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Ribosomal Proteins in the Spotlight

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ABSTRACT The assignment of specific ribosomal functions to individual ribosomal proteins is difficult due to the enormous cooperativity of the ribosome; however, important roles for distinct ribosomal proteins are becoming evident. Although rRNA has a major role in certain aspects of ribosomal function, such as decoding and peptidyl-transferase activity, ribosomal proteins are nevertheless essential for the assembly and optimal functioning of the ribosome. This is particularly true in the context of interactions at the entrance pore for mRNA, for the translation-factor binding site and at the tunnel exit, where both chaperones and complexes associated with protein transport through membranes bind.

KEYWORDS protein synthesis, ribosome, RNA, translation

BOTH PROTEIN AND RNA ARE ESSENTIAL FOR OPTIMAL RIBOSOME FUNCTION

In the 1970s, it was generally accepted that ribosomal proteins (r-proteins) constituted the functionally active part of the ribosome, whereas ribosomal RNA (rRNA) was essentially a scaffold that kept the proteins in a position for optimal functioning. By the 1980s, the pendulum had swung in the opposite direction, with r-proteins thought to be the scaffolding for the rRNA. The reason for this change was the discovery of catalytic RNAs, coupled with the impressive wealth of data showing the direct involvement of rRNAs in distinct ribosomal functions, for example, the Shine-Dalgarno interactions between mRNA and rRNA during initiation of bacterial protein synthesis, as well as the predominance of rRNA at the decoding and peptidyl-transferase centre (PTC) of the ribosome. The crystal structures of the ribosome confirmed this predominance of rRNA at these active sites, but also revealed that a number of r-proteins were located in positions of functional importance, for example, S12 at the decoding center, L11 and L10 $\times$ $(L7/L12)_4$ as components involved in translation factor binding, L4/L22 for interacting with nascent chains in the tunnel, as well as L23 as a factor docking site at the tunnel exit. The truth may lie somewhere in the middle, as is so often the case. Certainly, the interplay of both r-proteins and rRNA is a prerequisite for optimal functioning of the ribosomal machine, such that although "the ribosome is a ribozyme," one should add as Thomas Cech noted, "admittedly one dependent on structural support from protein components" (Cech, 2000). Since the importance of rRNA for ribosome function has been discussed extensively elsewhere (Bashan *et al.*, 2003b; Ramakrishnan, 2002; Steitz & Moore, 2003; Wilson & Nierhaus, 2003), we aim here to rectify

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the imbalance and to give the r-proteins their share of the limelight.[1]

COOPERATIVITY WITHIN THE RIBOSOME

It should be recognized that it is often difficult to attribute a specific function, in terms of ribosomal translational activity, to a single given r-protein. This difficulty is rooted in the highly cooperative nature of the interactions between rRNA and r-proteins and between the r-proteins themselves. This cooperative nature is evident from several different observations:

(i) Alterations in some r-proteins lead to antibiotic resistance, despite no direct contact between the drug and the r-protein. This is evident for r-proteins L4 and L22, alterations which can confer resistance to the macrolide erythromycin (as will be discussed later), probably through perturbation of the surrounding 23S rRNA, rather than through a direct interaction with the antibiotic (Gregory & Dahlberg, 1999). Furthermore, the erythromycin resistance conferred by mutations in L4 can be modulated by additional mutations in the small subunit (Saltzman & Apirion, 1976).

(ii) Mutations in r-proteins of the large subunit (L-proteins) can have an impact on the functions related to the small subunit; for example, alterations in the L-protein L6 confer resistance to the aminoglycoside gentamycin, an antibiotic that induces misreading by binding within the decoding center on the small subunit (Davies *et al.*, 1998). Analysis of erythromycin-resistant ribosomes bearing alterations in L4 and L22 reveal conformational rearrangements, not only in the large subunit, but also within the small subunit (Gabashvili *et al.*, 2001). This is consistent with the recent biochemical characterization of these mutant ribosomes, which demonstrated the L4 mutant ribosomes exhibited a twofold reduced translational fidelity and were more sensitive than wild-type ribosomes to a number of antibiotics that target the 30S subunit, such as spectinomycin, kasugamycin, and streptomycin (O'Connor *et al.*, 2004).

(iii) Perhaps one of the most impressive illustrations of cooperativity within the ribosome results from an analysis of a special set of streptomycin-independent mutants: Usually, the reversion from a dependent- to an independent-phenotype is not a true 'back mutation' that re-establishes the initial genotype, but instead arises from additional mutations that functionally counteract the effect of the mutation conferring dependence. Indeed, it was found that a specific streptomycin-dependent mutant (carrying in addition to a mutated S12 a second less-well defined mutation in S8) reverts to an independent phenotype by mutations located in any one of 50 r-proteins of *Escherichia coli* ribosomes (Dabbs, 1978), an observation that played an important role in mapping the genetic loci for some of the r-proteins.

THE RIBOSOMAL COMPONENTS

Ribosomes consist of a small number (3 or 4) of large rRNA molecules (5S–28S rRNA ranging from 120 to 4500 nucleotides) and a large number (50 to 80) of small r-proteins (typically 25 to 300 amino acids). The fact that r-proteins are short in comparison to the long rRNA molecules is probably related to the high accuracy of transcription compared to translation. The accuracy of transcription without proofreading is in the order of 1:60,000 to 1:120,000 (one wrong nucleotide per 120,000 incorporated nucleotides; Libby *et al.*, 1989) and thus allows the synthesis of accurate rRNAs of 3000 to 5000 nucleotides without problems. In contrast, the accuracy of translation is no better than 1:3000 (Bouadloun *et al.*, 1983). Thus, to attain the synthesis of accurate r-proteins, the length of the synthesized proteins should be restricted to a length of $\leq$300 amino acid residues in order to produce the majority of proteins with a correct sequence.

Sequences and structures of rRNAs from organisms of all kingdoms have been determined. Currently the Comparative RNA Web Site database (Cannone *et al.*, 2002) (http://www.rna.icmb.utexas.edu/) contains 765/26 9206/1150 and 439/81 primary sequences/secondary structures of the 5S, 16S, and 23S rRNA, respectively. The *E. coli* ribosome was the first organism for which the primary sequences of both the rRNA and r-protein components were elucidated (Brosius *et al.*, 1980; Brosius *et al.*, 1978; Wittmann, 1986). Two thirds of its mass is rRNA and one third as protein, whereas the other extreme is seen in mitochondrial ribosomes, which in contrast are two thirds protein and one third RNA. The 5S, 16S, and 23S rRNAs in *E. coli* are 120, 1542, and 2904 nucleotides in length,

[1]This is an extended and updated version of a review that appeared in ELS (Wilson & Nierhaus, 2005).

respectively, whereas the respective *Caenorhabditis elegans* mitochondrial rRNAs are absent (5S) or significantly shorter (697 and 953) (Mears *et al*., 2002). There are 21 r-proteins in the small subunit (S1–S21) and 33 r-proteins are found in the large subunit (L1–L36) of *E. coli* ribosomes. In contrast, mitochondrial (and, to a lesser extent, chloroplast) ribosomes have longer versions of these r-proteins but also contain additional organellar specific r-proteins (see Mears *et al*., 2002 and references therein). For example, the bovine mitochondrial ribosome has a total of 77 r-proteins, 29 in the small subunit and 48 in the large subunit–of these, almost half are specific for mitochondrial ribosomes (Koc *et al*., 2001a; Koc *et al*., 2001b; Suzuki *et al*., 2001a; Suzuki *et al*., 2001b). Thus, the loss of the rRNA in mitochondrial ribosomes is more than compensated for by the presence of additional r-proteins in the equivalent positions resulting in significantly larger mitochondrial ribosomes than the bacterial ones (Sharma *et al*., 2003). In this respect, it would seem that in mitochondrial ribosomes r-proteins have taken over the role of rRNA to some extent, particularly for many of the intersubunit bridges (Sharma *et al*., 2003). However, all proteins are present in only one copy per ribosome with the exception of L7/L12. L7 is the *N*-acetylated form of L12, and together with L10 forms in *E. coli* the pentameric complex L10 × $(L7/L12)_4$, which was once referred to as L8 before its multimeric nature was realized. Interestingly, thermophilic bacteria contain three $(L7/L12)_2$ dimers forming a heptameric complex L10 × $(L7/L12)_6$ (Ilag *et al*., 2005; Diaconu *et al*., 2005). Proteins S20 and L26 have identical sequences and are present in only one copy per 70S ribosome–L26 was later shown to be a mis-assignment, therefore only S20 exists, which binds at the bottom of the 30S subunit on the intersubunit surface. The r-proteins were originally numbered according to their arrangement on a two-dimensional polyacrylamide gel, and as a consequence large acidic proteins have small numbers and small basic proteins have large numbers (Kaltschmidt & Wittmann, 1970).

Ribosomal proteins are defined as those proteins present in stoichiometric amounts in the ribosome, whereas translation factors are present on the ribosome with a copy number less than one per ribosome. Erroneously, S22 was considered to be the 22nd protein of the small ribosomal subunit from *E. coli*. However, since this protein accumulates in stationary phase ribosomes, and is found only in minute amounts in log-phase ribosomes (Wada, 1998), it has been reassigned as the stationary-phase ribosome-associated factor, SRA (Izutsu *et al*., 2001).

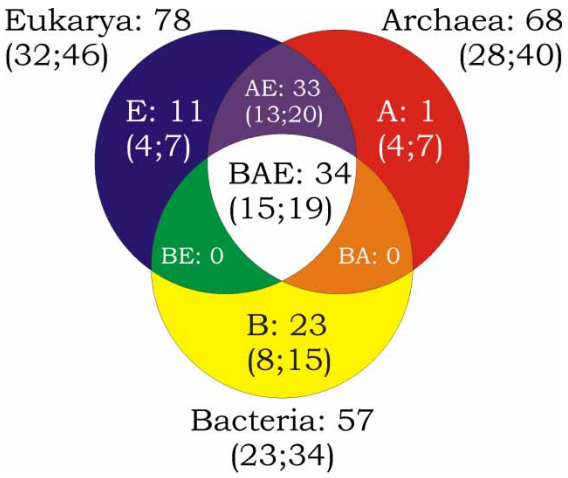

FIGURE 1 **Ribosomal proteins shared by the ribosomes from the three evolutionary domains: bacteria, archaea, and eukarya. modified from Lecompte *et al*., (2002). The numbers signify r-proteins present in each subunit of each species, e.g., "eukarya: 78 (32;46)" mean that an eukaryotic ribosome has on average 78 r-proteins, 32 in the small and 46 in the large ribosomal subunit. In addition, E, A and B mean ribosomes from eukarya, archaea and bacteria, respectively, such that "AE: 33 (13;20)," for example, means that archaeal and eukaryotic ribosomes share 33 r-proteins, 13 in the small and 20 in the large subunit. All three domains (BAE) have 34 r-proteins in common, 15 in the small and 19 in the large subunit.**

About 30% of the *E. coli* r-proteins, especially those critical for ribosomal function and assembly, have orthologous counterparts in higher eukaryotic and archaeal ribosomes (Lecompte *et al*., 2002; see Figure 1 and Table 1). Interestingly, archaeal ribosomes have an additional 30% of the r-proteins in common with eukaryotic ribosomes. In striking contrast, bacterial ribosomes do not share any r-proteins exclusively with archaeal or eukaryotic ribosomes, thus supporting the view that the common ancestor of archaea and eukarya separated from the bacteria before the separation of the archaea and eukarya. S11 and S12 show a sequence identity of 30% with respect to the corresponding proteins of cytoplasmic 80S ribosomes from the rat; the corresponding numbers for L14 and L2 are 35% and 30%, respectively (Müller & Wittmann-Liebold, 1997). These relatively high numbers indicate the functional importance of these proteins; however, what is noteworthy is that specific functions for most of the r-proteins have not been discovered.

