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Update on Gene Regulation

Dynamic Regulation of Chloroplast Transcription[1]

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PLASTID FUNCTIONS AND SPECIALIZATION

Plastids are ubiquitous DNA-containing organelles present in plant cells. These organelles carry out numerous metabolic functions including photosynthesis, starch synthesis, and steps in lipid, terpenoid, amino acid, tetrapyrrole, and plant hormone biosynthesis. Some of these functions are highly expressed in differentiated plastids. For example, chloroplasts are specialized for photosynthesis, chromoplasts for carotenoid biosynthesis and storage, and amyloplasts for starch biosynthesis. Although basal levels of plastid gene expression are observed in all plastid types, a large increase in plastid transcription is observed during chloroplast biogenesis. This suggests that one central regulatory point of chloroplast differentiation is the activation of chloroplast transcription. However, chloroplast development requires coordinated expression of plastid and nuclear genes, and, therefore, activation of plastid gene transcription must be coordinated with the activation of nuclear gene expression by signals that can be transmitted between plastids and the nucleus. The study of chloroplast gene expression also provides an opportunity to understand how plants sense and alter gene expression in response to light. Light is a substrate for photosynthesis, a trigger of plant developmental processes, and a potential source of injury that modulates chloroplast transcription.

CHANGING PERSPECTIVES OF PLASTID TRANSCRIPTION

Early studies of chloroplast genes revealed −10 (TATAA) and −35 (GTGACA) transcription promoter elements and putative ribosome binding sites (GGAGG) that resembled prokaryotic transcription and translation elements (for review, see Mullet, 1988; Igloi and Kössel, 1992). These sequence elements were consistent with the plastid's prokaryotic-like RNA polymerase and 70S ribosomes. Sequence homology between plastid and bacterial genes provided additional evidence of common origin. Even more striking and important relative to gene regulation was the presence of conserved plastid operons, often having the same gene order as their counterparts in *Escherichia coli* (for review, see Sugiura, 1992). Although some features of chloroplast gene expression, such as RNA splicing, are less commonly associated with bacterial systems, it was generally expected that regulation of chloroplast gene expression would follow bacterial paradigms. Thus, when differential expression of *rbc*L was observed in mesophyll versus bundle sheath cells of maize (Link et al., 1978) and selective accumulation of *psb*A mRNA was documented in illuminated maize leaves (Bedbrook et al., 1978), differential transcription was suggested as the likely mechanism involved.

Run-on transcription assays for plastids, developed in 1987, allowed the first direct analysis of the involvement of transcriptional regulation of plastid gene expression (Deng et al., 1987; Mullet and Klein, 1987). Early run-on transcription assays were limited to providing information on relative transcription rates. Even so, these assays revealed large differences in transcription rates among plastid genes and allowed a comparison of transcription rates with mRNA levels. Determination of relative mRNA levels and transcription rates during light-induced chloroplast development in barley showed that changes in *psa*A-*psa*B and *atp*B mRNA levels were paralleled by changes in transcription rate. However, for other genes such as *psb*A, changes in transcription and mRNA levels were not coupled, suggesting that mRNA stability contributed significantly to the determination of *psb*A mRNA levels. Similar studies of spinach chloroplasts, root amyloplasts, and tomato chromoplasts led to the conclusion that transcriptional regulation in plastids was "limited" (Deng and Gruissem, 1987, 1988). This conclusion paralleled ideas set forth earlier by Bendich (1987) when considering the reasons why plastids contain a variable but large number of plastid DNAs. Bendich observed that plastid DNA copy number and ribosome abundance increased dramatically during chloroplast development, and he hypothesized that rRNA synthesis was template limited and that the increase in plastid DNA copy number during chloroplast development was needed to activate rRNA synthesis. This hypothesis suggested that expression of other plastid genes (not encoding rRNA) was not limited by transcription and that regulation of expression occurred posttranscriptionally at the levels of mRNA stability, translation, or protein turnover.

