Minireview

Function and evolution of plastid sigma factors

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Abstract Chloroplasts are plant-specific organelles that perform photosynthesis and responsible for the world's primary productivity. Using light energy, chloroplasts produce many important products, including starch, amino acids, lipids, pigments and various secondary products. Therefore, chloroplasts are essential to the lives of all plants and animals alike. Chloroplast transformation is a unique technology to produce huge amount of valuable materials in chloroplasts using photosynthetic energy. In order to control chloroplasts at will, we need more information on molecular basis of chloroplast gene expression and communication between the chloroplast and nucleus. Chloroplast sigma factors are key regulators of the chloroplast gene expression and chloroplast differentiation. This review summarized recent findings on roles of chloroplast sigma factors in the chloroplast differentiation and environmental responses.

Key words: Chloroplast, plastid, sigma factor, transcription.

Plastids are unique plant organelles that exist in several different forms. Plastids are dynamic and can convert between types by external and developmental signals. The most common plastid type is the chloroplast responsible for photosynthesis in land plants, algae and some protists. All living things depend on energy produced from photosynthesis. Moreover, chloroplasts carry out other essential biochemical processes, including starch, amino acids, fatty acids and pigments synthesis, nitrogen and sulfate assimilation, hormone synthesis and various secondary metabolism. Not only plants but also human life is dependent on these important products from plastids.

Plastids are known to have originated from prokaryotic photosynthetic cyanobacteria. Consequently plastids are semi-autonomous and contain their own genetic system. In higher plants, plastids contain a small prokaryotic-type genome which encodes ~120 genes. Higher plant plastids contain two types of RNA polymerases, eubacterial-type PEP (plastid-encoded plastid RNA polymerase) and phage-type NEP (nuclear-encoded plastid RNA polymerase) (Hess and Börner, 1999). In order to recognize promoters, PEP requires an exchangeable subunit, sigma factor. In bacteria, a variety of sigma factors specifically recognize different promoter sequences. The sigma factor determines which genes are transcribed. Higher plants also encode multiple sigma factor genes (Kanamaru and Tanaka, 2004). Recent molecular and genetic analyses revealed the roles of plastid sigma factors in chloroplast differentiation and

environmental responses. In this review, we summarize plastid transcription and its regulation in higher plants, focused on plastid sigma factors.

Plastid genomes

Plastids have evolved from ancestral photosynthetic cyanobacteria, which were taken into the host cells as endosymbionts. It is believed that chloroplasts are monophyletic, with one primary endosymbiosis. Thus, plastids retain a lot of typical cyanobacterial features such as circular genome of 100–200 kbp and prokaryotic-type gene expression system. During plant cell evolution, a large fraction of the original endosymbiont genes has either been lost or transferred to the nucleus. In consequence, the higher plant plastomes contain a limited number of conserved genes. For example, Arabidopsis chloroplast genome encodes only 129 genes, including 47 photosynthesis-related genes, 25 ribosomal genes, four RNA polymerase subunit genes, nine other protein coding genes (total 85 protein genes), 34 tRNA genes and 4 rRNA genes (Sato et al. 1999). On the other hand, chloroplast is believed to contain more than 3,000 proteins. Recent proteome analysis identified 1325 proteins in chloroplasts (Zybailov et al. 2008). More than 90% of them are encoded on nuclear DNA, synthesized in cytosol and transported into chloroplasts. Consequently, chloroplast function is largely dependent on the nuclei via nuclear-encoded proteins. It should be noted that DNA transfer from plastids to the nucleus is

ongoing (Matsuo et al. 2005).

