Impaired function of the phage-type RNA polymerase RpoTp in transcription of chloroplast genes is compensated by a second phage-type RNA polymerase

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ABSTRACT

Although chloroplast genomes are small, the transcriptional machinery is very complex in plastids of higher plants. Plastidial genes of higher plants are transcribed by plastid-encoded (PEP) and nuclear-encoded RNA polymerases (NEP). The nuclear genome of Arabidopsis contains two candidate genes for NEP, RpoTp and RpoTmp, both coding for phage-type RNA polymerases. We have analyzed the use of PEP and NEP promoters in transgenic Arabidopsis lines with altered RpoTp activities and in Arabidopsis RpoTp insertion mutants lacking functional RpoTp. Low or lacking RpoTp activity resulted in an albino phenotype of the seedlings, which normalized later in development. Differences in promoter usage between wild type and plants with altered RpoTp activity were also most obvious early in development. Nearly all NEP promoters were used in plants with low or lacking RpoTp activity, though certain promoters showed reduced or even increased usage. The strong NEP promoter of the essential ycf1 gene, however, was not used in mutant seedlings lacking RpoTp activity. Our data provide evidence for NEP being represented by two phage-type RNA polymerases (RpoTp and RpoTmp) that have overlapping as well as gene-specific functions in the transcription of plastidial genes.

INTRODUCTION

Chloroplasts (plastids) are derived from free-living cyanobacteria that were engulfed as endosymbionts by a eukaryotic host cell, the ancestor of algae and plants. During chloroplast evolution, most genes of the ancestral cyanobacterial genome have been lost or transferred into the nucleus leaving a plastidial genome (plastome) that, in case of higher plants, harbors only about 120 genes for products that function primarily in photosynthesis and gene expression (1). Chloroplast genes coding for components of the plastid translation machinery are essential for tobacco and Arabidopsis since lack of plastid protein synthesis leads to embryo lethality (2–4). In striking contrast, lack of the plastid encoded RNA polymerase [PEP, the core subunits of which are coded for the plastid rpoA, rpoB, rpoC1 and C2 genes; (5,6)], although resulting in an albino phenotype and impaired photosynthesis, still allows for heterotrophic growth of tobacco plants (7,8). The comparatively mild effects of the loss of PEP activity are explained by the existence of a second, plastid-localized, nuclear-encoded transcription activity (NEP, nuclear-encoded plastid RNA polymerase) that supplements PEP to fully transcribe the genes encoded in the plastome (6,9). The plastid NEP transcription activity is proposed to be represented by nuclear-encoded, phage-type RNA polymerases (10–13). Genes encoding organelar phage-type RNA polymerases have been found in several higher plant genomes. Aside from mitochondrial targeting (RpoTm), it was shown that a second RpoT enzyme is targeted into plastids both in monocots and dicots [RpoTp; (11,12,14–20)]. Moreover, a third RpoT enzyme found exclusively in dicots (RpoTmp) has been shown to be dually targeted both into mitochondria and plastids of Arabidopsis and Nicotiana (14,18,21).

Promoters recognized by NEP have been characterized by using plants lacking (7,22,23) or with diminished PEP activity (24–30). Most NEP promoters have a core sequence motif (YRTA; type-Ia), similar to promoters of plant mitochondria (31–35). A subclass of NEP promoters shares a GAA-box motif upstream of the YRTA-motif [type-Ib; (36)]. Type-II NEP promoters, represented by dicot clpP promoters, lack these motifs and possess crucial sequences located downstream of the transcription initiation site (32,33,37). Furthermore, the existence

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of additional NEP promoters has been reported for the *rrn* operon in spinach, mustard and *Arabidopsis* (Pc promoter), and for the internal promoters of certain tRNAs (6).

