

Arabidopsis COP1 protein specifically interacts *in vitro* with a cytoskeleton-associated protein, CIP1

(signal transduction/light regulation/coiled coil/plant development/subcellular trafficking)

MINAMI MATSUI*, CHATANIKA D. STOOP, ALBRECHT G. VON ARNIM, NING WEI, AND XING-WANG DENG

Department of Biology, Yale University, New Haven, CT 06520-8104

Communicated by Winslow R. Briggs, Carnegie Institution of Washington, Stanford, CA, January 9, 1995

ABSTRACT *Arabidopsis* COP1 acts inside the nucleus to suppress photomorphogenic cellular development, and light inactivation of COP1 may involve a specific control of its nuclear activity in hypocotyls and cotyledons, but not in roots, of developing seedlings. To understand the molecular mechanisms of COP1 action during light-mediated development, we initiated a screen for *Arabidopsis* cDNAs encoding proteins which interact directly with COP1 *in vitro* as a step to identify the cellular components involved. We report here the isolation and characterization of a cDNA clone encoding a protein designated CIP1 (COP1-interactive protein 1). CIP1 is predominantly α -helical and most likely involved in coiled-coil formation. It interacts specifically with the putative coiled-coil region of COP1 *in vitro*. Further, CIP1 is encoded by a single gene in *Arabidopsis*, and its mRNA and protein levels are not regulated by light. Immunofluorescent labeling of CIP1 in *Arabidopsis* seedling protoplasts demonstrated that CIP1 is part of, or associated with, a cytoskeletal structure in hypocotyl and cotyledon cells, but not in roots. Our results are consistent with a possible role of CIP1 in mediating light control of COP1 nuclear activity by regulating its nucleocytoplasmic partitioning.

Arabidopsis seedlings are able to follow two distinct morphogenetic pathways dependent on the ambient light conditions (for review, see ref. 1). Light-grown *Arabidopsis* seedlings have a short hypocotyl and open and enlarged cotyledons with chloroplast development. In contrast, dark-grown seedlings have a long hypocotyl, an apical hook, and closed and undeveloped cotyledons with etioplasts. To identify genes involved in the light control of plant development, *Arabidopsis* mutants with altered seedling developmental patterns in response to light have been isolated (for review, see refs. 2 and 3). Among them, mutations in six genetic loci—*DET1*, *COP1*, and *COP8–COP11*—result in dark-grown seedlings with pleiotropic photomorphogenic characteristics, including short hypocotyls, open and enlarged cotyledons with differentiated cell types, and expression of light-inducible genes (2–4).

Four of the pleiotropic photomorphogenic genes have been cloned. While *COP11/FUS6* (5), *COP9* (6), and *DET1* (7) encode novel proteins without significant homology to any reported sequence in the gene bank, *COP1* encodes a protein with three recognizable domains (8, 9). From the N terminus, these are a ring-finger-type zinc-binding motif (10), a predicted coiled-coil domain, and a domain with multiple WD-40 repeats, homologous to the β subunit of the heterotrimeric guanine nucleotide-binding proteins (G proteins). Significant amino acid sequence homology between COP1, excluding the zinc-binding domain, and the entire TAFII80 subunit of the *Drosophila* TFIID transcription factor complex has been reported (11). The COP1 structure suggests not only a potential to interact with nucleic acids through its zinc-finger domain but

also contacts with other proteins through its coiled-coil and $G\beta$ homologous domains.

The recessive nature of the mutations in the *COP1*, *DET1*, and *COP8–COP11* loci implies that their wild-type gene products act as negative regulators of photomorphogenic seedling development in darkness and that light acts to abrogate their repressive function. Recently we demonstrated that overexpression of COP1 protein alone is sufficient to suppress many aspects of photomorphogenic seedling development, such as hypocotyl elongation and cotyledon enlargement (12). This result provides direct evidence for COP1 being a light-inactivatable molecular repressor for photomorphogenic seedling development. Furthermore, cytological studies with β -glucuronidase–COP1 fusion protein have indicated that COP1 acts in the nucleus to achieve the suppression of photomorphogenic development, and light inactivation may involve a cell-type-specific depletion of nuclear COP1 activity (13).