In contrast to photosynthesis-related genes and ribosome genes, which are retained on the plastid genomes, plastid genomes have selectively lost prokaryotic genes for transcription factors during evolution. For example, sigma factor genes that are involved in promoter recognition and transcription initiation of prokaryotic RNA polymerases have been transferred to the nucleus at an early stage of plant evolution (see below). No sigma factor gene has been identified in chloroplast genomes of extant plants. Moreover, plant cells have lost prokaryotic genes for DNA binding proteins during evolution. Cyanobacteria encodes ~150 transcription factors (Nakamura et al. 1998), but chloroplast genome of primitive red algae *C. melolae* encodes just four putative transcription factor genes (Matsuzaki et al. 2004). Moreover, no prokaryotic DNA binding protein gene has been identified in both chloroplast and nuclear genomes of higher plants, suggesting that plants have lost prokaryotic during plant evolution (Sato 2001). Instead of prokaryotic factors, plastids acquired eukaryotic transcription factors to control plastid gene expression. As a consequence, plastid transcription is highly dependent on the nuclear-encoded sigma factors and eukaryotic type transcription factors in higher plants.

Plastid RNA polymerases

Cyanobacteria contain a multi subunit-type prokaryotic RNA polymerase (RNAP). Its catalytic core is composed of 2α , 1β , $1\beta'$ and $1\beta''$ subunits. Unlike other eubacteria, the β' subunit is usually split into two parts in cyanobacteria, β' and β'' subunits. The plastid genome contains *rpo* genes encoding homologs of the cyanobacterial RNAP α (*rpoA*), β (*rpoB*), β' (*rpoC1*) and β'' (*rpoC2*) subunits. This cyanobacterial-type RNAP is widely conserved in almost all of plant cells, except for achlorophyllous parasitic plant such as *C. gronovii* (Funk et al, 2007) and *E. virginiana* (Wolfe et al. 1992). The expected molecular masses for the PEP subunits of *Arabidopsis* are 38 kD (α), 121 kD (β), 79 kD (β'), and 156 kD (β''). These subunits form the core of the plastid-encoded plastid RNA polymerase (PEP). Sigma factors interact with PEP core and confer promoter-specific binding and transcription specificity. Higher plants contain multiple nuclear-encoded sigma factors, which direct RNA polymerase to distinct sets of promoters in response to environmental and developmental signals (see below).

One of the most unique points of the plastid transcription system is that the T7 phage type RNA polymerases (NEP) are working together with the prokaryotic type RNA polymerase PEP (Hess and Börner, 1999). In spite of small size of plastome, the transcription system of plastids is much more complex

than that of prokaryotes. The drug sensitivity of NEP is different from bacterial-type RNA polymerase and similar to the phage T7 RNA polymerase. *Arabidopsis* contains two plastid-targeting phage-type NEP (RpoTp and RpoTm), aside from mitochondrial RNA polymerase (RpoTm). It has been shown that RpoTp is primarily targeted to chloroplasts, while RpoTm is dually targeted both into plastids and mitochondria (Hedtke et al, 2000). Plastid NEP genes might have derived from a gene duplication event of a mitochondrial NEP gene (RpoTm). Interestingly, plastid-target phage-type RNAP gene is absent in the green alga *Chlamydomonas*. On the other hand, two phage-type RNAP genes have been identified in moss *Physcomitrella patens*, suggesting that NEP has been evolved from RpoTm during the evolution of land plants (Kabeya et al. 2002).

PEP and NEP

Plastid-encoded genes are classified into three groups; type I photosynthesis genes that are transcribed only from PEP promoters, type II photosynthesis and housekeeping genes that are transcribed from both PEP and NEP promoters, and type III NEP-dependent genes, including PEP core subunit genes (*rpoA*, *rpoB* and *rpoC*), *accD* (acetyl-CoA carboxylase) and *ycf2* (Hajdukiewicz et al. 1997; Shiina et al. 2005). It was generally assumed that during plastid differentiation the building up of the NEP transcription system precedes the building up of the PEP transcription system. At first NEP is activated and transcribes the type II and type III housekeeping genes involved in PEP construction, ribosome biogenesis and lipid synthesis. And then newly synthesized PEP takes over transcription during later stages. PEP actively transcribes type I and type II photosynthesis genes, including PSI and PSII subunits and rubisco large subunit in developing chloroplasts, and supports the quick building up of photosynthesis apparatus (Mullet, 1993). However, recent detailed expression studies revealed that both PEP and NEP transcription systems are built up simultaneously during germination (Demarsy et al. 2006). Both NEP and PEP proteins are present in dry seeds of *Arabidopsis*. NEP (RpoTp and RpoTm) protein levels are transiently up-regulated at an early phase of chloroplast development (2 days after germination), and subsequently decreased up to 5 days (Demarsy et al. 2006; Emanuel et al. 2006). Similarly, not only NEP but also PEP core protein levels are also up-regulated quickly during germination. Roles of plastid sigma factors in the switch from predominant NEP to predominant PEP activity during plant development will be discussed later.

