Minireview

Translational regulations as specific traits of chloroplast gene expression

Yves Choquet*, Francis-André Wollman*

UPR-CNRS 1261, Institut de Biologie Physico-Chimique, 13 rue Pierre et Marie Curie, 75005 Paris, France

Received 13 August 2002; accepted 14 August 2002

First published online 23 August 2002

Edited by Gunnar von Heijne

Abstract Studies of protein synthesis in the chloroplast compartment have revealed a unique combination of translational autoregulations and trans-regulations due to the delivery of a variety of nuclear factors that act post-transcriptionally. We show how these two characteristics concur to set the major step in the regulation of chloroplast gene expression at the translational level, leading to a surprisingly low sensitivity of chloroplast protein synthesis in response to extensive changes in plastome copy number and transcript concentration. © 2002 Published by Elsevier Science B.V. on behalf of the Federation of European Biochemical Societies.

Key words: Chloroplast gene expression; Protein assembly; *Chlamydomonas reinhardtii*

1. Introduction

Chloroplasts are specific organelles that convert light energy into chemical energy sources that fuel plant cell metabolism. Their inner membrane system – the thylakoid membrane system – contains four major multimeric protein complexes that participate in light-driven ATP synthesis and NADPH production. These protein complexes comprise numerous subunits that are most often present in a 1:1 stoichiometric ratio, some of which being encoded within the nucleus while others are encoded within the chloroplast itself. How these two genetic compartments coordinate the expression of the various subunits of a same protein complex in the stoichiometry required for their functional assembly is a key issue in the study of chloroplast biogenesis. Here we review some recent data that emphasize the key role of the translational step in the regulation of chloroplast gene expression.

2. Specific regulatory steps in chloroplast gene expression

The assembly of organellar protein complexes that are made of subunits of dual genetic origin is an intriguing process given the tremendous unbalance in gene copy number between the nucleus and the organelle compartments: for instance, a plant cell may contain up to 10 000 copies of chloroplast DNA, while the nuclear genome is in most cases diploid. A co-transcriptional regulation of a set of chloroplast genes dedicated to the same protein complex is not expected to play a major role in limiting expression of the organelle-encoded subunits since the operonal organization – a typical prokaryotic feature of gene expression – has been largely lost during endosymbiosis of the prokaryote ancestors of organelles: organellar genes concurring to the same function are most often split between different transcription units and may even be translated independently from monocistronic RNA resulting from processing of primary transcripts.

How the extent of chloroplast polyploidy affects the level of organellar transcripts has been addressed recently in the unicellular green alga *Chlamydomonas reinhardtii* [1]. We obtained a 10-fold decrease in chloroplast gene copy number using cells treated for 48 h with 5′-fluoro-2′-deoxyuridine, a thymidine analogue that inhibits specifically chloroplast DNA replication. We observed a parallel decrease in the rate of transcription of chloroplast genes, but the steady state accumulation of mRNAs was only marginally affected. Thus, transcription is not limiting in the expression of chloroplast mRNAs. Rather the extent of their accumulation is governed by limiting amounts of nuclear-encoded factors that are involved in the maturation of chloroplast transcripts and protects them against exo/endo-nucleolytic degradation [2,3]. The action of these maturation factors explains why chloroplast mRNAs are long-lived species, a property that we have directly assessed by blocking transcription of chloroplast genes with specific inhibitors [1]. When treated for 6 h with rifampicin, Chlamydomonas cells grown in photoheterotrophic conditions still displayed chloroplast transcripts that accumulated from 12 to 70% of their initial level. The same rifampicin treatment applied to cells grown in photoautotrophic conditions had a more drastic effect: the accumulation of several transcripts dropped below 10% of their original level reaching 2% in the case of *petA* and *atpA* transcripts, encoding respectively cytochrome *f* from the cytochrome b_6f complex and the α-subunit of the ATP synthase complex. Still, the rate of translation of these chloroplast-encoded polypeptides, as determined by pulse labeling experiments, *did not decrease significantly*. Thus, accumulation of template messengers is not limiting in the expression of chloroplast genes. The poor sensitivity of chloroplast translation to extensive changes in gene copy number and transcript concentration demonstrates that, at variance with their prokaryotic ancestors, chloroplasts have developed their major regulatory steps in gene expression at the translational and post-translational level. The factors that govern the rate of translation of chloroplast-encoded proteins belong most likely to the class of nucleus-encoded translation-

