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Review

Plastid sigma factors: Their individual functions and regulation in transcription☆

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ABSTRACT

Sigma factors are the predominant factors involved in transcription regulation in bacteria. These factors can recruit the core RNA polymerase to promoters with specific DNA sequences and initiate gene transcription. The plastids of higher plants originating from an ancestral cyanobacterial endosymbiont also contain sigma factors that are encoded by a small family of nuclear genes. Although all plastid sigma factors contain sequences conserved in bacterial sigma factors, a considerable number of distinct traits have been acquired during evolution. The present review summarises recent advances concerning the regulation of the structure, function and activity of plastid sigma factors since their discovery nearly 40 years ago. We highlight the specialised roles and overlapping redundant functions of plastid sigma factors according to their promoter selectivity. We also focus on the mechanisms that modulate the activity of sigma factors to optimise plastid function in response to developmental cues and environmental signals. This article is part of a Special Issue entitled: Chloroplast Biogenesis.

1. Introduction

It is generally accepted that the plastids of vascular plants originated from an ancestral cyanobacterial endosymbiont. The plastids have retained bacterial-like gene expression machinery. However, the massive transfer of genes encoding components of the photosynthetic apparatus to the nucleus after endosymbiosis together with the integration of the organelles into plants with distinct developmental stages and cell types has necessitated new strategies to coordinate the expression of plastid and nuclear genes. As a result, eukaryotic features were also acquired after plastids were incorporated into eukaryotic cells [1]. In vascular plant plastids, for example, transcription is carried out by both a nuclear-encoded phage-type RNA polymerase (NEP) and the cyanobacterium-derived plastid-encoded RNA polymerase (PEP) [1,2]. Moreover, most homologues of bacterial transcription factors have been lost in plastids, whereas a number of non-bacterial eukaryotic type nucleic acid binding-proteins likely involved in plastid transcription have been acquired during evolution [3,4]. Therefore, the plastid transcriptional machinery represents a mixed system with prokaryotic and eukaryotic traits [3,4]. It is therefore a good model to study transcription mechanisms in an evolutionary context.

Transcription is roughly divided into the following three major stages: initiation, elongation and termination [5]. Initiation is a dynamic and highly regulated step of gene transcription in both bacteria and eukaryotes. In eubacteria, the key players in transcription initiation are sigma factors (σ), which associate with the catalytic core of RNA polymerase (RNAP) to guide it through the essential steps of initiation, which are promoter recognition and opening and synthesis of the first few nucleotides of the transcript [6]. After RNA synthesis has started, sigma factors are released from the RNAP. However, a population of sigma factors is retained throughout elongation [6]. The PEP has evolved from eubacterial-type RNAPs. Accordingly, sigma factors mediate transcription initiation for PEP. Nevertheless, more complicated mechanisms than those used by eubacteria have evolved to modulate the activity of sigma factors through which plastid transcription is finely regulated.

In past decades, the use of genetic and biochemical approaches together with the ability to manipulate the plastid genome in several species has significantly advanced our understanding of plastid transcription at the mechanistic level. The aim of this review is to summarise recent advances in understanding the structure, function and regulation of the plastid sigma factors. We also pay specific attention to the mechanism through which the activities of sigma factors are modulated to

Abbreviations: ALA, aminolevulinic acid; BLRP, blue-light-responsive *psbD* promoter; CK2, casein kinase 2; CR, conserved region; CSK, chloroplast sensor kinase; MEcPP, methylerythritol cyclodiphosphate; NEP, phage-type RNA polymerase; PAP, 3′-phosphoadenosine 5′-phosphate; PEP, plastid-encoded RNA polymerase; PhANG, photosynthesis-associated nuclear gene; ppGpp, nucleotide guanosine tetraphosphate; PQ, plastoquinone; RNAP, RNA polymerase; UCR, unconserved region

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optimise plastid function in response to developmental cues and environmental signals.

2. Overview of plastid sigma factors

The first report on a plastid sigma factor appeared almost 40 years ago [7]. In this report, a putative plastid sigma factor protein was purified from a green alga, *Chlamydomonas reinhardi*, using biochemical assays. Such proteins with sigma factor activity were subsequently identified in red alga and higher plants [8–12]. Proteins associated with sigma factor activity were initially named "sigma-like" factors [8, 9]. Sequence analysis of all cloned plastid sigma factors clearly revealed the presence of the typical sigma factor domains. Together with functional assays, these findings justify their assignment as true sigma factors [12–14]. In contrast to the PEP core subunits, all of the sigma factors from higher plants are nuclear encoded rather than plastid encoded. Bacteria have a single essential housekeeping sigma factor that promotes the transcription of thousands of genes and many alternative sigma factors that promote the transcription of a specialised gene set required for adaptive stress [15]. In agreement with the multiple sigma factors typically found in bacteria, more than one sigma factor is usually found in plastids of higher plants [16–20]. This was also revealed through the sequencing of the genomes of several plastid-containing species including *Arabidopsis* (6 sigma genes), rice (*Oryza sativa*) (6 genes), *Sorghum biocolor* (6 genes), *Vitis vinifera* (6 genes) and *Populus trichocarpa* (7 genes) [21]. In particular, the six *Arabidopsis* sigma factors designated SIG1–SIG6 were extensively studied. Recent work with sigma factor mutants or antisense plants revealed that multiple sigma factors can have specialised roles and overlapping redundant functions based on the promoters they recognise (see below for details).

