Critical Reviews in Biochemistry and Molecular Biology, 42:187–219, 2007

ISSN: 1040-9238 print / 1549-7798 online
DOI: 10.1080/10409230701360843

informa
healthcare

The Weird and Wonderful World of Bacterial Ribosome Regulation

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ABSTRACT In every organism, translation of the genetic information into functional proteins is performed on the ribosome. In *Escherichia coli* up to 40% of the cell's total energy turnover is channelled toward the ribosome and protein synthesis. Thus, elaborate networks of translation regulation pathways have evolved to modulate gene expression in response to growth rate and external factors, ranging from nutrient deprivation, to chemical (pH, ionic strength) and physical (temperature) fluctuations. Since the fundamental players involved in regulation of the different phases of translation have already been extensively reviewed elsewhere, this review focuses on lesser known and characterized factors that regulate the ribosome, ranging from processing, modification and assembly factors, unusual initiation and elongation factors, to a variety of stress response proteins.

KEYWORDS Assembly, biogenesis, Protein synthesis, Ribosome, RNA, Translation regulation

A. FACTOR-MEDIATED RIBOSOME REGULATION

Ribosomes are macromolecular machines of more than 2.3 MDa that translate the genetic information into functional proteins (Nierhaus and Wilson, 2004). Because up to 40% of an *Escherichia coli* cell's total energy turnover goes toward protein synthesis, the translational apparatus must be strictly regulated. In bacteria, an elaborate network of translation regulation pathways has been identified that modulates gene expression in response to growth rate or to environmental stimuli, such as nutrient deprivation, chemical (pH, ionic strength), and physical (temperature) fluctuations.

One fundamental way of modulating gene expression is by regulating the biogenesis of the translational apparatus itself. In bacteria, the 70S ribosome is composed of two subunits, a small 30S subunit and a large 50S subunit. Each subunit is composed of ribosomal RNA (rRNA) and ribosomal proteins (r-proteins). In *E. coli,* for example, the 30S subunit contains a single 1542 nucleotide (nt) 16S rRNA and 21 r-proteins, whereas the 50S subunit contains two RNAs, a 115 nt 5S rRNA and 2904 nt 23S rRNA, together with 33 r-proteins. Therefore, the challenge for the cell is to ensure the coordinated synthesis and binding of r-proteins to the rRNA in the correct manner so as to form an active ribosomal particle. An ever increasing number of protein factors has been identified as being associated with the assembly process, ranging

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from processing and modification enzymes, such as RNases and methylases, to unwinding and chaperoning factors, such as helicases and highly specific GTPases.

Protein synthesis itself occurs on the 70S ribosome, and can be divided into three distinct phases: initiation, elongation, and termination/recycling. Each of these phases has a specific set of translation factors that regulate the process. *In vitro* translation systems have demonstrated that a complement of three initiation factors (IF1, IF2, and IF3), three elongation factors (EF-G, EF-Tu, and EF-Ts), and three of the four termination factors (RF1 or RF2, RF3, and RRF) are necessary and sufficient for synthesis (Shimizu *et al.*, 2001), although RF3 does not appear to be essential *in vivo* and is missing from 72 of the 191 available bacterial genomes (Margus *et al.*, 2007). However, it appears that *in vivo* many additional factors may be required, for example, EF-P seems to stimulate the first peptide bond during initiation (Ganoza and Aoki, 2000; Swaney *et al.*, 2006), and LepA (EF4) appears to have a role in counteracting mistranslocated ribosomes (Qin *et al.*, 2006). Indeed, additional factors and regulation systems appear to be particularly important for efficient translation under certain "stress" conditions, such as the RelA mediated stringent response system that is activated in response to nutrient deprivation (Wendrich *et al.*, 2002), and the numerous factors that bind and protect the ribosome upon entering stationary phase (Ueta *et al.*, 2005) or under conditions of cold shock (Weber and Marahiel, 2003; Wilson and Nierhaus, 2004) and shifts in pH and/or ionic strength (Qin *et al.*, 2006; Karim and Nierhaus, unpublished data). Since the fundamental players involved in the different phases of translation have already been reviewed elsewhere (Gualerzi and Pon, 1990; Hirokawa *et al.*, 2006; Laursen *et al.*, 2005; Nierhaus and Wilson, 2004; Ramakrishnan, 2002; Vesper and Wilson, 2006; Wilson *et al.*, 2002), the focus here is on lesser known and characterized factors that regulate the ribosome. We have tried to classify factors as best as possible, but we recognize that some factors may fall into multiple categories. For example, RbfA appears to play a role in small subunit assembly, but whether this is general or in response to cold shock is as yet unclear. Furthermore, while we have tried to be as thorough as possible, we acknowledge that the list of protein factors discussed here is likely to be incomplete, especially with the discovery of new functions for unknown proteins occurring more frequently.

B. IN THE BEGINNING . . .: PROTEIN FACTORS INVOLVED IN RIBOSOME BIOGENESIS

The synthesis of ribosomes is one of the major tasks of the cell, particularly in actively growing bacteria, where ribosomes can constitute up to 30% of the dry mass of the cell, whereas in eukaryotes they represent no more than 5% (Nierhaus, 1991). Therefore the ability to rapidly regulate the synthesis of nascent ribosomal particles provides an important advantage to the cell. As mentioned, one of the challenges for the *E. coli* cell is to coordinate both the synthesis of r-proteins and rRNA as well as the binding of the >50 r-proteins to the >4500 nts rRNA in the correct manner and order to ensure formation of active particles. Indeed, the binding order is governed by a strict hierarchy: (i) Specific r-proteins act as so-called assembly initiator proteins, for example S4 and S7 on the 30S and L3 and L24 on the 50S, which bind directly to the rRNA and coordinate the binding of other r-proteins to the particle; and (ii) the second line of the assembly sequence for the large ribosomal subunit is seen in the r-proteins essential for the early assembly, *i.e.,* the proteins L4, L13, L20 and L22, which bind exclusively to the 5′-end of 23S rRNA. These proteins are essential and sufficient to form the first assembly intermediate particle, which cannot be circumvented during the course of assembly. It follows that *in vivo*, where the rRNA synthesis is coupled to the assembly of ribosomes (Lewicki *et al.*, 1993), the assembly process is much simpler than during total reconstitution, where the addition of the mature rRNA simultaneously offers all rRNA binding sites to the r-proteins. In contrast, *in vivo* the first intermediate of the large ribosomal subunit can already be formed shortly after the onset of rRNA synthesis, when only a few binding sites are available for those r-proteins essential for formation of this first intermediate. This entropic advantage of *in vivo* assembly over *in vitro* reconstitution underlies the term "assembly gradient," whereby the progress of rRNA synthesis determines the progress of assembly. The necessity of maintaining the entropic advantage is hitherto the only explanation as to why in all eukaryotes, ribosome assembly occurs in the nucleoli, the locus of rRNA synthesis (Nierhaus, 1991).

Although bacterial ribosomal subunits can be reconstituted *in vitro* from purified rRNA and r-protein

components, the conditions required to do this, namely high magnesium and long incubations at elevated temperatures, are far from physiological (see Nierhaus, 1991; Nomura *et al.*, 1984). Instead, it is likely that a plethora of protein factors are involved *in vivo* to facilitate the assembly process. Indeed, the assembly process of eukaryotic, particularly yeast, ribosomes has been well characterized, revealing that nearly 200 auxiliary proteins are associated with pre-ribosomal particles (reviewed by Dez and Tollervey, 2004; Dlakic, 2005; Fatica and Tollervey, 2002; Fromont-Racine *et al.*, 2003). In bacteria, a number of protein factors have been identified that are involved in processing, such as RNases and helicases, or modification of the ribosomal components by methylases, acetylases and pseudouridinylases; however, accumulating evidence suggests that there are in fact many more protein factors that appear to be directly involved in the assembly process. These include factors that bind to the small 30S subunit, such as RimM, RbfA, Era, as well as to the large 50S ribosomal subunit, including Obg, Der and YlqF. Since many of these factors appear to be highly conserved and in many cases specific for bacteria, they are considered as emerging targets for new antimicrobial drugs (Comartin and Brown, 2006).

BI. Processing and Modification, Unwinding and Chaperoning During Assembly

In bacteria, the small and large rRNAs are transcribed together in a single transcript, which is processed by a number of endonucleases, in particular RNase III, which releases the pre-16S and pre-23S rRNAs from the transcript (Bram *et al.*, 1980; Ginsburg and Steitz, 1975; Young and Steitz, 1978; see Deutscher and Li, 2001; Apirion and Miczak, 1993 for review of RNases). The pre-23S rRNA contains 3 to 7 and 8 nucleotides (nts) at the 5′ and 3′ ends, respectively (Liiv and Remme, 2004; Sirdeshmukh and Schlessinger, 1985a), while the pre-16S, termed 17S, contains 115 and 33 nts at the 5′ and 3′ ends respectively (Young and Steitz, 1978). Whereas processing of the 5′ end has been well characterized, consisting of the sequential action of RNase E and G (Li *et al.*, 1999), the RNase responsible for processing of the 3′ end has not yet been identified. It has been known since the early 1970s that 17S precursor containing 30S particles are inactive in translation, probably because they cannot associate with the 50S subunit (Lindahl, 1973; Lindahl, 1975; Wireman and Sypherd, 1974). Since the 5′ and 3′ ends of the 16S rRNA in the mature 30S particle are located far apart, whereas in the precursor the extra nucleotides are basepaired, this additional secondary structure may either directly prohibit binding of the 50S subunit, or induce conformational changes in the 30S subunit that indirectly prohibit large subunit association. Additionally, the 3′ end of the 16S rRNA contains the anti-Shine-Dalgarno (anti-SD) sequence that base-pairs with the SD sequence present in many mRNAs. Blocking this interaction could also prevent mRNA recruitment and thus translation. In contrast, processing of the pre-23S rRNA does not appear to be as important since the pre-23S is found in polysomes (Sirdeshmukh and Schlessinger, 1985b). Structurally this can be rationalized, since in the mature 50S subunit (Harms *et al.*, 2001), the 3′ and 5′ ends remain base-paired, and are displaced relative to the active site (interface side) of the ribosome.

Modification of the rRNA occurs predominantly post-transcriptionally and consists of two main types, methylation and pseudo-uridinylation. The *E. coli* ribosome contains 24 methylated nucleosides, 10 in the 16S and 14 in the 23S rRNA, and 10 pseudo-uridines (Ψ), one in the 16S and nine in the 23S rRNA (Table 1). In addition, there are a number of positions that can be modified to confer resistance to specific antibiotics, such as the dimethylation of A2058 to confer macrolide resistance, or methylation of G1408 of the 16S rRNA to confer resistance to some aminoglycosides. While all of the pseudouridine synthase genes have been identified in *E. coli* (Del Campo *et al.*, 2001), a number of methylases responsible for rRNA modification have yet to be identified (Table 1). The largest question remaining unanswered, however, is the exact role of the modifications. Many of the modifications are dispensable for cell viability, with little observable effect on cell growth or ribosome function. There are some exceptions, which include the loss of G2251 modification in yeast mitochondrial ribosomes, which leads to defects in subunit assembly (Sirum-Connolly and Mason, 1993), and the absence of 2′-O-methylation of U2552 affects subunit association (Bügl *et al.*, 2000; Caldas *et al.*, 2000). Of the 11 pseudouridine synthases in *E. coli*, only strains lacking RluD exhibit reduced growth rates and defects in ribosome biogenesis, such as accumulation of subunits (Gutgsell *et al.*, 2005).

TABLE 1 **Summary of the modifications in 16S and 23S rRNA**[*1]

Location	Enzyme	Modifies	Modification[*2]	Resistance
		A. 16S rRNA modifications		
516	RsuA (YejD)		Ψ	
527			m^7G	
966	RsmD (yhhF)	30S	m^2G	Lesnyak *et al.*, 2007
967	RsmB (RrmB)	16S	m^5C	
1207	RsmC (yjjT)	30S	m^2G	
1402			m^4Cm	
1405			m^7G	Aminogly.[*3]
1407	RsmF (YebU)	30S	m^5C	Andersen and Douthwaite, 2006
1408			m^1A	Aminogly.[*3]
1498	RsmE (yggJ)	30S	m^3U	
1516			m^2G	
1518	RsmA (KsgA)	30S	$m^6{}_2A$	Kasugamycin[*4]
1519	RsmA (KsgA)	30S	$m^6{}_2A$	Kasugamycin[*4]
		B. 23S rRNA Modifications		
745	RlmA(I) (RrmA and yebH)		m^1G	
746	RluA (YabO)		Ψ	
747	RumB (YbjF)		m^5U (T)	
748	RlmA(II) (TlrB)		m^1G	Tylosin[*5]
955	RluC (YceC)		Ψ	
1618			m^6A	
1835	RlmG (YgjO)		m^2G	Sergiev *et al.*, 2006
1911	RluD (YfiL)		Ψ	
1915	RluD (YfiL)		$m^3Ψ$	
1917	RluD (YfiL)		Ψ	
1939	RumA (YgcA)		m^5U (T)	
1962			m^5C	
2030			m^6A	
2058	Erm (TlrD)		m^6A	MLS_B[*6]
2069			m^7G	
2251	RlmB (YjfH)		Gm	
2445	RlmL (YcbY)		m^2G	Lesnyak *et al.*, 2006
2449			D	
2457	RluE (YmfC)		Ψ	
2479	AviRb		Um	Avilamycin[*7]
2498			Cm	PhLOPlS[*8]
2503	Cfr		m^2A	
2504	RluC (YceC)		Ψ	
2535	AviRa*		m^1G	Avilamycin[*7]
2552	RrmJ (FtsJ)		Um	
2580	RluC (YceC)		Ψ	
2604	RluF (YjbC)		Ψ	
2605	RluB (YciL)		Ψ	

[*1] Data taken from http://medlib.med.utah.edu/RNAmods/ (Rozenski *et al.*, 1999) and http://medlib.med.utah.edu/SSUmods/ (McCloskey and Rozenski, 2005), and Del Campo *et al.*, 2001, unless otherwise indicated.

[*2] m^xN refers to methylation (m) of rRNA nucleotide N at the x position of the base, whereas Nm indicates methylation of the sugar at the 2 position of nucleotide N.

[*3] Methylation of A1408 and G1405 leads resistance to some aminoglycosides (Beauclerk and Cundliffe, 1987).

[*4] Lack of dimethylation of A1518 and A1519 confers modest resistance to kasugamycin (Helser *et al.*, 1971; Helser *et al.*, 1972).

[*5] Methylation of G748 confers tylosin resistance (Douthwaite *et al.*, 2004).

[*6] (Di-)Methylation of A2058 confers resistance to the macrolide, lincosamide and streptogramin B classes of antibiotics (Poehlsgaard and Douthwaite, 2003)

[*7] Methylation of U2479 and G2535 confers resistance to avilamycin in *Streptomyces* (Treede *et al.*, 2003)

[*8] Absence of C2498 methylation causes resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics in *Staphylococcus aureus* and *Escherichia coli* (Kehrenberg *et al.*, 2005; Long *et al.*, 2006)

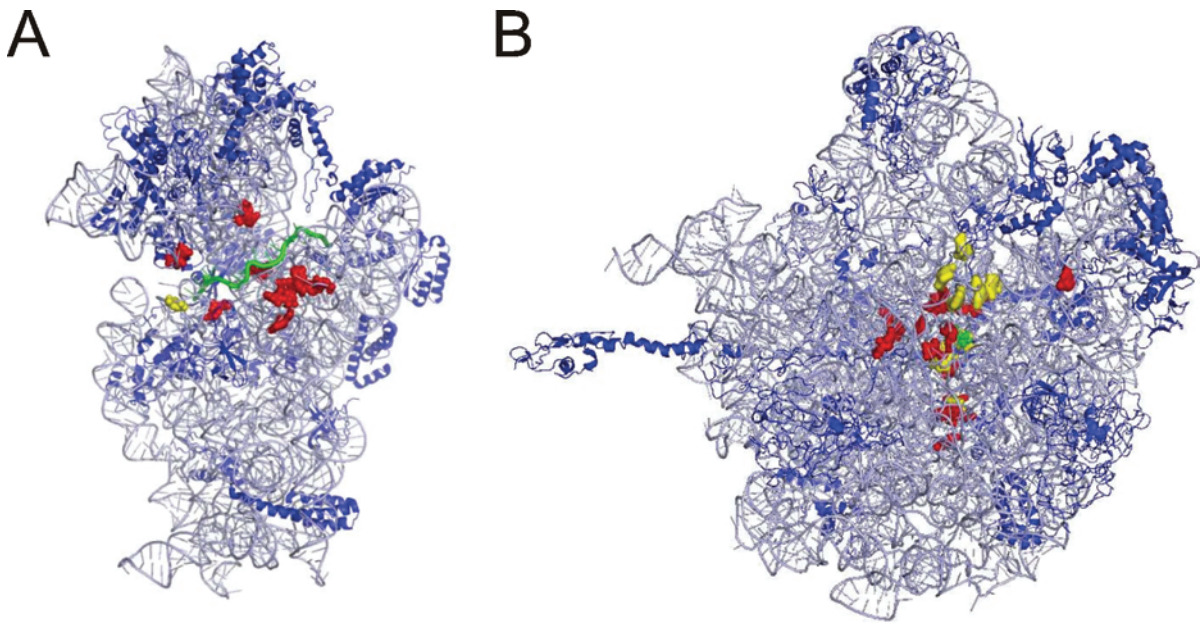


Figure 1 **Methylation and pseudouridinylation sites on the *E. coli* ribosome. Sites of methylation (*red*) and pseudourindinylation (*yellow*) are shown as space filled nucleotides on the (*A*) small and (*B*) large ribosomal subunits. rRNA and r-protein are shown as ribbons in light and dark blue, respectively. In (*A*) a green ribbon indicates the path of the mRNA through the small subunit, whereas in (*B*) the antibiotic chloramphenicol (*green*) acts as a reference for peptidyltransferase centre on the large subunit. This figure was assembled from PDB accessions numbers 2AW4/7 (30S and 50S; Schuwirth *et al.*, 2005), 1YL3 (mRNA; Jenner *et al.*, 2005) and 1K01 (antibiotic, Schlünzen *et al.*, 2001) and is based on figure from Decatur and Fournier, 2002.**

RluD is responsible for converting uridine to Ψ at the highly conserved positions 1911, 1915, and 1917 in 23S RNA (Table 1). The location of these nucleotides in the loop of helix 69, which forms part of universally conserved intersubunit bridge B2, is consistent with their influence on subunit association. Mapping all the methylations and pseudouridinylations onto the crystal structures of the 30S and 50S subunits reveals that they cluster around active sites of the ribosome, namely, the tRNA-mRNA binding site on the 30S subunit and the peptidyltransferase center and intersubunit bridge areas on the 50S subunit (Decatur and Fournier, 2002; Figure 1A and B). This has led to the suggestion that the modifications provide structural support to flexible regions in order to optimize ribosomal function. In some cases however, it appears that the modification enzymes themselves play a role in ribosome biogenesis independently of the modifications they catalyse, as exemplified by RlmA(I) (RrmA, Table 1), which is itself essential for cell viability, whereas the modification of m^1G in the 23S rRNA is not (Liu *et al.*, 2004). It should also be noted that a number of r-proteins are also modified, a classic example being the discovery that r-protein L12 is in fact just an acetylated version of L7. R-protein L11 is another example, which in *E. coli* is trimethylated at three positions (Met1, Lys3, and Lys29) by the methylase PrmA, however the roles of these modifications have yet to be elucidated (see Cameron *et al.*, 2004 and references therein).

E. coli cells have five DEAD-box genes, so-called because they contain nine conserved motifs, one of which is Asp-Glu-Ala-Asp (D-E-A-D). Of these five DEAD-box proteins, two have been shown to be involved in ribosome biogenesis, SrmB and CsdA, and a possible third is DbpA (reviewed by Iost and Dreyfus, 2006; Table 2). These families of proteins are ATP-dependent RNA helicases, although the specificity of the reaction differs among the members. Unlike CsdA and SrmB, DbpA requires a specific sequence present in the 23S rRNA, namely helix 92, to stimulate its unwinding activity. The region responsible for this specificity appears to be located in the C-terminal region of the protein, since for the DbpA homologue in *Bacillus subtilis* YxiN, deletion of the C-terminal domain removes all sequence specificity and appending this region to the catalytic core of the non-sequence specific RNA helicase, SrmB, confers sequence specificity (Karginov *et al.*, 2005). This suggests that the C-terminal tethers the helicase to H92 in the pre-50S subunit, allowing the catalytic core to unwind neighboring RNA duplexes to facilitate proper folding

TABLE 2 **Summary of the unusual protein factors that regulate ribosome biogenesis and protein synthesis**

Factor*	Possible functions
CsdA (DeaD)	**C**old **s**hock **D**eaD **A** (CsdA) is an ATP-dependent RNA helicase that binds to large ribosomal subunit to mediate unwinding of 23S rRNA during assembly. Previously called DeaD.
DbpA	**D**EAD **b**ox **p**rotein **A** (DbpA) is an ATP-dependent RNA helicase that mediates unwinding of 23S rRNA during assembly. Termed YxiN in *Bacillus subtilis*.
Der	See EngA
EF-3 (yeast)	**E**longation **F**actor **3** is a yeast specific ATPase that promotes release of the E-tRNA from the ribosome, upon binding of the ternary complex EF1·aa-tRNA·GTP to the A site.
EF4	(see LepA)
EF-P (IF-4)	**E**longation **F**actor **P** structurally mimics tRNA, binds to the ribosome to facilitate translation initiation by stimulating formation of the first peptide bond; is a homologue of eIF5A and has been proposed to be renamed as IF-4
EngA (Der/YphC)	Unique G-protein with tandem G-domains and RNA binding KH domain. Probably involved in assembly of the large ribosomal subunit. Also known as Der/YfgK (*E. coli*) or YphC (*B. subtilis*).
EngB (YihA, YsxC)	*E. coli* YihA and the *B. subtilis* homologue YsxC are essential proteins that appear to have a role in assembly of the large ribosomal subunit.
Era	E. coli Ras-like protein is a GTPase that binds to (pre-)30S subunit to facilitate processing of the 3′ end of the 16S rRNA precursor. Ortholog in *B. subtilis* termed Bex.
Hsp15	**H**eat **s**hock **p**rotein **15** (Hsp15) is encoded by the yrfH/hslR gene and is involved in recycling of nascent polypeptide containing free large ribosomal subunits.
LepA (EF4)	**L**eader **p**eptidase **A**, renamed to Elongation Factor 4 (EF4). Shown to bind to POST state ribosomes and induce back-translocation.
Obg (CgtA)	Spo**OB**-associated **G**TP-binding protein (OBG) binds ppGpp and appears to monitor levels of G-nucleotides in the cell. Obg also binds the large ribosomal subunit and may provide a link the stress response, DNA replication and ribosome assembly. Also known as CgtA or YhbZ.
pY (YfiA, RaiA)	**p**rotein **Y** binds and inactivates ribosomes under conditions of cold shock. Previously known as YfiA and Ribosome-associated inhibitor **A** (RaiA).
RbbA(YhiH, W)	**R**i**b**osome-**b**ound **A**TPase has been proposed to facilitate ejection of E-tRNA from the ribosome, analogous to yeast EF-3. The *yhi*H gene encodes RbbA and the truncated form was originally termed W.
RbfA (P15B)	**R**ibosome **b**inding **f**actor **A** binds to 30S subunit to facilitate subunit assembly. Overexpression suppresses cold sensitive C23U mutation in the 16S rRNA. Previously termed P15B.
RbgA (YlqF)	**R**ibosome **b**iogenesis **G**TPase **A** is involved in a late assembly step of *B. subtilis* 50S subunits. Previously called YlqF. Not present in *E. coli*.
RelA	The stringent factor RelA binds to ribosomes containing uncharged or deacylated tRNA at the A site and synthesizes the alarmone (p)ppGpp.
RelE	RelE is a toxin that binds to ribosomes and cleaves mRNA in the A site. The antitoxin RelB inactivates RelE.
RimM (21K, YfjA)	**R**ibosome **m**aturation factor **M** binds to head of small ribosomal subunit to facilitate assembly. Previously called 21K or YfjA.
RimN (YrdC)	**R**ibosome **m**aturation factor **N** has been suggested to bind the 16S rRNA to promote proper processing.
RMF	**R**ibosome **m**odulation **f**actor (RMF) binds to stationary phase 70S ribosomes to induce dimerization (100S formation).
RsgA (YjeQ/YloQ)	**R**ibosome **s**mall subunit-dependent **G**TPase **A** (RsgA) has a putative role in small subunit ribosomal assembly. Previously called YjeQ (*E. coli*). Homologues include YloQ/YqeH (*B. subtilis*) or YawG (yeast).
SRA (S22)	**S**tationary phase induced **r**ibosome-**a**ssociated protein (SRA) binds to ribosomes in stationary phase. Previously identified as ribosomal protein S22.

