

Review



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The plastid transcription machinery and its coordination with the expression of nuclear genome: Plastid-Encoded Polymerase, Nuclear-Encoded Polymerase and the Genomes Uncoupled 1-mediated retrograde communication

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Plastid genes in higher plants are transcribed by at least two different RNA polymerases, the plastid-encoded RNA polymerase (PEP), a bacteria-like core enzyme whose subunits are encoded by plastid genes (*rpoA*, *rpoB*, *rpoC1* and *rpoC2*), and the nuclear-encoded plastid RNA polymerase (NEP), a monomeric bacteriophage-type RNA polymerase. Both PEP and NEP enzymes are active in non-green plastids and in chloroplasts at all developmental stages. Their transcriptional activity is affected by endogenous and exogenous factors and requires a strict coordination within the plastid and with the nuclear gene expression machinery. This review focuses on the different molecular mechanisms underlying chloroplast transcription regulation and its coordination with the photosynthesis-associated nuclear genes (*PhANGs*) expression. Particular attention is given to the link between NEP and PEP activity and the GUN1- (Genomes Uncoupled 1) mediated chloroplast-to-nucleus retrograde communication with respect to the *Δrpo* adaptive response, i.e. the increased accumulation of NEP-dependent transcripts upon depletion of PEP activity, and the editing-level changes observed in NEP-dependent transcripts, including *rpoB* and *rpoC1*, in *gun1* cotyledons after norflurazon or lincomycin treatment. The role of cytosolic preproteins and HSP90 chaperone as components of the GUN1-retrograde signalling pathway, when chloroplast biogenesis is inhibited in *Arabidopsis* cotyledons, is also discussed.

This article is part of the theme issue 'Retrograde signalling from endosymbiotic organelles'.

1. Introduction

Compared to its cyanobacterial ancestor, the transcriptional apparatus of the land plant chloroplast is more complex and reflects the evolutionary integration of a prokaryotic gene expression system into a eukaryotic host cell. Unlike bacteria, chloroplasts of angiosperms and the moss *Physcomitrella patens* require at least two different RNA polymerases to ensure transcription of plastid genes: the plastid-encoded polymerase (PEP), a multimeric bacterial-type enzyme [1], and, in addition, the nuclear-encoded polymerase (NEP), a monomeric T3-T7 bacteriophage-type enzyme [2,3]. Correct plastid development and functionality necessitate the interplay of both PEP and NEP enzymes, together with nuclear-encoded factors, such as sigma-like factors (Sig) and PEP-associated proteins (PAPs) [4–8]. These are just a few examples of nuclear factors that exert control

over the plastid gene expression (PGE), since the nuclear genome encodes most of the plastid proteins [9]. On the other hand, chloroplast biogenesis and functionality also require tight coordination of the transcription of thousands of nuclear genes with the expression of the relatively few plastid genes. This coordination is achieved through an extensive flow of information from developing plastids to the nucleus, via biogenic retrograde signalling, and from mature chloroplasts to the nucleus, via operational retrograde signalling [10–12]. The GUN1 (genomes uncoupled 1) protein, a pentatricopeptide repeat protein that localizes to plastids, was proposed as the central node relaying information from multiple retrograde signalling pathways that regulate photosynthesis-associated nuclear genes (*PhANGs*) expression [13]. Interestingly, the involvement of GUN1 in relaying signals after norflurazon (NF) and lincomycin (Lin) treatments [13], and after impairment of protein import [14] and PGE [15–17] raises the possibility that each of the treatments or mutant genetic backgrounds may affect a similar process, thereby using GUN1 as a common signalling component [18]. Intriguingly, GUN1 and NEP proteins have some features in common including the fact that (i) NEP, as GUN1, should be found in plastid nucleoids, since plastid DNA is exclusively located in these domains; (ii) the NEP polymerase and the GUN1 protein have never been detected in plastid proteomics analysis [6,19,20], most probably as a consequence of their very low abundance; and (iii) they are highly active during early stages of chloroplast biogenesis [2,21]. This review focuses on the recently discovered molecular mechanisms at the basis of the regulation of chloroplast transcription and its coordination with *PhANGs* expression during early stages of chloroplast development and upon alteration of plastid protein homeostasis in *Arabidopsis* cotyledons. Particular emphasis is given to the link between NEP and PEP activity and the GUN1-mediated chloroplast-to-nucleus retrograde communication in *Arabidopsis* cotyledons [22–24].

