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Deconstructing ribosome construction

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Abstract

The ribosome is an essential ribonucleoprotein enzyme, and its biogenesis is a fundamental process in all living cells. Recent X-ray crystal structures of the bacterial ribosome and new technologies have allowed a greater interrogation of *in vitro* ribosome assembly; however, substantially less is known about ribosome biogenesis *in vivo*. Ongoing investigations are focused on elucidating the cellular processes that facilitate biogenesis of the ribosomal subunits, and many extraribosomal factors, including modification enzymes, remodeling enzymes and GTPases, are being uncovered. Moreover, specific roles for ribosome biogenesis factors in subunit maturation are now being elaborated. Ultimately, such studies will reveal a more complete understanding of processes at work in *in vivo* ribosome biogenesis.

Ribosome assembly beyond the test tube

The bacterial ribosome is an extremely complex macromolecular machine capable of performing a fundamental and instrumental cellular process, protein translation. The ribosome comprises two ribonucleoprotein subunits, defined by their sedimentation coefficients, which reflect their relative mass, structure and composition differences. The bacterial ribosome has been widely used in biochemical, structural and genetic analyses for many years, and *in vivo* biogenesis of bacterial ribosomes will be highlighted herein. The large, or 50S, subunit, which is responsible for the catalysis of peptide bonds linking the amino acid building blocks of protein, contains two RNA molecules, 23S and 5S, as well as 34 ribosomal proteins (r-proteins). The small, or 30S, subunit (SSU) contains a 16S RNA molecule and 21 r-proteins and is the site of mRNA decoding. Recent advances have provided X-ray crystal structures of the bacterial ribosome and its subunits [1–3] (Figure 1), which have shifted our view of the ribosome from a somewhat amorphous structure to an intricate and precise, protein-laced web of RNA. These structures are guiding the field in developing a detailed understanding of the well-coordinated process of translation, but they lack information about the impressive and coordinated integration of these rRNAs and r-proteins, which ultimately results in functional subunit production.

Initially, the RNA that constitutes both subunits is transcribed as a single primary transcript containing 16S, 23S and 5S RNA and intervening sequences [4]. Sequential processing of the primary transcript by endonucleases (Table 1) removes the intervening sequences and produces the mature RNA products; this processing is thought to occur in concert with other biogenesis processes, including nucleotide modification and r-protein addition and modification [4]. Many of the processing and modifying enzymes, and a growing number of assembly factors, have now been identified (Table 1). To date, however, a temporal framework for the function of these different factors is still lacking, and for many of the

extra-ribosomal assembly factors, precise functional roles in biogenesis have not been characterized (Figure 1). Further studies in the field will begin to unravel this complicated and elegant network that is essential for cell physiology.

Seminal experiments performed by Nomura and colleagues [5–7] have detailed an *in vitro* 30S subunit assembly map illustrating the hierarchical and cooperative incorporation of r-proteins to maturing 30S subunits. Nomura *et al.*'s *in vitro* studies exposed some of the complexity of 30S subunit assembly, and because several factors have been found to facilitate biogenesis processes (Table 1), our appreciation of this complexity has only deepened. Recent work has focused on uncovering how extra-ribosomal biogenesis factors work in concert to facilitate proper ribosome biogenesis and further define how ribosome assembly occurs *in vivo*.

Because many aspects of *in vitro* 30S subunit assembly have been extensively reviewed [8–11], we will mention only some recent highlights in this area. Instead, we will focus on recent advances regarding *in vivo* biogenesis of the two bacterial ribosomal subunits, giving the majority of our attention to the 30S subunit.

