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Sigma-like transcription factors from mustard (*Sinapis alba* L.) etioplast are similar in size to, but functionally distinct from, their chloroplast counterparts

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Abstract

Three proteins resembling bacterial sigma factors were previously isolated from mustard chloroplasts (K. Tiller, A. Eisermann and G. Link, Eur J Biochem 198: 93–99, 1991). These sigma-like factors (SLFs) confer DNA-binding and transcription specificity to a system consisting of *Escherichia coli* core RNA polymerase and cloned DNA regions that carry a chloroplast promoter. Sigma-like activity was now isolated also from etioplasts and could be assigned to three polypeptides of M_r 67000 (SLF^{67}), 52000 (SLF^{52}) and 29000 (SLF^{29}), i.e. the same sizes as for the chloroplast SLFs. The purification scheme for the factors from either plastid type included an initial heparin-Sepharose and a final gel filtration step. For the etioplast factors, however, an additional phosphocellulose step was required to release these polypeptides from the RNA polymerase. The etioplast SLFs have similar, but not identical, salt requirements for DNA binding as compared to their chloroplast counterparts. Under conditions of maximum binding activity there is overall preference of etioplast SLFs for the *psbA* promoter over the *trnQ* and *rps16* promoters.

Introduction

Apart from its role in photosynthesis, light acts as an environmental signal that affects plant growth and development. The possibly most intensely studied photoresponse at the cellular level is the conversion of photosynthetically inactive etioplasts into green chloroplasts upon illumination of dark-grown seedlings, a process involving distinct changes in gene expression (for review, see e.g. [1, 2, 3, 4, 5]). The expression of nuclear genes for plastid proteins is thought to be primarily controlled at the level of transcription, although post-transcriptional regulation at RNA level, during translation, and by differential protein stability has also been reported (for review, see [6]).

A common notion with regard to the expression of plastid genes is that most regulation appears to be post-transcriptionally both at the RNA and protein level, which does not, however, exclude the possibility for transcriptional control

of certain organelle genes in certain tissues and developmental situations [7, 8, 9].

For instance, transcripts of the *psbA* gene for the D1 reaction center protein of photosystem II were shown to be present in increased levels upon illumination of seedlings from various plant species [10, 11, 12, 13]. Although post-transcriptional mechanisms are clearly involved in the photoregulated *psbA* mRNA accumulation [7, 9, 14], it has become clear that differential transcription of the *psbA* gene also plays an important role [9, 15, 16, 17].

Plastid promoters in general are considered 'prokaryotic' [18, 19], since many of them contain conserved −35- and −10-like elements [20]. The *psbA* promoter as well as several other plastid promoters, however, were shown to contain an additional element resembling the 'eukaryotic' TATA box of many nuclear genes transcribed by RNA polymerase II [12, 21]. *In vitro* transcription studies with point mutants of the mustard (*Sinapis alba*) *psbA* promoter suggest differential usage of the −35 and TATA-like elements in chloroplast and etioplast transcription systems [15].

In prokaryotes, promoter elements are recognized by the sigma subunit of the RNA polymerase holoenzyme, which associates transiently with the catalytic core enzyme for correct transcription initiation. Different sigma factors are used for transcription of various classes of genes [22]. Protein factors showing sigma-like activity were also isolated from chloroplast lysates [23, 24, 25, 26]. These factors seem to interact *in vivo* with a multi-subunit RNA polymerase core enzyme which contains plastid DNA-encoded polypeptides that have sequence homology with the α, β and β' subunits of the bacterial enzyme [19].

In mustard chloroplasts there is evidence for at least three sigma-like factors, each one conferring specific DNA binding at the *psbA* promoter, although with different efficiency [26]. Thus far, no information is available on the possible existence of similar factors in etioplasts or other plastid types. It hence remains to be clarified if the differential usage of promoter elements in chloroplast versus etioplast transcription systems [15] might be related to stage-specific characteristics of the factors involved. Here we present the purification of three sigma-like factors from mustard etioplasts and a comparison with their chloroplast counterparts.

Materials and methods

Cloned DNA material

DNA fragments containing the *psbA*, *rpsl6* or *trnQ* promoter were prepared as described [25, 27, 15]. *PsbA*, *rps16*, *trnQ* are the plastid genes encoding the D1 reaction center protein of photosystem II, ribosomal protein CS16, and $tRNA^{Gln}$, respectively. Bam0.5 is a 0.5 kb fragment of pSA364 [12] containing sequences of the mustard *trnK* intron [25]. The *psbA* promoter fragment was 3′-end-labelled with [α-^{32}P]dATP (Amersham, 410 Ci/mmol) by the fill-in reaction of Klenow enzyme (BRL) [28] and then used as labelled probe in gel retardation assays. The *rps16* and *trnQ* promoter fragments were used as unlabelled competitors.

