Chapter 1
Plastid Transcription: A Major Regulatory Point in Chloroplast Biogenesis

Nora Flynn, Xuemei Chen, and Meng Chen

Abstract Plastids are endosymbionts that retain their own, minimal genome that must be transcribed. This genome is known as the plastome. The host cell nucleus exerts tight control over the plastome to ensure the accurate timing of key developmental events, such as chloroplast biogenesis. Therefore, communication from the nucleus to the plastid, or anterograde signaling, is a vital step in plastome expression. Regulation of plastid transcription by the nucleus is tied to two DNA-dependent RNA polymerases that transcribe the plastome: the nuclear-encoded polymerase (NEP) and the plastid-encoded polymerase (PEP), which contains numerous nuclear-encoded PEP-associated proteins (PAPs). This chapter will cover the fundamentals and regulation of plastome transcription, with a focus on the role of plastid transcription in chloroplast biogenesis. In particular, the chapter will highlight the regulatory strategy of the nucleus to comprehensively control plastome gene expression through the assembly and activation of the PEP supercomplex, which is coordinated by both environmental and developmental factors.

Keywords Chloroplast · Organelle gene expression · Plastid-encoded polymerase (PEP) · PEP-associated proteins (PAP) · Sigma factors · Nuclear-encoded polymerase (NEP) · Anterograde signaling · Phytochrome · Phytochrome interacting factors (PIF)

N. Flynn · M. Chen (✉)
Department of Botany and Plant Sciences, Institute for Integrative Genome Biology, University of California, Riverside, CA, USA
e-mail: meng.chen@ucr.edu

X. Chen (✉)
National Key Laboratory for Protein and Plant Gene Research, Peking-Tsinghua Joint Center for Life Sciences, School of Life Sciences, Peking University, Beijing, China

Beijing Advanced Center of RNA Biology (BEACON), Peking University, Beijing, China
e-mail: xuemei.chen@pku.edu.cn

T. M. Burch-Smith (ed.), *Chloroplast Gene Expression: Regulation, Stress Signaling and Biotechnology*, Nucleic Acids and Molecular Biology 37,
https://doi.org/10.1007/978-3-031-70098-9_1

1.1 Becoming Green: Not as Easy as it Seems

Emerging from its seed, a plant activates a sophisticated molecular network to develop green leaves. The greening of plants is due to the differentiation of a special plastid, the chloroplast. As a photosynthetic powerhouse, a factory for diverse chemical compounds, and an intricate signaling hub, the chloroplast is a critical organelle with a unique origin. The chloroplast arose from an endosymbiotic event that occurred over a billion years ago, where a photosynthetic, cyanobacterial-like ancestor was taken up by a eukaryotic cell (Mereschkowsky 1905; Sagan 1967; Martin and Kowallik 1999; Gould et al. 2008). Over evolutionary time, the cyanobacterial ancestor endosymbiont evolved into the plastids we see today, of which the chloroplast is only one of multiple specialized types (Kirk and Tilney-Bassett 1978).

As descendants of cyanobacteria, plastids maintain their own genomes (Ris and Plaut 1962; Sager and Ishida 1963), complete with transcriptional and translational activity (Kirk 1964; Zoschke and Bock 2018). However, this genome is subjected to nuclear control by the host cell, accomplished partially through the large-scale the biosynthesis of the photoprotection information to the nucleus (Arabidopsis Genome Initiative 2000; Kleine et al. 2009). Thus, a free-roaming bacterium transitioned to the modern-day chloroplast, with a minimal genome, known as the plastome, that contains less than 10% of the cyanobacterial genome (Martin and Herrmann 1998; Martin et al. 2002). With so many of its original genes transferred to the nucleus, and newly-evolved proteins targeted to the plastid, the chloroplast depends on protein import to obtain a majority of plastid-localized proteins (Abdallah et al. 2000; Kessler and Schnell 2009). Therefore, the chloroplast is genetically semi-autonomous, with all basic functions, such as transcription, translation, and photosynthesis, dependent on nuclear gene expression.

Similarly, chloroplast biogenesis during greening requires the interplay of both photosynthesis-associated nuclear genes (PhANGs) and plastome-encoded photosynthesis-associated genes (PhAPGs). For the purposes of this chapter, PhANGs are defined as a set of ~150 genes that were classified by Hwang et al. 2022, which directly relate to light harvesting complexes, chlorophyll biosynthetic enzymes, photosystem I and II complexes, electron transport, ATP synthase, and the Calvin cycle. During chloroplast biogenesis, PhANGs and PhAPGs are closely coordinated through signaling between the nucleus and the plastid, leading to the frequent synchronization of their expression. For this reason, PhANGs and PhAPGs are typically considered as coupled (Nott et al. 2006). The mirroring of the two genomes can be observed by applying chemical treatments to the chloroplast, such as lincomycin or norflurazon, to inhibit plastid translation or block the biosynthesis of the photoprotection pigment, carotenoid, respectively (Oelmüller 1989; Susek et al. 1993; Koussevitzky et al. 2007). By greatly disturbing chloroplast function and PhAPG transcription, PhANG transcription decreases in turn (Nott et al. 2006; Ruckle et al. 2012), demonstrating the dynamic communication between the plastid and the nucleus.

To achieve this tight coordination between the nucleus and the plastid, there must be an array of regulatory signals. From the plastid, retrograde signals update the nucleus on chloroplast functioning (Nott et al. 2006; de Souza et al. 2017). Meanwhile, from the nucleus, anterograde signals exert extensive influence on chloroplast activity, especially by controlling plastome transcription (Yoo et al. 2020). Compared to retrograde signals, determining the identity of the anterograde signals has proved difficult considering the sheer number of nuclear-encoded proteins essential for chloroplast functions. Nonetheless, in recent years, promising advances in this challenging field have begun to reveal some possible nucleus-to-plastid anterograde mechanisms to control plastid transcription.

This chapter will consider both a plastid and a nuclear perspective to review the current theories surrounding the regulation of plastome transcription for accurate timing of chloroplast biogenesis. We will begin by exploring the plastome and the genes that remain there, then will pivot to discuss the DNA-dependent RNA polymerases involved in transcribing these genes. Finally, we will delve into the regulation of chloroplast transcription and biogenesis with an emphasis on how these processes are influenced by light via photoreceptor signaling. In particular, we will highlight an important contrast between the strategies of transcriptional control in the nucleus and plastid: while the nucleus employs a gene-by-gene-based regulatory system, the plastid instead utilizes polymerase activity itself as a key point of control in plastome expression.

As a fascinating chimeric system, the chloroplast retains both its original bacterial machinery as well as learned eukaryotic methods to achieve regulated gene expression, creating a complex transcriptional landscape despite its overwhelmingly simple genome. This chapter will only focus on the importance of transcriptional regulation in the plastid, particularly during chloroplast biogenesis. However, it is worthwhile to note that post-transcriptional and post-translational processes are also critically important regulatory steps in plastid gene expression. For a review of these topics, we suggest a number of reviews (Stern et al. 2010; Barkan and Small 2014; Nawaz and Kang 2017; Zhao et al. 2020; Zeng et al. 2022; Zhang et al. 2023) and Chaps. 2 and 3 of this volume.

1.2 The Plastome: A Brief Review of Structure and Contents

Reflecting its small size, the plastome was one of the first genomes to be fully sequenced using first-generation sequencing approaches. The *Marchantia polymorpha* (liverwort) and *Nicotiana tabacum* (tobacco) plastomes were fully sequenced in the 1980s (Ohyama et al. 1986; Shinozaki et al. 1986) and were followed by the Arabidopsis plastome roughly a decade later (Sato et al. 1999). Generally, the plastome contains 100–200 genes, including rRNA, tRNA, and protein-coding genes. The plastid DNA displays a circular, quadripartite structure that is shared among both plant and algal lineages, with two single-copy regions and two inverted repeats, though there are some exceptions to this rule (Turmel et al. 2002; Wicke

et al. 2011). The inverted repeats provide duplicated regions to increase the transcription of highly abundant genes, including rRNA. Meanwhile, the two single-copy regions encode genes associated with photosynthesis, carbon assimilation, and housekeeping. These two single-copy regions are distinguished as the large single-copy region, which houses most of the photosynthesis-associated protein-coding genes, and the small single-copy region, which, at least for most land plants, is associated with *ndh* genes for the NAD(P)H dehydrogenase complex, a mediator of electron transport (Peltier et al. 2016; Shikanai 2016). However, *ndh* genes have been lost multiple times, such as in *Chlamydomonas* or in the highly-reduced plastomes of parasitic plants such as *Epifagus* (dePamphilis and Palmer 1990; Martín and Sabater 2010; Wicke et al. 2011). Finally, both regions encode numerous tRNAs to support plastid translation, including around 35 tRNA genes that represent all 20 amino acids (Sugiura et al. 1989; Sato et al. 1999; Mohanta et al. 2023).

Similar to bacterial genomes, the plastome is organized in defined operons, with the vast majority of plastid genes transcribed as polycistronic units (Sugita and Sugiura 1996; Shahar et al. 2019; Castandet et al. 2019). For example, the plastome encodes core genes for the two photosystems, photosystem I (designated as *psa* genes) and photosystem II (designated as *psb* genes), with each having unique light absorption spectra during photosynthesis (Wollman 2001). Many photosystem-related genes are transcribed as polycistronic units, such as *psbD-psbC*, *psaA-psaB*, and *psbE-psbF-psbL-psbJ* (Sugita and Sugiura 1996). Similarly, many *ndh* genes locate to an operon with *psaC*, (*ndhD-psaC-ndhE-ndhG-ndhI-ndhA-ndhH*) (Kanno and Hirai 1993) and rRNA genes form an operon with some tRNAs (*rrn16-trnI-trnA-rrn23-rrn4.5-rrn5*) (Strittmatter et al. 1985; Sugita and Sugiura 1996; Shahar et al. 2019). Only a minority of genes, such as the large subunit of Rubisco (*rbcL*), the critical reaction center D1 protein of photosystem II (*psbA*), and many tRNAs, are considered monocistronic (Sugita and Sugiura 1996; Shahar et al. 2019).

1.3 What Transcribes the Plastome?

On its own, the plastome cannot fully achieve transcription. The chloroplast relies on the nucleus to provide many necessary factors for plastome transcription. For this reason, plastid protein import and protein complex assembly mark critical points of plastid transcriptional regulation.

What enzymes are involved in the transcription of the plastome? There are two DNA-dependent RNA polymerases (RNAPs) that transcribe the plastome—a bacterial-type, multi-subunit, plastid-encoded RNA polymerase (PEP) and a phage-type, single-subunit, nuclear-encoded RNA polymerase (NEP) (Liere and Börner 2007). The next sections will explore the discovery, structure, and activity of each of the polymerases.

1.3.1 PEP

1.3.1.1 Discovery of PEP

The endogenous PEP enzyme was first partially purified from maize (Bottomley et al. 1971; Smith and Bogorad 1974), wheat (Polya and Jagendorf 1971a, b), and the single-celled microorganism *Euglena gracilis* (Hallick et al. 1976). PEP was shown to be a stable, multisubunit complex (Bottomley et al. 1971; Smith and Bogorad 1974). When purified, the PEP transcription complex remained bound to chloroplast DNA and was able to transcribe this associated DNA. Therefore, the purified DNA-RNA-protein complexes containing the PEP were termed plastid Transcriptionally Active Chromosome (pTAC) (Hallick et al. 1976; Briat et al. 1979).

Biochemical analysis demonstrated that the purified PEP contains core bacterial subunits and therefore represents a bacterial-type plastid-encoded RNAP (Little and Hallick 1988; Hu and Bogorad 1990; Hu et al. 1991). Sequencing of the plastome revealed the genes homologous to the bacterial α, β, and β′ subunits: *rpoA, rpoB, and rpoC*, respectively. However, in plastids, the β′ subunit is split into β′ and β″, which are encoded by *rpoC1* and *rpoC2* (Ohyama et al. 1986; Shinozaki et al. 1986)—a feature shared by the ancestral cyanobacterial RNAP (Bergsland and Haselkorn 1991). In terms of organization, for most plant and algal lineages, *rpoB*, *rpoC1*, and *rpoC2* form an operon, while *rpoA* is part of a different operon that includes genes encoding ribosomal proteins (Liere and Börner 2007). Exceptions to this rule do exist, such as for *Chlamydomonas*, which displays fragmentation and rearrangement of these genes (Smith and Purton 2002). Despite these exceptions, overall, the core subunits of PEP are widely conserved among members of the green lineage, particularly the sequences of the catalytic domains of β, β′ and β″ (Sutherland and Murakami 2018; Ruedas et al. 2022). The α subunit is more involved in complex assembly and promoter binding and is less conserved than the catalytic domains.

The PEP core rarely exists or functions on its own. More commonly, PEP exists as part of a much larger supercomplex. Mass spectrometry (MS)-based proteomic analyses on the isolated pTAC complex from tobacco (*Nicotiana tabacum*) (Suzuki et al. 2004; Wu et al. 2024), Arabidopsis (Pfalz et al. 2006), and mustard (Steiner et al. 2011; Ruedas et al. 2022; Vergara-Cruces et al. 2024; do Prado et al. 2024) eventually identified fifteen nucleus-encoded accessory proteins—named PEP-associated proteins (PAPs) (Steiner et al. 2011). Many of the PAPs follow a dual naming convention due to their progressive identification. The Arabidopsis PAPs and their common identifiers are as follows: PAP1/pTAC3, PAP2/pTAC2, PAP3/pTAC10, PAP4/FSD3, PAP5/pTAC12/HEMERA, PAP6/FLN1, PAP7/pTAC14, PAP8/pTAC6, PAP9/FSD2, PAP10/TrxZ, PAP11/MurE-like, PAP12/pTAC7/ω, PAP13/FLN2, PAP14/pTAC18 and PAP15/PRIN2 (Table 1.1).

The PAPs are mostly eukaryotic additions that are not present in the polymerases of bacteria. In *Chlamydomonas*, many PAP orthologues are similarly not found and the PAPs that are present might not associate with PEP due to the lack of conservation

Table 1.1 Constituents of the PEP supercomplex

Component	Accession	Phenotype	Potential Interactors
Core			
rpoA/α	ATCG00740	Albino (De Santis-Maclossek et al. 1999)	PAP5 (Ruedas et al. 2022; Wu et al. 2024) PAP6 (Ruedas et al. 2022; Wu et al. 2024) PAP13 (Wu et al. 2024)
rpoB/β	ATCG00190	Albino (Allison et al. 1996; De Santis-Maclossek et al. 1999)	RpoC1 (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024) RpoC2 (Wu et al. 2024) PAP3 (Wu et al. 2024) PAP5 (Vergara-Cruces et al. 2024) PAP1 (Wu et al. 2024)
rpoC1/β′	ATCG00180	Albino (De Santis-Maclossek et al. 1999)	RpoB (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024) RpoC2 (Wu et al. 2024) PAP5 (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024) PAP14 (Vergara-Cruces et al. 2024) PAP13 (Wu et al. 2024) PAP1 (Vergara-Cruces et al. 2024; Wu et al. 2024)
rpoC2/β″	ATCG00170	Untested, assumed albino	RpoB (Wu et al. 2024) RpoC1 (Wu et al. 2024) PAP3 (Wu et al. 2024) PAP5 (Wu et al. 2024) PAP11 (Vergara-Cruces et al. 2024) PAP9 (Vergara-Cruces et al. 2024; Wu et al. 2024) PAP1 (Wu et al. 2024) PAP2 (Wu et al. 2024)
PAP12/ω/ pTAC7	AT5G24314	Albino/ivory (Pfalz and Pfannschmidt 2013; Yu et al. 2013)	PAP3 (Yu et al. 2013; Chang et al. 2017) PAP5 (Yu et al. 2013) PAP7 (Yu et al. 2013) PAP6 (Yu et al. 2013) PAP1 (Vergara-Cruces et al. 2024)
Scaffold Module			
PAP3/ pTAC10	AT3G48500	Albino/ivory (Steiner et al. 2011; Jeon et al. 2012; Chang et al. 2017)	RpoB (Wu et al. 2024) RpoC2 (Wu et al. 2024) PAP12 (Yu et al. 2013; Chang et al. 2017) PAP5 (Wu et al. 2024) PAP7 (Chang et al. 2017) PAP4 (Vergara-Cruces et al. 2024; Wu et al. 2024) PAP9 (Wu et al. 2024) PAP6 (Wu et al. 2024) PAP10 (Chang et al. 2017)
PAP5/ pTAC12/ HMR	AT2G34640	Albino/ivory (Pfalz et al. 2006a, 2015; Chen et al. 2010; Kendrick et al. 2022)	RpoA (Ruedas et al. 2022; Wu et al. 2024) RpoB (Vergara-Cruces et al. 2024) RpoC1 (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024) RpoC2 (Wu et al. 2024) PAP12 (Yu et al. 2013) PAP3 (Wu et al. 2024)

in key interface residues (Pfalz and Pfannschmidt 2013; Díaz et al. 2018; Wu et al. 2024; do Prado et al. 2024). PAPs more frequently emerged in charophytes, the sister group of land plants, but the full PEP-PAP supercomplex evolved with land plants and most land plants have homologs of the PAPs (Wu et al. 2024). Therefore, PAPs evolved over time to become an important piece of the plastid transcriptional machinery in land plants, providing an additional layer of nuclear control over the plastid.