Ribosome assembly *in vitro*

Nearly 40 years after the pioneering work by Nomura and colleagues [5,7] that described a 30S subunit assembly map, new technologies are being used to probe *in vitro* assembly of the 30S subunit, and although many nuances and fundamentals are being uncovered, the Nomura 30S subunit assembly map has survived nearly intact [5,7,12]. New methods have provided considerable insight not only into how we think about ribosome assembly but also into the assembly of RNA- and protein-containing molecular structures and the folding of large RNAs in general. It should be noted that a more incomplete *in vitro* assembly map of the 50S subunit has been proposed [13]; however, it is significantly more complex than that of the 30S subunit and, probably as a consequence, 50S subunit assembly is not as well studied as its counterpart.

An informative approach in studying 30S subunit assembly has been the use of time-resolved X-ray hydroxyl radical footprinting [14] (Box 1). In this work, structural information was obtained at very early stages in assembly and during the assembly process as it proceeds to completion. Using this technique, Woodson and colleagues significantly advanced previous work [15] by suggesting that early events in 30S subunit assembly are simultaneously nucleated at different positions of the 16S rRNA molecule [14] and, more strikingly, that induced fit of r-protein–16S rRNA complexes can be observed during the assembly process.

Box 1

Time-resolved X-ray hydroxyl radical footprinting and pulse–chase labeling quantified by mass spectrometry studies of ribosome assembly

Time-resolved X-ray hydroxyl radical footprinting, developed in the laboratory of Sarah Woodson, involves RNA–protein complexes being reconstituted and allowed to assemble for short time periods before being irradiated with a synchrotron X-ray beam as a means of generating hydroxyl radicals capable of cleaving exposed regions of the RNA backbone. Subsequent primer extension experiments are performed, whereby a complimentary primer is annealed to the RNA and extended by reverse transcriptase. Positions of RNA cleavage are revealed by strong stops corresponding to sites of RNA cleavage on sequencing gels of cDNA products [14].

Pulse–chase labeling quantified by mass spectrometry (PC/QMS) allows probing of 30S subunit reconstitution by performing reconstitution initially with RNA and labeled r-protein (pulse), followed by the addition of excess unlabeled r-protein (chase) after varying incubation periods. The ratios of labeled to unlabeled proteins on the reconstituted subunits are quantified by mass spectrometry. These data provide binding kinetics of the individual r-proteins in the experiment [16].

Another technological advance that has allowed a detailed characterization of r-protein–16S rRNA affinities and kinetics during the assembly of the 30S subunit is pulse–chase labeling quantified by mass spectrometry (PC/QMS) (Box 1). This technique yields binding profiles of r-proteins during *in vitro* reconstitution of the 30S subunit [16], and this work, pioneered by Williamson and coworkers, elegantly suggests that 30S subunit assembly proceeds via a landscape pathway, akin to that observed for protein folding, rather than through discrete intermediates [16]. Thus, the available data overwhelmingly suggest that multiple nucleating events occur during the biogenesis cascade.

The *in vitro* analyses of subunit assembly suggest that rRNA falls victim to misfolding during assembly and that r-proteins might initially stabilize these conformations before rearrangement and appropriate folding. It is also likely that these trapped conformations are related to the assembly intermediates that were uncovered by the Nomura and Nierhaus groups many years ago [6,13] and further characterized by us and others [17–26]. Moreover, the isolation of cold-sensitive *Escherichia coli* strains, one bearing a mutation in 16S rRNA that was proposed to stabilize a non-productive 30S subunit intermediate structure [27] and another bearing a mutation in S5 that also results in altered 30S architecture and an assembly intermediate [18,28], suggests that improper folding of 16S rRNA can be detrimental to subunit biogenesis and cell growth.

Given the similarities between intermediates observed under certain experimental conditions *in vitro* and *in vivo*, continued studies of the intermediate states will be informative regarding crucial junctures in the cellular biogenesis cascade. Some discontinuity between *in vitro* and *in vivo* assembly has also been uncovered because deletion of the gene coding r-protein S15 (*rpsO*), which is required for *in vitro* incorporation of S18, S6, S11 and S21 [5,7,12], did not affect their incorporation *in vivo* [29], underscoring the importance of understanding the cellular processes of ribosome assembly.

