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Regulation of Isocitrate Lyase Gene Expression in Sunflower[1]

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ABSTRACT

A cDNA sequence that encodes a portion of sunflower (*Helianthus annuus* L.) seedling isocitrate lyase was selected from a lambda gt11 cDNA library derived from sunflower seedling cotyledon poly(A)$^+$ messenger RNA. The library was screened for bacteriophage recombinants that expressed antigens which reacted with antisera directed against cotton seed isocitrate lyase. The isolated cDNA hybridized with a 2 kilobase RNA species that was first detectable in maturing sunflower embryos 19 days after flowering and remained at a constant low level through seed desiccation. The prevalence of this transcript in sunflower cotyledons increased by about 10-fold within 2 days after inhibition in darkness, and transcript levels began to decrease by 5 days after imbibtion. During the first 2 days of germination and growth of sunflower seedlings in light, the rate of isocitrate lyase mRNA accumulation was greater than the rate observed during this period in dark-grown seedlings, giving peak levels about 2-fold higher than corresponding levels in dark-grown seedlings. Illumination of seedlings also promoted an earlier, and more rapid decline of isocitrate lyase transcripts. Peak levels of isocitrate lyase mRNA preceded a corresponding peak in immunologically detectable isocitrate lyase polypeptides by about 24 hours. Isocitrate lyase expression in sunflower cotyledons is developmentally regulated and is modulated, in seedlings, by exposure to light. Mechanisms that control these processes appear to function primarily at the level of mRNA accumulation and are likely to involve changes in transcription rates and/or mRNA stability.

Cotyledons of sunflower and other oilseeds accumulate and store large amounts of lipid and protein which serve as nutrition sources for the seedling after germination. During seedling growth, cotyledons transform from storage organs into the primary photosynthetic structures of the young plant. This transformation is a developmentally controlled process that is modulated by exposure to light and/or growth regulators (12, 22). ICL[3] (EC 4.1.3.1) and other glyoxylate cycle enzymes are contained in glyoxysomes of maturing and germinated oilseeds (11). In germinated seeds, these enzymes are involved in the conversion of acetyl CoA, derived from β-oxidation of fatty acids, into succinate which serves as substrate for carbohydrate synthesis (26). These enzymes are of interest, not only for their role in seedling metabolism, but because their expression is highly regulated and much is known about their developmental appearance and subcellular compartmentation in a variety of organisms (11, 14). Activities of glyoxylate cycle enzymes increase dramatically following oilseed germination then diminish within 2 or 3 d of growth in the light as storage lipid is depleted and the seedlings become photosynthetically competent (5). Our objective is to understand the transcriptional and posttranscriptional systems that control expression of these developmentally regulated enzymes and the mechanisms by which they are incorporated into glyoxysomes. Also, since ICL has proven to be recalcitrant to analysis by biochemical methods (19), sequence analysis of DNAs that encode ICL will provide considerable information about the characteristics of this protein.

We describe the isolation of a cDNA sequence derived from sunflower ICL mRNA. This recombinant was selected from a λ-gt11 library by screening with antibodies against cotton ICL. This cDNA was used as a probe to examine the expression of ICL genes in maturing sunflower embryos and in seedlings grown in light or darkness. The correspondence of changing levels of ICL mRNA with the amount of ICL protein in seedlings at various stages of growth indicate that ICL gene expression is regulated largely at the level of ICL mRNA accumulation. Similar hybridization analyses have recently been reported for MS of cucumber cotyledons (23) and ICL in the heterotrophic endosperm of castor beans (17).

MATERIALS AND METHODS

Plant Material. Sunflower (*Helianthus annus* L. cv Giant Grey Stripe) seeds were obtained locally. Embryos were removed from achenes and surface sterilized with 10% (v/v) Chlorox for 10 min and rinsed thoroughly with H_2O. Sterilized embryos were germinated in Petri dishes on one-half strength Murashige and Skoog salts (pH 5.7) solidified with 1% (w/v) agar either in darkness or under continuous illumination (25 μE/m^2·s) at 28°C. Cotyledons, harvested from seedlings at 24 h intervals, were frozen in liquid N_2 and stored at −80°C. Embryos of immature sunflower seeds were collected from field grown plants at specified periods after anthesis of individual flowers. These specimens were also frozen in liquid N_2 and stored at −80°C. Cotton seeds (*Gossypium hirsutum* L. cv Deltapine 62) were germinated and grown at 30°C as previously described (7).

