Plant Physiol. (1994) 105: 707–713

# Characterization and Expression of Two cDNAs Encoding Carbonic Anhydrase in *Arabidopsis thaliana*[1]

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**Two distinct cDNA clones encoding carbonic anhydrase (CA) were isolated from an *Arabidopsis thaliana* λYES library. One of these clones, CA1, encodes a 36.1-kD polypeptide and is essentially the same as a previously reported *Arabidopsis* CA cDNA (C.A. Raines, P.R. Horsnell, C. Holder, J.C. Lloyd [1992] Plant Mol Biol 20: 1143–1148). Comparison of the derived amino acid sequence from this clone with other plant CAs suggests the presence of a chloroplastic transit peptide, which, when cleaved, would render a mature protein of 24.3 kD. The other identified clone, CA2, encodes a 28.3-kD polypeptide, which in addition to other residue changes, is 78 amino acids shorter at the N terminus than the primary product of CA1. The two cDNAs exhibit 76.9% sequence similarity at the DNA level and 84.6% identity between the predicted amino acid sequences. A polyclonal antibody generated against pea CA (N. Majeau, J.R. Coleman [1991] Plant Physiol 100: 1077–1078) hybridized to two protein bands (25 and 28 kD) from a total leaf extract and to only one band (25 kD) from a chloroplastic protein extract. The data suggest that the CA2 protein is an extrachloroplastic form of CA, presumably localized in the cytoplasm. Southern analysis indicated that CA1 and CA2 are encoded by different genes. Northern analysis of total leaf RNA resulted in hybridization of CA1- and CA2-derived probes to two transcripts of 1.47 and 1.2 kb, respectively. These data provide additional evidence that the CA2 clone is a full-length cDNA and that two transcribed CA genes are present in the *Arabidopsis* genome. Transcript levels of CA1 and CA2 decreased 70 and 20%, respectively, when mature plants were transferred to dark for 24 h. Seedlings germinated in the dark showed CA1 and CA2 transcript abundance levels of 4 and 22%, respectively, when compared with light-germinated seedlings. These data suggest that expression of CA1 is light regulated and dependent on leaf and/or chloroplast development. A possible role for cytoplasmic CA in the plant cell is discussed.**

CA (EC 4.2.1.1) catalyzes the reversible hydration of $CO_2$ to bicarbonate and is one of the most abundant soluble proteins in the leaves of $C_3$ higher plants, representing up to 1 to 2% of the soluble leaf protein (Reed and Graham, 1981). Most localization studies indicate that CA is found in the chloroplasts of $C_3$ plants and primarily within the cytosol of mesophyll cells of $C_4$ species. However, there have been reports of cytosolic localization in $C_3$ plants (Kachru and Anderson, 1974; Reed and Graham, 1981). Within the $C_3$ chloroplast it has been postulated that CA activity could maintain the supply of $CO_2$ for Rubisco by speeding the dehydration of $HCO_3^-$ or by facilitating the diffusion of $CO_2$ across the chloroplast envelope via maintenance of the equilibrium between the inorganic carbon species (Reed and Graham, 1981). In $C_4$ plants, the cytosolic CA catalyzes the hydration of $CO_2$ to bicarbonate, the substrate of PEPcase (Hatch and Burnell, 1990). The potential role of a cytosolic CA in $C_3$ plants is not well established. Although enzyme activity data suggesting the presence of CA isoforms have been shown for a few species (Kachru and Anderson, 1974; Reed and Graham, 1981), there are no protein or DNA sequences reported for any plant cytosolic CA.

In this paper we have identified and characterized two *Arabidopsis thaliana* CA cDNA clones, one of which is an extrachloroplastic, and presumably cytosolic, isoform. We have also shown that the two isoforms are differentially regulated by light and that one of them requires leaf and/or chloroplast development to be expressed.

## MATERIALS AND METHODS

### Plant Material

*Arabidopsis thaliana* Columbia was grown in controlled environmental chambers under constant white fluorescent light (250 μmol photons $m^{-2}$ $s^{-1}$) at 22°C in autoclaved soil. Plants (3 weeks postgermination) transferred to dark conditions were kept under the same temperature and watering conditions as light-grown plants. For studies of developmental regulation of expression, seeds were sterilized, allowed to imbibe for 2 d at 4°C, plated on Petri plates containing 0.5× Murashige and Skoog (1962) salts, 5 mM Mes/KOH (pH 5.7), 0.8% agar, and $10^{-5}$ M $GA_3$, and germinated under dark or light conditions for 10 d at 22°C. Dark-adapted plants and etiolated seedlings were frozen in liquid nitrogen in the dark with the aid of a green safelight prior to RNA extraction. Light-grown tissue was also frozen in liquid nitrogen prior to RNA extraction.