Tab. 1.1 (continued)

			PAP7 (Gao et al. 2011; Song et al. 2023) PAP8 (Liebers et al. 2020; Vergara-Cruces et al. 2024) PAP6 (Ruedas et al. 2022)
PAP7/ pTAC14	AT4G20130	Albino/ivory (Gao et al. 2011; Grübler et al. 2017)	PAP12 (Yu et al. 2013) PAP3 (Chang et al. 2017) PAP5 (Gao et al. 2011; Song et al. 2023)
PAP8/pTAC6	AT1G21600	Albino/ivory (Pfalz et al. 2006a)	PAP5 (Liebers et al. 2020; Vergara-Cruces et al. 2024) PAP1 (Vergara-Cruces et al. 2024)
PAP11/ MurE-like	AT1G63680	Albino (Garcia et al. 2008; Kendrick et al. 2022)	RpoC2 (Vergara-Cruces et al. 2024) PAP13 (Vergara-Cruces et al. 2024) PAP1 (Ruedas et al. 2022) PAP2 (Wu et al. 2024)
PAP14/ pTAC18	AT2G32180	Untested, possible WT-like phenotype (Pfalz and Pfannschmidt 2013)	RpoC1 (Vergara-Cruces et al. 2024) PAP9 (Vergara-Cruces et al. 2024; Wu et al. 2024)
Protection Module			
PAP4/FSD3	AT5G23310	Pale, but *fsd2 fsd3* is albino (Myouga et al. 2008)	PAP3 (Chang et al. 2017; Vergara-Cruces et al. 2024; Wu et al. 2024) PAP9 (Myouga et al. 2008)
PAP9/FSD2	AT5G51100	Pale, but *fsd2 fsd3* is albino[1] (Myouga et al. 2008)	RpoC2 (Vergara-Cruces et al. 2024; Wu et al. 2024) PAP3 (Chang et al. 2017; Wu et al. 2024) PAP14 (Vergara-Cruces et al. 2024; Wu et al. 2024) PAP4 (Myouga et al. 2008)
Regulation Module			
PAP6/FLN1	AT3G54090	Albino/ivory (Arsova et al. 2010; Steiner et al. 2011; Gilkerson et al. 2012)	RpoA (Ruedas et al. 2022; Wu et al. 2024) PAP12 (Yu et al. 2013) PAP3 (Wu et al. 2024) PAP5 (Ruedas et al. 2022) PAP10 (Arsova et al. 2010; He et al. 2018; Wu et al. 2024) PAP13 (Huang et al. 2013)
PAP10/TrxZ	AT3G06730	Albino/ivory (Arsova et al. 2010; Schröter et al. 2010)	PAP3 (Chang et al. 2017) PAP6 (He et al. 2018; Wu et al. 2024) PAP13 (Arsova et al. 2010; Huang et al. 2013; He et al. 2018) PAP15 (Díaz et al. 2018)
PAP13/FLN2	AT1G69200	Albino/ivory (Huang et al. 2013) or pale (Arsova et al. 2010; Gilkerson et al. 2012)	RpoA (Wu et al. 2024) RpoC1 (Wu et al. 2024) PAP6 (Huang et al. 2013) PAP10 (Arsova et al. 2010; He et al. 2018) PAP11 (Vergara-Cruces et al. 2024)
RNA/DNA Module			
PAP1/pTAC3	AT3G04260	Albino/ivory (Yagi et al. 2012)	RpoB (Wu et al. 2024) RpoC1 (Vergara-Cruces et al. 2024; Wu et al. 2024) RpoC2 (Wu et al. 2024) PAP12 (Vergara-Cruces et al. 2024) PAP8 (Vergara-Cruces et al. 2024) PAP11 (Ruedas et al. 2022) PAP2 (Ruedas et al. 2022; Wu et al. 2024)
PAP2/pTAC2	AT1G74850	Pale (Pfalz et al. 2006a)	RpoC2 (Wu et al. 2024) PAP11 (Wu et al. 2024) PAP1 (Ruedas et al. 2022; Wu et al. 2024)
PAP15/ PRIN2	AT1G10522	Pale (Kindgren et al. 2012; Díaz et al. 2018)	PAP10 (Díaz et al. 2018)

Components are colored by proposed functional module. Potential interactions only include interactions determined by crosslinking-MS or yeast two-hybrid assay. See Fig. 1.1 for additional interactions indicated by the cryo-EM structures

1.3.1.2 PEP Structure

Recent structural studies using cryo-electron microscopy (cryo-EM) revealed the complete structure of PEP complexes from white mustard and tobacco (Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). PEP is a 21-subunit

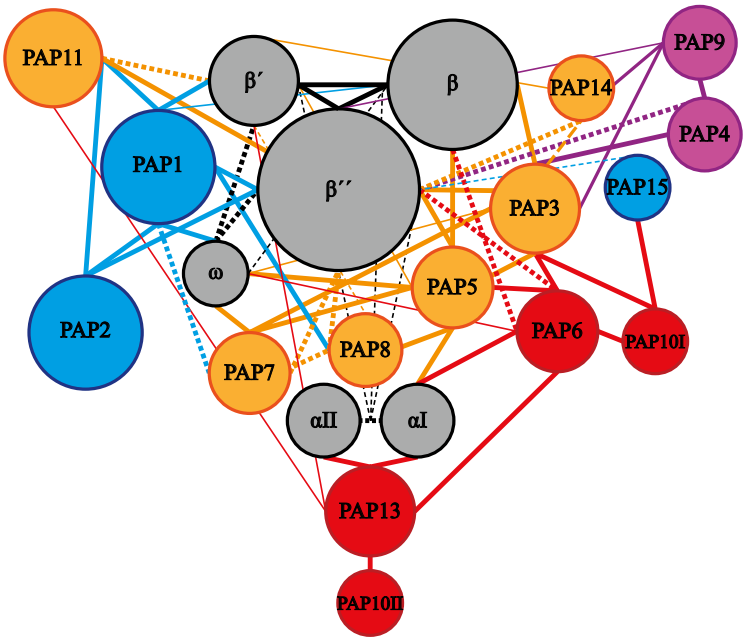

Fig. 1.1 The interaction network of the PEP supercomplex. PEP contains 21 subunits, 19 of which are unique. The colors correspond to the predicted functional modules in Table 1.1; the circle size is proportional to the size of the protein. Solid lines indicate that the interaction was confirmed by crosslinking-MS or yeast two-hybrid assay (Table 1.1). Dashed lines depict additional interactions that were found through cryo-EM (Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). Thinner lines pass behind at least one object, while thicker lines move directly between two objects

complex that contains six core subunits, $\alpha_2\beta\beta'\beta''$, which are similar in structure and sequence to bacterial RNAPs (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). A notable difference between bacterial RNAPs and the PEP core is the ω subunit. The core bacterial RNAP is $\alpha_2\beta\beta'\omega$ (Zhang et al. 1999; Murakami et al. 2002; Vassylyev et al. 2002), where the ω subunit serves structural and functional roles (Patel et al. 2020). In the PEP, the nuclear-encoded PAP12 may be homologous to the ω subunit due to its similarity in folding and positioning within the PEP complex, as well as the conservation of key residues (Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). For the remainder of this chapter, PAP12 will be considered part of the PEP core. However, surprisingly, PAP12 is not present in all chlorophytes and charophytes, which raises some skepticism about whether it fully encompasses the roles of the ω subunit or if it has more limited roles to promote stability (Wu et al. 2024; do Prado et al. 2024).

The PEP core evolved to handle extensive interactions with PAPs. Besides PAP12, PEP includes fourteen other PAPs, with thirteen unique PAPs and two copies of the thioredoxin subunit, PAP10. These PAPs encircle the interconnected core subunits, with each core subunit in close association with multiple PAPs (Fig. 1.1 and Table 1.1). To support these numerous interactions with eukaryotic PAPs, the

surface residues of the core are less conserved than the internal catalytic domains when compared with their bacterial counterparts (Ruedas et al. 2022). Further, PEP β″ contains a large lineage-specific region known as sequence insertion 3 (SI3) that may have roles in both protein interaction and polymerase activity (Lane and Darst 2010; Windgassen et al. 2014; Mazumder et al. 2020). In bacteria, SI3 is relatively short, with only a couple of sandwich-barrel hybrid-motif (SBHM) domains (Artsimovitch et al. 2003; Qayyum et al. 2024). Meanwhile, SI3 in cyanobacteria contains more SBHM domains (Qayyum et al. 2024), but is still approximately 100 residues shorter than the PEP SI3 (Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). The larger SI3 in PEP makes frequent contacts with PAPs, connecting the core with these essential eukaryotic proteins (Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). Overall, the PEP supercomplex forms a star-shape with five protruding arms created by the PAPs (Fig. 1.1) (Vergara-Cruces et al. 2024).

The positioning of PEP components is reflected in mutant phenotypes, with more peripheral PAPs demonstrating less severe phenotypes. The PEP core itself is critical for chloroplast development. Loss of any of the core subunits results in albino seedlings that require an external carbon source, such as sucrose, in order to survive (Allison et al. 1996; De Santis-MacIossek et al. 1999; Chateigner-Boutin et al. 2008; Pfannschmidt et al. 2015). Similarly, the loss of any of the PAPs results in a chloroplast-defective phenotype, though PAP14 remains untested (Table 1.1). Intermediate forms of the complex may be too unstable to achieve full PEP function, leading to the defects observed when even a single PAP is lost (Pfalz and Pfannschmidt 2013; Pfannschmidt et al. 2015). In particular, the PAPs that are most important for maintaining the integrity of the large complex demonstrate an albino phenotype upon their loss. These include PAP1, PAP3, PAP5, PAP6, PAP7, PAP8, PAP10, PAP11, PAP12, and, potentially, PAP13, many of which are also positioned close to the core (Pfalz et al. 2006; Garcia et al. 2008; Arsova et al. 2010; Schröter et al. 2010; Chen et al. 2010; Steiner et al. 2011; Gao et al. 2011; Jeon et al. 2012; Yagi et al. 2012; Gilkerson et al. 2012; Pfalz and Pfannschmidt 2013; Yu et al. 2013; Huang et al. 2013; Chang et al. 2017; Grübler et al. 2017; Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). Meanwhile, null mutants of *PAP2*, *PAP4*, *PAP9*, and *PAP15* are pale, implying that carotenoid biosynthesis remains intact. Following their slightly more mild phenotypes, these components are positioned towards the periphery of the complex, where they may function in transcriptional activity (Pfalz et al. 2006; Myouga et al. 2008; Steiner et al. 2011; Kindgren et al. 2012; Gilkerson et al. 2012; Pfalz and Pfannschmidt 2013; Díaz et al. 2018). For dark-grown plants, the *pap* mutants have no noticeable phenotype, suggesting that the addition of the PAPs serves as a switch for PEP transcription in light in some organisms (Ruedas et al. 2022).

The organization of the PAPs in the PEP complex can be divided into four modules based on their location and predicted functions (Steiner et al. 2011; Kindgren and Strand 2015; Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). First, the scaffold module (PAP3, PAP5, PAP7, PAP8, PAP11 and PAP14) contains the major PAPs for establishing PEP architecture. Second, the protection module

(PAP4 and PAP9) serves to shield the PEP from damaging reactive oxygen species. Third, the regulation module (PAP6, PAP10I, PAP10II, and PAP13) may be involved in redox-related processes. Finally, the RNA/DNA module (PAP1, PAP2, and PAP15) includes PAPs that may interact with nucleic acids during transcription. For a summary of the interactions of these modules and the core, see Fig. 1.1 and Table 1.1.

1.3.1.2.1 The Scaffold Module

The scaffold module serves as a docking site or bridge to connect the core with the other modules, with each PAP in the scaffold module fielding diverse interactions. Through its numerous interactions, the scaffold module is a major coordinator of PEP architecture, setting the correct positioning of PAPs around the core. However, the scaffolding PAPs could also have functions outside of PEP stability, as seen by their conserved domains. For example, PAP7 has a SET domain that is shared by enzymes with histone lysine methyltransferase function (Gao et al. 2011; do Prado et al. 2024). Meanwhile, PAP11 contains partially conserved residues for the substrate binding and catalytic activity of a Mur ligase (Garcia et al. 2008; do Prado et al. 2024). Whether these domains contribute to the activities of the PEP complex remains unknown and the specific roles of each of the PAPs requires further study.

1.3.1.2.2 The Protection Module

The protection module acts to defend the PEP complex from photosynthesis-related oxidation damage by converting precursors of reactive oxygen species to less-damaging forms. In support of this, both PAP4 and PAP9 belong to the iron superoxide dismutase family and loss of either protein leads to sensitivity to oxidative stress (Myouga et al. 2008; Steiner et al. 2011; Pfalz and Pfannschmidt 2013). In the PEP supercomplex, PAP4 and PAP9 form a heterodimer (Myouga et al. 2008) that is docked to the core through the scaffolding PAPs, PAP3 and PAP14 (Wu et al. 2024).

1.3.1.2.3 The Regulation Module

The regulation module may inform PEP activity through involvement in redox-related processes. PAP10 has a thioredoxin motif and disulfide-bond reductase activity, while PAP6 and PAP13 both have phosphofructokinase domains (Arsova et al. 2010; Chibani et al. 2011; Gilkerson et al. 2012; Wimmelbacher and Börnke 2014; Díaz et al. 2018). However, the mode of action of these components and the significance of these domains remain to be determined. For example, the albino phenotype of *pap10* could be rescued by a catalytically deficient PAP10, with slight chlorosis in the rescued plants (Wimmelbacher and Börnke 2014).

In the PEP supercomplex, two units of PAP10 (PAP10I and PAP10II) each form a heterodimer with PAP6 and PAP13, respectively (Arsova et al. 2010). The scaffolding PAP that is most involved in connecting the regulatory module with the core is PAP5. Specifically, PAP5 interacts with an α subunit to provide a bridge to attach the PAP6-PAP10I heterodimer to the PEP core, with the help of PAP3. On the other hand, the PAP13-PAP10II heterodimer is directly attached to the α subunits (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). PAP6 might also make direct contact with an α subunit, which could have interesting mechanistic implications in the movement and controlled release of the α subunits during transcription (Vergara-Cruces et al. 2024; Wu et al. 2024).