Immunological Procedures. Rabbit antibodies against purified cotton cotyledon ICL were prepared previously (7). The specificity of cotton anti-ICL for sunflower ICL was determined by double immunodiffusion, Western blot analysis, and immunotitration of sunflower ICL activity. Cotyledons of 3 DAI sunflower and cotton seedlings were homogenized with a motorized Teflon pestle in a medium containing 100 mM K-phosphate, 8 mM $MgCl_2$, 11 mM DTT, and 2 mM EDTA (pH 7.4) and the homogenates were centrifuged at 27,000g for 20 min at 4°C. Lipid layers were removed and the supernatants were recentrifuged at 190,000g for 1 h at 5°C. This supernatant was assayed for ICL

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[3] Abbreviations: ICL, isocitrate lyase; MS, malate synthase; PMSF, phenylmethylsulfonyl fluoride; DAI, days after inbibition; DAF, days after flowering; bp, base pair.

activity (6). Immunotitration of ICL activity was performed in 1 ml aliquots which contained 100 mM freshly prepared PMSF and increasing amounts of antiserum or preimmune serum. Samples were incubated at room temperature for 1 h then at 4°C overnight. Addition of 1% reconstituted *Staphylococcus* cells (Boehringer Mannheim) for 2 h at room temperature was required for precipitation of antibody-antigen complexes in sunflower samples. Samples were centrifuged for 15 min (4°C) in a microfuge and the supernatant was assayed for ICL activity. Relative ICL activity was computed as a percentage of control values (preimmune serum).

For Western blot analysis, cotyledon protein extracts were resolved on 12.5% SDS-polyacrylamide gels and the proteins were transferred to nitocellulose by electroblotting (24). Blots were probed with anticotton ICL (1:100 dilution of serum with a titer of 64:1) followed by reaction with ^{125}I-labeled protein A or donkey anti-rabbit IgG as previously described (1). Relative intensity of bands on autoradiograms of Western blots was determined by scanning densitometry (Joyce-Loebl scanning densitometer).

Preparation of cDNA Library. Total sunflower RNA was prepared, as described previously (1), by grinding tissues in a homogenization medium, extracting with phenol/chloroform/isoamyl alcohol (24:24:1) and precipitating with ethanol. RNA was then specifically precipitated with 2 M LiCl followed by 3 M K acetate. Poly(A)$^+$ RNA was prepared by oligo dT cellulose chromatography (2).

Methods for construction and screening of cDNA libraries in the bacteriophage vector, λ-gt11, have been described in detail elsewhere (1, 28). Therefore, only a brief outline of these procedures are given here. First strand cDNA was synthesized using avian myeloblastosis virus reverse transcriptase and oligo dT primers from poly(A)$^+$ RNA isolated from cotyledons of 3-DAI dark-grown sunflower seedlings. The second strand was produced using the Klenow fragment of DNA polymerase I and the two stands were cleaved with S1 nuclease. Synthetic *Eco*RI linkers were added, and the cDNAs were ligated into the unique *Eco*RI site in the Lac-Z gene of λ-gt11 and packaged *in vitro*. Packaged phage were plated on the host strain KM392 on media containing X-Gal as an indicator of β-galactosidase activity. Recombinant bacteriophage give colorless plaques on this media because they contain cDNA inserts that inactive the Lac-Z genes of these phage. The sunflower seedling cDNA library used in this study contained approximately 2×10^6 recombinants with a 25% background of nonrecombinant phage.

Screening of the λ-gt11 library was performed as previously described (1) by plating approximately 10^4 phage on KM392 in each of 12 large (15 cm) Petri dishes. Plaques were allowed to develop 6 to 8 h at 37°C, nitrocellulose filters were applied, and the plates were incubated at 37°C for an additional 12 h. Plates were then cooled to 4°C and the filters removed. The replica filters were probed with anticotton ICL, followed by ^{125}I-protein A, and exposed for autoradiography as described for Western blots. Immunoreactive plaques were selected and one, designated HaICL13, was rescreened to purity. Purified HaICL13 phage were amplified on the host strain KM392pMC9 (*lac*iq$^+$) and the phage DNA was isolated. The cDNA insert of HaICL13 was excised with *Eco*RI and subcloned into pUC19 and into M13mp19 for sequencing by the dideoxy-nucleotide method.

RNA Blot Experiments. Five μg of total sunflower cotyledon RNA was denatured with formaldehyde or glyoxal, then run on 1% agarose gels and transferred to nitrocellulose by blotting (16). Dot blots were prepared by applying 1 μg of total sunflower cotyledon RNAs directly to nitrocellulose using a dot blot manifold. Northern blots and RNA dot blots were prehybridized in a solution containing 50% (v/v) formamide, 5× SET (1× SET = 150 mM NaCl, 20 mM Tris-HCl pH 7.8, 1 mM EDTA), 25 mM Na phosphate, 10% (w/v) dextran sulfate, and 100 μg/ml denatured salmon sperm DNA and hybridized in the same solution plus 200 ng/ml HaICL13 cDNA insert DNA labeled to approximately 10^8 cpm/μg with [α^{32}P]dCTP and dATP by nick translation. Hybridization was carried out at 42°C for 15 h and blots were washed for 1 h each in: 2× SET wash (2× SET, 25 mM Na phosphate, 0.2% SDS), 1× SET wash and 0.1× SET wash at 65°C. Blots were exposed overnight to x-ray film at −70°C with intensifying screens. Relative intensity of hybridization signals on autoradiograms was determined by scanning densitometry. These experiments allow comparison of the relative fraction of ICL-specific RNA in total RNA of each stage.