Recently, increased understanding of the complexity of plastid operons, development of quantitative run-on transcription assays (Rapp et al., 1992), and examination of a wider range of developmental stages have led to a more dynamic view of chloroplast transcription. It is now known that transcription rates vary among plastid genes over 300-fold and that transcription rates are predictive of mRNA levels and protein abundance for many plastid genes. Furthermore, differential transcription of genes encoding the transcription/translation apparatus occurs early in barley chloroplast development (Baumgartner et al., 1993) and light-induced differential transcription of *psb*A, *psb*D-*psb*C (Chris-

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topher et al., 1992), and *petE* (Haley and Bogorad, 1990) have been documented. In this review, the dynamics of plastid transcription will be discussed and related to chloroplast development and biochemical mechanisms that mediate differential transcription. Detailed information on plastid genome structure, gene expression, and development can be found in other, more extensive reviews (Mullet, 1988; Palmer, 1990; Igloi and Kössel, 1992; Sugiura, 1992).

PLASTID GENOME ORGANIZATION

Most plastid genomes are circular and range in size from 120 to 217 kb (Palmer, 1990). Variation in genome size is related primarily to the size of an inverted repeat in the plastid genome and limited differences in coding capacity. Plastids contain from 22 to over 300 genome copies, depending on developmental stage (Bendich, 1987). The genome is membrane associated, complexed with protein, and resembles bacterial nucleoids. Plastid DNA encodes approximately 135 genes that fall into three major functional categories: genes encoding proteins and RNAs involved in transcription and translation of the plastid genome (RNA polymerase subunits, tRNAs, rRNAs, ribosomal proteins, initiation factor 1); genes encoding proteins of the photosynthetic apparatus (Rubisco, PSI, PSII, ATP synthase, Cyt complex); and genes encoding proteins of the NADH oxido-reductase complex. Whereas plastids function in nonphotosynthetic tissues, the plastid genome is clearly specialized for production of the photosynthetic apparatus.

Most plastid genes are organized in complex operons that are conserved among plastid genomes (Sugiura, 1992). The plastid operons listed in Table I show that genes with common functions are often co-localized in operons. For example, the *rpo*B-*rpo*C1-*rpo*C2 operon encodes subunits of the plastid-encoded RNA polymerase, whereas the *psb*I-*psb*K-*psb*D-*psb*C operon encodes proteins of PSII. Some operons contain genes involved only in transcription (*rpo*B operon), translation (*rrn* operon), or transcription and translation (*rpl*23 operon), but not photosynthesis. The localization of genes encoding proteins of a single protein complex in operons facilitates coordinated and stoichiometric accumulation of subunits. This organization also offers the opportunity to differentially regulate the transcription of some genes involved in transcription/translation independently of genes for photosynthesis. Other operons contain mixtures of genes encoding different functions. For example, *rps*14 (ribosomal protein) is co-transcribed with *psa*A-*psa*B (PSI subunits), *rpo*A (RNA polymerase subunit) with genes for ribosomal proteins, and *psb*B/*psb*H (PSII subunits) with *pet*B/*pet*D (Cyt complex), and tRNAs are included in several operons. Expression of specific genes within mixed function operons involves multiple levels of regulation including complex promoter/terminator combinations, selective RNA processing, differential RNA stability, regulated translation, and protein turnover. Not surprisingly, complex RNA populations are associated with most plastid operons. In some cases, the RNA complexity is due to the action of multiple promoters that allow differential transcription of selected genes within the same operon. For example, at least 12 different RNAs are produced from the barley *psb*I-*psb*K-*psb*D-*psb*C-*orf*6-*trn*G operon through the action of four different promoters and several RNA cleavage events (Sexton et al., 1990).

Table I. *Gene composition of selected plastid operons*

Operon	Gene Products
16S-*trn*I-*trn*A-23S-4.5S-5S rRNA	rRNAs, tRNAs
*rpl*23-*rpl*2-*rps*19-*rpl*22-*rps*3-*rpl*16-*rpl*14-*rps*8-*inf*A-*rpl*36-*rps*11-*rpo*A	ribosomal proteins, initiation factor 1, RNA polymerase subunit alpha
*rpo*B-*rpo*C1-*rpo*C2	RNA polymerase subunits
*psb*B-*psb*H-*pet*B-*pet*D	PSII and Cyt subunits
*psb*I-*psb*K-*psb*D-*psb*C-*orf*62-*trn*G	PSII subunits, orf62, tRNA
*psa*A-*psa*B-*rps*14	PSI subunits, ribosomal protein
*atp*I-*atp*H-*atp*F-*atp*A	ATP synthase