Of note, PAP15, which is currently placed in the RNA/DNA module, plays a critical role in the redox regulation of PEP activity. The light-dependent reduction of an intermolecular disulfide bond converts PAP15 from a dimeric form to a monomeric form that is required for PEP activation (Kindgren et al. 2012; Díaz et al. 2018). Continued research on the specific functions of the PAPs within the PEP complex may result in assignment of PAPs to different or multiple modules.

1.3.1.2.4 The RNA/DNA Module

The RNA/DNA module is split among several arms of the star-shaped structure (Fig. 1.1). These PAPs are thought to interact with nucleic acids. PAP1 was shown to bind chloroplast DNA and contains both a SAP domain and pentatricopeptide repeat (PPR) motifs (Yagi et al. 2012; Wang et al. 2024). While the SAP domain might interact with entering DNA during recruitment of PEP, the PPR motifs may instead be more involved in the scaffolding of the complex itself (Vergara-Cruces et al. 2024; Wang et al. 2024). Similarly, PAP2 has an SMR domain and PPR motifs, but the SMR domain may be more involved in PAP-PAP interaction than nuclease activity. (Vergara-Cruces et al. 2024). However, the PPR motif of PAP2 is positioned to interact with nascent RNA (Vergara-Cruces et al. 2024; Wu et al. 2024). As for PAP15, it does not contain a known domain for binding nucleic acids, but it can bind to DNA *in vitro* and may assist the PEP complex with DNA binding (Kremnev and Strand 2014; Vergara-Cruces et al. 2024). As for the other modules, further research is required to determine the functions of these domains within the complex.

The scaffolding proteins PAP8 and PAP7 dock PAP1 and PAP2, creating a region of PEP that could specialize in interacting with nucleic acids (Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). PAP1 interacts with PAP2 to position PAP2's PPR domain near where the nascent RNA exits the complex. PAP2's PPR domain could have sequence-specific RNA-binding properties to bind a consensus sequence and direct polymerase pausing and downstream RNA processing events (Ruedas et al. 2022; Vergara-Cruces et al. 2024; Wu et al. 2024; do Prado et al. 2024). Therefore, together, the interaction between PAP1 and PAP2 endows the complex the ability to interact with entering DNA through PAP1's SAP domain and

the ability to interact with nascent RNA through PAP2's PPR domain. As for PAP15, it binds directly to the β″-SI3 (Vergara-Cruces et al. 2024).

In addition, other PAPs outside of the RNA/DNA module could impact interactions with nucleic acids during transcription (Kindgren and Strand 2015; Pfannschmidt et al. 2015). For example, PAP3 contains an S1 domain with RNA-binding properties, but this domain might not be well-positioned in the PEP complex to interact with nucleic acids (Pfalz et al. 2006; Vergara-Cruces et al. 2024). Maize PAP5 has also demonstrated binding to single-stranded nucleic acids (Pfalz et al. 2015). Continued analysis of the activities of each of the PAPs is necessary to confirm their placement in each of these modules.

1.3.1.2.5 Sigma Factors and Promoter Recognition

The composition of PEP must accommodate the transient guidance by sigma (σ) factors. Similar to bacterial RNAPs, the PEP complex requires sigma factors for promoter binding and recognition (Burgess et al. 1969; Allison 2000; Schweer et al. 2010; Feklístov et al. 2014). The current models do not prohibit sigma factor binding and propose that sigma factors could associate with the complex by interacting with PAP11 (Vergara-Cruces et al. 2024).

Arabidopsis has six nucleus-encoded sigma factors (SIG1-SIG6) that recruit the PEP to similar consensus sequences as bacterial σ^{70}-type promoters, which have −35 (TGACA) and −10 (TATAAT) sequences (Gatenby et al. 1981; Liere and Börner 2007; Ortelt and Link 2021). The ancestors of plastid sigma factors, the bacterial σ^{70} family of proteins can be separated into three general categories in cyanobacteria: essential/nonessential proteins for viability, and alternative, stress-responsive proteins (Imamura and Asayama 2009; Yagi and Shiina 2016). Most, if not all, Arabidopsis sigma factors emerged from the essential sigma factor, SigA. The sigma factors that most likely branched off from SigA include SIG2, SIG3, SIG4 and SIG6, with the latter three emerging later from SIG2 itself (Shiina et al. 2009; Yagi and Shiina 2014, 2016; Pfannschmidt et al. 2015; Fu et al. 2021). While SIG3, SIG4 and SIG6 evolved in seed plants, SIG2 and SIG1 could have each emerged from a gene duplication event of SigA, or SIG1 might instead be derived from a different SigA homolog in the nonessential group of sigma factors (Pfannschmidt et al. 2015; Fu et al. 2021). Meanwhile, SIG5 is the most distinct among the six sigma factors. SIG5 may have evolved independently from the stress-responsive group in cyanobacteria, and appears orthologous to the *E. coli* sigma factor, RpoH, a stress-responsive protein (Fujiwara et al. 2000; Pfannschmidt et al. 2015; Yagi and Shiina 2016; Fu et al. 2021). Therefore, of the Arabidopsis sigma factors, SIG1, SIG2, and SIG5 are the most ancient, while SIG3, SIG4 and SIG6 evolved more recently.

Why does Arabidopsis contain so many sigma factors when the plastome itself is so small? One explanation is that sigma factors have been added over time in response to the high plastome mutation rate caused by photosynthetic stress. Point

mutations, such as in promoter regions, could hinder recruitment of the PEP and negatively impact plastid functioning, but the nucleus could accommodate these changes through the evolution of additional sigma factors. This theory is endearingly known as the "spoiled kid hypothesis", where the buildup of mutations in the troublesome plastome is compensated for by the nucleus, creating surprising transcriptional complexity (Lerbs-Mache 2011; Lefebvre-Legendre et al. 2014). Perhaps this explains the frequent functional redundancy among the sigma factors, because, outside of the delayed greening of *sig2* and *sig6* mutants, there are few observable phenotypic differences upon the loss of a single sigma factor. However, more severe phenotypes emerge if two or more sigma factors are lost, and triple mutants can be lethal (Hanaoka et al. 2003; Ishizaki et al. 2005; Woodson et al. 2013; Bock et al. 2014; Chi et al. 2015).

Despite this functional overlap, the sigma factors do not only maintain transcriptional efficiency by merely mitigating undesirable mutations. Later sections of this chapter will discuss how sigma factors have evolved specific roles as transcriptional regulators to influence PEP activity.

1.3.2 NEP

1.3.2.1 Discovery of NEP

Even without PEP, the plastome is transcriptionally active, indicating that an alternative RNA polymerase must be able to function in the plastid. For example, deletion of *rpoB* in tobacco does not prevent plastid transcription (Allison et al. 1996) and the plastid-ribosome-deficient barley mutant, *albostrians*, similarly still displays plastid transcription (Hess et al. 1993). An even more extreme example is the parasitic plant *Epifagus virginiana*, which lacks genes encoding PEP subunits, yet still maintains plastid transcription (Morden et al. 1991). These observations indicated that a nuclear-encoded RNA polymerase must be operational in plant plastids to perform transcription even when PEP is not active.

The presence of NEP in land plants was finally confirmed with the identification of organelle-targeted, nucleus-encoded proteins with strong sequence similarities to the RNA polymerase of T3/T7 bacteriophages (Hedtke et al. 1997; Emanuel et al. 2004). NEP is likely the newer polymerase for plastid transcription because it is absent in algae (Liere et al. 2011; Yagi and Shiina 2016). However, in land plants, monocotyledonous species can contain two NEP genes, while dicotyledonous plants can have three NEP genes. Monocots have both an NEP targeted to the plastid (RPOTp) and an NEP targeted to the mitochondria (RPOTm). The additional NEP in dicots can be targeted to either organelle (RPOTmp) (Hedtke et al. 1997, 2000; Ortelt and Link 2014). Conservation of sequences between the different NEPs suggests that the additional NEP genes arose from gene duplication events of *RPOTm* (Hedtke et al. 1997).

1.3.2.2 NEP Promoter Recognition

Unlike the PEP complex, NEP functions as a single-subunit enzyme. RPOTmp and RPOTp likely serve redundant roles because the loss of one can be tolerated, though it appears that RPOTp is the predominant NEP in plastids, since its loss results in a more severe phenotype (Hricová et al. 2006). The loss of both RPOTp and RPOTmp is lethal, demonstrating the importance of NEP in land plants.

Due to its bacteriophage origin, NEP recognizes distinct promoters from PEP. In general, NEPs recognize two types of promoters (Weihe and Börner 1999; Liere and Börner 2007; Zhelyazkova et al. 2012; Ortelt and Link 2014; Börner et al. 2015). The most common promoter type is the Type I promoter, which has a YRT-element (pyrimidine-purine-thymidine). In dicots, a subclass of this promoter can be found, which contains both a YRT motif and a GAA-box. Type II promoters have a completely different sequence from the YRT-motif and are considered nonconsensus promoters. RPOTp and RPOTm have the intrinsic ability to recognize these promoter sequences *in vitro*, without assistance from a cofactor (Kühn et al. 2007). However, *in vivo*, currently unknown cofactors could be involved in directing NEP activity. On the other hand, RPOTmp, the newest of the three polymerases, seems to have lost the ability to recognize promoters as only a single-polypeptide enzyme (Kühn et al. 2007; Yagi and Shiina 2016). Nonetheless, RPOTmp is still involved in transcription of the plastome because it affects the accumulation of plastid transcripts (Baba et al. 2004).

RPOTp and RPOTmp may exhibit some preferences between these promoter types (Courtois et al. 2007; Swiatecka-Hagenbruch et al. 2008). RPOTp appears to prefer the Type I motif, characteristic of it being the principal NEP in plastids. Meanwhile, RPOTmp prefers the nonconsensus Type II motif, found in promoters of *clpP* and the *rrn16* operon. However, despite the limited specificity between the two plastid-targeted NEPs, they likely serve redundant functions, as RPOTmp can rescue transcription from most NEP promoters upon the loss of RPOTp (Swiatecka-Hagenbruch et al. 2008).

1.3.3 Shared or Divided Labor of the Plastid RNA Polymerases?

NEP and PEP share the responsibility of transcribing the plastome, but do not seem to collaborate. Despite the range of proteins isolated in pTACs, NEP was not co-isolated, suggesting that the two polymerases work independently (Pfannschmidt et al. 2015). During leaf development, NEP is activated first and is typically considered the major RNA polymerase of housekeeping genes, while PEP is the major RNA polymerase of photosynthesis-associated genes (Hajdukiewicz et al. 1997; Emanuel et al. 2004). This division of labor is canonically used to divide genes into three classes, where genes transcribed by PEP are Class I, genes transcribed by NEP

are Class III, and genes transcribed by both enzymes are Class II (Hajdukiewicz et al. 1997).

However, it may be too simplistic to say that NEP and PEP have a strict division of labor for most genes. Most plastid genes have both PEP and NEP promoters (Zhelyazkova et al. 2012) and can be transcribed in the absence of PEP (Silhavy and Maliga 1998; Krause et al. 2000; Legen et al. 2002; Swiatecka-Hagenbruch et al. 2007; Ortelt and Link 2021). In fact, there are far more promoters than genes in the plastome (Zhelyazkova et al. 2012). There could be 400 transcription start sites in the Arabidopsis plastome of ~130 genes, averaging at a transcription start site every 600-nt or so (Castandet et al. 2019). These promoters can be found all over the plastome, with transcription start sites in regions not only preceding genes, but also within genes, antisense to genes, or in intergenic regions. The abundance of transcriptional events has led to speculation that the plastome can be entirely transcribed, though perhaps not equally (Barkan 2011; Shi et al. 2016; Sanitá Lima and Smith 2017; Palomar et al. 2022).

There are still some genes that are mostly or completely transcribed by only one of the polymerases. For example, NEP is essential for the transcription of the *rpoB-rpoC1-rpoC2* operon to initially generate the core subunits of PEP. NEP also largely transcribes other housekeeping genes like *accD*, part of the acetyl-CoA carboxylase for fatty acid biosynthesis, *clpP*, part of the Clp protease complex, and *ycf2*, an open reading frame that may be important in protein import (Hajdukiewicz et al. 1997; Silhavy and Maliga 1998; Börner et al. 2015; Ortelt and Link 2021; Puthiyaveetil et al. 2021; Palomar et al. 2022; Chang et al. 2023). On the other hand, PEP transcribes *psbA* and *rbcL*, the large subunit of ribulose bisphosphate carboxylase (Rubisco) (Ortelt and Link 2021). Considering the entirety of the plastome, PEP most frequently binds within the inverted repeats and large single-copy region (Palomar et al. 2022). However, PEP's presence is reduced in the small single-copy region, suggesting that NEP may be involved in transcribing these genes. Still, overall, plastid transcription by the polymerases is highly flexible and both can transcribe wide swaths of the plastome.

1.3.3.1 Regulation of the Plastid RNA Polymerases

NEP and PEP are regulated developmentally. Early in development, NEP is the predominant transcriber of the plastome (Emanuel et al. 2004) and NEP-associated transcripts, like *clpP*, tend to display the highest expression in younger tissues, like the base of a monocot leaf (Baumgartner et al. 1989, 1993; Sakai et al. 1992; Cahoon et al. 2004, 2008). Initial transcription of housekeeping genes prepares the plastid for chloroplast biogenesis because NEP is needed to transcribe the PEP core. PEP is canonically viewed as the predominant polymerase once it is produced and activated. However, the PEP core on its own has limited capabilities, and putting together the numerous components of the PEP supercomplex is complicated. Therefore, the assembly and activation of the PEP supercomplex itself is a major point of transcriptional control imposed on the plastid by the nucleus.

PEP assembly coordinates plastome transcription with light and developmental stage to accurately time chloroplast biogenesis. The remainder of this chapter will focus on the nuclear control of chloroplast development, which is accomplished through anterograde signaling. To explore plastid transcriptional control by the nucleus, we will discuss two models: chloroplast biogenesis during light-dependent seedling de-etiolation in dicots and chloroplast biogenesis during leaf development in monocots. Through these models, we will impart the importance of the PEP for collective control of the transcription of plastid photosynthesis genes.

1.4 Anterograde Signaling: Constant Dialogue Between the Nucleus and the Plastid

1.4.1 Anterograde Signaling for the Control of PEP by Light

Plastid gene expression is part of the developmental program of the host cell and, ultimately, is controlled by the host cell's nucleus (Taylor 1989; Pearce et al. 2017). However, the mechanism of nucleus-to-organelle communication, or anterograde signaling, has remained elusive for decades. The main challenge is the difficulty in teasing apart regulators from the battery of nuclear-encoded components required for organellar biogenesis and/or function (Taylor 1989). Finally, some anterograde signals may have been revealed by studying the role of photoreceptor signaling in the light-dependent regulation of PEP during de-etiolation of developing seedlings. This anterograde signaling not only controls the transcription of PhAPGs by PEP, but also synchronizes PhAPGs with PhANGs.

1.4.1.1 The Process of Greening: De-etiolation

The greening, or chloroplast biogenesis, of angiosperms (flowering plants) and some gymnosperms occurs only in the presence of light (Kirk and Tilney-Bassett 1978; Armstrong 1998). Light directly turns on chlorophyll biosynthesis in plastids by activating protochlorophyllide oxidoreductase to catalyze the final step of the biosynthesis pathway, the conversion of protochlorophyllide to chlorophyll a (Klein and Schiff 1972; Mapleston and Griffiths 1980; Santel and Apel 1981). In parallel, as an environmental signal, light reprograms hundreds of genes in the nucleus to initiate the developmental transition from heterotrophic growth, which is supported by seed-stored energy, to autotrophic growth, which relies on photosynthesis (Kirk and Tilney-Bassett 1978; Chen et al. 2004). In dicotyledonous plants, such as *Arabidopsis thaliana*, young seedlings that germinate underground adopt a dark-grown developmental program called skotomorphogenesis (or etiolation), which inhibits leaf development and promotes elongation of the embryonic stem (hypocotyl), allowing seedlings to emerge quickly from the soil into the light. Plastids in

the leaves of dark-grown seedlings differentiate into photosynthetically inactive, non-green etioplasts. Upon illumination, seedlings undergo de-etiolation, a developmental transition to a light-grown developmental program called photomorphogenesis, which restricts hypocotyl elongation and promotes leaf development and chloroplast biogenesis.

