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REVIEW

# Plant sigma factors and their role in plastid transcription

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**Abstract** Plant sigma factors determine the promoter specificity of the major RNA polymerase of plastids and thus regulate the first level of plastome gene expression. In plants, sigma factors are encoded by a small family of nuclear genes, and it is not yet clear if the family members are functionally redundant or each paralog plays a particular role. The review presents the analysis of the information on plant sigma factors obtained since their discovery a decade ago and focuses on similarities and differences in structure and functions of various paralogs. Special attention is paid to their interaction with promoters, the regulation of their expression, and their role in the development of a whole plant. The analysis suggests that though plant sigma factors are basically similar, at least some of them perform distinct functions. Finally, the work presents the scheme of this gene family evolution in higher plants.

**Keywords** Plastids · Promoters · RNA polymerases · Sigma factors · Transcription

**Abbreviations**

PEP Plastid encoded RNA polymerase
LRP-*psbD* Light-regulated promoter *psbD*

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## Introduction

Multisubunit RNA polymerases are the main enzymes that are responsible for transcription in the cells of living organisms. The composition of these transcriptional complexes is highly conservative: the proteins homologous to $\alpha$, $\beta$, $\beta'$ and $\omega$ subunits of *Escherichia coli* RNA polymerases form the catalytic core of multisubunit bacterial, archaeal, and eukaryal RNA polymerases (Langer et al. 1995; Minakhin et al. 2001; Shematorova and Shpakovski 2002). To bind promoter and to initiate transcription, core enzyme interacts with particular proteins which are not that conservative: the promoter recognition in bacteria is provided by $\sigma$-subunits of RNA polymerases (Helmann and Chamberlin 1988; Borukhov and Severinov 2002), whereas nuclear RNA polymerases use a big set of general transcription factors non-homologous to bacterial sigma factors (Paule and White 2000; Shematorova and Shpakovski 2002; Hahn 2004).

A decade ago genes coding sigma factors were discovered in genomes of eukaryotic organisms—the red alga *Cyanidium caldarium* (Tanaka et al. 1996; Liu and Troxler 1996) and the flowering plant *Arabidopsis thaliana* (Isono et al. 1997; Tanaka et al. 1997). The results of the previous investigations (Tiller et al. 1991; Tiller and Link 1993a, b) suggested that proteins homologous to bacterial sigma factors interact with a multisubunit chloroplast RNA polymerase [Plastid Encoded RNA Polymerase—PEP (Hajdukiewicz et al. 1997)]. The subsequent research confirmed this supposition—the plant and algal genomes encode sigma factors that determine promoter specificity of the major plastid RNA polymerase—PEP.

Plastids are thought to derive from ancient cyanobacteria (Dyall et al. 2004). Free-living cyanobacteria first entered as obligate endosymbionts in eukaryotic cells and

then transformed into plastids. Hypothetically, in the course of transformation of intracellular cyanobionts into chloroplasts and rhodoplasts, many cell structures and functions were lost and most of genes were either lost or transferred into nuclei. In contemporary higher plants, plastome,the set of genes and *cis*-elements encoded by plastid DNA,consists of a hundred genes approximately, whereas in contemporary cyanobacteria, genome comprises from three to six thousand genes. A huge number of genes were transferred into nuclei: about 18% of *A. thaliana* protein-encoding sequences originated from cyanobacteria (Martin et al. 2002). Evidently, genes of plant sigma factors got into nuclei with this torrent.

Plant sigma factors are encoded by the small family of nuclear *Sig* genes. Orthologs of *Sig1*, *Sig2*, and *Sig5* have been found in the moss *Physcomitrella patens* (Hara et al. 2001a, b; Ichikawa et al. 2004) and in many flowering plants (Isono et al. 1997; Tanaka et al. 1997; Kestermann et al. 1998; Tozawa et al. 1998; Lahiri et al. 1999; Morikawa et al. 1999; Tan and Troxler 1999; Oikawa et al. 2000; Fujiwara et al. 2000; Homann and Link, 2003; Kasai et al. 2004b); orthologs of *Sig3* and *Sig6* have been discovered only in flowering plants, both dicots (Isono et al. 1997; Tanaka et al. 1997; Fujiwara et al. 2000; Homann and Link 2003) and grasses (Lahiri and Allison 2000) and a number of sequences in GenBank listed in the supplementary materials); for the present, *Sig4* has been found only in *A. thaliana* (Fujiwara et al. 2000), though indirect data suggest that such a gene also occurs in another representative of the Brassicaceae family—mustard (Tiller et al. 1991; Tiller and Link 1993a, b). In 2000–2001 a unified nomenclature for sigma factor genes of *A. thaliana* and other plants was proposed (http://www.sfns.u-shizuoka-ken.ac.jp/pctech/sigma/proposal). Not to confuse the reader, the names of sigma factors are given hereinafter according to this unified nomenclature and may differ from the names given in the original articles published until 2000 inclusive. Table 1 introduces genes whose names were altered.

*Sig* genes have been found also in some algal lineages. The sole sigma factor gene (*RpoD*) of the green microalga *Chlamydomonas reinhardtii* (Carter et al. 2004) is not an ortholog of any terrestrial plant gene (Carter et al. 2004; Lysenko 2006). One gene was discovered in the representatives of cryptophytes (*Guillardia theta*) (Douglas et al. 2001) and one in glaucocystophytes (*Cyanophora paradoxa*, Accession number BAB87264). The red alga genomes comprise 3–4 *Sig* genes (A–D) (Oikawa et al. 1998; Minoda et al. 2005).

In a plant cell, sigma factors are destined for chloroplasts. This was experimentally confirmed for all *A. thaliana* sigma factors except AtSig4 (Isono et al. 1997; Kanamaru et al. 1999; Fujiwara et al. 2000; Yao et al. 2003; Privat et al. 2003), as well as for Sig1 in mustard (Kestermann et al. 1998), sigma factors 2A, 2B, and 6 in maize (Lahiri et al. 1999; Lahiri and Allison 2000; Beardslee et al. 2002), and sigma factors 1 and 2 in moss *P. patens* (Hara et al. 2001a, b). In maize, one of the sigma factors (ZmSig2B) is transported not only to chloroplasts, but also to mitochondria (Beardslee et al. 2002), though the sense of this event is still unclear. Sigma factors are the members of chloroplast transcriptional complex (Troxler et al. 1994; Liu and Troxler 1996). In vitro, purified plant sigma factors provide for the interaction of purified PEP and *E. coli* RNA polymerase with plastome gene promoters (Oikawa et al. 1998; Beardslee et al. 2002; Privat et al. 2003; Homann and Link, 2003; Suzuki et al. 2004). Knockout of *Sig* genes causes the repression of some plastome gene transcription (Kanamaru et al. 2001; Ishizaki et al. 2005). These data ensure that plant sigma factors implement the same function as bacterial sigma factors—they provide for the interaction of multisubunit RNA polymerase (PEP) with promoters.

**Table 1** List of renamed plant sigma factors

| Article | Name in an original article | Name in the unified nomenclature |
|---|---|---|
| Tanaka et al. (1997), Kanamaru et al. (1999) and Fujiwara et al. (2000) | *SigA* | *AtSig1* |
| | *SigB* | *AtSig2* |
| | *SigC* | *AtSig3* |
| Fujiwara et al. (2000) | *SigD* | *AtSig4* |
| | *SigE* | *AtSig5* |
| | *SigF* | *AtSig6* |
| Hakimi et al. (2000) and Isono et al. (1997) | *SIG1* | *AtSig2* |
| | *SIG2* | *AtSig1* |
| Tan and Troxler (1999) | *Sig1* | *ZmSig1a* |
| | *Sig2* | *ZmSig1b* |
| Lahiri et al. (1999) and Lahiri and Allison (2000) | *Sig1* | *ZmSig2A* |
| | *Sig2* | *ZmSig2B* |
| | *Sig3* | *ZmSig6* |
| Oikawa et al. (2000) | *SigA1* | *NtSig1A* |
| | *SigA2* | *NtSig1B* |
| Tozawa et al. (1998) | *SigA* | *OsSig1* |
| Morikawa et al. (1999) | *SigA* | *TaSig1* |
| Hara et al. (2001b) | *PpSig1* | *PpSig2* |

Bacteria use a set of sigma factors to regulate its genome transcription. Evidently, terrestrial plants and red algae use a set of sigma factors for the same purpose. This review is an attempt to comprehensively summarize all information on plant sigma factors, but the main task of this paper is to clarify whether all the plant sigma factors play the same role, or some of them perform specific functions. Some other aspects may be set out without going into detail,

therefore other articles may be useful to the reader: the first review on plant sigma factors (Allison 2000), the analysis of the role of Sig2 and Sig5 in *A. thaliana* (Kanamaru and Tanaka 2004), and the latest review on plastid transcription (Liere and Borner 2006).