PROTEIN STOICHIOMETRY, mRNA ABUNDANCE, AND TRANSCRIPTION RATES

Segregation of genes encoding proteins of different functions into separate operons could be related, in part, to a requirement for different levels of protein production. The abundance of plastid-encoded proteins varies over 1000-fold, with the large subunit of Rubisco being the most abundant protein, followed by proteins involved in electron transport, ribosomes, and subunits of the plastid-encoded RNA polymerase (Rapp et al., 1992). Among plastid genes, mRNA abundance also varies approximately 1000-fold and, in general, mRNA abundance parallels protein abundance (Rapp et al., 1992). A 300-fold range of transcription rate is observed among these same genes, and transcription activity varies in parallel with mRNA level and protein abundance (exceptions are discussed below). Whereas these data lead to the overall conclusion that transcription plays a central role in establishing the levels of many plastid mRNAs and proteins, variation in plastid mRNA stability also significantly influences plastid gene expression. Moreover, these data help explain the exclusion of genes encoding proteins of the photosynthetic apparatus from operons with low transcription rates (i.e. *rpo*B-*rpo*C1-*rpo*C2) because, without unusual mechanisms for RNA stabilization or translation, transcription rates would be insufficient for the required production of protein from these operons.

Additional levels of regulation are involved in the production of some plastid proteins. For example, *psb*A transcription and mRNA levels are elevated relative to other genes encoding electron transport proteins (Rapp et al., 1992). This is consistent with the relative instability of the protein encoded by *psb*A (D1, a reaction center protein of PSII), which necessitates higher rates of synthesis if protein stoichiometry in PSII is to be maintained (Mattoo et al., 1989). Higher than expected transcription and mRNA levels for *rpo*A and *rpl*16 relative to *rpo*B and *rps*16 suggest regulation at the level of translation or protein turnover (Rapp et al., 1992). It is interesting that bacteria coordinate the expression of ribosomal and RNA polymerase subunits through feedback inhibition of translation. Similar regulation could be operating in higher plant plastids.

SIGNIFICANCE OF PLASTID mRNA STABILITY

Recent assays of plastid mRNA stability revealed half-lives ranging from 6 h to over 40 h (Klaff and Gruissem, 1991; Kim et al., 1993). In contrast, the stability of most mRNAs in *E. coli* range from 20 s to several minutes. Thus, the stability of many plastid mRNAs is similar to the stability of mRNAs found in eukaryotic organisms. Long plastid mRNA half-lives mean that RNA stability is an important determinant of plastid mRNA levels. In addition, high mRNA stability limits the rate with which plastids can alter mRNA levels through a change in transcription rate. For example, illumination of 4.5-d-old dark-grown barley results in a 5-fold decrease in plastid transcription activity over a 24-h period (Mullet and Klein, 1987). In this situation, the abundance of *psa*A mRNA (half-life = 6 h) changes in parallel with the decrease in transcription activity, whereas *psb*A mRNA (half-life > 40 h) shows a much delayed decrease in abundance. The high stability of plastid mRNAs may help explain why some changes in plastid transcription induced by 12 h of illumination were not coupled to changes in plastid RNA populations (Krupinska and Apel, 1989). Furthermore, differences in plastid and cytoplasmic mRNA stability may lead to altered ratios of these mRNAs following changes in transcription. Although the general importance of plastid mRNA stability is well established, relatively few cases of regulated mRNA stability have been characterized in detail. The biochemical basis of differential mRNA stability has been related to stem loop structures located at the 5′ and 3′ termini of RNAs, translation activity, and RNA binding proteins (Stern et al., 1989; Klaff and Gruissem, 1991).