*Corresponding authors. Fax: (33)-1 58 41 50 22.
E-mail addresses: choquet@ibpc.fr (Y. Choquet), wollman@ibpc.fr (F.-A. Wollman).

0014-5793/02/$22.00
PII: S0014-5793(02)03260-X

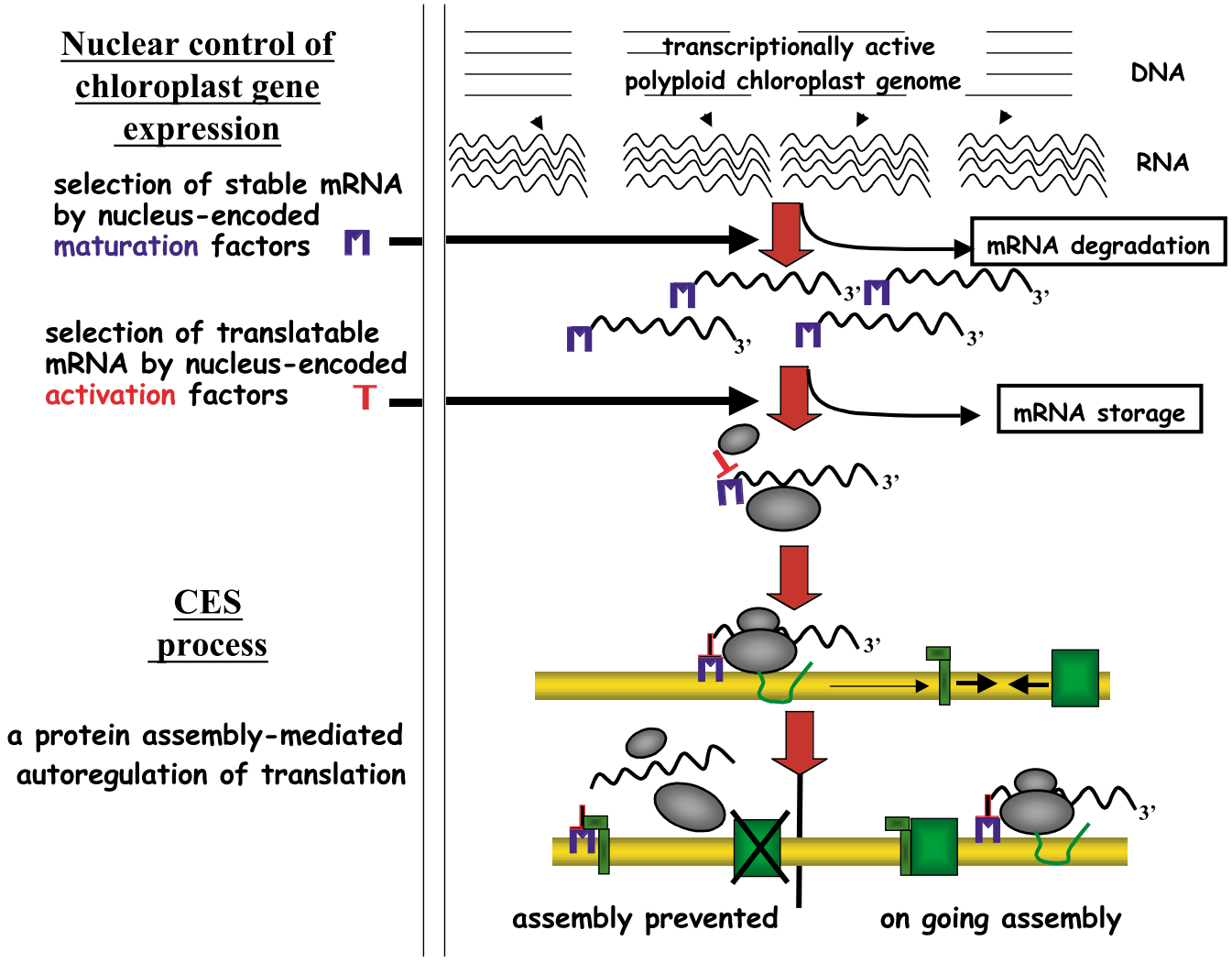

Fig. 1. Key steps in the expression of a chloroplast-encoded protein: we assume that most – if not all – copies of the chloroplast DNA are transcriptionally active. Only a fraction of the de novo made transcripts is selected for maturation/stabilization by nucleus-encoded M factors that bind to their 5′-untranslated region. The remaining transcripts are targeted to degradation. A second set of nuclear-encoded factors, the T factors, selects some of the mature mRNAs for translation, the remaining transcripts being stored by some presently unknown mechanism. The translational step is represented for a CES subunit, whose translation is autoregulated in an assembly-dependent manner. The unassembled form of the CES subunit represses translation of its own mRNA, probably through competitive trapping of a translational activator complex. The activator complex is released upon assembly of the CES subunit, allowing translation to resume.

al activators that have been identified through numerous genetic studies [2,4].

We summarized on Fig. 1 the major conclusions drawn from the experiments above, with special emphasis on the role of the M and T factors that should be mobilized for the expression of a chloroplast gene. Indeed, most photosynthetic mutants from *C. reinhardtii* that were characterized as specifically lacking synthesis of a single chloroplast-encoded polypeptide turned out to carry nuclear mutations instead of chloroplast mutations. These mutations identified a number of nuclear genes encoding factors acting on the expression of a specific chloroplast gene target. Some of these factors – the M factors – are required for the correct maturation and stabilization of a specific transcript [3], while others – the T factors – are required for its translation (reviewed in [2,4,5]). The two sets of factors bind to the 5′-untranslated region of their chloroplast