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Ribosome Biogenesis and the Translation Process in *Escherichia coli*

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INTRODUCTION

The ribosome is a nucleoprotein particle responsible for one of the key processes in every cell, the decoding of mRNA into protein. Although much research has been focused on the molecular mechanisms behind each step of translation, ribosome biogenesis per se and feedback signaling from ongoing protein synthesis to the biogenesis machinery have been somewhat neglected. Formation of the ribosomal particle involves a complex series of processes, i.e., synthesis, processing and modification of both rRNA and ribosomal proteins, and assembly of the components. The quality of the particle must also be checked and the amount of active ribosomes monitored. All of these events must be tightly regulated and coordinated to avoid energy losses and imbalances in cell physiology.

How, then, is synthesis of all of the ribosomal components coregulated? How is the ribosome actually put together from some 55 components into a mature and functional ribozyme? Recently, the field of ribosome biogenesis has been experiencing a boost, and yet the picture of the process remains unclear. Some of the information comes from work with eukaryotic ribosomes, e.g., *Dictyostelium* or *Saccharomyces*, and some from eubacterial species. This review concentrates on *Escherichia coli*, and we address questions such as the following. In what order are all of the ribosomal components put together? When does the final rRNA processing occur? How is the maturation of the two ribosomal subunits coordinated and their quality checked? Is this process somehow linked to translation? We also discuss the many accessory factors needed during the assembly process, the list of which has grown substantially during the last few years even though the precise mechanism and role for most of the proteins are not understood.

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TRANSLATION AND THE RIBOSOME

The Translation Process

The translation process is not within the scope of this review and thus is reviewed only briefly to provide the necessary background; for more detailed reviews, see references 72, 139, 159, 173, 197, and 213.

Initiation. Initiation of protein synthesis in *E. coli* is promoted by three initiation factors: IF1, IF2, and IF3. It involves interaction between the Shine-Dalgarno (SD) sequence in the

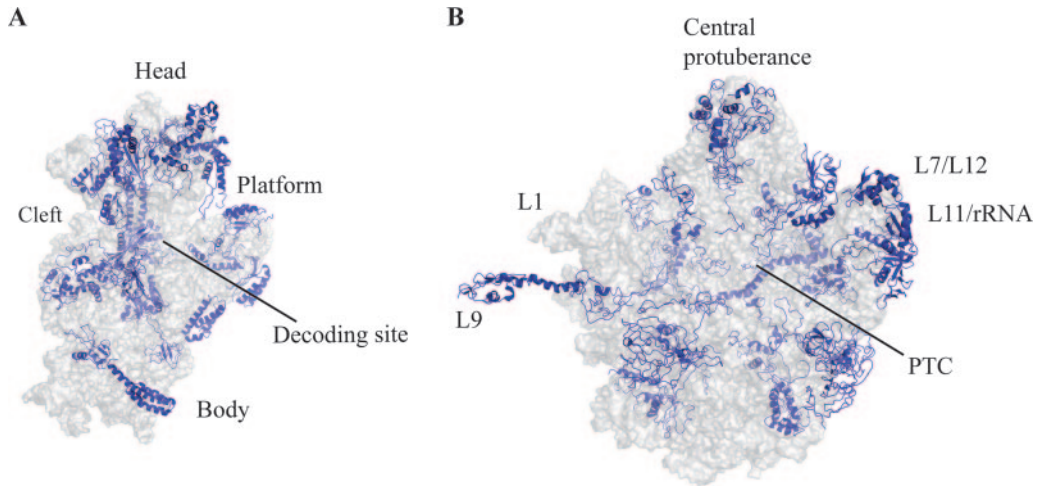

FIG. 1. Tertiary structures of the 30S (A) and 50S (B) subunits, seen from the interface side. The structures are adapted from the *E. coli* 3.5-Å crystal structure (187) and were modeled with PyMol (51). Features described in the text are indicated; L1 and L7/L12 are not present in the structure. rRNA is shown as translucent gray spheres, and ribosomal proteins are shown as blue ribbons.

mRNA, about 6 to 9 nucleotides (nt) upstream of the translation initiation codon, and the anti-SD sequence at the 3′ end of the 16S rRNA (192). After primary association of the mRNA to the 30S subunit via the SD–anti-SD interaction, the initiation factors are involved in start codon accommodation at the P site, contributing to the fidelity of translation initiation (72). Initiation is thought to be the rate-limiting step in protein synthesis, and several ways of regulating translation through initiation are used.

Elongation. After association of the 30S and 50S subunits at the end of the initiation step, the P site holds the aminoacylated initiator tRNA, while the A site is empty and ready to receive an aminoacylated tRNA. It is brought to the ribosome as a ternary complex with elongation factor Tu (EF-Tu) and GTP. After GTP hydrolysis, EF-Tu releases the aminoacyl end of the A site tRNA, allowing it to swing into the P site. Hence, the ends of the A and P site tRNAs are positioned at the peptidyl transferase center (PTC) on the 50S subunit, and peptide bond formation can occur (163). The deacylated tRNA is moved from the P site to the E site, to eventually be ejected from the ribosome, while the peptidyl tRNA repositions from the A site to the P site. The translocation reaction is driven by GTP hydrolysis and EF-G (176). The result of translocation is a ribosome ready for the next round of elongation.

Termination and recycling. Translation termination begins when a stop codon in the mRNA enters the ribosomal A site. The termination codon is recognized by either release factor 1 (RF1) or RF2; RF1 terminates at stop codons UAA and UAG, while RF2 terminates at UAA and UGA. Binding of RF1/RF2 to the ribosome triggers hydrolysis and release of the peptide chain from the P site tRNA (109). Upon peptide bond hydrolysis, the third release factor, RF3, which is a GTPase, binds to the ribosome and promotes dissociation of RF1/RF2 from the A site (63, 110).

After peptide release, the ribosome is left with bound mRNA and a deacylated tRNA in the P site. This complex needs to be disassembled to prepare the ribosomal subunits for a new round of protein synthesis. Ribosome recycling factor, along with EFG, is required for the process of subunit dissociation (105). Subsequently, IF3 replaces the deacylated tRNA on the 30S subunit and allows the mRNA to either detach from the complex or form a new stable SD–anti-SD interaction with the downstream ribosome-binding site (103).

The Ribosome and Its Biogenesis

Prokaryotic ribosomes sediment as 70S particles and are formed by two subunits, 30S and 50S. In *E. coli*, the 70S ribosome is a 210-Å particle that consists of roughly two-thirds RNA and one-third protein (187). The small subunit, 30S, is made of 16S rRNA (1,542 nt) and 21 ribosomal proteins (r-proteins), while the large subunit, 50S, is composed of two rRNAs, 23S (2,904 nt) and 5S (120 nt) rRNA, and 33 proteins (156).

The recently published *E. coli* 3.5-Å-resolution 70S crystal structure is highly similar to the structures obtained from other prokaryotes, though small species-specific divergences can be found (187). The low-resolution crystal structures of the 70S ribosome and the subunits from the eubacteria *Deinococcus radiodurans* and *Thermus thermophilus* and the archaeon *Haloarcula marismortui* (11, 76, 212, 219) provide insights into the universal mechanism of translation and the complex organization of rRNA, ribosomal proteins, and ligands (30, 32, 62, 113, 188). However, it is important to remember that although they are very helpful, the above-mentioned structures show only "snapshots" of the ribosomal conformation at the time of crystallization and do not always reflect the flexibility of the particle during translation, as has been illustrated by cryo-electron microscopy studies (2).

The ribosomal subunits have different functions in translation. The 50S particle is a hemisphere, with three structures protruding from its top (Fig. 1B). The view of the 50S interface, the side that interacts with the 30S particle, shows the central protuberance in the middle, which includes the 5S rRNA and its associated proteins, while the two arms that extend to the left and right are formed by protein L1 and

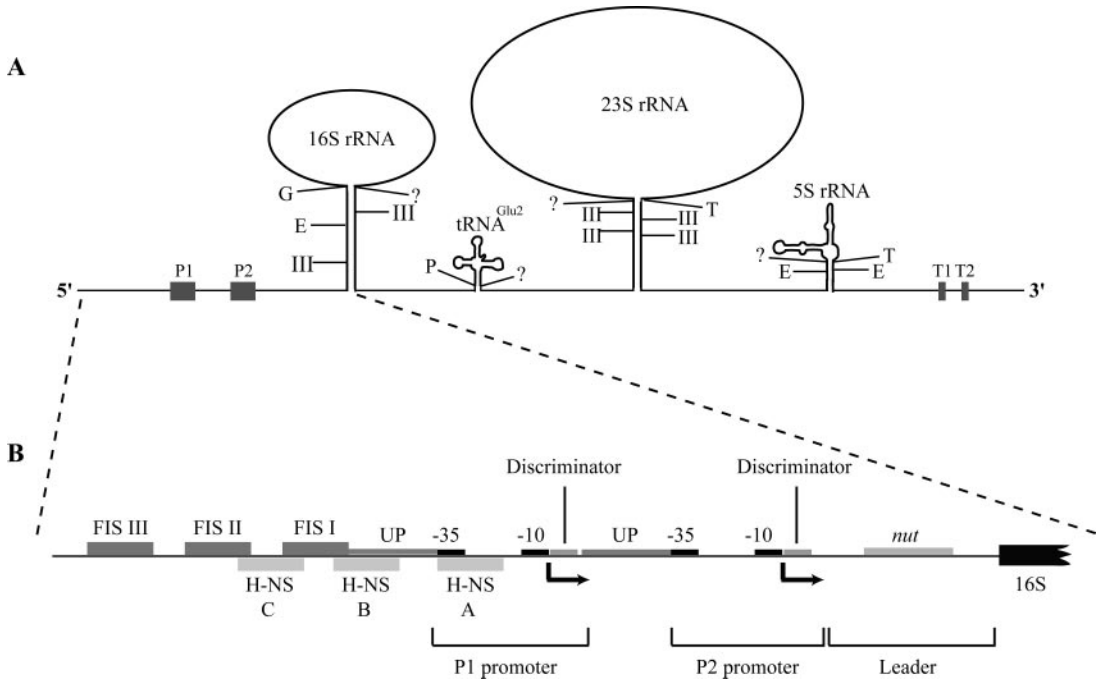

FIG. 2. Schematic drawing of the *rrnB* operon. (A) Nucleolytic processing of the *rrnB* primary transcript. The rRNA and tRNA species, promoters P1 and P2, and terminators T1 and T2 are indicated, as well as the processing sites of RNase III (III), RNase G (G), RNase E (E), RNase P (P), RNase T (T), and the unknown RNases (?). (B) Promoter region of the *rrnB* operon. Locations of FIS- and H-NS-binding sites and the UP, discriminator, and *nut* sequences are marked. Arrows show the start sites of transcription.

protein L11/rRNA, respectively. The protein tetramer L7/L12 is bound to the L11/rRNA complex but is not visualized in the crystals due to the structure's high flexibility. Another flexible feature is found just beneath the L1 arm and is formed by protein L9 (187). Also visible from this perspective is the PTC, a region where the acceptor arms of the A-site and P-site tRNAs come together, enabling rapid and spontaneous peptide bond formation. Beginning just below the PTC is the polypeptide exit tunnel, which provides the nascent peptides with a stable path through the subunit to the solvent side of the particle (146). The tunnel is about 100 Å long and up to 25 Å in diameter and can accommodate a peptide of approximately 40 amino acids (146, 217). It possesses a nonstick character to allow the passage of all peptides, even though, under certain circumstances, some peptides stall the ribosome to regulate the synthesis of downstream proteins (66, 146).

The two main functions of the small subunit are to initiate the interaction with mRNA during translation initiation and to decode the message. The 30S particle is approximately half the molecular weight of the 50S subunit, and its structure can be divided into an upper part, the head, and a lower, larger body (Fig. 1A). When viewed from the interface side, the head has a narrow neck region and is slightly bent to the left over the shoulder, forming a deep cleft (20). To the right, below the head, is the platform where the anti-SD sequence is found. The decoding site with the A and P sites is located at the bottom of the cleft. Here, the anticodon loops of accommodated tRNAs interact with codons in the mRNA. The E site, whose existence is no longer disputed, differs from the other two sites in that the mRNA and tRNA disconnect, preparing for the ejection of the tRNA from the ribosome (186, 188). The decoding region, being more flexible than the compact lower part of the subunit, facilitates the rearrangements that are needed for cognate tRNA selection at the A site. The flexibility has also been suggested to be important for control of mRNA and tRNA movements during translocation (113, 187, 188).

Upon translation initiation, the two subunits associate and become connected via a complex network of molecular interactions along the interface (80). This region is relatively free of ribosomal proteins and provides binding surfaces for many substrates and ligands (44, 219). The intersubunit RNA bridges have been shown to rearrange, or even break, as part of the translation elongation cycle (187), demonstrating high elasticity of the rRNA that is essential for both signal transmission between the ribosomal subunits and coordination of their relative movements during translation.

The assembly and maturation of the ribosomal subunits are very complex and involve a series of events, such as processing and modification of rRNA, ordered binding of ribosomal proteins and metal ions, and sequential conformational changes. In vivo this takes approximately 2 min at 37°C (127, 185). In the following sections, we describe the steps leading to the assembly of 70S ribosomes and mention the factors and enzymes that are thought to be involved in the maturation process.

Nucleolytic processing of rRNA. The biogenesis of ribosomes begins with transcription of the 16S, 23S, and 5S rRNA, which are synthesized as one primary transcript (Fig. 2A). Maturation of the transcript begins before transcription is completed, with instant formation of local secondary structures and, as soon as their binding sites emerge from the poly-

merase, binding of ribosomal proteins. Simultaneously, rRNA becomes chemically modified at a number of positions and is processed by several RNases to generate mature rRNA species (211).

The first endoribonuclease to cleave the primary rRNA transcript is RNase III, which separates precursor rRNA and tRNA. During transcription, the sequences flanking 16S and 23S rRNA come together, forming double helical structures whose hairpin loops include the entire 16S and 23S rRNAs, respectively. These double-stranded stems, and not any common sequence elements, are recognized by RNase III (17, 126, 218). The activity of the endoribonuclease involves separate reactions in each strand (107), and, although not directly dependent on ribosomal protein binding (16), the sequence specificity of the cleavage reactions changes in their presence (6). In a strain deficient in RNase III, 16S rRNA with mature ends will eventually form but the 23S rRNA will not be fully matured (106).

The rRNA leader sequence, defined as the region between the *rrn* P2 promoter and the first nucleotide of the 16S rRNA gene, facilitates correct 16S rRNA folding as it immediately interacts with the 5′ end of the 16S rRNA. This interaction is of a transient nature and is outcompeted by the stable complex between the leader and the sequence flanking the 3′ end of the 16S rRNA (218). The importance of the immediate correct folding of rRNA for ribosomal assembly can be exemplified by the fact that mutations in either the leader sequence or the 5′- or 3′-end-flanking regions of the 16S rRNA often confer a cold-sensitive phenotype and affect both the structure and function of the 30S subunits (14, 166, 184, 194).