OVERALL DYNAMICS OF CHLOROPLAST TRANSCRIPTION

Overall transcription rates change dramatically during chloroplast development, and in sorghum, RNA polymerase levels increase in parallel with transcription activity (Schrubar et al., 1990). However, in spite of the global changes in transcription rates, the relative ratio of transcription of many plastid genes is constant in different tissues and developmental stages (Deng and Gruissem, 1987, 1988; Rapp et al., 1992). This observation is not surprising for three reasons. First, the coding capacity of the plastid genome is limited, thus precluding the need for regulation in situations where plastid genes are not relevant. Second, with a few exceptions (i.e. *ndh* genes), plastid genes can be classified into two major groups: genes encoding proteins and RNAs directly involved in gene expression (i.e. transcription and translation) and genes encoding the photosynthetic apparatus. Expression of these two groups of genes is sequentially linked during chloroplast development in that high levels of the plastid's transcription and translation machinery are required only during synthesis and assembly of the photosynthetic apparatus. Third, large numbers of plastid genes encode proteins that accumulate in fixed stoichiometries in a few large protein complexes (RNA polymerase, ribosomes, photosynthetic electron transport complexes). Therefore, constant ratios of transcription of genes encoding a basic set of plastid proteins needed in fixed stoichiometry is not surprising.

Differential transcription of plastid genes is superimposed over the basic transcriptional plan described above. Altered ratios of plastid gene transcription have been reported in amyloplasts of sycamore cell culture and related to differential DNA methylation (Ngernprasirtsiri and Akazawa, 1990). Differential activation of *rpo*B transcription early in chloroplast development and light-modulated transcription of *psb*A and *psb*D-*psb*C further demonstrate the importance of regulated plastid transcription. The rationale for these latter two cases of differential transcription is described below within the context of chloroplast development.

DIFFERENTIAL TRANSCRIPTION DURING CHLOROPLAST DEVELOPMENT

Proplastids, the progenitors of chloroplasts, are small (0.1–1.0 μm diameter), nonphotosynthetic organelles with low DNA copy number, transcription rates, and RNA levels. Proplastids are converted into chloroplasts during development of mesophyll leaf cells from cells of leaf primordia. Steps in this developmental process have been studied intensively in monocotyledonous plants due to the restriction of meristematic activity to the leaf base early in leaf biogenesis. This results in an ontogenic series of cells and plastids starting from undeveloped cells and proplastids in the leaf base and extending to fully mature cells and chloroplasts in leaf tips. The events described below and summarized in Figure 1 are based on the study of cells and plastids as they move from the meristematic zone in the monocot leaf base, through a zone of cell enlargement, followed by final maturation of cells and chloroplasts in older apical regions of the leaf.

The first step in chloroplast development in monocot leaves involves the activation of plastid DNA synthesis and plastid replication (Fig. 1, stage I). This occurs while cells are in the leaf basal meristem and is required to maintain plastid number and DNA content in the dividing cells. At present, no plastid genes involved in plastid replication or DNA synthesis have been identified, indicating that this early phase of chloroplast development is controlled through nuclear gene expression. Plastid transcription activity and RNA levels remain low in cells of the leaf basal meristem, but they dramatically increase when cells enter the zone of cell enlargement (Fig. 1, stage II). In particular, transcription and RNA levels for *rpo*B-*rpo*C1-*rpo*C2, *rps*16, rRNA, and some tRNAs are differentially elevated at this stage relative to genes encoding proteins of the photosynthetic apparatus (Rapp et al., 1992). This step in chloroplast development is followed shortly by the activation of plastid and nuclear genes encoding proteins of the photosynthetic apparatus (Fig. 1, stage III). Coordinated expression of nuclear and plastid genes at this stage of development involves a "plastid signal," which is required for high expression of nuclear genes encoding proteins of the photosynthetic apparatus (for review, see Taylor, 1989). If chloroplast development is blocked either by inhibitors of translation (or mutants deficient in ribosomes) or through inhibition of transcription, then production of the plastid signal is limited. During normal development, activation of genes encoding the photosynthetic apparatus leads to the synthesis and assembly of the photosynthetic apparatus. Finally, as chloroplasts mature, overall transcription rates