Preparation of E. coli *core RNA polymerase*

E. coli core enzyme ($\alpha_2\beta\beta'$) was prepared from holoenzyme by phosphocellulose chromatography following standard procedures [29].

Etioplast preparation and lysis

1 kg of cotyledons from dark-grown 5-day-old seedlings were disrupted in 0.1 M Tris/HCl pH 8.0, 0.5 M sucrose, 10 mM $MgCl_2$, 0.1% BSA, 0.04 mM 2-mercaptoethanol, 50 μg/ml PMSF, 1 mM benzamidine, using a Waring blender. Etioplasts were prepared by differential centrifugation (12 min at $3500 \times g$) followed by density gradient centrifugation (45 min at $120000 \times g$) in the same buffer containing 20–70% (w/w) sucrose. They were collected from the

gradient and lysed in a hypotonic buffer (50 mM Tris/HCl pH 7.6, 4 mM EDTA, 40 mM 2-mercaptoethanol, 25% glycerol (v/v), 50 μg/μl PMSF, 1 mM benzamidine, 1.5% Triton X-100).

Purification of SLF

Heparin-Sepharose chromatography of the etioplast lysate was performed as described for chloroplast SLF preparation with modifications. The lysate was adjusted to a 8 ml heparin-Sepharose column equilibrated with 50 mM Tris/HCl pH 7.6, 0.1 mM EDTA, 5 mM 2-mercaptoethanol, 0.1% Triton X-100 (v/v), 10% glycerol (v/v), 0.1 M $(NH_4)_2SO_4$, 50 μg/ml PMSF and 1 mM benzamidine (buffer A). Proteins were eluted with a linear salt gradient from 0.1–1.5 M $(NH_4)_2SO_4$ in buffer A at a flow rate of 10 ml/h and 3.5 ml fractions were collected.

Fractions were assayed for DNA-binding and transcription activity after dialysis of each fraction against a buffer similar to buffer A but lacking $(NH_4)_2SO_4$ and containing 50% glycerol (buffer C). Transcriptionally active fractions were pooled, diluted with one volume of buffer A lacking glycerol, and then subjected to a 5 ml phosphocellulose column equilibrated with buffer B (identical to buffer A except for the concentration of 50 mM $(NH_4)_2SO_4$). Proteins were eluted at a flow rate of 8 ml/h using a linear salt gradient from 0.05–1.3 M $(NH_4)_2SO_4$ and 2.5 ml fractions were collected. Each fraction was dialysed separately and aliquots were tested for transcription and DNA-binding activity in the presence or absence of *E. coli* core RNA polymerase.

Fractions containing SLF activity were pooled, concentrated by ultrafiltration to a final volume of 0.4 ml, and subsequently subjected to FPLC gel filtration on a 0.45 cm × 38 cm Sephacryl S-300 column (Pharmacia) that had been equilibrated in buffer B. The flow rate was 6 ml/h and 0.8 ml fractions were collected, dialysed against buffer C, and then tested for SLF activity. SLF fractions could be stored at −20 °C for several months without loss of activity.

In vitro *transcription*

RNA polymerase activity was assayed in a 100 μl reaction mixture containing 50 mM Tris/HCl pH 8.0, 50 mM KCl, 10 mM $MgCl_2$, 2 mM DTT, 0.6 mM each of ATP, CTP and GTP, 12.5 μM [5,6-^{3}H]UTP (1 Ci/mmol; Amersham), 30 μl protein sample, and 25 μg heat-denatured calf thymus DNA as template. After incubation for 30 min at 30 °C, the total amount of RNA synthesized was determined by scintillation counting of trichloroacetic acid-precipitable material [30].

Gel retardation assays

Gel retardation assays were performed according to [31]. To detect DNA-binding activity of RNA polymerase fractions, 15 μl aliquots were incubated with 2.5 ng 3′-labelled *psbA* promoter fragment in 50 μl 30 mM Tris/HCl pH 7.0, 5 mM 2-mercaptoethanol, 0.5 mM EDTA, 5% (v/v) glycerol, 80 mM $(NH_4)_2SO_4$ at 25 °C for 15 min. Mixtures were then loaded on a 5% native polyacrylamide gel (30:0.8 acrylamide/bisacrylamide) containing 0.5 M Tris/HCl, 192 mM glycine and run at room temperature. Gels were dried and autoradiographed at −80 °C, using Kodak XAR-films.

For detection of SLF activity after column chromatography, assays were performed as described above, except that the binding reaction mixture contained 0.5 μg *E. coli* core enzyme and had only 5 mM $(NH_4)_2SO_4$. In competition experiments reaction mixtures contained 4 μg poly-(dI-dC) and 100 mM $(NH_4)_2SO_4$ [26].

