Transcription and transcriptional regulation in plastids

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Abstract

This chapter describes the components of the transcriptional apparatus in plastids (RNA polymerases, promoters, transcription factors) and their roles in transcription. The chromosomes of plastids from nearly all plants contain genes for core subunits of PEP, a bacterial-type RNA polymerase which might be responsible for transcription of all plastid genes in algae but shares responsibility for transcription with one or more nuclear encoded transcriptases (NEP) in higher plants. There is increasing evidence that the catalytic subunit of NEP is related to RNA polymerases of bacteriophages like T7. NEP and PEP are active throughout leaf development. Transcription of plastid genes and operons by multiple promoters is common. Promoter recognition by PEP is mediated by σ-factors. Factors supporting NEP in promoter binding are not known yet. Examples of regulation of transcription are described demonstrating promoter selection by σ-factors and activation/repression of gene activity by transcription factors.

1 Introduction

Plastids divide in a similar manner as bacteria. Each plastid in a plant contains identical circular copies of the plastid chromosome, the plastome. In addition to monomeric circles, dimers, trimers, and tetramers exist, but also numerous linear and even more complex molecules of different sizes. The number of plastids per cell and of plastomes per plastid changes species-specifically from cell-type to cell-type and may vary during the development of plants (Butterfass 1980; Herrmann and Possingham 1980; López-Juez and Pyke 2005; see Chapters 2, 3, 4). Adjusting the copy number of plastomes per cell could be a way to respond to different needs for plastid gene products, in particular of rRNAs as Bendich (1987) suggested. The striking increase of plastome copies at the beginning of the development of chloroplasts from proplastids is certainly a precondition for the biogenesis of the photosynthetic apparatus in young leaf cells. Other ways to control gene expression at the DNA level could be *via* alteration of the DNA conformation (Stirdivant et al. 1985; Gauly and Kössel 1989; Sekine et al. 2002) or differential methylation (Ngernprasirtsiri et al. 1989; Kobayashi et al. 1990; Ngernprasirtsiri and Akazawa 1990). Both ways are investigated in only a few

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studies and at least the latter one may be an exception rather than the rule (Hess et al. 1993; Isono et al. 1997a).

Early studies on gene expression in chloroplasts revealed specific effects of light on the expression of the *psbA* gene and of the cell type (mesophyll vs. bundle sheath cells) on the expression of *rbcL* (Bedbrook et al. 1978; Link et al. 1978) suggesting an important role of differential transcription like in bacteria. Further studies during the 1980's, however, revealed important contributions of posttranscriptional processes in controlling the levels of gene products (plastid RNAs and proteins) and only a minor role for transcription in the regulation of gene expression in plastids during plant development (Deng and Gruissem 1987; Gruissem et al. 1988). While the importance of posttranscriptional processes in the control of RNA and protein levels remained undisputed until today (see Chapters 6-10), the view on the role of transcription changed again during the 1990's with the discovery of differential transcription of house-keeping vs. photosynthesis genes, of light-induced differential transcription of several genes, and of additional promoters within operons (Mullet 1993). Moreover, the machinery for transcription in plastids of angiosperms unexpectedly turned out to be more complex as known from bacteria and to need different RNA polymerases, although plastids possess a much smaller genome than their cyanobacterial ancestors (Stern et al. 1997; Gray and Lang 1998; Hess and Börner 1999; Liere and Maliga 2001). This chapter describes the components of the transcriptional apparatus in plastids and their roles in transcription and its regulation.

2 RNA polymerases

2.1 NEP: nuclear-encoded plastid RNA polymerase

2.1.1 Evidence for the existence of PEP and NEP

Transcription of all bacterial genes is performed by one core RNA polymerase consisting of 4 subunits (two α, one β, and one β'; e.g. in *E. coli*), or 5 subunits in case of the cyanobacteria which have β' split into two subunits, β' and β" (Kaneko et al. 1996). The plastid chromosomes of algae and higher plants posses genes for core subunits of a cyanobacterial-type RNA polymerase, first reported for *Marchantia*, tobacco and spinach (Ohyama et al. 1986; Shinozaki et al. 1986; Sijben-Muller et al. 1986), which is commonly abbreviated as PEP (for *p*lastid-*e*ncoded *p*lastid RNA *p*olymerase; Hajdukiewicz et al. 1997; see Section 1.2; Fig. 1). The existence of one or more *n*uclear-*e*ncoded *p*lastid RNA *p*olymerase(s) (NEP) was suggested by comparing the effects of inhibitors of translation on cytoplasmic and plastidial ribosomes, respectively (Ellis and Hartley 1971). Detection of RNA polymerase in plastids with impaired protein synthesis implies a nuclear location of the gene(s) encoding this activity. Ribosome-deficient plastids isolated from heat-bleached rye leaves were found to exhibit RNA polymerase activity (Bünger and Feierabend 1980), and the detection of mature rRNAs in plastids that lack

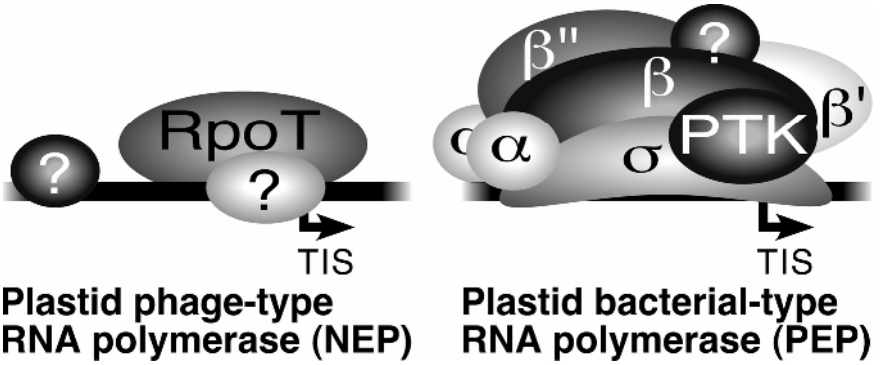

Fig. 1. RNA polymerases in plastids. The nuclear-encoded plastid RNA polymerase (NEP) is related to phage-type single-subunit enzymes and may need additional, yet unknown protein factors for promoter recognition. The plastid-encoded plastid RNA polymerase (PEP) is a multisubunit enzyme homologous to bacterial RNA polymerases and consists of the core α_2, β, β', and β" subunits and the nuclear-encoded σ-like factor required for promoter recognition. In chloroplasts, PEP associates with additional factors, which are thought to be involved in regulation of PEP transcription activity, including the plastid transcription kinase (PTK). The transcription initiation sites (TIS) are indicated by arrows.

ribosomes was reported as proof for accurately functioning nuclear-encoded RNA polymerase and rRNA processing activities in plastids of the barley mutant *albostrians* (Siemenroth et al. 1981). These early data have later been confirmed by demonstrating the expression of several genes in ribosome-free plastids of barley mutants (Hess et al. 1993), of heat-bleached rye leaves (Falk et al. 1993; Hess et al. 1993), and of the *iojap* mutant of maize (Han et al. 1993). Further evidence for NEP activity came from the detection of RNA synthesis in nonphotosynthetic plastids of the parasitic plant *Epifagus virginiana* (Ems et al. 1995). *E. virginiana* has a relatively small plastid genome that lacks genes for proteins involved in photosynthesis and, important in this context, for the core subunits of PEP (Morden et al. 1992). Similar observations have been made more recently with other parasitic plants, where transcription has to rely solely on NEP activity as their plastomes lack PEP genes (Lusson et al. 1998; Krause et al. 2003; Berg et al. 2004). The invention of genetic manipulation of plastid genes allowed for the directed inactivation of PEP genes. Plants with deleted PEP genes still were able to transcribe their plastid genes, i.e., provided additional evidence for the existence of NEP. Moreover, the albino phenotype of these plants indicated that NEP activity alone is not sufficient for the development of photosynthetically active chloroplasts (Allison et al. 1996; Hajdukiewicz et al. 1997; Krause et al. 2000; Legen et al. 2002).

2.1.2 Phage-type RNA polymerases in plants

There is increasing evidence that the catalytic subunit of NEP is related to RNA polymerases of bacteriophages like T3, T7, or SP6 (Fig. 1). Lerbs-Mache (1993)

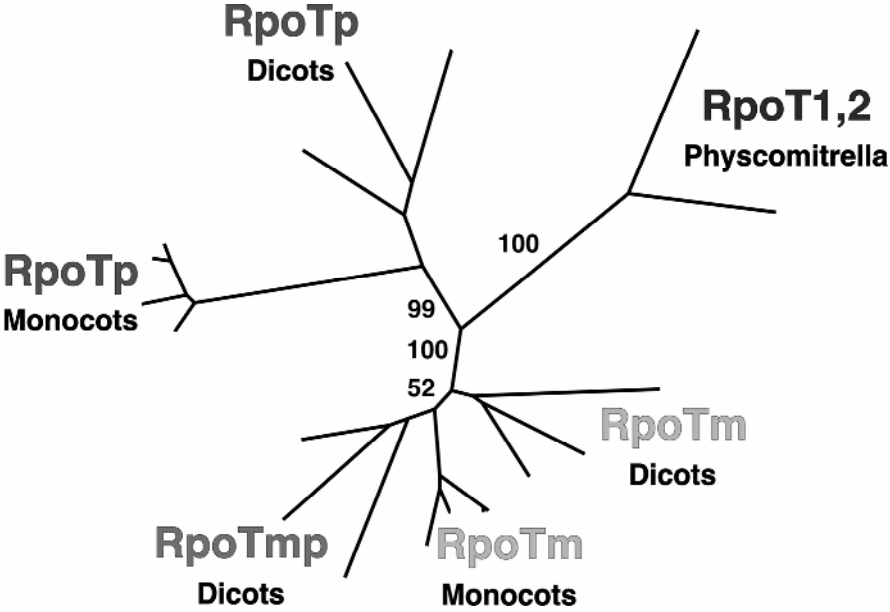

Fig. 2. Schematic representation of RpoT phylogeny. Distances are proportional to relative sequence divergence; numbers at nodes show branch support values. *RpoT* gene duplications occurred several times during the evolution of land plants. Reconstruction of phylogeny was done by quartet puzzling (based on data published in Emanuel et al. 2006).

observed an RNA polymerase activity of a 110 kDa protein (the size expected for RpoT products) that was prepared from spinach chloroplasts and initiated transcription from a T7 promoter but not from an *rbcL* PEP promoter. It has been known for many years that the mitochondrial genes of baker's yeast, *Saccharomyces cerevisiae*, are transcribed by a nuclear encoded phage-type RNA polymerase (Masters et al. 1987). It is now evident that related phage-type polymerases are responsible for mitochondrial transcription in nearly all eukaryotes. The only exceptions from this rule are freshwater protozoa like *Reclinomonas* belonging to the jakobids. These lower eukaryotes still possess genes for a bacterial-type RNA polymerase in their mitochondrial genomes which became lost during the evolution of this organelle in the other lineages of eukaryotes (Lang et al. 1997). Genes potentially coding for RNA polymerases of the phage-type are also found on so-called 'linear plasmids', double-stranded DNAs of around 10 kb that have been detected in the mitochondria of several protozoa, fungi, and plants. Neither the origin of these 'plasmids', that exhibit features of viral genomes, nor the functional roles of their genes are known (Meinhardt et al. 1997). Phylogenetic trees of phage-type RNA polymerases suggest that the nuclear gene for the mitochondrial RNA polymerase evolved independently of the plasmid-localized RNA polymerase genes probably from an ancestral bacteriophage gene (Lysenko and Kuznetsov 2005; Azevedo et al. 2006; Emanuel et al. 2006). Genes coding for phage-type RNA polymerases duplicated several times during the evolution of

plants (Fig. 2). They were first discovered in *Arabidopsis* and *Chenopodium* and designated as *RpoT* genes (for *R*NA *po*lymerase of the phage *T*3/*T*7 type; Hedtke et al. 1997; Weihe et al. 1997). Meanwhile it is evident that the nuclear genomes of dicotyledonous and monocotyledonous plants contain more than one *RpoT* gene (Fig. 3). The diploid genomes of the eudicots *Arabidopsis* (Hedtke et al. 1997, 2000), *Nicotiana silvestris* (Kobayashi et al. 2001a, 2001b, 2002), and *Populus* (deduced from the sequences data in http://genome.jgi-psf.org; Tuskan et al. 2006) contain 3 *RpoT* genes. The amphidiploid genome of tobacco, *N. tabacum*, contains 6 *RpoT* genes (two sets of three genes, one set each from the two diploid parental species; Hedtke et al. 2002).

