Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 321 (2004) 1–6
BBRC
www.elsevier.com/locate/ybbrc

Breakthroughs and Views

Transcription, translation, degradation, and circadian clock

Yuan Shu, Lin Hong-Hui *

College of Life Science, Sichuan University, 29 Wang-Jiang Road, Chengdu 610064, PR China

Received 10 May 2004
Available online 3 July 2004

Abstract

Synthesis and degradation of mRNA together with synthesis and degradation of corresponding protein, this four-step-expression confers great fitness to all organisms. Transcription rate and mRNA stability both are essential for circadian expression of clock genes. In many cases, transcription rates and half-lives of mRNAs and corresponding proteins are not necessarily tightly linked with each other. The methods for measuring four-step-expression should be carefully selected and the experimental conditions should be strictly controlled.

Keywords: mRNA; Protein; Half-life; Oscillation

After the genome sequences of many species were identified, more and more attention was paid to elucidating the functions of the vast majority of genes in genomes [1]. Regulated transcription, translation, and degradation of mRNA and corresponding proteins play a key role in all gene expressions during development. One significant instance is that the combination of appropriate transcription rate and degradation rate of mRNA controls endogenous rhythms and their adaptation to the environment [2].

Four steps of gene expression

In some cases, higher mRNA synthesis rates are accompanied with higher mRNA levels, but in other cases they are not. Fig. 1 shows the relationships between transcription rate and mRNA level, mRNA level and corresponding protein level, and translation rate and protein level. (Because few studies have been conducted about the whole relationship among this four-step-expression, we only list three major types of relationships.) Although the transcription rates and mRNA abundance of most genes change with the same direction, they usually have different ranges of variation (Fig. 1A). Translation rate with protein abundance and mRNA level with protein level vary in a similar manner (Figs. 1B and C). For some chloroplast and mitochondrial genes (Fig. 1B, conditions 1 and 4), severely changed transcripts still permit nearly unchanged protein levels. Some mitochondrial genes (cob, cox1, nad2, and nad4) in *Chlamydomonas reinhardtii* exhibiting increased mRNA abundance failed to show higher transcription rates (Fig. 1A, condition 7) [3]. Additionally, in one ATP synthase mutant of *C. reinhardtii*, an increased protein synthesis rate led to a decreased steady-state protein level (Fig. 1C, condition 7) [4].

A correlation between transcription and translation has been established in some instances [5,6]. In general, transcriptional activity is not necessarily tightly linked to corresponding mRNA levels and protein abundance, especially for mutant genes, chloroplast genes, and mitochondrial genes, pointing to extensive post-transcriptional and post-translational control of gene expression [4,7]. A little fluctuation in mRNA half-life or protein half-life could have significant effects on steady-state levels of mRNA or protein [4,8–10].

* Corresponding author. Fax: +86-028-85412571.
E-mail address: Honghuilin@hotmail.com (H.-H. Lin).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.06.093