To understand the molecular mechanisms of COP1 in repressing photomorphogenic development and its light inactivation, it will be necessary to identify other cellular components that directly interact with COP1. Toward this goal, we initiated a screen for *Arabidopsis* cDNAs encoding proteins which interact with COP1 *in vitro*. We report here the isolation and characterization of a cDNA clone encoding a protein, designated CIP1, which interacts with the putative coiled-coil domain of COP1 and is part of, or associated with, the cytoskeleton.†

MATERIALS AND METHODS

COP1 Probe, cDNA Library Screening, and Protein-Interaction Assay. The COP1 open reading frame was cloned as a *Bam*HI–*Bgl*II fragment into the *Bam*HI site of the vector pET3b (Novagen). Expression in *Escherichia coli* BL21(DE3)/pLysS (Novagen) resulted in a COP1 protein in which 11 vector-derived amino acids replaced the 6 residues at the N terminus of COP1. COP1 was purified from inclusion bodies of isopropyl β -D-thiogalactopyranoside (IPTG)-induced bacteria according to a published procedure (10), except that 0.1 mM $ZnCl_2$ was present in the final dialysis buffer.

The purified COP1 protein was biotinylated (14) with 25 μ g of COP1 protein and 2 μ g of biotin *N*-hydroxysuccinimide ester (BRL). Biotinylated COP1 was dialyzed for 16 hr at 4°C and used as a protein probe to screen *Arabidopsis* λ gt11 cDNA libraries made from poly(A)⁺ mRNAs of either dark-grown seedlings or leaves of adult *Arabidopsis thaliana* (Columbia) plants (8). The cDNA libraries were plated with *E. coli* Y1090 (r⁻) and screened with biotinylated COP1 essentially accord-

Abbreviations: BSA, bovine serum albumin; GST, glutathione *S*-transferase; MBP, maltose-binding protein.

*Present address: Molecular Biology Laboratory, Nippon Medical School, 1-396 Kosugicho, Nakahara, Kawasaki 211, Japan.

†The sequence reported in this paper has been deposited in the GenBank database (accession no. U20810).

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.

ing to a published protocol (14), except that biotinylated COP1 protein was used at 0.5 $\mu\text{g}/\text{ml}$.

For analysis of the interaction of COP1 with the protein encoded by the selected cDNA clone, CIP1, the 1.5-kb cDNA insert was subcloned to the *EcoRI* site of the pUR291 expression vector (24), which resulted in a β -galactosidase (LacZ)-CIP1 fusion protein. Total proteins from *E. coli* DH5 α F' carrying this plasmid, either uninduced or IPTG-induced, were separated by SDS/10% PAGE and blotted onto nitrocellulose filter for far-Western analysis according to the same procedure as for the cDNA screen. Various deletion versions (or fusions) of COP1 were biotinylated and used as probes for far-Western assay. The full-length COP1 fusion to the *E. coli* maltose-binding protein (MBP) and its derivatives, a deletion of the WD40 repeats, and a deletion of the zinc-binding motif have been described (10). The glutathione *S*-transferase (GST)-COP1 fusion was generated by expressing the COP1 cDNA in the pGEX vector (Pharmacia) and purified by glutathione affinity chromatography. A COP1 fragment encompassing aa 105–205 was expressed from a cDNA fragment adapted for cloning into the *Nco* I and *Bam*HI sites of pET15b (Novagen) by polymerase chain reaction. In this protein, COP1 residue 104 (leucine) is replaced by a methionine-valine dipeptide.

RNA and Protein Gel Blot Analysis. Six-day-old seedlings were used for RNA and protein analysis. Plant growth, RNA extraction, and Northern analysis (15) and protein extraction and analysis (9) were according to published procedures. Rabbit polyclonal antibodies produced against a purified *E. coli*-expressed GST-CIP1 fusion were affinity-purified against a MBP-CIP1 fusion and used in Western and cytological studies.

Immunocytological Localization of CIP1. Protoplasts were released from dissected organs of *Arabidopsis* seedlings by incubation in enzyme solution [10 mM Mes, pH 5.7/0.4 M mannitol/1% cellulase R10/0.25% macerozyme R10 (Yakult Pharmaceutical, Tokyo)/0.1% bovine serum albumin (BSA)/30 mM CaCl_2 /5 mM 2-mercaptoethanol] at 22°C under dark or light conditions for 1–3 hr. The protoplasts were filtered through 65- μm nylon mesh and pelleted by a brief centrifugation at 150 $\times g$. They were washed once with 4 mM Mes, pH 5.7/20 mM KCl/0.5 M mannitol and transferred to polylysine-coated slides to settle for about 3 hr at room temperature under the required light conditions. Bound protoplasts on the slides were fixed for 10 min with 2% formaldehyde in PHEM solution (60 mM Pipes/25 mM Hepes/10 mM EGTA, 2 mM MgCl_2 , pH 6.9) and then permeabilized for 5 min with 0.5% Nonidet P-40 in PHEM. The slides were soaked twice for 15 min each in methanol/acetone (1:1, vol/vol) at -20°C and air dried. They were rehydrated in phosphate-buffered saline (PBS) and blocked with 1% BSA in PHEM. After 2 hr of reaction with the CIP1 antibody at 6 $\mu\text{g}/\text{ml}$ in PHEM with 0.5% BSA, they were washed first with PBS, then with 0.1% Tween 20 in PBS, and lastly with PBS again. A fluorescein-labeled polyclonal antibody against rabbit IgG (Sigma) at a dilution of 1:100 was applied for 2 hr of incubation at room temperature. Slides were washed as above and mounted with 90% glycerol/1% *p*-phenylenediamine at pH 9.0 and 4',6-diamidino-2-phenylindole (1 $\mu\text{g}/\text{ml}$).

