contain proteins near 71 and 60 kD that are immunostained by pc480 and by four different MAb's known to bind to mammalian lamins, G2, G13, TIB 131, and C23 (Fig. 3). In addition, pc480, but none of the MAb's, immunostains bands at 47, 29, and 19 kD.

Immunofluorescence Assay of Pea Nuclei with pc480 and Anti-Lamin Antibodies

The MAb's G13 and TIB 131 and pc480 all show similar immunofluorescence staining patterns in pea cells (compare Fig. 4, G and H, both stained by pc480, with Fig. 4J, stained by TIB 131, and Fig. 4K, stained by G13). All MAb's stain both cytoplasmic and nucleoplasmic antigens, and both pc480 (Fig. 4, B and G) and G13 (Fig. 4K) show some enhanced staining around the nuclear periphery. Preimmune staining is weak and barely above the level of the autofluorescence given off by the cells and nuclei shown (Fig. 4, C, F, I, L). An immunostained whole cell (Fig. 4B) and isolated nuclei teased out from whole cells (Fig. 4E) are shown also as phase-contrast images (Fig. 4, A and D, respectively).

Comparative Assays of Proteolytic Activity in Pea and Rat Liver Nuclei

Because the 46-kD NTPase from rat NS appears to be derived from lamin A/C by a Ser protease activity that displays a chymotryptic-like specificity (Tokes and Clawson, 1989; Clawson et al., 1990), we examined a partially purified pea NTPase preparation for a similar protease activity using synthetic SB substrates (Table I). The tetrapeptide $AAPF_{SB}$ gave maximal activity, but Tyr_{SB} (Y_{SB}) was not hydrolyzed. In comparison, $AAPF_{SB}$ was the best substrate for the rat NS protease, whereas Y_{SB} was about 50% as active. In addition, the pea NS protease showed 21% of maximal activity with VLK_{SB}, similar to that (17%) shown by rat liver NS protease. The K_m for $AAPF_{SB}$ for the pea NS protease was 240 μM, which is slightly higher than the K_m measured for the rat liver NS protease (approximately 100 μM).

DISCUSSION

Both immunoprecipitation and western blot analyses reveal that the 47-kD calmodulin-stimulated NTPase in pea nuclei has at least one epitope in common with the 46-kD NTPase that has been characterized in rat liver nuclei. This epitope appears to be one found in mammalian lamins and in lamin-like proteins in pea nuclei. The postulate that the rat NTPase contains lamin epitopes is supported by the findings that its primary structure is essentially identical to that of the N-terminal portion of human lamin C (Clawson et al., 1988), that anti-lamin MAb's immunoprecipitate an a-ATP-labeled 46-kD peptide from rat NS preparations (Clawson et al., 1990), and that cloned lamin C preparations exhibit substantial ATP binding, albeit without ATPase activity (Clawson et al., 1990; Schwartz and Clawson, 1991). The evidence that the pea NTPase may be derived from a lamin precursor, as

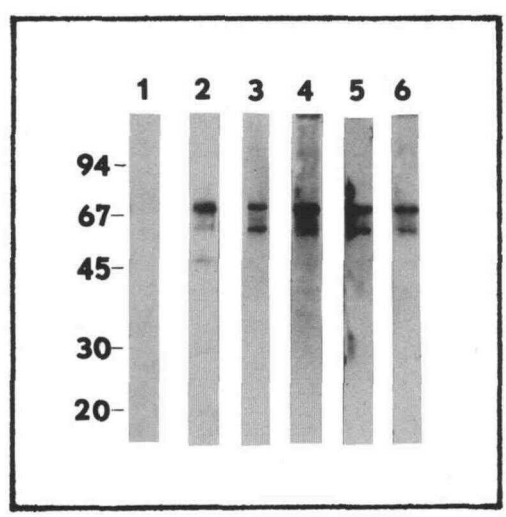

Figure 3. Western blot of envelope-matrix proteins of pea nuclei. Antibodies used for the immunostain were: lane 1, preimmune serum; lane 2, pc480; lane 3, MAb TIB 131; lane 4, MAb G13; lane 5, MAb G2; lane 6, MAb C23. A total of 8 μg of pea nuclear envelope-matrix proteins were loaded onto a 3-cm wide lane of a minigel, resolved by SDS-PAGE, and electroblotted onto a nitrocellulose sheet, which was then divided into six strips and probed with six different first antibodies as noted. The immunostain was developed by X-Omat AR film (Kodak) and ECL western blotting detection reagents (Amersham) with horseradish peroxidase-labeled secondary antibodies.