The products of the RNase III cleavage reactions are precursor 16S rRNA (17S rRNA), precursor 23S rRNA, precursor 5S rRNA (9S rRNA), and, depending on the rRNA operon, a few tRNA precursors. To generate mature 16S rRNA, 115 nt from the 5′ end and 33 nt from the 3′ end must be removed (93). These cleavages are not dependent on prior RNase III cleavage, as mature 16S rRNA is formed in RNase III-deficient cells. Final 16S rRNA ends are generated by three different enzymes: RNase E, RNase G, and one still-uncharacterized RNase. RNase E and RNase G jointly remove the extra 115 nt at the 5′ terminus of the 16S rRNA. First, RNase E generates a product with an extra 66 nt, forming the 16.3S precursor, whose final 5′ end is processed by RNase G (Fig. 2A) (124). RNase E and RNase G are homologous enzymes with a functional overlap (162). However, this overlap is limited, and even though the enzymes can replace each other in the processing of the 5′ 16S rRNA terminus, the cleavages are inaccurate and inefficient (124). The extra 33 nt at the 3′ end are removed by an unknown RNase (77), whose activity may depend on efficient 5′-end processing. It has been suggested that base pairing of the 16S rRNA 3′ and 5′ termini may have an inhibitory effect on the processing of the 3′ end. Once RNase E cleavage has occurred and the terminal stem can no longer form, 3′ maturation may be accelerated (124). In addition, data indicating that final maturation takes place during initiation or the first rounds of translation have been presented (77, 138).

Unlike that of the 16S rRNA, maturation of the 23S rRNA is strictly dependent on RNase III. In cells lacking this enzyme, a heterogeneous population of precursors with additional nucleotides at both ends is found. However, the correct cleavage is not essential, as strains deficient in RNase III are viable (108, 195). The rRNA product after cleavage by RNase III contains only 3 or 7 nt at the 23S rRNA 5′ end and 7 or 9 nt at the 3′ end (93). Final processing of the 5′ terminus is performed by an unknown enzyme, while maturation of the 3′ terminus requires the exoribonuclease RNase T, an enzyme capable of trimming residues close to stem structures (Fig. 2A) (122). Furthermore, as in the case of 16S rRNA maturation, the 3′-end trimming seems to be dependent on prior 5′-end maturation (122). There are also indications that final maturation of the 23S rRNA occurs during initiation or the first cycles of translation (33, 193).

Following cleavage by RNase III, the 3′-terminal part of the primary transcript contains 5S rRNA and additional sequences that may include one or two distal tRNAs. The 5′ termini of the tRNA sequences are processed by RNase P, which results in the release of 9S rRNA, the 5S rRNA precursor (93). The 9S rRNA includes an extra 84 nt at the 5′ end and 42 nt at the 3′ end. Both termini become rapidly processed by RNase E, which leaves 3 nt at both ends (180). Final maturation at the 3′ end has been shown to depend on the exoribonuclease RNase T (121), while the RNase active at the 5′ end is still unknown.

Chemical modification of rRNA. In *E. coli*, tRNA and rRNA (except for the 5S rRNA) are covalently modified during maturation. Both the base and ribose entities can be subject to more than 80 different modifications, e.g., isomerization of uridine to pseudouridine (Ψ) or addition of carbonyl, methyl, amino, or thio groups. The 16S rRNA contains 11 modified positions, of which 10 are methylations and 1 is a pseudouridine. The 23S rRNA contains 25 known modifications, of which 14 are methylations, 9 are pseudouridines, 1 is a methylated pseudouridine, and 1 is unknown (Table 1). Most of the modifications in both the 16S and 23S rRNA are clustered in the decoding region and the peptidyl transferase center, respectively (50, 158).

In the 16S rRNA, some modifications are added to naked RNA while others are added late during maturation of the 30S rRNA; modification of the 23S rRNA is mainly an early event (158). 30S subunits can be assembled with in vitro-transcribed 16S rRNA, i.e., independently of the presence of chemical modifications. Such particles differ to some extent from 30S subunits reconstituted with rRNA modified in vivo, showing only 50% tRNA-binding capacity (116). The large ribosomal subunit seems to depend more on chemical modifications for proper assembly than does the 30S subunit. Inhibited modification leads to formation of 50S particles that lack ribosomal protein L16 and that show reduced amounts of other ribosomal proteins, which results in functionally inactive subunits (4). However, not all modifications in the 23S rRNA are essential; Green and Noller (71) have shown that at least 17 are dispensable for both assembly and function in vitro. Only seven modifications found in the vicinity of the PTC in domain V (nt 2445 to 2523) are required for in vitro reconstitution of functional particles (Table 1) (71). In vivo 50S assembly, however, requires at least the three Ψ in helix 69 (1911, 1915, and 1917) and Um2552 (28, 74). Deletion of the *rrmJ* gene, which codes for the methyltransferase that modifies U2552, leads to a severe growth defect which can be rescued by two different

TABLE 1. Modified nucleosides in *E. coli* rRNA[a]

Site	Modification	Synthetase gene	Phenotype when lacking
16S rRNA			
516	Ψ	*rsuA*	
527	m^7G	*rsmG*[b]	
966	m^2G	*rsmD*, *yhhF*[c]	
967	m^5C	*rsmB*	
1207	m^2G	*rsmC*	
1402	m^4Cm		
1407	m^5C	*rsmF*[d]	
1498	m^3U	*rsmE*[e]	
1516	m^2G		
1518	$m^6{}_2A$	*ksgA*, *rsmA*	
1519	$m^6{}_2A$	*ksgA*, *rsmA*	
23S rRNA			
745	m^1G	*rrmA*	
746	Ψ	*rluA*	
747	m^5U	*rumB*	
955	Ψ	*rluC*	
1618	m^6A		
1835	m^2G	*rlmG*, *ygjO*[f]	
1911	Ψ	*rluD*	Effect on growth due to assembly defect
1915	$m^5Ψ$	*rluD*	Effect on growth due to assembly defect
1917	Ψ	*rluD*	Effect on growth due to assembly defect
1939	m^5U	*rlmD*	Effect on growth due to assembly defect
1962	m^5C		
2030	m^6A		
2069	m^7G		
2251	Gm	*rlmB*	
2445	m^2G	*rlmL*, *ycbY*[g]	Slow growth in competition experiments; necessary for in vitro assembly
2449	hU		Necessary for in vitro assembly
2457	Ψ	*rluE*	Necessary for in vitro assembly
2498	Cm		Necessary for in vitro assembly
2501	Unknown C		Necessary for in vitro assembly
2503	m^2A		Necessary for in vitro assembly
2504	Ψ	*rluC*	Necessary for in vitro assembly
2552	Um	*rlmE*, *rrmJ*	Deficiency in assembly
2580	Ψ	*rluC*	
2604	Ψ	*rluF*	
2605	Ψ	*rluB*	

[a] Data are from reference 158 except as indicated.
[b] The gene *rsmG* has been identified in *Streptomyces coelicolor* (154), and the corresponding protein in *E. coli*, GidB, has been crystallized (177), but its activity has not been shown.
[c] Data are from reference 117.
[d] Data are from reference 148.
[e] Data are from reference 13.
[f] Data are from reference 190.
[g] Data are from reference 118.

GTPases, ObgE and Der (EngA) (199).

Most of the modified nucleosides are highly conserved; however, our understanding of their function is poor. The chemical properties of the modifications may slightly alter the features of the nucleosides, providing an additional way to fine-tune the rRNA folding and its interactions. The hydrophobic methylations, for instance, have been suggested to modulate rRNA maturation and affect the stability of rRNA structures, and many have been found to be located at the subunit interface (28). The proposed function of the hydrophilic pseudouridines is to act as molecular glue and to tighten RNA conformations, which is of great importance for, e.g., proper fixation and orientation of ligands involved in translation (158). One possible function of the modifications could be to act as structural checkpoints. Some modifications are added to naked rRNA, while others require the presence of ribosomal proteins. It is possible that enzymes that require the presence of proteins actually require a certain structure. Only if the correct structure is present will modification occur and the assembly process proceed.

Chemical modification of ribosomal proteins. It is known that some ribosomal proteins are posttranslationally modified (Table 2) (8). Proteins S11, L3, L7/L12, L16, and L33 are all monomethylated (21, 37, 40, 201), while L11 has nine methyl groups (40). Proteins S5, S18, and L12 are acetylated (45, 92). When acetylated, the L12 protein becomes converted to L7, and the ratio between these two forms varies with the growth rate (172). Ribosomal protein S6 is modified by up to six glutamic acid residues (101). In addition to its methylation, about half of the S11 molecules contain an isoaspartate (49).

TABLE 2. Modifications of *E. coli* ribosomal proteins[a]

Protein	Modification
S5	Acetylation
S6	Glutamic acid residues
S11	Monomethylation; partial modification with isoaspartate
S12	Methylthio-aspartate
S18	Acetylation
L3	Monomethylation
L7/L12	Monomethylation
L12	Acetylation
L11	Nine methylations
L16	Monomethylation; unknown
L33	Monomethylation

[a] Data are from reference 7 and references therein.

Protein S12 has been found to have a methylthio-aspartic acid (114), and protein L16 has, in addition to the methyl group, one unknown modification (7). It should be pointed out that there may be some additional modifications that have not yet been found (7), and, as in the case with modifications of the RNA, the role of protein modifications is not clear. The fact that so many proteins are modified, and that many of the proteins carry more than one modification, indicates significance for ribosomal structure and/or function. First of all, the modifications may alter the efficiency and specificity of ribosomal protein binding to the rRNA but may also optimize the binding sites of translation ligands. The ones present in substoichiometric amounts may be a way of signaling the physiological state of the cell. The only modification known so far to lead to a phenotype when absent is the methylation of ribosomal protein L3. Such a mutant shows poor growth and cold sensitivity and accumulates ribosome subunit precursors (40, 120).

Assembly of ribosomal subunits. (i) Assembly of the 30S subunit. The secondary structure of the 16S rRNA can be divided into three major domains, of which domain I is formed by the 5′ end of the rRNA, domain II by the central part, and domain III by the 3′ end of the molecule. Domain III is subdivided into one major and one minor domain. The three domains can easily be recognized in the structure of the assembled 30S subunit; domain I comprises the body, domain II makes up the platform, and the 3′ major domain forms the head (196). The 3′ minor domain forms the longest single helix in the subunit, as it stretches all the way from the base of the head (the neck region) to the bottom of the body along the 30S interface (Fig. 3A) (212).

30S subunits can be reconstituted in vitro with 16S rRNA and a mixture of ribosomal proteins from a crude 30S protein fraction (TP30), a mixture of individually purified natural or recombinant proteins (44, 78). These studies have led to the conclusion that all of the information required for assembly is contained within the rRNA and ribosomal proteins. During reconstitution experiments, it was found that protein binding to rRNA is cooperative and hierarchical, with early binding events organizing the binding sites of late proteins. The small-subunit ribosomal proteins have been divided into three groups called the primary (1°), secondary (2°), and tertiary (3°) binding proteins. 1° proteins bind directly to the rRNA, initiating the nucleation of 30S domains (20). 2° proteins require the interaction of rRNA with primary proteins, while 3° proteins need at least one primary and one secondary protein for correct association. Reconstitution experiments have also demonstrated that the assembly proceeds with a 5′ to 3′ polarity, which suggests a cotranscriptional direction of protein binding (171). This occurs during transcription, as shown in vivo by Cowgill de Narvarez and Schaup (43). Taken together, these observations have resulted in an assembly map (78). The map has recently been modified and improved, but the general

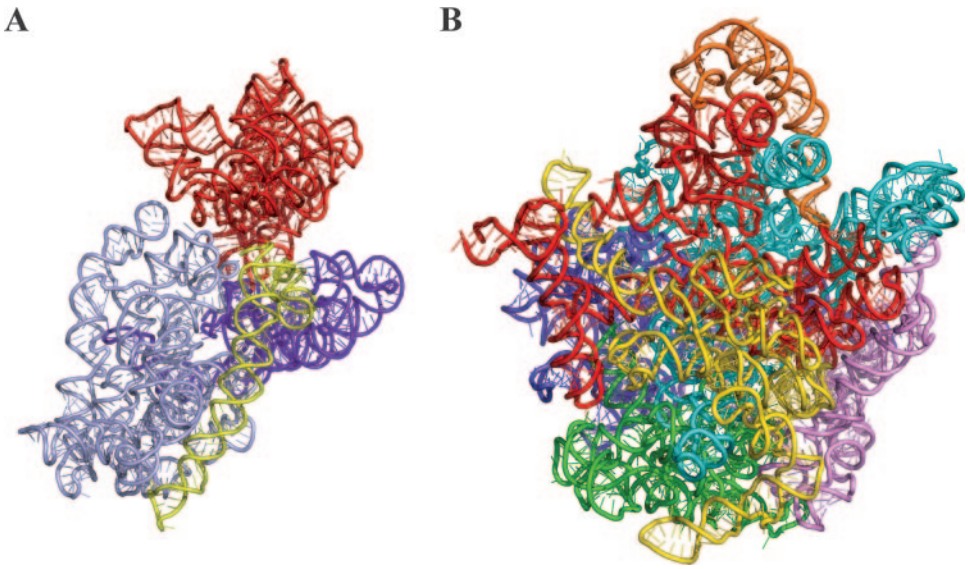

FIG. 3. Tertiary rRNA structures of the 30S and 50S subunits, seen from the interface side. The structures are adapted from the *E. coli* 3.5-Å crystal structure (187) and were modeled with PyMol (51). The division into domains is adapted from reference 73. (A) 16S rRNA and its four domains: 5′ (purple), central (gray), 3′ major (red), and 3′ minor (yellow). (B) The 5S subunit (orange) and the six domains of 23S rRNA: domain I (purple-blue), domain II (cyan), domain III (green), domain IV (yellow), domain V (red), and domain VI (violet).

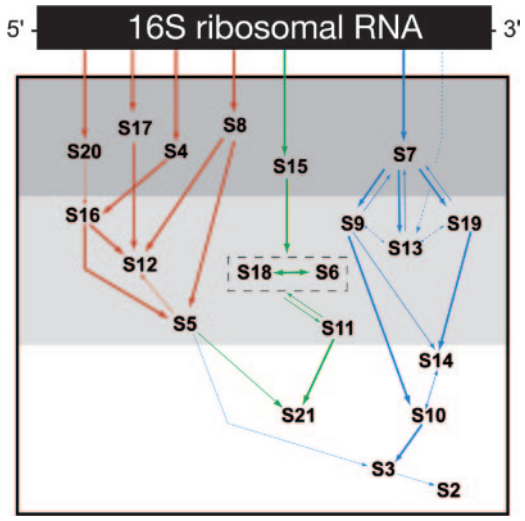

FIG. 4. Assembly map of the 30S subunit (a kind gift from G. Culver). The 16S rRNA is represented by a rectangle, and the binding order of the ribosomal proteins is shown. The dark gray area indicates the primary binding proteins, the light gray area indicates the secondary binding proteins, and the white area indicates the tertiary binding proteins. The thick, thin, and dashed arrows show strong, weak, and very weak interactions between the proteins, respectively. Proteins S6 and S18 bind as a complex and are therefore enclosed in a dashed box. Red arrows indicate the assembly of the body, green arrows indicate the platform, and blue arrows indicate the head.

outline still holds (Fig. 4) (1, 75, 87, 198).