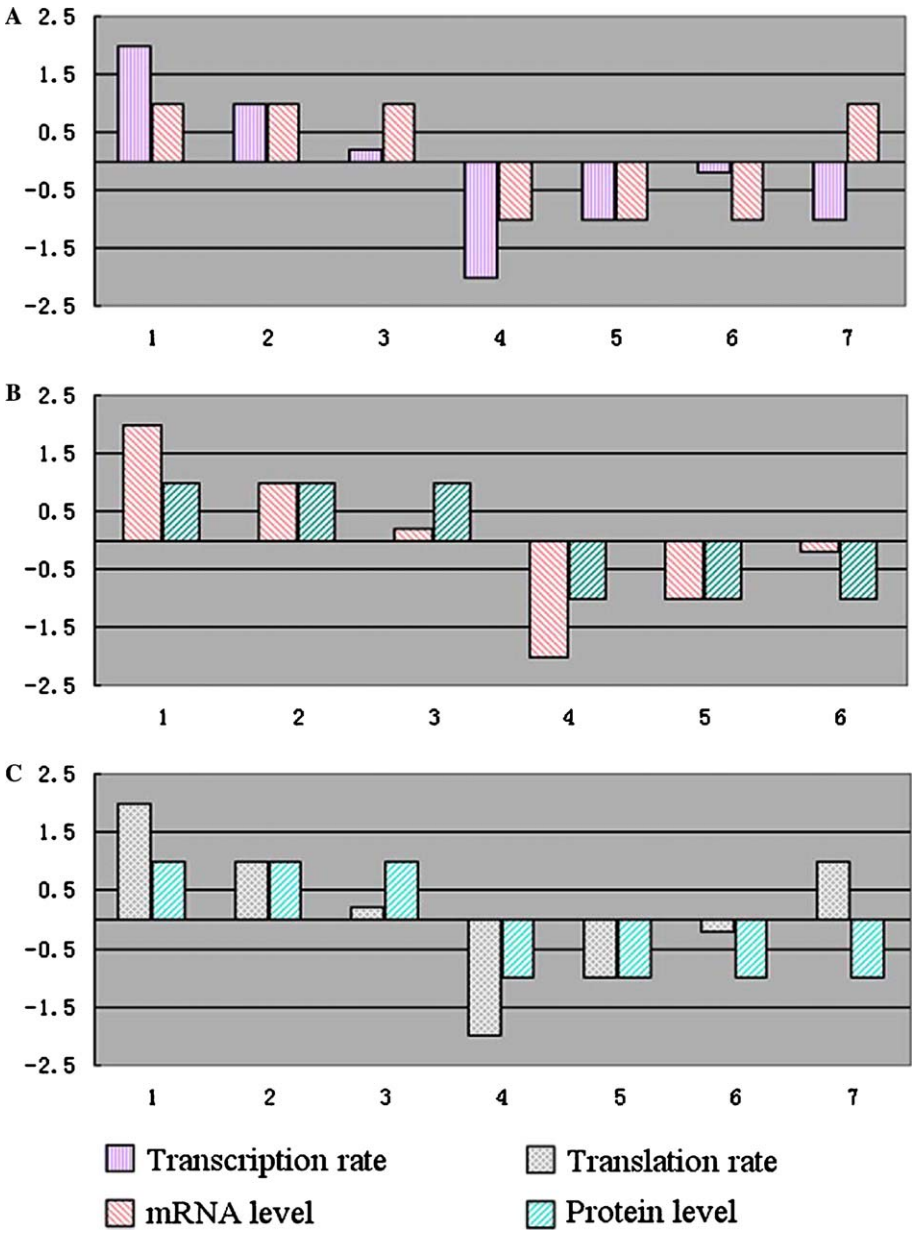

Fig. 1. Relationship between transcription rate and mRNA level (A), mRNA level and protein level (B), and translation rate and protein level (C). The *x*-axes are labeled by gene expression conditions. The color bars represent relative changes of transcription rate, mRNA level, translation rate, and protein level. The altitude of each bar does not mean the actual quantity, but the approximate fold change in abundance. Control transcription rate, mRNA level, translation rate, and protein level are all normalized to zero (not shown in the figure). (Example genes of (A): condition 1—psbD [34], 2—psaJ [3], 3—psaL [45], 4—psbB [3], 5—rps3 [3], 6—ORF112 [3], and 7—cox1 [3]; example genes of (B): condition 1—cab [46], 2—αAmy3 [32], 3—psbA [22], 4—petA [7], 5—Act [32], and 6—cab [47]; example proteins of (C): condition 1—β subunit of ATP synthase [48], 2—CP47 [28], 3—CP47 [28], 4—α subunit of ATP synthase [4], 5—CP43 [28], 6—D2 [28], and 7—β subunit of ATP synthase [4]. All examples come from plants and microbes, but it is easy to find other examples in other organisms.) (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)

Therefore, measuring transcriptional activity without focusing on protein level is imprecise, even false, especially for some genes with complicated regulation. Moreover, when no direct correlation between the transcripts level and protein abundance is found, it is advisable to examine the half-lives of mRNA and the corresponding protein.

