

Translation initiation factor 3 families: what are their roles in regulating cyanobacterial and chloroplast gene expression?

April D. Nesbit · Craig Whippo · Roger P. Hangarter · David M. Kehoe

Received: 21 May 2014 / Accepted: 2 January 2015 / Published online: 29 January 2015
© Springer Science+Business Media Dordrecht 2015

Abstract Initiation is a key control point for the regulation of translation in prokaryotes and prokaryotic-like translation systems such as those in plant chloroplasts. Genome sequencing and biochemical studies are increasingly demonstrating differences in many aspects of translation between well-studied microbes such as *Escherichia coli* and lesser studied groups such as cyanobacteria. Analyses of chloroplast translation have revealed its prokaryotic origin but also uncovered many unique aspects that do not exist in *E. coli*. Recently, a novel form of posttranscriptional regulation by light color was discovered in the filamentous cyanobacterium *Fremyella diplosiphon* that requires a putative stem-loop and involves the use of two different prokaryotic translation initiation factor 3s (IF3s). Multiple (up to five) putative IF3s have now been found to be encoded in 22 % of sequenced cyanobacterial genomes and 26 % of plant nuclear genomes. The lack of similar light-color regulation of gene expression in most of these species suggests that IF3s play roles in regulating gene expression in response to other environmental and

developmental cues. In the plant *Arabidopsis*, two nuclear-encoded IF3s have been shown to localize to the chloroplasts, and the mRNA levels encoding these vary significantly in certain organ and tissue types and during several phases of development. Collectively, the accumulated data suggest that in about one quarter of photosynthetic prokaryotes and eukaryotes, IF3 gene families are used to regulate gene expression in addition to their traditional roles in translation initiation. Models for how this might be accomplished in prokaryotes versus eukaryotic plastids are presented.

Keywords Light regulation · Transcription attenuation · RNA-binding protein · Stem-loop · 5' Leader

Overview of the variation in prokaryotic and plastid translation initiation

Initiation is the rate-limiting step in eubacterial translation and an important point of regulation of gene expression (Laursen et al. 2005). In essence, it is a multi-step process involving the assembly of three translation initiation factors, IF1, IF2, and IF3, GTP, the 30S and 50S subunits of the ribosome, an mRNA, and an initiator *N*-formylmethionyl-tRNA. Most of our understanding of this process has come from studies of *Escherichia coli*. Yet, the analysis of the first sequenced cyanobacteria genome, *Synechocystis* sp. PCC6803 (Kaneko et al. 1996), made it clear that the protein subunit composition of cyanobacterial ribosomes differs from *E. coli*. For some cyanobacteria with sequenced genomes, the variation in ribosomal protein composition and stoichiometry compared to *E. coli* has been substantiated at the protein level (Davydov et al. 2013; Mutsuda and Sugiura 2006), providing an initial

A. D. Nesbit · C. Whippo · R. P. Hangarter · D. M. Kehoe (✉)
Department of Biology, Indiana University, Bloomington,
IN 47405, USA
e-mail: dkehoe@indiana.edu

A. D. Nesbit
Department of Biology/Chemistry, Purdue University North
Central, 1401 S. US 421, Westville, IN 46391, USA

C. Whippo
Department of Natural Science, Dickinson State University,
Dickinson, ND 58601, USA

D. M. Kehoe
Indiana Molecular Biology Institute, Indiana University,
Bloomington, IN 47405, USA

indication of the degree to which cyanobacterial ribosomes have diverged in form and operation from those of *E. coli*. The possible functional consequences of heterogeneity between ribosomal populations, between and even within the same organism, are being increasingly recognized and demonstrated (Filipovska and Rackham, 2013; Gilbert 2011; Komili et al. 2007; Schippers and Mueller-Roeber 2010; Sharma et al. 2007).

The mechanics of translation initiation are more varied than initially believed, particularly in the correct initial positioning of the 30S subunit on the mRNA, which can be facilitated by a Shine–Dalgarno (SD) sequence that is usually located 7 ± 2 nucleotides upstream of the initiation codon (Shine and Dalgarno 1974, 1975; Sugiura et al. 1998). Typically identified as the sequence 5'-GGAGG-3', the SD sequence base pairs with the complementary, antiparallel sequence present at the 3' end of the 16S rRNA within the 30S subunit. Although this interaction has been widely adopted as an invariant feature of the prokaryotic translation initiation process (Schmeing and Ramakrishnan 2009), prokaryotes actually produce several classes of mRNAs that are distinguished by their 5' leaders: SD-containing mRNAs, non-SD-containing mRNAs, and mRNAs that lack a 5' leader (Malys and McCarthy 2011). The percentage of SD-containing mRNAs varies widely between prokaryotic groups. Remarkably, only 39 % of cyanobacterial mRNAs examined contained a recognizable SD element (Nakagawa et al. 2010), which is lower than many other eubacteria and similar to the percentage of chloroplast mRNAs from land plants with correctly positioned SD sequences (Peled-Zehavi and Danon 2007; Sugiura et al. 1998). These findings support the view that although classical SD–16S rRNA interaction can be used during cyanobacterial and plastid translation initiation, the absence or incorrect positioning of SD sequences in the majority of their mRNAs means that these two groups must also use additional mechanisms to insure the proper location and frequency of translation initiation (Barkan 2011; Nakagawa et al. 2010; Sugiura et al. 1998). One additional mechanism for the proper initial localization of the 30S preinitiation complex involves ribosomal protein S1, which has been shown to play a role in translation initiation in cyanobacteria and other eubacteria by providing an RNA chaperone function that helps dock and unfold the mRNA, and to correctly position the initiation codon (Duval et al. 2013; Hajnsdorf and Boni 2012; Mutsuda and Sugiura 2006). Further evidence for the dispensability of SD sequences has come from biochemical and *in vitro* translation experiments, which have demonstrated that translation initiation can require sequences other than the SD for many plastid and cyanobacterial mRNAs, such as AU-rich regions near the translation start sites that may be the sites for RNA-binding proteins (reviewed in Sugiura et al. 1998;

also see Hirose and Sugiura 1996, 2004; Mutsuda and Sugiura 2006; Plader and Sugiura 2003). The AU-rich regions can also be sites at which nucleases act to regulate mRNA stability (Horie et al. 2007).

Both chloroplasts and mitochondria use translation components that are closely related to those of eubacteria. For example, *Chlamydomonas* and land plant plastids contain some eubacterial ribosomal components like *E. coli*, but some components are absent or very diverged and some components are unique to plastid ribosomes (Beligni et al. 2004; Manuell et al. 2007; Subramanian 1993; Tiller et al. 2012; Yamaguchi et al. 2002, 2003; Yamaguchi and Subramanian 2003). Because of the similarities between plastid and eubacteria ribosomes, it is plausible that similar mechanisms of translation regulation exist in plastids, including chloroplasts, as in eubacteria.

For the regulation of translation in plastids, genetic screens have uncovered many nuclear-encoded RNA-binding proteins that, often together with elements primarily located within the 5' leader of mRNAs, influence the extent of translation and stabilization of specific chloroplast transcripts (reviewed in Peled-Zehavi and Danon 2007). Unlike eubacteria, transcription and translation are typically uncoupled in chloroplasts and mRNAs are generally very stable (Marin-Navarro et al. 2007; Rochaix 1996). Consequently, posttranscriptional control has emerged as the primary form of regulation of plastid gene expression. Stem-loops at various locations in the 5' leaders of chloroplast mRNAs have been identified as central structures in this control mechanism (Mayfield et al. 1994; Zerges and Rochaix, 1994). These stem-loops can be bound by one or several nuclear-encoded proteins, which typically activate translation (reviewed in Barkan 2011). The binding activities of such proteins are generally specific to one or a few chloroplast mRNA species in both the green alga *Chlamydomonas* and land plants (see Marin-Navarro et al. 2007; Peled-Zehavi and Danon 2007; Rochaix 1996). A major family of such proteins are the pentatricopeptide repeat (PPR) proteins, which bind 5' stem-loops in mRNAs and influence translation as well as many other aspects of chloroplast mRNA metabolism by blocking exoribonuclease activity and remodeling the structure of the ribosome binding site, possibly functionally mimicking the action of small bacterial RNAs (Barkan 2011; Stern et al. 2010). This family has not been found in cyanobacteria or other eubacteria thus far.