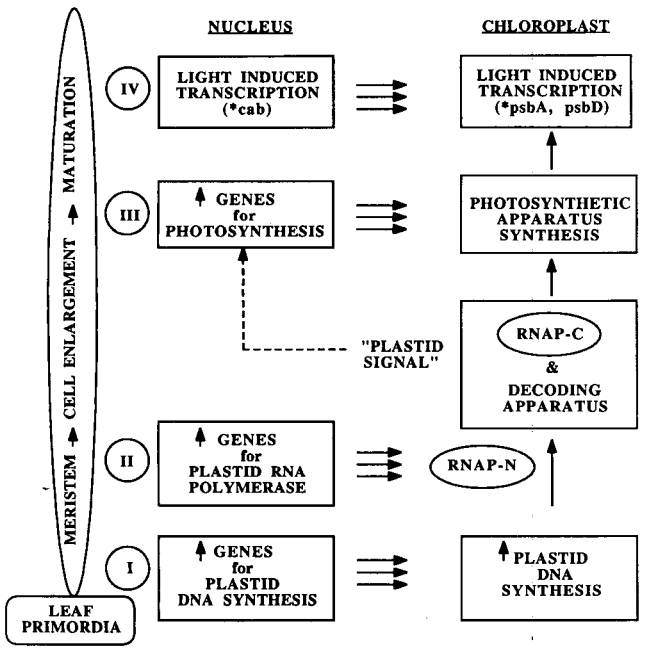

Figure 1. Cascade of events leading to chloroplast development. Stages of chloroplast development are designated I to IV. Chloroplast transcription is activated at stage II, rises to a maximum in stage III, and declines to a low level in mature chloroplast populations (stage IV). Differential light-induced transcription of the nuclear gene *Cab*, which encodes proteins of the light-harvesting complex of PSII, and the plastid genes *psb*A and *psb*D, which encode reaction center proteins of PSII, is shown in mature chloroplasts. Light-modulated transcription of these genes can also occur in developing chloroplasts. The nuclear-encoded RNA polymerase (RNAP-N) and the chloroplast-encoded RNA polymerase (RNAP-C) are shown as ovals.

decline (Fig. 1, stage IV). However, the need for continued synthesis of the PSII reaction center proteins (D1/D2) remains high due to light-mediated damage of these proteins. Differential light-induced transcription of *psb*A and *psb*D helps sustain synthesis of these proteins in mature chloroplasts.

SPECIAL ROLE FOR A NUCLEAR-ENCODED PLASTID-LOCALIZED RNA POLYMERASE

Differential transcription of *rpo*B-*rpo*C1-*rpo*C2 and other genes early in chloroplast development was hypothesized to be caused by increased expression of a nuclear-encoded plastid-localized RNA polymerase (Rapp et al., 1992). This hypothesis was based on several lines of evidence. First, plastid promoters recognized by the plastid-encoded RNA polymerase contain prokaryotic −10 and −35 sequence elements. In contrast, preliminary characterization of the *rpo*B and *rps*16 promoters did not reveal the presence of −35 promoter elements, indicating that additional protein factors or a different RNA polymerase was involved in transcribing these genes. Second, earlier biochemical studies described the characteristics of two plastid RNA polymerases (Greenberg et al., 1984). One of these polymerase activities corresponded to the RNA polymerase encoded by the plastid, whereas the other was of unknown origin.

Strong evidence for a nuclear-encoded plastid-localized RNA polymerase came from the study of *Epifagus*, a nonphotosynthetic plant (Morden et al., 1992). *Epifagus* plastids accumulate rRNA even though the plastid genome of *Epifagus* lacks *rpo*B, *rpo*C1, and *rpo*C2, which encode subunits of the plastid-encoded RNA polymerase. In addition, it was recently reported that mRNA for *rpo*B-*rpo*C1-*rpo*C2 accumulates in plastids of *albostrians*, a barley mutant that lacks ribosomes (Hess et al., 1993). This study showed that the nuclear-encoded plastid-localized RNA polymerase can transcribe the *rpo*B operon.

The hypothesis described above suggests that expression of the nuclear-encoded plastid-localized RNA polymerase is activated early in chloroplast development by as-yet unknown cell-specific factors. This results in the differential transcription of the *rpo*B operon and other loci involved in plastid gene expression early in chloroplast development. Transcription of the *rpo*B operon leads to increased levels of the plastid-encoded RNA polymerase, which, in turn, further activates transcription of genes needed for synthesis and assembly of the photosynthetic apparatus. This sequential cascade model for the activation of plastid gene transcription during chloroplast development can be directly tested once the nuclear-encoded plastid-localized RNA polymerase is isolated and characterized.

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