Assembly of Bacterial Ribosomes

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Abstract

The assembly of ribosomes from a discrete set of components is a key aspect of the highly coordinated process of ribosome biogenesis. In this review, we present a brief history of the early work on ribosome assembly in *Escherichia coli*, including a description of in vivo and in vitro intermediates. The assembly process is believed to progress through an alternating series of RNA conformational changes and protein-binding events; we explore the effects of ribosomal proteins in driving these events. Ribosome assembly in vivo proceeds much faster than in vitro, and we outline the contributions of several of the assembly cofactors involved, including Era, RbfA, RimJ, RimM, RimP, and RsgA, which associate with the 30S subunit, and CsdA, DbpA, Der, and SrmB, which associate with the 50S subunit.

Contents

INTRODUCTION

The ribosome is a ribonucleoprotein complex responsible for one of the key processes in cells—protein synthesis. The bacterial ribosome sediments during ultracentrifugation as a 70S particle composed of a small subunit (30S) and a large subunit (50S). The small subunit contains 21 ribosomal proteins (designated S1–S21) and a 16S ribosomal RNA (rRNA), which is 1,542 nucleotides (nt) in length, whereas the large subunit is made up of 33 proteins (designated L1–L36) and two rRNAs, the 23S, which is 2,904 nt in length, and 5S, which is 120 nt in length. The 30S subunit is responsible for association with mRNA during translation initiation and for mRNA decoding, deciphering the 3-nt codon that determines which amino acid to incorporate into the polypeptide chain next. The 50S subunit contains the peptidyl transferase center, which is the site of peptide-bond formation. The crystal structures of the complete 70S ribosome (**Figure 1**) (1), the 30S (2, 3), and 50S subunits (4) solved in the past decade confirmed that the catalytic sites actually lie within the rRNA components and that the ribosome is in fact a ribozyme (5). These structures have also shed light on many important aspects of the mechanism of translation (6–9), yet they provide limited information on how the ribosome assembles into a stable multicomponent complex.

Ribosome assembly is a central component of the overall program of ribosome biogenesis, which is a complex and highly coordinated process. The basic steps of ribosome assembly involve (*a*) the transcription, processing, and modification of rRNA; (*b*) the translation and modification of ribosomal proteins; (*c*) the proper folding of rRNA and ribosomal proteins; (*d*) the binding of ribosomal proteins; and (*e*) the binding and release of assembly factors. Many of these steps are coupled and occur in parallel during the transcription of the rRNAs. The assembly process is believed to progress through an alternating series of RNA conformational changes and protein-binding events, whereby RNA folding gains are stabilized by the binding of ribosomal proteins, incrementally driving the RNA structure to the final native state (10, 11).

The ribosome is a paradigm for RNA-protein recognition and the principles of RNA and protein folding and the assembly of multicomponent complexes in general. In addition, improperly assembled ribosomes have been implicated in several human diseases, and insights into ribosome biogenesis may lead to new therapeutic strategies (12). An understanding of both the physical and temporal pathways leading to intact ribosomes will yield valuable insight into how the assembly process is regulated and why errors in biogenesis sometime occur.

30S: *E. coli* small ribosomal subunit

50S: *E. coli* large ribosomal subunit

Studies focusing on how the ribosome assembles have been in progress for decades (13–15), and there are both several early (16–23) and more recent reviews describing ribosome biogenesis in bacteria (10, 24–27). Some recent reviews highlight a particular aspect of assembly, such as 30S assembly (28–30), ribosomal protein binding and function (31), assembly factors (32–34), rRNA maturation (35), and rRNA modifications (36), whereas others focus on emerging biophysical techniques for studying assembly (37). The aim of this review is to provide a comprehensive overview of the ribosome assembly process in *Escherichia coli*, with a particular focus on progress from the past few years and how it contributes to our global understanding of the assembly process. We first provide a historical introduction to the field and a description of the known in vitro and in vivo assembly intermediates. We include recent studies that aim to elucidate the assembly pathway(s) of the ribosome, specifically ribosomal protein binding dependencies, kinetics and the latest understanding of rRNA-ribosomal protein association. The interplay between rRNA conformational changes and protein binding events is discussed, followed by an account of ribosomal defects that accumulate in the absence of a single ribosomal protein. Finally, we provide an overview of the recent wealth of work detailing the specific role of ribosomal assembly cofactors.

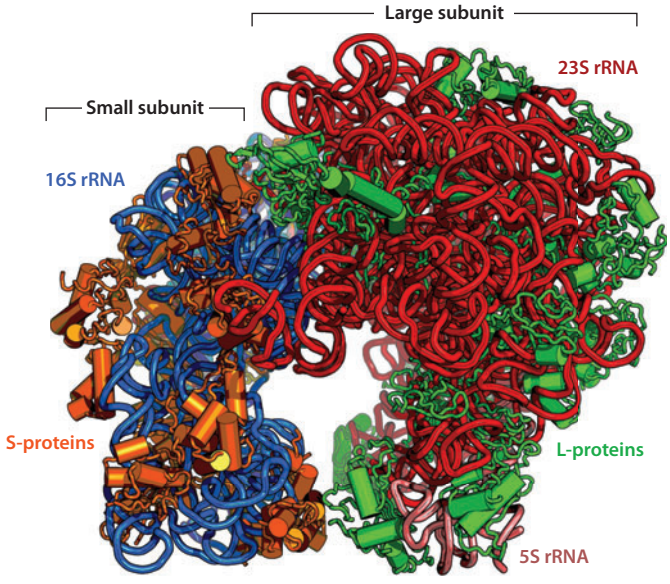

Figure 1

A rendering of the *E. coli* 70S ribosome. The small subunit is on the left, with the 16S rRNA shown in blue and the small subunit proteins (S-proteins) shown in orange. The large subunit is on the right, with the 23S rRNA shown in red, the 5S rRNA shown in pink, and the large subunit proteins (L-proteins) shown in green.

OVERVIEW OF 50 YEARS OF RIBOSOME ASSEMBLY

In Vitro Assembly Map and Assembly Intermediates

The high-resolution structures of the ribosome that emerged over the past decade have somewhat overshadowed the extraordinary body of work that was performed during the previous half century. Early studies aimed to determine the components of the ribosome, and in the late 1950s, researchers were concluding that the various components (30S, 50S, 70S, 100S) were different physical states of the same particle that could be altered by the concentration of magnesium ions. Tissieres & Watson (13) demonstrated that the bacterial ribosome is composed of a 70S particle that can be broken down into two smaller components, the small and large subunit, and that the 100S particle was an aggregate of the 70S.

A milestone occurred in the late 1960s, when Traub & Nomura (38) demonstrated that an active 30S subunit could be assembled in vitro from free rRNA and ribosomal proteins without any additional components. These in vitro reconstitution experiments demonstrated that all of the information necessary for assembly is encoded within the rRNA and proteins themselves. Active 30S particles can also be reconstituted from 16S rRNA in combination with individually purified proteins (39, 40), recombinant ribosomal proteins (41, 42), and unmodified 16S rRNA transcribed in vitro (43). The 30S subunit is divided into three domains: the body (5′ domain), the platform region (central domain), and the head (3′ domain), and each

