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Coordinate Expression of Transcriptionally Regulated Isocitrate Lyase and Malate Synthase Genes in *Brassica napus* L.

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We have analyzed the temporal and spatial expression of genes encoding the glyoxylate cycle enzymes isocitrate lyase and malate synthase in *Brassica napus* L. to determine whether they are coordinately expressed. Both enzymes participate in reactions associated with lipid mobilization in oilseed plant seedlings and are sequestered in a specialized organelle, the glyoxysome. We have identified an isocitrate lyase cDNA clone containing the complete protein coding region. RNA blot and in situ hybridization studies with isocitrate lyase and malate synthase cDNA clones from *B. napus* showed that the genes exhibit similar expression patterns. The mRNAs begin to accumulate during late embryogeny, reach maximal levels in seedling cotyledons, are not detected at significant amounts in leaves, and are distributed similarly in cotyledons and axes of seedlings. Furthermore, transcription studies with isolated nuclei indicate that the genes are controlled primarily although not exclusively at the transcriptional level. We conclude that glyoxysome biogenesis is regulated in part through the coordinate expression of isocitrate lyase and malate synthase genes.

INTRODUCTION

Isocitrate lyase (threo-D_s-isocitrate glyoxylate-lyase, EC 4.1.3.1) and malate synthase (L-malate glyoxylate-lyase [CoA-acetylating], EC 4.1.3.2) are key enzymes involved in storage lipid mobilization during the growth of higher plant seedlings (reviewed by Trelease and Doman, 1984). They participate in reactions of the glyoxylate cycle, a pathway responsible for the net conversion of two molecules of acetyl coenzyme A into succinate. The metabolic intermediate is ultimately converted into sucrose to serve as a primary nutrient source for growing seedlings until photosynthetic activity commences.

The two glyoxylate cycle enzymes are encoded by nuclear genes and compartmentalized in a specialized peroxisome, the glyoxysome, which contains glyoxylate cycle and β-oxidation enzymes (Breidenbach et al., 1967; Hutton and Stumpf, 1969). Biogenesis of the organelle is developmentally regulated; glyoxysomes are present primarily in developing seeds and in seedlings, although other peroxisomes are found in mature plant organs (reviewed by Huang et al., 1983; Trelease, 1984). In general, all peroxisomes appear to share a number of common characteristics: they are bounded by a single membrane, usually possess catalase and hydrogen peroxide-generating oxidases, and lack an organellar genome. However, the organelle fulfills different roles in distinct cell types that appear to be dictated by the environment or the differentiated state of the cell. For example, in contrast to glyoxysomes, leaf-type peroxisomes possess a different set of prevalent enzymes that are involved in photorespiration. Therefore, biogenesis appears to be controlled partially by the regulated accumulation of constituents unique to a particular class of peroxisomes.

To investigate the cellular processes controlling glyoxysome biogenesis, we have been studying the expression of isocitrate lyase and malate synthase genes in the oilseed plant *Brassica napus* L. In higher plants, the two enzymes appear to be associated exclusively with glyoxysomes and, therefore, they are excellent markers of organelle biogenesis. We are particularly interested in determining whether genes encoding the two enzymes are coordinately regulated. To this end, we have identified isocitrate lyase cDNA clones from *B. napus* and determined the complete primary structure of the polypeptide. This cloned mRNA and malate synthase cDNA clones were used to measure mRNA levels in embryos, seedlings, and leaves and to localize the mRNAs in cotyledons and axes. We also determined whether the genes are transcriptionally regulated by assaying nuclear RNA synthesis in isolated nuclei. Our results indicate that glyoxysome biogenesis is at least partly controlled by both transcriptional and posttranscriptional processes regulating the two glyoxylate cycle enzyme genes. The temporal and spatial patterns of mRNA accumulation indicate that isocitrate lyase and malate synthase genes are coordinately expressed.