2. The plastid-encoded RNA polymerase

The PEP catalytic core is made of α_2 (it occurs as a dimer and serves as a stabilizing agent of the PEP core), β (the catalytic subunit), β' (unknown function) and β'' (DNA binding function) subunits, encoded by the plastid *rpoA*, *rpoB*, *rpoC1* and *rpoC2* genes, respectively, and inherited from the cyanobacterial ancestor [25]. Studies performed in barley, tobacco and *Arabidopsis* showed that *rpo* genes are essential for proper chloroplast biogenesis, as mutants lacking any PEP subunit exhibit an albino or yellowish phenotype [26]. As in any other bacterial RNA polymerase, the PEP enzyme needs sigma factors (σ) for promoter recognition and initiation of transcription [27]. Genes coding for plastid sigma factors are located in the nuclear genome, thus, PGE is strictly controlled by the nucleus. In *Arabidopsis thaliana*, six different sigma factors, SIG1–SIG6, have been identified. Their specific functions in chloroplast physiology have not been fully understood yet, however, the investigation of knockout mutants has provided the first insights [5]. The PEP enzyme is located in the nucleoids and the Rpo core subunits jointly with one of the sigma factors form the holoenzyme that, upon light exposure, recruits additional subunits driving chloroplast biogenesis. Different nucleus-encoded proteins, unrelated to bacterial transcription factors, are consistently co-purified with PEP and collectively are named PEP-associated proteins

(PAPs) [6]. PAPs are an evolutionary conquest of solely land plants, as no PAP orthologues were found in *Chlamydomonas reinhardtii* [6,28]. Intriguingly, functional studies on PAP knock-out mutants indicate that PAP proteins are required for PEP-mediated transcription and regulation [29–36], contributing to establish a subdomain in the plastid nucleoid where PEP-mediated transcription takes place [6].

3. Origin and evolution of nuclear-encoded polymerase

In the early 1990s, researchers postulated the existence of a nuclear-encoded and plastid-located RNA polymerase, NEP. The first evidence came from the obligate parasitic angiosperm *Epifagus virginiana* that possesses plastids with a reduced genome (just nine genes are retained), deprived of all *rpo* genes [37], and from the barley mutant *albostrians* deficient in plastid ribosomes [38] and therefore unable to synthesize the PEP core subunits. Despite the absence of PEP, plastid transcripts were detectable both in *Epifagus virginiana* and in *albostrians* barley mutant, suggesting the existence of a nuclear-encoded plastid RNA polymerase. In the same period, a 110 kDa polypeptide was purified from spinach chloroplasts via sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The purified polypeptide actively synthesized RNA in the presence of supercoiled DNA template and nucleoside triphosphates, showing features of a single-subunit RNA polymerase of the T3-T7 bacteriophage type [39]. The plant genome era, inaugurated by the release of the *Arabidopsis thaliana* genome, allowed the identification of three nuclear genes, called *RPOT*, coding for phage-like RNA polymerases, targeted to mitochondria (*RPOT1/RpoTm*), chloroplasts (*RPOT3/RpoTp*) and to both organelles (*RPOT2/RpoTmp*) [40,41]. In general, the three *RPOT* genes are present in eudicotyledon plants, while *RPOT2*, encoding the dual-located RpoTmp, is absent in monocots and early-diverging angiosperms [42,43]. The basal angiosperm *Nuphar advena*, for instance, has two mitochondrial RNA polymerases and a proper NEP, which could be considered the phylogenetically earliest RpoTp enzyme in higher plants [44]. Unicellular green algae, such as *C. reinhardtii*, *Ostreococcus tauri* and *Thalassiosira pseudonana*, also have a single gene for the RpoTm and no RpoTmp enzyme [43,45]. This feature is in common with fungi and mammals, as well as the spike-moss *Selaginella moellendorffii* [43,46]. However, the nuclear genome of the moss *P. patens* bears three *RPOT* gene as in *Arabidopsis*. However, one gene codes for an exclusively mitochondrial polymerase, while the other two produce two dual-located RpoTmp enzymes [3]. In the light of these phylogenetic data, the NEP enzyme appeared to originate from duplication of the *RPOT1* gene, which could have occurred multiple times in the evolution of land plants, as three copies of *RPOT* genes are present in mosses and dicot plants but not in spike-mosses and monocots. Alternatively, the extra dual-located enzyme could have been lost during evolution [43,44].

NEP, like PEP, is essential for chloroplast transcription. Knocking out the *RPOT2* or *RPOT3* genes in *Arabidopsis* yields plants with delayed chloroplast biogenesis, while the *rpot2 rpot3* double mutant is characterized by chlorophyll deficiency and a complete arrest of growth early in development [47], indicating partially overlapping functions of RpoTp and RpoTmp in chloroplast gene transcription.