The role of RpoTp and RpoTmp in transcription of plastid genes is not understood yet. Recently, the so-called SCABRA mutants lacking RpoTp activity have been reported to show altered steady-state levels of the NEP-transcribed genes *rpoB*, *clpP* and *accD* (38). Evidence for a role of RpoTp in plastid transcription was provided by overexpression of the RpoTp enzyme in transgenic tobacco plants that revealed enhanced transcription from certain type-I NEP promoters but not from the type-II *PclpP*-53 (the number indicates here and for all other promoters the position of the transcription initiation site with respect to the translation initiation site, +1), an indication of the involvement of RpoTp in transcription from type-I NEP promoters (13). Recognition of a type-I promoter by RpoTp was observed in *in vitro* transcription assays (39). Although a role of RpoTmp in transcription of *Arabidopsis* chloroplast genes has been questioned (40) and this enzyme does not recognize NEP promoters in *in vitro* (39), several observations suggest a function of RpoTmp as plastidial RNA polymerase. The analysis of an *AthRpoTmp* homolog of spinach was detected in chloroplasts rather than mitochondria (41). T-DNA insertion mutant provided data, which corroborate a function of RpoTmp in plastid rather than mitochondrial transcription and suggest a role in early plant development (42). Analysis of an *rpoTp/rpoTmp* double mutant indicated that both RpoTmp and RpoTp might have redundant functions in plant development (38). However, no information is available yet about effects of the altered RpoTp and/or RpoTmp as plastidial RNA polymerases and if they transcribe the same set of plastid genes. Furthermore, the question remains, which RNA polymerase is responsible for recognition and transcription from ‘non-consensus-type’ NEP promoters.

To acquire information on their individual role in plastid transcription, we have studied transgenic *Arabidopsis* plants with lowered activity of RpoTp as well as *Arabidopsis* SCABRA mutants lacking functional RpoTp. We report here on differential effects of the altered RpoTp activities on the usage of NEP promoters. Our data provide evidence for the importance of RpoTp for transcription of a subset of NEP promoters and clearly demonstrate that a second phase-type RNA polymerase (RpoTmp) is involved in chloroplast transcription and might play a major role in transcription from *PclpP*-53 (type-II) and *Prnrn*-139 (Pc) NEP promoters.

**MATERIALS AND METHODS**

**Plant material**

*Arabidopsis thaliana* (ecotype Columbia) was grown on MS medium under an 8h/16h light/dark cycle at 20°C. After 14 days the seedlings were exposed to a light regime of 16h/8h light/dark. *AthRpoTp* insertion mutants *sca3*-2 and *sca3*-3, kindly provided by José Luis Micol, were grown as described by Hricová *et al.* (38).

**Plasmid construction**

Plasmid pMS1 was constructed by PCR amplification of the Ath*psbA* promoter sequence from *A. thaliana* using (NC_000932) primers 73 TACATTGGTGTGATG CTAT (1567) and 74 ATCCAGTTCAGAAGGCGACC (1383); pMS3 contains a PCR fragment (77 GAAATC CCATCTAGCCCG, 71 774 and 78 CCGGTTAGTCC ATAGGG, 72897) comprising the promoter region of Ath*clpP*; pMS4 contains a PCR fragment (81 GATTTA TCCGGGTATTGC, 26281 and 82 GAACTAAATTA GTAGTGGAAGTAAAG, 27626) comprising the promoter region of Ath*rrnB*, cloned into pGEM-T (Promega).

Plasmid pGPTV-sRpoTp was constructed by PCR amplification of a 2.99 kb PCR *AthRpoTp* fragment (AT2g24120) from an *Arabidopsis* cDNA library using primer ggtctcagctctgATGGGCTTCTCC (AS 1) and primer GACTCA GTTGAAAGATGACTGTTGGT (AS 993) and subsequently cloning it XbaI/SmaI into pGPTV (43). A 3.04 kb *AthRpoTmp* (At5g15700) PCR fragment (ggtctca GATTGAGTCCAGTG, AS 1; CTATACGTAGTTAGAAGAGGTTGATTAAG, AS 1011) was ligated into the XbaI and SmaI sites of the vector pGPTV resulting in pGPTV-sRpoTmp.

**Transformation of Arabidopsis**

*Arabidopsis thaliana* (ecotype Columbia) plants were transformed by the floral dip method according to Ref. (44) using *Agrobacterium tumefaciens* strain EHA105 transformed with plasmids pGPTV-sRpoTp and pGPTV-sRpoTmp. Plants were selected on plates containing 30 mg/l phosphinotricine (Sigma). Integration of the transgenes was confirmed by PCR and Southern hybridization (data not shown).

**Northern blot analysis**

Total leaf RNA was prepared using TRizol (Invitrogen) following the manufacturer’s protocol. Five microgram of total RNA was subjected to electrophoresis on 1% agarose-formaldehyde gels, transferred onto nylon membranes and hybridized with random-primed labeled DNA fragments overnight at 55°C. The DNA fragments were prepared by PCR using the primers 13 CATTCA TTGCTGTCCTGAGTA (1293) and 14 GAGGCTTC AACAAGCAGCTAGGTCT (400) for *psbA* and 17 GCTC TGGTGATTAAGGCGTCAG (54021) and 18 GCAG GTGCCGGGTTGCT (53138) for *atpB*.