In vitro, the 30S particle is assembled in three steps. Upon incubation of 16S rRNA and a complete set of proteins at low temperature (0 to 15°C), the assembly becomes stalled after formation of a distinct particle, termed the reconstitution intermediate (RI). This particle sediments at 21S, and its components are the 16S rRNA and the 1° and 2° binding proteins. When the temperature is changed to 42°C, the conformation of RI is rearranged, resulting in a 26S particle, RI*. When added, the 3° binding proteins associate with RI*, leading to formation of the 30S subunit (78, 88). Ribonucleoparticles similar to RI have been observed in vivo, suggesting that the above-described in vitro assembly process is genuine. Most of the conformational changes between RI and RI* are concentrated at the head structure of the 30S subunit. These changes, involving proteins S4, S5, S7, S8, and S9, play a substantial role in the rate-limiting steps of the assembly (88).

A rough order for the addition of proteins in vivo has been determined for the 30S subunit (43); in general, it agrees with that found in vitro, but there are discrepancies. One explanation for the observed differences, besides the methodology used, is that while assembled on precursor RNA in vivo, the proteins were mixed with mature 16S rRNA in the in vitro reaction. Experiments done in vitro showed that less energy was needed to form 30S subunits if starting with precursor 16S rRNA instead of mature 16S rRNA (137), suggesting that proper processing and maturation of precursor 16S rRNA is important for accelerating ribosomal protein binding and 30S subunit assembly. One additional explanation for the observed discrepancies is offered in a recent publication. The assembly pathway for the 30S platform, dependent on ribosomal protein S15, was studied in vivo by an in-frame deletion of the gene for S15, *rpsO* (24). Proteins S18, S6, S11, and S21, which according to the in vitro data are dependent on S15 for assembly (Fig. 4), were found in normal amounts in purified subunits. This result indicates that there are alternative assembly pathways in vivo.

(ii) Assembly of the 50S subunit. Even though the assembly of the 50S subunit has much in common with that of the 30S subunit, the series of events leading to formation of the 50S particle is much more complex. The 23S rRNA is almost twice the size of the 16S rRNA, it binds nearly twice as many proteins, and it must form correct interactions with the 5S rRNA. According to the 23S rRNA secondary structure, the rRNA is divided into six domains (I to VI) (73). These six domains do not, however, correspond well to the structural tertiary domains, suggesting that the assembly may not follow the 5′ to 3′ transcription direction (Fig. 3B) (211). Reconstitution experiments similar to those done to resolve the 30S assembly map were used to determine the 50S assembly map (Fig. 5) (see reference 82 and references therein).

Optimal in vitro 50S reconstitution from purified natural 50S ribosomal proteins and the 23S and 5S rRNA requires four steps with different reaction conditions. First the 23S rRNA, 5S rRNA, and a subgroup of proteins are incubated at 0°C with 4 mM Mg^{2+}, which results in the $RI_{50}(1)$ particle that sediments at 33S. Thereafter, the temperature must be increased to 44°C to enable conformational changes and to produce a new 41S particle, RI^*_{50}. The addition of the remaining proteins leads to the formation of $RI_{50}(2)$, sedimenting at 48S. Finally, $RI_{50}(2)$ is incubated at 50°C with 20 mM Mg^{2+}, resulting in 50S particles (Fig. 5) (82). The sequence of events during in vitro reconstitution involves three defined reconstitution intermediates with sedimentation coefficients 33S, 41S, and 48S. These precursor particles are very similar in their S values and compositions to the three 50S precursors found in vivo (82, 151). Five ribosomal proteins—L4, L13, L20, L22, and L24—located at the 5′ end of the 23S rRNA and protein L3, within the 3′ part, are essential for the formation of $RI^*_{50}(1)$. The binding of proteins L3 (domain IV) and L24 (domain I) seems to be especially important for proper assembly. It has been shown that during the folding of 23S rRNA, these proteins initiate the formation of two main and independent assembly centers (161a). Proteins L5, L18, and L25 mediate the principal interactions between the 5S and 23S rRNA and are necessary for the correct formation of the central protuberance (112).

Formation of the 50S subunit has also been studied in vivo, and as in the case of the 30S subunit, some discrepancies were found (43).

(iii) Role of metal ions and ribosomal proteins during assembly. To this point there has been no evidence of the involvement of metal ions in peptide bond formation, and their main role in rRNA folding seems to be stabilization of the compact tertiary rRNA structures. The structure of the *H. marismortui* large subunit has been shown to include 88 monovalent cations (Na^+ and K^+) and 117 Mg^{2+}, 22 Cl^-, and 5 Zn^{2+} ions. The most frequently occurring ion, Mg^{2+}, is often found within regions where the local density of phosphate groups is high and where the positively charged ion helps to stabilize structures by neutralizing the negative charge of the RNA (111, 146).

A general notion is that the overall folding of rRNA is

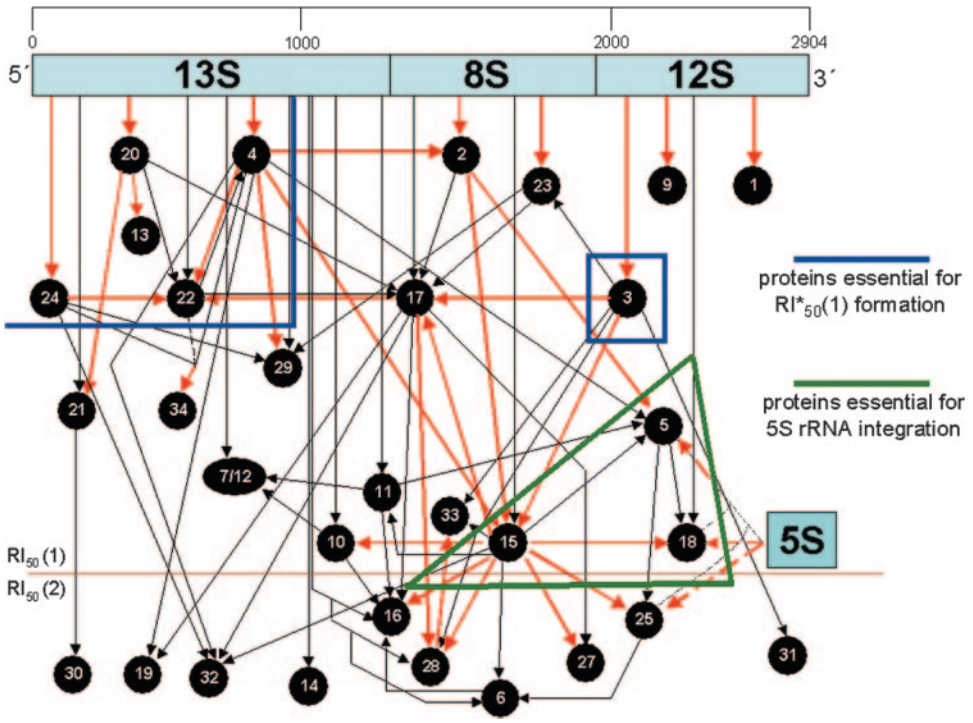

FIG. 5. Assembly map of the 50S subunit. The 23S rRNA is represented by a rectangle with its main fragments and the binding order of the ribosomal proteins and 5S rRNA. The red arrows indicate strong dependence for binding, and the black arrows indicate weaker dependence. The blue line encloses proteins essential for $RI*_{50}(1)$ complex formation, and the green triangle encloses proteins essential for 5S rRNA integration. The horizontal orange line shows the division between the $RI_{50}(1)$ and $RI_{50}(2)$ proteins. (Reproduced from reference 152 with permission of the publisher, Wiley-VCH Verlag GmbH & Co., KGaA.)

governed and maintained by its own intramolecular interactions and that the final folding and stabilization of the tertiary structure require ribosomal proteins (189, 212). The major function of the proteins is probably stabilization of rRNA structures, although some of the proteins are also involved in functionally important interactions with translation factors. Protein binding may also stabilize intermediate structures during ribosome assembly and help to overcome the conformational entropy. There are several examples of how the lack of one ribosomal protein affects assembly of the ribosome (see, among others, references 149, 160, and 181).

The most striking feature of many ribosomal proteins is a long, basic extension stretching from the otherwise typical acidic globular domain. The acidic residues allow the globular domains to associate with the exterior of the ribosome, while the extensions form structures that penetrate deeper into the particle. The extensions require a cofolding with the RNA to be able to fill the gaps and cracks formed by the rRNA (Fig. 1) (20).

The distribution of proteins and RNA in the two subunits is asymmetrical, with proteins concentrated at the top, sides, and back (Fig. 1). Most of the 50S proteins make contacts with sites in several rRNA domains, which are often separated in the nucleotide sequence (Fig. 1B and 3B). Therefore, the apparent function of most 50S proteins is to stabilize interdomain interactions that shape the particle as a whole. Two regions have higher concentration of proteins: one is the area just below the L7/L12 stalk, a binding site of many translation factors, and the other is the region surrounding the end of the polypeptide exit tunnel (146).

(iv) RNA chaperones, RNA helicases, ribosome-dependent GTPases, and other maturation factors. One of the problems faced during RNA folding is its structural promiscuity: the ease with which any given RNA sequence finds a reasonable complementarity. In fact, it has been predicted that about 50% of the bases in an RNA molecule with a randomly generated sequence form double helices (128). With the growing length of the RNA, both the flexibility and the number of obtained structures, many of which are inactive, increase. Such misfolded structures may be very stable (209), and transitions from kinetically trapped intermediates to the native state are slow because at least some of the interactions must be broken before the correct structure can be formed (214). The rate-limiting steps of in vitro reconstitution of ribosomes are likely to be such kinetic traps, since heating is required to complete the assembly (78, 82). Although it is clear that the ribosomal subunits can be reconstituted in vitro without exogenous factors, there is no doubt that there are proteins in the cell that facilitate and speed up ribosome assembly. Such maturation factors may lower the activation energy required for maturation and thereby omit the heating step (211).

Besides the ribosomal proteins, the importance of which was discussed above, there are at least three classes of proteins that may help manage RNA folding: RNA chaperones, RNA helicases, and ribosome-dependent GTPases (Table 3). The chaperones may help the RNA out of kinetic traps in the folding pathway by resolving and destabilizing RNA structures (reference 128 and references therein). The resolved RNA then becomes free to try again to reach the correct structure (209). Chaperones may also prevent or slow down formation of cer-

TABLE 3. Factors involved in ribosome maturation in *E. coli*

Factor	Suggested function and description	Reference(s)
DEAD-box RNA helicases		
CsdA (DeaD)	50S biogenesis; cold shock-inducible ATPase	97, 202
DbpA	50S biogenesis; helix 92-dependent ATPase	57, 58
SrmB	50S biogenesis; nucleic acid-dependent ATPase	35, 153
Ribosome-dependent GTPases		
Era	16S rRNA maturation, 30S biogenesis	19, 141, 183
Der (EngA)	50S biogenesis	89
ObgE ($CgtA_E$)	16S and 23S rRNA maturation, 50S biogenesis	94, 182, 215
RsgA (YjeQ)	30S biogenesis	85
Maturation factors		
EryC	Ribosome biogenesis	164
DnaK	Protein chaperone; ribosome biogenesis	5, 25, 59, 132
GroEL	Protein chaperone; ribosome biogenesis	60
RbfA	30S biogenesis	27, 48
RimB	Ribosome biogenesis	23
RimC	Ribosome biogenesis	23
RimD	Ribosome biogenesis	23
RimH	Ribosome biogenesis	96
RimM	30S biogenesis	27
RimN	30S biogenesis	99, 100
YbeB	Ribosome biogenesis	94, 95
YhbY	Ribosome biogenesis	94, 95
YibL	Ribosome biogenesis	94, 95
YjgA	Ribosome biogenesis	94, 95

tain intramolecular structures and bring together different RNAs, acting as matchmakers (83). There is at least one potential RNA chaperone candidate involved in translation in *E. coli*. IF1 has been suggested to have a chaperone activity, although which RNA may be the target for this activity is not yet known (43a).

RimN (YrdC) has been suggested to play a role during 30S subunit assembly, as a *rimN* mutant accumulates 17S rRNA (99, 100). The protein does not contain any known protein motifs characteristic of RNases or DEAD-box helicases, but it has a concave surface that can bind double-stranded RNA (200). The function of RimN is not known; it may possess a chaperoning activity, although this possibility needs to be studied further.

RNA helicases are proteins that modulate secondary and tertiary RNA structures. They play important roles in many cellular processes involving RNA, such as RNA processing, degradation, translation, and ribosome biogenesis (64). Many of the RNA helicases belong to a large and highly conserved family of proteins called the DEAD-box family. This family is further subdivided into DEAD, DEAH, and DExH subgroups according to their characteristic motifs. Some of the DEAD-box proteins possess RNA-dependent ATPase activity and are capable of melting short RNA duplexes (64). In *E. coli*, there are three DEAD-box helicases suggested to be involved in ribosome biogenesis: SrmB, CsdA (DeaD), and DbpA (34, 35, 58). CsdA is a ribosome-associated factor involved in a late step of 50S biogenesis; deletion of the *csdA* gene leads to an accumulation of 40S particles (34). CsdA was initially identified as a suppressor of a mutation in the gene encoding ribosomal protein S2 (202) and was later shown to be a cold shock-inducible ATP-independent RNA helicase (97). The SrmB protein was isolated as a multicopy suppressor of a temperature-sensitive mutant of ribosomal protein L24 and was suggested to be a nucleic acid-dependent ATPase involved in 50S assembly (153). Deletion of the *srmB* gene leads to accumulation of a 40S particle corresponding to an incompletely assembled 50S subunit. The protein composition of this particle suggests that SrmB is involved in an early step of 50S biogenesis, which fits with the fact that L24 is one of the assembly nucleation proteins (35). The RNA target(s) of CsdA and SrmB is not known, in contrast to the DbpA helicase. DbpA is a 3′ to 5′ RNA helicase (58), whose helicase and ATPase activities are dependent on a specific region in the 23S rRNA, helix 92, located within the PTC (58). Its precise role, however, is not known.

The third class of proteins significant for RNA folding includes the GTPases. Some of them have activities that are ribosome dependent and that lead to defects in ribosome assembly when malfunctioning (22, 29). A well-studied example is Era, a protein that possesses both 16S rRNA and 30S subunit-binding capacity and whose GTPase activity increases when bound to RNA (141). Era binds to the 30S subunit in the cleft between the head and the platform, a site overlapping the binding site for ribosomal protein S1, one of the last proteins to be added to the subunit (191). The Era protein is also in contact with the 3′ end of the 16S rRNA, and depletion of Era has been shown to affect maturation of the 16S rRNA. However, whether it is directly involved in 3′-end processing remains to be elucidated (183). It has also been shown that the cold-sensitive phenotype of an Era mutant can be suppressed by overexpression of the *ksgA* gene, coding for a 16S rRNA methyltransferase (129). This enzyme dimethylates two A's in helix 45 at the 3′ end of the 16S rRNA. KsgA binds to a pre-30S particle, but methylation of the 16S rRNA probably occurs later during assembly (54). All these data taken to-

gether suggest that Era is important for maturation of the 3′ end of the 16S rRNA and that this is probably a late event. To support this, mutants deficient in Era have been shown to become cell cycle arrested, a finding that indicates a link between ribosome biogenesis and the cell cycle (19).