The N-termini of the different RpoT polymerases (RpoTm, RpoTp, and RpoTmp) fused to GFP (green fluorescence protein) target the protein to mitochondria, plastids, and both organelles, respectively (Hedtke et al. 1997, 1999, 2000, 2002; Kobayashi et al. 2001a, 2001b, 2002). It has therefore been suggested that the *RpoT* genes encode mitochondrial (RpoTm; the *Arabidopsis* gene was originally designated as *RpoY* and *RpoT;1*; Hedtke et al. 1997, 2000), plastid (RpoTp; originally RpoZ and RpoT;3), and dual-targeted RNA polymerases (RpoTmp; originally RpoT;2). Targeting to one or the other organelle might be regulated at the level of translation as the *RpoTmp* mRNAs contain two potential start codons for translation, a feature which is conserved for all RpoTmp messengers of dicots and even *Physcomitrella* (see below). If the first start codon with a position more close to the 5′ end is used, a transit peptide is synthesized that allowed for transportation of the protein into both organelles. If the exclusive usage of the second start codon was forced by deletion of the first one, the smaller transit peptide imported GFP only into mitochondria (Hedtke et al. 2000, 2002; Kobayashi et al. 2001a; Richter et al. 2002).

Monocots (only cereals have so far been investigated) have only two *RpoT* genes, one coding for a mitochondrial (*RpoTm*), the other for a plastidial RNA polymerase (*RpoTp*; Chang et al. 1999; Ikeda and Gray 1999; Emanuel et al. 2004; Kusumi et al. 2004; Fig. 3). Also the moss *Physcomitrella patens* contains two *RpoT* genes (Kabeya et al. 2002; Richter et al. 2002). Other plants have not been studied so far. The *Physcomitrella* genes were named *RpoTmp1* and *RpoTmp2* by Richter et al. (2002), since it was observed that the putative transit peptides encoded by both genes mediated dual targeting of GFP to plastids and mitochondria like in the case of the RpoTmp polymerases of dicots. However, RpoTmp localization is still a matter of debate. Targeting of GFP to mitochondria but not to plastids was observed in *Arabidopsis* or *Physcomitrella* when the protein was fused not only with the putative RpoTmp targeting sequence but also with the 5′-flanking UTR. For yet unknown reasons, the presence of the 5′-UTR prevents usage of the first start codon during translation of the *Arabidopsis* and *Physcomitrella* RpoTmp mRNAs. As mentioned above, translation from the second start codon produces a transit peptide for import into mitochondria (Kabeya and Sato 2005). The authors proposed, therefore, that dual targeting of GFP fused to the putative RpoTmp transit peptide alone, i.e. without the 5′-UTR, was an experimental artifact and these genes encode mitochondrial RNA polymerases. On the other hand, exclusive targeting to mitochondria is not in agreement with the observation

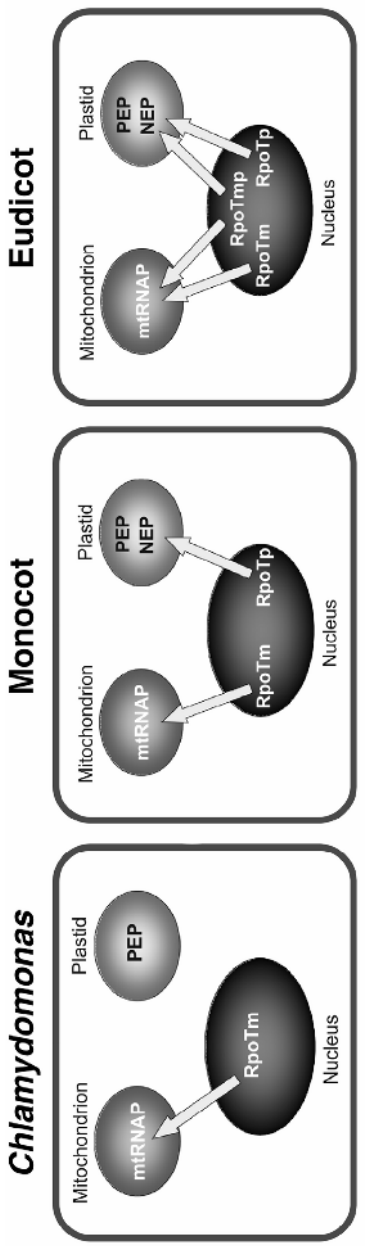

Fig. 3. *Chlamydomonas* possesses only one nuclear *RpoT* gene that is proposed to encode the mitochondrial RNA polymerase (mtRNAP). Cereals have two RpoT genes, one encoding the mtRNAP, the other a plastid RNA polymerase supposed to represent NEP, whereas *Arabidopsis* and other eudicots additionally acquired RpoTmp, which may contribute to transcription in mitochondria and plastids.

that the RpoTmp homolog of spinach was detected in chloroplasts but not in mitochondria with antibodies reacting specifically with this enzyme (Azevedo et al. 2006). Moreover, studies on mutants lacking RpoTmp suggested a function in plastid transcription (Baba et al. 2004; Hricova et al. 2006; see below). Obviously, more detailed investigations into the localization of RpoTmp are required and the possibility of a regulation of targeting to mitochondria and/or plastids at the level of translation should be considered (Christensen et al. 2005).

Whether algae need NEP in addition to PEP to transcribe their plastid genes is not known yet (see review by Smith and Purton 2002). Like higher plants, algae bear genes for the core subunits of PEP in their plastid genomes. In contrast to *Epifagus* (see above), even the nonphotosynthetic alga *Astasia longa*, the malaria parasite *Plasmodium falciparum*, and related organisms have plastid (apicoplast) PEP genes (Wilson et al. 1996; Gockel and Hachtel 2000; Sheveleva et al. 2002) suggesting that a NEP activity is lacking. The nuclear genome of *Chlamydomonas* contains only one *RpoT* gene (A. Weihe et al., unpublished data). There are no experimental data on the subcellular localization of the *Chlamydomonas RpoT* gene product, but it likely codes for the mitochondrial RNA polymerase as in the other eukaryotes that possess only a single gene for a phage-type RNA polymerase (Fig. 3). Inhibition of transcription in *Chlamydomonas* chloroplasts by inhibitors which are specific for the bacterial-type RNA polymerase and would not affect the activity of phage-type RNA polymerases led to a complete block of plastid gene expression arguing against the presence of NEP activity in this alga (Surzycki 1969; Guertin and Bellemare 1979). Also another alga, *Osteococcus tauri*, possesses only one *RpoT* gene, which likely encodes the mitochondrial RNA polymerase (W. Hess, T. Börner, H. Moreau, unpublished; Derelle et al. 2006).

2.1.3 Function of RpoT polymerases in higher plants

Only little information is available on the function of RpoT polymerases in plants. Heterologously expressed RpoTp, RpoTmp, and RpoTm enzymes of *Arabidopsis* are active RNA polymerases that prefer circular over linear template DNA. RpoTm and RpoTp (not RpoTmp) exhibit an inherent ability to recognize several mitochondrial and at least one NEP promoter *in vitro* (Kühn et al. 2007). In monocots with two *RpoT* genes, RpoTm is assumed to represent the catalytic subunit of the mitochondrial RNA polymerase and RpoTp the catalytic subunit of NEP. RpoTp has been detected by specific antibodies in the chloroplasts of rice and maize (Chang et al. 1999; Kusumi et al. 2004) and RpoTm in maize mitochondria (Chang et al. 1999). *RpoTp* mRNAs are particularly abundant in very young cells of cereal leaves (Chang et al. 1999; Emanuel et al. 2004; Kusumi et al. 2004) in agreement with the proposed importance of NEP activity early in chloroplast development for transcription of the PEP genes (Mullet 1993; see Section 4.1). Expression of *RpoTm* and *RpoTp* in monocots is under control of light (Chang et al. 1999) and plastid signal(s) (Emanuel et al. 2004). In *Arabidopsis*, *RpoTm* and *RpoTmp* promoters showed identical expression patterns with highest levels in tissues known for their requirement of high respiration activity (e.g. meristems, tapetum) suggesting a function of both polymerases in mitochondria, whereas

RpoTp expression was highest in green tissues of leaves, stems, and sepals (Emanuel et al. 2006). Like in monocots, transcription of the *RpoT* genes is stimulated by light in *Arabidopsis* leaves (T. Preuten, K. Liere, T. Börner, unpublished results), i.e., light-activated expression of phage-type RNA polymerases may be a general phenomenon in angiosperms. Evidence for NEP being represented by RpoTp (probably together with RpoTmp; see below) was provided by studies on transgenic *Nicotiana* and *Arabidopsis* plants that overexpressed RpoTp and exhibited an increased usage of certain NEP promoters (Liere et al. 2004). Mutation of the *Arabidopsis RpoTp* gene led to impaired chloroplast biogenesis and altered accumulation of plastid transcripts (Hricova et al. 2006). Similar observations were made on *Arabidopsis* plants with reduced RpoTp mRNA levels due to expression of antisense RNA (Emanuel et al., unpublished data). Although the localization of RpoTmp in mitochondria is not in doubt (Kabeya and Sato 2005), its function for this organelle remains obscure so far. RpoTmp was supposed, however, to play a role in plastid gene expression (Baba et al. 2004; Hricova et al. 2006). *Arabidopsis* lines with impaired *RpoTmp* function were delayed in chloroplast biogenesis and showed altered plastid transcript levels (Baba et al. 2004). *RpoTp/RpoTmp* double mutants exhibited a more severe phenotype than both of the single mutants and were extremely retarded in growth (Hricova et al. 2006).

Clearly, more studies are needed to exactly define the function of the different organellar RNA polymerases. First insights into the division of labor between PEP (the bacterial type RNA polymerase) and NEP (probably represented by RpoTp in monocots and RpoTp and RpoTmp in dicots) were obtained from investigations on the use of PEP *vs.* NEP promoters in different tissues and under the influence of different endogenous and exogenous factors as discussed below.

2.2 PEP: plastid-encoded plastid RNA polymerase

The chromosomes of plastids from nearly all plants contain genes for core subunits of PEP, a bacterial-type RNA polymerase, which might be responsible for transcription of all plastidial genes in algae but shares responsibility for plastid transcription with one or more NEP enzymes in higher plants (see above). The *rpoA* gene codes for the 38-kDa α-subunit of PEP (Little and Hallick 1988; Ruf and Kössel 1988; Hu and Bogorad 1990). Like in bacteria, it forms an operon together with several ribosomal protein-encoding genes (Purton and Gray 1989). The *Physcomitrella* plastome lacks this gene. Instead, *rpoA* is found in the nuclear genome (Sugiura et al. 2003). A similar situation was reported for the bacterial-type RNA polymerase in chloroplasts of several algae (Smith and Purton 2002) and in the plastid-like organelles (apicoplasts) of *Plasmodium* (Wilson et al. 1996). The ß- (120 kDa), ß'- (85 kDa), and ß"-subunits (185 kDa) are encoded by the *rpoB*, *rpoC1*, and *rpoC2* genes, respectively, which together form an operon, exactly as known from cyanobacteria (Ohyama et al. 1986; Hudson et al. 1988; Little and Hallick 1988; Hu et al. 1991; Kaneko et al. 1996; Shinozaki et al. 1986; reviewed in Lysenko and Kuznetsov 2005). The structural relationship of the *E. coli* RNA polymerase and PEP was confirmed by reconstituting a functional *E.*

coli enzyme with polypeptides truncated as in PEP (Severinov et al. 1996). The high degree of conservation kept by PEP during evolution from the bacterial RNA polymerase is also demonstrated by its sensitivity to tagetitoxin (e.g. Mathews and Durbin 1990; Sakai et al. 1998). Other potent inhibitors of transcription in bacteria, rifampicin, and its related drugs, were also shown to inhibit transcription by the *E. coli*-like form of PEP found in etioplasts but not by the more complex form in chloroplasts (Fig. 1; e.g. Surzycki 1969; Loiseaux et al. 1975; Pfannschmidt and Link 1997). Furthermore, replacing the PEP α-subunit with the *E. coli* homologue in transplastomic tobacco resulted in a non-functional PEP enzyme, indicating that the evolutionary conservation of both α-subunits is insufficient to allow such an exchange (Suzuki and Maliga 2000).

The *rpoBC* operon is under control of a NEP promoter in monocotyledonous and dicotyledonous plants (see Section 3.1). Transcript levels of *rpo* genes are low compared with genes for proteins involved in photosynthesis (e.g. Hess et al. 1993; Legen et al. 2002). For promoter recognition, the core subunits have to be complemented by a sigma (σ) factor. Sigma factors are encoded by nuclear genes in all embryophytes (see Section 4.2.2) ensuring together with NEP a control of plastid transcription by the nucleus.

While NEP activity (demonstrated by recognition of NEP promoters) could be found hitherto only in soluble fractions of plastid lysates, PEP can be isolated from plastids as a 'soluble' (DNA-dependent) enzyme and in a 'insoluble' (DNA-associated) form together with DNA and other proteins of unknown function as the so-called 'transcriptionally active chromosome' (TAC; e.g. Briat et al. 1979; Greenberg et al. 1984; Little and Hallick 1988; Suck et al. 1996; Krause and Krupinska 2000; Pfalz et al. 2006). In the case of *Euglena*, the soluble RNA polymerase fraction and TAC were reported to transcribe different sets of genes. If this is due to the presence of different RNA polymerases, as discussed by Little and Hallick (1988), different transcriptions factors, or has other reasons is unclear yet (Smith and Purton 2002). The soluble PEP fraction contains different proteins and exhibits different sensitivity against rifampicin if prepared from etioplasts *vs.* chloroplasts. PEP isolated from etioplasts of mustard seedlings consists mainly of the core subunits (Pfannschmidt and Link 1997), whereas PEP preparations from chloroplasts were found to be more complex and contain additional proteins that might be needed for transcription and regulation of transcription under the conditions of light and active photosynthesis (Pfannschmidt and Link 1994, 1997; Link 1996; Baginsky et al. 1999; Pfannschmidt et al. 2000; Ogrzewalla et al. 2002) as discussed below.