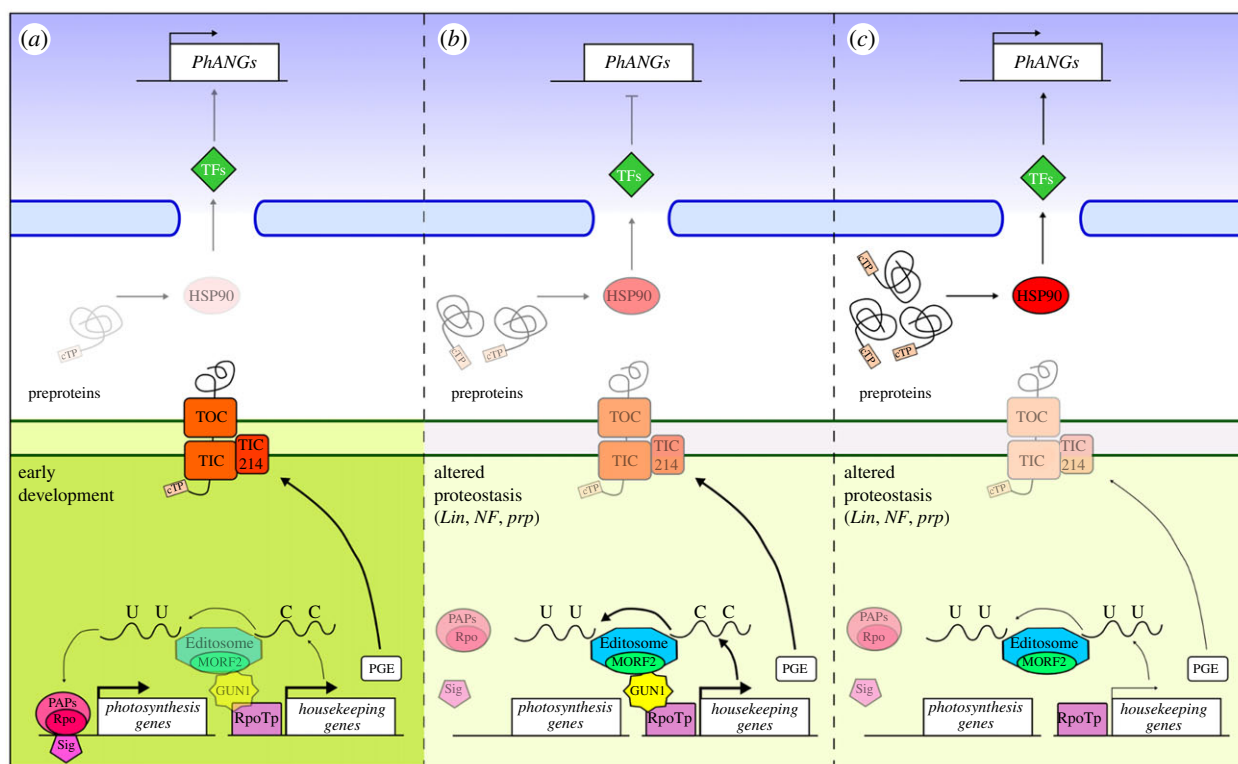


Figure 1. Schematic overview of the GUN1-dependent coordination of plastid and nuclear transcriptional machinery in three different scenarios: early stages of chloroplast development (a), altered protein homeostasis (b) and altered protein homeostasis in the absence of GUN1 protein (c). The role of GUN1 becomes evident when chloroplast biogenesis is altered either by chemical treatments (lincomycin or norflurazon) or genetic defects that impair plastid protein homeostasis (see panels indicated as altered proteostasis) [15,16,24]. Under these conditions, GUN1 has been reported to (i) interact with NEP and favour accumulation of NEP-dependent transcripts, including *ycf1* that encodes the Tic214 subunit of the protein translocon at the inner envelope membrane of chloroplasts [24]; (ii) interact with MORF2, a component of the Editosome protein complex, and edit NEP-dependent transcripts, including *rpoB* and *rpoC1*, but also *rps12*, *rps14*, *ndhB* and *ndhD* mRNAs [22]; (iii) interact with cpHSC70-1 and regulate plastid protein import (this detail is not shown) [23]. Overaccumulation of preproteins in the cytosol of *gun1* cotyledon cells induces the accumulation of cytosolic HSP90 proteins that favour *PhANGs* transcription [23,24]. This could be obtained by either 26S proteasome-dependent degradation of negative transcriptional regulators or activation, through HSP90-promoting folding, of positive ones (transcription factors, TFs). It is conceivable that a similar mechanism could be active even at early stages of chloroplast development [21], to fine-tune the chloroplast and nuclear transcription machineries with respect to the developmental needs. However, under these conditions the accumulation of preproteins and downstream components of the signalling pathway have not been described yet. Note that the thickness and brightness of lines and shapes is directly proportional to the activity of the specific process. cTP, chloroplast transit peptide; TOC, translocon at the outer chloroplast membrane; TIC, translocon at the inner chloroplast membrane; Lin, lincomycin; NF, norflurazon; *prp*, plastid ribosomal protein mutants; PGE, plastid gene expression, defined as transcription and translation rate of plastid-encoded genes.