Denaturing SDS/PAGE and protein staining

Polypeptide patterns were analysed by denaturing SDS/PAGE on 10–15% polyacrylamide gels [32], followed by silver staining [33].

Protein quantification

Protein concentration of samples was determined by the Bradford assay [34]. At less than 0.1 μg/

ml, comparative gel scanning of polypeptides in silver-stained gels was used.

Renaturation of SLF activity

A sample of the concentrated material after phosphocellulose chromatography containing SLF activity was loaded on a denaturing SDS gel and electrophoresed at 4 °C. The gel strip was then cut into 0.5 cm slices and polypeptides were eluted [35].

Results

Isolation of sigma-like factors from etioplasts and chloroplasts requires different purification schemes

To decide whether the etioplast transcription apparatus makes use of the same specificity factors as those described for chloroplasts from comparable tissue of the same age [26], we chose cotyledons from five-day-old dark-grown mustard seedlings as starting material. Etioplasts were isolated and lysed and the lysate then subjected to three chromatographic purification steps (Fig. 1). The transcription profile of the first column, heparin-Sepharose (Fig. 2A), shows a broad peak eluting within the range of 0.15–0.5 M $(NH_4)_2SO_4$. The fractions from this region were found to bind to a labelled fragment containing the *psbA* promoter, while no DNA binding activity was observed at salt concentrations higher than 0.5 M $(NH_4)_2SO_4$. When these latter high-salt fractions were assayed for sigma-like factors (SLFs) by carrying out DNA binding in the presence of *E. coli* RNA polymerase core enzyme, no SLF activity was observed (Fig. 2A). This is in contrast to the situation found for chloroplast lysates, where SLF activity was eluted at 1.0–1.3 M salt after heparin-Sepharose chromatography [26] (Fig. 1).

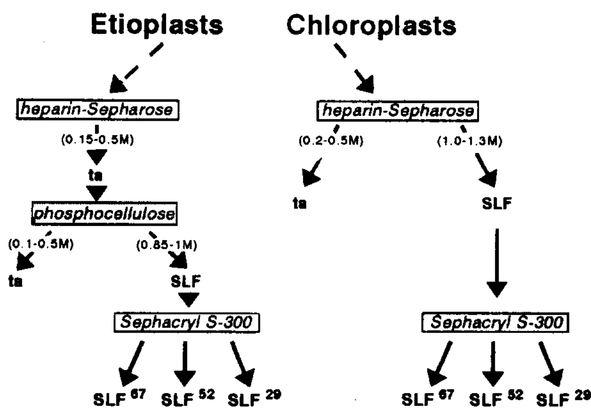

Fig. 1. Purification scheme for sigma-like transcription factors from etioplasts and chloroplasts. Indicated are the column materials (boxed) and the ammonium sulfate concentrations (M) at which fractions were eluted (ta, transcriptionally active fraction; SLF, sigma-like factor).

However, when the transcriptionally active material at 0.15–0.5 M salt was applied to phosphocellulose chromatography (Fig. 2B), two peaks eluting from 0.1–0.4 M and 0.85–1.0 M $(NH_4)_2SO_4$, respectively, were detected by DNA-binding assays. The first peak showed binding in the absence of *E. coli* core enzyme and was capable of transcribing exogenous DNA templates, whereas the second peak was observed only in the presence of core enzyme, indicating SLF activity. When the latter high-salt fraction was rechromatographed on heparin-Sepharose, it eluted at 1–1.3 M $(NH_4)_2SO_4$, i.e. at the salt concentration range reported for chloroplast SLF [26] (data not shown).

FPLC gel filtration (Fig. 2C) further separated the phosphocellulose-purified SLF fraction into three major activity peaks at positions consistent with the M_r values of 67000, 52000 and 29000 previously determined for the three chloroplast SLFs [26].

As summarized in Table 1, microgram amounts of each of the three etioplast SLFs were obtained from 1 kg of cotyledons. Both the yield and specific activity is within the range of values for the chloroplast SLFs.