Posttranscriptional regulation in cyanobacteria

The majority of posttranscriptional regulation described thus far in cyanobacteria has been at the level of mRNA stability and/or translation elongation, with the 5' leader

regions of the regulated genes often implicated as having a role in regulation. Regulation by antisense RNAs (e.g., Duhring et al. 2006), which appears to be very prevalent (Georg and Hess 2011), will not be addressed here. In *Microcystis aeruginosa* K-81, the 5' leader of *psbA2* confers posttranscriptional regulation, apparently by changing the stability of the transcript (Agrawal et al. 2001). Light regulation of *hliA* mRNA stability also occurs, perhaps due to ribosome stalling (Salem and van Waasbergen 2004), and iron-dependent stabilization of ferredoxin I mRNA was determined to act through the 5' end of the transcript in *Synechococcus* PCC 7942 (Bovy et al. 1993). Cyanobacterial responsiveness to cold shock also involves RNA stability changes of the *des* genes (Sakamoto and Bryant 1997) and the cold-regulated expression of *rbpA1* and *rbp2*, which encode RNA-binding proteins in many cyanobacteria (Hamano et al. 2004; Mutsuda et al. 1999). For *rbpA1*, this is controlled in part at the level of mRNA stability and involves the formation of a stem-loop structure in the 5' leader region (Ehira et al. 2003, 2005; Sato and Nakamura 1998). In *Synechococcus* PCC 7942, posttranscriptional regulation of *psbA* involves both the 5' leader and an element in the coding sequence (Kulkarni and Golden 1997; Kulkarni et al. 1992; Li and Golden 1993); this was found to be due to changes in mRNA stability and translation elongation rather than differential translation initiation (Mulo et al. 2012; Tyystjarvi et al. 2004). In *Synechocystis* PCC 6803, *psbA* is posttranscriptionally regulated at the level of translation elongation (Tyystjarvi et al. 2001) and stability (Horie et al. 2007), and the structure of the 5' leader accounts for the differential use of two different translation start sites of the *petH* gene (Omairi-Nasser et al. 2011; Thomas et al. 2006). The regulation of Photosystem I by light in cyanobacteria appears to act primarily during translation or assembly of the complex, and at least part of *psaA* regulation also occurs at the level of translation (Aizawa and Fujita 1997; Fujita 1997; Herranen et al. 2005).

A recently identified form of posttranscriptional regulation in cyanobacteria that is not the result of differential mRNA stability is the light-color responsiveness of the *cpeCDEST*R operon (hereafter *cpeC*), which encodes proteins involved in biosynthesis of the photosynthetic light harvesting-antennae of *Fremyella diplosiphon* UTEX481 (also called *Tolypothrix* PCC 7601). Along with a transcriptional control system, *cpeC* regulation is mediated by the Cgi (control of green light induction) pathway, a posttranscriptional regulatory system that represses *cpeC* expression during growth in red light but not in green light (Kehoe and Gutu 2006). The Cgi system does not influence the stability of *cpeC* mRNA and operates through a region of the *cpeC* mRNA 5' leader approximately 30 nucleotides 5' of the start codon for *CpeC* that is predicted to form a

stem-loop with a ΔG of $-3.4 \text{ kcal mol}^{-1}$ (Bezy et al. 2011). Nucleotide substitutions within either the stem or loop of this putative stem-loop structure abolish repression by the Cgi system. Collectively, these data suggest that *cpeC* mRNA levels may change in response to shifts in light color through modulation of transcription attenuation, with more attenuation occurring during growth in red light than green light (Bezy et al. 2011). Although it has not yet been shown in cyanobacteria, transcription attenuation is a relatively common form of gene regulation in prokaryotes and often controlled by the degree of coupling between the transcription and translation machinery (Gollnick and Babitzke 2002; Henkin and Yanofsky 2002). However, because chloroplast gene expression is mainly regulated at the translational level (Deng and Grussem 1987) and mostly uncoupled from transcription, a plastid gene regulation system that operates in a manner directly parallel to the putative transcription attenuation system controlling *cpeC* seems unlikely.

Use of multiple IF3s in *F. diplosiphon* and perhaps other bacteria

A transposon mutagenesis screen for Cgi pathway components in *F. diplosiphon* that failed to light regulate *cpeC* mRNA levels uncovered multiple mutants with insertions in *infCa*, which encodes an IF3 named IF3 α (Gutu et al. 2013). IF3s are essential in most bacteria. They are dumb-bell-shaped proteins, consisting of C- and N-terminal domains (Cheng and Kaiser 1989; Springer et al. 1977). However, an *F. diplosiphon infCa* deletion mutant was viable due to the presence of *infCb*, which encodes a second IF3 called IF3 β (Gutu et al. 2013). Each gene was capable of complementing an *Escherichia coli infC* mutant, demonstrating that both genes encoded *bona fide* IF3s (Gutu et al. 2013). Interestingly, deletion of *infCb* did not affect light regulation of *cpeC* mRNA, demonstrating that IF3 α and IF3 β have unique cellular roles even with their functional overlap as translation initiation factors (Gutu et al. 2013). Although the selective use of multiple IF3s has not been previously found, there have been reports of regulatory roles for other IF3s in addition to translation initiation and ribosome recycling. Cold shock causes the single *E. coli* IF3 to increase in abundance relative to ribosome abundance, causing the preferential translation of cold-shock mRNAs (Giuliodori et al. 2004). At low temperature, IF3 increases the binding rate of fMet-tRNA to ribosomes that are associated with cold-shock mRNAs, increases the pool size of 30S subunits, and also lowers the translation fidelity of non-cold-shock mRNAs (Giuliodori et al. 2007). In *Myxococcus xanthus*, the IF3 family member D signal (Dsg) is involved in both translation

initiation and developmental processes, including the control of sporulation and the development of fruiting bodies (Cheng et al. 1994; Kalman et al. 1994). In *Rhodobacter sphaeroides*, an IF3-like protein called PifC influences the production of bacteriochlorophyll and photosynthetic complexes (Babic et al. 1997). The mechanisms through which Dsg and PifC operate are unknown.

The absence of either IF3 α or IF3 β affects the expression of many genes in *F. diplosiphon*, and thus these initiation factors are more widely employed than simply regulating *cpeC* expression by light color. Recent microarray studies of $\Delta infCa$ and $\Delta infCb$ mutants suggest that the expression levels of 80 % of the 298 light-color-regulated genes in *F. diplosiphon* were directly or indirectly controlled by IF3 α , and that an additional 1,806 genes that were not light-color-regulated were differentially expressed when either IF3 α or IF3 β was missing (A. Nesbit and D. Kehoe, unpublished results). While it has not been established how many of these gene expression changes are direct, these results indicate that in *F. diplosiphon* these IF3s are important components of a previously unrecognized gene regulatory system. It is noteworthy that these gene expression differences were measured at the mRNA level because it raises the possibility that their regulation also could be via transcription attenuation.

Genome database searches of all sequenced non-cyanobacterial, eubacterial genomes revealed that 1 % apparently encode more than one IF3 (Gutu et al. 2013). In cyanobacteria, 22 % of all available sequenced genomes encode multiple putative IF3s. The majority of cyanobacteria with multiple IF3s contain at least two putative IF3s, while the greatest number of putative IF3s in any cyanobacterial species uncovered thus far is five (Gutu et al. 2013). Half of the genomes with multiple IF3s are members of the *Nostoc* clade, which typically have large genome sizes and extensive phenotypic plasticity in their environmental responsiveness and developmental pathways (Castenholz and Waterbury 1989). Because many of the species with multiple IF3s are not able to regulate *cpeC* gene expression in response to light color (Tandeu de Marsac 1977), it is likely that IF3 family members control different cellular responses in those cyanobacteria.