4. Nuclear-encoded polymerase and plastid-encoded polymerase and their role in plastid gene expression

Data obtained during the last two decades indicate that both PEP and NEP polymerases are active in all green and non-green tissues, but with different degrees of activity according to the developmental stages and physiological conditions (figure 1). As a matter of fact, *Arabidopsis* dry seeds already contain several transcripts of the plastid transcription machinery, such as the NEP *RPOT2* and *RPOT3*, the PEP core *rpoA*, *rpoB*, *rpoC1*, *rpoC2*, and the nuclear-encoded PEP-associated sigma factors *SIG2* and *SIG5* [48,49]. These PEP core transcripts derive from chloroplasts present in photosynthetically active *Arabidopsis* embryos, before dedifferentiation to small non-green plastids, termed eoplasts, occurs during late embryo and seed maturation. In addition, during stratification (72 h at 4°C in the dark) several plastid house keeping genes, including *rpoA*, *rpoB*, *rpoC1* and *rpoC2*, and many nuclear genes encoding *SIG1–6* factors and the RpoTp, RpoTm enzymes are actively transcribed [48,50].

At the protein level, *rpoB*, RpoTp and RpoTm are also present in dry seeds. Furthermore, a dramatic accumulation of RpoTp protein occurs shortly after imbibition, and its increased activity leads to a higher accumulation of PEP core proteins as well. Upon seed germination, NEP activities and transcripts increase and peak at 2–3 days after germination, leading to increased accumulation of NEP-dependent transcripts, followed by a severe drop as *RPOT3* gene expression decreases [51,52]. Moreover, gene expression studies revealed that *RPOT3* is mainly active in green photosynthetic tissues while *RPOT2* plays its role in dividing and non-green cells [53].

When seed germination takes place in darkness, the eoplasts turn into etioplasts, where a soluble inactive form of the PEP holoenzyme, termed PEP-B and made of core proteins only, is present. Upon illumination, the PEP complex starts interacting with several PAP proteins and becomes part of the membrane-bound plastid transcription active chromosome (pTAC) megadalton complex. This more complex enzyme, named PEP-A, increases the PEP activity and leads to the transcription of *Photosynthesis-Associated Plastid-Encoded Genes (PhAPGs)* and cotyledon greening [34,54,55].

NUCLEAR CONTROL OF PEP ACTIVITY (NCP), a dual-targeted nuclear/plastidial protein, also known as MRL7-L (Mesophyll-cell RNAi Library line 7-like) [56] and SVR4-like (Suppressor of Variegation 4-like) [57] because of its essential role in chloroplast biogenesis [56,57], is required for both the nuclear and plastidial signalling steps of *PhAPGs* activation, promoting the assembly of the PEP complex upon illumination [58]. In particular, NCP mediates the degradation of two repressors of chloroplast biogenesis in the nucleus, PIF1 and PIF3, thus participating in the phytochrome-mediated signalling at the basis of chloroplast biogenesis. NCP also has a paralog in *Arabidopsis*, At4g28590 also known as ECB1 (Early Chloroplast Biogenesis 1) [59], SVR4 (Suppressor of Variegation 4) [57] and MRL7 (Mesophyll-cell RNAi Library line 7), which was renamed Regulator of Chloroplast Biogenesis (RCB) [60]. Like NCP, RCB is also a dual-targeted nuclear/plastidial protein required for the degradation of the nuclear transcriptional regulators PIF1 and PIF3 and for PEP assembly and *PhAPGs* expression in the plastids. These data support a model in which phytochromes control *PhAPGs* expression through light-dependent double nuclear and plastidial switches that are linked by evolutionarily conserved and dual-localized regulatory proteins.

Following PEP-A assembly, the enzyme takes over most of the transcription activity, including mRNAs, rRNAs and most of the tRNAs [61–65], while the NEP enzyme remains active to perform the transcription of *rpoB* (forming an operon with *rpoC1* and *rpoC2*), *accD* (in dicots), *clpP*, *atpB*, *atpI*, genes coding for ribosomal proteins and a few tRNAs, and, in dicots, *ycf1* and *ycf2* until senescence.

5. Plastid-encoded polymerase regulation

PEP promoter recognition and transcription initiation are mediated by σ^{70} -like factors, as occurs in prokaryotes [27]. *Arabidopsis* has six different sigma factors, SIG1–SIG6. All *Arabidopsis sig* knockout mutants fail to accumulate wild-type levels of plastid transcripts; nevertheless, only *sig2* and *sig6* displayed a pale-green phenotype, suggesting a more relevant role in plastid transcription and chloroplast development [66]. Furthermore, only a partial functional redundancy among *Arabidopsis* SIG factors during early steps of seedling development has been revealed, indicating gene-specific functions [4,5].