Plastid promoters

Standard PEP promoters

According to the presence of multiple RNA polymerases and multiple sigma factors (promoter recognition subunit of PEP), different types of promoters have been mapped on the plastid genomes. Standard PEP promoters resemble *E. coli* σ^{70} -type promoters that are characterized by consensus -35 and -10 sequences spaced at 17~19 nt. In fact, purified *E. coli* RNA polymerase holoenzyme is able to initiate transcription from the standard σ^{70} -type PEP promoters, suggesting that plastid PEP promoters share common features with eubacterial consensus promoters. Meanwhile, relative PEP promoter strength varies more than 40 times. The PEP promoter strength is probably determined by the sequence similarity of the promoter elements to consensus sequences. Usually, PEP transcription activity is transiently up-regulated during chloroplast development and declines significantly later in mature leaves. However, *psbA*, *psbD*, and *rrnP1* promoters remain active in mature chloroplasts. These PEP promoters exhibit unique promoter structures.

The *psbA* promoter

Plastid *psbA* encodes D1 protein of the photosystem II core complex. The *psbA* promoter is one of the most active PEP promoters in chloroplasts. Plant cells counteract photoinhibition by increasing D1 protein turn over. It is assumed that the *psbA* promoter is responsible for the differential maintenance of high transcription activity in mature leaves to support the light-dependent rapid turn over of D1 protein. The *psbA* promoter represents a typical σ^{70} -type PEP promoter, but contains two additional motifs; TATA box-like motif and TGn motif, which regulate the transcription initiation efficiency. The TATA box-like motif (TATATA) locating -24 and -19 nt of the transcription initiation site is well conserved among Characeae and land plants. *In vitro* and *in vivo* experiments suggested that the TATA box-like motif contributed to the *psbA* promoter activity in mature chloroplasts (Eisermann et al. 1990; Kim et al. 1999). The TGn motif is found upstream of the -10 element of the *psbA* promoter of many higher plants and forms an extended -10 promoter. In wheat the TGn motif is essential for the -35 element-independent *psbA* transcription (Satoh et al. 1999).

The *psbD* light-responsive promoter

The *psbD* encode another PSII reaction center protein D2 that also shows a light-dependent rapid turnover (Christopher and Mullet, 1994). The *psbD* light-responsive promoter (*psbD* LRP) is a unique PEP promoter, which is specifically activated by high-irradiance blue and UV-A light (Christopher and Mullet,

1994). The *psbD* LRP also exhibited circadian rhythmicity (Nakahira et al. 1997). Cryptochromes and phytochrome A likely mediate blue light-dependent transcription from the *psbD* LRP (Thum et al. 2001; Chun et al. 2001). Furthermore, it should be noted that the *psbD* LRP is activated by various stresses, including low temperature, salt and osmotic stresses (Nagashima et al. 2004). Genome-wide analysis of plastome transcription in stressed plants revealed that the *psbD* LRP is the only plastid promoter whose activity is specifically regulated by environmental stresses (Kin et al. unpublished data).

