Complementary Expression of Two Plastid-Localized σ-Like Factors in Maize[1]

Sushmita D. Lahiri and Lori A. Allison*

Department of Biochemistry, University of Nebraska, N258 Beadle Center, Lincoln, Nebraska 68588–0664

The eubacterial-like RNA polymerase of plastids is composed of organelle-encoded core subunits and nuclear-encoded σ-factors. Families of σ-like factors (SLFs) have been identified in several plants, including maize (*Zea mays*) and Arabidopsis. In vitro import assays determined that at least two of the maize σ-like proteins have functional chloroplast transit peptides and thus are likely candidates for chloroplast transcriptional regulators. However, the roles of individual SLFs in chloroplast transcription remain to be determined. We have raised antibodies against the unique amino-terminal domains of two maize SLFs, ZmSig1 and ZmSig3, and have used these specific probes to examine the accumulation of each protein in different maize tissues and during chloroplast development. The expression of ZmSig1 is tissue specific and parallels the light-activated chloroplast development program in maize seedling leaves. Its accumulation in mature chloroplasts however, is not affected by subsequent changes in the light regime. It is interesting that the expression profile of ZmSig3 is complementary to that of ZmSig1. It accumulates in non-green tissues, including roots, etiolated seedling leaves, and the basal region of greening seedling leaves. The nonoverlapping expression patterns of these two plastid-localized SLFs suggest that they may direct differential expression of plastid genes during chloroplast development.

The plastids differentiate from proplastids into a variety of specialized types, including leaf chloroplasts, etiolated leaf etioplasts, fruit and petal chromoplasts, and root amyloplasts and leucoplasts. All plastids contain a polyploid circular genome encoding proteins of the photosynthetic apparatus as well as proteins and RNAs involved in transcription and translation of plastid genetic information. The expression of plastid genes is regulated by a number of nuclear-encoded gene products in response to both environmental and developmental cues (for review, see Somanchi and Mayfield, 1999. Although much of this regulation is imposed post-transcriptionally, the importance of transcriptional regulation has also been recognized (Mullet, 1993).

Plastid-encoded genes are transcribed by two different RNA polymerase enzymes, termed NEP (nuclear-encoded plastid RNA polymerase) and PEP (plastid-encoded plastid RNA polymerase) (for review, see Hess and Börner, 1999). NEP RNA polymerase is entirely nuclear encoded and resembles the single subunit RNA polymerase of mitochondria in both subunit structure (Lerbs-Mache, 1993) and promoter recognition properties (Hajdukiewicz et al., 1997; Kapoor et al., 1997). Recently, candidate genes for plastid-localized, single-subunit RNA polymerases were cloned from Arabidopsis (Hedtke et al., 1997), maize (*Zea mays*; Young et al., 1998; Chang et al., 1999), and wheat (Ikeda and Gray, 1999), and their deduced sequences confirmed the close relationship of NEP to mitochondrial and T3/T7 bacteriophage RNA polymerases (Gray and Lang, 1998). The NEP enzyme is thought to comprise the predominant transcription activity in undifferentiated proplastids (Hess and Börner, 1999) and may have an additional, more limited role in mature chloroplasts (Hajdukiewicz et al., 1997; Silhavy and Maliga, 1998; Chang et al., 1999).

The principal transcription activity in mature chloroplasts is provided by the PEP RNA polymerase. In contrast to NEP, the subunit composition of PEP has been well characterized in a variety of plant species (Igloi and Kössel, 1992; Hess and Börner, 1999). The core enzyme, similar to the RNA polymerases of eubacteria, is composed of the plastid-encoded subunits α, β, β′, and β″. The promoters recognized by the PEP RNA polymerase contain functional sequence elements resembling the "−35" and "−10" motifs of eubacterial σ-70-type promoters. In bacteria these promoter sequences are contacted by the principal housekeeping σ-subunit, σ-70, which assembles with core RNA polymerase to form a promoter-selective holoenzyme (Gross et al., 1992; Lonetto et al., 1992, 1998). In plastids, σ-like factors (SLFs) were detected immunologically in chloroplast transcription extracts from maize and rice (Troxler et al., 1994) and were purified from chloroplasts and etioplasts of mustard seedlings (Tiller et al., 1991; Tiller and Link, 1993a). Recently, database searches as well as cDNA library screens uncovered candidate sequences for plant SLFs from Arabidopsis (Isono et al., 1997; Tanaka et al., 1997; Yao and Allison, 1998), rice (Tozawa et al., 1998), mustard (Kestermann et al.,

[1] This work was supported by the National Research Initiative Competitive Grants Program/U.S. Department of Agriculture (grant no. 97–35301–4514 to L.A.A.). This paper is journal series no. 12,820, Agricultural Research Division, University of Nebraska.

* Corresponding author; e-mail lallison1@unl.edu; fax 402–472–7842.

1998), wheat (Ito et al., 1999), and maize (Lahiri et al., 1999; Tan and Troxler, 1999). These sequences are nuclear encoded and form small gene families with at least six members in Arabidopsis and five in maize (for review, see Allison, 2000).

Proteins in the eubacterial σ-70 family contain four general regions of sequence similarity defined as regions 1 through 4 (Helmann and Chamberlin, 1988; Gross et al., 1992; Lonetto et al., 1992). The regions are further subdivided to indicate the most highly conserved domains. Region 1 is the least well conserved in both length and sequence composition (Lonetto et al., 1992). Region 2 is typically the most highly conserved portion and is divided into five subdomains: 2.1, thought to be involved in core RNA polymerase binding (Malhotra et al., 1996); 2.2, essential for formation of a tightly packed hydrophobic core (Malhotra et al., 1996); 2.3, proposed to be involved in promoter melting (Helmann and Chamberlin, 1988; Malhotra et al., 1996); 2.4, which contacts the −10 promoter motif (Malhotra et al., 1996); and 2.5, important for recognition of extended −10 promoters (Barne et al., 1997). Region 3 is thought to be involved in core RNA polymerase binding (Lonetto et al., 1992; Severinov et al., 1994), whereas region 4 interacts with the −35 motif of the core promoter (Lonetto et al., 1992). Each of the plant SLFs exhibits substantial sequence similarity with the conserved regions of eubacterial σ-factors (for diagram, see Fig. 1A) As with the eubacterial σ-factors, there is a higher percentage amino acid identity between the conserved C-terminal domains of different plant SLFs than there is within their NH_2-terminal regions (percent identities indicated in Fig. 1A for two maize SLFs). Based on sequence similarity the plant SLFs are proposed to function as promoter selectivity factors for the plastid-localized PEP RNA polymerase.

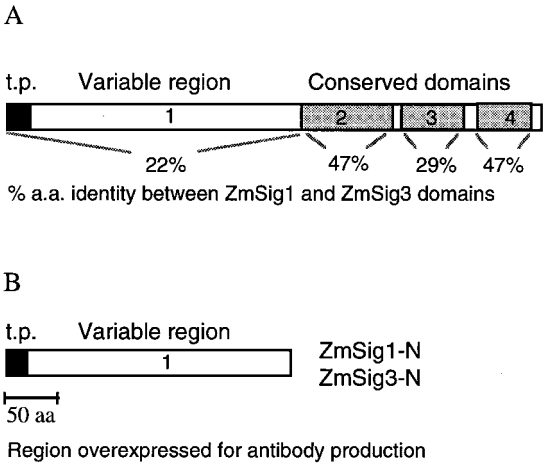

Figure 1. Diagram depicting features of plant polypeptides belonging to the σ-70 family. A, The domains of plant SLFs are indicated: The NH_2-terminal transit peptide sequence (t.p.) is indicated by a black box; the variable region 1 is depicted as a white box; conserved regions 2, 3, and 4 are diagrammed as gray boxes. Regions shown are as originally defined in *Escherichia coli* σ-70 by Lonetto et al. (1992). The percent amino acid identities between the analogous regions in ZmSig1 and ZmSig3 are shown. B, Diagram indicating the regions of ZmSig1 and ZmSig3 that were overexpressed for antibody production. Note that the highly conserved C-terminal region of each protein was not included in the recombinant protein antigens ZmSig1-N and ZmSig3-N.