## Comparative analysis of the conservative regions in plant sigma factors. Predictions and some confirmations

Plant sigma factors belong to the superfamily $\sigma^{A}/\sigma^{70}$ and have sequences homologous to conservative regions 1.2, 2, 3, 4 of bacterial sigma factors (Figs. 1a, 2). The most remarkable distinction between plant and bacterial sigma factors is that the former have a large non-conservative N-terminal region (200–300 amino acids). This region prevents plant sigma factors from integrating into *E. coli* transcription machinery; N-terminus of AtSig3 is probably toxic for *E. coli* cells (Hakimi et al. 2000). A small part of variable N-terminus is a transit peptide (30–60 amino acids) that provides for protein transport from cytosol to chloroplasts and is cut off after polypeptide is delivered into organelle. Nevertheless, mature plant sigma factors are bigger than their cyanobacterial ancestors. In size they are closer to *E. coli* $\sigma^{70}$ which also has a large non-conservative region, though differently located (Fig. 1a). Little is known of the role of regions 1.2–4 in plants. Conservative regions of some plant sigma factors can replace $\sigma^{70}$ regions in *E. coli* cells (Hakimi et al. 2000). Therefore we can assume that plant sequences form a similar spatial structure and perform a function analogous to that of their bacterial homologs. The spatial organization of the principal bacterial sigma factor and the functions of its certain regions have been thoroughly considered in numerous reviews (Helmann and Chamberlin 1988; Lonetto et al. 1992; Borukhov and Severinov 2002; Young et al. 2002; Paget and Helmann 2003; Dove et al. 2003), so only major facts and conclusions concerning bacterial sigma factors will be stated here.

In bacteria, conservative regions 1.2 and 2 form domain $\sigma_2$, regions 3.0 (earlier named 2.5. Barne et al. 1997) and 3.1—domain $\sigma_3$; region 3.2 forms a flexible loop, a linker that connects domains $\sigma_3$ and $\sigma_4$; and, finally, region 4 forms domain $\sigma_4$. All the three domains bind both to core enzyme and DNA.

In domain $\sigma_2$ regions 2.1 and 2.2 bind to $\beta'$ coiled-coil, while regions 1.2, 2.3, and 2.4 bind to DNA in the promoter area. Region 1.2 is essential for the effective transcription initiation (Wilson and Dombroski 1997; Hsu et al. 2004) and probably binds DNA non-template strand just downstream -10 box in a sequence-specific manner (Haugen et al. 2006). Region 2.3 participates in promoter melting and binds to DNA non-template strand, region 2.4 binds to template strand near –10 element of promoter.

Only a part of plant sequences that correspond to bacterial region 1.2 is conservative (Fig. 2). The conservative plant (and cyanobacterial) sigma factor sequences extend from C-terminal part of region 1.2 nearly to region 2.1, which was mentioned previously (Tanaka et al. 1997; Tan and Troxler 1999). Evidently, it is more correct to name this plant conservative region 1.3 which partially overlaps bacterial conservative region 1.2. The conservation degree of region 1.3 is rather low.

In plant sigma factors, amino acid sequences of region 2 are most conservative (Fig. 2). The conservation of this

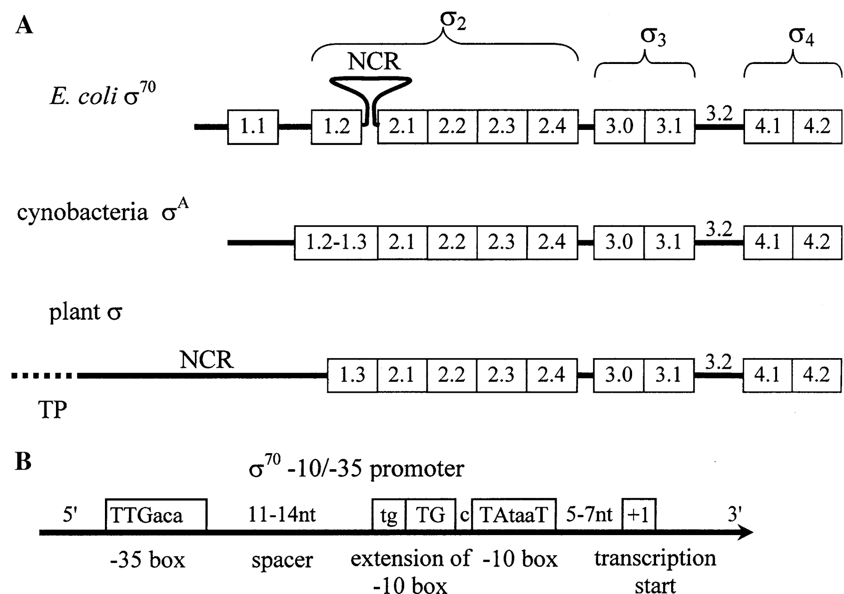


**Fig. 1** Scheme of sigma factors (**a**) and their consensus promoter (**b**). $\sigma_2$-$\sigma_4$, sigma domains; 1.1–4.2, conservative regions; *NCR*, non-conserved region; *TP*, transit peptide. In promoter sequence (**b**) *capital letters* stand for the more conservative nucleotides, *small letters* for the less conservative ones