Unlike their bacterial counterparts, the plastid sigma factors SIG1, SIG2 and SIG6 can be modified post-translationally by phosphorylation at the N-terminal variable region in fully mature chloroplasts [4,67]. SIG2 and SIG6 phosphorylation have been related to alterations in the promoter binding efficiency of PEP [68]. Similarly, SIG1 phosphorylation is regulated in a redox-dependent manner and serves to adapt PSII/PSI stoichiometry to light changes by modulating the relative transcription of the photosynthesis reaction centre genes *psbA* (photosystem II, PSII) and *psaA/B* (photosystem I, PSI), indicating that phosphorylation is also needed to adapt the PEP-dependent transcription to the redox state of the thylakoid electron transport chain [67]. Lastly, a role for SIG6 in the operational retrograde signalling during singlet oxygen stress has been reported [69]. In particular, a genetic screen uncovered a *sig6* mutant (*soldat8*) that was able to survive the high levels of singlet oxygen in the *Arabidopsis flu* mutant that has uncontrolled tetrapyrrole synthesis. This regulatory mechanism originated after the endosymbiosis event, since the N-terminal

of plastid SIG factors is not conserved among bacteria and chloroplasts, and makes plastid SIG factors incompatible with the transcription machinery of bacteria [70,71].

SIG factors also contribute to the construction of PEP-A holoenzyme, together with the 12 true PAP proteins, identified through genetic and biochemical studies, and shown to be essential for chloroplast biogenesis in *Arabidopsis* [6]. Indeed, all *pap* mutants display an albinotic/chlorotic phenotype, corroborating the hypothesis that PAP proteins are fundamental for PEP activity, as Rpo-core subunits. In particular, PAPs are involved in different regulatory functions [34], including:

- (i) DNA/RNA metabolism-related gene expression regulation, as in the case of PAP1, 2, 3, 5, 7 and 12. For instance, PAP1 and PAP2 display pentatricopeptide repeat (PPR) motifs, known to be involved in RNA metabolism [72] and PAP3 is predicted to interact with RNAs through its S1-like domain [35].
- (ii) Redox-dependent gene regulation and protection against oxidative stresses, as in PAP4, 6, 9 and 10. As an example, PAP10/TrxZ physically interacts with the PLASTID REDOX INSENSITIVE 2 (PRIN2) protein, a key regulator of PEP activity, capable of transducing the redox state of thylakoid membranes into regulation of PEP-dependent transcription [73,74]. In particular, PAP10 interacts with the PRIN2 dimer and through its thioredoxin domain causes the reduction of inter-molecular disulfide bridges and the release of PRIN2 monomers, that are then able to boost the transcription of PEP-dependent genes, providing a mechanistic link between photosynthetic electron transport and activation of photosynthetic gene expression.

Chloroplast transcription has also been reported to be under the control of phytohormones during the greening of etioplasts [75] and in fully developed chloroplasts of barley (*Hordeum vulgare* L.). In particular, stimulatory effects of cytokinin (6-benzyladenine BA) and repressive effects of methyl jasmonate (MeJA), auxin (indole-3-acetic acid, IAA) and gibberellic acid (GA3) on chloroplast gene expression at the levels of transcription and transcript accumulation of both NEP- and PEP-transcribed genes have been reported [76,77]. More detailed information is available for abscisic acid (ABA). In particular, it has been shown that exogenously supplied ABA represses the transcription of plastid genes during greening and in fully mature chloroplasts in barley leaves, with the exception of *psbA*, *psbD* and a few other genes that remain as active as in untreated leaves [78]. Based on these findings, ABA seems to coordinate the repression of photosynthesis genes in the nuclear and chloroplast genomes while leaving active those chloroplast genes that are needed for the protection of the PSII reaction centres from damage by reactive oxygen species (ROS) [78]. This adaptive response involves the ABA-activated expression of *RSH2* and *RSH3* nuclear genes, coding for enzymes in the chloroplast that synthesize guanosine-3', 5'-bisdiphosphate (ppGpp), an inhibitor of chloroplast transcription. In particular, ppGpp was shown to inhibit transcriptional activities of purified PEP preparations *in vitro* [79]. In addition, an assay based on the *in planta* incorporation of the base analogue 4-thiouridine into nascent chloroplast RNA demonstrated that the accumulation of ppGpp inhibits the transcription of PEP-dependent and, to a lesser extent, of NEP-dependent genes in developing seedlings [80], resembling the ancient bacterial stress-signalling pathway

known as the stringent response (for a review see [81]). Furthermore, relatively high ABA levels increase the amount of SIG5 by activating the expression of its gene in the nucleus leading to a subpopulation of SIG5–PEP. The SIG5–PEP transcribes chloroplast genes from specific promoters, resulting in the observed escape of a few plastid genes (*psbA*, *psbD*) from the otherwise general downregulation of transcription of chloroplast genes [78].

