Evolutionary aspects of plastid proteins involved in transcription

The transcription of a tiny genome is mediated by a complicated machinery

Yusuke Yagi[1] and Takashi Shiina[2,*]

[1]Faculty of Agriculture; Kyushu University; Fukuoka, Japan; [2]Graduate School of Life and Environmental Sciences; Kyoto Prefectural University; Kyoto, Japan

Chloroplasts in land plants have a small genome consisting of only 100 genes encoding partial sets of proteins for photosynthesis, transcription and translation. Although it has been thought that chloroplast transcription is mediated by a basically cyanobacterium-derived system, due to the endosymbiotic origin of plastids, recent studies suggest the existence of a hybrid transcription machinery containing non-bacterial proteins that have been newly acquired during plant evolution. Here, we highlight chloroplast-specific non-bacterial transcription mechanisms by which land plant chloroplasts have gained novel functions.

Evolutionary Footprint of the Chloroplast Genome

Chloroplasts are plant organelles that are responsible for photosynthesis. It is generally accepted that the chloroplasts are derived from a single endosymbiotic event in which the ancestor of the plant lineage engulfed a green photosynthetic cyanobacterium more than one billion years ago. Although green algal cells contain only chloroplasts, land plant plastids differentiate into specialized plastid types that can be distinguished by their structure, pigment composition (color) and function. Chloroplasts are found mainly in the leaves, whereas plastids convert into non-photosynthetic amyloplasts and chromoplasts that accumulate starch and a variety of secondary metabolic products in roots and fruits, respectively. All types of plastids are derived from undifferentiated proplastids in meristematic tissues, and plastid differentiation is controlled by environmental and tissue-specific cues.

Plastids have their own genome, whose size and number of genes decreased drastically during plant evolution as compared to genomes of living cyanobacteria. The unicellular cyanobacterium *Synechocystis* sp. PCC6803 genome is 3,573,471 bp and contains 3,317 genes, whereas the *Arabidopsis thaliana* plastid genome (154,478 bp) consists of only 87 protein-coding genes for photosynthesis, lipid metabolism, RNA polymerase and ribosome components, along with 37 tRNA and 4 rRNA genes.[1,2] Most cyanobacterial protein-coding genes have been lost or transferred to the host nuclear genome during plant evolution. On the other hand, chloroplast proteome analyses identified more than 3,000 proteins in chloroplasts, indicating that chloroplasts are semi-autonomous and largely dependent on nuclear-encoded proteins.[3]

Chloroplast gene expression is generally mediated by prokaryotic machinery analogous to cyanobacterial RNA polymerase and ribosomes. However, no homologues of bacterial transcription factors or bacterial nucleoid proteins have been found in higher plant genomes. On the other hand, recent proteomic analysis of higher plant chloroplasts has identified a number of plant-specific non-bacterial eukaryotic type proteins that are likely involved in plastid gene expression. These eukaryotic type chloroplast proteins might be newly acquired from the host genome after the endosymbiotic event, and might be involved in the regulation of plastid differentiation and the adaptation of plastids to the land environment. This implies that
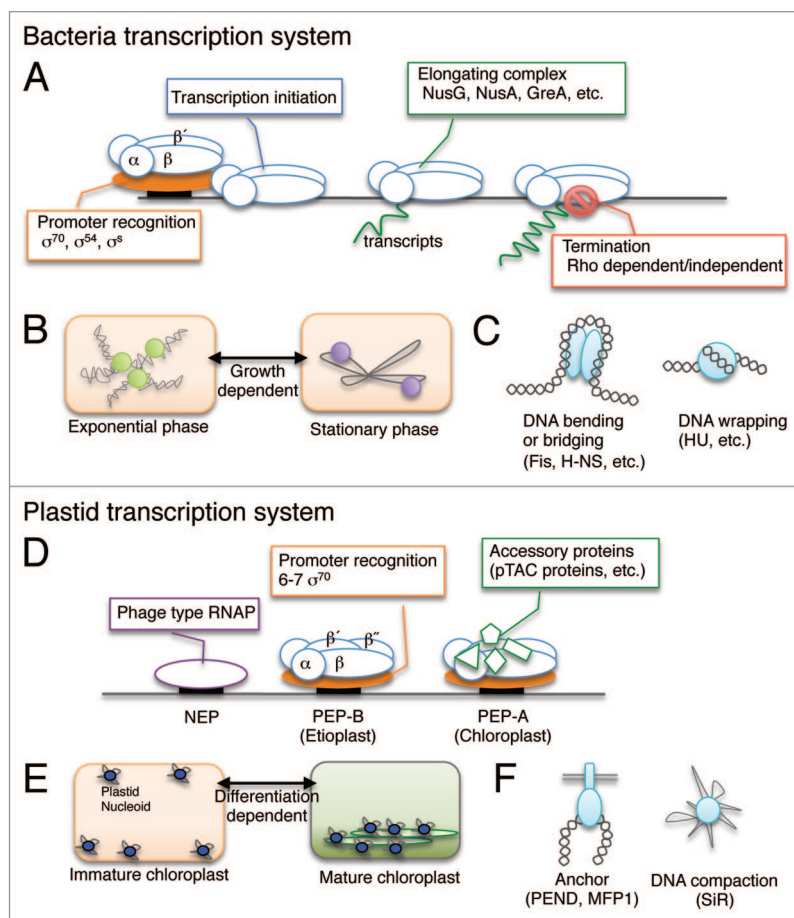
Figure 1. Comparison of transcription systems in bacteria and plastids. (**A**) Bacterial RNA polymerase traffics from a promoter to a terminator. (**B**) Changes in compaction and DNA supercoiling in bacterial nucleoids is dependent on growth conditions. (**C**) The roles of abundant DNA binding proteins in bacterial nucleoid organization. (**D**) Plastid transcription machineries. (**E**) Changes in the intra-plastid localization of nucleoids during plastid differentiation. (**F**) Plant-specific DNA binding proteins involved in plastid nucleoid function.