16S rRNA: a ribosomal RNA component of the 30S subunit

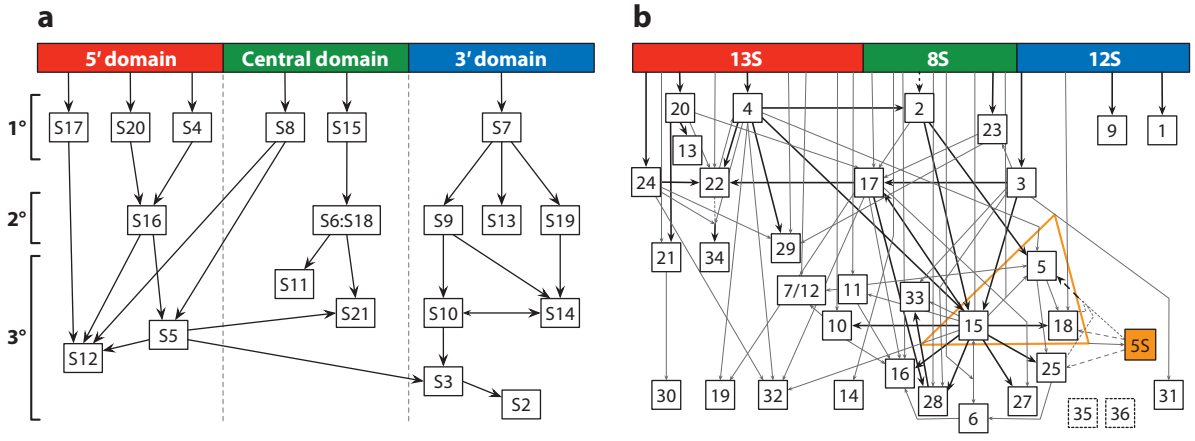

Figure 2

Assembly maps. (*a*) The Nomura assembly map depicts thermodynamic protein binding dependencies in the 30S subunit. There is a clear hierarchy of primary binding proteins (1°), which stably bind directly to the rRNA; secondary binding proteins (2°), which depend on primary binders; and tertiary binding proteins (3°), which depend on secondary binders. The map is further divided into 5′ (*red*), central (*green*), and 3′ (*blue*) domains on the basis of binding position relative to the 16S rRNA (39, 48, 49). (*b*) The Nierhaus assembly map depicts thermodynamic protein binding dependencies in the 50S subunit. The 5S rRNA (*orange*) depends on proteins L5, L15, and L18 for binding (L characters are omitted owing to space constraints). The 23S rRNA is divided into three sections analogous to the 5′, central, and 3′ domains of the 30S subunit (59).

domain can be reconstituted independently (44–46). The ability to reconstitute active subunits represented a major breakthrough as it now became possible to alter the various components to dissect ribosome assembly. Though recent results have called into question the somewhat simplified view of assembly that emerged from this early work, it nevertheless laid the foundation for an impressive and ongoing body of work on ribosome biogenesis and assembly.

By varying the order in which the proteins were added, Nomura and coworkers (38) determined that in vitro, protein-binding events are thermodynamically interdependent and that the ribosomal proteins bind to 16S rRNA in a hierarchical manner. A few proteins S4, S7, S8, S15, S17, S20 (referred to as primary binding proteins) stably bind directly to the nascent 16S rRNA. The remaining proteins (secondary and tertiary binding proteins) require prior binding of one or more proteins, respectively. This hierarchy of association is depicted in the classic Nomura Map, which remains in nearly the same form after 40 years (**Figure 2*a***) (39, 40, 47–49).

Reconstitution intermediate (RI): an in vitro intermediate observed at low temperature

Interestingly, some proteins, for example, S16 and S18, are important for assembly but not for function (50).

Two intermediates for assembly of the 30S subunit were identified by reconstitution at different temperatures (20, 47, 51, 52). At low temperatures (0°C to 15°C), assembly stalls, and a distinct particle, the reconstitution intermediate (RI) composed of 16S rRNA and 15 ribosomal proteins, is formed, which sediments at 21S-22S. Reconstitution cannot continue until the particle is heated to 40°C; at this point, the particle (RI*, an in vitro intermediate obtained from heating RI) is thought to rearrange so that it sediments at 25S-26S. Upon addition of the remaining proteins, this RI* particle is capable of forming an active 30S subunit (**Figure 3*a***) (47).

Nierhaus & Dohme (53) successfully reconstituted biologically active 50S subunits under rather harsh, nonphysiological conditions (**Figure 3*b***). They identified three reconstitution intermediates, the balance among them varying depending upon the temperature and ionic conditions of the reconstitution (21, 54).

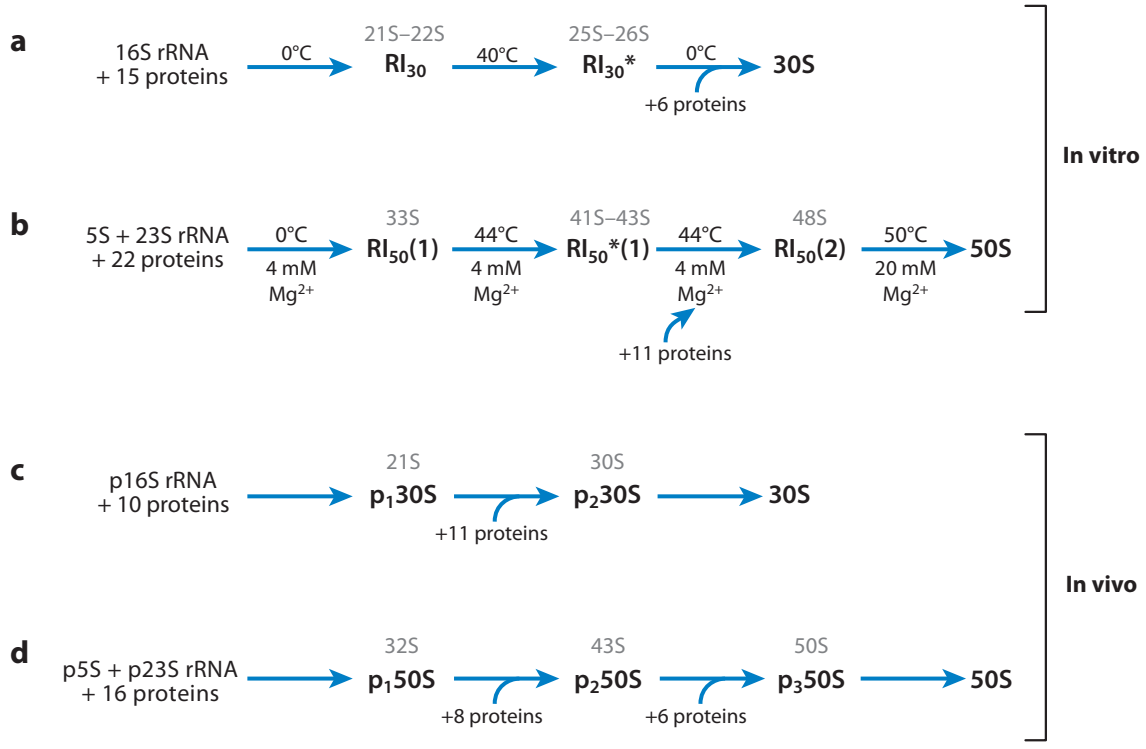

Figure 3

Ribosomal subunit assembly mechanisms. Asterisks denote species that differ from previous species by conformational change but not the addition of proteins. (*a*) An in vitro assembly mechanism for the 30S subunit. (*b*) An in vitro assembly mechanism for the 50S subunit. (*c*) An in vivo assembly mechanism for the 30S subunit. (*d*) An in vivo assembly mechanism for the 50S subunit.

The first intermediate, $RI_{50}(1)$ sediments at 33S and undergoes a high-temperature transition to the second intermediate $RI_{50}{}^*(1)$ that sediments at 41S–43S. The third intermediate is formed by the addition of the remaining proteins and contains the full complement of proteins but is inactive and sediments at 48S. Increased heat and high magnesium ion concentrations promote the transition to active 50S subunits (54).

Ribosomal proteins L4, L13, L20, L22, and L24 and 23S rRNA are essential for the $RI_{50}(1)$ to $RI_{50}{}^*(1)$ conformational change, which occurs independently of 5S rRNA (55), whereas L3 plays a stimulatory but nonessential role. The essential ribosomal proteins cluster at the 5′ of the 23S rRNA transcript. Interestingly, the ribosomal proteins L20 and L24, which are essential for the conformational change, do not play a role in the late steps of assembly and are not functionally important (56). The addition of the remaining proteins yields the inactive 48S particle [$RI_{50}(2)$], which becomes an active 50S subunit when the incubation temperature and magnesium concentration are increased to 50°C and 20 mM, respectively. 5S rRNA is necessary to proceed from the 48S intermediate to the 50S subunit, but dispensable for the earlier assembly steps (57). Because of the number of components involved, it is not surprising that the 50S subunit assembly map (**Figure 2*b***) is significantly more complex than that for the small subunit (**Figure 2*a***) (21, 58–60).