6. Nuclear-encoded polymerase regulation

Although PEP regulation has been widely characterized and several regulatory factors identified, only a few pieces of information on NEP activity regulation are currently available. Firstly, the tRNA-Glu is able to bind and inhibit the activity of NEP *in vitro*, displaying specificity compared to tRNA-Val, tRNA-Gly and tRNA-Trp, used as control [51]. The tRNA-Glu is different from the other plastid-encoded tRNAs, since it is not only involved in plastid protein synthesis but is the substrate for the synthesis of 5-aminolevulinic acid, an early step of the tetrapyrrole biosynthesis pathway that leads to chlorophyll, heme and phytochrome production. In particular, the tRNA-Glu, massively transcribed by PEP during the greening process, has a pivotal role in the switch between NEP and PEP activity, by inhibiting the NEP-dependent transcription in a threshold manner, once the greening has occurred [51]. A second regulatory mechanism of NEP activity was initially observed in tobacco leaves through the impairment of the plastid *rpoB* gene, encoding the β -subunit of the PEP enzyme. In particular, an entire class of plastid transcripts was found to accumulate at high level upon the complete loss of PEP activity [62,82]. Such an adaptation mechanism, termed as ‘*Drpo* phenotype’, was then reproduced in tobacco leaves upon depletion of the *rpoA*, *rpoB*, *rpoC1* and *rpoC2* genes [83], encoding the core complex of the PEP enzyme. Later, the *Drpo* adaptive response was described in nearly every mutant identified as PEP regulator and impaired in PEP-dependent gene expression in *Arabidopsis*, including mutants with *PAP* gene expression defects (for further details, see table 1), together with many other mutants affected in the accumulation of key players of chloroplast protein homeostasis, such as PPR proteins involved in mRNA metabolism (CRP1 and PPR4), plastid ribosomal proteins (PRPL11 and PRPS21), chaperones (CLPR1 and cpHSP70–1), and the iron superoxide dismutase FSD2 and FSD3 (table 1). Moreover, impairment of chloroplast translation upon lincomycin treatment activates the *Drpo* adaptive response in *Arabidopsis* seedlings [13,24]. More generally, it appears that every genetic- or chemical-induced impairment of plastid protein homeostasis leads to the upregulation of NEP-dependent transcripts, as an attempt by the PGE machinery to maintain non-photosynthetic plastid functions by expressing housekeeping genes. Strikingly, we have recently reported that GUN1 protein is able to physically interact with NEP in chloroplasts of *Arabidopsis* cotyledons and is required for the *Drpo* adaptive response, providing an important link between plastid transcription and the GUN1-mediated plastid-to-nucleus retrograde communication [24].

7. Interplay between plastid transcription regulation and retrograde signalling

Due to the chimeric nature of PEP enzyme, made of subunits encoded by both the plastid and the nuclear genome, and the

nuclear localization of NEP encoding genes, it is clear that the coordinated transcription of plastid and nuclear genes is essential for proper development of all plastid types. This coordination takes place through the nuclear control of PGE and the retrograde signalling pathways [10].

In the case of chloroplast biogenesis, multiple pieces of evidence on the coordination of plastid transcription and *PhANGs* expression have been reported. In particular, the status of plastid transcription and translation as a trigger for retrograde signalling has been demonstrated by repression of *PhANGs* expression upon chemical treatments, for instance with the plastid translation inhibitor lincomycin or by rifampicin, which selectively inhibit the PEP enzyme [12,66]. More specifically related to PEP-dependent transcription, increasing evidence indicates that several factors involved in the coordination of plastid and nuclear transcriptional machineries are characterized by the presence of both the chloroplast transit peptide (cTP) and the nuclear localization signal (NLS) in their peptide sequence, implying a plastid and nuclear dual localization [20]. These proteins have potential access to plastid and nuclear DNA and, therefore, they can be directly involved in the coordination of gene expression in both compartments. Among them are NCP and RCB, described above, and PAP1, –5, –7, –8, –9 and –12. Currently, only in the case of PAP5/pTAC12 has the dual localization been experimentally confirmed [88,100]. Indeed, PAP5 seems to be involved in the red light-mediated skotomorphogenesis-to-photomorphogenesis transition, through the physical interaction and degradation of phytochrome-interacting-factors PIF1 and PIF3, thereby affecting gene expression in the nucleus [101–103], similarly to NCP and RCB. Interestingly, genes coding for PAPs were found to be expressed in non-green tissues before the assembly of the PEP-A complex, which is induced in the light. It is, therefore, possible to speculate that the NLS-containing PAP proteins can form two different complexes, one in the nucleus and one in the chloroplast, and modulate gene expression in both compartments by interacting with different kinds of RNA polymerases [26]. In this scenario, a nuclear PAP complex could assemble in the dark at first, mediating the early steps of plastid development by interacting with RNA polymerase II, and upon illumination migrate to the chloroplast to form PEP-A, in a sort of NLS-cTP competition which could determine the PAP protein intracellular localization.

In addition to dual-located proteins, proteins specifically located in plastids are also able to coordinate plastid and nuclear gene expression. For instance, PRIN2 has been reported to be able to trigger retrograde signalling in response to light, besides its direct regulation of PEP activity, [73,74].