TABLE 2 **Summary of the unusual protein factors that regulate ribosome biogenesis and protein synthesis (*Continued*)**

Factor*	Possible functions
SmpB	**S**mall protein **B** (SmpB) binds tmRNA and is involved in the trans-translation system for rescue of ribosomes stalled on truncated mRNAs.
SrmB	**S**uppressor of temperature-sensitive mutation in ribosomal protein L24. SrmB is a DEAD box RNA helicase involved in ribosome biogenesis.
Tet(O)	A ribosomal protection protein (RPP) that binds to tetracycline-stalled ribosomes to release the drug and allow translation to continue.
YciH	Bacterial ortholog of eIF1/SUI1. Not present in many bacterial genomes.
YhbH	Found bound to 100S ribosome dimers in stationary phase cells. Putative role in stabilization and preservation of ribosomes.
YihA (YsxC)	See EngB
YhbY	Has a similar fold to the C-terminal domain of IF3, suggesting potential interaction with RNA. Reported to associate with 50S subunits.

**E. coli* nomenclature given unless indicated.

or interaction with r-proteins. Indeed, deletion of the either the *srmB* or *csdA* gene leads to accumulation of a pre-50S particles, which in former case (Δ*srmB*) are lacking r-protein L13, an early assembly protein (Charollais *et al.*, 2003), whereas in the latter (Δ*csdA*) only late assembly proteins are absent. This suggests that SrmB acts earlier in the assembly pathway than CsdA (Charollais *et al.*, 2004). It should be noted that CsdA is induced by cold shock and therefore may be more important under these stress conditions (Jones *et al.*, 1996).

Another set of protein factors that have been implicated in ribosome biogenesis are the chaperones DnaK/DnaJ/GrpE (Alix and Guerin, 1993), GroEL/GroES (El Hage *et al.*, 2001). DnaK and GrpE have been shown to be stably associated with pre-30S assembly particles and their presence has been thought to overcome the need for a high temperature activation step required for *in vitro* reconstitution of 30S particles at 37°C or lower temperatures (Maki *et al.*, 2002). In contrast, strains bearing temperature sensitive DnaK mutants do not display ribosomal assembly defects *in vivo*, except at non-permissive temperatures above 35°C (Alix and Guerin, 1993). This suggests that DnaK is only important for ribosomal assembly at high temperatures. Indeed, this finding is supported by the recent *in vitro* analysis monitoring the reconstitution of 30S particles in the presence and absence of DnaK at 30°C, where in both cases a dependence on the heat activation step remained (Alix and Nierhaus, 2003). Further investigation will be required to decipher the role and importance of chaperones for ribosomal assembly.

BII. The Hierarchy of Small Subunit Assembly

In addition to modification, processing enzymes, helicases and chaperones, an increasing number of non-ribosomal proteins have been found to be associated with the 30S (or pre-30S) subunit, and implicated in ribosomal assembly; RimM, RbfA, Era, RsgA, and RimN (Table 2).

I. RimM

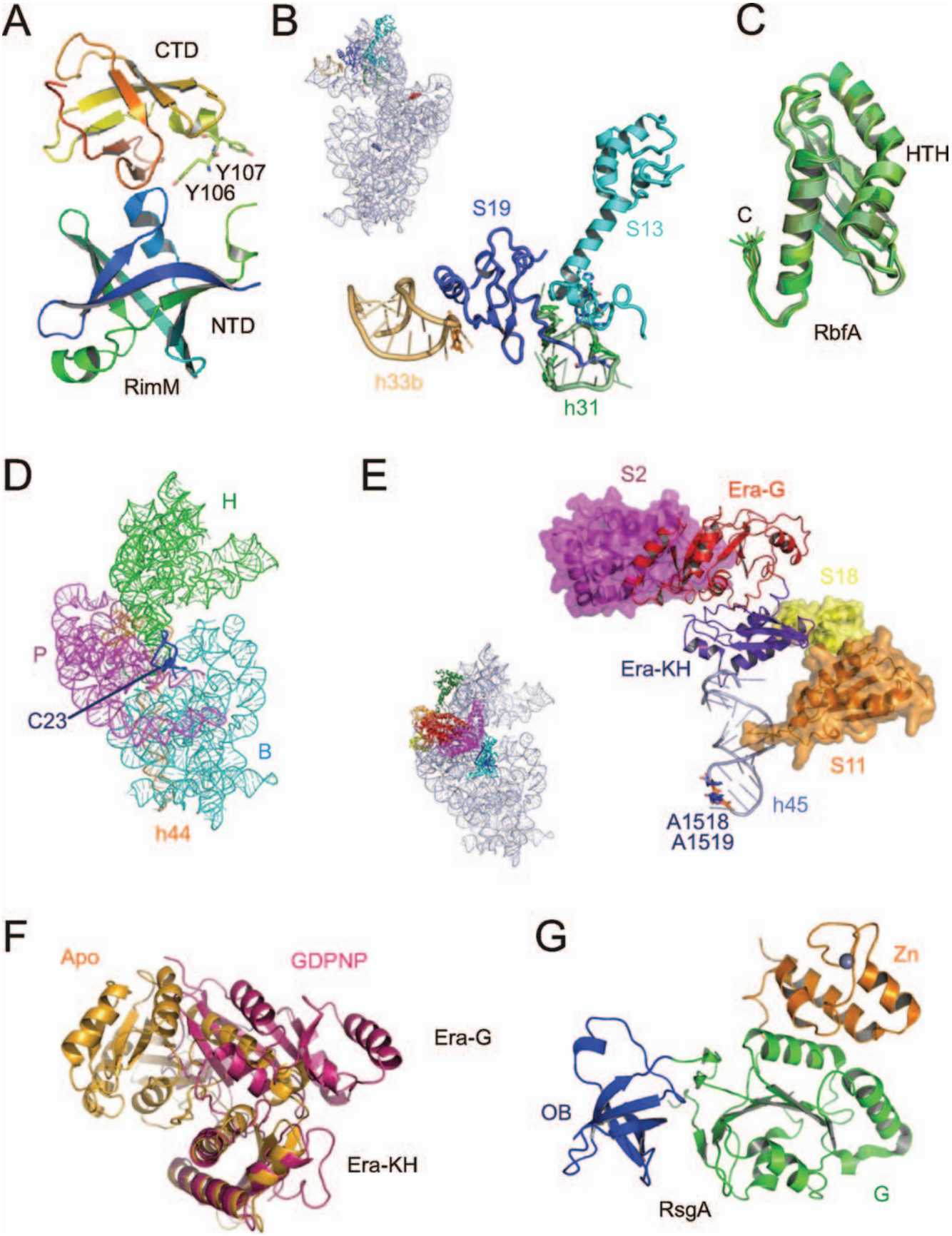
RimM (ribosome maturation factor M) is found associated with free 30S subunits, but not with 70S ribosomes (Bylund *et al.*, 1997). The first indication that RimM plays a role in assembly of the 30S subunit was the fact that deletion of the *rimM* gene (Δ*rimM*) effects translational efficiency (Bylund *et al.*, 1997) and leads to an accumulation of the precursor to the 16S rRNA, termed 17S (Bylund *et al.*, 1998). RimM, previously termed 21K since it is a 21 kD protein, has a two domain structure, with the N- and C-terminal domains (NTD and CTD, respectively) exhibiting PRC β-barrel-like topologies (Figure 2A). Conserved aromatic residues located at positions 106-108 (*E. coli* numbering) within the interdomain linker region (Figure 2A) have been proposed to be involved in protein binding (Anantharaman and Aravind, 2002). A double mutation, Y106A-Y107A, in RimM creates a strain with a phenotype similar to that seen for Δ*rimM* (Lövgren *et al.*, 2004). Suppressor mutations for the RimM mutant were identified as alterations in r-protein S13 (Δ89 to 99), S19 (H83Y) or in helices

31 and 33b of the 16S rRNA (Lövgren *et al.*, 2004). In the mature 30S structure, these ribosomal elements cluster within the head region on the interface side (Figure 2B). A GST-RimM fusion protein has been shown to bind to S19 in the 30S subunit, whereas the RimM mutant did not (Lövgren *et al.*, 2004). The fact that most of the rRNA mutations, as well as S19 (H83Y) mutant, were more efficient suppressors of the rRNA deficiency in the RimM mutant, but not the Δ*rimM* strain, might be thought to indicate that the suppressor mutations restore binding of the mutant RimM to the ribosome. Surprisingly, this was not observed to be the case, at least for the S19 suppressor (Lövgren *et al.*, 2004), although this may only indicate that the interaction is very weak and/or transient. Certainly, it is easy to envisage how alterations in S13, which interacts directly with S19, or rRNA nucleotides neighboring S19, could allow better binding of RimM by indirectly affecting the conformation of S19. Alternatively, the suppressor mutations may mediate conformational changes within the 30S that promote more efficient rRNA processing or 30S maturation, in the absence of RimM or presence of the RimM mutant. Either way, the direct interaction with the head of the 30S subunit supports a role for RimM in the correct maturation of this region. An important step to understanding the action of RimM will be the identification of the substrate for RimM. Initial experiments using the GST-RimM fusion protein as a bait to pull out interaction partners in a cellular extract suggest that a number of r-proteins are absent in the complex and that mature 16S rRNA is present (Lövgren *et al.*, 2004); however, these results require further investigation.

II. RbfA

Ribosome binding factor A (RbfA) was first discovered as a multicopy suppressor for a dominant cold-sensitive C23U mutation located in the 5′ end of the 16S rRNA (Dammel and Noller, 1993, 1995). Consistently, deletion of the *rbfA* gene results in a cold-sensitive phenotype for growth, a reduction in polysomes/70S ribosomes and a corresponding increase in 30S and 50S subunits (Dammel and Noller, 1995; Xia *et al.*, 2003). Similar to the Δ*rimM* phenotype, Δ*rbfA* also displays an accumulation of the 17S rRNA (Bylund *et al.*, 1998; Inoue *et al.*, 2003). Interestingly, overexpression of RbfA complements, at least partially, the slow growth phenotype of the Δ*rimM* strain, whereas the converse is not true, *i.e.*, RimM overexpression cannot complement a Δ*rbfA* phenotype (Bylund *et al.*, 1998). RbfA has been shown to associate specifically with the 30S subunits *in vivo*, and proposed to bind to the 5′ terminal helix 1 of the 16S rRNA (Dammel and Noller, 1995). NMR and crystal structures reveal that RbfA has a single characteristic RNA binding helix-turn-helix (HTH) containing KH-domain (Figure 2C), with a flexible C-terminal extension that was not fully visualized in any of the available structures (Huang *et al.*, 2003). The C-terminal extension may be important for RNA binding since *E. coli* RbfA lacking the C-terminal 25 amino acids, cannot bind stably to the 30S, nor complement the cold-sensitive Δ*rfbA* phenotype, although it can correct for the deficiency in rRNA processing (Xia *et al.*, 2003). If helix 1 of the 16S rRNA is really the binding site for RbfA, then this would require that either RbfA binds before r-protein S6, since this protein blocks access to the 5′ end of the 16S rRNA from

Figure 2 **The hierarchy of small subunit assembly. (*A*) Structure of RimM homologue from *Pseudomonas aeruginosa* (PDB2F1L), showing N- and C-terminal domains (NTD and CTD) with Y106 and Y107 indicated as sticks. (*B*) Location of RimM suppressor mutations in the *E. coli* 30S subunit (PDB2AW4; Schuwirth *et al.*, 2005). G1015A in h33b (*orange*), DA974 in h31 (*green*), D89-99 in S13 (*cyan*) and R81 in S19 (*blue*) are shown with stick representation. On the 30S subunit, the 5′ and 3′ termini of the 16S rRNA are indicated in blue and red, respectively. (*C*) An ensemble of ten NMR structures of RbfA (PDB1KKG; Huang *et al.*, 2003), with the helix-turn-helix (HTH) motif indicated in the KH-domain, as well as the flexible C-terminus. Note the 24 C-terminal residues were not visualized, probably due to their flexible and unstructured nature. (*D*) Helix 1 (*blue*) and nucleotide C23 *(blue spacefill)* in the 5′ end of the 16S rRNA occupy a pivotal position being sandwiched between the head (H, *green*), body (B, *cyan*), platform (P, *magenta*) and 3′ minor domain (h44, orange) on the *E. coli* 30S subunit (PDB2AW4; Schuwirth *et al.*, 2005). (*E*) Model for the binding site of Era on the 30S subunit derived from cryo-EM reconstructions (Sharma *et al.*, 2005). In this model, the G-domain of Era (*red*) interacts with r-protein S2 (*purple*), whereas the KH domain of Era (*blue*) contacts S11 (*orange*), S18 (*yellow*) and S7 (not shown for clarity). In addition, the helix-turn-helix motif in the KH domain approaches the 3′ end of the 16S rRNA. The relative positions of A1518 and A1519 in helix 45 are also indicated. Note: the position of the 5′ end of the 16S rRNA (*blue*) is covered by r-protein S2 (*cyan*) in the mature 30S subunit. (*F*) Comparison of the crystal structures of *E. coli* nucleotide-free (apo) form of Era (orange; Chen *et al.*, 1999) and *Thermus thermophilus* Era in complex with GDPNP (magenta). (*G*) Structure of RsgA (YjeQ from *Thermotoga maritima*; Shin *et al.*, 2004) highlighting the N-terminal OB-fold domain (*blue*), G-domain (*green*) and C-terminal zinc finger domain (*orange*). The Zn ion is shown as a gray sphere.**



the solvent side of the mature 30S subunit (compare position of nucleotide C23 in Figure 2D and 2E), or that the domain arrangement of the (pre-)30S subunit is different when RbfA is bound. In this regard it is interesting to note that helix 1 is pivotally positioned, located directly behind the 3′ minor domain (h44) and sandwiched between the body (B), head (H) and platform (P) regions (Figure 2D). Thus, the role of RbfA may be during a late assembly step, to facilitate bringing these domains together.

III. Era

Era (*E. coli* ras-like protein) is a highly conserved GTPase, essential in *E. coli*, which binds to 30S subunits *in vitro* (Sayed *et al.*, 1999; Sharma *et al.*, 2005). Depletion of Era from the cell produces phenotypes reminiscent of the Δ*rimM* and Δ*rbfA* strains, namely a reduction in 70S ribosomes and an accumulation of subunits and 17S precursor rRNA (Inoue *et al.*, 2003). The crystal structure of *E. coli* Era reveals a two domain protein, with GTP-binding and KH RNA-binding domains located at the N- and C-terminus of the protein, respectively (Chen *et al.*, 1999). A recent cryo-electron microscopy reconstruction of *Thermus thermophilus* Era bound to the 30S subunit reveals that Era binds in a cavity formed by r-proteins S2, S7, S11, and S18, located between the head and platform (Figure 2E) (Sharma *et al.*, 2005). Docking of the crystal structures for Era and the 30S subunit into the cryo-EM density suggests that Era contacts four of the five rRNA domains, with the RNA-binding helix-turn-helix motif located in the KH-domain in close proximity to the 3′ end of the 16S rRNA. This localization is also consistent with the fact that overexpression of KsgA, which dimethylates A1518 and A1519 located in the penultimate h45 of the 16S rRNA (Figure 2E), leads to suppression of a cold-sensitive Era mutant (Lu and Inouye, 1998). Since the methylation state of A1518 and A1519 has been shown to affect the conformation of the 3′ end of 16S rRNA (Micura *et al.*, 2001), it seems likely that methylation promotes formation of an optimal RNA secondary structure for the Era mutant to function. Collectively, these data led to the suggestion that Era may act as an RNA chaperone, facilitating the correct positioning of the rRNA for processing by the maturation RNase (Sharma *et al.*, 2005).

The GTPase activity of Era appears to be essential for Era function (Lerner *et al.*, 1992), although it cannot be ruled out that this is related to the role of Era in many other cellular processes, such as cell division (Britton *et al.*, 1998) and carbon metabolism (Pillutla *et al.*, 1996), rather than ribosome assembly. Certainly, the G-nucleotide appears to influence Era stability on the ribosome, since only the non-hydrolysable GTP form of Era could prevent association of 30S and 50S subunits *in vitro* (Sharma *et al.*, 2005; D.N.W, unpublished results). Thus it is tempting to speculate that processing of the rRNA could trigger the GTPase activity of Era, but this remains to be tested. The best fitting of the *E. coli* Era crystal structure into the cryo-EM density requires flexibility between the KH (CTD) and G-domains (NTD). This is supported by a recent crystal structure of *T. thermophilus* Era in the GTP form (GDPNP), which reveals an even more dramatic difference in the relative domain arrangement when compared to the nucleotide-free (apo) *E. coli* Era structure (Figure 2F). Therefore it is easy to envisage how GTP hydrolysis could alter the conformation of Era to destabilize and release it from the ribosome. Alternatively (or additionally), r-protein S1 may play a role in recycling of Era from the 30S, since the binding position of Era on the 30S subunit overlaps significantly with that determined for S1 (Sharma *et al.*, 2005). Since S1 is involved in mRNA recruitment to the 30S, Era may act as a control checkpoint, ensuring that only mature 30S subunits can enter into the translation initiation cycle, *i.e.,* Era would prevent binding of S1 to precursor containing 30S subunits. This model would suggest Era is involved in one of the last steps in 30S assembly. However, it should be noted that while Era can complement the Δ*rbfA* strain at high temperature (Inoue *et al.*, 2006), it cannot suppress Δ*rimM* or the cold sensitive C23U strains (Inoue *et al.*, 2003). Determination of the binding sites of RbfA and RimM on (pre-)30S particles will go someway to providing further insight in the relationship between these factors.

IV. RsgA (YloQ/YjeQ)

Another GTPase that associates with the 30S subunit is *E. coli* YjeQ (Daigle *et al.*, 2002) (YloQ in *B. subtillus*; Campbell *et al.*, 2005), recently renamed as RsgA (ribosome small subunit-dependent GTPase A) (Himeno *et al.*, 2004). RsgA has a low intrinsic GTPase activity that is stimulated by 30S subunits and 70S ribosomes, but not 50S subunits (Daigle and Brown, 2004; Himeno *et al.*, 2004). RsgA is found associated with ribosomes at very low stoichiometry (1:200) *in vivo* (Daigle and Brown, 2004), and *in vitro* binds stably

to 30S subunits in the presence of GDPNP, but not GTP or GDP (Daigle and Brown, 2004; Himeno *et al.*, 2004). In the presence of GDPNP, all 70S ribosomes were dissociated into subunits by RsgA, suggesting an intersubunit localization of the factor (Himeno *et al.*, 2004). Indeed, aminoglycoside antibiotics such as neomycin, paromomycin and gentamycin, which bind in the decoding center on the interface side of the 30S subunit, inhibit the ribosome-dependent GTPase of RsgA (Campbell *et al.*, 2005; Himeno *et al.*, 2004), possibly by preventing binding of RsgA to the ribosome (Himeno *et al.*, 2004). In contrast, chloramphenicol, which binds to the 50S subunit, as well as other 30S binding antibiotics, *e.g.*, tetracycline, kasugamycin and streptomycin, had no effect (Himeno *et al.*, 2004). The structure of RsgA is composed of three highly conserved domains, an N-terminal oligosaccharide/oligonucleotide-binding (OB)-fold, a central GTPase (G) domain that is circularly permutated (G4-G1-G2-G3 rather than G1-G2-G3-G4) and a C-terminal domain with a zinc (Zn) binding motif (Levdikov *et al.*, 2004; Shin *et al.*, 2004) (Figure 2G). N-terminal truncations indicate that the OB domain is important for interaction of RsgA with the 30S (Daigle and Brown, 2004). Since the initiation factor IF1 consists of an OB-fold domain and binds in the A site of the 30S subunit (Carter *et al.*, 2001), it will be interesting to investigate the interrelationship between these factors on the ribosome. Although RsgA is dispensable for viability of *E. coli* cells, loss of RsgA protein results in a slow growth phenotype and an altered ribosome profile (Campbell *et al.*, 2005). Surprisingly, in the absence of RsgA most of the 70S ribosomes are present as subunits, the 30S subunits contain 17S precursor rRNA and stimulate RsgA GTPase less efficiently than wild-type 30S subunits (Himeno *et al.*, 2004). Clearly, further experiments are required to understand whether RsgA is really involved in assembly of the 30S subunit, or in some other regulatory pathway.