higher plant chloroplasts have developed complicated gene expression systems supported by a number of eukaryotic proteins, despite their dramatically reduced genome information.

Basic Transcription Machinery for the Chloroplast Genome

In angyosperms, chloroplast transcription is mediated by two distinct types of RNA polymerases, plastid-encoded plastid RNA polymerase (PEP) and nuclear-encoded plastid RNA polymerase (NEP) (for review see ref. 4). PEP is a bacterial type enzyme that is composed of four core subunits (α, β, β′, β″) and a sigma factor allowing recognition of promoter sequences (**Fig. 1A and D**). Genes encoding PEP core subunits are retained in

the chloroplast genome as *rpoA*, *rpoB*, *rpoC1* and *rpoC2* (except in the moss *Physcomitrella patens* in which the *rpoA* gene has been transferred to the nuclear genome), whereas all genes for plastid sigma factors have been transferred to the nuclear genome. Interestingly, green algae including *Chlamydomonas reinhardtii*, *Chlorella* and the most primitive, *Ostreococcus tauri*, have only one plastid sigma factor, while land plants such as moss (five genes in Physcomitrella), ferns (three genes in Selaginella), dicots (six genes in Arabidopsis), and monocots (seven genes in rice) possess multiple sigma factors. Phylogenic analysis revealed that all chloroplast sigma factors fall into the σ^{70} family, members of which are responsible for transcription of housekeeping genes in bacteria during the exponential

growth phase. Thus, it is suggested that only the essential sigma factor σ^{70} has been retained in chloroplasts, while other non-essential alternative sigma factors were lost during the early evolution of the green plant lineage. The sigma factor gene copy number in chloroplasts may have increased as a result of genomic duplication to support plastid differentiation in land plants. The σ^{70} recognizes a consensus promoter sequence that is characterized by -10 and -35 core elements spaced at 17-19 nt. Such promoter sequences are commonly found upstream of chloroplast-encoded genes. Molecular characterization of Arabidopsis sigma factors AtSIG1-AtSIG6 demonstrated that they have specific and partially overlapping roles in transcription of photosynthesis and ribosomal RNA genes (For review see ref. 5).