The *psbD* LRP is characterized by a weakly conserved -35 element and a conserved 28-nt upstream sequence (AAG box: -36 ~ -64 nt). Aside from the standard PEP promoters that are transcribed only by PEP holoenzyme, the *psbD* LRP activity is dependent on the upstream AAG-box and the AAG-box binding factors (AGF) in various plants (Kim and Mullet, 1995; To et al. 1996; Nakahira et al. 1998). The AAG-box is well conserved among gymnosperms and angiosperms, but ferns and moss have different types of upstream elements (Shimmura et al. unpublished data). PTF1 is a putative AGF that has been identified by yeast one hybrid screening (Baba et al. 2001). However, recent study revealed the nuclear localization of PTF1, suggesting its possible function as a nuclear transcription factor (Kodama and Sano, 2007). Recently, it has been revealed that light-dependent activation of the *psbD* LRP is mediated by a special sigma factor AtSIG5 (see below).

The *rrn P1* promoter

The *rrn P1* promoter is a major promoter in higher plants that is responsible for transcription of the *rrn* operon encoding four rRNA molecules and two tRNA genes. The *rrn P1* promoter contains well conserved -35 and -10 elements. In addition to these consensus sequence elements, a hexameric sequence, named the rRNA operon upstream activator (RUA) has been identified just upstream of the -35 element (Suzuki et al. 2003). The RUA sequence is well conserved in higher plants. It has been shown that the RUA play a role in *rrnP1* promoter activity, although it is not clear whether the RUA is recognized directly by PEP or by specific DNA-binding proteins.

The NEP promoters

Most NEP promoters share a 10-nt consensus sequence overlapping the transcription initiation site. These consensus-type NEP promoters (Type I) contain a conserved YRTA motif just upstream of the transcription initiation site. This feature is similar to mitochondrial and T3/T7 phage type promoters (Hajdukiewicz et al. 1997). On the other hand, a non-consensus type NEP promoter (Type II) has been identified upstream of *clpP*

gene encoding the catalytic subunit of the ClpP ATP-dependent protease (Sriraman et al. 1998). The *clpP* promoter lacks a YRTA motif, but its activity is dependent on downstream sequences (−5 to +25) of the transcription initiation site.

Recent molecular genetic analyses of RpoTp and RpoTmp revealed that two NEP enzymes have overlapping as well as gene-specific functions in the transcription of plastid genes (Swiatecka-Hagenbruch et al. 2008; Courtois et al. 2008). RpoTp plays a major role in transcription of NEP promoters and RpoTmp might play a major role in transcription from the *clpP* and *rrn* Pc NEP promoters.

Functional characterization of plastid sigma factors

Sigma factors interact with PEP to confer promoter-specific binding and transcription specificity. Bacterial cells usually contain several sigma factors with different functions and play a central role in transcriptional control of cell proliferation and environmental responses. The sigma factors form a large family, the σ^{70} family, which can be divided into three groups with different structure and function (Lonetto et al. 1992). Under normal growth conditions, an RNA polymerase holoenzyme containing the principal sigma factors (Group 1) such as *E. coli* σ^{70} play a major role in the transcription of housekeeping genes. These principal sigma factors are essential for cell viability. Under nutrient starvation or under environmental stresses, additional non-essential sigma factors (group 2 and group 3) compete for the limited amount of RNA polymerase core enzyme. Since the amino acid sequence of the DNA-binding region is conserved among group 1 and group 2 sigma factors, both sigma factors likely recognize similar promoter sequences. The group 3 sigma factors comprises alternative sigma factors that are structurally different from group 1 and group 2 sigma factors, and recognize promoters with a different consensus sequence and play a role in stress-induced transcription of specific regulons.