mRNA targets and their possible interaction in the control of translation should be considered: a detailed study of the 5′-end maturation of the *psbD* transcript from Chlamydomonas chloroplasts has shown that only those transcripts that have been processed by M factors become translatable [6], i.e. can be selected by T factors for translation. Similar nucleus-encoded factors were identified in yeast, where they control the post-transcriptional steps of mitochondrial gene expression [7], and in higher plants, where they may act on multiple messenger targets and be less gene specific [2]. Although the expression of these nuclear factors is required for organelle genes to be properly expressed, it remains to be shown whether they merely participate constitutively in organellar gene expression or whether they actually play a regulatory role. The latter option implies that variations in the level of expression of these nuclear factors – whether it would be due to changes in their rates of synthesis or to changes in their half-life in the organelle – would quantitatively and qualitatively modify the pattern of organelle protein expression. A recent report that a translational activator specific for mitochondrial COX2 exerts its activity in limiting concentrations strongly argues for this regulatory role [8].

Thus, it appears that organelles have diverged from their prokaryotic ancestors, in devising new strategies to cope with the splitting of operons and the migration to the nucleus of some of the structural genes encoding subunits of the organelle-located oligomeric proteins. The salient feature of these new traits is a two-step selection of those mRNAs that should be translated, the bulk of mRNAs being for one part rapidly degraded after transcription is completed while another part would probably be stored in a non-translatable form (Fig. 1). Whereas the main features of chloroplast mRNA degradation are currently being worked out [3], storage complexes for mRNAs in the chloroplast have not been identified yet and should therefore still be considered as hypothetical.