Multiple IF3s in *Arabidopsis thaliana* chloroplasts

Many diatom, green algal, and land plant nuclear genomes also encode multiple putative prokaryotic-like IF3s, which are easily distinguished from their multi-subunit eukaryotic eIF3 counterparts (Gutu et al. 2013). For photosynthetic eukaryotes, it is likely that at least one of the nuclear-encoded IF3s is targeted to the mitochondria and one to the chloroplast. Therefore, only species encoding three or more

Fig. 1 Clustal Omega alignments (Goujon et al. 2010; Sievers et al. 2011) of the two *F. diplosiphon* IF3s (IF3 β , IF3 α), the mitochondria-localized (AtIF3-1), and chloroplast-localized (AtIF3-2 and AtIF3-4) putative IF3s from *Arabidopsis*. Numbering is from the start site of each translated protein. An asterisk indicates a position with a single, fully conserved residue; a colon indicates conservation between groups of strongly similar properties; a dot indicates conservation between groups of weakly similar properties. The color code is: red small (+hydrophobic), blue acidic, magenta basic, green hydroxy, sulfhydryl, amine, and glycine

putative IF3s are considered to possess “multiple” IF3s for an organelle. Genomes encoding multiple putative IF3s were more common in land plants (6 out of 23 genomes, or 26 %) than in cyanobacteria (22 %), while none have been found in algae (Gutu et al. 2013).

Because prokaryotic-like IF3s could localize to either the mitochondria and/or the chloroplast, it is of interest to know where such IF3 family members localize in photosynthetic eukaryotes. In some land plants, the ultimate subcellular destination of any nuclear-encoded putative IF3 can be predicted with reasonable certainty by analysis of the protein’s transit peptide sequence (Small et al. 2004). The nuclear genome of *A. thaliana* (*Arabidopsis*, thale cress), a model plant that is a member of the Brassicaceae, is predicted to encode three prokaryotic-like IF3s (Gutu et al. 2013). Encoded on chromosomes 1, 2, and 4, we have tentatively designated these genes *AtINFC-1* (At1g34360), *AtINFC-2* (At2g24060), and *AtINFC-4* (At4g30690) and their corresponding proteins AtIF3-1, AtIF3-2, and AtIF3-4. These are shown, aligned with the *F. diplosiphon* IF3s, in Fig. 1. AtIF3-1, with a predicted molecular mass of 58.6 kDa, consists of a slightly longer N-terminal region and a significantly longer C-terminal half than IF3 α (20.6 kDa), IF3 β (20.6 kDa), AtIF3-2 (35.1 kDa), or AtIF3-4 (31.8 kDa). Predotar N-terminal targeting sequence analysis (Small et al. 2004) predicted an 86 % chance that AtIF3-1 was mitochondrial localized and 0 % chance that it was chloroplast targeted. AtIF3-2 was predicted to have a 1 % chance of mitochondrial localization and a 96 % probability of chloroplast localization. AtIF3-4 also had a 1 % probability of mitochondrial localization and an 87 % chance of chloroplast localization. These predictions were tested by stably transforming *Arabidopsis* with the genes encoding AtIF3-2 or AtIF3-4, fused in frame to DNA sequence encoding yellow fluorescence protein (YFP) (Shaner et al. 2004), and determining the subcellular location of each using a Leica scanning confocal microscope. Based on the co-localization of the YFP and chlorophyll fluorescence signals, both AtIF3-2-YFP and AtIF3-4-YFP localized to the chloroplast (Fig. 2) (A. Nesbit, C. Whippo, R. Hangarter, and D. Kehoe, unpublished). Although the reporter gene encoding AtIF3-1-YFP could not be stably transformed into *Arabidopsis*, it was transiently expressed in *Nicotiana benthamiana*, where it

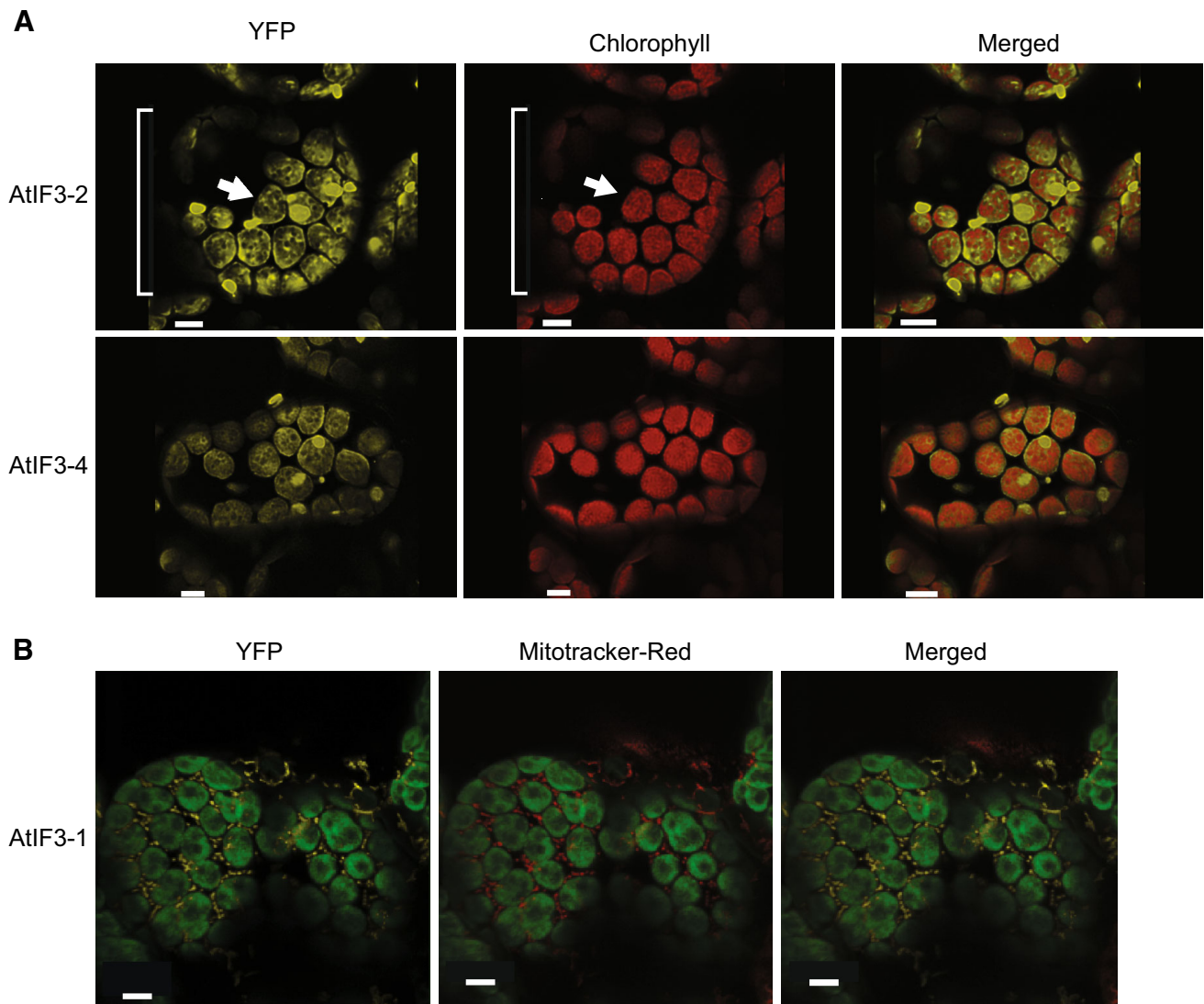


Fig. 2 a Micrograph of fluorescence from *Arabidopsis* cells stably transformed with a gene encoding AtIF3-2 (top row), AtIF3-4 (bottom row) fused at its C-terminus to the yellow fluorescent protein (YFP). In the first two panels of AtIF3-2, brackets denote cell diameter; arrows indicate individual chloroplasts. Fluorescence emission was measured from either YFP (left column), plastid-localized chlorophyll (middle column), or both emissions were merged (right column). Regions of intense yellow fluorescence for both AtIF3-2- and AtIF3-4-transformed cells may be aggregates of YFP-tagged proteins. **b** Micrograph

of fluorescence from *N. benthamiana* transiently transformed with AtIF3-1 fused at its C-terminus to the YFP and injected with Mitotracker-Red. Fluorescence emission was measured from either YFP (left column), Mitotracker-Red (middle column), or merge of both emissions (right column). White bars indicate 5 μ m. Excitation at 514 nm was provided by an argon laser; chlorophyll emission was measured from 650 to 720 nm and YFP emission measured from 525 to 600 nm, and sample preparation and microscopy was carried out as previously described (Ruppel et al. 2011)

did not localize to the chloroplast. Subsequent co-localization studies using AtIF3-1-YFP and Mitotracker-Red demonstrated that AtIF3-1-YFP was targeted to mitochondria in *N. benthamiana* (Fig. 2) (A. Nesbit, C. Whippo, R. Hangarter, and D. Kehoe, unpublished).