The chloroplast ancestor cyanobacteria also contain multiple sigma factors. The unicellular cyanobacterium *Synechocystis* sp. Strain PCC6803 possesses nine sigma factors; one group 1 (SigA), four group 2 and four group 3 sigma factors (Kaneko et al. 1996). The same is true for plastid sigma factors. In *Arabidopsis*, cDNAs encoding six different sigma factors have been isolated and sequenced (Isono et al. 1997; Tanaka et al. 1997; Fujiwara et al. 2000). Unlike cyanobacteria, all plastid sigma factors are related to group 1 and group 2 sigma factors and no group 3 sigma factor has been identified in plants. Phylogenetic analysis revealed that plastid sigma factors are grouped into five major sub groups (SIG1, SIG2, SIG3, SIG5 and SIG6). SIG5 is distinct from other plastid sigma factors in amino acid sequences

and intron insertion sites (Fujiwara et al. 2000). Multiple plastid sigma factors may be involved in differential gene expression in response to developmental and environmental signals. The specificity of each sigma factor has recently been revealed by extensive characterization of *Arabidopsis* sigma factor T-DNA insertion mutants.

Sigma factors responsible for early chloroplasts development

Photosynthesis genes are not transcribed efficiently in seed plastids and their expression is up-regulated during germination and subsequent seedling development. It has been shown that SIG2 and SIG6 play crucial roles in early chloroplast development during light-dependent seedling growth in *Arabidopsis*. *AtSIG6* T-DNA insertion mutants showed a pale green phenotype at an early stage of seedling development (3–5 days after imbibition: Figure 1), while the seedlings turn green at a later stage (7days) (Ishizaki et al. 2005; Loschelder, et al. 2006)). Light-dependent chloroplast development is significantly retarded in the *AtSIG6* mutants. The transcript level of most PEP-dependent genes, including photosynthesis genes, rRNAs and some tRNAs are greatly decreased in young cotyledons by inactivation of *SIG6*, while NEP-dependent transcripts mostly increased. All of the *SIG6*-dependent genes are preceded by typical σ^{70} -type promoters. These data suggest that *SIG6* is an early general sigma factor in plastids that plays a key role in chloroplast development at an early stage of seedling development. Detailed expression analysis of plastid sigma factors during germination revealed that *SIG6* expression is transiently up-regulated between 1-3 days after imbibition, and then drop down. In addition to these early roles, it should be noted that *SIG6* has the second (long term) role in mature leaves. Accumulation of 2.6 kb *atpB/atpE* transcripts was significantly reduced in *SIG6* knock down mutants in mature leaves (Loschelder, et al. 2006).

SIG2-deficient mutants also showed a pale-green phenotype in *Arabidopsis*. However, unlike *SIG6* mutants, the pale green phenotype has been observed in both young cotyledons and mature leaves (Figure 1), suggesting that *SIG2* play a role in all stages of plant development. The pale green phenotype of *AtSIG2*-deficient mutants is characterized by decreased accumulation of chlorophyll and plastid-encoded photosynthesis related proteins (Kanamaru et al. 2001). However, most of the photosynthesis-related transcripts were not reduced in the *AtSIG2*-deficient mutants, suggesting that *SIG2* is not responsible for transcription of plastid-encoded photosynthesis genes. On the other hand, the amount of several PEP-dependent tRNAs was significantly reduced in the *AtSIG2*-deficient mutants in both cotyledons and mature leaves. The *SIG2*-dependent

Figure 1. Characterization of the *Arabidopsis* SIG2 and SIG6 mutants. (A) Wild-type (WT) and SIG2 T-DNA insertion mutants (*sig2-1* and *sig2-2*). SIG2 mutants showed pale green phenotype in both young and older seedlings. (B) WT and SIG6 T-DNA insertion mutant (*sig6-1*). SIG6 mutants showed pale green phenotype in young seedlings, but the mutant phenotype was recovered in older seedlings. (C) Summary of functional diversity of plastid sigma factors in higher plants.

tRNAs include *trnV*-UAC, *trnM*-CAU, *trnE*-UUC and *trnD*-GUC. It should be noted that *trnE* is a precursor of 5'-aminolevulinic acid (5'-ALA) and chlorophyll biosynthesis. Taken together, SIG2 is probably a special sigma factor that is indispensable for transcription of a set of tRNA genes. Decreased accumulation of photosynthesis related proteins without affecting their mRNA levels might be caused by defects in chlorophyll biosynthesis and/or plastid translation activity in the *AtSIG2* mutants.