### cDNA Library Screening

A λYES cDNA library synthesized from above-ground parts of *A. thaliana* (Elledge et al., 1991) was screened three times, using different strategies. All DNA probes used for screening were labeled with [$^{32}$P]dCTP as described by Fein-

[1] J.P.F. was funded by a postgraduate scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnólogico (Brazil). This research was funded by a grant to J.R.C. from the Natural Science and Engineering Research Council of Canada.

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Abbreviations: CA, carbonic anhydrase; PEPcase, phospho*enol*-pyruvate carboxylase.

berg and Volgelstein (1984). Filters were prehybridized in hybridization ovens for 1 h in 0.5 M NaCl, 0.1 M $NaH_2PO_4$, 0.1 M Tris, 2 mM EDTA, 0.1% SDS and hybridized overnight in fresh solution plus the labeled probe. This was followed by three washes at room temperature for 15 min in 10 mM $NaPO_4$ (pH 6.5), 2 mM EDTA, 0.1% SDS and one wash for 45 min in 50 mM $NaPO_4$ (pH 6.5), 2 mM EDTA, 0.1% SDS (Lomax et al., 1987). The temperatures of hybridization and the final wash varied in the different screening strategies.

Initially, a total of $10^6$ recombinants were screened with a 617-bp *Sac*I-*Bam*HI internal restriction fragment of the pea CA cDNA (Majeau and Coleman, 1991). The hybridization was done at 65°C and the final washing at 55°C. Positively hybridizing phage were identified, rescreened, and transformed into *Escherichia coli* BNN132 to regenerate the LE 392 plasmid. Plasmid DNA was isolated, digested with *Eco*RI to release the cloned inserts, electrophoresed in an agarose gel, blotted onto nitrocellulose, and reprobed with the same probe. Clones were chosen for sequencing based on insert size and intensity of hybridization.

A second screen of $10^6$ recombinants was also performed by using a 515-bp *Sac*I-*Sac*I internal restriction fragment of one of the clones isolated on the first screen, identified as CA2. Both hybridization and final washing were done at 65°C. Following rescreening, positively hybridizing clones were transformed into BNN132, their plasmid DNA was analyzed on Southern blots, and three of the clones were partially sequenced. The longest one was completely sequenced and identified as CA1.

An additional screening was done in an attempt to identify any larger CA2 clones. Sixty positive clones selected on the first two screens were probed with a 5′ end 400-bp *Eco*RI-*Asp*I fragment of the CA2 clone. Hybridization was carried out at 69°C and washings at 65°C. Control plates with CA1 and CA2 clones were also hybridized, and only clones hybridizing to the probe with the intensity observed for authentic CA2-CA2 reactions were transformed into BNN132. Insert sizes were determined and the largest clones sequenced.

### DNA Sequencing

All cDNAs to be sequenced were first excised from LE392 with *Eco*RI and cloned in pBS (Stratagene). Double-stranded DNA sequencing of the inserts was performed according to the dideoxy method of Sanger et al. (1977), using either the −20 primer and the reverse primer for pBS or synthesized oligonucleotide primers.

### Chloroplast Isolation and Western Blotting Analysis

*Arabidopsis* plants were maintained in the dark for 12 h prior to chloroplast isolation. Protoplasts were prepared by incubating leaf segments with macerase and cellulysin (Calbiochem, La Jolla, CA) as described by Somerville et al. (1981). Rupture of protoplasts was done by passing the protoplast suspension through a small section of Miracloth (Calbiochem) attached to the end of a 5-mL syringe, as suggested by Nishimura et al. (1976). Microscopic examination indicated that about 90% of the protoplasts had been broken. Released chloroplasts were recovered by centrifugation at 270*g* for 35 s and resuspended in protein extraction buffer (50 mM Hepes/KOH [pH 7.1], containing 20 mM $MgCl_2$, 1 mM $Na_2EDTA$, 10 mM DTT, 0.1% [w/v] polyvinylpolypyrrolidone, 1 mM benzamidine, 1 mM PMSF). Both $Na_2CO_3$ and DTT were added to a final concentration of 0.1 M, and the protein samples were prepared for electrophoresis on denaturing 12% (w/v) polyacrylamide gels as described previously (Piccioni et al., 1982). Soluble proteins from total leaf tissue were extracted by grinding in ice-cold extraction buffer. After the sample was centrifuged (10 min at 12,000*g*), the supernatant was made 0.1 M $Na_2CO_3$/DTT, and the soluble protein samples were analyzed by SDS-PAGE. Acrylamide gels were blotted onto nitrocellulose according to the manufacturer's specifications (Pharmacia Multiphor II Systems Handbook 18–1013–42), and western analysis was performed by incubation with a polyclonal antibody against pea CA (Majeau and Coleman, 1991).