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RESULTS

Identification of Isocitrate Lyase cDNA Clones

To measure the prevalence of mRNA encoding isocitrate lyase, several cross-hybridizing cDNA clones were identified from a cDNA library of seedling mRNA sequences using an isocitrate lyase genomic clone from *Aspergillus nidulans* (Ballance and Turner, 1986) (see "Methods"). The identity of the cloned mRNAs was verified by determining the nucleotide sequence of the longest cDNA clone, pIL9, and comparing the primary structure of the deduced polypeptide with published sequences. Figure 1A shows representative restriction endonuclease sites present in the 2.0-kb insert fragment of pIL9 and the strategy used for sequence analysis. The longest open reading frame initiated by a methionine codon consists of 1728 bp that specify 576 amino acids flanked by 51 and 254 bp of the 5′ and 3′ untranslated region, respectively (data not shown). The presence of an in-frame translation stop codon upstream of the first methionine codon suggests that the cDNA clone contains the complete protein coding region. Comparison of the predicted amino acid sequence of *B. napus* isocitrate lyase with that deduced from a castor bean (*Ricinus communis*) cDNA clone (Beeching and Northcote, 1987) shows that the polypeptides contain the same number of residues and share 85% amino acid sequence identity (Figure 1B).

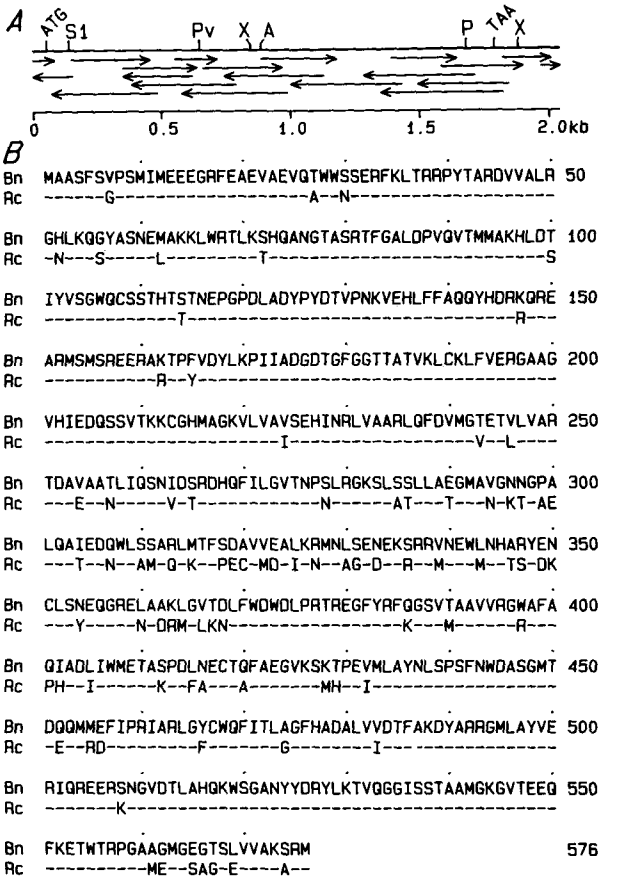

Figure 1. Characterization of an Isocitrate Lyase cDNA Clone from *B. napus*.

(A) Restriction map of isocitrate lyase cDNA clone pIL9. Arrows show sequenced regions of the insert. ATG and TAA represent the position of the deduced translation initiation and termination codons, respectively. Abbreviations: A, AvaI; P, PstI; Pv, PvuII; S1, SstI; X, XhoI.

(B) Comparison of the primary structure of isocitrate lyase from *B. napus* and castor bean. Amino acid sequence predicted from the nucleotide sequence of pIL9 from *B. napus* (Bn) and a castor bean (*Ricinus communis*) (Rc) cDNA clone (see text) were aligned. Amino acids are designated by the single letter code. Dashes indicate identical residues. Amino acids are numbered in the column to the right.