To date, SIG2, SIG3, SIG4, SIG5 and SIG6 T-DNA insertion mutants have been characterized in *Arabidopsis*. Only SIG2 and SIG6 T-DNA insertion mutants had pale green phenotypes in young cotyledons, whereas other mutants grew normally. Furthermore, detailed expression analysis of plastid sigma factors during seedling development in *Arabidopsis* revealed that SIG2 and SIG6 transcripts were detected within 1 day imbibition, while other sigma factor expression is induced after 2 days imbibition or later. The *OsSIG6* (rice *AtSIG6* homolog) transcripts was also more abundantly detected in the leaf base (young tissues) than in the leaf tip (matured tissue) (Kubota et al. 2007). Furthermore, the expression of maize ZmSIG6 protein was also detected in leaf base and root of light-grown seedlings (Lahiri and Allison, 2000). Taken together, these studies suggest that SIG6 and SIG2 cooperatively

support the early transcription of photosynthesis genes and a set of tRNA genes during seedling development, respectively. Interestingly, SIG2/SIG6 double mutant shows an albino phenotype (Ishizaki et al. unpublished data). It is conceivable that coordinated functions of SIG6 and SIG2 in developing chloroplasts are essential for normal chloroplast development in *Arabidopsis*.

Other General plastid sigma factors

It should be noted that the *AtSIG6*-deficient mutant phenotype is restored in the later stages of seedling development (Ishizaki et al. 2005). It is assumed that another sigma factor(s) that recognizes the standard σ^{70} -type PEP promoters and is involved in the transcription of photosynthesis genes may take over the SIG6 functions in the later stage of seedling development. Since plastid sigma factors are related to group 1 and group 2 bacterial sigma factors that recognize σ^{70} -type promoter elements, it is expected that all plastid sigma factors can initiate transcription from the standard-type PEP promoters harboring -35 and -10 promoter elements. In fact, it has been demonstrated that *psbA* and *rbcL* promoters are recognized by several sigma factors (Hakimi et al. 2000; Homann et al. 2003; Privat, 2003), suggesting overlapping specificity of plastid sigma factors.

On the other hand, it seems that plastid sigma factors

have specific as well as overlapping functions. *In vitro* transcription assays performed without competition by other sigma factors may have masked the overlapping functions. In fact, the specific functions of plant sigma factors have been obtained by analyses of *Arabidopsis* T-DNA insertion mutants that reflect *in vivo* competition conditions. For example, *OsSIG1*-deficient mutants have been characterized in rice (Tozawa et al. 2007). *OsSIG1* mRNA accumulates relatively late during leaf development. The *OsSIG1*-deficient mutants showed a reduced chlorophyll content (one third). Moreover, transcripts derived from the plastid-encoded *psaA* operon were markedly decreased in the *OsSIG1*-deficient mutants. These results suggest that *OsSIG1* is responsible for transcription of *psaA* operon genes for components of photosystem I in mature chloroplasts of rice. On the other hand, *SIG1*-deficient mutant has not been characterized in *Arabidopsis*.

On the other hand, *AtSIG3*-deficient mutants showed no visible phenotype (Zghidi et al. 2007). However, plastid genome microarray analysis revealed that *psbN* transcription was significantly reduced in the *AtSIG3*-deficient mutants. The *psbN* transcription produces antisense RNA to the *psbB* operon. Thus, the *SIG3*-dependent *psbN* transcription might serve a regulatory function for the expression of the *psbB* operon genes. *AtSIG4*-deficient mutants also showed no visible phenotype (Favory et al. 2005). It was shown that transcription of the plastid-encoded *ndhF* gene was specifically reduced in the *AtSIG4*-deficient mutants. *SIG4* may be involved in the regulation of the plastid NDH activity by regulating the *ndhF* transcription.