RESULTS

Isolation of a cDNA Clone Encoding a COP1-Interactive Protein. Biotinylated, *E. coli*-produced COP1 protein was used to screen three *Arabidopsis* $\lambda\text{gt}11$ cDNA libraries, representing a total of 3.5×10^6 independent recombinants made from mRNAs of dark-grown seedlings or leaves of light-grown adult plants. About 200 recombinant phages were selected in our primary screen. In the subsequent rescreening, duplicate filter sets of those phages were screened in parallel with either biotinylated COP1 or biotinylated GST as control. One re-

combinant phage which strongly bound to biotinylated COP1 but not to biotinylated GST was purified (Fig. 1). Further, this same phage also interacted with biotinylated MBP-COP1 fusion protein but not with biotinylated MBP, BSA, and LacZ (Fig. 1 and data not shown). This cDNA was designated CIP1, for COP1-interactive protein 1, and subcloned for sequence analysis.

CIP1 Encodes a Protein with a Potential for Participating in a Coiled-Coil Structure. Sequence analysis of the entire 1.5-kb CIP1 cDNA insert revealed one open reading frame with the *lacZ* gene in the recombinant $\lambda\text{gt}11$. The open reading frame continues to the very end of the cDNA insert without a stop codon (Fig. 2A). Chou-Fasman analysis predicted a predominantly hydrophilic protein rich in α -helix with only one break in almost 90% of the deduced sequence (Fig. 2B). Comparison of the CIP1 protein sequence to those available in the GenBank database revealed moderate homology to a variety of coiled-coil proteins [e.g., 18% identity in a 302-aa stretch to the *Acanthamoeba castellanii* myosin heavy chain (16) and 16% identity in a 330-aa stretch to the rat neuronal myosin heavy chain (17)]. Analysis of CIP1 by the COILED-COIL program (18) predicted a high probability for α -helix formation with a >90% probability to participate in a coiled-coil structure. The tendency of CIP1 to form a coiled-coil structure is also evident from its periodic pattern of leucine, isoleucine, or other hydrophobic or large-side-chain amino acids at 7-aa intervals (Fig. 2A), a commonly observed heptad repeat structure in coiled-coil proteins (19).

CIP1 Can Specifically Interact with the Putative Coiled-Coil Domain of COP1 *in Vitro*. To unambiguously demonstrate that CIP1 interacts with COP1 and, further, to reveal the COP1 protein domain responsible for the interaction, we created a *lacZ*-CIP1 fusion in a plasmid vector and analyzed the interaction of the LacZ-CIP1 fusion protein with COP1 after gel fractionation. Besides full-length COP1, several mutated COP1 proteins were made and tested to localize the COP1 domain responsible for the interaction with CIP1 (Fig. 3). Evidently, full-length COP1 and its fusion proteins with MBP or GST were able to interact with LacZ-CIP1. Since full-length COP1 did not bind LacZ itself and neither MBP nor GST alone bound LacZ-CIP1, we conclude that COP1 interacts specifically with CIP1. Deletion of the COP1 G_β -homologous domain alone or together with the zinc-binding

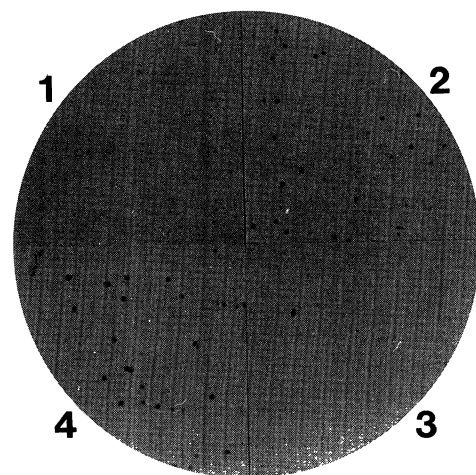


FIG. 1. Identification of CIP1 as a specific COP1-interactive clone. The purified recombinant phage containing the CIP1 cDNA were plated at low density and blotted to nitrocellulose membrane. The membrane was divided into four equal sections that were incubated with biotin-labeled GST (section 1), COP1 (section 2), MBP (section 3), or MBP-COP1 fusion protein (section 4). The specific interaction of CIP1 with biotin-labeled GST-COP1, but not BSA, was also confirmed with the same assay.

