Review

Raphael Trösch and Felix Willmund*

The conserved theme of ribosome hibernation: from bacteria to chloroplasts of plants

<https://doi.org/10.1515/hsz-2018-0436>

Received November 19, 2018; accepted January 3, 2019; previously published online January 17, 2019

Abstract: Cells are highly adaptive systems that respond and adapt to changing environmental conditions such as temperature fluctuations or altered nutrient availability. Such acclimation processes involve reprogramming of the cellular gene expression profile, tuning of protein synthesis, remodeling of metabolic pathways and morphological changes of the cell shape. Nutrient starvation can lead to limited energy supply and consequently, remodeling of protein synthesis is one of the key steps of regulation since the translation of the genetic code into functional polypeptides may consume up to 40% of a cell's energy during proliferation. In eukaryotic cells, downregulation of protein synthesis during stress is mainly mediated by modification of the translation initiation factors. Prokaryotic cells suppress protein synthesis by the active formation of dimeric so-called ‘hibernating’ 100S ribosome complexes. Such a transition involves a number of proteins which are found in various forms in prokaryotes but also in chloroplasts of plants. Here, we review the current understanding of these hibernation factors and elaborate conserved principles which are shared between species.

Keywords: 100S ribosomes; energy availability; hibernation factors; protein synthesis; starvation; stringent response.

Introduction

Conditions that allow constant logarithmic growth, such as in laboratory set-ups, are not prevalent in nature.

*Corresponding author: Felix Willmund, Department of Biology, Molecular Genetics of Eukaryotes, University of Kaiserslautern, Paul-Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany, e-mail: willmund@bio.uni-kl.de. <https://orcid.org/0000-0002-3988-4590>

Raphael Trösch: Department of Biology, Molecular Genetics of Eukaryotes, University of Kaiserslautern, Paul-Ehrlich-Straße 23, D-67663 Kaiserslautern, Germany

Under nutrient-limiting conditions, single-celled organisms like most bacteria transition from steady growth into a survival state termed the stationary phase. In fact, microorganisms in such resting phases seem to account for more than half of the biomass in our ecosystem (Gray et al., 2004). Survival under prolonged periods of nutrient-limiting conditions requires the acquisition of adaptation strategies in order to cope with fluctuating nutrient availabilities. Such an adaptation involves the modulation of gene expression, protein synthesis, metabolic pathways, the cell cycle and the formation of resistant cells (as for many Gram-negative bacteria) or even dormant spores (as for many Gram-positive bacteria) (Kolter et al., 1993). For heterotrophic bacteria, limiting carbon and hence energy supply is caused by external nutrition availability in the growth medium and it is the common trigger for the transition into the stationary phase. In contrast, photoautotrophic organisms, including cyanobacteria and plants, may suffer starvation by limitation of nitrogen and phosphate availability in the medium/soil while energy availability is directed by diurnal light-dark cycles.

Ribosome hibernation is one prominent molecular strategy to modulate protein synthesis during starvation and stress and is found in both prokaryotic and eukaryotic cells (Wilson and Nierhaus, 2007; Vila-Sanjurjo, 2008). Ribosomes are the ancient and highly conserved machineries that translate the genetic code into functional proteins. Protein synthesis normally involves three stages: initiation, elongation and termination. During prokaryotic-type translation, initiation is characterized by the binding of the small ribosomal subunit, also called 30S subunit, to the 5'-untranslated region of the messenger RNA (mRNA), forming a complex together with the three initiation factors IF1-3. Then the large ribosomal subunit, also called the 50S subunit, attaches to the initiation complex forming the full 70S ribosome. Elongation commences by the binding of the initiator-tRNA(Met) to the P-site of the 50S subunit, and then continues by the binding of further tRNAs according to the codon-anticodon pairing in the A-site of the 50S subunit, the formation of the peptide bond between the nascent chain on the P-site tRNA and the amino acid on the A-site tRNA, and the shift of P- and A-site tRNAs to

the E- and P-sites, respectively. Uncharged E-site tRNAs are released, and the A-site is free for binding of the next amino-acyl tRNA. Finally, translation is terminated when a stop codon reaches the A-site, which triggers the release of the nascent chain and disassembly of the 70S ribosome (reviewed in Green and Noller, 1997).

Under optimal growth of *Escherichia coli* (*E. coli*), it was estimated that the synthesis of proteins and rRNA consumes 80% of all ATP which is designated for biomass production (Stouthamer and Bettenhausen, 1973; Tempest and Neijssel, 1984; Maitra and Dill, 2015). Thus, reducing overall translation output is essential for survival under energy-limiting conditions. Over recent years, a number of so-called ‘hibernation factors’ were characterized which block the process of translation in two ways: either by the formation of 100S ribosomes by head-to-head joining of both 30S subunits of two ribosomes, or by the stabilization of ‘empty’ 70S ribosomes via conformational changes induced by the binding of the hibernation factor (reviewed in Gohara and Yap, 2018). In both cases no mRNA is bound to the 30S subunit, therefore these hibernating ribosomes are translationally silent. The stabilized state of the assembled ‘empty’ ribosome prevents initiation as that would require the disassembly of the ribosome in order for the 30S subunit to bind to mRNA. Translation is only resumed when the hibernation factors are removed, for example by the action of ribosome recycling factors which allow ribosome disassembly into 30S and 50S subunits and subsequent re-initiation. In this review we focus on the specific biological function of the individual hibernation factors and emphasize regulatory principles which are universally shared by bacteria, cyanobacteria and the chloroplast of plants. Of note, chloroplasts contain a prokaryotic-type gene expression machinery which evolved from the gene expression machinery of formerly free-living cyanobacteria and is now perfectly integrated and regulated in order to match the requirements within the plant cell (reviewed in Zoschke and Bock, 2018). Since the structural features of 100S formation were recently covered by a comprehensive review of Gohara and Yap (2018), mechanistical details of hibernation factor-induced 100S ribosome formation will be only briefly described unless new information is available.

Hibernation of translation in gammaproteobacteria

Escherichia coli is the best-understood system of how gene expression is reprogrammed during starvation (Ishihama,

2018). A major role is attributed to the transcription regulator sigma factor S (RpoS), which accumulates in the stationary phase and has classical sigma factor properties (Loewen and Triggs, 1984; Tanaka et al., 1993, 1995). Furthermore, global regulators such as cAMP-CRP, FIS, H-NS, IHF, OmpR and ppGpp regulate gene expression in the stationary phase (reviewed in Pletnev et al., 2015). Under nutrient replete conditions, RpoS is still expressed but quickly targeted for degradation by the AAA + ATPase ClpXP protease system (Baker and Sauer, 2012), a process which is mediated by the adaptor protein SprE (Muffler et al., 1996; Pratt and Silhavy, 1996). Degradation of RpoS via ClpXP requires high levels of ATP, and consequently it is the reduced ATP level caused by nutrient limitation at the beginning of the stationary phase which is the direct signal that leads to the repression of RpoS degradation (Peterson et al., 2012). In the stationary phase, RpoS competes with the housekeeping sigma factor RpoD in binding to the RNA polymerase complex and modulates its promoter recognition in order to target it to a specific set of genes that are required for survival under nutrient limiting conditions (Tanaka et al., 1993, 1995). This set is composed of roughly 500 proteins, or 10% of the *E. coli* proteome, including a number of ribosome hibernation factors, and many other genes that act during general stress survival (Weber et al., 2005) (Figure 1).

The expression of RpoS has been shown to be positively regulated by the intracellularly acting alarmone guanosine 5'-diphosphate 3'-diphosphate or guanosine 5'-triphosphate 3'-diphosphate [referred to as (p)ppGpp hereafter] (Gentry et al., 1993). During the so-called stringent response of starving *E. coli* cells, (p)ppGpp is synthesized at the ribosome via the (p)ppGpp synthetase, Relaxed (RelA), which is activated upon sensing the accumulation of uncharged tRNAs on the ribosome which in turn is attributed to low amino acid levels in cells (reviewed in Chatterji and Ojha, 2001) (Figure 1). RelA generates guanosine pentaphosphate by transfer of a pyrophosphate from ATP to GTP, which is then dephosphorylated to (p)ppGpp by the guanosine pentaphosphate hydrolase. The regulator (p)ppGpp then targets RNA polymerase in order to downregulate the transcription of rRNA and tRNA and thus reduce ribosome number (reviewed in Chatterji and Ojha, 2001), and it directly regulates a number of metabolic enzymes and signaling factors (reviewed in Kanjee et al., 2012). Additionally, increased levels of (p)ppGpp compete with GTP for the binding to the GTP-dependent translation initiation factor IF2 and the elongation factor EF-G (Milon et al., 2006; Mitkevich et al., 2010). There is a close connection between this stringent response and the stationary phase, and nutrient

Figure 1: Mechanism of ribosomal hibernation in the gammaproteobacterium *E. coli*.