V. YrdC (RimN)

Selection of suppressors of a strain bearing a temperature-sensitive termination release factor 1 (RF1) mutation identified a 12-nucleotide deletion that removed the initiation codon from the *yrdC* gene (Kaczanowska and Rydén-Aulin, 2004). Characterization of the Δ*yrdC* strain revealed an accumulation of immature 30S subunits containing 17S rRNA. The structure of YrdC exhibits a large concave surface with positive electrostatic potential and the protein preferentially binds to dsRNA (Teplova *et al.*, 2000); however, no direct evidence for stable binding of YrdC to 30S particles has been forthcoming (Kaczanowska and Rydén-Aulin, 2005). Nevertheless, incubation of cell extracts from the Δ*yrdC* strain with YrdC protein led to maturation of the 16S rRNA, implicating YrdC in the assembly process and prompting the renaming of this protein to RimN (Kaczanowska and Rydén-Aulin, 2005). Other members of the same protein family as YrdC include yeast homologue Sua5, which has been suggested to be involved in translation re-initiation (Na *et al.*, 1992).

BIII. GTPases That Affect Large Subunit Assembly

In light of the large amount of fully sequenced genomes, database analysis has revealed that bacteria contain 11 universally conserved GTPases (Caldon *et al.*, 2001). While this list includes canonical translation factors such as EF-G, EF-Tu, IF2, the recently characterized elongation factor LepA (EF4; Qin *et al.*, 2006), and the signal sequence recognition pathway proteins Ffh and FtsY, the list also identified new families of proteins, members of which, EngA (Der), EngB (YihA), and Obg (see Table 2), have been implicated in assembly of the large 50S subunit (Brown, 2005). In addition, although the GTPase YlqF is not present in *E. coli*, it is widely distributed in gram-positive bacteria and has been shown to be essential for growth in *B. subtilis* (Morimoto *et al.*, 2002). Two proteins implicated in 60S biogenesis in yeast, Nog2p and Nug1p, exhibit similarity to YlqF that extends beyond the G-domain (Bassler *et al.*, 2001; Saveanu *et al.*, 2001). Furthermore, humans contain several homologs for YlqF, GTPBP7 and the nucleolar GTPases nucleostemin and GNL2 (Uicker *et al.*, 2006). Two recent publications addressing the role of YlqF nicely complement each other to reveal a role for this protein in a late assembly step of the large 50S ribosomal subunit (Matsuo *et al.*, 2006; Uicker *et al.*, 2006), thus appearing to support the renaming of YlqF to RbgA (ribosome biogenesis GTPase A) (Uicker *et al.*, 2006).

I. Obg (CgtA/YhbZ)

Homologues to the *obg* gene have been identified in all genomes sequenced so far, ranging from the

smallest free living bacteria, to archaea (usually two genes, Nog1p and Rbg1p) and eukaryotes (usually four copies, including nucleolar Nog1p). The structure of the full-length Obg from *T. thermophilus* reveals three domains, a central G domain flanked by N- and C-terminal domains, termed OBG and OCT (Obg-C-terminal), respectively, that are unique to Obg (Figure 3A) (Kukimoto-Niino *et al.*, 2004). Obg has been implicated in many different cellular processes, ranging from DNA replication, protein synthesis and cellular differentiation (reviewed by Bénédicte, 2005; Brown, 2005; Czyz and Wegrzyn, 2005). Indeed, the name Obg derives from Spo**OB**-associated **G**TP-binding protein, since the *B. subtilis* protein was shown to co-express with SpoOB and be involved with sporulation (Trach and Hoch, 1989).

RrmJ is a methyltransferase that modifies position U2552 of the 23S rRNA (see Table 1), and loss of this modification in a Δ*rrmJ* strain leads to a reduction in 70S ribosomes as well as an accumulation of both 30S and 50S subunits (Tan *et al.*, 2002). Overexpression of Obg (or EngA, but not Era) can rescue the slow-growth phenotype and restore the polysome profile resulting from an *rrmJ*-knockout, without leading to modification of U2552 (Tan *et al.*, 2002). Direct binding of Obg to 50S subunits has been observed for bacteria; *E. coli* (Sato *et al.*, 2005; Wout *et al.*, 2004), *Caulobacter crescentus* (Lin *et al.*, 2004) and *Vibrio harveyi* (Sikora *et al.*, 2006b), as well as Obg homologs from yeast, namely, mitochondrial Mtg2p (Datta *et al.*, 2005) and nucleolar Nog1p (Jensen *et al.*, 2003; Kallstrom *et al.*, 2003). Obg has a moderate affinity for G-nucleotides, exhibits slow GTP hydrolysis rates and exchanges G-nucleotides rapidly (Lin *et al.*, 1999; Sikora *et al.*, 2006 a; Wout *et al.*, 2004). The nucleotide requirement of Obg for ribosome binding is unclear: *B. subtilis* Obg co-fractionates with ribosomes in a G-nucleotide-dependent fashion, exhibiting strongest interaction with GTP over GDP (Zhang and Haldenwang, 2004), whereas *E. coli* and *C. crescentus* Obg seem to migrate with 50S subunits regardless of the presence or absence of G-nucleotide (Lin *et al.*, 2004; Wout *et al.*, 2004). Interestingly, when subunits from purified 70S ribosomes were used instead of lysates, *E. coli* Obg bound both to 30S and 50S subunits, but only strongly in the presence of GDPNP (Jiang *et al.*, 2006). Similarly, *E. coli* Obg was found to interact with naked 16S and 23S rRNA in a nucleotide-specific manner, binding in the presence of GTP, but not GDP or absence of nucleotide (Sato *et al.*, 2005). Therefore, the G domain does appear to play a role in the binding of Obg to the ribosome, and furthermore, that under some conditions binding to the 30S subunit is also possible. However the significance of this latter finding is unclear at present.

Depletion of Obg from the cell leads to a decrease in 70S ribosomes, an increase in both 30S and 50S subunits, as well as the appearance of an intermediate pre-50S particle (Jiang *et al.*, 2006; Sato *et al.*, 2005). Furthermore, precursors to both the 16S and 23S rRNA are significantly increased, suggesting that RNA processing is impaired (Jiang *et al.*, 2006). It should be noted that defects in processing of the 16S can arise as secondary events due to from defects in 23S rRNA maturation (Charollais *et al.*, 2003; Gutgsell *et al.*, 2005), which may well be the case here. Analysis of the pre-50S particle reveals reduced levels of r-proteins L33, L34, and to a lesser extent L16 (Jiang *et al.*, 2006). These three proteins are late assembly proteins, suggesting that Obg is involved in a late step in 50S biogenesis, analogous to RrmJ (Hager *et al.*, 2002), CsdA (see section BI), EngA/B and RbgA (see section BIII, II to IV, respectively). In addition, RrmJ and RluC (a pseudouridinylase that modifies U955 of the 23S rRNA; Table 1) were also found in the pre-50S particle (Jiang *et al.*, 2006).

Pull-down assays using his-tagged *E. coli* Obg as bait, identified many interaction partners such as r-proteins from both 30S and 50S subunits, as well as CsdA, ClpA, hypothetical protein 274#5, and RNA polymerase β and β' subunits (Sato *et al.*, 2005). This latter finding is interesting since Obg interacts with RsbT, RsbW, and RsbX, regulators that mediate stress activation of σ^B (Scott and Haldenwang, 1999), as well as SpoT (Wout *et al.*, 2004; Raskin *et al.*, 2007), a (p)ppGpp synthetase/hydrolase involved in the stringent response (see section EI). Interestingly, in the crystal structure of *B. subtilis* Obg, one of the two molecules in the asymmetric unit was bound with ppGpp in the active site (Buglino *et al.*, 2002). Since ppGpp was not included in the crystallization conditions, the suggestion is that the molecule binds tightly to Obg and co-purifies with the protein. Comparing the conformations of nucleotide-free and GTP bound forms of Obg reveals a dramatic rearrangement of the G domain relative to the OBG domain (Figure 3B and C), supporting the suggestion that Obg senses and responds to the G-nucleotide state of the cell (Bénédicte, 2005).

II. EngA (Der/YphC)

The EngA family of GTP-binding (G) proteins is unique in that it contains two consecutive G-domains (G1 and G2), located at the N-terminus of the protein, followed by a C-terminal RNA binding KH domain (Muench *et al.*, 2006; Robinson *et al.*, 2002) (Figure 3D). *E. coli* EngA (also known as Der [**d**ouble **Er**a-like GTPase]) as well as many homologs, such as *Bacillus subtilis* YphC, have been shown to be essential for cell viability (Bharat *et al.*, 2006; Hwang and Inouye, 2006; Morimoto *et al.*, 2002). Consistently, EngA homologues are highly conserved in bacterial, but not archaeal or eukaryotic genomes (with the exception of *Arabidopsis thaliana*; Leipe *et al.*, 2002). Perhaps the first hint that EngA was involved in ribosome biogenesis was the report that overexpression of EngA, like that of Obg (see previous section), could rescue the slow-growth phenotype of a *rrmJ*-knockout strain (Tan *et al.*, 2002). Indeed, similar phenotypes are observed when the EngA itself is depleted from the *E. coli* cell (Bharat *et al.*, 2006; Hwang and Inouye, 2006; Schaefer *et al.*, 2006), with the growth rate correlating with the amount of EngA present (Hwang and Inouye, 2006). Furthermore, the accumulating 50S subunits in the EngA-depleted strain are unstable at low Mg^{2+} concentrations, had reduced levels of r-proteins L9 and L18 (and to a lesser extent L2 and L6) and contain pre-23S rRNA (with seven additional 5′ nucleotides) (Hwang and Inouye, 2006). In contrast, the 30S subunit had the full complement of r-proteins, and while the precursor 17S rRNA was also present, this is likely to be a secondary effect due to the defect in 23S rRNA maturation (Hwang and Inouye, 2006). Surprisingly, an independent study analysing the content of the 45S peak from EngA-depleted cells found that r-proteins L16, L27, and L36 were absent, reminiscent of the particles accumulating in the YsxC- and RbgA-depleted strains (Schaefer *et al.*, 2006).

E. coli EngA has been shown to associate with 50S subunits, rather than with 70S ribosomes or 30S subunits (Bharat *et al.*, 2006; Hwang and Inouye, 2006; Schaefer *et al.*, 2006). Furthermore, the binding was dependent on the presence of non-hydrolysable GDPNP, whereas little or no binding was observed with GDP or GTP (Hwang and Inouye, 2006; Schaefer *et al.*, 2006). Both G-domains of EngA appear to be important for function and cell viability. EngA with either mutation S16A in GD1, or K216A (or S217A) in GD2, has reduced GTP binding activity and is not able to rescue a Δ*engA* strain (Bharat *et al.*, 2006). Similarly, mutations N118D in GD1, or N321D in GD2, cannot rescue a Δ*engA* strain at low temperature (30°C). However, complementation is possible at higher temperatures (42°C), but not with a double N118D/N321D EngA mutant (Hwang and Inouye, 2006). Consistently, binding of the double mutant to 50S subunits is significantly diminished compared to wild-type and N118D/N213D mutants (Hwang and Inouye, 2006). This suggests that at high temperature, a single G-domain is sufficient for EngA function.

Two structures for EngA are available, from *T. maritima* with GDP bound in G2 and two phosphate molecules bound to G1 (Robinson *et al.*, 2002), and *B. subtilis*, with GDP in both G1 and G2 active sites (Muench *et al.*, 2006). Aligning the two molecules on the basis of the C-terminal KH domain, reveals that G2 is in the same relative orientation in the two structures, whereas G1 has undergone a dramatic conformational change (Figure 3E). The two phosphates in G1 of *T. maritima* EngA have been suggested to mimic the β and γ phosphates of GTP, inducing an "on" state in the factor, whereas the GDP in *B. subtilis* EngA represents the "off" state (Muench *et al.*, 2006). This is consistent with the open conformation representing the "on" state, such that the highly basic surface of the KH domain, which is responsible for RNA interaction, remains exposed (Figure 3F), whereas in the closed "off" state, G1 has shifted position to cover this region (Figure 3G) (Muench *et al.*, 2006). This model is in agreement with the idea that EngA binds to the pre-50S in the GTP state, and that hydrolysis to the GDP state could provide a mechanism for dissociating the factor from the mature subunit. Now it is important to determine whether EngA binds to the pre-50S particles and what the trigger for GTPase activation is.

III. EngB (YihA/YsxC)

The genes encoding EngB family of proteins, *ysxC* in *B. subtilis* and *yihA* in *E. coli*, have been shown to be essential for cell viability (Arigoni *et al.*, 1998; Dassain *et al.*, 1999; Prágai and Harwood, 2000). In *Bacillus*, *ysxC* is located within a bicistronic operon, downstream of the *lonA* gene, which encodes protease involved in protein degradation in response to environmental stress. YsxC has been shown to bind to 70S ribosomes and 50S, but not 30S subunits, with the strength of the interaction being increased by the presence of G

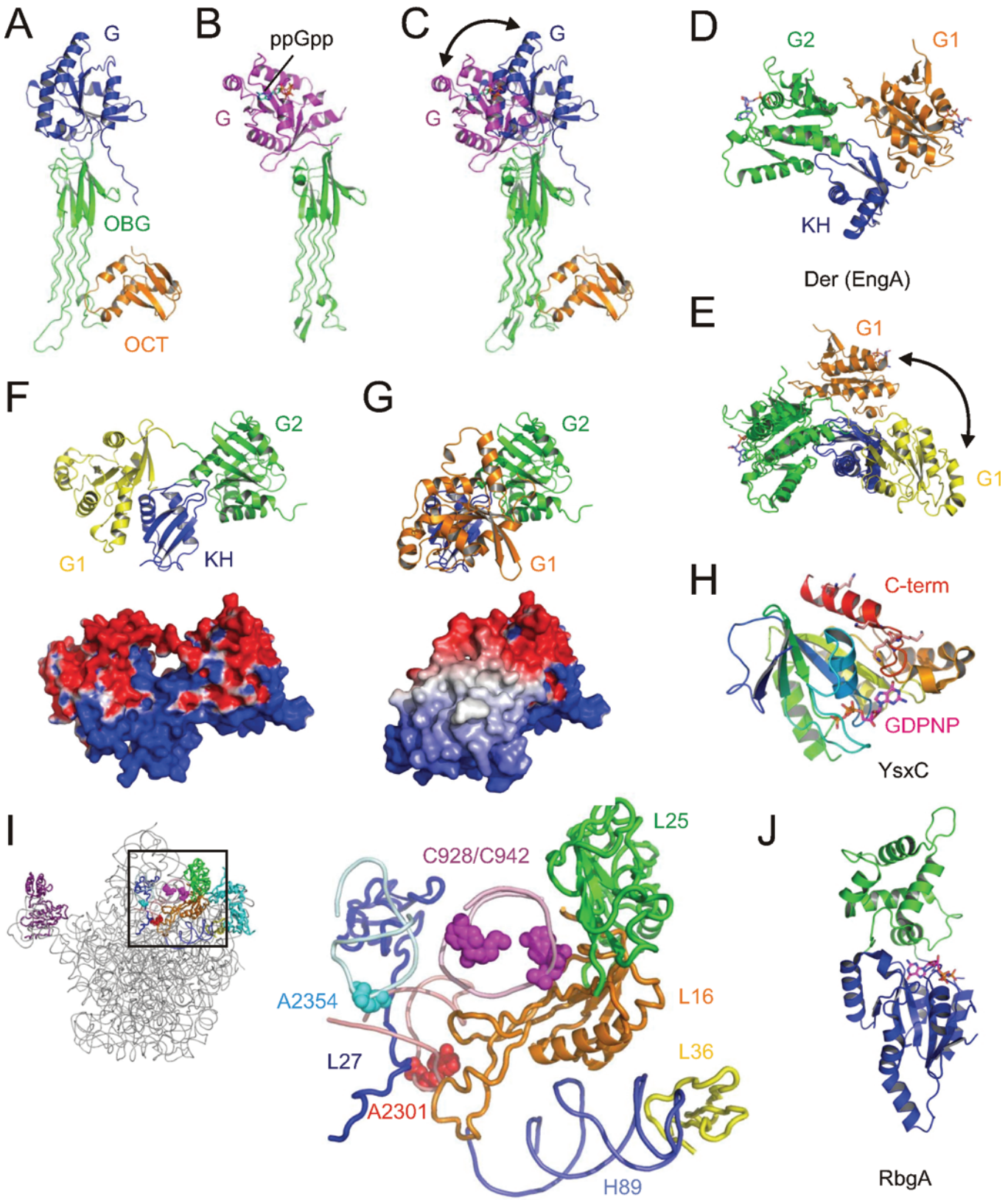
nucleotides (Schaefer *et al.*, 2006). The structure of YsxC in complex with GDPNP (Ruzheinikov *et al.*, 2004) reveals a single domain protein (Figure 3H), which, however lacks any classic RNA binding domains, such as the KH domains seen in Era or EngA. The highly basic C-terminal alpha helix could potentially mediate interaction with rRNA (Figure 3H), but this has yet to be confirmed (Ruzheinikov *et al.*, 2004). Depletion of YsxC from the cell leads to a reduction in the level of 70S ribosomes as well as a concomitant accumulation of subunits. Interestingly, the migration of the large ribosomal subunit migrates at 44.5S, rather than the usual 50S position (Schaefer *et al.*, 2006). A comparative analysis of the 44.5S particle reveals the absence of r-proteins L16, L27, and L36, analogous to the 45S particles that accumulate in cells depleted of EngA or RbgA (Schaefer *et al.*, 2006). These proteins are clustered together adjacent to the peptidyltransferase center of the mature 50S subunit, and are linked through common contacts with 23S rRNA: L16 and L36 both interact with H89, while L27 and L16 contact different regions of H38 (Figure 3I). Since these three proteins are late assembly proteins, the function of YsxC may be to convert the inactive 44.5S particle into an active 50S particle by recruiting these final group of late assembly proteins.

IV. RbgA (YlqF)

Gradual depletion of RbgA from *B. subtilis* cells leads to a corresponding decrease in growth rate (Matsuo *et al.*, 2006; Uicker *et al.*, 2006) and is characterized by a change in gene expression, namely an upregulation of genes involved in protein synthesis and downregulation of metabolic genes (Uicker *et al.*, 2006). Similar responses are observed during conditions of nutrient starvation (see section EI) as well as when sub-lethal concentrations of translation inhibitors are given to cells (Sabina *et al.*, 2003). The ribosome profiles of RbgA-depleted cells show greatly reduced levels of 70S ribosomes and concomitant accumulation of subunits (Matsuo *et al.*, 2006; Uicker *et al.*, 2006). In addition the large ribosomal subunit migrates at 45S, rather than 50S, suggesting it is an assembly intermediate (pre-50S) (Matsuo *et al.*, 2006; Uicker *et al.*, 2006). The appearance of the 45S particle is directly related to growth rate and the level of RbgA expression, as well as being specific for RbgA depletion since no 45S peak is observed when EF-Tu and IF2 are depleted (Uicker *et al.*, 2006).

RbgA has been shown to bind stably to the precursor 45S particles (Uicker *et al.*, 2006), however binding to the 50S subunit requires the presence of a non-hydrolysable GTP analogue (GDPγS), since no binding is detected in the presence of GTP or GDP (Matsuo *et al.*, 2006). Consistently, the GTPase activity of RbgA is stimulated by the presence of the 50S subunit; however, no comparison was made using 45S precursors. Analysis of the 45S particles reveals that r-protein L16, L27, and L36 are absent (Matsuo *et al.*, 2006; Schaefer *et al.*, 2006; Uicker *et al.*, 2006), and yeast two-hybrid and pull-down assays demonstrate that RbgA directly interacts with only one r-protein, namely L25 (Matsuo *et al.*, 2006). Binding of RbgA to the 50S subunit led to protection from DMS of C928 and C942 (H38), A2301 (H81), and A2354 (H85) in the 23S rRNA. In the mature 50S subunit, both L16, L25, and L27 interact with H38 and are in close proximity to C928 (Figure 3I). In addition, an extension from L16 reaches toward A2301. The crystals structure of *B. subtilis* RbgA (PDB1PUJ) has two domains, an N-terminal G-domain

Figure 3 **GTPases involved in large subunit assembly. Structures of Obg from (*A*) *T. thermophilus* (PDB1UDX; Kukimoto-Niino *et al.*, 2004) and (*B*) *B. subtilis* (PDB1LNZ; Buglino *et al.*, 2002) with (*C*) a superposition of both structures aligned on the basis of the unique OBG domain (*green*). In (*A*) the OCT domain (*orange*) was also visualized, whereas in (*B*) the signalling molecule, ppGpp was found in the G domain. The arrow in (*C*) indicates the different positions of the G domains relative to the OBG domain in the two structures. (*D*) Structure of *T. maritima* Der (PDB1MKY; Robinson *et al.*, 2002) coloured to highlight the C-terminal KH domain (blue) and the two G-domains (*green* and *orange*), which have GDP (pale blue) in the active sites. (*E*) Alignment of *T. maritima* Der with *B. subtilis* homologue (PDB2HJG; Muench *et al.*, 2006) on the basis of KH domain, revealing the dramatically different position of the G1 domains (arrowed). (*F*) Structure of Der in "open" conformation, shown as ribbons (above) and surface representation (below), highlighting regions of positive and negative electrostatic potential in blue and red, respectively. (*G*) Ribbons (above) and surface representation (below) of Der in the "closed" conformation, with coloring as in (*D*). Note the rearrangement of the G1 domain in the closed conformation covers the highly basic KH domain seen in the open conformation. (H) Structure of YsxC (rainbow coloured from N-terminal (*blue*) to C-terminal (*red*) in complex with GDPNP (*magenta*) (PDB1SVW; Ruzheinikov *et al.*, 2004). Note, the highly basic Lys and Arg residues located in the C-terminal (C-term) helix are also shown. (*I*) Binding site of RbgA (YlqF) on the large ribosomal subunit with enlargement of boxed area of inset subunit (with L1 (*magenta*) and L11 (*cyan*) included for reference). Ribosomal proteins L16 (*orange*), L25 (*green*) and L27 (*blue*) are highlighted, as are nucleotides C928 and C942 (*magenta*) in H38 (*pink*), A2301 (*red*) in H81 and A2354 (*cyan*) in H85. Figure uses *B. subtilis* numbering on *D. radiodurans* 50S structure (Wilson *et al.*, 2005) as outlined by Matsuo *et al.*, 2006. (*J*) Crystal structure of RbgA indicating two domain arrangement, with N-terminal G-domain (*blue*) with GTP molecule (magenta) and C-terminal acidic domain (*green*).**



connected through a conserved linker to an acidic C-terminal domain (CTD) (Figure 3J). Therefore it is tempting to speculate that it is the CTD of RbgA that interacts with the highly basic r-protein L25 and RbgA may even be involved in recruiting L16 and L27 to the ribosome. L16 and L27 are both late assembly proteins (Nierhaus, 1991) confirming that RbgA is involved in a late assembly step. The addition of L16 during assembly results in large conformational changes within the ribosome (Teraoka and Nierhaus, 1978), which is consistent with the conversion of the 45S to 50S particle. The inability of 45S to interact with 30S subunits to form 70S ribosomes suggests that RbgA may act as a control checkpoint, ensuring only mature 50S particles enter into the translation cycle (Uicker *et al.*, 2006).