A

1 GAATTCGGTGGAAAAACCGAGTGATCAGCTCAAAGATGAACTTCTAATCTCAAGCAGCAGCTTGAAGCATCAGAGCAGCGAGTTTCA
E F R W K T T S D Q L K D E T S N L K Q Q (L) E A S E Q R V S 30

91 GAGCTGACCAGCGGTATGAATAGTGCAGAGGAAGAGAACAATCTCTATCCTTGAAGTTTCGGAGATTTTCAGATGTGATCCAACAGGGA
E L T S G M N S A E E E N K S L S L K V S E I S D V (I) Q Q I H 60

181 CAGACCACCATACAAGAACTAATTTCCGAATTTGGGAGAGATGAAGAAAAGTACAAGAAAAAGAGAGTGAGCATTCTAGTTTGGTGGAG
Q T T (I) Q E L I S E (L) G E M K E K Y K E K E S E H S S L V E 90

271 TTACATAAGACCCATGAGAGAGAATCATCAAGTCAGGTGAAAGAATTAGAAGCACACATAGAATCATCAGAGAAGTTGGTTGCAGATTTTC
L H K T H E R E S S S Q V K E L E A H (I) E S S E K L V A D F 120

361 ACCCAAGCCCTGAACAATGCAGAGGAAGAGAAAAAAGTCTCTCAGAAAATAGCAGAACTCTTAACGAGATTTCAAGAGCGCAGAAC
T Q S (L) N N A E E E K K L L S Q K (I) A E L S N E (I) Q E A Q N 150

451 ACCATGCAAGAACTCATGTCTGAGTCTGGGCAGTTGAAAGAGAGCCACAGTGTGAAAGAGCGAGAACTTTTCAGTTTGGAGGACATCCAT
T M Q L M S E S G Q L K E S H S V K E R E (L) F S L R D I H 180

541 GAGATTTCAAAAGAGACTCATCCACAGAGCAAGTGAATTTAGAAGCTCAACTGGAGTCTCAAACAGCAGGTCTCAGATTTGAGTGGC
E I H Q R D S S T R A S E L E A Q (L) E S S K Q Q V S D L S A 210

631 AGTCTGAAAGCTGCAGAGGAAGAAAAAAGTATATCTCGAAAAACGTGAAACTATGAATAAACTCGAACAACGAGAACACCGATA
S (L) K A A E E E N K A I S S K N V E T M N K (L) E Q T Q N T (I) 240

721 CAGGAATCATGGCTGAATTTGGGAAAGAGAAAGACAGCCACAGAGAGAAAGAGAGTGAGCTTTCTAGTTTGGTGAAGTACACGAGACT
Q E L M A E (L) G K K K D S H R E K E S E (L) S S L V E V H E T 270

811 CACCAGAGAGATTCATCAATTCATGTGAAAGAATTAGAAGAGCAAGTGGAAATCATCAAAGAAAATTTGGTTGCGGAGTTGAACCAACCCCTG
H Q R D S S I H V K E L E E Q V E S S K K L V A E L N Q T (L) 300

901 AACAAATGCAGAGGAAGAGAAAAAAGTCTATCTCAGAAAATAGCCCAACTTTCTAACGAGATTAAAGAGGCACAAAACACCATACAAGAA
N N A E E E K K V L S Q K (I) A Q L S N E (I) K E A Q N T (I) Q E 330

991 CTCGTGTCTGAGTCTGGGCAGTTGAAAGAGAGCCACAGTGTAAAGGATAGAGATCTTTTCAGTTTGAAGGACATCCACGAGACTCATCAA
L V S E S G Q L K E S H S V K D R D (L) F S L R D I H E T H Q 360

1081 AGAGAATCATCCACTCGCGTGAGTGAATTTAGAAGCTCAACTGGAATCTCAGAACAGCGGATCTCAGATTTGACTGTGGATCTGAAGGAT
R E S S T R V V S E L E A Q (L) E S S E Q R (I) S D L T V D (L) K D 390

1171 GCAGAGGAAGAAAAAAGCTATCTCCTCGAAAAATTTGAAAATTTAGGACAAGCTTGAACAGGCTCAGAACACCGATAAAGAAGCTCATG
A E E E N K A I S S K N L E I M D K (L) E Q A Q N T (I) K E L M 420

1261 GATGAATTTGGGAGAGTTGAAAGAGCCGACACAAAGAGAGAGAGTGAGCTTTCTAGTTTGGTGAAGTACAGATCAACAGGTTGCAGAT
D E (L) G E L K D R H K E K E S E (L) S S L V K S A D Q Q V A D 450