Under nutrient-rich conditions (white background), a high energy state in form of ATP is maintained which allows a high activity of the ATP-dependent protease ClpXP (left part). The ClpXP target RpoS is thus degraded and cannot interfere with the transcription by the RNA polymerase (middle part). Under these conditions, ribosomes are fully active and protein synthesis can progress normally (right part). Under carbon starvation conditions (gray background), ATP levels drop due to energy limitation. This leads to an inefficient activity of ClpXP and thus to the accumulation of RpoS (left part). This stress-related sigma factor can now bind to the RNA polymerase and modify the set of transcribed genes to both stress-related genes and genes coding for hibernation factors such as HPF_{short} and pY (middle part). Hibernation factors bind to ribosomes and lead to the formation of inactive forms of ribosomes (both 100S ribosomes and inactive 70S ribosomes) and hence to translational silencing (right part). Additionally, RpoS-dependent RelA accumulates and converts GTP to (p)ppGpp (for simplicity 'ppGpp'). This (p)ppGpp then binds to RNA polymerase, promotes the transcription of RMF and SRA and inhibits the transcription of rRNA and tRNA leading to a reduction of ribosome biogenesis (stringent response).

starvation is the common theme (Navarro Llorens et al., 2010). Nutrient-starved stationary cultures induce translational shutdown by the stringent response via (p)ppGpp, and it is interesting that in gammaproteobacteria the very same regulator is also necessary for the expression of RpoS, the putative hibernation factor SRA and the hibernation factor RMF (Izutsu et al., 2001a,b) (see below). Thus, both the reduction of ribosome biogenesis and the hibernation of existing ribosomes are the effects of the same response.

The small (6.5 kDa) ribosome modulation factor (RMF) is only expressed in gammaproteobacteria but not found in other bacteria (Ueta et al., 2008) (Table 1). RMF is typically produced under nutrient starvation and stress conditions, and more recently it was observed that RMF expression is induced under nitrogen limitation in *E. coli* (Sanchuki et al., 2017) and under anoxic conditions in *Pseudomonas aeruginosa* (*P. aeruginosa*) (Williamson et al., 2012). Upon replenishment with fresh medium, the *rmf* transcript is rapidly degraded (Aiso et al., 2005). This degradation is dependent on protein *de novo* synthesis of, most likely, RNase E enzyme (Aiso et al., 2005). RMF binds at the backside of the ribosomal 30S subunit in a cavity which is formed by the structural proteins uS2, uS7, uS9 and bS21. It further contacts the 16S rRNA and stabilizes the large ribosomal protein bS1 in a compact conformation (Beckert et al., 2018). By this, RMF positions

the anti-Shine-Dalgarno sequence of the 16S rRNA in a conformation which is incapable to form Shine-Dalgarno hybrids between the leader of the translated mRNA and the anti-Shine-Dalgarno sequences of the 16S rRNA, which is essential for translation initiation of many transcripts in prokaryotes (Shine and Dalgarno, 1974). In addition, chemical crosslinking experiments and chemical probing by dimethyl sulfate postulated that RMF also blocks the entrance of the peptide exit tunnel (Yoshida et al., 2002, 2004). During ribosome hibernation through 100S formation, RMF works in concert with the YhbH protein, which was later called hibernation promoting factor (HPF) (Ueta et al., 2005). If a culture of stationary phase bacteria is diluted with fresh medium, RMF disassociates from 100S ribosomes within less than a minute (Aiso et al., 2005). RMF alone leads only to the formation of 90S ribosomes, which were thought to correspond to immature forms of the 100S ribosome, suggesting that HPF is needed to convert 90S ribosomes to mature 100S ribosomes (Ueta et al., 2005, 2008, 2013). HPF in gammaproteobacteria is only half the size (~11 kDa) of the orthologous form found in other prokaryotes and the chloroplasts of plants and is thus also termed HPF_{short} (Maki et al., 2000; Ueta et al., 2008) (Figure 2). *In vitro*, HPF_{short} binds ribosomes but does not lead to 100S formation in the absence of RMF (Ueta et al., 2008). Thus, both RMF and HPF_{short} are necessary for ribosome hibernation in *E. coli*, with RMF initiating

Table 1: Summary of hibernation factors.

Organism	Hibernating ribosome structure	Involved factors	Mutant phenotype	References
Gammaproteobacteria	100S during starvation	RMF	Loss of membrane integrity, poor survival of long-term starvation	Yoshida et al., 2002, 2004; Aiso et al., 2005; Ueta et al., 2008; Williamson et al., 2012; Sanchuki et al., 2017; Beckert et al., 2018
	100S during starvation	HPF _{short}	No 100S ribosome formation	Maki et al., 2000; Ueta et al., 2005, 2008, 2013; Beckert et al., 2018
	70S stabilization	pY/YfiA	Ribosome degradation	Agafonov et al., 1999, 2001; Agafonov and Spirin 2004; Maki et al., 2000; Vila-Sanjurjo et al., 2004; Sato et al., 2009; Di Pietro et al., 2013; Basu and Yap, 2017
	Inhibition of 70S formation	RsfA*	Reduced survival during starvation	Coe et al., 1988; Byrne and Taylor, 1996; Jiang et al., 2007; Häuser et al., 2012; Rorbach et al., 2012; Wanschers et al., 2012; Fung et al., 2013; Li et al., 2015
Firmicutes		SRA	Non-essential	Izutsu et al., 2001a,b
	100S during all growth phases	HPF _{long}	Ribosome degradation, reduced survival rate in long-term culture	Basu et al., 2018; Basu and Yap 2017; Franken et al., 2017; Khusainov et al., 2017; Matzov et al., 2017; Ueta et al., 2010
Cyanobacteria	100S during dark	LrtA	Delayed growth after starvation	Tan et al., 1994; Samartzidou and Widger 1998; Galmozzi et al., 2016; Contreras et al., 2018
Chloroplasts of algae and plants	70S stabilization	PSRP1	Unknown	Johnson et al., 1990; Sharma et al., 2007; Sharma et al., 2010; Ahmed et al., 2017; Bieri et al., 2017; Graf et al., 2017a,b; Boerema et al., 2018
Eukaryotic cytosol	80S	Stm1p	Reduced survival during starvation	Van Dyke et al., 2006
	110S	unknown		Krokowski et al., 2011

*Seems also present in mitochondria and chloroplasts of eukaryotes.

Figure 2: Comparison of domain topology between hibernation promoting factors and pY/YfiA.

Gammaproteobacteria contain the two homologous proteins short hibernation promoting factor (HPF_{short}) and pY/YfiA. HPF_{short} and pY/YfiA share a homologous domain (brown) with characteristic secondary structures of alpha-helices and beta-sheets. pY/YfiA carries an additional C-terminal extension (purple). HPF_{long} of Firmicutes, LrtA of cyanobacteria and PSRP1 of chloroplasts in plants share the characteristic HPF domain with HPF_{short} and pY/YfiA. In addition, the sequences are C-terminally extended by another domain (light blue). Chloroplast PSRP1 is translated as a precursor with an N-terminal chloroplast transit peptide (light green, cpTP) which is cleaved off upon successful import into the organelle.

dimerization and HPF_{short} taking over to complete 100S dimerization (Ueta et al., 2013). In fact, HPF_{short} has been shown to be essential for 100S formation in *E. coli*, as *hpf* mutants do not form 100S ribosomes any more (Ueta et al., 2005) (Table 1). Recent single-particle cryo-electron microscopy data of 70S hibernating *E. coli* ribosomes derived from dimeric ribosome complexes demonstrated that HPF_{short} binding takes place at the 30S ribosomal subunit within the A- and P-sites which occludes the binding of messenger RNAs (Beckert et al., 2018), a finding which is consistent with previous observations on low resolution structures (Vila-Sanjurjo et al., 2004; Polikanov et al., 2012). Furthermore, contacts are built with the anticodon stem loop of tRNAs in the E-site position. While it has been postulated that 100S dimerization happens via both 30S subunits of the two 70S ribosomes in gammaproteobacteria (Yoshida et al., 2002), the direct contribution of RMF and HPF_{short} in this process remained disputed for many years. A recent study demonstrated that both factors seem to indirectly support 70S dimerization through the stabilization of the ribosomal proteins bS1 and uS2 which then form bridges to uS4 and uS3 of the opposite 30S subunit (Beckert et al., 2018). Interestingly, Wilson and colleagues

also observed that their hibernating 100S ribosomes of *E. coli* frequently contained deacylated tRNAs. This matches well with previous reports that uncharged tRNAs accumulate at high levels in starving cells (Haurlyuk et al., 2015) and it suggests that the binding of deacylated tRNAs may label ribosomes for 100S formation (Beckert et al., 2018).

Gammaproteobacteria express an additional hibernation factor, the 13 kDa sized YfiA, or pY, which possesses a conserved region near the N-terminus with homology to short and long HPFs (Yoshida and Wada, 2014) (Table 1). YfiA/pY was discovered in *E. coli* to bind to the 30S subunit close to the subunit interface, and to stabilize ribosomes against dissociation (Agafonov et al., 1999). YfiA/pY accumulates in the stationary phase like HPF, but while HPF binds to 100S ribosomes, YfiA/pY could only be found in 70S particles (Maki et al., 2000). Low-resolution structures indicate that YfiA/pY binding shields the ribosomal A- and P-sites from initiator tRNA binding (Vila-Sanjurjo et al., 2004) and it was shown that YfiA/pY binding inhibits translation and stabilizes 70S particles against subunit dissociation (Agafonov et al., 1999; Vila-Sanjurjo et al., 2004). There is accumulating evidence that YfiA/pY does not participate in 100S dimerization but rather acts as an antagonist to HPF_{short}. First, YfiA/pY deficient mutants formed more 100S ribosomes than wild-type cells and, secondly, the binding sites of YfiA/pY and HPF_{short} overlap which suggests that they have competing roles (Ueta et al., 2005). This is surprising as the structures of YfiA/pY and HPF are very similar, both consisting mainly of a conserved β - α - β - β - α fold (Sato et al., 2009) (Figure 2). The only differences are in the second alpha helix at the C-terminus (Sato et al., 2009), and these differences might determine if the protein enforces or prevents 100S dimerization. Like RMF, YfiA/pY is absent in Firmicutes and other bacteria (Basu and Yap, 2017), and thus might play a more specialized role in gammaproteobacteria. Interestingly, YfiA/pY not only accumulated in the stationary phase but also under low temperature conditions, leading to the term ‘cold shock protein’ (Agafonov et al., 2001; Vila-Sanjurjo et al., 2004). Multiple findings suggest that YfiA/pY protects unused ribosomal subunits from degradation during unfavorable growth conditions (Maki et al., 2000; Vila-Sanjurjo et al., 2004; Di Pietro et al., 2013), and it was shown that translation reduction caused by YfiA/pY only affects a subset of mRNAs during a 10°C cold shock (Di Pietro et al., 2013). Although recent studies suggest that YfiA/pY containing 70S particles are translationally silent, Agafonov and Spirin (2004) reported an interesting observation that YfiA/pY may not directly inhibit translation in *in vitro* translation assays during temperature

