Review

Deconstructing ribosome construction

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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 hierarchal 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

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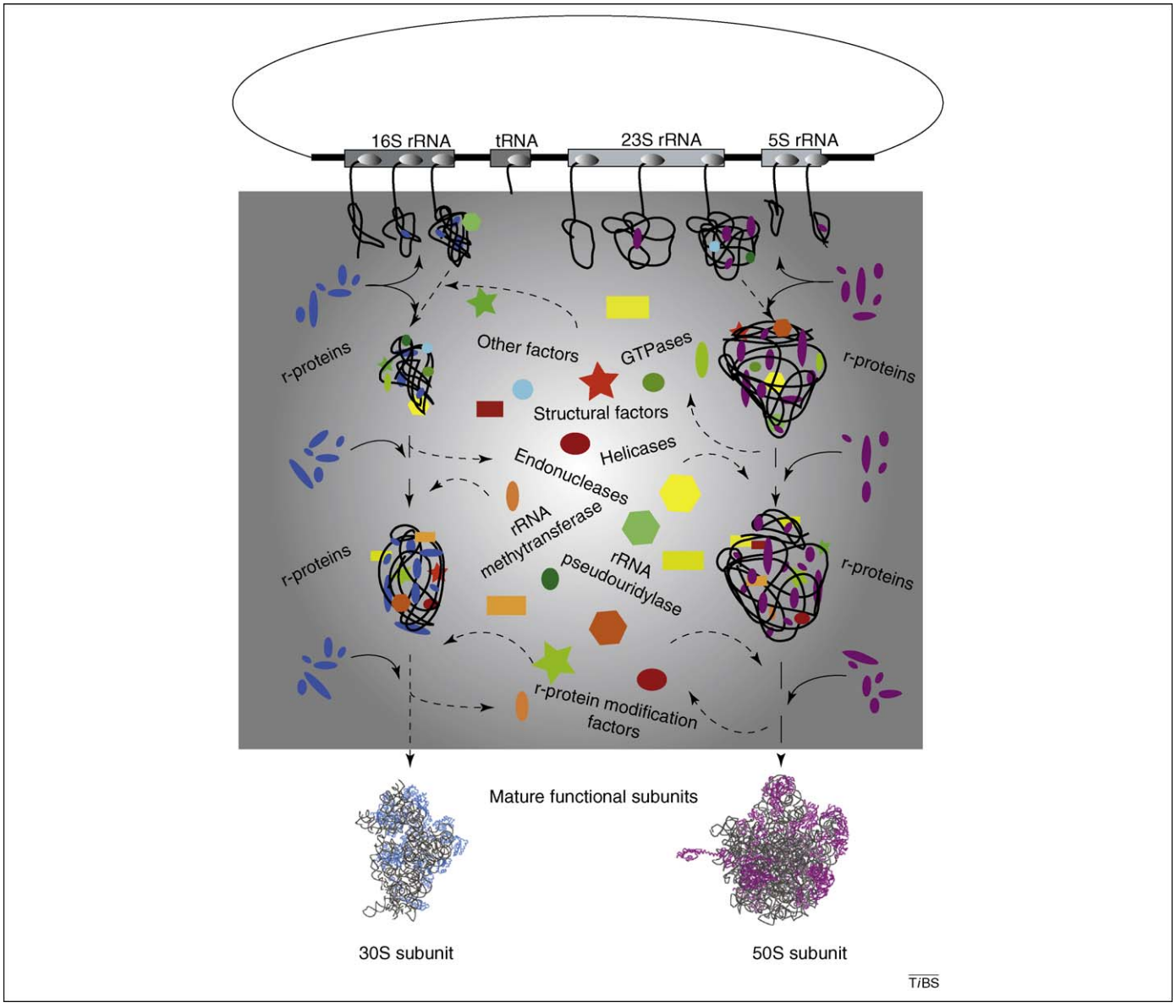

Figure 1. Bringing the maturation of ribosomal subunits into focus. Illustrated are the biogenesis pathways of the functional bacterial ribosomal subunits 30S (left; Protein Databank [PDB] code 2AVY; RNA colored gray and r-proteins colored blue) and 50S (right; PDB code 2AW4; RNA colored gray and r-proteins 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.

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.