### Genomic DNA Isolation and Analysis

Genomic DNA was extracted from *Arabidopsis* leaves according to the method of Dellaporta et al. (1983) and digested with restriction enzymes for 6 h (with addition of more enzyme after 3 h) at 37°C. Aliquots of 4 μg of digested DNA were eletrophoresed on 0.7% agarose gels and transferred to a Hybond-$N^+$ (Amersham) positively charged membrane in 0.4 M NaOH (Reed, 1986a). The membranes were hybridized to labeled probes generated from either a 723-bp *Hin*dIII-*Nde*I restriction fragment from CA1 or a 515-bp *Sac*I-*Sac*I fragment from CA2. Prehybridizations and hybridizations were done at 65°C in 1.5× sodium chloride/sodium phosphate/EDTA buffer, 1% SDS, 0.5% Blotto, and 0.5 mg/mL calf thymus DNA. The blots were washed with strong agitation at room temperature for 15 min each in 2× SSC/0.1% SDS, 0.5× SSC/0.1% SDS, and 0.1× SSC/0.1% SDS, followed by a final wash of 90 min in the hybridization oven at 50°C in 0.1× SSC/0.1% SDS (Reed, 1986b). Autoradiograms were developed following 72 h of exposure.

### RNA Isolation and Northern Hybridization

Total RNA was isolated from leaves of *Arabidopsis* plants according to the method described by Verwoerd et al. (1989). Two samples of 10 μg each were denatured and eletrophoresed on a 1.5% agarose gel containing 0.66 M formaldehyde. Transfer to nitrocellulose, prehybridization, and hybridization were as previously described (Owttrim and Coleman, 1989). Each of the samples was hybridized with one of the two probes used for the genomic DNA analysis. The hybridization pattern was determined by autoradiography, and the sizes of the transcripts were estimated by comparison with the five ribosomal bands, previously sized (Chang and Meyerowitz, 1986).

For slot-blot analysis, 5 μg of each sample were denatured with formaldehyde-formamide and blotted on nitrocellulose using a slot-blot apparatus (Minifold II Slot-Blotter, Schleicher & Schuell). Prehybridization, hybridization, and autoradiography were performed as previously described (Owttrim and Coleman, 1989). The samples were hybridized to each one of the following probes: a 723-bp *Hin*dIII-*Nde*I

restriction fragment of CA1 and a 515-bp *Sac*I-*Sac*I fragment of CA2. Hybridization with a 2.0-kb 18S soybean rDNA fragment was also performed and was used to normalize RNA aliquots loaded into each slot-blot well. The extent of hybridization of the probes to the immobilized *Arabidopsis* RNA was determined by phosphorimaging (model 400S; Molecular Dynamics, Sunnyvale, CA).

## RESULTS AND DISCUSSION

### Isolation and Characterization of cDNA Clones Encoding *Arabidopsis* CA

A cDNA library (Elledge et al., 1991) was screened three times, using different strategies, as described in "Materials and Methods." DNA from three positive clones obtained on the first screen exhibited strong hybridization with the pea probe on Southern blots, and partial sequence analysis showed that they were obtained from the same mRNA, although they were of different lengths. The longer clone was completely sequenced and is referred to as CA2 in this work (Fig. 1). The 1.05-kb cDNA is homologous to other plant CAs (Majeau and Coleman, 1991, 1992), but is shorter at the 5′ end. The sequence encodes an open reading frame for a polypeptide of 28.3 kD, starting at the Met that corresponds to $Met^{71}$, $Met^{65}$, and $Met^{66}$ in pea, tobacco, and spinach, respectively (Fawcet et al., 1990; Majeau and Coleman, 1991, 1992).