In Vivo Assembly Intermediates

Ribosomal assembly intermediates are not abundant under normal growth conditions, and early studies relied on conditional mutants (61,

5S rRNA: one of two ribosomal RNA components of the 50S subunit

23S rRNA: one of two ribosomal RNA components of the 50S subunit

$p_1$30S: the first of two 30S in vivo intermediates (sediments at 21S)

$p_2$30S: the second of two 30S in vivo intermediates (sediments at 30S); contains all the small subunit ribosomal proteins and untrimmed rRNA

$p_1$50S: the first of three 50S in vivo intermediates (sediments at 32S)

$p_2$50S: the second of three 50S in vivo intermediates (sediments at 43S)

$p_3$50S: the third of three 50S in vivo intermediates (sediments at 50S) and contains all of the large subunit proteins and untrimmed rRNA

17S rRNA: an untrimmed, immature 16S ribosomal RNA, also known as the 16S precursor rRNA

9S rRNA: an untrimmed 5S ribosomal RNA

62), temperature-sensitive strains (63, 64), or fragile growth mutants (65) to perturb growth and accumulate large amounts of potential ribosomal precursors, which are detectable on sucrose gradients. In normal cells, incomplete ribosomal particles have been characterized using pulse-labeling, allowing extremely small quantities to be detected (14, 66–68). A combination of pulse labeling and polyacrylamide gels determined that in vivo there are two 30S intermediates ($p_1$30S and $p_2$30S) (**Figure 3*c***) that sediment around 21S (67, 68) and 30S, respectively (66–68), and three 50S intermediates ($p_1$50S, $p_2$50S, and $p_3$50S) that sediment at 32S, 43S (66–68), and 50S, respectively (**Figure 3*d***) (67, 68). These precursors account for only 2–5% of total rRNA in exponentially growing cells. The protein composition of $p_1$50S and $p_2$50S are slightly different, with $p_2$50S containing eight more proteins (23, 69). The most abundant precursors ($p_2$30S and $p_3$50S) cosediment with mature 30S and 50S subunits and contain all the associated proteins of the mature subunits but have untrimmed forms of rRNA (precursor rRNA) (67). Final conversion of precursor rRNA is thought to take place in polysomes (67, 70–72), demonstrating that assembly and rRNA maturation are coupled.

rRNA Maturation and Modifications

In vivo the 16S, 23S, and 5S rRNA are synthesized as one primary transcript that is processed by at least five nucleases. RNase III cleaves the primary transcript to yield precursor 16S rRNA (17S rRNA), precursor 23S rRNA, and precursor 5S rRNA (9S rRNA), which contain the sequences for the mature 16S, 23S, and 5S rRNAs, respectively (73–75). 17S rRNA contains the 16S rRNA sequence with an additional 115 nt at the 5′ end and 33 nt at the 3′ end (76). The 5′ end is further cleaved by RNase E to yield a product with 66 extra nucleotides at the 5′ end, which is subsequently cleaved by RNase G (77). Maturation at the 5′ end can occur in the absence of RNase E, albeit more slowly (77). Maturation of the 5′ end occurs before the 3′ end (77, 78). At the 3′ end, a currently unknown RNase causes final maturation. Interestingly, mature 16S rRNA can still form in the absence of RNase III; however, mature 23S rRNA cannot be formed (79).

The 23S rRNA precursor formed by RNase III cleavage contains the mature 23S rRNA transcript with three or seven additional nucleotides at the 5′ end and seven to nine additional nucleotides at the 3′ end (80, 81). The enzyme responsible for final maturation of the 5′ end is still unknown, but final cleavage at the 3′ end is facilitated by RNase T (82). 5S rRNA is located at the 3′ end in the primary transcript and matures from a precursor 5S particle (9S rRNA) (83, 84) composed of the mature 5S transcript with 84 additional nucleotides at the 5′ end and 42 additional nucleotides at the 3′ end (85). RNase E is able to cleave the precursor at both ends to leave mature 5S rRNA and three additional nucleotides at each of the 5′ and 3′ ends (86). As with the 23S rRNA, final maturation at the 5′ end occurs by a still unknown enzyme, and RNase T causes final maturation at the 3′ end. Strains lacking RNase T cannot produce mature 5S rRNA. However, an intermediate form of rRNA, containing a mature 5′ end and two extra residues on the 3′ end, is incorporated into functioning ribosomes (**Figure 4**) (87).

In both subunits, most rRNA modifications occur on nucleotides that are conserved and that are in functionally important regions (88, 89). Modifications are thought to influence both ribosome structure and function, and the importance and role of each modification remain active areas of study. Currently, the 16S rRNA is known to undergo 11 modifications (1 pseudouridylation and 10 methylations), and the 23S rRNA undergoes 25 modifications (1 unknown, 14 methylations, 9 pseudouridylation, and 1 methylated pseudourdylation) (25, 36, 89). Remarkably, unmodified in vitro transcribed 16S rRNA and all of the natural 30S ribosomal proteins can reconstitute active 30S subunits that can participate to a lesser extent in tRNA binding and polyphenylalanine synthesis with somewhat reduced activity (43). Unlike the small subunit, in vitro transcripts

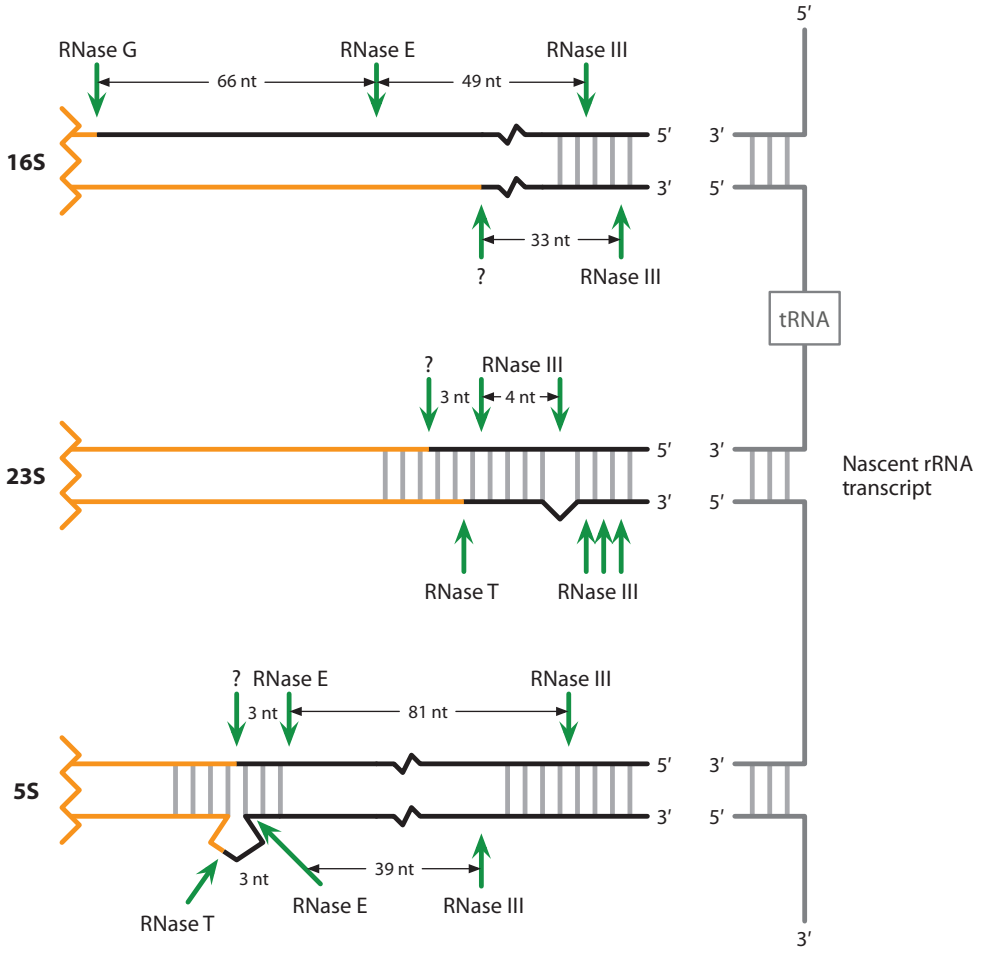

Figure 4

Maturation of ribosomal RNA (rRNA). 16S, 23S, and 5S rRNA are originally transcribed as a single transcript before cleavage by a series of RNases to obtain the mature product. Question marks indicate cleavage sites where the responsible enzyme has not yet been identified. Cleavage sites are indicated by green arrows, and mature rRNA is indicated in orange.

of 23S rRNA and natural 50S ribosomal subunit proteins cannot be reconstituted into catalytically active 50S subunits in *E. coli.* (90). It was determined that most of the 23S rRNA modifications are not necessary to reconstitute catalytically active particles but that a particular 80-nt region (nt 2445–2523) containing 7 of the known 25 modifications cannot be replaced by in vitro transcripts and must be natural (90), although this is not required in other organisms (91). In vivo modifications that take place outside of the 80-nt region may also be required, as assembly of the 50S subunit is significantly impaired when some of these modifications are absent (63, 92–95).