RESULTS

Isolation of Sunflower ICL cDNA. Rabbit antiserum prepared against ICL (purified from 3 d old cotton seedlings) precipitated ICL activity from clarified homogenates of sunflower seedling cotyledons (Fig. 1). Approximately 3-fold more antiserum was required to precipitate the same enzyme units of sunflower ICL as was needed to complex and remove all of the cotton ICL. This antiserum also detected subunits of sunflower ICL on western blots of sunflower seedling cotyledon protein extracts (Fig. 2A). Anticotton ICL reacted with a single band on these blots that migrated at 64 kD which corresponded with the mol wt of cotton ICL subunits. In other experiments, a single precipitin band formed between anticotton ICL serum and cotton and sunflower extracts in agarose double immunodiffusion analyses (data not shown). Partial serological identity was observed between cotton and sunflower ICL. These results indicate that the polyclonal antibodies prepared against cotton ICL specifically cross-react with sunflower ICL.

Because reaction of anticotton ICL with sunflower ICL was specific and reasonably intense, it was used to screen a λ-gt11 cDNA library prepared from poly(A)$^+$ RNA of 3 DAI sunflower seedlings. The λ-gt11 library was screened for bacteriophage recombinants that accumulate antigen immunoreactive with anti-ICL. Approximately 7×10^4 recombinants were screened and a single clone that specifically reacted with anti-ICL was isolated. This recombinant phage, designated HaICL13, contained a cDNA insert approximately 200 bp in length.

The specificity of reaction of the anticotton ICL with HaICL13

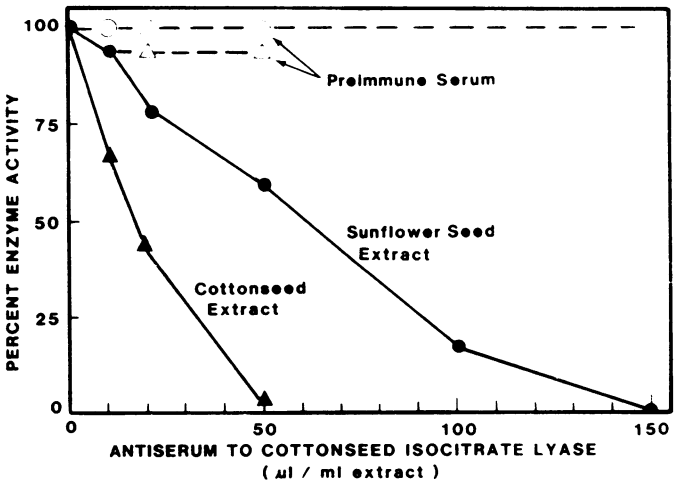

FIG. 1. Immunotitration of sunflower and cotton isocitrate lyase activity. Anticotton isocitrate lyase was used to immunoprecipitate isocitrate lyase from clarified homogenates of dark-grown, 3 DAI sunflower and cotton seedling cotyledons. All reactions initially contained equivalent units of ICL. Addition of *Staphylococcus* cells was required for precipitation of sunflower antibody-antigen complexes.

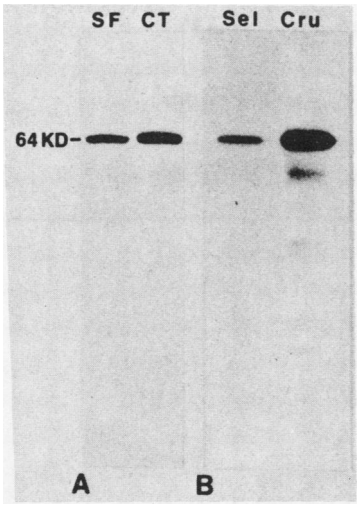

FIG. 2. Autoradiograph of Western blots. A, Sunflower (SF) and cotton (CT) seedling (3 DAI, dark-grown) cotyledon proteins were separated by SDS-PAGE and electroblotted to nitrocellulose. The blot was reacted with rabbit antiserum against cotton isocitrate lyase followed by secondary ^{126}I-labeled donkey anti-rabbit IgG. A 64 kD isocitrate lyase subunit band is present in both sunflower and cotton protein lanes. B, Blot of sunflower (3 DAI, dark grown) seedling cotyledon proteins reacted with crude rabbit anticotton isocitrate lyase (CRU) or with antibodies selected by affinity to HaICL13 bacteriophage filter screens (SEL). Antibodies that bind to HaICL13 plaques also react with genuine lyase polypeptides.