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

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^5C967
	RsmC	Generation of m^2G1207
	RsmD	Generation of m^2G966
	RsmE (YggJ)	Generation of m^3U1498
	RsmF (YebU)	Generation of m^5C1407
	RsmG (GidB)	Generation of m^7G527
	???	Generation of m^4Cm1402
	???	Generation of m^2G1516
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

[a]Deletion 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.

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.

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].

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

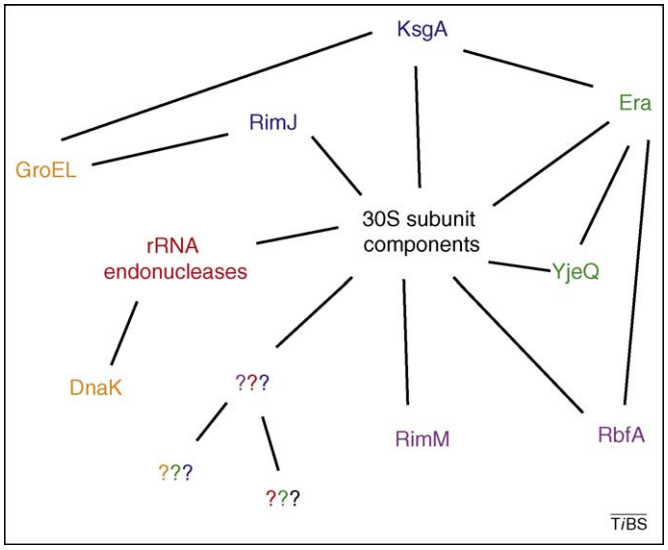

Figure 2. Interaction map of 30S subunit components and a subset of biogenesis factors. Data derived from references herein, as well as recent large-scale protein interaction studies [80,81], reveal an interaction network of 30S subunit biogenesis factors, including endonucleases (red), ATPases (orange), 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.

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 nonfunctional [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 pseudouridylations 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 m^3U1498 (*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 m^2G1207 [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 *rhlE* 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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References

1 Ban, N. *et al.* (2000) The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* 289, 905–920
2 Schuwirth, B.S. *et al.* (2005) Structures of the bacterial ribosome at 3.5 Å resolution. *Science* 310, 827–834
3 Wimberly, B.T. *et al.* (2000) Structure of the 30S ribosomal subunit. *Nature* 407, 327–339
4 Kaczanowska, M. and Ryden-Aulin, M. (2007) Ribosome biogenesis and the translation process in *Escherichia coli*. *Microbiol. Mol. Biol. Rev.* 71, 477–494
5 Held, W.A. *et al.* (1974) Assembly mapping of 30S ribosomal proteins from *Escherichia coli*. Further studies. *J. Biol. Chem* 249, 3103–3111
6 Held, W.A. and Nomura, M. (1973) Rate determining step in the reconstitution of *Escherichia coli* 30S ribosomal subunits. *Biochemistry* 12, 3273–3281
7 Traub, P. and Nomura, M. (1969) Studies on the assembly of ribosomes *in vitro*. *Cold Spring Harb. Symp. Quant. Biol.* 34, 63–67
8 Culver, G.M. (2003) Assembly of the 30S ribosomal subunit. *Biopolymers* 68, 234–249
9 Wilson, D.N. and Nierhaus, K.H. (2005) Ribosomal proteins in the spotlight. *Crit. Rev. Biochem. Mol. Biol.* 40, 243–267
10 Nierhaus, K.H. (2005) Bacterial ribosomes: assembly. In *Encyclopedia of Life Sciences*, DOI:10.1038/npg.els.0003947, John Wiley & Sons. (http://mrw.interscience.wiley.com/emrw/9780470015902/home/)
11 Woodson, S.A. (2008) RNA folding and ribosome assembly. *Curr. Opin. Chem. Biol.* 12, 667–673
12 Grondek, J.F. and Culver, G.M. (2004) Assembly of the 30S ribosomal subunit: positioning ribosomal protein S13 in the S7 assembly branch. *RNA* 10, 1861–1866
13 Rohl, R. and Nierhaus, K.H. (1982) Assembly map of the large subunit (50S) of *Escherichia coli* ribosomes. *Proc. Natl. Acad. Sci. U. S. A.* 79, 729–733