Circadian clock also involves mRNA stability

The circadian clock regulates hundreds of genes and allows organisms to anticipate daily changes in the environment [11]. Molecular mechanisms and components underlying clock function have been described for many years for several animals and prokaryotic organisms.

The emerging model of the clock consists of positive and negative feedback loops. In recent years, some presumed negative elements (TOC1, CCA1, and LHY) also have been found in *Arabidopsis* [12,13]. Much is known about the feedback regulation at the level of transcriptional activity, however, relatively little is known about other feedback regulations at the level of mRNA stability (Fig. 2). Methionine (Met) or its metabolites negatively regulate mRNA stability of cystathionine γ-synthase, which catalyzes a key step in Met biosynthesis [14]. The β-tubulin has a similar feedback mechanism in which the stability of β-tubulin mRNA is down-regulated by the unassembled β-tubulin subunits [15]. Besides these, recent studies indicated that KaiC, a member of the DNA recombinases, regulates genomewide gene expression (including its own expression) by changing the condensation and supercoiling status of chromosomes [16,17]. Similarly, in the mouse's circadian clock, the feedback loops of the *Cry* gene and *Per* gene involve the acetylation of the H3 histone, which has an overall effect on genomewide expression [18].

In summary, the mechanisms of circadian oscillation are complex and diversiform. Even in plants, the clock system is far from completely clear. However, from above, transcription rate and mRNA stability both are involved in circadian oscillation [2,19]. More analyses on synthesis and degradation of clock genes mRNAs and corresponding proteins may help to find new circadian pathways.

Accurate adjustment of transcription and translation

Synthesis and degradation of mRNA and synthesis and degradation of corresponding protein, this four-step-expression confers great fitness to microbes, plants, animals, and human beings [3,4,7,20,21]. An ideal mRNA level usually could not be reached by only alternating the transcription rate. None but a combination of regulated translation rate and protein half-life could lead protein to a high level instantly or stabilize protein to an appropriate level efficiently. When dark-grown *Synechocystis* was transferred to light, the psbA mRNA rapidly increased 2- to 3-fold, whereas D1 showed an at least 15-fold induction during the same period of illumination [22]. (D1 protein, encoded by psbA, constitutes part of the core of the photosystem II reaction center.) The plant needs D1 protein urgently when performing photosynthesis. In contrast, light affects neither the activity of the psbA promoter nor the accumulation of the psbA mRNA in mature chloroplasts, while the D1 synthesis is slightly regulated by light [23]. In mature plants, cells develop another system to balance the protein abundance in a narrow range for a normal function.

Circadian oscillations of clock-controlled genes are also controlled by four-step-expression. Simultaneous translational induction and transcriptional repression could narrow the peaks of clock proteins and increase the robustness and accuracy of circadian oscillations [24]. Variation in circadian period, phase, and amplitude

