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REVIEWS

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Plastid RNA Polymerases

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Abstract—Plastids have an interesting transcription machinery that makes it possible to study the interplay of mono- and multisubunit RNA polymerases (RNAPs) during intricate organelle biogenesis, requiring the concerted expression of genes located in different compartments of the cell. The past decade has been marked by a breakthrough in studies of chloroplast RNAPs. Nucleus-encoded monosubunit RNAP (NEP) was discovered; a nuclear gene family was found to code for σ subunits of plastid-encoded multisubunit RNAP (PEP); mutants knocked-out in PEP subunit genes were obtained; a reorganization of PEP was observed during plastid biogenesis; and a hypothesis was advanced to describe the division of functions between NEP and PEP. The review considers recent data on the organization, functions, and evolution of plastid RNAPs.

Key words: chloroplast, RNA polymerase, transcription, gene expression, evolution

INTRODUCTION

Chloroplasts have a unique transcription apparatus: their genes are transcribed by RNA polymerases (RNAPs) of two types, monosubunit and multisubunit. Moreover, these enzymes are encoded by genes localized in different compartments of the cell: plastid genes code for the core subunits of multisubunit plastid-encoded plastid RNAP (PEP), while nuclear genes code for monosubunit nucleus-encoded plastid RNAP (NEP), σ subunits of PEP, and, probably, other protein components of PEP. It is of particular interest how two different RNAPs interact with each other and how the concerted expression of their genes, located in different organelles, is achieved. Studies in the last seven or eight years have provided or suggested answers to these questions.

MULTISUBUNIT RNA POLYMERASE WITH A PLASTID-ENCODED CORE (PEP)

PEP belongs to the multisubunit RNAP family, which also includes archaebacterial and eubacterial RNAPs along with eukaryotic nuclear RNAPs I, II, and III [1]. The core of PEP is most similar to the RNAPs of bacteria, in particular, cyanobacteria. However, PEP of mature chloroplasts includes many additional proteins and is intermediate between eubacterial and nuclear RNAPs in the number of polypeptides in the whole complex [2–4].

PEP occurs in the chloroplasts of all photosynthesizing organisms. Plants defective in PEP genes are unviable, being virtually devoid of chlorophyll because of the distorted development of chloroplasts [5–7]. Nonphotosynthesizing parasitic plants quickly lose the PEP genes during evolution [8–10]. Data on the PEP activity in nonphotosynthesizing plastids are scarce. It is probable that PEP is active in nondifferentiated proplastids [11]. The RNAP responsible for transcription in specialized nonphotosynthesizing plastids has yet to be identified.

As in the cyanobacterial enzyme, the PEP core consists of five subunits: $(\alpha)_2$, β, β', and β", which are encoded by *rpo*A, *rpo*B, *rpo*C1, and *rpo*C2, respectively [12, 13]. These genes are components of the plastome in almost all algae and plants studied in this respect. The only exception is the moss *Physcomitrella patens*, in which *pro*A is relocated into the nucleus [14]. In addition, the genome of *Synechocystis* and the plastomes of some algae contain genes homologous to the genes for the *Escherichia coli* RNAP subunit ω and its orthologs, subunit K of archaebacterial RNAP, and the RPB6 subunit that is common for nuclear RNAPs I, II, and III [1, 15]. It is possible that the ω subunit is a component of PEP, although it has not thus far been found in plastids or isolated PEP complexes ([4]; Oelmuller, personal communication). Maize PEP includes the subunits α (38 kDa), β (120 kDa), β' (78 kDa), and β" (180 kDa) [16, 17]. The *rpo*C1 and *rpo*C2 genes correspond to the 5' and 3' regions of *rpo*C of *E. coli* and other eubacteria (Fig. 1a). The *rpo*C gene was also split in archaebacteria, but at a different site than in plastids (Fig. 1a) [12, 18]. The RNAP genes are structurally simple (only higher plant *pro*C1 has a single intron) and evolutionarily conserved. For instance, the deletion of a monocot-specific region from *rpo*C2 results in cytoplasmic male sterility in sorghum [19]. The genomic organization of the *rpo* genes is also con-

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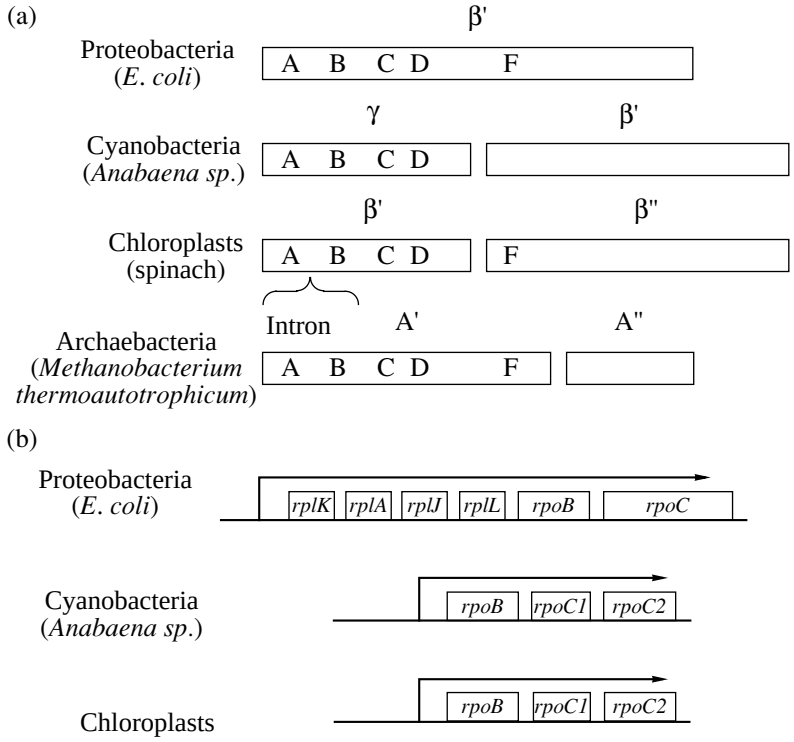


Fig. 1. Genes for the core subunits of multisubunit RNAPs. (a) Structure of *E. coli* RNAP subunit β and homologous subunits of cyanobacterial, plastid, and archaebacterial RNAPs. A, B, C, D, and F are conserved motifs. The position of the intron in the plastid RNAP β'-subunit gene is shown. Cited from [12] with modification. (b) Organization of the *rpo*B/C operon in proteobacteria, cyanobacteria, and plastids. The start and direction of transcription are shown with arrows.

served: *rpo*A is a component of an operon coding for ribosomal proteins while *rpo*B and *rpo*C (1 and 2) form another operon both in bacteria and in plastids (Fig. 1b) [12, 20–22].

RNAPs of the bacterial type differ from other multisubunit RNAPs in having σ subunits [1]. The σ subunits regulate the promoter specificity of the only RNAP in bacterial cells. The σ subunits interacting with the PEP core are encoded by a small family of the nuclear *Sig* genes. PEP lacking a σ subunit is incapable of correct transcription initiation [4]. The plant *Sig* genes code for polypeptides of 47–66 kDa. Nucleotide sequence comparison of the *Sig* genes and their mRNAs, along with our unpublished data, suggest the presence of short introns. Seven σ-subunit genes were found in *E. coli* and nine, in cyanobacteria [23–25]. Plants have five or six *Sig* genes (*Sig1–6*). Analysis of published data allows the following conclusions. The genes for chloroplast σ subunits ($_{cp}$σ) are expressed differently. *In vitro*, PEP with different σ subunits interacts with the same promoters of the σ^{70} type with different efficiencies. As with *E. coli* σ^{70}, $_{cp}$σ subunits alone do not bind to promoters [26–28]. Plants (*Arabidopsis thaliana*) knocked-out in one *Sig* gene are viable owing to the increased expression of the other *Sig* genes [29]. Still, the disruption of individual *Sig* genes suppresses the growth and development of plants [29, 30]. A protein interacting with SIG1, but not with the other σ subunits, is known in *A. thaliana* [31]. SIG2 and SIG5 ensure, respectively, the constitutive and inducible transcription of *psb*D from different promoters in *A. thaliana* [28, 30]. Thus, different $_{cp}$σ are homologous and interact with promoters of the same type, but they are still capable of differentially regulating the transcription of plastid genes. Apart from flowering plants, several *Sig* genes were found in the moss *P. patens* [32, 33] and in the red alga *Cyanidium caldarium* [34].

Two specific inhibitors, rifampicin and tagetin, are used to block the function of PEP. The antibiotic rifampicin, which binds to the β subunit of bacterial RNAP, suppresses the PEP activity as well [2, 36, 37]. Rifampicin prevents the formation of the first phosphodiester bonds, and its effect depends on the protein composition of the initiation complex [2, 38, 39]. Tagetin, also known as tagetitoxin, inhibits bacterial RNAP [40], PEP [2, 11, 40, 41], and nuclear RNAP III [42]. Tagetin acts at the stage of transcription elongation [43] and is independent of transcription initiation factors. However, its efficacy depends on the DNA template [43].

PEP is reorganized during plastid biogenesis. For instance, PEP-B is prevalent in mustard etioplasts [2]. PEP-B is probably a minimal transcription complex

consisting of the RNAP core and a σ subunit. The complex is sensitive to rifampicin. In mustard chloroplasts, PEP mostly occurs as PEP-A [9], an intricate complex containing not only the RNAP core, but also protective proteins, RNA-binding proteins, and a protein capable of binding to membranes [39]. A similar complication of PEP was observed in the plastids of pea [3], tobacco [4], and *Arabidopsis* (Oelmuller, personal communication). The binding of RNAP with the additional polypeptides is stronger than that with a σ subunit [4]. PEP-A consists of at least 13 polypeptides, is more than 900 kDa, and is insensitive to rifampicin. The difference in rifampicin sensitivity between PEP-A and PEP-B is most probably determined by the composition of transcription factors interacting with the RNAP core [38].