1351 ATGAAGCAGAGTCTGGACAATGCAGAAGAAGAGAAAAAATGTTATCTCAGAGAATCTTAGATATCTCTAATGAGATTCAGAAGCACAA
M K Q S (L) D N A E E E K K M L S Q R (I) L D I S N E (I) Q E A Q 480

1441 AAAACCATACAAGAACACATGTCTGAGTCTGAACAGTTGAAAGAGAGCCACGGTGTGAAAGGGAGGGCCGGAATTC 1516
K T (I) Q E H M S E S E Q L K E S H G V K G R A G I 505

B

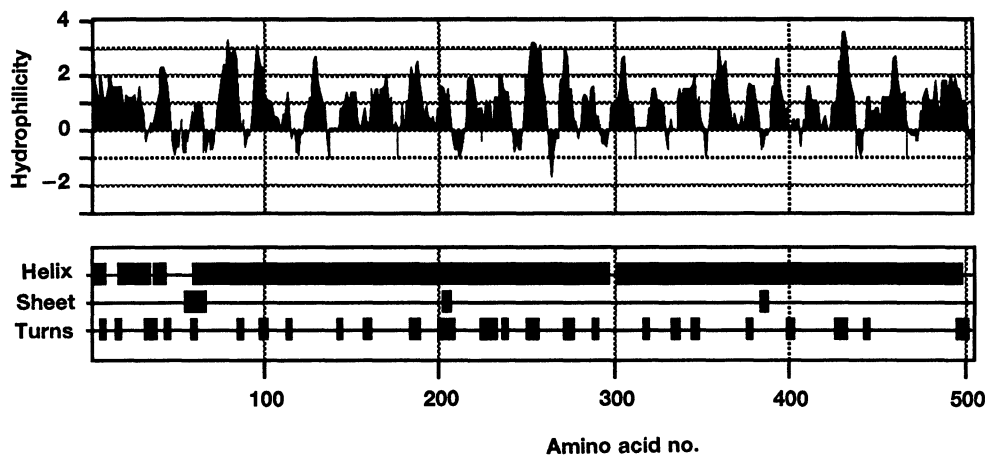


FIG. 2. (A) Nucleotide sequence of the CIP1 cDNA clone and the deduced protein sequence. The leucine and isoleucine residues at 7-aa intervals are circled, and other amino acids with large side chains that are frequently found in the seventh positions of coiled-coil proteins are underlined. (B) Hydrophilicity plot of the deduced CIP1 protein and the secondary structure predictions according to Chou and Fasman (25).

motif did not significantly affect the ability of COP1 to interact with LacZ-CIP1, suggesting that neither of these motifs is essential for CIP1 binding.

Since the CIP1 protein is rich in α -helix and potentially participates in a coiled-coil structure, CIP1 may interact with the predicted long α -helical region of COP1 (9). A 101-aa peptide corresponding to the α -helical region of COP1 was

produced and examined for its ability to interact with CIP1. It is evident from Fig. 3B (lane 9) that this α -helix domain alone is sufficient for binding to CIP1, although not as strong as the larger versions of COP1. This fragment of COP1 is highly charged, with 37% acidic and basic residues, and contains a putative bipartite nuclear translocation signal. Further, it shows moderate homology to the helical domains of several