downshift but rather counteracts the mis-incorporation of non-cognate aminoacyl-tRNAs during translation. At least *in vitro*, YfiA/pY seems to lower the affinity of charged tRNAs to the ribosomal A-site which facilitates ribosomes to better discriminate between cognate and non-cognate association of tRNAs to the A-site (Agafonov and Spirin, 2004). This fits well with the findings that lowering growth temperatures increases risk of miscoding during translation (Friedman and Weinstein, 1964; Manley and Gesteland, 1978). Interestingly, YfiA/pY is dispensable for translational silencing during cold adaptation as YfiA/pY mutants still show a shutdown of protein synthesis (Di Pietro et al., 2013). Thus, the role of YfiA/pY might rather be to partially inhibit translation initiation in order to fine-tune the cold response for specific mRNAs and thus increase translation fidelity, while other factors are responsible for a more general translational shutdown.

Early two-dimensional gel electrophoresis studies of *E. coli* ribosomes identified another small protein (5 kDa), originally termed protein D, S22 or RpsV, which associated exclusively with the small ribosomal subunit (Wada, 1986) (Table 1). The abundance of this protein was observed to be significantly increased in the stationary phase compared with the exponential phase, and transcription was shown to be also dependent on the stationary-phase specific sigma factor S, RpoS (Izutsu et al., 2001a,b; Lacour and Landini, 2004). Therefore, protein D was renamed stationary-phase-induced ribosome associated protein (SRA) (Izutsu et al., 2001a,b). So far, the exact function and binding-mode on ribosomes is not clear. SRA is not essential in *E. coli* but a quadruple knockout of the four components SRA, RMF, YfiA/pY and HPF_{short} shows an impaired survival in the stationary phase compared to the *rmf* knockout alone (Bubunenko et al., 2007). Thus, it was suggested that SRA supports RMF function and thus contributes to hibernation (Bubunenko et al., 2007).

Hibernation of translation in Firmicutes

Firmicutes, including the large subgroup of Gram-positive bacteria, share some features of gammaproteobacteria in terms of ribosome hibernation during stress or nutrient starvation. However, important differences were observed for the regulation of gene expression and the nature of hibernation factors. The transcription factor RpoS is not found in Firmicutes but instead the sigma factor B, SigB, regulates a similar general stress response (Hecker et al., 2007). SigB-regulated reprogramming of

gene expression is fairly well studied in *Bacillus subtilis* (*B. subtilis*) (reviewed in Hecker et al., 2007). SigB activity is tuned by reversible serine and threonine phosphorylation during regular growth or stress, and its activity is controlled by the anti-sigma factor RsbW and the antagonist protein RsbV. Under optimal growth conditions, RsbW forms a stable complex with SigB and thereby inactivates the transcription factor. At the same time RsbW inactivates RsbV through phosphorylation. When energy depletion causes a drop of subcellular ATP levels (Zhang and Haldenwang, 2005), RsbV is dephosphorylated and thus activates SigB by disrupting the complex with RsbW. Such RsbV dephosphorylation is achieved by RsbP which was shown to sense, together with RsbQ, cellular energy levels (Voelker et al., 1995; Moore et al., 2004; Zhang and Haldenwang, 2005). In addition, low levels of ATP seem to critically limit the kinase activity and thus inactivate RsbW function (Alper et al., 1996). Release of SigB complements the RNA polymerase for the specific transcription of stress-induced genes (Hecker et al., 2007) (Figure 3).

A hallmark for stringent control is the repression of rRNA transcription and the reduction of cellular ribosomal particles upon nutrient starvation. Such responses were also observed for Firmicutes, but targets of synthesized (p)ppGpp seem species-specific and show remarkable differences compared with gammaproteobacteria (reviewed in Wolz et al., 2010) (Figure 3). For example, repression of rRNA synthesis seems not mediated by the

direct interaction of (p)ppGpp with the RNA polymerase in *B. subtilis* (Krásny and Gourse, 2004), but rather affects gene expression indirectly via the modulation of intracellular nucleotide pools (Krásny and Gourse, 2004, Krásny et al., 2008). RelA-type (p)ppGpp synthetases also exist in Firmicutes. However, it is not clear to date if RelA is also activated by the binding of uncharged tRNAs to ribosomes (Wolz et al., 2010). Similar to the up regulation of hibernation factors in *E. coli*, (p)ppGpp promotes the increased synthesis of the hibernation factor HPF_{long} in *B. subtilis* and induces 100S ribosome formation (Tagami et al., 2012). Contrary to *E. coli*, Firmicutes possess a long variant of HPF (HPF_{long}) but RMF and YfiA/pY are absent (Ueta et al., 2008). Interestingly, most bacteria outside the gammaproteobacterial class achieve 100S formation solely through the action of HPF_{long} (Ueta et al., 2010), and it is likely that a part of HPF_{long} is able to adopt the function of RMF in Firmicutes (Ueta et al., 2013). HPF_{long} synthesis in Firmicutes is not only controlled by SigB but also by the sporulation regulator SigH and potentially also by the housekeeping transcription factor SigA (Drzewiecki et al., 1998; Majerczyk et al., 2010; Waters et al., 2016). Additionally, SigB is also controlled by the transcription factor CodY which acts upstream of SigB, and consequently 100S ribosomes are observed during all growth stages (Ueta et al., 2010; Basu and Yap, 2017; Basu et al., 2018). Deletion of HPF_{long} in *Staphylococcus aureus* (*S. aureus*) is not lethal; however, severe degradation

Figure 3: Mechanism of ribosomal hibernation in the Gram-positive *B. subtilis*.

Under nutrient-rich conditions (white background), a high energy state in form of ATP is maintained which allows the kinase RsbW to inactivate RsbV by phosphorylation, and at the same time to form a complex with the sigma factor SigB (left part). SigB is thus inactivated and cannot interfere with the transcription by the RNA polymerase (middle part). Under these conditions, ribosomes are fully active and protein synthesis can progress normally (right part). Under carbon starvation conditions (gray background), ATP levels drop due to energy limitation. This leads to dephosphorylation (via RsbP and RsbQ, not shown) and hence activation of RsbV which now breaks the complex between RsbW and SigB, thus activating SigB (left part). This stress-related sigma factor can now bind to the RNA polymerase and modify the set of transcribed genes to both stress-related genes and genes coding for hibernation factors such as HPF_{long} (middle part). HPF_{long} binds to ribosomes and leads to the formation of inactive 100S ribosomes and hence to translational silencing (right part). Additionally, the alarmone (p)ppGpp (for simplicity 'ppGpp') is synthesized by RelA. (p)ppGpp accumulation leads to a depleted GTP pool and indirectly to a reduced overall gene expression (stringent response).

of ribosomes was observed which reduces the survival rate of mutant cells during long-term cultivation (Basu and Yap, 2017). Furthermore, ribosome profiling experiments comparing *S. aureus* wild type and HPF_{long} mutants showed that loss of the hibernation factor leads to a profound increase of ribosome occupancy on the 5' end of certain mRNAs during starvation which could point to a function of HPF_{long} in suppressing translation initiation on hibernating ribosomes (Basu and Yap, 2017).

Cryo-EM studies of *Lactococcus lactis* and *S. aureus* could show that the C-terminus of HPF_{long} protrudes out of the 30S subunit and thus likely mediates dimerization while the N-terminus corresponds to the short HPF of gammaproteobacteria (Franken et al., 2017; Khusainov et al., 2017; Matzov et al., 2017). Thus, unlike RMF, the C-terminus of HPF_{long} directly contacts the dimer interface (Franken et al., 2017), which potentially is the cause for the more stable 100S ribosomes in Firmicutes compared with gammaproteobacteria (Ueta et al., 2013).

Taken together, 100S ribosome formation of ribosomes during starvation in Firmicutes seems mainly achieved by the HPF_{long} protein and hibernation seems not to require additional factors such as RMF and YfiA/pY as observed in gammaproteobacteria. The exclusive expression of RMF under starvation conditions in gammaproteobacteria suggests that this factor might have evolved specifically to delimit hibernation to starvation conditions and allow the full translational potential under optimal conditions.