If plant SLFs assemble with PEP RNA polymerase, the proteins must be targeted to the plastid compartment of plant cells. In addition to σ-like sequence regions, several plant SLFs have NH_2-terminal extensions with the sequence features of chloroplast transit peptides (for diagram, see Fig. 1A). Consistent with the presence of transit peptide sequences, three Arabidopsis SLFs were shown to be targeted to the plastid compartment (Isono et al., 1997; Kanamaru et al., 1999), two maize SLFs were demonstrated to encode NH_2-terminal chloroplast transit peptides that function in vitro (Lahiri et al., 1999), and two additional maize SLFs copurify with chloroplasts from maize leaf (Tan and Troxler, 1999).

Since they are encoded in the nuclear compartment, differential expression of plant SLFs could provide a mechanism for the plant nucleus to exert control over plastid gene transcription. To investigate this possibility, steady-state SLF transcript levels were measured in different tissues and under different light regimes. The five Arabidopsis SLFs exhibited very similar transcript accumulation patterns: mRNAs accumulated preferentially in leaf tissue compared to root tissue, and the accumulation in leaves was dramatically enhanced by light treatment (Isono et al., 1997; Tanaka et al., 1997; Yao and Allison, 1998; J. Yao and L.A. Allison, unpublished data). Reporter gene fusions to the promoters of two Arabidopsis SLF genes confirmed this similarity in expression (Kanamaru et al., 1999). Both promoters were similarly activated in cotyledons, hypocotyls, leaves, and siliques, but not in roots, seeds, or flowers.

Given the similarity of their expression profiles it is unclear how each Arabidopsis SLF contributes to regulation of transcription in chloroplasts. It is possible the gene family members are functionally redundant, or that each SLF recognizes a different version of the consensus PEP promoter. In contrast to Arabidopsis, investigations into maize SLF expression revealed significant differences in transcript accumulation among four of the SLF family members. Whereas transcripts for all of the maize *sig* genes accumulate in light-grown leaf tissue, two of the genes were also expressed in the etiolated leaves of dark-grown seedlings (Lahiri et al., 1999; Tan and Troxler, 1999), and transcripts of one of these genes were also detected in roots (Lahiri et al., 1999). These distinct expression profiles provided the first evidence that single maize SLFs may function in different tissues. In this paper, we establish distinct protein accumulation patterns for two of the maize SLF proteins, ZmSig1 and ZmSig3, providing evidence that these two SLFs accumulate in a nonoverlapping, or

complementary, profile in the maize seedling. These data support a model for differential SLF function during maize chloroplast development.

RESULTS

Antibodies against ZmSig1 Detect a Protein in Chloroplasts

The maize nuclear genome encodes a family of at least five genes whose protein products display sequence similarity to the principal σ-factors of eubacteria (Lahiri et al., 1999; Tan and Troxler, 1999). The three family members isolated by our group as cDNAs were named *sig1* (specifying the ZmSig1 protein), *sig2* (encoding the ZmSig2 protein), and *sig3* (encoding ZmSig3) (Lahiri et al., 1999). Two different family members were simultaneously isolated by Troxler and colleagues (1999) and named *sig1*, encoding the Sig1 protein, and *sig2*, specifying the Sig2 protein (Tan and Troxler, 1999). This confusion in nomenclature remains to be resolved, however for the purposes of this paper, *sig1* refers to the sequence deposited in GenBank under accession number AF099110, *sig2* under accession number AF099111, and *sig3* under accession number AF099112. We note that phylogenetic analysis of all maize and Arabidopsis SLFs characterized to date (Tan and Troxler, 1999; Allison, 2000) indicates that maize Sig1 and Sig2, reported by the Troxler group, are most closely related to the SigA protein of Arabidopsis (Tanaka et al., 1997; also referred to as SIG2 by Isono et al., 1997). In contrast, on the evolutionary tree (Allison, 2000) ZmSig1 and ZmSig2 cluster with the SigB protein of Arabidopsis (Tanaka et al., 1997; also referred to as SIG1 by Isono et al., 1997), whereas ZmSig3 clusters with a newly discovered Arabidopsis SLF, sig6 (GenBank accession no. AJ250812).

We had demonstrated previously that when fused to green fluorescent protein the NH_2-terminal 130 amino acids of either ZmSig1 or ZmSig3 directed the uptake of the heterologous protein into purified intact pea chloroplasts in vitro (Lahiri et al., 1999). Therefore, we anticipated that antibodies specific for each of these SLFs would detect immunoreactive protein in maize leaf chloroplasts. To generate antibodies specific to these two maize proteins the NH_2-terminal 295 amino acids of ZmSig1 (ZmSig1-N) and 297 amino acids of ZmSig3 (ZmSig3-N) were overexpressed in bacteria, purified, and used to inoculate rabbits (overexpressed regions indicated in Fig. 1B). The NH_2-terminal regions were chosen as antigens since the percent identity between the two proteins within the NH_2-terminal regions was significantly lower (22.6%) than within their C-terminal domain encompassing conserved regions 2 through 4 (Fig. 1A). The polyclonal antibodies raised against ZmSig1-N detected this protein on immunoblots, but did not cross-react with ZmSig3-N (data not shown). Antibodies against ZmSig3-N were similarly reactive against ZmSig3-N, but not against ZmSig1-N. To determine whether ZmSig1 and ZmSig3 proteins accumulate in maize leaf chloroplasts, protein extracts from seedling leaves and from purified chloroplasts were immunoblotted with anti-ZmSig1 and anti-ZmSig3 antibodies (Fig. 2). The anti-ZmSig1 antibodies revealed an immunoreactive protein in leaf extracts whose abundance was enhanced in chloroplasts (Fig. 2, lanes 3 and 4). The molecular mass of the immunoreactive protein was approximately 60 kD, which matched the predicted molecular mass of the ZmSig1 protein. These observations confirmed the chloroplast localization of the ZmSig1 protein. In contrast no immunoreactive protein of the expected size for ZmSig3 was seen in either leaf or chloroplast extract probed with anti-ZmSig3 antibodies (Fig. 2, lanes 7 and 8).

Detection of ZmSig1 in Bundle Sheath and Mesophyll Cells

Having demonstrated that ZmSig1 accumulated in leaf chloroplasts, we asked whether its expression was cell type specific. Leaves of C4 plants, such as maize, have agranal chloroplasts in the bundle sheath cells encircling the leaf vascular tissue, and granal chloroplasts in the mesophyll cells surrounding the bundle sheath (Langdale and Nelson, 1991; Furbank and Taylor, 1995). Consistent with their different roles in photosynthesis, bundle sheath and mesophyll chloroplasts differ in their expression of plastid-encoded genes (Link et al., 1978; Kubicki et al., 1994). Since these transcription differences may be mediated by different σ-factors in the two cell types, we asked whether ZmSig1 protein accumulated preferentially in one or the other cell type.

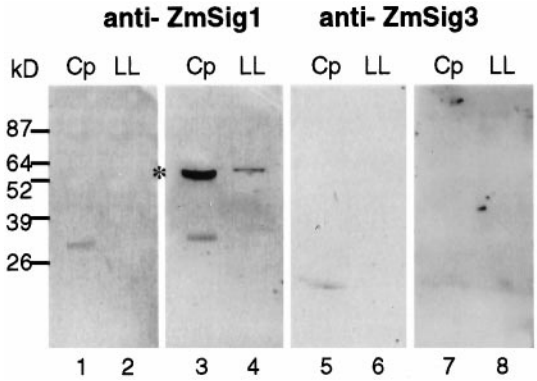

Figure 2. ZmSig1 is localized to maize leaf chloroplasts. Soluble proteins (20 μg) extracted from intact maize leaf chloroplasts (Cp) and from whole leaf tissue (40 μg, LL) were immunoblotted with either anti-ZmSig1 antisera (lanes 1 and 2, preimmune serum; lanes 3 and 4, immune serum) or with anti-ZmSig3 antisera (lanes 5 and 6, preimmune serum; lanes 7 and 8, immune serum). A chloroplast-localized immunoreactive protein was detected by anti-ZmSig1 as indicated by an asterisk. No immunoreactive proteins were detected in these tissues by anti-ZmSig3 antibodies. The positions of molecular mass standards are indicated to the left.