PEP is reorganized during the early development of chloroplasts: young greening plastids contain an intermediate enzyme, which is similar in molecular weight to PEP-A and in buffer salt requirements to PEP-B [37].

Many protein components of PEP-A have been identified ([4, 39]; Oelmuller, personal communication), yet their roles in transcription are still poorly understood. Most components are similar to proteins with unknown functions; some others show a similarity to nontranscription proteins such as phosphofructokinase, aldolase, acetylmuramyl-L-alanylglutamate–diaminopimelate ligase, superoxide dismutase (SOD), thioredoxin, and certain ribosomal proteins. Many of the proteins with unknown functions contain conserved motifs responsible for DNA binding, RNA binding, and protein–protein interactions. Disruption of the genes for three such proteins (an aldolase homolog and two proteins with unknown functions) is similar in effect to the disruption of the genes coding for components of the PEP core. Mutant plants fail to develop chloroplasts; the transcription of PEP-dependent genes is strongly suppressed and that of NEP-dependent genes is increased in leaf etioplasts (Oelmuller, personal communication). It is clear that these proteins are functionally important subunits of PEP-A.

PEP-A probably incorporates Fe-dependent SOD, an enzyme involved in protecting chloroplast from free radical damage ([39]; Oelmuller, personal communication). Such an association with SOD is unknown for nuclear and bacterial RNAPs [1, 44, 45]. Presumably, Fe-dependent SOD protects the transcription machinery of chloroplasts from free radicals generated in large amounts during intense photosynthesis.

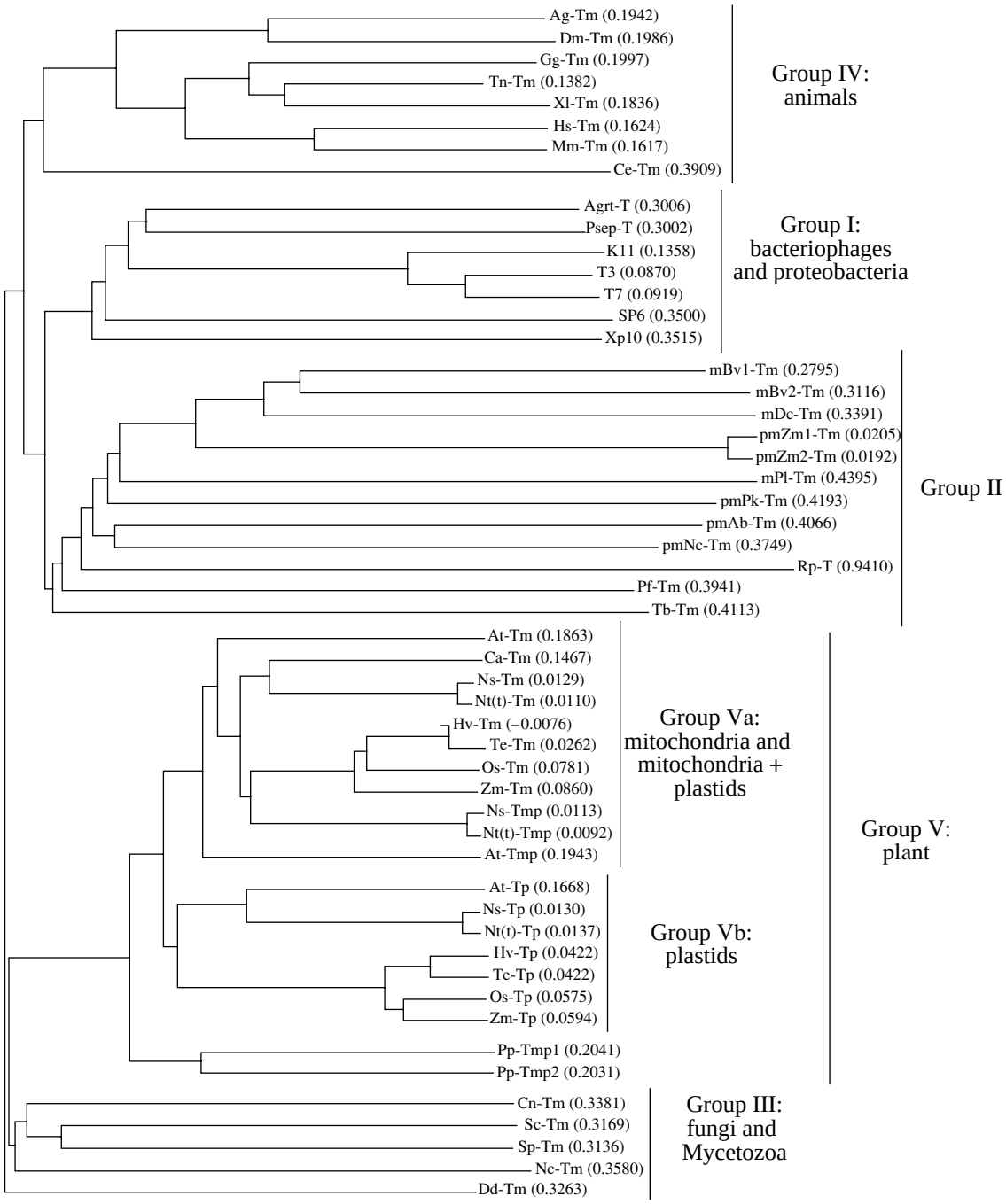
Another protein associated with PEP-A is casein kinase 2, which phosphorylates the transcription complex [46–48]. Phosphorylation/dephosphorylation affects the strength of the interaction between the RNAP core and σ subunits [49], the binding of the holoenzyme with a promoter [46, 47] and with rifampicin [39], and the activity of PEP-associated casein kinase 2 itself [46]. It is probable that the structural organization of PEP is also regulated by phosphorylation/dephosphorylation.

The PEP activity changes during chloroplast biogenesis. The observed changes in the amount of the *rpo*B mRNA for the PEP subunit β make it possible to assume that, as proplastids develop into chloroplasts, PEP synthesis is initiated, quickly reaches its maximum, and then declines with chloroplast maturation [50–52]. The NEP activity changes similarly but even more quickly. According to the results of inhibitor analysis, PEP activity accounts for 40–50% of the total RNAP activity in nondifferentiated proplastids and for 95–99% in mature chloroplasts [11].

As the synthesis of the PEP mRNA decreases and PEP is reorganized during chloroplast differentiation, transcription regulation by external factors becomes stronger. For instance, PEP-dependent *psb*A and *psb*C of photosystem II are actively transcribed regardless of illumination in young chloroplast but only in the light in mature chloroplasts [53]. The change is probably due to the reorganization of PEP, which differently interacts with the *psb*A promoter at different stages of chloroplast biogenesis, binding or not to the region 35 [53, 54].

Changes in PEP activity correspond to the program of chloroplast differentiation. The first stage, the build-up, is associated with the active synthesis of RNAs and proteins necessary for the formation of the internal structures of chloroplasts. PEP probably directs the active transcription of most genes without being distracted by any stimulus at this stage. At the second, maintenance, stage, mature chloroplasts require economical gene expression in order to maintain the existing structures. Hence, PEP is reorganized to drive inducible, rather than constitutive, transcription in mature chloroplasts. However, at least one gene, *psb*D, is transcribed in the inducible manner regardless of the stage of chloroplast differentiation [53, 55], which is probably explained by the specific structure of its light-responsive promoter [56–58].

The origin of PEP from bacterial RNAP is supported not only by their structural similarity, but also by specific features of their functioning. PEP recognizes the terminators of *E. coli thr*A, *rrn*B, and *rrn*C, as well as the T4 gene 32 *in vivo* [59]. On the other hand, the promoters of the plastid genes of wheat, maize (*rbc*L and *atp*B), and pea (*psb*A) function normally in *E. coli* cells [60–62]. The *psb*A promoter is recognized in chloroplasts *in vitro* by *E. coli* RNAP alone [63] or in complex with the plastid σ factor [26, 64]. However, RNAPs diverged in the course of evolution. For instance, *E. coli* RNAP initiates the transcription of the plastid ribosomal operon from a site that does not interact with plastid RNAPs *in vivo* or *in vitro* [65]. Unlike PEP, *E. coli* RNAP does not interact with the light-responsive *psb*D promoter [63]. The *E. coli*



*rpo*A gene for the RNAP subunit α fails to replace the corresponding plastid gene [66].

There are still no grounds for believing that the difference is determined by the evolution of the corresponding genes in the plant cells, rather than reflecting their divergence in γ-proteobacteria (*E. coli*) and cyanobacteria, which was the origin of chloroplasts. For instance, it was already in free-living cyanobacteria that *rpo*C was split into *rpo*C1 and *rpo*C2, and *rpo*B–*rpo*C1–*rpo*C2 were isolated in an operon with its own promoter (Fig. 1) [12, 13]. Structural and functional comparisons of PEP and cyanobacterial RNAP will provide further insight into the evolution of PEP in the eukaryotic cell.

NUCLEUS-ENCODED MONOSUBUNIT RNA POLYMERASE (NEP)

NEP was first isolated in 1993 [67], and its genes were identified in 1997 [68]. NEP was found in chloroplasts of higher plants: *P. patens* [69], *A. thaliana* [68, 70], three *Nicotiana* species [71–73], and four cereals [52, 74–76].

In flowering plants, NEP occurs in plastids of virtually all types: proplastids [52]; chloroplasts of cotyledons [73], leaves [52, 73, 74, 76], and stems [73]; etioplasts [6, 77]; root leucoplasts [52, 73, 74, 78, 79]; and amyloplasts of nondifferentiated cells [80, 81], as well as in floral tissues [74]. It is still unclear whether algae possess NEP. Unsuccessful attempts to disrupt the PEP genes in the *Chlamydomonas* plastome [82, 83] and the effect of rifampicin on transcription [84] testify, though indirectly, that PEP is the only RNAP in *Chlamydomonas* chlorotoplasts.