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     GG   A   A     C     A              G    C   G
 1   ACC AGA GGG ATG TTG GCT TAT GTG GAG AAG ATT CAA AGA
     Thr Arg Gly Met Leu Ala Tyr Val Glu Lys Ile Gln Arg
     Arg                                Arg

           A       A A         T       T C T   C
40   GAG GAG AGG AAG CAT GGG GTG GAC ACA TTG GCT CAT CAG
     Glu Glu Arg Lys His Gly Val Asp Thr Leu Ala His Gln
                    Asn

             C       T   C                   TAC   G  AA
79   AAA TGG TCA GGT GCA AAT TAT TAT GAT AGG GTG CTA AGG
     Lys Trp Ser Gly Ala Asn Tyr Tyr Asp Arg Val Leu Arg
                                              Tyr     Lys

       A   C   G   A       C T     C         C       G
118  ACT GTT CAA GGT GGG ATG ACT TCA ACT GCT GCT ATG GGC
     Thr Val Gln Gly Gly Ile Thr Ser Thr Ala Ala Met Gly
                           Ser

           G   G       A
157  AAA GGA GTT ACC GAG GAG
     Lys Gly Val Thr Glu Glu
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FIG. 3. Nucleotide and derived amino acid sequences of the cDNA insert of HaICL13. This sequence was compared with the corresponding region of an ICL cDNA sequence from castorbean endosperm and nucleotide and amino acid mismatches are indicated above and below, respecitively, those of the sunflower. These sequences are 90% homologous at the amino acid level.

was verified by elution of antibodies bound to filters prepared from plates containing 10^3 HaICL13 plaques. These filters were incubated in anti-ICL and washed under the same conditions as library screens. Bound antibodies were eluted with 5 mM glycine-HCl (pH 2.3) and immediately neutralized with 1 M Tris (pH 7.5). These selected antibodies reacted with the 64 kD ICL band on Western blots of sunflower seedling protein extracts (Fig. 2B). This reaction indicates that the antibodies which bind to the HaICL13 fusion protein specifically cross-react with genuine ICL and do not represent contaminating antibodies.

Positive identification of HaICL13 was provided by comparison of its nucleotide sequence with the corresponding region of an ICL cDNA sequence from castorbean (4). HaICL13 is homologous with castorbean ICL cDNA sequences located between 256 and 82 bp upstream of the termination codon (Fig. 3). These cDNAs are 78% conserved at the nucleotide level and the amino acid sequences derived from them are approximately 90% identical (51 of 57 amino acids).

Expression of ICL in Sunflower. The cDNA insert of HaICL13 was labeled with ^{32}P by nick translation and used as a probe for RNA gel blot analysis. HaICL13 hybridizes with a single RNA band of about 2 kb on sunflower seedling RNA gel blots (Fig. 4A). Accumulation of ICL mRNA during sunflower seed maturation and postgerminative growth was investigated by hybridization of HaICL13, with RNA dot blots (Fig. 4B). One μg of total RNA each from immature seeds (7, 9, 12, 15, 19, and 25 DAF), dry seed, light-grown seedling cotyledons (1, 2, 3, and 4 DAI), and leaves was applied to nitrocellulose and hybridized with HaICL13. Early-stage immature seed and leaf RNA did not contain ICL transcripts that were detectable above background. Low levels of ICL mRNA were first detected in mid- to late-stage maturing seeds (19 DAF) and this low level was maintained throughout seed maturation and desiccation. A large increase in the prevalence of ICL mRNA was apparent within 1 d after imbibition of sunflower seeds. ICL transcript levels peaked between 1 and 2 DAI and was still detectable at 4 DAI. The effect of light on the accumulation and disappearance of ICL mRNA in developing seedling cotyledons was investigated by RNA gel blot hybridizations (Fig. 5, A and B). Seedlings were grown under continuous light or in total darkness; cotyledons were harvested at d 0 (dry seeds) and at 24 h intervals on d 1 through 5. RNA purified from these cotyledons was run on formaldehyde-agarose gels, blotted to nitrocellulose, and hybridized with nick translated HaICL13 (Fig. 5A). The relative intensity of bands on these blots was determined by scanning densitometry and these values are plotted in Figure 5B. ICL transcripts were barely detectable in dry seeds and accumulated to peak levels in both light and dark-grown seedlings by 2 DAI. For dark-grown seedling RNA, the peak level represents approximately a 10-fold increase in hybridization signal over dry seed RNA. The prevalence of ICL transcripts in these cotyledons was constant through 4 DAI but dropped by about 50% during the next 24 h. ICL mRNA increased to greater levels in cotyledons of light-grown seedlings than in seedlings grown in darkness (Fig. 5B). Peak ICL transcript prevalence at 1 and 2 DAI in light-grown seedlings were approximately 2-fold higher than corresponding dark-grown seedlings. After 2 DAI, ICL transcript levels in light-grown seedlings declined rapidly and were at or below dry seed levels by 5 DAI.