Equal amounts of total protein from each purified cell type were immunoblotted with several different antisera (Fig. 3). To determine the purity of each preparation the extracts were probed for phospho*enol*pyruvate carboxylase (PEPC), which accumulates specifically in mesophyll cells, and for Rubisco, which accumulates preferentially in bundle sheath cells of green leaves. Anti-PEPC antibodies detected protein in mesophyll cells but not in bundle sheath extracts, confirming the purity of the bundle sheath cell preparation (Fig. 3, lanes 1 and 2). Antibodies against Rubisco, detected Rubisco large subunit (LSU) in the bundle sheath preparation as expected (Fig. 3, lane 3), but also detected low levels of LSU in the mesophyll extracts (Fig. 3, lane 4), indicating that the mesophyll cell preparation contained some bundle sheath cell protein. When both extracts were probed with the anti-ZmSig1 antibodies, the immunoreactive protein was clearly detected in both cell types, most predominantly in the mesophyll cells. These results indicated that ZmSig1 protein was not expressed differentially between the two photosynthetic cell types of the maize leaf but accumulated in both.

The western data were confirmed by in situ immunolocalization experiments on maize leaf-blade sections incubated with the anti-ZmSig1 antibody (Fig. 4). In these sections, the red chlorophyll autofluorescence signal (Fig. 4, A and D) indicates the location of chloroplasts. Hybridization of leaf tissues with either preimmune serum (Fig. 4, A–C) or anti-ZmSig1 antibodies (Fig. 4, D–F) was followed by staining with fluorescence-conjugated secondary antibody. The secondary antibody visualized as a green fluorescent signal (excitation at 488 nm and emission at 520 nm) gave rise to a diffuse background staining in sections treated with preimmune serum (Fig. 4B), and a strong, punctate signal in sections treated with anti-ZmSig1 serum (Fig. 4E). Merging the green antibody signal (Fig. 4E) with the red chlorophyll signal (Fig. 4D) resulted in a yellow image for sections stained with anti-ZmSig1 antibody (F). This colocalization of the two signals indicated that ZmSig1 was present in chloroplasts. ZmSig1 was detected in the chloroplasts of bundle sheath and mesophyll cells (Fig. 4F). Note that the merged image resulted in green rather than yellow signals for bundle-sheath chloroplasts since chlorophyll autofluorescence in these organelles is weaker than in mesophyll cell chloroplasts (Fig. 4D).

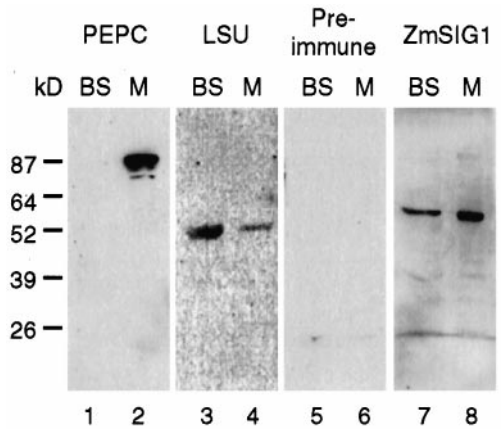

Figure 3. ZmSig1 is expressed in both bundle sheath and mesophyll cells of the maize leaf. In each panel 40 μg of proteins extracted from purified bundle sheath (BS) and mesophyll (M) cells was separated on SDS-PAGE and immunoblotted with the following antibodies: lanes 1 and 2, antibodies against maize PEPC; lanes 3 and 4, antibodies against maize Rubisco LSU; lanes 5 and 6, anti-ZmSig1 preimmune serum; and lanes 7 and 8, anti-ZmSig1 immune serum. The immunoreactive ZmSig1 protein was detected in pure preparations of bundle sheath cells (lane 7) as well as in the mesophyll cell preparation (lane 8). Note that, based on the LSU signals, the mesophyll cells were slightly contaminated with bundle sheath cell proteins (lane 3 versus lane 4). The positions of molecular mass standards are indicated to the left.

Leaf-Specific Expression of ZmSig1

Although ZmSig1 was not expressed in a cell type-specific manner in leaves, the protein accumulation is tissue specific (Fig. 5A). Total protein extracts were prepared from roots and leaves of greening maize seedlings grown for 2 d in darkness, followed by 2 d in cycling light conditions. The leaf tissues were harvested from two distinct sections of the seedling leaves: non-green leaf tissues encompassing the meristematic region 0 to 2 cm above the leaf base and green leaf tissues found 2 to 7 cm above the leaf base. In addition, etiolated leaf samples were harvested from 4-d-old maize seedlings grown in complete darkness. Equal amounts of protein from each extract were separated by SDS-PAGE and immunoblotted with anti-ZmSig1 antibodies. The antibodies detected ZmSig1 in greening leaf sections (Fig. 5A, lane 5), but not in any of the protein preparations for non-green plant tissues (Fig. 5A, lanes 6–8). Therefore, accumulation of ZmSig1 protein was specific for green tissues, consistent with its proposed role as a chloroplast SLF.

ZmSig1 Expression Correlates with Chloroplast Development

Since immunoblot analysis had shown that ZmSig1 protein accumulated in green sections of light-grown seedling leaves (LL in Fig. 5A) but was not detectable in the non-green leaf tissues adjacent to the leaf base (M in Fig. 5A), we asked whether the accumulation of ZmSig1 protein followed the chloroplast developmental gradient in light-grown maize seedling leaves (Fig. 5B). Maize exhibits a linear leaf development pattern with the youngest cells at the leaf base and the most mature cells at the leaf tip (Leech, 1984). Development and maturation of chloroplasts within the leaf cells follow the same linear gradient: Undif-