In general, r-proteins are very basic (average pI ∼10.1 compared to pI = 4 to 5 for most translation factors), suggesting that a general function of r-proteins may be to counteract the negative charges of the phosphate

TABLE 1 **Concordance between the universally conserved ribosomal proteins from the three kingdoms of life.**

Small Subunit				Large Subunit			
Bacteria: *E. coli**	Archaea *H. ma**	Low Eukarya: *Yeast**	High Eukarya: *Rat**	Bacteria: *E. coli**	Archaea *H. ma**	Low Eukarya: *Yeast*	High Eukarya: *Rat**
S2	S2	S0	Sa	L1	L1	L1	L10a
S3	S3	S3	S3	L2	L2	L2	L8
S4	S4	S9	S9	L3	L3	L3	L3
S5	S5	S4	S2	L4	L4	L4	L4
S7	S7	S5	S5	L5	L5	L11	L11
S8	S8	S22	S15a	L6	L6	L9	L9
S9	S9	S9	S16	L10	L10	P0(A0)	P0
S10	S10	S20	S20	L11	L11	L12	L12
S11	S11	S14	S14	L12	L12	P1/P2	P1/P2
S12	S12	S28	S23	L13	L13	L16	L13a
S13	S13	S18	S18	L14	L14	L23	L23
S14	S14	S29	S29	L15	L15	L28	L27a
S15	S15	S13	S13	L16	L10e	L10	L10
S17	S17	S18	S11	L18	L18	L5	L5
S19	S19	S15	S15	L22	L22	L17	L17
				L23	L23	L25	L23a
				L24	L24	L26	L26
				L29	L29	L35	L35
				L30	L30	L7	L7

**E. coli*, *Escherichia coli*; *H. ma.* = *Haloarcula marismortui*; *Sc. cer.* = *Saccharomyces cerivisiae*; *Rat* = *Rattus norvegicus*.
Source: http://www.expasy.org/cgi-bin/lists?ribosomp.txt

residues in the rRNA backbone. The main exceptions are S1 and S6 in the small subunit and the L7/L12 proteins in the large subunit; the latter interact directly with r-protein L10 rather than with rRNA, and of the former, S6 makes extensive contact with S18, while S1 interacts with S21, S11 and S18 (discussed later). Another function exerted by a surprisingly large number of ribosomal proteins is an RNA chaperone activity that might play a decisive role during the assembly of ribosomal proteins. The proteins of the large ribosomal subunit have been systematically analyzed *in vitro* for RNA chaperone activity by measuring the ability of the r-proteins to stimulate trans-splicing of a group I intron (Semrad *et al*., 2004). Of the r-proteins identified, the strongest effects were seen with L19 > L1 = L15 > L3 = L13 = L18 > L16. This observation is certainly among the most important recent advances concerning the mechanisms of assembly of prokaryotic ribosomes.

STRUCTURE, LOCATION AND INTERACTION OF RIBOSOMAL PROTEINS WITHIN THE RIBOSOME

Structures for a number of isolated r-proteins have been determined by NMR and crystallography (reviewed by Ramakrishnan & White, 1998) and their topographical neighbourhoods revealed by neutron scattering (Capel *et al*., 1987; Willumeit *et al*., 2001, and references therein) and immuno-electron microscopy (Stöffler-Meilicke & Stöffler, 1988), as well as using a plethora of biochemical techniques, such as cross-linking and hydroxyl-radical probing (for a compilation, see Baranov *et al*., 1998). Detailed analysis of the high resolution crystal structures of the *Thermus thermophilus* small subunit (Brodersen *et al*., 2002) and the *Haloarcula marismortui* large subunit (Klein *et al*., 2004) have revealed an abundance of new insights and interesting features of the r-proteins that we summarize below.

The 16S rRNA can be divided into four domains, which together with the r-proteins, constitute the structural landmarks of the 30S subunit (Figure 2A and B). Thus, the locations of the small subunit r-proteins can be to some extent defined by the rRNA domain with which they interact:

(i) the 5′ domain and 3′ minor (h44[2]) domain with proteins S4, S5, S12, S16, S17 and S20 constitute the body of the 30S subunit,

[2]Helices of the 16S rRNA are denoted with "h" and those of the 23S rRNA with "H."

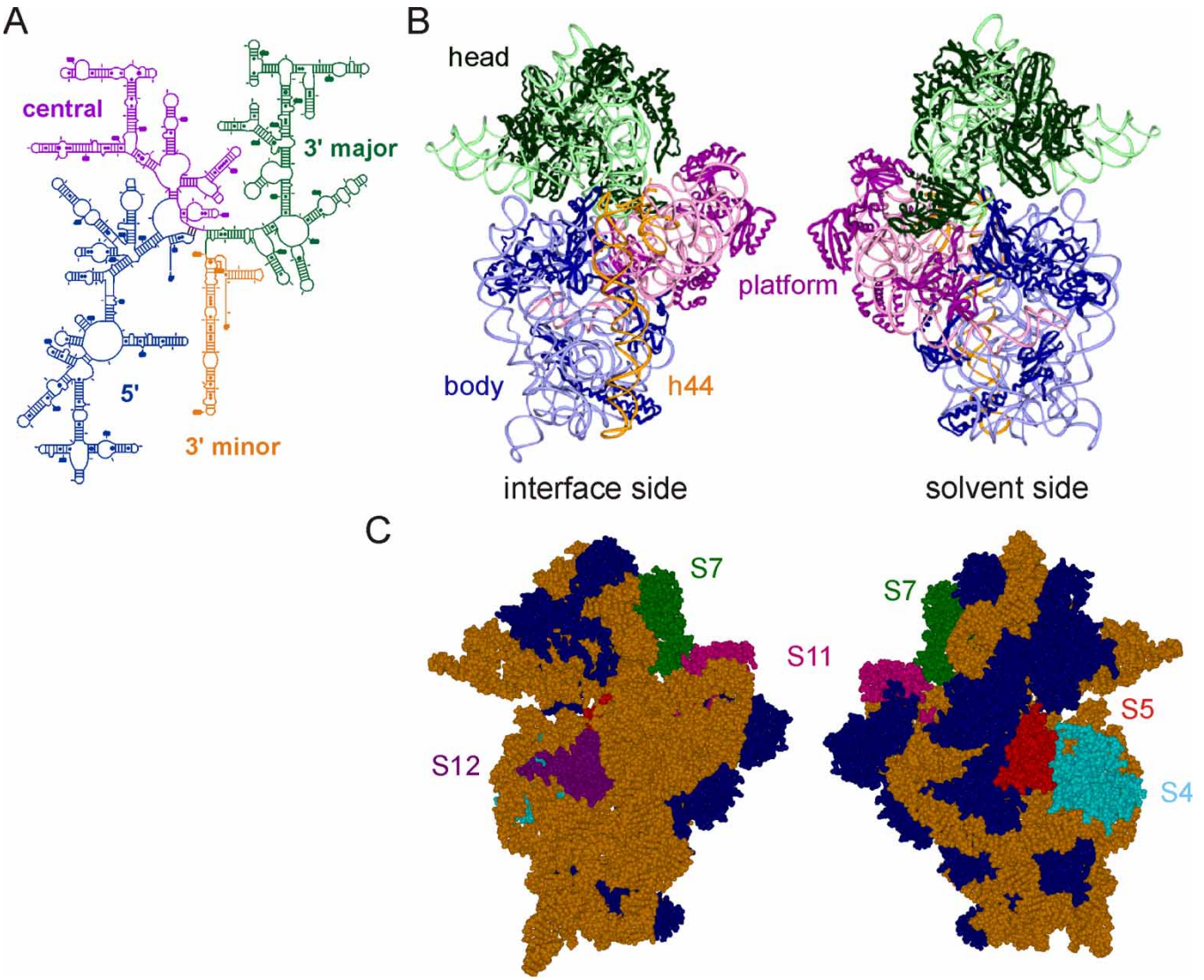

FIGURE 2 **Position of ribosomal proteins in the *Thermus thermophilus* 30S subunit. (*A*) Secondary structure of 16S rRNA with domain coloring: 5′ domain, blue; central domain, magenta; 3′ major, green; and 3′ minor, yellow. (*B*) Domain distribution of r-proteins (*dark*) and rRNA (*light*) in the 30S subunit with coloring coded by domain as in (A). (*C*) Spacefill representation of 30S subunit highlighting positions of r-proteins S4 (*cyan*), S5 (*red*), S7 (*green*), S11 (*pink*) and S12 (*purple*), while other r-proteins and rRNA coloured blue and yellow, respectively. Both interface (which contacts the 50S subunit) and solvent (cytoplasmic) views are shown.**

(ii) the 3′ major domain forms the head, which is protein rich, containing S2, S3, S7, S9, S10, S13, S14, and S19, whereas
(iii) the central domain makes up the platform by interacting with proteins S1, S6, S8, S11, S15, and S18.

The rRNA of the large ribosomal subunit can be divided into seven domains (including the 5S RNA as domain VII; Figure 3A and B), which–in contrast to the small subunit–are intricately interwoven with the r-proteins as well as each other. The complexity of the large subunit is evident from the fact that each r-protein in the *H. marismortui* 50S contacts on average 2.6 rRNA domains (L1 and L10 × $(L7/L12)_4$ excluded), whereas in the *T. thermophilus* 30S, each r-protein contacts on average only 1.2 domains. One extreme example is r-protein L22, which interacts with all 6 domains of the 23S rRNA.

The r-proteins are unevenly scattered over the ribosome. This is most conspicuous on the small subunit, where only one protein (S12) is found on the interface side, whereas all the other r-proteins are located on the cytoplasmic or solvent side (Figure 2C). This leads to a significant difference in the centers of mass of the r-proteins and the RNA components, being located 25.4 Å from one another. Most of the r-proteins are found in the head (3′ major) domain, whereas almost

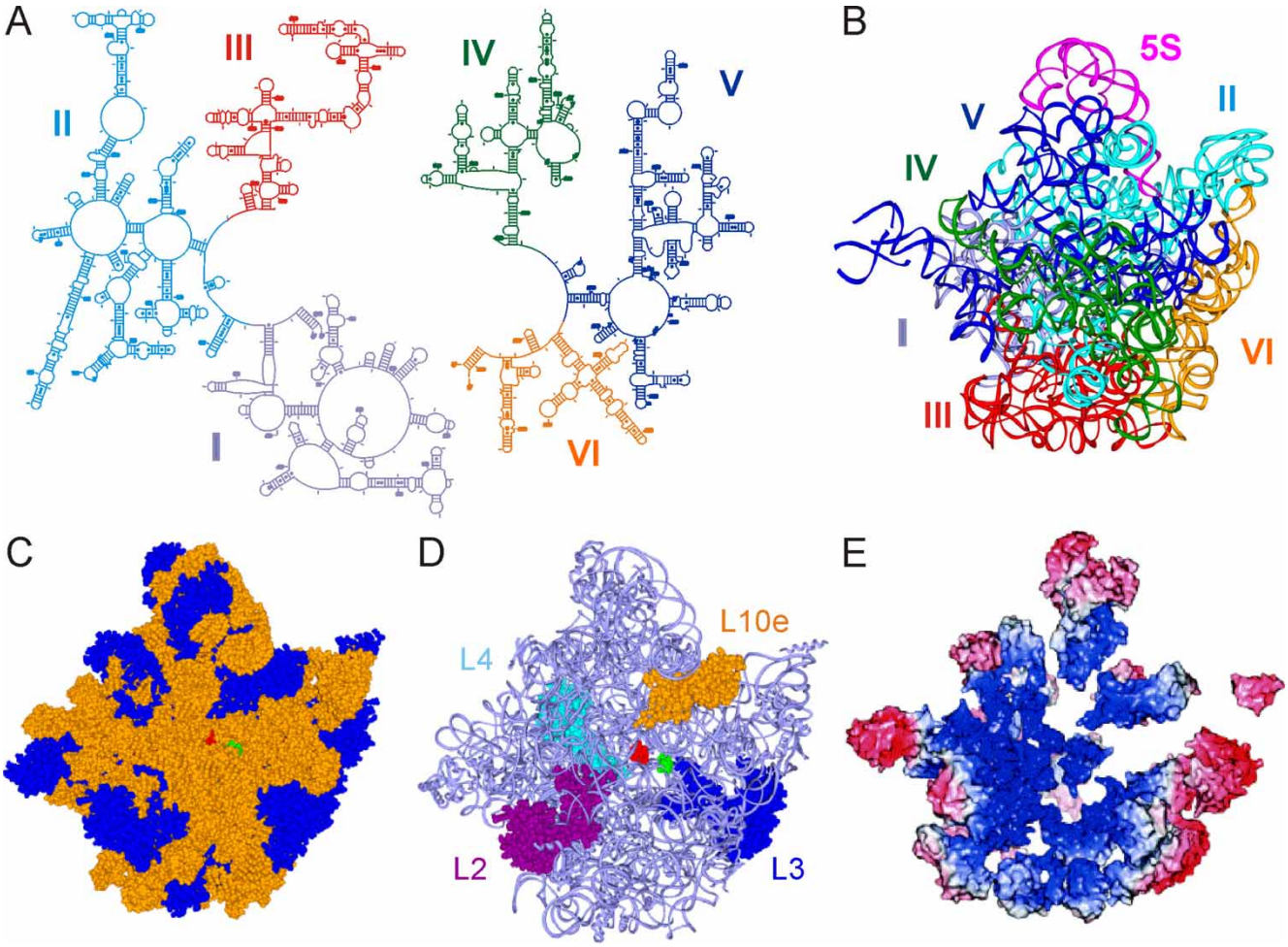

FIGURE 3 **Position of ribosomal proteins in the *Haloarcula marismortui 50S subunit.* (*A*) Secondary structure diagram of 23S rRNA with domains I to VI highlighted in different colors. (*B*) Tertiary structure of the rRNA of the large subunit with domain colors as in (*A*). Here also the 5S rRNA is included (domain VII). (*C*) Locations of ribosomal proteins (*blue*) and rRNA (*yellow*), (*D*) Ribosomal proteins, the extensions of which reach towards the peptidyl-transferase center (L2, purple; L3, blue; L4, cyan and L10e (L16), *yellow*) and (*E*) charge distribution of the r-proteins, where blue and red indicate positively and negatively charged regions, respectively. In (*C*) and (*D*) the positions of the CCA-end of an A- (*green*) and P-site (*red*) tRNA are given for reference. (*E*) was modified from Klein and coworkers (2004).**

no proteins interact with the functionally important 3′ minor domain (helices 44 and 45) containing the decoding center at the A site and the anti-Shine-Dalgarno sequence (with the exception of S1, discussed later). The interface side of the large subunit is also poor in r-proteins (Figure 3C and D), whereas the mRNA entry site, the binding site for translation factors around the L7/L12 stalk and the exit of the tunnel are, in contrast, particularly rich in proteins (see the respective sections below).