On the other hand, NEP is a phage-type chloroplast RNA polymerase that originated by gene duplication of a mitochondrial RNA polymerase gene. Although higher plants have one or two NEPs, green algae have none. NEP is required for transcription of housekeeping genes such as genes encoding PEP core subunits, ribosomal proteins and a lipid metabolism protein (accD). It is well known that PEP and NEP activities are inversely regulated during chloroplast development from seeds (white) to leaves (green).[6,7] Briefly, NEP becomes active to produce the PEP transcription system and chloroplast translation machinery at an early stage of seed germination. Subsequently, PEP actively transcribes photosynthesis genes to construct and maintain the photosynthesis system, while NEP activity gradually declines during the greening process. High NEP and low PEP activities are characteristic of non-green plastids, such as amyloplasts and chromoplasts in roots and colored fruits, respectively. During tomato fruit ripening, a subset of NEP-dependent genes including *accD*, *trnA* and *rpoC2* are specifically up-regulated.[8] This coordinated functioning of the two RNA polymerases is essential for plastid differentiation. Thus, chloroplasts inherited the bacterial-type transcription system from their cyanobacterial ancestor, but likely have evolved a complex transcription network with multiple RNA polymerases to control plastid differentiation (**Fig. 1D**).

Missing Parts for Chloroplast Transcription

The molecular understanding of transcriptional regulation in bacteria is well advanced. The transcription cycle comprises multiple steps including initiation, elongation and termination (**Fig. 1A**). RNA polymerase complexes with different types of σ factors recognize different promoter sequences and initiate transcription of specific sets of genes. Gene-specific transcriptional activation and repression are also regulated by DNA-binding transcription factors, although they are not part of the holoenzyme (RNAP core-σ complex). The sigma factor is released from the RNA polymerase holoenzyme during the transition from initiation to elongation, and the RNA polymerase complex is converted into an elongation complex (EC). The EC slides along DNA with the assistance of elongation regulators such as NusA, NusG and GreA. Bacterial transcription is terminated by two mechanisms: Rho protein-dependent termination and intrinsic terminator sequence-dependent (Rho-independent) termination.

In chloroplasts, it has been clearly shown that promoter recognition by PEP is also conferred by sigma factors in a similar manner to that in bacteria. Several promoters in higher plant chloroplasts have unique cis elements.[4] However, no transcription factors or DNA-binding proteins that are conserved between bacteria and higher plants have been identified. In contrast to the initiation steps, much less is known about the post-initiation steps. Recently, we have developed a chloroplast ChIP assay, and analyzed the binding of PEP core subunit, α, along chloroplast DNA.[9] The association of PEP is enriched at promoter-proximal regions, and its signal attenuated toward termination regions, similar to the distribution patterns of *E. coli* RNAP, suggesting that PEP-dependent transcription initiation, elongation and termination steps are regulated by mechanisms similar to those of bacteria. However, for PEP and NEP elongation and termination factors remain unidentified. Plastid transcriptionally active chromosome 13 (pTAC13) and Etched 1 (ET1) are candidates to be

plastid elongation regulators, since they possess similarity to the NusG domain and the nuclear transcription elongation factor TFIIS, respectively.[10,11] Molecular characterization of these factors in PEP-dependent transcription will provide insight into the PEP elongation mechanisms. Termination of chloroplast RNA polymerase activity was found to occur at intrinsic bacterial-like terminators in vitro.[12] However, most chloroplast 3' termini are generated by RNA processing rather than by termination at accurate positions in vivo.[13]

Novel Plant-Specific Transcriptional and Post-Transcriptional Regulators in Plastids

It is known that the molecular size of the PEP complex changes during leaf development.[14] In mustard, two distinct PEP complexes have been identified in chloroplasts: PEP-A and PEP-B, which differ in terms of subunit composition, functional properties and abundance during etioplast-to-chloroplast conversions. PEP-B consists of four proteins corresponding to the predicted sizes of PEP core subunits, whereas PEP-A is larger than PEP-B and contains at least 13 additional polypeptides. Etioplasts in dark-grown seedlings contain PEP-B, whereas PEP-A is predominant in chloroplasts. The PEP complex likely alters in size and activity through PEP-A-associated proteins during chloroplast development.

Plastid transcriptionally active chromosomes (pTACs) have been isolated from the chloroplast membrane by treatment with Triton X-100 followed by gel filtration.[15] Electron microscopic observation revealed huge protein-DNA complexes with a mesh-like structure.[16,17] Thus, pTACs are assumed to be a part of plastid nucleoids. Proteomic analysis of pTAC proteins has identified 18 novel non-bacterial proteins that are named pTAC1—pTAC18, together with PEP core subunits, DNA gyrase, DNA polymerase and some ribosomal proteins.[10] Interestingly, most pTAC gene-inactivated mutants display a chlorophyll-deficient phenotype and reduced PEP transcription activity, suggesting that PEP transcription requires not only core

RNA polymerase subunits but also pTAC proteins. However, their primary functions still remain unclear. Several pTAC proteins have also been identified as components of PEP-A or co-immunoprecipitated with PEP subunits, suggesting that some pTAC proteins interact directly with PEP (for review see ref. 18). For example, pTAC3 binds directly to the PEP complex and its defect results in decreased transcriptional activity of PEP.[9] Furthermore, chloroplast ChIP analysis demonstrated that the pTAC3 association pattern along the PEP-transcribed region is the same as that of the PEP core alpha subunit. These data suggest that pTAC3 associates with the PEP complex during transcription and is essential for PEP activity.