Ribosomal subunit biogenesis *in vivo*

It is clear that studies utilizing an *in vitro* system to study ribosome biogenesis have yielded incredible information, and to date no similar, facile system is available for studying eukaryotic ribosome assembly. Yet it is also becoming clear that the *in vivo* biogenesis cascade is likely to be coupled to the transcription of long primary rRNA transcripts containing rRNA and intervening sequences, which are liberated in a series of endonucleolytic reactions (Figure 1, Table 1) [4]. It also seems that these endonucleases, rRNA and r-protein modification enzymes, GTPases, helicases and additional proteins of unknown function are intimately involved in the biogenesis cascade (Figure 1). Although an enzymatic function can be attributed to some of these processing and modification factors, functional relevance is understood in only a few cases.

A major obstacle in understanding the functional impact of ribosomal biogenesis factors in bacteria is the apparent redundancy of processes. Indeed, the majority of factors associated with ribosome biogenesis in *E. coli* are non-essential, despite the absolute necessity of ribosome synthesis. Moreover, the few essential genes involved in this cascade have roles in additional cellular processes, and it remains unclear for which cellular process the factor is

absolutely required [30–32]. The rate of bacterial ribosomal subunit biogenesis also makes it difficult not only to isolate *in vivo* intermediates in wild-type strains but also to identify specific factors that facilitate the process. In addition, the specific functions of identified factors have, in many cases, remained enigmatic. Investigators have therefore sought out clever and informative ways to manipulate biogenesis to uncover events and participants of the biogenesis process that would otherwise be undetectable (see Ref. [33] for an example). Some recent advances in identifying and functionally characterizing ribosome biogenesis factors will be highlighted below.

Ribosomal subunit biogenesis factors

GTPases

GTPases regulate diverse cellular processes [34], particularly within the context of protein translation [35]. Therefore, it is no surprise that some GTPases have been implicated in ribosome biogenesis [30,36–38]. The surprise, however, might lie in the complicated network of interactions between different GTPases and between GTPases and other biogenesis factors (Figure 2). The identification of the GTPases involved in ribosomal subunit biogenesis suggests an intricate level of coordination and control within the biogenesis pathway, whereby biogenesis factors are recruited or extricated according to the maturation state of the developing subunit (Figure 1).

Genetic interactions between some of these GTPases and other genes has facilitated not only the identification of additional biogenesis factors but also of genetic interaction networks between biogenesis participants [36,38] (Figure 2). Era (*E. coli* ras-like protein), an essential GTPase, has been implicated in 30S subunit biogenesis [36]. *era* mutant and suppressor studies [36,39,40] have uncovered interactions between Era and another biogenesis protein, RbfA [33,36]. Cryo-electron microscopy studies have positioned Era on the 30S subunit, and its location seems to overlap sites occupied by tRNAs (small RNAs responsible for transferring amino acids to growing polypeptide chains) and r-protein S1 in a translating ribosome, suggesting that Era-bound 30S particles are non-functional [41]. GTPase YjeQ (also called RsgA) was then genetically linked to Era (as well as other biogenesis factors, including KsgA and RimM [see below]) through a detailed interaction study using *E. coli* genomic resources that facilitate experiments in which specific gene deletion growth phenotypes can be examined in parallel with either other gene deletions or protein overexpression [38]. Uncovering genetic interactions allows the identification of biogenesis factors and the formulation of networks of genetically interacting biogenesis factors (Figure 2). Interestingly, there is both congruence and separation in the factors that interact with Era and YjeQ; thus, these two GTPases do not seem to have completely overlapping functions, although they share some interacting partners (Figure 2). Such interaction maps will be helpful in positioning the GTPases Der [30] and CgtA [37] within the biogenesis cascade of the 50S subunit in conjunction with ongoing studies of the potential substrates of these GTPases. A remaining major challenge is to clearly define the specific activities and appropriate *in vivo* substrates for GTPases involved in ribosome biogenesis and expose the stages of the biogenesis cascade in which they participate. It is easy to speculate that these GTPases regulate the temporal and cooperative biogenesis system. They could act to fold (or refold) important assembly intermediates, to allow dissociation and/or association of other assembly factors, to allow specific association of r-proteins and/or to allow elaborate architectural changes that are crucial for the processing and modification of biogenesis intermediates.