Many r-proteins are composed of at least one globular domain, which is generally located on the surface of the ribosome, from which a long extended region penetrates into the ribosome's interior. In fact, all S-proteins (except S4 and S15) and about 50% of the L-proteins contain extensions, which are either "random coil" located at N- or C-termini or α-helix or β-sheet in the central region of the protein. The globular regions usually correspond well with the structures determined for the isolated protein, whereas the extended regions were generally not observed in the isolated structures, probably because their structure requires interaction with rRNA. Indeed, the extensions have distinctive amino acid compositions, containing multiple glycine residues to allow flexibility and tight packing, and are rich in basic amino acids to interact with rRNA. This latter feature is exemplified by the charge segregation of r-proteins of the archaeal halophilic 50S subunit (Figure 3E; Klein *et al.*, 2004), where positive charges are located on the protein regions inside the ribosome, counteracting the negatively charged phosphate residues, whereas the negative charges dominate the surface regions being necessary for halophilic adaptation, *i.e.*, to cope with the intracellular salt concentration of up to 4M monovalent cations

(Eisenberg *et al*., 1992). Consistently, the content of the basic amino acids Arg/Lys in the extensions of the L-proteins is larger (27%) than in the globular part (19%), and even though extensions represent only 18% of the protein mass of the large subunit, they are responsible for 44% of total RNA surface area buried. Overall, the interactions with the 23S rRNA are extensive: an L-protein buries on average ~3,400 Å^2 of the rRNA surface, and 13 of 28 L-proteins have 23S rRNA contacts that utilize 40% of their surface. Nevertheless, there are also protein-protein contacts: in the small subunit, for example, S6 and S18 form a tight complex that assembles as a heterodimer on the outer edge of the platform, and S3, S10, and S14 cluster tightly together at the back of the 30S head. In the large subunit the previously mentioned pentameric L10 × $(L7/L12)_4$ or heptameric L10 × $(L7/L12)_6$ complex are classic examples.

Among the longest and most complex extensions are those of L2 and L3, which come to within 20 Å of the PTF center (Figure 3D), explaining why early reconstitution experiments implicated these proteins with PTF activity (Schulze & Nierhaus, 1982). On the 30S subunit, the extremely long extension of S12 reaches from the globular domain, located adjacent to the decoding center on the intersubunit side, to the back or solvent side of the 30S, where it interacts with S8 and S17. Another extreme example is the small 61 amino acid r-protein S14, which is completely devoid of any globular domain. Thus, the function of the majority of r-proteins may ensure that the ribosome achieves a correctly folded state during the complex assembly process, and may support the correct conformation of the rRNA in the final assembled complex. In fact, cutting off the extensions of, for example, L20 severely impairs the assembly of the large subunit specifically (Guillier *et al*., 2005). At least in the small subunit, the r-proteins that initiate subunit assembly are generally globular proteins and bind to multi-stem junctions to fix the conformation of the region and bring together different parts of the ribosome. For example, S4, which is an assembly initiator protein that nucleates the 5′ domain (body) of the 30S subunit, binds to the 5-way junction of the 5′ domain formed by h3, h4, h16, h17, and h18, whereas in the 50S subunit, L3 makes interactions with domains 2 to 6. Consistent with its role as the assembly initiator protein, L24 binds only to domain I; however, the secondary binding proteins that are in the vicinity of L24 include L22, which as previously mentioned contacts all 6 domains.

Although individual motifs of the r-protein structures presently available are found in other non-ribosomal proteins, the overall fold of a complete r-protein is unique. There are however two interesting cases where one r-protein displays an identical fold to another r-protein suggesting that they share a common ancestry. These are the r-proteins S6 and S10 in the small subunit and L15 and L18e in the archaeal 50S subunit (Brodersen *et al*., 2002; Klein *et al*., 2004, respectively). Note that while L15 is a universally conserved r-protein, L18e is specific to archaea, signifying that L18e arose through an archaeal-specific duplication of the L15 gene.

SPECIFIC FUNCTIONS OF RIBOSOMAL PROTEINS

Some r-proteins have a clear and essential function in the assembly of ribosomal subunits, but are dispensable for function after the ribonucleoprotein particle is fully assembled (reviewed by Nierhaus, 1991). These r-proteins remain in the mature particle and might improve the stability of ribosomes; examples include the r-proteins S16, L15, L16, L20, and L24. Furthermore, it seems that proteins S6, S9, S13, S17, S20, L1, L9, L11, L15, L19, L24, L27 to L30, and L33 are not essential for the translational function of the ribosome either, because *E. coli* strains lacking these r-proteins are viable (Dabbs, 1986; Herr *et al*., 2001). In contrast to the original report by Dabbs, S1 is essential in the bacteria *E. coli* (Sorenson *et al*., 1998). Some of these mutants, however, are severely retarded, as seen by their conditional lethality (temperature sensitivity) and/or 2- to 10-fold reduction in growth rate. Despite this general ambiguity as to the importance of these r-proteins, there are nevertheless many r-proteins for which an involvement in distinct ribosomal functions is known (see Table 2). In the following sections, a few of these r-proteins are discussed in detail with respect to their known functions: On the small subunit, these include:

(i) S1 in delivery of the mRNA as well as tmRNA-mediated trans-translation;
(ii) S3, S4 and S5 forming the entry pore for the mRNA and exerting helicases activity; and
(iii) S4, S5 and S12 in the decoding and fidelity of translation.

On the large subunit:

(iv) L1 and L16/L27 may play roles in the release and binding of tRNAs to the ribosome, respectively;

TABLE 2 **Summary of the functions of selected ribosomal proteins.**

Protein	Functions
S1	Suggested to bring the mRNA into the proximity of the ribosome during initiation. Translational feedback regulation of S1 operon.
S3, S4 and S5	Form the mRNA entry pore and may have a helicase activity to unwind mRNA secondary structure encountered during translation.
S4	Mutations (*ram*) increase the error during the decoding process; role in rRNA transcription antitermination and translational feedback regulation of alpha operon.
S5	Probably facilitates changes of rRNA conformations that alters the selection mode of the ribosome from accurate to error prone and vice versa; mutations confer resistance against streptomycin and spectinomycin; *ram* mutations.
S12	Involved in decoding of the second and third codon positions at the A site. Mutations in S12 confer resistance against streptomycin, increase accuracy of the decoding process and, in most cases, concomitantly decrease the rate of translation. The lack of S12 in reconstituted particles also increases accuracy.
L1	Probably involved in the removal of deacylated tRNA from the E site. Translational feedback regulation of L11 operon.
L4	Mutations in L4 can confer resistance against macrolide antibiotics such as erythromycin by indirectly interfering with drug binding; role in rRNA transcription antitermination.
L7/L12	Involved in elongation-factor binding and GTPase activation. Together with L10, involved in translational feedback regulation of L10 operon.
L9	Mutations in L9 effect the efficiency of translational bypassing.
L11	Mutations in L11 or lack of the complete protein confer resistance against thiostrepton, an antibiotic that blocks the ribosomal transition from the pre- to post-translocational state and vice versa. During the stringent response this protein senses the presence of a deacylated tRNA in the A site; mutations or the absence of the protein can cause a relaxed phenotype (*relC*) resulting from loss of stringent control.
L16	May be involved in correct positioning of the acceptor stem of A- and P-site tRNAs as well as RRF on the ribosome. Mutations in L16 confer resistance to the orthomycins avilamycin and evernimicin. Homologue is L10e in archaea (and L10 in eukaryotes; Table 1).
L22	May interact with specific nascent chains to regulate translation. Furthermore, deletion of three amino acids in L22 confers erythromycin resistance without interfering with the binding of the drug.
L23	Present at the tunnel exit site and has been shown to be a component of the chaperone trigger factor binding site on the ribosome.
L27	Bacterial-specific protein implicated in the placement of the acceptor stem of P-site tRNA and binding of the ribosome recycling factor on the 50S subunit.
L29	Is located close to the tunnel exit site and may constitute part of the binding site for the signal recognition particle.

(v) L4 and L22, which protrude into the ribosomal tunnel, confer resistance to macrolide antibiotics. In addition, L22 may also interact with nascent chains to control translation of particular proteins.

(vi) L9 has been suggested to influence tRNA stability at the P site and mRNA movement, and mutations in L9 affect the efficiency of translational bypassing.

Two regions of the large ribosomal subunit have an above average concentration of r-proteins and are associated with translation factor binding:

(vii) The stalk region, composed of amongst others the r-proteins L11 and L10 $\times$ $(L7/L12)_4$, is associated with binding of the elongation factors EF-G and EF-Tu to the ribosome.

(viii) On the back of the 50S subunit, the r-proteins L17, L22, L23, L24, L29, and L32 form a ring around the tunnel exit site, of which L23 and L29 have been implicated in signal recognition particle and trigger factor binding.

S1 AND mRNA BINDING

S1 is the largest *E. coli* r-protein, with 557 amino acids and a molecular weight of 61,558. S1 is important but not essential for viability in *E. coli*. An S1 homolog is present in all Gram-negative bacteria and has also been reported in Gram-positive bacterium (see, for example,

Karlin *et al.*, 2004). S1 is able to interact with both the ribosome and mRNA. This would allow it to function in the initiation of translation possibly by catching the mRNA and directing it to the ribosome. S1 is weakly bound to ribosomes ($K_a = \sim 1.7 \times 10^8\ M^{-1}$), although this is still 50 times higher than the affinity for the 'naked' rRNA and thus can readily exchange between free ribosomes. In accordance with the low binding constant, a pool of about 10 to 20% of the cellular S1 is found in the post-ribosomal supernatant, whereas most of the r-proteins do not have a detectable pool (Ulbrich & Nierhaus, 1975). In contrast to polysomes, free ribosomes do not contain a full complement of S1 (for an comprehensive review on the early S1 literature, see Subramanian, 1983).

S1 can be cleaved into three large fragments using cyanogen bromide; an N-terminal fragment (NTD; residues 1 to 193), a middle fragment (224 to 309), and a C-terminal fragment (CTD, 332 to 547) (see Subramanian, 1983). The primary sequence of S1 suggests that it contains six repeats of a so-called S1 motif, two in the NTD (21-87, 105-171) and four in the CTD (192 to 260, 277 to 347, 364 to 434 and 451 to 520). Although no crystal structure for S1 is available, the S1 motif present in polynucleotide phosphorylase has been determined by NMR, revealing a five-stranded antiparallel β-barrel RNA binding motif (Bycroft *et al.*, 1997). The S1 motif is also found in the translation initiation factors, bacterial IF1 and eukaryotic eIF2α.

S1 is known to directly bind to RNA, even in the absence of the ribosome, although with little specificity and a relatively low binding constant ($\sim 3 \times 10^6\ M^{-1}$) (Subramanian, 1983). The NTD fragment of S1, containing the first two S1 motifs, has been shown to be devoid of RNA-binding ability yet still binds strongly to 30S subunit. In contrast, removal of the NTD using trypsin produces a CTD fragment (172 to 557) that retains RNA-binding ability but no longer binds to the 30S subunit. Deletion of the last S1 motif (451 to 520) does not impair ribosome or mRNA binding but abolishes autoregulation of S1 expression (discussed later). Collectively, this suggests that the NTD is important for ribosome binding, while the middle three S1 motifs appear to have a role in mRNA binding.