ObgE ($CgtA_E$) is a conserved GTPase with many suggested functions. It has been found to bind to ribosomal subunits and to affect ribosome maturation (182, 215). Recently it was shown that ObgE binds to 50S subunits in its GTP form, and its deletion affects maturation of not only 23S rRNA but also 16S rRNA (94). Interestingly, overexpression of ObgE or Der (described below) can suppress the slow growth caused by the lack of the Um2552 methyltransferase RrmJ (199).

Der (EngA) is a GTPase with tandem GTP-binding domains that is essential for 50S subunit formation (89).

RsgA (YjeQ) is a GTPase that is activated by 30S subunits (46, 85). The protein is probably involved in 30S subunit assembly, as a Δ*rsgA* mutant accumulates 17S rRNA (85).

Finally, there is a group of proteins that affects ribosome synthesis in an as-yet-unknown way; we refer to these proteins as maturation factors. When mutated, many of these proteins are known to affect ribosome biogenesis negatively. RbfA and RimM are proteins essential for efficient processing of 16S rRNA. Both have been shown to bind to the 30S subunit, and RbfA and RimM mutants show a cold-sensitive phenotype (27). RbfA was identified as a multicopy suppressor of a C23U mutation in the first helix at the 5′ end of the 16S rRNA, and it may be involved in late maturation of 30S subunits or in translation initiation (48). RimM mutants show incorrectly matured 30S subunits and impaired translational efficiency (27).

In summary, it has been shown that (i) a Δ*rimM* mutation can be suppressed by increased synthesis of RbfA (26, 27), (ii) a Δ*rbfA* mutation can be suppressed by overexpression of the Era protein (90), and (iii) a cold-sensitive Era mutant can be suppressed by overexpression of KsgA (129). These results indicate a functional order for the four proteins, RimM→RbfA→Era→KsgA, with KsgA perhaps being the last to exert its function before ribosomal protein S1 binds to the small subunit.

As mentioned above, overexpression of Der or ObgE can suppress the lack of RrmJ (199), which places Der and ObgE after RrmJ in the assembly process (94).

DnaK is a protein chaperone that belongs to the Hsp70 family. It stabilizes the hydrophobic regions of extended polypeptide segments in an ATP-dependent manner upon heat shock induction (25). At 45°C, temperature-sensitive DnaK mutants give rise to the accumulation of 30S and 50S precursor particles, implying that DnaK is involved in ribosome biogenesis at high temperatures (3, 59). However, the involvement of the protein at temperatures below 30°C is more controversial. Maki et al. have shown that at 15 to 20°C, DnaK, together with its cochaperones GrpE and DnaJ, is sufficient to convert 21S RI particles to 30S particles, omitting the heating activation required in vitro (132–134). This finding has been questioned, and the issue is still unresolved (5).

Another chaperone and heat shock protein, GroEL, has been shown to be required for a late step in 50S biogenesis at high temperatures, although it is dispensable for 30S assembly (60).

There are also a few not very well mapped genes which, when mutated, affect ribosome maturation. These genes are *eryC* (164); *rimB*, *rimC*, and *rimD* (23); and *rimH* (96). Also, a few proteins—YbeB, YhbY, YibL, and YjgA—have been found by a proteomic approach to be ribosome associated and possibly involved in ribosome biogenesis (94, 95).

REGULATION OF THE SYNTHESIS OF RIBOSOMAL COMPONENTS

Bacteria live in habitats with frequently changing conditions; to be able to adapt rapidly, they have evolved a wide array of mechanisms to regulate every cellular process from transcription initiation to protein inactivation and degradation. Regulation of gene expression on the transcriptional level relies on signals transferred to the RNA polymerase (RNAP) that alter the enzyme's activity or specificity. Different regulatory proteins, molecules, and small RNAs can modulate RNAP. Besides the σ and anti-σ factors, an additional 100 to 150 transcription factors and 240 to 260 DNA-binding proteins in *E. coli* are also involved in the fine-tuning of expression (91).

In this section, we focus on global regulatory networks that affect rRNA transcription: the stringent response and steady-state (growth rate) regulation. Both regulations respond to cytoplasmic concentrations of (p)ppGpp to regulate the synthesis of stable RNA (rRNA and tRNA). Stringent control leads to a reduction of stable RNA synthesis in response to amino acid starvation, while the growth rate control leads to adjustment of stable RNA synthesis in response to changes in the nutritional quality of the growth medium. The general belief is that these two processes are functionally distinct, something that recently has been questioned (53). We also discuss feedback regulation of rRNA synthesis in response to the amount of active ribosomes.

The Transcription Process

The transcription process per se is reviewed briefly; for more details, see references 169 and 175.

Transcription initiation. The DNA-dependent RNAP is a multisubunit entity consisting of at least four subunits ($\alpha_2\beta\beta'$), with a total molecular mass of 378.8 kDa, and it contains all of the catalytic activity required for RNA synthesis. However, the RNAP core enzyme (E) is unable to recognize promoter sequences required for transcription initiation without an additional subunit, the σ factor. There are seven different σ factors in *E. coli*: σ^{70}, σ^{N} (σ^{54}), σ^{S} (σ^{38}), σ^{H} (σ^{32}), σ^{F} (σ^{28}), σ^{E} (σ^{24}), and σ^{FecI} (σ^{19}). Most of the housekeeping and growth-related genes in exponentially growing cells are transcribed by RNAP containing σ^{70} (130), while $E\sigma^{S}$ is essential for transcription of many stationary phase-specific genes (208). The remaining five σ factors—σ^{H} and σ^{E} (206), σ^{F} (8), σ^{N} (142), and σ^{FecI} (61)—respond to different stimuli and alter gene expression accordingly.

Transcription initiation is a multistep process that starts with the RNAP sliding along the DNA in search of a promoter. At least four intrinsic elements are part of a promoter recognition sequence: (i) a hexamer at position −10 upstream from the transcription start site, (ii) a hexamer at position −35, (iii) the spacer region (the DNA region between these two elements), and (iv) the UP element (the region located between positions −40 and −60) (144). After binding of RNAP to the promoter

sequence, an unstable closed binary complex is formed. Melting of the double-stranded DNA leads to the placement of the template strand in the active-site channel and formation of an open complex. With the binding of the first (initiating) nucleoside triphosphate (iNTP), the initiation complex is formed (145).

Transcription elongation and termination. Formation of the stable elongation complex is probably facilitated by the release of the σ factor in order to enclose the RNA-DNA hybrid within the active-site channel. During elongation, a transiently open transcription bubble (about 18 bp in length) moves through the otherwise double-stranded DNA. The catalytic site contains a substrate-binding site, at which the incoming NTP (supplied via the secondary channel) is bound, and a product-binding site, at which the 3′ end of the growing RNA chain is positioned (205). The transcribed RNA forms an RNA-DNA hybrid with the template strand as it leaves the catalytic site before it exits via the RNA exit channel. One important feature of the elongation complex is its stability; the problem is how to bring about sequence-specific destabilization of the complex at terminator sites (79). Such destabilizing signals may be either intrinsic (factor independent) or factor dependent, which requires participation of the Rho protein.

Intrinsic terminators, which represent about half of the termination sites in *E. coli*, include a GC-rich sequence that forms a stable termination hairpin, followed by a 7- to 9-nt U-rich sequence. It appears that upon interaction with the hairpin, the exit channel is opened and the unstable U/A RNA-DNA hybrid becomes disrupted.

Rho-dependent terminators are defined by a C-rich 60- to 100-nt segment that lacks secondary structures. Binding of Rho to the terminator induces its RNA-dependent ATPase activity, resulting in a movement in the 5′ to 3′ direction along the nascent RNA. When the Rho protein catches up with the polymerase, the helicase activity triggers the release of the transcript (70, 174).

Regulation of Expression of *rrn* Operons

In bacteria, the ribosomes determine the capacity of translation; because this is directly linked to cell growth, control of rRNA transcription and ribosome biogenesis is of great importance for the adjustment to environmental changes.

In *E. coli*, there are seven rRNA operons: *rrnA*, *rrnB*, *rrnC*, *rrnD*, *rrnE*, *rrnG*, and *rrnH*. Transcription of these operons accounts for more than half of the cell's total RNA synthesis under rapid growth conditions (42). The operons are almost identical in sequence, with only minor sequence heterogeneities, containing the 16S, 23S, and 5S genes in that order (Fig. 2A). The spacer region separating the 16S and 23S genes contains one or two tRNA genes; two of the operons (*rrnH* and *rrnC*) also have distal tRNA genes located downstream of the 5S gene. The *rrnD* operon has two 5S rRNA genes, separated by a tRNA gene. Over the years, many of the studies of rRNA expression have been performed with the *rrnB* operon, which has been chosen as a representative example of rRNA operons (Fig. 2); however, there are minor differences in the regulation of the seven operons (84).

rrn operons contain two promoters, P1 and P2, which are arranged in tandem and separated by ~120 bp. Both promoters are recognized by $E\sigma^{70}$, and even though none contains a perfect match to the consensus sequence, they are among the most efficient in the cell. Changes in the P1 sequence, making it more similar to the σ^{70} consensus sequence, produce an even stronger promoter; however, they also lead to insensitivity to alterations in the growth rate or (p)ppGpp concentration (56, 167). This indicates that the P1 promoter sequences have not evolved for maximal initiation efficiency but have instead been optimized for intricate regulation.

There are two characteristic intrinsic features of the core *rrn* promoters (besides the −10 and −35 regions) that are important for the response to stringent and growth rate regulation. One of them is a suboptimal spacer region of 16 bp (instead of the most frequent 17 bp) between the −10 and −35 hexamers. The second feature is a GC-rich discriminator sequence found directly downstream of the −10 hexamer (Fig. 2B) (68). While P1 is most active at high growth rates, the activity of P2 seems to be homeostatic and more important at low growth rates. It has been suggested that the activity observed for P2 could be masked by transcription originating from the upstream P1 promoter, an effect known as promoter occlusion (67).

Two features contribute to efficient initiation of transcription: the UP element and the transcription factor FIS, a protein that binds as a dimer to the DNA upstream of UP (Fig. 2B). Together these two stimulate *rrn* transcription from all P1 promoters up to 200-fold in vivo (86). The UP element is an AT-rich DNA sequence, directly flanking the −35 hexamer and extending to the −60 region. UP elements have been found at P1, P2, and non-rRNA promoters, and the degree of similarity to the consensus sequence correlates with the transcription induction (86, 178). The α subunits of RNAP interact with the UP element to enhance RNAP binding to the promoter region (12).

FIS is a small, 11.2-kDa DNA-binding protein that serves as a transcription regulator of many genes, including rRNA and tRNA genes (135). Three to five FIS-binding sites have been identified upstream of all P1 promoters (Fig. 2B). The promoter-proximal site I, which accounts for most of the activation in vivo, is required for RNAP recruitment to the region; sites II and III increase transcription only marginally (20 to 30%) (161, 179). The FIS protein bends the DNA and untwists the −10 region, enhancing open complex formation (10).

H-NS is another DNA-bending protein involved in the regulation of *rrn* expression. It acts as a repressor, specifically antagonizing FIS-dependent activation. Repression is caused by entrapment of RNAP in a stable DNA loop formed by H-NS (47).

The difference in the rate of transcription of rRNA and mRNA genes has been attributed to the *rrn* antitermination system. All rRNA operons in *E. coli* have antiterminator sequences, called *nut*-like sequences, in their leader (downstream of P2) and spacer (between the 16S and 23S rRNA genes) regions. These sequences are important for the assembly of antitermination complexes, which allow RNAP to transcribe through Rho-dependent terminators within the long untranslated rRNA operons. Several components of an antitermination complex have been identified, i.e., NusA, NusB, NusG, and NusE (ribosomal protein S10). Other ribosomal proteins—S1, S4, L3, L4, and L13—have also been suggested to play a role in antitermination (203).

It seems that the *nut*-like sequences are located in close proximity to the 16S rRNA RNase III processing sites. Mutations in these sites affect RNase III processing and lead to misfolding of rRNA (147, 184). It is therefore possible that interactions between RNAP, rRNA, and Nus factors bring together the 5′ and 3′ ends of the RNase III targets, facilitating 16S rRNA processing (69). The antitermination system is also thought to maintain a transcription elongation rate that is optimized for both speed and folding of the transcript. A too-high elongation rate has been shown to result in improper folding of the rRNA transcript and misassembly of 50S subunits (119).

The involvement of ribosomal proteins in antitermination complexes renders an additional way to coregulate transcription and ribosome biogenesis. The ribosomal protein S1 has been suggested to decrease antitermination, while the S4, L3, L4, and L13 proteins increase terminator readthrough about 11-fold. This raises a possibility for the ribosomal proteins to increase the rRNA synthesis and, at the same time, to be delivered to their binding sites on the nascent transcript (203). Therefore, the antitermination system may have a role in promoting the first steps of correct folding and processing of rRNA, and the involvement of ribosomal proteins in the antitermination complex may be a way to prevent production of incomplete rRNAs and unequal titration of ribosomal proteins (155).

The stringent response and (p)ppGpp. When the availability of amino acids in the cell is lower than that required for continuous protein synthesis, a complex set of pleiotropic responses termed stringent control is initiated. The induced cellular changes result in an upregulation of protein degradation, amino acid synthesis, and carbohydrate metabolism and a downregulation of nucleic acid and protein synthesis. The major regulators of the stringent response are two small nucleotides, guanosine 3′,5′-bis(diphosphate) (ppGpp) and guanosine 3′-diphosphate 5′-triphosphate (pppGpp). These molecules are not known to differ much in function and are therefore collectively denoted (p)ppGpp. The nucleotides are synthesized by enzymatic phosphorylation of GDP and GTP to ppGpp and pppGpp, respectively, using ATP as a phosphate donor (131). During growth-favorable conditions, low basal steady-state levels of (p)ppGpp are maintained in the cell. However, upon induction of the stringent response, the cellular concentrations of (p)ppGpp increase by 10- to 100-fold (36). In *E. coli*, two closely related enzymes, RelA and SpoT, are responsible for synthesis and degradation of (p)ppGpp. RelA is a synthetase, whereas SpoT is a bifunctional enzyme with both a hydrolase and a synthetase activity.

RelA is a low-abundance ribosome-bound protein whose (p)ppGpp synthetase activity is triggered when a cognate, uncharged (deacylated) tRNA is bound to the A site on the ribosome (131).

Not much is known about the other enzyme, SpoT. It is a cytosolic protein responsible for maintenance of the basal levels of (p)ppGpp during balanced and slow growth. The synthetase activity seems to be triggered by carbon and energy source starvation, but the enzyme's primary function is pyrophosphohydrolase activity. The ability of SpoT to degrade high cellular (p)ppGpp levels is important for recovery from the starvation-induced stringent response (31).

During amino acid deprivation, bacterial cells react by an immediate shutdown of rRNA and tRNA transcription (31). In P1, the core promoter (−41 to +1), which contains the important and highly conserved GC-rich discriminator, appears to be the target for inhibition. Both the UP elements and the FIS protein-binding sites seem to be dispensable for stringent regulation (98).

The crystal structure of ppGpp bound to RNAP reveals that the regulator binds to a single site at the RNAP surface near the secondary channel and close to the active center (9). The binding of ppGpp may thus affect the addition of the NTP substrates to the catalytic site. The competition between iNTP and (p)ppGpp for the same binding site on RNAP may lead to increased instability of open complexes of P1 promoters. ppGpp has also been proposed to base pair with a cytosine in the nontemplate strand of the discriminator, negatively affecting interactions within the transcription bubble (9).