C. ON YOUR MARKS, GET SET, GO: FACTORS INFLUENCING TRANSLATION INITIATION

Compared with eukaryotic translation initiation that involves many multi-subunit factors (Hinnebusch *et al.*, 2004), the initiation process in bacteria appears by comparison to be relatively simple having only three essential factors IF1, IF2, and IF3. Indeed, *in vitro* translation systems using purified factors revealed that these three factors were necessary and sufficient for translation initiation (Shimizu *et al.*, 2001). However, *in vivo* other bacterial factors appear to be necessary for this process, such as elongation factor P (EF-P), and possibly YciH and YhbY.

I. EF-P May Stimulate Formation of the First Peptide Bond

EF-P was originally identified in the 1970s by Ganoza and coworkers as a soluble protein factor in *E. coli* that stimulated the peptidyltransferase activity of 70S ribosomes (Glick and Ganoza, 1975, 1976). Specifically, it has been reported that EF-P stimulates *in vitro* dipeptide formation between fMet-tRNA in the P site and puromycin in the A site of the ribosome, suggesting it is involved in the formation of the first peptide bond (Ganoza and Aoki, 2000; Ganoza *et al.*, 1985; Swaney *et al.*, 2006). Although EF-P does not appear to be essential for *in vitro* translation, the *efp* gene is universally conserved in bacteria (Kyrpides and Woese, 1998) and is essential for cell viability in *E. coli* (Aoki *et al.*, 1997).

E. coli EF-P is 188 amino acids and shares significant homology with eukaryotic initiation factor 5A (eIF-5A), although eIF-5A is generally shorter in length. Crystal structures of *T. thermophilus* EF-P (Hanawa-Suetsugu *et al.*, 2004) and three archaeal eIF-5As (Kim *et al.*, 1998; Peat *et al.*, 1998; Yao *et al.*, 2003) have been determined. EF-P and eIF-5A consist of three and two β-barrel domain proteins, respectively. Domain I of EF-P is topologically similar to the N-terminal domain of eIF-5A, whereas domain II and III of EF-P appear to have arisen by duplication and are equivalent to the C-terminal domain of eIF-5A (Hanawa-Suetsugu *et al.*, 2004) (Figure 4A and B, respectively). The N-terminal domain of eIF-5A contains a conserved lysine residue at the tip of the loop, which is modified post-translationally to hypusine (Figure 4B) (Park *et al.*, 1993). The enzyme that modifies lysine to hypusine is essential for viability in yeast (Park *et al.*, 1993). While hypusine is not present in bacteria (Magdolen *et al.*, 1994), the strict conservation of either lysine or arginine at the equivalent position in EF-P (Figure 4A) hints at an important functional role for this residue.

The three domains of EF-P are arranged in an L-shape with 65 Å and 56 Å arms at an angle of 95°, which is reminiscent of overall dimensions of a tRNA (compare Figure 4A and C) (Hanawa-Suetsugu *et al.*, 2004). The data on the binding site of EF-P are inconclusive. EF-P has been reported to bind to the both 30S and 50S subunit, near the streptomycin binding site on the 30S, and interacting with domains II and V of the 23S rRNA near the peptidyltransferase centre on the 50S subunit (Aoki *et al.*, 1997; Ganoza *et al.*, 2002). Furthermore, L16 or at least the N-terminal 47 amino acids appear to be important for EF-P mediated peptide bond formation (Baxter *et al.*, 1987; Ganoza *et al.*, 1985). If the structurally homology to tRNA is used as a guide, then EF-P may bind within the E site, since the A and P sites would be occupied by tRNA to form the first dipeptide. However, it should be noted that the ribosome-recycling factor (RRF) also exhibited structural similarities with tRNA (Selmer *et al.*, 1999) and was found to bind in a quite different orientation than would be expected from the mimicry (Vesper and Wilson, 2006).

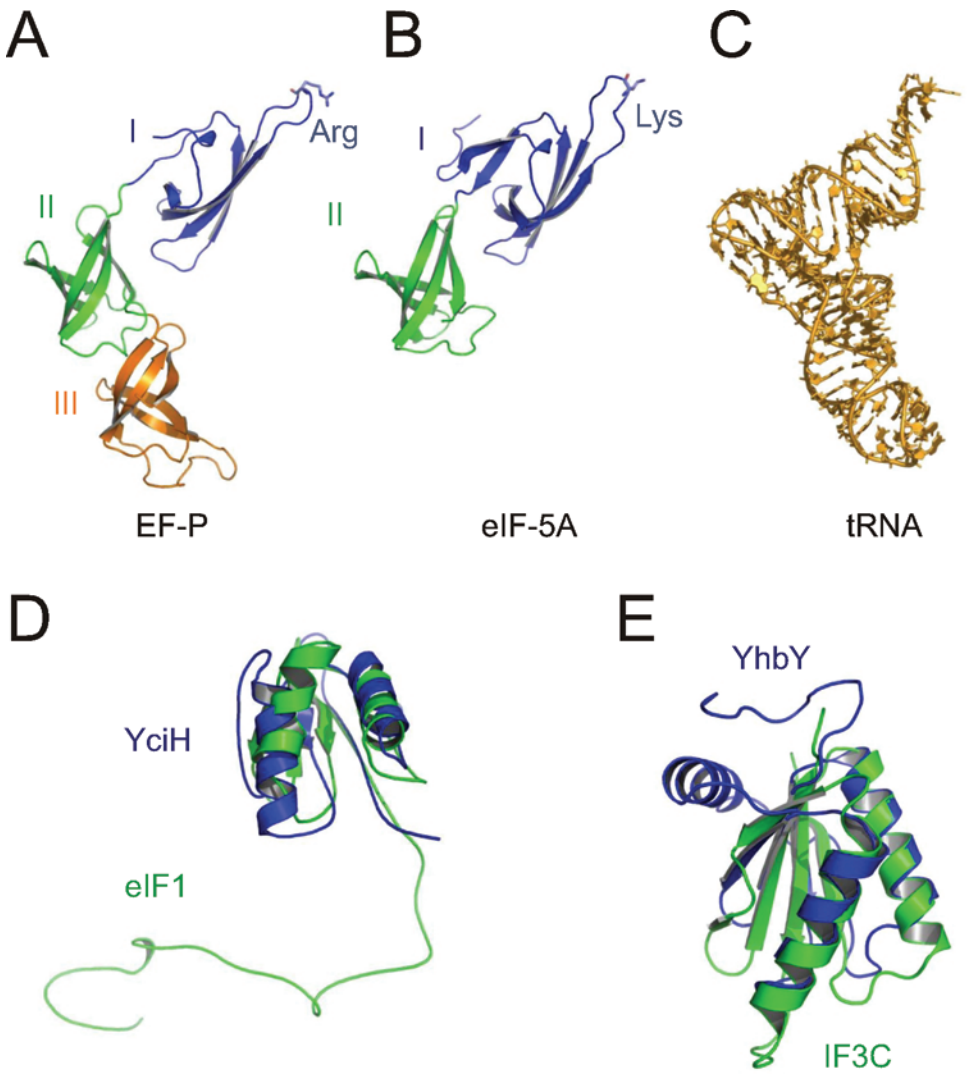


Figure 4 Factors involved during initiation of protein synthesis. Structures of (*A*) EF-P (PDB1UEB; Hanawa-Suetsugu *et al.*, 2004), (*B*) eIF-5A (PDB1EIF; Kim *et al.*, 1998), and (*C*) tRNA (PDB1TTT; Nissen *et al.*, 1995). The conserved Arg (or Lys) residue in EF-P and Lys residue that is hypusine modified in eIF-5A are shown as sticks. Superposition of structures for (*D*) YciH (blue, PDB1D1R; Cort *et al.*, 1999) and eIF1 (*green*, PDB2IF1; Fletcher *et al.*, 1999), and (*E*) YhbY (*blue*; PDB1LN4; Osthelmer *et al.*, 2002) and C-terminal domain of initiation factor 3 (IF3 C, green; PDB1TIG; Biou *et al.*, 1995).

II. A Role for YciH and YhbY in Bacterial Translation Initiation?

Sequence alignments suggest that YciH is an ortholog of yeast SUI1, the human homologue of which is eukaryotic initiation factor 1 (eIF-1; note that eIF-1 is not related to bacterial IF1). Yeast eIF1 is essential, and plays an important role in assembly of translation initiation complexes as well as AUG start codon selection (reviewed by Hinnebusch *et al.*, 2004). The structures of both eIF1 (Fletcher *et al.*, 1999) and YciH (Cort *et al.*, 1999) have been determined and are remarkably similar (Figure 4D). The topology is consistent with an oligonucleotide-binding fold, although neither YciH nor eIF1 has been shown to bind RNA (or DNA) specifically. Other proteins with similar folds include r-protein S6, EF-G, and a number of DNA binding proteins, such as polymerases and transcription factors (Cort *et al.*, 1999). *YhiH* is present in all completely sequenced archaeal genomes, and although this factor is not found in many bacterial genomes, the role it plays in *E. coli* may help to understand the function in higher organisms.

In at least two archaeal lineages, the *yhbY* homologues are found within operons encoding r-proteins (L20, L31, and L39), eIF6 and RNase P subunit (Osthelmer *et al.*, 2002). Although in *E. coli yhbY* is most likely monocistronically transcribed, the growth rate

dependence of *yhbY* mRNAs parallels those of mRNAs for translational components, and YhbY has been shown to co-sediment with 50S subunits (Osthelmer *et al.*, 2002). Indeed, YhbY was detected in 40S precursors to the 50S subunit that accumulate when Obg is depleted from the cell (Jiang *et al.*, 2006). The crystal structure of *E. coli* YhbY reveals extensive similarity with the CTD of IF3 (Figure 4E) despite no detectable sequence homology, as well as with many other oligonucleotide-binding proteins involved in intron-splicing or RNA modification (Osthelmer *et al.*, 2002). Much work needs to be done to elucidate whether this protein is involved in biogenesis, initiation or has another role during translation.

D. AT THE HEART OF THE MATTER: SPECIAL FACTORS THAT ENTER INTO THE ELONGATION PHASE OF TRANSLATION

The elongation cycle entails the ribosome moving between two main states: The pre-translocational state (PRE), with tRNAs at the A and P sites, and the post-translocation state (POST), where the tRNAs have moved together with the mRNA from the A and P sites to the P and E sites, respectively. Both states are separated by high energy barriers of about 120 kJ/mol (Schilling-Barteztko *et al.*, 1992), which are reduced by two universal elongation factors, EF-Tu (EF1) and EF-G (EF2) (seen in Figure 5A and B, respectively). Thus, the transition between PRE and POST states is catalyzed by these two elongation factors, with EF-G pushing the equilibrium towards the POST state, and EF-Tu, which forms a ternary complex with aa-tRNA and GTP, pushing the equilibrium to the PRE state by delivering the next tRNA to the A site of a POST state ribosome on the basis of the codon of the mRNA displayed there. Binding of ternary complex at the A site leads to concomitant release of the E-tRNA, thus maintaining two tRNAs on the ribosome (see Wilson and Nierhaus, 2006). The elongation cycle is universally conserved, with eukaryotes and archaea having homologous factors to EF-G and EF-Tu, termed EF2 and EF1, respectively. However, an exception exists: Yeast have an additional elongation factor EF-3, which is a necessary prerequisite for release of E-tRNA rather than causing the release (Triana-Alonso *et al.*, 1995). In addition, there are a number of factors that utilize their high sequence similarity to elongation factors to enter into the elongation cycle, such as LepA and Tet(O). LepA (EF4) is a protein present in all bacteria (with three exceptions, see below), including mitochondria and chloroplasts. LepA appears to improve the fidelity of translation by back-translocating mistranslocated ribosomes (Qin *et al.*, 2006). Lastly, some bacteria have additional ribosomal protection proteins (RPPs), such as the Tet(O) family of proteins. Tet(O) binds to ribosomes stalled during translation due to the presence of the antibiotic tetracycline, and chases the drug from the ribosome, allowing protein synthesis to continue unhindered (reviewed by Connell *et al.*, 2003a; Roberts, 2002, 2005).

I. LepA (EF4) is an Elongation Factor that Back-Translocates the Ribosome

LepA is one of the most conserved proteins known, ranging from 55% to 68% amino acid identity between bacterial homologs. Such conservation is higher than that observed for other essential translation factors, such as IF3 or exchange factor EF-Ts. LepA is not found in archaea, nor in eukaryotic 80S systems, but is present as one copy in all bacteria with three exceptions: (i) *Streptococcus pyogenes* MGAS8232 NC 003485, which has no *lepA* gene, whereas four other *Streptococci pyogenes* as well as other *Streptococci* strains do have a *lepA* gene, (ii) *Carsonella ruddii*, an obligate symbiont living in lice with the smallest genome (182 genes) known to date (Nakabachi *et al.*, 2006) also contains no *lepA* gene, and (iii) *Pirellula*, which has two copies (Margus *et al.*, 2007). LepA has been retained in *Mycoplasma*, which has a reduced genome of only ~500 genes, in the small free-living bacteria *Rickettsia*, as well as being found in chloroplasts and all mitochondria from yeast to human. Despite this conservation, deletion of the *lepA* gene in *E. coli*, as well as yeast mitochondria appears to have little effect on viability (Dibb and Wolfe, 1986; Kiser and Weinert, 1995). However, recently it could be shown that at a low pH of $\leq$6.5 exaggerated by higher concentrations of K^+ and Mg^{2+} LepA becomes essential for growth (Karim and Nierhaus, unpublished data). These observations are in agreement with a systematic randomized knock-out study of *Helicobacter pylori* demonstrating that the intact *lepA* gene is essential for survival of this strain at low pH (Bijlsma *et al.*, 2000). Therefore, at least for some bacterial species LepA could be an attractive target for new classes of antibiotics.

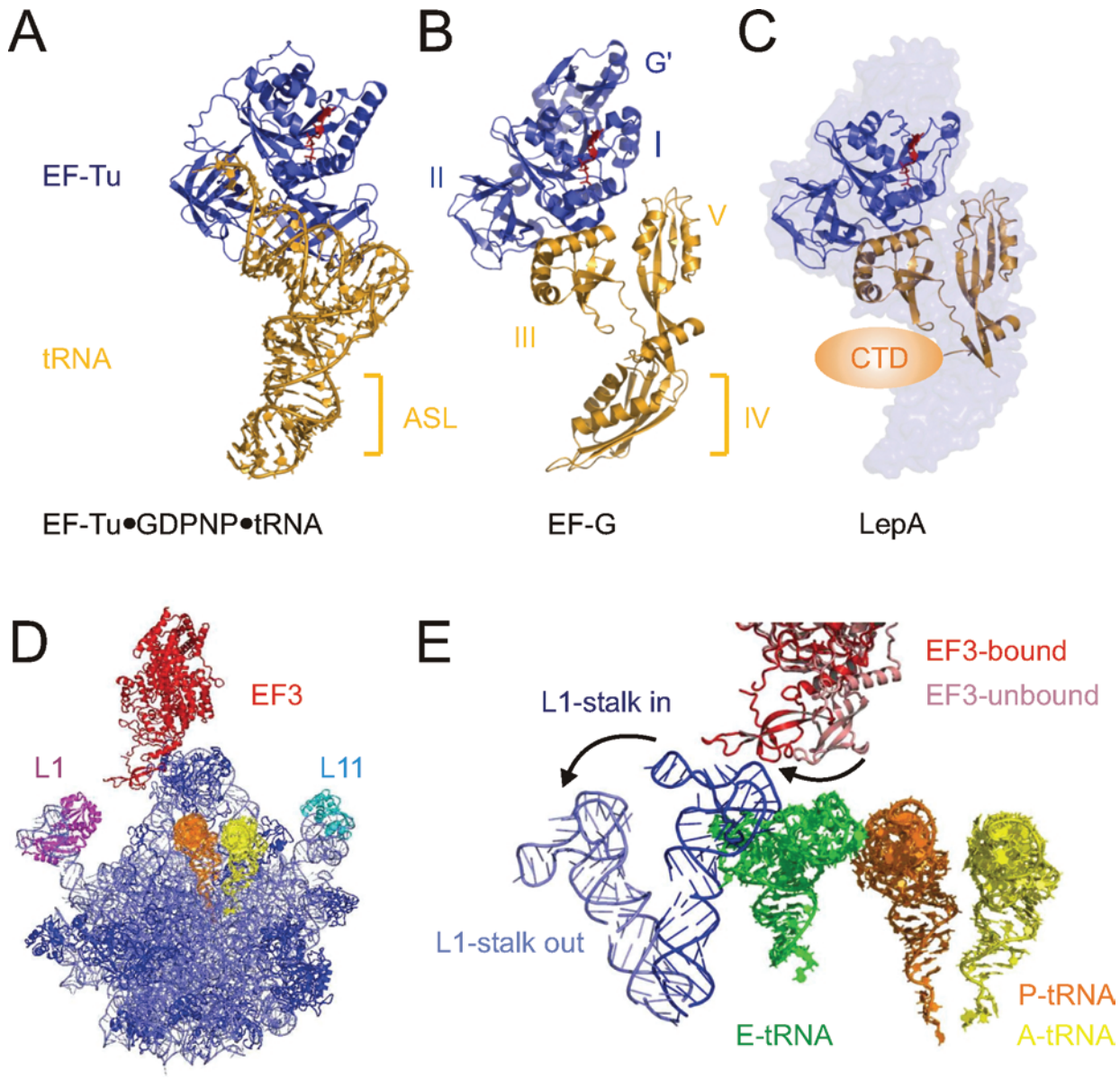


Figure 5 **Unusual factors in translation elongation. Comparison of the structures of (*A*) the ternary complex aa-tRNA·EF-Tu·GTP (PDB1TTT; Nissen *et al.*, 1995) with that of (*B*) EF-G (PDB1WDT) and (*C*) a homology model for *E. coli* LepA (Qin *et al.*, 2006). The domains of EF-G are indicated with roman numerals, except the G′ subdomain of domain I. Note that the EF-G domain IV corresponds to the anticodon stem-loop (*ASL*) of the aa-tRNA within the ternary complex and that LepA lacks the G′ domain and domain IV, but has a LepA specific C-terminal domain (CTD). (D) Model for EF3 (*red*) bound to the yeast 80S ribosome (rRNA and r-proteins in *pale blue* and *dark blue*, respectively) based on cryo-EM reconstruction (PDB2IX8; Andersen *et al.*, 2006). R-proteins L1 (*magenta*) and L11 (*cyan*), and A- (*yellow*) and P-tRNAs (*orange*) are highlighted for reference. (E) Model for EF3 action. Binding of EF3 to the ribosome involves a shift in the chromodomain from the "unbound" (*pale red*) to the "bound" (*red*) position, and induces movement of the L1 stalk from the "in" position (*dark blue*), where it contacts the E-tRNA (*green*), to the "out" position (*pale blue*), where the E-tRNA can be released from the ribosome upon binding of tRNA at the A site. Figure constructed from PDB2IX8 (Andersen *et al.*, 2006), PDB1S1I (Spahn *et al.*, 2004) and 1GIX (Yusupov *et al.*, 2001).**

Sequence comparison suggests that LepA is a G-protein with high homology to EF-G, having equivalents to domains I-V of EF-G, with the exception of domain IV and the absence of the G′ domain within domain I (compare Figure 5B and C). LepA binds to ribosomes *in vivo* (Colca *et al.*, 2003) and *in vitro* (Qin *et al.*, 2006), and has a low intrinsic GTPase activity that is stimulated by 70S ribosomes, to levels paralleling those of EF-G (Qin *et al.*, 2006). Overexpression of LepA *in vivo* is toxic to the cell, and consistently addition of increasing amounts of LepA protein progressively inhibits *in vitro* translation. Surprisingly, however, addition of low amounts of LepA increases the fidelity of translation *in vitro*, and can counteract the

translation misreading induced by increased Mg^{2+} ion concentration, but not the inaccuracies resulting from the presence of misreading antibiotics such as paromomycin, streptomycin or edeine (Qin *et al.*, 2006). Multiple biochemical experiments, such as puromycin reaction, footprinting, toeprinting and Pb^{2+} cleavage, reveal that binding of LepA to a POST ribosome, induces a back-translocation, moving the P and E site tRNAs back into the A and P sites, respectively. Collectively, the results suggest that LepA is a stress response elongation factor. Under conditions of low pH ($\leq$6.5) or high ionic strength (K^+ or Mg^{2+}), translocation on the ribosome becomes defective. This leads to incorrect display of the A site codon, which in turn promotes binding of near-cognate tRNAs and therefore misincorporation of amino acids. In the current model, LepA detects these defective POST ribosomes and induces a back-translocation, thus providing EF-G another chance to translocate the ribosomes correctly. Cryo-electron microscopy reconstructions of EF-G-70S complexes reveal that domain IV occupies the position of the A-site, supporting the suggestion that EF-G may act as a doorstop to prevent back-movement of the tRNAs. The back-translational ability of LepA and the absence of an equivalent to domain IV are certainly consistent with this proposal.