Furthermore, depletion of SIG2 and SIG6 in *Arabidopsis* allowed demonstration that the activity of both sigma factors is the source of retrograde signals that promote *PhANGs* expression [66]. The addition of the *gun1* mutation in *sig6* mutant led to a global reduction of 72 plastid transcripts, both PEP- and NEP-dependent, suggesting a possible involvement of GUN1 in the regulation of the plastid and nuclear transcriptional machineries. The physical interaction between PAP8/pTAC6 and GUN1 observed by Tadini *et al.* [15] points further to a direct link between PEP activity modulation and retrograde signalling.

More recently, Tadini and co-workers demonstrated that GUN1 plays a central role in the regulation of NEP activity, thus creating a direct link between retrograde signalling and

Table 1. List of genes/proteins whose impairments result in the activation of the Δrpo adaptive response, i.e. increase accumulation of NEP-dependent transcripts upon depletion of PEP activity.

gene	accession	phenotype	molecular defect	refs
plastid transcription regulation				
<i>SIG2</i>	AT1G08540	pale	reduced PEP activity	[66]
<i>SIG6</i>	AT2G36990	pale	reduced PEP activity	[66,84]
<i>DG1</i>	AT5G67570	delayed greening	reduced PEP activity	[84,85]
<i>PAP2/pTAC2</i>	AT1G74850	pale	reduced PEP activity; RNA maturation	[29]
<i>PAP3/pTAC10</i>	AT3G48500	albino	reduced PEP activity	[86]
<i>PAP4/FSD3</i>	AT5G23310	pale	reduced ROS scavenging; reduced PEP activity	[87]
<i>PAP5/pTAC12/HMR</i>	AT2G34640	pale	reduced PEP activity; altered phytochrome signalling	[88,89]
<i>PAP6/FLN1</i>	AT3G54090	albino	reduced PEP activity	[89]
<i>FLN2</i>	AT1G69200	virescent	reduced PEP activity	[89,90]
<i>PAP7/pTAC14</i>	AT4G20130	albino	reduced PEP activity	[90]
<i>PAP8/pTAC6</i>	AT1G21600	albino	reduced PEP activity	[29]
<i>PAP9/FSD2</i>	AT5G51100	pale	reduced ROS scavenging; reduced PEP activity	[87]
<i>PAP10/TrxZ</i>	AT3G06730	albino	reduced PEP activity	[34]
<i>PAP12/pTAC7</i>	AT5G24314	albino	reduced PEP activity	[91]
<i>pTAC5</i>	AT4G13670	pale	reduced PEP activity	[92]
<i>PRIN2</i>	AT1G10522	albino	reduced PEP activity; impaired redox-mediated retrograde signalling	[73,74]
<i>ECB1/MRL7/SVR4/RCB</i>	AT4G28590	tall/albino	reduced PEP activity; altered phytochrome signalling	[56,57,60]
<i>MRL7-L/SVR4-L/NCP</i>	AT2G31840	tall/albino	reduced PEP activity; altered phytochrome signalling	[57,58]
plastid transcripts maturation				
<i>CRP1</i>	AT5G42310	albino	impaired plastid mRNA maturation	[93]
<i>PPR4</i>	AT5G04810	embryo-lethal	impaired plastid mRNA maturation	[94]
<i>PDM1</i>	AT4G18520	albino	impaired plastid mRNA maturation	[95]
plastid translation				
<i>PRPS21</i>	AT3G27160	pale	reduced plastid ribosome activity	[24,96]
<i>PRPL11</i>	AT1G32990	pale	reduced plastid ribosome activity	[15,24,97]
plastid proteostasis maintenance				
<i>HSP21</i>	AT4G27670	none (22°C); albino (30°C)	reduced PEP activity under heat stress	[92]
<i>cpHSC70-1</i>	AT4G24280	variegated	reduced plastid import capacity; impaired protein quality control	[24,98]
<i>CLPR1</i>	AT1G49970	virescent	impaired protein quality control	[99]

PEP/NEP-dependent transcription (figure 1). In particular, GUN1 was shown to interact physically with RpoTp and to have a direct role in the upregulation of NEP-dependent transcripts upon perturbation of plastid protein homeostasis, i.e. during the Δrpo adaptive response [24]. Concomitantly, GUN1 has been reported to have a direct role in RNA editing by physically interacting with the MULTIPLE ORGANELLAR RNA EDITING FACTOR 2 (MORF2), a member of the so-called plastid RNA Editosome, which is involved in editing nearly all sites

of plastid RNA [22], in agreement with previous studies where a link between plastid signalling and RNA editing was shown [18,104]. Consistent with this, *gun1* mutant cotyledons have differential efficiency of RNA editing (C→U) levels of 11 sites in the plastid transcriptome, after norflurazon or lincomycin treatment, compared to wild type. Intriguingly, the target genes were NEP-dependent, including transcripts of PEP core subunits β and β' [22]. The *rpoB* and *rpoC1* editing sites have been observed in previous studies and lead to amino acid

