Posttranscriptional Control of Chloroplast Gene Expression. From RNA to Photosynthetic Complex

Jean-David Rochaix*

Departments of Molecular Biology and Plant Biology, University of Geneva, 30 Quai Ernest Ansermet, 1211 Geneva 4, Switzerland

Twenty-five years ago it was well established that chloroplasts contain their own DNA and protein synthesizing system, but little was known of how this organellar genome is expressed. As a result of their endosymbiotic origin, plastids contain a protein synthesizing system that displays several prokaryotic features. Its 70S ribosomes resemble those of bacteria and are sensitive to the same set of antibiotics. However, it has become apparent that the chloroplast gene expression system is unique, differing in many respects from bacterial systems and using a variety of unusual posttranscriptional steps.

MAJOR TECHNICAL ADVANCES

Scientific progress is often driven by new technology. A particularly striking example was the establishment of a chloroplast transformation system in 1988 by Boynton et al. (4) in *Chlamydomonas*, which was later extended to tobacco (18). These were major technical breakthroughs that coincided with the first determinations of the entire sequence of chloroplast genomes (17) and opened the door for the in vivo study of chloroplast gene expression. Because of the efficient chloroplast homologous recombination system, it became possible to perform precise DNA manipulations on any chloroplast gene of interest, in particular, specific gene disruptions and site-directed mutagenesis. This powerful technology also allowed one to dissect chloroplast promoter regions, to introduce chimeric genes in the chloroplast genome, and to identify novel functions by screening or selecting for specific phenotypes. The development of an efficient nuclear transformation system in *Chlamydomonas* and of gene tagging methods in *Chlamydomonas*, maize, and Arabidopsis provided also important tools that led to major advances in our understanding of chloroplast biogenesis (1).

MOLECULAR CROSS TALK BETWEEN NUCLEUS AND CHLOROPLAST

One of the most original features of the chloroplast protein synthesizing system is that it cooperates with the nucleocytosolic system in the biosynthesis of the photosynthetic apparatus. The subunits of the photosynthetic complexes are encoded by chloroplast and nuclear genes that need to be coordinately expressed. The study of this molecular crosstalk between chloroplast and nucleus was greatly helped by genetic approaches (7). The genetic analysis of photosynthetic mutants of *Chlamydomonas reinhardtii* and maize revealed a large number of nuclear and chloroplast loci involved in several posttranscriptional steps of chloroplast gene expression such as RNA stability, RNA processing, splicing, and translation. A characteristic feature of the nucleus-encoded factors, at least in the case of *Chlamydomonas*, is that they are specifically required for a single posttranscriptional step in the synthesis of an individual plastid gene product. Thus genetic analysis of mutants deficient in the accumulation of single chloroplast mRNAs identified a distinct nuclear locus in each case. In a similar manner, mutations affecting chloroplast translation define one to three nuclear loci specifically required for the translation of a single chloroplast mRNA. A particularly striking example is provided by the genetic analysis of the maturation of the *psaA* mRNA of *Chlamydomonas*, a process that requires at least 14 nuclear loci. If these findings are extrapolated to the entire chloroplast genome with a total number of approximately 120 genes, one can estimate that several hundred nucleus-encoded factors are required for the expression of the entire set of plastid genes. The first specific factors of this type were recently cloned in *Chlamydomonas*, Arabidopsis, and maize using gene tagging or genomic complementation by transformation (1). Several are involved in chloroplast trans-splicing, RNA stability and processing, and translation. As chloroplast RNA processing, stability, and translation are closely coupled, a defect in any of these processes could affect the others.

ESTABLISHING IN VITRO SYSTEMS FOR SPECIFIC STEPS OF CHLOROPLAST GENE EXPRESSION

Major advances in our understanding of chloroplast gene expression, particularly the enzymatic machinery involved in chloroplast 3' end processing,

* E-mail Jean-David.Rochaix@molbio.unige.ch; fax 41–22–702–6868.

were achieved through the development of in vitro systems with chloroplast extracts (13). As it turns out, most chloroplast 3′ ends are produced by RNA processing rather than by transcription termination. In most cases, an endonucleolytic cleavage downstream of the mature 3′ end is followed by 3′-exonucleolytic resection to a stem-loop at the 3′ end. These processing steps are catalyzed by a chloroplast degradosome similar to that of *Escherichia coli,* consisting of several nucleus-encoded RNA-binding proteins, including exo- and endonucleases (9). As in bacteria, polyadenylation appears to play an important role in chloroplast RNA turnover (9). Evidence based on the existence of low abundant polyadenylated chloroplast mRNA fragments is compatible with a model in which the RNAs are endonucleolytically cut and extended with a short polyA tail. These tails may provide a foothold for the degradosome.