3 Plastidial Promoters

3.1 NEP promoters

Unambiguous identification of transcription initiation sites for a nuclear-encoded transcription activity (i.e. NEP) became feasible in plants with reduced or elimi-

nated transcriptional activity by PEP. Such systems comprise the ribosome-deficient plastids of the monocot *albostrians* barley and *iojap* maize mutants (Hübschmann and Börner 1998; Silhavy and Maliga 1998a), tobacco Δ*rpo* plants (Allison et al. 1996; Hajdukiewicz et al. 1997; Serino and Maliga 1998), *Arabidopsis* lacking PEP due to the action of spectinomycin which blocks plastidial protein synthesis (Swiatecka-Hagenbruch et al. 2007), and photosynthetically inactive tobacco and rice suspension cultures, with elevated levels of NEP activity (Vera et al. 1996; Kapoor et al. 1997; Miyagi et al. 1998; Silhavy and Maliga 1998b).

Most non-photosynthetic genes involved in housekeeping functions such as transcription and translation have promoters for both RNA polymerases NEP and PEP. NEP transcripts of these genes are, with a few exceptions, rarely detectable in chloroplasts and were therefore mostly analyzed in PEP-deficient plants (see above). Only a few genes are known to be transcribed exclusively from a NEP promoter, i.e. *accD,* encoding a subunit of the acetyl-CoA carboxylase in dicots; *ycf2*, encoding a protein with a yet unknown function; *rpl23*, encoding a ribosomal protein; *clpP*, encoding the proteolytic subunit of the Clp ATP-dependent protease, in monocots; and, most interestingly, the *rpoB* operon encoding three of the four PEP core subunits in all higher plants (Hajdukiewicz et al. 1997; Hübschmann and Börner 1998; Silhavy and Maliga 1998a; Swiatecka-Hagenbruch et al. 2007). Consequently, PEP abundance and activity depends on the nuclear-encoded RNA polymerase.

NEP promoters analyzed thus far resemble mitochondrial and phage promoters in their structural organization. Based on their sequence properties they can be grouped into three types (Fig. 4; Weihe and Börner 1999; Liere and Maliga 2001). Type-I promoters are characterized by a conserved YRTa-motif critical for *rpoB* promoter recognition embedded in a small DNA fragment (-15 to +5) upstream of the transcription initiation site (+1) (P*atpB*-289; Kapoor and Sugiura 1999; Xie and Allison 2002; P*accD*-129; Liere and Maliga 1999b; P*rpoB*-345; Liere and Maliga 1999a). Transient expression of chimeric *Arabidopsis rpoB* 5'-flanking region::GUS deletion-constructs in cultured tobacco cells suggested upstream regulatory regions for *rpoB* expression (Inada et al. 1997). However, no additional sequence elements outside the promoter core altered *rpoB* transcription *in vitro* (Liere and Maliga 1999a). Similar transient transcription assays to examine the 5'-flanking region of the tobacco *accD* gene revealed putative sequence elements up- and downstream of the promoter to determine its strength (Hirata et al. 2004). A subset of Type-I NEP promoters possesses a second conserved sequence motif ($ATAN_{0\text{-}1}GAA$) ~18 to 20 bp upstream of the YRTa-motif, designated box II or GAA-box (Fig. 4; Silhavy and Maliga 1998a; Kapoor and Sugiura 1999). Mutational analyses of the tobacco P*atpB*-289 promoter in *in vitro* and *in vivo* transcription experiments suggested a functional role of this element in promoter recognition (Kapoor and Sugiura 1999; Xie and Allison 2002). Hence, Type-I promoters are grouped into two subgroups: Ia, with only the YRTa-motif, and Ib, carrying both YRTa- and GAA-box (Weihe and Börner 1999; Liere and Börner 2007).

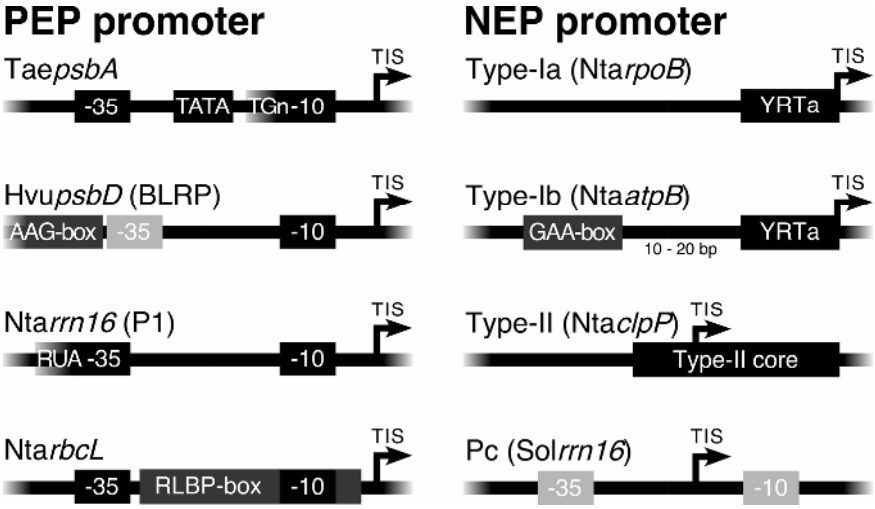

Fig. 4. Schematic overview of different types of PEP and NEP promoters. PEP promoter: the wheat *psbA* (Tae*psbA*), barley *psbD* BLRP (Hvu*psbD*), tobacco *rrn16* (Nta*rrn16*), and the tobacco *rbcL* PEP promoters (Nta*rbcL*) are shown. Conserved -10/-35 consensus elements, as well as individual promoter elements as the TATA-box (Eisermann et al. 1990), extended -10 sequence (TGn; Satoh et al. 1999), AAG-box (Kim et al. 1999), RUA-element (Suzuki et al. 2003), and RLBP-binding region (Kim et al. 2002) are indicated. The less conserved -35 element in the barley *psbD* BRLP is shown in grey. NEP promoter: typical architectures of Type-I, Type-II, and Pc NEP promoters from tobacco and spinach are shown with their names in brackets. The YRTa promoter core and GAA-box are marked (Hübschmann and Börner 1998; Kapoor and Sugiura 1999; Liere and Maliga 1999). TIS: transcription initiation site, indicated by arrows. The -35 and -10 elements not used in spinach *rrn16* promoter recognition are shown in grey.

Type-II NEP promoters lack the YRTa-motif and differ completely in sequence and organization from Type-I promoters. So far this class is represented by a single example, a promoter of the ClpP protease subunit gene (Fig. 4). The tobacco P*clpP*-53 was characterized using a transplastomic *in vivo* approach demonstrating that critical promoter sequences are located mainly downstream of the transcription initiation site (–5 to +25; Sriraman et al. 1998a). The *clpP*-53 promoter motif and transcription initiation site are conserved among monocots, dicots, conifers, and liverworts. But, although present, the tobacco P*clpP*-53 sequence motif is not used as a promoter in rice and *Chlamydomonas*. If the rice sequence is introduced into tobacco plastids, the tobacco NEP recognizes this conserved Type-II promoter. Therefore, the lack of transcription in rice from the P*clpP*-53 homologue may be resulting from either the lack of a Type-II specificity factor or to the lack of a distinct NEP enzyme not present in monocots (e.g. RpoTmp, see Chapter 1.1; Sriraman et al. 1998a; Liere et al. 2004). However, experimental data supporting one or the other of these scenarios are still missing.

Another non-YRTa-type promoter, which is attributed to be recognized by a NEP transcription activity is the *rrn* operon Pc promoter in spinach and *Arabidop-*

sis (Fig. 4, 6; Pc promoter; Baeza et al. 1991; Iratni et al. 1994, 1997; Sriraman et al. 1998a; Swiatecka-Hagenbruch et al. 2007). In spinach, the Pc promoter solely drives *rrn* operon transcription. Although it contains typical σ^{70}-elements which are active as the *rrn* operon promoter in other species, transcription initiates from a site between the conserved –10/–35 hexamers. However, sequences relevant for transcription initiation from Pc have yet to be identified.

3.2 PEP promoters

Having coevolved with the bacterial-type RNA polymerase (PEP), many plastidial promoters contain a variant of the -35 (TTGaca) and -10 (TAtaaT) consensus sequences of typical σ^{70}-type *E. coli* promoters (Reznikov et al. 1985; for reviews see Gruissem and Tonkyn 1993; Link 1994; Hess and Börner 1999; Liere and Maliga 2001; Weihe 2004). In fact, the *E. coli* RNA polymerase is able to accurately recognize plastidial σ^{70}-type promoters (e.g. Gatenby et al. 1981; Bradley and Gatenby 1985; Boyer and Mullet 1988; Eisermann et al. 1990). Since plastidial σ^{70}-type promoters are recognized by PEP, they are also often termed PEP promoters. In addition to the core motifs, some PEP promoters contain regulatory *cis*-elements. One of the best-characterized PEP promoters ensures transcription of the *psbA* gene, which encodes the D1 photosystem II reaction center polypeptide (Link 1984; Gruissem and Zurawski 1985; Boyer and Mullet 1986, 1988). *In vivo psbA* transcription is developmentally timed and activated by light (Klein and Mullet 1990; Schrubar et al. 1990; Baumgartner et al. 1993). *In vitro* characterization of the mustard *psbA* promoter identified a TATATA promoter element between the –10 and –35 hexamers resembling the TATA-box of nuclear genes transcribed by RNA polymerase II (Fig. 4; Eisermann et al. 1990; Link 1994). Basic transcription levels in plastidial extracts prepared from both dark and light grown plants were obtained *in vitro* with both the TATATA element together with the -10 region. Nonetheless, presence of the -35 element was essential for enhanced transcription rates characteristic of chloroplasts of light-grown plants (Link 1984; Eisermann et al. 1990). In barley, the *psbA* promoter also contains the TATA-motif between the -35/-10-elements. But, unlike in mustard, the –35 sequence is absolutely required for transcription *in vitro* (Kim et al. 1999b). Similarly, such TATA-box is also present in the wheat *psbA* promoter, but does not seem to be important. Light-independent (constitutive) transcription by PEP isolated from the leaf base (base-type PEP; young plastids) required both the –10 and –35 elements for promoter activity. However, PEP isolated from the leaf tip (tip-type PEP; mature plastids) employed only the –10 region with an additional TGn motif upstream of the –10 element (Fig. 4; extended –10 sequence; Bown et al. 1997; Satoh et al. 1999). The extended –10 sequence may be involved in promoter recognition by the tip-type PEP in mature plastids indicating that basal- and tip-type PEPs may differ by their associated transcription factors (Satoh et al. 1999). Since the mustard, barley and wheat *psbA* promoter sequences are highly conserved, differences in the utilization of *cis*-elements possibly are the result of a divergent evolution of *trans*-factors in these species.

Interestingly, it seems that most plastidial promoters in *Chlamydomonas* do not possess a valid -35 element, but rather a downstream extended -10 box (Klein et al. 1992). Furthermore, even remote sequences such as the coding regions are needed for full promoter strength of the *rbcL* and *psbA* but not *psbD*, *atpA*, and *atpB* genes (Blowers et al. 1990; Klein et al. 1994; Ishikura et al. 1999; Kasai et al. 2003). However, the mechanism of transcriptional enhancement by these *cis*-acting elements within the coding regions is not yet examined and might be unique for *Chlamydomonas* (Shiina et al. 1998; Kasai et al. 2003). Further regulatory sequences in addition to the core promoter regions were identified in the proximity of the *psbD-psbC* and *rbcL* promoters in higher plants (Fig. 4; see Section 4.1 for details).

3.3 Internal promoters of tRNAs

Most plastidial tRNAs are transcribed by the PEP from upstream σ^{70}-type promoters. However, transcription from internal promoters is assumed for some tRNA genes such as the spinach *trnS*, *trnR*, and *trnT* (Gruissem et al. 1986; Cheng et al. 1997b) as well as *trnS*, *trnH*, and *trnR* from mustard (Neuhaus and Link 1990; Nickelsen and Link 1990; Liere and Link 1994), and the *Chlamydomonas trnE* gene (Jahn 1992). Transcription of the spinach *trnS* gene is initiated twelve nucleotides upstream of the mature tRNA coding region (Wu et al. 1997). *In vitro* assays demonstrated that the coding region (+1/+93) promoted basal levels (8%) of transcription. Inclusion of an AT-rich sequence stretch between -31 and -11 upstream of the coding region restored wild type promoter strength. However, no sequences resembling either NEP or PEP promoters were found in this region. As most tRNAs, the *trnS* coding region contains sequences resembling the A and B blocks of nuclear tRNA promoters transcribed by the eukaryotic RNA polymerase III (Galli et al. 1981; Geiduschek et al. 1995). The $tRNA^{Arg}$(ACG) gene from *Pelargonium zonale* was efficiently transcribed in *Xenopus* oocyte nuclei (Hellmund et al. 1984), suggesting that the plastidial tRNAs may be transcribed by an RNA polymerase III-type enzyme. The biochemical properties and enzyme composition of such a transcription activity, however, remain to be determined. Thus far, *in silico* analyses of the *Arabidopsis* genome did not reveal a plastid-targeted polymerase of this type (Liere and Börner, unpublished). Alternatively, such tRNAs may be transcribed by specialized NEP or PEP enzymes associated with distinct transcription factors recognizing internal promoter structures.