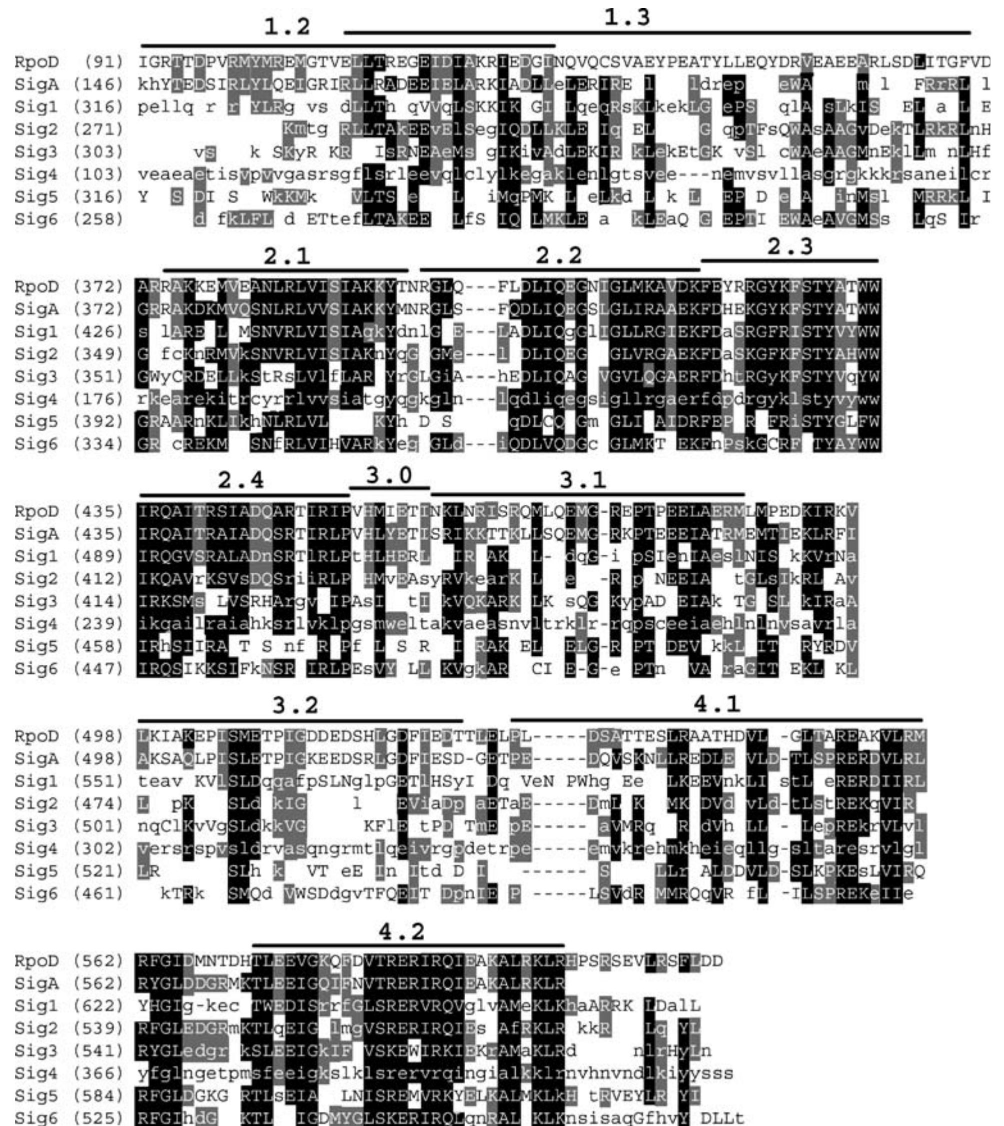


**Fig. 2** Aligning of consensus amino acid sequences of plant and cyanobacterial sigma factors. Consensus sequences are based on the aligning of the sigma factors presented in the supplementary materials and available on request. RpoD—sequence of *E. coli* principal sigma factor (BAB37373), SigA—consensus sequence of cyanobacterial principal sigma factors, Sig1–3, 5–6—consensus sequences of the corresponding plant sigma factors, Sig4—AtSig4 sequence (AAC97954). Symbols: in consensus sequences, *capital letters* stand for highly conservative amino acids in ortologous genes, *small letters* low conservative amino acids, *blank* non-conservative amino acids, *dash* break of sequence. The only known sequence of Sig4 is presented in full in small letters. *White letters on a black background* stand for amino acids that are conservative in most paralogs, *white letters on a grey background* amino acids conservative in some paralogs, *black letters on a white background* amino acids non-conservative in different paralogs. Conservative regions of *E. coli* $\sigma^{70}$ sequence are presented the way they are given in Vassylyev et al. (2002) and Borukhov and Severinov 2002)

region in Sig1 and Sig2 is the highest one, whereas sequence of Sig5 has the faintest similarity to the common consensus. In Sig5 interfacing sequences of regions 2.1 and 2.2 are non-conservative (Fig. 2). In bacteria, regions 2.1 and 2.2 form the main section binding to core enzyme (Murakami et al. 2002; Vassylyev et al. 2002). It is probable that in most plant sigma factors, interfacing sequences of regions 2.1 and 2.2 form the section binding to core and therefore are conservative, whereas in Sig5 they are variable as they perform no important functions and consequently do not participate in core binding. If this assumption is right, the interaction between Sig5 and core is weaker than the interactions between core and other sigma factors. In this case Sig5 is less competitive in the struggle for core binding, but holoenzyme comprising Sig5 provides for a lower level of abortive transcription (see below). Such a property may be very useful for stress inducible transfactors to which Sig5 belongs, at least, in *A. thaliana* (Nagashima et al. 2004b).

The sequence of region 2.4 that provides for binding –10 element is nearly the same in cyanobacterial SigA and plant Sig1 (Fig. 2). Sig2 differs only in two positions that are non-conservative in other plant sigma factors too. In Sig5 more than a half of region 2.4 has no similarity to the consensus sequence. The substitution of one amino acid in region 2.4 of mustard Sig1 has been proven to affect its binding to –10 box of promoter (Homann and Link 2003). Therefore it is quite probable that sigma factors 1 and 2 preferably recognize consensus sequence of –10 element (5′-TATAAT–3′), whereas binding of sigma factor 5 to consensus sequence is weaker, and it is more likely to bind to some deviating promoter sequence. Conservative part of Sig2 can even interact with $\sigma^{70}$ promoters in *E. coli* (Hakimi et al. 2000).

In domain $\sigma_3$ region 3.0 binds the upstream extension of –10 element, and region 3.1 participates in the binding of core (Sharp et al. 1999; Murakami et al. 2002).

Plant region 3.0 is low-conservative (Fig. 2). Amino acids H and E essential for the recognition of TG-extension of –10 element (Barne et al. 1997) (Fig. 1b) are present in Sig1 and Sig2 (in cereal Sig1 and in one of tobacco genes, H is substituted by a similar amino acid Y); in AtSig4 only E is present. In sigma factors 3, 5, and 6 these positions are occupied by different amino acids. Evidently, Sig1, 2 and, perhaps, AtSig4 are able to bind to 5′-extension of –10 element, whereas Sig3, 5, and 6 lost conservative region 3.0 as well as the ability to bind 5′-extension of –10 element. This prediction has been partially confirmed: the substitution of nucleotide G in 5′-extension of –10 element has been proven to affect the binding of mustard sigma factors 1 and 2 to promoter and to have no influence on the binding of sigma factor 3 (Homann and Link 2003). Not only does the binding of extended –10 element have an impact on the strength of interaction between holoenzyme and promoter, but also provides for the binding of bacterial sigma factors to promoters that lack –35 element (Barne et al. 1997). Probably, plant sigma factors 1 and 2 can bind core enzyme to promoters that have an extended –10 box and lack –35 box, whereas sigma factors 3, 5, and 6 have no such ability. However, this has not been confirmed experimentally yet.

Plant region 3.1 comprises conservative and variable sections (Fig. 2). The most conservative bacterial amino acids (*E. coli* $\sigma^{70}$ G475, P478, E482; Cashel et al. 2003) are conservative in plants as well (Fig. 2). Little is known of the interaction of this sigma factor section with core in bacteria, thus any supposition concerning the properties of a plant homologous section can hardly be made.

In bacteria the loop made by region 3.2 winds through the RNAP active-site channel formed by $\beta$ and $\beta'$ subunits and out through the RNA exit channel formed by $\beta$ flap domain. A part of region 3.2 is located in close vicinity to active enzyme centre and, evidently, plays some role in transcription initiation (Severinov et al. 1994; Sen et al. 1998; Young et al. 2002; Campbell et al. 2002; Darst et al. 2002; Murakami et al. 2002). Perhaps, region 3.2 participates in stabilization of open promoter state and/or in stabilization of the first nucleotide placement. It was proposed, that such a feature blesses RNA polymerases to initiate synthesis without primers, but curses them with abortive transcription (Borukhov and Severinov 2002). In bacteria, the rate of synthesis of the short abortive products can vastly exceed that of the full-length transcript. The position of $\sigma_3$–$\sigma_4$ linker inside RNA exit channel promotes a firm binding of sigma subunit to core and hinders processive elongation (Shuler et al. 1995; Sen et al. 1998; Murakami et al. 2002; Cashel et al. 2003). The following model has been proposed (Borukhov and Severinov 2002; Young et al. 2002; Murakami et al. 2002; Cashel et al. 2003): the growing RNA chain competes with the linker for binding to the exit channel (Fig. 3b). If the linker wins, the initiation ends in abortive synthesis—dissociation of short RNA (2–12 nt). If RNA wins, the linker leaves the channel, the binding of $\sigma$-subunit to core enzyme and DNA weakens, core escapes a promoter and processively elongates transcript—transcription shifts from the initiation stage to the elongation stage (Fig. 3a). This model suggests that the stronger a sigma factor binds to core enzyme the more frequently an abortive transcription occurs, and vice versa.