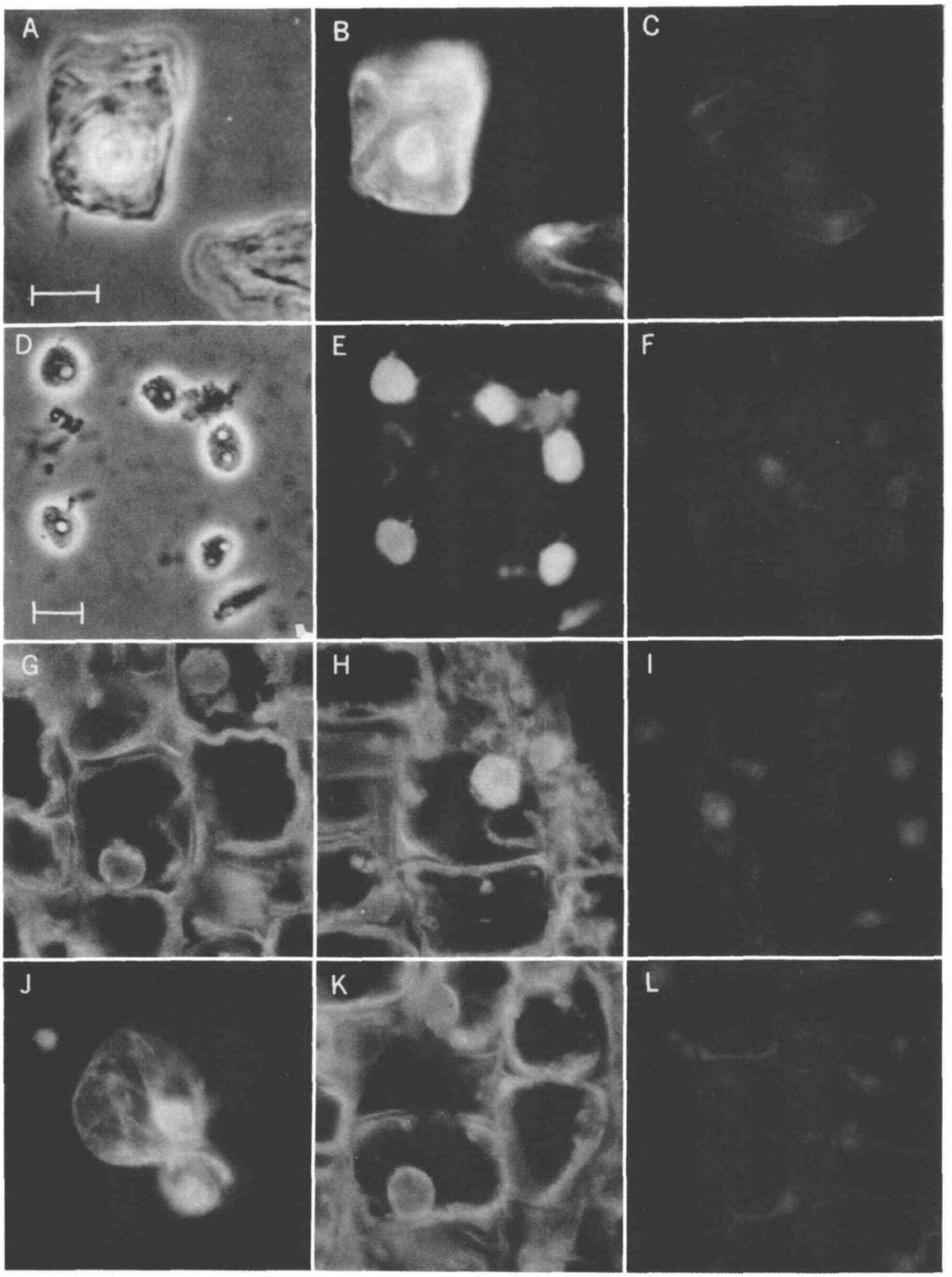

Figure 4. Immunocytochemical localization of NTPase/lamin in pea cells with: pc480 (B, E, G, and H); TIB 131 (J); G13 (K), and preimmune sera (C, F, I, L). Cells or organelles teased from fixed tissues are shown in A, B, C, D, E, F, and J. Embedded and sectioned cells are shown in G, H, I, K, and L. A and D are the phase-contrast images of the structures shown in B and E, respectively. Cells were fixed, immunostained, and examined as described in "Materials and Methods." A and B are ×1000; all others are ×750. Bar = 10 μm.