1.4.1.2 Phytochromes and the Sensing of Light

Light is perceived by a suite of photoreceptors that play an essential role in initiating chloroplast biogenesis in both monocots and dicots (Takano et al. 2009; Strasser et al. 2010; Hu et al. 2013; Yoo et al. 2019). These include the red and far-red light-sensing phytochromes (phys) and the blue-light-absorbing cryptochromes (CRYs) (Chen et al. 2004). Here we will focus on phy signaling, because the mechanistic link between phy signaling and chloroplast biogenesis has been extensively studied. Phy is a bilin-containing pigment or a biliprotein that senses light using a covalently linked phytochromobilin as the chromophore (Rockwell et al. 2006; Burgie and Vierstra 2014). Light induces the isomerization of the C15-C16 carbon-carbon double bond of phytochromobilin between a C15-*Z* (*trans*) and a C15-*E* (*cis*) configuration, which in turn photoconverts phys between two relatively stable forms: a red light-absorbing, inactive phytochrome, Pr (C15-*Z*), and a far-red light-absorbing, active phytochrome, Pfr (C15-*E*) (Rockwell et al. 2006; Burgie and Vierstra 2014). In Arabidopsis, phys are encoded by five genes, *PHYA-E*, among which the gene products of *PHYA* and *PHYB* are the predominant sensors of continuous far-red and red light, respectively (Sharrock and Quail 1989; Parks and Quail 1993; Reed et al. 1993; Whitelam et al. 1993).

One of the earliest light responses at the cellular level is the translocation of photoactivated phys from the cytoplasm to the nucleus, where phys are further compartmentalized to photosensory subnuclear domains named photobodies (Sakamoto and Nagatani 1996; Yamaguchi et al. 1999; Kircher et al. 1999; Chen et al. 2003, 2005; Van Buskirk et al. 2012). Nuclear-localized phys initiate photomorphogenesis, including chloroplast biogenesis, by inducing transcription of nuclear photosynthesis genes, the PhANGs (Leivar et al. 2008a, b; Shin et al. 2009; Li et al. 2012; Luo et al. 2014). Light-based signaling by phys also synchronously induces the transcription of PhAPGs (Link 1982; Thompson et al. 1983; Bennett et al. 1984). As photoactivated phys and CRYs localize only to the nucleus and not to plastids (Fankhauser and Chen 2008; Wang et al. 2018), they must regulate plastid gene expression via anterograde signaling.

1.4.1.3 Light-regulated PEP Assembly and Transcription

Although it was observed that plastid mRNA levels were influenced by light, these light-induced changes could result from altered transcription of plastid genes or altered mRNA stability. Plastid run-on transcription assays were developed to

distinguish between the two mechanisms (Mullet and Klein 1987; Deng et al. 1987). Using plastid run-on transcription assays, it was demonstrated that light-inducible transcription itself is a major control step during chloroplast biogenesis. However, intriguingly, the overall plastid transcription rates of individual plastid genes were similar. Therefore, differential accumulation of individual transcripts was mostly attributable to post-transcriptional regulation of RNA stability. These results raised the hypothesis that, unlike gene regulation in the nucleus, where cell signaling regulates individual genes via gene-specific transcription regulators, light regulates PhAPGs by controlling the general, collective transcriptional activity of plastids (Deng and Gruissem 1987; Klein and Mullet 1990).

Biochemical studies of the pTAC complex in mustard (*Sinapis alba* L.) first indicated that light regulates plastid transcription through the assembly of the PEP complex. While a 420-kDa PEP complex—made of only the core subunits—formed in etioplasts in dark-grown mustard seedlings, a 700-kDa PEP complex was enriched in chloroplasts in the light (Pfannschmidt and Link 1994). Both complexes were shown to be transcriptionally active (Pfannschmidt and Link 1994, 1997). The assembly of a 1 MDa PEP complex could also be induced by light in Arabidopsis, though no smaller intermediate complex was detected (Yang et al. 2019; Yoo et al. 2019). The discrepancies in the presence of the PEP core complex may be attributable to differences in the stability of the PEP core between the two plant species.

Light signaling to trigger PEP assembly is initiated by photoreceptors. Genetic analysis of PEP assembly in photoreceptor mutants in Arabidopsis seedlings under monochromatic far-red, red, or blue light conditions demonstrated that phys and CRYs can each individually initiate PEP assembly and PhAPG activation (Yang et al. 2019; Yoo et al. 2019; Hwang et al. 2022). PEP assembly can be blocked in *phyA-211* in far-red light, in *phyB-9* in red light, and in *cry1/cry2* in blue light (Yoo et al. 2019; Hwang et al. 2022). We discuss some of the mechanisms of this light-induced signaling below.

1.4.1.4 Degradation of Nuclear PIFs Triggers PEP Assembly

Chloroplast biogenesis is repressed in darkness by a smaller family of basic/helix-loop-helix transcription factors named PHYTOCHROME-INTERACTING FACTORs (PIFs). Both phys and CRYs regulate nuclear gene expression by inhibiting the stability and activity of PIFs (Al-Sady et al. 2006; Leivar and Quail 2011; Pedmale et al. 2016; Yoo et al. 2021). In Arabidopsis, PIFs include eight members (PIF1-8), of which PIF1, PIF3, PIF4, and PIF5 collectively repress PhANGs in the nucleus (Leivar et al. 2008b, 2009; Shin et al. 2009). In parallel, nuclear PIFs remotely repress the assembly of the PEP complex (Yang et al. 2019; Yoo et al. 2019). As such, PIFs act as master repressors of chloroplast biogenesis by synchronously turning off the expression of both PhANGs and PhAPGs. Consistent with this model, a dark-grown *pif1pif3pif4pif5* quadruple mutant (*pifq*) becomes de-etiolated, morphologically mimicking wild-type Col-0 seedlings grown in the light. In *pifq*, plants show early signs of chloroplast biogenesis, such as the disappearance

of the prolamellar bodies, the development of rudimentary pro-thylakoid membranes, and the activation of PhANGs and PhAPGs (Leivar et al. 2008b, 2009; Shin et al. 2009; Yang et al. 2019; Yoo et al. 2019; Hwang et al. 2022).

How do phys orchestrate massive transcriptional reprogramming in both the nuclear and plastid genomes? To trigger light responses, phytochromes must first change conformation and subcellular location. In the dark, phys locate to the cytosol, where they are present in an inactive, red-light absorbing Pr form. With light, phys convert to an active Pfr form that moves into the nucleus (Yamaguchi et al. 1999; Kircher et al. 1999; Fankhauser and Chen 2008). In the nucleus, photoactivated phys, primarily phyB, initiate chloroplast biogenesis by releasing the PIF-dependent repression of PhANGs and PhAPGs (Fig. 1.2). The active form of phyB interacts directly with all PIFs to promote their degradation (Legris et al. 2019; Cheng et al. 2021), attenuate their DNA binding (Park et al. 2012, 2018), and repress their transactivation activity (Yoo et al. 2021).

PIF1, PIF3, PIF4, and PIF5 are rapidly degraded during the dark-to-light transition in a phyB-dependent manner (Shen et al. 2005, 2007; Al-Sady et al. 2006; Lorrain et al. 2008; Pham et al. 2018). Binding of phyB promotes PIF3 phosphorylation by PHOTOREGULATORY PROTEIN KINASEs (PPKs) and subsequent ubiquitin-proteasome-dependent degradation (Al-Sady et al. 2006; Ni et al. 2014, 2017; Dong et al. 2017). Two E3 ubiquitin ligases have been identified that

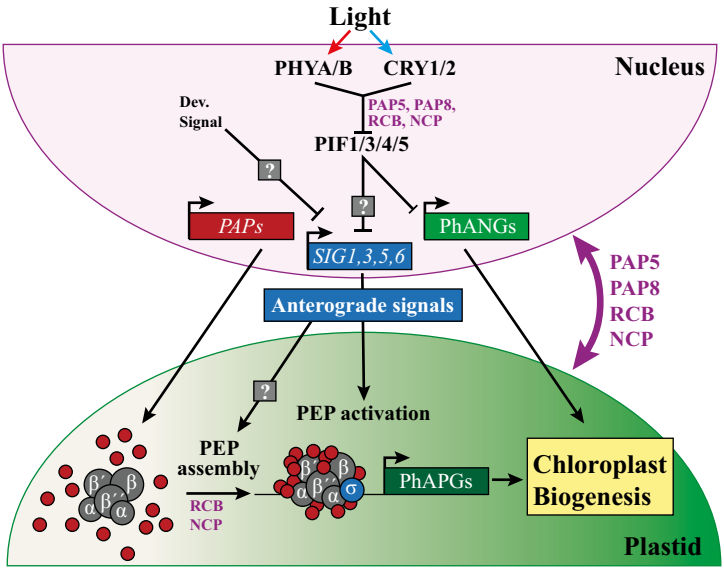

Fig. 1.2 A model of chloroplast biogenesis during light-dependent seedling de-etiolation in dicots. Photoreceptors trigger a cascade of light-induced signaling that leads to the degradation of PIFs and the activation of PhANGs and PhAPGs. To activate PhAPGs, PEP assembly and activation are major regulatory steps that are informed by anterograde signals and the accumulation of plastid-localized, nuclear-encoded proteins. Once PhANGs and PhAPGs are expressed, chloroplast biogenesis is triggered

ubiquitylate PIF3, including the **c**ullin3-**R**ING E3 ubiquitin **l**igase (CRL), with LIGHT-RESPONSE BRIC-A-BRAC/TRAMTRACK/BROADs (LRBs) as the substrate recognition subunits ($CRL3^{LRB}$), and the cullin1 E3, with EIN3-BINDING F BOX PROTEINs (EBFs) as the substrate recognition subunits ($CRL1^{EBF}$) (Ni et al. 2014; Dong et al. 2017). In addition to regulating PIFs via direct interaction, phyB also promotes PIF degradation indirectly by inhibiting factors that stabilize PIFs, such as the CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1), SUPPRESSOR OF PHYTOCHROME A-105 proteins (SPAs), and DE-ETIOLATED 1(DET1), which constitute the $CRL4^{COP1/SPA}$ and $CRL4^{COP1/DET1}$ E3 ubiquitin ligases (Saijo et al. 2003; Sheerin et al. 2015; Lu et al. 2015). Phys, and also CRYs, interact directly with COP1 and SPAs to block the formation of the COP1-SPA complex in the light (Lian et al. 2011; Liu et al. 2011; Sheerin et al. 2015; Lu et al. 2015). The classic *constitutive photomorphogenic/de-etiolated/fusca* (*cop/det/fus*) mutants, such as *cop1-4*, *det1-1*, and *spa*, exhibit similar phenotypes as *pifq,* including constitutively expressed PhANGs and PhAPGs and assembled PEP complexes in darkness (Reed and Chory 1994; Wei and Deng 1996; Hwang et al. 2022). Therefore, phyB-mediated degradation of PIFs is a major switch to initiate chloroplast biogenesis and serves as a synchronization mechanism that coordinates the expression of nuclear and plastid photosynthesis genes (Fig. 1.2).

1.4.1.5 Nucleus-and-Plastid Dual-Localized Regulators

The anterograde signaling mechanism downstream of PIFs to control the activity of the PEP remains elusive. The anterograde signal is likely encoded by a light-inducible gene repressed in darkness by PIFs, either directly or indirectly. PIFs do not regulate PAPs at the transcript level (Yoo et al. 2019; Hwang et al. 2022); therefore, it is unlikely that PIFs regulate PEP assembly by transcriptionally regulating the expression of PAPs.

The discovery of positive regulators of chloroplast biogenesis has been hindered by the difficulty to distinguish chloroplast-deficient regulator mutants from other albino mutants for genes encoding essential components of the chloroplast (Taylor 1989). Fortunately, recent genetic studies of early phy signaling have serendipitously uncovered a new class of light mutants in Arabidopsis that exhibit a combination of albino and long-hypocotyl seedling phenotypes (Chen and Chory 2011). The founding member of this new mutant class, *hemera* (*hmr*), was identified originally as a mutant defective in the localization of phyB to subnuclear foci called photobodies (Chen et al. 2010). The *hmr* mutant turns out to be defective in phyB signaling and chloroplast biogenesis (Pfalz et al. 2006; Chen et al. 2010). Surprisingly, HMR encodes the PAP5 subunit of the PEP, and it is dual-targeted to both plastids and the nucleus (Chen et al. 2010; Nevarez et al. 2017). In the nucleus, HMR/PAP5 is an acidic-type transcriptional activator that interacts directly with phys as well as PIFs to regulate the stability and activity of PIF1, PIF3, and PIF4 (Galvão et al. 2012; Qiu et al. 2015, 2019). The discovery of HMR/PAP5 revealed an unexpected link between nuclear phyB signaling and plastid transcription, showing that these two

spatially separated processes share common functional components (Fig. 1.2). In addition to HMR/PAP5, PAP8 is also dual-targeted to the nucleus and plastids. Similar to HMR/PAP5, PAP8 is required for phyB signaling in the nucleus (Liebers et al. 2020). Intriguingly, PAP8 forms a nuclear protein complex that may contain other PEP components (Liebers et al. 2020; Chambon et al. 2022).

Albino mutants had been ignored historically in the context of light signaling because chlorophyll-deficient mutants were thought to retain normal phy-mediated hypocotyl responses (Borthwick et al. 1951; Jabben and Deitzer 1979). As a result, the entire class of tall-and-albino mutants, like *hmr,* was overlooked (Chen and Chory 2011). A forward genetic screen for *hmr*-like tall-and-albino mutants identified two additional, previously uncharacterized phy signaling components: REGULATOR OF CHLOROPLAST BIOGENESIS (RCB) and NUCLEAR-CONTROL-OF-PEP-ACTIVITY (NCP) (Yang et al. 2019; Yoo et al. 2019). RCB and NCP are two paralogous thioredoxin-like proteins that lack thioredoxin reductase activity (Yang et al. 2019). Intriguingly, like HMR, RCB and NCP also localize to both the nucleus and plastids. Although the biochemical functions of RCB and NCP remain unclear, nuclear RCB and NCP likely work in concert with HMR to regulate photobody formation and the PIFs (Yang et al. 2019; Yoo et al. 2019; Qiu et al. 2021). The key difference between RCB and NCP is that RCB regulates the PEP primarily from the nucleus, whereas NCP is required for both PIF degradation in the nucleus as well as PEP assembly in plastids. This conclusion is supported by the genetic evidence that the *rcb-10/pifq* mutant could rescue the albino phenotype of *rcb-10*, whereas the *ncp-10/pifq* mutant remained albino, like *hmr-5/pifq* (Qiu et al. 2015; Yang et al. 2019; Yoo et al. 2019). Therefore, the *rcb* mutant represents a unique type of albino mutant whose chloroplast defect is due to the accumulation of nuclear repressors of chloroplast biogenesis.