All plastid sigma factors belong to the superfamily of σ^A/σ^{70} and have sequences homologous to the conserved regions 1.2, 2, 3, and 4 of bacterial sigma factors (Fig. 1). In addition, conserved regions of some plant sigma factors can replace σ^{70} regions of *Escherichia coli* [12]. It was therefore proposed that plastid sigma factors could adopt a similar spatial configuration and perform a function analogous to that of their bacterial homologues. However, plastid factors lack the 1.1 region of bacterial factors [22] (Fig. 1). In addition, plant sigma factors also display considerable variation in conserved domains [22–24]. These changes might confer different affinities for core enzyme, diverse ratios of abortive and processive transcription, and different preferences in choosing sequences of σ^{70}-type promoters to plastid sigma factors compared to those of *E. coli*. Region 2 of SIG5 is one such example. In bacteria, regions 2.1 and 2.2 (two sub-regions of region 2) form the main domain that binds to the RNAP core [25,26]. Regions 2.1 and 2.2 of most plant sigma factors display high similarity to *E. coli* sigma factors except for SIG5, which has the lowest similarity to the common consensus in these two regions. Due to this difference, it has been proposed that the interaction between SIG5 and the core enzyme is weaker than the interactions between core enzyme and other sigma factors. Consequently, SIG5 is less competitive in binding the core, but a holoenzyme comprising SIG5 has a lower level of abortive transcription [22]. Such a property may be critical for stress-inducible trans-factors like SIG5 [27]. For details on the similarities and differences in structure and function between bacterial and plastid sigma factors, we refer readers to the review of Lysenko [22].

The conserved region (CR), which is localised in the C-terminal region of sigma factors, is strikingly uniform in both sequence and length. However, the N-terminal half of the plant sigma factors does not share significant homology with bacterial factors. This region was named the UCR (unconserved region), and its role is only starting to be elucidated [23,24,28]. Schweer et al. identified two regions containing cpCK2 phosphorylation sites in the UCR of SIG6 [29]. One covers the region marked by Ser-94 and Ser-95, while the other spans several serine residues between Ser-174 and Ser-180. Similarly, Thr-170 in the UCR of SIG1 was also demonstrated to be a phosphorylation site [30]. By studying various phosphorylation-site mutants both in planta and in vitro, they demonstrated that the phosphorylation sites within UCR are critical for the activity and specificity of SIG6 [29]. In addition, the UCR of SIG6 was found to be responsible for the interaction of SIG6 with other partners [31]. The UCR function of other plastid sigma factors has not yet been elucidated.

3. Sigma-associated plastid promoters

Although sigma factors do not specifically bind promoters on their own, all specific recognition of promoters by RNAP is mediated by sigma factors [6]. In accordance with the cyanobacterial origin of PEP, despite many variable features, two conserved sequence motifs commonly known as the −35 region (TGACA) and −10 region (TATAAT) of typical bacterial sigma-recognised promoters are found upstream of many plastid genes [32,33]. Because such plastid σ^{70}-type promoters are used by PEP, they are also called PEP promoters. The plastid σ^{70}-type promoter can be fully recognised by *E. coli* RNAP in vitro, suggesting its similarity to the eubacterial σ^{70} promoter [34–36]. It is interesting that the plastid genes of *Chlamydomonas* do not contain valid −35 elements [37], whose function might be served by a more remote sequence in the coding region of some plastid genes of *Chlamydomonas* [38–40].

The function of these elements was dissected by in vitro transcription experiments. Several of these studies were carried on the *psbA*