(p)ppGpp-dependent regulation is not only restricted to the steps of transcription initiation. During elongation of mRNA, RNAP pausing at several sites is enhanced, which significantly increases the efficiency of termination (9, 115); interestingly, (p)ppGpp has no effect on rRNA elongation (204).

Recently, the role of the DksA protein in the inhibition of *rrn* expression was reported. DksA is a small protein that binds to RNAP and affects the rate of open complex decay, enhancing the sensitivity to the small variation in iNTP concentrations. It also is thought to enhance the sensitivity to (p)ppGpp (168, 170).

Steady-state regulation (growth rate regulation). Bacterial growth is directly linked to the capacity for protein synthesis. Both the fraction of ribosomes engaged in translation (~80%) and the rate of peptide chain elongation are independent of the growth rate. With an upper translation limit of ca. 22 amino acids per second, an increase in total protein synthesis can be achieved only by an increase in the number of ribosomes, which in a rapidly growing cell can be as many as 70,000. At lower growth rates this number is reduced to 2,000 (18, 157). The rate of stable RNA synthesis is commonly determined as the ratio of stable RNA (r_s) to total RNA (r_t) synthesis (r_s/r_t). The synthesis rates of mRNAs remain approximately constant, while the synthesis of rRNA is growth rate dependent. Under good growth conditions, nearly all RNA transcription is devoted to stable RNA synthesis, and the r_s/r_t ratio approaches 1. In slow growth, it reaches a minimum level of 0.25, with most transcripts originating from (p)ppGpp-insensitive promoters (53). Many experiments have been carried out to answer the question of whether (p)ppGpp is the main growth rate regulator. The P1 promoters have been suggested both to respond (65) and not to respond (81) to growth rate control in strains lacking (p)ppGpp (so-called $ppGpp^0$ strains). A thorough study of published results and additional experiments have led to the suggestion that ppGpp alone is the growth rate control regulator (53).

(i) Translational feedback of ribosomal proteins. During ribosome biogenesis, the rate of ribosomal protein synthesis is dictated by the activity of ongoing rRNA expression. In *E. coli*, there are 19 r-protein operons. In most cases, the ribosomal protein genes are clustered either with other r-proteins or with proteins involved in translation, e.g., elongation factors EF-G,

EF-Tu, and EF-Ts. Interestingly, the genes encoding the α, β, β′, and σ^{70} subunits of RNAP are also clustered among r-proteins (104). The main mechanism for coordinating the rate of ribosomal protein synthesis to other r-proteins, and to the available amount of rRNA, is the autogenous feedback regulation. This mechanism works at the translational level through one of the r-proteins in the operon that binds not only directly to the target rRNA but also to its own mRNA at a site called the operator. The operator may be located either upstream of the first gene or between genes in the operon. When in excess, the repressor r-protein binds to the operator and inhibits translation of the proteins encoded in the mRNA (104). The repressor may either compete with ribosomes for the mRNA or, upon binding to the message, induce formation of structures that prevent the ribosome from accessing the translation start site (140). Ten repressor proteins have been identified to date: S1, S4, S7, S8, S15, S20, L1, L4, L10, and L20, regulating the S1, α, *str*, *spc*, S15, S20, L11, S10, L10, and L35 operons, respectively (104).

Binding of a repressor r-protein may lead not only to reduced translation initiation but also to degradation of the mRNA. However, not all operons containing r-proteins are subjects of autogenous translational feedback regulation. Operons that encode r-proteins that do not bind directly to rRNA cannot utilize this kind of regulation and are instead repressed at the transcriptional level or through protein degradation (210).

(ii) Feedback regulation of rRNA synthesis. One of the proposed mechanisms explaining how the amount of ribosomes is regulated to meet the cell's demand for protein synthesis is the feedback mechanism (42). It allows maintenance of the balance between the energy devoted to biosynthetic pathways and to the protein synthesis machinery at any given growth rate (125). One example of when such regulation is needed is during rapid growth, when several rounds of replication of the chromosome are initiated simultaneously. The DNA will be partly polyploid, and the number of rRNA operons will be notably higher than seven. In this case, the feedback regulation prevents production of more ribosomes than needed (207).

The targets of the feedback signal are the *rrn* promoters. It has been shown that deletion of four *rrn* operons induces expression from the remaining three by several times and that this increase is induced at the level of transcription initiation (41). Yamagishi et al. were able to demonstrate that an alteration of three bases in the anti-SD region in a 16S rRNA gene on a plasmid abolishes feedback regulation of *rrn* operons (216). Also, a decrease in the concentration of translation initiation factor 2 significantly disrupts regulation, which is observed as increased accumulation of rRNA and nontranslating ribosomes. Based on these observations, it was proposed that ribosomes must be active in translation to be subject to feedback regulation (39).

Even though feedback regulation, as a way of regulating rRNA synthesis, is no longer controversial, our understanding of the identity of the molecular signal that senses the presence of functional ribosomes, and how it becomes transferred to the initiating RNAP, is minimal. There are two models for explaining feedback regulation, and both involve sensing the concentration(s) of small effector molecules linked to the translation process: (i) iNTP and ppGpp (169) or (ii) ppGpp only (53).

70S QUALITY CONTROL AND TRANSLATION INITIATION

The importance of ribosomal function and quality cannot be questioned. Not only does the cell need the correct amount of ribosomes but those produced must also function properly. The ribosome, when correctly assembled, is resistant to nuclease activity and is reused during many translation cycles. Making a ribosome is costly for the cell, requiring many components and factors. Therefore, it is better to eliminate faulty particles during the early stages of maturation than during the later stages, especially before they are finally matured and used in translation. The question can therefore be raised: how is the quality of the ribosome checked? In this section we argue in favor of a hypothesis.

More than a few times in this review, we have referred to results indicating a coupling between ribosome biogenesis and the translation process. The most straightforward is the stringent control, induced by a shortage of charged aminoacyl tRNAs. This triggers the production of (p)ppGpp, which turns off rRNA transcription and hence ribosome production. The mechanism behind the feedback control is less well understood, but its effects are clear: if the number of actively translating ribosomes is for some reason lowered, production of rRNA and synthesis of ribosomal proteins is increased. If, on the other hand, the ribosomes are in excess, the activity of ribosome biogenesis is reduced. Thus, there seems to be a signal in the cell that senses the number of active ribosomes and transfers this to the ribosome biogenesis machinery.

There are also indications of a link between maturation of the two ribosomal subunits. In cases when biogenesis of one of the subunits is affected, delays in maturation of the other are often observed. Two ribosomal proteins, S5 and L22, shown to be important for the assembly of their respective subunit, when mutated, affect maturation of both the 30S and the 50S particles (149, 150, 165). Deletion of the gene *rluD*, which codes for a pseudouridine synthase that modifies the 23S rRNA, leads not only to immature 50S subunits but also to immature 30S subunits (74). The assembly factors SrmB and CsdA, which interact with 50S, have been shown, when deleted, to be important for assembly of the 30S subunit, as judged from the presence of 17S rRNA (34, 35). Finally, the antibiotic chloramphenicol, although binding to the 50S subunit, has been shown to affect assembly of the 30S subunit (reference 99 and references therein). In the presence of chloramphenicol, the amount of *rrn* transcripts accumulates in the cell: a phenotype of affected feedback regulation (52). At first, it was believed that the defect in rRNA maturation was due to affected translation of ribosomal proteins. However, it is more likely that it is the reduced translation itself that affects the regulatory loop between translation and final maturation of the subunits (195).

In view of the fact that rRNA maturation is such a minute and complex process, one may ask why the RNA is transcribed with immature termini that eventually have to be removed. One purpose may be that the sequential conformational changes of the precursors during processing keeps the RNA folding within the correct assembly pathway. It has also been

suggested that the processing is part of an additional mechanism: function verification and quality control of newly assembled ribosomes. The idea that rRNA processing and protein synthesis may be interdependent has been proposed by Mangiarotti et al., who state that newly synthesized particles that cannot fulfill the requirements for structural reasons become disassembled in the eukaryotic slime mold *Dictyostelium discoideum* (136). For *E. coli*, data that suggest that final maturation of 16S rRNA, as well as the 5′ ends of both 23S and 5S rRNA, is dependent on conditions that favor protein synthesis have been presented (33, 77, 138, 193).

If maturation takes place during 70S initiation complex formation, or during the first cycles of translation, all of the above-mentioned results make sense. It has been found that formation of the 70S ribosome during translation initiation involves sequential contacts between the two subunits (80), which may be a way of probing the structure of the particles. Hence, if one of the subunits is affected in assembly, this leads to a delay in maturation of the other.

If there is quality control of the type described above, there must be a machinery to degrade the faulty subunits. Most studies of ribosome degradation have been done with starving cells in stationary phase, and very little is known about the sequence of events; however, knowledge of RNases involved in ribosome turnover is steadily growing (55). The initial cleavage reactions take place on free 30S and 50S subunits, possibly by RNase E, and once the degradation process starts, it proceeds rapidly until completion (15, 102).

RNase E is an enzyme that plays a major role in mRNA turnover (and probably also rRNA turnover), recognizing and cleaving sites located within AU-rich single-stranded regions. RNase E exists both as a free molecule in the cell and as part of a multiprotein complex called the degradosome (15). Whether RNase E degrades rRNA as a free enzyme or as part of the degradosome is still uncertain, although fragments of both 16S and 23S rRNA have been found associated with the degradosome in vivo (15). Following endonucleolytic cleavage is the exonucleolytic digestion of rRNA fragments by at least two enzymes, PNPase and RNase R (38).

It has also been shown that rRNA in *E. coli* can be polyadenylated, especially in the absence of processing exoribonucleases that cannot compete with the binding of poly(A) polymerase I (PAPI) to the exposed 3′ ends of the rRNA (123). Polyadenylation is an important signal for mRNA degradation in eubacteria, but it is still unknown whether the short poly(A) tails added to stable RNA promote degradation (143).

Normally, when finally matured, ribosomes are very stable and resistant to RNases. However, mistakes in the formation of ribosomes do occur, and there must be a way to identify defective ribosomal particles. Such a signal may include immature 3′ ends of rRNA, which, when exposed, could be polyadenylated by PAPI and recognized and degraded by the degradosome, PNPase, and RNase R.

Whether final maturation indeed takes place during translation initiation is under investigation. The studies mentioned above were performed in vitro; however, our experiments will show what happens in vivo. If maturation occurs during initiation or shortly thereafter, anything that slows down initiation will lead to an accumulation of immature rRNA. We have used two strains, mutated in either Fmt (methionyl tRNA formyltransferase) or IF2. Our preliminary results support the hypothesis (data not shown).

Even if we sort out when the final maturation of the subunits occurs, and hence when the quality of the ribosome is checked, that finding will not reveal the mechanism behind the control. We want to focus on this problem, but there are many other aspects of ribosome biogenesis to study: in what order are the proteins added, what are the roles of all of the accessory factors, and what are the identities of the missing RNases? In summary, ribosome biogenesis in bacteria is a field that calls for much research and promises an interesting scientific future.

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REFERENCES

1. **Adilakshimi, T., P. Ramaswamy, and S. A. Woodson.** 2005. Protein-independent folding pathway of the 16S rRNA 5′ domain. J. Mol. Biol. **351:** 508–519.
2. **Agrawal, R. K., R. K. Lata, and J. Frank.** 1999. Conformational variability in *Escherichia coli* 70S ribosome as revealed by 3D cryo-electron tomography. Int. J. Biochem. Cell Biol. **31:**243–254.
3. **Alix, J. H., and M. F. Guerin.** 1993. Mutant DnaK chaperones cause ribosome assembly defects in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **90:**9725–9729.
4. **Alix, J. H., D. Hayes, and K. N. Nierhaus.** 1979. Properties of ribosomes and RNA synthesized by *Escherichia coli* grown in the presence of ethionine. V. Methylation dependence of the assembly of *E. coli* 50S ribosomal subunits. J. Mol. Biol. **127:**375–395.
5. **Alix, J. H., and K. N. Nierhaus.** 2003. DnaK-facilitated ribosome assembly in *Escherichia coli* revisited. RNA **9:**787–793.
6. **Allas, Ü., A. Liiv, and J. Remme.** 2003. Functional interaction between RNase III and the *Escherichia coli* ribosome. BMC Mol. Biol. **4:**8.
7. **Arnold, R. J., and J. P. Reilly.** 1999. Observation of *Escherichia coli* ribosomal proteins and their posttranslational modifications by mass spectrometry. Anal. Biochem. **269:**105–112.
8. **Arnosti, D. N., and M. J. Chamberlin.** 1989. Secondary σ factor controls transcription of flagellar and chemotaxis genes in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **86:**830–934.
9. **Artsimovitch, I., V. Patlan, S.-I. Sekine, M. N. Vassylyeva, T. Hosaka, K. Ochi, S. Yokoyama, and D. M. Vassylyev.** 2004. Structural basis for transcription regulation by alarmone ppGpp. Cell **117:**299–310.
10. **Auner, H., M. Buckle, A. Deufel, T. Kutateladze, L. Lazarus, R. Mavathur, G. Muskhelishvili, I. Pemberton, R. Schneider, and A. Travers.** 2003. Mechanism of transcriptional activation by FIS: role of core promoter structure and DNA topology. J. Mol. Biol. **331:**331–344.
11. **Ban, N., P. Nissen, J. Hansen, P. B. Moore, and T. A. Steitz.** 2000. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. Science **289:**905–920.
12. **Barnard, A., A. Wolfe, and S. J. W. Busby.** 2004. Regulation at complex bacterial promoters: how bacteria use different promoter organizations to produce different regulatory outcomes. Curr. Opin. Microbiol. **7:**102–108.
13. **Basturea, G. N., K. E. Rudd, and M. P. Deutscher.** 2006. Identification and characterization of RsmE, the founding member of a new RNA base methyltransferase family. RNA **12:**426–434.
14. **Besancon, W., and R. Wagner.** 1999. Characterization of transient RNA-RNA interactions important for the facilitated structure formation of bacterial ribosomal 16S RNA. Nucleic Acids Res. **27:**4353–4362.
15. **Bessarab, D. A., V. R. Kaberdin, C.-L. Wei, G.-G. Liou, and S. Lin-Chao.** 1998. RNA components of *Escherichia coli* degradosome: evidence for rRNA decay. Proc. Natl. Acad. Sci. USA **95:**3157–3161.
16. **Birenbaum, M., D. Schlessinger, and S. Hashimoto.** 1978. RNase III cleavage of *Escherichia coli* rRNA precursors: fragment release and dependence on salt concentration. Biochemistry **17:**298–306.
17. **Bram, R. J., R. A. Young, and J. A. Steitz.** 1980. The ribonuclease III site flanking 23S sequences in the 30S ribosomal precursor RNA of *E. coli*. Cell **19:**393–401.
18. **Bremer, H., and P. P. Dennis.** 1996. Modulation of chemical composition and other parameters of the cell by growth rate, p. 1553–1569. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, DC.
19. **Britton, A. R., B. S. Powell, S. Dasgupta, Q. Sun, P. Margolin, J. R. Lupski, and D. L. Court.** 1998. Cell cycle arrest in Era GTPase mutants: a potential growth rate-regulated checkpoint in *Escherichia coli*. Mol. Microbiol. **27:** 739–750.