**Primer extension analysis**

Primer extension reactions were carried out with 5 µg (*psbA*), 10 µg (*clpP*), 15 µg (*atpB*, *rrnB*, *ycfI*) and 20 µg (*rpoB*) of total cotyledon and leaf RNA according to standard protocols (45). Briefly, primers PEA*psbA* (97), TCCAGT TACAGAAGCCAAGCCATAG (5′ position in the *Arabi- dopsis* plastid genome at 1384), PEA*clpP* (104), GGTA CTTTTGGGTTAGCCACATAG (5′ position 71857),
PE4AtatpB(5), CAAGCGATGTGGAATTCATTTT (5’ position 54 281), PE3AttRNA16(6), CTAGACAAAG CTGATTGGAATTG (5’ position 100 979/137 670), P3-ycf1(23), GGCGCCATGGAAGATGTGG (5’ position 109 524/129 125) and PE4rpoB(2), TACTGAATC ACAGAAATTATATTCAAATCC (5’ position 26 445) were end-labeled with [γ-32P]ATP and T4 polynucleotide kinase (Fermentas). Primer extensions were performed using Superscript III MMLV reverse transcriptase (Invitrogen) at 50°C and the resulting products analyzed on 5% sequencing gels. To map transcript 5’-ends, sequence ladders were generated using the same primers as listed above (USB Sequenase 2). Quantification of obtained signals was done for three independent experiments with a PhosphoImaging system using the complementary software (BioRad).

Mapping of transcription initiation sites

To map the transcription initiation site of rpoB in Arabidopsis, a PCR-based method was employed (46). In a volume of 100 μl, 5 μg of total RNA, 10 U TAP (tobacco acid pyrophosphatase, Epicentre) and 40 U RNasin (Fermentas) were incubated for 1 h at 37°C (+TAP). As a control, 5 μg of RNA was similarly incubated without TAP (−TAP). After precipitation, 150 ng of RNA linker (CGAAUUCGGUAGAACAGAACACUAGAGA) was added and ligated with 50 U RNA ligase (Epicentre) for 1 h at 37°C. cDNA was synthesized using primer PE1rpoB (CCTCTTTTTCATCCCCAAAGCATC; 5’ position 26 308) with 200 U Superscript III MMLV reverse transcriptase (Invitrogen) for 1 h at 55°C. Two microliter of cDNA was used in a first PCR using an rpoB gene-specific primer (PE1rpoB) and an adaptor primer (TGTAGAACAAAGCAGAACATGAGAGA), followed by a second PCR with 1/50th of the first PCR as a template, using the adaptor primer and a nested rpoB gene-specific primer PE4rpoB. The products were analyzed on 1.8% agarose gels and bands prominent in the +TAP samples were isolated and sequenced.

RESULTS

Reduced amounts of AthRpoTp transcripts lead to delayed plant development

To investigate the role of RpoTp in plant development, we created Arabidopsis plants overexpressing AthRpoTp. Plants expressing AthRpoTp with 1 transgene copy (Tp1x, Figure 1a) showed no phenotypical differences compared to the wild type. However, the seeds of plants with five copies of the AthRpoTp transgene (Tp5x) were end-labeled with [γ-32P]ATP and T4 polynucleotide kinase (Fermentas). Primer extensions were performed using Superscript III MMLV reverse transcriptase (Invitrogen) at 50°C and the resulting products analyzed on 5% sequencing gels. To map transcript 5’-ends, sequence ladders were generated using the same primers as listed above (USB Sequenase 2). Quantification of obtained signals was done for three independent experiments with a PhosphoImaging system using the complementary software (BioRad).