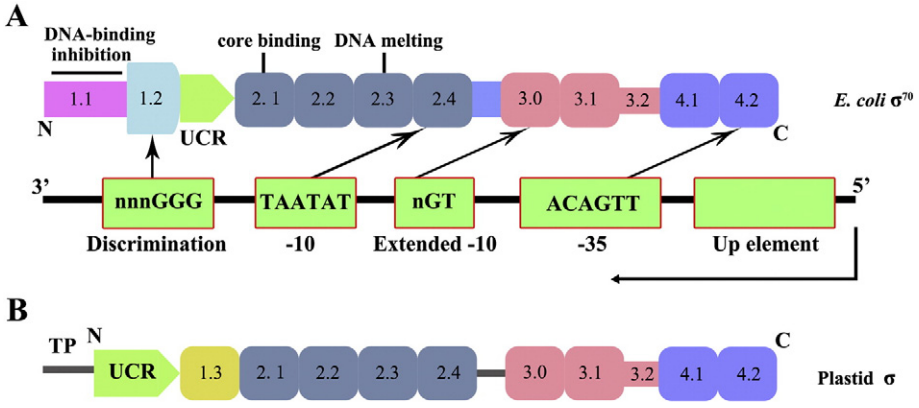

Fig. 1. Structural organisation of sigma factors. (A) Domains and associated functions of *E. coli* σ^{70} proteins. 1.1-4.2 indicate different conserved regions of *E. coli* σ^{70} proteins. UCR indicates an unconserved region. Consensus for the −35 hexamer (−35 to −30), the extension −10 element (−15 to −13), the −10 hexamer −12 to −7), and discriminator DNA (−6 to −1, with an optimal GGG −6 to −4), relative to the transcriptional start are shown in the lower lane. The vertical arrow indicates the transcription initiation site. (B) Structural scheme of plastid sigma factors. TP indicates the transit peptide.

gene, which encodes the D1 polypeptide of photosystem II. Data from mustard and spinach barely showed that *psbA* is transcribed from a typical σ^{70}-type promoter [41–43]. In addition to −35 and −10 elements, a TATATA promoter element between the −10 and −35 hexamers resembling the TATA-box of nuclear genes transcribed by RNA polymerase II was identified in the *psbA* promoter of mustard [41]. The TATA TA and −10 elements were sufficient to drive basic transcription in plastid extracts from both dark- and light-grown plants. However, enhanced rates of *psbA* transcription in light-grown plants were obtained only if the −35 element was included in the *psbA* promoter [44]. Therefore, the −35 element does not seem to play an essential role for basic *psbA* promoter activity. Another study showed that the function of the −10 element of the *rrn* P1 PEP promoter could be replaced (or modified) by a cis-element called RUA (rRNA operon upstream activator) [45]. Therefore, the roles of the −10/35 elements of plastid genes might not be as critical as those in *E. coli*. Many regulatory elements that collaborate with −10/−35 elements to regulate individual promoter activity have also been identified in different plastid promoters. Those regulatory elements contribute to modulate the transcription activity of plastid genes in response to exogenous signals, especially light [46–52].

The σ^{70}-type promoter displays some variations among different species. The *psbA* promoter of barley and wheat contains a TATATA motif between the −10 and −35 elements. Similar to that of mustard, the TATATA element of barley is important for full promoter activity. In contrast, the TATATA element of wheat does not seem to be critical for *psbA* transcription [53]. Unlike mustard, the −35 element of barley is absolutely required for transcription activity [52]. These studies indicate differences in the utilisation of *cis* elements of the plastid σ^{70} promoter, which probably reflect the divergent evolution of trans-factors in these species.

4. Function of plastid sigma factors

The roles of plastid sigma factors in the regulation of plastid transcription have been extensively explored by the characterisations of knockout mutants as well as anti-sense lines of different plastid sigma factors. The specific roles of individual sigma factors in plastid gene expression have been described in several reviews in great detail [21–23]. Here, we will summarise the major facts and conclusions. We focus on how the sigma factor activity is modulated to optimise plastid function in response to developmental cues and environmental signals through adjustments of plastid gene transcription.

4.1. SIG1

The recombinant SIG1 protein from *Arabidopsis* and mustard binds to the promoters of *rbcL* and *psbA*, although this binding ability is rather weak compared to that of SIG2 and SIG3 [12,14,54]. The abundance of transcripts derived from the *psaA* operon was markedly reduced in a rice *SIG1* knock-out mutant (*OsSIG1*), suggesting that OsSIG1 specifically participates in the transcription of the *psaA* operon [55]. A reduction in the abundance of the core protein complex of photosystem I was also observed in this mutant [55]. Further studies showed that SIG1 is phosphorylated in vivo and that the phosphorylation levels of SIG1 change in response to the redox state of the plastoquinone pool [30]. Changes in phosphorylated amino acids of SIG1 alter the ratio of *psbA* to *psaAB* transcription in vivo. In addition, *SIG1* mRNA is rapidly and strongly induced by red light as well as by blue light [56]. Therefore, it has been proposed that SIG1 phosphorylation might play an important role in the adjustments of photosystem stoichiometry to daily changes in light intensity.

In the *OsSIG1* mutant, reduced mRNA levels were also observed for all the genes of the *psbB* and *psbE* operons, although to a lesser extent [55]. SIG1 was also found to bind additional plastid promoters besides the *psaA* promoter using a chloroplast chromatin immunoprecipitation assay [57]. Thus, SIG1 seems to be involved in the regulation of other plastid genes besides *psaA*, and the overall transcriptional regulation by SIG1 should be further clarified in the future.