Temporal Patterns of Isocitrate Lyase and Malate Synthase mRNA Accumulation

Isocitrate lyase and malate synthase enzyme activities are prevalent in cotyledons of *B. napus* seedlings, present at low levels in dry seeds, and not detected in leaves (W. F. Ettinger and J. J. Harada, unpublished results). To determine whether the modulation in enzymatic activity corresponds to changes in mRNA amounts and to compare precisely the accumulation of isocitrate lyase and malate synthase mRNA, we measured the prevalence of each mRNA using cloned probes. The isolation and characterization of malate synthase cDNA clones from *B. napus* has been described previously (Comai et al., 1989). The reaction of the cDNA clones with polyadenylated RNA isolated from seedlings grown for 2 days after the start of imbibition (2 DAI) was assessed in the RNA gel blots shown in Figure 2A. Isocitrate lyase and malate synthase cDNA clones each react with a single size class mRNA, approximately 2.3 kb and 2.1 kb in size, respectively. Identical results have been obtained using mRNA from other embryonic and postgerminative stages of growth (data not shown). mRNA levels were quantified by reacting a sequence excess of each cDNA clone with total polyadenylated RNA from embryos, seedlings, and leaves in RNA dot blot experiments. As shown in Figure 2B and summarized in Figure 2C, the mRNAs are prevalent in seedlings, preferentially accumulating in cotyledons, and are at very low levels in leaves ($<2.5 \times 10^{-4}$% of polyadenylated RNA; Harada et al., 1988). In agreement with the finding that the enzyme activities are detected in dry seeds, we also found that the mRNAs begin to accumulate late in embryogeny at approximately 35 to 40 days post-anthesis (DPA) and are stored in the dry seed. Similar results were obtained with both total and polysomal mRNA preparations (data not shown).

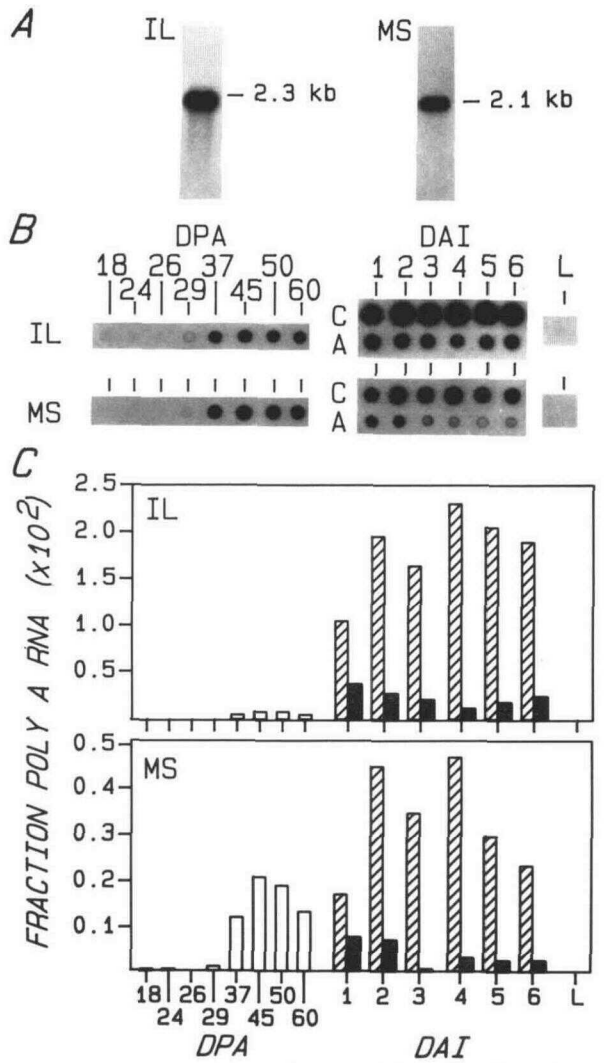

Figure 2. Coordinate Temporal Accumulation of Isocitrate Lyase and Malate Synthase mRNA.

(A) mRNA size. RNA gel blots containing 0.2 μg of total polyadenylated RNA from 2 DAI seedlings were reacted with isocitrate lyase cDNA clone pIL1 (IL) and malate synthase cDNA clone pMS19 (MS). mRNA sizes were estimated relative to RNA standards (Bethesda Research Laboratories).

(B) RNA dot blot hybridization reactions. Total polyadenylated RNA was isolated from embryos at the indicated days postanthesis (DPA), from dissected cotyledons (C) and axes (A) of seedlings at 1 to 6 days after the start of imbibition (DAI), and from leaves (L). Two-tenths microgram of each RNA was applied to replicate filters and the blots were reacted with a sequence excess of either ^{32}P-labeled pIL1 or pMS19.