14 Adilakshmi, T. *et al.* (2008) Concurrent nucleation of 16S folding and induced fit in 30S ribosome assembly. *Nature* 455, 1268–1272
15 Nowotny, V. and Nierhaus, K.H. (1988) Assembly of the 30S subunit from *Escherichia coli* ribosomes occurs via two assembly domains which are initiated by S4 and S7. *Biochemistry* 27, 7051–7055
16 Talkington, M.W. *et al.* (2005) An assembly landscape for the 30S ribosomal subunit. *Nature* 438, 628–632
17 Foster, C. and Champney, W.S. (2008) Characterization of a 30S ribosomal subunit assembly intermediate found in *Escherichia coli* cells growing with neomycin or paromomycin. *Arch. Microbiol.* 189, 441–449
18 Guthrie, C. *et al.* (1969) Structure and function of *E. coli* ribosomes. 8. Cold-sensitive mutants defective in ribosome assembly. *Proc. Natl. Acad. Sci. U. S. A* 63, 384–391
19 Nashimoto, H. *et al.* (1971) Structure and function of bacterial ribosomes. XII. Accumulation of 21 s particles by some cold-sensitive mutants of *Escherichia coli*. *J. Mol. Biol* 62, 121–138
20 Nierhaus, K.H. *et al.* (1973) Ribosomal proteins. 43. *In vivo* assembly of *Escherichia coli* ribosomal proteins. *J. Mol. Biol* 74, 587–597
21 Lindahl, L. (1975) Intermediates and time kinetics of the *in vivo* assembly of *Escherichia coli* ribosomes. *J. Mol. Biol.* 92, 15–37
22 Alix, J.H. and Guerin, M.F. (1993) Mutant DnaK chaperones cause ribosome assembly defects in *Escherichia coli*. *Proc. Natl. Acad. Sci. U. S. A.* 90, 9725–9729
23 Holmes, K.L. and Culver, G.M. (2004) Mapping structural differences between 30S ribosomal subunit assembly intermediates. *Nat. Struct. Mol. Biol.* 11, 179–186
24 Tam, M.F. *et al.* (1981) Physical characteristics of 23 S rRNA from the 50 S ribosomal subunit of *Escherichia coli*. *FEBS Lett.* 130, 217–220
25 Tam, M.F. *et al.* (1981) Physical characteristics of 16 S rRNA under reconstitution conditions. *J. Biol. Chem.* 256, 6430–6434
26 Tam, M.F. and Hill, W.E. (1981) Physical characteristics of the reconstitution intermediates (RI30 and RI30*) from the 30S ribosomal subunit of *Escherichia coli*. *Biochemistry* 20, 6480–6484
27 Dammel, C.S. and Noller, H.F. (1993) A cold-sensitive mutation in 16S rRNA provides evidence for helical switching in ribosome assembly. *Genes Dev.* 7, 660–670
28 Roy-Chaudhuri, B. *et al.* (2008) Suppression of a cold-sensitive mutation in ribosomal protein S5 reveals a role for RimJ in ribosome biogenesis. *Mol. Microbiol.* 68, 1547–1559
29 Bubunenko, M. *et al.* (2006) 30S ribosomal subunits can be assembled in vivo without primary binding ribosomal protein S15. *RNA* 12, 1229–1239
30 Hwang, J. and Inouye, M. (2006) The tandem GTPase, Der, is essential for the biogenesis of 50S ribosomal subunits in *Escherichia coli*. *Mol. Microbiol.* 61, 1660–1672
31 Britton, R.A. *et al.* (1998) Cell cycle arrest in Era GTPase mutants: a potential growth rate-regulated checkpoint in *Escherichia coli*. *Mol. Microbiol.* 27, 739–750
32 Li, Z. *et al.* (1999) RNase G (CafA protein) and RNase E are both required for the 5′ maturation of 16S ribosomal RNA. *EMBO J.* 18, 2878–2885
33 Dammel, C.S. and Noller, H.F. (1995) Suppression of a cold-sensitive mutation in 16S rRNA by overexpression of a novel ribosome-binding factor, RbfA. *Genes Dev* 9, 626–637
34 Bourne, H.R. *et al.* (1990) The GTPase superfamily: a conserved switch for diverse cell functions. *Nature* 348, 125–132
35 Caldon, C.E. *et al.* (2001) Evolution of a molecular switch: universal bacterial GTPases regulate ribosome function. *Mol. Microbiol.* 41, 289–297
36 Inoue, K. *et al.* (2006) Era and RbfA have overlapping function in ribosome biogenesis in *Escherichia coli*. *J. Mol. Microbiol. Biotechnol.* 11, 41–52
37 Jiang, M. *et al.* (2006) The *Escherichia coli* GTPase CgtA is involved in late steps of large ribosome assembly. *J. Bacteriol.* 188, 6757–6770
38 Campbell, T.L. and Brown, E.D. (2008) Genetic interaction screens with ordered overexpression and deletion clone sets implicate the *Escherichia coli* GTPase YjeQ in late ribosome biogenesis. *J. Bacteriol.* 190, 2537–2545
39 Awano, N. *et al.* (2007) Complementation analysis of the cold-sensitive phenotype of the *Escherichia coli csdA* deletion strain. *J. Bacteriol.* 189, 5808–5815