Two additional clones from the first screen (pea CA probe) and three from the second screen (CA2 probe) were partially sequenced and found to be identical with each other. The longest one (CA1) was completely sequenced, and the 1.32-kb insert was found to encode a predicted polypeptide of 36.1 kD. If we assume processing of the transit peptide at a similar site as that described for both the spinach (Burnell et al., 1990) and pea (Roeske and Ogren, 1990) proteins, the mature product would be a 24.3-kD polypeptide, with an N terminus at $Ala^{114}$. Both cited studies based their identification of the cleavage site on the N-terminal amino acid sequence of the mature chloroplast protein when isolated from leaf tissue.

The size discrepancy between CA2 and CA1, as well as other plant CA sequences, initially suggested that the CA2 clone may be incomplete. Sixty positive clones that had been identified on the first two screens were plated, and plate lifts hybridized under highly stringent conditions to a radiolabeled 400-bp 5′ end fragment of CA2. Twelve clones that hybridized to the probe as strongly as a control CA2 clone (and significantly more strongly than a CA1 control) were

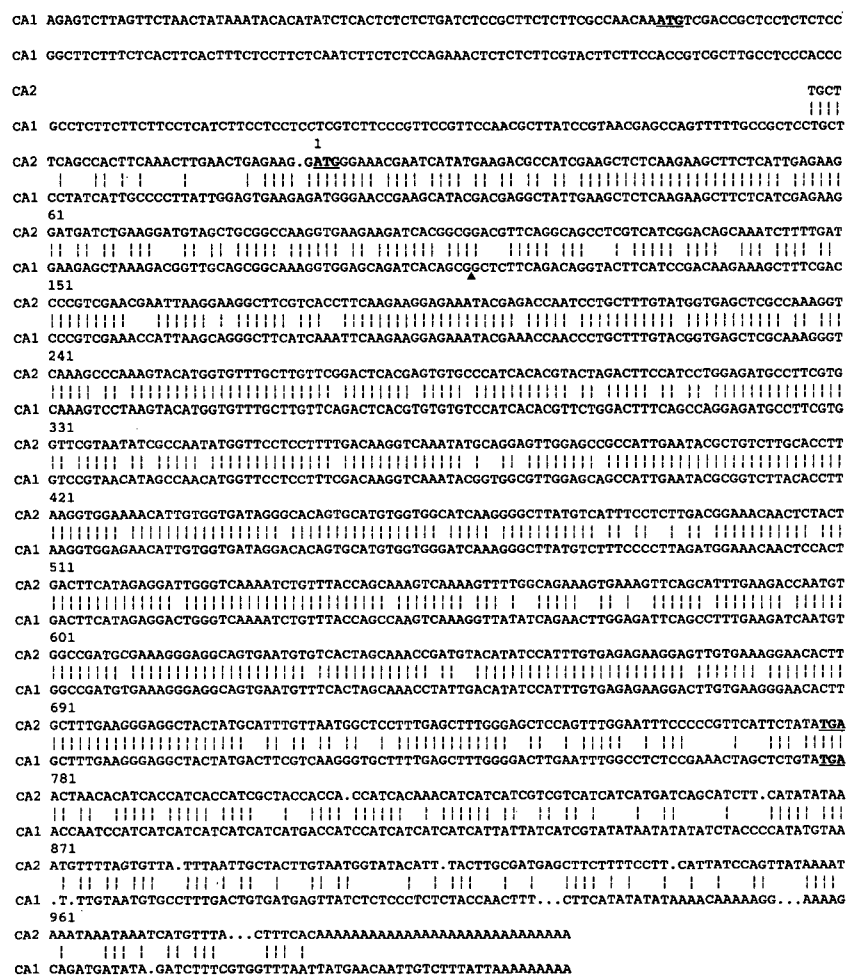


**Figure 1.** Alignment of the nucleotide sequences of two *A. thaliana* cDNAs coding for CA. Numbering starts at the presumed start site for translation of CA2. The proposed initiation codons and stop codons for both CA proteins are indicated in boldface and are underlined. The putative cleavage site for the removal of the CA1 transit peptide is indicated with an arrowhead.

```
Ara CA2   CFSHFKLELRRM[1]
Ara CA1   APIIAPYWSEEM[78]
Spi CA    APIITPTLKEDM[66]
Pea CA    SPIITPVLREEM[71]
Tob CA    TPIINPILREEM[65]
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**Figure 2.** Predicted amino acid sequences of plant chloroplastic CA proteins corresponding to the proposed untranslated portion of the extrachloroplastic localized CA2. Numbers indicate amino acid positions of Met residues in the respective proteins. Sequence in italics represents amino acids that would be encoded by CA2 if the respective region were translated. Ara, *Arabidopsis*; Spi, spinach; Pea, pea; Tob, tobacco.

identified. Five of those were partially analyzed and all were shown to have the same sequence as CA2. Although they were of different sizes, not one had a longer 5′ sequence than CA2. We suggest that the sequence shown in Figure 1 contains the full open reading frame of a second form of CA in *Arabidopsis*. This hypothesis is supported by the significant decrease in sequence homology between CA2 and CA1 (as well as other plant CAs) in the region 5′ to the proposed initiation codon for CA2. If CA2 were simply a truncated, incomplete cDNA clone and thus missing a portion of the open reading frame, it is unlikely that this region would be completely dissimilar to the corresponding amino acid sequence of CA1 and other sequenced CAs in plants (Fig. 2).