(C) Isocitrate lyase and malate synthase mRNA prevalence. Following autoradiography, each dot was excised and counted by liquid scintillation spectrometry. For postgermination stages, crosshatched and filled bars represent mRNA levels in cotyledons and axes, respectively. Maximal counts/minute hybridized minus background (probe binding to 0.2 μg of tRNA) for pIL1 and pMS19 are 53,100 and 17,000, respectively.

Isocitrate lyase and malate synthase mRNAs accumulate at the same stages of embryogeny and postgerminative growth, but they attain different absolute levels (Figure 2C). Malate synthase mRNA is more prevalent than isocitrate lyase in dry seeds (0.13% and 0.07% of polyadenylated RNA, respectively), whereas isocitrate lyase mRNA reaches approximately fivefold higher levels than malate synthase in seedlings. The results show that genes encoding the two glyoxylate cycle enzymes display qualitatively similar but quantitatively distinct patterns of expression.

Distribution of Isocitrate Lyase and Malate Synthase mRNA in Seedlings

We examined the possibility that isocitrate lyase and malate synthase genes are coordinately expressed by determining the cellular location of the two mRNAs in seedlings. Transverse sections through a cotyledon pair from 2 DAI seedlings, shown in the bright-field photomicrograph in Figure 3A, were hybridized with in vitro synthesized RNA labeled with ^{35}S. Probes complementary (antisense RNA) and identical (sense RNA) to isocitrate lyase and malate synthase mRNA sequences were used to localize mRNA and to monitor nonspecific binding of labeled nucleic acids to the sections. Figure 3, B and C, shows dark-field micrographs of sections hybridized with isocitrate lyase and malate synthase ^{35}S-labeled antisense RNA, respectively. The distribution of autoradiographic silver grains which appear as white dots in the photomicrographs indicate that both mRNAs are relatively evenly distributed throughout the cotyledon. The autoradiographic signals most likely represent specific hybridization of the probes with mRNA, since neither isocitrate lyase nor malate synthase sense RNA bound appreciably to the sections (Figure 3D and data not shown). The experiment confirms our previous results showing that isocitrate lyase mRNA is prevalent throughout the cotyledons of 2 DAI seedlings (Dietrich et al., 1989) and shows that malate synthase mRNA also accumulates in similar locations.

Because the two mRNAs were detected in RNA isolated from axes (see Figure 2), we also examined their distribution in this seedling part. Longitudinal sections through the hypocotyl of a 2 DAI seedling, shown in the bright-field photomicrograph in Figure 3E, were hybridized with the probes. As shown in Figure 3, F and G, hybridization of isocitrate lyase and malate synthase antisense RNA probes, respectively, confirmed the presence of the mRNAs in axes. The silver grains are localized primarily at the periphery of the cells because the cytoplasm is compacted into the region by the vacuole. Strikingly, the results show that the two mRNAs are similarly distributed in axes. Both mRNAs accumulate primarily in the cortex but not vascular cylinder, and they are more prevalent in the apical end of the hypocotyl (oriented to the left in Figure 3, E-H) than in the basal region. Sense RNA probes did not bind

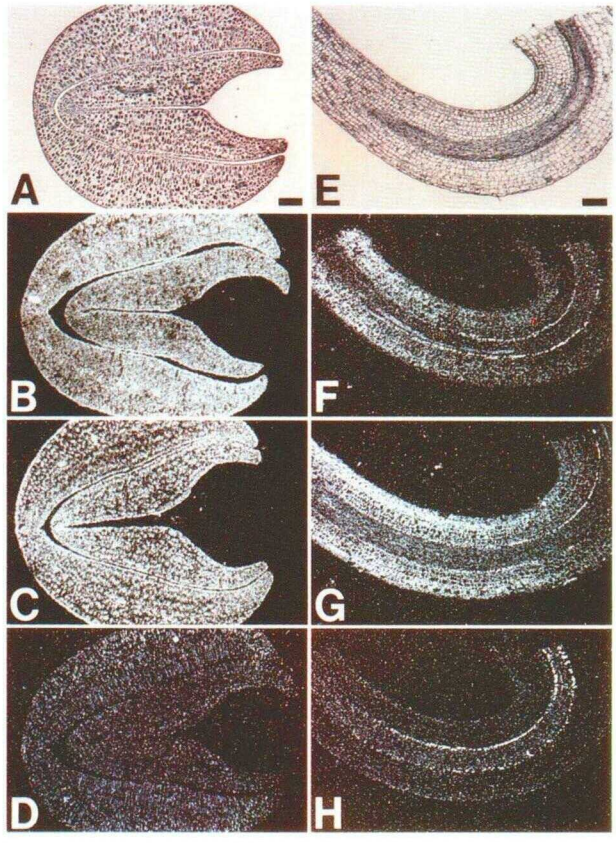

Figure 3. Distribution of Isocitrate Lyase and Malate Synthase mRNA in Seedlings.