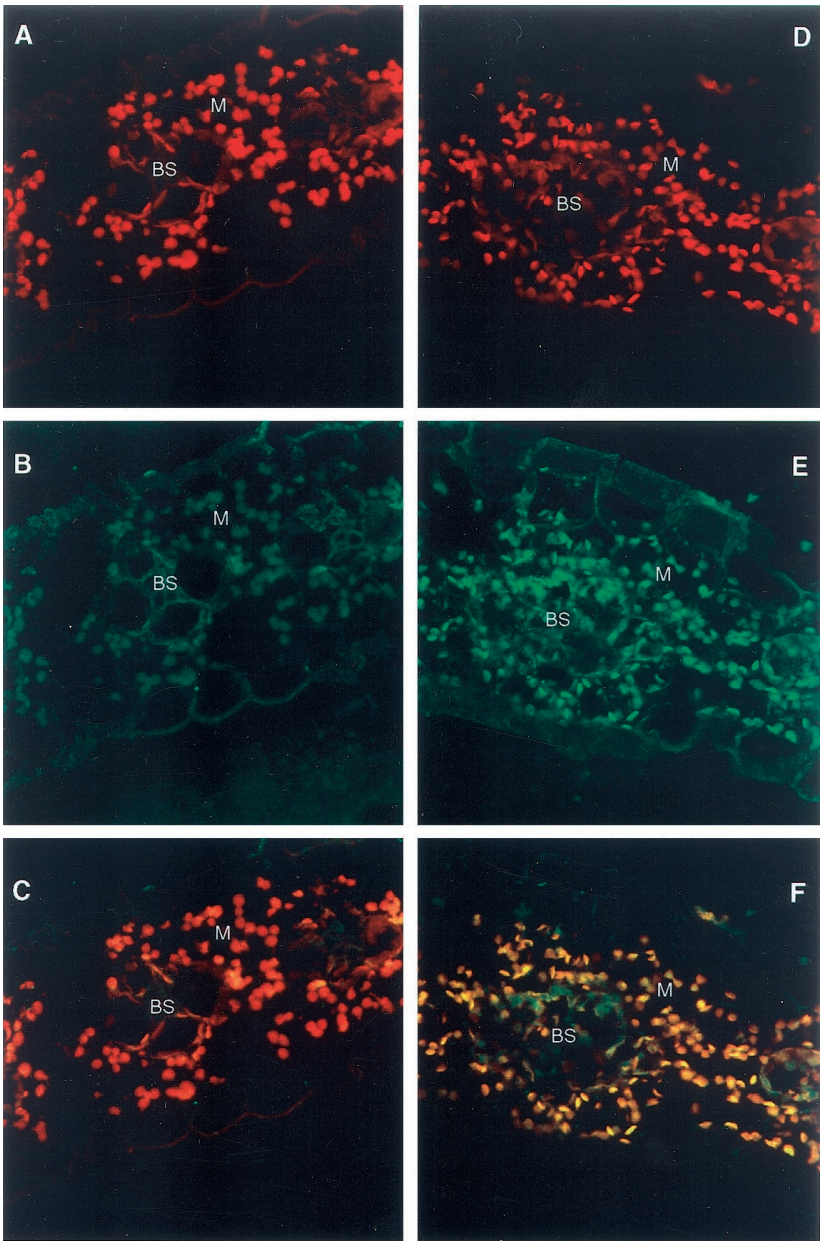

Figure 4. Immunocalization of ZmSig1 in mesophyll (M) and bundle-sheath (BS) cells of a maize leaf. Maize leaf sections from the mid-leaf region of 21-d-old seedlings were probed with preimmune (A–C) or anti-ZmSig1 (D–F) antisera and subsequently detected with an anti-IgG secondary antibody conjugated to a fluorescent probe. The signals were visualized using a Bio-Rad MRC600 confocal microscope. The red signals in A and D are due to chlorophyll autofluorescence within the chloroplast (excitation at 647 nm and emission at 666 nm); whereas the green fluorescence in B and E (excitation at 488 nm and emission at 520 nm) is due to the secondary antibody and thus represents immunoreactive protein. The immunoreactive protein is localized mainly in chloroplasts, since merging the red and green signals results in a yellow image (F). Note that, since bundle sheath chloroplasts have a less intense autofluorescent (red) signal than mesophyll cell chloroplasts, the immunoreactive protein signal in BS cells appears green in the merged image (F).

ferentiated proplastids are found in cells at the leaf base, whereas fully developed chloroplasts occupy cells at the leaf tip. Run-on transcription assays with plastids isolated from segments of developing barley leaves established that plastid genes are differentially transcribed during chloroplast development (Baumgartner et al., 1989, 1993; Mullet, 1993; Satoh et al., 1999), possibly due to differential σ-factor expression. To examine the expression of ZmSig1 protein along the chloroplast development gradient, leaves were cut into sections that measured 0 to 2, 2 to 4, 4 to 6, or 6 to 8 cm from the leaf base. Equal amounts of total protein extracted from each section were immunoblotted with anti-ZmSig1 antibodies (Fig. 5B). The principal immunoreactive protein of approximately 60 kD was detected in extracts from sections close to the leaf tip (from 4–8 cm; Fig. 5B, lanes 7 and 8, asterisk) but was not detectable in sections harvested less than 4 cm from the leaf base (Fig. 5B, lanes 5 and 6). These results suggest that accumulation of the ZmSig1 SLF in maize leaves parallels the development of mature, photosynthetically active chloroplasts. In addition, a band of higher apparent molecular mass was detected by the ZmSig1 antibodies (also detected in Fig. 6). This band may represent a post-translationally modified form of ZmSig1 or may be cytosolic ZmSig1 with its chloroplast transit peptide intact. An alternate possibility is that the anti-ZmSig1 antibodies cross-react with other chloroplast-localized maize SLFs, such as the Sig1 and Sig2 proteins characterized by Tan and Troxler (1999).

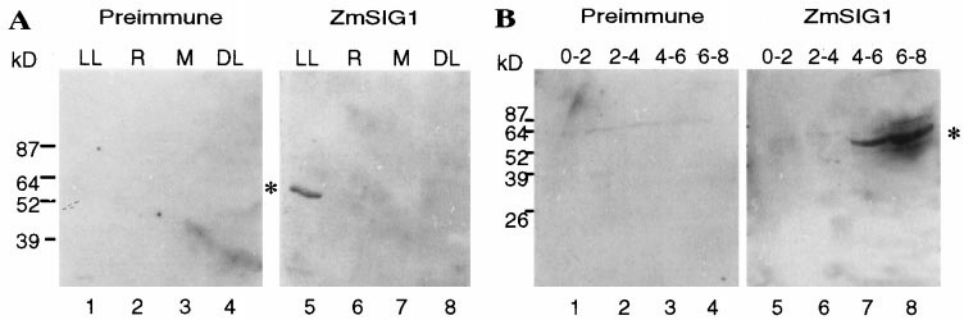

Figure 5. Tissue-specific and developmentally regulated expression of the maize ZmSig1 protein. A, Total proteins were extracted from the top portions (2–7 cm above the leaf base) of 4-d-old greening maize seedlings (LL), from the roots of the same seedlings (R), from the leaf base (0–2 cm region) of the seedling leaves (M), and from the etiolated leaves of 4-d-old seedlings grown in complete darkness (DL). Forty micrograms of protein was separated and immunoblotted with either preimmune (lanes 1–4) or immune (lanes 5–8) antisera against ZmSig1. The immunoreactive 60-kD ZmSig1 protein was detected only in the upper regions of light-grown leaves (asterisk). B, Leaves from 5-d-old light-grown maize seedlings were cut into sections that measured 0 to 2, 2 to 4, 4 to 6, or 6 to 8 cm from the leaf base. Total proteins (40 μg) extracted from each section were immunoblotted with either preimmune (lanes 1–4) or immune (lanes 5–8) antisera against ZmSig1. The immunoreactive 60-kD ZmSig1 protein was detected only in the upper regions of light-grown leaves, from 4 to 8 cm above the leaf base (asterisk). This discrete expression profile indicates that ZmSig1 accumulates preferentially in mature chloroplasts. The positions of molecular mass standards are indicated.

ZmSig1 Accumulation in Mature Chloroplasts Is Not Dramatically Influenced by Light

Dissection of tobacco chloroplast promoters in vivo has revealed that promoter regions encompassing the σ-factor-interaction sequences, from -37 to $+9$ with respect to the transcription start site, are activated by light (Shiina et al., 1998). Therefore, we asked whether the accumulation of the ZmSig1 protein in mature chloroplasts was significantly enhanced by light treatment. Maize seedlings, grown in cycling light for 7 d, were placed in complete darkness for 24 h, then transferred into constant illumination for 24 h. Protein extracts prepared from the upper portions of green leaves exposed to each light treatment were immunoblotted with anti-ZmSig1 antibodies (Fig. 6). Each tissue sample was harvested at the same hour of the day to avoid possible circadian-linked changes in protein accumulation (Ito et al., 1999). The immunoblots demonstrated that the levels of ZmSig1 protein present in mature chloroplasts are not dramatically influenced by light treatment. When plants grown in cycling light were placed in complete darkness no decrease was detected in the level of ZmSig1 protein (Fig. 6, lane 4 versus lane 5). Placing the dark-treated seedlings into constant illumination caused only a minor increase in the levels of ZmSig1 (Fig. 6, lane 5 versus lane 6). These results indicate that the accumulation of ZmSig1 in the mature chloroplasts of light-grown leaves is controlled principally by a light-responsive developmental program, rather than by light cues themselves.