Etioplasts contain three SLF polypeptides with molecular sizes similar to those of chloroplast SLFs

Protein fractions obtained at various stages of SLF purification (Fig. 1) were analysed by SDS/

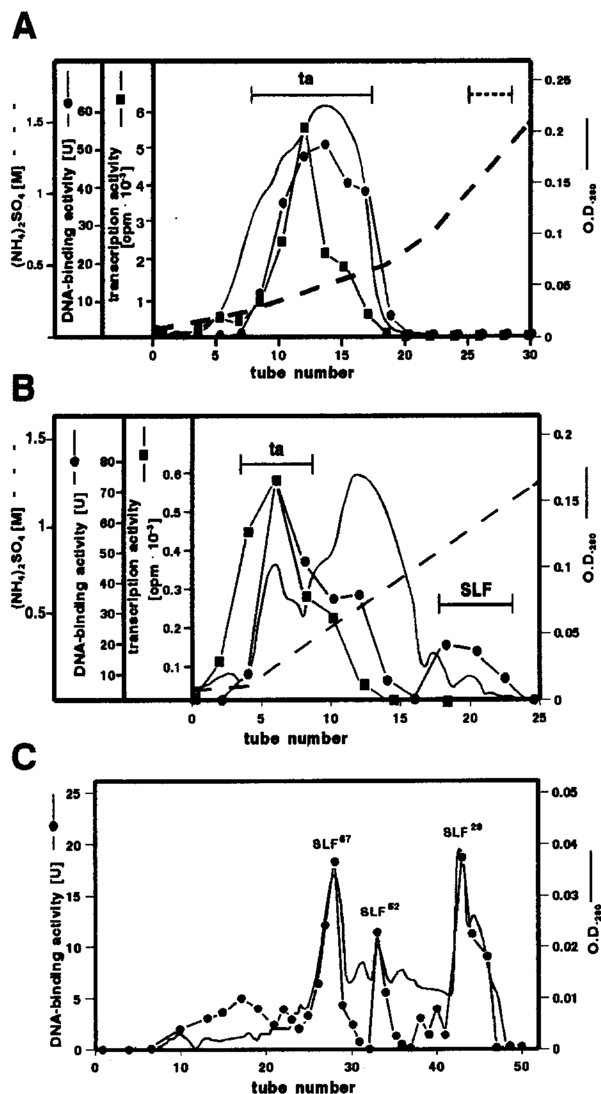

Fig. 2. Chromatography of etioplast sigma-like factors on heparin-Sepharose (A), phosphocellulose (B), and Sephacryl S-300 (C). Profiles show the protein and ammonium sulfate concentrations as well as DNA binding (SLF) and transcription activity (the latter in A and B only). SLF activity was determined by gel retardation assays using dialysed fractions in the presence of *E. coli* core RNA polymerase and a chloroplast DNA fragment containing the *psbA* promoter [26]. The positions of RNA polymerase (ta) and SLF activity are indicated by horizontal bars. The broken bar in (A) marks the expected position for free (chloroplast) SLF [26], where no activity is eluted in the etioplast system.

PAGE (Fig. 3). The heparin-Sepharose fraction containing etioplast RNA polymerase activity (HS-ta) showed multiple polypeptides (Fig. 3, lane 1), a number of which (labelled by asterisks) had M_r values similar to those reported for the subunits of purified chloroplast RNA polymerase from pea [36, 37] and spinach [24]. In addition, fraction HS-ta contained three polypeptides mi-

Table 1. Summary of SLF purification from etioplasts (ET) and chloroplasts (CP).

SLF fraction	Protein (μg)		Activity (U)	
	ET	CP	ET	CP
Heparin-Sepharose		620		8370
Phosphocellulose	90		5500	
Sephacryl S-300				
SLF^{29}	2	3	1900	1020
SLF^{52}	1	4	600	790
SLF^{67}	3	7	1200	740

Protein and activity values for typical preparations from 1 kg of cotyledons from etiolated (Figs. 1 and 2) or light-grown [26] mustard seedlings. One unit (U) of SLF activity is defined as 1% of the total DNA (2 ng) present in the gel retardation assay being in the core-SLF-DNA complex.

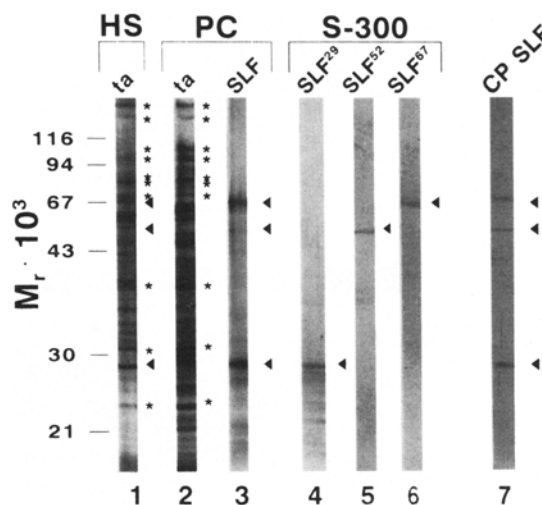

Fig. 3. SDS/PAGE analysis of polypeptides from the heparin-Sepharose (HS), phosphocellulose (PC), and S-300 gel filtration (S-300) stage of purification. The positions of SLFs (lanes 4–6) among the multiple polypeptides of the transcriptionally active fraction (ta) at the HS stage are marked by triangles (lane 1). Asterisks denote the positions of putative subunits of the plastid RNA polymerase core enzyme (lanes 1 and 2). The M_r values of marker proteins are indicated at the left.