Cotyledons (A to D) and axes (E to H) from 2 DAI seedlings were fixed, sectioned, and hybridized with ^{35}S-labeled RNA probes. Sections were exposed to emulsion for 2 days (B,D,F), 4 days (G,H), or 8 days (C).

(A) Bright-field photomicrograph of a transverse section through the cotyledon. Scale bar equals 200 μm.

(B, C) Sections hybridized with an isocitrate lyase **(B)** or malate synthase **(C)** antisense RNA probe as viewed with dark-field microscopy. White grains indicate the location of isocitrate lyase or malate synthase mRNA.

(D) Binding of an isocitrate lyase sense RNA probe with a section.

(E) A longitudinal section through the hypocotyl is shown in the bright-field micrograph. The enlarged basal hypocotyl region is oriented to the right. Scale bar equals 200 μm.

(F, G) Dark-field photomicrograph of sections hybridized with an isocitrate lyase **(F)** or malate synthase **(G)** antisense RNA probe. White regions at the border between the vascular tissue and the cortex are not silver grains (see **H**).

(H) Background binding of an isocitrate lyase sense RNA probe with the section.

significantly to axis sections (e.g. Figure 3H). The results suggest that isocitrate lyase and malate synthase mRNAs exhibit identical spatial accumulation patterns in 2 DAI seedlings.

Transcriptional Activity of Isocitrate Lyase and Malate Synthase Genes

Our previous results indicated that accumulation of isocitrate lyase and malate synthase mRNA is highly regulated. We next asked whether the genes are controlled at the transcriptional and/or posttranscriptional level using transcription assays with isolated nuclei (see "Methods"). In theory, "run-on" transcription experiments measure the density of RNA polymerase molecules on a particular gene, and, therefore, provide an estimate of relative transcriptional activity in vivo. In several cases, conclusions based upon assays with isolated nuclei have been verified using independent methods (Darnell, 1982, Morelli et al., 1985).

Figure 4 shows that ^{32}P-labeled nuclear RNA elongated in nuclei isolated from 37 DPA embryos and 2 DAI seedlings hybridize intensely with insert fragments from isocitrate lyase and malate synthase cDNA clones. In contrast, relatively little reaction of the cDNA probes with nuclear RNA synthesized in 24 DPA embryo and leaf nuclei is detected. The results suggest that the two genes are either inactive or transcribed at very low relative rates in 24 DPA embryos, are actively transcribed in 37 DPA embryos and 2 DAI seedlings, and are repressed in leaves. As a control, hybridization of nuclear RNA with a constitutively expressed gene represented by cDNA clone GS43 (Harada et al., 1988) demonstrates that the four sets of nuclei were competent to elongate nascent transcripts. The specificity of the transcription reactions is supported by the observation that the cDNA insert of a storage protein cruciferin cDNA clone (kindly provided by M. Crouch; Simon et al., 1985) only reacts to an appreciable extent with nuclear RNA from 37 DPA embryos. Others have observed similar patterns of transcriptional activity for cruciferin (A. DeLisle and M. Crouch, personal communication) and other storage protein genes (Walling et al., 1986). In addition, the nuclear RNAs did not react with pBR322 DNA. We conclude that isocitrate lyase and malate synthase genes are regulated primarily at the transcriptional level, although posttranscriptional processes must also influence the level of mRNA accumulation (see "Discussion").

DISCUSSION

Eucaryotic organisms often utilize the strategy of regulating unlinked sets of genes that participate in related developmental processes by a common mechanism (discussed in Britten and Davidson, 1969). To determine whether glyoxysome biogenesis is controlled through the coordinate expression of genes encoding glyoxylate cycle enzymes, we characterized the expression of isocitrate lyase and malate synthase genes.