4.2. SIG2

The *sig2* mutant and anti-sense RNA plants display a chlorophyll-deficient phenotype, presumably as a result of impaired chloroplast development, suggesting that SIG2 acts as an essential sigma factor for plastid function [54,58]. The molecular characterisation of *sig2* mutants revealed that the transcript levels of *psaJ*, *psbD* (initiated from promoter P_{psbD}-256) and several tRNAs (*trnV-UAC*, *trnM-CAU* and *trnE-UUC*) were reduced in these mutants [54,58,59]. In anti-sense *sig2* plants, the amounts of *psbA* transcripts also decreased, and they could not be detected in knock-out plants. The recognition of the *psaJ* and *trnV-UAC* promoters and the P_{psbD}-256 promoter seems to be specific to SIG2, whereas the *trnE-UUC* and *trnM-CAU* promoters may also be recognised by other sigma factors given that the corresponding transcript levels of *trnE-UUC* and *trnM-CAU* were only diminished and not completely eliminated [54,58]. SIG2 can also recognise P_{atpI}-229/225 promoter and an internal promoter of *atpE* (P_{atpE}-431), which might be a mechanism to coordinate the synthesis of a basal level of *atp* mRNAs coding for the different ATP synthase subunit [60].

The function of SIG2 in transcriptional switching from mainly NEP to mainly PEP transcription during early plant development and chloroplast biogenesis has been proposed [61]. The two types of RNA polymerases have distinct roles in the plastid transcription of vascular plants according to the genes they transcribe. PEP is mainly responsible for the transcription of photosynthetic genes, while NEP is responsible for the transcription of housekeeping and PEP genes [62]. Consequently, an initial model for the response of plastid transcription to developmental cues has been suggested. This model postulates that during early chloroplast development, NEP is activated for the accumulation of PEP, which then transcribes photosynthetic genes in later stages of chloroplast development accompanied by repression of NEP activity [1,2]. However, the mechanism of NEP repression is unknown. One of the tRNAs regulated by SIG2, *trnE-UUC*, functions both in plastid protein synthesis and aminolevulinic acid (ALA, a chlorophyll precursor) synthesis. It has been shown that *trnE-UUC* directly binds to the RPOTp (one of NEP RNA polymerases) and inhibits its transcriptional activity in vitro [61]. The repression of NEP-dependent gene transcription does not occur in the *sig2* mutant due to the lack of *trnE-UUC*. However, this model has been challenged. Bohne et al. showed that other tRNAs also bind to RPOTp and inhibit transcription [63]. Further experiments are needed to answer the question of whether RPOTp transcriptional activity is inhibited by all tRNAs or only by some tRNAs and whether these tRNAs are all transcribed by SIG2.

4.3. SIG3 and SIG4

Unlike the *sig2* mutant, neither the *sig3* nor the *sig4* mutant shows any visible phenotype. However, obvious defects in plastid gene expression occur in these mutants. The microarray analysis by Zhhidi et al. revealed two significantly reduced mRNAs in the *sig3* mutant, *psbN* and *atpH*. The *psbN* gene is transcribed from a single promoter, and this study also showed that the transcription of *psbN* completely depends on SIG3 [64]. In addition, by specific transcription initiation at the *psbN* promoter, SIG3 could also influence the expression of the *psbB* operon (in particular the accumulation of the PSBT protein) transcribed from the opposite DNA strand by producing *psbT* antisense RNA [64,65]. Similarly, the microarray analysis of the *Arabidopsis sig4* mutant showed a strong and specific diminution of one mRNA, *ndhF*, which encodes a subunit of the plastid NADH dehydrogenase-like complex [66]. This study demonstrated that SIG4 is involved in the transcription of *ndhF*.

4.4. SIG5

SIG5 of *Arabidopsis* can modulate the transcription of *psbD* by specifically recognising the blue-light-responsive *psbD* promoter (BLRP), which is present in various plant species. The *psbD–psbC* operon encodes the reaction centre protein D2 and the chlorophyll-binding antenna protein CP43 of photosystem II [67]. The *psbD/C* operon can be transcribed from multiple PEP promoters. However, in mature leaves, *psbD/C* is transcribed predominantly from the blue-light-responsive promoter BLRP, which is selectively activated by illumination with high-fluence blue/UV-A light [47–52,68,69]. This might serve to maintain high rates of synthesis of D2 and PSII activity under high-light conditions. In vivo mutational analyses have demonstrated that the *psbD* BLRP contains σ^{70}-type core promoter elements. However, the *psbD* BLRP is a structurally unique PEP promoter with a typical −10 region but a less conserved and nonessential −35 region [52].

SIG5 is induced by blue-light irradiation as well as various stress conditions and directs *psbD* BLRP transcription. It is thus considered a multiple stress-responsive sigma factor that enhances the turnover of damaged PSII reaction centre proteins [27,70–72]. A protoplast transient expression assay with a SIG5–SIG1 chimeric gene showed that SIG5 contains determinants for activating the *psbD* BLRP in region 4.2 rather than region 2.4, and Asn484 functions as a key residue for the activation of *psbD* BLRP [56]. However, it was reported that SIG5 can also recognise the promoter of other genes, i.e., *psbA* [71]. In addition, one mutant line of SIG5 was reported to be embryonic lethal [73]. These studies suggest multiple roles of SIG5 in plastid transcription and plant development. Interestingly, SIG5 also exists in the liverwort *Marchantia polymorpha*, and its expression was induced by blue-light irradiation and under various stress conditions as well. However, the relevant blue-light-responsive promoter was not found in *M. polymorpha*, and *psbD* transcript accumulation did not occur upon blue-light treatment [74]. The physiological role of SIG5 seems to vary among plant phyla.