Recent work in *Bacillus subtilis* has extended our understanding of GTPases involved in biogenesis beyond the *E. coli* system [42–46]; these findings might lend insight to molecular mechanisms that are less obvious in *E. coli*. For example, YqeH is a GTPase implicated in

30S subunit biogenesis in *B. subtilis* and indeed its deletion results in impaired 70S ribosome formation [45]. Surprisingly, instead of an accumulation of both large and small subunits concomitant with 70S monosome reduction, as is typically observed in *E. coli* strains with ribosome assembly defects, only 50S subunits accumulate, whereas 30S or 30S precursors seem to be degraded for reasons that are as yet undetermined. The Britton laboratory has identified crucial roles for the GTPases YphC, YsxC and YlqF (RbgA) in 50S subunit assembly by deletion analysis and characterization of biogenesis defects [42–44]. Further study of YlqF using a dominant negative mutation that disrupts GTPase activity suggests that the GTPase activity is important for YlgF dissociation from the 50S subunit [46]. As would be expected for a role in the regulation of proper biogenesis, improper YlgF dissociation prevented the addition of several late binding r-proteins. Finally, multiple experiments have implicated YsxC as a GTPase involved in 50S biogenesis as a result of its interaction with 50S subunits and/or r-proteins [42,44]. These data present an interesting path because the comparison of conserved GTPases could reveal shared steps in the process, whereas differences in the GTPase requirements for subunit biogenesis in different species could reveal evolved differences between bacterial organisms, perhaps caused by different environmental challenges placed on ribosome biogenesis.

rRNA modification systems

Modification of rRNA is common to all organisms from bacteria to man; however, the exact position of the modifications and the overall number of modified nucleotides are generally not conserved. Although most of these modifications are positioned near translationally important sites [47], functional roles of the majority of these modifications have remained a mystery. Recent studies have begun to define clear roles for rRNA modifications and to extend into the arena of ribosome biogenesis the roles of not only the modifications themselves but also of the responsible enzymes. Some rRNA modifications can be related to antibiotic drug resistance [48–50], so these studies could result in important findings relevant to the study and design of drug targets.

Base methylations are the most common rRNA modification in 16S rRNA [51]. To date, seven methyltransferases and one pseudouridine synthase have been identified that modify 16S rRNA in *E. coli* [51] (some methyltransferases are yet to be identified, see Table 1). Similarly, recent studies have identified eight methyltransferases and six pseudouridine synthases that modify 23S rRNA [51]. The Fournier laboratory provides a complete and up-to-date electronic resource for rRNA modifications and modification enzymes [51] (<http://people.biochem.umass.edu/fournierlab/3dmodmap/main.php>). For the most part, these enzymes have been identified by deletion analyses and have not been well characterized. However, recent experiments showed that three particular 23S rRNA pseudouridylation by RluD, at positions 1911, 1915 and 1917, are important for effective recruitment of the translation termination factor RF2 (release factor 2). Indeed, the absence of these modifications prompted the misreading of stop codons [52]. In addition, the absence of methylation modifications on specific residues within 16S rRNA is deleterious in the accurate selection of initiator tRNA [53]. Thus, detailed functional roles for a few *E. coli* rRNA modifications have recently been demonstrated, yet in the majority of cases, the functional significance of the modifications in translation or ribosome biogenesis remains unclear.