II. Is the Function of RbbA Really Analogous to That of EF3?

The discovery of a third elongation factor in yeast was a surprise, especially considering that this factor, termed EF3, could hydrolyze ATP, unlike other translational factors that have a strict requirement for GTP. *In vitro* studies to determine the function of this factor revealed that EF3 is an E site factor, the presence of which is necessary for release of the E-tRNA upon binding of ternary complex EF1α·GTP·aa-tRNA to the A site (Triana-Alonso *et al.*, 1995). In these experiments, ternary complex of EF1α·GTP·aa-tRNA can only bind in the absence of EF3 to ribosomes with a free E site, *i.e.*, to initiation-type (Pi) ribosomes (fMet-tRNA in P site only), but not to POST state ribosomes (tRNAs at both P and E sites). However, the presence of EF3 stimulates ternary complex binding to the A site with concomitant release of the E-tRNA. This reaction is dependent on ATP hydrolysis, since no stimulation by EF3 is observed in the presence of ADP or a non-hydrolysable ATP analog (AMPPNP). EF3 directly affects the binding stability of E-tRNA, since radiolabelled E-tRNA can be chased by unlabelled tRNA cognate to the E-tRNA, but only in the presence of EF3 and ATP (Triana-Alonso *et al.*, 1995). The fact that the E-tRNA cannot be chased by non-cognate tRNA suggests that codon-anticodon interaction must exist at the E site, as has been proposed for bacteria (see Blaha and Nierhaus, 2001). A recent article presenting the crystal structure of *Saccharomyces cerevisiae* EF3 as well as a cryo-EM reconstruction of EF3 bound to POST state 80S ribosome has provided structural insight into the mechanism of EF3 action (Andersen *et al.*, 2006). Elongation factor 3 (EF3) belongs to the family of ABC (ATP-binding cassette) proteins, together with proteins involved in membrane transport and DNA repair. The crystal structure of *Saccharomyces cerevisiae* EF3 comprises five structural domains, an N-terminal HEAT repeat domain linked by a flexible linker to a four-helix bundle, with the C-terminal region containing two ABC cassettes, ABC1 and ABC2, into the latter of which a chromodomain is inserted. In the cryo-EM reconstruction, EF3 binds at novel site on the 80S, distinct from the binding sites of EF1α and EF2, with the HEAT domain contacting the head of the small 40S subunit and the ABC2 domain interacting with the central protuberance of the large 60S subunit (Figure 5D and E). Comparison of the ribosome-bound and free forms of EF3 suggest that EF3 undergoes dramatic conformational change upon binding to the ribosome. In particular, it appears likely that a switch in the chromodomain position correlates with an opening of the L1 stalk from a closed to an open position, which would unlock the E site and allow release of the E-tRNA upon A site binding (Andersen *et al.*, 2006) (Figure 5E).

A homologue for EF-3 has been proposed to exist in *E. coli*, termed ribosome-bound ATPase (RbbA) and encoded by the *yhiH* gene (Kiel *et al.*, 1999). The sequence of the RbbA gene indicates that it has two ATP-motifs in the N-terminal region and an RNA-binding motif (Kiel *et al.*, 1999). RbbA has a low intrinsic ATPase activity, which is stimulated in the presence of 70S ribosomes (Xu *et al.*, 2006). Indeed, RbbA has been shown to bind to both 30S subunits and 70S ribosomes, and to stimulate *in vitro* poly(Phe) synthesis (Kiel and Ganoza, 2001; Xu *et al.*, 2006). A truncated stable form of RbbA, lacking the C-terminal transmembrane domain, has been demonstrated to function similar to full-length RbbA by promoting binding of ternary complex EF-Tu·GTP·aa-tRNA to

the A site of ribosomes, as well as stimulating release of deacylated tRNA from the E site (Xu *et al.*, 2006). However, these experiments were performed with mini-mRNAs such as $AUGU_3$, therefore it will be important in the future to analyze more physiological mRNAs as well. Binding of RbbA to the ribosome leads to strong protection of A937 against DEP treatment, and lesser protection at A915 and A949, in contrast to the protections observed around G889, G890, or G925, on the naked 16S rRNA (Xu *et al.*, 2006). Both A937 and A949 are located in close proximity to where the anticodon stem-loop of an E-tRNA binds, suggesting that RbbA may indeed bind within the ribosomal E site, consistent with the competition with E-tRNA. However, A915 is totally inaccessible, being located behind h44 within the body of the 30S subunit. It still remains unclear as to which conditions necessitate the presence of RbbA, since it is not required for *in vitro* translation systems using purified factors (Shimizu *et al.*, 2001) and release of the E-tRNA from *E. coli* ribosomes has been shown to occur upon binding of the ternary complex to the A site in the absence of RbbA (Dinos *et al.*, 2005). Furthermore, the homology with EF3 may be a "red herring," since it is predominantly to the ABC cassettes located in the C-terminal of EF3. In fact, RbbA exhibits no homology with the N-terminal HEAT domain region of EF3, which has been shown by cryo-EM to be the main site of contact with the head of the 40S (Andersen *et al.*, 2006). This does not exclude that RbbA plays an analogous role to EF-3 but in a different manner; however, further experiments will be required to confirm this.

III. Tet(O) Enters the Elongation Cycle to Confer Tetracycline Resistance

The tetracycline conferring resistance RPPs represent one of the subfamilies known to be derived from the EF-G family (Roberts, 2005). These RPPs are likely to have originated from *otrA*, an RPP-determinant found in the producer of tetracycline, *Streptomyces rimosus* (Connell *et al.*, 2003a). A recent genome analysis identified Tet-coding genes in only 20 genomes, predominantly in bacterial lineages that are likely to have been exposed to the drug and therefore have acquired the resistance genes, such as symbionts in the mammalian gut or mammalian pathogens (Margus *et al.*, 2007). RPPs confer resistance to tetracycline by binding to the drug-inhibited ribosomes, for example, the addition of purified Tet(O) to a tetracycline inhibited *in vitro* poly(Phe) system can shift the half-inhibitory concentration (IC_{50}) from 100 μM to over 500 μM (Trieber *et al.*, 1998). A cryo-EM reconstruction of Tet(O) bound to the *E. coli* 70S ribosomes reveals Tet(O) binds in a common site with EF-G and has an overall similar shape, differing in the vicinity of domain IV (Spahn *et al.*, 2001). In EF-G, domain IV contacts h44 of the 16S rRNA and is important for the translocation activity of EF-G (see LepA section above), whereas the equivalent region of Tet(O) approaches h34 instead, consistent with the lack of translocation ability of this factor. Protection experiments suggest that binding of Tet(O) induces long-range conformational changes in the ribosome that release tetracycline from it's primary binding site in minor groove of h34 (Connell *et al.*, 2003b; Connell *et al.*, 2002). Interestingly, this conformational change persists after GTP hydrolysis and dissociation of Tet(O) from the ribosome, thus protecting the ribosome from rebinding of tetracycline and providing an advantage for ternary complex binding (Connell *et al.*, 2003b).

E. COPING WITH STRESS: TRANSLATION REGULATION UNDER DIVERSE ENVIRONMENTAL CONDITIONS

I. RelA, RelBE and tmRNA: A Three Pronged Response to Nutrient Deprivation

The three regulatory systems RelA, RelBE, and tmRNA form an intricate network representing a very efficient "SOS" system for the bacterial cell under a variety of emergency situations (reviewed by Condon, 2006; Wilson and Nierhaus, 2005). The most complicated component of this network is the stringent reponse governed by RelA, which will be discussed first followed by a description of the astonishing RelBE toxin-antitoxin system. After mentioning the function of the tmRNA system, we will comment on how these three systems are interwoven.

One of the most important regulatory circuits in bacteria is the stringent response. This term means a tight coupling of protein and RNA synthesis: If protein synthesis stops due to a curtailment in nutrient supply, the synthesis of rRNA is stopped within a fraction of a minute. In contrast, relaxed mutants

continue to synthesize rRNA for >1 hour following a translational block (Yang and Ishiguro, 2001), thus destroying the finely tuned balance between synthesis of r-proteins and rRNA that normally exists in the cell. Even if the growth conditions improve, the cell has serious difficulties in recovering and–dependent on the strength of the starvation period–might even fail to recover at all. The stringent response provides the cell with a prominent regulatory means to control gene expression (for review see Iskakova *et al.*, 2004). The effect is twofold: (i) transcriptional repression of genes associated with the translational apparatus, for example genes encoding tRNAs, rRNAs, ribosomal proteins, translational factors, and synthetases; (ii) and up-regulation of genes encoding metabolic enzymes, especially those involved in amino acid biosynthesis. Activation of the stringent response initially stems from the shortage of one (or more) amino acid(s), which in turn produces a significant increase in the pool of uncharged-tRNA (termed deacylated-tRNA) for the corresponding amino acid(s). In log-phase bacterial cells, deacylated-tRNA constitutes ~15% of the total tRNA, the majority of which is present in a bound state, *viz.* bound either to ribosomes or synthetases. Under conditions of amino acid starvation, the deacylated-tRNA fraction can increase to >80% of the total tRNA. The scarcity of the specific aminoacylated-tRNA, compounded by the large pools of the free deacylated-tRNA, enables the deacylated-tRNA to bind to the empty ribosomal A site, conditional to presence of a cognate codon in the A site (Jenvert and Schiavone, 2005). The presence of a deacylated-tRNA in the A site triggers RelA-dependent synthesis of guanosine 5′-triphosphate 3′ diphosphate (pppGpp) and guanosine 3′, 5′ bisphosphate (ppGpp) from ATP and GTP or GDP, respectively (Haseltine and Block, 1973; Haseltine *et al.*, 1972; Sy and Lipmann, 1973). The products, collectively referred to as (p)ppGpp, most likely exert a regulatory effect on transcription via an interaction with the β-subunit of the transcriptase (Chatterji *et al.*, 1998; Travers, 1976; van Ooyen *et al.*, 1976). (p)ppGpp is a more general effector also being important for general stress conditions, *e.g.*, when pathogenic bacteria attack their host as well as for bacterial multicellular behavior (reviewed by Braeken *et al.*, 2006). The relaxed phenotype that removes the stringent coupling between translation and transcription arises from mutations in either *relA*, the gene encoding the stringent factor RelA (Block and Haseltine, 1975; Stent and Brenner, 1961), or in *relC*, the gene for ribosomal protein L11 (Friesen *et al.*, 1974; Parker *et al.*, 1976; *relC* = *rplK*). Early studies demonstrated that binding of RelA to 70S ribosomes is essential for the production of (p)ppGpp synthesis (Ramagopal and Davis, 1974; Richter, 1976; Richter *et al.*, 1975) and that RelA binding is enhanced by the presence of a poly(U)-mRNA (Wagner and Kurland, 1980). The synthesis of (p)ppGpp has been shown to be dependent on a deacylated-tRNA at the A site (Haseltine and Block, 1973) and inhibited *in vivo* when L11 is absent (Friesen *et al.*, 1974). The binding of RelA to the ribosome is predominantly influenced by mRNA and not by deacylated-tRNA or L11 (Wendrich *et al.*, 2002). In contrast, RelA-catalyzed (p)ppGpp synthesis is strictly dependent on L11 and is coupled to RelA release from the ribosome. Furthermore, it is the release of RelA, not deacylated-tRNA, from the ribosome that is concomitant with (p)ppGpp synthesis. The resulting model describing the mode of action of RelA during the stringent response shows RelA hopping between blocked ribosomes (Figure 6A; Wendrich *et al.*, 2002), providing an explanation for how low intracellular concentrations of RelA (1/200 ribosomes) can synthesize (p)ppGpp at levels that accurately reflect the starved ribosome population. There also appears to be a link between the stringent response and ribosome biogenesis, since the Obg gene (section BIII), which is normally essential, can be deleted in a RelA-knock-out strain (Raskin *et al.*, 2007). Furthermore, depletion of Obg leads to global changes in gene expression reminescent of the stringent response (Raskin *et al.*, 2007) and Obg has been shown to interact with SpoT, an enzyme that degrades ppGpp (Wout *et al.*, 2004; Raskin *et al.*, 2007),

The second regulatory circuit present in bacteria, and at least some archaea, belongs to the astonishing "suicidal systems." Each system consists of a stable toxin and an unstable anti-toxin that neutralizes the killing effect of the toxin. As long as the toxin exists in the cell at a significant concentration, the anti-toxin must be synthesized to prevent cell death. Both proteins are encoded in a single operon with the toxin gene located downstream of the anti-toxin gene. Both the toxin, as well as the anti-toxin/toxin complex, negatively regulate the promoter of the operon, thus providing a feedback autoregulation control. What could be the importance for such a seemingly exotic system? One explanation is the maintenance of plasmids: When plasmid born products do not provide a selective advantage, random

mutations of the plasmid replication apparatus causes loss of the replicon, thus leaving most bacteria on this planet without plasmids. However, the presence of an toxin/anti-toxin system ensures survival of the plasmid, since the initial loss of the plasmid leaves the cell with the stable toxin and no way to continue production of the labile antitoxin. In fact, the *pem* locus on the plasmid R100 encoding the *E. coli* chromosomal homologue *chpBIK* is responsible for the stable maintenance in the host cell (Masuda *et al.*, 1993) and in lambda phage an antitoxin has also been found serving the same function (Engelberg-Kulka *et al.*, 1998). The term "addiction module" for such a system seems therefore to be an appropriate name. Linking such a system with antibiotic resistance genes would provide a devilish combination. However, these systems are also present in the genome of archaea and bacteria, and in *E. coli* five such systems have been identified including the *relBE* systems, in addition to *chpBIK* (Bech *et al.*, 1985; Gotfredsen and Gerdes, 1998; Pedersen *et al.*, 2003). The crystal structure of an archaeal RelBE complex at 2.3 Å resolution derived from the archeon *Pyrococcus horikishii* provides insight into the mechanism of action of the complementary partners (Takagi *et al.*, 2005). Both the toxin and anti-toxin are small proteins (8 and 11 kDa, respectively) and their structures bear no resemblance to known RNases or RNase inhibitors. Indeed, the RelBE complex presents a bundle of surprises relating to both the structure and function of these proteins. The toxin RelE contains a single domain with an unusual $\beta\alpha_3\beta_4$ fold, whereas the anti-toxin has no hydrophobic core and is wrapped around the toxin by more than one turn (Figure 6B). It follows that the antitoxin has a defined structure only in complex with the toxin, thus when unbound is an easy prey for the ATP-dependent Lon protease, explaining the short half-life of this protein. The toxin has an overall shape of a prolate ellipsoid with the dimension of 40Å × 26Å × 30Å composed of three α-helices and a five stranded β-sheet. The toxin thus mimics, in overall dimension and folding topology, domain IV of the EF-G (Figure 6C–E) that interacts with the decoding center at the ribosomal A site (dimensions of 46Å × 32Å × 25Å). This feature implies how RelE might interact with mRNA at the ribosomal A site. The RelE toxin mediates a highly specific cleavage of the codon of the mRNA located at the ribosomal A site, predominantly after the second nucleotide (Pedersen *et al.*, 2003) (Figure 6F). There is a preference for stop codons with UAG being the most efficient to be cleaved and UGA the worst, and with strong cleavage also observed with the sense codons CAG and UCG. When a deacylated tRNA is at the P site then cleavage at A and E sites is observed with comparable efficiency, and without ribosomes the mRNA is not cleaved (Pedersen *et al.*, 2003). A standard way of blocking nucleases by their respective inhibitors is by binding to the active center of the enzyme, as is true for barnase (Buckle *et al.*, 1994) and the colicins (Graille *et al.*, 2004; Kolade *et al.*, 2002), but the structure and inhibition mechanism of the aRelBE complex is different: RelE has to "sneak" into the A site in order to attack the A-site codon. Therefore, RelB may block the action of RelE by wrapping around the toxin and thus making the toxin too large to enter the A site. This prevents the toxin binding to the target, the ribosome, and thus there is no need for the antitoxin to interact directly with the active site of the toxin. One remaining question for the future is how RelE mediates cleavage of the mRNA: Since RelE does not exhibit RNase activity *per se*, this prompts the question as to what extent the ribosome is involved in the cleavage process.

The main player of the third system is tmRNA (SsrA RNA or 10S RNA), a small stable RNA, which is present in all eubacteria as well as in some chloroplasts and mitochondria. The 3′- and 5′-ends of tmRNA are folded into a tRNA-like structure with an amino-acid acceptor stem that possesses identity elements of $tRNA^{Ala}$ and enables specific aminoacylation of the tmRNA by the alanyl-tRNA synthetase. tmRNA has a short open reading frame in the middle of the molecule that encodes a degradation signal (*tag*-peptide) (Figure 6F), which is recognized by certain cellular proteases (ClpXP, ClpAP). The combination of properties of both tRNA and mRNA results in an unusual translational mechanism for this molecule known as "*trans*-translation"–switching translation from cellular mRNAs lacking stop codons to the coding part of tmRNA, thus adding the *tag*-peptide to the truncated polypeptide chain and targetting it for degradation (reviewed by Haebel *et al.*, 2004; Saguy *et al.*, 2005). Several proteins have been shown to function during initiation of tmRNA-mediated *trans*-translation. EF-Tu is important for the initial binding of tmRNA to the ribosome and may also facilitate the structural arrangement of the tmRNA molecule. In addition, ribosomal protein S1 may bind to the mRNA part of tmRNA in a manner similar to cellular mRNAs. Small protein B (SmpB) is the only key protein known

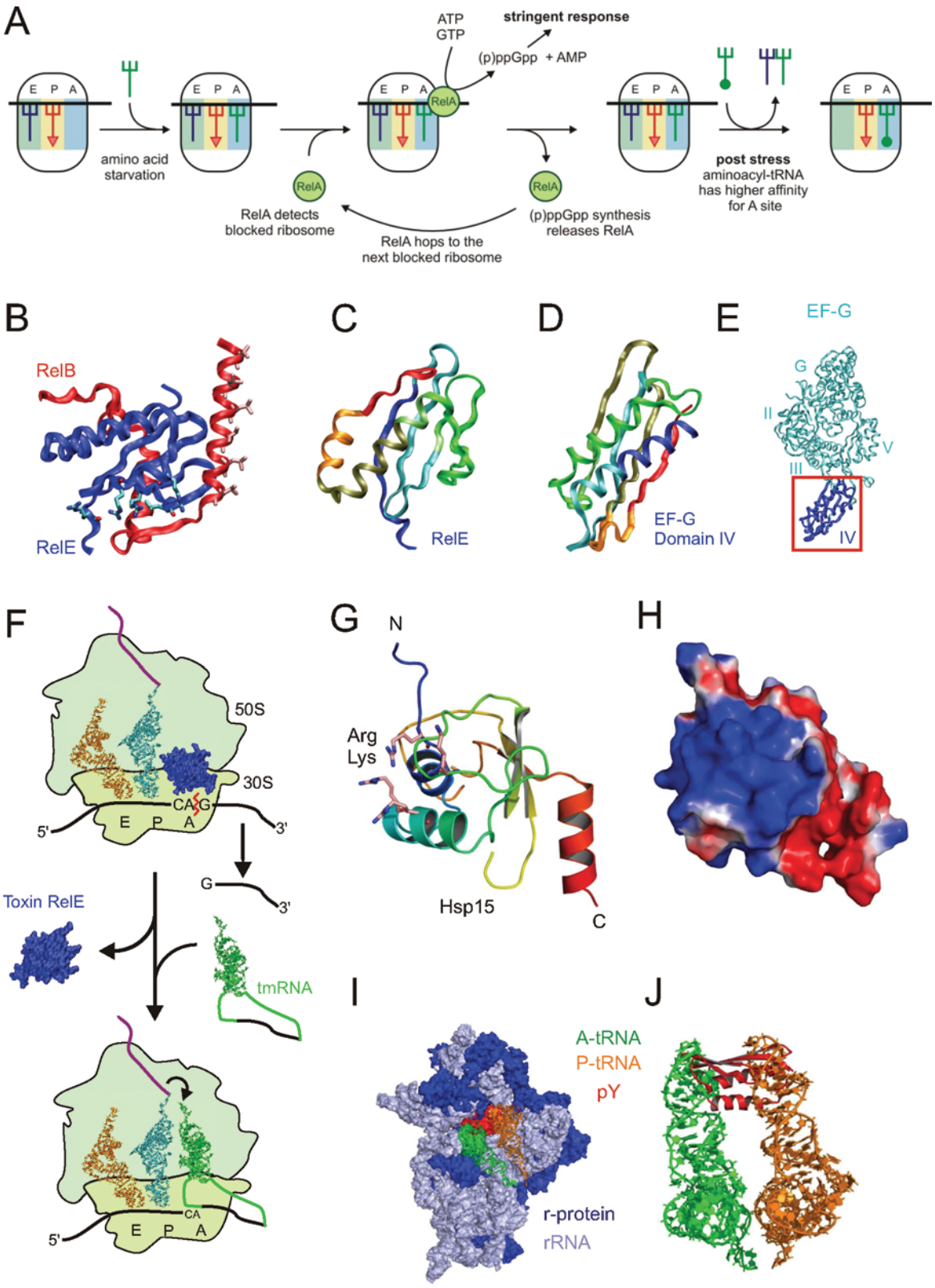
to be essential and specific for *trans*-translation. Both *in vivo* and *in vitro* studies have shown that *trans*-translation cannot occur in the absence of SmpB (Hanawa-Suetsugu *et al.*, 2002; Karzai *et al.*, 1999). SmpB also facilitates tmRNA aminoacylation. One ribosome can bind two SmpB molecules, one on the small subunit and a second on the large. Binding to the large subunit appears to be weaker in comparison to the small subunit (see also for references Gillet *et al.*, 2007). Furthermore, SmpB has been shown to remain on the ribosome during translation of the mRNA module of tmRNA (Shpanchenko *et al.*, 2005).

So how do these three layers of regulation, the RelA-mediates stringent response, the RelBE toxin/antitoxin and tmRNA rescue systems, combine to form a larger stress response? Let us, for example, consider amino acid starvation that generates a pool of free uncharged tRNA and allows deacylated tRNA to bind at the A site, triggering the stringent response. The increased concentration of (p)ppGpp synthesized by the stringent factor can completely use up the cellular content of GTP and GDP, reducing the activities of processes requiring these G nucleotides, which involves all steps of translation, initiation (IF2), elongation (EF-G, EF-Tu, and EF-Ts) and termination (RF3). This is in addition to the aforementioned upregulation of metabolic enzymes and downregulation of translation components. Furthermore, if the inhibition of translational activity leads to a marked reduction in the concentrations of the termination release factors, the second layer of regulation is invoked: With the halt of translation, the labile antitoxin RelB is no longer produced and the levels of RelB drop due to the Lon protease (Christensen *et al.*, 2001). The remaining RelE toxin binds to the stalled ribosomes and cleaves the A site stop-codon (Figure 6F). This generates a ribosome·mRNA·peptidyl-tRNA complex that is ready for third level of regulation, *viz*. the action of the monster tmRNA that tags the nascent peptide chain with a protease signal (Figure 6F) and allows recycling of the stalled ribosomes via a canonical termination procedure. The overall result of this intricate network is a strikingly increased degradation of the nascent peptide chains present on stalled ribosomes and thus improves the supply of amino acids, which in turn improves the survival chance of the cell in such harsh conditions.