Figure 1. Overexpression and partially silencing the native AthRpoTp gene by insertion of ectopic AthRpoTp gene copies leads to different phenotypes. (a) Single copy plants (Tp1x) are not distinguishable from wild-type plants. Multiple copy plants (Tp5x) germinate into white seedlings. The white seedlings will eventually become green reaching wild-type levels after two weeks (b). (c) In northern blot analyses, 50 μg total RNA of Tp1x (lane 1), Tp5x (lane 2) and wild-type plants (lane 3) was separated in 1% agarose-formaldehyde gels. Ethidium bromide stained gel image is shown as a loading control (bottom panel). The RNA blot was hybridized with a single-stranded full-length AthRpoTp antisense DNA probe. Positions of rRNAs are given on the margin. AthRpoTp RNA accumulates to highest amounts in overexpressor plants with only one AthRpoTp transgene (Tp1x, lane 1). However, in multiple copy plants AthRpoTp RNA levels (Tp5x, lane 2) are reduced to lower than wild-type RNA levels (wt, lane 3).

than in the wild type (Figure 1c, lanes 1 and 3), in Tp5x less than wild-type levels of AthRpoTp transcripts were observable (lane 2). Both Tp1x and Tp5x AthRpoTp transcripts showed lower molecular degradation products, indicating that the transgene mRNAs were subjected to a faster turnover and might be less stable than the wild-type AthRpoTp transcripts. Obviously, high copy numbers of the AthRpoTp transgene may lead to delayed greening in early seedling development due to higher AthRpoTp transcript turnover and/or gene silencing. We therefore considered Tp5x plants as partially silenced in their AthRpoTp gene expression.

To analyze the delayed seedling development in Tp5x plants, we performed northern analysis of the PEP-transcribed psbA (Figure 2a) and the atpB gene (Figure 2b), which is transcribed by NEP and PEP (30), with total RNA isolated from 6-day-old cotyledons and 3-week-old leaves. In white cotyledons of Tp5x plants, only negligible amounts of psbA messages and reduced atpB transcript levels were detectable, which returned to wild-type levels in older leaves. To further investigate the effect of reduced AthRpoTp expression on Arabidopsis PEP and NEP promoter activity in Tp5x plants, we determined transcript levels by primer extension analysis mapping transcript 5’-ends in 4- and 9-day-old cotyledons and 6-week-old leaves from both, transgenic and wild-type plants (Figure 3). Reminiscent to the northern data, the transcript 5’-ends of the PsbA-77 PEP promoter (30) accumulated to only ~10–30% of wild-type levels.
in 4- and 9-day-old Tp5x cotyledons (see also Figure 4b, lane 6). Similarly, transcript 5'-ends of the type-I NEP promoter of the rpoB operon (PrpoB-300; 30) were barely detectable in Tp5x cotyledons (see also Figure 4a, lane 6). The Arabidopsis clpP gene is transcribed from a weak PEP promoter (PclpP-115) and a strong type-II NEP promoter (PclpP-58; 30). While PclpP-115 followed the same pattern as observed with PpsbA-77 and PrpoB-300 (see also Figure 4d, lane 6), examination of PclpP-58 revealed no significant change in its 5'-end accumulation in Tp5x when compared to the wild type (see also Figure 4d, lane 6). Reduced expression of AthRpoTp in Tp5x plants may therefore directly affect transcription from type-I, but not from type-II NEP promoters.

Comparison of PEP and NEP initiated transcript 5’-ends in wild-type and rpoTp mutants indicates transcriptional compensation during plant development

To confirm the suggested role of RpoTp in plastid transcription, we compared PEP and NEP initiated transcript 5'-ends in wild-type and rpoTp mutants. The rpoTp mutants sca3-2 and sca3-3 displayed pale-green cotyledons and leaves as described by Hricova et al. (38). Compared to the wild type, they were retarded both in growth and development, i.e. 4-day-old rpoTp seedlings developmentally equal 2-day-old wild-type seedlings. We therefore chose these developmental stages to analyze the accumulation of transcript 5'-ends of plastid genes transcribed from a PEP promoter (psbA), a consensus type (type-I) NEP promoter (rpoB), by both PEP and type-II NEP promoters (atpB, ycf1), and by both PEP and ‘non-consensus-type’ NEP promoters (clpP, rrn16; 30). To compare the effects of the reduction with the loss of AthRpoTp, we additionally examined the accumulation of these transcript 5'-ends in 4-day-old Tp1x and Tp5x plants (Figures 2 and 3). Unlike Tp5x plants, rpoTp mutants showed generally no reduction but rather enhanced accumulation of PEP initiated transcript 5'-ends from PpsbA-77, PatpB-520 (Figure 4b and c, lanes 2 and 3), Prrn16-112, and Pycf1-34/33 (Figure 5a and c, lanes 2 and 3).