NEP belongs to the monosubunit RNAP family, which includes RNAPs of bacteriophages (T3, T7, SP6, K11) and mitochondria (Fig. 2) (for review, see [85, 86]). A knock-out of one of the two NEP genes suppresses the growth of *A. thaliana* [87]. A specific NEP inhibitor has not been found, but it is known that phage T7 lysozyme inhibits T7 RNAP [88].

Little continues to be known about the NEP structure. NEP is 107–108 kDa in cereals [52, 75, 76] and 113 kDa in *A. thaliana* [68], including a transit peptide of approximately 8 kDa [68]. The C-terminal region is conserved to the greatest extent and the N-terminal region is most variable in RNAPs of this type [68, 74]. The PEP core subunits are not contained in the NEP complex, because NEP is active when the PEP genes are silent or disrupted in plants [5, 6, 89, 90]. In higher plants, the C-terminal region of monosubunit RNAPs contain a conserved amino acid sequence that differs between plastid NEP (**R**-T-S-L-Q-x-L**A**-L-x-R-E-**G**-D-x-x-x) and its mitochondrial counterpart (**K**-T-x-L-Q-V-L-**T**-L-x-x-x-**T**-D-**K**-V-**M**) (the conserved residues specific for the plastid and mitochondrial enzymes are in bold) [52, 91]. This sequence corresponds to the specificity loop of T7 RNAP; this loop is responsible for the interaction with a promoter [74]. Although this region displays only a low similarity between RNAPs of bacteriophage T7 and plants [74, 92], NEP recognizes T7 promoters [41] and T7 RNAP recognizes NEP-dependent promoters [93].

Unlike bacteriophage RNAPs, mitochondrial enzymes require accessory proteins for specific transcription initiation (for review, see [94, 95]). These proteins are termed σ-like, because they are functionally and structurally similar to σ subunits of bacterial-type RNAPs, although there is no similarity in the amino acid sequences interacting with a promoter [75]. It is of interest that one of the maize σ subunits is delivered both in chloroplasts and in mitochondria [64].

Since mitochondrial and plastid RNAPs are encoded by homologous, if not the same, genes, it is probable that NEP also requires accessory proteins for specific transcription initiation. The existence of partner proteins essential for the NEP functional activity is indirectly supported by the fact that antibodies against the region conserved among plant monosubunit RNAPs interact with functionally inactive, but not with active, NEP in chloroplasts [41]. It is possible that these antibodies compete for the binding site with a protein that is required for NEP to interact with a promoter. The binding of NEP to the promoter of the ribosomal operon is ensured by a *trans*-acting factor, CDF2, the composition of which is obscure [41].

Monosubunit RNAPs of plant plastids and mitochondria are encoded by the *Rpo*T genes. The genes are located on different chromosomes [52, 74, 76, 96], are more than 6 kb in size, and are complex in struc-

Fig. 2. Phylogenetic analysis of monosubunit RNAPs. Cluster analysis (ClustalW) was carried out using the Vector NTI package [97]. Both monosubunit RNAP genes and homologous open reading frames were included in the analysis. Group I includes RNAPs of bacteriophages and proteobacteria. Group II includes RNAPs whose genes are on mitochondrial chromosomes and in plasmids along with RNAPs of α-proteobacteria and protozoa. Group III includes RNAPs of fungi and their close relatives Mycetozoa. Group IV includes animal RNAPs. Group V includes plant RNAPs, with individual clusters formed by RNAPs of mitochondrial or mitochondrial–plastid targeting (Va), RNAPs of plastid targeting (Vb), and RNAPs of mosses. RNAPs of bacteriophages: K11 (CAA37330), T3 (NP_523301), T7 (AAP33914), SP6 (NP_853568), and Xp10 (AAP58699). RNAPs of α-proteobacteria: Agrt-T (*Agrobacterium tumefaciens*, NP_531879) and Rp-T (*Rhodopseudomonas palustris*, NP_947869). RNAP of a γ-proteobacterium: Psep-T (*Pseudomonas putida*, NP_744415). RNAPs encoded by the mitochondrial chromosome: mBv1-Tm (*Beta vulgaris*, BAD66791), mBv2-Tm (*B. vulgaris*, T14558), mDc-Tm (*Daucus carota*, AAS15052), and mP1-Tm (*Pylaiella littoralis*, AAC23956). RNAPs encoded by mitochondrial plasmids: mpAb-Tm (*Agaricus bitorquis*, P33539), pmNc-Tm (*Neurospora crassa*, P33540), pmPk-Tm (*Pichia kluyveri*, CAA72339), pmZm1-Tm (*Zea mays*, AAR91041), and pmZm2-Tm (*Z. mays*, S22768). Other RNAPs, encoded by nuclear genes: protozoan Pf-Tm (*Plasmodium falciparum*, NP_701124) and Tb-Tm (*Trypanosoma brucei*, AAK97228); Mycetozoa Dd-Tm (*Dictyostelium discoideum*, AAK73754); fungal Cn-Tm (*Cryptococcus neoformans*, AAN75608), Nc-Tm (*Neurospora crassa*, XP_326163), Sc-Tm (*Saccharomyces cerevisiae*, S56218), and Sp-Tm (*Schizosaccharomyces pombe*, CAB16197); animal Ce-Tm (*Caenorhabditis elegans*, NP_740951), Ag-Tm (*Anopheles gambiae*, EAA12908), Dm-Tm (*Drosophila melanogaster*, NP_608565), Tn-Tm (*Tetraodon nigroviridis*, CAG01374), Xl-Tm (*Xenopus laevis*, AAF19376), Gg-Tm (*Gallus gallus*, XP_418204), Mm-Tm (*Mus musculus*, NP_766139), and Hs-Tm (*Homo sapiens*, AAB58255); plant mitochondrial At-Tm (*A. thaliana*, CAA69331), Ca-Tm (*Chenopodium album*, CAA69305.1), Hv-Tm (*Hordeum vulgare*, CAE52834), Ns-Tm (*Nicotiana sylvestris*, CAC95019), Nt(t)-Tm (*N. tabacum*, the gene inherited from *N. tomentosiformis*, CAC82574), Os-Tm (*Oryza sativa*, BAC76604), Ta-Tm (*Triticum aestivum*, AAF32492), and Zm-Tm (*Z. mays*, AAD22976); plant mitochondrial–plastid Pp-Tmp1 (*P. patens*, CAC95163), Pp-Tmp2 (*P. patens*, CAC95164), At-Tmp (*A. thaliana*, CAC17120), Ns-Tmp (*N. sylvestris*, CAC82575), and Nt(t)-Tmp (*N. tabacum*, the gene inherited from *N. tomentosiformis*, CAC95020); and plant plastid At-Tp (*A. thaliana*, CAA69717), Hv-Tp (*H. vulgare*, CAD45445), Ns-Tp (*N. sylvestris*, CAC82576), Nt(t)-Tp (*N. tabacum*, the gene inherited from *N. tomentosiformis*, CAC95021), Os-Tp (*O. sativa*, BAC98394), Ta-Tp (*T. aestivum*, AAB01085), and Zm-Tp (*Z. mays*, AAD22977).

ture: the *Rpo*T genes of different plants have 18 common introns; some genes have one or two additional introns. The intron size varies from 76 to 816 bp [68, 73], but *N. silvestris Rpo*Tm contains a 17-kb intron [92]. The mature *Rpo*T mRNA is more than 3 kb and codes for 950–1100 amino acid residues. The *Rpo*T genes are evolutionarily conserved; the locations of the 18 common introns are the same in all genes examined in flowering plants; and some introns are located similarly in plant and human *Rpo*T [86].

The similar arrangement of introns in plant and human *Rpo*T suggest that the gene arose early in the evolution of the eukaryotic cell. Genes homologous to the bacteriophage monosubunit RNAP genes were detected in the genomes of some α- and γ-proteobacteria (Fig. 2, groups I and II) and in representatives of the most ancient groups of eukaryotes [98]. Since α-proteobacteria are regarded as evolutionary ancestors of mitochondria [99], it is possible to assume that *Rpo*T found its way into the eukaryotic cell as a component of the α-proteobacterial genome. Fungal and plant mitochondrial DNAs still harbor genes homologous to the monosubunit RNAP genes, but their functions are unknown. Cluster analysis showed that these genes are most similar to the proteobacterial and protozoan genes (Fig. 2, group II), yet they cluster separately from the corresponding genes of bacteriophages and multicellular eukaryotes. These findings support the assumption that the eukaryotic cell acquired the monosubunit RNAP gene as a component of the α-proteobacterial genome. Then, as the bacterium was transformed into an organelle (mitochondrion), the gene was relocated into the nucleus like many other genes of α-proteobacteria and cyanobacteria. The bacterial origin of the gene is indirectly supported by the fact that, in contrast to bacteriophage RNAP, eukaryotic monosubunit RNAP interacts with transcription factors homologous to the σ subunits of bacterial RNAP [94, 95]. Thus, it is possible to assume that the function of monosubunit RNAP was initially associated with mitochondria in the eukaryotic cell.

NEP, a monosubunit RNAP functioning in plastids, originated no later than terrestrial plants, because it is present in the moss *P. patens* [69]. Yet its origin was hardly earlier according to circumstantial evidence [82–84]. Hence, NEP probably originated when terrestrial plants were arising. Development of the terrestrial flora was accompanied by a substantial reorganization of plant morphology and physiology and inevitably involved changes in the structure or expression of many genes. At that time, algal chloroplasts were transformed into plastids with intricate biogenesis. The products of many genes coding for proteins of other cell compartments probably appeared in plastids during this transformation.