In summary, *SIG1*, *SIG3* and *SIG4* likely have the specific functions in addition to the overlapping functions. It is conceivable that these sigma factors may support active transcription of photosynthesis genes in mature leaves together with *SIG6*, and may also be involved in the transcriptional regulation of specific genes in response to the developmental and environmental signals.

Stress responsive sigma factor SIG5

As mentioned above, the *psbD* light-responsive promoter (*psbD* LRP) is the only promoter that is selectively activated by environmental stresses, including high salinity, high osmolarity, low temperature and high irradiance of blue light (Nagashima et al. 2004; Tsunoyama et al. 2002). Since the *psbD* LRP exhibits unusual promoter architecture (*psbD* LRP activity is dependent on the -10 element and AAG box, but not the -35 element), it is assumed that the *psbD* LRP might be recognized by a special sigma factor. Among higher plant sigma factors, *AtSIG5* is structurally distinct from other sigma factors, and *AtSIG5* expression is also specifically up-regulated by high-irradiance blue light

(Tsunoyama et al. 2002) and various environmental stresses (Nagashima et al. 2004). Analyses of *Arabidopsis AtSIG5* T-DNA insertion mutants revealed that *AtSIG5* is essential for the light-dependent *psbD* LRP activity (Tsunoyama et al. 2004; Nagashima et al. 2004). Moreover, over-expression of *AtSIG5* in protoplasts (Tsunoyama et al. 2004) and in transgenic *Arabidopsis* and tobacco plants (Nozoe et al. unpublished data) resulted in the selective elevation of *psbD* LRP activity. Furthermore, high-intensity light-induced *psbD* transcription was also dependent on the *PpSIG5* in the moss *Physcomitrella patense* (Ichikawa et al. 2008). These findings demonstrated that *SIG5* acts as a special sigma factor in chloroplasts that selectively recognizes the *psbD* LRP in response to various environmental stresses.

The germination rate of the *AtSIG5* T-DNA insertion mutants decreased significantly under salinity conditions and the recovery process of PSII photochemical efficiency after exposure to high light was also delayed in the *AtSIG5* mutants (Nagashima et al. 2004). On the other hand, over expression of *AtSIG5* resulted in the higher germination rates in high salinity conditions (Figure 2; Kin et al. unpublished data). These data suggest that *SIG5*-dependent *psbD* LRP activation is directly or indirectly involved in the stress responses of *Arabidopsis*.

The expression of *SIG5* is differentially induced by blue light but not red light (Tsunoyama et al. 2002) and the blue light-induced *SIG5* expression is mediated by cryptochrome 1 and 2 (Mochizuki et al. 2004). The blue light-dependency of *SIG5* expression is biphasic, the lower-light induction and higher-light induced further enhancement of *SIG5* expression (Onda et al. 2008). Interestingly, the lower light induction is dependent on both cryptochrome 1 and 2, while stronger light dependent expression is mediated by only cryptochrome 1. On the other hand, *SIG5*, but not other sigma factors is quickly induced by red light in a Phytochrome-A-dependent manner during de-etiolation of dark grown seedlings (Tepperman et al. 2006). Moreover, *AtSIG5* T-DNA insertion mutant displayed significant defects in the phytochrome-A-induced inhibition of hypocotyl elongation and stimulation of cotyledon growth, suggesting another direct or indirect role of *SIG5* in the phytochrome-A-induced de-etiolation (Khanna et al. 2006). Furthermore, blue light-induced *SIG5* expression is mediated by a nuclear localized protein phosphatase PP7 that has been identified as a positive regulator of blue light signaling (Müller et al. 2003). Finally, it should be noted that activation of the *psbD* LRP requires light even in the *AtSIG5* overexpressing plants (Nozoe et al. unpublished data). The specific function of *SIG5* beautifully illustrates how the nucleus holds control over plastid gene expression. Figure 2 represents a schematic

Figure 2. Characterization of the *Arabidopsis* SIG5 mutants. (A) Schematic representation of a possible mechanism of the blue light-induced activation of *psbD* LRP. (B) Effects of high salinity on wild type (WT), SIG5 T-DNA insertion mutant (*sig5.1*) and SIG5 overexpressing mutant (SIG5ox) plants. The germination rate of the SIG5-deficient mutants decreased significantly, while over expression of SIG5 resulted in the higher germination rates in high salinity conditions.

model of SIG5-mediated activation of *psbD* LRP.