Interestingly, the very low abundant transcripts from the PclpP-115 PEP promoter were absent in 4-day-old rpoTp mutants (Figure 4d, lanes 2 and 3), but reappeared in 3-week-old leaves (lane 4). By and large, we observed a similar pattern with all other examined type-I NEP transcripts as well. Similar to Tp5x, transcripts initiated from PrpoB-300 (Figure 4a) and the weak Pycf1-104 (Figure 5c) type-I NEP promoters showed in comparison to the 2-day-old wild type reduced levels in the 4-day-old rpoTp mutants (lanes 1–3), which in 3-week-old leaves of the rpoTp mutants returned to levels close to those found in wild-type and rpoTp mutants.
in the wild type (lanes 1 and 4). In case of PatpB-318 (Figure 4c) and Pycf1-39 (Figure 5c), no corresponding 5'-ends were observed in the primer extension analyses with RNAs from 4-day-old rpoTp mutants (lanes 2 and 3). However, in 3-week-old leaves of sca3-2 and sca3-3 RNA from these promoters accumulated close to wild-type levels (lanes 1 and 4).

Analyses of the accumulation of the two NEP promoters PclpP-58 (Figure 4d) and Prnrn16-139 (Figure 5a), however, revealed a different picture. The 5'-ends initiated from both promoters did not decrease in the rpoTp mutants (lanes 2 and 3). While accumulation from PclpP-58 seemed not to be affected (Figure 4d, lanes 2 and 3), Prnrn16-139 5'-ends accumulated to much higher levels in sca3-2 and sca3-3 (lanes 2 and 3) than in the wild type (lane 1). Interestingly, although to much lower extent, this was also observable in Tp5x plants (lane 6).

We therefore conclude that, to some extent, an additional RNA polymerase is able to compensate for the loss of AthRpoTp, by recognizing and initiating from type-I NEP promoters. AthRpoTmp, as the only known second phage-type RNA polymerase in plastids, is the best candidate to maintain the observed transcript initiation as suggested by Hricová et al. (38). Since transcription from non-consensus (type-II) promoters was not negatively, but rather positively affected in the rpoTp mutants, one may speculate that AthRpoTmp might be responsible for transcription of these promoters in wild-type plants.

**DISCUSSION**

Plastid genes in _Arabidopsis_ are transcribed by at least three RNA polymerases. PEP uses bacterial-type promoters, which are found upstream of genes encoding components of the photosynthetic apparatus, and often together with NEP promoters upstream of housekeeping genes. NEP activity is required for the expression of most housekeeping genes and most important, for the transcription of the genes for the core subunits of PEP (6).

Although most yet described NEP promoters contain an YRTA consensus motif (type-I NEP promoters), several others do not, which in _Arabidopsis_ are most prominently represented by PclpP-58 (type-II NEP promoter) and the Pe Prnrn16-139 promoter (30). _In vitro_ transcription assays have shown, that for accurate recognition of the majority of promoters by NEP enzymes _in vivo_, additional yet unknown factors are needed (31,39). So far, individual roles of RpoTp and RpoTmp in NEP promoter recognition are unknown. An indication for a role of RpoTp transcription from type-I NEP promoters was provided by overexpression of the RpoTp enzyme in transgenic tobacco plants (13). In this study, we present for the first time evidence that an additional phage-type RNA polymerase other than RpoTp is involved in transcription of plastid genes by recognizing NEP promoters. Obviously, with RpoTmp so far shown to be exclusively mitochondrial localized, RpoTmp might represent the additional RNA polymerase activity in plastids.
To investigate the individual role of RpoTp in plastid transcription, we analyzed Arabidopsis mutants lacking RpoTp (sca3-2, sca3-3: rpoTp; 38), with partially silenced AthRpoTp gene expression (Tp5x) and Arabidopsis plants overexpressing the AthRpoTp gene (Tp1x). Although we detected an enhanced level of RpoTp transcripts in Tp1x overexpressor plants, they did not differ from the wild type. Since we found also indications for a fast degradation of the RpoTp transcripts in Tp1x, these plants may not possess markedly higher RpoTp amounts and activity than the wild type. In contrast, rpoTp plants displayed a pale-green phenotype throughout their development, whereas Tp5x plants with reduced AthRpoTp transcript levels germinated into seedlings with white cotyledons, which eventually greened up after nine days, and subsequently developed normally (Figure 1). The sca3-2 and sca3-3 RpoTp insertion mutants were previously shown to lack functional RpoTp (38). Analysis of the transcript 5'-ends of NEP and PEP promoters in 4-day-old plants showed striking similarities for the effect on NEP but not on PEP promoter usage between Tp5x and the rpoTp mutants. It seems likely that reduction of AthRpoTp transcript levels leads to lower AthRpoTp enzyme amounts, which may be responsible for the observed negative effect on transcription of the rpoB operon. This possibly results in decreased amounts of PEP, which in turn results in delayed accumulation of PEP transcripts. After 9 days AthRpoTp amounts may build up to levels that allow for sufficient expression of PEP-transcribed photosynthesis genes such as psbA and finally facilitating greening up of the cotyledons. In rpoTp plants, however, transcript accumulation from PrpoB-300 is not as strongly affected as in Tp5x plants and levels of the PEP PsbA-77 5'-ends seem not to be affected (Figure 4). In contrast to partially silenced Tp5x plants, rpoTp plants completely lack AthRpoTp. Therefore, different control mechanisms of maintaining plastid gene expression in interaction with the nucleus may take place, e.g. stronger expression of RpoTmp, the second phage-type RNA polymerase in plastids, or of transcription factors responsible for specific promoter recognition, or by increasing the transcript stability of plastid genes transcribed by PEP.