grating at M_r 67000, 52000 and 29000, i.e. the values previously determined for mustard chloroplast SLF^{67}, SLF^{52} and SLF^{29} [26]. The relative abundance of these three bands was lower in the leading portion than in the trailing portion of the ta peak (Fig. 2A), and none of them could be detected in the fractions eluting at >0.7 M $(NH_4)_2SO_4$ where the chloroplast SLFs were eluted [26] (data not shown).

The pattern of the transcriptionally active fraction after phosphocellulose chromatography (Fig. 3, lane 2; PC-ta) showed multiple bands including those that could be putative core RNA polymerase subunits [24, 36, 37] (asterisks), whereas the three polypeptides with M_r values similar to those of chloroplast SLFs [26] appeared to be absent. The high-salt phosphocellulose fraction with SLF activity (Figs. 1 and 2B; SLF) contained two major bands at M_r 67000 and 29000 as well as 8–10 minor bands within the M_r range 20000–52000 (Fig. 3, lane 3). FPLC gel filtration of this fraction led to further separation of these polypeptides (Fig. 2C). Each peak contained a prominent band at either M_r 67000, 52000 or 29000, suggesting that the latter are the etioplast counterparts of chloroplast SLF^{67}, SLF^{52} and SLF^{29} (Fig. 3, lane 4–6).

To further assign SLF activity to distinct polypeptides, renaturation experiments were performed [35]. An aliquot of the high-salt phosphocellulose fraction (SLF) was subjected to SDS/PAGE, the gel strip cut into slices, and proteins were then eluted and renatured. Gel retardation assays using this material showed SLF activity for proteins from slices consistent with M_r values of 67000, 52000 and 29000 (Fig. 4), indicating