The binding position of S1 has been determined by comparing cryo-EM reconstructions of *E. coli* 30S subunits (containing S1) with the *T. thermophilus* 30S subunit crystal structures that lack the S1 protein (Sengupta *et al.*, 2001). This study reveals that S1 is located at the junction between the head, platform, and body of the 30S subunit (Figure 4), a finding that supports previous immuno-EM (Walleczek *et al.*, 1990), neutron scattering (Capel *et al.*, 1988) and crosslinking data (Golinska *et al.*, 1981). In this position, S1 is surrounded by a number of r-proteins, S2, S6 to S9, S11, and S18, with extensive contacts with r-proteins S21, S11, and S18 envisaged. S1 also contacts 16S rRNA, predominantly with helices h26 and the penultimate h45 that contains the anti-Shine-Dalgarno sequence.

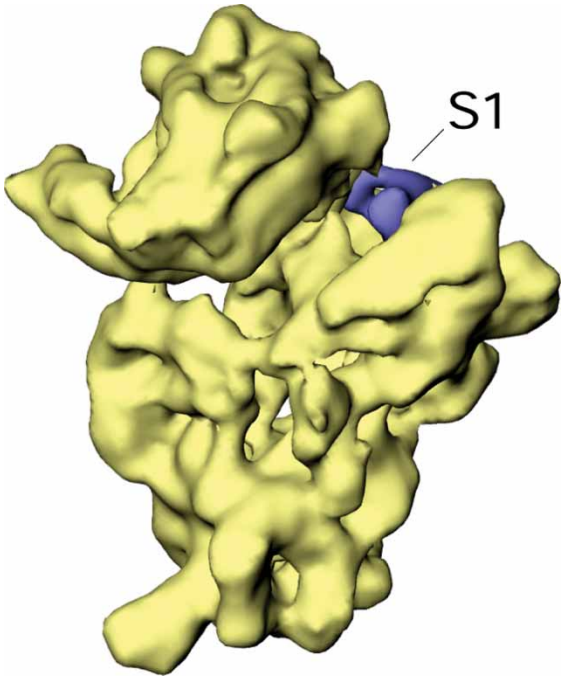

FIGURE 4 **Position of S1 (*blue*) determined by comparing the cryo-EM reconstruction of an *E. coli* 70S ribosome with the *T. thermophilus* 30S crystal structure that is lacking the S1 protein. Modified from Sengupta *et al.* (2001).**

This placement at the platform of the 30S subunit is consistent with the observation that S1 interacts with the 3′ end of the 16S rRNA (Dahlberg & Dahlberg, 1975) and crosslinks to the 5′ end of an mRNA (Czernilofsky *et al.*, 1975). Therefore, it appears that the major function of S1 might be to bring the mRNA onto the 30S subunit, thereby assisting subsequent mRNA interactions between the Shine-Dalgarno (SD) sequence in the mRNA with the 3′ end (anti-SD) of 16S rRNA in order to position the AUG at the ribosomal P site (Subramanian, 1983). This is consistent with the observation that translation of leaderless mRNAs (those starting directly with 5′ AUG) does not require S1 (or S2; Moll *et al.*, 2002), since it does not depend on SD interactions.

S3, S4 AND S5: THREADING THE mRNA THROUGH THE ENTRY PORE

The ribosome covers approximately 35 to 40 nucleotides of an mRNA (Beyer *et al.*, 1994). The path of the mRNA through the ribosome can be separated into three regions, (i) the entry pore with the following upstream tunnel of the mRNA housing ~10 nucleotides of the mRNA, (ii) a region where at least six nucleotides are exposed to the interface of the ribosomal subunit *i.e.* either A- and P-site codons before translocation or at P- and E-site codons after translocation, and (iii) an upstream region encompassing ~15 nucleotides. The convention is that the first nucleotide of the P-site codon is the +1 nucleotide. Upstream nucleotides are counted as −1, −2, −3, (namely the three codons located at the E site) and downstream +1, +2, +3 for the P site and +4, +5, +6 for the A site codons.

The ribosomal entry site for the mRNA and the following upstream tunnel is a functional region dominated by ribosomal proteins, *viz*. S3, S4, and S5 (Figure 5A). Basic residues (mainly Arg and Lys) from these r-proteins protrude into the tunnel lumen conferring a diameter of ~15 Å (Figure 5B; Yusupova *et al.*, 2001, Takyar *et al.*, 2005).

All mRNAs form some secondary structure—a point illustrated by the early observation that ≈50% of the nucleotides in a random RNA sequence participate in hairpin or other secondary structures (Doty *et al.*, 1959). Since the minimum secondary structure of RNA, a double helix, itself has a diameter of 20 Å, it is immediately clear that any secondary structure present in a mRNA has to be melted in order to allow the mRNA to pass through the entry pore. Indeed, a translating ribosome has long been known to be able to unwind RNA/RNA duplexes with a stability stronger than $\Delta G = -60$ kcal·mol^{-1} (Kozak, 1989), yet until recently it has remained unclear whether this was an innate ability of the ribosome or is conferred by the presence of an additional factor.

Using an *in vitro* translation system composed of purified components, Takyar and coworkers (2005) demonstrated that the ribosome itself is capable of melting double-stranded RNA with a length of up to 27 base-pairs ($T_m = 70^\circ$C). Interestingly, melting was shown to start at nucleotide position +11 in the mRNA, which would be located in the middle of the tunnel gated by the mRNA entry pore. Consistent with this finding was the observation that mutations of the basic residues in S3 and S4 (but not S5) that protrude into the lumen

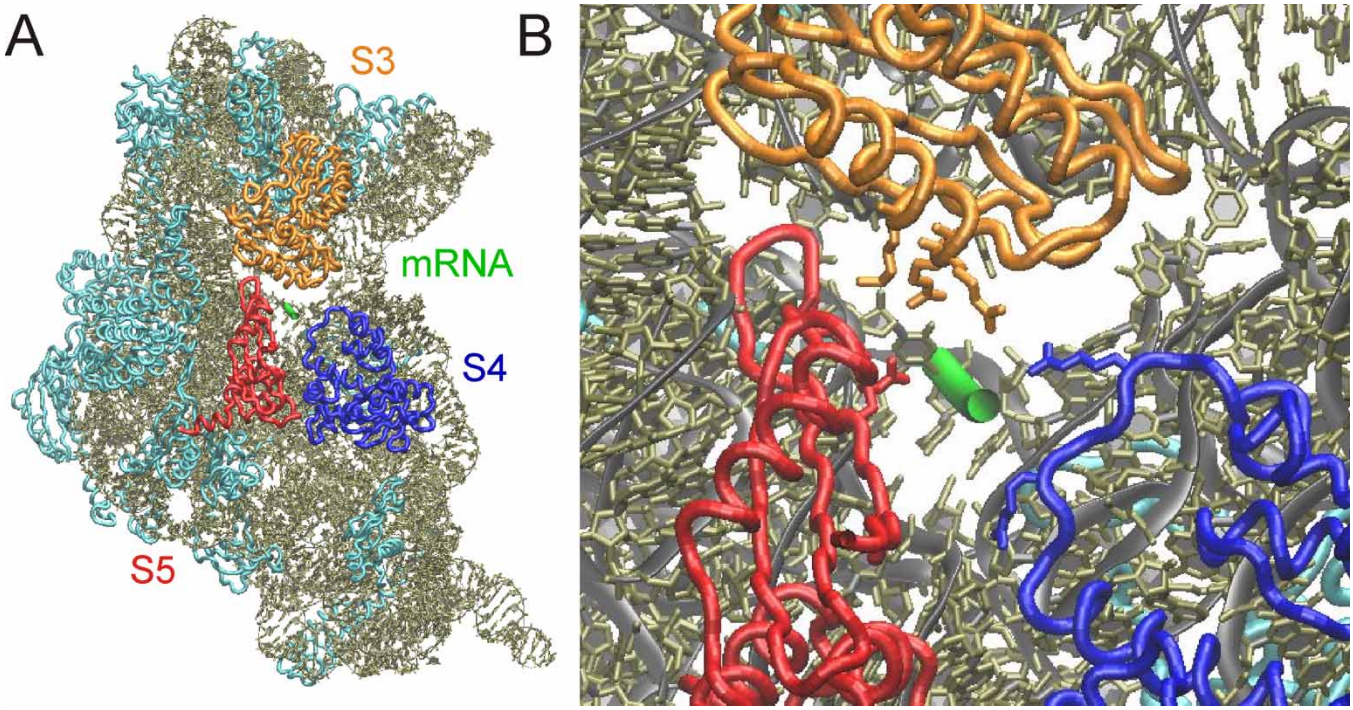

FIGURE 5 **The proteins S3, S4, and S5 form the entry pore and the downstream tunnel for the mRNA. (*A*) View of the *Thermus thermophilus* 30S subunit from solvent side with r-proteins that form the mRNA pore, S3 (*orange*), S4 (*blue*) and S5 (*red*). A three nucleotide section of the mRNA in the A site (*green*) is shown for reference. (*B*) Close-up of (*A*) showing the basic Arg and Lys residues that when mutated in S3 and S4 reduce the helicase activity of the ribosome (Takyar *et al*., 2005).**

of the mRNA entrance pore severely impaired the ribosome helicase activity. Furthermore, the ribosomal helicase activity was shown not to require energy in the form of GTP or ATP, instead the driving force was the translocation reaction, *i.e.*, the movement of the mRNA $\times$ $(tRNA)_2$ complex from the A-P to the P-E sites was sufficient to unwind secondary structure in the mRNA (Takyar *et al*., 2005).

S12, S4 AND S5: ACCURACY OF PROTEIN SYNTHESIS

Accuracy in ribosomal translation is optimal, but not maximal, and varies depending on growth rate. Obviously, a translational error rate is sustainable that does not significantly affect the function of proteins due to substitutions of conserved amino acid during misincorporation. This means that a mistake in amino-acid selection during decoding preferentially incorporates amino acids that are chemically related to the correct (or cognate) one. In fact, according to a rough estimate, 1 in 400 misincorporations abolish the function of a protein (Kurland *et al*., 1990). In theory, accuracy could be increased by either decreasing the speed of a reaction, such as elongation, or increasing the expenditure of energy, such as GTP. However, translation does not seem to follow this simple scheme. S12 mutants are known that combine increased accuracy with a normal rate of growth (Piepersberg *et al*., 1979), which is proof that protein biosynthesis is not maximized with respect to accuracy.

Since the ribosome is an extremely cooperative machine, it is not unexpected that mutations in many ribosomal components can impair ribosomal accuracy. In fact, mutations in tRNAs, EF-Tu, rRNAs (16S and 23S) and r-proteins (S7, S11, S17, L6, and L7/L12) have been shown to negatively affect fidelity. However, the strongest effects have been observed through mutations in r-proteins S12, S4, and S5 and in h27 of 16S rRNA.

The classical S12 mutations were isolated as streptomycin resistance mutants (reviewed by Kurland *et al*., 1990). Streptomycin is best known as an error-inducing antibiotic, and mutants conferring resistance to, or even dependence on, streptomycin have an increased accuracy and, in a most cases, a slower elongation rate. A pseudo-dependent strain can grow in the absence of streptomycin; however, the drug stimulates the growth and translation rate. Both mutations of S12 and the omission of this protein from *in vitro* reconstituted 30S subunits lead to an increase in accuracy, suggesting that S12 increases the rate of translation at the cost of accuracy. On the other hand, the highly conserved residue Ser46 (*E. coli* nomenclature) participates in the recognition of the middle base-pair of codon-anticodon interaction at the A site (see Figure 6A, left, and 6B) and Pro44 indirectly monitors the third or wobble base-pair position (Ogle *et al*., 2002).

A 10-fold reduction in accuracy, analogous to the extent of increased misincorporation induced by streptomycin, results from mutations in the S4 and S5 genes (Kurland *et al*., 1990). The resulting phenotype is termed *ram* (ribosomal ambiguity mutants) and cells displaying the *ram* phenotype remain viable despite a high error rate in protein synthesis. S4 mutants are also characterized by ribosomal assembly defects, which could explain the reduced growth rate that accompanies the increased ambiguity in translation. The *ram* mutations were obtained as revertants of hyperaccurate phenotypes that compensate for mutations in S12. These revertants, termed streptomycin-independent to distinguish them from the streptomycin-sensitive wild-type, show normal accuracy and/or a relief of the streptomycin dependency.