II. Entering Stationary Phase: The role of RMF, SRA, pY, and YhbH

A number of protein factors have been identified that are specifically expressed as the cell moves from exponential growth phase into the stationary phase, and that several of them, such as ribosome modulation factor (RMF), stationary phase-induced ribosome-associated protein (SRA), YfiA and YhbH, are ribosome binding proteins. RMF is a very small basic protein that is found in *E. coli*, but has been identified in only a few other enterobacteria (Wada *et al.*, 2000). RMF stimulates the dimerization of 70S ribosomes to form 100S dimers (Wada *et al.*, 1990). The 100S particles exhibit no translational activity (Wada *et al.*, 1995) and therefore are thought to represent a protection and/or

Figure 6 **Factors that bind to the ribosome during stress conditions. (*A*) Model for mechanism of RelA-mediated (p)ppGpp Synthesis. Amino acid starvation generates large pools of deacylated tRNAs (*green fork*), which bind to the ribosomal A site with low affinity and block the ribosome. RelA detects a blocked ribosome with a 3′ extension of the mRNA, mediates the conversion of ATP and GTP(GDP) to AMP and (p)ppGpp. Release of RelA, but not the A site-bound deacylated tRNA, occurs simultaneously with RelA-mediated (p)ppGpp synthesis. RelA "hops" to the next blocked ribosome, and the synthesis of (p)ppGpp is repeated. High levels of (p)ppGpp activate the stringent response. Aminoacylated tRNAs are replenished following post-stress conditions. The higher affinity of an aminoacylated tRNA over deacylated tRNAs for the A site enables displacement of the deacylated tRNAs, which rescues blocked ribosomes and reactivates translation. (*B*) Crystal structure of the RelB- (*red*) RelE (*blue*) complex (*C*) Structures of RelE compared with (*D*) domain IV of elongation factor G (EF-G) aligned to illustrate their similarity. (*E*) The region of EF-G in (*D*) is boxed in red on the complete EF-G structure. (*F*) RelE binds to stalled ribosomes and cleaves at the second position of the A site codon of the mRNA, in this case, CAG. Blocked ribosomes with truncated mRNAs in the A site are the substrate for the tmRNA rescue system. The tmRNA molecule binds to the A site, initially without codon-anticodon interaction, and the tRNA-like part of the molecule (*green*) accepts the nascent chain (*purple*) from the P site tRNA (*cyan*). Translation then proceeds of the mRNA-like part (*black*) of the tmRNA, thus tagging the nascent chain for degradation. Figures B–F are modified from Wilson and Nierhaus, 2005. (G) Ribbon structure of Hsp15 (*blue* to *red* from N- to C-terminus, respectively), with conserved Arg and Lys residues on the putative RNA-binding surface highlighted (PDB1DM9; Staker *et al.*, 2000). (H) Surface representation of Hsp15 (same orientation as [*G*]) showing electrostatic potential, in particular the highly positive (*blue*) RNA-binding surface. (I) Protein Y (pY, red) binds to the interface side of the 30S subunit (rRNA and r-protein, *light blue* and *dark blue*, respectively) of a 70S ribosome (PDB1VOQ; Vila-Sanjurjo *et al.*, 2004). The A- (*green*) and P-tRNA (orange) positions are shown for reference. (*J*) Relative position of pY (*red* ribbons) compared to A- and P-tRNA (taken from PDB1GIX; Yusupov *et al.*, 2001).**



storage state, a process termed "ribosome hibernation" (Yoshida *et al.*, 2002). Indeed, Δ*rmf* strains lose viability in the stationary phase earlier than wild-type strains, particularly in low pH (3.5) medium (El-Sharoud and Niven, 2005, 2007), and transfer of stationary phase cells into fresh medium leads to release of RMF from the 100S particles, which rapidly dissociate into translationally active 70S ribosomes (Yamagishi

et al., 1993). Chemical footprinting and crosslinking assays suggest that RMF binds to the 50S subunit in the vicinity of the peptidyltransferase center (PTC) (Yoshida *et al.*, 2002; Yoshida *et al.*, 2004). Specifically, RMF crosslinks to r-proteins L2, L13 and S13 (Yoshida *et al.*, 2002) and protects nucleotides, such as A2451 in the heart of the PTC but also nucleotides located in the ribosomal tunnel, adjacent to the PTC, such as A2058, A2059, and A2062 (Yoshida *et al.*, 2004). This suggests that RMF directly prevents binding of the tRNAs at the PTC, explaining the observed inhibition. However, it raises the question as to whether RMF could bind to an elongating ribosome that contains tRNAs and a nascent polypeptide chain in the tunnel? It would also be interesting to analyze the effect of antibiotics that bind at the PTC, such as chloramphenicol, or in the tunnel, such as erythromycin, on 100S formation, since the binding sites appear to overlap with RMF. Early reports indicate that 100S dimerization occurs through a 30S:30S interaction (Yoshida *et al.*, 2002), which raises the question as to how RMF binding at the PTC on the 50S subunit can induce dimerization through the 30S?

Ribosome hibernation appears to be linked to the stringent control mechanism (see previous section on RelA), since expression of RMF requires the signaling molecule (p)ppGpp (Izutsu *et al.*, 2001a). Furthermore, RMF is expressed in exponential phase when growth rates are reduced due to transfer into minimal medium (Yamagishi *et al.*, 1993). However, under these conditions no evidence for 100S dimer formation was observed. Indeed, the role of dimerization for protection has been questioned, at least under stress conditions such as heat (Niven, 2004) or acidity (El-Sharoud and Niven, 2005, 2007).

SRA was transiently called r-protein S22 because of its small size (5 kDa) and its strong association with ribosomes (Wada, 1998). In exponentially growing cells, low levels (0.1) of SRA are found associated with ribosomes indicating that this protein is a translational factor rather than a ribosomal protein; however, this increases (to 0.4) as the cells enter stationary phase (Izutsu *et al.*, 2001b). The role of SRA has remained elusive. Δ*sra* strains appear similar to wild-type strains in terms of growth rate, and the levels of 100S ribosomes are also similar. Like RMF, SRA is not widely spread, so far being identified only in enterobacteria, such as *E. coli* and *Salmonella typhimurium* (Izutsu *et al.*, 2001b).

Two other *E. coli* proteins found associated preferentially with stationary-phase, rather than log-phase, ribosomes are YhbH and YfiA (Maki *et al.*, 2000). Although both proteins are related in terms of amino acid sequence (40% sequence homology), they have different ribosome localizations and functions. While YhbH is detected exclusively on 100S dimers, YfiA is found more on 70S ribosomes rather than 100S (Maki *et al.*, 2000). Deletion of *yhbH* or *yfiA* genes or a double deletion (Δ*yhbH*-Δ*yfiA*) exhibits very little difference in terms of growth rate compared to the wild-type, or viability during stationary phase (Ueta *et al.*, 2005). However, the Δ*yfiA* strain shows elevated levels of YhbH binding and more 100S particles than the wildtype, whereas the Δ*yhbH* strain shows the presence of 90S particles, which are considered as immature 100S particles. This led to the conclusion that RMF promotes dimerization of 70S particles to form 90S precursors, which are converted by YhbH into mature 100S particles (Ueta *et al.*, 2005). In contrast, YfiA prevents dimerization by RMF, hence the absence of the factor leads to elevated levels of 100S. Thus YfiA and YhbH seem to have antagonistic actions, and have been proposed to occupy the same binding site on the ribosome, with the former blocking the binding of RMF, whereas the latter promotes it (Ueta *et al.*, 2005).

III. Hsp15 Is a Ribosome-Associated Heat Shock Protein

The *yrfH* gene encoding heat shock protein 15 (Hsp15) is the fifth most highly inducible gene in *E. coli*, being more responsive to heat shock than many well characterized Hsp genes, such as *groEL/ES*, *dnaJ/K*, *clpA/P*, *rpoD/H,* and *lon* (Richmond *et al.*, 1999). Hsp15 is highly conserved among eubacteria and the structure of Hsp15 reveals a highly basic novel RNA-binding motif, termed αL, which is also found in r-protein S4 and threonyl-tRNA synthetase (Staker *et al.*, 2000) (Figure 6G and H). Hsp15 has been shown to bind specifically to the free large ribosomal subunit, but only with high affinity when the 50S subunit bears a nascent polypeptide chain (Korber *et al.*, 2000). However, Hsp15 cannot bind to 70S ribosomes or polysomes, suggesting that the binding site is located to the subunit interface (Korber *et al.*, 2000). The current model suggests that under heat shock conditions Hsp15 binds to erroneously dissociated nascent polypeptide chain bearing large subunits and mediates removal of

the nascent chain, enabling the free 50S subunit to re-enter the translation cycle (Korber *et al.*, 2000).

IV. Cold Shock Inhibition of Translation by YfiA/pY

In addition to the abovementioned role during stationary phase, YfiA, also known as protein Y (pY), has also been identified as a cold shock protein (Agafonov *et al.*, 2001; reviewed by Wilson and Nierhaus, 2004). Protein Y is not found associated with ribosomes at 37°C, however appears in the ribosomal fraction when cells are chilled to 15°C. *In vitro* analysis reveals that pY associates with 30S subunits or 70S ribosomes, but not with 50S subunits. In fact, the presence of pY on 70S ribosomes stabilizes the ribosomes against dissociation at low Mg^{2+} ion concentrations (Agafonov *et al.*, 1999). pY has been proposed to interfere with translation by decreasing the accuracy of translation and by preventing binding of amino-acyl tRNAs to the A site, prompting the suggestion to rename pY as ribosome-associated inhibitor A (RaiA) (Agafonov *et al.*, 2001; Agafonov and Spirin, 2004). NMR structures of pY (Rak *et al.*, 2002; Ye *et al.*, 2002) and the pY homologue from *Haemophilus influenzae* (Parsons *et al.*, 2001), reveal a single N-terminal globular domain, adopting a topology reminiscent of dsRNA binding proteins, with a flexible C-terminal tail. Crystal structures of pY bound to the 70S ribosome at 11 Å determine the binding site of pY to be on the interface side of the 30S (Vila-Sanjurjo *et al.*, 2004) (Figure 6I), consistent with earlier tritium bombardment experiments (Agafonov *et al.*, 1999). In this location, pY would be predicted to overlap with anticodon stems of A- and P-tRNAs (Vila-Sanjurjo *et al.*, 2004) (Figure 6J). Consistently, footprinting and binding assays demonstrate competition between pY and tRNAs at both A and P sites, with pY being much more competitive at lower temperatures when chasing the initiator fMet-tRNA from the ribosomal P site (Vila-Sanjurjo *et al.*, 2004). The initiation factor IF3 binds to the 30S subunit and prevents 70S formation, and under certain conditions IF3 can even promote 70S dissociation. Interestingly, in the presence of polyamines, pY can also counteract the dissociate effects of IF3 (and IF1), collectively suggesting that pY prevents translation initiation under stress conditions. Therefore the model is that the pY regulatory pathway is stimulated by cold shock or stationary phase conditions resulting in pY overexpression. pY binds and stabilizes 70S ribosomes, effectively inactivating them by preventing binding of IFs and tRNAs. When the stress conditions are relieved, the initiation machinery overcomes the pY effect since under these conditions, IFs and tRNAs are better competitors for ribosome binding. Moreover, the levels of pY are lower in exponentially growing cells than cold shock or stationary phase cells, providing an additional advantage to translation initiation.

Homologues for pY have also been found in chloroplasts and some cyanobacteria. The spinach pY homologue was originally assigned as r-protein S22 of plastid ribosomes, but later reassigned as plastid-specific ribosomal protein 1 (Psrp1) (Yamaguchi *et al.*, 2000). Interestingly, when mature spinach chloroplast Psrp1 protein was overexpressed in *E. coli* cells, the protein was found bound to 30S subunits and 70S ribosomes, but not present in polysomes (Bubunenko and Subramanian, 1994). Thus it seems likely that Psrp1 is a chloroplast homologue of pY and not an r-protein at all. However, the C-terminal extensions of chloroplast and cyanobacterial pY homologs are much longer than those of *E. coli* pY and thus may confer additional functions. Indeed, the pY homologue from the cyanobacterium *Synechococcus*, termed LrtA (light repressed transcript A), has been shown, as its name suggests, to be repressed by light, *i.e.*, the LrtA protein would operate in dark stress conditions, where the translational machinery is less active (Tan *et al.*, 1994).

F. THE TIP OF THE ICEBERG OF RIBOSOME REGULATION

Here we have merely touched the tip of the iceberg in terms of the complexity of regulating the ribosome; however; we hope to have at least illustrated the diverse array of protein factors, mechanisms and regulatory pathways that exist to modulate translation. The focus here has been on novel protein factors that bind to the bacterial ribosome and that exert their action in a more or less mRNA-independent manner. However, it should be recognized that a vast area of research into various mRNA-specific regulatory systems exist that operate directly on ribosomes, ranging from autoregulatory feedback mechanisms, such as that seen for r-protein S15 (Springer and Portier, 2003), effector-mediated translational arrest, for example, TrpC and SecM systems as well as antibiotic protection and peptide inhibition systems, such as the ermC and cat-86 (reviewed by Tenson and Ehrenberg, 2002),

translational recoding mechanisms such as frameshifting, hopping and bypassing to incorporation of unusual amino acids (Baranov *et al.*, 2002). Indeed, it appears that the deeper we delve, the more we find, illustrating the central role, importance and complexity of the ribosome and the process of translation.

ACKNOWLEDGMENTS

We would like to thank Jaanus Remme for comments on this manuscript, and to Thomas Becker and Roland Beckmann for making the pdb of the model for EF3 bound to the yeast 80S ribosome available for Figure 5D and 5E. D.N.W. would like to thank the DFG and CiPSM for financial support.

REFERENCES

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.

Agafonov, D.E. and Spirin, A.S. 2004. The ribosome-associated inhibitor A reduces translation errors. *Biochem Biophys Res Commun* 320:354–358.

Alix, J.H. and Guerin, M.F. 1993. Mutant DnaK chaperones cause ribosome assembly defects in *Escherichia coli*. *Proc Natl Acad Sci USA* 90:9725–9729.

Alix, J.H. and Nierhaus, K.H. 2003. DnaK-facilitated ribosome assembly in *Escherichia coli* revisited. *RNA* 9:787–793.

Anantharaman, V. and Aravind, L. 2002. The PRC-barrel: A widespread, conserved domain shared by photosynthetic reaction center subunits and proteins of RNA metabolism. *Genome Biol* 3:61–69.

Andersen, B.F., Becker, T., Blau, M., Anand, M., Halic, M., Balar, B., Mielke, T., Boesen, T., Pedersen, J.S., Spahn, C.M.T., *et al.* 2006. Structure of eEF3 and the mechanism of transfer RNA release from the E-site. *Nature* 433:663–668.

Andersen, N. and Douthwaite, S. 2006. YebU is a m5 C methyltransferase specific for 16S rRNA nucleotide 1407. *Mol Biol* 359:777–786.

Apirion, D. and Miczak, A. 1993. RNA processing in prokaryotic cells. *Bioessays* 15:113–120.

Aoki, H., Adams, S.L., Turner, M.A., and Ganoza, M.C. 1997. Molecular characterization of the prokaryotic efp gene product involved in a peptidyltransferase reaction. *Biochimie* 79:7–11.

Arigoni, F., Talabot, F., Peitsch, M., Edgerton, M.D., Meldrum, E., Allet, E., Fish, R., Jamotte, T., Curchod, M.L., and Loferer, H. 1998. A genome-based approach for the identification of essential bacterial genes. *Nature Biotechnol* 16:851–856.

Baranov, P.V., Gesteland, R.F., and Atkins, J.F. 2002. Recoding: translational bifurcations in gene expression. *Gene* 286:187–201.

Bassler, J., Grandi, P., Gadal, O., Lessmann, T., Petfalski, E., Tollervey, D., and al., E. 2001. Identification of a 60S preribosomal particle that is closely linked to nuclear export. *Mol Cell* 8:517–529.

Baxter, R.M., Ganoza, M.G., Zahid, N., and Chung, D.-G. 1987. Reconstitution of peptidyltransferase activity on 50S and 70S ribosomal particles by peptide fragments of protein L16. *Eur. J Biochem* 163:473–479.

Beauclerk, A.A.D. and Cundliffe, E. 1987. Sites of action of two ribosomal RNA methylases responsible for resistance to aminoglycosides. *J Mol Biol* 193:661–671.

Bech, F.W., Jorgensen, S.T., Diderichsen, B., and Karlstrom, O.H. 1985. Sequence of the *relB* transcription unit from *Escherichia coli* and identification of the *relB* gene. *EMBO J* 4:1059–1066.

Bénédicte, M. 2005. Obg/CgtA, a signalling protein that controls replication, translation and morphological development. *Dev Cell* 9:300–301.

Bharat, A., Jiang, M., Sullivan, S.M., Maddock, J.R., and Brown, E.D. 2006. Cooperative and critical roles for both G domains in the GTPase activity and cellular function of ribosome-associated *Escherichia coli* EngA. *J Bacteriol* 188:7992–7996.

Bijlsma, J.J., Lie, A.L.M., Nootenboom, I.C., Vandenbroucke-Grauls, C.M., and Kusters, J.G. 2000. Identification of loci essential for the growth of *Helicobacter pylori* under acidic conditions. *J Infect Dis* 182:1566–1569.

Biou, V., Shu, F., and Ramakrishnan, V. 1995. X-ray crystallography shows that translational initiation factor IF3 consists of two compact alpha/beta domains linked by an alpha-helix. *EMBO J* 14:4056–4064.

Blaha, G. and Nierhaus, K.H. 2001. Features and functions of the ribosomal E site. *Cold Spring Harbor Symposia on Quantitative Biology* 65:135–145.

Block, R. and Haseltine, W. 1975. Purification and properties of stringent factor. *J Biol Chem* 250:1212–1217.

Braeken, K., Moris, M., Daniels, R., Vanderleyden, J., and Michiels, J. 2006. New horizons for (p)ppGpp in bacterial and plant physiology. *Trends Microbiol* 14:45–54.

Bram, R.J., Young, R.A., and Steitz, J.A. 1980. The ribonuclease III site flanking 23S sequences in the 30S ribosomal precursor RNA of *E. coli*. *Cell* 19:393–401.

Britton, R.A., Powell, B.S., Dasgupta, S., Sun, Q., Margolin, W., Lupski, J.R., and Court, D.L. 1998. Cell cycle arrest in Era GTPase mutants: a potential growth rate-regulated checkpoint in *Escherichia coli*. *Mol Microbiol* 27:739–750.

Brown, E.D. 2005. Conserved P-loop GTPases of unknown function in bacteria: an emerging and vital ensemble in bacterial physiology. *Biochem Cell Biol* 83:738–746.

Bubunenko, M.G. and Subramanian, A.R. 1994. Recognition of novel and divergent higher plant chloroplast ribosomal proteins by *Escherichia coli* ribosome during *in vivo* assembly. *J Biol Chem* 269:18223–18231.

Buckle, A., Schreiber, G., and Fersht, A. 1994. Protein-protein recognition: crystal structural analysis of a barnase-barstar complex at 2.0 Å resolution. *Biochemistry* 33:8878–8889.

Bügl, H., Fauman, E.B., Staker, B.L., Zheng, F., Kushner, S.R., Saper, M.A., Bardwell, J.C., and Jakob, U. 2000. RNA methylation under heat shock control. *Mol Cell* 6:349–360.

Buglino, J., Shen, V., Hakimian, P., and Lima, C.D. 2002. Structural and biochemical analysis of the Obg GTP binding protein. *Structure* 10:1581–1592.

Bylund, G., Wipemo, L., Lundberg, L., and Wikstrom, P. 1998. RimM and RbfA are essential for efficient processing of 16S rRNA in *Escherichia coli*. *J Bacteriol* 180:73–82.

Bylund, G.O., Persson, B.C., Lundberg, L.A.C., and Wikstrom, P.M. 1997. A novel ribosome-associated protein is important for efficient translation in *Escherichia coli*. *J Bacteriol* 179:4567–4574.

Caldas, T., Binet, E., Bouloc, P., and Richarme, G. 2000. Translational defects of *Escherichia coli* mutants deficient in the Um(2552) 23S ribosomal RNA methyltransferase RrmJ/FtsJ. *J Biochem Biophys Res Commun* 271:714–718.

Caldon, C.E., Yoong, P., and March, P.E. 2001. Evolution of a molecular switch: universal bacterial GTPases regulate ribosome function. *Mol Microbiol* 41:289–297.

Cameron, D., Gregory, S., Thompson, J., Suh, M., Limbach, P., and Dahlberg, A. 2004. *Thermus thermophilus* L11 methyltransferase, PrmA, is dispensable for growth and preferentially modifies free ribosomal protein L11 prior to ribosome assembly. *J Bacteriol* 186:5819–5825.