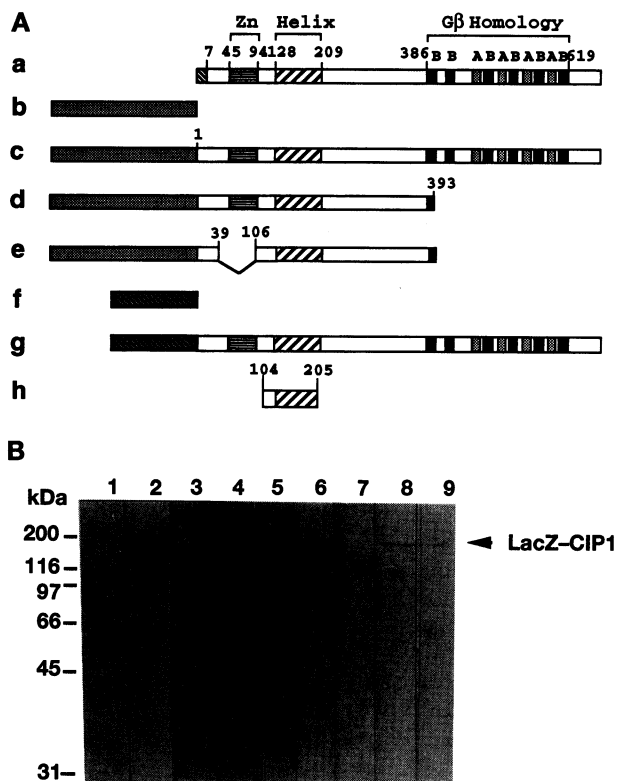


FIG. 3. CIP1 protein interacts specifically with the helix domain of COP1 *in vitro*. (A) Diagrams of various proteins used for the *in vitro* filter binding assay. The positions of the three structural domains of COP1 (zinc-binding, helix, G_{β} homology), and the subrepeats A and B of the WD40 repeats (9) in the G_{β} -homologous region are indicated. The fusion partners were either MBP (stippled boxes in *b*, *c*, *d*, and *e*) or GST (hatched boxes in *f* and *g*). (B) *E. coli* lysates with plasmids containing the *lacZ-CIP1* fusion in either antisense (lane 1) or sense (lanes 2–9) orientation were fractionated by SDS/PAGE and blotted to nitrocellulose membrane. Membrane strips corresponding to individual lanes were incubated separately with biotinylated proteins shown in A. Lanes 1 and 2, *a*; lanes 3–9, *b–h*, respectively. Positions of protein size markers are indicated at left. In lanes 2 and 6, interaction of probes with putative degradation products of the LacZ–CIP1 fusion protein is also visible.

known coiled-coil proteins, including keratins [28% identity over 42 aa in human keratin 8 (20)], myosin heavy chains [28% identity over 52 aa in the type II myosin heavy chain of *Schistosoma mansoni* (21)], and intermediate-filament proteins [25% identity over 55 aa in a mouse neurofilament protein (22)]. Indeed, the COILED COIL program predicted a 60% probability of this COP1 domain to participate in a coiled-coil structure. A circular dichroism spectrum of the N-terminal half of COP1, containing this domain, was consistent with this region being in α -helical conformation in solution at the physiological range of pH (C.D.S. and X.-W.D., unpublished data).

To rule out the possibility that the interaction between COP1 and CIP1 was simply a nonspecific coil–coil association, we examined whether COP1 would interact with two other coiled-coil proteins, type II myosin of chicken embryo skeletal muscle and type V myosin of chicken brain. We did not detect any nonspecific interaction between COP1 and the selected coiled-coil proteins, although the COP1/CIP1 interaction was clearly observed under the same conditions (data not shown). This result suggested that the ability of COP1 to bind to CIP1 is not a random coil–coil interaction, although we cannot rule out the possibility that COP1 may be able to interact with a specific class of coiled-coil proteins.

CIP1 Is Encoded by a Single Gene and Its Expression Is Not Regulated by Light. Southern blot analysis of total *Arabidopsis* DNA with the CIP1 cDNA as a hybridization probe at high stringency revealed a single band in three different restriction digests, indicating that CIP1 is encoded by a single-copy gene. However, two or three weakly hybridizing bands were observed under low-stringency conditions (data not shown). It is possible that several CIP1-related genes exist in the genome.

To examine the possible light regulation of CIP1 expression, total RNA or protein from 6-day-old dark- and light-grown wild-type and *cop1-1* seedlings was isolated and subjected to gel blot analysis with the CIP1 cDNA or CIP1-specific antibodies. The CIP1 mRNA is around 5.5 kb long, and its expression level is affected neither by light nor by the *cop1-1* mutation (Fig. 4A). The CIP1 protein appears as a band at ≈ 205 kDa, and its level is not affected by light either (Fig. 4B). This expression pattern is similar to that of COP1 (8, 9).

CIP1 Is Associated with the Cytoskeleton in Hypocotyl and Cotyledon Cells but Not in Root Cells. Since the nucleocytoplasmic partitioning of COP1 may be regulated by light, information on the subcellular localization of CIP1 may provide a clue to its role *in vivo*. We therefore examined the cellular localization of CIP1 by immunofluorescent labeling of protoplasts from *Arabidopsis* seedlings with CIP1-specific antibodies. Similar CIP1 localization patterns were observed with both dark- and light-grown seedlings. As shown in Fig. 5, CIP1 is cytoplasmic and is clearly excluded from the nucleus. Interestingly, CIP1 exhibited a fibrillar pattern typical for cytoskeletal structures (Fig. 5A) in a majority of protoplasts isolated from hypocotyls or cotyledons, with the remaining fraction (up to 50%) of the protoplasts exhibiting either a uniform background staining or no staining at all. In contrast, in almost all stained protoplasts isolated from roots, CIP1 was distributed as disconnected speckles of variable size (Fig. 5B). It is interesting that this organ-specific localization pattern of CIP1 mirrors the nuclear depletion of β -glucuronidase–COP1 staining in the hypocotyl and cotyledon cells but a persistent β -glucuronidase–COP1 nuclear staining in the root cells of light-grown *Arabidopsis* seedlings (13). Therefore, the ability of CIP1 to interact specifically with COP1 *in vitro* implies a possible role in mediating the hypothesized light regulation of COP1 nuclear activity.