The *P. patens* genome harbors two *Rpo*T genes, whose products are delivered into both mitochondria and plastids (*Rpo*Tmp) [69]. *Rpo*T of *P. patiens* was duplicated after the divergence of mosses and vascular plants [69]; i.e., plants had the only *Rpo*Tmp before these two groups were separated. The dicots *N. sylvestris* and *A. thaliana* each have three *Rpo*T. One gene (*Rpo*T1 = *Rpo*Tm) codes for a product transported into mitochondria; another (*Rpo*T2 = *Rpo*Tmp), for a product transported both into mitochondria and plastids; and the third one (*Rpo*T3 = *Rpo*Tp), for a product transported only into plastids [68, 70–73]. Cereals (wheat, barley, rice, and maize), which represent the youngest monocot family, each have two *Rpo*T genes, *Rpo*Tm and *Rpo*Tp, which respectively code for mitochondrial and plastid RNAPs [52, 74–76].

It seems that *Rpo*Tm was transformed into *Rpo*Tmp in the course of evolution, and its product came to drive transcription not only in mitochondria, but also in plastids. The transformation of *Rpo*Tm into *Rpo*Tmp might have been due to the accidental insertion of a fragment of 100–150 bp into the 5'-terminal region of the ancestral gene. The *Rpo*Tmp mRNA contains two active translation initiation sites. When translation starts from the first site, the product is directed into plastids; when the second site is involved, the product is directed into mitochondria [69, 70, 72]. The distance between the two sites is 100–150 bp and corresponds to 35–50 codons. Some other nuclear genes also code for proteins functioning both in plastids and in mitochondria (for review, see [64, 100]).

As with mosses, a similar scenario can be assumed for vascular plants: *Rpo*Tmp was duplicated, and the duplicated copies each lost one of the two translation initiation sites to become *Rpo*Tm and *Rpo*Tp, which is more advantageous for the fine regulation of gene expression in plastids and mitochondria. It is still difficult to tell whether *Rpo*Tmp, whose product functions in both organelles, is widespread in dicots. However, cereals, the youngest and most advanced group of plants, have only two genes, *Rpo*Tm for mitochondrial RNAP and *Rpo*Tp for plastid RNAP [52, 74–76].

Thus, the following evolutionary scenario can be assumed for monosubunit RNAPs. A T7-like RNAP gene found its way in a proteobacterium (it cannot be excluded, however, that bacteriophages acquired a bacterial RNAP gene). As a component of the α-proteobacterial genome, the monosubunit RNAP gene was incorporated in the eukaryotic cell. As bacteria were transformed into mitochondria, the gene was transferred into the nucleus, but the product preserved its mitochondrial function. It was presumably during the formation of terrestrial plants that the monosubunit RNAP gene was slightly changed (see above) so that its product came to be transported not only into mitochondria, but also in plastids. Then the gene was

duplicated (probably, more than once), and the resulting copies gradually evolved to the state in which one gene serves one organelle. A scheme of the putative evolution of the *Rpo*T genes is shown in Fig. 3.

The regulation of *Rpo*T gene expression in plants is still poorly understood, yet some differences between *Rpo*Tp and *Rpo*Tm are known. The amount of the *Rpo*Tp mRNA is almost always greater than that of the *Rpo*Tm (and *Rpo*Tmp) mRNA [52, 71, 74]. An opposite proportion is observed only in etiolated plants [74]. Mutant plants defective in chloroplast development display a higher level of *Rpo*Tp expression; the expression of *Rpo*Tm (and *Rpo*Tmp) remains unchanged or is also increased, but later and to a lesser extent [52, 71]. The maximal activity of the *Rpo*T genes and their products is characteristic of the earliest stage of plant development, *Rpo*Tp being activated later than *Rpo*Tm [74, 76].

NEP synthesis is most intense in young leaves and is considerably suppressed during cell differentiation and aging [41, 52, 73, 74, 76, 101]. Maize is the only known exception: the amount of the NEP mRNA increases along the gradient of leaf cell differentiation (up to 5 cm) and then decreases, although not so quickly as in barley [52, 74]. The NEP activity is much the same as the PEP activity in young leaves, while NEP accounts for only 1–5% of total transcription in chloroplasts of mature tissues [11, 41]. However, NEP is capable of transcribing the whole of the plastid DNA in the absence of PEP [102, 103].

NEP is utilized not only by the plant itself, but also by its parasites. For instance, the avocado viroid is replicated in plastids with the use of NEP [104].

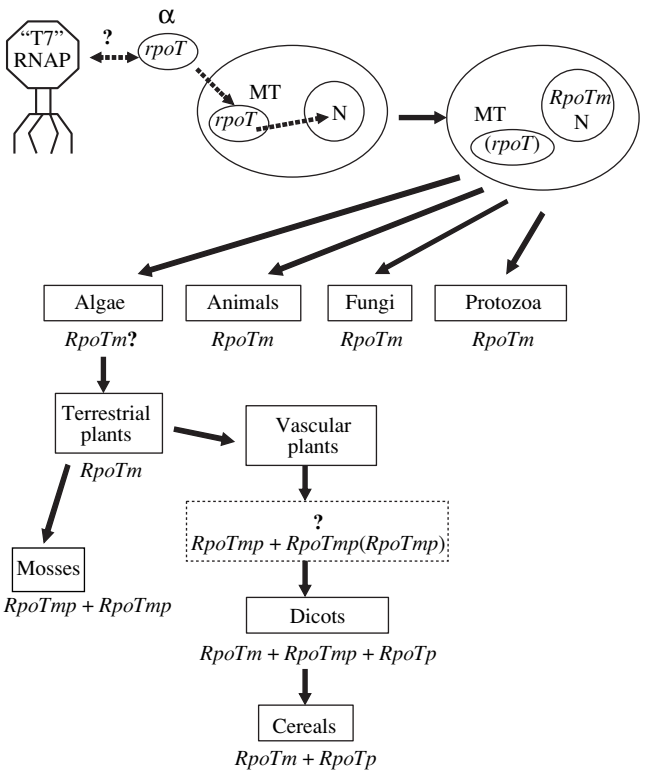


Fig. 3. Hypothetical evolution of eukaryotic genes for monosubunit RNAPs. "T7" RNAP, protein similar to bacteriophage T7 RNAP; *rpo*T, gene for monosubunit RNAP of bacteria and mitochondria; *Rpo*T, nuclear genes for monosubunit RNAPs targeted to mitochondria (*Rpo*Tm), both mitochondria and plastids (*Rpo*Tmp), or plastids (*Rpo*Tp); α, α-proteobacterium; MT, mitochondrion; N, nucleus. Putative steps of RNAP evolution are indicated with (?) and dashed lines. See text for comments.

OTHER PLASTID RNA POLYMERASES

Pea plastids were shown to possess a primase activity, which is involved in the synthesis of 4- to 60-nt ribonucleuotide primers during the replication of plastid DNA [105]. Pea chloroplast primase is 115–120 kDa in size and is not inhibited by tagetin. The primase function in plastids is possibly performed by NEP, the more so as RNAPs of the same type play a similar role in bacteriophage T7 [106] and in mitochondria [107, 108].

Spinach and pea chloroplasts contain template-independent RNAP–polynucleotide phosphorylase, which polyadenylates RNA molecules and plays a role in their degradation [109, 110].

DIVISION OF FUNCTIONS BETWEEN RNA POLYMERASES

The functions of both RNAPs are closely associated with the biogenesis of plastids. NEP and PEP are most active during the transformation of proplastids into chloroplasts. The development of chloroplasts requires the expression of the photosynthesis genes. Key proteins of photosynthetic complexes are encoded by the plastome, and special machinery must be generated preliminarily to ensure the expression of the corresponding genes. In addition, the plastome codes for many proteins (and RNAs) involved in transcription, RNA processing, translation, and protein degradation in plastids [111]. Their genes are transcribed and the transcripts translated at the initial stage of proplastid differentiation.

During the early differentiation of leaf cells, NEP synthesis is activated before PEP synthesis [52]. NEP efficiently transcribes the plastome transcription/translation genes and is almost inactive with the photosynthesis genes [77, 86]. In the absence of PEP, NEP can ensure the function of the gene expression machinery in plastids [7], but not the formation of the functional photosynthetic system [5–7]. We have further developed the existing hypothesis of the division of RNAP functions during chloroplast differentiation [86]. This hypothesis is illustrated in Fig. 4. Transcription of plastid genes is preceded by the activation

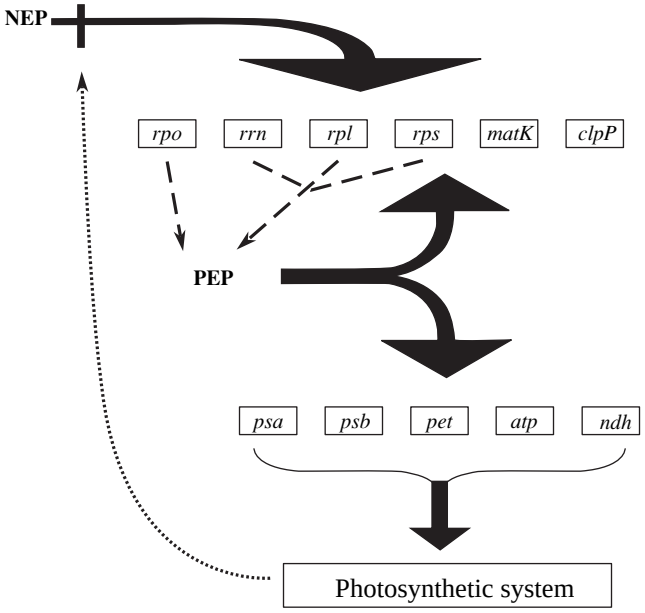


Fig. 4. Interplay of NEP and PEP during chloroplast differentiation. Solid arrows show the role of RNAP in the transcription of plastome genes and the formation of the photosynthetic system as a result of the expression of the corresponding plastid genes. Dashed arrows show the role of plastome genes in PEP synthesis. A dotted arrow shows the presumable role of the photosynthetic system of chloroplasts in the inhibition of the expression of the NEP gene. The *mat*K gene codes for maturase, which is involved in the maturation of plastid RNAs. The proteins encoded by the other genes are indicated in the table.

of nuclear *Rpo*Tp [52]. Newly synthesized NEP is transported into proplastids and begins to transcribe the transcription/translation genes, including those coding for PEP [50, 52]. In turn, PEP transcribes both transcription/translation and photosynthesis genes. As its activity increases, the formation of the gene expression machinery is continued and that of the photosynthesis system begins [52].