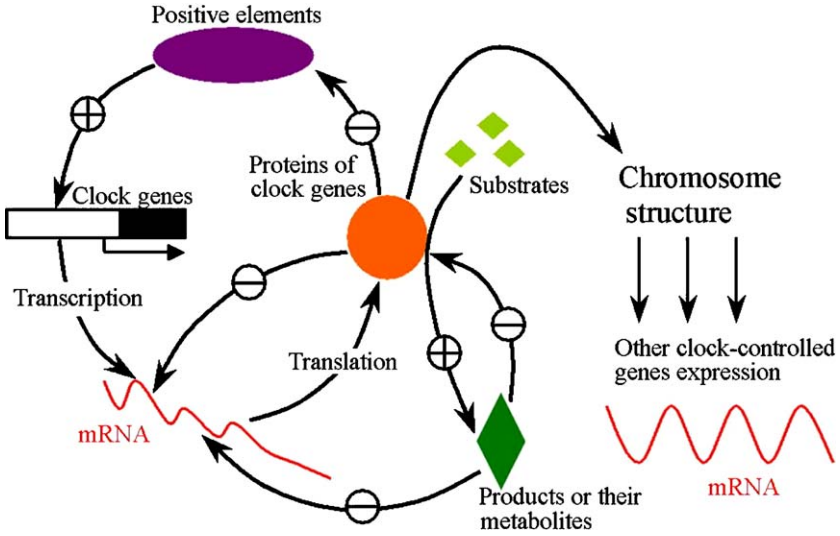

Fig. 2. Molecular model of current circadian oscillators. Positive elements (CLOCK and CYC in *Drosophila*, WC-1 and WC-2 in *Neurospora*, and CLOCK and BMAL in mice, possibly PIF-PHY13 in *Arabidopsis*) act as transcriptional activators to induce clock gene expressions. Protein products of clock genes (PER and TIM in *Drosophila*, FRQ in *Neurospora*, and mCRYs and mPERs in mice, possibly CCA-1, LHY, and TOC1 in *Arabidopsis*) act as negative elements and block the action of positive elements [12,13]. Proteins of clock genes include some enzymes (cystathionine γ-synthase in *Arabidopsis*) catalyzing the substrates to form corresponding products (methionine in *Arabidopsis*), and these products or their metabolites in turn down-regulate activities and mRNA stability of enzymes [14]. Some unassembled proteins of clock genes (β-tubulin subunits in animals) act in *cis* to down-regulate their own mRNA stability [15]. Other protein oscillators (KaiC proteins in *Synechococcus*, Per and Cry in mice) regulate a large number of genes (including their own transcription) by changing the structure of the chromosome [16–18].

contributes to seasonal time measurement [25] and changes in fitness within specific environments [26]. Studies on the relationship between transcription and translation would help to clarify the dynamic regulation of rhythmic expression.

Measuring four-step-expression

Transcription, translation, and degradation of mRNA and protein are vital for sophisticated gene expressions. However, the methods for this four-step-expression are used confusedly sometimes.

Measuring initiation of transcription and translation

Run-on transcription is a standard method for transcription rate determination. Another method is shot-time transcription (constructing a plasmid containing promoter of the selected gene and a reporter gene) [27]. Studies on translation rate determination are relatively short and the common method is protein pulse-labeling [4,28]. All of these methods have a common problem which is how to decide an appropriate pulse time. Since different mRNAs or proteins have different half-lives, we suggest that the pulse time should be much shorter than the minimum half-life of interested mRNAs but also ensure that a readable expression signal can be acquired.

Measuring degradation rate of mRNA

Relatively more methods have been used for measuring the half-life of mRNA. Cells which are simply cultured with an inhibitor and harvested at different times is the simplest technique. 5,6-Dichloro-1-β-ribofuranosylbenzimidazole (DRB), cordycepin, and α-amanitin are usually used as inhibitors in bacteria and animals [8]. Rifampicin [3] and tagetitoxin, a selective inhibitor of chloroplast transcription [29–31], are used for chloroplasts in plants. Actinomycin D is the most frequently used and the most effective inhibitor for all organisms. It can inhibit all transcriptions of chloroplasts, mitochondria, and nuclei [3,8,32], although it is very noxious to cells and possibly changes some mRNA stability [33]. Noticeably, every gene has a variety of reactions to different inhibitors [3] and mRNA half-lives measured with inhibitors differ considerably from this inhibitor to other inhibitors [8]. Therefore, transcription inhibitors should be used cautiously to fit different genes. Besides these, developmental stage and many abiotic conditions (light, nutrilite, etc.) could change mRNA stability dramatically [3,29,34]. Experimental conditions should be strictly controlled when the inhibitors are used for measuring mRNA stability.

The pulse-chase method (pulse-labeling with nucleosides then chasing with unlabeled nucleotides) overcomes the limitations of inhibitors, although it is often difficult to label the mRNA of interest. The approach to the steady-state method needs a constant transcription rate, and the short-term promoter activation method needs a suitably inducible promoter. We should select suitable methods according to different situations. Determining a correct half-life for mRNA may not be feasible using only one method, in fact, it may require several different methods until a consensus value emerges [35]. The detailed information of these methods has been reviewed by Jeff Ross (see [8], for review).