In plant region 3.2 there is sequence SLd that is conservative both in plants and bacteria (Fig. 2). The other part of plant $\sigma_3$–$\sigma_4$ linker is non-conservative. In Sig1 this region is very conservative but it bears no similarity to the corresponding sections of bacterial sigma factors or other plant sigma factors (Fig. 2). Amino acid sequence of the linker plays a big role in the interaction between core and sigma factors and affects the transition from initiation to elongation (Zhou et al. 1992; Sen et al. 1998; Murakami et al. 2002; Cashel et al. 2003). Therefore it is quite probable that plant sigma factors bind to core with different strength and determine various ratio of abortive and processive transcription. It would be interesting to clarify what role the conservative sequence of Sig1 $\sigma_3$–$\sigma_4$ linker plays here.

Domain $\sigma_4$ binds $\beta$-flap and –35 element of promoter. Two conservative regions 4.1 and 4.2 participate in binding core (Sharp et al. 1999; references therein) and promoter (Campbell et al. 2002). Many proteins regulating holoenzyme binding to promoter interact with region 4.2 (Dove et al. 2003) and with region 4.1 (Sharma and Chatterji 2006).

The sequences of plant regions 4.1 and 4.2 are rather conservative, though in a less degree than the sequence of region 2 (Fig. 2). Each plant sigma factor has amino acid substitutions in regions 4.1 and 4.2, and these substitutions are conservative inside one of the groups of orthologs. Amino acid substitutions in this part may affect binding either with –35 promoter element or with $\beta$-flap of RNA polymerase. The substitution of one amino acid in region 4.2 of mustard sigma factor 1 has been shown to influence its binding to –35 promoter box (Homann and Link 2003). The strength of $\sigma_4$ binding to $\beta$-flap affects the interaction between holoenzyme and promoter. *E. coli* $\sigma^S$ binds to $\beta$-flap much stronger than $\sigma^{70}$ (Kuznedelov et al. 2002), which may be the reason for holoenzyme with $\sigma^S$ being more tolerant to the deviation of –35 box nucleotides from the consensus sequence than holoenzyme with $\sigma^{70}$ (Gaal et al. 2001). This suggests that plant sigma factors bind to sequences that deviate from –35 box consensus sequence

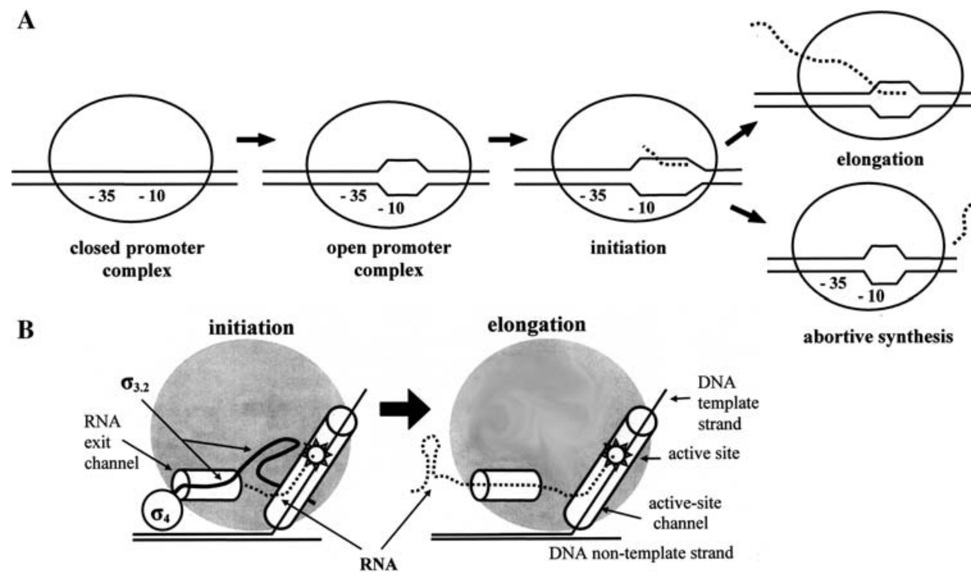


**Fig. 3** Scheme of the early stages of transcription. **a** RNAP binds DNA and forms complex with closed promoter—closed complex. Then holoenzyme separates DNA strands around -10 box of promoter and transcription start site; this stage is called open (promoter) complex. Afterwards RNAP initiates transcription, escapes a promoter and enters the elongation stage. If it cannot leave promoter then RNAP releases short RNA chain (2–12 nt) without dissociating from the promoter—the process called an abortive synthesis. **b** After a transcription initiation, growing RNA chain has to go out of RNAP through the RNA exit channel. However, while RNAP initiates transcription, this channel is occupied by region 3.2 of sigma factor. For a sufficient elongation process, growing RNA must force this part of sigma factor out from the channel. Otherwise initiation will result in abortive synthesis

(5′-TTGACA-3′) with different strength. Besides, variability of region 4 in plant sigma factors allows differential regulation of their activities by other proteins. For example, in *A. thaliana*, protein Sib1 (sigma binding 1) in vitro specifically binds to region 4 of AtSig1 and does not bind to the corresponding region of AtSig2 (Morikawa et al. 2002). Screening in yeast two-hybrid assay has shown that Sib1 and homologous protein T3K9.5 interact with region 4 of AtSig1 and do not interact with AtSig2, AtSig4, and AtSig5 (Morikawa et al. 2002). This interaction could modify Sig1 binding to promoter but its role is unclear.

It seems probable that another transcription factor interacts with region 4. In Fig. 4b you can see the scheme of the light-regulated promoter of chloroplast *psbD*-operon (LRP-psbD) conservative in flowering plants and black pine (Hoffer and Christopher 1997). The initiation of synthesis from this promoter is provided by protein complex AGF specifically binding to AAG-box (Kim and Mullet 1995). –35 box has no influence on RNA polymerase binding to promoter (Nakahira et al. 1998; Kim et al. 1999; Thum et al. 2001). Moreover, non-functionality of –35 box is essential for the inducible activation of this promoter (Nakahira et al. 1998). Since Sig5 provides for this promoter activation (Tsunoyama et al. 2004; Nagashima et al. 2004b), this sigma factor, evidently, binds to –10 box. The direct interaction between AGF and Sig5 has not been shown; nevertheless, such interaction is quite probable. Sig5 does not comprise conservative region 3.0, therefore there are no grounds to believe that it binds holoenzyme to promoter by interacting with extended –10 box only. RNA polymerase of *E. coli* interacts with promoter in a similar way: protein MotA binds to a specific operator located near –35 position, $\sigma^{70}$, as a member of holoenzyme, binds to –10 box, whereas domain $\sigma_4$ binds not to –35 box but to protein MotA (reviewed in Dove et al. 2003) (Fig. 4a). It is probable that plant Sig5 interacts with LRP-psbD in a similar way: domain $\sigma_2$ binds to –10 box, whereas domain $\sigma_4$ binds to AGF (Fig. 4b).

Summing up I will touch upon two points. Firstly, it is quite probable that plant sigma factors interact with core enzyme and promoter in the same way as their bacterial homologs. Secondly, plant paralogs have a considerable variability of amino acid sequences in conservative and thus, evidently, functional regions. One can assume that plant sigma factors have a different affinity for core enzyme, diverse ratio of abortive and processive transcriptions, different preferences in choosing sequence of –10/–35 $\sigma^{70}$-type promoter, and finally, their interaction with promoters could be differentially regulated by other proteins.