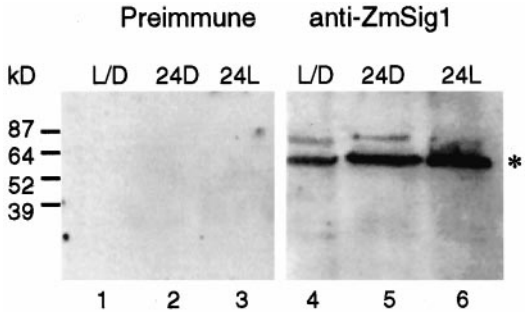

Figure 6. The accumulation of ZmSig1 protein in mature chloroplasts is not influenced by light. Proteins were extracted from the upper region of leaf tissues of seedlings grown for 7 d in cycling light (L/D), from a similar batch of seedlings transferred to complete darkness for 24 h (24D), and from the same batch of seedlings transferred from the 24-h dark treatment to constant illumination for 24 h (24L). The tissues were harvested at the same time of day (10:30 AM) to avoid possible circadian fluctuations in protein accumulation. Extracted proteins (40 μg) were immunoblotted with either preimmune sera (lanes 1–3) or anti-ZmSig1 antisera (lanes 4–6). No dramatic changes in levels of ZmSig1 protein (asterisk) were detected. The positions of molecular mass standards are indicated to the left.

ZmSig1 and ZmSig3 Exhibit Complementary Expression Profiles

Although the protein expression data for ZmSig1 correlated well with its proposed role as a chloroplast σ-factor, the role of ZmSig3 was less clear since no immunoreactive proteins were detected with anti-ZmSig3 antibodies in extracts from green leaf tissues or purified intact chloroplasts (Fig. 2, lanes 7 and 8). However, when extracts from other maize tissues were probed with this antibody an immunoreactive protein was detected with a slightly slower mobility than the protein detected in chloroplasts by the anti-ZmSig1 antibody. The anti-ZmSig3-reactive protein band was present in all non-green tissues tested, including root, meristematic region of light-grown leaf, and etiolated leaf tissue from dark-grown seed-

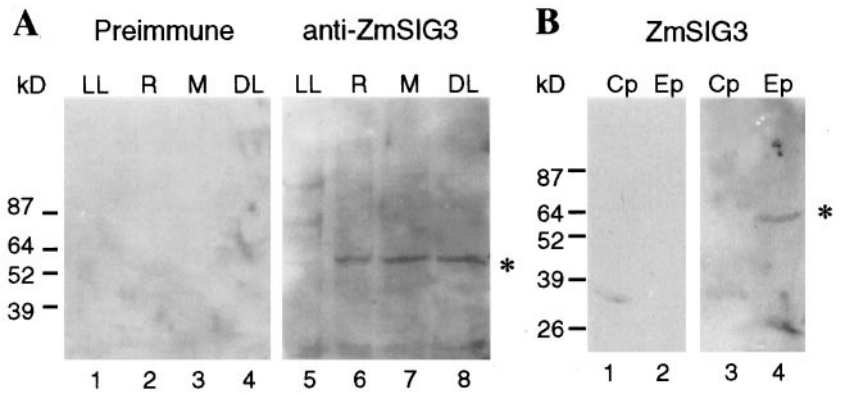

Figure 7. ZmSig3 accumulates in non-green plastids. A, Total proteins (40 μg) from leaf sections 2 to 7 cm above the base of 4-d-old greening maize seedlings (LL), from the roots of the same seedlings (R), from the leaf base of the seedling leaves (M), and from leaves of 4-d-old etiolated seedlings (DL) were immunoblotted with either preimmune (lanes 1–4) or immune (lanes 5–8) antisera against ZmSig3. The immunoreactive 62-kD ZmSig3 protein was detected in the non-green tissues (asterisk) but not in the upper portion of the light-grown leaves. B, Total proteins (25 μg) extracted from chloroplasts (Cp) purified from light-grown leaves, or etioplasts (Ep) purified from etiolated seedling leaves were immunoblotted with either preimmune sera (lanes 1 and 2) or immune sera raised against ZmSig3 (lanes 3 and 4). The immunoblot shows that ZmSig3 protein accumulates in etioplasts (asterisk) rather than chloroplasts. The positions of molecular mass standards are indicated to the left.

lings (Fig. 7A, lanes 6–8). Since the apparent molecular mass of the immunoreactive protein was similar to the predicted molecular mass of ZmSig3, these results suggested that this SLF is expressed exclusively in non-green tissues. To investigate the subcellular location of ZmSig3, protein extracts were prepared from intact etioplasts purified from dark-grown seedling leaves and from chloroplasts purified from light-grown leaves. In contrast to ZmSig1, which accumulates in chloroplasts, (Fig. 2, lane 3) the ZmSig3 protein was detectable in etioplasts but not chloroplasts (Fig. 7B, lanes 3 and 4). Therefore, both ZmSig1 and ZmSig3 are plastid-localized SLFs and exhibit distinct patterns of expression within maize tissue.

DISCUSSION

We have generated specific tools to investigate the expression of two SLFs, ZmSig1 and ZmSig3, in different maize tissues and during chloroplast development. Based on in vitro import data (Lahiri et al., 1999), we had anticipated that both ZmSig1 and ZmSig3 would localize to maize leaf chloroplasts. We found that, although both appear to be plastid-localized proteins, the two SLFs have complementary expression profiles. Whereas ZmSig1 accumulated in green leaf tissue, ZmSig3 was found in the non-green tissues of the plant, including roots, etiolated leaves, and the meristematic region of green leaves. In addition, we determined that the accumulation of ZmSig1 in green leaf tissues is responsive to the light-induced chloroplast development program. Although this SLF was not present at detectable levels in undifferentiated proplastids in the leaf base, it accumulated to significant levels in the upper half of the leaf where it was found within the mature chloroplasts. In contrast, ZmSig3 could be detected in the meristematic regions of green seedling leaves, however it did not accumulate in the upper one-half of the leaves where mature chloroplasts are found. Interestingly, these protein accumulation profiles do not parallel the transcript accumulation previously reported for the two maize SLFs (Lahiri et al., 1999). Reverse transcriptase-PCR analysis had indicated that transcripts for *sig1* accumulate in all tissues examined (etiolated seedling leaves, green seedling leaves, and roots), whereas *sig3* transcripts were detected most abundantly in green seedling leaves and were at much lower levels in root and etiolated leaf tissues. The reason for this discrepancy is not yet clear, but it may represent the semiquantitative nature of the reverse transcriptase-PCR technique or post-transcriptional control of ZmSig1 and ZmSig3 accumulation. We note that there are many examples of plant proteins whose changes in abundance in different tissues or environmental conditions do not reflect changes in transcript levels (e.g. Crete et al., 1997; Germain et al., 1997; Kircher et al., 1998; Mittler et al., 1998; Crosatti et al., 1999; Percy et al., 1999).

These data begin to address the question of why plants encode so many SLFs. It had been speculated that the plant nucleus regulates plastid gene expression through a family of σ-factor genes each with a different expression profile. Transcript accumulation data supported this model for the maize SLF gene family (Lahiri et al., 1999; Tan and Troxler, 1999). Moreover, a polyclonal antiserum raised against the two maize SLFs isolated by Troxler and colleagues, detected two cross-reacting proteins in chloroplasts, one of which was also present in etioplasts, and

neither of which was found in root (Tan and Troxler, 1999). Although the antisera were not able to discriminate between these two highly conserved SLF family members, they did indicate that one SLF was expressed in both etiolated and green tissues, whereas the other was specifically localized to chloroplasts. These data, combined with the expression studies presented here, begin to build a profile of the maize SLF protein family. Two family members accumulate preferentially in chloroplasts (Tan and Troxler, 1999; ZmSig1, this study), one SLF is detected in both etioplasts and chloroplasts (Tan and Troxler, 1999), and one member of the family, ZmSig3, accumulates preferentially in the plastids of non-green tissues (this study).

Given that the plant SLFs may program the promoter specificity of the PEP RNA polymerase, it is of interest to ask how promoter usage by PEP differs within the plastids of different tissue types. Recent studies by Toyoshima and colleagues (Satoh et al., 1999) have addressed this question using in vitro transcription assays on different plastid gene promoters. Transcription extracts, prepared from the leaf tip region or the leaf base region of 7-d-old wheat seedlings, were tested for their ability to transcribe several plastid promoters in a light-dependent fashion (Satoh et al., 1999). These experiments demonstrated that the PEP RNA polymerase present in the immature chloroplasts of the leaf base required the "−35" promoter element for promoter recognition, and was not activated by light. In contrast, the PEP RNA polymerase located within the mature chloroplasts of the leaf tip was reversibly induced by light, and could recognize promoters in the absence of a "−35" sequence, as long as an "extended −10" box was present. The most likely explanation for these results was that the PEP RNA polymerase was complexed with different σ-factors in the leaf tip compared to the leaf base (Satoh et al., 1999). The expression data presented here are consistent with such a model (for a diagram, see Fig. 8) and would predict that the promoter preference for ZmSig1 expressed in the leaf tip may differ from the promoter specificity of ZmSig3, expressed in the leaf base.