The dual localization of HMR/PAP5, PAP8, RCB, and NCP directly links the nuclear and plastid signaling mechanisms to the control of PhAPG transcription. All four proteins possess putative nuclear localization signals (NLSs) and transit peptides for chloroplast targeting. Intriguingly, the nuclear fractions of HMR/PAP5, PAP8, RCB, and NCP share the same molecular masses as their respective chloroplast counterparts, which are smaller than the mature proteins—i.e., both nuclear and chloroplast fractions lack the transit peptides, which are presumably cleaved upon chloroplast import—suggesting that they localize to the plastids first before being translocated to the nucleus (Nevarez et al. 2017; Yang et al. 2019; Yoo et al. 2019; Liebers et al. 2020). This dual targeting mechanism has been demonstrated for another nuclear and plastidial localized protein, Whirly1 (WHY1) (Grabowski et al. 2008), which is loosely associated with PEP in the nucleoid (Pfalz et al. 2006; Melonek et al. 2010) and required for chloroplast biogenesis and plastid genome repair (Prikryl et al. 2008; Cappadocia et al. 2010, 2012). Plastome-expressed WHY1 in tobacco could translocate to the nucleus, providing strong evidence supporting the existence of a plastid-to-nucleus protein transport mechanism (Isemer et al. 2012). One possible mechanism is via stromules—narrow tubular structures that emanate from plastids (Caplan et al. 2015; Kumar et al. 2018; Lee et al. 2024). Further investigations are needed to elucidate the dual nucleus-plastid

protein-targeting mechanism and the functional significance of these dual-localized proteins in PEP assembly and activation by light.

1.4.1.6 Other Nucleus-to-Plastid Signals: Sigma Factors

Beyond proteins that are dual-localized to the nucleus and the plastid, sigma factors—due to their function in promoter selection—are critical nucleus-encoded regulators of PEP activity. Each sigma factor demonstrates unique roles in fine-tuning PEP recruitment, including serving as nucleus-to-plastid anterograde signals during chloroplast biogenesis. This chapter will only briefly review the sigma factors and discuss their role in anterograde signaling. For more extensive details, refer to the following reviews (Lerbs-Mache 2011; Börner et al. 2015; Chi et al. 2015; Ortelt and Link 2021; Puthiyaveetil et al. 2021).

1.4.1.6.1 The Role of SIG1 in Photosystem Stoichiometry

Expression of the sigma factors is controlled by light (Allison 2000) and sigma factors can display a circadian rhythm (Oikawa et al. 2000; Noordally et al. 2013; Schneider et al. 2019). The interplay between the sigma factors and light could serve to protect the plastid during changing light conditions. Though not the only sigma factor involved in delivering light-based information, SIG1 specifically plays a role in adjusting photosystem stoichiometry during fluctuating light (Lerbs-Mache 2011; Chi et al. 2015). SIG1 rapidly accumulates in light (Onda et al. 2008) and regulates the *psaAB* operon (Tozawa et al. 2007) to balance the abundance of the two photosystems, thereby maintaining proper electron transfer. Shorter wavelengths of light favor photosystem II, creating reducing conditions, while longer wavelengths of light favor photosystem I, creating oxidizing conditions. The redox conditions generated by this imbalance influence the phosphorylation status of SIG1 to direct it in the regulation of *psaA* (Shimizu et al. 2010; Macadlo et al. 2020). In addition to its regulation of the *psaAB* operon, SIG1 displays a light-influenced binding pattern with a preference for the promoters of the *psbEFLJ* and *psbBT* operons (Hanaoka et al. 2012). Overall, this demonstrates that, along with being an important general housekeeping sigma factor, SIG1 is a light-controlled and redox-responsive sigma factor that might specifically intervene to influence the transcription of photosystem core subunits and protect the photosynthetic apparatus from light-induced damage.

1.4.1.6.2 The Developmental Roles of SIG2 and SIG6

SIG2 and SIG6 are seen as critical developmental sigma factors that are involved in the transcription of a wide assortment of PEP-dependent genes. The loss of even one of the two sigma factors results in an initial, PEP-deficient-like, pale green

phenotype, signifying the importance of the factors for proper chloroplast development and functioning (Hanaoka et al. 2003; Ishizaki et al. 2005). SIG2 is associated with the transcription of tRNA genes such as *trnV-UAC*, *trnM-CAU* and *trnE-UUC*, as well as photosynthesis-associated genes such as *psaJ* and *psbD* from the −256 promoter (Kanamaru et al. 2001; Hanaoka et al. 2003; Nagashima et al. 2004a; Kanamaru and Tanaka 2004). Meanwhile, SIG6 activates many PEP-dependent photosynthesis-associated genes, especially early in development (Ishizaki et al. 2005; Loschelder et al. 2006). Comparison of PEP binding in *sig2* and *sig6* revealed their widespread presence along the plastome, but simultaneously displayed their specificity, with the *sig2* mutation causing a particularly strong reduction for *trnE*, *trnY*, *trnD*, *trnL2*, *trnV1*, and *ndhC* (Palomar et al. 2022). On the other hand, the *sig6* mutation caused an overall strong reduction for most genes, but particularly *trnI1*.

1.4.1.6.3 The Narrow Roles of SIG3 and SIG4

Two of the more recently evolved sigma factors, SIG3 and SIG4, have specific roles in the transcription of *psbN* and *ndhF*, respectively (Favory et al. 2005; Zghidi et al. 2007). For SIG3, its role in the transcription of *psbN* has implicated it in being involved in the regulation of the *psbB* operon (*psbB-psbT-psbH-petB-petD*). Interestingly, *psbN* is located within *psbT* but on the antisense strand, leading to the generation of RNA antisense to *psbT* (Zghidi et al. 2007). The frequent occurrence of antisense transcription in the plastid is unusual, but could have regulatory roles that remain to be deeply explored (Sharwood et al. 2011; Mitschke et al. 2011; Zghidi-Abouzid et al. 2011; Hotto et al. 2011). As for SIG4, the specificity of this sigma factor for *ndhF* can be highlighted by tracing occurrences of SIG4 gene loss and concurrent absence or truncations of the *ndhF* gene (Pfannschmidt et al. 2015; Fu et al. 2021). The activity of SIG4 on *ndhF* may regulate the amount of NDH complexes present in the plastid (Favory et al. 2005).

1.4.1.6.4 The Stress-Responsive Roles of SIG5

SIG5 is induced by a variety of stresses (Nagashima et al. 2004b; Cano-Ramirez et al. 2023). In particular, SIG5 is involved in monitoring both the intensity and quality of light, which is demonstrated by its regulation of *psbD*. SIG5 controls alternative promoter usage of *psbD* during high light and other stress conditions (Christopher 1996; Nagashima et al. 2004b; Kanamaru and Tanaka 2004; Cano-Ramirez et al. 2023). *psbD* can be transcribed from several promoters, including two promoters −256 and −948 from the translation initiation site (Hoffer and Christopher 1997; Christopher and Hoffer 1998; Nagashima et al. 2004b). While SIG2 is involved in transcription initiation at the −256 position (Hanaoka et al. 2003), SIG5 controls initiation from the −948 site, known as the *psbD* light-responsive promoter (*psbD*-LRP). In support of this, transcription from this site is

abolished in *sig5* (Tsunoyama et al. 2004; Nagashima et al. 2004b; Shimmura et al. 2017). The extended 5′ untranslated region that is transcribed in high light could be involved in bolstering photosystem functioning during stress by boosting the replacement of the critical D2 protein of photosystem II. Further, not only does SIG5 direct alternative promoter recognition of *psbD*, it also informs the circadian rhythm of this operon and others by promoting oscillating transcription patterns (Noordally et al. 2013; Belbin et al. 2017; Cano-Ramirez et al. 2023).

1.4.1.6.5 Sigma Factors Are Anterograde Signals During Chloroplast Biogenesis

During de-etiolation, four sigma factors—SIG1, SIG3, SIG5, and SIG6—serve as nucleus-to-plastid signals for triggering PEP activation (Fig. 1.2) (Hwang et al. 2022). In etiolated seedlings, the expression of these sigma factors is repressed by PIFs. PhyB-dependent proteolysis of PIFs induces their expression in the light to promote PEP activity (Fig. 1.2). Current evidence indicates that PIFs are transcription activators (Yoo et al. 2021), so it is likely that PIFs repress sigma factors indirectly by transactivating unknown transcription repressor(s). In addition to their involvement in light-induced anterograde signaling, sigma factors could also influence developmentally-regulated anterograde signaling. During wheat leaf development, the genes of multiple sigma factors, including *SIG1*, *SIG2-1*, *SIG2-2*, *SIG3*, *SIG5*, and *SIG6*, are acutely induced at defined leaf segments, indicating that sigma factors also play pivotal roles in the anterograde control of plastid transcription during the proplastid-to-chloroplast developmental transition (Loudya et al. 2021).

1.4.1.7 Modeling Chloroplast Biogenesis in Light

An overview of the steps of chloroplast biogenesis in light can be seen in a pluripotent inducible cell line of single Arabidopsis cells (Dubreuil et al. 2018). Upon light exposure, these cells demonstrate two key regulatory phases as they shift from carrying proplastids (plastid progenitors) to containing mature chloroplasts. The first phase is associated with large-scale gene expression changes in response to light, as the photoreceptors trigger a cascade of downstream signals, with more than 3000 genes showing differential expression. The second phase revolves around activation and development of the photosynthetic chloroplast, which similarly displays a massive shift of gene expression of more than 12,000 genes. During this time, nuclear signals control PEP assembly and, thereby, plastid transcription to achieve chloroplast biogenesis.

The pivotal communication between the nucleus and the chloroplast throughout this process is highlighted by plastid positioning during the second regulatory phase. Plastids start this phase in clusters around the nucleus to, presumably, maximize efficient communication between the two organelles. As chloroplasts develop, they shift away from the nucleus to the cell cortex. The anterograde signaling that

takes place to initiate the process of chloroplast biogenesis is critical to give plants their characteristic green color. However, light is not the only factor that regulates chloroplast biogenesis. Developmental cues can play an even greater role in this process. Our final section will briefly discuss progress in this field.

1.4.2 Control of Plastid Transcription During Monocot Leaf Development

The linear developmental gradient of monocot leaves provides an ideal model to investigate the proplastid-to-chloroplast developmental transition. Across the developmental gradient of each leaf blade, there are basal, proliferating progenitor cells near the shoot apical meristem and distal, differentiated cells towards the tip of the leaf (Leech et al. 1973; Chotewutmontri and Barkan 2016). In a single leaf blade, plastids gradually differentiate from non-photosynthetic proplastids at the leaf base, to developing chloroplasts, then to young chloroplasts, and finally to mature chloroplasts near the leaf tip. In contrast to the environmental control of chloroplast biogenesis during de-etiolation, because monocot leaf development occurs in the presence of light, the proplastid-to-chloroplast transition across the leaf blade represents a developmental control of chloroplast biogenesis.

In all monocot models examined, including maize, barley, and wheat, the proplastid-to-chloroplast transition follows two distinct developmental phases (Baumgartner et al. 1989, 1993; Chotewutmontri and Barkan 2016; Loudya et al. 2021). In the initial phase—where the host cell transitions from proliferation to differentiation—plastids also differentiate from proplastids to developing chloroplasts, replicating their DNA, dividing rapidly and growing in size. At this point, transcription in plastids is carried out mainly by the NEP, RPOTp (Loudya et al. 2021), which activates plastid genes associated with plastid transcription and translation. These include *rpoB-rpoC1-rpoC2* and genes encoding ribosomal proteins (Baumgartner et al. 1989, 1993; Chotewutmontri and Barkan 2016; Loudya et al. 2021). Concurrently, nuclear-encoded sigma factors and PAPs, such as PAP2, PAP3, PAP5, and PAP6, are activated to promote plastid gene expression by the PEP (Loudya et al. 2021). After the initial phase—when cells undergo a transition from cell differentiation to expansion—developing plastids fully differentiate to young chloroplasts, which is accompanied by build-up of the photosynthesis machinery. The PEP gradually replaces the NEP as the major RNAP in plastids and transcripts of PhAPGs, the primary targets of the PEP, peak in this phase. However, as chloroplasts continue to mature, plastome transcription declines.

Intriguingly, RCB and NCP are activated at distinct stages during wheat leaf development (Loudya et al. 2021). RCB is activated in the initial phase of chloroplast biogenesis, corroborating its central role in the nucleus to initiate chloroplast biogenesis (Yoo et al. 2019; Loudya et al. 2021). In contrast, NCP is activated only at the leaf tip, suggesting a role during chloroplast maturation (Yang et al. 2019;

Loudya et al. 2021). Together, the studies using monocot models highlight the importance of controlling plastid transcription during proplastid-to-chloroplast differentiation and reveal that developmental cues control plastid transcription by orchestrating two distinct anterograde mechanisms to activate the NEP and PEP sequentially during the two phases of chloroplast biogenesis.

1.5 Concluding Thoughts

Plastid transcription is a key part of energy production, biosynthesis, and development in photosynthetic organisms. As such, the nucleus strictly regulates the plastid genome. Using anterograde signaling, the nucleus directs chloroplast biogenesis during de-etiolation and leaf development through the comprehensive regulation of plastid transcription itself. To do this, PEP assembly and activity are key points of control to establish plastome expression.

We have only begun to listen in on the light-based conversations that take place during the etioplast-to-chloroplast conversion and the developmental proplastid-to-chloroplast conversion. Many questions remain surrounding both PEP structure and overall nucleus-plastid communication. The cryo-EM structure of the PEP supercomplex revealed a complicated web of interactions between the PEP core and PAP subunits, but further research is needed to determine the specific roles of each PAP and how these roles support PEP activation and nuclear control of chloroplast function. The order of assembly of the massive PEP complex also remains a mystery, as does the existence of alternate assemblies during different stages of transcription. Overall, the construction of such a large complex for the transcription of a relatively small genome underscores the conclusion that the assembly of PEP itself is a major point of control for plastid transcription. Additionally, the identities and mechanisms of anterograde signals that trigger light-based and developmentally-controlled PEP assembly remain unresolved. An array of nucleus-encoded proteins, such as sigma factors, PAP5, PAP8, RCB and NCP, seem to be involved in the communication pathways that lead to chloroplast biogenesis, but their specific roles are unknown.

On its surface, transcription of the relatively small plastome appears deceptively simple. In actuality, an exciting array of regulatory pathways are required for the activation of these PhAPGs to spur chloroplast biogenesis. Future work in this field will continue to unravel inter-organellar signaling mechanisms to broaden our understanding of a critical, bacterial transcription system within eukaryotic cells.

Acknowledgements This work was supported by National Institute of General Medical Sciences grant R01GM132765 to M.C. and National Science Foundation grant IOS-2034015 to X.C. and M.C. N.F. was supported by the US Department of Education GAANN grant P200A210029, National Science Foundation Research Traineeship grant 1922642, and a Howard Hughes Medical Institute bridge fund to X.C.