Comparing Ribosome Assembly In Vitro and In Vivo

The reconstitution reaction for ribosome assembly in vitro occurs much slower than assembly in vivo (68), and the activation energy

for assembly is lower if precursor rRNA is used instead of mature 16S rRNA, implying that proper processing and maturation of precursor 16S rRNA is necessary for 30S assembly (25, 96). Reconstitution experiments, protein-binding kinetic data, and time-resolved fingerprinting have also demonstrated that assembly proceeds in the 5′–3′ direction (97–100). The 5′ domain folds first, even in the absence of ribosomal proteins (101), suggesting a cotranscriptional direction to protein binding. In addition, in vitro reconstitutions have demonstrated that 30S subunit proteins bind the rRNA significantly faster in the presence of the cofactors Era, RimM, and RimP (102). Overall, these suggest that various cofactors and the cotranscriptional nature of assembly in vivo likely allow the assembly process to progress more quickly by preventing the kinetic traps that often arise in larger RNAs (27).

Despite the similar sedimentation coefficients, the rRNA components are also different for both in vivo and in vitro intermediates. Reconstitution in vitro takes place with fully processed rRNA, whereas in vivo the 16S, 23S, and 5S rRNA are synthesized as one primary transcript, where local secondary structures can form once the binding sites emerge, and ribosomal proteins can bind even before transcription is completed (65, 68, 99, 100). It is not difficult to imagine that maturation differences between the rRNA components can lead to differences in protein composition or protein levels, particularly because protein binding and rRNA modification and maturation are coupled. The 30S and 50S ribosomal protein composition of both the in vivo (23, 69, 103) and in vitro particles (23, 47, 55, 103, 104) are similar but not identical (**Table 1**), and still unknown is the extent to which particles generated during in vitro assembly follow the same pathway as those found during in vivo assembly.

The protein composition of assembly intermediates was typically qualitatively detected using two-dimensional gel electrophoresis. However, the ability to precisely measure relative protein levels is a recent advance. To date, the protein levels of in vivo intermediates have been measured for only a few cases (105–107). In each case, normal cell growth was perturbed by a deletion (107) or mutation (106) of a particular gene or by the addition of a chemical inhibitor (105) to allow precursor particles to accumulate in the amounts necessary for quantitation. In one example, the addition of the antibiotic neomycin generated a precursor particle that sediments at ~21S (108). Using mass spectrometry, the protein composition of neomycin-induced 21S particles and RI particles, which also sediment at 21S, were determined to be somewhat different, with the central domain proteins being comparatively overrepresented in the neomycin-generated particle compared to the RI particle (105).

ASSEMBLY PATHWAYS AND KINETIC STUDIES

On the basis of the 30S and 50S assembly maps, ribosome assembly is believed to be cooperative, where binding of ribosomal proteins induces structural changes in the rRNA (10, 11). Early studies of in vitro reconstitution of the 30S and 50S ribosomal subunits have described the formation of global rate-limiting intermediates that sediment at 21S and 33S, respectively (47). Recently there has been much discussion of whether there is a single rate-limiting step and pathway that leads to the intact ribosome or if assembly can proceed through multiple pathways with similar energetics (28, 48, 98, 109).

The Nomura and Nierhaus assembly maps are not kinetic mechanisms but rather diagrams detailing the protein-binding interdependence during assembly at equilibrium. Although there is a wealth of information contained in the assembly maps, to elucidate the assembly pathway(s), one needs to measure the kinetics of protein-RNA association to determine the preferred pathways and the populations of intermediates. Using time-resolved hydroxyl-radical footprinting, Woodson and coworkers (110) mapped changes in the structure of the 16S rRNA within 20 ms after the addition of 30S ribosomal proteins. They demonstrated that assembly is not cooperative at every stage.

Table 1 Protein content of in vitro and in vivo intermediates

Protein[a]	In vitro reconstitution intermediate (21S)[b]	In vivo $p_1$30S (21S)	Protein	In vitro (33/41S)	In vivo $p_1$50S (32S)	In vivo $p_2$50S (43S)
S1	**–**	**+**	L1	+	+	+
S2	–	–	**L2**	**+**	**–**	**–**
S3	–	–	**L3**	+	–	+
S4	+	+	L4	+	+	+
S5	+	+	L5	+	+	+
S6	**+**	**–**	L6	–	–	–
S7	**+**	**–**	L7	+	–	+
S8	+	+	L9	+	+	+
S9	**+**	**–**	L10	+	+	+
S10	–	–	L11	+	–	+
S11	**+**	**–**	L13	+	+	+
S12	**+**	**–**	L14	–	–	+
S13	+	+	L15	+	–	+
S14	–	–	L16	–	–	–
S15	+	+	L17	+	+	+
S16	+	+	L18	+	+	+
S17	+	+	L19	–	–	+
S18	**+**	**–**	L20	+	+	+
S19	**+**	**–**	L21	+	+	+
S20	+	+	L22	+	+	+
S21	**–**	**+**	L23	+	–	+
			L24	+	+	+
			L25	**–**	**+**	**+**
			L27	**–**	**+**	**+**
			L28	–	–	–
			L29	+	+	+
			L30	**–**	**+**	**+**
			L31	–	–	–
			L32	–	–	–
			L33	+	–	+
			L34	+	U	U
			L35	U	U	U
			L36	U	U	U

[a]Bold highlights difference between in vivo and in vitro.

[b]+, protein is present; –, protein is absent; U, unknown; $p_1$30S, a small subunit in vivo intermediate; $p_1$50S, the first large subunit in vivo intermediate; $p_2$50S, the second large subunit in vivo intermediate.

Instead, at fast timescales, assembly of the 30S subunit nucleates from different points along the rRNA at the same time, presenting many early assembly intermediates. Interestingly, most nucleotides exhibited two binding phases: the first, a quick phase (<100 ms) forming initial encounter complex, followed by a slow refolding event (~seconds long). This suggests that assembly takes place in two stages—an early step, wherein concurrent nucleation leads

to multiple early pathways, and a later step, wherein assembly is thought to be cooperative and take place by protein binding-induced RNA folding (111, 112). Different subsets of nucleotides that were contacted by the same ribosomal protein exhibited different rates of protection, implying that some RNA folding events follow the formation of an initial encounter complex with a ribosome protein (110).

In order to characterize the mechanism of 30S assembly, the Williamson lab (98) monitored the binding rates and activation energies of all the 30S ribosomal proteins simultaneously, using matrix-assisted laser desorption/ionization mass spectrometry. Again, there was no evidence of a single rate-limiting step, but rather there was an ensemble of multiple intermediate states from distinct assembly pathways. The temperature dependency of the individual protein binding rates did not reveal any changes in the rate-limiting step for any protein. Hence, the last steps of assembly are not more dependent on temperature than the earlier steps (98). In addition, the binding rates and activation energies are poorly correlated, disputing the idea that RI is the only bottleneck in the assembly pathway. If two proteins have similar binding rates but different activation energies, they face different kinetic barriers. This suggests that assembly can proceed along alternate pathways (10, 98). Recently, time-resolved electron microscopy has identified and visualized several in vitro 30S intermediates (113).

The existence of multiple pathways has also been implied from in vivo studies (105, 109). The precursors to both 30S and 50S subunits upon treatment of *E. coli* with neomycin were a heterogeneous collection of particles with similar sedimentation coefficients but different protein compositions, suggesting the existence of alternate assembly routes. Multiple parallel assembly pathways would generate a wide variety of particles that have distinct protein compositions as the particles build up in different ways (105).

