REPORT

Staphylococcus aureus domain V functions in *Escherichia coli* ribosomes provided a conserved interaction with domain IV is restored

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ABSTRACT

Domain V of *Escherichia coli* 23 S rRNA (residues 2023–2630) was replaced by that from *Staphylococcus aureus*, thereby introducing 132 changes in the rRNA sequence. The resulting ribosomal mutant was unable to support cell growth. The mutant was rescued, however, by restoring an interaction between domains IV and V (residues 1782 and 2586). Although the importance of this interaction, U/U in *E. coli*, C/C in *S. aureus*, is therefore demonstrated, it cannot be the only tertiary interaction important for ribosomal function as the rescued hybrid grew more slowly than the wild type. Additionally, although the single-site mutations U1782C and U2586C in *E. coli* are viable, the double mutant is lethal.

Keywords: hybrid ribosomes; large ribosomal subunit; pathogenic bacteria; peptidyl transferase center; ribosomal RNA

INTRODUCTION

The notion that ribosomal RNA (rRNA) folds into discrete domains first emerged during the early studies on the assembly of ribosomal subunits (Nomura & Held, 1974; Röhl & Nierhaus, 1982). This idea was extended by the development of the maps of the secondary structures of rRNAs from all kingdoms of life (Noller & Woese, 1981; Gutell et al., in prep.), and was very recently confirmed directly in the atomic-level crystal structures of both subunits (Ban et al., 2000; Wimberly et al., 2000). It is strikingly evident, most particularly in the 50S subunit, that the vast majority of interactions involving two or more hydrogen bonds occur within, rather than between, domains. Of course, not all these interactions are of functional significance; indeed, the majority may be associated chiefly with assembly or even, simply, result from proximity. On the other hand, interactions between domains and even between subunits may be crucially important in long-range signaling during the

elongation cycle. One way to explore which interdomain interactions are crucial for ribosome function is to replace domains in *Escherichia coli* with those from other organisms and to ask what functions, if any, the resulting hybrid particles possess.

A number of studies over the years, both in vitro (Nomura et al., 1968; Higo et al., 1973) and in vivo (Asai et al., 1999), have indicated that ribosomes composed of RNA from one organism and proteins from another can be assembled and are functional. However, the successful replacement of a complete structural domain of rRNA has not previously been reported in any organism. The closest approach has been to make chimeric rRNAs containing foreign GTPase centers. The replacement of the GTPase center in yeast with that from *E. coli* resulted in functional hybrid 60 S subunits (Musters et al., 1991). Equally, the reverse exchange, the replacement of the L11 binding region in domain II of *E. coli* with that from yeast, yielded ribosomes that were active both in vivo and when assayed in vitro for their ability to support EF G-dependent GTP hydrolysis (Thompson et al., 1993). The same rRNA region was also successfully transferred from each of the developmentally controlled types of rRNA in the

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human malarial parasite, *Plasmodium falciparum*, into yeast (Velichutina et al., 1998). A probable reason for the success of these exchanges, aside from the homologies between elongation factors, is that this region of the ribosome occupies a discrete and largely autonomous site in the 50S subunit, readily visualized in cryoelectron micrographs (Gabashvili et al., 2000). Indeed, the complex of protein L11 and the residues to which it binds has been crystallized and its structure resolved at the atomic level (Conn et al., 1999; Wimberly et al., 1999). The potential barriers to replacing a whole domain in the 50S subunit, even between closely related species are, however, much more severe. For example, a substantial number of proteins must recognize and fold the RNA correctly; the RNA itself must be able to interact correctly with the other domains in the subunit; the ribosomal factors must be able to recognize and effect their changes not only during the elongation cycle itself but also at initiation, termination and recycling of the subunits. In the present study, therefore, the conservative decision was made to construct a hybrid between bacterial species.