The above results do not address two central issues: are these three *Arabidopsis* proteins *bona fide* IF3s, and do AtIF3-2 and AtIF3-4 play unique roles in regulating chloroplast gene expression? We do not yet have direct answers for either of these questions. However, we reasoned that if

AtIF3-2 and AtIF3-4 have different roles in *Arabidopsis* chloroplasts, their expression patterns should differ at different developmental stages and in different tissue and organ types. Using the *Arabidopsis* eFP Browser developed at the University of Toronto (Winter et al. 2007), we examined *AtINFC-2* and *AtINFC-4* mRNAs ratios in many locations and under many conditions and found that these varied widely, with an *AtINFC-2*: *AtINFC-4* mRNA ratio of 12.2 in the cotyledon tissue of the heart embryo (see Fig. 3) and an *AtINFC-4*: *AtINFC-2* mRNA ratio of 50.0 in four-hour post-

germination pollen (Fig. 3; Table 1). These data strongly support the hypothesis that *Arabidopsis* optimizes the expression and use of its IF3s throughout development and in response to various abiotic cues. The results of this analysis are therefore consistent with the proposal that AtIF3-2 and AtIF3-4 have unique roles in regulating chloroplast gene expression throughout the plant life cycle. It can also be predicted that other photosynthetic eukaryotes containing multiple chloroplast-targeted IF3s may also use them to regulate chloroplast gene expression.

How might the use of multiple IF3s control gene expression?

Although the process(es) by which multiple IF3s are able to influence gene expression remains to be discovered, in

this section, we will provide some hypotheses that may be useful starting points for mechanistic investigations. At the outset, it should be noted that the use of multiple IF3s in either prokaryotes or chloroplasts is not an essential capacity, but may provide significantly more variation in the ways that gene expression can be regulated. For cyanobacteria, this additional regulatory layer may be particularly important for species with large genomes and complex responses to environmental cues. A second issue is that because transcription and translation are coupled in cyanobacteria and predominantly uncoupled in plastids, it is hard to imagine that there could be a common regulatory process in cyanobacteria and plastids which involves a translation initiation factor despite their evolutionary relatedness. We propose that the commonly regulated process is the differential efficiency of translation initiation between IF3 family members. We predict that in

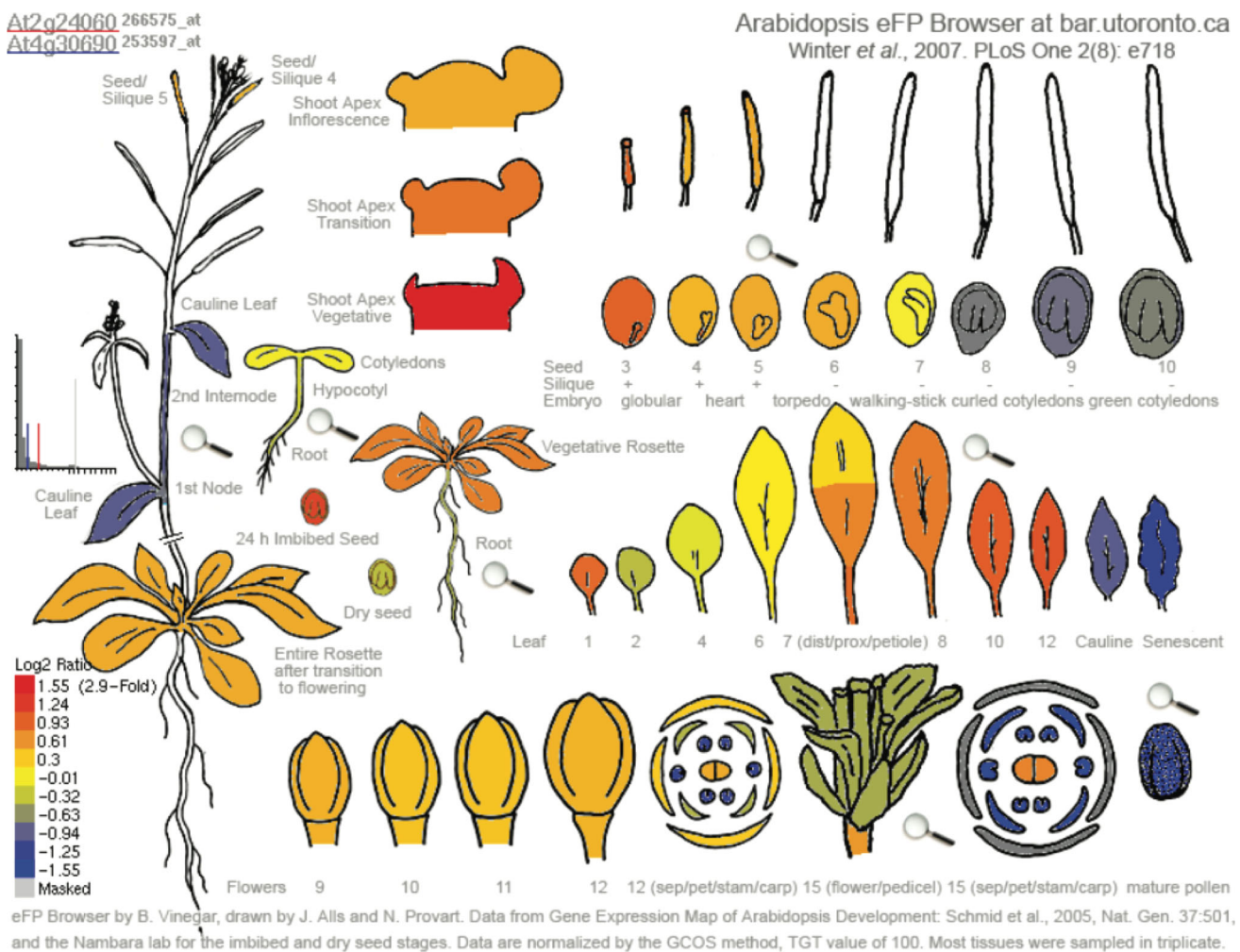


Fig. 3 Using the *Arabidopsis* eFP Browser at the University of Toronto (Winter et al. 2007), *AtINFC-2* (At2g24060) and *AtINFC-4* (At4g30690) mRNAs ratios were calculated in many locations and conditions, a subset of which is shown here. For heat map, a higher

AtINFC-2: AtINFC-4 mRNA ratio (Log2) is represented by red and a higher *AtINFC-4: AtINFC-2* mRNA ratio (Log2) is represented by blue (see scale in lower left corner)

Table 1 *Arabidopsis AtINFC-2* and *AtINFC-4* mRNA Ratios (greater than 4-fold marked in italics)