3. An assembly-dependent regulation of cytochrome *f* synthesis: the CES process

Studies of photosynthesis mutants from *C. reinhardtii* lacking in the synthesis of only one polypeptide provided unique insights into the assembly process leading to the stoichiometric accumulation of the various subunits of chloroplast protein complexes. These mutants often show a dramatic drop in the accumulation of all constitutive subunits from that complex, which suggests a contrario that these subunits accumulate through some concerted process in the wild-type strain (reviewed in [9,10]). Some subunits, when not assembled, show rapid degradation: for instance cytochrome b_6 and subunit IV from the cytochrome b_6f complex show unaltered rates of synthesis whether cytochrome *f* is made or not but they are rapidly degraded in its absence [11]. In marked contrast, cytochrome *f* shows a reduced rate of synthesis in the absence of cytochrome b_6 and subunit IV but no change in its half-life [11]. We referred to the latter phenomenon, which is observed for a variety of chloroplast-encoded subunits, as 'a control by epistasy of synthesis' (*CES* process), to stress the idea that there is some order in the expression of subunits from the same protein complex: the CES subunits are those whose rate of synthesis is dependent upon the presence of their assembly partners that we refer to as 'dominant' subunits.

We found that this assembly-dependent control of cytochrome *f* synthesis relied on an autoregulation of translation initiation of its own *petA* mRNA. The regulation depends on the 5′-untranslated region of *petA* mRNA which is sufficient to confer the CES behavior to a reporter gene [12]. It is mediated by a repressor motif carried by the C-terminal domain of the unassembled protein that is able to inhibit further translation of its own messenger [12–14]. This motif, that we further characterized recently by site-directed mutagenesis, comprises a tetrapeptide cluster $K_{305}QFE_{308}$ from the stromal extension of cytochrome *f*, together with an upstream residue, Q_{297}, buried in the transmembrane helix of the protein. Various substitutions of these critical residues deeply altered the CES process: they lead to an enhanced translation of cytochrome *f* and these elevated rates of translation become independent of the presence or absence of assembly partners, i.e. the dominant subunits SUIV and cytochrome b_6. Because the repressor motif contains a residue embedded in the thylakoid membrane, it is unlikely that it would interact directly with the *petA* 5′-untranslated region. The CES process would require a ternary effector associated with the membrane (see Fig. 1). This CES effector would act as a translational activator for *petA* mRNA, able of competitive binding to the 5′-untranslated region of *petA* mRNA and to the regulatory motif: when bound to the repressor motif exposed by unassembled cytochrome *f* it would be unavailable for translation activation of *petA* mRNA. Once released upon assembly of cytochrome *f* with the rest of the cytochrome b_6f complex, it would allow cytochrome *f* synthesis to resume. We have identified two nuclear genes, encoding factors specifically involved in the expression of cytochrome *f*, the 'M' factor MCA1 involved in the stability of *petA* mRNA [15] and the 'T' factor TCA1 that is required for *petA* mRNA translation [16]. Both MCA1 and TCA1 target the *petA* 5′-leader, share some of the major characteristics expected from the CES ternary effector, but their role in the CES process has still to be proven.

4. Generality of the CES process

The CES process is not restricted to cytochrome *f* since examination of the chloroplast literature about photosynthesis mutants shows that each protein complex of the thylakoid membrane exhibits at least one CES subunit (reviewed in [9,10,12]). ATP synthase mutants lacking the β-subunit show a decreased synthesis of the α-subunit [18]. In the absence of the small subunit of Rubisco, translation of its chloroplast-encoded large subunit decreases [19]. Photosystem II mutants unable to express D1 show a reduced synthesis of CP47 and of the phosphoprotein PsbH [17,20,21], while mutants lacking D2 show reduced synthesis of D1, CP47 and PsbH [17,22]. This can be described as a CES cascade, where D2 is required for the synthesis of D1 that is itself required for the translation of CP47 and PsbH. The biogenesis of photosystem I also involves a CES cascade: in the absence of one of the two large reaction center subunits, PsaB, the synthesis of the other subunit, PsaA, becomes undetectable [23,24], while the absence of either of the two large subunits results in a decreased synthesis of a third subunit, PsaC ([25]; Wostrikoff, K. and Choquet, Y., unpublished results).