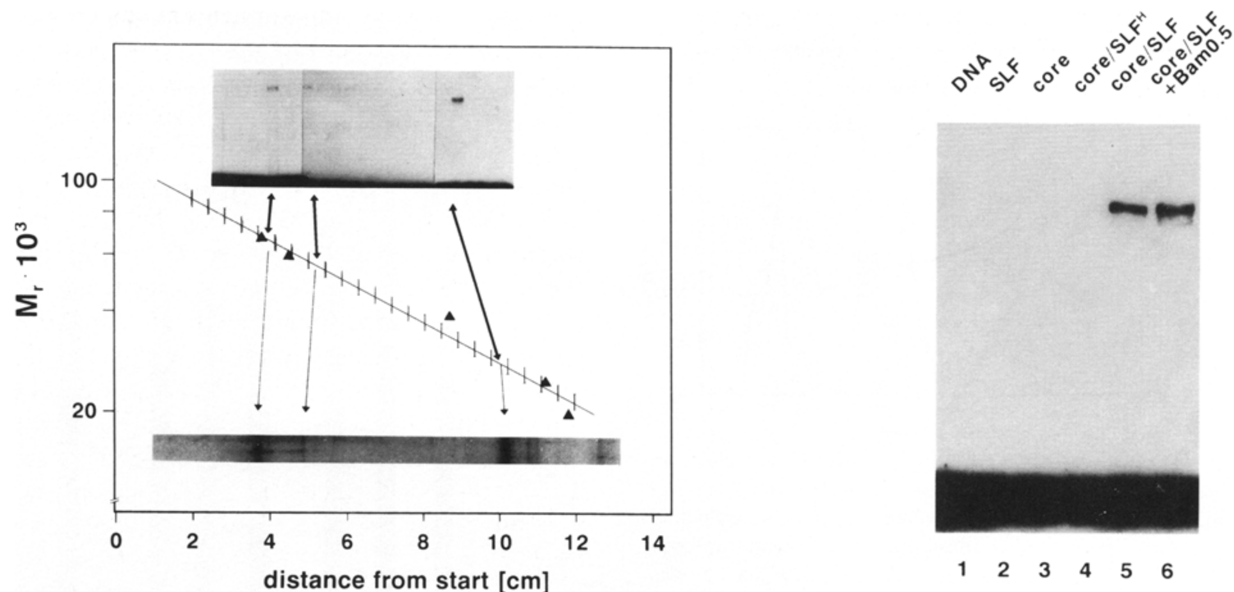

Fig. 4. Left panel: elution and renaturation of etioplast SLF activity after SDS/PAGE. Phosphocellulose-stage SLF (Fig. 3, lane 3) was subjected to SDS/PAGE and the gel strip then cut into slices. Protein was eluted and renatured [35], and aliquots were then used in gel retardation assays with *E. coli* core enzyme and the *psbA* promoter fragment (top inset). Gel slices (borders marked by vertical bars) that correspond to SLF activity are aligned by arrows. The bottom inset shows the silver-stained polypeptides of the phosphocellulose SLF fraction, with arrows pointing to SLF^{67}, SLF^{52} and SLF^{29}. The marker proteins used for calibration of the gel (arrowheads) were bovine serum albumin, katalase, ovalbumin, chymotrypsin and soybean trypsin inhibitor (M_r scale at the left margin). Right panel: controls showing specificity of gel retardation experiments with etioplast phosphocellulose-stage SLF, *E. coli* core RNA polymerase and labelled *psbA* promoter fragment (lane 5): DNA fragment alone (lane 1), DNA plus SLF (lane 2), DNA plus core enzyme (lane 3), DNA plus core and heat-treated (10 min 100 °C) SLF (lane 4), DNA plus core and SLF in the presence of 100-fold molar excess of unlabelled fragment Bam0.5 containing a portion of the *trnK* intron [25]. The latter fragment was previously shown not to act as a competitor in the same system containing chloroplast SLF [25]. In contrast, unlabelled *psbA* promoter fragment did act as competitor (see Fig. 6).

that indeed three etioplast SLFs exist which have the same, or very similar, molecular weight as their chloroplast counterparts. It is not known if the bands migrating ahead of SLF^{29} represent unrelated polypeptides, degradation products, or otherwise modified forms that lack SLF activity.

Etioplast and chloroplast SLFs differ in their salt dependence and promoter specificity

Since the three chloroplast SLFs have defined salt requirements for specific binding [26], this criteria was also used for further analysis of the etioplast SLFs. As shown in Fig. 5 and Table 2, maximal DNA binding to the *psbA* promoter fragment occurs at 80–100 mM $(NH_4)_2SO_4$, regardless of which etioplast factor was present, thus reflecting the situation for the chloroplast SLFs [26]. For each etioplast SLF, however, a second peak of binding activity was observed at 200 mM (Fig. 5), whereas among the chloroplast factors only SLF^{29} was found to give a second

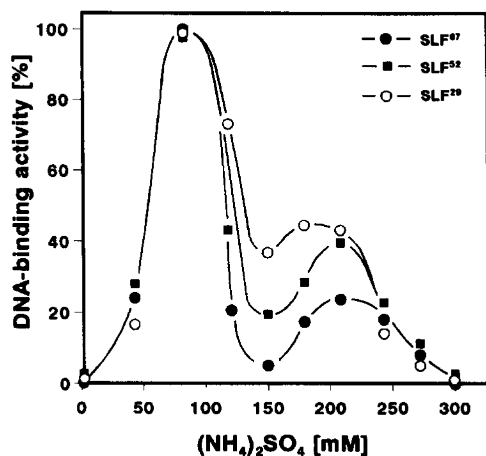

Fig. 5. Salt dependence of DNA-protein binding in the presence of etioplast SLFs. Aliquots of the FPLC-purified SLF fractions (Fig. 3, lanes 4–6) were tested in gel retardation assays with *E. coli* core enzyme and the *psbA* promoter fragment at the indicated salt concentrations. To suppress the salt-dependent unspecific binding of the ^{32}P-labelled *psbA* promoter fragment by the core enzyme [26], 4 μg poly(dI-dC) were added to the incubation mixtures. After electrophoresis, gel drying and autoradiography, DNA-binding activity was determined by comparative gel scanning.

Table 2. Salt characteristics of purified SLFs from etioplasts (ET) and chloroplasts (CP).

SLF fraction	Salt optima	
	ET	CP
SLF^{29}	LS + HS	LS + HS
SLF^{52}	LS + HS	LS
SLF^{67}	LS + HS	LS*

The optima for SLF activity were at 80–120 mM (LS = low salt) and 180–220 mM (HS = high salt) ammonium sulphate, respectively (Fig. 5 and [26]). * Chloroplast SLF^{67} has activity over a broad range of salt concentrations but lacks a distinct high-salt optimum [26].

activity peak at high salt concentrations (Table 2 and [26]). This suggests the possibility of plastid type-specific differences at least in the case of SLF^{67} and SLF^{52}.