Although it has been established that SIG5 acts as a mediator of blue light signalling to activate *psbD* BLRP transcription, how this signal is perceived and transmitted in plastids remains unknown. The signal-transduction pathway is assumed to involve the perception of blue light by cryptochromes and phytochrome A, transfer of the signal by the protein phosphatase PP7, and subsequent induction of SIG5 expression [69,75,76]. A possible downstream signal component of cryptochromes/phytochrome is DET1 (de-etiolated 1), a repressor of photomorphogenesis, because the accumulation of the *psbD* BLRP transcript was down-regulated in young *det1* seedlings [77].

A recent study revealed a novel function of SIG5 in the circadian control of plastid transcription [78]. Circadian oscillations in the abundance of many plastid-encoded transcripts occur, but how the circadian clock regulates the transcription of plastid genes is largely unknown. Many studies have shown that expression of several plastid sigma factors in algae and higher plants is regulated by circadian or diurnal rhythms [19,79–84], but whether plastid sigma factors directly participate in circadian control of plastid transcription was not addressed. Noordally et al. showed that SIG5 controls the circadian rhythms of transcription of several plastid genes and revealed one pathway by which the nuclear-encoded circadian oscillator controls the rhythms of chloroplast gene expression [78]. SIG5 might therefore play a critical role in mediating the circadian gating of light input for chloroplast-encoded genes. The integration of light and circadian signals has been demonstrated in higher plants [85], and SIG5 seems to provide a clue to study the integration of light and circadian signalling in the regulation of plastid gene transcription [84].

4.5. SIG6

Similar to *sig2*, the *sig6* mutant displays a chlorophyll deficiency phenotype but specifically in cotyledons. The accumulation of most PEP-dependent transcripts including mRNAs encoding thylakoid proteins, some tRNAs and rRNA are reduced in an early developmental stage but restored to wild-type levels in a later developmental stage in *sig6* seedlings [86,87]. These data indicate that SIG6 acts as a principal sigma factor during early plant development. However, Schweer et al. also showed that transcripts of certain plastid operons (*atpB/E*, *ndhC/psbG/ndhJ*) are negatively affected until the rosette stage of the *sig6* mutant, suggesting a second, longer-term role of SIG6 during plant development [88]. Based on the (transient) appearance and disappearance of the 4.3 kb *atpB/E* transcript in *sig6* during different chloroplast developmental stages, a promoter switch mechanism that can rescue a plant sigma factor mutant has been proposed [88]. In the seedling stage of *Arabidopsis*, a far-upstream NEP promoter cluster is activated. This activation counteracts the loss of SIG6/PEP-dependent transcription and ensures that RNA levels are those normally obtained by the proximal NEP promoter. However, this promoter activation disappears at the mature stage, when the proximal NEP promoter alone is sufficient for transcription [88].

A role of SIG6 and SIG2 in plastid-nucleus communication has emerged. The compartmentalisation between genes and gene products in plastids and nucleus requires the coordination of plastid and nuclear gene expression to maintain plastid development and function. The plastid development and gene expression are largely under nuclear control, referred to as anterograde control. However, the coordination is also mediated by retrograde control, which functions in the opposite direction [89,90]. Distinct putative plastid retrograde signalling pathways have been recognised based on the sources of the signals, including intermediates in the tetrapyrrole biosynthetic pathway, plastid gene expression, plastid redox state and reactive oxygen species (ROS) and several novel plastid retrograde signalling pathways mediated by stress-induced metabolites such as PAP (3′-phosphoadenosine 5′-phosphate) and MEcPP (methylerythritol cyclodiphosphate) [90]. Woodson et al. showed that SIG2 and SIG6 have partially redundant roles in retrograde signalling to control nuclear gene expression [91]. They demonstrated that these sigma factors are involved in at least two retrograde signalling pathways. One involves transcription of plastid tRNA Glu by SIG2 to increase tetrapyrrole synthesis and promote photosynthesis-associated nuclear gene expression [91]. The tetrapyrrole intermediate Mg-ProtoIX not only controls expression of photosynthesis-associated nuclear genes but also controls the expressions of plastid genes, and the latter might occur by regulation of plastid sigma factors [92]. Nevertheless, the role of Mg-ProtoIX in plastid signalling has been disputed [93,94]. SIG6 and SIG2 were also found to be involved in the phytochrome-dependent coordinated control of plastid gene expression in anterograde signalling during photomorphogenesis [95,96]. SIG6 and SIG2 seem to have dual functions in plastid-nucleus communication (Fig. 2.).