The activities of plastid RNAPs are probably determined by the internal developmental program during the early differentiation of leaf cells. In this period, the transcription of many plastid photosynthesis genes is light-independent [53, 112, 113] and the transcription of the transcription/translation genes is activated regardless of whether the plant is capable of generating chloroplasts [52].

Chloroplast formation is accompanied by a decrease in the amount of the NEP-coding *Rpo*Tp mRNA in leaf cells. In barley mutants defective in chloroplast formation, the amount of the *Rpo*TP mRNA fails to decrease for a long time and then even increases, suggesting that the *Rpo*Tp transcription in greening leaves is suppressed by a plastid signal [52]. Such a signal can be provided by the accumulation of chlorophyll synthesis intermediates or by the reduction/oxidation of some molecules (e.g., glutathione or plastoquinones) [114]. Some proteins can act as signal molecules [115]. Being transduced from chloroplasts to the nucleus, these signals regulate the expression of many genes.

Thus, during chloroplast differentiation, NEP presumably initiates the expression of the PEP and ribosomal genes; PEP supports the generation of components of the plastid gene expression machinery and ensures the formation of the photosynthetic system; and unidentified factors generated together with the photosynthetic system (possibly, as a result of photosynthesis) suppress the synthesis of NEP.

In differentiated chloroplasts, PEP transcribes most genes [11] while the NEP activity is extremely low [41, 67]. What is the role of NEP in mature plastids?

According to the hypothesis of the division of RNAP functions in differentiated chloroplasts, PEP predominantly transcribes the photosynthesis genes and NEP transcribes the transcription/translation genes [77, 86]. Yet the situation is not so simple. Data on the roles of PEP and NEP in the transcription of plastid genes are summarized in the table. The genes are divided into three groups: group I (PEP) harbors the genes that are transcribed predominantly (or exclusively) by PEP; group II (PEP/NEP), the genes that are transcribed by both enzymes; and group III (NEP), the genes that are transcribed predominantly (or exclusively) by NEP. The contribution of either RNAP to the transcription of particular genes was inferred from the results of promoter analysis or by a comparison of the transcription intensity and the amount of the corresponding mRNA between PEP-lacking mutants and wild-type plants.

As the table demonstrates, the photosynthesis genes are transcribed predominantly by PEP in mature chloroplasts. Yet PEP transcribes many transcription/translation genes as well: the rRNA and tRNA genes are transcribed by PEP or by both RNAPs. Analysis of the *A. thaliana* Δ*Sig2* mutant suggest the important role of PEP in the transcription of the tRNA genes [29]. Some genes for ribosomal proteins are also transcribed by both RNAPs or mostly by PEP.

NEP is important for the transcription of the genes coding for PEP, Clp protease, and many ribosomal proteins in mature chloroplasts (table). This conclusion is based on the fact that these genes are transcribed more intensely in PEP-lacking plastids. Other factors can also increase the transcription of these genes in etioplasts. However, the currently available data make it possible to believe that the *Rpo* operon (the genes coding for the PEP subunits β, β', and β'') and cereal *clp*P (protease Clp) are transcribed exclusively by NEP.

It is still unclear why it is that many genes are transcribed by both RNAPs in mature chloroplasts (table).

Transcription of plastome genes by RNAPs

Gene	I PEP	II PEP/NEP	III NEP
rbcL	[5, 11, 90, 102, 103, 116, 118, 120, 124]		
psb	**A** [5, 11, 90, 102, 103, 116] **B** [77] **D-C** [5, 102, 103, 124] **E-F-L-J** [77, 103]		
psa	**A-B** [77, 103]	**A-B** [11, 102, 103]	
pet	**B** [11]	**B-D** [103, 119]	
atp	**B** [118] **F** [116]	**B-E** [11, 77, 102, 117, 117] **I-H-F-A** [5, 11, 77, 90, 103, 119, 121]	
ndh	**A** [77]	**F** [77] **J** [103]	**B** [77, 103]
rpo		**A** [102]	**B-C1-C2** [90, 102, 103, 116, 117]
rrn 16-23-5-4.5	[116, 118, 122, 124]	[5, 11, 80, 90, 102, 103, 122]	
trn	**F** [103] **G** [119] **H** [103] **M** [103] **N** [90] **Q** [103] **R** [90] **V** [119]	**E** [116] **G** [103] **I** [103] **R** [103] **V** [103]	
rps	**14** [77, 103]	**2** [116] **4** [116] **15** [90] **16** [77]	**3** [103] **7** [103] **12** [103, 123] **15** [116] **18** [77, 103]
rpl	**16** [11]	**16** [5] **32** [81]	**23-2** [116, 122, 123] **20** [103] **33** [77, 103]
clpP		[77]	[77, 103, 116, 117, 118]
accD		[102, 103]	[102, 77]
ycf		**1** [77]	**2** [77, 103] **3** [103] **10** [103] **15** [103]

Note: Group I (PEP), genes transcribed predominantly or exclusively by PEP; group II (PEP/NEP), genes transcribed by both enzymes; and group III (NEP), genes transcribed predominantly or exclusively by NEP. The *rbcL* gene codes for the large subunit of ribulose bisphosphate carboxylase; *psb*, for a photosystem II subunit; *psa*, for a photosystem I subunit; *pet*, for a subunit of the cytochrome b_6/f complex; *atp*, for an ATP synthase subunit; *ndh*, for an NADH dehydrogenase subunit; *rpo*, for a PEP subunit; *rrn*, for an rRNA; *trn*, for a tRNA; *rps*, for a small-subunit ribosomal protein; *rpl*, for a large-subunit ribosomal protein; *clpP*, for the proteolytic subunit of protease Clp; and *accD*, for the β subunit of acetyl-CoA carboxylase-carboxytransferase; *ycf* are conserved plastid genes with unknown functions. The results were obtained with tobacco [5, 11, 77, 80, 81, 102, 013, 120, 121], barley [90, 116, 119, 122, 123, 124], maize [116, 117], rice [118], and rye [90].

Possibly, such a mode of transcription allows for a fine regulation of gene expression. An example of differential expression regulation is provided by *psb*D. In *A. thaliana*, SIG2-containing PEP ensures the constitutive transcription of *psb*D from one promoter [28] while SIG5-containing PEP ensures inducible transcription from another promoter [30].

Thus, the functions of PEP and NEP in mature chloroplasts are as follows. PEP is responsible for more than 90% of total transcription and transcribes most photosynthesis and many translation genes. Both NEP and PEP transcribe some photosynthesis genes (e.g., all ATP synthase genes of the plastome) and many translation genes. Some transcription/translation genes are transcribed exclusively by NEP. In the absence of PEP, NEP is capable of transcribing the whole of the plastid DNA, but only to a low level [102, 103]. However, such transcription may be due to nonspecific initiation, since bacteriophage monosubunit RNAPs are capable of directing transcription in the absence of promoters [125].

It is possibly NEP that ensures transcription in nonphotosynthesizing plastids, because NEP is synthesized in all organs while almost nothing is known on the presence of PEP in nonphotosynthesizing organs.

The mechanisms regulating the expression of the PEP and NEP genes are still poorly understood. The RNAP functions are probably associated with photosynthesis in chloroplasts. The development of photosynthesizing chloroplasts probably suppresses the NEP activity [52]. The following sequence of events was demonstrated for mature chloroplasts: increase in photosynthesis ⟶ reduction of the glutathione pool ⟶ inactivation of casein kinase 2 ⟶ activation of PEP [47, 126]. Further study will provide for a better understanding of the mechanisms regulating the activities of the two RNAPs and their interaction in plastids.