To test the three etioplast SLFs for promoter specificity, and to compare their binding characteristics with those of their chloroplast counterparts, competition gel retardation experiments were carried out, using three different chloroplast promoters. Each etioplast SLF was incubated at 100 mM salt with *E. coli* core enzyme and labelled DNA fragments containing the *psbA* promoter in the absence or presence of unlabelled *psbA*, *rps16* or *trnQ* promoter fragments. As shown in Fig. 6, the (unlabelled) *psbA* promoter acted as a strong competitor with either of the three SLFs. Competition by the *trnQ* promoter fragment was efficient in the case of SLF^{67} and SLF^{52}, but there was little effect with SLF^{29}. The *rps16* promoter fragment did not significantly act as a competitor for *psbA* binding in the presence of either SLF.

As shown in Fig. 7, the results obtained with the etioplast SLFs differ from those with the chloroplast factors under the same assay conditions at 100 mM salt [26]. All three etioplast SLFs confer strong binding to the *psbA* promoter-containing fragment, whereas among the chloroplast factors, SLF^{67} does this to a lesser extent than SLF^{52} and SLF^{29}. The weak affinity of etioplast SLF^{29} for both the *trnQ* and *rps16* promoters (as inferred from their low competition efficiency) is in contrast to the strong affinity of

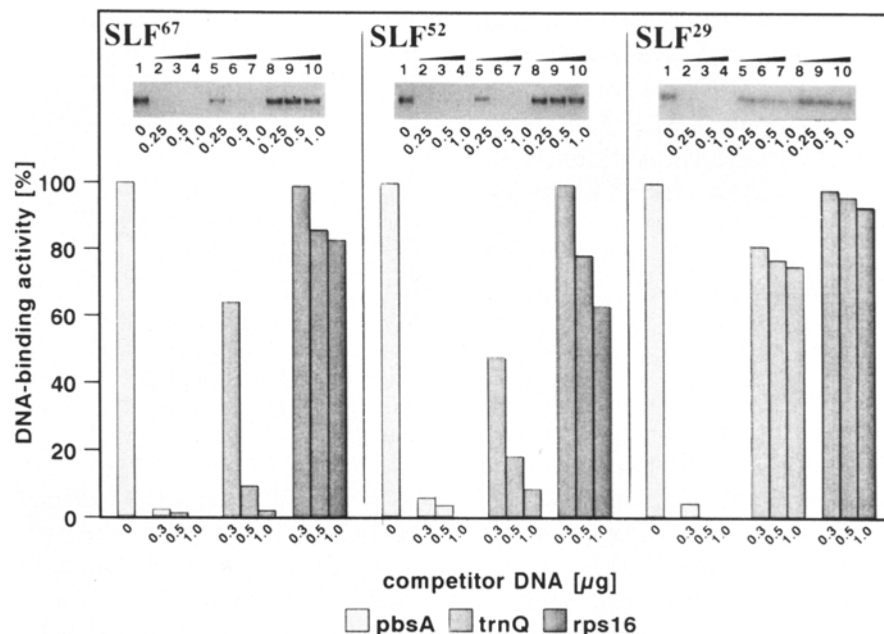

Fig. 6. Promoter preference of etioplast SLFs assessed by competition gel retardation assays. FPLC-purified polypeptides (Fig. 3, lanes 4–6) were incubated at 100 mM $(NH_4)_2SO_4$ with *E. coli* core enzyme, poly(dI-dC) and either the ^{32}P-end-labelled *psbA* promoter fragment alone or in the presence of the indicated anounts of unlabelled *psbA*, *trnQ* or *rps16* promoter fragments. Autoradiographs were scanned and the DNA-binding activity expressed as a percentage of the intensity of DNA-protein complex bands (insets at top) in the absence of competitor DNA.

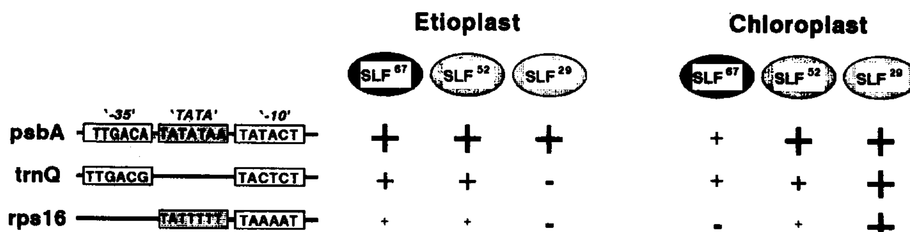

Fig. 7. Scheme showing the promoter binding characteristics of etioplast versus chloroplast SLFs. The relative affinity for a given promoter as inferred from competition efficiency in gel retardation assays (Fig. 6) is indicated by + symbols of various sizes and by − (i.e., no significant competition).

chloroplast SLF[29]. It is interesting to note that at higher salt concentrations (200 mM), chloroplast SLF[29] was found to show weak affinity for these two promoters [26], which resembles the situation with etioplast SLF[29] at 100 mM salt.