References

Abdallah F, Salamini F, Leister D (2000) A prediction of the size and evolutionary origin of the proteome of chloroplasts of *Arabidopsis*. Trends Plant Sci 5:141–142

Allison LA (2000) The role of sigma factors in plastid transcription. Biochimie 82:537–548

Allison LA, Simon LD, Maliga P (1996) Deletion of *rpoB* reveals a second distinct transcription system in plastids of higher plants. EMBO J 15:2802–2809

Al-Sady B, Ni W, Kircher S et al (2006) Photoactivated phytochrome induces rapid PIF3 phosphorylation prior to proteasome-mediated degradation. Mol Cell 23:439–446

Arabidopsis Genome Initiative (2000) Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature 408:796–815

Armstrong GA (1998) Greening in the dark: light-independent chlorophyll biosynthesis from anoxygenic photosynthetic bacteria to gymnosperms. J Photochem Photobiol B 43:87–100

Arsova B, Hoja U, Wimmelbacher M et al (2010) Plastidial thioredoxin z interacts with two fructokinase-like proteins in a thiol-dependent manner: evidence for an essential role in chloroplast development in *Arabidopsis* and *Nicotiana benthamiana*. Plant Cell 22:1498–1515

Artsimovitch I, Svetlov V, Murakami KS, Landick R (2003) Co-overexpression of *Escherichia coli* RNA polymerase subunits allows isolation and analysis of mutant enzymes lacking lineage-specific sequence insertions. J Biol Chem 278:12344–12355

Baba K, Schmidt J, Espinosa-Ruiz A et al (2004) Organellar gene transcription and early seedling development are affected in the *rpoT;2* mutant of *Arabidopsis*. Plant J 38:38–48

Barkan A (2011) Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold. Plant Physiol 155:1520–1532

Barkan A, Small I (2014) Pentatricopeptide repeat proteins in plants. Annu Rev Plant Biol 65:415–442

Baumgartner BJ, Rapp JC, Mullet JE (1989) Plastid transcription activity and DNA copy number increase early in barley chloroplast development. Plant Physiol 89:1011–1018

Baumgartner BJ, Rapp JC, Mullet JE (1993) Plastid genes encoding the transcription/translation apparatus are differentially transcribed early in barley (*Hordeum vulgare*) chloroplast development. Plant Physiol 101:781–791

Belbin FE, Noordally ZB, Wetherill SJ et al (2017) Integration of light and circadian signals that regulate chloroplast transcription by a nuclear-encoded sigma factor. New Phytol 213:727–738

Bennett J, Jenkins GI, Hartley MR (1984) Differential regulation of the accumulation of the light-harvesting chlorophyll a/b complex and ribulose bisphosphate carboxylase/oxygenase in greening pea leaves. J Cell Biochem 25:1–13

Bergsland KJ, Haselkorn R (1991) Evolutionary relationships among eubacteria, cyanobacteria, and chloroplasts: evidence from the *rpoC1* gene of *Anabaena sp.* strain PCC 7120. J Bacteriol 173:3446–3455

Bock S, Ortelt J, Link G (2014) *AtSIG6* and other members of the sigma gene family jointly but differentially determine plastid target gene expression in *Arabidopsis thaliana*. Front Plant Sci 5:667

Börner T, Aleynikova AY, Zubo YO, Kusnetsov VV (2015) Chloroplast RNA polymerases: role in chloroplast biogenesis. Biochim Biophys Acta 1847:761–769

Borthwick HA, Hendricks SB, Parker MW (1951) Action spectrum for inhibition of stem growth in dark-grown seedlings of albino and nonalbino barley (*Hordeum vulgare*). Bot Gaz 113:95–105

Bottomley W, Smith HJ, Bogorad L (1971) RNA polymerases of maize: partial purification and properties of the chloroplast enzyme. Proc Natl Acad Sci USA 68:2412–2416

Briat JF, Laulhere JP, Mache R (1979) Transcription activity of a DNA-protein complex isolated from spinach plastids. Eur J Biochem 98:285–292

Burgess RR, Travers AA, Dunn JJ, Bautz EK (1969) Factor stimulating transcription by RNA polymerase. Nature 221:43–46

Burgie ES, Vierstra RD (2014) Phytochromes: an atomic perspective on photoactivation and signaling. Plant Cell 26:4568–4583

Cahoon AB, Harris FM, Stern DB (2004) Analysis of developing maize plastids reveals two mRNA stability classes correlating with RNA polymerase type. EMBO Rep 5:801–806
Cahoon AB, Takacs EM, Sharpe RM, Stern DB (2008) Nuclear, chloroplast, and mitochondrial transcript abundance along a maize leaf developmental gradient. Plant Mol Biol 66:33–46
Cano-Ramirez DL, Panter PE, Takemura T et al (2023) Low-temperature and circadian signals are integrated by the sigma factor SIG5. Nat Plants 9:661–672
Caplan JL, Kumar AS, Park E et al (2015) Chloroplast stromules function during innate immunity. Dev Cell 34:45–57
Cappadocia L, Maréchal A, Parent J-S et al (2010) Crystal structures of DNA-Whirly complexes and their role in *Arabidopsis* organelle genome repair. Plant Cell 22:1849–1867
Cappadocia L, Parent J-S, Zampini E et al (2012) A conserved lysine residue of plant Whirly proteins is necessary for higher order protein assembly and protection against DNA damage. Nucleic Acids Res 40:258–269
Castandet B, Germain A, Hotto AM, Stern DB (2019) Systematic sequencing of chloroplast transcript termini from Arabidopsis thaliana reveals >200 transcription initiation sites and the extensive imprints of RNA-binding proteins and secondary structures. Nucleic Acids Res 47:11889–11905
Chambon L, Gillet F-X, Chieb M et al (2022) PAP8/pTAC6 is part of a nuclear protein complex and displays RNA recognition motifs of viral origin. Int J Mol Sci 23. https://doi.org/10.3390/ijms23063059
Chang SH, Lee S, Um TY et al (2017) pTAC10, a key subunit of plastid-encoded RNA polymerase, promotes chloroplast development. Plant Physiol 174:435–449
Chang C-Y, Chen L-J, Li H-M (2023) Chloroplast import motor subunits FtsHi1 and FtsHi2 are located on opposite sides of the inner envelope membrane. Proc Natl Acad Sci USA 120:e2307747120
Chateigner-Boutin A-L, Ramos-Vega M, Guevara-García A et al (2008) CLB19, a pentatricopeptide repeat protein required for editing of rpoA and clpP chloroplast transcripts. Plant J 56:590–602
Chen M, Chory J (2011) Phytochrome signaling mechanisms and the control of plant development. Trends Cell Biol 21:664–671
Chen M, Schwab R, Chory J (2003) Characterization of the requirements for localization of phytochrome B to nuclear bodies. Proc Natl Acad Sci USA 100:14493–14498
Chen M, Chory J, Fankhauser C (2004) Light signal transduction in higher plants. Annu Rev Genet 38:87–117
Chen M, Tao Y, Lim J et al (2005) Regulation of phytochrome B nuclear localization through light-dependent unmasking of nuclear-localization signals. Curr Biol 15:637–642
Chen M, Galvão RM, Li M et al (2010) Arabidopsis HEMERA/pTAC12 initiates photomorphogenesis by phytochromes. Cell 141:1230–1240
Cheng M-C, Kathare PK, Paik I, Huq E (2021) Phytochrome signaling networks. Annu Rev Plant Biol 72:217–244
Chi W, He B, Mao J et al (2015) Plastid sigma factors: their individual functions and regulation in transcription. Biochim Biophys Acta 1847:770–778
Chibani K, Tarrago L, Schürmann P et al (2011) Biochemical properties of poplar thioredoxin z. FEBS Lett 585:1077–1081
Christopher DA (1996) Leaf development and phytochrome modulate the activation of *psbD-psbC* transcription by high-fluence blue light in barley chloroplasts. Photosynth Res 47:239–251
Chotewutmontri P, Barkan A (2016) Dynamics of chloroplast translation during chloroplast differentiation in maize. PLoS Genet 12:e1006106
Christopher DA, Hoffer PH (1998) DET1 represses a chloroplast blue light-responsive promoter in a developmental and tissue-specific manner in *Arabidopsis thaliana*. Plant J 14:1–11
Courtois F, Merendino L, Demarsy E et al (2007) Phage-type RNA polymerase RPOTmp transcribes the *rrn* operon from the PC promoter at early developmental stages in *Arabidopsis*. Plant Physiol 145:712–721

De Santis-MacIossek G, Kofer W, Bock A et al (1999) Targeted disruption of the plastid RNA polymerase genes *rpoA*, *B* and *C1*: molecular biology, biochemistry and ultrastructure. Plant J 18:477–489

Deng XW, Gruissem W (1987) Control of plastid gene expression during development: the limited role of transcriptional regulation. Cell 49:379–387

Deng XW, Stern DB, Tonkyn JC, Gruissem W (1987) Plastid run-on transcription. Application to determine the transcriptional regulation of spinach plastid genes. J Biol Chem 262:9641–9648

dePamphilis CW, Palmer JD (1990) Loss of photosynthetic and chlororespiratory genes from the plastid genome of a parasitic flowering plant. Nature 348:337–339

Díaz MG, Hernández-Verdeja T, Kremnev D et al (2018) Redox regulation of PEP activity during seedling establishment in *Arabidopsis thaliana*. Nat Commun 9:50

Dong J, Ni W, Yu R et al (2017) Light-dependent degradation of PIF3 by SCFEBF1/2 promotes a photomorphogenic response in *Arabidopsis*. Curr Biol 27:2420–2430.e6

Dubreuil C, Jin X, de Dios Barajas-López J et al (2018) Establishment of photosynthesis through chloroplast development is controlled by two distinct regulatory phases. Plant Physiol 176:1199–1214

Emanuel C, Weihe A, Graner A et al (2004) Chloroplast development affects expression of phage-type RNA polymerases in barley leaves. Plant J 38:460–472

Fankhauser C, Chen M (2008) Transposing phytochrome into the nucleus. Trends Plant Sci 13:596–601

Favory J-J, Kobayshi M, Tanaka K et al (2005) Specific function of a plastid sigma factor for *ndhF* gene transcription. Nucleic Acids Res 33:5991–5999

Feklístov A, Sharon BD, Darst SA, Gross CA (2014) Bacterial sigma factors: a historical, structural, and genomic perspective. Ann Rev Microbiol 68:357–376

Fu X-X, Zhang J, Zhang G-Q et al (2021) Insights into the origin and evolution of plant sigma factors. J Syst Evol 59:326–340

Fujiwara M, Nagashima A, Kanamaru K et al (2000) Three new nuclear genes, *sigD*, *sigE* and *sigF*, encoding putative plastid RNA polymerase sigma factors in *Arabidopsis thaliana*. FEBS Lett 481:47–52

Galvão RM, Li M, Kothadia SM et al (2012) Photoactivated phytochromes interact with HEMERA and promote its accumulation to establish photomorphogenesis in *Arabidopsis*. Genes Dev 26:1851–1863

Gao Z-P, Yu Q-B, Zhao T-T et al (2011) A functional component of the transcriptionally active chromosome complex, Arabidopsis pTAC14, interacts with pTAC12/HEMERA and regulates plastid gene expression. Plant Physiol 157:1733–1745

Garcia M, Myouga F, Takechi K et al (2008) An *Arabidopsis* homolog of the bacterial peptidoglycan synthesis enzyme MurE has an essential role in chloroplast development. Plant J 53:924–934

Gatenby AA, Castleton JA, Saul MW (1981) Expression in *E. coli* of maize and wheat chloroplast genes for large subunit of ribulose bisphosphate carboxylase. Nature 291:117–121

Gilkerson J, Perez-Ruiz JM, Chory J, Callis J (2012) The plastid-localized pfkB-type carbohydrate kinases FRUCTOKINASE-LIKE 1 and 2 are essential for growth and development of *Arabidopsis thaliana*. BMC Plant Biol 12:102

Gould SB, Waller RF, McFadden GI (2008) Plastid evolution. Annu Rev Plant Biol 59:491–517

Grabowski E, Miao Y, Mulisch M, Krupinska K (2008) Single-stranded DNA-binding protein Whirly1 in barley leaves is located in plastids and the nucleus of the same cell. Plant Physiol 147:1800–1804

Grübler B, Merendino L, Twardziok SO et al (2017) Light and plastid signals regulate different sets of genes in the albino mutant Pap7-1. Plant Physiol 175:1203–1219

Hajdukiewicz PT, Allison LA, Maliga P (1997) The two RNA polymerases encoded by the nuclear and the plastid compartments transcribe distinct groups of genes in tobacco plastids. EMBO J 16:4041–4048

Hallick RB, Lipper C, Richards OC, Rutter WJ (1976) Isolation of a transcriptionally active chromosome from chloroplasts of *Euglena gracilis*. Biochemistry 15:3039–3045

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

Hanaoka M, Kato M, Anma M, Tanaka K (2012) SIG1, a sigma factor for the chloroplast RNA polymerase, differently associates with multiple DNA regions in the chloroplast chromosomes in vivo. Int J Mol Sci 13:12182–12194

He L, Zhang S, Qiu Z et al (2018) Fructokinase-like protein 1 interacts with TRXz to regulate chloroplast development in rice. J Integr Plant Biol 60:94–111

Hedtke B, Börner T, Weihe A (1997) Mitochondrial and chloroplast phage-type RNA polymerases in *Arabidopsis*. Science 277:809–811

Hedtke B, Börner T, Weihe A (2000) One RNA polymerase serving two genomes. EMBO Rep 1:435–440

Hess WR, Prombona A, Fieder B et al (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

Hoffer PH, Christopher DA (1997) Structure and blue-light-responsive transcription of a chloroplast *psbD* promoter from *Arabidopsis thaliana*. Plant Physiol 115:213–222

Hotto AM, Schmitz RJ, Fei Z et al (2011) Unexpected diversity of chloroplast noncoding RNAs as revealed by deep sequencing of the *Arabidopsis* transcriptome G3(1):559–570

Hricová A, Quesada V, Micol JL (2006) The *SCABRA3* nuclear gene encodes the plastid RpoTp RNA polymerase, which is required for chloroplast biogenesis and mesophyll cell proliferation in *Arabidopsis*. Plant Physiol 141:942–956

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

Hu J, Troxler RF, Bogorad L (1991) Maize chloroplast RNA polymerase: the 78-kilodaiton polypeptide is encoded by the plastid *rpoC1* gene. Nucleic Acids Res 19:3431–3434

Hu W, Franklin KA, Sharrock RA et al (2013) Unanticipated regulatory roles for Arabidopsis phytochromes revealed by null mutant analysis. Proc Natl Acad Sci USA 110:1542–1547

Huang C, Yu Q-B, Lv R-H et al (2013) The reduced plastid-encoded polymerase-dependent plastid gene expression leads to the delayed greening of the *Arabidopsis fln2* mutant. PLoS One 8:e73092

Hwang Y, Han S, Yoo CY et al (2022) Anterograde signaling controls plastid transcription via sigma factors separately from nuclear photosynthesis genes. Nat Commun 13:7440

Imamura S, Asayama M (2009) Sigma factors for cyanobacterial transcription. Gene Regul Syst Biol 3:65–87

Isemer R, Mulisch M, Schäfer A et al (2012) Recombinant Whirly1 translocates from transplastomic chloroplasts to the nucleus. FEBS Lett 586:85–88

Ishizaki Y, Tsunoyama Y, Hatano K et al (2005) A nuclear-encoded sigma factor, *Arabidopsis* SIG6, recognizes sigma-70 type chloroplast promoters and regulates early chloroplast development in cotyledons. Plant J 42:133–144

Jabben M, Deitzer GF (1979) Effects of the herbicide San 9789 on photomorphogenic responses. Plant Physiol 63:481–485

Jeon Y, Jung HJ, Kang H et al (2012) S1 domain-containing STF modulates plastid transcription and chloroplast biogenesis in *Nicotiana benthamiana*. New Phytol 193:349–363

Kanamaru K, Tanaka K (2004) Roles of chloroplast RNA polymerase sigma factors in chloroplast development and stress response in higher plants. Biosci Biotechnol Biochem 68:2215–2223

Kanamaru K, Nagashima A, Fujiwara M et al (2001) An Arabidopsis sigma factor (SIG2)-dependent expression of plastid-encoded tRNAs in chloroplasts. Plant Cell Physiol 42:1034–1043

Kanno A, Hirai A (1993) A transcription map of the chloroplast genome from rice (*Oryza sativa*). Curr Genet 23:166–174

Kendrick R, Chotewutmontri P, Belcher S, Barkan A (2022) Correlated retrograde and developmental regulons implicate multiple retrograde signals as coordinators of chloroplast development in maize. Plant Cell 34:4897–4919

Kessler F, Schnell D (2009) Chloroplast biogenesis: diversity and regulation of the protein import apparatus. Curr Opin Cell Biol 21:494–500

Kindgren P, Strand Å (2015) Chloroplast transcription, untangling the Gordian Knot. New Phytol 206:889–891

Kindgren P, Kremnev D, Blanco NE et al (2012) The plastid redox insensitive 2 mutant of Arabidopsis is impaired in PEP activity and high light-dependent plastid redox signalling to the nucleus. Plant J 70:279–291