The first real insights into how S12, S4, and S5 affect fidelity were concurrent with a better understanding of the ribosomal changes associated with tRNA selection. A comparison of 30S subunit crystal structure bound with a codon–anticodon mRNA-tRNA duplex, in one case a cognate interaction, while in the other near-cognate, led to a model whereby selection of the correct or cognate tRNA by the ribosome triggers a transition of the 30S subunit from an open to a closed form (Figure 6A; Ogle *et al*., 2002). The closed form is thought to be prerequisite for binding the aa-tRNA to the A site (accommodation, see Wilson & Nierhaus, 2003 for details) after the decoding step. Transition into the closed form involves (i) disruption of multiple interactions at the interface between S4 and S5, and (ii) establishment of salt-bridge interactions between S12 and either h44 or h27 of the 16S rRNA. Consistently, (i) mutations in S4 and S5 that promote formation of the ram state would also lead to disruptions at this interface (Figure 6A, right, and 6C), suggesting that the observed error-prone phenotype stems from partially inducing the closed form, and (ii) mutations in S12 that block salt-bridge formation may destabilize the closed form and thus confer resistance to streptomycin (or in some cases, even dependence on the drug). The antagonistic

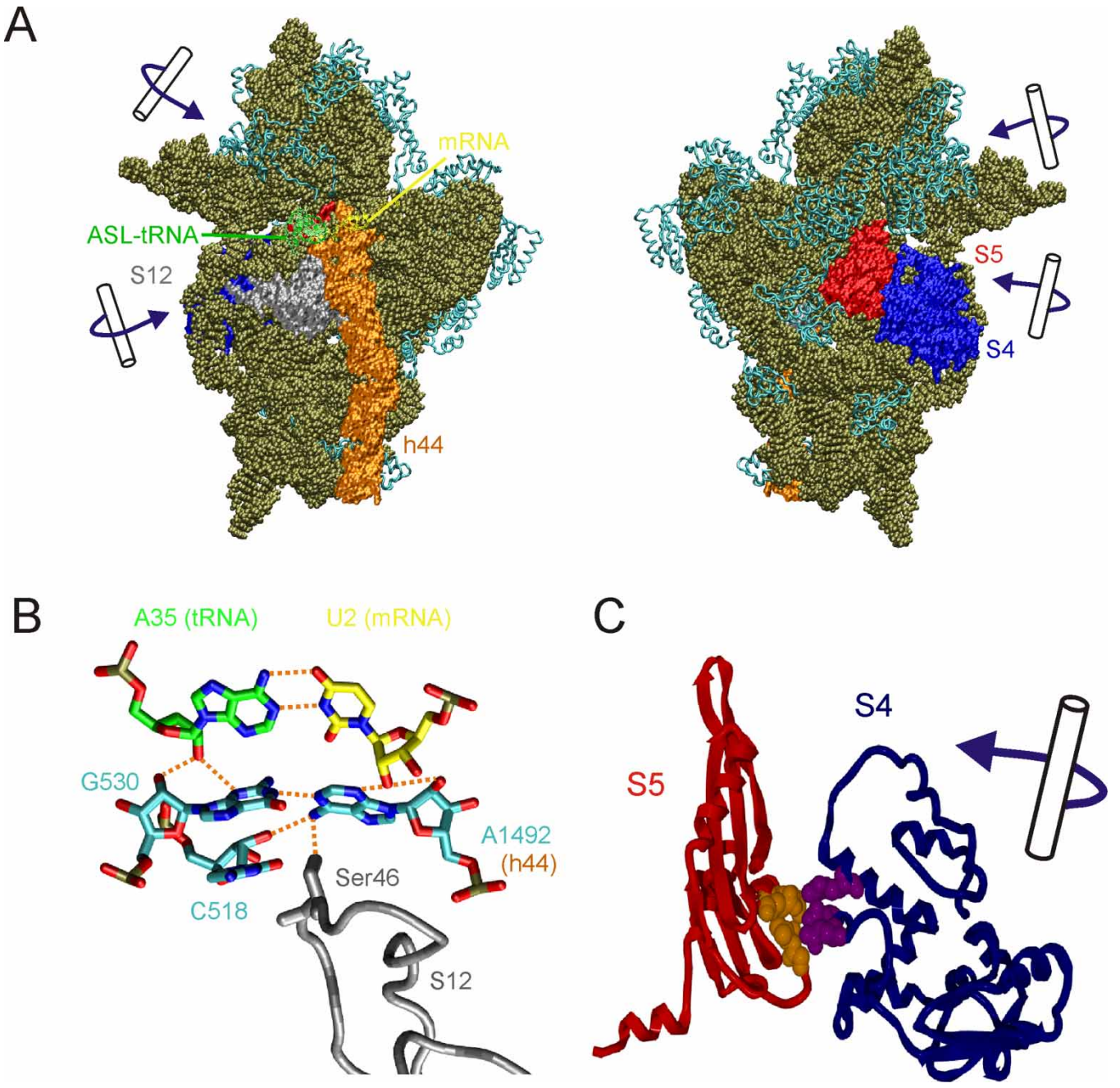

FIGURE 6 **Involvement of S4, S5 and S12 during decoding. (*A*) Binding of cognate tRNA to the A site induces a transition in the 30S subunit from the open to the closed form. This involves a rotation of the head and movement of body (see arrows) towards the decoding site (anticodon-stem loop [ASL] of tRNA indicated (*green*) to show A site position). The closed form brings elements of S12 (*gray*) and h44 (*orange*) into contact. (*B*) Serine 46 (Ser46) in the loop of S12 monitors the correctness of the second position base-pair of the A site codon-anticodon complex (U2 in the mRNA (*yellow*) and A35 in the tRNA (*green*)) by hydrogen bonding with A1492 of the 16S rRNA. (*C*) Ram mutations shown in orange and purple spacefill representation that disrupt the interface between r-proteins S4 (*blue*) and S5 (*red*) facilitate transition from the open to the closed form.**

effects of the S4/S5 ram mutants to destabilize the closed form, and the S12 streptomycin resistant mutants to stabilize it, rationalizes the compensatory effects observed on translational fidelity.

Interestingly, it was shown recently that r-proteins S7 and S11 can influence translational fidelity from their position in the E site. The interface of S7 and S11 forms part of the binding site of the anticodon loop of the E-tRNA, and mutations that disrupt the interface between S7 and S11 led to readthrough, frameshifting and mis-incorporation events similar in extent as those seen in the presence of streptomycin (Robert & Brakier-Gingras, 2003). In fact, the presence of an E-site tRNA has also been shown to influence the accuracy of

A-site decoding (Geigenmüller & Nierhaus, 1990) and maintenance of the ribosomal reading frame (Marquez *et al.*, 2004), suggesting that interactions in the E site may also contribute to the transition between open to closed forms. This allosteric linkage between A and E sites may also explain how the binding of the antibiotic edeine within the E site of 70S ribosomes can cause severe translational misreading at the ribosomal A site (Dinos *et al.*, 2004).

NOT TO BE BYPASSED: L9 AND tRNA STABILITY AT THE P SITE

Crystallography and NMR studies of L9 have revealed a protein with two globular RNA binding domains, separated by a remarkably long and invariant nine-turn α-helix, with the NTD of L9 (1 to 52) showing structural homology to r-proteins L7/L12 and L30 (Hoffman *et al.*, 1996; Hoffman *et al.*, 1994). In the 70S $\times$ $(tRNA)_3$ structure, L9 binds at the base of the L1 stalk utilizing the NTD domain to contact predominantly H76, whereas the CTD (59 to 149) extends more than 50 Å away from the surface of the subunit (Yusupov *et al.*, 2001). This localization is consistent with (i) the position determined by immuno-EM (Nag *et al.*, 1991), the fact that (ii) L1 can bind a 778-nucleotide fragment of 23S rRNA (containing H76) in domain V (Adamski *et al.*, 1996) and (iii) both full-length L9 as well as the NTD of L9 protect positions 2093 to 2097 (H76) and 2194 to 2199 (at the junction of H76 and H79) of the 23S rRNA from chemical modification in L9-deficient 50S subunits whereas no protection is observed for the CTD alone (Lieberman *et al.*, 2000).

The first insight into the role of L9 during translation came from studies into recoding events on the T4 gene 60 mRNA. For the translation of this mRNA, ribosomes have to bypass a 50-nucleotide coding gap: The mRNA disengages from the peptidyl-$tRNA^{Gly}$ at codon 46 (GGA), the take-off site, and the tRNA and mRNA associate again at a landing-site GGA triplet located 50 nucleotides downstream, where translation resumes (Adamski *et al.*, 1996). Substitutions of certain amino acids in the CTD of L9 have been shown 'stimulate' translational bypassing by 10-fold (*hop-1* phenotype). It should be noted, however, that the control construct was a mutant form of gene 60 with a bypass rate reduced to 0.35% of the wild-type, therefore the overall stimulation in bypassing seen with the L9 mutants correlates to only 3.5% of the wild-type level (Adamski *et al.*, 1996). Subsequently, Herr and coworkers (2001) have shown that the complete absence of L9 promotes hopping over stop codons at the expense of -1 framshifting, leading to the suggestion that L9 may influence mRNA movement through the ribosome, rather than P-site tRNA stability, as first thought.

Certainly, on the basis of L9 in the crystal structure, it does seem improbable that L9 can directly contact the P site tRNA: L9 is tucked-in behind the L1 stalk and, despite the long linker region, it is hard to envisage the CTD being able to reach around the L1 stalk to approach the tRNA binding sites. One possibility is that the CTD of L9 makes contact with the head of the L1 stalk and influences tRNA stability by altering the orientation of the stalk relative to the ribosome (see next section on L1). This finding is consistent with the observation that the CTD of L9 was found by toeprinting to bind 5′ to nucleotide 2179 (H77) near to the L1 binding site (Adamski *et al.*, 1996). Therefore, L9 may adopt different orientations during translation to influence tRNA stability. However, it should be noted that this is probably a fine-tuning mechanism specific for bacteria, since as mentioned *E. coli* L9 deletion mutants exist (Herr *et al.*, 2001; Lieberman *et al.*, 2000) and L9 is not present in archaea or eukaryotes.

L16/L27 AND tRNA BINDING; L1 AND tRNA RELEASE

L16 and L27 are globular r-proteins that have extensions that reach toward the PTC. Interestingly, both proteins have been associated with proper placement of tRNA ligands at the PTC: L27 has been cross-linked to A- and P-site bound tRNAs (Wower *et al.*, 1998) and truncations of L27 suggest that it is the N-terminal amino acids that can come within close proximity of the aminoacyl moiety of the P-tRNA (Maguire *et al.*, 2003). Consistently, the oxazolidinone class of antibiotics that bind within the PTC also crosslink to L27 (Colca *et al.*, 2003). In the *D. radiodurans* 50S subunit, the flexible N-terminus of L27 would be expected to contact the elbow region of a P-tRNA, specifically the highly conserved lysine residues at positions 4 and 5 of L27 contact the backbone of G4-A5 of the acceptor stem (Figure 7A). L16 makes potential contacts with elbow region (TΨC arm) of both A- and P-site tRNAs (Figure 7A): Arg9 (*E. coli* Arg10) and Phe12 (His13) in the N-terminal of L16 come within 5 Å of the backbone of C63 (P-tRNA) and