Hibernation of translation in cyanobacteria

Comparable to Firmicutes, cyanobacteria contain sigma factor B, SigB, which is multifunctional and regulates gene expression in a large variety of stresses such as heat, osmotic or oxidative stress as well as nitrogen starvation (Imamura and Asayama, 2009). Interestingly, in day-night adapted cyanobacteria, SigB is also induced in the oxidative state of the dark phase and might contribute to the accommodation to darkness (Imamura and Asayama, 2009). Under light, SigB is inactive through the repressive action of SigC on SigB (Imamura et al., 2003) (Figure 4). In the cyanobacterium *Synechococcus* sp. PCC 7002, a transcript was detected that accumulates in the dark, but is rapidly degraded in light (Tan et al., 1994). This transcript, called *light repressed transcript A* (*LrtA*), is translated only upon reillumination of dark-adapted cells (Tan et al., 1994), suggesting that the transcripts are translated just before degradation. Protein synthesis seems to be required

for light-induced degradation of the *LrtA* transcript as degradation is partially inhibited by chloramphenicol (Samartzidou and Widger, 1998). A phylogenetic analysis concluded that LrtA most likely corresponds to the long HPF of Firmicutes (Galmozzi et al., 2016). Additionally, LrtA alone can trigger 100S formation in cyanobacteria in the dark which is consistent with its homologous form in Firmicutes (Hood et al., 2016) (Table 1). Accordingly, the N-terminus of LrtA adopts the conserved β - α - β - β - α fold of bona fide HPF proteins while the C-terminus is disordered (Contreras et al., 2018) (Figure 2). SigB is also responsible for the expression of this cyanobacterial ribosome hibernation factor LrtA in the dark (Imamura et al., 2003, 2004), suggesting that in photosynthetic cyanobacteria, accommodation to darkness might be regulated in an analogous way to the response to nutrient limitation in the stationary phase of heterotrophic bacteria. Furthermore, the circadian clock is necessary for LrtA transcript accumulation as clock mutants show an aberrant accumulation of LrtA transcripts in the light and less accumulation in the dark (Dörrich et al., 2014). Interestingly, the levels of the alarmone (p)ppGpp increase in the dark, resembling the situation in stationary phase *E. coli* (Hood et al., 2016). In mutants unable to synthesize (p)ppGpp, growth defects occur specifically in darkness, and LrtA fails to accumulate in the dark while it even accumulates in the light when (p)ppGpp is added externally (Hood et al., 2016). This suggests that in cyanobacteria starvation is firmly integrated in the circadian rhythm in the form of darkness, and that the mechanisms to suppress translation in the dark starvation phase have been adapted from the corresponding starvation responses of heterotrophic bacteria (Figure 4). But as the absence of LrtA in mutants does not result in drastic phenotypes (Galmozzi et al., 2016), it is difficult to define a role for 'dark-hibernation' in photosynthetic cyanobacteria. Prolonged starvation leads to delayed recovery of mutants compared with wild type (Galmozzi et al., 2016), suggesting that LrtA and dark hibernation might serve as an energy saving mechanism to prevent wasteful translation in the dark. In addition, LrtA might play a role in stabilization of 70S ribosomes since mutants lacking LrtA in *Synechocystis* have an increased amount of dissociated ribosomal subunits (Galmozzi et al., 2016).

Hibernation of translation in the chloroplast of eukaryotes

After endosymbiosis and the conversion of free-living organism to plant organelles, chloroplasts retained small,

Figure 4: Mechanism of ribosomal hibernation in photosynthetic cyanobacteria.

Under light conditions (day, white background), a reduced state is maintained by the activity of photosynthesis, which allows the sigma factor SigC to repress the expression of SigB (left part). SigB is thus inactivated and cannot interfere with the transcription by the RNA polymerase (middle part). Under these conditions, ribosomes are fully active and protein synthesis can progress normally (right part). Under dark conditions (night, gray background), the oxidized state inhibits the repressive action of SigC on SigB, and SigB accumulates (left part). This stress-related sigma factor can now bind to the RNA polymerase and modify the set of transcribed genes to both stress-related genes and genes coding for hibernation factors such as *LrtA* (middle part). *LrtA* binds to ribosomes and leads to the formation of inactive 100S ribosomes and hence to translational silencing (right part). Note that due to the regularity of light-dark cycles, *LrtA* expression is also regulated by the circadian clock (indicated by a clock symbol). Additionally, RelA converts GTP to (p)ppGpp (for simplicity 'ppGpp') and (p)ppGpp accumulation leads to an increased expression of *LrtA* and possibly other stress-related genes (stringent response, white background).

circular genomes which contain 50–200 protein-coding genes (Bock, 2007). Accordingly, expression of these genes is achieved by a machinery which is still homologous to the prokaryotic machinery (Gray, 1993). However, gene expression within plastids is tightly coordinated with processes in the plant cell. This is achieved through the action of multiple nuclear-encoded factors, which are imported into chloroplasts and mainly control gene expression on the post-transcriptional and translational levels (Barkan, 2011; Zoschke and Bock, 2018). These factors are remarkably diverse between morphologically different plant species (such as algae and land plants), but their action leads to an overall highly conserved chloroplast translation output (Trösch et al., 2018). Consistent with its prokaryotic character, a putative hibernation factor associated with the 30S subunit was discovered already at the beginning of the 1990s (Johnson et al., 1990; Bisanz-Seyer and Mache, 1992). As bacterial hibernation factors were discovered only later, this chloroplast ribosomal protein was thought to be plastid specific, and hence was named plastid-specific ribosomal protein 1 (PSRP1) (Table 1). Two-dimensional gel electrophoresis and proteomics of chloroplast ribosome 30S subunits in spinach confirmed the presence of PSRP1 in 30S subunits, and it was hypothesized that PSRP1 might form a plastid translational regulatory module (Yamaguchi and Subramanian, 2000, 2003). However, later cryo-EM studies of spinach chloroplast ribosomes showed that PSRP1 binds

to the inter-subunit space of the 70S ribosome and blocks the binding of tRNAs to the A- and P-sites, suggesting that PSRP1 inactivates ribosomes in a similar way as bacterial hibernation factors (Sharma et al., 2007, 2010). Indeed, the N-terminus of PSRP1 shows homology to YfiA/pY, and homology modeling showed the conserved β - α - β - β - α fold (Sharma et al., 2010; Ahmed et al., 2017) (Figure 2). Therefore, some studies renamed PSRP1 to pY, accounting for this structural homology. However, PSRP1 also contains an extended ~110 amino acid C-terminus which appears to be unstructured and is therefore not detectable in most recent cryo-EM data (Ahmed et al., 2017; Bieri et al., 2017; Graf et al., 2017a,b). Alignment of hibernation factor sequences from different species suggests that PSRP1, just like *LrtA*, rather corresponds to a long HPF (Galmozzi et al., 2016), a notion which was confirmed by the most recent structural study of chloroplast ribosomes (Boerema et al., 2018). PSRP1 stabilizes the 70S ribosomes by attaching the 30S subunit firmly to the 50S subunit in a non-rotated state (Ahmed et al., 2017; Bieri et al., 2017). This prevents subunit dissociation in the idle state (Sharma et al., 2010), as it has also been shown for *LrtA* (Galmozzi et al., 2016). Both PSRP1 and *LrtA* can be released from the ribosome by the ribosome recycling factor (RRF) and the elongation factor EF-G, leading to subunit dissociation and new translation initiation (Sharma et al., 2010; Galmozzi et al., 2016). It is particularly interesting that both PSRP1 and *LrtA* have been

Figure 5: Mechanism of ribosomal hibernation in plant chloroplasts.

Under light conditions (day, white background), the circadian clock leads to the expression of a subset of nuclear-encoded genes (blue shading, transcription) that are targeted to the chloroplast by post-translational protein targeting (green shading) and play roles in light-dependent processes such as photosynthesis. Under these conditions, ribosomes are fully active and protein synthesis can progress normally (right part). Under dark conditions (night, gray background), the circadian clock leads to the expression of a subset of nuclear-encoded genes such as *PSRP1* that potentially play a role in inactivating chloroplast ribosomes during the dark. Additionally, the different redox status of the chloroplast stroma during light (reduced) and dark (oxidized) could play a role in the regulation of ribosome activity. Chloroplast-targeted RelA homologues convert GTP to (p)ppGpp (for simplicity 'ppGpp'). The accumulation of (p)ppGpp leads to a depleted GTP pool and indirectly to a reduced chloroplast gene expression (stringent response). Potential further effects of the plastid (p)ppGpp pool on nuclear gene expression via retrograde signaling are marked with a dashed arrow.

shown to stabilize 70S ribosomes in the light, while LrtA has additionally been shown to be able to trigger 100S formation in the dark (Hood et al., 2016). This clearly argues against a functional homology of LrtA with pY but rather with long HPF and based on the alignment with LrtA (Galmozzi et al., 2016), it might be speculated that this is also the case for PSRP1. However, the formation of hibernating 100S ribosomes has not been observed within chloroplasts, and the conformation of the plastidic bS1c and bS2c suggests that plastidic ribosome dimer formation might not exist or involve other structural features compared with 100S ribosome formation in prokaryotes (Boerema et al., 2018).