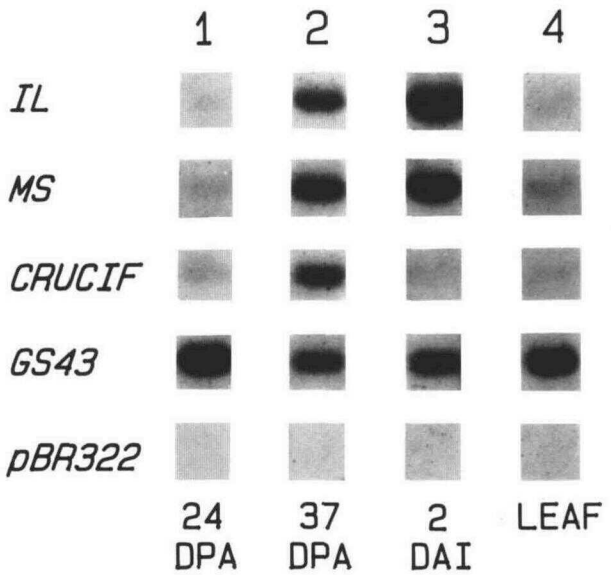

Figure 4. Transcriptional Regulation of Isocitrate Lyase and Malate Synthase Genes.

^{32}P-labeled nuclear RNA (1.5×10^7 cpm) synthesized in nuclei isolated from 24 DPA (lane 1) and 37 DPA (lane 2) embryos, 2 DAI seedlings (lane 3), and leaves (lane 4) were reacted with gel blots containing the following DNAs: 2.0-kb cDNA insert fragment from pIL9 (IL), 1.9-kb insert fragment from pMS1 (MS), 1.6-kb insert from the storage protein cruciferin cDNA clone pC1 (Crucif), 1.5-kb cDNA insert corresponding to the constitutively expressed gene GS43, and pBR322 DNA. The intensity of hybridization signals in 24 DPA and 37 DPA embryos, 2 DAI seedlings, and leaves, respectively, was estimated by densitometry and is given in arbitrary units. IL: 0.02, 0.40, 1.0, 0.06; MS: 0.08, 0.51, 0.85, 0.08; Crucif: 0.06, 0.35, 0.09, 0.07; GS43: 0.67, 0.45, 0.53, 0.53.

Identification and Structure of Isocitrate Lyase cDNA Clones

The identity of cloned isocitrate lyase mRNAs was confirmed by demonstrating that a cDNA clone specifies a predicted polypeptide which shares 85% sequence similarity with the enzyme from castor bean and contains an identical number of residues (Figure 1B). This percent sequence identity is significantly above the 25% level considered to indicate homology between proteins (Doolittle, 1986). Moreover, the *B. napus* and *Escherichia coli* (Matsuoka and McFadden, 1988) polypeptides are 38% similar.

Knowledge of the primary structure of isocitrate lyase may aid in defining structural determinants responsible for targeting the polypeptide to glyoxysomes/peroxisomes. Many peroxisomal proteins, including isocitrate lyase, are not detectably processed during posttranslational import into the organelle, suggesting that the sequences involved in targeting must be located within the mature polypeptide (Riezman et al., 1980; Roberts and Lord, 1981; Lazarow and Fujiki, 1985; Borst, 1986). We did not detect extensive regions of amino acid sequence similarity between isocitrate lyase and other plant peroxisomal proteins, i.e. malate synthase (Comai et al., 1989), soybean uricase (Nguyen et al., 1985), and spinach glycolate oxidase (Volokita and Somerville, 1987). However, we note that the carboxy-terminal three amino acids (Ser-Arg-Met) of isocitrate lyase are similar to those implicated to be both necessary and sufficient to target other proteins to peroxisomes (Ser-Lys/His-Leu; Gould et al., 1988). A role for the carboxy-terminal tripeptide in the trafficking of isocitrate lyase remains to be determined.