In the dark, the *flu* mutant of *Arabidopsis thaliana* overaccumulates protochlorophyllide, which generates singlet oxygen upon illumination [97]. The discovery of one suppressor mutation of the $^1O^2$-mediated stress response of young *flu* seedlings, *soldat8* (*s*inglet *o*xygen-*l*inked *d*eath *a*ctivator8), suggested an unexpected role of SIG6 in $^1O_2$-mediated cell-death responses [98]. The *soldat8* mutant was shown to impair *SIG6* gene expression. The suppression of $^1O_2$-mediated stress responses by *soldat8* mainly occurred at the seedling stage [98]. No obvious change in the plastid transcript level was detected in the *soldat8* mutant. However, the amount of D1 protein was strongly reduced in young *soldat8* seedlings compared to wild-type plants. The mechanism through which the *sig6* mutation predominantly blocks the $^1O_2$-mediated cell death response of *flu* seedlings is unknown. It might be related to the enhanced light sensitivity and acclimation response [98].

The functional role and temporal/spatial distribution of sigma factors were also characterised in other photosynthetic organisms, including mustard, maize, tobacco and *Physcomitrella patents* [23]. One particularly interesting sigma factor is ZmSIG2B, which can undergo dual targeting to both plastids and mitochondria [99]. SIG5 was also

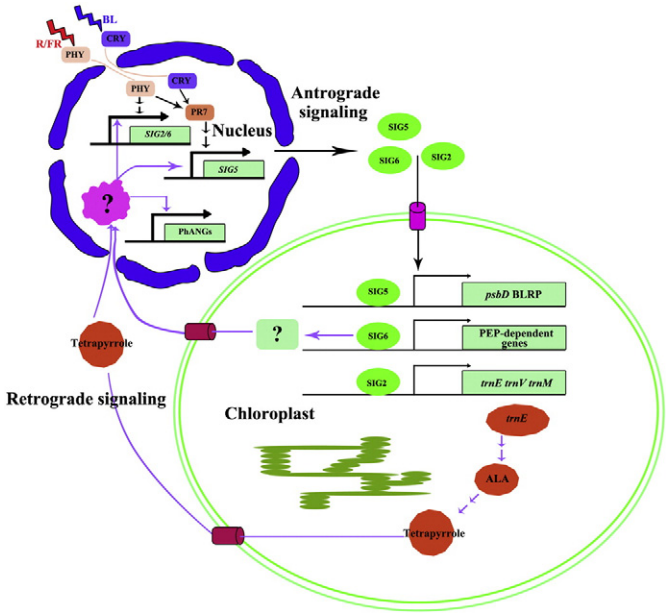

Fig. 2. Involvement of plastid sigma factors in chloroplast-nucleus communication during chloroplast development. In the anterograde signalling pathway, the light signal is perceived by the photoreceptors then transmitted to the *SIG* genes through a largely unknown pathway(s) and promotes the transcription of *SIG* genes. SIG proteins are synthesised in the cytosol and subsequently imported into chloroplasts where they control the transcription of chloroplast genes. In the retrograde signal pathway, SIG2 and SIG6 generate two plastid retrograde signals. One results from transcription of the plastid *trnE-UUC* by SIG2 to increase tetrapyrrole synthesis and promote PhANG expression in the nucleus, while the nature of the other retrograde signal is unknown. Tetrapyrroles also regulate the expression of SIG genes; however, the mechanism remains unknown. The different circle colours indicate the distinct factors involved in anterograde and retrograde signalling. The black and purple arrows indicate the anterograde and retrograde signalling pathway, respectively. BL: blue light; R/FR: red/far red light; PHY: phytochrome; CRY: cryptochrome; ALA: aminolevulinic acid.

found to be localised to mitochondria [100]. One might speculate that ZmSIG2B is involved in mitochondrial transcription mediated by a phage-type RNA polymerase. However, experimental data to link the activities of bacterial-type sigma factors to phage-type RNA polymerases are still lacking.

5. Regulation of plastid sigma factor activities

Modulation of the levels and/or activities of different sigma factors and, consequently, the levels of cognate RNAP holoenzymes provide a simple yet versatile means to control transcription initiation [15]. In bacteria, the mechanisms that regulate the activity of sigma factors include binding to partner proteins that target sigma factor for destruction, proteolytic cleavage of inactive precursors to remove initiation N-terminal extension and use of alternative start codons to generate high-molecular weight variants that are vulnerable to rapid proteolytic turnovers [101]. In many other cases, the primary level of control is achieved by anti-sigma factors that can sequester sigma factors to prevent their association with the core RNAP [15]. In addition, the modulation of sigma factor activity involves regulatory molecules such as the nucleotide guanosine tetraphosphate (ppGpp) and proteins such as DksA that directly target the active site of σ-RNAP [15,102]. Therefore, the regulation of bacterial sigma factors is relatively diverse and occurs at distinct levels. In plants, anti-sigma factors have not yet been identified, and very little is known regarding the presence and function of ppGpp in plastids. However, the activity of plastid sigma factors is regulated by direct phosphorylation, which does not exist in bacteria [24]. In addition, several sigma-interacting partners were identified in plastids. However, they do not seem to act as anti-sigma factors [21,22].