Measuring degradation rate of protein

Translation inhibitors measure protein half-life in the same way. However, this method is unreliable, because the cell is clearly not in a normal physiological condition, and stability of some components of multisubunit complexes depends on assembly with other proteins [36]. Additionally, none of the inhibitors (lincomycin, chloramphenicol, cycloheximide, and anisomycin) could inhibit all proteins effectively [37,38]. Protein pulse-labeling is a more precise method [4,7,39–41]. After pulse-chasing, radiolabeled proteins were usually separated on polyacrylamide gels. However, it is difficult to analyze these proteins, due to the large number of radiolabeled proteins in a sample. According to recent studies, immunoprecipitation [40–43] and sucrose gradient centrifugation [42,44] before electrophoresis could enhance distinguishability of labeled proteins.

Whereas, the experiments about four-step-expression should be carefully designed and more effective and convenient methods should be created. We also hope for more meticulous analyses on four-step-expression.

Acknowledgments

This work was supported by National Natural Science Foundation of China (30170557 and 30170081) and Sichuan Sci&Tech Foundation. We thank Dr. Zhang Nian-Hui for helpful discussion and Dr. Adam S. Wilkins, Dr. Li Jia, and Dr. Rebecca Schuler for critical review of the manuscript.

References

[1] K. Yamada et al., Empirical analysis of transcriptional activity in the *Arabidopsis* genome, Science 302 (2003) 842–846.

[2] M.L. Salvador, U. Klein, L. Bogorad, Light-regulated and endogenous fluctuations of chloroplast transcript levels in *Chlamydomonas*. Regulation by transcription and RNA degradation, Plant J. 3 (1993) 213–219.