These observations are based on comparisons of the relative amount of ICL RNA in total RNA. However, the amount of total RNA extracted from dark-grown seedling cotyledons increased approximately 4-fold between 1 and 4 DAI and remained constant through 5 DAI. Total RNA from light-grown seedling cotyledons increased similarly, but yields were 10 to 20% greater from 2 through 5 DAI light-grown seedlings than from corresponding dark-grown specimens. Because the amount of RNA in growing sunflower seedlings increases during growth, estimations of ICL transcript prevalence underrepresent the total increase in ICL transcripts on a per cotyledon basis.

Differences were also seen in the amount of sunflower ICL polypeptide in cotyledons of light- and dark-grown seedling as detected on Western blots with antibodies against cotton ICL (Fig. 6, A and B). The patterns of ICL polypeptide accumulation and decline in these seedlings were similar to those for the prev-

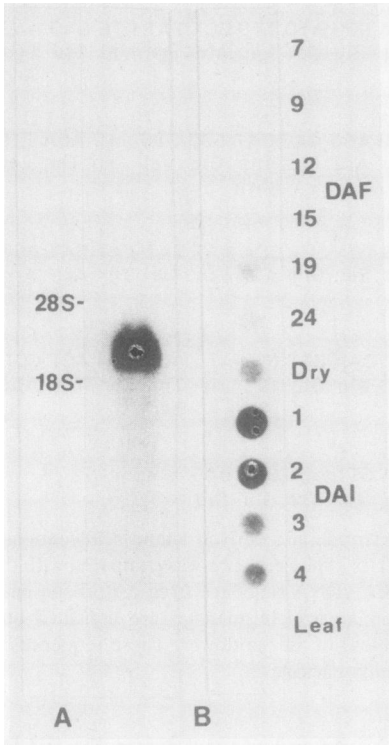

FIG. 4. A, Autoradiogram of sunflower seedling RNA (3 DAI) gel blot. Total RNA was denatured with glyoxal, electrophoresed on an agarose gel, and hybridized with ^{32}P-labeled HaICL13 cDNA. A single band about 2 kb in length is detected with this probe. Positions of major ribosomal RNA bands are indicated. B, Autoradiogram of sunflower RNA dot blot hybridized with ^{32}P-labeled cDNA insert of HaICL13. One μg of total RNA was applied from immature seeds (DAF), dry seeds, light-grown seedlings (DAI), and leaves. Hybridization is not detecable between HaICL13 and leaf RNA or immature-seed RNA before 19 DAF. Peak hybridization signals were seen with RNA samples from 1 and 2 DAI seedlings.

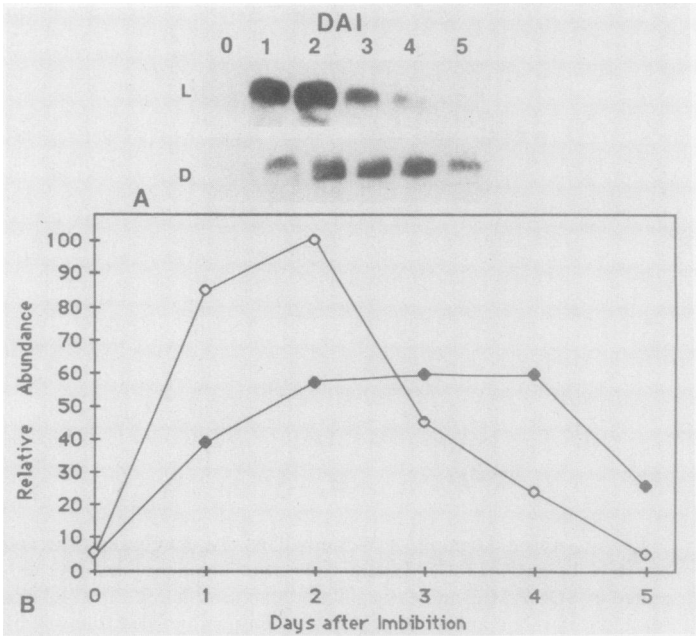

FIG. 5. A, Autoradiograph of gel blot of RNA from sunflower cotyledons of dry seeds (O DAI) and seedlings (1–5 DAI) germinated and grown in darkness (D) or in continuous light (L). Denatured RNA was run on formaldehyde-agarose gels, transferred to nitrocellose and hybridized with ^{32}P-labeled HaICL13 cDNA. B, Intensity of hybridization signals from autoradiographs similar to those in Figure 5A were quantitated by scanning densitometry. Relative levels of hybridization are presented as percentages of the highest signal obtained. Open symbols indicate light grown seedlings, and closed symbols indicate dark grown seedlings.