Post-transcriptional regulation of plastid sigma factors

There is some evidence to suggest that plant sigma factors might be regulated post-translationally. SIB1 is a nuclear-encoded SIG1-binding protein that targets to chloroplasts. The activity of SIG1 might be controlled by its interaction with SIB1 (Morikawa *et al.* 2002). On the other hand, the activity of SIG3 might be regulated by proteolytic cleavage (Hkimi *et al.* 2000; Hoffmann *et al.* 2003). Furthermore, biochemical evidence suggests that plastid sigma factors might be regulated by protein phosphorylation in mustard (Baginsky *et al.* 1997)

Evolution of plastid sigma factors

As mentioned above, the cyanobacterial ancestor of the plastids has group 3 sigma factors, besides group 1 and group 2 sigma factors. On the other hand, no sigma factor of the group 3 has been found in plant genomes. Primitive red algae *Cyanidioschyzon merolae* genome encodes four plastid sigma factors (*CmSig1-4*) (Matsuzaki *et al.* 2005), while green algae *Chlamydomonas reinhardtii* encodes a single sigma

factor (*CrRpoD*) (Bohne *et al.* 2006). Phylogenetic analysis of plastid sigma factors suggested that plant sigma factors of red and green algal plastids, and the group 1 sigma factors of cyanobacteria form a monophyletic group (Minoda *et al.* 2005). Considering monophyletic nature of land plants and green algae, it is also likely that all plastid sigma factors are derived from cyanobacterial group1 (principal) sigma factor. It is assumed that the principal sigma factor genes were transferred from the ancestral plastid to the host nuclear genome. Subsequently, the nuclear encoded principal sigma factors were diversified during plant evolution. Figure 3 shows the possible evolution of plastid sigma factors in higher plants.

There are four conserved intron insertion sites in higher plant sigma factors, except for SIG5. These introns are not conserved in green algal (*CrRpoD*) and red algal (*CmSig1-4*) sigma factors. Thus, it is assumed that the sigma factors of green and red algae were evolved independently from higher plant sigma factors. Moreover, the amino acid sequence of SIG5 is different from other plastid sigma factors in higher plants, suggesting that Sig5 was firstly diverged from the common ancestral plastid sigma factor and evolved

Figure 3. Schematic representation of the plastid sigma factor gene evolution. Dotted lines represent unidentified route of Sig5 origin.

independently. Furthermore, the analysis of intron-exon structure suggested that the common ancestor of higher plant sigma factors first duplicated into *SIG1* and *SIG2*. In fact, the moss *Physcomitrella patens* (Rensing et al. 2008) and early divergent vascular land plant *Selaginella nvolvens* (see *Selaginella* Genomics: <http://selaginella.genomics.purdue.edu/>) has *SIG1*, *SIG2* and *SIG5* homologs, but not *SIG3*, *SIG4* and *SIG6*. These ancient type sigma factors may have emerged in *Streptophyta* phylum, the common ancestors of charophytes and higher plants. Subsequently *SIG3* and *SIG6* likely emerged from *SIG2* during the evolution of angiosperms, before the separation of monocots and dicots. Finally AtSIG4 was evolved in dicots after the separation of monocots and dicots, since *SIG4* homolog was not found in the rice genome. On the other hand, four sigma factors in *Cyanidioschyzon merolae* probably evolved independently in primitive red algae.

A sharp increase in diversity of plastid sigma factors may be involved in the increasing complexity of plant body plans and meet the increasing demand for stress tolerance during evolution of higher plants. Further detailed functional and expression analysis of each sigma factor will shed light on the role of multiple plastid sigma factors in higher plants.

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