```
          1        10        20        30        40        50        60
ARA1CA    MSTAPLSGFFLTSLSPSQSSLQKLSLRTSSTVACLPPASSSSSSSSSSSSRSVPTLIRNE
ARA2CA

                  70        80        90       100       110       120
ARA1CA    PVFAAPAPIIAPYWSEEMGTEAYDEAIEALKKLLIEKEELKTVAAAKVEQITAALQTGTS
                                                          ▼
ARA2CA                    MGNESYEDAIEALKKLLIEKDDLKDVAAAKVKKITADVQAASS
                          1        10        20        30        40

          130       140       150       160       170       180
ARA1CA    SDKKAFDPVETIKQGFIKFKKEKYETNPALYGELAKGQSPKYMVFACSDSRVCPSHVLDF
ARA2CA    SDSKSFDPVERIKEGFVTFKKEKYETNPALYGELAKGQSPKYMVFACSDSRVCPSHVLDF
               50        60        70        80        90       100

          190       200       210       220       230       240
ARA1CA    QPGDAFVVRNIANMVPPFDKVKYGGVGAAIEYAVLHLKVENIVVIGHSACGGIKGLMSFP
ARA2CA    HPGDAFVVRNIANMVPPFDKVKYAGVGAAIEYAVLHLKVENIVVIGHSACGGIKGLMSFP
               110       120       130       140       150       160

          250       260       270       280       290       300
ARA1CA    LDGNNSTDFIEDWVKICLPAKSKVISELGDSAFEDQCGRCEREAVNVSLANLLTYPFVRE
ARA2CA    LDGNNSTDFIEDWVKICLPAKSKVLAESESSAFEDQCGRCEREAVNVSLANRCTYPFVRE
               170       180       190       200       210       220

          310       320       330   336
ARA1CA    GLVKGTLALKGGYYDFVKGAFELWGLEFGLSETSSV
ARA2CA    GVVKGTLALKGGYYAFVNGSFELWELQFGISPVHSI
               230       240       250       259
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**Figure 3.** Comparison of the deduced amino acid sequences for the two *Arabidopsis* CA cDNAs. Numbering starts at the deduced initiation Met residue for each cDNA. The putative cleavage site for the removal of the CA1 transit peptide is indicated with an arrowhead.

The comparison of the two cDNAs revealed the presence of 76.9% homology, which is increased to 84.9% when the entire putative coding region of CA2 is compared with the comparable portion of CA1. There are 96 base-pair differences between CA1 and CA2 in the protein-coding region (bp 1–780 in CA2, Fig. 1), 59 (61.5%) of which result in a codon for the same amino acid. Given that the nucleotide changes are spread throughout the sequence, it is unlikely that the differences between the two clones could be accounted for by differential processing of one primary transcript.

An *Arabidopsis thaliana* cDNA encoding CA has been previously reported (Raines et al., 1992) and is essentially the same as CA1. The CA1 clone reported here is shorter by 18 bp at the 5′ end and is also missing an 11-bp fragment (5′-ATTTGTGTATC-3′) at the 3′ end, positioned immediately before the poly(A) tail. There is also 1 base-pair difference at position 414 (Fig. 1), where a G to A transition has occurred, although this does not modify the encoded amino acid (TTG and TTA, encoding Leu).

Comparison of the predicted amino acid sequences of CA1 and CA2 indicated that they are 84.6% identical and that 12 of the 40 amino acid changes are conservative (Fig. 3). All of the highly conserved blocks of homology between plant CAs appear to be maintained. It is interesting, however, that an additional Cys residue was introduced into each of the CA forms: $Cys^{34}$ in CA1 and $Cys^{216}$ in CA2 (Fig. 3). Previous studies using site-directed mutagenesis of various amino acids in pea chloroplastic CA have indicated that some Cys residues are required for activity and/or correct protein folding (Provart et al., 1993).