Domain V, a critical functional region of the ribosome, was chosen for the exchange. Over 30 years of genetic, biochemical and crosslinking studies have demonstrated that the peptidyl transferase center lies at the heart of this region of 23 S rRNA, at the highly conserved junction of four helices (for a compilation of the evidence, see Garrett et al., 2000). *Staphylococcus aureus* was selected as the donor organism for the domain V exchange because of its almost complete similarity in secondary structure to that of *E. coli* in this region (Gutell web site <http://www.rna.icmb.utexas.edu>); indeed, in the precise region chosen for the cloning, of the total of 607 residues to be replaced, there are only 132 differences between the primary sequences (bold lettering in Fig. 1). Additionally, and almost certainly of importance for the viability of the hybrid ribosomes, phylogenetic analyses suggest *S. aureus* rRNA has relatively few interdomain interactions differing from those in *E. coli*. One which is of particular interest is the covariation between residues 1782 (in domain IV) and 2586 (in domain V). This 1782/2586 noncanonical pairing was proposed in some of the earliest models and has withstood the addition of hundreds of new sequences to the database (Gutell & Woese, 1990; Gutell web site <http://www.rna.icmb.utexas.edu>). In *E. coli*, these two residues are both uridines; in *S. aureus*, and in the majority of other bacteria, these two residues are both cytidines. To explore the contribution of this and other, less obvious, interdomain interactions, we constructed the chimeric 23S rRNA and explored the effects of the mutations on cell viability.

Additionally, *S. aureus* was chosen for the hybrid construction because of its pathogenicity and, particularly, in the context of the rapid emergence of antibiotic-resistant strains of serious clinical impact (Plouffe, 2000).

The possibility of being able to direct drug development and testing to specific targets in the ribosome, without having to employ a serious pathogen for screening, would be of obvious advantage.

RESULTS AND DISCUSSION

Replacement of domain V of *E. coli* 23 S rRNA with that from *S. aureus*

The broad strategy for the replacement of an entire domain involved the creation of two unique restriction sites at either end of domain V in *E. coli* by site-directed mutagenesis and their inclusion in the primers used to amplify the *S. aureus* domain V by PCR. The chimera was then constructed by direct cloning. A *SpeI* site (recognition sequence 5'-ACTAGT-3') was created at the 5' end of the domain by mutation of residue C2023 to A and residue C2025 to U. At the same time, residue G2630 at the 3' end was mutated to C, creating a *XhoI* site (recognition sequence 5'-CTCGAG-3'). For completeness, and to assist in resolving whether any of the mutations introduced during the construction of the hybrid might contribute to its inviability, each of the two mutations was also created separately. Domain V of *S. aureus* was then amplified by PCR, with primers containing *SpeI* and *XhoI* sites at the 5' and 3' ends, respectively. Finally, domain V was excised from the *E. coli* sequence and replaced by the *S. aureus* amplified fragment by double restriction digestion and ligation. Verification of the accuracy of the amplification and of the final cloning step was made by complete sequencing of the insert and extensive analytical restriction digests.

Viability of cells carrying the mutant rRNAs

To test the ability of the mutant rRNAs to support growth, advantage was taken of a strain of *E. coli* lacking all seven chromosomal rRNA operons and expressing rRNA from a plasmid-borne, wild-type operon ($\Delta 7prn$). The strain carries a recessive streptomycin-resistance mutation in S12 (*rpsL*), but the resident *rrn* plasmid also carries a wild-type S12 gene, thereby conferring dominant streptomycin sensitivity. Replacement of the resident *rrn* plasmid by one lacking *rpsL* results in streptomycin resistance. Consequently, any of the mutated plasmids that are capable of displacing the resident *rrn* plasmid, and supplying the total rRNA requirements of the cell, can support growth on streptomycin-containing medium (O'Connor et al., 2001). Using this approach, we were able to show that not only was the strain containing the *XhoI* mutant (G2630C) viable, but that its doubling time was almost indistinguishable from that of the strain carrying the wild-type *rrn* plasmid (Table 1, step 2). We conclude, therefore, that despite the high conservation of identity of this residue in bacteria

FIGURE 1. Secondary structure of domain V in 23S rRNA (Gutell web site <http://www.rna.icmb.utexas.edu>). The structures for *E. coli* and *S. aureus* are superimposable; residues in bold are those in the *S. aureus* sequence that differ from those in *E. coli*. The solid line indicates the covariation with residue 1782 in domain IV, a part of which is shown with the *E. coli* sequence and structure. Dotted lines indicate residues interacting with at least two hydrogen bonds to the residues indicated. Large arrows indicate the junctions between the *E. coli* and *S. aureus* sequences. The residue at position 2630, shown as an italic C, was mutated to generate the *Xho*I cloning site but was a silent mutation and was therefore not repaired.