[3] J.W. Lilly, J.E. Maul, D.B. Stern, The *Chlamydomonas reinhardtii* organellar genomes respond transcriptionally and post-transcriptionally to abiotic stimuli, Plant Cell 14 (2002) 2681–2706.
[4] D. Drapier, J. Girard-Bascou, F.A. Wollman, Evidence for nuclear control of the expression of the *atpA* and *atpB* chloroplast genes in *Chlamydomonas*, Plant Cell 4 (1992) 283–295.
[5] J.E. Mullet, Dynamic regulation of chloroplast transcription, Plant Physiol. 103 (1993) 309–313.
[6] T. Pfannschmidt, A. Nilsson, J.F. Allen, Photosynthetic control of chloroplast gene expression, Nature 397 (1999) 625–628.
[7] S. Eberhard, D. Drapier, F.A. Wollman, Searching limiting steps in the expression of chloroplast-encoded proteins: relations between gene copy number, transcription, transcript abundance and translation rate in the chloroplast of *Chlamydomonas reinhardtii*, Plant J. 31 (2002) 149–160.
[8] J. Ross, mRNA stability in mammalian cells, Microbiol. Rev. 59 (1995) 423–450.
[9] J.E. Mullet, P.G. Klein, R.R. Klein, Chlorophyll regulates accumulation of the plastid-encoded chlorophyll apoproteins of CP43 and D1 by increasing apoprotein stability, Proc. Natl. Acad. Sci. USA 87 (1990) 4038–4042.
[10] D.A. Christopher, J.E. Mullet, Separate photosensory pathways co-regulate blue light/ultraviolet-a-activated psbD-psbC transcription and light-induced D2 and CP43 degradation in barley (*Hordeum vulgare*) chloroplasts, Plant Physiol. 104 (1994) 1119–1129.
[11] S.L. Harmer et al., Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock, Science 290 (2000) 2110–2113.
[12] S. Barak, E.M. Tobin, C. Andronis, S. Sugano, R.M. Green, All in good time: the *Arabidopsis* circadian clock, Trends Plant Sci. 5 (2000) 517–522.
[13] D.E. Somers, The physiology and molecular bases of the plant circadian clock, Plant Physiol. 121 (1999) 9–19.
[14] Y. Chiba et al., Evidence for autoregulation of cystathionine γ-synthase mRNA stability in *Arabidopsis*, Science 286 (1999) 1371–1374.
[15] T.J. Yen, P.S. Machlin, D.W. Cleveland, Autoregulated instability of polysome-bound b-tubulin mRNAs is achieved through recognition of the nascent amino-terminus of tubulin, Nature 334 (1988) 580–585.
[16] M. Ishiura et al., Expression of a gene cluster *kaiABC* as a circadian feedback process in cyanobacteria, Science 281 (1998) 1519–1523.
[17] Y. Nakahira et al., Global gene repression by *KaiC* as a master process of prokaryotic circadian system, Proc. Natl. Acad. Sci. USA 101 (2004) 881–885.
[18] J.P. Etchegaray, C. Lee, P.A. Wade, S.M. Reppert, Rhythmic histone acetylation underlies transcription in the mammalian circadian clock, Nature 421 (2003) 177–182.
[19] S. Hwang, R. Kawazoe, D.L. Herrin, Transcription of *tufA* and other chloroplast-encoded genes is controlled by a circadian clock in *Chlamydomonas*, Proc. Natl. Acad. Sci. USA 93 (1996) 996–1000.
[20] R. Hayes, J. Kudla, W. Gruissem, Degrading chloroplast mRNA: the role of polyadenylation, Trends Biochem. Sci. 24 (1999) 199–202.
[21] H. Hellmann, M. Estelle, Plant development: regulation by protein degradation, Science 297 (2002) 793–797.
[22] Q.F. He, V. Vermaas, Chlorophyll a availability affects psbA translation and D1 precursor processing in vivo in *Synechocystis* sp. PCC 6803, Proc. Natl. Acad. Sci. USA 95 (1998) 5830–5835.
[23] J. Satoh, K. Baba, Y. Nakahira, T. Shniia, Y. Toyoshima, Characterization of dynamics of *psbD* light-induced transcription in mutant wheat chloroplasts, Plant Mol. Biol. 33 (1997) 267–278.
[24] J.Y. Kim, H.R. Song, B.L. Taylor, I.A. Carre, Light-regulated translation mediates gated induction of the *Arabidopsis* clock protein LHY, EMBO J. 22 (2003) 935–944.
[25] M.J. Yanovsky, S.A. Kay, Molecular basis of seasonal time measurement in *Arabidopsis*, Nature 419 (2002) 308–312.
[26] T.P. Michael et al., Enhanced fitness conferred by naturally occurring variation in the circadian clock, Science 302 (2003) 1049–1053.
[27] Y. Nakahira, K. Baba, A. Yoneda, T. Shiina, Y. Toyoshima, Circadian-regulated transcription of *psbD* light-responsive promoter in wheat chloroplasts, Plant Physiol. 118 (1998) 1079–1088.