Ribosomal protein S15 is a primary binding protein in the 30S that organizes a region of the central domain protein, and in vitro it is required for the subsequent binding of proteins S6, S18, S11, and S21. Interestingly, an *E. coli* strain with an in-frame deletion of the gene coding S15 is viable, although it is compromised for growth and has a cold-sensitive phenotype (109). More importantly, the secondary and tertiary binding proteins in the central domain still bind, suggesting that the strong thermodynamic hierarchy observed in vitro can be circumvented by alternate assembly pathways in vivo (109). Each of these findings suggests the potential for a series of parallel assembly pathways in the ribosome. Furthermore, the assembly intermediates, often described in earlier work, may correspond to a single prominent intermediate or a heterogeneous collection of particles of similar size and shape.

RIBOSOMAL PROTEINS DRIVE THE BINDING OF MORE RIBOSOMAL PROTEINS

The results that led to the Nomura and Nierhaus assembly maps demonstrate that ribosomal protein binding is hierarchical, but the exact mechanism by which ribosomal proteins and rRNA organize themselves remains unknown. Can assembly proceed in the absence of individual ribosomal proteins? Do some ribosomal proteins have a specific influence on the assembly pathway? Several groups have tried to determine the relationship between binding of ribosomal proteins and rRNA folding as well as the interdependencies among binding of ribosomal proteins, and these studies are summarized in the following section.

RNA-Protein Interactions in the Ribosome

The ability of the 5′ domain of the 16S rRNA to form predicted tertiary backbone contacts in the absence of proteins indicates that the initial steps of rRNA folding are driven purely by the RNA (101). Driven by base pairing, stable RNA secondary structures form easily, and

competition among variant secondary structures can result in kinetic trapping of intermediates in misfolded states. Two of the important roles that ribosomal proteins hold in the assembly process are guiding the rRNA into the proper conformations and stabilizing the native secondary structure. Footprinting studies demonstrated that the 5′ primary binding proteins S4 and S17 organize not just their binding sites but the entire 5′ domain, whereas S20 binding has a more local effect (114). Binding of the 5′ domain primary binding proteins S4, S20, and S17 preorganizes the binding site for the secondary binding protein S16, and following this, assembly passes through either a native or nonnative pair of states that are in equilibrium. Binding of protein S16 stabilizes the native conformation and destabilizes the structure of a nonnative conformation, thus directing the assembly toward the native folded state (115).

Kinetic Dependencies of 30S Proteins

To elucidate the binding relationships between the ribosomal proteins during assembly, various subsets of the ribosomal proteins were prebound to 16S rRNA in vitro, and the kinetics of remaining proteins was monitored using pulse-chase quantitative mass spectrometry (116). Prebinding of the primary binding proteins resulted in a dramatic increase in binding rates for central domain proteins but smaller changes in the 3′ and 5′ domains. Prebinding of the 5′ and central domain proteins did not accelerate binding of the 3′ domain ribosomal proteins, demonstrating that there is little to no interdomain cooperativity. Interestingly, within the 3′ domain, secondary binding proteins S9, S13, and S19 all depend on prior binding of protein S7. Prebinding of S7 caused only S9 to bind faster, and the combination of S7 and S19 caused dramatic acceleration in the binding of S9, S13, and S10. All other combinations of secondary binding ribosomal proteins and S7 resulted in little or no change for the other proteins. Despite having equivalent thermodynamic dependencies according to the Nomura map, secondary binding proteins S9 and S19 differ in the extent to which they direct subsequent assembly into alternative pathways in vitro (116).

Effect of a Single Ribosomal Protein Deletion on Assembly

As described above, ribosome assembly still proceeds in vivo in the absence of S15, although both assembly and cell growth are compromised (109). Ribosomes that lack the gene *rpsT* (ribosomal protein S20) are viable but deficient in their ability to form 70S ribosomes (117, 118). *E. coli* strains with in-frame deletions of the S6 ribosomal protein are also viable and show no defects in 30S protein composition or protein level (Z. Shajani & J.R. Williamson, unpublished observations).

5S rRNA binding proteins L5 and L18 are essential, whereas a strain with a deletion of *rplY* (L25) is viable and functional but displays a slow-growth phenotype (119). In-frame deletions of ribosomal protein L33 and ribosomal protein L35 were both viable, although 50S subunit precursors accumulated (Z. Shajani & J.R. Williamson, unpublished observations). A deletion of *rpmA*, which encodes ribosomal protein L27, displays severe growth defects as well as deficiencies in both the activity and assembly of the 50S subunit. In addition, a 50S precursor forms that is deficient in L16, L20, and L21 (120). A mutant with a defective *rpmB,G* operon (which codes for L28 and L33) is unable to form complete ribosomes, and 50S precursors accumulate and sediment at 47S. These precursor particles lack proteins L28 and L33 and have reduced amounts of ribosomal proteins L16, L25, and L27 (121). Although most ribosomal proteins are essential, several proteins appear to be dispensable for ribosome assembly and translation. Nevertheless, these nonessential ribosomal proteins can still convey a selective advantage in growth rate and are important for efficient assembly and protein synthesis capacity.

RIBOSOMAL COFACTORS AND ASSEMBLY

A large RNA can easily form multiple distinct secondary structures, and as its length increases, so does the number and stability of possible nonnative structures. Many of these local misfolded rRNA regions are quite stable, and such misfolding is thought to be one reason why the assembly process is significantly slower in vitro than in vivo. Ribosome assembly factors could potentially help limit the folding landscape and the number of alternate conformations by facilitating proper rRNA folding and protein-RNA interactions, and additionally, these factors may serve as sensors of checkpoints during the assembly process. Assembly factors are especially important for assembly of the more complex 50S subunit. A summary of the effects of deletion or overexpression of various ribosomal assembly cofactors is listed in **Table 2**.

DEAD-Box Proteins

DEAD-box proteins are a large family of RNA helicases that are conserved from bacteria and viruses to humans (122) and characterized by a core of ~350 amino acids, each containing at least 9 conserved amino acid motifs, one of which is D-E-A-D box (122, 123). Studies in vitro have demonstrated that they possess RNA-dependent ATPase activity. DEAD-box proteins and the related family DExD/H are believed to have multiple roles in ribosome biogenesis, ranging from helicase activity, where they unwind local RNA secondary structures, to acting as RNA chaperones by assisting proper RNA folding or rearranging or dissociating RNA-protein interactions (124–126). DEAD-box proteins are essential for eukaryotic ribosome assembly, but in *E. coli*, they are often dispensable under normal growth conditions. The *E. coli* ribosome has been associated with

Table 2 Effects of assembly cofactor deletion and overexpression[a]

Protein	Subunit, rRNA or particle that protein cofactor associates with	Overexpression of protein suppresses defects in	Deletion or depletion of protein		
			Precursor	Immature 16S rRNA	Immature 23S rRNA
$CgtA_E$	30S, 50S,16S, and 23S rRNA	Δ*rrmJ*	40S (high salt)	Increase	Increase
CsdA	40S	Δ*srmB*	40S		Increase
DbpA	Fragment of 23S rRNA				
Der	YihI and 50S	Δ*rrmJ*	21S, 32S, 45S	Increase	Increase
DnaK-DnaJ				Increase	
Era	16S rRNA and 30S	Δ*rbfA* and Δ*rsgA*		Increase	
GroEL-GroES		Δ*dnaK*/Δ*dnaJ*			
KsgA		Mutant of Era			
RbfA	30S	Δ*rimM*		Increase	
RhlE	70S	Δ*deaD*			
RimJ	Pre-30S	S5(G28D) mutation			
RimM	30S			Increase	
RimP	30S			Increase	
RrmJ			40S		Increase
RsgA	30S			Increase	
SrmB	40S		40S	Increase	Increase
YihI	Der				

[a]Blank space, currently unknown or no data available.

five members of the DEAD-box helicase family (SrmB, CsdA, DbpA, RhlE, and RhlB) of which the first four have been implicated directly in ribosome biogenesis (106, 107, 127–129).