The "extended −10" promoter, first identified in eubacteria, consists of a 5′-TG-3′ sequence located one base upstream of the −10 hexamer element (for review, see Bown et al., 1997). Analyses of mutant σ-factors in *E. coli* demonstrated that extended −10 regions are recognized by conserved region 2.5 of the RNA polymerase σ-70 subunit (Barne et al., 1997). Mutations at two highly conserved residues in region 2.5, Glu-458 and His-455, reduced the activity of the holoenzyme from extended −10 promoters (Barne et al., 1997). Therefore, one would expect that a plastid-localized σ-factor capable of recognizing an extended −10 sequence in a plastid promoter should contain a conserved region 2.5. Interestingly, of the five maize SLFs identified to date, ZmSig1 and ZmSig2 contain

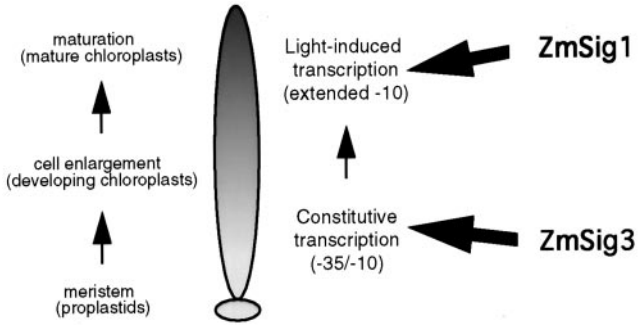

Figure 8. A model for SLF function in the developing maize leaf. A maize seedling leaf is diagrammed with three different zones of development indicated (based on Mullet, 1993): the meristematic region at the leaf base, containing proplastids; the zone of cell enlargement, containing early developing chloroplasts; and the maturation zone in the upper region of the leaf, containing mature chloroplasts. The PEP RNA polymerase promoter usage and light dependence derived from experiments with wheat seedlings (Satoh et al., 1999) are indicated to the right. We speculate that ZmSig1, due to its conservation of region 2.5, should be capable of recognizing extended −10 promoters and thus may confer this property upon the PEP RNA polymerase located in the zone of mature chloroplasts. In contrast ZmSig3, whose expression is restricted to the meristematic zone, does not have region 2.5 and thus may restrict the PEP RNA polymerase in developing chloroplasts to recognize promoters containing a −35 sequence element.

the conserved residues of region 2.5 (Lahiri et al., 1999) and thus may recognize extended −10 promoters. As shown in this study, ZmSig1 is expressed in the tips of green maize seedling leaves. Based on the data presented for promoter selectivity by the wheat PEP RNA polymerase (Satoh et al., 1999), we speculate that accumulation of ZmSig1 in maize leaf tips allows the PEP RNA polymerase of mature chloroplasts to recognize extended −10 promoters, whereas in the leaf base the accumulation of ZmSig3, missing region 2.5, causes the PEP RNA polymerase to be dependent on a −35 box for promoter utilization (Fig. 8). An investigation of ZmSig1 and ZmSig3 promoter binding characteristics will help to verify this model.

It is well documented that the activity of some chloroplast promoters is dramatically enhanced by light in mature chloroplasts (for review, see Mullet, 1993). Recently, both in vivo (Shiina et al., 1998) and in vitro (Nakahira et al., 1998; Satoh et al., 1999) evidence supported a role for the core promoter sequences in this regulation. Since in eubacteria, the equivalent sequences interact with σ-factors, it is plausible that mature chloroplasts contain a σ-factor whose abundance and/or activity is enhanced by light. Whereas ZmSig1 is present in mature chloroplasts of maize leaf tips, its abundance is not reduced by growth of plants in darkness, nor increased dramatically by exposure of dark-grown plants to light. However, the activity of this SLF may be regulated by a light-dependent post-translational modification

such as phosphorylation or dephosphorylation. Biochemical analysis of SLFs purified from mustard revealed that the phosphorylation state of these proteins differed in etioplasts and chloroplasts, and influenced their relative affinity for a subset of plastid promoters (Tiller and Link, 1993b). Alternatively, ZmSig1 could play the role of a housekeeping σ-factor in mature chloroplasts, whereas one of the other members of the maize SLF family may mediate light-dependent activity of a subset of plastid core promoters (Tan and Troxler, 1999). Ultimately, to uncover the role of each SLF in vivo, reverse genetics approaches to identify transposon disruptions each SLF gene will be instrumental (Bensen et al., 1995; Fisk et al., 1999).

MATERIALS AND METHODS

Growth and Extraction of Plant Material

Seeds of maize (*Zea mays* cv B53) were soaked overnight in tap water with continuous aeration, then planted in flats of greenhouse mix (40% [v/v] Canadian peat, 40% [v/v] coarse vermiculite, 15% [v/v] masonry sand, and 5% [v/v] screened top soil [v/v]). For experiments on light-grown leaves, seedlings were grown at 23°C in cycling light (16 h light/8 h dark). For experiments requiring etiolated seedling tissues, seedlings were grown at 23°C in complete darkness. When seedlings grown in cycling light were used the tissues were harvested in the first one-half of the light period. For extraction of total protein from light-grown leaf sections, roots, and etiolated leaves, the tissues were harvested from 7-d-old seedlings and immediately frozen in liquid nitrogen, then ground in a mortar and pestle. Approximately 3 g of ground tissue were further homogenized with 1 mL of crude protein extraction buffer (20 mM Tris [tris(hydroxymethyl)aminomethane], pH 7.6, 1 mM EDTA, 0.1% [v/v] SDS, 0.1% [v/v] Triton X-100, 0.02 mM phenylmethylsulfonyl fluoride, and 0.05 mM dithiothreitol) to make a paste. The homogenate was centrifuged for 10 min at top speed in a table-top centrifuge, and the supernatant containing the soluble protein was collected and stored at −80°C. All determinations of protein concentration were determined using the Bio-Rad Standard Protein Assay (Bio-Rad Laboratories, Hercules, CA). Chloroplasts were purified on Percoll density gradients from the upper one-half (5–15 cm portion as measured from the leaf base) of 7-d-old light-grown seedling leaves using standard protocols (Lahiri et al., 1999). The same protocols were used to purify etioplasts from the aerial portions of 7-d-old seedlings grown in complete darkness. For all manipulation of dark-grown seedling tissues, a green safe-light was used.

Isolation of Bundle Sheath and Mesophyll Cells

Bundle sheath or mesophyll cells were isolated from the third leaf of 21-d-old maize seedlings grown in cycling light, using a protocol provided by Jane Langdale (University of Oxford). For mesophyll cell preparations 5 g of leaves were cut perpendicular to the midrib in 0.5- to 1.0-mm strips. The strips were infiltrated under vacuum with 80 mL of enzyme buffer (0.02 M MES [2-(*N*-morpholino)ethanesulfonic acid], pH 5.5, 0.6 M Sorbitol, 1 mM $MgCl_2$, 2% [w/v] cellulase, and 0.1% [w/v] macerase) and digested 3 to 5 h at room temperature. Cellulase and macerase were purchased from Sigma (St. Louis). After digestion, any broken cells were separated by filtration through a 135-μm nylon mesh (Millipore, Bedford, MA) and discarded. The residual partially-digested leaf strips left on the filter were resuspended in 50 mL of wash buffer (0.05 M Tris-HCl, pH 7.5, 0.6 M Sorbitol, 1 mM $MgCl_2$, and 0.1 M β-mercaptoethanol) in a Petri dish. The leaf strips were pressed gently with a spatula to release the protoplasts, then filtered through a 60-μm nylon mesh (Millipore). The mesophyll protoplasts in the filtrate were subsequently collected by centrifugation at 300*g* for 5 min. The final pellet was resuspended in 0.5 mL of crude protein extraction buffer (recipe above) and stored at −80°C.