TABLE 1. Growth rates of strains carrying the various plasmids used in the construction of the *Escherichia coli*/*Staphylococcus aureus* domain V chimerae.

Step	Characteristics of plasmids created during the chimerae construction	Doubling time (min)	Residue 1782 (Domain IV)	Residue 2586 (Domain V)
	<i>E. coli</i> wild type (plasmid pLK35)	70.7 ± 0.9	U	U
1	Create <i>SpeI</i> site at 5' end of domain V (mutations C2023A and C2025U)	NV	U	U
2	Create <i>XhoI</i> site at 3' end of domain V (mutation G2630C)	75.2 ± 1.5	U	U
3	Combination of <i>SpeI</i> site at 5' end plus <i>XhoI</i> site at 3' end of domain V (mutations C2032A and C2025U plus G2630C)	NV	U	U
4	Exchange <i>E. coli</i> for <i>S. aureus</i> domain V (<i>SpeI</i> - <i>XhoI</i> fragment exchange)	NV	U	C
5	Repair disrupted <i>E. coli</i> / <i>S. aureus</i> 5' junction (mutations G2024C and U2025C)	NV ^a	U	C
6	Clone in mutation U1782C (domain IV)	138.7 ± 6.2	C	C

NV: Not viable in liquid culture.

^aPin-prick colonies appear on plates after 2–3 days at 37 °C.

(87% G), the G2630C mutation has little effect on ribosome function and so no further changes were made at this residue. On the other hand, the *SpeI* mutant (C2023A, C2025U) was incapable of displacing the resident *rrn* plasmid (Table 1, step 1), so it was hardly surprising that the double restriction site mutant (C2032A, C2025U plus G2630C) was also lethal (Table 1, step 3). In fact, a single-site mutation at residue C2025 (also C to U) in a wild-type background gives rise to the conditional loss of expression of erythromycin resistance at high temperature (K. Triman, pers. comm.), indicating that maintenance of the base pairing between residues C2025 and G2038 in helix 72 (see Fig. 1) is important for ribosome function. Indeed, a plasmid carrying the C2025U mutation was introduced into the $\Delta 7\text{prn}$ strain, but was incapable of displacing the resident *rrn* plasmid (data not shown), indicating that disruption of helix 72 results in an inviable mutant. Thus, it was no surprise that the straightforward exchange of the *E. coli SpeI-XhoI* fragment encompassing domain V for that amplified from *S. aureus* resulted in a lethal construct, also incapable of displacing the resident *rrn* plasmid (Table 1, step 4). The next step, therefore, was to attempt to rescue the chimera by further mutation at the 5' junction site in helix 72.

Clearly, it seemed crucial to restore residue 2025 to a C, which, in any case, is its identity in both *E. coli* and *S. aureus*. At the same time, residue G2024 was mutated to a C to restore the canonical base pairing with residue G2039. The whole of helix 72, encompassing residues A2023 to U2040 now had the identical sequence to that of *S. aureus* (Fig. 1). Thus, the 5' junction of the chimera comprised a simple splice between residues 2022 and 2023; residue U2022 and 5' of it is *E. coli* sequence, residue A2023 and 3' of it derives from *S. aureus*. At the 3' junction, the splice is at res-

idue 2630. Bases 5' of this residue derive from *S. aureus* and 3' of it is *E. coli* sequence. Residue 2630 itself is now a C, which is present in neither sequence, but which was shown to be a silent mutation. Nevertheless, the resultant mutant construct (Table 1, step 5) was still incapable of displacing the resident *rrn* plasmid from the deletion strain. Only sparse and extremely slow-growing colonies were seen on plates containing streptomycin and these did not grow either in liquid medium or after restreaking on solid medium.