On the other hand, the maize whirly 1 (WHY1/pTAC1) protein has been identified in the Chloroplast RNA Splicing 1 (CRS1) protein complex, which promotes the splicing of the chloroplast *atpF* group II intron.[19] ZmWHY1 is part of the plant-specific non-bacterial 'Whirly' protein family, members of which have been described as DNA-binding proteins and are localized at nucleoids in chloroplasts and mitochondria. Genome-wide DNA or RNA immunoprecipitation assays showed that ZmWHY1 is associated with maize chloroplast DNA and with a subset of plastid RNAs including *atpF* transcripts. Moreover, ZmWHY1 is required for PEP-dependent transcription, but not directly involved in either DNA replication or global plastid transcription. Although it is not clear whether the WHY1 protein is associated with PEP, its DNA binding pattern and association with the chloroplast RNA splicing complex suggest that WHY1 is involved in post-transcriptional regulation at chloroplast nucleoids and the coupling of transcription and splicing. In addition to the role of WHY1 in chloroplasts, it has been reported that AtWHY1 also acts as a nuclear transcription factor regulating the salicylic-acid dependent defense system.[20] Likewise, pTAC12/HEMERA protein is also localized to both nuclei and chloroplasts, and is involved in phytochrome light signaling.[21] These dual-localized pTAC proteins might be involved in the crosstalk between chloroplast and nuclear gene expression. genome uncoupled 1 (GUN1) has been

identified as a key player involved in the plastid-to-nucleus signaling that coordinates nuclear gene expression with the chloroplast status via signaling molecules such as chloroplast-generated ROS and Mg-ProtoIX.[22] Interestingly, GUN1 is colocalized with pTAC2 in chloroplasts and has both DNA- and RNA-binding activities in vitro. Genetic analysis has implied that GUN1 integrates multiple signaling pathways responsible for recognition of aberrant chloroplasts, which leads to ABI4-mediated repression of nuclear-encoded genes, but the transcriptional roles of GUN1 and pTAC2 in chloroplast gene expression are still unknown.

Taken together, transcription by the bacterial type chloroplast RNA polymerase PEP is mediated by a number of pTAC proteins, which are not conserved in bacteria and have been likely acquired during higher plant evolution. pTAC proteins might be involved in plastid maintenance processes such as plastid differentiation and the recognition of aberrant chloroplasts.

Plastid Nucleoid Proteins

In addition to gene-specific transcriptional regulatory mechanisms, it has also been shown that global plastid transcription activity is under the control of the spatial architecture of the genome. Bacterial DNA is packed into a protein-DNA complex, a bacterial chromosome termed a nucleoid. DNA binding proteins such as HU, Fis, and H-NS induce compaction and supercoiling of DNA through their DNA binding activity (**Fig. 1C**).[23] Chloroplast transcription also occurs at nucleoids. In bacteria, nucleoid compaction and DNA supercoiling are differentially regulated depending on the growth phase and transcription status (**Fig. 1B**). Similarly, plastid nucleoids drastically change in size, morphology and localization during chloroplast development.[24] The plastid nucleoid is located in the envelope membrane of immature proplastids, whereas it relocates to the thylakoids in mature chloroplasts (**Fig. 1E**). Relocation of plastid nucleoids might be involved in transcriptional regulation of plastid-encoded genes during chloroplast development.