Evidence for a control of translation by protein assembly is not restricted to Chlamydomonas chloroplasts. In the *vir*^{115} mutant of barley, primarily impaired in the expression of D1, the synthesis of CP47 is also reduced [26,27]. In tobacco antisense plants with reduced expression of the small subunit of the Rubisco, the proportion of *rbcL* mRNAs associated with polysomes is decreased [28]. The CES process may also participate in the biogenesis of respiratory membranes in mitochondria: in yeast, the rate of synthesis of the mitochondrion-encoded cytochrome oxidase COXI subunit, but not its half-life, is reduced when the cytochrome oxidase complex does not assemble due to the absence of other COX subunits [29,30]. Also, mutants deficient for the synthesis of subunit 9 of the ATP synthase complex show reduced synthesis of Atp6 and Atp8 [31,32].

5. The core mechanism of the CES process is an autoregulation of translation initiation

The molecular mechanism we described for the control of cytochrome *f* expression, i.e. an autoregulation of translation initiation mediated by the unassembled protein, seems to be the general feature of the CES process in *C. reinhardtii* chloroplasts. First, the 5′-untranslated region of the mRNA for each CES subunit that we tested (PsaA, PsaC, CP47 and sub-

unit α of the ATP synthase complex), was found to be sufficient to confer a CES behavior to a reporter gene (Wostrikoff, K., Minai, M., Rimbault, B., Drapier, D., Choquet, Y. and Wollman F.-A., unpublished results). An assembly-dependent regulation of translation initiation is also responsible for the CES behavior of the large subunit of the Rubisco in higher plant chloroplasts where the absence of the small subunit results in a decreased association of *rbcL* mRNAs with polysomes [19,28]. Second, we found evidence for a autoregulation of translation, rather than a trans-regulation, for the CES subunits PsaA, PsaC and CP47 since they were able, when unassembled, to inhibit translation of their own messenger RNA (Wostrikoff, K., Minai, M., Wollman F.-A. and Choquet, Y., unpublished results). How these unassembled CES subunits interact with the 5′-leader of their mRNAs is now a challenging question. The membrane proteins in which they assemble play a role in energy transduction and have no part in RNA metabolism and therefore to functional requirement to develop RNA binding domains. It is highly unlikely that each of these CES subunits, the sequence of which is conserved through evolution from photosynthetic prokaryotes to eukaryotes, has evolved RNA binding domains able to interact specifically with the 5′-leader of their chloroplast mRNA. Thus, as we have concluded from our study of the CES behavior of cytochrome *f* [16], the assembly-dependent autoregulation of translation of all these CES subunits probably involves ternary protein effectors that correspond to these M and T factors that tightly control the post-transcriptional fate of chloroplast genes.