alence of ICL mRNA but changes in the amount of ICL protein were delayed by about 24 h (*cf.* Figs. 5B and 6B). A small amount of ICL polypeptide was detected in protein extracts of dry seed and in 1 DAI dark-grown and light-grown seedling cotyledons. A 5-fold increase in autoradiographic signal was detected for ICL protein extracted from 2 DAI cotyledons grown in darkness, and the ICL protein level increased slightly through d 5. ICL polypeptides in light-grown seedling cotyledons reached peak levels on d 3 that were nearly 2-fold greater than in 3 DAI dark-grown seedlings. After reaching peak values, ICL polypeptides in light-grown seedlings decreased to about 40% of the maximum observed level by 5 DAI.

DISCUSSION

We describe the isolation of a cDNA recombinant, HaICL13, that represents a sunflower seedling ICL mRNA. HaICL13 was selected from a λ-gt11 cDNA library by screening with antibodies made against cotton ICL that cross-react with sunflower ICL. Specific reaction of this antiserum with HaICL13 indicates that this clone contains an insert that encodes a portion of the ICL protein and that this insert is expressed as an antigen during bacteriophage replication. We have used this approach previously to isolate cDNAs that represent major sunflower seed storage proteins (1). The cDNA insert of HaICL13, which has strong sequence homology with an ICL cDNA derived from castorbean endosperm, hybridized with an RNA species about 2 kb in length. This transcript appeared in immature and germinated sunflower seeds at specific stages of maturation (10) and postgerminative growth (8, 21). The changing levels of ICL mRNA corresponded well with the accumulation and decline of ICL polypeptides (Figs. 5 and 6).

Polypeptides that correspond to ICL subunits were detected in extracts of dry seeds by Western blot analysis, but we were unable to detect ICL polypeptides in immature seeds up to 25 DAF. Activities of glyoxysomal enzymes other than ICL have been detected in crude extracts of immature and dry seeds of a number of species (18). However, ICL activity has not been detected in crude homogenates of any oilseed species, suggesting that glyoxysomes in late stage maturing and mature seeds contain all glyoxylate cycle enzymes except ICL, and were therefore metabolically inactive (15). However, immature cucumber seeds were shown to contain immunologically detectable ICL but its activity could be measured only after partial purification by sucrose gradient centrifugation (9). These authors have suggested that crude homogenates of immature seeds contain an inhibitor which can block ICL activity, because extracts from ripening seeds strongly inhibit the activity of purified ICL. Activity of ICL has also been detected in partially purified extracts of mature and nearly mature sunflower seeds (10); this activity is associated with glyoxysomes in mature seeds. These data indicate that glyoxysomes in dry seeds contain all of the glyoxylate cycle enzymes, but the activity of ICL may be blocked until after germination. ICL mRNAs, on the other hand, were present at very low levels during late sunflower seed maturation (19 DAF) through seed desiccation. The presence of ICL mRNA has also been demonstrated in immature cucumber seed by *in vitro* translation of RNA (13). The appearance of ICL mRNA in maturing sun-

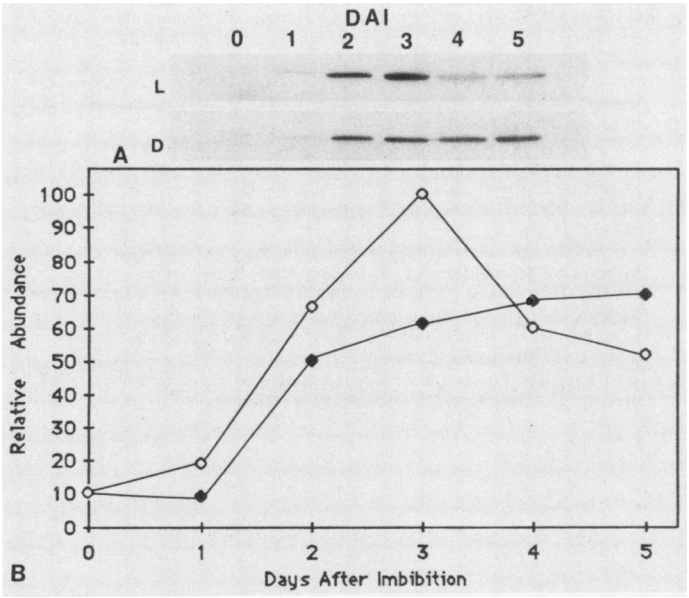

FIG. 6. A, Autoradiograph of Western blots of sunflower dry seed (0) and cotyledon (1–5 DAI) extracts of seedling grown in light (L) and dark (D). Proteins were resolved by SDS-PAGE, transferred electrophoretically to nitrocellulose, and reacted with rabbit anticotton isocitrate lyase and ^{125}I-donkey anti-rabbit IgG. B, Relative intensity of autoradiographic signals from autoradiographs as in 6(A) were determined by scanning densitometry, and are presented as percentages of the highest observed level. Symbols are the same as in Figure 5B.