changes in the protein sequence [105]. However, the biological meaning of such editing events and the impact on the transcripts is not always clear. RNA editing is usually associated with the restoration of conserved codons or aims to recreate start/stop codons serving as a correction for otherwise defective transcripts [106]. In this specific case, GUN1 is responsible for three amino acid changes in *rpoB* sequence (S113F, S184 L and S811 L) and one in *rpoC1* (S163 L) [22]. The edited residues are highly conserved in several species, suggesting that the lack of GUN1 leads to the production of altered forms of PEP core proteins. In this scenario (figure 1), GUN1 appears to be part of a protein complex that act as a positive regulator of both NEP- and PEP-dependent transcription, and that could contribute to the increase of cellular RNA amount observed during germination or even to the doubling of plant cell RNA detected within 48 h under stress conditions, i.e. in response to herbicide-induced Mg-protoporphyrin and heme accumulation or a high level of sugar treatment, as previously reported [107]. According to this model, GUN1-dependent closure of the photosynthetic apparatus, by repressing photosynthesis-associated nuclear gene expression, would contribute further to protection from oxidative stress [107]. Alternatively, the GUN1-dependent increased accumulation of NEP-dependent transcripts upon depletion of PEP activity would serve to maintain the house keeping functions of plastids, reducing to a minimum the photosynthesis-dependent oxidative damage.

Interestingly, the over-accumulation of unimported precursor proteins (preproteins) observed in the cytosol of *gun1* cotyledon cells upon growth in the presence of lincomycin (figure 1) induces upregulation of cytosolic Heat Shock Protein 90 (HSP90) and in turn sustains the expression of *PhANGs*, indicating that this pathway might play a key role in the coordination of plastid and nuclear gene transcription [23,24]. In particular, HSP90 could mediate retrograde communication by either repressing negative regulators of transcription (for example, through delivery to the 26S proteasome for degradation) such as abscisic acid insensitive 4 (*ABI4*) [13], although its role in retrograde signalling has been questioned recently [108], or by activating a positive regulator of transcription (for example, through promoting folding or refolding of a transcription factor) such as Elongated Hypocotyl 5 (*HY5*) [109] and Golden-Like 1/2 (*GLK1/2*) [110], reported to be involved in retrograde communication and required for coordinated expression of key genes in chloroplast biogenesis [111,112].

8. Concluding remarks and future perspectives

The field of plastid-to-nucleus signalling has been very dynamic over the last few years, and there have been several major breakthroughs leading to a much more advanced understanding of the mechanisms involved in plastid and nuclear genome crosstalk. Three recent studies have identified GUN1-interacting proteins in *Arabidopsis* cotyledons, contributing to unravelling

the mechanism by which the activity of plastid transcription machinery is coordinated with the nuclear gene expression apparatus [22–24]. A direct connection between GUN1 and the NEP-PEP plastid RNA polymerases has been provided by both the physical interaction of GUN1 with RpoTp and its role in favouring the NEP-dependent transcript accumulation upon alteration of plastid protein homeostasis [24], and by the editing-level changes observed in *rpoB* and *rpoC1* transcripts, which encode subunits of plastid-encoded RNA polymerase [22]. In agreement with previous studies [66,74], it is conceivable that the alteration of the activity of the plastid RNA polymerases results in abnormal transcription of a specific set of plastid genes and, possibly, in the source of the GUN1-dependent retrograde signalling. Furthermore, the altered transcription of the NEP-dependent *ycf1* gene, which encodes the Tic214 subunit of the 1 MDa TIC complex [24], together with the GUN1-cpHSC70-1 (chloroplast heat shock protein 70-1) interaction [23], have provided the connection between cytosolic folding stress and the GUN1-dependent retrograde signalling. HSP90, induced by cytosolic preproteins, has been verified to sustain the expression of nuclear photosynthesis-related genes [23], pointing to a possible connection of GUN1-retrograde signalling and the chloroplast unfolded protein response (cpUPR) [113,114].

These recent findings offer a novel point of view to elucidate the mechanisms by which the plastid transcriptional machinery and, more generally, the PGE apparatus communicates with the nucleus; however, several questions still remain. Critical future directions for the research field include efforts to understand whether cytosolic folding stress only occurs in the *gun1* mutant. Also, the identity of the downstream signal components of HSP90 require further exploration. Another question is whether plastid RNA editing and cytosolic folding stress are also connected with each other. Finally, the intriguing dual localization of a few PAP proteins and their role in the coordination of plastid and nuclear transcription deserves further investigation. Nuclear proteomics analyses, and the in-depth biochemical analyses of the main players identified, could provide important clues on the molecular mechanisms at the root of plastid-nucleus transcription machinery coordination, essential during early stages of chloroplast biogenesis and upon alteration of plastid protein homeostasis.

Data accessibility. This article has no additional data.

Authors' contributions. L.T. and P.P. designed the study. N.J. and M.C. took care of table 1 and figure 1. All authors helped draft the manuscript. P.P. coordinated the study and took care of the final version of the manuscript. All authors gave final approval for publication and agree to be held accountable for the work performed therein.

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