References

[1] Eberhard, S., Drapier, D. and Wollman, F.A. (2002) Plant J. 31, 149–160.
[2] Barkan, A. and Goldschmidt-Clermont, M. (2000) Biochimie 82, 559–572.
[3] Monde, R.A., Schuster, G. and Stern, D.B. (2000) Biochimie 82, 573–582.
[4] Zerges, W. (2000) Biochimie 82, 583–601.
[5] Rochaix, J.D. (1996) Plant Mol. Biol. 32, 327–341.
[6] Ossenbuhl, F. and Nickelsen, J. (2000) Mol. Cell. Biol. 20, 8134–8142.
[7] Fox, T.D. (1996) in: Translational Control (Sonenberg, N., Ed.), pp. 733–758, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
[8] Green-Willms, N.S., Butler, C.A., Dunstan, H.M. and Fox, T.D. (2001) J. Biol. Chem. 276, 6392–6397.
[9] Wollman, F.A., Minai, L. and Nechushtai, R. (1999) Biochim. Biophys. Acta 1411, 21–85.
[10] Choquet, Y. and Vallon, O. (2000) Biochimie 82, 615–634.
[11] Kuras, R. and Wollman, F.-A. (1994) EMBO J. 13, 1019–1027.
[12] Choquet, Y., Stern, D.B., Wostrikoff, K., Kuras, R., Girard-Bascou, J. and Wollman, F.A. (1998) Proc. Natl. Acad. Sci. USA 95, 4380–4385.
[13] Kuras, R., Wollman, F.A. and Joliot, P. (1995) Biochemistry 34, 7468–7475.
[14] Choquet, Y., Wostrikoff, K., Rimbault, B., Zito, F., Girard-Bascou, J., Drapier, D. and Wollman, F.A. (2001) Biochem. Soc. Trans. 29, 421–426.
[15] Gumpel, N.J., Ralley, L., Girard-Bascou, J., Wollman, F.A., Nugent, J.H. and Purton, S. (1995) Plant Mol. Biol. 29, 921–932.
[16] Wostrikoff, K., Choquet, Y., Wollman, F.A. and Girard-Bascou, J. (2001) Genetics 159, 119–132.
[17] de Vitry, C., Olive, J., Drapier, D., Recouvreur, M. and Wollman, F.A. (1989) J. Cell Biol. 109, 991–1006.
[18] Drapier, D., Girard-Bascou, J. and Wollman, F.-A. (1992) Plant Cell 4, 283–295.
[19] Khrebtukova, I. and Spreitzer, R.J. (1996) Proc. Natl. Acad. Sci. USA 93, 13689–13693.
[20] Bennoun, P., Spierer-Herz, M., Erickson, J., Girard-Bascou, J., Pierre, Y., Delosme, M. and Rochaix, J.D. (1986) Plant Mol. Biol. 6, 151–160.
[21] Jensen, K.H., Herrin, D.L., Plumley, F.G. and Schmidt, G.W. (1986) J. Cell Biol. 103, 1315–1325.
[22] Erickson, J.M., Rahire, M., Malnoe, P., Girard-Bascou, J., Pierre, Y., Bennoun, P. and Rochaix, J.-D. (1986) EMBO J. 5, 1745–1754.
[23] Stampacchia, O., Girard-Bascou, J., Zanasco, J.L., Zerges, W., Bennoun, P. and Rochaix, J.D. (1997) Plant Cell 9, 773–782.
[24] Girard-Bascou, J., Choquet, Y., Schneider, M., Delosme, M. and Dron, M. (1987) Curr. Genet. 12, 489–495.
[25] Takahashi, Y., Goldschmidt-Clermont, M., Soen, S.Y., Franzen, L.G. and Rochaix, J.D. (1991) EMBO J. 10, 2033–2040.
[26] Gamble, P.E. and Mullet, J.E. (1989) J. Biol. Chem. 264, 7236–7243.
[27] Kim, J., Klein, P.G. and Mullet, J.E. (1994) Plant Mol. Biol. 25, 459–467.
[28] Rodermel, S., Haley, J., Jiang, C.Z., Tsai, C.H. and Bogorad, L. (1996) Proc. Natl. Acad. Sci. USA 93, 3881–3885.
[29] Calder, K.M. and McEwen, J.E. (1991) Mol. Microbiol. 5, 1769–1777.
[30] Nakai, T., Yasuhara, T., Fujiki, Y. and Ohashi, A. (1995) Mol. Cell. Biol. 15, 4441–4452.
[31] Payne, M.J., Schweizer, E. and Lukins, H.B. (1991) Curr. Genet. 19, 343–351.
[32] Ooi, B.G., Lukins, H.B., Linnane, A.W. and Nagley, P. (1987) Nucleic Acids Res. 15, 1965–1977.