flower seeds much earlier than ICL protein may indicate that ICL mRNA is not translated until very late stages of ripening, or that ICL mRNA or protein is unstable before seed maturity.

The prevalence of sunflower ICL mRNAs increased dramatically during germination, then diminished after a few days of seedling growth in the light (Fig. 5). A similar pattern of regulation has been reported for transcripts of several glyoxysomal enzymes, including ICL, using *in vitro* translation of RNA from cotyledons of germinating cucumber seed cotyledons (20, 27). Exposure of sunflower seedlings to light promotes the accumulation of ICL transcripts in the cotyledons, and ICL mRNAs disappear from illuminated seedling more quickly than dark-grown seedlings. Light regulation of MS in cucumber cotyledons also appears to function at the level of transcript prevalence (23). The reported effects of light on MS mRNA accumulation correlate with our observations for sunflower ICL mRNAs. In both cases, a rapid increase in mRNA levels, probably due to an increased rate of transcription, is followed by a rapid drop in transcript abundance under continuous light conditions. On the other hand, ICL and MS mRNAs accumulate more slowly in dark-grown seedlings, but elevated levels of these transcripts persist for longer periods than in seedlings grown in light. In castor bean endosperm, exogenously applied GA_3 was shown to promote the accumulation of ICL; these transcripts disappeared more rapidly from GA_3-treated tissues than from untreated control specimens (17). The kinetics of this effect are similar to those of the light regulation response of ICL mRNA in sunflower cotyledons and MS mRNA in cucumber cotyledons.

Previously reported data on light regulation of glyoxylate cycle enzyme mRNAs indicated a more rapid decline in mRNA abundance in light-grown seedlings compared to dark grown seedlings, but these studies failed to show increased levels of these transcripts during early stages of illuminated growth (3, 27). It is important to note that mRNA levels were determined in these experiments by *in vitro* translation of seedling RNAs while our results and those of Smith and Leaver (23) and Martin *et al.* (17) were determined by hybridization of cloned cDNAs to RNA blots. The differences between these observations may indicate that the absolute amount of transcript for these enzymes (detected by hybridization) in seedlings does not directly correlate with the level of translatable mRNAs. Becker *et al.* (3) have also shown that the recovery of glyoxylate cycle enzyme mRNAs by oligo dT cellulose chromatography is low, possibly indicating degradation of poly(A) tails of these transcripts. These data seem to indicate that mRNAs for glyoxysomal enzymes have a very rapid turnover rate which suggests that transcript stability may play an important role in regulating the expression of these enzymes.

The phototropic transformation of cotyledons of seedlings that germinate epigeneally appears to be promoted by illumination (22). Because cotyledons serve as storage sites as well as photosynthetic organs, rapid removal of stored materials from cotyledons exposed to light (26) may enable these organs to assume their phototrophic role more quickly. Conversely, in cotyledons of seedlings grown in darkness, food reserves are utilized more slowly (26). In this way nutrients are conserved until cotyledons are exposed to light.

Considerable uncertainty exists about the mechanisms of uptake of proteins into glyoxysomes (25). Import of ICL into glyoxysomes appears to occur posttranslationally, and apparently does not involve proteolytic cleavage of a 'transit peptide' because the mature protein has the same mol wt as the *in vitro* translation product (14, 25). Availability of cloned DNAs that encode glyoxysomal enzymes will be a valuable resource for further investigation of this important system. Sequence analysis of full length cDNAs may provide clues about polypeptide domains that are important for targeting to glyoxysomes and/or translocation into or across their membrane. Using HaICL13 as a probe, we have isolated longer sunflower ICL cDNAs and are currently sequencing these molecules. We plan to use these recombinants to continue our investigations of the regulatory systems that control the expression of ICL during seed maturation and seedling growth.