20. **Brodersen, D. E., W. M. Clemons, A. P. Carter, B. T. Wimberly, and V. Ramakrishnan.** 2002. Crystal structure of the 30 S ribosomal subunit from *Thermus thermophilus*: structure of the proteins and their interactions with 16S RNA. J. Mol. Biol. **316:**725–768.
21. **Brosius, J., and R. Chen.** 1976. The primary structure of protein L16 located at the peptidyltransferase center of *Escherichia coli* ribosomes. FEBS Lett. **68:**105–109.
22. **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.
23. **Bryant, R. E., and P. S. Sypherd.** 1974. Genetic analysis of cold-sensitive ribosome maturation mutants of *Escherichia coli*. J. Bacteriol. **117:**1082–1092.
24. **Bubunenko, M., A. Korepanov, D. L. Court, I. Jagannathan, D. Dickinson, B. R. Chaudhuri, M. B. Garber, and G. M. Culver.** 2006. 30S ribosomal subunits can be assembled *in vivo* without primary binding ribosomal protein S15. RNA **12:**1229–1239.
25. **Bukau, B., and A. L. Horwich.** 1998. The Hsp70 and Hsp60 chaperone machines. Cell **92:**351–366.
26. **Bylund, G. O., J. M. Lövgren, and P. M. Wikström.** 2001. Characterization of mutations in the *metY-nusA-infB* operon that suppress the slow growth of a Δ*rimM* mutant. J. Bacteriol. **183:**6095–6106.
27. **Bylund, G. O., L. C. Wipemo, L. A. C. Lundberg, and P. M. Wikström.** 1998. RimM and RbfA are essential for efficient processing of 16S rRNA in *Escherichia coli*. J. Bacteriol. **180:**73–82.
28. **Caldas, T., E. Binet, P. Bouloc, and G. Richarme.** 2000. Translational defects of *Escherichia coli* mutants deficient in the Um_{2552} 23S ribosomal RNA methyltransferase RrmJ/FtsJ. Biochem. Biophys. Res. Commun. **271:**714–718.
29. **Caldon, C. E., P. Yoong, and P. E. March.** 2001. Evolution of a molecular switch: universal bacterial GTPases regulate ribosome function. Mol. Microbiol. **41:**289–297.
30. **Carter, A. P., W. M. Clemons, D. E. Brodersen, R. J. Morgan-Warren, B. T. Wimberly, and V. Ramakrishnan.** 2000. Functional insights from the structure of the ribosomal subunit and its interactions with antibiotics. Nature **407:**340–348.
31. **Cashel, M., D. R. Gentry, V. J. Hernandez, and D. Vinella.** 1996. The stringent response, p. 1458–1496. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC.
32. **Cate, J. H., M. M. Yusupov, G. Z. Yusupova, T. N. Earnest, and H. F. Noller.** 1999. X-ray crystal structures of 70S ribosome functional complexes. Science **285:**2095–2104.
33. **Ceccarelli, A., G. P. Dotto, F. Altruda, L. Silengo, E. Turco, and G. Mangiarotti.** 1978. Immature 50S subunits in *Escherichia coli* polyribosomes. FEBS Lett. **93:**348–350.
34. **Charollais, J., M. Dreyfus, and I. Iost.** 2004. CsdA, a cold-shock RNA helicase from *Escherichia coli*, is involved in the biogenesis of the biogenesis of 50S ribosomal subunit. Nucleic Acids Res. **323:**2751–2759.
35. **Charollais, J., D. Pflieger, J. Vinh, M. Dreyfus, and I. Iost.** 2003. The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. Mol. Microbiol. **48:**1253–1265.
36. **Chatterji, D., and A. K. Ojha.** 2001. Revisiting the stringent response, ppGpp and starvation signaling. Curr. Opin. Microbiol. **4:**160–165.
37. **Chen, R., J. Brosius, B. Wittmann-Liebold, and W. Schäfer.** 1977. Occurrence of methylated amino acids as N-termini of proteins from *Escherichia coli* ribosomes. J. Mol. Biol. **111:**173–181.
38. **Cheng, Z.-F., and M. P. Deutscher.** 2003. Quality control of ribosomal RNA mediated by polynucleotide phosphorylase and RNase R. Proc. Natl. Acad. Sci. USA **100:**6388–6393.
39. **Cole, J. R., C. L. Olsson, J. W. B. Hershey, M. Grunberg-Manago, and M. Nomura.** 1987. Feedback regulation of rRNA synthesis in *Escherichia coli*. Requirement for initiation factor IF2. J. Mol. Biol. **198:**383–392.
40. **Colson, C., J. Lhoest, and C. Urlings.** 1979. Genetics of ribosomal protein methylation in *Escherichia coli*. III. Map position of two genes, *prmA* and *prmB*, governing methylation of proteins L11 and L3. Mol. Gen. Genet. **169:**245–250.
41. **Condon, C., S. French, C. Squires, and C. L. Squires.** 1993. Depletion of functional ribosomal RNA operons in *Escherichia coli* causes increased expression of the remaining intact copies. EMBO J. **12:**4305–4315.
42. **Condon, C., D. Liveris, C. Squires, I. Schwartz, and C. L. Squires.** 1995. rRNA operon multiplicity in *Escherichia coli* and the physiological implications of *rrn* inactivation. J. Bacteriol. **177:**4152–4156.
43. **Cowgill de Navarez, C., and H. W. Schaup.** 1979. *In vivo* transcriptionally coupled assembly of *Escherichia coli* ribosomal subunits. J. Mol. Biol. **134:**1–22.

43a. **Croitoru, V., K. Semrad, S. Prenninger, L. Rajkowitsch, M. Vejen, B. B. Søgaard Laursen, H. U. Sperling-Petersen, and L. A. Isaksson.** 2006. RNA chaperone activity of translation initiation factor IF1. Biochimie **88:**1875–1882.

44. **Culver, G. M., and H. F. Noller.** 1999. Efficient reconstitution of functional *Escherichia coli* 30S ribosomal subunits from a complete set of recombinant small subunit ribosomal proteins. RNA **5:**832–843.
45. **Cumberlidge, A. G., and K. Isono.** 1979. Ribosomal protein modification in *Escherichia coli*. I. A mutant lacking the N-terminal acetylation of protein S5 exhibits thermosensitivity. J. Mol. Biol. **131:**169–189.
46. **Daigle, D. M., and E. D. Brown.** 2004. Studies of the interaction of *Escherichia coli* YjeQ with the ribosome in vitro. J. Bacteriol. **186:**1381–1387.
47. **Dame, R. T., C. Wyman, R. Wurm, R. Wagner, and N. Goosen.** 2002. Structural basis for H-NS-mediated trapping of RNA polymerase in the open initiation complex at the *rrn* P1. J. Biol. Chem. **277:**2146–2150.
48. **Dammel, C. S., and H. F. Noller.** 1995. Suppression of a cold-sensitive mutation in 16S rRNA by overexpression of a novel ribosome-binding factor, RbfA. Genes Dev. **9:**626–637.
49. **David, C., J. Keener, and D. W. Aswad.** 1999. Isoaspartate in ribosomal protein S11 of *Escherichia coli*. J. Bacteriol. **181:**2871–2877.
50. **Decatur, W. A., and M. J. Fournier.** 2002. rRNA modifications and ribosome function. Trends Biochem. Sci. **27:**344–351.
51. **Delano, W. L.** 2002. The PyMOL molecular graphics system. http://pymol.sourceforge.net/.
52. **Dennis, P. P.** 1976. Effects of chloramphenicol on the transcriptional activities of ribosomal RNA and ribosomal protein genes in *Escherichia coli*. J. Mol. Biol. **108:**535–546.
53. **Dennis, P. P., M. Ehrenberg, and H. Bremer.** 2004. Control of rRNA synthesis in *Escherichia coli:* a systems biology approach. Microbiol. Mol. Biol. Rev. **68:**639–668.
54. **Desai, P. M., and J. P. Rife.** 2006. The adenosine dimethyltransferase KsgA recognizes a specific conformational state of the 30S ribosomal subunit. Arch. Biochem. Biophys. **449:**57–63.
55. **Deutscher, M. P.** 2006. Degradation of RNA in bacteria: comparison of mRNA and stable RNA. Nucleic Acids Res. **34:**659–666.
56. **Dickson, R. R., T. Gaal, H. A. deBoer, P. L. deHaseth, and R. L. Gourse.** 1989. Identification of promoter mutants defective in growth-rate-dependent regulation of rRNA transcription in *Escherichia coli*. J. Bacteriol. **171:**4862–4870.
57. **Diges, C. M., and O. C. Uhlenbeck.** 2005. *Escherichia coli* DbpA is a 3′ to 5′ RNA helicase. Biochemistry **44:**7903–7911.
58. **Diges, C. M., and O. C. Uhlenbeck.** 2001. *Escherichia coli* DbpA is an RNA helicase that requires hairpin 92 of 23S rRNA. EMBO J. **20:**5503–5512.
59. **El Hage, A., and J. H. Alix.** 2004. Authentic precursors to ribosomal subunits accumulate in *Escherichia coli* in the absence of functional DnaK chaperone. Mol. Microbiol. **51:**189–201.
60. **El Hage, A., M. Sbai, and J. H. Alix.** 2001. The chaperonin GroEL and other heat-shock proteins, besides DnaK, participate in ribosome biogenesis in Escherichia coli. Mol. Gen. Genet. **264:**796–808.
61. **Enz, S., V. Braun, and J. H. Crosa.** 1995. Transcription of the region encoding the ferric dicitrate-transport system in *Escherichia coli*: similarity between promoters for *fecA* and for extracytoplasmic function sigma factors. Gene **163:**13–18.
62. **Ferbitz, L., T. Maier, H. Patzelt, B. Bukau, E. Deuerling, and N. Ban.** 2004. Trigger factor in complex with the ribosome forms a molecular cradle for nascent proteins. Nature **431:**590–596.
63. **Freistroffer, D. V., M. Y. Pavlov, J. MacDougall, R. H. Buckingham, and M. Ehrenberg.** 1997. Release factor RF3 in *E. coli* accelerates the dissociation of release factors RF1 and RF2 from the ribosome in a GTP-dependent manner. EMBO J. **16:**4126–4133.
64. **Fuller-Pace, F. V.** 1994. RNA helicases: modulators of RNA structure. Trends Cell Biol. **4:**271–274.
65. **Gaal, T., and R. L. Gourse.** 1990. Guanosine 3′-diphosphate 5′-diphosphate is not required for growth rate-dependent control of rRNA synthesis in *Escherichia coli*. Proc. Natl. Acad. Sci. USA **87:**5533–5537.
66. **Gabashvili, I. S., S. T. Gregory, F. Valle, R. Grassucci, M. Worbs, M. C. Wahl, A. E. Dahlberg, and J. Frank.** 2001. The polypeptide tunnel system in the ribosome and its gating in erythromycin resistance mutants of L4 and L22. Mol. Cell **8:**181–188.
67. **Gafny, R., S. Cohen, N. Nachaliel, and G. Glaser.** 1994. Isolated P2 rRNA promoters of *Escherichia coli* are strong promoters that are subject to stringent control. J. Mol. Biol. **243:**152–156.
68. **Gourse, R. L., T. Gaal, M. S. Bartlett, J. A. Appleman, and W. Ross.** 1996. rRNA transcription and growth rate-dependent regulation of ribosome synthesis in *Escherichia coli*. Annu. Rev. Microbiol. **50:**645–677.
69. **Gourse, R. L., and W. Ross.** 1996. Control of rRNA and ribosome synthesis, p. 521–545. *In* C. C. Lin and A. S. Lynch (ed.), Regulation of gene expression in *Escherichia coli.* R. G. Landes Company, Austin, TX.
70. **Graham, J. E.** 2004. Sequence-specific Rho-RNA interactions in transcription termination. Nucleic Acids Res. **32:**3093–3100.
71. **Green, R., and H. F. Noller.** 1996. *In vitro* complementation analysis localizes 23S rRNA posttranscriptional modifications that are required for *Escherichia coli* 50S ribosomal subunit assembly and function. RNA **2:**1011–1021.
72. **Gualerzi, C. O., L. B. Brandi, E. Caserta, A. La Teana, R. Spurio, J. Tomsic, and C. L. Pon.** 2000. Translation initiation in bacteria, p. 477–494. *In* R. A. Garrett, S. R. Douthwaite, A. Liljas, A. T. Matheson, P. B. Moore, and H. F. Noller (ed.), The ribosome: structure, function, antibiotics, and cellular interactions. ASM Press, Washington, DC.