One of the major bacterial nucleoid proteins, HU, has been identified in the red alga *Cyanidioschyzon merolae* and in apicomplexa. However, mosses and higher plants have lost all bacterial-type nucleoid proteins, including HU,[25] suggesting that higher plants have adopted novel eukaryotic-type proteins as nucleoid proteins to compact plastid DNA and regulate nucleoid function. It should be noted that chloroplasts in land plants contain several unique proteins as plastid nucleoid proteins (**Fig. 1F**). Sulfite reductase (SiR) is a 70 kDa soluble protein and one of the most abundant proteins in the plastid nucleoid. SiR induces the compaction of plastid DNA and effectively represses chloroplast transcription activity in vitro. These results suggest that SiR may regulate the global transcriptional activity of chloroplast nucleoids through changes in DNA compaction.[26] Moreover, small molecular proteins containing a eukaryotic SWIB (SWI/SNF complex B) domain have been recently identified in TAC fractions.[27] Among them, SWIB4, which is localized in both plastid nucleoids and cellular nuclei, has a histone H1 motif and could functionally complement an *E. coli* mutant lacking the histone-like nucleoid structuring protein H-NS, indicating that SWIB4 might be a counterpart of the bacterial nucleoid proteins that is involved in the maintenance of nucleoid structure.

It has been proposed that plastid envelope DNA-binding protein (PEND) and MAR-binding filament-like protein (MFP1) are also unique nucleoid anchor proteins that bind both DNA and chloroplast membranes. PEND is composed of a cbZIP domain and a C-terminal hydrophobic domain. The cbZIP domain is involved in dimerization of PEND and sequence-specific DNA binding, whereas the C-terminal hydrophobic domain is required for targeting the PEND protein to the chloroplast envelope membrane.[28] In contrast to PEND, MFP1 has been shown to be localized to the thylakoid membranes and its C-terminal domain has DNA binding activity.[29] Thus, it is assumed that two anchor proteins are likely involved in the relocation of plastid nucleoids from the envelope to thylakoid membranes during chloroplast

development. Furthermore, recent proteomic analysis of maize chloroplast nucleoids during chloroplast development identified not only basic proteins involved in DNA replication, repair and transcription, but also a number of proteins involved in post-transcriptional events such as RNA processing and editing.[30] It is suggested that the plastid transcription system is spatially and functionally coupled to post-transcriptional events at plastid nucleoids. Interestingly, most of the proteins involved in post-transcriptional processes in plastid nucleoids are not related to bacterial proteins. Further characterization of plastid nucleoid proteins should provide insights into the co-evolution of non-bacterial proteins with the basic plastid transcription system.

Summary

Proteomic analysis of plastid transcription machinery such as pTACs and plastid nucleoids has raised the question of why plastid transcription requires many additional factors besides the basic transcription machinery to transcribe a small genome encoding only a hundred genes. It is likely that almost all pTAC and plastid nucleoid proteins were acquired when plants became adapted for life on land, as evidenced by the absence of homologous proteins in green algae such as *Chlamydomonas reinhardtii*. It is reasonable that green algae have simplified their transcription regulatory system in chloroplasts by reducing the number of cyanobacterium-derived regulatory factors, since green alga chloroplasts exist in a stable environment, the cytoplasm of the host cells. Furthermore, chloroplasts of green algae stay green even in the dark, suggesting a lack of light-dependent regulation of chloroplast development. Indeed, chloroplasts of green algae such as Chlamydomonas retain only one sigma factor and have acquired no NEP. By contrast, chloroplasts in land plants differentiate into several different types of plastids in response to cell differentiation. To gain a plastid differentiation system, chloroplasts were forced to develop both repression (green to white) and activation (white to green) systems for photosynthetic machinery. The acquisition of the

NEP type novel plastid RNA polymerase likely enabled a transcriptional switching system in chloroplasts, which allows selective transcription of a set of housekeeping genes in non-photosynthetic tissues, such as roots and flowers. In addition, PEP accessory proteins, including a number of pTAC proteins, may be required for the establishment and maintenance of active transcription of photosynthesis genes through PEP. These non-bacterial factors have been probably acquired from host cells, since plant cells lost most of the cyanobacterium-derived regulatory proteins early during evolution of green algae. Finally, nucleoid architecture and/or intra-chloroplast localization may be involved in the regulation of plastid transcription activity in response to plastid differentiation, and the regulatory proteins likely have been developed during plant evolution.

Taken together, land plants have acquired several novel non-bacterial proteins that are involved in transcriptional and post-transcriptional regulation of plastid gene expression during chloroplast differentiation and adaptation to the environment. Further molecular characterization of pTACs and plastid nucleoid proteins will provide insights into the complex regulatory mechanisms of plastid gene expression in response to plastid differentiation and environmental cues.

References

1. Sugiura M. The chloroplast genome. Plant Mol Biol 1992; 19:149-68; PMID:1600166; http://dx.doi.org/10.1007/BF00015612.

2. Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, et al. Sequence analysis of the genome of the unicellular cyanobacterium Synechocystis sp. strain PCC6803. II. Sequence determination of the entire genome and assignment of potential protein-coding regions. DNA Res 1996; 3:109-36; PMID:8905231; http://dx.doi.org/10.1093/dnares/3.3.109.

3. Martin W, Herrmann RG. Gene transfer from organelles to the nucleus: how much, what happens, and Why? Plant Physiol 1998; 118:9-17; PMID:9733521; http://dx.doi.org/10.1104/pp.118.1.9.

4. Shiina T, Tsunoyama Y, Nakahira Y, Khan MS. Plastid RNA polymerases, promoters, and transcription regulators in higher plants. Int Rev Cytol 2005; 244:1-68; PMID:16157177; http://dx.doi.org/10.1016/S0074-7696(05)44001-2.

5. Shiina T, Ishizaki Y, Yagi Y, Nakahira Y. Function and evolution of plastid sigma factors. Plant Biotechnol 2009; 26:57-66; http://dx.doi.org/10.5511/plantbiotechnology.26.57.

6. Baumgartner BJ, Rapp JC, Mullet JE. Plastid Genes Encoding the Transcription/Translation Apparatus Are Differentially Transcribed Early in Barley (Hordeum vulgare) Chloroplast Development (Evidence for Selective Stabilization of psbA mRNA). Plant Physiol 1993; 101:781-91; PMID:12231729.

7. Demarsy E, Courtois F, Azevedo J, Buhot L, Lerbs-Mache S. Building up of the plastid transcriptional machinery during germination and early plant development. Plant Physiol 2006; 142:993-1003; PMID:16963522; http://dx.doi.org/10.1104/pp.106.085043.

8. Kahlau S, Bock R. Plastid transcriptomics and translatomics of tomato fruit development and chloroplast-to-chromoplast differentiation: chromoplast gene expression largely serves the production of a single protein. Plant Cell 2008; 20:856-74; PMID:18441214; http://dx.doi.org/10.1105/tpc.107.055202.

9. Yagi Y, Ishizaki Y, Nakahira Y, Tozawa Y, Shiina T. Eukaryotic-type plastid nucleoid protein pTAC3 is essential for transcription by the bacterial-type plastid RNA polymerase. Proc Natl Acad Sci U S A 2012; 109:7541-6; PMID:22529394; http://dx.doi.org/10.1073/pnas.1119403109.

10. Pfalz J, Liere K, Kandlbinder A, Dietz KJ, Oelmüller R. pTAC2, -6, and -12 are components of the transcriptionally active plastid chromosome that are required for plastid gene expression. Plant Cell 2006; 18:176-97; PMID:16326926; http://dx.doi.org/10.1105/tpc.105.036392.

11. da Costa e Silva O, Lorbiecke R, Garg P, Müller L, Wassmann M, Lauert P, et al. The Etched1 gene of Zea mays (L.) encodes a zinc ribbon protein that belongs to the transcriptionally active chromosome (TAC) of plastids and is similar to the transcription factor TFIIS. Plant J 2004; 38:923-39; PMID:15165185; http://dx.doi.org/10.1111/j.1365-313X.2004.02094.x.

12. Chen LJ, Orozco EM Jr. Recognition of prokaryotic transcription terminators by spinach chloroplast RNA polymerase. Nucleic Acids Res 1988; 16:8411-31; PMID:2843817; http://dx.doi.org/10.1093/nar/16.17.8411.

13. Stern DB, Gruissem W. Control of plastid gene expression: 3' inverted repeats act as mRNA processing and stabilizing elements, but do not terminate transcription. Cell 1987; 51:1145-57; PMID:3690662; http://dx.doi.org/10.1016/0092-8674(87)90600-3.

14. Pfannschmidt T, Link G. Separation of two classes of plastid DNA-dependent RNA polymerases that are differentially expressed in mustard (Sinapis alba L.) seedlings. Plant Mol Biol 1994; 25:69-81; PMID:8003698; http://dx.doi.org/10.1007/BF00024199.

15. Hallick RB, Lipper C, Richards OC, Rutter WJ. Isolation of a transcriptionally active chromosome from chloroplasts of Euglena gracilis. Biochemistry 1976; 15:3039-45; PMID:821516; http://dx.doi.org/10.1021/bi00659a016.