Studies aimed at determining possible substrates of modification enzymes have yielded clues regarding the specificity of these enzymes and the temporal nature in which they act. For example, RsmE, which modifies U1498 to m³U1498 (*E. coli* numbering [54]), can methylate fully assembled, active 30S subunits but not sub-30S particles, suggesting that this enzyme is active very late in biogenesis or during an early step in translation initiation [55]. By contrast, studies implicate an intermediate in the 30S subunit biogenesis cascade as

the appropriate substrate for RsmC-mediated production of m²G1207 [56]. Moreover, the crystal structures of RsmC in complex with a guanosine and of 30S subunits support the contention that RsmC acts during biogenesis because appropriate substrate–enzyme interactions seem to be precluded within the mature 30S structure [57]. Thus, substrate analysis has enabled a cursory understanding of some temporal aspects of rRNA modification during the course of 30S subunit biogenesis (i.e. RsmC is likely to act before RsmE).

Many rRNA modifications are not conserved, and great divergence has occurred within the catalytic modification systems of prokaryotes and eukaryotes, but one notable exception is the dimethylation of A1518 and A1519 within the SSU. In fact, the dimethylation of two adjacent adenosines in SSU rRNA is conserved in all cytoplasmic ribosomes studied to date [51], and the KsgA and/or Dim1p enzyme family, which is responsible for methylation of both residues, is conserved from bacteria to mammals [58]. A second function of KsgA in *E. coli*, apart from rRNA modification, is the regulation of 30S subunit participation in the translation cycle, a function that was proposed based on an understanding of its substrate requirements and interaction [59]. Recent findings demonstrate that KsgA interacts with the same region of the 30S subunit as 50S subunits and initiation factor 3 [60], suggesting a mechanism by which association of KsgA could prevent translation. Indeed, when a mutant allele of *ksgA*, which is incapable of modification, was used to decouple the methyl transfer and regulation properties of the protein, a dominant negative phenotype was uncovered [61]. This finding and subsequent analysis suggest that KsgA release from the 30S subunit, perhaps as a result of methylation, is crucial for normal cellular growth, and this discovery further illustrates a possible regulatory function of KsgA, whereby dissociation of KsgA allows the maturing subunit to progress to later stages of 30S subunit biogenesis [61]. This regulatory role is strikingly similar to the GTPase RbgA mentioned earlier. Thus, this modification system seems to have functionality in biogenesis, and because the presence of the methyl groups has only a small impact on the translation process, this new role is likely to be its dominant function [62]. This model serves to introduce rRNA modification systems as part of a possible regulatory cascade.

The functions of rRNA modifications have long been enigmatic. The clustering of these modified residues near functional sites in the ribosome has only increased the curiosity surrounding the significance of these alterations. Recent work suggests that the modifications and the responsible enzymes might be crucial for biogenesis of these regions. If true, this would be fundamentally important for accurate and appropriate translation. The ongoing identification and characterization of many modification enzymes will undoubtedly lead to a more complete understanding of both the modifications and their cellular significance.

Remodeling

In light of the extreme complexity of ribosomal subunit assembly, it is not surprising that many factors are needed to facilitate this process. Cold sensitivity has been an important and common phenotype of ribosome biogenesis defects and is undoubtedly caused by kinetic traps that arise during the folding of such a complicated macromolecule [27]. How then do bacteria synthesize enough active ribosomes to sustain growth under conditions less than ideal for ribosome assembly? Clues to this question came with the identification of cold shock proteins that possess possible helicase activity as potentiators of ribosome biogenesis. Helicase CsdA was recently shown to be important for 50S subunit biogenesis at low temperature [63]. Additionally, CsdA binds precursor 50S subunits [64]. Complementation studies of the Δ *csdA* phenotype identified *rhIE* as another putative helicase capable of facilitating ribosome biogenesis at low temperature [39,65]. Helicase SrmB has also been implicated in 50S subunit biogenesis and indeed its deletion prompts accumulation of a

stalled 50S subunit precursor [66]. Finally, DbpA has been implicated in 50S subunit biogenesis based on substrate analysis [67], although its deletion does not seem to affect 50S subunit biogenesis [64]. At least one cold shock protein (RbfA – see below) has been implicated in 30S subunit assembly [68]. To date, the exact function of this class of proteins *in vivo* remains unclear; nonetheless, it seems likely that further studies will illuminate our understanding of orchestrated rRNA refolding during the course of ribosomal subunit assembly.