Kircher S, Kozma-Bognar L, Kim L et al (1999) Light quality-dependent nuclear import of the plant photoreceptors phytochrome A and B. Plant Cell 11:1445–1456

Kirk JT (1964) Studies on RNA synthesis in chloroplast preparations. Biochem Biophys Res Commun 16:233–238

Kirk JTO, Tilney-Bassett RAE (1978) The plastids: their chemistry, structure. Growth Inheritance 6

Klein RR, Mullet JE (1990) Light-induced transcription of chloroplast genes. *psbA* transcription is differentially enhanced in illuminated barley. J Biol Chem 265:1895–1902

Klein S, Schiff JA (1972) The correlated appearance of prolamellar bodies, protochlorophyll(ide) species, and the shibata shift during development of bean etioplasts in the dark. Plant Physiol 49:619–626

Kleine T, Maier UG, Leister D (2009) DNA transfer from organelles to the nucleus: the idiosyncratic genetics of endosymbiosis. Annu Rev Plant Biol 60:115–138

Koussevitzky S, Nott A, Mockler TC et al (2007) Signals from chloroplasts converge to regulate nuclear gene expression. Science 316:715–719

Krause K, Maier RM, Kofer W et al (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

Kremnev D, Strand A (2014) Plastid encoded RNA polymerase activity and expression of photosynthesis genes required for embryo and seed development in Arabidopsis. Front Plant Sci 5:385

Kühn K, Bohne A-V, Liere K et al (2007) Arabidopsis phage-type RNA polymerases: accurate in vitro transcription of organellar genes. Plant Cell 19:959–971

Kumar AS, Park E, Nedo A et al (2018) Stromule extension along microtubules coordinated with actin-mediated anchoring guides perinuclear chloroplast movement during innate immunity. elife 7:e23625. https://doi.org/10.7554/eLife.23625

Lane WJ, Darst SA (2010) Molecular evolution of multisubunit RNA polymerases: sequence analysis. J Mol Biol 395:671–685

Lee J-H, Doan TM, Senthilkumar S, Yoo CY (2024) Mechanism of nucleus-chloroplast communication by alternative promoter usage and stromules to establish photomorphogenesis in *Arabidopsis*. bioRxiv 2024.05.13.593997

Leech RM, Rumsby MG, Thomson WW (1973) Plastid differentiation, acyl lipid, and fatty acid changes in developing green maize leaves. Plant Physiol 52:240–245

Lefebvre-Legendre L, Merendino L, Rivier C, Goldschmidt-Clermont M (2014) On the complexity of chloroplast RNA metabolism: *psaA* trans-splicing can be bypassed in *Chlamydomonas*. Mol Biol Evol 31:2697–2707

Legen J, Kemp S, Krause K et al (2002) Comparative analysis of plastid transcription profiles of entire plastid chromosomes from tobacco attributed to wild-type and PEP-deficient transcription machineries. Plant J 31:171–188

Legris M, Ince YÇ, Fankhauser C (2019) Molecular mechanisms underlying phytochrome-controlled morphogenesis in plants. Nat Commun 10:5219. https://doi.org/10.1038/s41467-019-13045-0

Leivar P, Quail PH (2011) PIFs: pivotal components in a cellular signaling hub. Trends Plant Sci 16:19–28

Leivar P, Monte E, Al-Sady B et al (2008a) The *Arabidopsis* phytochrome-interacting factor PIF7, together with PIF3 and PIF4, regulates responses to prolonged red light by modulating phyB levels. Plant Cell 20:337–352

Leivar P, Monte E, Oka Y et al (2008b) Multiple phytochrome-interacting bHLH transcription factors repress premature seedling photomorphogenesis in darkness. Curr Biol 18:1815–1823

Leivar P, Tepperman JM, Monte E et al (2009) Definition of early transcriptional circuitry involved in light-induced reversal of PIF-imposed repression of photomorphogenesis in young *Arabidopsis* seedlings. Plant Cell 21:3535–3553

Lerbs-Mache S (2011) Function of plastid sigma factors in higher plants: regulation of gene expression or just preservation of constitutive transcription? Plant Mol Biol 76:235–249

Li L, Ljung K, Breton G et al (2012) Linking photoreceptor excitation to changes in plant architecture. Genes Dev 26:785–790

Lian H-L, He S-B, Zhang Y-C et al (2011) Blue-light-dependent interaction of cryptochrome 1 with SPA1 defines a dynamic signaling mechanism. Genes Dev 25:1023–1028

Liebers M, Gillet F-X, Israel A et al (2020) Nucleo-plastidic PAP8/pTAC6 couples chloroplast formation with photomorphogenesis. EMBO J 39:e104941

Liere K, Börner T (2007) Transcription and transcriptional regulation in plastids. In: Bock R (ed) Cell and molecular biology of plastids. Springer, Berlin, Heidelberg, pp 121–174

Liere K, Weihe A, Börner T (2011) The transcription machineries of plant mitochondria and chloroplasts: composition, function, and regulation. J Plant Physiol 168:1345–1360

Link G (1982) Phytochrome control of plastid mRNA in mustard (*Sinapis alba* L.). Planta 154:81–86

Little MC, Hallick RB (1988) Chloroplast *rpoA*, *rpoB*, and *rpoC* genes specify at least three components of a chloroplast DNA-dependent RNA polymerase active in tRNA and mRNA transcription. J Biol Chem 263:14302–14307

Liu B, Zuo Z, Liu H et al (2011) *Arabidopsis* cryptochrome 1 interacts with SPA1 to suppress COP1 activity in response to blue light. Genes Dev 25:1029–1034

Lorrain S, Allen T, Duek PD et al (2008) Phytochrome-mediated inhibition of shade avoidance involves degradation of growth-promoting bHLH transcription factors. Plant J 53:312–323

Loschelder H, Schweer J, Link B, Link G (2006) Dual temporal role of plastid sigma factor 6 in *Arabidopsis* development. Plant Physiol 142:642–650

Loudya N, Mishra P, Takahagi K et al (2021) Cellular and transcriptomic analyses reveal two-staged chloroplast biogenesis underpinning photosynthesis build-up in the wheat leaf. Genome Biol 22:151

Lu X-D, Zhou C-M, Xu P-B et al (2015) Red-light-dependent interaction of phyB with SPA1 promotes COP1-SPA1 dissociation and photomorphogenic development in *Arabidopsis*. Mol Plant 8:467–478

Luo Q, Lian H-L, He S-B et al (2014) COP1 and phyB physically interact with PIL1 to regulate its stability and photomorphogenic development in *Arabidopsis*. Plant Cell 26:2441–2456

Macadlo LA, Ibrahim IM, Puthiyaveetil S (2020) Sigma factor 1 in chloroplast gene transcription and photosynthetic light acclimation. J Exp Bot 71:1029–1038

Mapleston RE, Griffiths WT (1980) Light modulation of the activity of protochlorophyllide reductase. Biochem J 189:125–133

Martin W, Herrmann RG (1998) Gene transfer from organelles to the nucleus: how much, what happens, and why? Plant Physiol 118:9–17

Martin W, Kowallik KV (1999) Annotated English translation of Mereschkowsky's 1905 paper "Über Natur und Ursprung der Chromatophoren im Pflanzenreiche". Eur J Phycol 34:287–295

Martín M, Sabater B (2010) Plastid *ndh* genes in plant evolution. Plant Physiol Biochem 48:636–645

Martin W, Rujan T, Richly E et al (2002) Evolutionary analysis of *Arabidopsis*, cyanobacterial, and chloroplast genomes reveals plastid phylogeny and thousands of cyanobacterial genes in the nucleus. Proc Natl Acad Sci USA 99:12246–12251

Mazumder A, Lin M, Kapanidis AN, Ebright RH (2020) Closing and opening of the RNA polymerase trigger loop. Proc Natl Acad Sci USA 117:15642–15649

Melonek J, Mulisch M, Schmitz-Linneweber C et al (2010) Whirly1 in chloroplasts associates with intron containing RNAs and rarely co-localizes with nucleoids. Planta 232:471–481

Mereschkowsky C (1905) Über natur und ursprung der chromatophoren im pflanzenreiche. Biol Centralbl 25:593

Mitschke J, Georg J, Scholz I et al (2011) An experimentally anchored map of transcriptional start sites in the model cyanobacterium *Synechocystis* sp. PCC6803. Proc Natl Acad Sci USA 108:2124–2129

Mohanta TK, Mohanta YK, Sharma N (2023) Anticodon table of the chloroplast genome and identification of putative quadruplet anticodons in chloroplast tRNAs. Sci Rep 13:760

Morden CW, Wolfe KH, dePamphilis CW, Palmer JD (1991) Plastid translation and transcription genes in a non-photosynthetic plant: intact, missing and pseudo genes. EMBO J 10:3281–3288

Mullet JE, Klein RR (1987) Transcription and RNA stability are important determinants of higher plant chloroplast RNA levels. EMBO J 6:1571–1579

Murakami KS, Masuda S, Campbell EA et al (2002) Structural basis of transcription initiation: an RNA polymerase holoenzyme-DNA complex. Science 296:1285–1290

Myouga F, Hosoda C, Umezawa T et al (2008) A heterocomplex of iron superoxide dismutases defends chloroplast nucleoids against oxidative stress and is essential for chloroplast development in *Arabidopsis*. Plant Cell 20:3148–3162

Nagashima A, Hanaoka M, Motohashi R et al (2004a) DNA microarray analysis of plastid gene expression in an *Arabidopsis* mutant deficient in a plastid transcription factor sigma, SIG2. Biosci Biotechnol Biochem 68:694–704

Nagashima A, Hanaoka M, Shikanai T et al (2004b) 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

Nawaz G, Kang H (2017) Chloroplast- or mitochondria-targeted DEAD-Box RNA helicases play essential roles in organellar RNA metabolism and abiotic stress responses. Front Plant Sci 8:871

Nevarez PA, Qiu Y, Inoue H et al (2017) Mechanism of dual targeting of the phytochrome signaling component HEMERA/pTAC12 to plastids and the nucleus. Plant Physiol 173:1953–1966

Ni W, Xu S-L, Tepperman JM et al (2014) A mutually assured destruction mechanism attenuates light signaling in *Arabidopsis*. Science 344:1160–1164

Ni W, Xu S-L, González-Grandío E et al (2017) PPKs mediate direct signal transfer from phytochrome photoreceptors to transcription factor PIF3. Nat Commun 8:15236

Noordally ZB, Ishii K, Atkins KA et al (2013) Circadian control of chloroplast transcription by a nuclear-encoded timing signal. Science 339:1316–1319

Nott A, Jung H-S, Koussevitzky S, Chory J (2006) Plastid-to-nucleus retrograde signaling. Annu Rev Plant Biol 57:739–759

Oelmüller R (1989) Photooxidative destruction of chloroplasts and its effect on nuclear gene expression and extraplastidic enzyme levels. Photochem Photobiol 49:229–239

Ohyama K, Fukuzawa H, Kohchi T et al (1986) Chloroplast gene organization deduced from complete sequence of liverwort *Marchantia polymorpha* chloroplast DNA. Nature 322:572–574

Oikawa K, Fujiwara M, Nakazato E et al (2000) Characterization of two plastid sigma factors, SigA1 and SigA2, that mainly function in matured chloroplasts in *Nicotiana tabacum*. Gene 261:221–228

Onda Y, Yagi Y, Saito Y et al (2008) Light induction of Arabidopsis SIG1 and SIG5 transcripts in mature leaves: differential roles of cryptochrome 1 and cryptochrome 2 and dual function of SIG5 in the recognition of plastid promoters. Plant J 55:968–978

Ortelt J, Link G (2014) Plastid gene transcription: promoters and RNA polymerases. In: Maliga P (ed) Chloroplast biotechnology: methods and protocols. Humana Press, Totowa, NJ, pp 47–72

Ortelt J, Link G (2021) Plastid gene transcription: an update on promoters and RNA polymerases. In: Maliga P (ed) Chloroplast biotechnology: methods and protocols. Springer, New York, NY, pp 49–76

Palomar VM, Jaksich S, Fujii S et al (2022) High-resolution map of plastid-encoded RNA polymerase binding patterns demonstrates a major role of transcription in chloroplast gene expression. Plant J 111:1139–1151

Park E, Park J, Kim J et al (2012) Phytochrome B inhibits binding of phytochrome-interacting factors to their target promoters. Plant J 72:537–546

Park E, Kim Y, Choi G (2018) Phytochrome B requires PIF degradation and sequestration to induce light responses across a wide range of light conditions. Plant Cell 30:1277–1292

Parks BM, Quail PH (1993) *hy8*, a new class of arabidopsis long hypocotyl mutants deficient in functional phytochrome A. Plant Cell 5:39–48

Patel UR, Gautam S, Chatterji D (2020) Validation of omega subunit of RNA polymerase as a functional entity. Biomol Ther 10:1588. https://doi.org/10.3390/biom10111588

Pearce SF, Rebelo-Guiomar P, D'Souza AR et al (2017) Regulation of mammalian mitochondrial gene expression: recent advances. Trends Biochem Sci 42:625–639

Pedmale UV, Huang S-SC, Zander M et al (2016) Cryptochromes Interact directly with PIFs to control plant growth in limiting blue light. Cell 164:233–245

Peltier G, Aro E-M, Shikanai T (2016) NDH-1 and NDH-2 plastoquinone reductases in oxygenic photosynthesis. Annu Rev Plant Biol 67:55–80

Pfalz J, Pfannschmidt T (2013) Essential nucleoid proteins in early chloroplast development. Trends Plant Sci 18:186–194

Pfalz J, Liere K, Kandlbinder A et al (2006) pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. Plant Cell 18:176–197

Pfalz J, Holtzegel U, Barkan A et al (2015) ZmpTAC12 binds single-stranded nucleic acids and is essential for accumulation of the plastid-encoded polymerase complex in maize. New Phytol 206:1024–1037

Pfannschmidt T, Link G (1994) Separation of two classes of plastid DNA-dependent RNA polymerases that are differentially expressed in mustard (*Sinapis alba* L.) seedlings. Plant Mol Biol 25:69–81

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

Pfannschmidt T, Blanvillain R, Merendino L et al (2015) Plastid RNA polymerases: orchestration of enzymes with different evolutionary origins controls chloroplast biogenesis during the plant life cycle. J Exp Bot 66:6957–6973

Pham VN, Kathare PK, Huq E (2018) Dynamic regulation of PIF5 by COP1-SPA complex to optimize photomorphogenesis in *Arabidopsis*. Plant J 96:260–273

Polya GM, Jagendorf AT (1971a) Wheat leaf RNA polymerases. I. Partial purification and characterization of nuclear, chloroplast and soluble DNA-dependent enzymes. Arch Biochem Biophys 146:635–648

Polya GM, Jagendorf AT (1971b) Wheat leaf RNA polymerases. II. Kinetic characterization and template specificities of nuclear, chloroplast, and soluble enzymes. Arch Biochem Biophys 146:649–657

do Prado PFV, Ahrens FM, Liebers M et al (2024) Structure of the multi-subunit chloroplast RNA polymerase. Mol Cell 84:910–925.e5

Prikryl J, Watkins KP, Friso G et al (2008) A member of the Whirly family is a multifunctional RNA- and DNA-binding protein that is essential for chloroplast biogenesis. Nucleic Acids Res 36:5152–5165

Puthiyaveetil S, McKenzie SD, Kayanja GE, Ibrahim IM (2021) Transcription initiation as a control point in plastid gene expression. Biochim Biophys Acta Gene Regul Mech 1864:194689

Qayyum MZ, Imashimizu M, Leanca M et al (2024) Structure and function of the Si3 insertion integrated into the trigger loop/helix of cyanobacterial RNA polymerase. Proc Natl Acad Sci USA 121:e2311480121