Importantly, chloroplast translation is spatially separated from the expression of regulatory factors, which are mostly encoded in the nucleus. Thus, hibernation of chloroplast ribosomes requires different strategies compared to prokaryotic cells (Figure 5). PSRP1 is nuclear-encoded and thus subject to eukaryotic-type transcription regulation. Consequently, it is rather unlikely that *PSRP1* transcription is directly controlled by the diurnal redox-state of the chloroplast stroma. However, *PSRP1* expression seems to be directly controlled by the circadian clock, as *PSRP1* transcripts peak directly after onset of night in synchronized diurnally grown *Chlamydomonas reinhardtii* cells (Zones et al., 2015) and also show clock-dependence in higher plants (Graf et al., 2017a,b). Interestingly, components of the stringent response seem also

conserved in plastids (reviewed in Boniecka et al., 2017; Field, 2018), suggesting that chloroplast metabolism is also regulated by the stringent response. Homologues of RelA have also been found in plants and seem to localize predominantly to chloroplasts (reviewed in Boniecka et al., 2017). Already in 1974 it was shown that chloroplast ribosomes may also function as the origin of (p)ppGpp production in plants (Margulies and Michaels, 1974). And there is accumulating evidence by now that the plastidic stringent response directly affects transcription (Nomura et al., 2014; Sugliani et al., 2016), protein synthesis (Nomura et al., 2012) and the synthesis of nucleotides, hormones (Sugliani et al., 2016), lipids and metabolites (Maekawa et al., 2015) within plastids. Of note, regulation of transcription seems not mediated by the direct binding of (p)ppGpp to the plastidic-encoded polymerase itself but – comparable to *B. subtilis* – through the depletion of the GTP pool upon (p)ppGpp synthesis (reviewed in Boniecka et al., 2017). Interestingly, *Arabidopsis thaliana* *RSH3oe* mutants with increased (p)ppGpp synthesis in plastids displayed altered expression of a number of nuclear genes, suggesting that the chloroplast stringent response is communicated to the nucleus via retrograde signaling (Abdelkefi et al., 2018). It remains to be shown in future studies whether ribosome hibernation via PSRP1 is coupled with the stringent response in chloroplasts or if alternative pathways exist for ribosome hibernation in diurnal cycles and during nutrient starvation.

Ribosomal silencing factor A

Ribosome hibernation not only involves stabilization of 70S, formation of dimeric 100S ribosomes and the inactivation of translation through the competitive binding between GTP and (p)ppGpp to IF2 and EF-G (see above). An additional factor was identified that seems to specifically prevent the association of the small and large ribosomal subunits. In *E. coli*, YbeB was found to be associated with ribosomal components (Butland et al., 2005) (Table 1). This protein appears to be conserved, and it specifically co-sediments with the 50S subunit (Galperin and Koonin, 2004; Jiang et al., 2007). Mutants of this factor show strong defects in the viability during the stationary phase as well as under nutrient limitation (Häuser et al., 2012). It was shown that this protein interacts with the uL14 protein of the ribosomal large subunit and interferes with subunit joining and thus translational initiation, hence the factor was renamed ribosomal silencing factor A (RsfA) (Häuser et al., 2012). It is particularly interesting that the interaction between RsfA homologues and uL14 seems to be conserved not only in several bacterial species but also in mitochondria and chloroplasts (Häuser et al., 2012). A recent cryo-EM structure of *Mycobacterium tuberculosis* shows that the RsfA homologue binds to uL14 as a monomer, although RsfA forms dimers in solution, and that the binding position of RsfA indeed sterically inhibits subunit joining (Li et al., 2015). Thus, fast reduction of translation under nutrient-limiting conditions might be also achieved through the inhibition of subunit joining. In plastids and mitochondria, the RsfA homologues have been called iojap and C7orf30, respectively. In fact, iojap has been already discovered in 1979 as a mutant in *Zea mays* that lacks plastid ribosomes (Walbot and Coe, 1979). Despite the lack of plastid ribosomes, it has been shown that some cells are able to support chloroplast development even in the absence of the iojap gene product Ij (Byrne and Taylor, 1996), which phenotypically leads to green sectors in an otherwise white, non-photosynthetic mutant (Coe et al., 1988). However, as mutants lack plastid ribosomes, it appears that the plastid RsfA homolog is rather involved in ribosome biogenesis than in the regulated inhibition of subunit joining under stress conditions. In human mitochondria, C7orf30 has been shown to associate with 50S subunits of mito-ribosomes, and knockdown show reduced mitochondrial gene expression (Wanschers et al., 2012). The cause for this reduced gene expression appears to be a defect in the assembly of the 50S subunit and thus reduced formation of 70S ribosomes in the mutants (Rorbach et al., 2012; Fung et al., 2013). Therefore, both plastid and mitochondrial RsfA

homologues appear to be involved in ribosome biogenesis, suggesting that despite the conservation of RsfA and its binding partner uL14 a functional divergence occurred during evolution away from the bacterial, starvation-induced translational shutdown.

Conclusion and outlook

The past two decades revealed the widely conserved phenomenon of translation hibernation by inactivation of 70S ribosomes. In heterotrophic bacteria, hibernation of translation occurs as a direct consequence of the limited availability of external resources such as carbon, amino acids or nitrogen and protects the protein synthesis machinery during the stationary phase. Interestingly, this theme is also conserved in photoautotrophic bacteria and eukaryotes (i.e. plants) in which these mechanisms were adapted to modulate translation during the dark phase when external energy is missing. Here, the two factors LrtA and PSRP1 are responsible for orchestrating hibernation and ribosome stability during diurnal cycles of photoautotrophic cyanobacteria and plants, respectively. The stabilization of ribosomes during nutrient starvation and potentially during the night is an important aspect for cell fitness and long-term survival. In *E. coli*, it was observed that nutrient limitation leads to widespread ribosome degradation (Ben-Hamida and Schlessinger, 1966; Jacobson and Gillespie, 1968) which most likely serves as important source of nutrients. However, uncontrolled ribosome degradation leads to severe disadvantages and the disability to recover from starvation, and the prevention of this uncontrolled ribosome degradation requires the action of stabilizing hibernation factors. For example, biofilm forming *P. aeruginosa* lacking hibernation factors HPF and RMF were unable to divide after transition to rich media and showed decreased membrane integrity. Moreover, *P. aeruginosa* mutants lacking the stringent response were unable to exit from dormancy (Williamson et al., 2012; Akiyama et al., 2017). These findings make hibernation factors and di-ribosome disassembly factors (reviewed in Gohara and Yap, 2018) putative targets for future antibiotics that may prevent dormant cells in biofilms from becoming pathogenic.

Remarkably, homologues of hibernation factors exist also in organelles of eukaryotes. However, their exact function is not known yet, and it might well be that they have acquired novel functions. Thus, the homologue of RsfA seems to play a novel role in ribosome biogenesis in mitochondria and chloroplasts. Also, plastidic PSRP1 might

play a more specialized role as 100S formation has not been observed in plastids despite the homology of PSRP1 to LrtA and other forms of HPF_{long}. It will require in-depth studies to unravel if ribosome hibernation exists in plants and what the exact role of PSRP1 is in this process. Interestingly, few studies about ribosome hibernation also exist for the 80S ribosome system in eukaryotic cells. In *Saccharomyces cerevisiae* the clamping protein Stm1p was found to associate with free 80S ribosomes, and deletion of this factor results in rapamycin hypersensitivity and reduced survival during nutrient limitation (Van Dyke et al., 2006). Furthermore, 110S ribosomes, the equivalent forms of 100S ribosomes of bacteria, were observed under nutrient starvation of mammalian cells, although in this case a hibernation factor has not been identified to date (Krokowski et al., 2011).

While the understanding of structural and mechanistic details of ribosome hibernation in bacterial systems is very advanced, it will be now interesting to investigate the biological significance and mechanism of these processes in eukaryotic organelles and the cytosolic 80S protein synthesis system.

Acknowledgments: This work was supported by the German Research Foundation grant TRR175-A05 and the Forschungsschwerpunkt BioComp to F.W.