4 Regulation of transcription in plastids

Expression of nuclear-encoded plastid-localized gene products is thought to be managed by transcriptional control (Kuhlemeier 1992). While posttranscriptional events contribute significantly to regulation of plastidial gene expression (see Chapters 6, 7; Deng and Gruissem 1987; Stern et al. 1997; Barkan and Gold-

schmidt-Clermont 2000; Monde et al. 2000), transcription of plastid genes was also shown to react to exogenous and endogenous factors such as light and plastid type (Rapp et al. 1992; Mullet 1993; Mayfield et al. 1995; Link 1996).

The circadian rhythm of plastidial gene expression in *Chlamydomonas* is regulated by transcriptional activity (Salvador et al. 1993; Hwang et al. 1996). Kawazoe et al. (2000) could show that the circadian clock-induced transcription is sensitive to cycloheximide, an inhibitor of cytoplasmic translation. However, basal plastidial transcription activity was still maintained. The identity of the cycloheximide-sensitive factor(s) needed for circadian peaks of plastidial transcription is still unknown. Expression of the sole σ-factor gene *CreRpoD* (Section 3.2.2; Carter et al. 2004; Bohne et al. 2006) seems also to be under circadian control (Carter et al. 2004). Therefore, a possible dual role of CreRpoD, which might be assisted by topological fluctuations of the plastome (Thompson and Mosig 1990; Salvador et al. 1998), in regulating plastidial gene transcription in *Chlamydomonas* has been discussed (Misquitta and Herrin 2005).

Transcription activities of most plastid-encoded genes in higher plants increase at an early stage of light-induced plastid development to support rapid construction of the photosynthesis apparatus. Moreover, light-dependent plastid transcription occurs in mature leaves as well as leaves under greening (Greenberg et al. 1989; Schrubar et al. 1990; Baumgartner et al. 1993; DuBell and Mullet 1995; Hoffer and Christopher 1997; Shiina et al. 1998; Satoh et al. 1999; Baena-Gonzalez et al. 2001; Chun et al. 2001; Nakamura et al. 2003). Most prominent examples are photosynthesis-related genes as *psbA*, *psbD-psbC*, *petG*, *rbcL*, but also housekeeping genes as *atpB* (Klein et al. 1988; Haley and Bogorad 1990; Klein and Mullet 1990; Sexton et al. 1990; Isono et al. 1997a). Distinctive photoreceptors involved in transcriptional activation of photosynthesis-related genes have been analyzed (Chun et al. 2001; Thum et al. 2001). The developmental stage may influence perception of the light quality. While red light only partially increased plastid transcription, blue light further enhanced overall plastid transcription activity in dark-adapted mature leaves. Therefore, global activation of plastidial transcription after dark adaptation is likely to be mediated by cryptochromes. When exposed to blue light/UV-A an *Arabidopsis phyA*-mutant displayed lower *psbA* and *rrn16* transcript activities than the wild type suggesting a further role for PhyA in light reception (Chun et al. 2001). Recently, Dhingra et al. (2006) furthermore showed that green light plays a balancing/antagonistic role in controlling gene expression during early photomorphogenic development by downregulating plastidial transcription of genes normally induced by light. As illustrated before, transcriptional response to developmental and environmental changes is likely to involve interaction of the core RNA polymerase with specific regulatory molecules (e.g. σ-factors), which may be available only under certain conditions. *In silico* analyses of nuclear *Arabidopsis* and rice genes with putative chloroplast transit peptides revealed many putative transcription factors likely to be imported into plastids (Wagner and Pfannschmidt 2006; Schwacke et al. 2007).

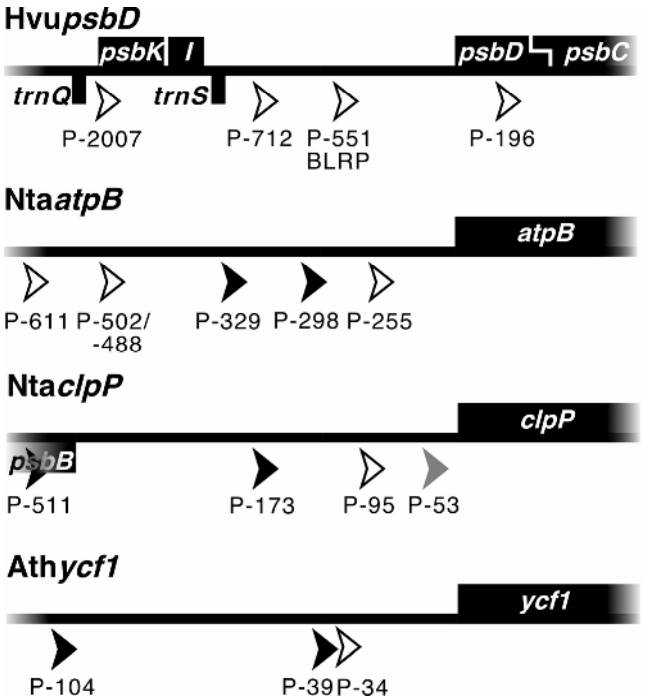

Fig. 5. Genes with multiple promoters. Schematic synopsis which shows the multiple PEP promoters of the barley *psbD/C* operon (Hvu*psbD*), as well as PEP and NEP promoters of the tobacco *atpB* (Nta*atpB*), *clpP* (Nta*clpP*), and *Arabidopsis ycf1* genes (Ath*ycf1*). Boxes below the line represent genes on the opposite strand, while open arrowheads denote PEP promoters, filled black arrowheads Type-I NEP promoters, and filled gray arrowheads Type-II NEP promoters. The promoters are named based on their transcription initiation sites in respect to the translation initiation site (+1).

These factors may represent such candidates to expand the actually known capacity of the chloroplast to regulate its transcription machinery. Additionally, various pathways routing developmental and environmental cues may regulate these factors.

4.1 Role of multiple and diverse promoters

Although genes exist that are transcribed from a single promoter, transcription of plastidial genes and operons by multiple promoters seems to be a common feature. For example, the *psbD-psbC* operon is transcribed from up to three different PEP promoters (Fig. 5; Yao et al. 1989; Berends Sexton et al. 1990; Christopher et al. 1992; Wada et al. 1994; To et al. 1996; Hoffer and Christopher 1997) and the tobacco *rpl32* gene from two promoters far upstream of the coding region (NEP-P*rpl32*-1101, PEP-P*rpl32*-1030; Vera et al. 1996). Similarly, the tobacco *atpB*

Table 1. Diversity of promoter usage in different species.

Gene	Arabidopsis	tobacco	maize	barley
accD	NEP: P*accD*-251; P*accD*-172	NEP: P*accD*-129	n.d.	n.d.
atpB	PEP: P*atpB*-520 NEP: P*atpB*-318	PEP: P*atpB*-611; P*atpB*-502; P*atpB*-255 NEP: P*atpB*-329; P*atpB*-289	PEP: P*atpB*-298 NEP: P*atpB*-601	PEP: n.d. NEP: P*atpB*-593
atpI	PEP: P*atpI*-229	PEP: P*atpI*-130 NEP: P*atpI*-207	n.d.	n.d.
clpP	PEP: P*clpP*-115 NEP II: P*clpP*-57	PEP: P*clpP*-95 NEP: P*clpP*-511; P*clpP*-173 NEP II: P*clpP*-53	NEP: P*clpP*-111	NEP: P*clpP*-133
psaA	PEP: P*psaA*-188	PEP: P*psaA*-194	PEP: P*psaA*-175	n.d.
psbA	PEP: P*psbA*-77	PEP: P*psbA*-85	PEP: P*psbA*-86	PEP: P*psbA*-80
rpoB	NEP: P*rpoB*-300	NEP: P*rpoB*-345	NEP: P*rpoB*-147	NEP: P*rpoB*-147
rrn16	PEP: P*rrn16*-112 NEP Pc: P*rrn16*-139	PEP: P*rrn16*-114 NEP: P*rrn16*-62	PEP: P*rrn16*-117	PEP: P*rrn16*-118
ycf1	PEP: P*ycf1*-34 NEP: P*ycf1*-39; P*ycf1*-104	NEP: P*ycf1*-41	n.d.	n.d.

PEP denotes PEP promoters, NEP represents NEP Type-I promoters, NEP II indicates NEP Type-II promoters, and NEP Pc denotes Pc promoters; n.d. indicates not yet identified promoters.

gene is transcribed from at least three NEP (P*atpB*-255, -502/-488, -611) and two PEP promoters (Fig. 5; P*atpB*-289, -329; Hajdukiewicz et al. 1997), but only one PEP and one NEP promoter are driving this gene in *Arabidopsis* (PEP-P*atpB*-520, NEP-P*atpB*-318; Swiatecka-Hagenbruch et al. 2007) and maize (NEP-P*atpB*-601, PEP-P*atpB*-298; Silhavy and Maliga 1998a). In case of *clpP*, the tobacco gene has two Type-I NEP (P*clpP*-173, -511), one PEP (P*clpP*-95) and the main Type-II NEP initiation sites (Fig. 5; P*clpP*-53; Hajdukiewicz et al. 1997; Sriraman et al. 1998a). The *Arabidopsis clpP* gene has a PEP (P*clpP*-115) and a Type-II NEP initiation site (P*clpP*-58; Sriraman et al. 1998a; Swiatecka-Hagenbruch et al. 2007). The maize gene, however, is transcribed from a sole Type-I NEP promoter (P*clpP*-111; Silhavy and Maliga 1998a) indicating a high diversity in promoter usage in different species (see Table 1 for a comparison of promoters of more plastidial genes in different plants). Furthermore, an increasing number of genes are reported to be co-transcribed with other genes within an operon and to additionally possess an individual promoter upstream of their coding region (e.g. *trnG* and *psbA*; Meng et al. 1991; Nickelsen and Link 1991; Kapoor et al. 1994; Liere and Link 1994; Liere et al. 1995).

The *rrn16* promoters are an interesting and well investigated example of the diversity of promoter usage within a highly conserved DNA sequence even in closely related species. The main *rrn* operon promoter in tobacco is a σ^{70}-type PEP promoter (P1 or Nt-P*rrn*-114; Vera and Sugiura 1995; Allison et al. 1996). In barley, maize, and pea the *rrn* operon is also transcribed from the P1 σ^{70}-type PEP promoter (Fig. 6; Strittmatter et al. 1985; Sun et al. 1989; Hübschmann and Börner 1998). Additionally, the *rrn* operon in tobacco has a NEP promoter (Fig. 6; P2 or Nta-P*rrn*-62), inactive in chloroplasts, but functional in BY2 tissue culture cells and in plants lacking PEP (Vera and Sugiura 1995; Allison et al. 1996). Conversely, there is no active NEP promoter directly upstream of the *rrn* operon in maize plastids (Silhavy and Maliga 1998a). In spinach chloroplasts transcription of the *rrn* operon initiates within a region between the promoter elements of P1 (Fig. 4, 6; Pc promoter; Baeza et al. 1991; Iratni et al. 1994, 1997). However, the σ^{70}-type promoter sequences are not utilized *in vivo*. Interestingly, the Pc site appears to be faithfully recognized by partially purified mustard PEP *in vitro* (Pfannschmidt and Link 1997). A good candidate for the Pc activating factor in spinach is CDF2 (see Section 4.2.1; Iratni et al. 1994, 1997; Bligny et al. 2000).

In *Arabidopsis*, *rrn* operon transcripts were mapped to both the major PEP P1 and the spinach Pc initiation sites (Fig. 6; Sriraman et al. 1998b; Swiatecka-Hagenbruch et al. 2007). A study of *rrn* promoters in heterologous plastids indicates that tobacco plastids lack the factor required for transcription from Pc, while spinach has an intact P1 promoter but lacks the cognate P1 activator (Sriraman et al. 1998b). However, in tobacco an rRNA operon upstream activator region (RUA) that is conserved in monocot and dicot species has been identified (Fig. 4; Suzuki et al. 2003). It has been suggested that the -10 element plays only a limited role in *rrn16* P1 recognition and that σ-factor interaction is replaced in part by direct PEP-RUA (protein-DNA) interaction or by protein-protein interaction between the PEP and a putative RUA-binding transcription factor.