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REFERENCES

1. Shematorova E.K., Shpakovsky G.V. 2002. Structure and functions of eukaryotic DNA-dependent RNA polymerase I. *Mol. Biol*. **36**, 1–17.
2. Pfannschmidt T., Link G. 1994. Separation of two classes plastid DNA-dependent RNA polymerases that are differently expressed in mustard. *Plant Mol. Biol*. **25**, 69–81.
3. Rajasekhar V.K., Sun E., Meeker R., Wu B.W., Tewari K.K. 1991. Highly purified pea chloroplast RNA polymerase transcribes both rRNA and mRNA genes. *Eur. J. Biochem*. **195**, 215–228.
4. Suzuki J.Y., Ytterberg J., Beardslee T.A., Allison L.A., Wijk K.J., Maliga P. 2004. Affinity purification of the tobacco plastid RNA polymerase and *in vitro* reconstitution of the holoenzyme. *Plant J*. **40**, 164–172.
5. Allison L.A., Simon L.D., Maliga P. 1996. Deletion of *rpo*B reveals a second distinct transcription system in plastids of higher plants. *EMBO J*. **15**, 2802–2809.
6. Serino G., Maliga P. 1998. RNA polymerase subunits encoded by the plastid *rpo* genes are not shared with the nucleus encoded plastid enzyme. *Plant Physiol*. **117**, 1165–1170.
7. DeSantis-Maciossek G., Kofer W., Bock A., Rudiger W., Koop H.U., Herrmann R.G. 1999. Targeted disruption of the plastid RNA polymerase genes *rpoA, B* and *C1*: Molecular biology, biochemistry and ultrastructure. *Plant J*. **18**, 477–489.
8. Wolfe K.H., Morden C.W., Palmer J.D. 1992. Function and evolution of a minimum plastid genome from a nonphotosynthetic parasitic plant. *Proc. Natl. Acad. Sci. USA*. **89**, 10648–10652.
9. Berg S., Krupinska K., Krause K. 2003. Plastids of three *Cuscuta* species differing in plastid coding capacity have a common parasite-specific RNA composition. *Planta*. **218**, 135–142.
10. Krause K., Berg S., Krupinska K. 2003. Plastid transcription in the holoparasitic plant genus *Cuscuta*: parallel loss of the *rrn16* PEP-promoter and of the *rpoA* and *rpoB* genes coding for the plastid-encoded RNA polymerase. *Planta*. **216**, 815–823.
11. Sakai A., Saito C., Inada N., Kuroiwa T. 1998. Transcriptional activities of the chloroplast-nuclei and proplastid-nuclei isolated from tobacco exhibit different sensitivities to tagetitoxin: Implication of the presence of distinct RNA polymerases. *Plant Cell Physiol*. **39**, 928–934.
12. Bergsland K.J., Haselkorn R. 1991. Evolutionary relationships among eubacteria, cyanobacteria, and chloroplasts: Evidence from the *rpoC1* gene of *Anabaena* sp. strain PCC7120. *J. Bacteriol*. **173**, 3446–3455.
13. Kaneko T., Sato S., Kotani H., Tanaka A., Asamizu E., Nakamura Y., Miyajima N., Hirosawa M., Sugiura M., Sasamoto S., Kimura T., Hosouchi T., Matsuno A., Muraki A., Nakazaki N., Naruo K., Okumura S., Shimpo S., Takeuchi C., Wada T., Watanabe A., Yamada M., Yasuda M., Tabata S. 1996. Sequence analysis of the genome of the unicellular cyanobacterium *Synechocystis* sp. strain PCC6803: 2. Sequence determination of the entire genome and assignment of potential protein-coding regions. *DNA Res*. **3**, 109–136.
14. Sugiura Ch., Kobayashi Yu., Aoki S., Sugita Ch., Sugita M. 2003. Complete chloroplast DNA sequence of the moss *Physcomitrella patens*: Evidence for the loss and relocation of *rpoA* from the chloroplast to the nucleus. *Nucleic Acids Res*. **31**, 5324–5331.
15. Minakhin L., Bhagat S., Brunning A., Campbell E.A., Darst S.A., Ebright R.H., Severinov K. 2001. Bacterial RNA polymerase subunit ω and eucaryotic RNA polymerase subunit RPB6 are sequence, structural, and functional homologs and promote RNA polymerase assembly. *Proc. Natl. Acad. Sci. USA*. **98**, 892–897.
16. Hu J., Bogorad L. 1990. Maize chloroplast RNA polymerase: The 180-, 120-, and 38-kilodalton polypeptides are encoded in chloroplast genes. *Proc. Natl. Acad. Sci. USA*. **87**, 1531–1535.
17. Hu J., Troxler R.F., Bogorad L. 1991. Maize chloroplast RNA polymerase: The 78-kilodalton polypeptide is encoded by the plastidic *rpoC1* gene. *Nucleic Acids Res*. **19**, 3431–3434.
18. Severinov K., Mustaev A., Kukarin A., Muzzin O., Bass I., Darst S.A., Goldfarb A. 1996. Structural modules of the large subunits of RNA polymerase. *J. Biol. Chem*. **271**, 27969–27974.
19. Chen Z., Muthukrishnan S., Liang G.H., Schertz K.F., Hart G.E. 1993. A chloroplast DNA deletion located in RNA polymerase gene *rpoC2* in CMS lines of sorghum. *Mol. Gen. Genet*. **236**, 251–249.
20. Sugiura M. 1992. The chloroplast genome. *Plant Mol. Biol*. **19**, 149–168.
21. Kanno A., Hirai A. 1993. A transcription map of the chloroplast genome from rice (*Oryza sativa*). *Curr. Genet*. **23**, 166–174.
22. Downing W.L., Dennis P.P. 1987. Transcription products from the *rplKAJL–rpoBC* gene cluster. *J. Mol. Biol*. **194**, 609–620.
23. Ishihama A. 2000. Functional modulation of *Escherichia coli* RNA polymerase. *Annu. Rev. Microbiol*. **54**, 499–518.
24. Ozoline O.N., Purtov Yu.A., Brok-Volchanski A.S., Deev A.A., Lukyanov V.I. 2004. Specificity of DNA–protein interactions within transcription complexes of *Escherichia coli*. *Mol. Biol*. **38**, 786–797.
25. Imamura S., Yoshihara S., Nakano S., Shiozaki N., Yamada A., Tanaka K., Takahashi H., Asayama M., Shirai M. 2003. Purification, characterization, and gene expression of all sigma factors of RNA polymerase in a cyanobacterium. *J. Mol. Biol*. **325**, 857–872.
26. Kestermann M., Neukirchen S., Kloppstech K., Link G. 1998. Sequence and expression characteristics of a nuclear-encoded chloroplast sigma factor from mustard (*Sinapis alba*). *Nucleic Acids Res*. **26**, 2747–2753.
27. Homann A., Link G. 2003. DNA-binding and transcription characteristics of three cloned sigma factors from mustard (*Sinapis alba* L.) suggest overlapping and distinct roles in plastid gene expression. *Eur. J. Biochem*. **270**, 1288–1300.
28. Hanaoka M, Kanamaru K, Takahashi H, Tanaka K. 2003. Molecular genetic analysis of chloroplast gene promoters dependent on SIG2, a nucleus-encoded sigma factor for the plastid-encoded RNA polymerase, in *Arabidopsis thaliana*. *Nucleic Acids Res*. **31**, 7090–7098.

29. Kanamaru K., Nagashima A., Fujiwara M., Shimada H., Shirano Y., Nakabayashi K., Shibata D., Tanaka K., Takahashi H. 2001. An *Arabidopsis* sigma factor (SIG2)-dependent expression of plastid encoded tRNAs in chloroplasts. *Plant Cell Physiol*. **42**, 1034–1043.

30. Nagashima A., Hanaoka M., Shikanai T., Fujiwara M., Kanamaru K., Takahashi H., Tanaka K. 2004. The multiple-stress responsive plastid sigma factor, SIG5, directs activation of the *psbD* blue light-responsive promoter (BLRP) in *Arabidopsis thaliana*. *Plant Cell Physiol*. **45**, 357–368.

31. Morikawa K., Shiina T., Murakami S., Toyoshima Y. 2002. Novel nuclear-encoded proteins interacting with a plastid sigma factor, Sig1, in *Arabidopsis thaliana*. *FEBS Lett*. **514**, 300–304.

32. Hara K., Morita M., Takahashi R., Sugita M., Kato S., Aoki S. 2001. Characterization of two genes, *Sig1* and *Sig2*, encoding distinct plastid sigma factors (1) in the moss *Physcomitrella patens*: Phylogenetic relationships to plastid sigma factors in higher plants. *FEBS Lett*. **499**, 87–91.

33. Hara K., Sugita M., Aoki S. 2001. Cloning and characterization of the cDNA for a plastid sigma factor from the moss *Physcomitrella patens*. *Biochem. Biophys. Acta*. **1517**, 302–306.

34. Oikawa K., Tanaka K., Takahashi H. 1998. Two types of differently photo-regulated nuclear genes that encode sigma factors for a chloroplast RNA polymerase in the red alga *Cyanidium caldarium* strain RK-1. *Gene*. **210**, 277–285.

35. Severinov K., Soushko M., Goldfarb A., Nikiforov V. 1993. Rifampicin region revisited. New rifampicin-resistant and streptolydigin-resistant mutants in the β subunit of *Escherichia coli* RNA polymerase. *J. Biol. Chem*. **268**, 14820–14825.

36. Surzycki S.J. 1969. Genetic functions of the chloroplasts of *Chlamydomonas reinchardtii*: Effect of rifampin on chloroplast DNA-dependent RNA polymerase. *Proc. Natl. Acad. Sci. USA*. **63**, 1327–1334.

37. Pfannschmidt T., Link G. 1997. The A and B form of plastid DNA-dependent RNA polymerases from mustard (*Sinapis alba* L.) transcribe the same genes in a different developmental context. *Mol. Gen. Genet*. **257**, 35–44.

38. Wegrzyn A., Szalewska-Palasz A., Blaszczak A., Liberek K., Wegrzyn G. 1998. Differential inhibition of transcription from σ^{70}- and σ^{32}-dependent promoters by rifampicin. *FEBS Lett*. **440**, 172–174.

39. Pfannschmidt T., Ogrzewalla K., Baginsky S., Sickmann A., Meyer H.E., Link G. 2000. The multisubunit RNA polymerase A from mustard (*Sinapis alba* L.): Integration of procaryotic core into a larger complex with organelle specific functions. *Eur. J. Biochem*. **267**, 253–261.

40. Mathews D.E., Durbin R.D. 1990. Tagetitoxin inhibits RNA synthesis directed by RNA polymerase from chloroplasts and *Escherichia coli*. *J. Biol. Chem*. **265**, 493–498.

41. Bligny M., Courtois F., Thaminy S., Chang C.-C., Lagrange T., Baruah-Wolff J., Stern D.B., Lerbs-Mache S. 2000. Regulation of plastid rDNA transcription by interaction of CDF2 with two different RNA polymerases. *EMBO J*. **19**, 1851–1860.