Isocitrate Lyase and Malate Synthase Genes Exhibit Qualitatively Similar Temporal and Spatial Patterns of Expression

Our results indicate that the level of isocitrate lyase and malate synthase mRNA is modulated during the plant's life cycle (Figure 2C). The mRNAs and enzyme activities (W. F. Ettinger and J. J. Harada, unpublished results) are detected in late maturation stage embryos and dry seeds, but they are most abundant in seedlings, consistent with their primary role in lipid mobilization during postgerminative growth. Others have reported similar temporal changes in isocitrate lyase and malate synthase mRNA levels (Weir et al., 1980; Martin et al., 1984; Smith and Leaver, 1986; Rodriguez et al., 1987; Turley and Trelease, 1987; Allen et al., 1988).

Genes that display similar temporal patterns of expression can be expressed in different tissues and, therefore, not coordinately regulated (Dietrich et al., 1989). We obtained additional support for the coordinate expression of isocitrate lyase and malate synthase genes by showing that the two mRNAs accumulate in similar locations in seedlings (Figure 3). The prevalence of both mRNAs in storage parenchyma cells of cotyledons is consistent with the activities of the enzymes because the majority of lipids are stored in these cells (Figure 3, B and C). Detection of the mRNAs in axes was also expected (see Figure 2B and Figure 3, F and G) since lipids are mobilized in this seedling part after imbibition (Kubacka et al., 1976). However, both mRNAs are distributed unequally in the cortex of hypocotyls (Figure 3, F and G). The accumulation pattern is not characteristic of all genes expressed in axes since we have shown that other unrelated mRNAs are distributed differently (Dietrich et al., 1989). Although the physiological processes that dictate this pattern of isocitrate lyase and malate synthase mRNA accumulation are not known, the results clearly show that two genes encoding enzymes participating in the same metabolic pathway and targeted to a common organelle appear to be coordinately expressed.

It is unclear whether all glyoxysomal constituents exhibit the same accumulation patterns as isocitrate lyase and malate synthase. Catalase appears to be present in all peroxisomes and, therefore, the gene(s) may be constitutively expressed (Huang et al., 1983). Miernyk and Trelease (1981) have shown that the activities of other glyoxylate cycle enzymes and β-oxidation enzymes accumulate before malate synthase activity becomes detectable in maturing cotton seeds. We have not yet studied other glyoxysomal/peroxisomal protein genes to determine whether they are expressed similarly to isocitrate lyase and malate synthase genes.

Accumulation of Isocitrate Lyase and Malate Synthase mRNA Is Transcriptionally and Posttranscriptionally Regulated

The coordinate accumulation of isocitrate lyase and malate synthase mRNA implies that the genes may be controlled through a common mechanism. Our results indicate that the genes appear to be transcribed actively only at stages of the life cycle when the mRNAs are prevalent (Figures 2 and 4), suggesting that transcriptional initiation is a primary control point regulating their expression. A corollary of the conclusion is that glyoxysome biogenesis is regulated in part by factors that control the transcriptional activity of isocitrate lyase and malate synthase genes

Although the genes display similar patterns of transcriptional activation and repression, there are quantitative differences in transcriptional activity. The ratio of relative transcription rates for isocitrate lyase and malate synthase genes in 37 DPA embryos is 0.8, whereas in 2 DAI seedlings it is 1.2 (Figure 4). The corresponding ratios of mRNA prevalence at the same stages are 0.6 and 4.3, respectively (Figure 2). We interpret the lack of correlation between transcriptional activity and mRNA level in 2 DAI seedlings to indicate that, although the genes are primarily regulated at the transcriptional level, posttranscriptional processes, e.g. nuclear RNA processing, translocation into the cytosol, and/or mRNA stability, also affect the level of mRNA accumulation. Thus, as has been shown for other plant genes (Beach et al., 1985; Berry et al., 1985; Walling et al, 1986; Lincoln and Fischer, 1988), a variety of mechanisms appear to influence expression of the two genes.

In summary, we have shown that isocitrate lyase and malate synthase genes display similar qualitative patterns of temporal and spatial expression and appear to be regulated predominately although not exclusively at the transcriptional level. Definitive proof for coordinate regulation will come through identification and comparison of *cis*-acting nucleotide sequences and *trans*-acting factors involved in regulating the genes. We anticipate that the information will also provide insight into the physiological signals that control glyoxysome biogenesis.

METHODS

Plant Material

Brassica napus L. (rapid cycling base population, CrGC5) embryos and seedlings were grown and staged as previously described (Harada et al., 1988). One- to four-centimeter leaves from plants 3 weeks old were used for RNA and nuclei isolations.