Table I. *Substrate specificity of protease activity associated with preparations of pea NTPase and rat liver NS proteins*

Colorimetric protease assays were conducted as described by Clawson et al. (1990) using pea NTPase and rat liver NS preparations that were purified as described in "Materials and Methods." The values given are minus the background absorbance, and represent the mean of triplicate assays. In all cases, the SD was less than 15% of the mean given.

Substrate	Proteolytic Activity (A_{324})[a]	
	Pea NTPase preparation	Rat liver NS preparation
$AAPF_{SB}$	2.4	0.60
VLK_{SB}	0.51	0.10
Y_{SB}	n.s.[b]	0.28
$FGAL_{SB}$	n.s.	n.s.

[a] Absorbance reading taken after incubation of sample for 5.5 h at 37°C. [b] Reading is below 0.06 and considered insignificantly above background.

described above, is thus far all immunological: polyclonal antibodies raised to the 47-kD NTPase, pc480, cross-react with pig lamins and with lamin-sized proteins in pea nuclear envelope-matrix fractions, and a monoclonal anti-lamin antibody, G2, immunoprecipitates the pea NTPase and immunostains it in western blot analysis.

In Figure 1B, the immunoprecipitated bands labeled by a-ATP that are above and below 47 kD are seen only when autoradiograms are overdeveloped, and thus represent minor and/or weakly labeled components of the immunoprecipitate. Their identity is unknown, but the band near 60 kD in Figure 1B may be related to lamins. Lamins from rat NS have been shown to label with a-ATP, although at a much lower efficiency than the NTPase (Schwartz and Clawson, 1991). The low level of labeled peptides immunoprecipitated by preimmune serum in Figure 1B may reflect the presence of anti-intermediate filament antibodies in the preimmune serum of the guinea pigs used to raise pc480. The presence of such antibodies in nonimmunized rabbits is commonly observed (Osborn et al., 1977).

One or more of the lamin-like epitopes in the pea NTPase are also present on 29- and a 19-kD peptides apparent in Figures 1 and 3. These peptides may represent proteolytic breakdown products of the NTPase, although their appearance in nuclei cannot be prevented by a cocktail of standard protease inhibitors. Alternatively, these peptides may be other NTP-binding proteins in the pea nucleus that share only this site in common with the 47-kD NTPase. In Figure 1C, the bands at 56 and 92 kD that are weakly stained by G2 may correspond to lamin-like bands of these molecular masses that have been reported in pea nuclei by others (Bevan et al., 1991; McNulty and Saunders, 1992).

The site of cleavage in lamin A/C that yields the 46-kD NTPase in rat liver nuclei has been identified as the Y residue at amino acid 376 (Clawson et al., 1990). Specific assays with a variety of thiobenzyl ester substrates, including the Tyr-containing Y_{SB}, show that there is a Ser-active site neutral protease activity in rat nuclei that cleaves at Tyr residues (Clawson et al., 1990). Experiments with the same artificial substrates indicate that pea nuclei also contain a protease that, like the rat liver nuclear protease, hydrolyzes $AAPF_{SB}$ best among the substrates tested. However, the pea nuclear protease does not hydrolyze the Y_{SB} substrate. These results suggest that the Y^{376} site may not be conserved in the pea lamin-like proteins. Sequencing studies are now underway to test this possibility.

The major lamin-hydrolyzing proteolytic activity in rat nuclei requires Ca, is stimulated by micromolar Ca, and is inhibited by calmodulin antagonists (Madsen et al., 1990). Whether the rat NTPase itself, like the pea NTPase, can be stimulated by calmodulin has not been determined, nor is it known whether the protease activity found in pea nuclei can be activated by Ca or calmodulin. The intriguing possibility that calmodulin could serve to stimulate both the proteolytic production of nuclear NTPases and their activity needs to be investigated.