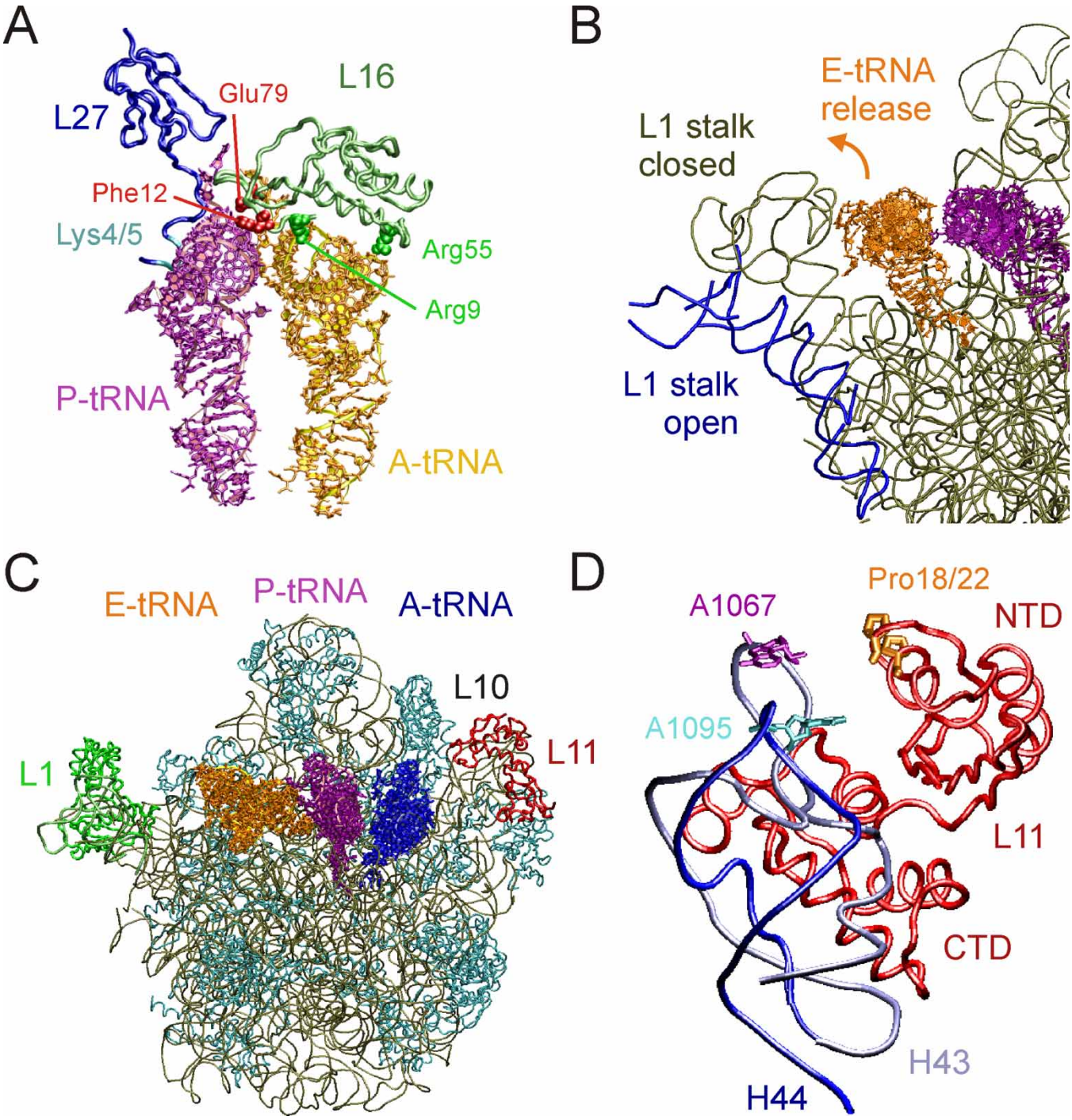

FIGURE 7 Function of ribosomal proteins in tRNA binding and release. (*A*) L16 (*green*) and L27 (*blue*) have extensions that approach the A- (*yellow*) and P- (*pink*) tRNAs. Lys4/5 (*cyan*) of L27 contact G4-A5 of the P-tRNA whereas L16 makes potential contacts with both A- (*green*) and P-site (*red*) tRNAs, specifically, Arg9 (*E. coli* Arg10) and Phe12 (His13) in the N-terminal of L16 come within 5 Å of the backbone of C63 (P-tRNA) and U52 (A-tRNA), respectively, whereas Arg55 and Glu79 (Ala79) are within hydrogen bonding distance the backbone of U52-G53 (A-tRNA) and G1 (P-tRNA), respectively. (*B*) The flexibility of the L1 stalk. The more open position of the L1 stalk in the *D. radiodurans* 50S subunit (*blue*) compared with that present in the *T. thermophilus* 70S ribosome (*tan*) suggests that opening and closing of the L1 stalk may regulate release of the E site tRNA (*orange*). The P-tRNA is shown in magenta. (*C*) The 50S subunit from *D. radiodurans* showing the relative positions of L11 (*red*), L1 (*green*) as well as A-(*blue*), P-(*magenta*) and E-site (*orange*) tRNAs. The approximate position of L10 near to the L11 binding site is as indicated. Note the L1-rRNA complex structure (Nikulin *et al*., 2003) has been aligned to the 50S subunit on the basis of H76. (*D*) L11-rRNA complex (Wimberly *et al*., 1999), indicating the two rRNA helices H43 (*light blue*) and H44 (*dark blue*), the N- and C-terminal domains (NTD/CTD) of L11 (*red*) as well as positions in the rRNA, A1067 (*magenta*) and A1095 (*cyan*), and in L11, P22 (*orange*), that when mutated confer resistance to thiostrepton.

U52 (A-tRNA), and Arg55 is within hydrogen bonding distance to the backbone of U52-G53 of an A-tRNA (Nishimura *et al*., 2004). In addition, Glu79 (*D. radiodurans*) located in the flexible loop between β4 and β5 can potentially hydrogen bond with G1 (P-tRNA) (Bashan *et al*., 2003a), although this interaction may be species-specific, since a few organisms, such as *E. coli*, have Gly or Ala at the equivalent position.

Recently, it was shown that the extensions of L16 and L27 interact with the ribosome recycling factor (RRF) (Wilson *et al*., 2005). Since both RRF and L27 are only present in bacteria (and organelles), the function of L27 may be associated with ribosome recycling rather than with tRNA binding. It should be noted, however, that L27 gene knock-outs are viable in *E. coli* (Dabbs, 1986), suggesting that the role of L27 may only be needed for fine-tuning of interactions. In contrast, L16 has homolog in achaea and eukarya called L10e (see Table 1), and therefore may play a more fundamental role in translation (note, however, that the loop in L16 that contacts RRF has no equivalent in L10e). Interestingly, mutations in L16 give rise to evernimicin resistance, an antibiotic that has been proposed to inhibit 70S initiation complex formation in an IF2-dependent manner (Belova *et al*., 2001), suggesting a possible link between L16 and translation initiation.

Ribosomes obtained from *E. coli* strains lacking L1 display a 40% to 60% reduced activity in polypeptide synthesis, which can be cured by the addition of L1. These ribosomes show reduced peptidyl tRNA binding and a decrease in EF-G-dependent GTPase activity. L1 and its rRNA binding region constitute one of the landmarks of the large ribosomal subunit, the so-called L1 stalk (Figure 7B). Crystallographic (Harms *et al*., 2001; Yusupov *et al*., 2001) and cryo-electron microscopic (EM) studies (Agrawal *et al*., 2000) have suggested that the movement of the L1 stalk controls removal of deacylated tRNA from the E site of the ribosome: In the *T. thermophilus* 70S $\times$ $tRNA_3$ crystal structure (Yusupov *et al*., 2001) the L1 stalk is in a closed form, where it makes contact with the elbow of the E-site tRNA and blocks tRNA release from this site, whereas in the *D. radiodurans* 50S structure (Harms *et al*., 2001), the L1 stalk is in a more open conformation, which would allow release of the E-tRNA (Figure 7B). This mechanism may be universal, since in yeast, when comparing the P-tRNA bound 80S ribosome (pseudo PRE state) with a stalled translating ribosome (artificially induced POST state), there is an enormous 70 Å difference in the tip position of the L1 stalk (Beckmann *et al*., 2001; Spahn *et al*., 2001).

The flexibility of the L1 stalk is supported by the lack of order within this region of the available ribosome crystal structures (Ban *et al*., 2000; Harms *et al*., 2001; Yusupov *et al*., 2001). The contact of L1 with tRNA is consistent with the biochemical data such that the elbow region of a tRNA (position 8) has been cross-linked near the L1-binding region (at nucleotide position 2111, 2112 of 23S rRNA) (Rinke-Appel *et al*., 1995) and three of the four tRNA-dependent E-site protections (against modifying agents) are found in (A2169) or near (G2112, G2116) the L1-binding site (Moazed & Noller, 1989). On the basis of the 70S $\times$ $tRNA_3$ crystal structure, it was suggested that protection of G2112 and G2116 might result from stacking interactions with the G19-C56 base-pair at the top of the tRNA elbow (Yusupov *et al*., 2001).

The crystal structure of the L1 protein bound to a specific 55-nucleotide fragment of 23S rRNA (Nikulin *et al*., 2003) has enabled this region to be modelled onto the ribosome (Figure 7C). The structure of the free L1 reveals two domains separated by a hinge region that has been observed in both an open and closed conformation. When bound to an rRNA fragment containing the L1 binding site, the open form of L1 is found. The majority of contacts with rRNA involve strictly conserved residues in domain I of L1 (Phe26, Thr208-Gly210) that contact highly conserved nucleotides in H77 and one strand of H78 (for example, G2124-2125, G2127, and C2175). This conservation explains why *E. coli* L1 can bind to large subunit rRNAs from all three kingdoms. Additional interactions from domain II are observed, mainly with the backbone of residues G2165-A2171 in the loop B following H78 (Nikulin *et al*., 2003). Recently, the structure of L1 in complex with its own mRNA has been determined, revealing a remarkable similarity with the L1-rRNA complex (Nevskaya *et al*., 2005). However, the mRNA has a shortened loop B compared to the equivalent region in the rRNA, which removes the potential for a number of hydrogen bonds with L1, which may explain the 5- to 10-fold higher affinity of L1 for the rRNA than the mRNA.

PROTEINS OF THE RIBOSOMAL STALK: FACTOR BINDING

The acidic L7/L12 (pI $\sim$4.9) is the only protein present in four copies per *E. coli* ribosome: Two dimers

bind independently to the C-terminal region of L10 (positions 71 to 164) to form the pentameric complex L10 × $(L7/L12)_4$. The pentameric complex forms a clearly defined morphological feature of the ribosome, which is referred to as the L7/L12 stalk. The complex has a high stability that partially resists even 6 M urea and was considered to be an individual protein termed L8 in the early days of ribosome research. Despite being perhaps one of the most studied r-proteins, there remains much to be unravelled about both the structure and function of these essential ribosomal proteins (reviewed by Brot & Weissbach, 1981; Gudkov, 1997; Wahl & Moller, 2002).

L7/L12 can be separated into three functional regions: (i) an N-terminal domain (NTD: residues 1 to 37) responsible for protein–protein interaction and dimerization. The NTD anchors L7/L12 to r-protein L10, and dimerization is essential for this process; (ii) a very acidic C-terminal domain (CTD: residues 50 to 120), which improves ribosome binding of the elongation factors and stimulates their GTPase activities (Dey *et al*., 1995; Kischa *et al*., 1971). Consistently, L7/L12 lacking the CTD (generated via mild proteolysis) bind to the ribosome but inhibit protein synthesis, and antibodies against the CTD also inhibit protein synthesis and factor binding as well; and (iii) a flexible hinge-region (residues 38 to 49) that facilitates independent movements of the N- and CTD domains.

Based on many intensive physico-chemical studies, a number of models for L7/L12 dimer were proposed, with monomers aligned in parallel or antiparallel configurations (see Gudkov, 1997). In contrast to these elongated models for the L7/L12 dimer, the crystal structure of *Thermatoga maritima* L12 reveals a compact hetero-tetrameric structure with parallel configurations containing two full-length L12 molecules and two N-terminal L12 fragments (Wahl *et al*., 2000). The two L12 dimers interact through their NTDs as expected, however, the hinge region has adopted a helical structure bringing the CTDs into close contact with the NTDs. Interestingly, the two additional N-terminal fragments of L12 present in the crystal structure have unstructured hinge regions, thus demonstrating the ability of the hinge region to alternate between different structural conformers (see Wahl & Moller, 2002).

In the 70S × $(tRNA)_3$ crystal structure, only one dimer of L7/L12 has been modelled onto the ribosome (Yusupov *et al*., 2001), tentatively placed at the base of the stalk in the compact conformation. The remaining L7/L12 copies are thought to form the stalk, the flexibility of which may prevent their structure determination. Indeed, NMR studies of intact 70S ribosomes reveal the dynamic nature of only two of the L7/L12 molecules suggesting that the other molecules are in the compacted conformation (Christodoulou *et al*., 2004). Interestingly, even these signals are lost upon EF-G binding, suggesting that the factor binding that induces a fixed conformation of the stalk (Christodoulou *et al*., 2004; Mulder *et al*., 2004). Consistently, cryo-EM reconstructions of 70S ribosomes reveal that the pentameric complex becomes partially ordered upon EF-G binding (Agrawal *et al*., 1998; Agrawal *et al*., 1999).

L7/L12 can be selectively removed from ribosomes by washing with 50% ethanol and 1 M ammonium chloride, a process which results in loss of factor-dependent GTPase activity. L7/L12 can then be rebound to restore activity (Hamel *et al*., 1972; Kischa *et al*., 1971). Similar results are seen with EF-Tu and initiation factor 2, and both elongation factors EF-G and EF-Tu have been cross-linked to L7/L12, as has been seen for the IF-2 (also a GTPase) (see Brot & Weissbach, 1981; and Wahl & Moller, 2002, and references therein). More work will be required to determine exactly the role L7/L12 plays during the initiation and termination phases of translation.

It was possible to generate a 70S ribosome *via* reconstitution that contained a normal L7/L12 dimer and second dimer lacking the L7/L12 CTD. Such a ribosome could support protein synthesis at levels approaching the two-headed wild-type dimer (Oleinikov *et al*., 1998). More strikingly, ribosomes containing only a single L7/L12 dimer (made by removing 10 amino acids from the C-terminus of L10) supported EF-G–dependent GTP hydrolysis and protein synthesis *in vitro* with the same activity as that of two-dimer particles (Griaznova & Traut, 2000). Collectively, this suggests that at least one dimer is needed during translation.