Accumulation of 5'-ends from NEP promoters in 4-day-old rpoTp plants showed remarkable differences to the wild type (Figures 4 and 5). While transcript 5'-ends of some type-I NEP promoters such as PrpoB-300 and PycfJ-104 showed reduced levels, no accumulation could be observed for PatpB-318 and PycfJ-39. However, in all cases, accumulation of these promoters returned to wild-type levels in 3-week-old rpoTp plants. With AthRpoTmp being the second phage-type RNA polymerase in plastids, we propose that AthRpoTmp is able to take over the transcription from type-I NEP promoters.

The T7 RNA polymerase is a single-polypeptide polymerase and does not need any transcription factor (47) whereas mitochondrial phage-type RNA polymerases of animals and yeasts need two transcription factors for promoter recognition and to optimal transcriptional efficiency (48). Such general transcription factors are not known yet in plants but predicted to exist from data obtained by in vitro transcription assays with the Arabidopsis phage-type RNA polymerases (39). Moreover, our data indicate the existence of more promoter-specific factors acting only at certain developmental stages. The differential recognition of NEP promoters in mutants and wild type, in particular the inability to use Pcycf1-39 and PatpB-318 only in very young seedlings of the mutants, points to missing interaction between activator(s) and RpoTmp. Interestingly, accumulation of 5'-ends from so-called non-consensus type NEP promoters (type-II) such as PclpP-58 and the Prrn16-139 (Pc promoter) was not affected and even enhanced, respectively, in 4-day-old rpoTp plants. We therefore conclude that AthRpoTmp may have a special function in maintaining transcription from this type of promoters in wild-type plants. AthRpoTp, in turn, seems to be important for recognition and transcription from type-I NEP promoters, as suggested by Liere et al. (13). Beside a division of labor between RpoTp and RpoTmp with regards to promoter usage, the two polymerases may be of variant importance in different tissues. It was recently reported that RpoTp shows maximal expression in green tissues whereas the highest RpoTmp promoter activity was observed in meristematic and young cells containing non-green plastids (49).

In conclusion, we have analyzed the usage of PEP promoters (recognized by the bacterial-type plastid-encoded RNA polymerase) and NEP promoters (recognized by phage-type nuclear-encoded RNA polymerases) in Arabidopsis plants with altered and lacking RpoTp activity. Low or lacking activity of RpoTp led to lower levels of transcripts originating from NEP promoters with the consensus YRTA motif. The strong NEP promoter that drives transcription of the essential ycfl gene in wild-type chloroplasts was even not used at all in very young mutant seedlings without functional RpoTp. This data demonstrates on one hand the importance of RpoTp for chloroplast transcription. On the other hand, most NEP promoters were active even in the absence of RpoTp activity, thus providing clear evidence for the participation of another phage-type RNA polymerase (RpoTmp) in the transcription of plastid genes from NEP promoters. Interestingly, usage of the non-consensus promoters of the clpP gene and the rrn operon was not affected and even enhanced, respectively, by the lack of RpoTp activity. Our data indicate that RpoTp and RpoTmp together form the NEP activity and play overlapping as well as specific roles in the transcription of plastid genes. During the reviewing process of this manuscript, Courtois et al. (50) published data, which are in agreement with our results. Further studies including also plants with altered RpoTmp activity have to show if the two phage-type polymerases play different roles in different tissues and at certain stages of plant development.

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