Location or condition	Fold expression difference		Location or condition	Fold expression difference	
	<i>AtINFC-2:</i> <i>AtINFC-4</i>	<i>AtINFC-4:</i> <i>AtINFC-2</i>		<i>AtINFC-2:</i> <i>AtINFC-4</i>	<i>AtINFC-4:</i> <i>AtINFC-2</i>
<i>Development</i>			<i>Circadian</i>		
Vegetative shoot apex	2.9		LL + 12 °C temp cycle, then constant temp (4 h)		4.0
Mature pollen		10.0	LL + 12 °C temp cycle, then constant temp (8 h)		14.3
Cauline leaf		2.0	LL + 12 °C temp cycle, then constant temp (12 h)		6.3
Senescent leaf		27	LL + 12 °C temp cycle, then constant temp (16 h)		5.0
2nd Internode		2.0	LL + 12 °C temp cycle, then constant temp (20 h)		5.9
Petal (stage 15)		4.5	LL + 12 °C temp cycle, then constant temp (24 h)		5.9
Stamen (stage 15)		4.2	LL + 12 °C temp cycle, then constant temp (28 h)		11.1
Seed (early)	1.9		LL + 12 °C temp cycle, then constant temp (36 h)		12.5
(late)		17	LL + 12 °C temp cycle, then constant temp (40 h)		7.7
<i>Abiotic</i>			LL + 12 °C temp cycle, then constant temp (44 h)		
Cold (3 h)	2.9		<i>Tissue Specific</i>		
Cold (6 h)	2.5		Microgametogenesis		
Osmotic (6 h)	4.0		Pollen (tricellular)		
Osmotic (12 h)	2.9		(Mature)		
Drought (0.25 h)	1.8		(Pollen tubes)		
(6 h)		2.2	(4 h Post-germination)		
UV-B (3 h)	2.3		Embryo (globular/apical)	3.0	
Heat(4 h)		1.9	Embryo (heart/cotyledon)	12.2	
<i>Guard Cell</i>			(Heart/root)	2.1	
Alone		2.6	Embryo (torpedo/root)	5.0	
+50 μM ABA	2.2		(Torpedo/cotyledon)	7.3	
gpa1 leaf +50 μM ABA		6.3	(Torpedo/meristem)	2.4	
<i>Light Sensing</i>			(Torpedo/apical)	4.6	
UV-A or UV-B (5 min)	2.1		(Torpedo/basal)	5.9	
For red light (4 h)	2.5		Xylem		4.5
Blue light (4 h)	2.4		Cork		3.6
White light (4 h)	1.9		<i>Seeds</i>		
			Heart stage (seed coat)		9.9

cyanobacteria, the consequence of this would be variation in the extent of transcriptional/translational coupling, leading to changes in the amount of transcriptional attenuation, while in plastids it would simply alter the degree of translational activity of a specific mRNA or a set of mRNAs (Fig. 4). These hypotheses, and the data supporting them, are explored further below.

Cyanobacteria

In *F. diplosiphon*, the post-transcription initiation regulation of *cpeC* is strongly dependent on a region of the 5' leader approximately 30 nucleotides upstream of the translation start site (Bezy et al. 2011). Mfold predicts that this region folds into a stem-loop with a ΔG of $-3.4 \text{ kcal mol}^{-1}$, sufficiently strong to be folded virtually

always (de Smit 1998) but not strong enough to cause attenuation of transcription by DNA-dependent RNA polymerase. Yet *cpeC* post-transcription initiation regulation appears to be at the level of transcriptional attenuation, since *cpeC* mRNA stability differences were not involved, and differences in extent of translation elongation would have required differences in mRNA stability, which were not detected (Bezy et al. 2011).

Our data also indicate that the amount of IF3 α present in the cell, rather than the IF3 α :IF3 β ratio, was the factor determining *cpeC* mRNA levels, since *infCa* overexpression lowered *cpeC* levels while deletion of *infCb*, which dramatically shifted the IF3 α :IF3 β ratio, had no effect (Gutu et al. 2013). Interestingly, cryo-EM structures show that IF3 is positioned on the 30S subunit in a location relatively close to the 5' leader region of the mRNA

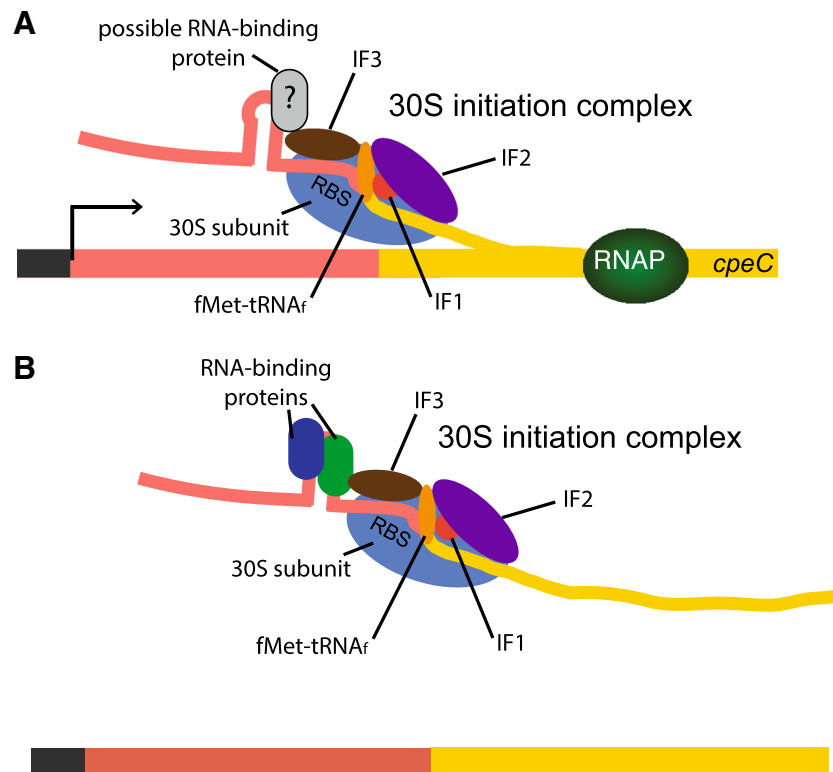


Fig. 4 Model of the possible similarities and differences in the use of IF3 family members in prokaryotes versus eukaryotic chloroplasts. Both groups use IF3 (brown oval), IF2 (purple oval) and IF1 (red circle) to correctly position the initiator fMet-tRNA_f (orange oval) within the 30S subunit (light blue oval) along the mRNA (pink 5' untranslated region, yellow translated region). Some also may use stem-loops 5' of the ribosome binding site (RBS); these have been shown to be binding sites for RNA-binding proteins in chloroplasts (dark blue and green) (see text) and may or may not also bind protein(s) in prokaryotes (gray question mark). **a** In prokaryotes such

as cyanobacteria, translation initiates during transcription by RNA polymerase (RNAP), providing the potential for translational coupling/uncoupling, possibly through the differential usage of IF3 family members and specific RNA-binding protein(s), and regulation of gene expression by transcriptional attenuation. **b** In chloroplasts, significant regulation of gene expression occurs at the level of translation initiation, which often involves stem-loops and RNA-binding proteins that may interact with different IF3 family members to further modify the translatability of specific mRNAs

(Fig. 4) (Julian et al. 2011), and recent single-molecule FRET studies have demonstrated that there is movement of the C-terminal and N-terminal domains relative to each other while IF3 is bound to the 30S subunit (Elvekrog and Gonzalez 2013). Thus, both domains of IF3 are near the 5' leader of the mRNA and could directly or indirectly interact with secondary structures in that region, such as the putative stem-loop adjacent to the start codon of *cpeC* (Fig. 4). IF3s are quite variable in the length and composition of both their N- and C-terminal ends within and between cyanobacterial and plant species (Gutu et al. 2013) (Fig. 5), providing significant potential for variation in how different IF3s might interact with the 5' leader of any given mRNA.

Proposed model

Our model proposes a mechanism for post-transcription initiation regulation of gene expression by alternative IF3s

in cyanobacteria in which the efficiency of translation initiation of each IF3, either by direct interactions with the 5' mRNA leader or with one or more gene-specific RNA-binding proteins as in plants, alters the extent of transcriptional-translational coupling and thus transcriptional attenuation. In addition to controlling such a system by changing IF3 levels, IF3 could be posttranslationally modified and there could be changes in ribosomal protein structure or abundance, or changes in the structure or abundance of RNA 5' leader-binding proteins. This last alternative would provide an explanation for why, in *F. diplosiphon*, our unpublished microarray data show that some genes are both light color and IF3 regulated, while many others genes are only IF3 regulated, since there could be differences in the extent to which the abundance or activity of different RNA-binding proteins is regulated by light color. Also, because many cyanobacterial genes do not contain a canonically positioned SD sequence, 5' stem-loop interactions with IF3 could also help correctly