16. Yoshida Y, Laulhere JP, Rozier C, Mache R. Visualization of Folded Chloroplast DNA from Spinach. Biol Cell 1978; 32:187-90.

17. Briat JF, Gigot C, Laulhere JP, Mache R. Visualization of a Spinach Plastid Transcriptionally Active DNA-Protein Complex in a Highly Condensed Structure. Plant Physiol 1982; 69:1205-11; PMID:16662371; http://dx.doi.org/10.1104/pp.69.5.1205.

18. Lerbs-Mache S. Function of plastid sigma factors in higher plants: regulation of gene expression or just preservation of constitutive transcription? Plant Mol Biol 2011; 76:235-49; PMID:21107995; http://dx.doi.org/10.1007/s11103-010-9714-4.

19. Prikryl J, Watkins KP, Friso G, van Wijk KJ, Barkan A. A member of the Whirly family is a multifunctional RNA- and DNA-binding protein that is essential for chloroplast biogenesis. Nucleic Acids Res 2008; 36:5152-65; PMID:18676978; http://dx.doi.org/10.1093/nar/gkn492.

20. Desveaux D, Subramaniam R, Després C, Mess JN, Lévesque C, Fobert PR, et al. A "Whirly" transcription factor is required for salicylic acid-dependent disease resistance in Arabidopsis. Dev Cell 2004; 6:229-40; PMID:14960277; http://dx.doi.org/10.1016/S1534-5807(04)00028-0.

21. Chen M, Galvão RM, Li M, Burger B, Bugea J, Bolado J, et al. Arabidopsis HEMERA/pTAC12 initiates photomorphogenesis by phytochromes. Cell 2010; 141:1230-40; PMID:20603003; http://dx.doi.org/10.1016/j.cell.2010.05.007.

22. Koussevitzky S, Nott A, Mockler TC, Hong F, Sachetto-Martins G, Surpin M, et al. Signals from chloroplasts converge to regulate nuclear gene expression. Science 2007; 316:715-9; PMID:17395793; http://dx.doi.org/10.1126/science. 1140516.

23. Dillon SC, Dorman CJ. Bacterial nucleoid-associated proteins, nucleoid structure and gene expression. Nat Rev Microbiol 2010; 8:185-95; PMID:20140026; http://dx.doi.org/10.1038/nrmicro2261.

24. Sato N, Misumi O, Shinada Y, Sasaki M, Yoine M. Dynamics of localization and protein composition of plastid nucleoids in light-grown pea seedlings. Protoplasma 1997; 200:163-73; http://dx.doi.org/10.1007/BF01283292.

25. Sato N, Terasawa K, Miyajima K, Kabeya Y. Organization, developmental dynamics, and evolution of plastid nucleoids. Int Rev Cytol 2003; 232:217-62; PMID:14711120; http://dx.doi.org/10.1016/S0074-7696(03)32006-6.

26. Sekine K, Hase T, Sato N. Reversible DNA compaction by sulfite reductase regulates transcriptional activity of chloroplast nucleoids. J Biol Chem 2002; 277:24399-404; PMID:11997391; http://dx.doi.org/10.1074/jbc.M201714200.

27. Melonek J, Matros A, Trosch M, Mock HP, Krupinska K. The Core of Chloroplast Nucleoids Contains Architectural SWIB Domain Proteins. Plant Cell 2012; 24:3060-73; doi: http://dx.doi.org/10.1105/tpc.112.099721.

28. Sato N, Ohshima K, Watanabe A, Ohta N, Nishiyama Y, Joyard J, et al. Molecular characterization of the PEND protein, a novel bZIP protein present in the envelope membrane that is the site of nucleoid replication in developing plastids. Plant Cell 1998; 10:859-72; PMID:9596643.

29. Jeong SY, Rose A, Meier I. MFP1 is a thylakoid-associated, nucleoid-binding protein with a coiled-coil structure. Nucleic Acids Res 2003; 31:5175-85; PMID:12930969; http://dx.doi.org/10.1093/nar/gkg693.

30. Majeran W, Friso G, Asakura Y, Qu X, Huang M, Ponnala L, et al. Nucleoid-enriched proteomes in developing plastids and chloroplasts from maize leaves: a new conceptual framework for nucleoid functions. Plant Physiol 2012; 158:156-89; PMID:22065420; http://dx.doi.org/10.1104/pp.111.188474.