The DEAD-box protein SrmB is a multicopy suppressor of a temperature-sensitive mutant of the gene that encodes ribosomal protein L24 (130). Deletion of the *srmB* gene causes a slow-growth phenotype at low temperatures (<30°C) and an altered sucrose gradient ribosome profile compared to wild-type strains. Strains missing the SrmB protein factor have fewer 70S particles, an increased number of free subunits, as well as 50S precursor particles that sediment at approximately 40S (107). These changes in the ribosome profile become more significant as the temperature decreases but are minor at 37°C (128). SrmB associates with the 40S particle, but not with 30S and 50S subunits or 70S particles. Precursor particles exhibited reduced amounts of ribosomal proteins L6, L7/L12, L14, L16, L25, L27, L31, L32, and L33, and they were missing ribosomal proteins L13, L28, and L34 (107). Ribosomal protein L13 is essential for the formation of the first 50S in vitro intermediate (55), hence, SrmB is thought to have a role in the early steps of ribosome biogenesis (**Figure 5*a***) (107, 131). Further evidence supporting this comes from recent studies that indicate that SrmB forms a quaternary complex with the ribosomal proteins L4 and L24, which are also essential for the first 50S in vitro intermediate, and a fragment from the 5′ end of 23S rRNA that contains the binding site for L4 and L24 (131).

The composition of the 40S particle is different from the composition of wild-type in vivo precursors (23, 69, 103), implying that assembly of the 40S particle occurs along an alternate pathway (107) or that the 40S particle is a misassembled 50S subunit. The 40S particle contains immature 23S rRNA; interestingly, the 30S particle contains the precursor 16S rRNA. Processing of both the 23S and 16S rRNA is impaired, although 5S rRNA processing is not. The effect on 30S subunits is considered to be indirect; final maturation is thought to take place in polysomes, and the incorporation of 30S particles into 70S particles and polysomes is impaired owing to the defects in 50S biogenesis that lead to a shortage of properly assembled 50S subunits (107).

The cold-shock DEAD-box protein A (CsdA or DeaD) is a multicopy suppressor of a temperature-sensitive mutant of the ribosomal protein S2 (132) and is required for growth at temperatures below 30°C (133). Deletion of the

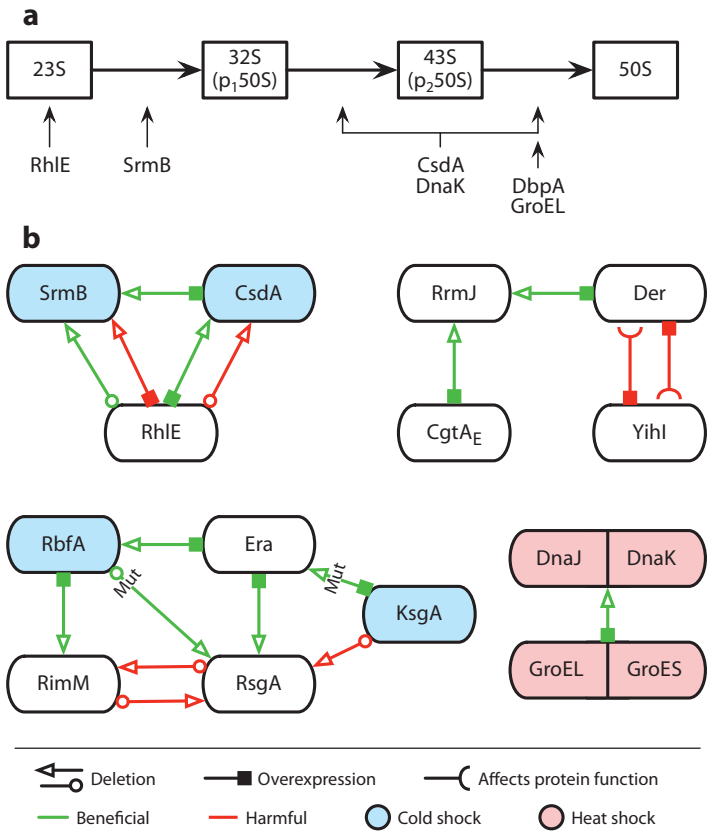

Figure 5

Ribosome assembly cofactors. (*a*) A schematic of 50S assembly in vivo, indicating the approximate point at which various cofactors are believed to act. (*b*) Cofactor interaction networks. Overexpression or deletion of cofactors can have enhancing or suppressing effects on other cofactors. A shaded box indicates overexpression, whereas open circles or arrows indicate deletion. Effects of a double deletion are indicated by both an open circle and open arrow. A mutation is indicated with the label Mut. An effect on a protein's function is indicated by a semicircle. Green arrows indicate effects that are beneficial to *E. coli* (suppression of deletion), and red arrows indicate effects that are harmful (enhancement of deletion, suppression of protein function). Blue shaded cofactors are known cold shock proteins, pink shaded cofactors are known heat shock proteins.

deaD gene leads to a slow-growth phenotype at low temperatures and an increased accumulation of unassociated subunits, a decrease in the amount of polysomes (133) compared to wild-type strains, and the emergence of 50S precursor particles that sediment at approximately 40S (127, 134). The growth rate of this strain slows as the temperature decreases, although there is only a small difference in growth rate and ribosomal profile between the strains at 37°C (127, 128, 133, 134). The 40S precursor particles are inactive but are able to slowly mature into 70S particles (134).

CsdA associates with the pre50S particles, and all of the detectable large subunit ribosomal proteins are present in the precursor, but in varying amounts. Ribosomal proteins L9, L10, L14, L17, L21, L22, L23, and L30 were present at intermediate levels, and ribosomal proteins L6, L16, L25, L28, L32, L33, and L34 were present at very reduced levels, implying that the particles are either heterogeneous or loosely assembled (127). The composition of the 40S particle is different from the composition of wild-type in vivo precursors (23, 69, 103) and from the *srmB* deletion strain (107). Most of the ribosomal proteins that are present in reduced amounts in the precursor particles are assembled at a late stage in the assembly process, suggesting that the role of CsdA in 50S biogenesis takes place at a later stage than SrmB (**Figure 5*a***) (127), although it is possible that CsdA also participates in multiple assembly steps (134). Overexpression of CsdA corrects the *srmB* deletion defect (**Figure 5*b***), but the phenotypes for the *srmB* and *deaD* deletions are not additive, which suggests that when they are expressed at normal levels SrmB and CsdA are involved in different stages of 50S biogenesis. When overexpressed, it has been suggested that the specificity of CsdA is relaxed so that it can replace SrmB (127). The 40S particles are composed of 23S rRNA with immature 5′ ends; however, the 23S rRNA in the polysomes is mature (127, 134). As with the SrmB deletion strain, 5S rRNA is correctly processed and present in normal amounts in the CsdA deletion strain (127).

At temperatures below 30°C, CsdA and SrmB are essential for proper cell growth and cellular function; cells deficient in these proteins exhibit severe ribosomal defects. As the temperature decreases, the thermal energy available for rRNA fluctuations also decreases, and rRNA is more likely to be trapped in misfolded states. CsdA and SrmB are thought to facilitate escape from these misfolded states, although the molecular basis of this escape is still unknown.

Overexpression of *rhlE* partially suppresses the growth defect in Δ*deaD* strains (129, 135, 136) but has an additive effect on Δ*srmB* strains, causing a substantial (threefold) increase in the doubling time. At temperatures below 20°C, a mutant strain containing the double-deletion Δ*deaD*Δ*rhlE* grew slower and displayed an increased number of 50S precursor particles than the single-mutant strain Δ*deaD*, implying that the absence of both proteins has a negative effect on ribosome assembly. At the same temperature, the double-deletion Δ*srmB*Δ*rhlE* grew faster and had less precursor particles than the single-mutant strain Δ*srmB* (**Figure 5*b***) (129). On the basis of these results, a model was proposed where the presence/absence of the RhlE protein shifts the equilibrium precursor forms of 23S rRNA to one of two pathways, one where CsdA matures the 50S subunit and an alternate pathway where SrmB matures the 50S subunit. In this model, the concentration of RhlE would determine the predominant conformation for the precursor. At low levels, the conformation requiring CsdA is dominant, but at high levels, the conformation that requires SrmB is dominant (129). This model does not provide a mechanism of how overexpression of CsdA corrects Δ*srmB* defects, and the actual interplay between these proteins is likely to be significantly more complex. The effect of the interactions between all of the DEAD-box proteins and their effects on assembly adds another layer of complexity. At 25°C, strains with deletions of Δ*deaD*Δ*srmB* plus one of the other three DEAD-Box genes were found to have additive effects on growth rate, but the largest effect on growth rate was

the absence of *deaD*. A quintuplet mutant of Δ*deaD*Δ*srmB*Δ*dbpA*Δ*rhlE*Δ*rhlB* had the greatest defect but was only 30% slower than the Δ*deaD* mutant (128).