Discussion

The work reported here has provided evidence for the occurrence of SLF polypeptides in mustard etioplasts which, based on their apparent sizes, are likely to be direct counterparts of the three chloroplast factors [26]. During the course of this work, however, an unexpected difference between the etioplast and chloroplast systems was noted. In contrast to the efficient separation of chloroplast SLF on heparin-Sepharose, none of the etioplast protein fractions obtained by this chromatographic step exhibited sigma-like activity (Fig. 2A).

Phosphocellulose chromatography was re-

quired as an additional step to separate free SLF activity from the etioplast polymerase (Fig. 2B). This activity could then be resolved by FPLC gel filtration into three fractions, each of which contained a major polypeptide resembling either chloroplast SLF^{67}, SLF^{52} or SLF^{29} (Figs. 2C and 3). The renaturation experiments (Fig. 4) further support the notion that the purified etioplast and chloroplast SLFs have similar molecular weights. Although the amino acid sequences for all these factors remain to be established, the similarity in size suggests that closely related sets of SLFs occur in both chloroplasts and etioplasts. Nevertheless, it appears possible that there might be additional plastid stage-specific factors that were not resolved by our purification scheme. It is notable that factors with SLF activity but different molecular size were reported for spinach chloroplasts [24].

The detection of chloroplast [26] but not etioplast SLF (Fig. 2A) activity on heparin-Sepharose is not an invariable feature of the SLFs from either plastid type, since re-chromatography of phosphocellulose-purified etioplast SLF on heparin-Sepharose resulted in activity that eluted in the same high-salt region of the gradient as did chloroplast SLF (data not shown). It is therefore likely that the differences in the initial chromatographic behaviour reflect the extent to which SLF polypeptides are associated with the (core) polymerases from either plastid type. The etioplast holoenzyme could be more stable than its chloroplast counterpart as a result of a different polypeptide composition and/or post-translational modification.

Despite the similarity in size of chloroplast versus etioplast SLFs, the etioplast factors show distinct functional characteristics as compared to their chloroplast counterparts. For instance, etioplast SLF^{67}, SLF^{52} and SLF^{29} all promote specific DNA-binding at both lower (100 mM) and higher (200 mM) $(NH_4)_2SO_4$ concentrations (data not shown), whereas chloroplast SLF^{52} shows significant affinity only at the lower salt concentration. In addition, chloroplast SLF^{29} shows preference for the *psbA* promoter only at high-salt conditions [26].

At 100 mM salt, i.e. under conditions of maximal DNA binding activity (Fig. 5), the overall preference of etioplast SLFs for the *psbA* promoter is at least as high as that revealed by their chloroplast counterparts [26]. The question emerging from these data is how this preference of both the etioplast and chloroplast SLFs for the *psbA* promoter can be reconciled with plastid type-specific differential transcription of this gene. It could be argued that the binding studies were performed in a heterologous system using *E. coli* core enzyme and plastid factors. However, similar results were also obtained in a homologous system using either etioplast or chloroplast RNA polymerase holoenzyme at the heparin-Sepharose stage. On the other hand, we found a 5–10-fold higher transcription activity for the chloroplast holoenzyme (stage ta supplemented with SLF; Fig. 1) as compared to the etioplast enzyme, which contains tightly bound SLF at this stage (Figs. 1 and 2) (K. Tiller and G. Link, unpublished data). Taken together, this may indicate negative control of transcription efficiency in the etioplast system in the presence of SLF.

Since functional differences were observed for highly purified SLFs from etioplasts versus chloroplasts, these are likely related to inherent properties of these polypeptides themselves, rather than to the presence or absence of undefined contaminants. Hence, these differences might be the result of variations in primary sequence or of post-translational modifications or both. An answer to this question can ultimately be expected from the cloning of the SLF genes for both the chloroplast and etioplast factors. This would also provide information on the intracellular coding site for the various SLFs. The genes for polypeptides resembling the bacterial β, β' and α subunits were shown to be located on the chloroplast DNA from several plant species (for review, see [19, 39]).

However, no evidence is available thus far for a chloroplast open reading frame with sequence similarity to genes for prokaryotic sigma factors. This could be related to the overall low degree of sequence homology among sigma factors from bacteria [22], including cyanobacteria [38], or to

the complex post-transcriptional regulation of chloroplast gene expression including *trans*-splicing [39] and RNA editing [40]. It appears equally likely, however, that the plastid SLFs are products of nuclear genes. This would not be in contradiction to their physical and functional relationship to bacterial sigma factors since there are precedents in other eukaryotic cells. Several nuclear sigma-like transcription factors have been reported [41, 42] and the nuclear-coded transcription factor MTF1 from yeast mitochondria was shown to reveal sequence similarity with bacterial sigma factors [43].

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