The interaction between residues 1782 in domain IV and 2586 in domain V

The sequence exchange in the hybrid rRNA introduced a total of 132 changes (highlighted in Fig. 1). Close inspection of the 50S crystal structure (Ban et al., 2000) to determine which hydrogen bonds may have been disrupted by any of these changes revealed only four potential mismatches (Fig. 1). The first of these, and the only one we have tested here, is the well-documented co-identity between residues 1782 (in domain IV) and 2586. In *E. coli* the pairing is U/U and in *S. aureus*, as in two-thirds of all bacterial species, it is C/C. In the chimera, however, the pairing is U1782/C2586, a combination that has not yet been found in the current total of 1,045 sequences from all three phylogenetic domains plus mitochondria and chloroplasts in the Gutell database (<http://www.rna.icmb.utexas.edu>).

Rescue of the hybrid plasmid was achieved by changing U1782 to a C to introduce the 1782C/2586C interaction that occurs in *S. aureus* (Table 1, step 6), although our preliminary investigations had indicated such a mutation might be unsuccessful. Previously, single-site mutations in the *E. coli* sequence, either U1782C or U2586C, had yielded ribosomes that were capable of

TABLE 2. Growth characteristics of strains carrying mutations in *Escherichia coli* 23S rRNA.

Plasmid	Doubling time (min)	Residue 1782 (Domain IV)	Residue 2586 (Domain V)
pKK3535 (wild type)	56.0 ± 1.1	U	U
pKK1782C	66.3 ± 1.4	C	U
pKK2586C	78.5 ± 0.1	U	C
pKK1782C/2586C	NV	C	C

NV: Not viable in liquid culture, although pin-prick colonies appear on plates after 2–3 days at 37 °C.

supporting protein synthesis, with the U2586C mutation being more deleterious (Table 2), but to our surprise, the double mutant 1782C/2586C was lethal. However, when the U1782C mutation was introduced into the *S. aureus* chimera using the unique I-*CeuI* site located between domains II and IV, the C/C hybrid construct was able to displace the resident *rm* plasmid from the deletion strain, although restoration to wild-

type growth rates was not achieved. The converse mutation, C2586U, was not constructed as the intention was to transplant the intact domain V from *S. aureus* into *E. coli*. Mutation of any residue within this domain would consequently disrupt its integrity. Thus, *E. coli* ribosomes containing the peptidyl transferase domain V from a foreign organism, *S. aureus*, are capable of functioning sufficiently well to support cell growth with restoration of only one, noncanonical interdomain pairing. Clearly, however, additional structural distortions remain, as the growth rate of the chimera is still impaired.

The 1782/2586 interaction in *Haloarcula marismortui* is, as in *E. coli*, U/U, and is visualized in the atomic resolution crystal structure (Ban et al., 2000). The bases are coplanar and form a U•U 4-carbonyl-N3 symmetric pairing (Tinoco, 1993), with the N3 imino group of each uridine hydrogen bonding with the N4 carbonyl group of the base opposite (Fig. 2). The lethality of the C/C mutation is therefore puzzling, as both the number of hydrogen bonds and the spatial orientation of the equivalent C•C N3-amino symmetric pairing are identical to

FIGURE 2. Left: Crystal structure of the interaction of residues U1782 and U2586 and adjacent region in *H. marismortui* (Ban et al., 2000). Broken lines indicate probable hydrogen bonds. Right: Potential hydrogen bonding between U/U, C/U, and C/C pairs (Tinoco, 1993).

the original U/U combination (Fig. 2). Clearly, however, the nature of the functional groups presented to the solvent face for each pairing is directly opposite: for the U/U pair these are the hydrogen-bond-accepting carbonyl groups whereas for the C/C pair they are the hydrogen-bond-donating amino groups. The nearest residues observed in the crystal structure are A2587 and G2608, also from domain V, and identical in *H. marismortui*, *E. coli*, and *S. aureus*. There remains the distinct possibility, therefore, that other tertiary interactions may occur at this site during the elongation cycle.

Functional implications

It is clear from numerous functional studies that domains IV and V work in concert during protein synthesis (Garrett et al., 2000). Thus, it is not surprising that there would be a close structural association between these domains. Such an association has been vividly demonstrated by the atomic level structures of the 50S subunit, where domains IV and V are visualized to be intimately associated (Ban et al., 2000). What recent structures have not indicated, however, are the dynamic interactions either within or between domains. For the 2586 region of domain V, there is functional evidence to suggest that there may be more than one interacting partner (Green et al., 1997). Our studies dramatically emphasize the importance of the 1782/2586 partners and lend support to the notion that additional residues may be involved.