40 Inoue, K. *et al.* (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
41 Sharma, M.R. *et al.* (2005) Interaction of Era with the 30S ribosomal subunit implications for 30S subunit assembly. *Mol. Cell* 18, 319–329
42 Schaefer, L. *et al.* (2006) Multiple GTPases participate in the assembly of the large ribosomal subunit in *Bacillus subtilis*. *J. Bacteriol.* 188, 8252–8258
43 Uicker, W.C. *et al.* (2006) The essential GTPase RbgA (YlqF) is required for 50S ribosome assembly in *Bacillus subtilis*. *Mol. Microbiol.* 59, 528–540
44 Wicker-Planquart, C. *et al.* (2008) Interactions of an essential *Bacillus subtilis* GTPase, YsxC, with ribosomes. *J. Bacteriol.* 190, 681–690
45 Loh, P.C. *et al.* (2007) The GTP-binding protein YqeH participates in biogenesis of the 30S ribosome subunit in *Bacillus subtilis*. *Genes Genet. Syst.* 82, 281–289
46 Matsuo, Y. *et al.* (2007) Isolation and characterization of a dominant negative mutant of *Bacillus subtilis* GTP-binding protein, YlqF, essential for biogenesis and maintenance of the 50 S ribosomal subunit. *J. Biol. Chem.* 282, 25270–25277
47 Decatur, W.A. and Fournier, M.J. (2002) rRNA modifications and ribosome function. *Trends Biochem. Sci.* 27, 344–351
48 Helser, T.L. *et al.* (1972) Mechanism of kasugamycin resistance in *Escherichia coli*. *Nat. New Biol.* 235, 6–9
49 Sparling, P.F. (1970) Kasugamycin resistance: 30S ribosomal mutation with an unusual location on the *Escherichia coli* chromosome. *Science* 167, 56–58
50 Okamoto, S. *et al.* (2007) Loss of a conserved 7-methylguanosine modification in 16S rRNA confers low-level streptomycin resistance in bacteria. *Mol. Microbiol.* 63, 1096–1106
51 Piekna-Przybylska, D. *et al.* (2008) The 3D rRNA modification maps database: with interactive tools for ribosome analysis. *Nucleic Acids Res.* 36, D178–D183
52 Ejby, M. *et al.* (2007) Pseudouridylation of helix 69 of 23S rRNA is necessary for an effective translation termination. *Proc. Natl. Acad. Sci. U. S. A.* 104, 19410–19415
53 Das, G. *et al.* (2008) Role of 16S ribosomal RNA methylations in translation initiation in *Escherichia coli*. *EMBO J.* 27, 840–851
54 Brimacombe, R. (1995) The structure of ribosomal RNA: a three-dimensional jigsaw puzzle. *Eur. J. Biochem.* 230, 365–383
55 Basturea, G.N. and Deutscher, M.P. (2007) Substrate specificity and properties of the *Escherichia coli* 16S rRNA methyltransferase, RsmE. *RNA* 13, 1969–1976
56 Sergiev, P.V. *et al.* (2007) Ribosomal RNA guanine-(N2)-methyltransferases and their targets. *Nucleic Acids Res.* 35, 2295–2301
57 Demirci, H. *et al.* (2008) Crystal structure of the *Thermus thermophilus* 16 S rRNA methyltransferase RsmC in complex with cofactor and substrate guanosine. *J. Biol. Chem.* 283, 26548–26556
58 O'Farrell, H.C. *et al.* (2006) Recognition of a complex substrate by the KsgA/Dim1 family of enzymes has been conserved throughout evolution. *RNA* 12, 725–733
59 Thammana, P. and Held, W.A. (1974) Methylation of 16S RNA during ribosome assembly *in vitro*. *Nature* 251, 682–686
60 Xu, Z. *et al.* (2008) A conserved rRNA methyltransferase regulates ribosome biogenesis. *Nat. Struct. Mol. Biol.* 15, 534–536
61 Connolly, K. *et al.* (2008) Mechanistic insight into the ribosome biogenesis functions of the ancient protein KsgA. *Mol. Microbiol.* 70, 1062–1075
62 Poldermans, B. *et al.* (1979) Studies on the function of two adjacent N6,N6-dimethyladenosines near the 3′ end of 16 S ribosomal RNA of *Escherichia coli*. II. The effect of the absence of the methyl groups on initiation of protein biosynthesis. *J. Biol. Chem* 254, 9090–9093
63 Charollais, J. *et al.* (2004) CsdA, a cold-shock RNA helicase from *Escherichia coli*, is involved in the biogenesis of 50S ribosomal subunit. *Nucleic Acids Res.* 32, 2751–2759
64 Peil, L. *et al.* (2008) Ribosome assembly in *Escherichia coli* strains lacking the RNA helicase DeaD/CsdA or DbpA. *FEBS J.* 275, 3772–3782
65 Jain, C. (2008) The *E. coli* RhlE RNA helicase regulates the function of related RNA helicases during ribosome assembly. *RNA* 14, 381–389
66 Charollais, J. *et al.* (2003) The DEAD-box RNA helicase SrmB is involved in the assembly of 50S ribosomal subunits in *Escherichia coli*. *Mol. Microbiol.* 48, 1253–1265