42. Steinberg T.H., Mathews D.E., Durbin R.D., Burgess R.R. 1990. Tagetitoxin: A new inhibitor of eucaryotic transcription by RNA polymerase III. *J. Biol. Chem*. **265**, 499–505.

43. SteinbergT.H., Burgess R.R. 1992. Tagetitoxin inhibition of RNA polymerase III transcription results from enchanced pausing at discrete sites and is template-dependent. *J. Biol. Chem*. **267**, 20204–20211.

44. Knorre D.G. 1999. RNA polymerase II. *Mol. Biol*. **32**, 129–134.

45. Paule M.R., White R.J. 2000. Survey and summary: Transcription by RNA polymerase I and III. *Nucleic Acids Res*. **28**, 1283–1298.

46. Baginsky S., Tiller K., Link G. 1997. Transcription factor phosphorylation by a protein kinase associated with chloroplast RNA polymerase from mustard (*Sinapis alba*). *Plant Mol. Biol*. **34**, 181–189.

47. Baginsky S., Tiller K., Pfannschmidt T., Link G. 1999. PTK, the chloroplast RNA polymerase-associated protein kinase from mustard (*Sinapis alba*), mediates redox control of plastid *in vitro* transcription. *Plant Mol. Biol*. **39**, 1013–1023.

48. Ogrzewalla K., Piotrowski M., Reinbothe S., Link G. 2002. The plastid transcription kinase from mustard (*Sinapis alba* L.). A nuclear encoded CK2 type chloroplast enzyme with redox-sensitive function. *Eur. J. Biochem*. **269**, 3329–3337.

49. Tiller K., Link G. 1993. Phosphorylation and dephosphorylation affect functional characteristics of chloroplast and etioplast transcription system from mustard (*Sinapis alba*). *EMBO J*. **12**, 1745–1753.

50. Baumgartner B.J., Rapp J.C., Mullet J.E. 1993. Plastid genes encoding the transcription/translation apparatus are differentially transcribed early in barley (*Hordeum vulgare*) chloroplast development (Evidence for selective stabilization of *psbA* mRNA). *Plant Physiol*. **101**, 781–791.

51. Inada H., Kusumi K., Nishimura M., Iba K. 1996. Specific expression of the chloroplast gene for RNA polymerase (*rpoB*) at an early stage of leaf development in rice. *Plant Cell Physiol*. **37**, 229–232.

52. Emanuel C., Weihe A., Graner A., Hess W.R., Borner T. 2004. Chloroplast development affects expression of phage-type RNA polymerases in barley leaves. *Plant J*. **38**, 460–472.

53. Satoh J., Baba K., Nakahira Y., Tsunoyama Y., Shiina T., Toyoshima Y. 1999. Developmental stage-specific multi-subunit plastid RNA polymerase (PEP) in wheat. *Plant J*. **18**, 407–415.

54. Eisermann A., Tiller K., Link G. 1990. *In vitro* transcription and DNA binding characteristics of chloroplast end etioplast extract from mustard (*Sinapis alba*) indicate differential usage of *psbA* promoter. *EMBO J*. **9**, 3981–3987.

55. Gamble P.E., Mullet J.E. 1989. Blue light regulates the accumulation of two *psbD–psbC* transcripts in barley chloroplasts. *EMBO J*. **8**, 2785–2794.

56. Nakahira Y., Baba K., Yoneda A., Shiina T., Toyoshima Y. 1998. Circadian-regulated transcription of the *psbD* light-responsive promoter in wheat chloroplsts. *Plant Physiol*. **118**, 1079–1088.

57. Kim M., Thum K.E., Morishige D.T., Mullet J.E. 1999. Detailed architecture of the barley chloroplast *psbD–psbC* blue light-responsive promoter. *J. Biol. Chem*. **274**, 4684–4692.
58. Thum K.E., Kim M., Morishige D.T., Eibl C., Koop H.-U., Mullet J.E. 2001. Analysis of barley chloroplast *psbD* light responsive promoter elements in transplastomic tobacco. *Plant. Mol. Biol*. **47**, 353–366.
59. Chen L.J., Orozco E.M., Jr. 1988. Recognition of prokaryotic transcription terminators by spinach chloroplast RNA polymerase. *Nuceic Acids Res.* **16**, 8411–8431.
60. Gatenby A.A., Castleton J.A., Saul M.W. 1981. Expresssion in *E. coli* of *Z. mays* and wheat chloroplast genes for large subunit of ribulose biphosphate carboxylase. *Nature*. **291**, 117–121.
61. Bradley D., Gatenby A.A. 1985. Mutational analysis of the maize chloroplast ATPase-β subunit gene promoter: The isolation of promoter mutants in *E. coli* and their characterisation in a chloroplast *in vitro* transcription system. *EMBO J*. **4**, 3641–3648.
62. Boyer S.K., Mullet J. 1986. Characterisation of *Pisum sativum* chloropast *psbA* transcript produced *in vivo*, *in vitro* and in *E. coli*. *Plant. Mol. Biol*. **6**, 229–243.
63. Kim M., Mullet J.E. 1995. Identification of a sequence-specific DNA binding factor required for transcription of the barley chloroplast blue light-responsive *psbD–psbC* promoter. *Plant Cell*. **7**, 1445–1457.
64. Beardslee T.A., Roy-Chowdhury S., Jaiswal P., Buhot L., Lerbs-Mache S., Stern D.B., Allison L.A. 2002. A nucleus-encoded maize protein with sigma factor activity accumulates in mitochondria and chloroplasts. *Plant J*. **31**, 199–209.
65. Lerbs-Mache S. 2000. Regulation of rDNA transcription in plastids of higher plants. *Biochimie*. **82**, 525–535.
66. Suzuki J.Y., Maliga P. 2000. Engineering of the *rpl23* gene cluster to replace the plastid RNA polymerase alpha subunit with the *Escherichia coli* homologue. *Curr. Genet*. **38**, 218–225.
67. Lerbs-Mache S. 1993. The 110-kDa polypeptide of spinach DNA-dependent RNA polymerase: Single-subunit enzyme or catalytic core of multimeric enzyme complexes? *Proc. Natl. Acad. Sci. USA*. **90**, 5509–5513.
68. Hedtke B., Borner T., Weihe A. 1997. Mitochondrial and chloroplast phage-type RNA polymerases in *Arabidopsis. Science*. **277**, 809–811.
69. Richter U., Kiessling J., Hedtke B., Decker E., Reski R., Borner T., Weihe A. 2002. Two *RpoT* genes of *Physcomitrella patens* encode phage type RNA polymerases with dual targeting to mitochondria and plastids. *Gene*. **290**, 95–105.
70. Hedtke B., Borner T., Weihe A. 2000. One RNA polymerase serving two genomes. *EMBO Rep*. **1**, 435–440.
71. Hedtke B., Legen J., Weihe A., Herrmann R.G., Borner T. 2002. Six active phage-type RNA polymerase genes in *Nicotiana tabacum. Plant J*. **30**, 625–637.
72. Kobayashi Y., Dokiya Y., Sugita M. 2001. Dual targeting of phage-type RNA polymerase to both mitochondria and plastids is due to alternative translation initiation in single transcripts. *Biochem. Biophys. Res. Commun*. **289**, 1106–1113
73. Kobayashi Y., Dokiya Y., Kumazawa Y., Sugita M. 2002. Non-AUG translation initiation of mRNA encoding plastid-targeted phage-type RNA polymerase in *Nicotiana sylvestris. Biochem. Biophys. Res. Commun*. **299**, 57–61.
74. Chang C.-C., Sheen J., Bligny M., Niwa Y., Lerbs-Mache S., Stern D.B. 1999. Functional analysis of two maize cDNA encoding T7-like RNA polymerases. *Plant Cell*. **11**, 911–926.
75. Ikeda T.M., Gray M.W. 1999. Identification and characterization of T3/T7 bacteriophage-like RNA polymerase sequences in wheat. *Plant. Mol. Biol*. **40**, 567–578.
76. Kusumi K., Yara A., Mitsui N., Tozawa Yu., Iba K. 2004. Characterisation of a rice nuclear-encoded plastid RNA polymerase gene *OsRpoTp. Plant Cell Physiol*. **45**, 1194–1201.
77. Hajdukiewicz P.T.J., Allison L.A., Maliga P. 1997. The two RNA polymerase encoded by the nuclear and the plastid compartments transcribe distinct groups of genes in tobacco plastids. *EMBO J*. **16**, 4041–4048.
78. Iratni R., Diedrich L., Harrak H., Bligny M., Lerbs-Mache S. 1997. Organ-specific transcription of the *rrn*-operon in spinach plastids. *J. Biol.Chem.* **272**, 13676–13682.
79. Hedtke B., Meixner M., Gillandt S., Richter E., Borner T., Weihe A. 1999. Green fluorescent protein as a marker to investigate targeting of organellar RNA polymerases of higher plants *in vivo. Plant J*. **17**, 557–561.
80. Vera A., Sugiura M. 1995. Chloroplast rRNA transcription from structurally different tandem promoters: An additional novel-type promoter. *Curr. Genet*. **27**, 280–284.
81. Vera A., Hirose T., Sugiura M. 1996. A ribosomal protein gene (*rpl32*) from tobacco chloroplast DNA is transcribed from alternative promoters: Similarities in promoter region organization plastid housekeeping genes. *Mol. Gen. Genet*. **251**, 518–525.
82. Goldschmidt-Clermont M. 1991. Transgenic expression of aminoglicoside adenine transferase in the chloroplast: A selectable marker for site-directed transformation of *Chlamydomonas. Nucleic Acids Res*. **19**, 4083–4090.
83. Fisher N., Stampacchia O., Redding K., Rochaix J.D. 1996. Selectable marker recycling in the chloroplast. *Mol. Gen. Genet*. **251**, 373–380.
84. Eberhard S., Drapier D., Wollman F.-A. 2002. Searching limiting steps in the expression of chloroplast-encoded proteins: Relations between gene copy number, transcription, transcript abundance and translation rate in the chloroplast of *Chlamydomonas reinhardtii. Plant J*. **31**, 149–160.
85. Cermakian N., Ikeda T.M., Miramontes P., Lang B.F., Gray M.W., Cedergren R. 1997. On the evolution of the single-subunit RNA polymerases. *J. Mol. Evol*. **45**, 671–681.
86. Hess W.R., Borner T. 1999. Organellar RNA polymerases of higher plants. *Int. Rev. Cytol*. **190**. 1–59.
87. Baba K., Schmidt J., Espinosa-Ruiz A., Villarejo A., Shiina T., Gardestrom P., Sane A.P., Bhalerao R.P. 2004. Organellar gene transcription and early seedling development are affected in the *rpoT2* mutant of *Arabidopsis. Plant J*. **38**, 38–48.