73. **Gutell, R. R.** 1996. Comparative sequence analysis and the structure of 16S and 23S rRNA, p. 111–128. *In* R. A. Zimmermann and A. E. Dahlberg (ed.), Ribosomal RNA. Structure, evolution, processing, and function in protein biosynthesis. CRC Press, New York, NY.
74. **Gutgsell, N. S., M. P. Deutscher, and J. Ofengand.** 2005. The pseudouridine synthetase RluD is required for normal ribosome assembly and function in *Escherichia coli*. RNA **11:**1141–1152.
75. **Hamacher, K., J. Trylska, and J. A. McCammon.** 2006. Dependency map of proteins in the small ribosomal subunit. PLoS Comp. Biol. **2:**e10.
76. **Harms, J., F. Schluenzen, R. Zarivach, A. Bashan, S. Gat, I. Agmon, H. Bartels, F. Franceschi, and A. Yonath.** 2001. High resolution structure of the large ribosomal subunit from a mesophilic bacterium. Cell **107:**679–688.
77. **Hayes, F., and M. Vasseur.** 1976. Processing of the 17-S *Escherichia coli* precursor RNA in the 27-S pre-ribosomal particle. Eur. J. Biochem. **61:** 433–442.
78. **Held, W. A., B. Ballou, S. Mizushima, and M. Nomura.** 1974. Assembly mapping of 30S ribosomal proteins from *Escherichia coli*. J. Biol. Chem. **249:**3103–3111.
79. **Henkin, T. M.** 2000. Transcription termination control in bacteria. Curr. Opin. Microbiol. **3:**149–153.
80. **Hennelly, S. P., A. Antoun, M. Ehrenberg, C. O. Gualerzi, W. Knight, J. S. Lodmell, and W. E. Hill.** 2005. A time-resolved investigation of ribosomal subunit association. J. Mol. Biol. **346:**1243–1258.
81. **Hernandez, V. J., and H. Bremer.** 1993. Characterization of RNA and DNA synthesis in *Escherichia coli* strains devoid of ppGpp. J. Biol. Chem. **268:** 10851–10862.
82. **Herold, M., and K. N. Nierhaus.** 1987. Incorporation of six additional proteins to complete the assembly map of the 50S subunit from *Escherichia coli* ribosomes. J. Biol. Chem. **262:**8826–8833.
83. **Herschlag, D.** 1995. RNA chaperones and the RNA folding problem. J. Biol. Chem. **270:**20871–20874.
84. **Hillebrand, A., R. Wurm, A. Menzel, and R. Wagner.** 2005. The seven *E. coli* ribosomal RNA operon upstream regulatory regions differ in structure and transcription factor binding efficiencies. Biol. Chem. **366:**523–534.
85. **Himeno, H., K. Hanawa-Suetsugu, T. Kimura, K. Takagi, W. Sugiyama, S. Shirata, T. Mikami, F. Odagiri, Y. Osani, D. Watanabe, S. Goto, L. Kalachnyuk, C. Ushida, and A. Muto.** 2004. A novel GTPase activated by the small subunit of the ribosome. Nucleic Acids Res. **32:**5303–5309.
86. **Hirvonen, C. A., W. Ross, C. E. Wozniak, E. Marasco, J. R. Anthony, S. E. Ayar, V. H. Newburn, and R. L. Gourse.** 2001. Contributions of UP elements and the transcription factor FIS to expression from the seven *rrn* P1 promoters in *Escherichia coli*. J. Bacteriol. **183:**6305–6314.
87. **Holmes, K. L., and G. M. Culver.** 2005. Analysis of conformational changes in 16S rRNA during the course of 30S subunit assembly. J. Mol. Biol. **354:**340–357.
88. **Holmes, K. L., and G. M. Culver.** 2004. Mapping structural differences between 30S ribosomal subunit assembly intermediates. Nat. Struct. Mol. Biol. **11:**179–186.
89. **Hwang, J., and M. Inouye.** 2006. The tandem GTPase, Der, is essential for the biogenesis of 50S ribosomal subunits in *Escherichia coli*. Mol. Microbiol. **61:**1660–1672.
90. **Inoue, K., J. Alsina, J. Chen, and M. Inouye.** 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.
91. **Ishihama, A.** 2000. Functional modulation of *Escherichia coli* RNA polymerase. Annu. Rev. Microbiol. **54:**499–518.
92. **Isono, I., and K. Isono.** 1980. Ribosomal protein modification in *Escherichia coli*. II. Studies of a mutant lacking the N-terminal acetylation of protein S18. Mol. Gen. Genet. **177:**645–651.
93. **Jemiolo, D. K.** 1996. Processing of prokaryotic ribosomal RNA, p. 453–468. *In* R. A. Zimmermann and A. E. Dahlberg (ed.), Ribosomal RNA. Structure, evolution, processing, and function in protein synthesis. CRC Press, New York, NY.
94. **Jiang, M., K. Datta, A. Walker, J. Strahler, P. Bagamasbad, P. C. Andrews, and J. R. Maddock.** 2006. The *Escherichia coli* GTPase $CgtA_E$ is involved in late steps of large ribosome assembly. J. Bacteriol. **188:**6757–6770.
95. **Jiang, M., S. M. Sullivan, A. K. Walker, J. R. Strahler, P. C. Andrews, and J. R. Maddock.** 2007. Identification of novel *Escherichia coli* ribosome-associated proteins using isobaric tags and multidimensional protein identification techniques. J. Bacteriol. **189:**3434–3444.
96. **Johnson, S. C., N. Watson, and D. Apirion.** 1976. A lethal mutation which affects the maturation of ribosomes. Mol. Gen. Genet. **147:**29–37.
97. **Jones, P. G., M. Mitta, Y. Kim, W. Jiang, and M. Inouye.** 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.
98. **Josaitis, C. A., T. Gaal, and R. L. Gourse.** 1995. Stringent control and growth-rate-dependent control have nonidentical promoter sequence requirements. Proc. Natl. Acad. Sci. USA **92:**1117–1121.
99. **Kaczanowska, M., and M. Rydén-Aulin.** 2004. Temperature sensitivity caused by mutant release factor 1 is suppressed by mutations that affect 16S rRNA maturation. J. Bacteriol. **186:**3046–3055.
100. **Kaczanowska, M., and M. Rydén-Aulin.** 2005. The YrdC protein—a putative ribosome maturation factor. Biochim. Biophys. Acta **1727:**87–96.
101. **Kang, W.-K., T. Icho, S. Isono, M. Kitakawa, and I. Isono.** 1989. Characterization of the gene *rimK* responsible for the addition of glutamic acid residues to the C-terminus of ribosomal protein S6 in *Escherichia coli* K12. Mol. Gen. Genet. **217:**281–288.
102. **Kaplan, R., and D. Apirion.** 1975. The fate of ribosomes in *Escherichia coli* starved for a carbon source. J. Biol. Chem. **250:**1854–1863.
103. **Karimi, R., M. Y. Pavlov, R. H. Buckingham, and M. Ehrenberg.** 1999. Novel roles for classical factors at the interface between translation termination and initiation. Mol. Cell **3:**601–609.
104. **Keener, J., and M. Nomura.** 1996. Regulation of ribosome synthesis. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, DC.
105. **Kiel, M. C., V. S. Raj, H. Kaji, and A. Kaji.** 2003. Release of ribosome-bound ribosome recycling factor by elongation factor G. J. Biol. Chem. **278:**48041–48050.
106. **King, C. K., and D. Schlessinger.** 1983. S1 nuclease mapping analysis of ribosomal RNA processing in wild type and processing deficient *Escherichia coli*. J. Biol. Chem. **258:**12034–12042.
107. **King, T. C., R. Sirdeshmukh, and D. Schlessinger.** 1986. Nucleolytic processing of ribonucleic acid transcripts in procaryotes. Microbiol. Rev. **50:** 428–451.
108. **King, T. C., R. Sirdeshmukh, and D. Schlessinger.** 1984. RNase III cleavage is obligate for maturation but not for function of *Escherichia coli* pre-23S rRNA. Proc. Natl. Acad. Sci. USA **81:**185–188.
109. **Kisselev, L., M. Ehrenberg, and L. Y. Frolova.** 2003. Termination of translation: interplay of mRNA, rRNAs and release factors? EMBO J. **22:**175–182.
110. **Klaholtz, B. P., A. G. Myasnikov, and M. van Heel.** 2004. Visualization of release factor 3 on the ribosome during termination of protein synthesis. Nature **427:**862–865.
111. **Klein, D. J., P. B. Moore, and J. A. Steitz.** 2004. The contribution of metal ions to the structural stability of the large ribosomal subunit. RNA **10:**1366–1379.
112. **Korepanov, A., G. M. Gongadze, M. B. Graber, D. L. Court, and M. Bubunenko.** 2007. Importance of the 5S rRNA-binding ribosomal proteins for cell viability and translation *Escherichia coli*. J. Mol. Biol. **366:**1199–1208.
113. **Korostelev, A., S. Trakhanov, M. Laurberg, and H. F. Noller.** 2006. Crystal structure of a 70S ribosome-tRNA complex reveals functional interactions and rearrangements. Cell **126:**1–13.
114. **Kowalak, J. A., and K. A. Walsh.** 1996. β-Methylthio-aspartic acid: identification of a novel posttranslational modification in ribosomal protein S12 from *Escherichia coli*. Protein Sci. **5:**1625–1632.
115. **Krohn, M., and R. Wagner.** 1996. Transcriptional pausing of RNA polymerase in the presence of guanosine tetraphosphate depends on the promoter and gene sequence. J. Bacteriol. **271:**23884–23894.
116. **Krzyzosiak, W., R. Denman, K. Nurse, W. Hellmann, M. Boublik, C. W. Gehrke, P. F. Agris, and J. Ofengand.** 1987. In vitro synthesis of 16S ribosomal RNA containing single base changes and assembly into a functional 30S ribosome. Biochemistry **26:**2353–2364.
117. **Lesnyak, D. V., J. Osipiuk, T. Skarina, P. V. Sergiev, A. A. Bogdanov, A. Edwards, A. Savchenko, A. Joachimiak, and O. A. Dontsova.** 2007. Methyltransferase that modifies guanine 966 of the 16S rRNA: functional identification and tertiary structure. J. Biol. Chem. **282:**5880–5887.
118. **Lesnyak, D. V., P. V. Sergiev, A. A. Bogdanov, and O. A. Dontsova.** 2006. Identification of *Escherichia coli* m^2G methyltransferases. I. The ycbY gene encodes a methyltransferase specific for G2445 of the 23S rRNA. J. Mol. Biol. **364:**20–25.
119. **Lewicki, B. T. U., T. Margus, J. Remme, and K. N. Nierhaus.** 1993. Coupling of rRNA transcription and ribosomal assembly *in vivo*. J. Mol. Biol. **231:**581–593.
120. **Lhoest, J., and C. Colson.** 1981. Cold-sensitive ribosome assembly in an *Escherichia coli* mutant lacking a single methyl group in ribosomal protein L3. Eur. J. Biochem. **121:**33–37.
121. **Li, Z., and M. P. Deutscher.** 1995. The tRNA processing enzyme RNase T is essential for maturation of 5S RNA. Proc. Natl. Acad. Sci. USA **92:**6883–6886.
122. **Li, Z., S. Pandit, and M. P. Deutscher.** 1999. Maturation of 23S ribosomal RNA requires the exoribonuclease RNase T. RNA **5:**139–146.
123. **Li, Z., S. Pandit, and M. P. Deutscher.** 1998. Polyadenylation of stable RNA precursors *in vivo*. Proc. Natl. Acad. Sci. USA **95:**12158–12162.
124. **Li, Z., S. Pandit, and M. P. Deutscher.** 1999. RNase G (CafA protein) and RNase E are both required for the 5′ maturation of 16S ribosomal RNA. EMBO J. **18:**2878–2885.
125. **Liang, S. T., Y. C. Xu, P. Dennis, and H. Bremer.** 2000. mRNA composition and control of bacterial gene expression. J. Bacteriol. **182:**3037–3044.
126. **Liiv, A., and J. Remme.** 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.

127. **Lindahl, L.** 1975. Intermediates and time kinetics of the *in vitro* assembly of *Escherichia coli* ribosomes. J. Mol. Biol. **92:**15–37.
128. **Lorsch, J. R.** 2002. RNA chaperones exist and DEAD box proteins get a life. Cell **109:**797–800.
129. **Lu, Q., and M. Inouye.** 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.
130. **Maeda, H., N. Fujita, and A. Ishihama.** 2000. Competition among seven *Escherichia coli* σ subunits: relative binding affinities to the core RNA polymerase. Nucleic Acids Res. **28:**3497–3503.
131. **Magnusson, L. U., A. Farewell, and T. Nyström.** 2005. ppGpp: a global regulator in *Escherichia coli*. Trends Microbiol. **13:**236–242.
132. **Maki, J. A., and G. M. Culver.** 2005. Recent developments in factor-facilitated ribosome assembly. Methods **36:**313–320.
133. **Maki, J. A., D. J. Schnobrich, and G. M. Culver.** 2002. The DnaK chaperone system facilitates 30S ribosomal subunit assembly. Mol. Cell **10:**129–138.
134. **Maki, J. A., D. R. Southworth, and G. M. Culver.** 2003. Demonstration of the role of the DnaK chaperone system in assembly of 30S ribosomal subunits using a purified in vitro system. RNA **9:**1418–1421.
135. **Mallik, P., T. S. Pratt, M. B. Beach, M. D. Bradley, J. Undamatla, and R. Osuna.** 2004. Growth phase-dependent regulation and stringent control of *fis* are conserved processes in enteric bacteria and involve a single promoter (*fis* P) in *Escherichia coli*. J. Bacteriol. **186:**122–135.
136. **Mangiarotti, G., S. Chiaberge, and S. Bulfone.** 1997. rRNA maturation as a "quality" control step in ribosomal subunit assembly in *Dictyostelium discoideum*. J. Biol. Chem. **272:**27818–27822.
137. **Mangiarotti, G., E. Turco, C. Perlo, and F. Altruda.** 1975. Role of precursor 16S RNA in assembly of *E. coli* 30S ribosome. Nature **253:**569–570.
138. **Mangiarotti, G., E. Turco, A. Ponzetto, and F. Altruda.** 1974. Precursor 16S RNA in active 30S ribosomes. Nature **247:**147–148.
139. **Marintchev, A., and G. Wagner.** 2004. Translation initiation: structures, mechanisms and evolution. Q. Rev. Biophys. **37:**197–284.
140. **Mathy, N., O. Pellegrini, A. Serganov, D. J. Patel, C. Ehresmann, and C. Portier.** 2004. Specific recognition of *rpsO* mRNA and 16S rRNA by *Escherichia coli* ribosomal protein S15 relies on both mimicry and site differentiation. Mol. Microbiol. **52:**661–675.
141. **Meier, T. I., R. B. Peery, K. A. McAllister, and G. Zhao.** 2000. Era GTPase of *Escherichia coli*: binding to 16S rRNA and modulation of GTPase activity by RNA and carbohydrates. Microbiology **146:**1071–1083.
142. **Merrick, M. J.** 1993. In a class of its own—the RNA polymerase sigma factor σ^{54} (σ^{N}). Mol. Microbiol. **10:**903–909.
143. **Mohanty, B. K., and S. R. Kushner.** 1999. Analysis of the function of Escherichia coli poly(A) polymerase I in RNA metabolism. Mol. Microbiol. **34:**1094–1108.
144. **Mooney, R. A., I. Artsimovitch, and R. Landick.** 1998. Information processing by RNA polymerase: recognition of regulatory signals during RNA chain elongation. J. Bacteriol. **180:**3265–3275.
145. **Mooney, R. A., and R. Landick.** 1999. RNA polymerase unveiled. Cell **98:**687–690.
146. **Moore, P. B., and T. A. Steitz.** 2003. The structural basis of large ribosomal subunit function. Annu. Rev. Biochem. **72:**813–850.
147. **Morgan, E. A.** 1986. Antitermination mechanisms in rRNA operons of *Escherichia coli*. J. Bacteriol. **168:**1–5.
148. **Møller Andersen, N., and S. R. Douthwaite.** 2006. YebU is a m^5C methyltransferase specific for 16S rRNA nucleotide 1407. J. Mol. Biol. **359:**777–786.
149. **Nashimoto, H., W. A. Held, E. Kaltschmidt, and M. Nomura.** 1971. Structure and function of bacterial ribosomes. XII. Accumulation of 21S particles by some cold-sensitive mutants of *Escherichia coli*. J. Mol. Biol. **62:**121–138.
150. **Nashimoto, H., and M. Nomura.** 1970. Structure and function of bacterial ribosomes. XI. Dependence of 50S ribosomal assembly on simultaneous assembly of 30S subunits. Proc. Natl. Acad. Sci. USA **67:**1440–1447.
151. **Nierhaus, K. H., K. Bordasch, and H. E. Homann.** 1973. Ribosomal proteins. XLIII. *In vivo* assembly of *Escherichia coli* ribosomal proteins J. Mol. Biol. **74:**587–597.
152. **Nierhaus, K. N.** 2004. Ribosome assembly: assembly of the prokaryotic ribosome, p. 85–105. *In* K. N. Nierhaus and D. N. Wilson (ed.), Protein synthesis and ribosome structure. Wiley-VCh, Einheim, Germany.
153. **Nishi, K., F. Morel-Deville, J. W. B. Hershey, T. Leighton, and J. Schnier.** 1988. An eIF-4A-like protein is a suppressor of an *Escherichia coli* mutant defective in 50S ribosomal subunit assembly. Nature **336:**496–498.
154. **Nishimura, K., T. Hosaka, S. Tokuyama, S. Okamoto, and K. Ochi.** 2007. Mutations in *rsmG*, encoding a 16S rRNA methyltransferase, result in low-level streptomycin resistance and antibiotic overproduction in *Streptomyces coelicolor* A3(2). J. Bacteriol. **189:**3876–3883.
155. **Nodwell, J. R., and J. Greenblatt.** 1993. Recognition of *boxA* antiterminator RNA by the *E coli* antitermination factors NusB and ribosomal protein S10. Cell **72:**261–268.
156. **Noller, H. F., and M. Nomura.** 1996. Ribosomes. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. ASM Press, Washington, DC.
157. **Nomura, M.** 1999. Regulation of ribosome biosynthesis in *Escherichia coli* and *Saccharomyces cerevisiae*: diversity and common principles. J. Bacteriol. **181:**6857–6864.
158. **Ofengand, J., and M. del Campo.** 29 December 2004, posting date. Chapter 4.6.1, Modified nucleosides of *Escherichia coli* ribosomal RNA. *In* G. R. Björk (ed.), EcoSal—*Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC. http://www.ecosal.org.
159. **Ogle, J. M., and V. Ramakrishnan.** 2005. Structural insights into translational fidelity. Annu. Rev. Biochem. **74:**129–177.
160. **Olsson, M. O.** 1979. Analysis of *rpsD* mutations in *Escherichia coli.* II: Physiology of some representative mutants. Mol. Gen. Genet. **169:**259–269.
161. **Opel, M. L., K. A. Aeling, W. M. Holmes, R. C. Johnson, C. J. Benham, and G. W. Hatfield.** 2004. Activation of transcription initiation from a stable RNA promoter by a FIS protein-mediated DNA structural transmission mechanism. Mol. Microbiol. **53:**665–674.