Other factors that seem to be important for the integrity of the ribosome assembly cascade are the chaperone protein DnaK [22,69,70], the S5 acetyltransferase RimJ [28] and three proteins with as yet unknown function: RbfA [33,36,71,72], RimM [71,73] and RimN [74]. Although specific enzymatic activities in ribosome biogenesis have not been described for all of these factors, much *in vivo* and *in vitro* data support a role for these proteins in biogenesis, although only under a discrete subset of cellular conditions in some cases. RimJ, a known r-protein S5 acetyltransferase [75], seems to have additional function(s) in ribosome biogenesis apart from r-protein modification; recent work has demonstrated that an acetyltransferase-deficient allele of *rimJ* is capable of supporting its function in 30S subunit biogenesis [28] (however, this specific function remains unknown). A cryo-electron microscopy study by Agrawal and colleagues [72] identified a marked conformational rearrangement of the 30S subunits in the presence of RbfA, suggesting that when this protein is bound, the 30S subunits would be non-functional. Thus, it is possible that RbfA release must be achieved before biogenesis is complete in a similar manner as proposed for KsgA (see above). Numerous studies present a recurring theme: that is, biogenesis factors associate with pre-30S subunits during biogenesis. However, these factors must be extricated at a specific assembly stage before biogenesis can proceed and/or before the subunit is capable of translation. Indeed, such interactions have now been proposed for RbfA [72], KsgA [60,61] and, to a lesser extent, RsmC [57] and Era [41].

The level of complexity of ribosome biogenesis is not easily resolved; indeed, there are many examples where redundancy can be found in this biogenesis cascade. Further complexity arises when considering that many of the factors involved perform multiple cellular functions (e.g. Era [31]). Additionally, some factors seem to have multiple functions related to ribosomes and their biogenesis (e.g. KsgA and RimJ). Thus, it seems likely that many of these factors, once recruited to the biogenesis machinery, perform additional functions that helped to attenuate and facilitate this process as it evolved.

Concluding remarks

Although many advances in understanding the components and the structural architecture that make up the functional ribosome have occurred in recent years, we are still far from an exact blueprint of the complex process of translation and even further from a detailed examination of ribosomal subunit maturation. The recent structural analyses of the bacterial ribosome have painted a precise and detailed picture of the translating ribosome, yet the genesis of the ribosome from RNA and protein components remains a fascinating question. The recent identification of many factors involved in the ribosome biogenesis process has moved us closer to understanding how the ribosomal subunits are synthesized and constructed. The previously enigmatic incorporation of modified residues is beginning to be linked to specific processes in biogenesis and translation. Additionally, the mechanism(s) of modulating and the components involved in these processes are beginning to come into view.

However, a complete blueprint for construction of the ribosome is far from complete. There are many factors with known important roles for biogenesis processes, but parsing out their

exact functions has proven difficult. In addition, factors with established roles in biogenesis are now being further interrogated and new roles are being prescribed to them. It is becoming clear that there are stages in the biogenesis pathway and that specific processes facilitated by specific factors occur during these stages. It is the challenge of investigators to deconstruct the ribosome biogenesis process by further characterization and identification of factors and processes to more precisely define the stages of biogenesis and create a temporal map of processes involved. Taken together, a comprehensive map of the network of factors that participate in bacterial ribosome biogenesis seems within reach and, further, a more clear illustration of the stages that take place from the start of transcription to the initial round of translation can begin to be fully unveiled.