For preparation of bundle sheath cells 3 g of leaves were cut into 2 × 2 mm squares. The cut tissue was homogenized in 25 mL of disruption buffer (0.05 M Tris-HCl, pH 8.0, 0.6 M Sorbitol, 1 mM $MgCl_2$, and 0.1 M β-mercaptoethanol) using a Polytron for 40 s at speed 15,000 rpm. The homogenate was filtered through a 60-μm nylon mesh (Millipore). The residue was resuspended in 25 mL of disruption buffer and the homogenization step was repeated twice more. The bundle sheath cells retained on the mesh after the third homogenization step were collected, washed once in disruption buffer, resuspended in 0.5 mL of crude protein extraction buffer, and stored at −80°C.

Expression and Purification of ZmSig1 and ZmSig3 Proteins

The cDNA sequences encoding the unique NH_2-terminal 295 amino acids of ZmSig1 (ZmSig1-N) and 297 amino acids of ZmSig3 (ZmSig3-N) were amplified by PCR, using primers designed with restriction sites for subsequent subcloning of the PCR products. Primers used for amplification of ZmSig1-N were UNL160 (5′-ccggatccGCGTGCCTGGCGCCGCAG-3′) and UNL161 (5′-cgactgcagACCATAATTC AAGCGCTTGCGC-3′) in which gene-specific sequences are indicated by uppercase letters. For amplification of ZmSig3-N the primers used were UNL162 (5′-ccgcatgcAATTCCAGCAGAAGCCTCCTCTC-3′) and UNL163 (5′-cccaagcttTCCGG TGCGCACACAGGATTGC-3′). The amplified DNA fragments were subcloned into the polylinker region of expression vector pET28A (Novagen, Madison, WI) using the engineered primer restriction sites (*Bam*HI and *Pst*I for subcloning the ZmSig1-N fragment, and *Sph*I and *Hind*III for introducing the ZmSig3 insert). Primers were designed to place the introduced coding regions of each SLF in frame with the NH_2-terminal hexa-His tag sequence of the expression vector. Insert sequences were confirmed by DNA sequencing.

For expression and purification of the His-tagged recombinant proteins, plasmids were transformed into BL21 (DE3) cells (Novagen) and grown at 37°C to an optical density (OD_{600}) of between 0.4 and 0.8. Recombinant protein expression was induced by addition of isopropyl β-D-thiogalactopyranoside to a final concentration of 1 mM, followed by growth at 37°C for 3 h. His-tagged protein from cell lysate was bound to Talon resin (CLONTECH Laboratories, Palo Alto, CA) in denaturing buffer and purified according to the manufacturer's protocols. Rabbit polyclonal antisera were prepared against each purified recombinant protein by the Core Research Facilities of the University of Nebraska-Lincoln.

Immunodetection of ZmSig1 and ZmSig3

Except where indicated in the figure legends, western blots were performed on 40 μg of plant protein separated on 12.5% (v/v) polyacrylamide gels. Separated proteins were transferred onto Hybond-ECL membranes (Amersham-Pharmacia Biotech, Uppsala) using a semidry Transblot-SD apparatus (Bio-Rad Laboratories) following the manufacturer's suggestions. For detection of ZmSig1 protein, an antibody dilution of 1 in 15,000 was used; whereas for ZmSig3, the antibody dilution was 1 in 5,000. Immunoreactive proteins were detected using the ECL western-blotting detection kit and Hyperfilm ECL (Amersham-Pharmacia Biotech). Film exposures ranged from 15 min to 2 h.

For in situ immunolocalizations 10-mm thin sections from the leaf blades of 21-d-old maize seedlings grown under cycling light conditions (16-h light/8-h dark) were hybridized with either preimmune or immune anti-ZmSig1 sera at a 1 in 200 dilution. Immunoreactive proteins were detected with a 1 in 100 dilution of goat anti-rabbit CY2-conjugated secondary antibody exhibiting green fluorescence. The hybridized sections were observed using a Bio-Rad MRC600 confocal microscope at the University of Nebraska Core Microscopy Facility. Chlorophyll autofluorescence was observed at an excitation wavelength of 647 nm and emission at 666 nm. Signals from immunoreactive material were detected with excitation at 488 nm and emission at 520 nm.

ACKNOWLEDGMENTS

We thank Dr. Ray Chollet for antisera against maize Rubisco and PEP carboxylase, Dr. Jane Langdale for advice in preparation of maize bundle sheath and mesophyll cells, Michelle Mathiesen and Dr. Joe Zhou (University of Nebraska Center for Biotechnology Core Facilities, Lincoln) for assistance with the sample preparation and imaging for in situ immunolocalization studies, and Dr. Gautham Sarath, Junlan Yao, and Tom Beardslee for helpful discussion.

Received October 13, 1999; accepted March 15, 2000.

LITERATURE CITED

Allison LA (2000) The role of σ factors in plastid transcription. Biochimie (in press)

Barne KA, Bown JA, Busby SJW, Minchin SD (1997) Region 2.5 of the *Escherichia coli* RNA polymerase σ^{70} subunit is responsible for the recognition of the 'extended −10' motif at promoters. EMBO J **16:** 4030–4040

Baumgartner BJ, Rapp JC, Mullet JE (1989) Plastid transcription activity and DNA copy number increase early in barley chloroplast development. Plant Physiol **89:** 1011–1018

Baumgartner BJ, Rapp JC, Mullet JE (1993) Plastid genes encoding the transcription/translation apparatus are differentially transcribed early in barley (*Hordeum vulgare*) chloroplast development. Plant Physiol **101:** 781–791

Bensen RJ, Johal GS, Crane VC, Tossberg JT, Schnable PS, Meeley RB, Briggs SP (1995) Cloning and characterization of the maize *An1* gene. Plant Cell **7:** 75–84

Bown J, Barne K, Minchin S, Busby S (1997) Extended −10 promoters. Nucleic Acids Mol Biol **11:** 41–52

Chang C-C, Sheen J, Bligny M, Niwa Y, Lerbs-Mache S, Stern DB (1999) Functional analysis of two maize cDNAs encoding T7-like RNA polymerases. Plant Cell **11:** 911–926

Crete P, Caboche M, Meyer C (1997) Nitrite reductase expression is regulated at the post-transcriptional level by the nitrogen source in *Nicotiana plumbaginifolia* and *Arabidopsis thaliana*. Plant J **11:** 625–634

Crosatti C, Polverino de Laureto P, Bassi R, Cattivelli L (1999) The interaction between cold and light controls the expression of the cold-regulated barley gene *cor14b* and the accumulation of the corresponding protein. Plant Physiol **119:** 671–680

Fisk DG, Walker MB, Barkan A (1999) Molecular cloning of the maize gene crp1 reveals similarity between regulators of mitochondrial and chloroplast gene expression. EMBO J **18:** 2621–2630

Furbank RT, Taylor WC (1995) Regulation of photosynthesis in C_3 and C_4 plants: a molecular approach. Plant Cell **7:** 797–807

Germain V, Raymond P, Ricard B (1997) Differential expression of two tomato lactate dehydrogenase genes in response to oxygen deficit. Plant Mol Biol **35:** 711–721

Gray MW, Lang BF (1998) Transcription in chloroplasts and mitochondria: a tale of two polymerases. Trends Microbiol **6:** 1–3

Gross CA, Chan C, Dombroski A, Gruber T, Sharp M, Tupy J, Young B (1998) The functional and regulatory roles of σ factors in transcription. Cold Spring Harbor Symp Quant Biol **63:** 141–155

Gross CA, Lonetto M, Losick R (1992) Bacterial σ factors. *In* SL McKnight, KR Yamamoto, eds, Transcription Regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, pp 129–176