## Regulation of plant sigma factor affinity for core

Bacterial sigma factors have different affinity for core enzyme, which is very important for the competition of sigma

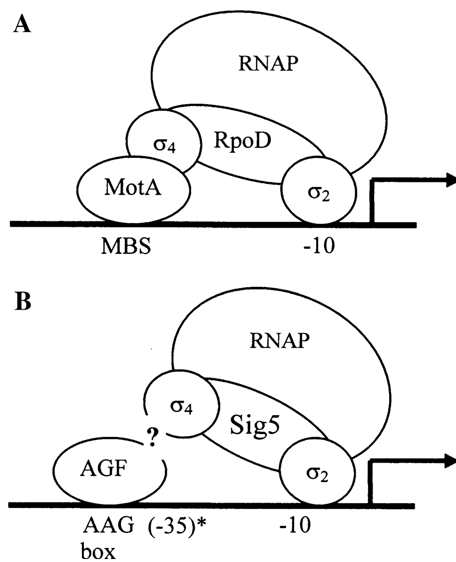


**Fig. 4** Scheme of RNAP interaction with some non-canonical promoters. **a** Interaction of *E. coli* (core + $\sigma^{70}$) holoenzyme with bacteriophage T4 middle genes promoters. **b** Presumed interaction of *A. thaliana* (core + Sig5) with LRP-psbD. *MBS*,MotA binding site, –10 –10 box, (–35)*—non-functional –35 box, $\sigma_2$, $\sigma_4$—corresponding sigma factor domains, ?—sign indicating the presumed interaction of Sig5 with AGF that is not experimentally confirmed. Explanations are in the text

factors for binding to core (Ishihama 2000). In the previous chapter it was concluded that plant sigma factors possess different affinity for core as well. This prediction may be supported by the data obtained by Homann and Link (2003). In vitro, holoenzyme comprising mustard sigma factors 1–3 bind to chloroplast promoter PpsbA. SaSig1, and SaSig2 initiate transcription from the promoter, whereas SaSig3 could do it only after removing variable N-terminus and regions 2.1 and 2.2 (SaSig3–374). Probably, holoenzyme containing full-size Sig3 was able to provide for abortive transcription only, and after the deletion of regions 2.1 and 2.2 (the main section for core binding–see previous chapter), its binding to core weakened, which allowed RNA polymerase to escape promoter and elongate transcripts processively. Evidently, SaSig3 has a considerably higher affinity for core than SaSig1 and SaSig2, at least in vitro.

Bacteria have various mechanisms to change the affinity of sigma factors for core; for example, alarmone (p)ppGpp decreases the binding of main sigma factor to core and increases the portion of RNA polymerases with the alternative sigma factors (Jishage et al. 2002). It was proved recently that (p)ppGpp-generating signaling system works in plant chloroplasts in a similar way (Kasai et al. 2004a; references therein). It is quite probable that plant cells also can regulate the transcription in chloroplasts by changing the affinity of various sigma subunits for core.

Plant sigma factor affinity is regulated by plastid protein kinases. Core and sigma factor phosphorylation strengthens their binding to each other and holoenzyme binding to promoter (Tiller and Link 1993a). Phosphorylation of RNA polymerase components mostly occurs in etioplasts, whereas in photosynthesizing chloroplasts the activity of the corresponding protein kinases is depressed (Tiller et al. 1991; Tiller and Link 1993a, b; Baginsky et al. 1999; Baena-Gonzalez et al. 2001). It is not yet clear if this way could be used for the differential regulation of core binding to $\sigma$-subunits, nevertheless, phosphorylation is known to differentially affect binding to different promoters: it strengthens the related binding of SLF29 to plastid promoter psbA, as compared to other plastid promoters—trnQ and rps16 (Tiller and Link 1993a).

## Interaction of plant sigma factors with promoters

It has been experimentally shown that free sigma factors 1–3, like bacterial homologs, do not bind to DNA (Kestermann et al. 1998; Beardslee et al. 2002; Homann and Link 2003; Hanaoka et al. 2003). The deletion of the entire non-conservative N-terminus does not affect this property: truncated AtSig3 is able to bind DNA only together with core (Hakimi et al. 2000). Evidently, the reason why free sigma factors are unable to bind to DNA is that their domains $\sigma_2$ and $\sigma_4$ are located too close to each other and cannot bind to –10 and –35 promoter boxes simultaneously. After sigma factor binds to core, the distance between $\sigma_2$ and $\sigma_4$ increases and sigma subunit can bind to both promoter elements at the same time (Callaci et al. 1999).

It has been demonstrated that plant sigma factors 1, 2, and 3 together with *E. coli* core enzyme are able to specifically bind to plastid promoters and initiate transcription (Beardslee et al. 2002; Hakimi et al. 2000; Kestermann et al. 1998; Homann and Link 2003; Privat et al. 2003). Red alga *C. caldarium* sigma factors have been shown to be able to initiate transcription from bacterial promoters tac and RNA I together with *E. coli* core (Oikawa et al. 1998). The transcription from chloroplast promoter psbA by holoenzyme consisting of purified tobacco PEP and purified maize sigma factors 2A or 2B was obtained in vitro recently (Suzuki et al. 2004). Nothing is known of the direct interaction between sigma factors 4–6 and promoters.

The experiments in vitro showed that sigma factors 1–3 interact with the same promoters of $\sigma^{70}$-type but do so differently. *A. thaliana* proteins together with *E. coli* core provide for transcription from chloroplast promoters rbcL, psbA, rrn P1, and P2 in different ways: Sig3 initiates transcription from each of these promoters, Sig1 and Sig2

initiate transcription from promoters rbcL and psbA only, Sig1 doing it much worse than Sig2 and Sig3 (Hakimi et al. 2000; Privat et al. 2003). Homann and Link (2003) applied one of the strongest plastid promoters—psbA—and studied in vitro its exclusion from the complex *E. coli* core + mustard sigma factor 1, 2, or 3. The experimental results prove that Sig2 interacts with promoters trnK, trnQ, rrn16, and rps16 more weakly than Sig1 and Sig3, and Sig3 binds to promoter rbcL more weakly than Sig1 and Sig2. Promoter ycf3 hardly excludes promoter psbA from all the complexes, though the sequence of promoter ycf3 bears great similarity to the sequences of promoters rbcL, trnK, trnQ, and rrn16.

The affect of mononucleotide substitutions on promoter psbA binding by Sig1–3 has also been studied in this work: all the three sigma factors interact with –35 box in a similar way; Sig1 and Sig2 interact with TATA-box in spacer, 5′-extension of –10 box, and 5′-terminus of –10 box more strongly than Sig3; and Sig2 and Sig3 interact with 3′-terminus of –10 box more strongly than Sig1 (Homann and Link 2003).

We can see that plant sigma factors interact with promoters of $\sigma^{70}$-type but do so in different ways. The same results have been obtained in bacteria: $\sigma^{70}$ and $\sigma^{S}$ are more likely to bind to the same nucleotide sequence—consensus sequence of $\sigma^{70}$-type promoter (5′-CTTGACaa–10nt-TGTGCTATAa/cT-3′) (Gaal et al. 2001); nevertheless, inside the cell each of them directs transcription of a distinct set of genes. The authors' explanation is that $\sigma^{70}$ and $\sigma^{S}$ are more likely to bind to various deviations from the consensus sequence. Moreover, both sigma factors have proved to initiate transcription from the complete consensus promoter sequence much worse than from a 'less consensus' one (Gaal et al. 2001). Evidently, holoenzyme binds to the consensus promoter sequence too strongly and is hardly able to realize the elongation process. This discovery lets us make a fundamental conclusion: deviation from the consensus sequence may be essential for the effective work of $\sigma^{70}$-type promoters in a cell. Probably, various plant sigma factors, just like bacterial ones, recognize 'their own' deviations from the consensus sequence of $\sigma^{70}$-type promoters, which allows them to regulate plastid gene transcription differentially.

## Roles of sigma factors in plastid gene transcription

A number of approaches allow us to judge the roles of various sigma factors in plastid gene transcription. Protoplasts isolated from *A. thaliana* leaves were adapted to darkness for 16 h and then transformed by vectors bearing genes *AtSig2* or *AtSig5* under the control of 35S promoter; transcription of 12 plastid operons and monocistronic genes was studied. The effect obtained in darkness looks like light induction: transient expression of both vectors increases the transcription intensity of *psbA* gene, Sig2 overexpression stimulates *trnEYD* operon transcription, whereas the expression of Sig5 intensifies the transcription of genes *psaA*, *psbB*, and *psbD*, the later growing about 10 times (Tsunoyama et al. 2004).