Campbell, T.L., Daigle, D.M., and Brown, E.D. 2005. Characterization of the *Bacillus subtillis* GTPase YloQ and its role in ribosome function. *Biochem J* 389:843–852.
Carter, A.P., Clemons, W.M. Jr., Brodersen, D.E., Morgan-Warren, R.J., Hartsch, T., Wimberly, B.T., and Ramakrishnan, V. 2001. Crystal structure of an initiation factor bound to the 30S ribosomal subunit. *Science* 291:498–501.
Charollais, J., Dreyfus, M., and Iost, I. 2004. CsdA, a cold-shock RNA helicase from *Escherichia coli*, is involved in the biogenesis of 50S ribosomal subunit. *Nucleic Acids Ress* 32:2751–2759.
Charollais, J., Pflieger, D., Vinh, J., Dreyfus, M., and Iost, I. 2003. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. *Mol Microbiol* 48:1253–1265.
Chatterji, D., Fujita, N., and Ishihama, A. 1998. The mediator for stringent control, ppGpp, binds to the beta-subunit of *Escherichia coli* RNA polymerase. *Genes Cells* 3:279–287.
Chen, X., Court, D., and Ji, X. 1999. Crystal structure of ERA: a GTPase-dependent cell cycle regulator containing an RNA binding motif. *Proc Natl Acad Sci USA* 96:8396–8401.
Christensen, S., Mikkelsen, M., Pedersen, K., and Gerdes, K. 2001. RelE, a global inhibitor of translation, is activated during nutritional stress. *Proc Natl Acad Sci USA* 98:14328–14333.
Colca, J.R., McDonald, W.G., Waldon, D.J., Thomasco, L.M., Gadwood, R.C., Lund, E.T., Cavey, G.S., Mathews, W.R., Adams, L.D., Cecil, E.T., *et al.* 2003. Crosslinking in the living cell locates the site of action of oxazolidinone antibiotics. *J Biol Chem* 278:21972–21979.
Comartin, D.J. and Brown, E.D. 2006. Non-ribosomal factors in ribosome subunit assembly are emerging targets for new antibacterial drugs. *Curr Opin Pharmacol* 6:453–458.
Condon, C. 2006. Shutdown decay of mRNA. *Mol Microbiol* 61:573–583.
Connell, S.R., Tracz, D.M., Nierhaus, K.H., and Taylor, D.E. 2003a. Ribosomal protection proteins and their mechanism of tetracycline resistance. *Antimicrob Agents Chemother* 47:3675–3681.
Connell, S.R., Trieber, C.A., Dinos, G.P., Einfeldt, E., Taylor, D.E., and Nierhaus, K.H. 2003b. Mechanism of Tet(O)-mediated tetracycline resistance. *EMBO J* 22:945–953.
Connell, S.R., Trieber, C.A., Stelzl, U., Einfeldt, E., Taylor, D.E., and Nierhaus, K.H. 2002. The tetracycline resistance protein Tet(O) perturbs the conformation of the ribosomal decoding centre. *Mol Microbiol* 45:1463–1472.
Cort, J., Koonin, E., Bash, P., and Kennedy, M. 1999. A phylogenetic approach to target selection for structural genomics: solution structure of YciH. *Nucleic Acids Res* 27:4018–4027.
Czyz, A. and Wegrzyn, G. 2005. The Obg subfamily of bacterial GTP-binding proteins: essential proteins of largely unknown functions that are evolutionarily conserved from bacteria to humans. *Acta Biochemica Polonica* 52:35–43.
Daigle, D.M. and Brown, E.D. 2004. Studies of the interaction of *Escherichia coli* YjeQ with the ribosome *in vitro*. *J Bacteriol* 186:1381–1387.
Daigle, D.M., Rossi, L., Berghuis, A.M., Aravind, L., Koonin, E.V., and Brown, E.D. 2002. YjeQ, an essential, conserved, uncharacterized protein from *Escherichia coli*, is an unusual GTPase with circularly permuted G-motifs and marked burst kinetics. *Biochemistry* 41:11109–11117.
Dammel, C.S. and Noller, H.F. 1993. A cold-sensitive mutation in 16S rRNA provides evidence for helical switching in ribosome assembly. *Genes Dev* 7:660–670.
Dammel, C.S. and Noller, H.F. 1995. Suppression of a cold-sensitive mutation in 16S rRNA by overexpression of a novel ribosome-binding factor, RbfA. *Genes Dev* 9:626–637.
Dassain, M., Leroy, A., Colosetti, L., Carole, S., and Bouche, J.P. 1999. A new essential gene of the 'minimal genome' affecting cell division. *Biochimie* 81:889–895.
Datta, K., Fuentes, J.L., and Maddock, J.R. 2005. The yeast GTPase Mtg2p is required for mitochondrial translation and partially suppresses an rRNA methyltransferase mutant, *mrm2*. *Mol Biol Cell* 16:954–963.
Decatur, W. and Fournier, M. 2002. rRNA modifications and ribosome function. *TIBS* 27:344–351.
Del Campo, M., Kaya, Y., and Ofengand, J. 2001. Identification and site of action of the remaining four putative pseudouridine synthases in *Escherichia coli*. *RNA* 7:1603–1615.
Deutscher, M.P. and Li, Z. 2001. Exoribonucleases and their multiple roles in RNA metabolism. *Prog Nucleic Acid Res Mol Biol* 66:67–105.
Dez, C. and Tollervey, D. 2004. Ribosome synthesis meets the cell cycle. *Curr Opin Microbiol* 7:631–637.
Dibb, N.J. and Wolfe, P.B. 1986. lep operon proximal gene is not required for growth or secretion by *Escherichia coli*. *J Bacteriol* 166:83–87.
Dinos, G., Kalpaxis, D.L., Wilson, D.N., and Nierhaus, K.H. 2005. Deacylated tRNA is released from the E site upon A site occupation but before GTP is hydrolyzed by EF-Tu. *Nucleic Acids Res* 33:5291–5296.
Dlakic, M. 2005. The ribosomal subunit assembly line. *Genome Biol* 6:234.
Douthwaite, S., Crain, P., Liu, M., and Poehlsgaard, J. 2004. The tylosin-resistance methyltransferase RlmA(II) (TlrB) modifies the N-1 position of 23S rRNA nucleotide G748. *J Mol Biol* 337:1073–1077.
El-Sharoud, W.M. and Niven, G.W. 2005. The activity of ribosome modulation factor during growth of *Escherichia coli* under acidic conditions. *Arch Microbiol* 184:18–24.
El-Sharoud, W.M. and Niven, G.W. 2007. The influence of ribosome modulation factor on the survival of stationary-phase *Escherichia coli* during acid stress. *Microbiology* 153:247–253.
El Hage, A., Sbai, M., and Alix, J.H. 2001. The chaperonin GroEL and other heat-shock proteins, besides DnaK, participate in ribosome biogenesis in *Escherichia coli*. *Mol Gen Genet* 264:796–808.
Engelberg-Kulka, H., Reches, M., Narasimhan, S., Schoulaker-Schwarz, R., Klemes, Y., Aizenman, E., and Glaser, G. 1998. rexB of bacteriophage lambda is an anti-cell death gene. *Proc Natl Acad Sci USA* 95:15481–15486.
Fatica, A. and Tollervey, D. 2002. Making Ribosomes. *Curr Opin Cell Biol* 14:313–318.
Fletcher, C.M., Pestova, T.V., Hellen, C.U.T., and Wagner, G. 1999. Structure and interactions of the translation initiation factor eIF1. *EMBO J* 18:2631–2637.
Friesen, J.D., Fiil, N.P., Parker, J.M., and Haseltine, W.A. 1974. A new relaxed mutant of *Escherichia coli* with an altered 50S ribosomal subunit. *Proc Natl Acad Sci USA* 71:3465–3469.
Fromont-Racine, M., Senger, B., Seaveanu, C., and Fasiolo, F. 2003. Ribosome assembly in eukaryotes. *Gene* 313:17–42.
Ganoza, M.C. and Aoki, H. 2000. Peptide bond synthesis: function of the efp gene product. *Biol Chem* 381:553–559.
Ganoza, M.C., Kiel, M.C., and Aoki, H. 2002. Evolutionary conservation of reactions in translation. *Microbiol Mol Biol Rev* 66:460–485.
Ganoza, M.C., Zahid, N., and Baxter, R.M. 1985. Stimulation of peptidyltransferase reactions by a soluble protein. *Eur J Biochem* 146:287–294.
Gillet, R., Kaur, S., Li, W., Hallier, M., Felden, B., and Frank, J. 2007. Scaffolding as an organizing principle in trans-translation: The roles of small protein B and ribosomal protein S1. *J Biol Chem.* Epub.
Ginsburg, D. and Steitz, J.A. 1975. The 30S ribosomal precursor RNA from *E. coli.* A primary transcript containing 23S, 16S, and 5S sequences. *J Biol Chem* 250:5647–5654.
Glick, B.R. and Ganoza, M.C. 1975. Identification of a soluble protein that stimulates peptide bond synthesis. *Proc Natl Acad. Sci USA* 72:4257–4260.
Glick, B.R. and Ganoza, M.C. 1976. Characterization and site of action of a soluble protein that stimulates peptide-bond synthesis. *Eur J Biochem* 71:483–491.
Gotfredsen, M. and Gerdes, K. 1998. The *Escherichia coli relBE* genes belong to a new toxin-antitoxin gene family. *Mol Microbiol* 29:1065–1076.
Graille, M., Mora, L., Buckingham, R., Van Tilbeurgh, H., and De Zamaroczy, M. 2004. Structural inhibition of the colicin D tRNase by the tRNA-mimicking immunity protein. *EMBO J* 23:1474–1482.

Gualerzi, C.O. and Pon, C.L. 1990. Initiation of messenger-RNA translation in prokaryotes. *Biochemistry* 29:5881–5889.

Gutgsell, N.S., Deutscher, M.P., and Ofengand, J. 2005. The pseudouridine synthase RluD is required for normal ribosome assembly and function in *Escherichia coli*. *RNA* 11:1141–1152.

Haebel, P.W., Gutmann, S., and Ban, N. 2004. Dial tm for rescue: tmRNA engages ribosomes stalled on defective mRNAs. *Curr Opin Struct Biol* 14:58–65.

Hager, J., Staker, B.L., Bügl, H., and Jakob, U. 2002. Active site in RrmJ, a heat shock-induced methyltransferase. *J Biol Chem* 277:41978–41986.

Hanawa-Suetsugu, K., Sekine, S., Sakai, H., Hori-Takemoto, C., Terada, T., Unzai, S., Tame, J.R., Kuramitsu, S., Shirouzu, M., and Yokoyama, S. 2004. Crystal structure of elongation factor P from *Thermus thermophilus* HB8. *Proc Natl Acad Sci USA* 101:9595–9600.

Hanawa-Suetsugu, K., Takagi, M., Inokuchi, H., Himeno, H., and Muto, A. 2002. SmpB functions in various steps of trans-translation. *Nucleic Acids Res* 30:1620–1629.

Harms, J., Schluenzen, F., Zarivach, R., Bashan, A., Gat, S., Agmon, I., Bartels, H., Franceschi, F., and Yonath, A. 2001. High resolution structure of the large ribosomal subunit from a mesophilic eubacterium. *Cell* 107:679–688.

Haseltine, W.A. and Block, R. 1973. Synthesis of guanosine tetra- and pentaphosphate requires the presence of a codon-specific, uncharged transfer ribonucleic acid in the acceptor site of ribosomes. *Proc Natl Acad Sci USA* 70:1564–1568.

Haseltine, W.A., Block, R., Gilbert, W., and Weber, K. 1972. MSI and MSII made on ribosome in idling step of protein synthesis. *Nature* 238:381–384.

Helser, T.L., Davies, J.E., and Dahlberg, J.E. 1971. Change in methylation of 16S ribosomal RNA associated with mutation to kasugamycin resistance in *Escherichia coli*. *Nat New Biol* 233:12–14.

Helser, T.L., Davies, J.E., and Dahlberg, J.E. 1972. Mechanism of kasugamycin resistance in *Escherichia coli*. *Nat New Biol* 235:6–9.

Himeno, H., Hanawa-Suetsugu, K., Kimura, T., Takagi, K., Sugiyama, W., Shirata, S., Mikami, T., Odagiri, F., Osanai, Y., Watanabe, D., *et al.* 2004. A novel GTPase activated by the small subunit of ribosome. *Nucleic Acids Res* 32:5303–5309.

Hinnebusch, A.G., Dever, T.E., and Sonenberg, N. 2004. Mechanism and regulation of protein synthesis initiation in eukaryotes. In *Protein synthesis and ribosome structure: translating the genome*. pp. 241–322. Nierhaus, K.H. and Wilson, D.N. eds. WILEY-VCH Verlag GmbH&Co., Weinheim, Germany.

Hirokawa, G., Demeshkina, N., Iwakura, N., Kaji, H., and Kaji, A. 2006. The ribosome-recycling step: consensus or controversy?. *Trends Biochem Sci* 31:143–149.

Huang, Y., Swapna, G., Rajan, P., Ke, H., Xia, B., Shukla, K., Inouye, M., and Montelione, G. 2003. Solution NMR structure of ribosome-binding factor A (RbfA), a cold-shock adaptation protein from *Escherichia coli*. *J Mol Biol* 327:521–536.

Hwang, J. and Inouye, M. 2006. The tandem GTPase, Der, is essential for the biogenesis of 50S ribosomal subunits in *Escherichia coli*. *Mol Microbiol* 61:1660–1672.

Inoue, K., Chen, J., and Inouye, M. 2006. Era and RbfA have overlapping function in ribosome biogenesis in *Escherichia coli*. *J Mol Microbiol Biotechnol* 11:41–52.

Inoue, K., Alsina, J., Chen, J., and Inouye, M. 2003. Suppression of defective ribosome assembly in a rbfA deletion mutant by overexpression of Era, an essential GTPase in *Escherichia coli*. *Mol Microbiol* 48:1005–1016.

Iost, I. and Dreyfus, M. 2006. DEAD-box RNA helicases in *Escherichia coli*. *Nucleic Acids Res* 34:4189–4197.

Iskakova, M., Connell, S.R., and Nierhaus, K.H. 2004. Regulation of ribosome biosynthesis in *Escherichia coli*. In *Protein synthesis and ribosome structure: translating the genome*. pp. 429–448. Nierhaus, K.H. and Wilson, D.N. eds. WILEY-VCH Verlag GmbH&Co., Weinheim, Germany.

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.

Jenner, L., Romby, P., Rees, B., Schulze-Briese, C., Springer, M., Ehresmann, C., Ehresmann, B., Moras, D., Yusupova, G., and Yusupov, M. 2005. Translational operator of mRNA on the ribosome: How repressor proteins exclude ribosome binding. *Science* 308:120–23.

Jensen, B.C., Wang, Q., Kifer, C.T., and Parsons, M. 2003. The NOG1 GTP-binding protein is required for biogenesis of the 60S ribosomal subunit. *J Biol Chem* 278:32204–32211.

Jenvert, R. and Schiavone, L. 2005. Characterization of the tRNA and ribosome-dependent pppGpp-synthesis by recombinant stringent factor from *Escherichia coli*. *FEBS J* 272:685–695.

Jiang, M., Datta, K., Walker, A., Stahler, J., Bagamasbad, P., Andrews, P.C., and Maddock, J.R. 2006. The *Escherichia coli* GTPase CgtAE is involved in late steps of large ribosome assembly. *J Bacteriol* 188:6757–6770.

Jones, P.G., Mitta, M., Kim, Y., Jiang, W.N., and Inouye, M. 1996. Cold shock induces a major ribosomal-associated protein that unwinds double-stranded RNA in *Escherichia coli*. *Proc Natl Acad Sci USA* 93:76–80.

Kaczanowska, M. and Rydén-Aulin, M. 2004. Temperature sentivitiy caused by mutant release factor 1 is suppressed by mutations that affect 16S rRNA maturation. *J Bacteriol* 186:3046–3055.

Kaczanowska, M. and Rydén-Aulin, M. 2005. The YrdC protein—a putative ribosome maturation factor. *Biochim Biophys Acta* 1727:87–96.

Kallstrom, G., Hedges, J., and Johnson, A. 2003. The putative GTPases Nog1p and Lsg1p are required for 60S ribosomal subunit biogenesis and are localized to the nucleus and cytoplasm, respectively. *Mol Biol Cell* 23:4344–4355.

Karginov, F., Caruthers, J., Hu, Y., McKay, D., and Uhlenbeck, O. 2005. YxiN is a modular protein combining a DExD/H core and a specific RNA-binding domain. *J Biol Chem* 280:35499–35505.

Karzai, A.W., Susskind, M.M., and Sauer, R.T. 1999. SmpB, a unique RNA-binding protein essential for the peptide-tagging activity of SsrA (tmRNA). *EMBO J* 18:3793–3799.

Kehrenberg, C., Schwarz, S., Jacobsen, L., Hansen, L., and Vester, B. 2005. A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. *Mol. Microbiol.* 57:1064–1073.

Kiel, M.C., Aoki, H., and Ganoza, M.C. 1999. Identification of a ribosomal ATPase in *Escherichia coli* cells. *Biochemie* 81:1097–1108.

Kiel, M.C. and Ganoza, M.C. 2001. Functional interactions of an *Escherichia coli* ribosomal ATPase. *Eur J Biochem* 268:278–286.

Kim, K.K., Hung, L.W., Yokota, H., Kim, R., and Kim, S.H. 1998. Crystal structures of eukaryotic translation initiation factor 5A from *Methanococcus jannaschii* at 1.8 Å resolution. *Proc Natl Acad Sci USA* 95:10419–10424.

Kiser, G.L. and Weinert, T.A. 1995. GUF1, a gene encoding a novel evolutionarily conserved GTPase in budding yeast. *Yeast* 11:1311–1316.

Kolade, O.O., Carr, S.B., Kuhlmann, U.C., Pommer, A., Kleanthous, C., Bouchcinsky, C.A., and Hemmings, A.M. 2002. Structural aspects of the inhibition of DNase and rRNase colicins by their immunity proteins. *Biochimie* 84:439–446.

Korber, P., Stahl, J.M., Nierhaus, K.H., and Bardwell, J.C. 2000. Hsp15: a ribosome-associated heat shock protein. *EMBO J* 19:741–748.

Kukimoto-Niino, M., Murayama, K., Inoue, M., Terada, T., Tame, J.R., Kuramitsu, S., Shirouzu, M., and Yokoyama, S. 2004. Crystal structure of the GTP-binding protein Obg from *Thermus thermophilus* HB8. *J Mol Biol* 337:761–770.

Kyrpides, N.C. and Woese, C.R. 1998. Universally conserved translation initiation factors. *Proc Natl Acad Sci USA* 95:224–228.

Laursen, B.S., Sorensen, H.P., Mortensen, K.K., and Sperling-Petersen, H.U. 2005. Initiation of protein synthesis in bacteria. *Microbiol. Mol Biol Rev* 69:101–123.

Leipe, D.D., Wolf, Y.I., Koonin, E.V., and Aravind, L. 2002. Classification and evolution of P-loop GTPases and related ATPases. *J Mol Biol* 15:41–72.

Lerner, C.G., Sood, P., Ahnn, J., and Inouye, M. 1992. Cold sensitive growth and decreased GTP-hydrolytic activity from substitution of Pro17 for Val in Era, an essential *Escherichia coli* GTPase. *FEMS Microbiol Lett* 74:137–142.

Lesnyak, D., Osipiuk, J., Skarina, T., Sergiev, P., Bogdanov, A., Edwards, A., Savchenko, A., Joachimiak, A., and Dontsova, O. 2007. Methyltransferase that modifies guanine 966 of the 16S rRNA: Functional identification and tertiary structure. *J Biol Chem* 282:5880–5887.

Lesnyak, D., Sergiev, P., Bogdanov, A., and Dontsova, O. 2006. Identification of *Escherichia coli* m(2)G methyltransferases: I. The ycbY Gene encodes a methyltransferase specific for G2445 of the 23S rRNA. *J Mol Biol* 364:20–25.

Lewicki, B.T., Margus, T., Remme, J., and Nierhaus, K.H. 1993. Coupling of rRNA transcription and ribosomal assembly *in vivo*—formation of active ribosomal subunits in Escherichia coli requires transcription of rRNA genes by host RNA polymerase which cannot be replaced by bacteriophage-T7 RNA polymerase. *J Mol Biol* 231:581–593.

Levdikov, V.M., Blagova, E.V., Brannigan, J.A., Cladiere, L., Antson, A.A., Isupov, M.N., Seror, S.J., and Wilkinson, A.J. 2004. The crystal structure of YloQ, a circularly permuted GTPase essential for *Bacillus subtilis* viability. *J Mol Biol* 340:767–782.

Li, Z., Pandit, S., and Deutscher, M.P. 1999. RNase G (CafA protein) and RNase E are both required for the 5′ maturation of 16S ribosomal RNA. *EMBO J* 18:2878–2885.

Liiv, A. and Remme, J. 2004. Importance of transient structures during post-transcriptional refolding of the pre-23S rRNA and ribosomal large subunit assembly. *J Mol Biol* 342:725–741.

Lin, B., Covalle, K.L., and Maddock, J.R. 1999. The *Caulobacter crescentus* CgtA protein displays unsual guanine nucleotide binding and exchange properties. *J Bacteriol* 181:5825–5832.

Lin, B., Thayer, D.A., and Maddock, J.R. 2004. The *Caulobacter crescentus* CgtAC protein cosediments with the free 50S ribosomal subunit. *J Bacteriol* 186:481–489.

Lindahl, L. 1973. Two new ribosomal precursor particles in *E. coli*. *Nat New Biol* 243:170–172.

Lindahl, L. 1975. Intermediates and time kinetics of the *in vivo* assembly of *Escherichia coli* ribosomes. *J Mol Biol* 92:15–37.

Liu, M., Novotny, G.W., and Douthwaite, S. 2004. Methylation of 23S rRNA nucleotide G745 is a secondary function of the RlmAI methyltransferase. *RNA* 10:1713–1720.

Long, K., Poehlsgaard, J., Kehrenberg, C., Schwarz, S., and Vester, B. 2006. The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrob Agents Chemother* 50:2500–2505.

Lövgren, J., Bylund, G., Srivastava, M., Lundberg, L., Persson, O., Wingsle, G., and Wikström, P. 2004. The PRC-barrel domain of the ribosome maturation protein RimM mediates binding to ribosomal protein S19 in the 30S ribosomal subunits. *RNA* 10:1798–1812.

Lu, Q. and Inouye, M. 1998. The gene for 16S rRNA methyltransferase (ksgA) functions as a multicopy suppressor for a cold-sensitive mutant of era, an essential RAS-like GTP-binding protein in *Escherichia coli*. *J Bacteriol* 180:5243–5246.

Magdolen, V., Klier, H., Wohl, T., Klink, F., Hirt, H., Hauber, J., and Lottspeich, F. 1994. The function of the hypusine-containing proteins of yeast and other eukaryotes is well conserved. *Mol Gen Genet* 244:646–652.

Maki, J.A., Schnobrich, D.J., and Culver, G.M. 2002. The DnaK chaperone system facilitates 30S ribosomal subunit assembly. *Mol Cell* 10:129–138.

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.

Margus, T., Remm, M., and Tenson, T. 2007. Phylogenetic distribution of translational GTPases in bacteria. *BMC Genomics* 8:15.

Masuda, Y., Miyakawa, K., Nishimura, Y., and Ohtsubo, E. 1993. chpA and chpB, *Escherichia coli* chromosomal homologs of the pem locus responsible for stable maintenance of plasmid R100. *J Bacteriol* 175:6850–6856.

Matsuo, Y., Morimoto, T., Kuwano, M., Loh, P.C., Oshima, T., and Ogasawara, N. 2006. The GTP-binding protein YlqF participates in the late step of 50S ribosomal subunit assembly in *Bacillus subtilis*. *J Biol Chem* 281:8110–8117.