References

- Abdelkefi, H., Sugliani, M., Ke, H., Harchouni, S., Soubigou-Taconat, L., Citerne, S., Mouille, G., Fakhfakh, H., Robaglia, C., and Field, B. (2018). Guanosine tetraphosphate modulates salicylic acid signalling and the resistance of *Arabidopsis thaliana* to turnip mosaic virus. *Mol. Plant Pathol.* **19**, 634–646.
- Agafonov, D.E. and Spirin, A.S. (2004). The ribosome-associated inhibitor A reduces translation errors. *Biochem. Biophys. Res. Commun.* **320**, 354–358.
- Agafonov, D.E., Kolb, V.A., Nazimov, I.V., and Spirin, A.S. (1999). A protein residing at the subunit interface of the bacterial ribosome. *Proc. Natl. Acad. Sci. USA* **96**, 12345–12349.
- Agafonov, D.E., Kolb, V.A., and Spirin, A.S. (2001). Ribosome-associated protein that inhibits translation at the aminoacyl-tRNA binding stage. *EMBO Rep.* **2**, 399–402.
- Ahmed, T., Shi, J., and Bhushan, S. (2017). Unique localization of the plastid-specific ribosomal proteins in the chloroplast ribosome small subunit provides mechanistic insights into the chloroplastic translation. *Nucleic Acids Res.* **45**, 8581–8595.
- Aiso, T., Yoshida, H., Wada, A., and Ohki, R. (2005). Modulation of mRNA stability participates in stationary-phase-specific expression of ribosome modulation factor. *J. Bacteriol.* **187**, 1951–1958.
- Akiyama, T., Williamson, K.S., Schaefer, R., Pratt, S., Chang, C.B., and Franklin, M.J. (2017). Resuscitation of *Pseudomonas aeruginosa* from dormancy requires hibernation promoting factor (PA4463) for ribosome preservation. *Proc. Natl. Acad. Sci. USA* **114**, 3204–3209.
- Alper, S., Dufour, A., Garsin, D.A., Duncan, L., and Losick, R. (1996). Role of adenosine nucleotides in the regulation of a stress-response transcription factor in *Bacillus subtilis*. *J. Mol. Biol.* **260**, 165–177.
- Baker, T.A. and Sauer, R.T. (2012). ClpXP, an ATP-powered unfolding and protein-degradation machine. *Biochim. Biophys. Acta* **1823**, 15–28.
- Barkan, A. (2011). Studying the structure and processing of chloroplast transcripts. *Methods Mol. Biol.* **774**, 183–197.
- Basu, A. and Yap, M.N. (2017). Disassembly of the *Staphylococcus aureus* hibernating 100S ribosome by an evolutionarily conserved GTPase. *Proc. Natl. Acad. Sci. USA* **114**, E8165–E8173.
- Basu, A., Shields, K.E., Eickhoff, C.S., Hoft, D.F., and Yap, M.F. (2018). Thermal and nutritional regulation of ribosome hibernation in *Staphylococcus aureus*. *J. Bacteriol.* **200**, e00426–e00418.
- Beckert, B., Turk, M., Czech, A., Berninghausen, O., Beckmann, R., Ignatova, Z., Plitzko, J.M., and Wilson, D.N. (2018). Structure of a hibernating 100S ribosome reveals an inactive conformation of the ribosomal protein S1. *Nat. Microbiol.* **3**, 1115–1121.
- Ben-Hamida, F. and Schlessinger, D. (1966). Synthesis and breakdown of ribonucleic acid in *Escherichia coli* starving for nitrogen. *Biochim. Biophys. Acta* **119**, 183–191.
- Bieri, P., Leibundgut, M., Saurer, M., Boehringer, D., and Ban, N. (2017). The complete structure of the chloroplast 70S ribosome in complex with translation factor pY. *EMBO J.* **36**, 475–486.
- Bisanz-Seyer, C. and Mache, R. (1992). Organization and expression of the nuclear gene coding for the plastid-specific S22 ribosomal protein from spinach. *Plant Mol. Biol.* **18**, 337–341.
- Bock, R. (2007). *Cell and Molecular Biology of Plastids* (Berlin/Heidelberg: Springer).
- Boerema, A.P., Aibara, S., Paul, B., Tobiasson, V., Kimanius, D., Forsberg, B.O., Wallden, K., Lindahl, E., and Amunts, A. (2018). Structure of the chloroplast ribosome with chl-RRF and hibernation-promoting factor. *Nat. Plants* **4**, 212–217.
- Boniecka, J., Prusinska, J., Dabrowska, G.B., and Goc, A. (2017). Within and beyond the stringent response-RSH and (p)ppGpp in plants. *Planta* **246**, 817–842.
- Bubunenko, M., Baker, T., and Court, D.L. (2007). Essentiality of ribosomal and transcription antitermination proteins analyzed by systematic gene replacement in *Escherichia coli*. *J. Bacteriol.* **189**, 2844–2853.
- Butland, G., Peregrin-Alvarez, J.M., Li, J., Yang, W., Yang, X., Canadian, V., Starostine, A., Richards, D., Beattie, B., Krogan, N., et al. (2005). Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* **433**, 531–537.
- Byrne, M. and Taylor, W.C. (1996). Analysis of Mutator-induced mutations in the iojap gene of maize. *Mol. Gen. Genet.* **252**, 216–220.
- Chatterji, D. and Ojha, A.K. (2001). Revisiting the stringent response, ppGpp and starvation signaling. *Curr. Opin. Microbiol.* **4**, 160–165.
- Coe, E.H. Jr., Thompson, D., and Walbot, V. (1988). Phenotypes mediated by the iojap genotype in maize. *Am. J. Bot.* **75**, 634–644.
- Contreras, L.M., Sevilla, P., Cámara-Artigas, A., Hernández-Cifre, J.G., Rizzuti, B., Florencio, F.J., Muro-Pastor, M.I., García de la

- Torre, J., and Neira, J.L. (2018). The cyanobacterial ribosomal-associated protein LrtA from *Synechocystis* sp. PCC 6803 is an oligomeric protein in solution with chameleonic sequence properties. *Int. J. Mol. Sci.* 19, pii: E1857.
- Di Pietro, F., Brandi, A., Dzeladini, N., Fabbretti, A., Carzaniga, T., Piersimoni, L., Pon, C.L., and Giuliodori, A.M. (2013). Role of the ribosome-associated protein PY in the cold-shock response of *Escherichia coli*. *Microbiologyopen* 2, 293–307.
- Dörrich, A.K., Mitschke, J., Siadat, O., and Wilde, A. (2014). Deletion of the *Synechocystis* sp. PCC 6803 *kaiAB1C1* gene cluster causes impaired cell growth under light-dark conditions. *Microbiology* 160, 2538–2550.
- Drzewiecki, K., Eymann, C., Mittenhuber, G., and Hecker, M. (1998). The *yyvD* gene of *Bacillus subtilis* is under dual control of sigmaB and sigmaH. *J. Bacteriol.* 180, 6674–6680.
- Field, B. (2018). Green magic: regulation of the chloroplast stress response by (p)ppGpp in plants and algae. *J. Exp. Bot.* 69, 2797–2807.
- Franken, L.E., Oostergetel, G.T., Pijning, T., Puri, P., Arkhipova, V., Boekema, E.J., Poolman, B., and Guskov, A. (2017). A general mechanism of ribosome dimerization revealed by single-particle cryo-electron microscopy. *Nat. Commun.* 8, 722.
- Friedman, S.M. and Weinstein, I.B. (1964). Lack of fidelity in the translation of synthetic polyribonucleotides. *Proc. Natl. Acad. Sci. USA* 52, 988–996.
- Fung, S., Nishimura, T., Sasarman, F., and Shoubridge, E.A. (2013). The conserved interaction of C7orf30 with MRPL14 promotes biogenesis of the mitochondrial large ribosomal subunit and mitochondrial translation. *Mol. Biol. Cell.* 24, 184–193.
- Galmozzi, C.V., Florencio, F.J., and Muro-Pastor, M.I. (2016). The cyanobacterial ribosomal-associated protein LrtA is involved in post-stress survival in *Synechocystis* sp. PCC 6803. *PLoS One* 11, e0159346.
- Galperin, M.Y. and Koonin, E.V. (2004). ‘Conserved hypothetical’ proteins: prioritization of targets for experimental study. *Nucleic Acids Res.* 32, 5452–5463.
- Gentry, D.R., Hernandez, V.J., Nguyen, L.H., Jensen, D.B., and Cashel, M. (1993). Synthesis of the stationary-phase sigma factor sigma s is positively regulated by ppGpp. *J. Bacteriol.* 175, 7982–7989.
- Gohara, D.W. and Yap, M.F. (2018). Survival of the drowsiest: the hibernating 100S ribosome in bacterial stress management. *Curr. Genet.* 64, 753–760.
- Graf, A., Coman, D., Uhrig, R.G., Walsh, S., Flis, A., Stitt, M., and Grisse, W. (2017a). Parallel analysis of *Arabidopsis* circadian clock mutants reveals different scales of transcriptome and proteome regulation. *Open Biol.* 7, pii: 160333.
- Graf, M., Arenz, S., Huter, P., Dönhöfer, A., Nováček, J., and Wilson, D.N. (2017b). Cryo-EM structure of the spinach chloroplast ribosome reveals the location of plastid-specific ribosomal proteins and extensions. *Nucleic Acids Res.* 45, 2887–2896.
- Gray, M.W. (1993). Origin and evolution of organelle genomes. *Curr. Opin. Genet. Dev.* 3, 884–890.
- Gray, J.V., Petsko, G.A., Johnston, G.C., Ringe, D., Singer, R.A., and Werner-Washburne, M. (2004). ‘Sleeping beauty’: quiescence in *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* 68, 187–206.
- Green, R. and Noller, H.F. (1997). Ribosomes and translation. *Annu. Rev. Biochem.* 66, 679–716.
- Hauryliuk, V., Atkinson, G.C., Murakami, K.S., Tenson, T., and Gerdes, K. (2015). Recent functional insights into the role of (p)ppGpp in bacterial physiology. *Nat. Rev. Microbiol.* 13, 298–309.
- Häuser, R., Pech, M., Kijek, J., Yamamoto, H., Titz, B., Naeve, F., Tovchigrechko, A., Yamamoto, K., Szaflarski, W., Takeuchi, N., et al. (2012). RsfA (YbeB) proteins are conserved ribosomal silencing factors. *PLoS Genet.* 8, e1002815.
- Hecker, M., Pané-Farré, J., and Völker, U. (2007). SigB-dependent general stress response in *Bacillus subtilis* and related Gram-positive bacteria. *Annu. Rev. Microbiol.* 61, 215–236.
- Hood, R.D., Higgins, S.A., Flamholz, A., Nichols, R.J., and Savage, D.F. (2016). The stringent response regulates adaptation to darkness in the cyanobacterium *Synechococcus elongatus*. *Proc. Natl. Acad. Sci. USA* 113, E4867–E4876.
- Imamura, S. and Asayama, M. (2009). Sigma factors for cyanobacterial transcription. *Gene Regul. Syst. Biol.* 3, 65–87.
- Imamura, S., Asayama, M., Takahashi, H., Tanaka, K., Takahashi, H., and Shirai, M. (2003). Antagonistic dark/light-induced SigB/SigD, group 2 sigma factors, expression through redox potential and their roles in cyanobacteria. *FEBS Lett.* 554, 357–362.
- Imamura, S., Asayama, M., and Shirai, M. (2004). *In vitro* transcription analysis by reconstituted cyanobacterial RNA polymerase: roles of group 1 and 2 sigma factors and a core subunit, RpoC2. *Genes Cells* 9, 1175–1187.
- Ishihama, A. (2018). Building a complete image of genome regulation in the model organism *Escherichia coli*. *J. Gen. Appl. Microbiol.* 63, 311–324.
- Izutsu, K., Wada, A., and Wada, C. (2001a). Expression of ribosome modulation factor (RMF) in *Escherichia coli* requires ppGpp. *Genes Cells* 6, 665–676.
- Izutsu, K., Wada, C., Komine, Y., Sako, T., Ueguchi, C., Nakura, S., and Wada, A. (2001b). *Escherichia coli* ribosome-associated protein SRA, whose copy number increases during stationary phase. *J. Bacteriol.* 183, 2765–2773.
- Jacobson, A. and Gillespie, D. (1968). Metabolic events occurring during recovery from prolonged glucose starvation in *Escherichia coli*. *J. Bacteriol.* 95, 1030–1039.
- Jiang, M., Sullivan, S.M., Walker, A.K., Strahler, J.R., Andrews, P.C., and Maddock, J.R. (2007). Identification of novel *Escherichia coli* ribosome-associated proteins using isobaric tags and multidimensional protein identification techniques. *J. Bacteriol.* 189, 3434–3444.
- Johnson, C.H., Kruff, V., and Subramanian, A.R. (1990). Identification of a plastid-specific ribosomal protein in the 30 S subunit of chloroplast ribosomes and isolation of the cDNA clone encoding its cytoplasmic precursor. *J. Biol. Chem.* 265, 12790–12795.
- Kanjee, U., Ogata, K., and Houry, W.A. (2012). Direct binding targets of the stringent response alarmone (p)ppGpp. *Mol. Microbiol.* 85, 1029–1043.
- Khusainov, I., Vicens, Q., Ayupov, R., Usachev, K., Myasnikov, A., Simonetti, A., Validov, S., Kieffer, B., Yusupova, G., Yusupov, M., et al. (2017). Structures and dynamics of hibernating ribosomes from *Staphylococcus aureus* mediated by intermolecular interactions of HPF. *EMBO J.* 36, 2073–2087.
- Kolter, R., Siegle, D.A., and Tormo, A. (1993). The stationary phase of the bacterial life cycle. *Annu. Rev. Microbiol.* 47, 855–874.
- Krásny, L. and Gourse, R.L. (2004). An alternative strategy for bacterial ribosome synthesis: *Bacillus subtilis* rRNA transcription regulation. *EMBO J.* 23, 4473–4483.