Arabidopsis1	MAGITSSTVGFNAVFTGITKTVSSHSLSFVDSKLCSLRLSKT-ELSFTNLTPSPRRFAFA	58
glycine	MAGITTFPFK-----LSHCPLFSTSDSKLFLPLSNPYSIKFDPSSLSSTSLPY	49
Nostoc71201	-----	0
Ecoli	-----	0
Nostoc71202	-----MLLRQTNQNKAIKSTLHHLTKKLPQ	25
Arabidopsis1	VTCRF-GGGGGYRFSGDNRG--RPKEAIDEALDISSIRSATVRLIDGQQNMLGLVSK	115
glycine	VTARYYGGGGGASPRSPNDPRSRSKSSDSDDDDKALDLSTLRSDTVRLIDQSQNMVGVVSI	109
Nostoc71201	-----MIDHENNRGLIDT	14
Ecoli	----MKGGKRV-----QT-----ARPNRINGEIRAQEVRLTGLEGEQLGIVSL	39
Nostoc71202	I--IMPVIEKK-----RT-----RDLPQINERIRFPKIRVIDTDSQLGILTP	66
	: . . *::	
Arabidopsis1	DEAVRMADDAELDLVILSPDADPPVVKMMDYSKYRYEQQKRKKDQQKK--TTRMDLKELK	173
glycine	DDAIQMAEDVELDLVILSPDADPPVVRIMDYSKYRYEQQKKKREQQKSAANRMDLKELK	169
Nostoc71201	YEALQLAQSVELDLVVVSQSKDTPVAKILNYGKLQYQKKRQGG-----SARPTVKEVR	68
Ecoli	REALEKAAEEAGVDLVEISPNAEPPVCRIMDYGKFLYEKSSSKEQKKK--QKVIQVKEIK	97
Nostoc71202	QEALQLAEEKELDLVLLSDKADPPVCRIMDYGKYKFEQEKKAREARKK--QHTADVKEVK	124
	*: . * . : *** : * . : * * : : : * . * : : : * : : * : : *	
Arabidopsis1	MGYNIDQHDYSVRLRAAQKFLQDGDVKVIVSMKGRENEFRNIAIELLRRFQTEIGELAT	233
glycine	MGYNIDEHDYSVRLKAAQKFLKDGDKVKVIVNLKRENEFRNMAIGLITRFQNDVVGELGI	229
Nostoc71201	FRPNVGAADYNLRIEQALQWLSKGDVVKFAIRLRGRENQYREQAGMLERIVTDLSQVGK	128
Ecoli	FRPGTDEGDYQVKLRSLIRFLEEGDKAKITLRFGRREMAHQQIGMEVLNRVKDDLQELAV	157
Nostoc71202	MRYKIEEHHDYNVRVKQAEERFLKDGDKVKATVMFRGREIQHSDLAEDLLKRMATDLEPFGE	184
	: * . : : : . : : * . * . * . * : : * * * . : : . : : * . : : . .	
Arabidopsis1	EESK--NFRDRNMFIILVFNKEMIRKQEPPTRKKKKTAENEASASAAEITAEPEPEPEP	291
glycine	EETK--NFRDRNIFIILVFNKAVVQKTQEPKPKRDKSAADEVTTISAQA-----	275
Nostoc71201	VQSLDK---RSLIVQIMPA-----	144
Ecoli	VESFPTKIEGRQMIMVLAPKKKQ-----	180
Nostoc71202	VQQAP-KKEGRNMMLISPCK-----	204
	: * . : : : *	
Arabidopsis1	EPEPEPEPEPEPEPEPLQIDS	312
glycine	-----	275
Nostoc71201	-----	144
Ecoli	-----	180
Nostoc71202	-----	204

Fig. 5 Clustal Omega alignments (Goujon et al. 2010; Sievers et al. 2011) of a sampling of putative chloroplast and prokaryotic IF3s showing the variability in lengths of the N- and C-terminal regions of these proteins. The protein designations are: *Arabidopsis1*, the *Arabidopsis* mitochondria-localized IF3 (AtIF3-1); glycine, *Glycine max* (soybean) (Glyma02g36710.1); Nostoc71201, *Nostoc sp.* PCC 7120 (BAB74067.1); *Ecoli*, *E. coli infC*; and Nostoc71202, *Nostoc sp.*

PCC 7120 (BAB76322.1). Numbering is from the start site of each translated protein. An *asterisk* indicates a position with single, fully conserved residues; a *colon* indicates conservation between groups of strongly similar properties; a *period* indicates conservation between groups of weakly similar properties. The color code is: *red* small (+hydrophobic), *blue* acidic, *magenta* basic, *green* hydroxy, sulfhydryl, amine, and glycine

position the ribosome on the mRNA, augmenting SD usage or replacing it entirely.

Chloroplasts of land plants

The general absence of transcriptional and translational coupling in plastids strongly indicates that the overall

mechanism(s) through which multiple IF3s operate in chloroplasts is likely to be different from those in cyanobacteria (Fig. 4). One way in which IF3s might function to regulate translation of mRNAs in chloroplasts is by increasing the frequency of translation initiation of specific mRNAs. The variety of stem-loops found in the 5' leader regions of plastid mRNAs (Marin-Navarro et al. 2007) and

the many proteins that bind to these regions (Barkan 2011) have the potential to provide tremendous complexity to the regulation of plastid gene expression.

Plastids with multiple IF3s may use one particular IF3 to boost the translation initiation of a specific mRNA as a result of its tighter interactions with the 5' RNA-binding protein(s) and/or stem-loops of that mRNA than exist for the other IF3s. This would result in the creation of more efficient translation initiation complexes and greater protein production levels overall. These IF3–RNA-binding protein interactions may also partially or completely replace the function of the SD sequence in properly positioning the 30S subunit on the mRNA.

Summary

The discovery that cyanobacteria and photosynthetic eukaryotes have multiple IF3s adds an interesting, new layer of complexity to translation initiation in these organisms by expanding the possible roles of IF3s (Gualerzi et al. 2010) as regulators of gene expression (Gutu et al. 2013). How such regulation is achieved mechanistically is an important question, and the models presented in this review hopefully will help to guide such studies. Another central issue is why the capacity to use multiple IF3s exists predominantly in photosynthetic organisms. One possibility is that multiple IF3s and stem-loops in the 5' leader regions of mRNAs existed in the progenitor cyanobacteria that were engulfed during the creation of photosynthetic eukaryotes.

Our proposals for how IF3 families posttranscriptionally regulate gene expression in prokaryotes and eukaryotes also may provide an answer to the interesting question of why “translational modulators in chloroplasts are consistently activators of chloroplast translation, (while) translational regulation in bacteria is generally mediated by negative regulators” (Barkan 2011). In our models, plastid translation is weak unless it is positively amplified by increasing the recruitment of the translation initiation machinery to a completed mRNA, which in many cases may involve specific RNA-binding proteins and, in some species, the “correct” IF3 family member. Conversely, in prokaryotes such as cyanobacteria, strong transcriptional activity from genes such as *cpeC* (Gutu et al. 2013) is acted upon by the translation initiation machinery shortly after transcription initiation and exerts its effect negatively, by attenuating the recently initiated transcription.

Finally, unraveling the roles of IF3 family members in chloroplasts may be of significant agricultural importance, since several economically important crop plants, including soybean and many members of the Brassicaceae family, appear to have multiple chloroplast-localized IF3s. In

Arabidopsis, the genes encoding these proteins have very different patterns of expression during the plant life cycle and in response to many different environmental cues. Some of the strongest differences are during seed development, a critical stage of the life cycle for many commercial crops. The modulation of the expression of different IF3s may allow powerful modifications of plant development in key production stages that lead to improved crop quality and yield.