Another breakthrough was the establishment of an in vitro translation system from tobacco chloroplasts (10). This system promotes accurate initiation of translation from several chloroplast RNAs and revealed important cis-acting elements within the chloroplast 5′-untranslated regions (UTRs). The in vitro translation system also provided new insights on how polypeptides of the photosynthetic apparatus are targeted and inserted into the thylakoid membrane. It is generally assumed that this process occurs cotranslationally based on run-on translations with thylakoid-bound ribosomes and detection of translation intermediates in the membrane (7). Using the in vitro translation system, it was possible to produce stable ribosome nascent chain complexes and to show that one polypeptide of the chloroplast signal reduction particle (SRP) particle, SRP54, interacts with the nascent polypeptide chain and thus represents a soluble component of the targeting machinery (14). Because of their prokaryotic origin, it is not surprising that several other homologs of *E. coli* besides SRP54 are involved in membrane protein targeting and insertion, including the chloroplast SecA, SecB, and SecY proteins. Disruption of the *SecY* gene in maize leads not only to a severe reduction of thylakoid membranes, but also to a deficiency in chloroplast translation, thus revealing a link between thylakoid membrane biogenesis and chloroplast translation (16).

A characteristic feature of chloroplast protein synthesis is its strong stimulation by light. A major advance occurred when a correlation was shown between the light-stimulated binding of a multiprotein complex to the 5′-UTR of the chloroplast *psbA* mRNA and its translation (6). Binding of this complex to the 5′-UTR was proposed to be controlled by the redox potential and ATP levels that are modulated by photosynthetic activity (5). The characterization of this complex revealed that it contains a 47-kD protein that is homologous to polyA-binding proteins and a 60-kD protein that appears to be a protein disulfide

isomerase, usually found in the endoplasmic reticulum (5). It thus appears that several cytoplasmic proteins have been recruited by the chloroplast for novel regulatory functions in plastid gene expression.

COORDINATE EXPRESSION OF CHLOROPLAST PROTEINS

All photosynthetic complexes consist of several subunits, the accumulation of which needs to be coordinated in a stoichiometric fashion. This appears to be achieved in two ways. The first involves proteolytic degradation of most of the unassembled subunits. The proteases required for this process are still largely unknown, although recent evidence indicates that the ATP-dependent ClpP protease is partly involved (20). The second mechanism was elegantly demonstrated with the cytochrome *b6f* complex of *Chlamydomonas* (20). In this process, referred to as control by epistasy of synthesis, translation of the cytochrome *f* subunit of the cytochrome *b6f* complex is strongly attenuated when other subunits from the same complex are absent. This control is mediated by a direct or indirect interaction between the 5′-UTR of the cytochrome *f* mRNA and the C-terminal domain of the unassembled cytochrome *f* subunit. Whether this process is also valid for other chloroplast genes in *Chlamydomonas* and higher plants remains to be determined.

UNUSUAL FEATURES OF CHLOROPLAST POSTTRANSCRIPTIONAL PROCESSES

The chloroplast *psaA* gene of *Chlamydomonas* consists of three exons flanked by group II intron sequences that are widely separated on the plastid genome and transcribed independently. The assembly of the mature *psaA* mRNA depends on two trans-splicing reactions that require several trans-acting factors (7). Factors involved in this process were recently characterized. One of them resembles ψ-uridine synthases, although this enzyme activity is not required for trans-splicing (15). Another trans-acting factor required for *psaA* trans-splicing is an RNA (8). This RNA, called *tscA* RNA, was identified as part of the group II *psaA* intron 1 structure, a first example of a tri-partite intron. This finding has important evolutionary implications given the fact that group II introns with their cis-acting catalytic domains are considered to be the precursors of nuclear introns with their trans-acting snRNPs. The tripartite *psaA* group II intron may thus represent an intermediate stage in this evolutionary process. The *tscA* RNA has recently been found to be part of a protein complex that may represent a chloroplast counterpart of snRNPs (C. Rivier, unpublished data). It will be particularly interesting to determine whether any evolutionary relationship is apparent between the proteins of this complex and those of eukaryotic

snRNPs. These studies should provide new insights into the evolution of gene expression systems.