Knockout mutants for the majority of *Sig* genes have been obtained. The disruption of *Sig* genes has different impacts on a plant phenotype. ΔSig4 plants have no visible differences from wild type plants (Favory et al. 2005). In ΔSig6 the primary stage of chloroplast biogenesis is impaired: as a result of the delayed chloroplast differentiation, cotyledons and the tips of first leaves have low chlorophyll content; then mutants catch up with wild type plants and either have no apparent distinctions from them (Ishizaki et al. 2005) or are a little delayed in development (Loschelder et al. 2006). Under optimal conditions, mutant ΔSig5 has no evident differences from wild type plants but mutant plants are much less viable when under stress (Nagashima et al. 2004b). Under optimal conditions mutant ΔSig2 is of pale-green color and has a defect in chloroplast ultrastructure at all stages of development (Kanamaru et al. 2001).

In mutant ΔSig2, of all chloroplast mRNA only the level of transcript *psaJ* falls for certain (Nagashima et al. 2004a); decrease also occurs in the amount of transcript *psbD* transcribed from constitutive promoter (–256) (Hanaoka et al. 2003). In *A. thaliana* anti-sense Sig2 plants, the amount of transcript *psbA* decreases (Privat et al. 2003). Besides, in mutant ΔSig2 and in an anti-sense Sig2 plant, the content of some tRNA (trnM-CAU, trnV-UAC, and trnEYD-operon) is lowered (Kanamaru et al. 2001; Privat et al. 2003; Hanaoka et al. 2003). tRNA Glu (E) catalyzes the first stage of synthesizing chlorophyll and other tetrapyrrole compounds and inhibits the second plastid RNA polymerase (Hanaoka et al. 2005). Therefore, the impairments that occur in ΔSig2-mutants may be caused by three factors: a defect in plastid translation, insufficient chlorophyll synthesis, and/or imperfection in RNA polymerase switching.

At the early development stages in mutant ΔSig6, the amount of great many PEP-dependent transcripts is considerably less: *rbcL*, *psbA*, *B, C, D, H, N, T*, and all rRNA (Ishizaki et al. 2005). All other *Sig* genes are expressed at this stage (see the next chapter). Probably, at the early stages Sig6 plays its role much better than other plant sigma factors. Anyway, in maize, Sig6 protein is mainly present in non-photosynthesizing tissues. One of the possible explanations could be a difference in physical and chemical parameters of stroma between mature chloroplasts and developing/non-photosynthesizing plastids. In vitro, osmotic parameters and an ionic compound of

reaction buffer differentially affect the binding of different *E. coli* sigma factors to promoters (Ding et al. 1995; Rajkumari et al. 1996) and influence promoter binding by a mustard sigma factor (Tiller and Link 1993a). However, AtSig6 performs a specific function not only at the early developmental stages. In *A. thaliana* operon *atpBE* is represented by the two major transcripts: 2.6 kb and 2.0 kb. Both seedlings and adult plants of ΔSig6-mutant lack transcript 2.6 kb, whereas the amount of 2.0 kb-transcript is nearly the same as in the wild type plants (Loschelder et al. 2006). In tobacco and spinach *atpB* is known to be preceded by several PEP-dependent promoters and the distance between the upper and the lower promoters is approximately 0.4–0.5 kb (Chen et al. 1990; Orozco et al. 1990; Hajdukiewicz et al. 1997). Evidently, transcription from the upper *atpB* promoter in *A. thaliana* is initiated by Sig6 only.

In mutant ΔSig4, only *ndhF* mRNA decreases twice, the steady-state level of all other chloroplast mRNA changes less than twice (Favory et al. 2005). No analysis of plastome gene expression in ΔSig5-mutants has been made; nevertheless, these mutants have been shown to have no induction of LRP-psbD (Tsunoyama et al. 2004; Nagashima et al. 2004b). In Table 2 the information concerning the influence of inactivation and overexpression of certain sigma factors on chloroplast gene expression is summed up.

Apparently, in *A. thaliana* some promoters are recognized by a single sigma factor: LRP(–948)*psbD*—by Sig5 (Nagashima et al. 2004b), constitutive P(–256)*psbD*—by Sig2 (Hanaoka et al. 2003), upper P*atpB*—by Sig6 (Loschelder et al. 2006). Beside *psbD* and *atpB*, some other plastid genes are preceded by several PEP-dependent promoters. It has been shown for a ribosomal operon (Lerbs-Mache 2000) and *psaA* (Chen et al. 1993). Probably, the transcription of these genes is also regulated by several sigma factors, each of them initiating transcription from its own promoter.

Inactivation of various plant sigma factors influences chloroplast gene expression and plant development differentially. One should mind that the revealed changes in the amount of certain transcripts are no more than the tip of the iceberg. A plant tries to compensate for the loss of one of transcription factors: the expression of other sigma factors increases (Nagashima et al. 2004a), and they may initiate transcription of the corresponding genes from other promoters (see above); transcription directed by the second plastid RNA polymerase increases (Loschelder et al. 2006); huge abilities of the chloroplast system of RNA stability regulation could be applied (Barkan and Goldschmidt-Clermont 2000; Monde et al. 2000). In ΔSig-mutants the changes that a plant cannot compensate for have been mainly revealed. It is probable that the influence of certain sigma factors on transcription initiation is more extensive and diverse.

**Table 2** Sig-dependent changes of *A. thaliana* chloroplast gene transcription in vivo

| I (↓) | | | | II(↑) | |
|---|---|---|---|---|---|
| –Sig2 | –Sig4 | –Sig5 | –Sig6 | +Sig2 | +Sig5 |
| trnEYD | ndhF | LRP-psbD[b] | atpBE-2.6kb[b] | trnEYD | psaA |
| trnV | | | psbA[c] | psbA | psbA |
| trnM | | | psbB[c] | | psbB |
| psaJ | | | psbC[c] | | psbD |
| psbA[a] | | | psbD[c] | | |
| const-psbD[b] | | | psbH[c] | | |
| | | | psbN[c] | | |
| | | | psbT[c] | | |
| | | | rbcL[c] | | |
| | | | rrn16[c] | | |
| | | | rrn23[c] | | |
| | | | rrn5[c] | | |
| | | | rrn4.5[c] | | |

*I* Decrease of corresponding gene transcripts in knockout mutant tissues (–Sig). *II* Increase in corresponding gene transcription caused by *Sig* gene transient expression in protoplasts (+Sig). Data compiled from: Kanamaru et al. (2001), Hanaoka et al. (2003), Privat et al. (2003), Nagashima et al. (2004a, b), Tsunoyama et al. (2004), Favory et al. (2005), Ishizaki et al. (2005), and Loschelder et al. (2006)

[a] Only in an anti-sense Sig2 plant

[b] Transcript initiated from one of the promoters

[c] Only at the early stage of plant development

## Expression of plant sigma factors and their regulation

Evidently, all genes of plant sigma factors are expressed. At mRNA level this has been proved for all the six *A. thaliana* genes (Tsunoyama et al. 2002; Nagashima et al. 2004b), three known *P. patens* genes (Hara et al. 2001a; Ichikawa et al. 2004), and some cereal *Sig* genes (Morikawa et al. 1999; Lahiri et al. 1999; Kasai et al. 2004b). RT-PCR data confirm the transcription of seven maize *Sig* genes (1a, 1b, 2A, 2B, 3, 5, 6) (Lysenko, unpublished results). At protein level the expression of *A. thaliana* and mustard genes Sig1–3 (Privat et al. 2003; Homann and Link 2003), maize Sig2A and Sig2B (Lahiri and Allison 2000; Beardslee et al. 2002), *A. thaliana* Sig5 (Yao et al. 2003), and maize Sig6 (Lahiri and Allison 2000) has been confirmed. A small-size protein (29 kDa) that possesses sigma factor properties (Tiller et al. 1991; Tiller and Link 1993a, b) and is of the same size as the product of AtSig4-gene alternative splicing (Fujiwara et al. 2000) has been detected in mustard leaves. Therefore, it is highly probable that all plant *Sig* genes are expressed and are not pseudogenes.