It seems likely that the CA1 protein is a chloroplastic form of CA, because its N-terminal region is similar to the transit peptides previously identified in other plant CAs, and abundant Ser and Thr residues, characteristic of chloroplast transit peptides, are conserved (Heijne et al., 1989). Comparison with the N-terminal protein sequence from spinach (Burnell et al., 1990) and pea (Roeske and Ogren, 1990) chloroplastic CAs suggests that the cleavage site is between $Ala^{113}$ and $Ala^{114}$. Such processing of the polypeptide would render a mature protein of 223 residues, with a molecular mass of 24.3 kD. In contrast, if we assume that CA2 is processed to generate a similarly sized mature protein, it seems unlikely that the first 36 amino acids on CA2 could function as a transit peptide. The N termini of chloroplast transit peptides so far identified are Ser/Thr rich and rarely have any charged residues (Heijne et al., 1989). For CA2, there is only one Ser and there are eight acidic and seven basic residues in this 36-amino acid fragment (Fig. 3). Moreover, it has been reported that turn-inducing residues (Gly, Ser, Asp, Asn, Pro) appear to be largely absent from positions −3 to +1 in relation to the stromal cleavage site (Heijne et al., 1989). CA2 (in comparison to CA1) has a point mutation resulting in an Asp residue at position +1, which could result in impaired processing. If it is assumed that CA2 encodes an extrachloroplastic protein, the full-length polypeptide, without any processing, would have 259 residues and a molecular mass of 28.3 kD.

### Western Analysis of *A. thaliana* CA

Further evidence for the existence of two CA proteins of different sizes in *Arabidopsis* comes from western blots of

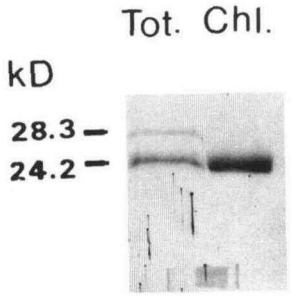


**Figure 4.** Western analysis of total soluble proteins (Tot.) and soluble chloroplastic proteins (Chl.) from *Arabidopsis* leaves. Approximately 5 μg of soluble protein per lane, from total leaf extract and from isolated chloroplasts, were separated by SDS-PAGE, transferred to nitrocellulose, and hybridized with a polyclonal antibody against pea CA.

*Arabidopsis* leaf proteins. Following SDS-gel electrophoresis, transfer to nitrocellulose, and probing with pea CA antisera, two protein bands of 24.9 and 28.1 kD were observed (Fig. 4, lane 1). The proteins detected are very similar in size to the 24.3- and 28.3-kD polypeptides predicted from the deduced amino acid sequences of CA1 and CA2, respectively. When proteins extracted from isolated chloroplasts were subjected to western blot analysis, only the lower band was detected (Fig. 4, lane 2). Therefore, it seems that the CA2 protein is not present in the chloroplast and is perhaps a cytoplasmic form of CA.

### Southern Blot Analysis

Total genomic DNA was extracted from *Arabidopsis* leaves, digested with the enzymes *Hin*dIII, *Sac*I, *Nsi*I, *Sal*I, and *Bcl*I, and subjected to Southern analysis. Hybridization with radiolabeled fragments of either CA1 or CA2 resulted in significantly different banding patterns for some of the DNA digests (Fig. 5). For example, the CA1 probe hybridized to a 10.6-kb *Sac*I genomic DNA fragment, whereas the CA2 probe identified *Sac*I fragments of 2.9 and 1.3 kb (Fig. 5, cf. lanes a2 and b2). Different hybridization patterns were also obtained with *Nsi*I, *Sal*I, and *Bcl*I digests. The genomic DNA hybridization data reinforce the idea that there are at least two CA genes in *A. thaliana* and, in addition, show that the two cDNAs sequenced are derived from the *Arabidopsis* genome.