The other three potential mismatches, inferred from the 50S crystal structure, involve hydrogen bonding between residues 2358/835, 2622/2824, and 2489/1127. For the first two pairs, the identity of the residue in domain V (A2358 and U2622) is actually unchanged in the hybrid; the difference lies in the nature of the paired residue (A835 in *S. aureus* versus C in *E. coli* and U2824 versus C). Conversely, in the third pair, the residue outside domain V is identical between *E. coli* and *S. aureus* (A1127 in domain II) but different in the peptidyl transferase center (G2489 in *S. aureus* versus U in *E. coli*). Thus, it is less likely that alterations in hydrogen bonding between these three less highly conserved pairs of residues would contribute to the non-functioning of the chimeric ribosomes.

This is the first demonstration that the substitution of a complete ribosomal domain from one organism into the rRNA of another is feasible, and opens up the possibility of establishing in a direct manner those inter-domain interactions that are of functional significance. Additionally, protein-RNA contacts can now be investigated because, despite the introduction of well over 100 altered residues, the hybrid rRNA is still recognized by the 15 or so ribosomal proteins and translation factors (e.g., EF Tu, EF G, IF2) that interact with domain V. Importantly, the L1 binding region of *S. aureus*

domain V must be sufficiently recognizable that the protein can bind in a manner appropriate to initiate the assembly cascade of that part of the ribosome (Röhl & Nierhaus, 1982). The recognition elements crucial for protein L1 binding comprise helices 77 and 78 and some residues between them. Mutation of residues G2123, G2125, A2126, and C2174 all reduce L1 binding (Said et al., 1988). More recently, a detailed analysis of the ribose-phosphate backbone contacts with the protein have led to the proposal that helix 77 (comprising residues 2120–2124/2174–2178) binds in the cleft between the two domains of L1 (Drygin & Zimmermann, 2000). The residues of this helix are largely conserved between *S. aureus* and *E. coli* (Fig. 1), presumably, therefore, preserving the recognition site for L1 in the chimera. Unfortunately, visualizing the details of these contacts in the crystal structure is not possible as none of the protein side chain coordinates are available nor is the protein L1 binding region ordered sufficiently in the crystal for it to be modeled at atomic resolution (Ban et al., 2000). Nevertheless, the atomic structure of the 50S subunit will aid in identifying nucleotide residues that can be implicated in supporting the functional interaction between domains IV and V. Subsequent efforts will be directed towards attempting to discern which step(s) in the elongation cycle are impaired in the chimeric subunits in order to establish the functional implications of this interaction.

MATERIALS AND METHODS

Bacterial strains and plasmids

Mutations at residues U1782 and U2586 in *E. coli* 23 S rRNA were cloned into plasmid pKK3535, a pBR322 derivative containing the intact *rrnB* operon under control of the native P₁P₂ promoters (Brosius et al., 1981). All remaining constructs were cloned in plasmid pLK35 in which the *rrnB* operon is transcribed from the inducible λ P_L promoter. In the presence of the thermolabile λ cl repressor, transcription of the *rrnB* operon is induced by a temperature shift to 42 °C. The host strain for pKK3535 and derivatives was XL1. pLK35 and derivatives were maintained in *E. coli* strain pop2136 (Gregory et al., 1998), which carries the λ cl repressor on the chromosome. To test for the viability of the mutants in the absence of wild-type ribosomes, plasmids were transformed into strain MC250 (O'Connor et al., 2001), a derivative of strain TA542 in which all seven rRNA operons have been deleted/inactivated (Asai et al., 1999), containing the *rpsL121* streptomycin-resistance mutation and carrying *prnS12* (*rrnB* and *rpsL*) as the sole source of rRNA genes.