The recent detection that thermophilic bacteria such as *Thermotoga maritima*, *Thermus aquifex* and *Thermus thermophilus* contain a heptameric complex L10 × $(L7/L12)_6$ instead of the canonical pentameric one was a surprise (Ilag *et al.*, 2005; Diaconu *et al.*, 2005). A simple explanation is not at hand; it might be that the high-energetic movement of the elongation factors at temperatures above 60°C calls for an amplified "factor-catching device."

Hybrid ribosomes were constructed where the proteins at the GTPase center from *E. coli*, L7/L12, L10, and

L11, were replaced with their eukaryotic counterparts from rat P1/P2, P0 and L12, respectively (Uchiumi *et al.*, 2002). Both the *in vitro* translation and GTPase activity of the resultant hybrid ribosomes was strictly dependent on the presence of the eukaryotic elongation factors, EF2 and EF1a. This reflects not only the specificity of the interaction between the stalk proteins and the elongation factors from each kingdom, but also the importance of the stalk proteins L7/L12 for elongation factor recognition and GTPase stimulation. Since both EF-Ts, which is a guanine exchange factor for EF-Tu, and L12 interact with EF-Tu, the similarity between the NTD of EF-Ts and L12 CTD suggests a region in the CTD, which may interact with EF-Tu to stimulate GTP hydrolysis.

Deletions within the hinge region lead to translational misreading, deceased factor binding and a lowered rate of protein synthesis (Gudkov *et al.*, 1991). Therefore, the hinge region seems to be important in maintaining the flexibility of the CTD of L7/L12 required during elongation (Peske *et al.*, 2000). Evidence has been provided for multiple possible locations of the CTD: Ribosomes were reconstituted using an L7/L12 variant in which a single cysteine at position 89 in the C-terminal domain was modified with goldcluster (Montesano-Roditis *et al.*, 2001). Cryo-EM and particle reconstructions revealed the gold to be positioned at four different positions on the interface, including on the head of the 30S subunit. This illustration of the flexibility of the L7/L12 dimers is supported by previous data showing the localisation of L7/L12 at (1) the tip of the ribosomal stalk by immunoelectron microscopy; (2) near the base of the stalk, for example, cross-links to L6, L10, L11, and L14; or (3) near the peptidyltransferase centre (cross-links to L2 and L5) (Traut *et al.*, 1995). All three sites are close to the intersubunit region of the ribosome, therefore crosslinks are found including 30S proteins: L7/L12 to S2, S3, S7, S14, and S18, which were stimulated by the presence of EF-Tu (Traut *et al.*, 1995). All positions of the CTD may be of functional significance. Thus, models explaining the function of L7/L12 ought to consider the high flexibility of the CTD and the presence of two dimers of L7/L12 on the *E. coli* ribosome, a fact that has as yet no explanation.

L11 AND THE 'GTPase-ASSOCIATED CENTRE'

The pentameric L10 × $(L7/L12)_4$ complex and protein L11 bind in a cooperative way to a short sequence in domain II of 23S rRNA (nucleotides 1041 to 1114, *E. coli* numbering). Cryo-EM reconstructions suggest that the binding sites of L10 and L11 are immediately adjacent to each other. Although the L11 region is poorly ordered in the high-resolution ribosome structure, crystal structures of L11 in complex with an rRNA fragment containing the L11 binding site have been solved to 2.6 to 2.8 Å resolution (Figure 7D) (Conn *et al.*, 1999; Wimberly *et al.*, 1999). Protein L11 consists of two domains: a C-terminal domain (CTD) responsible for the tight interaction with the rRNA-binding region for L11 that is connected through a flexible linker to the N-terminal domain (NTD). It is the latter NTD that is required for the co-operative binding of antibiotic thiostrepton. Thiostrepton is one of the most effective blockers of both the transition from the pre- to the post-translocational state of the ribosome (translocation) and the reverse reaction, *viz.* A-site occupation of the e-type (elongation- or e-type ribosomes have P and E sites occupied; Hausner *et al.*, 1988). Since thiostrepton also blocks the factor-dependent GTPase, the L11-binding site on 23S rRNA was termed the 'GTPase-associated center'. (Note: the phrase 'GTPase center' that is sometimes used is certainly incorrect because the ribosome cannot bind GTP and does not contain a GTPase centre.) Mutations in the NTD (specifically, substitutions of P22S/T in *E. coli* L11) confer resistance to thiostrepton, although not by affecting interaction of thiostrepton with the rRNA, but perhaps by allowing L11 the freedom to move despite the presence of thiostrepton (Porse *et al.*, 1998). Interestingly, in bacterial (and archaeal) ribosomes, on which thiostrepton is active, Pro22 (Pro18 in archaea) in L11 is conserved, whereas in the equivalent position in eukaryotic L11 the proline is not conserved, consistent with the natural resistance of eukaryotic ribosomes to thiostrepton. The L11–rRNA interaction is conserved throughout all phylogenetic kingdoms, but not the interaction with thiostrepton. 2′-*O*-methylation (natural resistance mechanism of thiostrepton-producing *Streptomyces* strains) or mutation of A1067 (as well as 1095) confers resistance to thiostrepton (Thompson *et al.*, 1982; Cameron *et al.*, 2004 and references therein). It has been proposed that the sugar of A1067 and the base A1095 interact directly with thiostrepton by forming a binding pocket in conjunction with the prolyl residues of the NTD of L11 (Figure 7D; Lentzen *et al.*, 2003). Consistently, EF-G · GTP protects A1067 and, more weakly, A1096 from chemical modification;

furthermore, A1067U mutation impairs the function of EF-Tu and EF-G. Thiostrepton has been shown to reduce the binding affinity of EF-G for the ribosome and therefore decreases the EF-G dependent GTPase activity (Cameron *et al*., 2002). Studies using ribosomes depleted of L11 and L10 × $(L7/L12)_4$ demonstrate that the latter has a much more dramatic effect on the GTPase activities of EF-G and EF-Tu, whereas L11 only stimulated the rate of protein synthesis (Uchiumi *et al*., 2002). This is consistent with the recent cryo-EM reconstructions of yeast 80S-EF2 complexes where the yeast homolog of L11 (L12) is proposed to interact with domain V of EF2 (R760-G762), whereas the sarcin-ricin loop (H95) and the P proteins (eukaryotic L7/L12, see Table 1) interact with the G domain of EF2 (Spahn *et al*., 2004).

Another role for L11 in bacteria has been discovered during conditions of starvation. When nutrient levels are low, uncharged or deacylated tRNA binds to the A site of the ribosome and stalls translation. L11 detects the presence of the uncharged tRNA and signals this event to the stringent factor, RelA, which responds by catalyzing the synthesis of the alarmone (p)ppGpp (see Wendrich *et al*., 2002 and references therein). This signalling molecule downregulates transcription of components of the translational apparatus and upregulates the transcription of metabolic enzymes. This tight coupling of translation and RNA synthesis is termed the stringent control. Mutants lacking L11 (*relC*) do not show the stringent response under starvation conditions, a phenotype that is called 'relaxed.' How L11 detects the presence of uncharged tRNA and stimulates RelA has yet to be determined.

L4 AND L22: TUNNEL VISION

The crystal structures of the large ribosomal subunits have revealed the tunnel at high resolution (Harms *et al*., 2001; Ban *et al*., 2000) and confirmed the previously determined dimensions—a length of 100 Å with a diameter of 20 Å at the widest point (Frank *et al*., 1995; Milligan and Unwin, 1986; Yonath *et al*., 1987). These structures show that the wall of the tunnel is composed of nucleotides from domains I through V of the 23S rRNA, as well as of the extensions of r-proteins L4 and L22 that reach into the interior to form part of the narrowest region of the tunnel, near to the PTC (Figure 8A and B). Note that the extention of the bacterial-specific protein L32 also reaches the same tunnel region. This region of the tunnel is the binding site of the macrolide antibiotics that inhibit translation by blocking the path of the growing polypeptide chain through the tunnel. Mutations in L4 (K63E) and deletions in L22 ($\Delta_{82}MKR_{84}$) can confer resistance to some macrolides, such as erythromycin. However, since there is no direct contact between these proteins and the drug (Schlünzen *et al*., 2001) (Figure 8B), resistance most likely results indirectly through perturbation of the rRNA that constitutes the macrolide binding site (Gregory & Dahlberg, 1999; Gabashvili *et al*., 2001).

Interestingly, it is this section of the tunnel that has also been singled out as a region that in special cases may regulate translation *via* interaction with specific sequences within the nascent chain under translation. Such sequence-specific interactions between the exit tunnel and nascent peptides suggest that the ribosome can recognize signals in the nascent chain and use them for translational regulation. Indeed some nascent peptides in prokaryotes and eukaryotes contain special sequence motifs or so-called effector sequences, which, when situated in the tunnel of translating ribosomes, can significantly affect both protein elongation and peptide termination. Notably, in all documented cases, the effector sequence-containing peptides act only in *cis* and thus only affect the ribosome on which they are synthesized. Several of these active peptides have a co-effector and the interplay between the effector motif and the co-effector is key to the intracellular control. The co-effector can, for example, be an antibiotic (leading to expression of resistance genes), an amino acid (leading to induction of an amino acid degradation operon), or a polyamine (leading to repression of polyamine synthesis; summarized in Tenson & Ehrenberg, 2002).

An example of a peptide with an effector sequence is the secretion monitor (*secM*) gene of *E. coli* encoding a unique secretory protein that monitors cellular activity for protein export and accordingly regulates translation of the *secA* cistron downstream of the *secM* cistron on the bi-cistronic mRNA (reviewed by Nakatogawa *et al*., 2004). The SecM protein is exported to the periplasm, where it is rapidly degraded by a tail-specific protease. The regulation works via translational arrest using a signal sequence of the SecM protein with the consensus motif FXXXX**W**IXXXXGIRAG**P**. Stalled ribosomes have a polypeptide-Pro(**P**)-$tRNA^{Pro}$ positioned at the P-site and a Trp (**W**) residue located 12 amino acid residues toward the N-terminal end. Arrest will only be relieved,