161a. **Østergaard, P., H. Phan, L. B. Johansson, J. Egebjerg, L. Østergaad, B. T. Porse, and R. A. Garrett.** 1998. Assembly of proteins and 5S rRNA to transcripts of the major structural domains of 23S rRNA. J. Mol. Biol. **284:**227–240.

162. **Ow, M. C., T. Perwez, and S. R. Kushner.** 2003. RNase G of *Escherichia coli* exhibits only limited functional overlap with its essential homologue, RNase E. Mol. Microbiol. **49:**607–622.
163. **Pape, T., W. Wintermeyer, and M. V. Rodnina.** 1998. Complete kinetic mechanism of elongation factor Tu-dependent binding of aminoacyl-tRNA to the A site of the *E. coli* ribosome. EMBO J. **17:**7490–7497.
164. **Pardo, D., and R. Rosset.** 1977. A new ribosomal mutation which affects the two ribosomal subunits in *Escherichia coli*. Mol. Gen. Genet. **153:**199–204.
165. **Pardo, D., C. Vola, and R. Rosset.** 1979. Assembly of ribosomal subunits affected in a ribosomal mutant of *E coli* having an altered L22 protein. Mol. Gen. Genet. **174:**53–58.
166. **Pardon, B., and R. Wagner.** 1995. The *Escherichia coli* ribosomal RNA leader *nut* region interacts specifically with mature 16S RNA. Nucleic Acids Res. **23:**932–941.
167. **Park, W. J., Y. Jung, S. J. Lee, D. J. Jin, and Y. Lee.** 2002. Alteration of stringent response of the *Escherichia coli rnpB* promoter by mutations in the −35 region. Biochem. Biophys. Res. Commun. **290:**1183–1187.
168. **Paul, B. J., M. M. Barker, D. A. Schneider, C. Webb, J. W. Foster, and R. L. Gourse.** 2004. DksA: a critical component of the transcription machinery that potentiates the regulation of rRNA promoters by ppGpp and the initiating NTP. Cell **118:**311–322.
169. **Paul, B. J., W. Ross, T. Gaal, and R. L. Gourse.** 2004. rRNA transcription in *Escherichia coli*. Annu. Rev. Genet. **38:**749–770.
170. **Perederina, A., V. Svetlov, M. N. Vassylyeva, T. H. Tahirov, S. Yokoyama, I. Artsimovitch, and D. M. Vassylyev.** 2004. Regulation through the secondary channel—structural framework for ppGpp-DksA synergism during transcription. Cell **118:**297–309.
171. **Powers, T., G. Daubresse, and H. F. Noller.** 1993. Dynamics of *in vitro* assembly of 16S rRNA into 30S ribosomal subunits. J. Mol. Biol. **232:**362–374.
172. **Ramagopal, S., and A. R. Subramanian.** 1975. Growth-dependent regulation in production and utilization of acetylated ribosomal protein L7. J. Mol. Biol. **94:**633–641.
173. **Ramakrishnan, V.** 2002. Ribosome structure and the mechanism of translation. Cell **108:**557–572.
174. **Richardson, J. P.** 2002. Rho-dependent termination and ATPases in transcript termination. BBA Gene Struct. Expr. **1577:**251–260.
175. **Richardson, J. P., and J. Greenblatt.** 1996. Control of RNA chain elongation and termination, p. 822–848. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology. ASM Press, Washington, DC.
176. **Rodnina, M. V., A. Savelsbergh, V. I. Katunin, and W. Wintermeyer.** 1997. Hydrolysis of GTP by elongation factor G drives tRNA movement on the ribosome. Nature **385:**37–41.
177. **Romanowski, M. J., J. B. Bonanno, and S. K. Burley.** 2002. Crystal structure of the *Escherichia coli* glucose-inhibited division protein B (GidB) reveals a methyltransferase fold. Proteins Struct. Funct. Genet. **47:**563–567.
178. **Ross, W., S. E. Aiyar, J. Salomon, and R. L. Gourse.** 1998. *Escherichia coli* promoters with UP elements of different strengths: modular structure of bacterial promoters. J. Bacteriol. **180:**5375–5383.
179. **Ross, W., J. F. Thompson, J. T. Newlands, and R. L. Gourse.** 1990. *E. coli* Fis protein activates ribosomal RNA transcription *in vitro* and *in vivo*. EMBO J. **9:**3733–3742.
180. **Roy, M. K., B. Singh, B. K. Ray, and D. Apirion.** 1983. Maturation of 5S rRNA: ribonuclease E cleavages and their dependence on precursor sequences. Eur. J. Biochem. **131:**119–127.
181. **Rydén-Aulin, M., S. Zhang, P. Kylsten, and L. A. Isaksson.** 1993. Ribosome activity and modification of 16S RNA are influenced by deletion of ribosomal protein S20. Mol. Microbiol. **7:**983–992.
182. **Sato, A., G. Kobayashi, H. Hayashi, H. Yoshida, A. Wada, H. Maeda, S. Hiraga, K. Takeyasu, and C. Wada.** 2005. The GTP binding protein Obg homolog ObgE is involved in ribosome maturation. Genes Cells **10:**393–408.
183. **Sayed, A., S.-I. Matsuyama, and M. Inouye.** 1999. Era, an essential *Esch-*

erichia coli small G-protein, binds to the 30S ribosomal subunit. Biochem. Biophys. Res. Commun. **264:**51–54.
184. **Schäferkordt, J., and R. Wagner.** 2001. Effects of base change mutations within an *Escherichia coli* ribosomal RNA leader region on rRNA maturation and ribosome formation. Nucleic Acids Res. **29:**3394–3403.
185. **Schlessinger, D.** 1974. Ribosome formation in *Escherichia coli*, p. 393–416. *In* M. Nomura (ed.), Ribosomes. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
186. **Schluenzen, F., A. Tocilj, R. Zarivach, J. Harms, M. Gluehmann, D. Janell, A. Bashan, H. Bartels, I. Agmon, F. Franceschi, and A. Yonath.** 2000. Structure of functionally activated small ribosomal subunit at 3.3 Å resolution. Cell **102:**615–623.
187. **Schuwirth, B. S., M. A. Borovinskaya, C. W. Hau, W. Zhang, A. Vila-Sanjurjo, J. M. Holton, and J. H. D. Cate.** 2005. Structures of the bacterial ribosome at 3.5 Å resolution. Science **310:**827–834.
188. **Selmer, M., C. M. Dunham, F. V. Murphy IV, A. Weixlbaumer, S. Petry, A. C. Kelley, J. R. Weir, and V. Ramakrishnan.** 2006. Structure of the 70S ribosome complexed with mRNA and tRNA. Science **313:**1935–1942.
189. **Serdyuk, I. N., S. C. Agalarov, S. E. Sedelnikova, and A. S. Spirin.** 1983. Shape and compactness of the isolated ribosomal 16S RNA and its complexes with ribosomal proteins. J. Mol. Biol. **169:**409–425.
190. **Sergiev, P. V., D. V. Lesnyak, A. A. Bogdanov, and O. A. Dontsova.** 2006. Identification of *Escherichia coli* m^2G methyltransferases. II. The *ygjO* gene encodes a methyltransferase specific for G1835 of the 23S rRNA. J. Mol. Biol. **364:**26–31.
191. **Sharma, M. R., C. Barat, D. N. Wilson, T. M. Booth, M. Kawazoe, C. Hori-Takemoto, P. Fucini, and R. K. Agrawal.** 2005. Interaction of Era with the 30S ribosomal subunit: implications for 30S subunit assembly. Mol. Cell **18:**319–329.
192. **Shine, J., and L. Dalgarno.** 1974. The 3′-terminal sequence of *Escherichia coli* 16S ribosomal RNA: complementarity to nonsense triplets and ribosome binding sites. Proc. Natl. Acad. Sci. USA **71:**1342–1346.
193. **Srivastava, A. K., and D. Schlessinger.** 1988. Coregulation of processing and translation: mature 5′ termini of *Escherichia coli* 23S ribosomal RNA form in polysomes. Proc. Natl. Acad. Sci. USA **85:**7144–7148.
194. **Srivastava, A. K., and D. Schlessinger.** 1989. *Escherichia coli* 16S rRNA 3′-end formation requires a distal transfer RNA sequence at a proper distance. EMBO J. **8:**3159–3166.
195. **Srivastava, A. K., and D. Schlessinger.** 1990. Mechanism and regulation of bacterial ribosomal RNA. Annu. Rev. Microbiol. **44:**105–129.
196. **Stagg, S. M., J. A. Mears, and S. C. Harvey.** 2003. A structural model for the assembly of the 30S subunit of the ribosome. J. Mol. Biol. **328:**49–61.
197. **Søgaard Laursen, B., H. P. Sørensen, K. K. Mortensen, and H. U. Sperling-Petersen.** 2005. Initiation of protein synthesis in bacteria. Microbiol. Mol. Biol. Rev. **69:**101–123.
198. **Talkington, M. W. T., G. Siuzdak, and J. R. Williamson.** 2005. An assembly landscape for the 30S ribosomal subunit. Nature **438:**628–632.
199. **Tan, J., U. Jakob, and J. C. A. Bardwell.** 2002. Overexpression of two different GTPases rescues a null mutation in a heat-induced tRNA methyltransferase. J. Bacteriol. **184:**2692–2698.
200. **Teplova, M., V. Tereshko, R. Sanishvili, A. Joachimiak, T. Bushueva, W. F. Anderson, and M. Egli.** 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.
201. **Terhorst, C., B. Wittmann-Liebold, and W. Möller.** 1972. 50S ribosomal proteins. Peptide studies on two acidic proteins, A_1 and A_2, isolated from 50S ribosomes of *Escherichia coli.* Eur. J. Biochem. **25:**13–19.
202. **Toone, W. M., K. E. Rudd, and J. D. Friesen.** 1991. *deaD*, a new *Escherichia coli* gene encoding a presumed ATP-dependent RNA helicase, can suppress a mutation in *rpsB*, the gene encoding ribosomal protein S2. J. Bacteriol. **173:**3291–3302.
203. **Torres, M., C. Condon, J. M. Balada, C. Squires, and C. L. Squires.** 2001. Ribosomal protein S4 is a transcription factor with properties remarkably similar to NusA, a protein involved in both non-ribosomal and ribosomal RNA antitermination. EMBO J. **20:**3811–3820.
204. **Vogel, U., and K. F. Jensen.** 1995. Effects of the antiterminator BoxA on transcription elongation kinetics and ppGpp inhibition of transcription elongation in *Escherichia coli*. J. Biol. Chem. **270:**18335–18340.
205. **von Hippel, P. H.** 1998. An integrated model of the transcription complex in elongation, termination, and editing. Science **281:**660–665.
206. **Wade, J. T., D. C. Roa, D. C. Grainger, D. Hurd, S. J. W. Busby, K. Struhl, and E. Nudler.** 2006. Extensive functional overlap between σ factors in *Escherichia coli*. Nat. Struct. Mol. Biol. **13:**806–814.
207. **Wagner, R.** 2000. Transcription regulation in prokaryotes. Oxford University Press, New York, NY.
208. **Weber, H., T. Polen, J. Heuveling, V. F. Wendlisch, and R. Hengge.** 2005. Genome-wide analysis of the general stress response network in *Escherichia coli*: σ^S-dependent genes, promoters, and sigma factor selectivity. J. Bacteriol. **187:**1591–1603.
209. **Weeks, K. M.** 1997. Protein-facilitated RNA folding. Curr. Opin. Struct. Biol. **7:**336–342.
210. **Wikstrom, P. M., A. S. Byström, and G. R. Björk.** 1988. Non-autogenous control of ribosomal protein synthesis from the *trmD* operon in *Escherichia coli*. J. Mol. Biol. **203:**141–152.
211. **Williamson, J. R.** 2003. After the ribosome structure: how are the subunits assembled? RNA **9:**165–167.
212. **Wimberly, B. T., D. E. Brodersen, W. M. Clemons, Jr., R. J. Morgan-Warren, A. P. Carter, C. Vonrhein, T. Hartsch, and V. Ramakrishnan.** 2000. Structure of the 30S ribosomal subunit. Nature **407:**327–339.
213. **Wintermeyer, W., F. Peske, M. Beringer, K. B. Grodmadski, A. Savelsbergh, and M. V. Rodnina.** 2004. Mechanisms of elongation on the ribosome: dynamics of a macromolecular machine. Biochem. Soc. Trans. **32:**733–737.
214. **Woodson, S. A.** 2000. Recent insights on RNA folding mechanisms from catalytic RNA. Cell Mol. Life Sci. **57:**796–808.
215. **Wout, P., K. Pu, S. M. Sullivan, V. Reese, S. Zhou, B. Lin, and J. R. Maddock.** 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.
216. **Yamagishi, M., H. A. de Boer, and M. Nomura.** 1987. Feedback regulation of rRNA synthesis. A mutational alteration in the anti-Shine-Dalgarno region of the 16S rRNA gene abolishes regulation. J. Mol. Biol. **198:**547–550.
217. **Yonath, A., K. R. Leonard, and H. G. Wittmann.** 1987. A tunnel in the large ribosomal subunit revealed by three-dimensional image reconstruction. Science **236:**813–816.
218. **Young, R. A., and J. A. Steitz.** 1978. Complementary sequences 1,700 nucleotides apart from a ribonuclease III cleavage site in *Escherichia coli* ribosomal precursor RNA. Proc. Natl. Acad. Sci. USA **75:**3593–3597.
219. **Yusupov, M. M., G. Z. Yusupova, A. Baucom, K. Lieberman, T. N. Earnest, J. H. D. Cate, and H. F. Noller.** 2001. Crystal structure of the ribosome at 5.5 Å resolution. Science **292:**883–896.