- Krásny, L., Tiserová, H., Jonák, J., Rejman, D., and Sanderová, H. (2008). The identity of the transcription +1 position is crucial for changes in gene expression in response to amino acid starvation in *Bacillus subtilis*. *Mol. Microbiol.* 69, 42–54.
- Krokowski, D., Gaccioli, F., Majumder, M., Mullins, M.R., Yuan, C.L., Papadopoulou, B., Merrick, W.C., Komar, A.A., Taylor, D., and Hatzoglou, M. (2011). Characterization of hibernating ribosomes in mammalian cells. *Cell Cycle* 10, 2691–2702.
- Lacour, S. and Landini, P. (2004). SigmaS-dependent gene expression at the onset of stationary phase in *Escherichia coli*: function of sigmaS-dependent genes and identification of their promoter sequences. *J. Bacteriol.* 186, 7186–7195.
- Li, X., Sun, Q., Jiang, C., Yang, K., Hung, L.W., Zhang, J., and Sacchettini, J.C. (2015). Structure of ribosomal silencing factor bound to *Mycobacterium tuberculosis* ribosome. *Structure* 23, 1858–1865.
- Loewen, P.C. and Triggs, B.L. (1984). Genetic mapping of *katF*, a locus that with *katE* affects the synthesis of a second catalase species in *Escherichia coli*. *J. Bacteriol.* 160, 668–675.
- Maekawa, M., Honoki, R., Ihara, Y., Sato, R., Oikawa, A., Kanno, Y., Ohta, H., Seo, M., Saito, K., and Masuda, S. (2015). Impact of the plastidial stringent response in plant growth and stress responses. *Nat. Plants* 1, 15167.
- Maitra, A. and Dill, K.A. (2015). Bacterial growth laws reflect the evolutionary importance of energy efficiency. *Proc. Natl. Acad. Sci. USA* 112, 406–411.
- Majerczyk, C.D., Dunman, P.M., Luong, T.T., Lee, C.Y., Sadykov, M.R., Somerville, G.A., Bodi, K., and Sonenshein, A.L. (2010). Direct targets of CodY in *Staphylococcus aureus*. *J. Bacteriol.* 192, 2861–2877.
- Maki, Y., Yoshida, H., and Wada, A. (2000). Two proteins, YfiA and YhbH, associated with resting ribosomes in stationary phase *Escherichia coli*. *Genes Cells* 5, 965–974.
- Manley, J.L. and Gesteland, R.F. (1978). Suppression of amber mutants *in vitro* induced by low temperature. *J. Mol. Biol.* 125, 433–447.
- Margulies, M.M. and Michaels, A. (1974). Ribosomes bound to chloroplast membranes in *Chlamydomonas reinhardtii*. *J. Cell Biol.* 60, 65–77.
- Matzov, D., Aibara, S., Basu, A., Zimmerman, E., Bashan, A., Yap, M.F., Amunts, A., and Yonath, A.E. (2017). The cryo-EM structure of hibernating 100S ribosome dimer from pathogenic *Staphylococcus aureus*. *Nat. Commun.* 8, 723.
- Milon, P., Tischenko, E., Tomsic, J., Caserta, E., Folkers, G., La Teana, A., Rodnina, M.V., Pon, C.L., Boelens, R., and Gualerzi, C.O. (2006). The nucleotide-binding site of bacterial translation initiation factor 2 (IF2) as a metabolic sensor. *Proc. Natl. Acad. Sci. USA* 103, 13962–13967.
- Mitkevich, V.A., Ermakov, A., Kulikova, A.A., Tankov, S., Shyp, V., Soosaar, A., Tenson, T., Makarov, A.A., Ehrenberg, M., and Haurlyuk, V. (2010). Thermodynamic characterization of ppGpp binding to EF-G or IF2 and of initiator tRNA binding to free IF2 in the presence of GDP, GTP, or ppGpp. *J. Mol. Biol.* 402, 838–846.
- Moore, C.M., Nakano, M.M., Wang, T., Ye, R.W., and Helmann, J.D. (2004). Response of *Bacillus subtilis* to nitric oxide and the nitrosating agent sodium nitroprusside. *J. Bacteriol.* 186, 4655–4664.
- Muffler, A., Fischer, D., Altuvia, S., Storz, G., and Hengge-Aronis, R. (1996). The response regulator RssB controls stability of the sigma(S) subunit of RNA polymerase in *Escherichia coli*. *EMBO J.* 15, 1333–1339.
- Navarro Llorens, J.M., Tormo, A., and Martinez-Garcia, E. (2010). Stationary phase in Gram-negative bacteria. *FEMS Microbiol. Rev.* 34, 476–495.
- Nomura, Y., Takabayashi, T., Kuroda, H., Yukawa, Y., Sattasuk, K., Akita, M., Nozawa, A., and Tozawa, Y. (2012). ppGpp inhibits peptide elongation cycle of chloroplast translation system *in vitro*. *Plant Mol. Biol.* 78, 185–196.
- Nomura, Y., Izumi, A., Fukunaga, Y., Kusumi, K., Iba, K., Watanabe, S., Nakahira, Y., Weber, A.P., Nozawa, A., and Tozawa, Y. (2014). Diversity in guanosine 3',5'-bisdiphosphate (ppGpp) sensitivity among guanylate kinases of bacteria and plants. *J. Biol. Chem.* 289, 15631–15641.
- Peterson, C.N., Levchenko, I., Rabinowitz, J.D., Baker, T.A., and Silhavy, T.J. (2012). RpoS proteolysis is controlled directly by ATP levels in *Escherichia coli*. *Genes Dev.* 26, 548–553.
- Pletnev, P., Osterman, I., Sergiev, P., Bogdanov, A., and Dontsova, O. (2015). Survival guide: *Escherichia coli* in the stationary phase. *Acta Naturae* 7, 22–33.
- Polikanov, Y.S., Blaha, G.M., and Steitz, T.A. (2012). How hibernation factors RMF, HPF, and YfiA turn off protein synthesis. *Science* 336, 915–918.
- Pratt, L.A. and Silhavy, T.J. (1996). The response regulator SprE controls the stability of RpoS. *Proc. Natl. Acad. Sci. USA* 93, 2488–2492.
- Rorbach, J., Gammage, P.A., and Minczuk, M. (2012). C7orf30 is necessary for biogenesis of the large subunit of the mitochondrial ribosome. *Nucleic Acids Res.* 40, 4097–4109.
- Samartzidou, H. and Widger, W.R. (1998). Transcriptional and post-transcriptional control of mRNA from lrtA, a light-repressed transcript in *Synechococcus* sp. PCC 7002. *Plant Physiol.* 117, 225–234.
- Sanchuki, H.B., Gravina, F., Rodrigues, T.E., Gerhardt, E.C., Pedrosa, F.O., Souza, E.M., Raittz, R.T., Valdameri, G., de Souza, G.A., and Huergo, L.F. (2017). Dynamics of the *Escherichia coli* proteome in response to nitrogen starvation and entry into the stationary phase. *Biochim. Biophys. Acta Proteins Proteom.* 1865, 344–352.
- Sato, A., Watanabe, T., Maki, Y., Ueta, M., Yoshida, H., Ito, Y., Wada, A., and Mishima, M. (2009). Solution structure of the *E. coli* ribosome hibernation promoting factor HPF: implications for the relationship between structure and function. *Biochem. Biophys. Res. Commun.* 389, 580–585.
- Sharma, M.R., Wilson, D.N., Datta, P.P., Barat, C., Schlutzenzen, F., Fucini, P., and Agrawal, R.K. (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.
- Sharma, M.R., Dönhöfer, A., Barat, C., Marquez, V., Datta, P.P., Fucini, P., Wilson, D.N., and Agrawal, R.K. (2010). PSRP1 is not a ribosomal protein, but a ribosome-binding factor that is recycled by the ribosome-recycling factor (RRF) and elongation factor G (EF-G). *J. Biol. Chem.* 285, 4006–4014.
- Shine, J. and 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.
- Stouthamer, A.H. and Bettenhausen, C. (1973). Utilization of energy for growth and maintenance in continuous and batch