88. Zhang X., Studier F.W. 1997. Mechanism of inhibition of bacteriophage T7 RNA polymerase by T7 lysozyme. *J. Mol. Biol*. **269**, 10–27.

89. Han C.D., Patrie W., Polacco M., Coe E.H. 1993. Aberrations in plastid transcripts and deficiency of plastid DNA in striped and albino mutants in maize. *Planta*. **191**, 552–563.

90. Hess W.R., Prombona A., Fieder B., Subramanian A.R., Börner T. 1993. Chloroplast *rps15* and the *rpoB/C1/C2* gene cluster are strongly transcribed in ribosome deficient plastids: Evidence for a functioning non-chloroplast encoded RNA polymerase. *EMBO J*. **12**, 563–571.

91. Kabeya Y., Hashimoto K., Sato N. 2002. Identification and characterization of two phage-type RNA polymerase cDNAs in the moss *Physcomitrella patens*: Implication of recent evolution of nuclear encoded RNA polymerase of plastids in plants. *Plant Cell Physiol*. **43**, 245–255.

92. Kobayashi Y, Dokiya Y, Sugiura M., Niwa Y., Sugita M. 2001. Genomic-organization and organ-specific expression of a nuclear gene encoding phage-type RNA polymerase in *Nicotiana sylvestris. Gene*. **279**, 33–40.

93. Magee A.M., Kavanagh T.A. 2002. Plastid genes transcribed by the nucleus-encoded plastid RNA polymerase show increased transcript accumulation in transgenic plants expressing a chloroplast-localized phage T7 RNA polymerase. *J. Exp. Bot*. **53**, 2341–2349.

94. Shadel G.S., Clayton D.A. 1993. Mitochondrial transcription initiation. Variation and coservation. *J. Biol. Chem*. **268**, 16083–16086.

95. Ikeda T.M., Gray M.W. 1999. Characterization of a DNA-binding protein implicated in transcription in wheat mitochondria. *Mol. Cell Biol*. **19**, 8113–8122.

96. *Arabidopsis* Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana. Nature*. **408**, 796–815.

97. Lu G., Moriyama E.N. 2004. Vector NTI, a balanced all-in-one sequence analysis suite. *Brief. Bioinform*. **5**, 378–388.

98. Cermakian N., Ikeda T.M., Cedergren R., Gray M.W. 1997. Sequences homologous to yeast mitochondrial and bacteriophage T3 and T7 RNA polymerases are widespread throughout the eukaryotic lineage. *Nucleic Acids Res*. **24**, 648–654.

99. Gray M.W. 1992. The endosymbiont hypothesis revisited. *Int. Rev. Cytol*. **141**, 233–357.

100. Small I., Wintz H., Akashi K., Mireau H. 1998. Two birds with one stone: Gene that encode products targeted to two or more compartments. *Plant Mol. Biol*. **38**, 265–277.

101. Cahoon A.B., Harris F.M., Stern D.B. 2004. Analyzing of developing plastids reveals two mRNA stability classes correlating with RNA polymerase type. *EMBO Rep*. **5**, 801–806.

102. Krause K., Maier R.M., Kofer W., Krupinska K., Herrmann R.G. 2000. Disruption of plastid encoded RNA polymerase genes in tobacco: Expression of only a distinct set of genes is not based on selective transcription of the plastid chromosome. *Mol. Gen. Genet*. **263**, 1022–1030.

103. Legen J., Kemp S., Krause K., Profanter B., Herrmann R.G., Maier R. 2002. Comparative analysis of plastid transcription profiles of entire plastid chromosomes from tobacco attributed to wild-type end PEP-deficient transcription machineries. *Plant J*. **31**, 171–188.

104. Navarro J.A., Vera A., Flores R. 2000. A chloroplastic RNA polymerase resistant to tagetitoxin is involved in replication of avocado sunblotch viroid. *Virology*. **268**, 218–225.

105. Nielsen B.L., Rajasekhar V.K., Tewari K.K. 1991. Pea chloroplast DNA primase: Characterization and role in initiation of replication. *Plant Mol. Biol*. **16**, 1019–1034.

106. Romano L.G., Tamanoi F., Richardson C.C. 1981. Initiation of DNA replication at the primary origin of bacteriophage T7 by purified proteins: Requirement for T7 RNA polymerase. *Proc. Natl. Acad. Sci. USA*. **78**, 4107–4111.

107. Edwards J.C., Levens D., Rabinowitz M. 1982. Analysis of transcriptional initiation of yeast mitochondrial DNA in a homologous *in vitro* transcription. *Cell*. **31**, 337–346.

108. Schinkel A.H., Groot-Koerkamp M.J.A., Tabak H.F. 1988. Mitochondrial RNA polymerase of *Saccharomyces cerevisiae*: Composition and mechanism of promoter recognition. *EMBO J*. **7**, 3255–3262.

109. Li Q.-S., Gupta J.D., Hunt A.G. 1998. Polynucleotide phosphorylase is a component of a novel plant poly(A) polymerase. *J. Biol. Chem*. **273**, 17539–17543.

110. Yehudai-Resheff S., Hirsh M., Schuster G. 2001. Polynucleotide phosphorylase functions as both an exonuclease and a poly(A) polymerase in spinach chloroplasts. *Mol. Cell. Biol*. **21**, 5408–5416.

111. Odintsova M.S., Yurina N.P. 2003. Plastid genomes of higher plants and fungi: Structure and finctions. *Mol. Biol*. **37**, 768–783.

112. Kusumi K., Komori H., Satoh H., Iba K. 2000. Characterization of a *zebra* mutant of rice with increased susceptibility to light stress. *Plant Cell Physiol*. **41**, 158–164.

113. Kusumi K., Mizutani A., Nishimura M., Iba K. 1997. A virescent gene VI determines the expression timing of plastid genes for transcription/translation apparatus during early leaf development in rice. *Plant J*. **12**, 1241–1250.

114. Surpin M., Larkin R.M., Chory J. 2002. Signal transduction between the chloroplast and the nucleus. *Plant Cell*. **14**, 327–338.

115. Lagrange T., Hakimi M.A., Pontier D., Courtois F., Alcaraz J.P., Grunwald D., Lam E., Lerbs-Mache S. 2003. Transcription factor IIB (TFIIB)-related protein (pBrp), a plant-specific member of the TFIIB-related protein family. *Mol. Cell. Biol*. **23**, 3274–3286.

116. Zubko M.K., Day A. 2002. Differential regulation of genes transcribed by nucleus-encoded plastid RNA polymerase, and DNA amplification, within ribosome-deficient plastids in stable phenocopies of cereal albino mutants. *Mol. Genet. Genomics*. **267**, 27–37.

117. Silhavy D., Maliga P. 1998. Mapping of promoters for the nuclear-encoded plastid RNA polymerase (NEP) in the *iojap* maize mutant. *Curr. Genet*. **33**, 340–344.

118. Silhavy D., Maliga P. 1998. Plastid promoter utilization in a rice embryogenic cell culture. *Curr. Genet*. **34**, 67–70.

119. Vogel J., Börner T., Hess W.R. 1999. Comparative analysis of splicing of the complete set of chloroplast group II introns in three higher plant mutants. *Nucleic Acids Res*. **27**, 3866–3874.

120. Kapoor S., Suzuki J.Y., Sugiura M. 1997. Identification and functional significance of a new class of non-consensus-type plastid promoters. *Plant J*. **11**, 327–337.

121. Miyagi T., Kapoor S., Sugita M., Sugiura M. 1998. Transcript analysis of the tobacco plastid operon *rps2/atpI/H/F/A* reveals the existence of a non-consensus type II (NCII) promoter upstream of *atpI* coding sequence. *Mol. Gen. Genet*. **257**, 299–307.

122. Hübschmann T., Börner T. 1998. Characterisation of transcript initiation sites in ribosome-deficient barley plastids. *Plant Mol. Biol*. **36**, 493–496.

123. Hess W.R., Hoch B., Zeltz P., Hübschmann T., Kössel H., Börner T. 1994. Inefficient *rpl2* splicing in barley mutants with ribosome-deficient plastids. *Plant Cell*, **6**, 1455–1465.

124. Dunford R., Walden R.M. 1991. Plastid genome structure and plastid-related transcript levels in albino barley plants derived from author culture. *Curr. Genet*. **20**, 339–347.

125. Rusakova E.E., Tunitskaya V.L., Kochetkov S.N. 1999. RNA polymerase of bacteriophage T7. *Mol. Biol*. **33**, 353–367.

126. Baena-Gonzalez E., Baginsky S., Mulo P., Summer H., Aro E.-M., Link G. 2001. Chloroplast transcription at different light intensities. Glutathione-mediated phosphorylation of the major RNA polymerase involved in redox-regulated organellar gene expression. *Plant Physiol*. **127**, 1044–1052.