References

- Agrawal GK, Kato H, Asayama M, Shirai M (2001) An AU-box motif upstream of the SD sequence of light-dependent *psbA* transcripts confers mRNA instability in darkness in cyanobacteria. *Nucleic Acids Res* 29:1835–1843
- Aizawa K, Fujita Y (1997) Regulation of synthesis of PSI in the cyanophytes *Synechocystis* PCC6714 and *Plectonema boryanum* during the acclimation of the photosystem stoichiometry to the light quality. *Plant Cell Physiol* 38:319–326
- Babic S, Hunter CN, Rakhlin NJ, Simons RW, PhillipsJones MK (1997) Molecular characterization of the *pifC* gene encoding translation initiation factor 3, which is required for normal photosynthetic complex formation in *Rhodobacter sphaeroides* NCIB 8253. *Eur J Biochem* 249:564–575
- Barkan A (2011) Expression of plastid genes: organelle-specific elaborations on a prokaryotic scaffold. *Plant Physiol* 155:1520–1532
- Beligni MV, Yamaguchi K, Mayfield SP (2004) The translational apparatus of *Chlamydomonas reinhardtii* chloroplast. *Photosynth Res* 82:315–325
- Bezy RP, Wiltbank L, Kehoe DM (2011) Light-dependent attenuation of phycoerythrin gene expression reveals convergent evolution of green light sensing in cyanobacteria. *Proc Natl Acad Sci USA* 108:18542–18547
- Bovy A, de Vrieze G, Lugones L, van Horssen P, van den Berg C, Borrias M, Weisbeek P (1993) Iron-dependent stability of the ferredoxin I transcripts from the cyanobacterial strains *Synechococcus* species PCC 7942 and *Anabaena* species PCC 7937. *Mol Microbiol* 7:429–439
- Castenholz RW, Waterbury JB (1989) Oxygenic photosynthetic bacteria. Group I. Cyanobacteria. In: Bryant MP, Pfenning N, Holt JG (eds) *JT Staley. Bergey's manual of systematic bacteriology Williams and Wilkins, Baltimore*, pp 1710–1789
- Cheng Y, Kaiser D (1989) *dsg*, a gene required for *Myxococcus* development, is necessary for cell viability. *J Bacteriol* 171:3727–3731
- Cheng YL, Kalman LV, Kaiser D (1994) The *dsg* gene of *Myxococcus xanthus* encodes a protein similar to translation initiation factor IF3. *J Bacteriol* 176:1427–1433
- Davydov II, Wohlgemuth I, Artamonova II, Urlaub H, Tonevitsky AG, Rodnina MV (2013) Evolution of the protein stoichiometry in the L12 stalk of bacterial and organellar ribosomes. *Nat Commun* 4:1387
- de Smit MH (1998) Translational control by mRNA structure in eubacteria: Molecular biology and physical chemistry. In *RNA Structure and Function*, Cold Spring Harbor Press, Cold Spring Harbor, p 495–540
- Deng XW, Grussem W (1987) Control of plastid gene expression during development: the limited role of transcriptional regulation. *Cell* 49:379–387

- Duhring U, Axmann IM, Hess WR, Wilde A (2006) An internal antisense RNA regulates expression of the photosynthesis gene *isiA*. *Proc Natl Acad Sci USA* 103:7054–7058
- Duval M, Korepanov A, Fuchsbaauer O, Fechter P, Haller A, Fabbretti A, Choulier L, Micura R, Klaholz BP, Romby P et al (2013) *Escherichia coli* ribosomal protein S1 unfolds structured mRNAs onto the ribosome for active translation initiation. *Plos Biol* 11:e1001731
- Ehira S, Hamano T, Hayashida T, Kojima K, Nakamoto H, Hiyama T, Ohmori M, Shivaji S, Sato N (2003) Conserved temperature-dependent expression of RNA-binding proteins in cyanobacteria with different temperature optima. *FEMS Microbiol Lett* 225:137–142
- Ehira S, Ohmori M, Sato N (2005) Role of the 5'-UTR in accumulation of the *rhpA1* transcript at low temperature in the cyanobacterium *Anabaena variabilis* M3. *FEMS Microbiol Lett* 251:91–98
- Elvekrog MM, Gonzalez RL (2013) Conformational selection of translation initiation factor 3 signals proper substrate selection. *Nat Struct Mol Biol* 20:628–635
- Filipovska A, Rackham O (2013) Specialization from synthesis: how ribosome diversity can customize protein function. *FEBS Lett* 587:1189–1197
- Fujita Y (1997) A study on the dynamic features of photosystem stoichiometry: accomplishments and problems for future studies. *Photosynth Res* 53:83–93
- Georg J, Hess WR (2011) Cis-antisense RNA, another level of gene regulation in bacteria. *Microbiol Mol Biol Rev* 75:286
- Gilbert WV (2011) Functional specialization of ribosomes? *Trends Biochem Sci* 36:127–132
- Giuliodori AM, Branki A, Gualerzi CO, Pon CL (2004) Preferential translation of cold-shock mRNAs during cold adaptation. *RNA* 10:265–276
- Giuliodori AM, Brandi A, Giangrossi M, Gualerzi CO, Pon CL (2007) Cold-stress-induced *de novo* expression of *infC* and role of IF3 in cold-shock translational bias. *RNA* 13:1355–1365
- Gollnick P, Babitzke P (2002) Transcription attenuation. *Bba-Gene Struct Expr* 1577:240–250
- Goujon M, McWilliam H, Li WZ, Valentin F, Squizzato S, Paern J, Lopez R (2010) A new bioinformatics analysis tools framework at EMBL-EBI. *Nucleic Acids Res* 38 (Suppl):W695–W699
- Gualerzi CO, Fabbretti A, Brandi L, Milon P, Pon CL (2010) Role of the initiation factors in mRNA start site selection and fMet-tRNA recruitment by bacterial ribosomes. *Isr J Chem* 50:80–94
- Gutu A, Nesbit AD, Alverson AJ, Palmer JD, Kehoe DM (2013) Unique role for translation initiation factor 3 in the light color regulation of photosynthetic gene expression. *Proc Natl Acad Sci USA* 110:16253–16258
- Hajnsdorf E, Boni IV (2012) Multiple activities of RNA-binding proteins S1 and Hfq. *Biochimie* 94:1544–1553
- Hamano T, Murakami S, Takayama K, Ehira S, Maruyama K, Kawakami H, Morita EH, Hayashi H, Sato N (2004) Characterization of RNA-binding properties of three types of RNA-binding proteins in *Anabaena* sp PCC 7120. *Cell Mol Biol* 50:613–624
- Henkin TM, Yanofsky C (2002) Regulation by transcription attenuation in bacteria: how RNA provides instructions for transcription termination/antitermination decisions. *BioEssays* 24:700–707
- Herranen M, Tyystjarvi T, Aro EM (2005) Regulation of photosystem I reaction center genes in *Synechocystis* sp strain PCC 6803 during light acclimation. *Plant Cell Physiol* 46:1484–1493
- Hirose T, Sugiura M (1996) Cis-Acting elements and trans-acting factors for accurate translation of chloroplast *psbA* mRNAs: development of an in vitro translation system from tobacco chloroplasts. *EMBO J* 15:1687–1695
- Hirose T, Sugiura M (2004) Multiple elements required for translation of plastid *atpB* mRNA lacking the Shine-Dalgarno sequence. *Nucleic Acids Res* 32:3503–3510
- Horie Y, Ito Y, Ono M, Moriwaki N, Kato H, Hamakubo Y, Amano T, Wachi M, Shirai M, Asayama M (2007) Dark-induced mRNA instability involves RNase E/G-type endoribonuclease cleavage at the AU-box and SD sequences in cyanobacteria. *Mol Genet Genomics* 278:331–346
- Julian P, Milon P, Agirrezabala X, Lasso G, Gil D, Rodnina MV, Valle M (2011) The cryo-EM structure of a complete 30S translation initiation complex from *Escherichia coli*. *PLoS Biol* 9:e1001095
- Kalman LV, Cheng YL, Kaiser D (1994) The *Myxococcus xanthus* Dsg gene product performs functions of Translation Initiation Factor If3 in vivo. *J Bacteriol* 176:1434–1442
- Kaneko T, Sato S, Kotani H, Tanaka A, Asamizu E, Nakamura Y, Miyajima N, Hirosawa M, Sugiura M, Sasamoto S et al (1996) 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* 3:109–136
- Kehoe DM, Gutu A (2006) Responding to color: the regulation of complementary chromatic adaptation. *Annu Rev Plant Biol* 57:127–150
- Komili S, Farny NG, Roth FP, Silver PA (2007) Functional specificity among ribosomal proteins regulates gene expression. *Cell* 131:557–571
- Kulkarni RD, Golden SS (1997) mRNA stability is regulated by a coding-region element and the unique 5' untranslated leader sequences of the three *Synechococcus psbA* transcripts. *Mol Microbiol* 24:1131–1142
- Kulkarni RD, Schaefer MR, Golden SS (1992) Transcriptional and posttranscriptional components of *psbA* response to high light intensity in *Synechococcus* sp. Strain PCC 7942. *J Bacteriol* 174:3775–3781
- Laursen BS, Sorensen HP, Mortensen KK, Sperling-Petersen HU (2005) Initiation of protein synthesis in bacteria. *Microbiol Mol Biol Rev* 69:101–123
- Li R, Golden SS (1993) Enhancer activity of light-responsive regulatory elements in the untranslated leader regions of cyanobacterial *psbA* genes. *Proc Natl Acad Sci USA* 90:11678–11682
- Malys N, McCarthy JEG (2011) Translation initiation: variations in the mechanism can be anticipated. *Cell Mol Life Sci* 68:991–1003
- Manuell AL, Quispe J, Mayfield SP (2007) Structure of the chloroplast ribosome: novel domains for translation regulation. *PLoS Biol* 5:1785–1797
- Marin-Navarro J, Manuell AL, Wu J, Mayfield SP (2007) Chloroplast translation regulation. *Photosynth Res* 94:359–374
- Mayfield SP, Cohen A, Danon A, Yohn CB (1994) Translation of the *psbA* mRNA of *Chlamydomonas reinhardtii* requires a structured RNA element contained within the 5' untranslated region. *J Cell Biol* 127:1537–1545
- Mulo P, Sakurai I, Aro EM (2012) Strategies for *psbA* gene expression in cyanobacteria, green algae and higher plants: from transcription to PSII repair. *BBA-Bioenergetics* 1817:247–257
- Mutsuda M, Sugiura M (2006) Translation initiation of cyanobacterial *rbcS* mRNAs requires the 38-kDa ribosomal protein S1 but not the Shine-Dalgarno sequence - Development of a cyanobacterial in vitro translation system. *J Biol Chem* 281:38314–38321
- Mutsuda M, Sugiura M, Sugita M (1999) Physiological characterization of RNA-binding protein-deficient cells from *Synechococcus* sp strain PCC7942. *Plant Cell Physiol* 40:1203–1209
- Nakagawa S, Niimura Y, Miura K, Gojobori T (2010) Dynamic evolution of translation initiation mechanisms in prokaryotes. *Proc Natl Acad Sci USA* 107:6382–6387