5.1. Phosphorylation regulation of plastid sigma factors

The mechanisms and the significance of phosphorylation were studied in SIG1 and SIG6. This type of regulatory mechanism is clearly different from what is known about the regulation of sigma factor activity in prokaryotes, and the regulatory phospho-acceptor sites seem to reside exclusively within the highly variable UCRs of plastid sigma factors (also see Overview of Plastid Sigma Factor). Phosphorylation can have both positive and negative effects on transcription depending on the member of the sigma family and which promoter it recognises [24]. For instance, phosphorylation of SIG6 is critical for *atpB* transcription whereas it has no effect on *psbA* transcription in vivo and in vitro [29]. However, mutation of the phosphorylated Thr-170 of SIG1 reduced the transcription of both *psaA* and *psbA* [30]. These differences in response to phosphorylation of sigma factors likely reflect the differences in promoter architecture, including the presence of the TATA box elements and extended −10 elements.

A PEP-associated Ser/Thr protein kinase named plastid transcription kinase (PTK) was proposed to participate in plastid sigma factor phosphorylation [103]. The catalytic component of PTK is closely related to the subunit of casein kinase 2 (CK2) and was subsequently named cpCK2 [104]. PTK/cpCK2 itself responds to phosphorylation and the thiol redox state in vitro. The target phosphorylation sites are considered to be unique in terms of their acidic (rather than basic) phosphoacceptor motif S/TxxE/D, where E/D at the $n + 3$ position can be substituted by phosphoserine, and their ability to use GTP as donor [105]. cpCK2 is involved in numerous signalling chains in plants cells and is proposed to be part of a signalling pathway controlling PEP activity [106]. Light-dependent reduction of GSSG would inactivate cpCK2, while dephosphorylation of PEP under high light conditions would enhance PEP-dependent transcription. It has been demonstrated that SIG6 is a substrate for regulatory phosphorylation by cpCK2 both in vivo and in vitro [29]. One of the most sensitive phosphorylation sites of SIG6 (Ser174) is not a typical cpCK2 phosphoacceptor motif. It was therefore suggested that sigma factor phosphorylation by cpCK2 might be assisted by other "pathfinder" kinase(s) that generate CK2 sites by pre-phosphorylation [29]. This might be used to increase the flexibility of transcriptional regulation *via* sigma factor phosphorylation. Nevertheless, this potential "pathfinder" kinase has not yet been identified.

A different signalling pathway for SIG1 phosphorylation is used. It is proposed that, depending on its oxidation state, plastoquinone (PQ) generates a 'priming signal', which might be generated as an early step in the signal transduction pathway by a chloroplast sensor kinase (CSK) [107,108]. CSK is a bacterial-type sensor histidine kinase found in plastids. CSK interacts with cpCK2 and SIG1 based on a yeast two-hybrid assay [109]. In addition, a correlation between CSK autophosphorylation, SIG1 phosphorylation and the reduced expression of *psaAB* was found when the PQ pool was oxidised [110]. These results indicate a role of CSK in the redox regulation of SIG1 activity. However, the redox-sensing mechanism of CSK has yet to be revealed. CSK might be able to sense the oxidised PQ through an unknown redox-responsive cofactor and/or redox-sensitive cysteine residues [110]. It is also possible that the activity of CSK is regulated by the redox state of PQ pool through the STN7 kinase [111]. The STN7 kinase is involved in both short-term response such as state transition and long-term response that comprises changes in nuclear and plastid gene expression [112]. It plays an important role in the maintenance of the PQ redox poise under fluctuating light levels [112]. A working model linking the redox state of the photosynthetic electronic chain with SIG1 phosphorylation via STN7 and CSK kinases has been proposed [111]. Nevertheless, the relationship between STN7 and CSK is still an open question.

The bacterial sigma-dependent signal pathway is a partner-switching cascade that utilises phosphorylation to alter the binding-partner specificity of proteins that are ultimately responsible for the activation of sigma factors [113]. In contrast to plastids, the direct

phosphorylation of sigma factors does not exist in bacteria, and it represents a novel mechanism acquired by higher plants after the ancestral cyanobacterial endosymbiosis. However, the significance of direct phosphorylation of sigma factors remains to be determined. It might have evolved as an adaption to the lack of anti-sigma factors or to the necessity to shorten the signal transduction pathway to respond rapidly to environmental stresses [21].

5.2. Interaction proteins of plastid sigma factors

Although anti-sigma factors were not found in plastids, several interaction partners specifically interacting with sigma factors have been identified. A SIG1 interaction protein that specifically binds to region 4 of SIG1 was isolated. SIB1 is a member of the large plant-specific VQ (FxxxVQxLTG) motif-containing protein family [114]. However, deletion of *SIB1* in *Arabidopsis* did not lead to any remarkable change in *psaA/B* expression in the rice *sig1* mutant. In addition, SIB1 was reported to play a role in the salicylate and jasmonate-mediated defence response [115]. Therefore, whether SIB1 is really involved in the regulation of SIG1 activity remains an open question.