[28] J. Yu, W.F.J. Vermaas, Transcript levels and synthesis of photosystem II components in cyanobacterial mutants with inactivated photosystem II genes, Plant Cell 2 (1990) 315–322.
[29] M. Kim, D.A. Christopher, J.E. Mullet, Direct evidence for selective modulation of psbA, rpoA, rbcL and 16S RNA stability during barley chloroplast development, Plant Mol. Biol. 22 (1993) 447–463.
[30] A. Sakai, C. Saito, N. Inada, T. Kuroiwa, Activities of the chloroplast-nuclei and proplastid-nuclei isolated from tobacco exhibit different sensitivities to tagetitoxin: implication of the presence of distinct RNA polymerases, Plant Cell Physiol. 39 (1998) 928–934.
[31] T.B. Sexton, D.A. Christopher, J.E. Mullet, Light-induced switch in barley *psbD-psbC* promoter utilization: a novel mechanism regulating chloroplast gene expression, EMBO J. 9 (1990) 4485–4494.
[32] S.L. Ho, Y.C. Chao, W.F. Tong, S.M. Yu, Sugar coordinately and differentially regulates growth- and stress-related gene expression via a complex signal transduction network and multiple control mechanisms, Plant Physiol. 125 (2001) 877–890.
[33] S.R. Helms, F.M. Rottman, Characterization of an inducible promoter to investigate decay of stable mRNA molecules, Nucleic Acids Res. 18 (1990) 255–259.
[34] J.C. Rapp, B.J. Baumgartner, J. Mullet, Quantitative analysis of transcription and RNA levels of 15 barley chloroplast genes, J. Biol. Chem. 267 (1992) 21404–21411.
[35] S. Harrold, C. Genovese, B. Kobrin, S.L. Morrison, C. Milcarek, A comparison of apparent mRNA half-life using kinetic labeling techniques vs decay following administration of transcriptional inhibitors, Anal. Biochem. 198 (1991) 19–29.
[36] D.F. Steele, C.A. Butler, T.D. Fox, Expression of a recoded nuclear gene inserted into yeast mitochondrial DNA is limited by mRNA-specific translational activation, Proc. Natl. Acad. Sci. USA 93 (1996) 5253–5257.
[37] R. Kawazoe, S. Hwang, D.L. Herrin, Requirement for cytoplasmic protein synthesis during circadian peaks of transcription of chloroplast-encoded genes in *Chlamydomonas*, Plant Mol. Biol. 44 (2000) 699–709.
[38] J. Kim, L.A. Eichacker, W. Rudiger, J.E. Mullet, Chlorophyll regulates accumulation of the plastid-encoded chlorophyll proteins P700 and D1 by increasing apoprotein stability, Plant Physiol. 104 (1994) 907–916.
[39] J. Yu, W.F.J. Vermaas, Synthesis and turnover of photosystem II reaction center polypeptides in cyanobacterial D2 Mutants, J. Biol. Chem. 268 (1993) 7407–7413.
[40] Z. Xu, J. Choi, W. Lu, J.H. Ou, Hepatitis C virus F protein is a short-lived protein associated with the endoplasmic reticulum, J. Virol. 77 (2003) 1578–1583.
[41] L. Casalino, D.D. Cesare, P. Verde, Accumulation of Fra-1 in *ras*-transformed cells depends on both transcriptional autoregulation and MEK-dependent posttranslational stabilization, Mol. Cell. Biol. 23 (2003) 4401–4415.
[42] B. Muller, L.A. Eichacker, Assembly of the D1 precursor in monomeric photosystem II reaction center precomplexes precedes chlorophyll *a*—triggered accumulation of reaction center II in barley etioplasts, Plant Cell 11 (1999) 2365–2377.
[43] A. Lardans, N.W. Gillham, J.E. Boynton, Site-directed mutations at residue 251 of the photosystem II D1 protein of *Chlamydomonas* that result in a nonphotosynthetic phenotype and impair D1 synthesis and accumulation, J. Biol. Chem. 272 (1997) 210–216.

[44] L. Zhang, V. Paakkarinen, K.J.V. Wijk, E.M. Aro, Co-translational assembly of the D1 protein into photosystem II, J. Biol. Chem. 274 (1999) 16062–16067.
[45] L. Tang, S. Bhat, M.E. Petracek, Light control of nuclear gene mRNA abundance and translation in tobacco, Plant Physiol. 133 (2003) 1979–1990.
[46] D.P. Maxwell, D.E. Laudenbach, N.P.A. Huner, Redox regulation of light-harvesting complex II and cab mRNA abundance in *Dunaliella salina*, Plant Physiol. 109 (1995) 787–795.
[47] S. Yuan, N.H. Zhang, H.H. Lin, unpublished data.
[48] D. Drapier et al., The chloroplast atpA gene cluster in *Chlamydomonas reinhardtii*, Plant Physiol. 117 (1998) 629–641.