Identification of Isocitrate Lyase cDNA Clones

A *B. napus* genomic DNA library in Charon 35 (M. Gomez-Pedrozo, D. J. Maslyar, C. S. Baden, and J. J. Harada, in preparation) was reacted in low criterion hybridization experiments (50% formamide, 1 M Na^+, 30°C) with an isocitrate lyase genomic clone, pDJB11, from *Aspergillus nidulans* (generously provided by G. Turner; Ballance and Turner, 1986). One genomic clone, λIL116, identified with the *A. nidulans* probe, was then used to isolate cDNA clones from a library of *B. napus* seedling mRNA sequences (Harada et al., 1988). Two clones, pIL1 and pIL9, with cDNA insert fragments of 1.7 kb and 2.0 kb, respectively, were used in this study.

DNA Sequencing

pIL9 was recloned into plasmids containing an M13 intergenic region (Bluescribe, Stratagene, and pUC128, kindly provided by S. Tamaki). Deletion derivatives of the clones were generated using exonuclease III digestion protocols (Henikoff, 1984). Nucleic acid sequence was determined using the dideoxynucleotide chain-termination method (Sanger et al., 1977) with single-stranded (Dente et al., 1983) or double-stranded (Chen and Seeburg, 1985) DNA templates. The nucleotide sequence used to obtain the deduced amino acid sequence shown in Figure 1B has been submitted to GenBank (Los Alamos, NM).

RNA Isolation and Blot Hybridization

Isolations of total and polysomal polyadenylated RNA were performed as described by Harada et al. (1988). RNA gel and dot blot hybridizations and quantification of mRNA were done as described previously (Harada et al., 1988) using isocitrate lyase cDNA clone pIL1 and malate synthase cDNA clone pMS19 (Comai et al., 1989).

In Situ Hybridization

In situ hybridization was performed as described previously (Dietrich et al., 1989). To generate RNA probes for the hybridization reactions, cDNA insert fragments from pIL9 and pMS1 were recloned in a plasmid with T3 and T7 RNA polymerase promoters arranged in opposite orientations (Bluescribe, Stratagene). The 1.9-kb cDNA insert fragment of pMS1 contains the entire protein coding region of the malate synthase cDNA clone (Comai et al., 1989). ^{35}S-labeled RNA probes were synthesized in vitro using plasmid DNA templates and were hydrolyzed to a size of approximately 0.15 kb (Cox et al., 1984). Sections 7 μm thick were

prepared from 3-mm slices of 2 DAI seedlings that had been fixed in 10% formalin, 5% acetic acid, and 50% ethanol and embedded in paraffin. The cleared sections were hybridized with the RNA probes for 16 hr, treated with 50 μg/ml ribonuclease A, washed, and exposed to autoradiographic emulsion. Following development and staining with toluidine blue, the sections were photographed with an Olympus Vanox AHBT photomicroscope fitted with a dry dark-field and bright-field condenser.

Transcription Assays in Isolated Nuclei

Nuclei were isolated using the methods of Luthe and Quatrano (1980a; 1980b) except that buffers were adjusted to pH 8.5. Nuclear RNA synthesis in isolated nuclei was done in the presence of ^{32}P-UTP for 20 min as described by Walling et al. (1986). ^{32}P-UTP incorporation was linear for at least 20 min, dependent on the presence of all four nucleotides, and reduced 47% to 54% in the presence of 2 μg/ml α-amanitin. ^{32}P-labeled nuclear RNA was isolated using the procedures of Groudine et al. (1981) except that the final proteinase K digestion and phenol-chloroform extraction steps were omitted.

Gel blots containing 5 μg of plasmid DNA digested with restriction endonucleases that excise the cDNA insert were hybridized with ^{32}P-labeled nuclear RNA using conditions described by Lincoln and Fischer (1988). Filter-bound DNA is in sequence excess in hybridization reactions. Following hybridization, blots were washed extensively and treated with ribonuclease A prior to autoradiography. Hybridization intensity was measured by densitometry of autoradiograms using a Kontes Model 800 Fiber Optic Scanner and a Hewlett Packard 3390A Integrator.

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