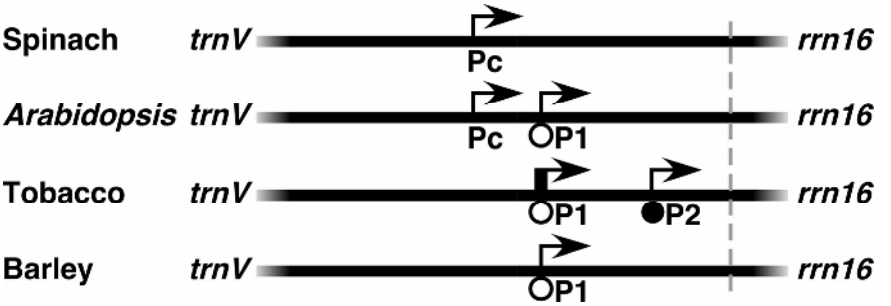

Fig. 6. Diverse promoters of the *rrn* operon in spinach, *Arabidopsis*, tobacco, and barley. The distinct promoters that are used in different species are shown by the schematic representation of the transcription initiation sites between *trnV* and *rrn16* (marked by arrows). P1 and P2 mark transcription initiation sites by PEP (open circle) and NEP (filled circle). Transcript initiation in spinach and *Arabidopsis* from a yet uncharacterized NEP promoter is indicated by Pc. A dashed vertical line indicates an RNA processing site.

Interestingly, *ycf1* in *Arabidopsis* is transcribed from a strongly conserved NEP promoter as in tobacco (NEP-AthP*ycf1*-39; Swiatecka-Hagenbruch et al. 2007). However, a PEP promoter located at the NEP promoter position takes over transcription in green leaves (PEP-AthP*ycf1*-34). With the *rrn16* Pc and PEP promoters in *Arabidopsis*, this is a rare incident where a defined DNA sequence serves as a promoter for both NEP and PEP.

The role of most multiple promoters upstream of plastidial genes and operons is not fully understood, however, some are well characterized. The blue-light-responsive promoter (BRLP) amongst the three PEP promoters of the *psbD-psbC* operon, for example, is thought to differentially maintain the ability to re-synthesize and replace damaged D2 and CP43 photosystem components in mature chloroplasts.

In spite of the observed diversity of plastidial promoter usage between different species of higher plants, the data support also the existence of common themes in promoter usage that have been deduced mainly from studies on transcription in tobacco plastids. Mixed NEP and PEP promoters typically are found upstream of housekeeping genes which need to be transcribed during full plastidial development (Maliga 1998). Consequently, both promoter types are believed to differentially express their cognate gene during plant development (reviewed in Liere and Maliga 2001). NEP promoters are generally recognized in youngest and non-green tissues early in plant development, while PEP takes over in maturating, photosynthetically active chloroplasts (Bisanz-Seyer et al. 1989; Baumgartner et al. 1993; Hajdukiewicz et al. 1997; Kapoor et al. 1997; Emanuel et al. 2004).

This simple model has been challenged by results from transcriptional re-analyses of tobacco Δ*rpo* mutants lacking PEP (Krause et al. 2000; Legen et al. 2002). Large spurious transcripts initiated by NEP cover the entire plastome in these mutants, suggesting that besides selective promoter utilization, posttranscriptional processes also determine the transcript pattern of plastids. Furthermore, it has been shown in maize, that although the NEP enzyme becomes less abundant

as chloroplasts mature its transcriptional activity increases (Cahoon et al. 2004). The stability of the RNA generated by NEP, however, declines during chloroplast development. For transcripts generated by PEP, transcription rates increase as chloroplasts develop, whereas RNA stability remains constant or increases. Hence, in a proposed model for maize plastidial biogenesis, NEP-controlled transcript accumulation changes little during plastidial development while PEP-controlled transcript accumulation increases (Cahoon et al. 2004). In other species, a strong correlation between the transcribing enzyme (NEP or PEP) and the pattern of transcript accumulation was not observed (Zoschke et al. 2007).

Since genes exclusively transcribed by NEP encode housekeeping functions like the *rpoB* gene/operon and *rps15*, NEP should be still necessary for proper gene expression and regulation also in mature chloroplasts. Furthermore, an additional role of NEP as an SOS-enzyme in plastidial transcription has been proposed by Schweer et al. (2006). Analyses of transcript accumulation of *atpB* in an AthSig6 knockout mutant suggested that a further upstream located NEP promoter compensates for failing transcription from the main PEP promoter. Indeed, NEP and PEP are active throughout leaf development in *Arabidopsis*, although PEP plays a major role in mature leaves (Cahoon et al. 2004; Zoschke et al. 2007). Interestingly, exclusively PEP-transcribed genes code for proteins with a role in photosynthesis. As the major active polymerase in mature chloroplasts, present data point to PEP as a prominent target for regulation signals including redox control, not yet determined for NEP (for review see Forsberg et al. 2001; Liere and Maliga 2001; Pfannschmidt and Liere 2005). Since plants that turn to a parasitic lifestyle lost photosynthetic genes as well as PEP promoters (Wolfe et al. 1992a; Wolfe et al. 1992b; Krause et al. 2003; Berg et al. 2004), transcription and regulation of plastidial gene expression by PEP might be connected to photosynthesis.

4.2 Transcription factors involved in promoter recognition in plastids

4.2.1 NEP transcription factors

Recent *in vitro* studies of the yeast mitochondrial transcription machinery unexpectedly revealed promoter specificity to be conferred by the core RNAP rather than mtTFB (Matsunaga and Jaehning 2004). Similarly, *in vitro* transcription assays with recombinant AthRpoTm and AthRpoTp enzymes showed accurate initiation of transcription from overlapping subsets of mitochondrial and plastidial promoters without auxiliary factors, therefore retaining a characteristic feature of the T7 RNAP. However, AthRpoTm and AthRpoTp failed to recognize some of the investigated promoters and AthRpoTmp displayed no significant promoter specificity while showing high non-specific transcription activity. Therefore, it is evident that the *Arabidopsis* enzymes need auxiliary factors for transcription *in organello* like the mitochondrial RNA polymerases of other organisms (Kühn et al. 2007).

Thus far, identification of factors involved in specific promoter recognition and transcription initiation by NEP has failed. Based on information on such factors

interacting with the related mitochondrial phage-type RNA polymerases from humans, mice, *Xenopus laevis*, and yeast one can only speculate. These mitochondrial transcription complexes consist of a minimum of two components: the catalytic core enzyme (mtRPO, ~ 120–150 kDa), and a specificity factor, which confers promoter recognition (mtTFB, ~ 40–45 kDa). Despite poor overall sequence similarity, it recently has been shown that mtTFB factors belong to a family of RNA-methyltransferases (Falkenberg et al. 2002; McCulloch et al. 2002; Rantanen et al. 2003; Seidel-Rogol et al. 2003). An additional component, which binds the DNA further upstream, enhances mitochondrial transcription *in vitro* (mtTFA, 20–25 kDa). This DNA-binding protein belongs to the HMG (high mobility group) family and may also facilitate the interaction with other trans-acting factors (reviewed in Jaehning 1993; Shadel and Clayton 1993; Tracy and Stern 1995; Hess and Börner 1999). To date, no functional mtTFA or mtTFB homologues have been isolated from plant mitochondria or plastids, and the presence of such proteins in plant organelles is unclear. BLAST searches of the *Arabidopsis* genome revealed a TFB-like dimethyladenosine transferase gene, which possesses an N-terminal transit peptide mediating protein import into plastids of isolated tobacco protoplasts (B. Kuhla, K. Liere, T. Börner; unpublished data). This gene corresponds to the previously characterized *PFC1* gene encoding a plastid 16S rRNA dimethylase homologous to the yeast nucleolar 18S rRNA dimethylase Dim1 (Tokuhisa et al. 1998). The phenotype of *PFC1*-knockout mutants, however, does not support the idea that this TFB-like dimethyladenosine transferase may act as a primary transcription factor for the phage-type RNA polymerases (M. Swiatecka-Hagenbruch, K. Liere, T. Börner, unpublished data).

A good candidate for an activating factor for NEP transcription in spinach is CDF2, which has been reported to stimulate transcription of the *rrn* operon Pc promoter by NEP-2, a yet to be characterized nuclear-encoded transcription activity (Table 3; Bligny et al. 2000). CDF2 is supposed to exist in two distinct forms, CDF2-A and CDF2-B. CDF2-A might repress transcription initiation of PEP at the *rrn16* P1 promoter (termed P2 in spinach), while CDF2-B possibly binds NEP-2 and initiates specific transcription from the *rrn16* Pc promoter.

Another factor that is discussed to be involved in NEP transcription is the plastidial ribosomal protein L4 (RPL4; encoded by the nuclear *Rpl4* gene). A role for RPL4 in NEP transcription was proposed, as it co-purifies with the T7-like transcription complex in spinach (Trifa et al. 1998). In prokaryotes the ribosomal protein L4 was shown to have extra-ribosomal functions in transcriptional regulation (Zengel et al. 1980). The spinach and *Arabidopsis Rpl4* genes have acquired remarkable 3' extensions during evolutionary transfer to the nuclear genome, which resemble highly acidic C-terminal ends of certain transcription factors. A function for this protein in NEP or PEP transcription, however, has yet to be demonstrated.

Besides, some nucleus-encoded σ-factors for the bacterial-type PEP in plastids were found to additionally localize to mitochondria (see Section 4.2.2). So far phage-type RNA polymerases are the sole transcription activity in mitochondria of higher plants. One may speculate that these σ-factors may have an additional role in regulating mitochondrial transcription by these RNA polymerases (H. Tandara and K. Liere, unpublished data; Beardslee et al. 2002; Yao et al. 2003). Yet, ex-

perimental data to link the activity of the bacterial-type plastidial σ-factors to the phage-type enzymes in mitochondria or plastids are still lacking.

4.2.2 Nuclear-encoded plastidial σ-factors

Specific transcription initiation in bacteria requires a transcription factor (σ), which is responsible for promoter recognition and contributes to DNA melting around the initiation site. Most bacterial genomes contain genes for several σ-factors recognizing distinct promoters. Bacterial σ-factors possess conserved functional regions and are grouped into two families, σ^{70} and σ^{54} (Wösten 1998; Ishihama 2000). The σ^{70}-factors are furthermore categorized into primary (group 1, essential for cell growth), non-essential primary (group 2), and alternative σ-factors (group 3), responsible for recognition of certain promoters in response to environmental signals (Lonetto et al. 1992; Gruber and Bryant 1997). Cyanobacteria, the ancestors of plastids, have also multiple σ-factors with distinct promoter specificity (Kaneko et al. 1996).

Early on, biochemically purified σ-like activities in plant plastids were reported in *Chlamydomonas* (Surzycki and Shellenbarger 1976), spinach (Lerbs et al. 1983), and mustard (Bülow and Link 1988; Tiller and Link 1993b). Furthermore, immunological evidence for σ-like factors was obtained in chloroplast RNA polymerase preparations of maize, rice, *Chlamydomonas reinhardtii*, and *Cyanidium caldarium* (Troxler et al. 1994). Moreover, multiple nuclear-encoded genes encoding bacterial σ^{70}-type factors were identified in the red algae *Cyanidium caldarium* (*CcaA-C*; Liu and Troxler 1996; Tanaka et al. 1996) and *Cyandioschyzon merolae* (*CmeSig1-4*; Matsuzaki et al. 2004) suggesting specialized promoter recognition as in bacteria. Correspondingly, σ-factor families were identified in genomes of land plants such as *Arabidopsis* (*AthSig1-6*; Isono et al. 1997b; Tanaka et al. 1997; Fujiwara et al. 2000; Hakimi et al. 2000), mustard (*SalSig1-3*; Kestermann et al. 1998; Homann and Link 2003), tobacco (*NtaSigA1*, *-A2*; Oikawa et al. 2000), rice (*OsaSig1-4*; Tozawa et al. 1998; Kasai et al. 2004), maize (*ZmaSig1-5*; Lahiri et al. 1999; Tan and Troxler 1999; Lahiri and Allison 2000), *Physcomitrella patens* (*PpaSig1, -2, -5*; Hara et al. 2001a, 2001b; Ichikawa et al. 2004), as well as wheat (*TaeSigA*; Morikawa et al. 1999), and *Sorghum* (*SbiSig1*; Kroll et al. 1999). Interestingly, the genome of the unicellular green algae *Chlamydomonas reinhardtii* harbors only a single gene encoding a σ-factor (*CreRpoD*; Carter et al. 2004; Bohne et al. 2006). The N-termini of these σ-factors show sequences typical for plastid-targeting transit peptides and indeed have been demonstrated to confer plastidial targeting either of GFP-fusion proteins *in vivo* (Isono et al. 1997b; Tanaka et al. 1997; Kanamaru et al. 1999; Fujiwara et al. 2000; Lahiri and Allison 2000; Oikawa et al. 2000; Hara et al. 2001a) or with radio-labeled proteins *in vitro* (Kestermann et al. 1998). Surprisingly, targeting of some plant σ-factors occurred not only into plastids but also into mitochondria. Alternative splicing of *AthSig5* transcripts within intron 1 establishes two initiation methionines (M1 and M2). Shorter peptides starting with M2 showed exclusive GFP targeting into plastids. However, GFP fusion proteins starting with M1 were localized to mitochondria.