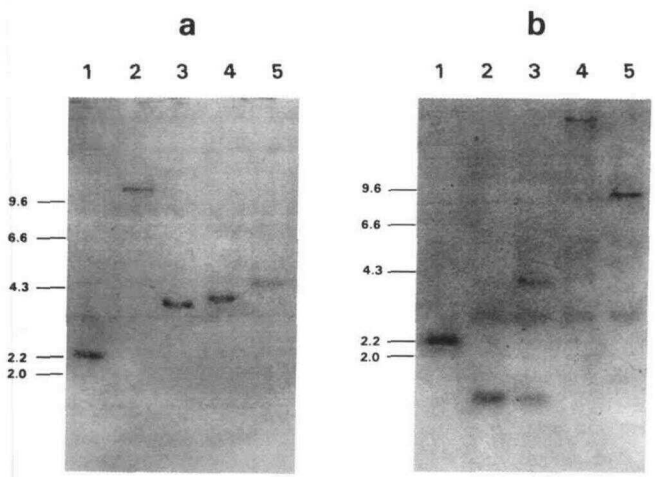


**Figure 5.** Southern blot analysis of *Arabidopsis* leaf genomic DNA digested with *Hin*dIII (lane 1), *Sac*I (lane 2), *Nsi*I (lane 3), *Sal*I (lane 4), and *Bcl*I (lane 5) and hybridized with a radiolabeled 723-bp *Hin*dIII-*Nde*I restriction fragment from the CA1 cDNA (a) or a radiolabeled 515-bp *Sac*I-*Sac*I restriction fragment from the CA2 cDNA (b).

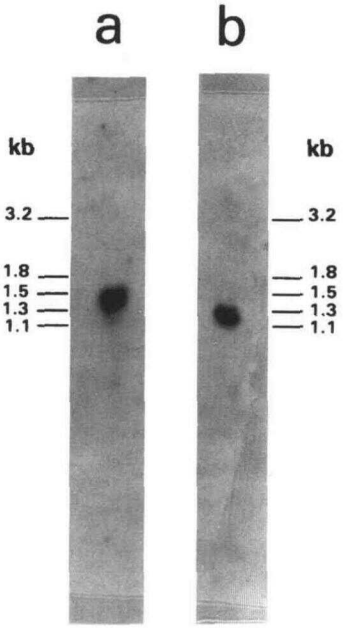


**Figure 6.** Northern blot analysis of total RNA from *A. thaliana* leaves. Equal amounts (10 μg) of RNA were probed with a 723-bp *Hin*dIII-*Nde*I restriction fragment from the CA1 cDNA (a) or a 515-bp *Sac*I-*Sac*I restriction fragment from the CA2 cDNA (b).

### RNA Blot Hybridization with cDNA Clones

To determine whether both CAs are expressed in *Arabidopsis* leaves (the library screened was made from all aboveground parts, including flowers and siliques), total leaf RNA was subjected to northern analysis. Radiolabeled fragments of both CA1 and CA2 hybridized to leaf RNA, identifying 1.47- and 1.2-kb transcripts, respectively (Fig. 6). Because the two hybridization signals were somewhat similar in size, the presence of two distinct transcripts was confirmed by stripping the blots and reprobing with the opposite CA gene. This resulted in the identification of the expected transcript size on each blot (data not shown). These data reinforce our hypothesis that the CA2 gene product is shorter than the CA1 transcript and presumably does not encode a transit peptide. As such, CA2 would be the first cytoplasmic plant CA gene to be identified and sequenced in $C_3$ plants. Sugiharto et al. (1992) have reported unpublished results in which two maize mRNA bands were detected on northern blots probed with a CA cDNA clone. This suggests that *Arabidopsis* may not be the only plant with more than one CA gene.

### Differential Expression of the Two CAs in *Arabidopsis*

Both CA1 and CA2 transcript levels (steady state) were reduced when 3-week-old plants were transferred to dark conditions (Fig. 7). The decrease, however, was much more pronounced for the CA1 transcript than for the CA2 transcript, accounting for 70 and 20% reductions in transcript abundance after 24 h, respectively. It was also apparent that CA mRNA levels stabilized after 24 h, because there was no further reduction following 48 h of dark exposure. It is interesting to note that, although CA2 transcript levels under light correspond to only 35% of the CA1 levels (Fig. 7, light), the reduction of CA1 transcript abundance in the dark is so severe that the CA2 transcript now represented the more abundant CA mRNA species.

The significant decline in CA1 transcript abundance following transfer to dark conditions was not unexpected. It has been shown previously that accumulation of nucleus-encoded mRNAs of proteins involved in photosynthesis, such as cab and rbcS, decreases in the dark (Giuliano et al., 1988; Chory et al., 1989). We have shown previously that CA and Rubisco expression in pea is coordinated during development and that CA expression is modulated with respect to levels of Rubisco activity in mature plants (Majeau and Coleman, 1994).