RNA editing, the mechanism of posttranscriptional nucleotide modification, is one of the most striking chloroplast oddities and also occurs in plant mitochondria. Chloroplast RNA editing involves mostly cytidine-to-uridine conversions with the reverse change occurring only in few cases (3). It thus adds a novel posttranscriptional step in chloroplast gene expression besides RNA 5'- and 3'- end processing, cleavage of polycistronic into monocistroinic mRNAs, and group I and group II splicing. Only 25 sites in the entire tobacco plastid genome are edited (11). How the editing machinery selectively modifies these sites remains an intriguing question, although recent studies indicate that both mRNA sequences flanking the editing site and specific trans-acting factors play an important role (3). The existence of chloroplast editing requires caution in the evaluation of chloroplast DNA sequence data. In particular, plastid open reading frames may be missed because of the editing of cryptic ACG initiation codons to AUG.

PERSPECTIVES

Although the nucleus influences the expression of the chloroplast genome through a large set of factors, the chloroplast can also influence nuclear gene activity. Plants devoid of carotenoids photobleach when exposed to light. This condition leads to the selective inhibition of transcription of a selected set of nuclear genes including the genes of the light-harvesting chlorophyll *a/b* proteins (19). The chloroplast signal involved in this response has remained enigmatic for many years. However, recent studies with *C. reinhardtii* indicate that intermediates in the porphyrin pathway such as magnesium protochlorophyllide methyl ester play a crucial role in this response (12). An important task for the future is to identify the targets of these porphyrin intermediates.

Mitochondria interact in many ways with the chloroplast through metabolic pathways. Recent work strongly suggests that genetic interactions also exist between these two organelles. Genetic data indicate that informational suppressors of chloroplast nonsense mutations suppress mitochondrial mutations, suggesting exchange of tRNAs between these two organelles (2). A molecular analysis of this intriguing process should prove particularly rewarding.

LITERATURE CITED

1. **Barkan A, Goldschmidt-Clermont M** (2000) Biochimie **82:** 559–572
2. **Bennoun P, Delosme M** (1999) Mol Gen Genet **262:** 85–89
3. **Bock R** (1998) Methods: A Companion to Methods in Enzymology **15:** 75–83
4. **Boynton JE, Gillham, Harris EH, Hosler JP, Johnson AM, Jones AR, Randolph-Anderson BL, Robertson D, Klein TM, Shark KB, Sanford JC** (1988) Science **240:** 1534–1538
5. **Bruick RK, Mayfield SP** (1999) Trends Plant Sci **19:** 190–195
6. **Danon A, Mayfield SP** (1991) EMBO J **10:** 3993–4001
7. **Goldschmidt-Clermont M** (1998) Int Rev Cytol **177:** 115–180
8. **Goldschmidt-Clermont M, Choquet Y, Girard-Bascou J, Michel F, Schirmer-Rahire M, Rochaix JD** (1991) Cell **65:** 135–143
9. **Hayes R, Kudla J, Gruissem** (1999) Trends Biochem Sci **24:** 199–202
10. **Hirose T, Kusumegi T, Tsudzuki T, Sugiura M** (1998) Mol Gen Genet **262:** 462–467
11. **Hirose T, Sugiura M** (1996) EMBO J **15:** 1687–1695
12. **Kropat J, Oster U, Rudiger W, Beck, CF** (1997) Proc Natl Acad Sci USA **94:** 14168–14172
13. **Monde R, Schuster G, Stern D** (2000) Biochimie **82:** 573–582
14. **Nilsson R, Brunner J, Hoffman NE, van Wijk KL** (1999) EMBO J **18:** 733–742
15. **Perron K, Goldschmidt-Clermont M, Rochaix JD** (1999) EMBO J **18:** 6481–6490
16. **Roy LM, Barkan A** (1998) J Cell Biol **141:** 385–395
17. **Sugiura M, Hirose T, Sugita M** (1998) Annu Rev Genet **32:** 437–459
18. **Svab Z, Hajdukiewicz P, Maliga P** (1990) Proc Natl Acad Sci USA **87:** 8526–8530
19. **Taylor WC** (1989) Annu Rev Plant Physiol Plant Mol Biol **40:** 211–233
20. **Wollman FA, Minai L, Nechushtai R** (1999) Biochim Biophys Acta **141:** 21–85