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Figure 1.

Bringing the maturation of ribosomal subunits into focus. Illustrated are the biogenesis pathways of the functional bacterial ribosomal subunits 30S (left; Protein Databank [PDB] code 2AVY; RNA colored gray and r-proteins colored blue) and 50S (right; PDB code 2AW4; RNA colored gray and r-proteins colored purple). The structures were generated using Pymol (<http://pymol.sourceforge.net>). The biogenesis of these subunits commences with transcription of primary rRNA transcripts, which contain 16S, 23S and 5S rRNA sequences and intervening sequences, and proceeds through a series of ill-defined stages. Although a precise X-ray crystal structure of the ribosomal subunits has been solved [1–3], the cellular processes involved in ribosome maturation (presented within the gray box) remain incompletely understood. It is known that an array of ribosomal biogenesis factors facilitates processes through a coordinated series of maturation events. Some of these factors are listed in the middle of the gray box, but the temporal aspects of their function have not been resolved. Elucidating a more complete understanding of the specific processes involved in the biogenesis of ribosomes remains a formidable challenge in the field but would result in a lessening of the ‘gray haze’ overlaying the biogenesis cascade at this time.

Figure 2.

Interaction map of 30S subunit components and a subset of biogenesis factors. Data derived from references herein, as well as recent large-scale protein interaction studies [80,81], reveal an interaction network of 30S subunit biogenesis factors, including endonucleases (red), ATPases (orange), rRNA and r-protein modification enzymes (blue), GTPases (green), other factors (purple) and yet to be identified factors (represented as various colored question marks). This map illustrates the complexity of the process and yet is still likely to be incomplete. Continued work on identifying biogenesis factors and characterizing their functions and interactions will shed light on both how and when biogenesis factors facilitate the maturation of ribosomal subunits.

Table 1

Extraribosomal 30S subunit biogenesis factors

Role	Factor	30S biogenesis function and/or activity ^a
RNA transcription [76–78]	RNA polymerase	Transcription of rRNA
	DksA	Regulation of rDNA promoter activity
	NusA	Antitermination of rRNA transcription
	NusB	Antitermination of rRNA transcription
	NusG	Antitermination of rRNA transcription
RNA processing [4]	RNase III	Double-stranded endonucleolytic cleavage of primary transcript, yielding 16S precursor (17S) rRNA
	RNase E	Endonucleolytic cleavage in 5' leader region of 17S rRNA, yielding 16S precursor (16.3S) rRNA
	RNase G (CafA)	Endonucleolytic cleavage of 16.3S rRNA, yielding mature 5' end of 16S rRNA
	Unknown	Endonucleolytic cleavage, yielding mature 3' end of 16S rRNA
RNA modification [51]	RsuA	Generation of pseudouridine at U516
	RsmA (KsgA)	Generation of m ⁶ ₂ A1518 and m ⁶ ₂ A1519; late-stage biogenesis checkpoint
	RsmB	Generation of m ⁵ C967
	RsmC	Generation of m ² G1207
	RsmD	Generation of m ² G966
	RsmE (YggJ)	Generation of m ³ U1498
	RsmF (YebU)	Generation of m ⁵ C1407
	RsmG (GidB)	Generation of m ⁷ G527
	???	Generation of m ⁴ Cm1402
	???	Generation of m ² G1516
r-protein modification [51,79]	RimJ	Acetylation of S5; unknown
	???	Addition of glutamic acid residues to S6
	???	Monomethylation of S11
	???	Addition of methylthio-aspartate to S12
	RimI	Acetylation of S18
GTPases [4,38]	Era	Unknown
	RsgA (YjeQ)	Unknown
Other [10,74]	RbfA	Unknown
	RimM	Unknown
	RimN	Unknown

^aDeletion and/or mutation of many factors alters 16S rRNA processing but direct catalytic processing functions are not thought to be associated with these factors, so these phenotypic changes are not listed.