The reduced expression of CA1 and the continued expression of the extrachloroplastic CA2 in the dark is similar to that observed for *Arabidopsis* glyceraldehyde-3-phosphate dehydrogenase, a protein with two chloroplastic and one cytoplasmic isoform (Yang et al., 1993). Transfer of plants from light to dark conditions for a period of 5 d resulted in the elimination of *GapB* and a significant decline in *GapA* mRNAs, which encode the chloroplastic isoforms, whereas the cytoplasmic *GapC* mRNA was still abundant. The addition of Suc to dark-adapted plants resulted in a significant increase in the expression of *GapC* but had little impact on expression of the chloroplastic isoforms, suggesting a role for cytoplasmic glyceraldehyde-3-phosphate dehydrogenase in glycolytic carbon metabolism. In a similar manner, the continued expression of CA2 in dark-adapted leaves suggests a role for the extrachloroplastic CA that is distinct from photosynthesis. In a role similar to that evoked for the mesophyll cell-localized CA in $C_4$ plants, it is possible that the enzyme is required for catalysis of $HCO_3^-$ synthesis, the substrate for a cytoplasmic PEPcase (Hatch and Burnell, 1990). The anaplerotic synthesis of carbon skeletons by a $C_3$ PEPcase in the cytoplasm is required for amino acid biosynthesis as well as replenishment of Krebs cycle intermediates (Champigny and Foyer, 1992).

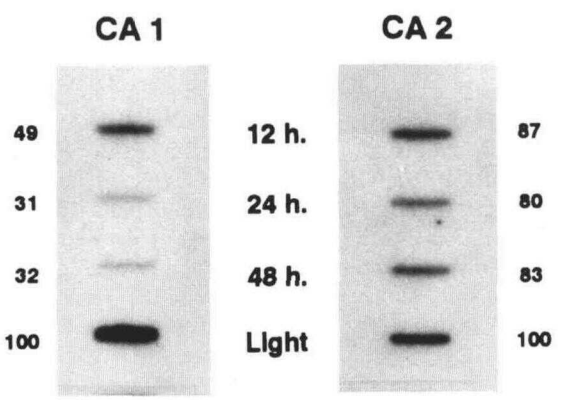


**Figure 7.** Slot-blot analysis of total RNA isolated from *Arabidopsis* grown for 3 weeks under light and transferred to dark for 12, 24, and 48 h. Slot blots were hybridized to radiolabeled fragments of CA1 and CA2. Numbers represent percentage of hybridization relative to control plants kept under light for each of the times, as determined by phosphorimaging.

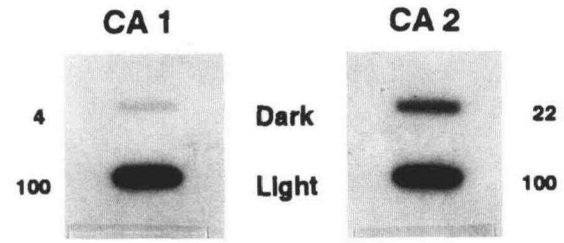


**Figure 8.** Slot-blot analysis of total RNA isolated from *Arabidopsis* seedlings germinated and grown for 10 d under light or dark. Slot blots were hybridized to radiolabeled restriction fragments of CA1 and CA2. Numbers represent percentage of hybridization relative to that obtained from RNA extracted from seedlings germinated under light, as determined by phosphorimaging.

It has been suggested that the primary effect of light on gene expression is mediated by the activation of leaf development (Chory et al., 1989). To determine whether CA1 or CA2 expression is controlled by leaf development, *Arabidopsis* seeds were germinated under dark and light conditions. Following 10 d of growth, total RNA was extracted and subjected to slot-blot northern analysis. Figure 8 shows that expression is certainly lower in dark-germinated seedlings; however, CA2 transcript levels are significantly higher than those of CA1 (22 and 4% of those of light-grown seedlings, respectively). These data suggest that CA1 expression is dependent on leaf and/or chloroplast development. The constitutive expression of CA2 in etiolated seedlings strengthens the argument for a nonphotosynthetic role for this isoform. The low levels of CA2 expression (relative to those in light-grown seedlings) may be a function of the limited metabolic activity of etiolated seedlings, which rely totally on cotyledon reserves for energy. Higher levels of expression would be achieved in mature tissues that are fully metabolically active.

#### ACKNOWLEDGMENT

The authors thank Dr. P. McCourt (Department of Botany, University of Toronto) for the use of the *A. thaliana* cDNA library and for much helpful advice and discussion.

Received November 12, 1993; accepted February 24, 1994.
Copyright Clearance Center: 0032-0889/94/105/0707/07.
The GenBank accession number for the sequence reported in this article is L18901.

#### LITERATURE CITED

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