RNA analyses revealed that the longer (plastidial) *AthSig5* transcripts are exclusively located in flowers, whereas the shorter (mitochondrial) transcripts were detectable in both flower and leaf tissue (Yao et al. 2003). Furthermore, AthSig1::GFP fusion proteins as well are co-localized to both plastids and mitochondria in tobacco protoplast import assays (H. Tandara and K. Liere, unpublished data). Similarly, dual targeting was shown for the maize ZmaSig2B protein by immunological and GFP-fusion protein import studies. Interestingly, ZmaSig2B was biochemically co-purified with RpoTm, the mitochondrial phage-type RNA polymerase (Beardslee et al. 2002), suggesting a possible role of these mitochondrial localized σ-factors in regulation of plant mitochondrial transcription.

Historically, plastidial σ-factors were designated either alphabetically or by numbers. Thus, in *Arabidopsis* SigA, SigB, and SigC (Tanaka et al. 1997) were also named SIG2, SIG1, and SIG3 (Isono et al. 1997b), respectively. In an effort to unify the nomenclature, σ-factors sequences were subjected to phylogenetic analyses and distinguished by numbers (http://sfns.u-shizuoka-ken.ac.jp/pctech; Shiina et al. 2005). Higher plant σ-factors belong into a monophyletic group (Lysenko 2006). They are related to bacterial primary (group 1) and non-essential primary (group 2) σ^{70}-factors. However, none fit into alternative group 3 nor are related to σ^{54}-factors. Phylogenetic analyses revealed that plastidial σ-factors are split into at least 5 subgroups: Sig1, Sig2, Sig3, Sig5, and Sig6. Interestingly, the monocot and dicot σ-factors within the Sig1 and Sig2 groups are located on separate branches. Most sequenced higher plant and moss genomes contain at least one gene for a Sig1-type σ-factor. Since the *Arabidopsis Sig1* homologues are highly expressed during chloroplast biogenesis, it is assumed that Sig1 represents the principal σ-factor in chloroplasts (Tanaka et al. 1997; Kestermann et al. 1998; Tozawa et al. 1998; Kanamaru et al. 1999; Morikawa et al. 1999). Similarly, *Sig2*, *Sig3*, and *Sig5* genes have been identified in various plant organisms, suggesting a correspondingly important role in plastidial transcription. Conversely, to date *AthSig4* is the only *Arabidopsis Sig* gene without known ortholog in other plants, and in comparison to the other σ-factors its transcription is rather low in light-grown plants (Tsunoyama et al. 2002). Supported by the observation that intron sites of *AthSig1*, *AthSig2*, *AthSig3*, *AthSig4*, and *AthSig6* are almost identical (Fujiwara et al. 2000), phylogenetic analysis suggests that the Sig3, AthSig4, and Sig6 groups are related to Sig2 (Shiina et al. 2005; Lysenko 2006). Although closely related, the Sig1 and Sig2 groups possess different number of introns. These σ-factors, therefore, may originate from gene duplication events of one or more ancestral genes. Albeit only partially, the Sig5 group seems to be phylogenetically related to the bacterial alternative σ-factors (Tsunoyama et al. 2002; Shiina et al. 2005; Lysenko 2006). *AtSig4* is suggested to have originated from partly processed transcript of *AthSig2*, *AthSig3*, or *AthSig6* inserted as cDNA into the genome, since it is the only *Sig* gene in higher plants that has lost an intron (Lysenko 2006).

Bacterial σ^{70}-factors contain three conserved domains involved in binding the core RNA polymerase (domains 2.1 and 3), hydrophobic core formation (2.2), DNA melting (2.3), recognition of the -10 promoter motif (2.4), and recognition of the -35 promoter motif (Section 4.1, 4.2; Wösten 1998; Paget and Helmann 2003).

Since these domains are as well present in all known plastidial σ-factors it is to be expected that they are responsible for transcription from σ^{70}-type promoters in plastids. However, structural analysis seems not to provide answers if the role of the different plastidial σ-factors is to selectively activate promoters and if they possess distinct or overlapping promoter specificities. Based on the phylogenetic analyses one might presume that plastidial σ-factors group into general σ-factors involved in transcription of standard σ^{70}-type promoters and specialized σ-factors responsible for recognition of exceptional promoters in response to developmental and/or environmental cues (Shiina et al. 2005; Lysenko 2006).

4.2.3 Role of σ-factor diversity in transcriptional regulation

To address the question of a specific role of σ-factor diversity in transcriptional regulation (see Table 2 summarizing putative roles of σ-factors), *in vitro* reconstitution and transcription experiments using recombinant σ-factors and the *E. coli* core RNA polymerase were carried out by several groups. These again demonstrated that plant σ-factor genes encode functional plastidial σ-factors (Kestermann et al. 1998; Hakimi et al. 2000; Beardslee et al. 2002; Homann and Link 2003; Privat et al. 2003). While the three mustard σ-factors SalSig1, SalSig2, and SalSig3 recognized the *psbA* promoter, only SalSig1 and SalSig2 recognized the *rbcL* promoter. However, *trnK*, *trnQ*, *rps16*, and *rrn16* (PEP-P1) promoters were rather recognized by SalSig1 and SalSig3, but less efficiently by SalSig2 (Homann and Link 2003). Similar experiments with *Arabidopsis* σ-factors suggested that rather AthSig2 and AthSig3 confer specific recognition of the *rbcL* and *psbA* promoters than AthSig1 (Hakimi et al. 2000; Privat et al. 2003). The observed discrepancies in promoter recognition may be due to the heterologous transcription systems with hindered abilities to identify species- and/or PEP-specific regulatory elements at *cis*- and *trans*-factor level.

Further efforts to specify distinct functionality of plant σ-factors in regulation of plastidial gene expression employed characterization of their expression profiles. Profiling of light-dependent transcription in the red algae *Cyanidioschyzon merolae* and *Cyanidium caldarium* revealed light induced accumulation of the mRNAs of σ-factor genes (*CmeSig1-4*; *CcaSigB,C*; Oikawa et al. 1998; Minoda et al. 2005). Furthermore, *CmeSig2* transcript accumulation was additionally increased by high light, indicating that CmeSig2 might be a high-light responsive σ-factor (Minoda et al. 2005). Consistent with a prominent role of PEP in leaves, most plastidial σ-factor genes of higher plants are expressed in light-dependent manner in green tissue but are silent in non-photosynthetic roots (Isono et al. 1997b; Tanaka et al. 1997; Fujiwara et al. 2000; Oikawa et al. 2000). Moreover, expression of plastidial σ-factors seems to be differentially regulated during early *Arabidopsis* development. *AthSig2, AthSig3, AthSig4,* and *AthSig6* but not *AthSig1* and *AthSig5* transcripts accumulate in four day old seedlings (Ishizaki et al. 2005), while in eight day old seedlings transcript levels increase for all σ-factors (Nagashima et al. 2004a). Additionally, expression of *Sig2* transcripts prior to *Sig1*

Table 2. Roles of σ-factors in higher plants.

σ-factor	Target	Function	Plant	Gene	Reference
Sig1	general, *rbcL*	alternative σ-factor, may possibly need activating factor(s)	*Arabidopsis*	At1g64860	(Hakimi et al. 2000) (Privat et al. 2003)
			mustard		(Kestermann et al. 1998) (Homann and Link 2003)
Sig2	general, *psbA*, *trnV/E*, sole σ-factor to control *psaJ*	primary / alternative σ-factor (?)	*Arabidopsis*	At1g08540	(Hakimi et al. 2000) (Hanaoka et al. 2003) (Kanamaru et al. 2001) (Privat et al. 2003) (Shirano et al. 2000) (Tsunoyama et al. 2002)
			mustard		(Homann and Link 2003)
Sig3	general, sole σ-factor to control *psbN*	light-independent early primary / alternative σ-factor (?)	*Arabidopsis*	At3g53920	(Privat et al. 2003) (Zghidi et al. 2006)
			mustard		(Homann and Link 2003)
Sig4	sole σ-factor to control *ndhF*	σ-factor in plant stress response (?)	*Arabidopsis*	At5g13730	(Favory et al. 2005)
Sig5	*psbA*, sole σ-factor to control *psbD*	σ-factor in plant stress response (?) and regulating *psbD* BLRP *via* blue- /UVA-light	*Arabidopsis*	At5g24120	(Nagashima et al. 2004a) (Tsunoyama et al. 2002, 2004)
Sig6	general, *psbA*, *rbcL*, *atpB*, *trnV/E*, *ndhC*	light-independent early primary / alternative σ-factor (?)	*Arabidopsis*	At2g36990	(Ishizaki et al. 2005) (Loschelder et al. 2006)
			maize		(Lahiri and Allison 2000)

Question marks signify a proposed yet unproved function.

in developing leaves was reported for both *Arabidopsis* and rice, suggesting an early function of Sig2 in seedling development (Kanamaru et al. 1999; Kasai et al. 2004). This was supported by recent findings by Demarsy et al. (2006) showing that the mRNAs of AthSig2 and AthSig5 are already present in dry *Arabidopsis* seeds. Interestingly, unlike AthSig1 and AthSig2, AthSig3 protein accumulates in seeds and during early germination (Homann and Link 2003; Privat et al. 2003) as was shown for SolSig2 in spinach (Demarsy et al. 2006). A similar expression pattern was observed for the mustard SalSig3 factor, which accumulates rather in the dark than in light grown seedlings (Homann and Link 2003). Hence, Sig3 may play a distinctive role in regulation of gene expression in etio- and/or proplastids, and might be regulated by posttranslational processes (Homann and Link 2003; Privat et al. 2003). Similarly, ZmaSig6 was detected in root, leaf base, and etiolated leaf tissue in maize (Lahiri and Allison 2000). Therefore, it might be possible that Sig3 and Sig6 represent light-independent, early σ-factors regulating plastid gene expression during seedling growth and development. In opposite, AthSig5 transcripts are expressed later in plant development, controlled *via* the plastidial redox state (Fey et al. 2005). Furthermore, *AthSig5* is rapidly induced by blue, but not red light, which coincides with the blue-light-activated expression of *psbD* (Tsunoyama et al. 2002, 2004). *AthSig5* expression is also activated by various stress cues (Nagashima et al. 2004b). Expression of some plastid genes in higher plants seems to be regulated by circadian rhythms (Nakahira et al. 1998). Circadian timing of plastid gene expression is expected to be mediated by nuclear factors. σ-factors are good candidates to represent such factors. Indeed, *TaeSig1*, *NtaSig1*, *AthSig1*, *AthSig2*, and *PpaSig5* transcripts were shown to exhibit circadian or diurnal expression patterns (Kanamaru et al. 1999; Morikawa et al. 1999; Oikawa et al. 2000; Ichikawa et al. 2004).

Increasingly, functions of σ-factor genes in plants are investigated by analyses of knockout mutants, overexpression, or anti-sense lines. If plastidial gene expression would be controlled by a principal σ-factor similar to the situation in most bacteria, one would assume that inactivation of this gene would result in a drastic, most likely albino phenotype by causing defects in PEP-dependent transcription of photosynthesis related genes. However, examination of various *Arabidopsis* mutants of *AthSig2*, *AthSig3*, *AthSig4*, *AthSig5*, and *AthSig6* did not reveal such a severe phenotype (Shirano et al. 2000; Kanamaru et al. 2001; Hanaoka et al. 2003; Privat et al. 2003; Nagashima et al. 2004b; Tsunoyama et al. 2004; Favory et al. 2005; Ishizaki et al. 2005; Loschelder et al. 2006; Zghidi et al. 2006). Yet, a major break-through in revealing the specificity of σ-factors in transcription came by characterization of these plants.

***AthSig2* knockout mutants.** *AthSig2* mutants displayed a pale green phenotype accompanied by reduced accumulation of some plastid-encoded photosynthesis genes (Shirano et al. 2000; Kanamaru et al. 2001; Privat et al. 2003; Nagashima et al. 2004a). Furthermore, several PEP-transcribed tRNAs including *trnD*-GUC, *trnE*-UUC, *trnM*-CAU, and *trnV*-UAC were prominently reduced in *AthSig2* knockout mutants (Kanamaru et al. 2001; Hanaoka et al. 2003) and anti-sense plants (Privat et al. 2003). *Vice versa*, overexpression of AthSig2 enhanced transcription of *trnE-trnD* (Tsunoyama et al. 2004). It has been suggested that reduc-

tion of the photosynthesis-related components is caused by defects in chlorophyll biosynthesis and plastid translation due to the decrease of *trnE*, an initiator of ALA and consequently chlorophyll synthesis. Hence, AthSig2 may have a primary role in driving transcription of certain plastid tRNAs. It cannot be excluded, however, that Sig2 is able to recognize other PEP promoters as suggested for *psbA, psbD,* and *rbcL* (Kanamaru et al. 2001; Hanaoka et al. 2003; Tsunoyama et al. 2004).