- Omairi-Nasser A, de Gracia AG, Ajlani G (2011) A larger transcript is required for the synthesis of the smaller isoform of ferredoxin: NADP oxidoreductase. *Mol Microbiol* 81:1178–1189
- Peled-Zehavi H, Danon A (2007) Translation and translational regulation in chloroplasts, vol: Topics in Current Genetics. Springer, Berlin
- Plader W, Sugiura M (2003) The Shine-Dalgarno-like sequence is a negative regulatory element for translation of tobacco chloroplast *rps2* mRNA: an additional mechanism for translational control in chloroplasts. *Plant J* 34:377–382
- Rochaix J-D (1996) Post-transcriptional regulation of chloroplast gene expression in *Chlamydomonas*. *Plant Mol Bio* 32:327–341
- Ruppel NJ, Logsdon CA, Whippo CW, Inoue K, Hangarter RP (2011) A mutation in *Arabidopsis* SEEDLING PLASTID DEVELOPMENT1 affects plastid differentiation in embryo-derived tissues during seedling growth. *Plant Physiol* 155:342–353
- Sakamoto T, Bryant DA (1997) Temperature-regulated mRNA accumulation and stabilization for fatty acid desaturase genes in the cyanobacterium *Synechococcus* sp. strain PCC 7002. *Mol Microbiol* 23:1281–1292
- Salem K, van Waasbergen LG (2004) Light control of *hliA* transcription and transcript stability in the cyanobacterium *Synechococcus elongatus* Strain PCC 7942. *J Bacteriol* 186:1729–1736
- Sato N, Nakamura A (1998) Involvement of the 5′-untranslated region in cold-regulated expression of the *rbpA1* gene in the cyanobacterium *Anabaena variabilis* M3. *Nucleic Acids Res* 26:2192–2199
- Schippers JHM, Mueller-Roeber B (2010) Ribosomal composition and control of leaf development. *Plant Sci* 179:307–315
- Schmeing TM, Ramakrishnan V (2009) What recent ribosome structures have revealed about the mechanism of translation. *Nature* 461:1234–1242
- Shaner NC, Campbell RE, Steinbach PA, Giepmans BNG, Palmer AE, Tsien RY (2004) Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp red fluorescent protein. *Nat Biotechnol* 22:1567–1572
- Sharma MR, Wilson DN, Datta PP, Barat C, Schlutzenzen F, Fucini P, Agrawal RK (2007) Cryo-EM study of the spinach chloroplast ribosome reveals the structural and functional roles of plastid-specific ribosomal proteins. *Proc Natl Acad Sci USA* 104:19315–19320
- Shine J, Dalgarno L (1974) The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. *Proc Natl Acad Sci USA* 71:1342–1346
- Shine J, Dalgarno L (1975) Determinant of cistron specificity in bacterial ribosomes. *Nature* 254:34–38
- Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li WZ, Lopez R, McWilliam H, Remmert M, Soding J et al (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539
- Small I, Peeters N, Legeai F, Lurin C (2004) Predotar: a tool for rapidly screening proteomes for N-terminal targeting sequences. *Proteomics* 4:1581–1590
- Springer M, Graffe M, Grunbergmanago M (1977) Characterization of an *Escherichia coli* mutant with a thermolabile Initiation Factor-If3 activity. *Mol Gen Genet* 151:17–26
- Stern DB, Goldschmidt-Clermont M, Hanson MR (2010) Chloroplast RNA metabolism. *Annu Rev Plant Biol* 61:125–155
- Subramanian AR (1993) Molecular genetics of chloroplast ribosomal proteins. *Trends Biochem Sci* 18:177–181
- Sugiura M, Hirose T, Sugita M (1998) Evolution and mechanism of translation in chloroplasts. *Annu Rev Genet* 32:437–459
- Tandeu de Marsac N (1977) Occurrence and nature of chromatic adaptation in cyanobacteria. *J Bacteriol* 130:82–91
- Thomas JC, Ughy B, Lagoutte B, Ajlani G (2006) A second isoform of the ferredoxin : NADP oxidoreductase generated by an in-frame initiation of translation. *Proc Natl Acad Sci USA* 103:18368–18373
- Tiller N, Weingartner M, Thiele W, Maximova E, Schottler MA, Bock R (2012) The plastid-specific ribosomal proteins of *Arabidopsis thaliana* can be divided into non-essential proteins and genuine ribosomal proteins. *Plant J* 69:302–316
- Tyystjarvi T, Herranen M, Aro EM (2001) Regulation of translation elongation in cyanobacteria: membrane targeting of the ribosome nascent-chain complexes controls the synthesis of D1 protein. *Mol Microbiol* 40:476–484
- Tyystjarvi T, Sirpio S, Aro EM (2004) Post-transcriptional regulation of the *psbA* gene family in the cyanobacterium *Synechococcus* sp PCC 7942. *FEBS Lett* 576:211–215
- Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ (2007) An “Electronic Fluorescent Pictograph” browser for exploring and analyzing large-scale biological data sets. *PLoS One* 2:e718
- Yamaguchi K, Subramanian AR (2003) Proteomic identification of all plastid-specific ribosomal proteins in higher plant chloroplast 30S ribosomal subunit - PSRP-2 (U1A-type domains), PSRP-3 alpha/beta (ycf65 homologue) and PSRP-4 (Thx homologue). *Eur J Biochem* 270:190–205
- Yamaguchi K, Prieto S, Beligni MV, Haynes PA, McDonald WH, Yates JR, Mayfield SP (2002) Proteomic characterization of the small subunit of *Chlamydomonas reinhardtii* chloroplast ribosome: identification of a novel S1 domain-containing protein and unusually large orthologs of bacterial S2, S3, and S5. *Plant Cell* 14:2957–2974
- Yamaguchi K, Beligni MV, Prieto S, Haynes PA, McDonald WH, Yates JR, Mayfield SP (2003) Proteomic characterization of the *Chlamydomonas reinhardtii* chloroplast ribosome—identification of proteins unique to the 70 S ribosome. *J Biol Chem* 278:33774–33785
- Zerges W, Rochaix JD (1994) The 5′ leader of a chloroplast mRNA mediates the translational requirements for two nucleus-encoded functions in *Chlamydomonas reinhardtii*. *Mol Cel Biol* 14:5268–5277