In *A. thaliana*, *Sig* genes are expressed at the early stages of development. In dry seeds, mRNAs of *Sig2* and *Sig5* have been found whereas mRNAs of the rest of *Sig* genes have not (Demarsy et al. 2006). However, protein Sig3 has been detected in dry seeds, but Sig1 and Sig2 have not (Privat et al. 2003). All the six *Sig* mRNAs are present in seeds after 72-hour imbibition and vernalisation and up to the 6-th day of plant development (Demarsy et al. 2006). These results have been confirmed by other researchers for 4-day plants (Nagashima et al. 2004a) and for 6-day plants (Loschelder et al. 2006). Proteins Sig2 and Sig1 appear on the second and third day after imbibition, respectively (Privat et al. 2003). Early expression of *Sig* genes is not characteristic of *A. thaliana* only: in spinach dry seeds, mRNAs of *Sig2* and *Sig3*, as well as protein Sig2, have been detected (Demarsy et al. 2006). When seedlings begin developing the amount of these mRNAs and the protein increases. The data obtained by professor Lerbs-Mache's group indicate that not only sigma factors are present at the early stages of *A. thaliana* development but also other PEP subunits, and that PEP activity plays some role in seedling development (Demarsy et al. 2006).

However, PEP-dependent plastome expression mostly occurs in photosynthesizing tissues and is light-activated. This suggests that sigma factors are mostly expressed in photosynthesizing tissues and are light-regulated. Let us consider the information on tissue specific plant sigma factor expression in detail.

In *A. thaliana Sig1* is mostly expressed in leaves (Isono et al. 1997; Tozawa et al. 1998; Tan and Troxler 1999; Kasai et al. 2004b), whereas in *A. thaliana* roots the amount of *Sig1* mRNA is very small (Tozawa et al. 1998). *Sig3* expression has been discovered in seeds, cotyledons and leaves (Isono et al. 1997; Hakimi et al. 2000; Privat et al. 2003). No mRNA of *Sig3* has been found in roots (Isono et al. 1997). Messenger RNA of *AtSig4* has been detected in leaves and has not been found in roots (Fujiwara et al. 2000). *Sig5* expression has been discovered in leaves, stems, flowers, pods, and roots of seedlings (Tsunoyama et al. 2002, 2004; Yao et al. 2003; Nagashima et al. 2004b).

*A. thaliana Sig2* is also mostly expressed in leaves and cotyledons and is not expressed in roots (Isono et al. 1997; Kanamaru et al. 1999; Privat et al. 2003). In grasses *Sig2* is presented by two copies: 2A and 2B. In rice, mRNAs of *Sig2A* and *Sig2B* have been discovered in leaves but have not been discovered in roots (Kasai et al. 2004b). In maize, mRNA of *Sig2A* has been found in all tissues (Lahiri et al. 1999), protein Sig2A has been detected only in the green part of a leaf, its amount increasing with cell maturation (Lahiri and Allison 2000), protein Sig2B has been found mostly in non-photosynthesizing root and leaf base tissues, with leaf cell greening and maturation the amount of protein Sig2B decreases (Beardslee et al. 2002).

In *A. thaliana* and maize, *Sig6* mRNA has been mostly discovered in leaves (Lahiri et al. 1999; Ishizaki et al. 2005). In maize, though, protein Sig6 has been found in non-photosynthesizing tissues only (roots, etiolated leaves, white base of green leaves) and has not been detected in chloroplasts (Lahiri and Allison 2000). In *A. thaliana*, Sig6 function is mostly connected with the initial stage of plant development, but Sig6-dependent synthesis of *atpBE* 2.6 kb mRNA occurs in green tissues—cotyledons and rosette leaves (Loschelder et al. 2006).

Thus the majority of *Sig* genes—1, 2(A), 3, 4, and 5—are expressed in green leaves and hardly ever expressed in roots. However, many sigma factors are expressed in non-photosynthesizing tissues. To illustrate, *Sig3* is expressed in seeds, etiolated seedlings, the content of Sig3 being higher in leaf etioplasts as compared to chloroplasts (Privat et al. 2003). *Sig5* is expressed in the roots of seedlings and in flowers (Yao et al. 2003; Nagashima et al. 2004b). Maize *Sig2B* and *Sig6* are mostly expressed in non-photosynthesizing tissues (Lahiri and Allison 2000; Beardslee et al. 2002). Therefore, $\sigma$-dependent plastid transcription occurs mostly in chloroplasts but takes place in non-photosynthesizing plastids too.

Besides, another important conclusion should be made: Sig gene expression may vary in plants belonging to different systematic groups; for example, protein Sig2 has been detected in spinach dry seeds (Demarsy et al. 2006) but has not been found in *A. thaliana* dry seeds (Privat et al. 2003); protein Sig6 has not been detected in maize chloroplasts (Lahiru and Allison 2000), but Sig6-dependent transcript has been found in green tissues of *A. thaliana* (Loschelder et al. 2006). The expression of some Sig genes, mostly in non-photosynthesizing tissues, has been demonstrated for maize but has not been shown for *A. thaliana* (see above).

In some cases we can see drastic differences in the distribution and/or amount of mRNAs and proteins of the same genes. These discrepancies indicate that the expression of at least some plant sigma factors is regulated at the level of translation, but these mechanisms have not been studied yet.

Apparently, light increases the steady-state level of mRNAs of all *Sig* genes both in etiolated and dark-adapted green plants (Isono et al. 1997; Tanaka et al. 1997; Tozawa et al. 1998; Tan and Troxler 1999; Lahiri et al. 1999; Morikawa et al. 1999; Hara et al. 2001a; Tsunoyama et al. 2002; Kasai et al. 2004b). Most probably, there exists a general mechanism increasing the amount of all Sig-transcripts under the influence of light. Evidently anoter signaling pathway induces *Sig5* only. Light intensity increase from 50 to 1000 µmol $m^{-2}$ $s^{-1}$ (photosystem protein degradation occurs at 200 µmol $m^{-2}$ $s^{-1}$ and higher (Christopher and Mullet 1994)) intensifies *Sig5* expression but does

not affect the amount of mRNAs of other *A. thaliana* sigma factors (Nagashima et al. 2004b). Cryptochromes and Ser/Thr phosphotase PP7 (CryA/B → PP7 → ··· → Sig5 → LRP-psbD) are involved in this signaling pathway (Tsunoyama et al. 2004; Nagashima et al. 2004b; Möller et al. 2003). Red alga *Cyanidioschyzon merolae* has a similar regulation system: light activates the expression of all the four *Sig* genes, whereas higher intensity activates the expression of one gene only (Minoda et al. 2005). The similarity of regulatory systems is convergent, as higher plant *Sig* genes are not the orthologs of red alga *Sig* genes (Minoda et al. 2005; Lysenko 2006).

*Sig5* expression is activated not only by light but also by different stress factors. After *A. thaliana* plants were placed on a medium containing 250 mM of NaCl, *Sig5* expression was induced, but the amount of mRNA of the rest *Sig* genes remained the same (Nagashima et al. 2004b). Osmotic stress (250 mM of mannitol) and low temperature (4°C) also induce *Sig5* expression (Nagashima et al. 2004b). *Sig5* activation under osmotic stress is cryptochrome-independent (Nagashima et al. 2004b). Evidently, Sig5 is a highly inducible plastid transcription factor regulated by different signaling pathways. The analysis of amino acid sequences presented in the chapter ''Comparative analysis...''gives grounds to assume that Sig5 binds to core more weakly than the other plant sigma factors, and holoenzyme with Sig5 provides for a lower level of abortive transcription. This may be the reason why plants use Sig5 as a stress inducible transcription factor.