Mutagenesis

Mutations at U1782 and U2586 in *E. coli* rRNA were constructed directly in pKK3535 using the QuickChange™ kit from Stratagene. Otherwise, site-directed mutagenesis was performed essentially as described by Kunkel et al. (1991)

using an M13mp18 clone carrying the *EcoRI*-*Bam*HI fragment of plasmid pLK35, encoding the 3' half of 23S rRNA as a template. Double-stranded replicative form DNA from mutant M13 clones was digested with *Sph*I and *Bam*HI and ligated to *Sph*I/*Bam*HI/I-*Ceul*-digested pLK35. Primers were designed to introduce a *Spe*I site (primer sequence: 5'-ATCTTCACAACACTAGTTCAATTTTAC-3') at residues 2020–2025 (G2023A, C2025U) or a *Xho*I site (primer sequence: 5'-AGGAGCAGCCCCCTCAGTTCTCGAGCGCCCCACG-3') at positions 2628–2633 (G2630C). The double restriction site mutant (G2023A, C2025U plus G2630C) was constructed using both mutagenic primers simultaneously. Successful construction of the mutants was confirmed by sequencing across the mutation sites and diagnostic restriction digestion.

Replacement of *E. coli* domain V with that from *S. aureus* and further manipulation of the hybrid construct

DNA from *S. aureus* strain ATCC 33591 (kindly supplied by V. Lyamichev) was amplified by PCR using the same primer used to introduce the *Xho*I site and one complementary to that used to introduce the *Spe*I site (primer sequence: 5'-GTGAAATTGAACACTAGTTGTGAAGAT-3'). Amplification was for 35 cycles (initial denaturation: 94 °C 5 min; cycling: 94 °C 45 s, 45 °C 45 s, 72 °C 80 s; final 72 °C 10 min) using *Taq* polymerase. Amplified DNA was restricted with *Spe*I and *Xho*I and ligated to the gel-purified backbone fragment of the double restriction site pLK mutant plasmid. Successful cloning of the *S. aureus* amplified fragment to generate the chimera still carrying the *Spe*I and *Xho*I sites was confirmed by diagnostic restriction digests and then the whole of domain V (from residue 2080 to 2716) was sequenced.

Construction of a simple splice between the *E. coli* and *S. aureus* sequences at the 5' junction between residues 2022 and 2023 was achieved by mutating residues at positions 2024 and 2025 both to C by another round of site-directed mutagenesis. The *Bam*HI-*Sph*I fragment from the chimera cloned into M13mp19 was used as the starting material. Finally, mutation U1782C was introduced by cloning from the single-site mutant in pKK3535 using the I-*Ceul* site located between domains IV and V.

Growth rates

The growth rates of those plasmid constructs that were able to displace the resident plasmid *rrn* operon from strain MC250 were measured by monitoring growth at 37 °C in LB medium containing 200 µg/mL ampicillin and 60 µg/mL streptomycin in a Klett-Summerson colorimeter. Loss of the resident plasmid was confirmed by primer extension analysis of the rRNA (Sigmund et al., 1988).