McCloskey, J.A. and Rozenski, J. 2005. The small subunit rRNA modification database. *Nucleic Acids Res* 33:D135–D138.

Micura, R., Pils, W., Hobartner, C., Grubmayr, K., Ebert, M., and Jaun, B. 2001. Methylation of the nucleobases in RNA oligonucleotides mediates duplex-hairpin conversion. *Nucleic Acids Res* 29:3997–4005.

Morimoto, T., Loh, P., Hirai, T., Asai, K., Kobayashi, K., Moriya, S., and Ogasawara, N. 2002. Six GTP-binding proteins of the Era/Obg family are essential for cell growth in *Bacillus subtilis*. *Microbiology* 148:3539–3552.

Muench, S.P., Xu, L., Sedelnikova, S.E., and Rice, D.W. 2006. The essential GTPase YphC displays a major domain rearrangement associated with nucleotide binding. *Proc Natl Acad Sci USA* 103:12359–12364.

Na, J.G., Pinto, I., and Hampsey, M. 1992. Isolation and characterisation of SUA5, a novel gene required for normal growth in *Saccharomyces cerevisiae*. *Genetics* 131:791–801.

Nakabachi, A., Yamashita, A., Toh, H., Ishikawa, H., Dunbar, H.E., Moran, N.A., and Hattori, M. 2006. The 160-kilobase genome of the bacterial endosymbiont Carsonella. *Science* 314:267.

Nierhaus, K.H. 1991. The assembly of prokaryotic ribosomes. *Biochimie* 73:739–755.

Nierhaus, K.H. and Wilson, D.N. 2004. *Protein synthesis and ribosome structure: translating the genome*. WILEY-VCH Verlag GmbH&Co., Weinheim, Germany.

Nissen, P., Kjeldgaard, M., Thirup, S., Polekhina, G., Reshetnikova, L., Clark, B.F., and Nyborg, J. 1995. Crystal structure of the ternary complex of Phe-tRNA^{Phe}, EF-Tu, and a GTP analog. *Science* 270:1464–1472.

Niven, G.W. 2004. Ribosome modulation factor protects *Escherichia coli* during heat stress, but this may not be dependent on ribosome dimerization. *Arch Microbiol* 182:60–66.

Nomura, M., Gourse, R., and Baughman, G. 1984. Regulation of the synthesis of ribosomes and ribosomal components. *Ann Rev Biochem* 53:75–117.

Osthelmer, G.J., Barken, A., and Matthews, B.W. 2002. Crystal structure of *E. coli* YhbY: A representative of a novel class of RNA binding proteins. *Structure* 10:1593–1601.

Park, M.H., Wolff, E.C., and Folk, J.E. 1993. Is hypusine essential for eukaryotic cell proliferation?. *Trends Biochem Sci* 18:475–479.

Parker, J., Watson, R.J., Friesen, J.D., and Fiil, N.P. 1976. A relaxed mutant with an altered ribosomal protein L11. *Mol Gen Genet* 144:111–114.

Parsons, L., Eisenstein, E., and Orban, J. 2001. Solution structure of HI0257, a bacterial ribosome binding protein. *Biochemistry* 40:10979–10986.

Peat, T.S., Newman, J., Waldo, G.S., Berendzen, J., and Terwilliger, T.C. 1998. Structure of translation initiation factor 5A from Pyrobaculum aerophilum at 1.75 Å resolution. *Structure* 6:1207–1214.

Pedersen, K., Zavialov, A.V., Pavlov, M.Y., Elf, J., Gerdes, K., and Ehrenberg, M. 2003. The bacterial toxin RelE displays codon-specific cleavage of mRNAs in the ribosomal A site. *Cell* 112:131–140.

Pillutla, R.C., Ahnn, J., and Inouye, M. 1996. Deletion of the putative effector region of Era, an essential GTP-binding protein in *Escherichia coli*, causes a donminant-negatiove phenotype. *FEMS Microbiol Lett* 143:47–55.

Poehlsgaard, J. and Douthwaite, S. 2003. Macrolide antibiotic interaction and resistance on the bacterial ribosome. *Curr Opin Investig Drugs* 4:140–148.

Prágai, Z. and Harwood, C. 2000. YsxC, a putative GTP-binding protein essential for growth of *Bacillus subtilis* 168. *J Bacteriol* 182:6819–6823.

Qin, Y., Polacek, N., Vesper, O., Staub, E., Einfeldt, E., Wilson, D.N., and Nierhaus, K.H. 2006. The highly conserved LepA is a ribosomal elongation factor that back-translocates the ribosome. *Cell* 127:721–733.

Rak, A., Kalinin, A., Shcherbakov, D., and Bayer, P. 2002. Solution structure of the ribosome-associated cold shock response protein Yfia of *Escherichia coli*. *Biochem Biophys Res Commun* 299:710–714.

Ramagopal, S. and Davis, B.D. 1974. Localization of the stringent protein of *Escherichia coli* on the 50S ribosomal subunit. *Proc Natl Acad Sci USA* 71:820–824.

Ramakrishnan, V. 2002. Ribosome structure and the mechanism of translation. *Cell* 108:557–572.

Raskin, D.M., Judson, N., and Mekalanos, J.J. 2007. Regulation of the stringent response is the essential function of the conserved bacterial G protein CgtA in *Vibrio cholerae*. *Proc Natl Acad Sci USA*. 104:4636–46341.

Richmond, C.S., Glasner, J.D., Mau, R., Jin, H., and Blattner, F.R. 1999. Genome-wide expression profiling in *E. coli* K-12. *Nucleic Acids Res* 27:3821–3835.

Richter, D. 1976. Stringent factor from *Escherichia coli* directs ribosomal binding and release of uncharged tRNA. *Proc Natl Acad Sci USA* 73:707–711.

Richter, D., Nowak, P., and Kleinert, U. 1975. *Escherichia coli* stringent factor binds to ribosomes at a site different from that of elongation factor Tu or G. *Biochemistry* 14:4414–4420.

Roberts, M.C. 2002. Resistance to tetracycline, macrolide-lincosamide-streptogramin, trimethoprim, and sulfonamide drug classes. *Mol Biotechnol* 20:261–283.

Roberts, M.C. 2005. Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* 245:195–203.

Robinson, V., Hwang, J., Fox, E., Inouye, M., and Stock, A. 2002. Domain arrangement of Der, a switch protein containing two GTPase domains. *Structure* 10:1649–1658.

Rozenski, J., Crain, P.F., and McCloskey, J.A. 1999. The RNA Modification Database: 1999 update. *Nucleic Acids Res* 27:196–197.

Ruzheinikov, S.N., Das, S.K., Sedelnikova, S.E., Baker, P.J., Artymiuk, P.J., Garcıa-Lara, J., Foster, S.J., and Rice, D.W. 2004. Analysis of the open and closed conformations of the GTP-binding protein YsxC from *Bacillus subtilis*. *J Mol Biol* 339:265–278.

Sabina, J., Dover, N., Templeton, L.J., Smulski, D.R., Soll, D., and LaRossa, R.A. 2003. Interfering with different steps of protein synthesis explored by transcriptional profiling of *Escherichia coli* K-12. *J Bacteriol* 185:6158–6170.

Saguy, M., Gillet, R., Metzinger, L., and Felden, B. 2005. tmRNA and associated ligands: a puzzling relationship. *Biochimie* 87:897–903.

Sato, A., Kobayashi, G., Hayashi, H., Yoshida, H., Wada, A., Maeda, M., Hiraga, S., Takeyasu, K., and Wada, C. 2005. The GTP binding protein Obg homolog ObgE is involved in ribosome maturation. *Genes Cells* 10:393–408.

Saveanu, C., Bienvenu, D., Namane, A., Gleizes, P.E., Gas, N., Jacquier, A., and Fromont-Racine, M. 2001. Nog2p, a putative GTPase associated with pre-60S subunits and required for late 60S maturation steps. *EMBO J* 20:6475–6484.

Sayed, A., Matsuyama, S., and Inouye, M. 1999. Era, an essential Escherichia coli small G-protein, binds to the 30S ribosomal subunit. *Biochem. Biophys Res Commun* 264:51–55.

Schaefer, L., Uicker, W.C., Wicker-Planquart, C., Foucher, A., Jault, J., and Britton, R.A. 2006. Multiple GTPases participate in the assembly of the large ribosomal subunit in *Bacillus subtilis*. *J Bacteriol* 188:8252–8258.

Schilling-Bartetzko, S., Bartetzko, A., and Nierhaus, K.H. 1992. Kinetic and thermodynamic parameters for tRNA binding to the ribosome and for the translocation reaction. *J Biol Chem* 267:4703–4712.

Schlünzen, F., Zarivach, R., Harms, J., Bashan, A., Tocilj, A., Albrecht, R., Yonath, A., and Franceschi, F. 2001. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. *Nature* 413:814–821.

Schuwirth, B., Borovinskaya, M., Hau, C., Zhang, W., Vila-Sanjurjo, A., Holton, J., and Cate, J. 2005. Structures of the bacterial ribosome at 3.5 Å resolution.. *Science* 310:827–834.

Scott, J.M. and Haldenwang, W.G. 1999. Obg, an essential GTP binding protein of *Bacillus subtilis*, is necessary for stress activation of transcription factor σ^B. *J Bacteriol* 181:4653–4660.

Selmer, M., Al-Karadaghi, S., Hirakawa, G., Kaji, A., and Liljas, A. 1999. Crystal structure of *Thermotoga maritima* ribosome recycling factor: A tRNA mimic. *Science* 286:2349–2352.

Sergiev, P., Lesnyak, D., Bogdanov, A., and Dontsova, O. 2006. Identification of *Escherichia coli* m(2)G methyltransferases: II. The ygjO gene encodes a methyltransferase specific for G1835 of the 23S rRNA. *J Mol Biol* 364:26–31.

Sharma, M.R., Barat, C., Wilson, D.N., Booth, T.M., Kawazoe, M., Hori-Takemoto, C., Shirouzu, M., Yokoyama, S.P.F., and Agrawal, R.K. 2005. Interaction of Era with the 30S Ribosomal subunit: implications for 30S subunit assembly. *Mol Cell* 18:319–329.

Shimizu, Y., Inone, A., Tomari, Y., Suzuki, T., Yokogawa, T., Nishikawa, K., and Ueda, T. 2001. Cell-free translation reconstituted with purified components. *Nature Biotechnology* 19:751–755.

Shin, D.H., Lou, Y., Jancarik, J., Yokota, H., Kim, R., and Kim, S. 2004. Crystal structure of YjeQ from *Thermotoga maritima* contains a circularly permuted GTPase domain. *Proc Natl Acad Sci USA* 101:13198–13203.

Shpanchenko, O.V., Zvereva, M.I., Ivanov, P.V., Bugaeva, E.Y., Rozov, A.S., Bogdanov, A.A., Kalkum, M., Isaksson, L.A., Nierhaus, K.H., and Dontsova, O.A. 2005. Stepping tmRNA through the ribosome. *J Biol Chem* 280:18368–18374.

Sikora, A.E., Datta, K., and Maddock, J.R. 2006a. Biochemical properties of the *Vibrio harveyi* $CgtA_V$ GTPase. *Biochem Biophys Res Commun* 339:1165–1170.

Sikora, A.E., Zielke, R., Datta, K., and Maddock, J.R. 2006b. The *Vibrio harveyi* GTPase $CgtA_V$ is essential and is associated with the 50S subunit. *J Bacteriol* 188:1205–1210.

Sirdeshmukh, R. and Schlessinger, D. 1985a. Ordered processing of *Escherichia coli* 23S rRNA *in vitro*. *Nucleic Acids Res* 13:5041–5054.

Sirdeshmukh, R. and Schlessinger, D. 1985b. Why is processing of 23S ribosomal RNA in Escherichia coli not obligate for its function?. *J Mol Biol* 186:669–672.

Sirum-Connolly, K. and Mason, T.L. 1993. Functional requirement of a site-specific ribose methylation in ribosomal RNA. *Science* 262:1886–1889.

Spahn, C.M., Blaha, G., Agrawal, R.K., Penczek, P., Grassucci, R.A., Trieber, C.A., Connell, S.R., Taylor, D.E., Nierhaus, K.H., and Frank, J. 2001. Localization of the ribosomal protection protein Tet(O) on the ribosome and the mechanism of tetracycline resistance. *Mol Cell* 7:1037–1045.

Spahn, C.M., Gomez-Lorenzo, M.G., Grassucci, R.A., Jorgensen, R., Andersen, G.R., Beckmann, R., Penczek, P.A., Ballesta, J.P., and Frank, J. 2004. Domain movements of elongation factor eEF2 and the eukaryotic 80S ribosome facilitate tRNA translocation. *EMBO J* 23:1008–1019.

Springer, M. and Portier, C. 2003. More than one way to skin a cat: translational autoregulation by ribosomal protein S15. *Nature Struct Biol* 10:420–422.

Staker, B.L., Korber, P., Bardwell, J.C., and Saper, M.A. 2000. Structure of Hsp15 reveals a novel RNA-binding motif. *EMBO J* 19:749–757.

Stent, G.S. and Brenner, S. 1961. A genetic locus for the regulation of ribonucleic acid synthesis. *Proc Natl Acad Sci USA* 47:2005–2014.

Swaney, S., McCroskey, M., Shinabarger, D., Wang, Z., Turner, B.A., and Parker, C.N. 2006. Characterization of a high-throughput screening assay for inhibitors of elongation factor P and ribosomal peptidyltransferase activity. *J Biochem Screen* 11:736–742.

Sy, J. and Lipmann, F. 1973. Identification of the synthesis of guanosine tetraphosphate (MS I) as insertion of a pyrophosphoryl group into the 3′-position in guanosine 5′-diphosphate. *Proc Natl Acad Sci USA* 70:306–309.

Takagi, H., Kakuta, Y., Okada, T., Yao, M., Tanaka, I., and Kimura, M. 2005. Crystal structure of archaeal toxin-antitoxin (RelE-RelB) complex: implications for toxin activity and antitoxin effects. *Nat Struct Mol Biol* 12:327–331.

Tan, J., Jakob, U., and Bardwell, J.C. 2002. Overexpression of two different GTPases rescues a null mutation in a heat-induced rRNA methyltransferase. *J Bacteriol* 184:2692–2698.

Tan, X., Varughese, M., and Widger, W. 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.

Tenson, T. and Ehrenberg, M. 2002. Regulatory nascent peptides in the ribosomal tunnel. *Cell* 108:591–594.

Teplova, M., Tereshko, V., Sanishvili, R., Joachimiak, A., Bushueva, T., Anderson, W.F., and Egli, M. 2000. The structure of the yrdC gene product from *Escherichia coli* reveals a new fold and suggests a role in RNA binding. *Protein Sci* 9:2557–2566.

Teraoka, H. and Nierhaus, K.H. 1978. Protein L16 induces a conformational change when incorporation into an L16-deficient core derived from *E. coli* ribosomes. *FEBS Lett* 88:223–226.

Trach, K. and Hoch, J.A. 1989. The *Bacillus subtilis* spoOB stage 0 sporulation operon encodes an essential GTP-binding protein. *J Bacteriol* 171:1362–1371.

Travers, A. 1976. Modulation of RNA polymerase specificity by ppGpp. *Mol Gen Genet* 147:225–232.

Treede, I., Jakobsen, L., Kirpekar, F., Vester, B., Weitnauer, G., Bechthold, A., and Douthwaite, S. 2003. The avilamycin resistance determinants AviRa and AviRb methylate 23S rRNA at the guanosine 2535 base and the uridine 2479 ribose. *Mol Microbiol* 49:309–318.

Triana-Alonso, F.J., Chakraburtty, K., and Nierhaus, K.H. 1995. The elongation factor 3 unique in higher fungi and essential for protein biosynthesis is an E site factor. *J Biol Chem* 270:20473–20478.

Trieber, C.A., Burkhardt, N., Nierhaus, K.H., and Taylor, D.E. 1998. Ribosomal protection from tetracycline mediated by Tet(O) interaction with ribosomes Is GTP-dependent. *Biol Chem* 379:847–855.

Ueta, M., Yoshida, A., 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.

Uicker, W.C., Schaefer, L., and Britton, R.A. 2006. The essential GTPase RbgA (YlqF) is required for 50S ribosome assembly in *Bacillus subtilis*. *Mol Microbiol* 59:528–540.

van Ooyen, A.J.J., Gruber, M., and Jorgensen, P. 1976. The mechanism of action of ppGpp on rRNA synthesis *in vitro*. *Cell* 8:123–128.

Vesper, O. and Wilson, D.N. 2006. Ribosome recycling revisited. *Mol Biol (Moscow)* 40:742–50.

Vila-Sanjurjo, A., Schuwirth, B.S., Hau, C.W., and Cate, J.H.D. 2004. Structural basis for the control of translational initiation during stress. *Nature Struct Mol Biol* 11:1054–1059.

Wada, A. 1998. Growth phase coupled modulation of *Escherichia coli* ribosomes. *Genes Cells* 3:203–208.

Wada, A., Igarashi, K., Yoshimura, S., Aimoto, S., and Ishihama, A. 1995. Ribosome modulation factor: Stationary growth phase-specific inhibitor of ribosome functions from *Escherichia coli*. *Biochem Biophys Res Commun* 214:410–417.

Wada, A., Mikkola, R., Kurland, C.G., and Ishihama, A. 2000. Growth-phase coupled changes of the ribosome profile in natural isolates and laboratory strains of *Escherichia coli*. *J Bacteriol* 182:2893–2899.

Wada, A., Yamazaki, Y., Fujita, N., and Ishihama, A. 1990. Structure and probable genetic location of a ribosome modulation factor associated with 100S ribosomes in stationary-phase *Escherichia coli* cells. *Proc Natl Acad Sci USA* 87:2657–2661.

Wagner, E.G. and Kurland, C.G. 1980. *Escherichia coli* elongation factor G blocks stringent factor. *Biochemistry* 19:1234–1240.

Weber, M.H. and Marahiel, M.A. 2003. Bacterial cold shock responses. *Sci Prog* 86:9–75.

Wendrich, T.M., Blaha, G., Wilson, D.N., Marahiel, M.A., and Nierhaus, K.H. 2002. Dissection of the mechanism for the stringent factor RelA. *Mol Cell* 10:779–788.

Wilson, D.N., Harms, J., Nierhaus, K., Schlünzen, F., and Fucini, P. 2005. Species-specific antibiotic-ribosome interactions: Implications for drug development. *Biol Chem* 386:1239–1252.

Wilson, D.N., Blaha, G., Connell, S.R., Ivanov, P.V., Jenke, H., Stelzl, U., Teraoka, Y., and Nierhaus, K.H. 2002. Protein synthesis at atomic resolution: mechanistics of translation in the light of highly resolved structures for the ribosome. *Curr Protein Pept Sci* 3:1–53.

Wilson, D.N. and Nierhaus, K.H. 2004. The how and Y of cold shock. *Nat Struct Mol Biol* 11:1026–1028.

Wilson, D.N. and Nierhaus, K.H. 2005. RelBE or not to be. *Nat Struct Mol Biol* 12:282–284.

Wilson, D.N. and Nierhaus, K.H. 2006. The E-site Story: The importance of maintaining two tRNAs on the ribosome during protein synthesis. *Cell Mol Life Sci* 63:2725–2737.

Wireman, J.W. and Sypherd, P.S. 1974. *In vitro* assembly of 30S ribosomal particles from precursor 16S RNA of *E. coli*. *Nature* 247:552–554.

Wout, P., Pu, K., Sullivan, S., Reese, V., Zhou, S., Lin, B., and Maddock, J. 2004. The *Escherichia coli* GTPase $CgtA_E$ cofractionates with the 50S ribosomal subunit and interacts with SpoT, a ppGpp synthetase/hydrolase. *J Bacteriol* 186:5249–5257.

Xia, B., Ke, H., Shinde, U., and Inouye, M. 2003. The role of RbfA in 16S rRNA processing and cell growth at low temperature in *Escherichia coli*. *J Mol Biol* 332:575–584.

Xu, J., Kiel, M.C., Golshani, A., Chosay, J.G., Aoki, H., and Ganoza, M.C. 2006. Molecular localisation of a ribosome-dependent ATPase on *Escherichia coli* ribosomes. *Nucleic Acids Res* 34:1158–1165.

Yamagishi, M., Matsushima, H., Wada, A., Sakagami, M., Fujita, N., and Ishihama, A. 1993. Regulation of the *Escherichia coli* rmf gene encoding the ribosome modulation factor—growth phase-dependent and growth rate-dependent control. *EMBO J* 12:625–630.

Yamaguchi, K., von Knoblauch, K., and Subramanian, A.R. 2000. The plastid ribosomal proteins. Identification of all the proteins in the 30S subunit of an organelle ribosome (chloroplast). *J Biol Chem* 275:28455–28465.

Yang, X. and Ishiguro, E. 2001. Involvement of the N terminus of ribosomal protein L11 in regulation of the RelA protein of *Escherichia coli*. *J Bacteriol* 183:6532–6537.

Yao, M., Ohsawa, A., Kikukawa, S., Tanaka, I., and Kimura, M. 2003. Crystal structure of hyperthermophilic archaeal initiation factor 5 A: a homologue of eukaryotic initiation factor 5A (eIF-5A). *J Biochem (Tokyo)* 133:75–81.

Ye, K., Serganov, A., Hu, W., Garber, M., and Patel, D.J. 2002. Ribosome-associated factor Y adopts a fold resembling a double-stranded RNA binding domain scaffold. *Eur. J Biochem* 269:5182–5191.

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 (Tokyo)* 132:983–989.

Yoshida, H., Yamamoto, H., Uchiumi, 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.

Young, R.A. and Steitz, J.A. 1978. Complementary sequences 1700 nucleotides apart form a ribonuclease III cleavage site in *Escherichia coli* ribosomal precursor RNA. *Proc Natl Acad Sci USA* 75:3593–3597.

Yusupov, M.M., Yusupova, G.Z., Baucom, A., Lieberman, K., Earnest, T.N., Cate, J.H., and Noller, H.F. 2001. Crystal structure of the ribosome at 5. 5 Å resolution.. *Science* 292:883–896.

Zhang, S. and Haldenwang, W.G. 2004. Guanine nucleotides stabilize the binding of *Bacillus subtilis* Obg to ribosomes. *Biochem Biophys Res Commun* 322:565–569.

Editor: Michael M. Cox