DEAD-box protein A (DbpA) is an ATP-dependent helicase that is activated by a specific sequence (helix 92 in 23S rRNA) to stimulate unwinding (137, 138). Deletion of *dbpA* does not result in a growth defect (134), but an active site mutant, *dpbA R331A*, yields a dominant slow-growth phenotype when overexpressed in a wild-type background that is defective in ribosome assembly (139). The ribosome profile displays increased amounts of 30S and 50S subunits, decreased amounts of 70S particles, and a 50S precursor that sediments at 45S. It appears as if the presence of inactive DpbA leads to defects in assembly, although the mechanistic details of its role are still unknown. DbpA is thought to have a role during the late stages of ribosome assembly (**Figure 5*a***) (106).

Chaperones and Maturation Factors

The chaperones DnaJ and DnaK are part of the heat shock protein 70 (HSP 70) chaperone machine DnaK-DnaJ-GrpE. In the assembly process, DnaJ, DnaK, and GrpE are cochaperones (140). At temperatures below 30°C, strains with a deletion of the *dnaJ* or *dnaK* gene exhibit impaired growth rates compared to wild-type strains and little or no assembly defects. Ribosome biogenesis was partially defective at intermediate temperatures. At temperatures above 42°C, assembly halts, and 30S precursor particles accumulate that sediment at 21S, and two 50S precursor particles accumulate that sediment at 32S and 45S. These particles were able to mature slowly into complete ribosomal subunits, suggesting that they are authentic precursors (78).

Assembly defects can be partially overcome by overexpression of GroEL-GroES heat shock proteins at temperatures up to 42°C (78, 140), suggesting overlapping functions between DnaK-DnaJ-GrpE and GroEL-GroES (**Figure 5*b***). GroEL is required for the conversion of 45S to 50S subunits in these mutants, but it does not appear to be involved in 30S assembly (140). Overall, DnaK-DnaJ-GrpE are implicated in the later steps of ribosome assembly (**Figure 5*a***) (140, 141). The mechanism by which DnaK-DnaJ-GrpE facilitates ribosome assembly is undetermined, although a direct effect on 30S subunits has been suggested (142, 143) and debated (144). Analysis of the 30S subunit rRNA component determined that the rRNA was mostly composed of precursor 16S rRNA (17S rRNA), with a minor smaller species of a different 16S rRNA precursor that is unprocessed/partially processed at the 5′ end and unprocessed at the 3′ end (78).

Ribosome-binding factor A (RbfA) is a cold shock protein (145) essential for cell growth at low temperatures (133, 146). RbfA interacts with the 5′ terminal helix region of the 16S rRNA, and overexpression of RbfA suppresses the cold-sensitive dominant phenotype of the 16S rRNA C23U mutation (146). Cells with this mutation have decreased polysome levels and accumulate unassociated 30S and 50S subunits. RbfA has been found to associate with free 30S subunits but not with 70S or polysomes (146). RbfA is induced upon cold shock, and cells lacking RbfA are unable to adapt to low temperatures, are cold sensitive, and have impaired growth rates and altered ribosome profiles. The ribosome profile is nearly identical to what is observed in the 16S rRNA C23U mutation, where unassociated 30S and 50S subunits accumulate and fewer polysomes are present (146). Analysis of the 16S rRNA determined that the Δ*rbfA* strain contained more 16S precursor rRNA than wild-type strains. As the temperature decreases, both the amount of 16S precursor rRNA and ribosome profile defects increased (147). The Δ*rbfA* knockout strain is partially suppressed by overexpression of the essential GTPase Era (**Figure 5*b***) (148). RbfA is thought to be a late maturation factor that is essential for aiding efficient processing of 17S rRNA to 16S rRNA. It has been suggested that RbfA may also be involved in maturation after the formation of 16S rRNA because it binds to the 5′ terminal helix region of 16S rRNA, a helix that is found only in mature 16S rRNA (149).

Ribosome maturation factor M (RimM) is also essential for aiding efficient processing of 16S rRNA as deletion of *rimM* leads to an accumulation of precursor 16S rRNA, but it is not the final unknown 3′ end processing enzyme (149). Overexpression of RbfA partially suppresses the slow growth of the Δ*rimM* strain (**Figure 5*b***) but only causes a slight increase in the amount of mature 16S rRNA (150). As with RbfA, RimM associates only with the free 30S subunits, and the ribosome profile of the knockout strain Δ*rimM* shows the accumulation of unassociated 30S and 50S subunits and a depletion of polysomes compared to wild-type strains (149). RimM binds to ribosomal protein S19 and is thought to be involved in the maturation of the 3′ region, encompassing 16S rRNA helices 31 and 33b and ribosomal proteins S13 and S19. It has been suggested that its role in 30S biogenesis might be facilitating the interactions of S13 with helix 31 and S19 with helix 33b (149). During in vitro binding studies, the preincubation of RimM with 16S rRNA caused an acceleration in binding rates for the 3′ domain ribosomal proteins S9, S19, S10, and S3 but, surprisingly, a decrease in the binding extent for ribosomal protein S13 (102).

RimP (formerly known as YhbC or P15a) is encoded on the same operon as RbfA and is important for maturation of 30S subunits (151). The RimP protein was found to associate with free 30S subunits. In strains devoid of RimP, immature 16S rRNA accumulated, and the ribosomal profile displayed fewer polysomes and an increased amount of unassociated 30S and 50S subunits compared to wild-type strains (151). These differences were exacerbated as the temperature increased. During in vitro binding studies, the preincubation of RimP caused the binding rates of the 5′ domain ribosomal proteins S5 and S12 to accelerate along with almost all of the 3′ domain proteins (S9, S3, S7, S10, S13, and S14) (102).

The RimJ protein is a high-copy suppressor of a cold-sensitive phenotype associated with an S5 (G28D) mutation, which causes an increase in unassociated 30S and 50S subunits, a decrease in 70S ribosomes, and the formation of a 30S precursor (152). Overexpression of RimJ partially suppresses these growth defects, whereas deletion of *rimJ* exacerbates the effects of the S5(G28D) mutant. The RimJ protein associates with pre-30S subunits, but not with the 30S and 50S subunits or 70S particles, and is thought to bind to the precursor at an intermediate stage that occurs after binding of the 5′ primary binding ribosomal protein S4 but before binding of the 3′ tertiary binding ribosomal protein S3 (152).

KsgA modifies two adjacent adenosines (A1518 and A1519) in the 3′ terminal helix of 16S rRNA (153). These modifications are two of three modifications that are conserved throughout the three kingdoms and present in nearly all known ribosomes. Interestingly, archaeal and eukaryotic orthologs of KsgA are able to methylate *E. coli* rRNA both in vivo and in vitro, demonstrating that KsgA itself is also universally conserved (154). Deletion of *ksgA* results in a cold-sensitive phenotype, an altered ribosome profile, processing defects in 16S rRNA, and a decrease in cell growth rate at 25°C that becomes more prominent as the temperature decreases (155). The ribosome profile of the Δ*ksgA* strain had more unassociated small subunits and virtually no polysomes compared to the parent strain. At 37°C, overexpression of the KsgA protein has a negative effect on cell growth; however, at low temperatures, overexpression of the KsgA protein decreased the doubling time of the knockout strain, but also led to an increased relative amount of unassociated small subunits in the ribosomal profile. It has been suggested that these two pieces of data can be reconciled in the model where induction of KsgA rescues the defect of the knockout strain but inhibits the incorporation of the small subunit into 70S ribosomes. When KsgA is overexpressed and present in concentrations above its basal levels in Δ*ksgA* and parent strains, more precursor 16S rRNA accumulates in both Δ*ksgA* and parent strains, implying that proper processing of 16S rRNA stalls (155).