Hajdukiewicz PTJ, Allison LA, Maliga P (1997) Transcription by two distinct RNA polymerases is a general regulatory mechanism of plastid gene expression in higher plants. EMBO J **16:** 4041–4048

Hedtke B, Börner T, Weihe A (1997) Mitochondrial and chloroplast phage-type RNA polymerases in *Arabidopsis*. Science **277:** 809–811

Helmann JD, Chamberlin MJ (1988) Structure and function of bacterial σ factors. Annu Rev Biochem **57:** 839–872

Hess WR, Börner T (1999) Organellar RNA polymerases of higher plants. Int Rev Cytol **190:** 1–59

Igloi GL, Kössel H (1992) The transcriptional apparatus of chloroplasts. Crit Rev Plant Sci **10:** 525–558

Ikeda TM, Gray MW (1999) Identification and characterization of T3/T7 bacteriophage-like RNA polymerase sequences in wheat. Plant Mol Biol **40:** 567–578

Isono K, Shimuzu M, Yoshimoto K, Niwa Y, Satoh K, Yokota A, Kobayashi H (1997) Leaf-specifically expressed genes for polypeptides destined for chloroplasts with domains of σ^{70} factors of bacterial RNA polymerases in *Arabidopsis thaliana*. Proc Natl Acad Sci USA **94:** 14948–14953

Ito MK, Tsunoyama Y, Makahira Y, Shiina T, Toyoshima Y (1999) Circadian-regulated expression of a nuclear-encoded plastid σ factor gene (sigA) in wheat seedlings. FEBS Lett **451:** 275–278

Kanamaru K, Fujiwara M, Seki M, Katagiri T, Nakamura M, Mochizuki N, Nagatani A, Shinozaki K, Tanaka K, Takahashi H (1999) Plastidic RNA polymerase σ factors in *Arabidopsis*. Plant Cell Physiol **40:** 832–842

Kapoor S, Suzuki JY, Sugiura S (1997) Identification and functional significance of a new class of non-consensus-type plastid promoters. Plant J **11:** 327–337

Kestermann M, Neukirchen S, Kloppstech K, Link G (1998) Sequence and expression characteristics of a nuclear-encoded chloroplast σ factor from mustard (*Sinapis alba*). Nucleic Acids Res **26:** 2747–2753

Kircher S, Ledger S, Hayashi H, Weisshaar B, Schafer E, Frohnmeyer H (1998) CPRF4a, a novel plant bZIP protein of the CPRF family: comparative analyses of light-dependent expression, post-transcriptional regulation, nuclear import and heterodimerisation. Mol Gen Genet **257:** 595–605

Kubicki A, Steinmuller K, Westhoff P (1994) Differential transcription of plastome-encoded genes in the mesophyll and bundle-sheath chloroplasts of the monocotyledonous NADP-malic enzyme-type C_4 plants maize and *Sorghum*. Plant Mol Biol **25:** 669–679

Lahiri SD, Yao J, McCumbers C, Allison LA (1999) Tissue-specific and light-dependent expression within a family of nuclear-encoded σ-like factors from *Zea mays*. Mol Cell Biol Res Commun **1:** 14–20

Langdale JA, Nelson T (1991) Spatial regulation of photosynthetic development in C4 plants. Trends Genet **7:** 191–196

Leech RM (1984) Chloroplast development in angiosperms: current knowledge and future prospects. *In* NR Baker, J Barber, eds, Topics in Photosynthesis: Chloroplast Biogenesis, Vol V. Elsevier Science Publishing, New York, pp 1–21

Lerbs-Mache S (1993) The 110-kDa polypeptide of spinach plastid DNA-dependent RNA polymerase: single subunit enzyme or catalytic core of multimeric enzyme complexes? Proc Natl Acad Sci USA **90:** 5509–5513

Link G, Coen DM, Bogorad L (1978) Differential expression of the gene for the large subunit of ribulose bisphosphate carboxylase in maize leaf cell types. Cell **56:** 241–246

Lonetto M, Gribskov M, Gross CA (1992) The σ70 family: sequence conservation and evolutionary relationships. J Bacteriol **174:** 3843–3849

Malhotra A, Severinova E, Darst SA (1996) Crystal structure of a σ70 subunit fragment from *E. coli* RNA polymerase. Cell **87:** 127–136

Mittler R, Feng X, Cohen M (1998) Post-transcriptional suppression of cytosolic ascorbate peroxidase expression during pathogen-induced programmed cell death in tobacco. Plant Cell **10:** 461–473

Mullet JE (1993) Dynamic regulation of chloroplast transcription. Plant Physiol **103:** 309–313

Nakahira Y, Baba K, Yoneda A, Shiina T, Toyoshima Y (1998) Circadian-regulated transcription of the *psbD* light-responsive promoter in wheat chloroplasts. Plant Physiol **118:** 1079–1088

Percy JD, Philip R, Vodkin LO (1999) A defective seed coat pattern (Net) is correlated with the post-transcriptional abundance of soluble proline-rich cell wall proteins. Plant Mol Biol **40:** 603–613

Satoh J, Baba K, Nakahira Y, Tsunoyama Y, Shiina T, Toyoshima Y (1999) Development stage-specific multisubunit plastid RNA polymerases (PEP) in wheat. Plant J **18:** 407–415

Severinov K, Fenyo D, Severinova E, Mustaev A, Chait BT, Goldfarb A, Darst SA (1994) The σ subunit conserved region 3 forms "5′ face" of active center *of Escherichia coli* RNA polymerase. J Biol Chem **269:** 20826–20828

Shiina T, Allison L, Maliga P (1998) *rbcL* transcript levels in tobacco plastids are independent of light: reduced dark transcription rate is compensated by increased mRNA stability. Plant Cell **10:** 1713–1722

Silhavy D, Maliga P (1998) Mapping of promoters for the nucleus-encoded plastid RNA polymerase (NEP) in the *iojap* maize mutant. Curr Genet **33:** 340–344

Somanchi A, Mayfield SP (1999) Nuclear-chloroplast signalling. Curr Opin Plant Biol **2:** 404–409

Tan S, Troxler RF (1999) Characterization of two chloroplast RNA polymerase σ factors from *Zea mays*: photoregulation and differential expression. Proc Natl Acad Sci USA **96:** 5316–5321

Tanaka K, Tozawa Y, Mochizuki N, Shinozaki K, Nagatani A, Wakasa K, Takahashi H (1997) Characterization of three cDNA species encoding plastid RNA polymerase σ factors in *Arabidopsis thaliana*: evidence for the σ factor heterogeneity in higher plant plastids. FEBS Lett **413:** 309–313

Tiller K, Eisermann A, Link G (1991) The chloroplast transcription apparatus from mustard (*Sinapis alba* L.). Eur J Biochem **198:** 93–99

Tiller K, Link G (1993a) σ-Like transcription factors from

mustard (*Sinapis alba* L.) etioplast are similar in size to, but functionally distinct from, their chloroplast counterparts. Plant Mol Biol **21:** 503–513

Tiller K, Link G (1993b) Phosphorylation and dephosphorylation affect functional characteristics of chloroplast and etioplast transcription systems from mustard (*Sinapis alba* L.). EMBO J **12:** 1745–1753

Tozawa Y, Tanaka K, Takahashi H, Wakasa K (1998) Nuclear encoding of a plastid σ factor in rice and its tissue- and light-dependent expression. Nucleic Acids Res **26:** 415–419

Troxler RF, Zhang F, Hu J, Bogorad L (1994) Evidence that σ factors are components of chloroplast RNA polymerase. Plant Physiol **104:** 753–759

Yao J, Allison LA (1998) The cDNA sequence of *AtSIG4*, a new member of the nuclear-encoded σ-like factor gene family in *Arabidopsis thaliana* (accession no. AF101075) (PGR 98–212). Plant Physiol **118:** 1533

Young DA, Allen RL, Harvey AJ, Lonsdale DM (1998) Characterization of a gene encoding a single-subunit bacteriophage-type RNA polymerase from maize which is alternatively spliced. Mol Gen Genet **260:** 30–37