Qiu Y, Li M, Pasoreck EK et al (2015) HEMERA couples the proteolysis and transcriptional activity of PHYTOCHROME INTERACTING FACTORs in *Arabidopsis* photomorphogenesis. Plant Cell 27:1409–1427

Qiu Y, Li M, Kim RJ-A et al (2019) Daytime temperature is sensed by phytochrome B in *Arabidopsis* through a transcriptional activator HEMERA. Nat Commun 10:140

Qiu Y, Pasoreck EK, Yoo CY et al (2021) RCB initiates *Arabidopsis* thermomorphogenesis by stabilizing the thermoregulator PIF4 in the daytime. Nat Commun 12:2042

Reed JW, Chory J (1994) Mutational analyses of light-controlled seedling development in *Arabidopsis*. Semin Cell Biol 5:327–334

Reed JW, Nagpal P, Poole DS et al (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout *Arabidopsis* development. Plant Cell 5:147–157

Ris H, Plaut W (1962) Ultrastructure of DNA-containing areas in the chloroplast of *Chlamydomonas*. J Cell Biol 13:383–391

Rockwell NC, Su Y-S, Lagarias JC (2006) Phytochrome structure and signaling mechanisms. Annu Rev Plant Biol 57:837–858

Ruckle ME, Burgoon LD, Lawrence LA et al (2012) Plastids are major regulators of light signaling in *Arabidopsis*. Plant Physiol 159:366–390

Ruedas R, Muthukumar SS, Kieffer-Jaquinod S et al (2022) Three-dimensional envelope and subunit interactions of the plastid-encoded RNA polymerase from *Sinapis alba*. Int J Mol Sci 23:9922. https://doi.org/10.3390/ijms23179922

Sagan L (1967) On the origin of mitosing cells. J Theor Biol 14:255–274

Sager R, Ishida MR (1963) Chloroplast DNA in *Chlamydomonas*. Proc Natl Acad Sci USA 50:725–730

Saijo Y, Sullivan JA, Wang H et al (2003) The COP1-SPA1 interaction defines a critical step in phytochrome A-mediated regulation of HY5 activity. Genes Dev 17:2642–2647

Sakai A, Kawano S, Kuroiwa T (1992) Conversion of proplastids to amyloplasts in tobacco cultured cells is accompanied by changes in the transcriptional activities of plastid genes. Plant Physiol 100:1062–1066

Sakamoto K, Nagatani A (1996) Nuclear localization activity of phytochrome B. Plant J 10:859–868

Sanitá Lima M, Smith DR (2017) Pervasive, genome-wide transcription in the organelle genomes of diverse plastid-bearing protists. G3 7:3789–3796

Santel HJ, Apel K (1981) The protochlorophyllide holochrome of barley (*Hordeum vulgare* L.). The effect of light on the NADPH:protochlorophyllide oxidoreductase. Eur J Biochem 120:95–103

Sato S, Nakamura Y, Kaneko T et al (1999) Complete structure of the chloroplast genome of Arabidopsis thaliana. DNA Res 6:283–290

Schneider T, Bolger A, Zeier J et al (2019) Fluctuating light interacts with time of day and leaf development stage to reprogram gene expression. Plant Physiol 179:1632–1657

Schröter Y, Steiner S, Matthäi K, Pfannschmidt T (2010) Analysis of oligomeric protein complexes in the chloroplast sub-proteome of nucleic acid-binding proteins from mustard reveals potential redox regulators of plastid gene expression. Proteomics 10:2191–2204

Schweer J, Türkeri H, Kolpack A, Link G (2010) Role and regulation of plastid sigma factors and their functional interactors during chloroplast transcription – recent lessons from Arabidopsis thaliana. Eur J Cell Biol 89:940–946

Shahar N, Weiner I, Stotsky L et al (2019) Prediction and large-scale analysis of primary operons in plastids reveals unique genetic features in the evolution of chloroplasts. Nucleic Acids Res 47:3344–3352

Sharrock RA, Quail PH (1989) Novel phytochrome sequences in *Arabidopsis thaliana*: structure, evolution, and differential expression of a plant regulatory photoreceptor family. Genes Dev 3:1745–1757

Sharwood RE, Hotto AM, Bollenbach TJ, Stern DB (2011) Overaccumulation of the chloroplast antisense RNA AS5 is correlated with decreased abundance of 5S rRNA in vivo and inefficient 5S rRNA maturation in vitro. RNA 17:230–243

Sheerin DJ, Menon C, zur Oven-Krockhaus S et al (2015) Light-activated phytochrome A and B interact with members of the SPA family to promote photomorphogenesis in Arabidopsis by reorganizing the COP1/SPA complex. Plant Cell 27:189–201

Shen H, Moon J, Huq E (2005) PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in *Arabidopsis*. Plant J 44:1023–1035

Shen Y, Khanna R, Carle CM, Quail PH (2007) Phytochrome induces rapid PIF5 phosphorylation and degradation in response to red-light activation. Plant Physiol 145:1043–1051

Shi C, Wang S, Xia E-H et al (2016) Full transcription of the chloroplast genome in photosynthetic eukaryotes. Sci Rep 6:30135

Shiina T, Ishizaki Y, Yagi Y, Nakahira Y (2009) Function and evolution of plastid sigma factors. Plant Biotechnol 26:57–66

Shikanai T (2016) Chloroplast NDH: a different enzyme with a structure similar to that of respiratory NADH dehydrogenase. Biochim Biophys Acta 1857:1015–1022

Shimizu M, Kato H, Ogawa T et al (2010) Sigma factor phosphorylation in the photosynthetic control of photosystem stoichiometry. Proc Natl Acad Sci USA 107:10760–10764

Shimmura S, Nozoe M, Kitora S et al (2017) Comparative analysis of chloroplast *psbD* promoters in terrestrial plants. Front Plant Sci 8:1186

Shin J, Kim K, Kang H et al (2009) Phytochromes promote seedling light responses by inhibiting four negatively-acting phytochrome-interacting factors. Proc Natl Acad Sci USA 106:7660–7665

Shinozaki K, Ohme M, Tanaka M et al (1986) The complete nucleotide sequence of the tobacco chloroplast genome: its gene organization and expression. EMBO J 5:2043–2049

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

Smith HJ, Bogorad L (1974) The polypeptide subunit structure of the DNA-dependent RNA polymerase of *Zea mays* chloroplasts. Proc Natl Acad Sci USA 71:4839–4842

Smith AC, Purton S (2002) The transcriptional apparatus of algal plastids. Eur J Phycol 37:301–311

Song S, Wang Y, Ding X et al (2023) A plastid RNA polymerase-associated protein is involved in early chloroplast development in rice. Agronomy 13:1424

de Souza A, Wang J-Z, Dehesh K (2017) Retrograde signals: integrators of interorganellar communication and orchestrators of plant development. Annu Rev Plant Biol 68:85–108

Steiner S, Schröter Y, Pfalz J, Pfannschmidt T (2011) Identification of essential subunits in the plastid-encoded RNA polymerase complex reveals building blocks for proper plastid development. Plant Physiol 157:1043–1055

Stern DB, Goldschmidt-Clermont M, Hanson MR (2010) Chloroplast RNA metabolism. Annu Rev Plant Biol 61:125–155

Strasser B, Sánchez-Lamas M, Yanovsky MJ et al (2010) *Arabidopsis thaliana* life without phytochromes. Proc Natl Acad Sci USA 107:4776–4781

Strittmatter G, Gozdzicka-Jozefiak A, Kössel H (1985) Identification of an rRNA operon promoter from *Zea mays* chloroplasts which excludes the proximal $tRNA^{Val}_{GAC}$ from the primary transcript. EMBO J 4:599–604

Sugita M, Sugiura M (1996) Regulation of gene expression in chloroplasts of higher plants. Plant Mol Biol 32:315–326

Sugiura M, Wakasugi T, Kung S (1989) Compilation and comparison of transfer RNA genes from tobacco chloroplasts. CRC Crit Rev Plant Sci 8:89–101

Susek RE, Ausubel FM, Chory J (1993) Signal transduction mutants of *Arabidopsis* uncouple nuclear *CAB* and *RBCS* gene expression from chloroplast development. Cell 74:787–799

Sutherland C, Murakami KS (2018) An introduction to the structure and function of the catalytic core enzyme of *Escherichia coli* RNA polymerase. EcoSal Plus 8. https://doi.org/10.1128/ecosalplus.ESP-0004-2018

Suzuki JY, Ytterberg AJ, Beardslee TA et al (2004) Affinity purification of the tobacco plastid RNA polymerase and in vitro reconstitution of the holoenzyme. Plant J 40:164–172

Swiatecka-Hagenbruch M, Liere K, Börner T (2007) High diversity of plastidial promoters in *Arabidopsis thaliana*. Mol Gen Genomics 277:725–734

Swiatecka-Hagenbruch M, Emanuel C, Hedtke B et al (2008) Impaired function of the phage-type RNA polymerase RpoTp in transcription of chloroplast genes is compensated by a second phage-type RNA polymerase. Nucleic Acids Res 36:785–792

Takano M, Inagaki N, Xie X et al (2009) Phytochromes are the sole photoreceptors for perceiving red/far-red light in rice. Proc Natl Acad Sci USA 106:14705–14710

Taylor WC (1989) Regulatory interactions between nuclear and plastid genomes. Annu Rev Plant Physiol Plant Mol Biol 40:211–233

Thompson WF, Everett M, Polans NO et al (1983) Phytochrome control of RNA levels in developing pea and mung-bean leaves. Planta 158:487–500

Tozawa Y, Teraishi M, Sasaki T et al (2007) The plastid sigma factor SIG1 maintains photosystem I activity via regulated expression of the *psaA* operon in rice chloroplasts. Plant J 52:124–132

Tsunoyama Y, Ishizaki Y, Morikawa K et al (2004) Blue light-induced transcription of plastid-encoded *psbD* gene is mediated by a nuclear-encoded transcription initiation factor, AtSig5. Proc Natl Acad Sci USA 101:3304–3309

Turmel M, Otis C, Lemieux C (2002) The chloroplast and mitochondrial genome sequences of the charophyte *Chaetosphaeridium globosum*: insights into the timing of the events that restructured organelle DNAs within the green algal lineage that led to land plants. Proc Natl Acad Sci USA 99:11275–11280

Van Buskirk EK, Decker PV, Chen M (2012) Photobodies in light signaling. Plant Physiol 158:52–60

Vassylyev DG, Sekine S-I, Laptenko O et al (2002) Crystal structure of a bacterial RNA polymerase holoenzyme at 2.6 A resolution. Nature 417:712–719

Vergara-Cruces Á, Pramanick I, Pearce D et al (2024) Structure of the plant plastid-encoded RNA polymerase. Cell 187:1145–1159.e21

Wang Q, Zuo Z, Wang X et al (2018) Beyond the photocycle-how cryptochromes regulate photoresponses in plants? Curr Opin Plant Biol 45:120–126

Wang J, Miguel Palomar V, Min J-H, Wierzbicki AT (2024) pTAC3 and pTAC14 are required for binding of plastid-encoded RNA polymerase to DNA. bioRxiv 2024.03.01.582956

Wei N, Deng XW (1996) The role of the COP/DET/FUS genes in light control of arabidopsis seedling development. Plant Physiol 112:871–878

Weihe A, Börner T (1999) Transcription and the architecture of promoters in chloroplasts. Trends Plant Sci 4:169–170

Whitelam GC, Johnson E, Peng J et al (1993) Phytochrome A null mutants of *Arabidopsis* display a wild-type phenotype in white light. Plant Cell 5:757–768

Wicke S, Schneeweiss GM, dePamphilis CW et al (2011) The evolution of the plastid chromosome in land plants: gene content, gene order, gene function. Plant Mol Biol 76:273–297

Wimmelbacher M, Börnke F (2014) Redox activity of thioredoxin z and fructokinase-like protein 1 is dispensable for autotrophic growth of *Arabidopsis thaliana*. J Exp Bot 65:2405–2413

Windgassen TA, Mooney RA, Nayak D et al (2014) Trigger-helix folding pathway and SI3 mediate catalysis and hairpin-stabilized pausing by *Escherichia coli* RNA polymerase. Nucleic Acids Res 42:12707–12721

Wollman FA (2001) State transitions reveal the dynamics and flexibility of the photosynthetic apparatus. EMBO J 20:3623–3630

Woodson JD, Perez-Ruiz JM, Schmitz RJ et al (2013) Sigma factor-mediated plastid retrograde signals control nuclear gene expression. Plant J 73:1–13

Wu X-X, Mu W-H, Li F et al (2024) Cryo-EM structures of the plant plastid-encoded RNA polymerase. Cell 187:1127–1144.e21

Yagi Y, Shiina T (2014) Recent advances in the study of chloroplast gene expression and its evolution. Front Plant Sci 5:61

Yagi Y, Shiina T (2016) The chloroplast gene-expression system. In: Khan MS, Khan IA, Barh D (eds) Applied molecular biotechnology. CRC Press, pp 91–125

Yagi Y, Ishizaki Y, Nakahira Y et al (2012) Eukaryotic-type plastid nucleoid protein pTAC3 is essential for transcription by the bacterial-type plastid RNA polymerase. Proc Natl Acad Sci USA 109:7541–7546

Yamaguchi R, Nakamura M, Mochizuki N et al (1999) Light-dependent translocation of a phytochrome B-GFP fusion protein to the nucleus in transgenic *Arabidopsis*. J Cell Biol 145:437–445

Yang EJ, Yoo CY, Liu J et al (2019) NCP activates chloroplast transcription by controlling phytochrome-dependent dual nuclear and plastidial switches. Nat Commun 10:2630

Yoo CY, Pasoreck EK, Wang H et al (2019) Phytochrome activates the plastid-encoded RNA polymerase for chloroplast biogenesis via nucleus-to-plastid signaling. Nat Commun 10:2629

Yoo CY, Han S, Chen M (2020) Nucleus-to-plastid phytochrome signalling in controlling chloroplast biogenesis. Ann Plant Rev online 251–280

Yoo CY, He J, Sang Q et al (2021) Direct photoresponsive inhibition of a p53-like transcription activation domain in PIF3 by *Arabidopsis* phytochrome B. Nat Commun 12:5614

Yu Q-B, Lu Y, Ma Q et al (2013) TAC7, an essential component of the plastid transcriptionally active chromosome complex, interacts with FLN1, TAC10, TAC12 and TAC14 to regulate chloroplast gene expression in *Arabidopsis thaliana*. Physiol Plant 148:408–421

Zeng C, Jiao Q, Jia T, Hu X (2022) Updated progress on group II intron splicing factors in plant chloroplasts. Curr Issues Mol Biol 44:4229–4239

Zghidi W, Merendino L, Cottet A et al (2007) Nucleus-encoded plastid sigma factor SIG3 transcribes specifically the *psbN* gene in plastids. Nucleic Acids Res 35:455–464

Zghidi-Abouzid O, Merendino L, Buhr F et al (2011) Characterization of plastid *psbT* sense and antisense RNAs. Nucleic Acids Res 39:5379–5387

Zhang G, Campbell EA, Minakhin L et al (1999) Crystal structure of *Thermus aquaticus* core RNA polymerase at 3.3 A resolution. Cell 98:811–824

Zhang Y, Tian L, Lu C (2023) Chloroplast gene expression: recent advances and perspectives. Plant Commun 4:100611

Zhao X, Huang J, Chory J (2020) Unraveling the linkage between retrograde signaling and RNA metabolism in plants. Trends Plant Sci 25:141–147

Zhelyazkova P, Sharma CM, Förstner KU et al (2012) The primary transcriptome of barley chloroplasts: numerous noncoding RNAs and the dominating role of the plastid-encoded RNA polymerase. Plant Cell 24:123–136

Zoschke R, Bock R (2018) Chloroplast translation: structural and functional organization, operational control, and regulation. Plant Cell 30:745–770