LITERATURE CITED

1. ALLEN RD, CL NESSLER, TL THOMAS 1985 Developmental regulation of sunflower 11S storage protein genes. Plant Mol Biol 5: 165–173
2. AVIV H, P LEDER 1977 Purification of biologically active mRNA by chromatography on oligothymadilic acid-cellulose. Proc Natl Acad Sci USA 69: 1408–1412
3. BECKER WM, H RIEZMAN, EM WEIR, DE TITUS, CJ LEAVER 1982 *In vitro* synthesis and compartmentalization of glyoxysomal enzymes from cucumber. Ann NY Acad Sci 386: 329–349
4. BEECHING JR, DR NORTHCOTE 1987 Nucleic acid (cDNA) and amino acid sequences of isocitrate lyase from castor bean. Plant Mol Biol 8: 471–475
5. BEEVERS H 1979 Microbodies in higher plants. Annu Rev Plant Physiol 30: 159–197
6. COOPER TG, H BEEVERS 1969 Mitochondria and glyoxysomes from castor bean endosperm. J Biol Chem 224: 3507–3513
7. DOMAN DC, RN TRELEASE 1985 Protein A-gold immunocytochemistry of isocitrate lyase in cotton seeds. Protoplasma 124: 157–167
8. FRANZISKET U, B GERHARDT 1980 Synthesis of isocitrate lyase in sunflower cotyledons during the transition in cotyledonary microbody function. Plant Physiol 65: 1081–1084
9. FREVERT J, W KÖLLER, H KINDL 1980 Occurrence and biosynthesis of glyoxysomal enzymes in ripening cucumber seeds. Z Physiol Chem 361: 1557–1565
10. FUSSEDER A, RR THEIMER 1964 Lipolytic and glyoxysomal enzyme activities in cotyledons of ripening and germinating sunflower (*Helianthus annuus*) seeds. Z. Pflanzenphysiol 114: 403–411
11. HUANG AHC, RN TRELEASE, TS MOORE JR 1983 Plant Peroxisomes. Academic Press, New York
12. ILAN I, S GEPSTEIN 1980 Hormonal regulation of food reserve breakdown in germinating dicotyledonous seeds. Isr J Bot 29: 192–206
13. KINDL H 1982 Glyoxysome biogenesis via cystolic pools in cucumber. Ann NY Acad Sci 386: 314–328
14. KINDL H, PB LAZAROW 1982 Peroxisomes and glyoxysomes. Ann NY Acad Sci 386
15. KÖLLER W, J FREVERT, H KINDL 1979 Incomplete glyoxysomes appearing at a late stage of maturation of cucumber seeds. Z Naturforsch 34: 1232–1236

16. MANIATIS T, EF FRITSCH, J SAMBROOK 1982 Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
17. MARTIN C, JR BEECHING, DH NORTHCOTE 1984 Changes in levels of transcripts in endosperms of castor beans treated with exogenous gibberellic acid. Planta 162: 68–76
18. MIERNYK JA, RN TRELEASE, JS CHOINSKI 1979 Malate synthase activity in cotton and other ungerminated oilseeds: a survey. Plant Physiol 63: 1068–1071
19. PINZAUTI G, E GIACHETTI, G CAMICHI, G MANAO, G CAPPUGI, P VANNI 1986 An isocitrate lyase of higher plants: analysis and comparison of some molecular aspects. Arch Biochem Biophys 244: 85–93
20. REIZMAN H, EM WEIR, CJ LEAVER, DE TITUS, WM BECKER 1980 Regulation of glyoxysomal enzymes during germination of cucumber 3. *In vitro* translation and characterization of four glyoxysomal enzymes. Plant Physiol 65: 40–46
21. SCHNARRENBERGER C, A OESER, NE TOLBERT 1971 Development of microbodies in sunflower cotyledons and castor bean endosperm during germination. Plant Physiol 48: 566–574
22. SHROPSHIRE W, K APEL 1983 Photomorphogenesis. *In* W Shropshire, H Mohr, eds, Encyclopedia of Plant Physiology. Springer-Verlag, Heidelberg, pp 270–277
23. SMITH SM, CJ LEAVER 1986 Glyoxysomal malate synthase of cucumber: molecular cloning of a cDNA and regulation of enzyme synthesis during germination. Plant Physiol 81: 762–767
24. TOWBIN H, T STAEHELIN, J GORDON 1979 Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4350–4353
25. TRELEASE RN 1984 Biogenesis of glyoxysomes. Annu Rev Plant Physiol 35: 321–347
26. TRELEASE RN, DC DOMAN 1984 Mobilization of oil and wax reserves. *In* DL Murray, ed, Seed Physiology, Vol 2. Academic Press, Sydney, pp 201–245
27. WEIR EM, H REIZMAN, JM GRIENENBERGER WM BECKER, CJ LEAVER 1980 Regulation of glyoxysomal enzymes during germination of cucumber. Temporal changes in translatable mRNAs for isocitrate lyase and malate synthase. Eur J Biochem 112: 469–477
28 YOUNG RA, RW DAVIS 1983 Efficent isolation of genes by using antibody probes. Proc Natl Acad Sci USA 80: 1194–1198