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REFERENCES

- Asai T, Zaporozhets D, Squires C, Squires CL. 1999. An *Escherichia coli* strain with all chromosomal rRNA operons inactivated: Complete exchange of rRNA genes between bacteria. *Proc Natl Acad Sci USA* 96:1971–1976.
- Ban N, Nissen P, Hansen J, Moore PB, Steitz TA. 2000. The complete atomic structure of the large ribosomal subunit at 2.4 Å resolution. *Science* 289:905–920.
- Brosius J, Dull TJ, Sleeter DD, Noller HF. 1981. Gene organization and primary structure of a ribosomal RNA operon from *Escherichia coli*. *J Mol Biol* 148:107–127.
- Conn GL, Draper DE, Lattman EE, Gittis AG. 1999. Crystal structure of a conserved ribosomal protein–RNA complex. *Science* 284:1171–1174.
- Drygin D, Zimmermann RA. 2000. Magnesium ions mediate contacts between phosphoryl oxygens at positions 1222 and 1276 of the 23S rRNA and ribosomal protein L1. *RNA* 6:1717–1726.
- Gabashvili IS, Agrawal RK, Spahn CM, Grassucci RA, Svergun DI, Frank J, Penczek P. 2000. Solution structure of the *E. coli* 70S ribosome at 11.5 Å resolution. *Cell* 100:537–549.
- Garrett RA, Douthwaite SR, Liljas A, Matheson AT, Moore PB, Noller HF (eds). 2000. *The ribosome: Structure, function, antibiotics, and cellular interactions*. Washington, DC: ASM Press.
- Gregory ST, Brunelli CA, Lodmell JS, O'Connor M, Dahlberg AE. 1998. Genetic selection of rRNA mutations. *Methods Mol Biol* 77:271–281.
- Green R, Samaha RR, Noller HF. 1997. Mutations at nucleotides G2251 and U2585 of 23S rRNA perturb the peptidyl transferase center of the ribosome. *J Mol Biol* 266:40–50.
- Gutell RR, Woese CR. 1990. Higher order structural elements in ribosomal RNAs: Pseudo-knots and the use of non-canonical pairs. *Proc Natl Acad Sci USA* 87:663–667.
- Higo K, Held W, Kahan L, Nomura M. 1973. Functional correspondence between 30S ribosomal proteins of *Escherichia coli* and *Bacillus stearothermophilus*. *Proc Natl Acad Sci USA* 70:944–948.
- Kunkel TA, Bebenek K, McClary J. 1991. Efficient site-directed mutagenesis using uracil-containing DNA. *Methods Enzymol* 204:125–139.
- Musters W, Concalves PM, Boon K, Raué HA, van Heerikhuizen H, Planta RJ. 1991. The conserved GTPase center and variable region V9 from *Saccharomyces cerevisiae* 26S rRNA can be replaced by their equivalents from other prokaryotes or eukaryotes without detectable loss of ribosomal function. *Proc Natl Acad Sci USA* 88:1469–1473.
- Noller HF, Woese CR. 1981. Secondary structure of 16S ribosomal RNA. *Science* 212:403–411.
- Nomura M, Held WA. 1974. Reconstitution of ribosomes: Studies of ribosome structure, function and assembly. In: Nomura M, Tissières A, Lengyel P, eds. *Ribosomes*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 193–223.
- Nomura M, Traub P, Bechmann H. 1968. Hybrid 30S ribosomal particles reconstituted from components of different bacterial origins. *Nature* 219:793–799.
- O'Connor M, Lee W-C M, Mankad A, Squires CL, Dahlberg AE. 2001. The single dihydrouridine modification in *Escherichia coli* 23S rRNA is dispensable. *Nucleic Acids Res* 29:710–715.
- Plouffe JF. 2000. Emerging therapies for serious Gram-positive bacterial infections: A focus on linezolid. *Clin Infect Dis* 31(Suppl 4):S144–S149.
- Röhl R, Nierhaus KH. 1982. Assembly map of the large subunit (50S) of *Escherichia coli* ribosomes. *Proc Natl Acad Sci USA* 79:729–733.
- Said B, Cole JR, Nomura M. 1988. Mutational analysis of the L1

- binding site of 23S rRNA in *Escherichia coli*. *Nucleic Acids Res* 16:10529–10545.
- Sigmund CD, Ettayebi M, Borden A, Morgan EA 1988. Antibiotic resistance mutations in ribosomal RNA genes of *Escherichia coli*. *Methods Enzymol* 164:673–690.
- Thompson J, Musters W, Cundliffe E, Dahlberg AE. 1993. Replacement of the L11 binding region within *E. coli* 23S ribosomal RNA with its homologue from yeast: In vivo and in vitro analysis of hybrid ribosomes altered in the GTPase center. *EMBO J* 12: 1499–1504.
- Tinoco I. 1993. Structures of base pairs involving at least two hydrogen bonds. In: Gesteland RF, Atkins JF, eds. *The RNA world*. Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press. pp 603–607.
- Velichutina IV, Rogers MJ, McCutchan TF, Liebman SW. 1998. Chimeric rRNAs containing the GTPase centers of the developmentally regulated ribosomal RNAs of *Plasmodium falciparum* are functionally distinct. *RNA* 4:594–602.
- Wimberly BT, Brodersen DE, Clemons WM, Morgan-Warren RJ, Carter AP, Vonnrhein C, Hartsch T, Ramakrishnan V. 2000. The structure of the 30S ribosomal subunit. *Nature* 407:327–339.
- Wimberly BT, Guymon R, McCutcheon JP, White SW, Ramakrishnan V. 1999. A detailed view of a ribosomal active site: The structure of the L11-RNA complex. *Cell* 97:491–502.