- cultures of microorganisms. A reevaluation of the method for the determination of ATP production by measuring molar growth yields. *Biochim. Biophys. Acta* 301, 53–70.
- Sugliani, M., Abdelkefi, H., Ke, H., Bouveret, E., Robaglia, C., Caffari, S., and Field, B. (2016). An ancient bacterial signaling pathway regulates chloroplast function to influence growth and development in *Arabidopsis*. *Plant Cell* 28, 661–679.
- Tagami, K., Nanamiya, H., Kazo, Y., Maehashi, M., Suzuki, S., Namba, E., Hoshiya, M., Hanai, R., Tozawa, Y., Morimoto, T., et al. (2012). Expression of a small (p)ppGpp synthetase, YwaC, in the (p)ppGpp(0) mutant of *Bacillus subtilis* triggers YvyD-dependent dimerization of ribosome. *Microbiologyopen* 1, 115–134.
- Tan, X., Varughese, M., and Widger, W.R. (1994). A light-repressed transcript found in *Synechococcus* PCC 7002 is similar to a chloroplast-specific small subunit ribosomal protein and to a transcription modulator protein associated with sigma 54. *J. Biol. Chem.* 269, 20905–20912.
- Tanaka, K., Takayanagi, Y., Fujita, N., Ishihama, A., and Takahashi, H. (1993). Heterogeneity of the principal sigma factor in *Escherichia coli*: the *rpoS* gene product, sigma 38, is a second principal sigma factor of RNA polymerase in stationary-phase *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* 90, 3511–3515.
- Tanaka, K., Kusano, S., Fujita, N., Ishihama, A., and Takahashi, H. (1995). Promoter determinants for *Escherichia coli* RNA polymerase holoenzyme containing sigma 38 (the *rpoS* gene product). *Nucleic Acids Res.* 23, 827–834.
- Tempest, D.W. and Neijssel, O.M. (1984). The status of YATP and maintenance energy as biologically interpretable phenomena. *Annu. Rev. Microbiol.* 38, 459–486.
- Trösch, R., Barahimpour, R., Gao, Y., Badillo-Corona, J.A., Gotsmann, V.L., Zimmer, D., Mühlhaus, T., Zoschke, R., and Willmund, F. (2018). Commonalities and differences of chloroplast translation in a green alga and land plants. *Nat. Plants* 4, 564–575.
- Ueta, M., Yoshida, H., Wada, C., Baba, T., Mori, H., and Wada, A. (2005). Ribosome binding proteins YhbH and YfiA have opposite functions during 100S formation in the stationary phase of *Escherichia coli*. *Genes Cells* 10, 1103–1112.
- Ueta, M., Ohniwa, R.L., Yoshida, H., Maki, Y., Wada, C., and Wada, A. (2008). Role of HPF (hibernation promoting factor) in translational activity in *Escherichia coli*. *J. Biochem.* 143, 425–433.
- Ueta, M., Wada, C., and Wada, A. (2010). Formation of 100S ribosomes in *Staphylococcus aureus* by the hibernation promoting factor homolog SaHPF. *Genes Cells* 15, 43–58.
- Ueta, M., Wada, C., Daifuku, T., Sako, Y., Bessho, Y., Kitamura, A., Ohniwa, R.L., Morikawa, K., Yoshida, H., Kato, T., et al. (2013). Conservation of two distinct types of 100S ribosome in bacteria. *Genes Cells* 18, 554–574.
- Van Dyke, N., Baby, J., and Van Dyke, M.W. (2006). Stm1p, a ribosome-associated protein, is important for protein synthesis in *Saccharomyces cerevisiae* under nutritional stress conditions. *J. Mol. Biol.* 358, 1023–1031.
- Vila-Sanjurjo, A. (2008). Modification of the ribosome and the translational machinery during reduced growth due to environmental stress. *EcoSal Plus* 3. Doi: 10.1128/ecosalplus.2.5.6.
- Vila-Sanjurjo, A., Schuwirth, B.S., Hau, C.W., and Cate, J.H. (2004). Structural basis for the control of translation initiation during stress. *Nat. Struct. Mol. Biol.* 11, 1054–1059.
- Voelker, U., Voelker, A., Maul, B., Hecker, M., Dufour, A., and Haldenwang, W.G. (1995). Separate mechanisms activate sigma B of *Bacillus subtilis* in response to environmental and metabolic stresses. *J. Bacteriol.* 177, 3771–3780.
- Wada, A. (1986). Analysis of *Escherichia coli* ribosomal proteins by an improved two dimensional gel electrophoresis. II. Characterization of four new proteins. *J. Biochem.* 100, 1595–1605.
- Walbot, V. and Coe, E.H. (1979). Nuclear gene iojap conditions a programmed change to ribosome-less plastids in *Zea mays*. *Proc. Natl. Acad. Sci. USA* 76, 2760–2764.
- Wanschers, B.F., Szklarczyk, R., Pajak, A., van den Brand, M.A., Gloerich, J., Rodenburg, R.J., Lightowers, R.N., Nijtmans, L.G., and Huynen, M.A. (2012). C7orf30 specifically associates with the large subunit of the mitochondrial ribosome and is involved in translation. *Nucleic Acids Res.* 40, 4040–4051.
- Waters, N.R., Samuels, D.J., Behera, R.K., Livny, J., Rhee, K.Y., Sadykov, M.R., and Brinsmade, S.R. (2016). A spectrum of CodY activities drives metabolic reorganization and virulence gene expression in *Staphylococcus aureus*. *Mol. Microbiol.* 101, 495–514.
- Weber, H., Polen, T., Heuveling, J., Wendisch, V.F., and Hengge, R. (2005). Genome-wide analysis of the general stress response network in *Escherichia coli*: sigmaS-dependent genes, promoters, and sigma factor selectivity. *J. Bacteriol.* 187, 1591–1603.
- Williamson, K.S., Richards, L.A., Perez-Osorio, A.C., Pitts, B., McInerney, K., Stewart, P.S., and Franklin, M.J. (2012). Heterogeneity in *Pseudomonas aeruginosa* biofilms includes expression of ribosome hibernation factors in the antibiotic-tolerant subpopulation and hypoxia-induced stress response in the metabolically active population. *J. Bacteriol.* 194, 2062–2073.
- Wilson, D.N. and Nierhaus, K.H. (2007). The weird and wonderful world of bacterial ribosome regulation. *Crit Rev Biochem. Mol. Biol.* 42, 187–219.
- Wolz, C., Geiger, T., and Goerke, C. (2010). The synthesis and function of the alarmone (p)ppGpp in firmicutes. *Int. J. Med. Microbiol.* 300, 142–147.
- Yamaguchi, K. and Subramanian, A.R. (2000). The plastid ribosomal proteins. Identification of all the proteins in the 50 S subunit of an organelle ribosome (chloroplast). *J. Biol. Chem.* 275, 28466–28482.
- Yamaguchi, K. and Subramanian, A.R. (2003). Proteomic identification of all plastid-specific ribosomal proteins in higher plant chloroplast 30S ribosomal subunit. PSRP-2 (U1A-type domains), PSRP-3 α/β (ycf65 homologue) and PSRP-4 (Thx homologue). *Eur. J. Biochem.* 270, 190–205.
- Yoshida, H. and Wada, A. (2014). The 100S ribosome: ribosomal hibernation induced by stress. *Wiley Interdiscip Rev RNA*. 5, 723–732.
- Yoshida, H., Maki, Y., Kato, H., Fujisawa, H., Izutsu, K., Wada, C., and Wada, A. (2002). The ribosome modulation factor (RMF) binding site on the 100S ribosome of *Escherichia coli*. *J. Biochem.* 132, 983–989.
- Yoshida, H., Yamamoto, H., Uchiyumi, T., and Wada, A. (2004). RMF inactivates ribosomes by covering the peptidyl transferase centre and entrance of peptide exit tunnel. *Genes Cells* 9, 271–278.
- Zhang, S. and Haldenwang, W.G. (2005). Contributions of ATP, GTP, and redox state to nutritional stress activation of the

- Bacillus subtilis* sigmaB transcription factor. J. Bacteriol. 187, 7554–7560.
- Zones, J.M., Blaby, I.K., Merchant, S.S., and Umen, J.G. (2015). High-resolution profiling of a synchronized diurnal transcriptome from *Chlamydomonas reinhardtii* reveals continuous cell and metabolic differentiation. Plant Cell 27, 2743–2769.
- Zoschke, R. and Bock, R. (2018). Chloroplast translation: structural and functional organization, operational control and regulation. Plant Cell 30, 745–770.