67 Diges, C.M. and Uhlenbeck, O.C. (2001) *Escherichia coli* DbpA is an RNA helicase that requires hairpin 92 of 23S rRNA. *EMBO J.* 19, 5503–5512
68 Jones, P.G. and Inouye, M. (1996) RbfA, a 30S ribosomal binding factor, is a cold-shock protein whose absence triggers the cold-shock response. *Mol. Microbiol.* 21, 1207–1218
69 Al Refaii, A. and Alix, J.H. (2008) Ribosome biogenesis is temperature-dependent and delayed in *Escherichia coli* lacking the chaperones DnaK or DnaJ. *Mol. Microbiol.* 71, 748–762
70 Maki, J.A. *et al.* (2002) The DnaK chaperone system facilitates 30S ribosomal subunit assembly. *Mol. Cell* 10, 129–138
71 Bylund, G.O. *et al.* (1998) RimM and RbfA are essential for efficient processing of 16S rRNA in *Escherichia coli*. *J. Bacteriol.* 180, 73–82
72 Datta, P.P. *et al.* (2007) Structural aspects of RbfA action during small ribosomal subunit assembly. *Mol. Cell* 28, 434–445
73 Suzuki, S. *et al.* (2007) Structural characterization of the ribosome maturation protein. *RimM. J. Bacteriol.* 189, 6397–6406
74 Kaczanowska, M. and Ryden-Aulin, M. (2005) The YrdC protein – a putative ribosome maturation factor. *Biochim. Biophys. Acta* 1727, 87–96
75 Janda, I. *et al.* (1985) Gene *rpmF* for ribosomal protein L32 and gene *rimJ* for a ribosomal protein acetylating enzyme are located near *pyrC* (23.4 min) in *Escherichia coli*. *Mol. Gen. Genet* 201, 433–436
76 Torres, M. *et al.* (2004) *In vivo* effect of NusB and NusG on rRNA transcription antitermination. *J. Bacteriol.* 186, 1304–1310
77 Quan, S. *et al.* (2005) Transcriptional polarity in rRNA operons of *Escherichia coli nusA* and *nusB* mutant strains. *J. Bacteriol.* 187, 1632–1638
78 Paul, B.J. *et al.* (2004) rRNA transcription in *Escherichia coli*. *Annu. Rev. Genet.* 38, 749–770
79 Arnold, R.J. and Reilly, J.P. (1999) Observation of *Escherichia coli* ribosomal proteins and their posttranslational modifications by mass spectrometry. *Anal. Biochem.* 269, 105–112
80 Butland, G. *et al.* (2005) Interaction network containing conserved and essential protein complexes in *Escherichia coli*. *Nature* 433, 531–537
81 Arifuzzaman, M. *et al.* (2006) Large-scale identification of protein–protein interaction of *Escherichia coli* K-12. *Genome Res.* 16, 686–691