DISCUSSION

In this report we describe the isolation and characterization of an *Arabidopsis* cDNA clone, encoding a protein, CIP1, that

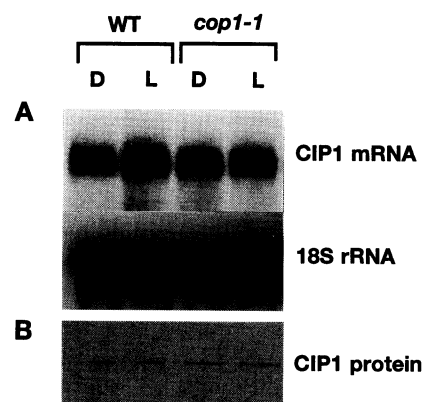


FIG. 4. CIP1 mRNA and protein levels are not affected by light or the *cop1-1* mutation. (A) RNA blot analysis of CIP1 mRNA and 18S rRNA. Equal amounts of total RNA (30 μ g for CIP1, 1 μ g for rRNA) from dark (D)- or light (L)-grown wild-type (WT) or *cop1-1* mutant seedlings were probed with radioactive CIP1 probe or rRNA probe. (B) Protein blot analysis of CIP1. Equal amounts of total proteins (5 μ g) from dark (D)- or light (L)-grown wild type (WT) or *cop1-1* mutant seedlings were used in each lane and probed with affinity-purified polyclonal antibodies against CIP1.

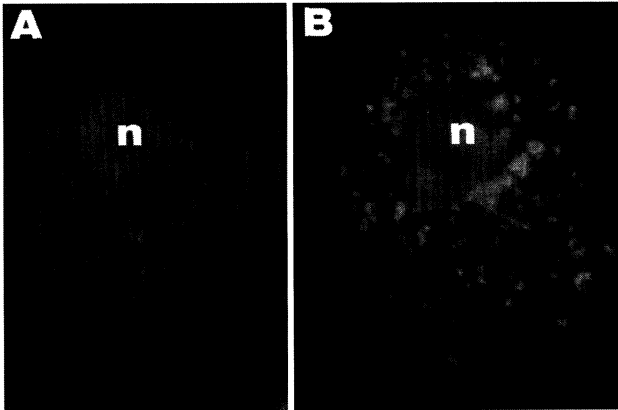


FIG. 5. Subcellular localization of CIP1 in *Arabidopsis* hypocotyl (A) and root (B) cells. Protoplasts isolated from dissected hypocotyls, roots, and cotyledons of light-grown *Arabidopsis* seedlings were fixed, permeabilized, and subjected to immunofluorescent labeling with CIP1 polyclonal antibodies as the primary antibody and fluorescein-labeled polyclonal antiserum against rabbit IgG as the secondary antibody. The localization of CIP1 in the cotyledon protoplasts is similar to that in the hypocotyl protoplasts. Positions of the nuclei (n) are indicated by the fluorescence of DNA staining (with 4',6-diamidino-2-phenylindole), which leaks through the green filter used during microphotography. ($\times 1550$.)

specifically interacts with the light-regulatory protein COP1 under our assay conditions. The CIP1 protein sequence predicts a high degree of α -helical structure and high likelihood of participating in a coiled-coil structure. Furthermore, deletion analysis suggested that a putative coiled-coil domain of ≈ 100 aa in COP1 mediates the interaction with CIP1. Because a partial COP1 protein containing this domain is highly α -helical, the interaction between COP1 and CIP1 is probably through a specific coiled-coil association. Our results suggested that this putative coiled-coil association is rather specific, since we have obtained only one coiled-coil protein after screening 3.5×10^6 independent cDNA clones, and COP1 was not able to interact with two other control coiled-coil proteins tested in our *in vitro* assay.

Immunofluorescent labeling of CIP1 in cells from upper parts (hypocotyl and cotyledon) of light-grown *Arabidopsis* seedlings revealed a fibrillar structure extending through the whole cytoplasm. This pattern, together with the homology to cytoskeletal proteins and the tendency for coiled-coil formation, strongly suggests that CIP1 participates in or associates with the cytoskeleton. However, the cytoskeleton-like fibrils are replaced by apparently disconnected speckles in cells originating from the root. This tissue-specific subcellular localization pattern is consistent with a role of CIP1 in the light control of plant development by COP1. We recently proposed that COP1 acts in the nucleus to suppress photomorphogenic development in darkness and that light inactivation of COP1 involves a specific depletion of nuclear COP1 activity in hypocotyls and possibly cotyledons, but not in roots, where COP1 is constitutively nuclear (13). CIP1 could regulate the light control of nuclear COP1 activity by sequestering COP1 in the cytoplasm under light conditions, thereby impeding its nuclear import in the cells of upper parts of *Arabidopsis* seedlings. The affinity between CIP1 and COP1 would have to