***AthSig3* knockout mutants.** In opposite, characterization of *AthSig3* knockout mutants revealed a distinct reduction of transcript levels of the plastid *psbN* gene (Zghidi et al. 2006). Further analyses of transcript initiation sites in these mutants not only showed a loss of transcription initiation from AthP*psbN*-32 but also from AthP*atpH*-413, one of the two PEP promoters upstream of *atpH* in *Arabidopsis*. Therefore, it seems likely that AthSig3 directly controls *psbN* and partially *atpH* gene expression. The function of PsbN is still unknown and its suggested presence in photosystem II has been challenged (Kashino et al. 2002).

***AthSig4* knockout mutants.** Similarly, characterization of an *AthSig4* knockout mutant revealed a specific reduction in transcription of the plastid *ndhF* gene resulting in a strong downregulation of the plastid NDH activity (Favory et al. 2005). Therefore, *ndhF* expression and thus NDH activity seems to be regulated at transcriptional level, controlled by specific σ-factor AthSig4. Interestingly, NDH is involved in plant stress response (Casano et al. 2001) and leaf senescence (Zapata et al. 2005). Whether AthSig4 expression is modulated by such environmental or developmental parameters remains to be investigated.

***AthSig5* knockout mutants.** Apart from AthSig3 and AthSig4, AthSig5 might be an additional σ-factor tied to a specific function in regulation of plastid gene expression. As shown by analyses of transcription in light-treated plants (Tsunoyama et al. 2002; Nagashima et al. 2004b), *AthSig5* knockout plants, and overexpression studies (Nagashima et al. 2004b; Tsunoyama et al. 2004), AthSig5 is regulated by blue light and specifically activates transcription from the *psbD* blue-light responsive promoter (BLRP). Interestingly, analysis of a further *AthSig5* knockout mutant showed embryo lethality (Yao et al. 2003). *AthSig5* has recently been identified as one of 250 genes required for normal embryo development in *Arabidopsis* (Tzafrir et al. 2004) and its mRNA is present in seeds (Demarsy et al. 2006) indicating a substantial role of AthSig5 in seed development. However, it is not yet understood why the different *AthSig5* mutants exhibit these diverse phenotypes.

***AthSig6* knockout mutants.** Cotyledons of *AthSig6* knockout mutants displayed a transient pale green phenotype during early plant development combined with a delay in light-dependent chloroplast development (Ishizaki et al. 2005; Loschelder et al. 2006). During this developmental stage the transcript pattern was found to be similar to that of Δ*rpo* mutants, since transcript levels of most PEP-dependent genes for photosynthesis components, rRNAs, and some tRNAs were decreased. Since the maize homologue ZmSig6 is expressed exclusively in tissue containing immature plastids (Lahiri and Allison 2000), it was proposed that (Ath)Sig6 might be a general σ-factor serving PEP in an early, initial developmental stage. Nonetheless, given that after eight days the mutant phenotype is restored

to wild type it is plausible that other σ-factor(s) are able to take over AthSig6 function later in seedling development and plant growth (Shiina et al. 2005). However, characterization of a second *Arabidopsis* knockout line with a *Sig6* mutant allele throughout leaf development (*sig6-2*) suggested a second (persistent or long-term) role of AthSig6 (Loschelder et al. 2006). While transcript accumulation of genes such as *psbA* and *rbcL* was only affected early in development, RNA levels of *atpB* and *ndhC* originating from their corresponding PEP promoters declined during plant development. Interestingly, emerging transcripts which originated further upstream of *atpB* suggested a SOS promoter switch (Schweer et al. 2006).

***AthSig1* overexpressing mutants.** Knockout or anti-sense mutants of *AthSig1* have yet to be characterized. Thus far, data on the role of AthSig1 in plastidial gene expression have been derived from mutant plants overexpressing the *AthSig1* gene (Tsunoyama et al. 2001). Investigation of transcription activity by run-on analyses revealed enhanced initiation from *psaA*, *psbB*, *psbE*, and *rbcL* promoters indicating a more general role of this σ-factor in transcription of genes encoding components of the photosynthesis complexes.

Taken together, only five genes in *Arabidopsis* seem to be controlled by a distinct σ-factor with specific function: *psaJ* by AthSig2, *psbN* by AthSig3, *ndhF* by AthSig4, and *psbD* (BLRP) by AthSig5 (Table 2). However, some other genes appear to be controlled by several σ-factors thereby possessing overlapping functions. Most prominent are genes such as *psbA*, controlled by AthSig2, AthSig5, and AthSig6; *rbcL* controlled by AthSig1 and AthSig6; *trnV*-UAC and *trnE*-UUC, controlled by AthSig2 and AthSig6. Consequently, overlapping functions of σ-factors are generally believed to be the reason for the weak phenotype of σ-factor knockout mutants.

Regulation of σ-factors. PEP activity depends on the developmental stage of the plastids: it is down regulated in etioplasts and is more active in chloroplasts (Rapp et al. 1992; DuBell and Mullet 1995). Furthermore, rates of PEP transcription are higher in the light than in the dark (Shiina et al. 1998). Changes in PEP transcription activity have been suggested to be partly resulting from changes in the phosphorylation state of σ-factors. Phosphorylation of σ-factors and the PEP enzyme itself have been shown to be an important regulatory event in chloroplast transcription (Tiller and Link 1993a; Baginsky et al. 1997; Christopher et al. 1997). In mustard, a CK2-type kinase has been identified to be part of the chloroplast PEP-A complex (Ogrzewalla et al. 2002). This plastid transcription kinase activity (PTK), termed cpCK2, is able to phosphorylate purified sigma-like factors (SLFs) as well as subunits of the PEP-A complex *in vitro*. Based on the observation that cpCK2 itself is differentially regulated by phosphorylation and redox state, cpCK2 was proposed to be part of a signaling pathway controlling PEP activity (Baginsky et al. 1999). Phosphorylation and SH-group redox state were shown to work antagonistically. A non-phosphorylated cpCK2 appears to be more active, but is inhibited by treatment with reduced glutathione (GSH). *Vice versa*, a phosphorylated non-active enzyme could be re-activated by adding GSH. In opposite to cpCK2 isolated from plants grown under high light conditions, cpCK2 iso-

lated from plants grown under moderate light conditions effectively phosphorylated the associated PEP-A, therefore corroborating these findings (Baena-Gonzalez et al. 2001). Thus, light dependent reduction of GSH would inactivate cpCK2, while dephosphorylation of PEP under high light conditions would enhance PEP-dependent transcription. It remains unknown whether cpCK2 is also regulated *via* extraplastidic signal chains mediated by phyto- and/or chryptochromes. Since cpCK2 orthologs have been identified in various plant species (Loschelder et al. 2004) it might well be that this kinase has an evolutionary conserved role in plastid redox-sensitive signal transduction.

In bacteria, σ-factor activity is controlled by anti-σ factors (Ishihama 2000). Plastid σ-factor AthSig1 associated proteins with plastid localization were identified in *Arabidopsis* (SibI and T3K9.5; Morikawa et al. 2002). They are not related to any proteins of known function and are light-dependent, developmental, and tissue-specifically expressed, and thus may be involved in regulation of AthSig1 activity.

4.3 Exogenous and endogenous factors controlling plastidial transcription

Plant development is highly influenced by environmental factors. Plastid gene expression was shown to differentially respond to environmental cues (Chory et al. 1995; Link 1996; Goldschmidt-Clermont 1998; Barkan and Goldschmidt-Clermont 2000). Therefore, *cis*- and *trans*-elements regulating differential gene expression in plastids were in the center of attention in the last decades (see Table 3 for summary). Regulatory sequence motifs upstream the -35 core promoter region were found in the promoters of *rbcL* and *psbD-psbC*. The *rbcL* gene is transcribed from a single PEP promoter with well conserved -35 and -10-elements and canonical spacing by 18 nucleotides (Shinozaki and Sugiura 1982; Mullet et al. 1985; Reinbothe et al. 1993; Isono et al. 1997a). *In vitro* studies demonstrated the importance of both the -35/-10 box spacing and sequence for *rbcL* promoter strength (Gruissem and Zurawski 1985; Hanley-Bowdoin et al. 1985). An upstream element, conserved between maize, pea, spinach, and tobacco was proposed to function as a binding site for the chloroplast DNA-binding factor 1 (CDF1) in maize (Lam et al. 1988). Interestingly, a segment of CDF1, region II, is reminiscent of the AT-rich UP element stimulating transcription in *E. coli* (Ross et al. 1993). However, analyses of transplastomic plants expressing chimeric P*rbcL::uidA* constructs demonstrated, that the *rbcL* core promoter is sufficient to obtain wild type rates of transcription (Shiina et al. 1998). Interestingly, another DNA-binding protein (RLBP, *rbcL* promoter-binding protein) binds specifically to the *rbcL* promoter core in tobacco (Fig. 4; -3 to -32; Kim et al. 2002). Only detectable in light-grown seedlings, RLBP is suggested to play a role in light-dependent *rbcL* transcription. However, stabilization of the *rbcL* mRNA *via* its 5' UTR is compensating for reduced rates of transcription in the dark and leads to light-dependent transcript accumulation (Shiina et al. 1998).

Table 3. Transcription regulating factors in higher plants.

Protein	Target	Function	Plant	Gene	Reference
PEP-regulating factors					
AGF	*psbD* BLRP	binds AAG-box; transcription enhancer	barley wheat		(Kim and Mullet 1995) (Nakahira et al. 1998)
PTF1	*psbD* BLRP	binds AAG-box; transcription enhancer part of AGF	*Arabidopsis*	At3g02150	(Baba et al. 2001)
PGTF	*psbD* BLRP	binds to PGT-box	barley		(Kim and Mullet 1995)
CDF1	*rbcL*	DNA-bdg.; transcription regulation	pea maize		(Lam et al. 1988)
CDF2-A	*rrn16*(P1)	DNA-bdg.; transcription repression	spinach		(Baeza et al. 1991) (Bligny et al. 2000)
Region U-bdg. protein	*psaA*	DNA-bdg.; transcription regulation	spinach		(Cheng et al. 1997a)
Region D-bdg. protein	*psaA*	DNA-bdg.; transcription regulation	spinach		(Cheng et al. 1997a)
RLBP	*rbcL*	DNA-bdg.; transcription regulation	tobacco		(Kim et al. 2002)
SibI		AthSig1-bdg. protein	*Arabidopsis*	At3g56710	(Morikawa et al. 2002)
T3K9.5		AthSig1-bdg. protein	*Arabidopsis*	At2g41180	(Morikawa et al. 2002)
cpCK2 (PTK)		plastid transcription kinase, phosphorylates sigma-like factors (SLFs) and subunits of PEP-A	*Arabidopsis* mustard	At5g67380	(Baginsky and Gruissem 2002) (Baginsky et al. 1997) (Ogrzewalla et al. 2002)
NEP-regulating factors					
RPL4		interaction with CDF2 (?); transcription regulation	spinach	X93160 (gi2792019)	(Trifa et al. 1998)
CDF-2B	*rrn16*	interaction with a NEP-2 transcription activity (?)	spinach		(Bligny et al. 2000)
$tRNA^{Glu}$		DNA-bdg.; transcription regulation inactivates NEP activity by binding to RpoTp	*Arabidopsis*	*trnE* (ArthCt097)	(Hanaoka et al. 2005)

Question marks signifies a proposed yet unproved function.

Blue-light control of the *psbD-psbC* operon. Contrary to most photosynthetic genes, the rate of transcription of *psbD-psbC* remains high in mature chloroplasts (Klein and Mullet 1990; Baumgartner et al. 1993; DuBell and Mullet 1995). Responsible is a specific activation of one of the *psbD* promoters, the blue light-responsive promoter (BLRP; Sexton et al. 1990), which is found upstream of the *psbD* gene of various species (Fig. 4; Christopher et al. 1992; Wada et al. 1994; Allison and Maliga 1995; Kim and Mullet 1995; To et al. 1996; Hoffer and Christopher 1997; Kim et al. 1999b; Thum et al. 2001). The architecture of the *psbD* BLRP promoter consists of two conserved upstream elements (PGT-box, AAG-box) and poorly conserved and closely spaced -35/-10-elements. *In vivo* studies in transplastomic tobacco revealed that deletion of parts of the PGT-box reduced mRNA levels, while subsequent deletion the AAG-box sequences even further reduced transcript levels (Allison and Maliga 1995). In tobacco, therefore, the conserved sequence elements upstream of the *psbD* promoter core are accountable for light-activated transcript accumulation. *In vitro* transcription from the *psbD* promoter in rice, wheat, and barley depends on the -10, but not on the -35 promoter element (To et al. 1996; Satoh et al. 1997; Nakahira et al. 1998; Kim et al. 1999b). The AAG-box of the barley promoter was shown to be the binding site for a nuclear-encoded AAG-binding complex *in vitro* (AGF; Kim and Mullet 1995). However, binding activity of AGF to the AAG-box is not correlated with transcriptional activation of the *psbD* BLRP (Nakahira et al. 1998). One of the components of AGF of *Arabidopsis* was cloned and designated plastid transcription factor 1 (PTF1; Baba et al. 2001). Studies on *PTF1*-deficient mutants revealed that PTF1 is rather involved in general transcriptional enhancement than in light-dependent activation of *psbD* transcription. Correspondingly, the PGT-box is the binding site for PGTF, the PGT-binding factor. Its DNA-binding activity is regulated by an ADP-dependent kinase (Kim et al. 1999a). A model based on these *in vitro* experiments in barley explains that constitutively binding of AGF to the upstream AAG-element may assist promoter recognition by PEP, whereas light-dependent transcriptional activation of *psbD* transcription is mediated by binding of PGTF to the PGT-box. In the dark, PGTF is phosphorylated and loses its affinity for the PGT element, thereby decreasing transcription. Although the *psbD* promoter architecture is highly conserved, it is unlikely that PGT is required for light-dependent transcription in various other plants. It was shown for rice (To et al. 1996), wheat (Satoh et al. 1997), and barley *in vitro* (Kim et al. 1999b) and in transplastomic tobacco *in vivo* (Thum et al. 2001) that the PGF-box is not required for light-dependent activation in these plants. Therefore, the roles of PGT and PGTF remain largely unknown.