A methyltransferase-inactive form of the KsgA protein is more deleterious for cell

growth than the absence of the KsgA protein (155). Overexpression of the catalytically inactive KsgA protein resulted in greater alterations in the ribsome profiles of both the wild-type and Δ*ksgA* strains than overexpression of *ksgA*, causing a dramatic increase in the proportion of unassociated small subunits. Both the active and inactive forms of the KsgA protein associated with the free small subunits, but the inactive form bound in much greater amounts, implying that methylation is required for the release of KsgA. A model was proposed whereby methylation activates the release of the KsgA protein, allowing for assembly to proceed and final 16S rRNA maturation to take place (155). The KsgA protein thus acts as a regulator for ribosome biogenesis by limiting maturation of immature 30S subunits and restricting the entry into fully formed 70S ribosomes. It has been suggested that the role of this methylation in KsgA function and its implications for biogenesis may explain the strict conservation of this particular rRNA modification (155).

GTPases

GTPases regulate a range of cellular processes, and several are implicated in the biogenesis of individual subunits. The role of GTPases in ribosome assembly has recently been well reviewed (33, 34), and readers are referred to those reviews for an additional perspective.

The *E. coli* Ras-like protein (Era) is a highly conserved, essential GTPAse that specifically binds to both 16S rRNA and 30S ribosomal subunits in vitro (156). Depletion of Era causes a loss of polysomes, the accumulation of precursor 16S rRNA, and an increase in the relative amount of 30S and 50S subunits compared to 70S ribosomes (148). A cold-sensitive mutation of Era can be suppressed by overexpression of KsgA (**Figure 5*b***) (157). During in vitro binding studies, preincubation of Era caused a twofold increase in the binding rate of ribosomal proteins S9, S11, S5, and S12, as well as subtler increases in 3′ domain proteins S7, S10, S13, S14, and S19 (102). These effects show that the addition of Era does not appear to facilitate the binding of particular proteins, and it has been speculated that Era may have a more general role in assembly by aiding a global maturation event (102).

The ribosome small subunit-dependent GTPase A (RsgA, formerly YjeQ) is a nonessential GTPase whose activity is extensively enhanced by the 30S subunit and 70S ribosomes, but not the 50S subunit (158). Only a small amount of RsgA associates with the 30S subunit or the 70S ribosome without the presence of guanine nucleotides, GTP or GDP. In the presence of a nonhydrolyzable analog of GTP (GMP-PNP), most of the ribosome dissociates, and RsgA cosediments with the 30S subunit (158). It appears that the GTP form of RsgA can bind to the 30S subunit and then induce a conformational change that causes the ribosome to dissociate into subunit components (159). Deletion of RsgA results in a slow-growth phenotype with an altered ribosome profile, where most of the 70S ribosomes dissociate into 30S and 50S subunits. Deletion also leads to the accumulation of the 16S precursor rRNA (159). Interestingly, either the deletion of RsgA or inactivation of its GTPase activity gives *E. coli* a resistance to high salt stress. High salt stress, in turn, suppresses both the assembly and processing defects (160). Overexpression of Era partially suppresses the slow-growth phenotype, causing an increase in the amount of 70S ribosomes and a reduction in the amount of free subunits (161). Overexpression of various mutants of *rbfA* (162) suppresses the defects in the RsgA deletion strain, but overexpression of *rbfA* does not. These mutations allowed RbfA to be released from the 30S subunit without RsgA (162). Deletion of RimM and KsgA enhances the effects of the RsgA deletion (161).

The double-Era-like GTPase (Der) is an essential *E. coli* GTPase that contains two GTP-binding domains located at the N-terminal regions (163). Homologs of Der are conserved in eubacteria but not in archaea or eukaryotes. At 30°C, both domains are important for function, but at 42°C, either domain is expendable (164). Der interacts with the 50S in presence of a nonhydrolyzable GTP analog but not in

the presence of GDP and plays a role in 50S ribosome maturation.

Depletion of Der leads to both ribosomal profile and rRNA maturation defects. Ribosome profiles exhibit a reduction in polysomes and monosomes and an increase in 30S and 50S subunits, whereas precursors of both 23S rRNA and 16S rRNA accumulate. The 16S rRNA is in the form of 17S rRNA, and the 23S rRNA has an extra seven bases at the 5′ end and possibly an unprocessed 3′ end. No 5S precursors have been detected (164). When Der-depleted cells are subject to low-magnesium conditions, a 50S precursor accumulates that has significantly reduced amounts of ribosomal proteins L9 and L18 (164). Overexpression of Der rescues the slow-growth phenotype of Δ*rrmJ* (**Figure 5*b***) (165). RrmJ causes methylation of U252 in 23S rRNA, and deletion of *rrmJ* destabilizes 70S ribosomes, leading to an accumulation of 30S and 50S subunits. Der is thought to overcome the destabilizing effects of the absent modification by stabilizing the 70S ribosome (165).

Recently it was determined that the protein YihI associates with Der at a stochiometric ratio of 1:1 and activates its GTP activity (166). YihI appears to act as a regulator of Der, whereby overexpression of YihI inhibits the cellular function of Der (**Figure 5*b***), and inhibits the assembly of the 50S subunits, which causes the accumulation of partially unprocessed 16S rRNA and 23S rRNA and a 50S precursor that contains reduced levels of L9, L18, and L25. Overproduction of Der reduces the effects of YihI overexpression (166). A model was proposed whereby YihI associates with Der in lag phase, causing Der to dissociate and resulting in incomplete 50S subunits. At its exponential phase, growth of YihI is suppressed, and GTP-bound Der is free to complete the assembly of the 50S subunit (166).

$CgtA_E$ is part of a family of highly conserved GTPases. It binds to both 30S and 50S subunits in the presence of GMP-PNP. In vitro purified $CgtA_E$ also cosediments with 16S rRNA and 23S rRNA in the presence of GTP and interacts with several ribosomal proteins (S3, S4, S5, S13, S16, L2, L4, L16, and L17) as well as the DEAD-box protein CsdA (167). Depletion of $CgtA_E$ leads to a decrease in polysomes and 70S particles, an increase in 30S and 50S subunits, and defects in the processing of 16S rRNA and 23S rRNA (167, 168). In the 50S subunit, several late-binding ribosomal proteins were absent or present at reduced levels (L16, L33, L34), implying that the subunit is immature and that $CgtA_E$ has role in the assembly of the 50S subunit. In addition, as with Der, overexpression of $CgtA_E$ rescues the slow-growth phenotype Δ*rrmJ*, overcoming the destabilizing effects of the absent modification by stabilizing the 70S ribosome (**Figure 5*b***) (165). Furthermore, in both a Δ*csdA* strain and Δ*srmB* strain, $CgtA_E$ binds only to the 50S subunit and not the 50S-precursors, suggesting that it has a role in the later assembly steps (168).

CONCLUDING REMARKS

The assembly of the ribosome has been studied for over half a century, and work from several decades ago remains of central importance today. Going forward, it is both important and necessary to synthesize this early research with ongoing studies of increasing sophistication and complexity to form a global view of ribosome assembly.

SUMMARY POINTS

1. The ribosomal intermediates found in vitro are not identical to the ones found in vivo.
2. Ribosome assembly likely takes place through multiple parallel pathways with a number of assembly intermediates.

3. Both rRNA and ribosomal proteins direct the assembly pathways that are populated.
4. In vivo ribosome assembly can proceed even in the absence of particular ribosomal proteins.
5. Both 30S and 50S subunits are assembled with the help of a diverse set of interconnected factors that increase the rate of ribosome assembly under specific conditions.

FUTURE ISSUES

1. The relevance of the body of in vitro work to assembly in vivo needs to be determined.
2. Investigations should be undertaken to determine the number and composition of in vivo assembly intermediates and how they relate to parallel assembly pathways.
3. Investigators should determine the identity of the final rRNA processing enzymes and when each of the rRNA processing steps takes place.
4. The roles of assembly cofactors need to be studied in more detail, including determining the precise molecular mechanism of each cofactor and the interactions between them.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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