DG1 (Delay Greening 1), a pentatricopeptide-repeat (PPR) protein is involved in the regulation of early chloroplast development and plastid gene expression in *Arabidopsis* [116]. DG1 was demonstrated to be a partner of SIG6 [31]. Increased SIG6 levels can promote PEP-dependent gene transcript accumulation in the *dg1* mutant, suggesting an association between DG1 and SIG6 in the control of plastid transcription [31]. However, the mechanism of DG1–SIG6 interaction in the regulation of PEP-dependent transcription remains elusive. The proposed mechanism of DG1 action is thought to involve enhanced promoter binding and/or reduced initiation arrest of SIG6. However, the *sig6 dg1* double mutant displayed a more severe chlorotic phenotype compared to each single mutant, suggesting that DG1 has a SIG6-independent function [31].

5.3. ppGpp

In *E. coli*, the nucleotide ppGpp is the primary mediator of the stringent response to amino acid starvation where translational capacity is balanced to reduce demand through down-regulation of transcription from tRNA and rRNA operon promoters (stringent σ^{70} promoters) [15]. The elevated intracellular levels of ppGpp result in decreased association of σ^{70} and core-RNAP (but not decreased σ^{70} levels *per se*), so that less σ^{70}-RNAP is available to occupy cognate σ^{70} promoters. It was therefore proposed that ppGpp plays a determining role in the outcome of sigma factor competition to favour holoenzyme formation with alternative sigma factors [15]. ppGpp molecules and ppGpp synthetase homologues, designated RSHs, have been identified in plants, and some of them are found to be targeted to plastids [117–120]. PpGpp is produced in plastids in response to various stress conditions, and it binds predominantly to the β subunit of PEP, inhibiting plastid RNA synthesis in vitro [121,122]. Nevertheless, a role of ppGpp in plastid transcription in vivo has not yet been determined. Whether it acts in a similar way in bacteria and in plants remains to be demonstrated.

6. Conclusions and perspectives

Although biochemical and genetic approaches have notably contributed to our understanding of the roles of sigma factors in the regulation of plastid transcription, we are still far from an integrated understanding of the molecular mechanism underlying this regulatory system. Much of the current knowledge is deduced from the related mechanisms in bacteria due to the similarity between plastid and bacterial transcription, but many of the processes occurring in bacteria have not yet been proven experimentally in plastids. Therefore, the degree to which these related mechanisms operate in the plastids of higher plants remains unclear. In this regard, the crystal structure of plastid sigma factors associated with RNAPs and/or promoters could provide new insights into structural domains and motifs as well as enable comparisons of interactions with RNPA and promoters and those in bacteria. However, no crystal structure of plastid sigma factors has been determined to date. It should also be emphasised that the plastome is organised into discrete units consisting of DNA/RNA/protein complexes called nucleoids, collectively known as TAC [123,124]. Many nucleoid proteins have important regulatory roles in plastid gene transcription and plastid development. SIG2 has been detected in maize nucleoids in recent proteomic studies [125]. It therefore would be instructive to study the action of sigma factors through their relationship with nucleoid proteins.

Most plastid transcription units are preceded by multiple promoters, allowing transcription by PEP as well as NEP. In addition, some sigma factors appear to be functionally redundant, though this has never been demonstrated in vivo. In this context, multiple sigma factors and multiple promoters could ensure constitutive gene expression without significant regulation and specificity. However, this scenario is not in accordance with the restricted, highly gene-promoter-specific specialised functions of sigma factors revealed by genetic results. The multiplicity of sigma factors could also be a strategy of the cell to overcome the high frequency of mutations accumulating in the promoter regions of the chloroplast genome [21,126]. This hypothesis is now called the “spoiled kid hypothesis” and has been used to explain the remarkable complexity of chloroplast RNA metabolism [127]. The purpose of having several sigma factors with both specific and redundant roles in the plastids of higher plants still remains unknown. A homeostatic equilibrium network of all 6 members of the *Arabidopsis* sigma factor family has been proposed to explain the necessity of having multiple plastid sigma factors [23]. However, there is no direct evidence supporting this hypothesis yet.

Bacteria have developed sigma factor-dependent signal transduction systems to respond to widely fluctuating environmental stresses because sigma factors can regulate a significant subset of genes in a rapid and straightforward manner [113]. The involvement of SIG5/6 in multiple signalling pathways suggests that this scenario might exist in the plant kingdom as well. However, the components of these sigma factor-dependent signal pathways in higher plants remain largely unknown. For instance, we currently know little about how the signals are sensed, integrated and propagated to the sigma factors. We also do not know how the activities of plastid sigma factors are regulated in response to distinct stress signals. To obtain a more detailed picture of SIG-dependent signalling pathways within the context of plant growth and development, it is important to identify more regulatory components and functional partners of plastid sigma factors.

Transparency document

The Transparency document associated with this article can be found, in the online version.

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