It has been proposed that AthSig5 might act as a mediator of blue-light signaling in activating *psbD* BLRP transcription in blue light (see Section 4.2.3; Tsunoyama et al. 2002; Nagashima et al. 2004b; Tsunoyama et al. 2004), whereas AGF enhances *psbD* BLRP transcription by constitutively binding to the AAG-box (Shiina et al. 2005). It is assumed that the signal transduction pathway involves reception of blue light by cryptochromes and PhyA (Thum et al. 2001; Mochizuki et al. 2004), further mediation by a protein phosphatase PP7 (Moller et al. 2003), and subsequent induction of *Sig5* expression (Mochizuki et al. 2004).

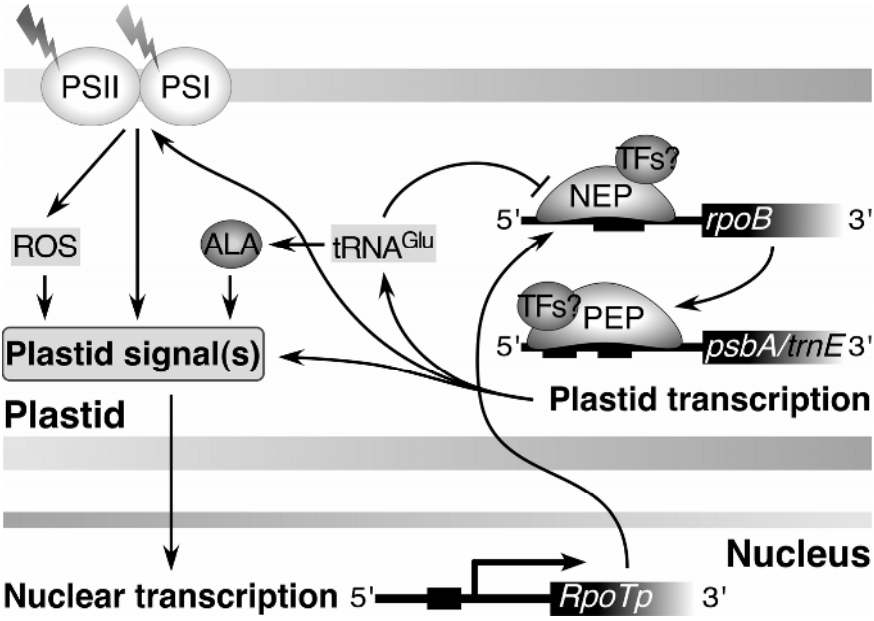

Fig. 7. The role of nuclear-encoded phage-type RNA polymerases in regulation of plastidial gene expression. NEP transcription activity is in part represented by a phage-type RNA polymerase encoded by the nuclear located *RpoTp* gene. NEP transcribes and therefore may regulate expression of the plastidial *rpoB* operon encoding subunits of the plastid-encoded RNA polymerase (PEP). PEP in turn transcribes genes encoding components of the photosynthetic complexes (PSI, PSII) that regulate nuclear transcription by generating diverse 'plastid signals' (ROS, reactive oxygen species). The *trnE* gene encoding $trnA^{Glu}$ which is required for the synthesis of δ-aminolevulinic acid (ALA) is also transcribed by PEP (Hess et al. 1992; Walter et al. 1995). ALA is a precursor of the chlorophyll and heme biosynthesis thought to provide 'plastid signals' which influence nuclear transcription. Furthermore, $tRNA^{Glu}$ is assumed to developmentally inhibit NEP transcription by binding to RpoTp (Hanaoka et al. 2005). In turn, the expression and activity of nuclear-encoded, plastid phage-type RNA polymerase regulates the transcription of plastidial genes and consequently the developmental stage of the plastid (RpoTp; Emanuel et al. 2004). Thus, the regulated network of the nuclear and plastidial transcription machineries may be a key element for a concerted expression of genes located within compartments of the plant cell.

After import into plastids, Sig5 associates with AGF (PTF1) and initiates *psbD* transcription. Furthermore, *psbD* BLRP activity is also regulated in a developmental and tissue-specific manner, since the *Arabidopsis* DET1 gene product downregulates the activity of *psbD* BLRP in young seedlings (Christopher and Hoffer 1998).

Plastid-to-nucleus signaling. Environmental control of plastidial gene expression is most intense in differentiation from proplastids to either etioplasts (dark) or chloroplasts (light). Analyses of photomorphogenic mutants established the existence of different pathways to communicate light perception to plastids in order to

control their development (Leon et al. 1998; Rodermel 2001; Gray et al. 2003; López-Juez and Pyke 2005). However, these analyses also showed that retrograde or 'plastid signals' are controlling nuclear gene expression depending on the developmental status of the plastid (Fig. 7; see Chapter 13; Rodermel 2001; Gray 2003; Beck 2005; Leister 2005; Nott et al. 2006). Both plastid transcription and translation are necessary for the production of a 'plastid signal'. However, it is not an immediate translational product of a plastid gene (Oelmüller et al. 1986; Lukens et al. 1987), but rather part(s) of signal transduction pathways in plastids.

The barley mutant *albostrians*, with alternating stripes of white and green tissue, contains no detectable ribosomes in plastids of white tissue cells (Siemenroth et al. 1981; Hess et al. 1993). Transcript levels of some photosynthesis-related plastidial and nuclear genes are reduced or missing suggesting the existence of 'plastid signals' controlling nuclear gene expression (Bradbeer et al. 1979; Hess et al. 1994). Recently, transcript levels of the nuclear-encoded *RpoTp*, which is likely to represent NEP activity, and its plastidial target genes were analyzed throughout the developmental gradient of *albostrians* leaves (Emanuel et al. 2004). The results revealed a significant influence of the developmental stage of plastids on expression and activity of RpoTp, indicating a plastid-to-nucleus signaling to coordinate expression of plastidial and nuclear-encoded RNA polymerases as a prerequisite of a concerted gene expression in both plastids and nucleus (Fig. 7).

Redox control of plastid gene expression. Light is not only the energy source for photosynthesis, but also an environmental signal to regulate plant biogenesis and environmental adaptation. Apart from blue/UVA-light, illumination has been early hypothesized to control plastid gene expression *via* the physiological status of the plastid, e.g., redox conditions (Link 2003; Pfannschmidt and Liere 2005). Redox control of plastidial gene expression has been interpreted as a selection force throughout evolution to retaining their genomes (Allen 1993). First confirmation for such a redox control was obtained by demonstrating that light supported incorporation of radioactive-labeled NADH into the RNA fraction of lettuce plastids (Pearson et al. 1993). Plastidial gene expression is controlled at different levels by photosynthetic activity such as RNA maturation (Deshpande et al. 1997; Liere and Link 1997; Salvador and Klein 1999) and translation (Danon and Mayfield 1994; Bruick and Mayfield 1999; Trebitsh et al. 2000; Zhang et al. 2000). Effects of the redox state on plastidial gene transcription were furthermore demonstrated by growing plants under light conditions generating an imbalance in excitation energy distribution between photosystems (PSII- and PSI-light, 680 and 700 nm, respectively; Pfannschmidt et al. 1999a, 1999b; Fey et al. 2005). Preferential excitation of PSII results in a reduction of the electron transport chain while a preferential excitation of PSI results in its oxidation. The change in photosystem stoichiometry correlated with respective changes in the transcriptional rates and transcript amounts of the plastidial genes for the reaction centre proteins of PSII and PSI, *psbA* and *psaAB*. Indeed, the redox state of the plastoquinone pool (PQ) is the major determinant for the changes in gene expression. A reduced PQ pool promotes transcription of the *psaAB* operon. In reverse, an oxidized PQ pool increases *psbA* transcription. Opposite regulation of these genes has been recently

found also in pea (Tullberg et al. 2000), *Chlamydobotrys stellata* (Kovacs et al. 2000) and *Synechocystis* PCC 6803 (Li and Sherman 2000; El Bissati and Kirilovsky 2001) suggesting that this mechanism represents an evolutionary old means of regulating gene expression. These data provide a first model on how plants adapt to light quality gradients occurring in natural environments under low light intensities. Still, the signal transduction pathway connecting the PQ pool with transcription is yet unknown. However, a long-term response may represent an extended branch of the short-term response (the state transition), which is also regulated by the redox state of the PQ pool (Allen and Forsberg 2001; Pursiheimo et al. 2001). The PQ oxidation site at the cyt b_6f complex functions as a sensor for the PQ redox state during state transition (Vener et al. 1997; Zito et al. 1999). A putative DNA-binding protein of PS II, TSP9, is partially released from PSII upon PQ reduction in spinach and may represent such a signal transducer towards transcription (Carlberg et al. 2003; Zer and Ohad 2003). Identification of an additional protein of 31 kDa capable of sequence-specific binding between positions + 64 to +83 (region D) of the light dependent *psaAB* PEP promoter region (Chen et al. 1993; Cheng et al. 1997a) suggests the existence of yet unidentified transcription factors that transmit redox signals. Furthermore, the *Arabidopsis* high chlorophyll fluorescence mutant *hcf145* shows decreased mRNA stability and transcription of *psaA* (Lezhneva and Meurer 2004). Thus, HCF145 might be involved in transcriptional regulation of the *psaA* operon. Further analysis of this promoter has yet to be reported.

PEP is not only responsible for the redox regulation at the *psbA* and *psaAB* promoters, but apparently is also regulated *via* redox control. A regulatory impact on steady-state levels of transcripts of genes for PEP components was observed by microarray analyses (Fey et al. 2005): *rpoB* (plastid-encoded β-subunit), *AthSig5* (nuclear-encoded σ-factor), and *SibI* (nuclear-encoded Sig1-binding protein; Morikawa et al. 2002). Interestingly, *rpoB* is transcribed by a nuclear-encoded phage-type RNA polymerase (Fig. 7, RpoTp; Liere et al. 2004), suggesting a redox regulation of this enzyme (see Chapter 13).

Developmental switch from NEP to PEP. A regulatory role, which links chlorophyll synthesis and the developmental switch from nucleus-encoded RNA polymerases to the plastid-encoded bacterial-type enzyme, has been proposed for the plastid-encoded $tRNA^{Glu}$ in *Arabidopsis* (Hanaoka et al. 2005). $tRNA^{Glu}$ is not only required for translation, but also for synthesis of δ-aminolevulinic acid, a precursor of chlorophyll (Schön et al. 1986). In gel mobility shift experiments recombinant RpoTp specifically bound this tRNA. Additionally, transcription from a putative plastidial *accD* NEP promoter sequence was inhibited by addition of $tRNA^{Glu}$ to *in vitro* transcription reactions with proplastid extracts from *Arabidopsis*. Hence, the authors suggested $tRNA^{Glu}$ to developmentally inhibit transcription by RpoTp (Fig. 7).

Bacterial-like stringent control. In bacteria, one of the most important processes to regulate gene expression is the so-called 'stringent control' enabling adaptation to nutrient-limiting conditions (Cashel et al. 1996). The effector molecule is guanosine 5'-diphosphate 3'-diphosphate (ppGpp), which binds to the core RNA polymerase modifying its promoter specificity (Toulokhonov et al. 2001). Stress-

induced synthesis is mediated by ppGpp synthetases, RelA and SpoT, homologues of which were found in *Chlamydomonas reinhardtii* (Kasai et al. 2002), *Arabidopsis* (van der Biezen et al. 2000), and tobacco (Givens et al. 2004). Plastidial targeting has been demonstrated for some of these RSH termed proteins, suggesting an implication in ppGpp signaling in plastids. RSH expression and plastidial ppGpp levels are clearly elevated by light and various abiotic and biotic stress conditions. Furthermore, PEP activity is inhibited by ppGpp *in vitro* (Givens et al. 2004; Takahashi et al. 2004). Thus, it is conceivable that PEP might indeed be under control of a bacterial-like stringent response mediated by ppGpp. Interestingly, stress signals specifically induce transcription initiation from the *psbD* BRLP conferred by a special σ-factor, AthSig5 (see Section 3.2.3; Nagashima et al. 2004b; Tsunoyama et al. 2004). However, target genes that are regulated by a plastidial stringent control have yet to be identified, which might help to elucidate the molecular mechanisms of transcriptional responses to plant hormones and environmental stress situations.

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