## Evolution of the *Sig* gene family in plants

The evolution of plant sigma factors was recently analyzed in detail (Lysenko 2006), therefore only major facts and conclusions will be stated here. As it has already been mentioned in the previous parts, regions 1.3–4.2 of plant sigma factors bear similarity to the corresponding sections of bacterial sigma factors of $\sigma^{70}$ family. The aligning of amino acid sequences lets us advance the hypothesis that all plant sigma factors are descended from the cyanobacterial principal sigma factor $\sigma^{A}$ (Lysenko 2006).

As compared to other plant sigma factors, PpSig2 has the strongest similarity to cyanobacterial SigA (Carter et al. 2004; Ichikawa et al. 2004; Lysenko 2006). Evidently, the orthologs of Sig2 preserved the strongest resemblance to the ancestor protein. Anyway, conservative regions 2–4 of Sig2 can efficiently replace the homologous conservative $\sigma^{70}$ regions in *E. coli* but Sig1 and Sig3 cannot (Hakimi et al. 2000). Amino acid sequence of Sig5 has the weakest similarity to other sequences of plant sigma factors, which testifies to the early isolation of this sigma factor in the course of evolution. The least variable amino acid sequences among the orthologous genes are found in Sig1 subfamily (Lysenko 2006). Knockout mutants of all *A. thaliana Sig* genes except *Sig1* have been obtained (Kanamaru et al. 2001; Nagashima et al. 2004b; Favory et al. 2005; Ishizaki et al. 2005; and Kan Tanaka, personal communication on ΔSig3 mutant). The function of *Sig1* may be very important for plant viability.

The analysis of intron-exon structure showed the following: (1) the only sigma factor gene (*RpoD*) of the green microalga *C. reinhardtii*, plant *Sig5* and other plant *Sig* genes, have no common introns; (2) there are 4 introns in genes *Sig1–4*, *6*, their positions coinciding in the corresponding genes of *P. patens*, *A. thaliana*, and *O. sativa* (Kanamaru et al. 1999; Fujiwara et al. 2000; Ichikawa et al. 2004; Lysenko 2006); the positions of introns ''b'' coincide in genes *Sig1–3*, *6*, the positions of introns ''a'', ''c'', and ''d'' coincide in genes *Sig2–4*, *6*.

On the basis of these facts, the following model of plant sigma factor evolution may be advanced (Fig. 5). *Sig* genes of higher plants that belong to *Streptophyta* phylum evolved independently of homologous genes in the majority of green algae belonging to *Chlorophyta* phylum. Most probably, all the higher plant sigma factors are descended from the cyanobacterial principal sigma factor. Gene *Sig5* either was the first to diverge from the common nuclear gene or emerged independently. The data on gene migration frequency from plastids to nucleus (Stegemann et al. 2003; Matsuo et al. 2005) suggest that the transfer of cyanobacterial gene SigA into nucleus may have occurred more than once, therefore it is probable that *Sig5* and the rest of plant *Sig* genes originated from different copies of

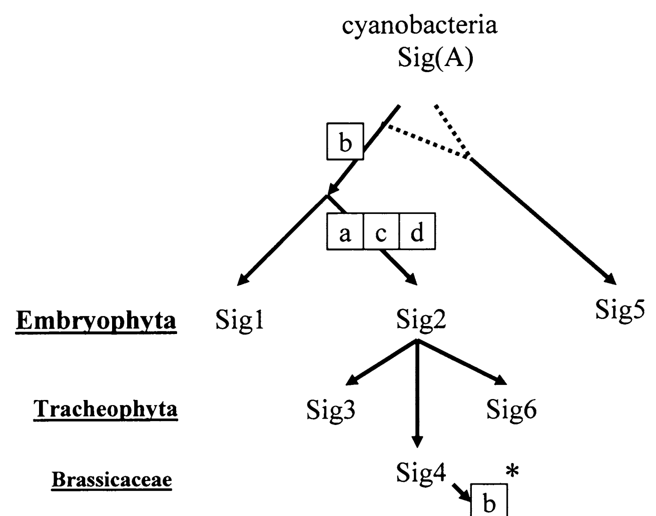


**Fig. 5** Scheme of plant *Sig* gene evolution. Dotted line stands for alternative variants of gene *Sig5* origin, letters a-d stand for introns whose location is conservative in most plant Sig genes, *AtSig4 lost intron b after it diverged from an ancestor gene. Explanations are in the text

one cyanobacterial ancestor gene that migrated to the nucleus at different times. The common ancestor of *Sig* genes 1–4 and 6 acquired intron ''b'' (gene *AtSig4* lost this intron later) and then was duplicated, which resulted in emergence of genes *Sig1* and *Sig2*. Later on *Sig2* acquired introns 'a', 'c', and 'd' and originated genes *Sig3*, *Sig6*, and *AtSig4*.

Genes *Sig1*, *Sig2*, and *Sig5* have been found in moss and flowering plants, each group of orthologs having its own conservative introns (except a–d) whose positions coincide in *P. patens*, *O. sativa*, and *A. thaliana* (Ichikawa et al. 2004; Lysenko 2006); consequently, these genes emerged long before mosses and vascular plants diverged. It is quite probable that these genes were forming as far back as in algae from *Streptophyta* phylum, the common ancestors of charophytes and higher plants. The identification of *Sig* genes of charophytes will help to clarify the evolutionary age of genes *Sig1*, *Sig2*, and *Sig5*. The orthologs of genes *Sig3* and *Sig6* are less conservative than more 'ancient' *Sig1*, *Sig2*, and *Sig5* (Lysenko 2006). Evidently, *Sig3* and *Sig6* emerged later but before flowering plants divided into monocotyledons and dicotyledons. It is interesting that gene *Sig3* has been discovered in maize but has not been found in the complete rice genome. Rice ancestors may have lost this gene. Cereal genes *Sig2A* and *Sig2B*, *Z. mays Sig1a* and *Sig1b*, and *A. thaliana Sig4*, most probably, emerged after monocotyledons and dicotyledons diverged.

Thus sigma factor pool of higher plants changed throughout the evolution process, from the earliest till rather late stages. Evidently, plants and red algae independently realized a single strategy and propagated the only sigma gene they got from cyanobacteria. There is still another strategy: in the genome of green alga *C. reinhardtii* belonging to *Chlorophyta* phylum, sigma factor is encoded by the only gene (Carter et al. 2004). This may suggest that terrestrial plants and red algae regulate plastome gene expression at the transcriptional level much more actively than algae that have only one sigma factor.

## Conclusion

Genes coding plant sigma factors were discovered in 1997 and have been studied throughout the last decade. Today we know that terrestrial plants have a range of sigma factors they use to regulate plastid transcription. Plant sigma factors recognize promoters of $\sigma^{70}$-type and direct the transcription of plastid multisubunit RNA polymerase (PEP). Probably, all plant sigma factors were descended from the cyanobacterial principal sigma factor $\sigma^{A}$, though contemporary paralogs differ much. They differ: (1) in affinity for various plastid promoters and, perhaps, in affinity for core enzyme; (2) in 'preferably transcribed' genes; (3) in expression and the ways of its regulation; (4) in the roles in the life and development of a plant. Some (or, perhaps, even many) plastid genes have several PEP-dependent promoters; probably, the initiation of transcription from these promoters is provided for by different sigma factors. Plant sigma factors are regulated not only at the level of expression, but also at the level of binding to core and promoters. Evidently, plants, like bacteria, use a set of sigma factors to differentially regulate plastid gene expression.

We are still too far from the integrated understanding of how this regulatory system works. Much is not clear: does phosphorylation of core and sigma factors have a differential influence on the transcription of each single gene? How does (p)ppGpp regulate interaction between core and sigma factors? What impact do physical and chemical properties of stroma have on binding to core and promoters, since in leaf chloroplasts, flower chromoplasts, and root leucoplasts sigma factors have to work in different 'reaction buffers'? For the present, most of investigations have been carried out using *A. thaliana* as an object. Moreover, nothing is known of sigma factors of ferns and conifers or what sigma factors the closest relatives of terrestrial plants—charophytes—have. To fill up these gaps is the task of the future research.

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