be regulated by light in these cells. It is possible that the disconnected cytoplasmic speckles of CIP1 in the root cells were unable to sequester COP1 under both dark and light conditions. Consistent with this hypothesis, the putative nuclear localization signals of COP1 reside either within or adjacent to the putative CIP1-interactive domain (13). Interestingly, a similar cytoskeleton-mediated cytoplasmic sequestering of the Cbl protein encoded by the *c-cbl* protooncogene has been proposed on the basis of its cytoskeletal distribution in the cytoplasm (23). Further, the Cbl protein shares the same type of zinc-binding domain with COP1. Thus it is possible that CIP1 may act as a cell type-specific and light-regulated cytoplasmic sequesterer for COP1 and may be involved in light control of nuclear COP1 activity through affecting its nucleocytoplasmic partitioning.

We thank Drs. M. Mooseker and R. Chain for the gift of chicken myosins and Dr. J. Sheen for advice on *Arabidopsis* protoplast isolation. This work was supported by National Institutes of Health Grant 1-R29-GM47850 (to X.-W.D.). A.G.v.A. was a recipient of a postdoctoral fellowship from the Deutsche Forschungsgemeinschaft.

- Kendrick, R. E. & Kronenberg, G. H. M., eds. (1993) *Photomorphogenesis in Plants* (Nijhoff, Dordrecht, The Netherlands).
- Chory, J. (1993) *Trends Genet.* **9**, 167–172.
- Deng, X.-W. (1994) *Cell* **76**, 423–426.
- Wei, N., Kwok, S. F., von Arnim, A. G., Lee, A., McNellis, T. W., Piekos, B. & Deng, X.-W. (1994) *Plant Cell* **6**, 629–643.
- Castle, L. A. & Meinke, D. W. (1994) *Plant Cell* **6**, 25–41.
- Wei, N., Chamovitz, D. A. & Deng, X.-W. (1994) *Cell* **78**, 117–124.
- Pepper, A., Delaney, T., Washburn, T., Poole, D. & Chory, J. (1994) *Cell* **78**, 109–116.
- Deng, X.-W., Matsui, M., Wei, N., Wagner, D., Chu, A. M., Feldmann, K. A. & Quail, P. H. (1992) *Cell* **71**, 791–801.
- McNellis, T. W., von Arnim, A. G., Araki, T., Komeda, Y., Miséra, S. & Deng, X.-W. (1994) *Plant Cell* **6**, 487–500.
- von Arnim, A. G. & Deng, X.-W. (1993) *J. Biol. Chem.* **268**, 19626–19631.
- Dynlacht, B. D., Weinzierl, R. O. J., Admon, A. & Tjian, R. (1993) *Nature (London)* **363**, 176–179.
- McNellis, T. W., von Arnim, A. G. & Deng, X.-W. (1994) *Plant Cell* **6**, 1391–1400.
- von Arnim, A. G. & Deng, X.-W. (1994) *Cell* **79**, 1035–1045.
- Macgregor, P. F., Abate, C. & Curran, T. (1990) *Oncogene* **5**, 451–458.
- Deng, X.-W., Caspar, T. & Quail, P. H. (1991) *Genes Dev.* **5**, 1172–1182.
- Hammer III, J. A., Bowers, B., Paterson, B. M. & Korn, E. D. (1987) *J. Cell Biol.* **105**, 913–925.
- Sun, W. & Chantler, P. D. (1992) *J. Mol. Biol.* **224**, 1185–1193.
- Lupas, A., van Dyke, M. & Stock, J. (1991) *Science* **252**, 1162–1164.
- Kolling, R., Nguyen, T., Chen, E. Y. & Botstein, D. (1993) *Mol. Gen. Genet.* **237**, 359–369.
- Leube, R. E., Bosch, F. X., Romano, V., Zimbelmann, R., Hoefler, H. & Franke, W. W. (1986) *Differentiation* **33**, 69–85.
- Soisson, L. M., Masterson, C. P., Tom, T. D., McNally, M. T., Lowell, G. H. & Strant, M. (1992) *J. Immunol.* **149**, 3612–3620.
- Julien, J. P., Meyer, D., Flavell, D., Hurst, J. & Grosveld, F. (1986) *Brain Res. Mol. Brain Res.* **1**, 243–250.
- Blake, T. J., Heath, K. G. & Langdon, W. Y. (1993) *EMBO J.* **12**, 2017–2026.
- Rüther, U. & Müller-Hill, B. (1983) *EMBO J.* **2**, 1791–1801.
- Chou, P. Y. & Fasman, G. D. (1978) *Adv. Enzymol. Relat. Areas Mol. Biol.* **47**, 45–148.