The pc480 antibodies were raised to a highly purified preparation of pea NTPase that was excised from a denaturing acrylamide gel as a stained 47-kD band after SDS-PAGE. Although the protein in this band was shown to produce two silver-stained spots after two dimensional gel analysis, both spots showed identical one-dimensional peptide patterns on SDS-PAGE after V8-protease digestion by the method of Cleveland et al. (1977) (data not shown), indicating that they had primary structures that were very similar if not identical. Thus, the polyclonal antibodies in pc480 were raised to a single protein species, although some of them may recognize epitopes common to a number of different NTP-binding and/or intermediate filament proteins.

The MAb's TIB 131 and G13 show a pattern of immunostaining in plant cells and nuclei that is virtually indistinguishable from that produced by pc480 (Fig. 4). This may reflect the fact that all three antibodies recognize lamin proteins, which share epitopes in common with intermediate filament proteins. Members of the intermediate filament family of proteins are known to be distributed both in the cytoplasm and in the nucleus of plant cells (Galcheva-Gargova et al., 1988; Goodbody et al., 1989), and both of these compartments stain with all three antibodies (Fig. 4). Other laboratories that have carried out immunolocalization studies of lamin-like intermediate filament proteins in plant nuclei have also found that their distribution is throughout the nucleus, and that anti-lamin antibodies stain the cytoplasm of plant cells (Bevan et al., 1991; Frederick et al., 1992, McNulty and Saunders, 1992), so the results presented in Figure 4 are in close agreement with previous studies.

It is interesting that neither TIB 131 nor G13 bind to the 47-kD NTPase in western blot analyses (Fig. 3), so the epitope on the lamin-like pea proteins recognized by these MAb's cannot be the NTP binding site, and not all lamin epitopes are present on the NTPase. Structural studies now in progress on the NTPase and on the lamin-like proteins in pea nuclei may reveal epitopes unique to the NTPase and/or to the lamin precursor from which it may be derived. Antibodies directed to these epitopes could then be used to discover whether the locale of the NTPase is truly general in plant cells, as suggested by Figure 4, or whether it is more restricted to the nucleus or even to the nuclear envelope.

If the 47-kD NTPase from pea nuclei resembles 46-kD rat

liver NTPase in function as well as in structure, this could have significance for understanding one possible mode of regulation of gene expression by phytochrome. The 46-kD NTPase is the major NTPase in rat liver nuclei (Clawson et al., 1984) and it has been strongly implicated as the principal NTPase that functions in the control RNA export from nuclei (Agutter et al., 1979; Clawson et al., 1980; Melese, 1990). The 47-kD NTPase, which is the major one in pea nuclei (Chen et al., 1987), is almost certainly the one regulated by phytochrome (see introduction). If it is also involved in the regulation of RNA export, this would provide a molecular basis for the observation of Sagar et al. (1988) that phytochrome controls the nuclear:cytoplasmic ratio of mRNA for certain genes, and it would give experimental support for their postulate that phytochrome may achieve this control in part by regulating the export of mRNA from nuclei.

Reports on the role of Ca in regulating gene expression have steadily increased in recent years (Salehi and Niedel, 1990; Vodnar-Filipowicz and Moroni, 1990). Because the 47-kD NTPase in pea nuclei is also regulated by calmodulin, it is a specific target of Ca action in nuclei that should be investigated further for its potential to transduce the Ca signal into changes in mRNA availability for expression.

There are only a few other reports on lamin-like proteins in plants (Galcheva-Gargova et al., 1988; Bevan et al., 1991; Frederick et al., 1992; Li and Roux, 1992; McNulty and Saunders, 1992). However, none of these reports suggest that there may be a relationship between lamins and NTPases in plant nuclei. If our immunochemical evidence that the pea NTPase may also be derived from a lamin-like precursor can be confirmed by more direct sequence data, this will indicate that the protease reaction that generates the major NTPase in nuclei is a highly conserved and fundamental process in nuclear metabolism.

ACKNOWLEDGMENTS

We thank Drs. R.M. Brown, Jr., and K. Sheshan for help with photography.

Received July 27, 1992; accepted December 9, 1992.

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