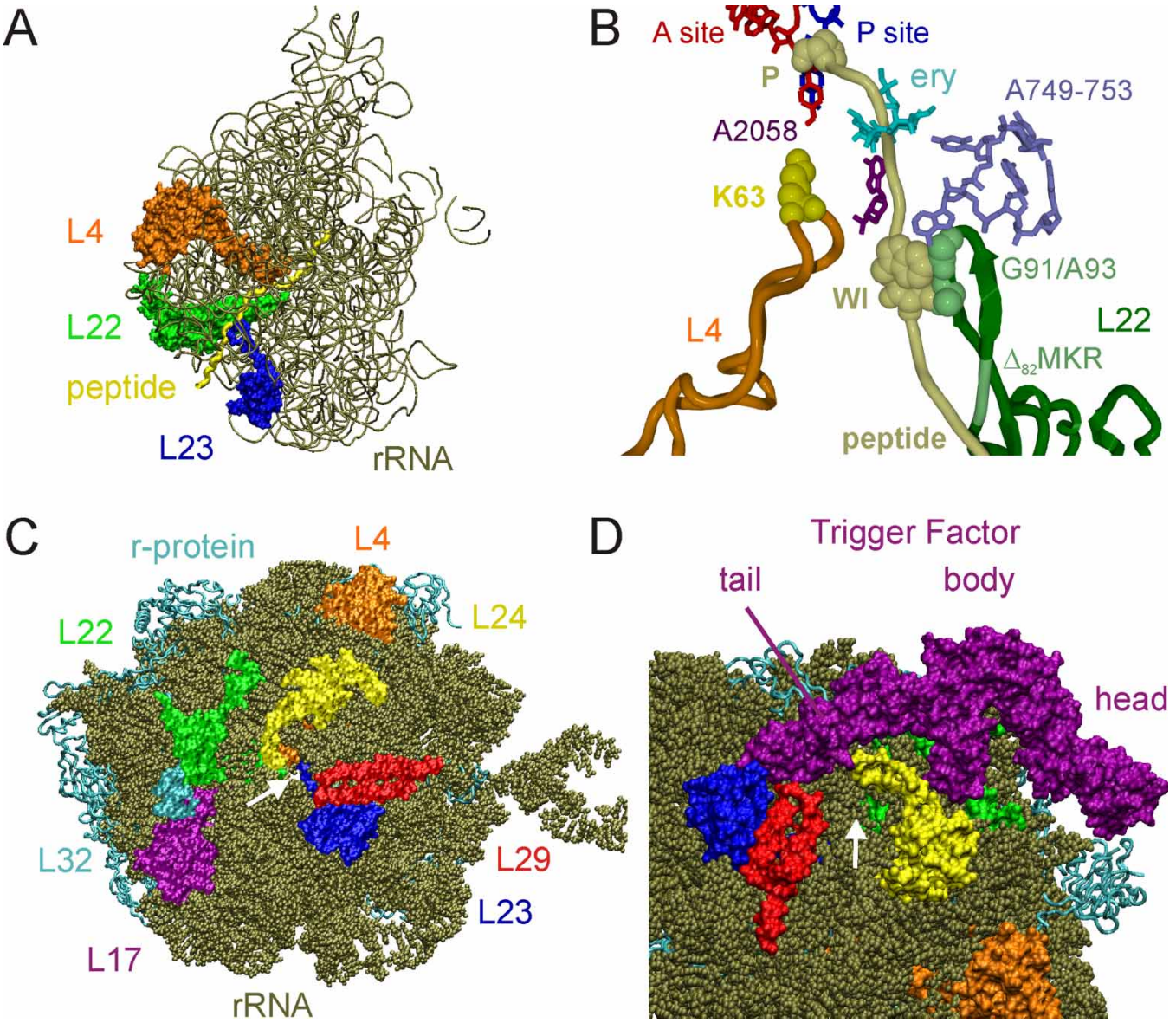

FIGURE 8 **Ribosomal proteins located in the ribosomal tunnel and at the exit site. (*A*) Sideview (from L1 side) of 50S subunit highlighting r-proteins L4, L22 and L23, the extensions of which reach into the tunnel (indicated by theoretical nascent polypeptide chain (*yellow*). (*B*) Close-up of the ribosomal components at the tunnel kink, located adjacent to the peptidyl transferase centre (indicated with A- and P-site ligands). The CCA-ends of the A-tRNA is in red and that of the P-tRNA in blue. The extensions of r-proteins L4 (*orange*) and L22 (*green*) reach into the interior of the ribosome, but do not come into contact with the macrolide erythromycin (*ery*) despite the fact that mutations (K63E in L4) or deletions ($_{82}$MKR$_{84}$ in L22) in these proteins confer resistance to this drug. Relief of the translational arrest caused by secM resulting from mutation at positions Gly91 and Ala93 in L22 (*space filled in light green*) and five-nucleotide insertion at position A749 of the 23S rRNA. Mutations at position A2058 (*purple*) of the 23S rRNA confer resistance to erythromycin and relieve secM translational arrest. The path of a hypothetical secM nascent chain is shown with Pro (*P*) at the active site and Trp/Ile (*WI*) located in vicinity of the β-hairpin of L22. (*C*) View through the tunnel from the cytoplasmic side of the 50S subunit showing the positions of the r-proteins located at the exit site. (*D*) The trigger factor (*TF*) interacts with r-protein L23 at the exit site of the tunnel. The full-length TF crystal structure has been docked onto the H50S subunit on the basis of the binding position of the N-terminal binding domain.**

if the ribosomal complex can contact the signal recognition particle (SRP)-SecA complex, thus triggering the export of nascent SecM. Only stalled ribosomes display the ribosomal binding site necessary for the translation of the downstream *secA* gene. In contrast, relief of the blocked SecM translation allows folding of the *secM-secA* mRNA, which hides the translational-initiation site of *secA*-mRNA region, thus preventing synthesis of the SecA protein.

Translational arrest can also be suppressed by insertion of five nucleotides at position 749 or mutation of A2048G in the 23S rRNA, as well as mutation at either of two positions in L22, namely, Gly91 or Ala93, (Nakatogawa & Ito, 2002). These two residues

are located in the β-hairpin of L22 that protrudes into the exit tunnel at the constricted region. Modelling of the signal-sequence-containing polypeptide in the tunnel revealed that the conserved Trp-Ile (WI) of the motif would be placed within close proximity to the tip of the β-hairpin of L22 (Figure 8B). Furthermore, binding position of the macrolide troleandomycin within the tunnel of the *D. radiodurans* 50S subunit coincides with the position of the β-hairpin of L22, which is consequently pushed across the tunnel lumen to contact the surface on the other side of the tunnel (Berisio *et al.*, 2003). This led to the suggestion that this "swung conformation" is related to a gating mechanism that could be involved in the secM-induced translational stalling; interaction of the Trp-Ile (both relatively bulky residues) may induce similar structural rearrangements in L22 such that the tunnel is temporarily closed and therefore translation blocked. The known ribosomal arrest suppression mutations of L22 (at Gly91 and Ala93) may therefore stabilize the swung conformation, however, confirmation will require structures of stalled nascent chain-ribosome complexes.

THE LIGHT AT THE END OF THE TUNNEL: RIBOSOMAL PROTEINS AS DOCKING SITES FOR CHAPERONES

The exit of the ribosomal tunnel is surrounded by a ring of r-proteins, including L22, L23, L24, and L29 (see Figure 8A and C) as well as L19e, L31e, and L39e in archaea. As nascent chains emerge from the ribosomal tunnel they are met by a cascade of chaperone systems that direct the unfolded chain towards its native folded state. In bacteria, the trigger factor (TF) is thought to be the first of these chaperones, because it binds to ribosomes and can be crosslinked to nascent chains as short as 57 amino acids, of which 30 to 40 amino acids would be still present in the ribosomal tunnel. Neutron scattering and cross-linking results suggest that TF is in close proximity to r-proteins L23 and L29 (Blaha *et al.*, 2003; Kramer *et al.*, 2002), two proteins that form part of the exit site of the tunnel. Direct interaction between L23 and TF has been demonstrated in the absence of the ribosome, and mutation of E13K in L23 abolishes TF binding (Kramer *et al.*, 2002). Although L29 is in close proximity to TF, strains where the gene encoding L29 was deleted are still viable and ribosomes isolated from these strains are still capable of binding TF, suggesting that L29 is not essential for TF interaction. Recently, the crystal structure of 35 amino acids of the N-terminal binding domain of TF in complex with the 50S subunit confirmed these results (Figure 8D), revealing that indeed the major interactions were with L23, and specifically, that E13K establishes important interactions with TF (Ferbitz *et al.*, 2004). What was perhaps surprising about this study was that bacterial TF was found to bind specifically to archaeal ribosomes, despite the fact that archaea do not have TF; however, this said, it should be noted that L23 is universally conserved and the region that interacts with TF including the E13 (of L23) is conserved in all known L23 sequences.

Another factor that has recently been shown to interact with the ribosome in the vicinity of the tunnel exit site is the signal recognition particle (SRP). This ribonucleoprotein complex recognizes nascent chain-bearing specific signal sequences as they emerge from the tunnel. SRP binds to the ribosome and interacts with the signal sequence and directs the translating ribosome to the docking site on the membrane. The universally conserved protein component of the SRP complex, SRP54 (termed Ffh in bacteria), has been crosslinked to L23 in eukaryotes (Pool *et al.*, 2002), suggesting that L23 may represent a general docking site at the tunnel exit for auxiliary factors. Cryo-EM of eukaryotic ribosomes containing a nascent chain with a signal sequence and bound to the SRP complex has revealed that the N-terminal domain of SRP54 interacts with the ribosome in close proximity to L23 and L29 (Halic *et al.*, 2004). Surprisingly, recent biochemical data demonstrate that in bacteria, at least, both TF and SRP can be present on the ribosome simultaneously (Raine *et al.*, 2004), suggesting that they do not have identical binding sites on the ribosome.

FUNCTIONS OF RIBOSOMAL PROTEINS BEYOND THE RIBOSOME

Besides functioning in protein biosynthesis within the ribosome, some r-proteins are involved in other cellular processes (Wool, 1996). One of the most conspicuous examples is the fact that ribosomal proteins feedback-regulate their own synthesis at the translational level (Nomura *et al.*, 1984). The principle is that an rRNA-binding protein coded for by a cistron of a polycistronic mRNA binds to the first ribosomal initiation site of its polycistronic mRNA, thus preventing the translation of the whole mRNA. The involvement

in translational regulation has been proven for the proteins S1 (S1 operon), S2 (S2 operon), S4 (alpha operon), S7 (*str* operon), S8 (*spc* operon), S15 (S15 operon), S20 (S20 operon), L1 (L11 operon), L4 (S10 operon), L10 and L12 (L10 operon), and L20 (thrS/L20/pheS operon). The S10 operon seems to be the only one that is also transcriptionally regulated by r-protein L4 in addition to the role of L4 in translational regulation (Zengel & Lindahl, 1994).

In prokaryotes, transcription and translation are coupled. This means that the first translating ribosome immediately follows the transcriptase (RNA polymerase). In the case of a significant gap between the first ribosome and the transcriptase, premature termination of transcription occurs (rho factor-dependent termination). rRNAs are not translated, and thus rRNA synthesis can be terminated in a rho-factor-dependent manner. In order to prevent premature termination, an anti-termination mechanism exists: defined short sequences called *box A*, *box B* and *box C* are found in the transcribed leader sequence of the rRNAs (*nut* sites). These boxes are needed to form an anti-termination complex with the RNA polymerase and Nus factors that act in concert with the r-proteins S1 and S10 (NusE; Squires & Zaporojets, 2000). In addition, there is sequence similarity between transcription elongation factor NusG and the bacterial r-protein L24, one of the two assembly initiation proteins. The detailed molecular mechanism of the anti-termination process is still under investigation.

OUTLOOK

It should be noted that while we have presented a wealth of information concerning the possible roles of ribosomal proteins, most is derived from studies using a single bacterial system, namely, that of *Escherichia coli*. Whether the properties of these r-proteins can be transferred to the r-proteins of other organisms remains to be seen. A classic example of the dangers of generalizing results obtained with *E. coli* to other organisms has been recently illustrated for the ribosomes of *Thermatoga maritima* and *Thermus thermophilus*. These thermophilic bacteria have been recently found to have, not four, but six copies of L7/L12 per ribosome (Ilag *et al*., 2005; Diaronu *et al.*, 2005). This opens the question as to how many copies other organisms have and what advantage having multiple copies of these r-proteins would confer?

In reality the ribosomes of very few organisms have actually been analysed in detail and therefore there may be even more ribosomal protein-functions to discover. This is particularly true for eukaryotic ribosomes: Proteomic analysis of yeast and human ribosomes identified a new small subunit ribosomal protein, termed RACK1 (Link *et al*., 1999). RACK1 has been shown by cryo-EM to bind to the head of the 30S subunit in close proximity to the mRNA exit pore (Sengupta *et al*., 2004). RACK1 interacts with a number of different signalling molecules, such as Src, intergrin-β and protein kinase C (hence, the name, **R**eceptor for **A**ctivated **C**-**K**inase), and through this interaction is thought to mediate translation regulation (reviewed by Nilsson *et al*., 2004).

Another point to consider that has not been discussed here relates to the possible role of post-translational modification of r-proteins. A number of r-proteins are modified in *E. coli* (Arnold & Reilly, 1999) and, in some cases, the modifications are developmental regulated, for example the number of C-terminal glutamic acid residues in S6 is reduced under stress conditions. Likewise, S6 phosphorylation has been implicated in a signaling pathway regulating translation (reviewed by Thomas, 2002). Phosphorylated L12 was found to be present in intact 70S ribosomes from *T. thermophilus*, in contrast to the presence of unmodified L12 in the unbound L10 $\times$ $(L7/L12)_6$, thus suggesting a role for r-protein modifications in stability of the stalk complex *in situ* (Ilag *et al*., 2005). However, in many cases the role of post-translational modifications of r-proteins is unclear, a classic example is the highly conserved methylations of r-protein L11: So far no detectable phenotype is evident for deletion of the gene encoding the L11 methylase PrmA, in both *E. coli* (Vanet *et al*., 1994) and *T. thermophilus* strains (Cameron *et al*., 2004). The latter study found that free L11 is a better substrate for *in vitro* methylation than ribosome-bound L11, leading the authors to speculate that methylation may play a role during ribosome assembly. This is reminiscent to the role of modifications of rRNA, for example pseudouridinylation, individual sites of which do not seem to be important per se, but collectively seem to be important for stabilization of rRNA tertiary structure (reviewed by Ofengand *et al*., 2001).

In eukaryotes, the presence of multiple genes encoding the same r-protein compounds the problem. This is exemplified by plants, especially those of *Arabidopsis thaliana*, where each ribosomal protein is encoded for on average by four separate genes, many isoforms of which have been shown to be present in the 80S

ribosome (Giavalisco *et al*., 2005). In eukaryotes, such as the slime mold *Dictyostelium discoideum*, the composition of the ribosome has been shown to change depending of the developmental stage, both in qualitative and quantitative terms as well as in terms of modifications (reviewed by Ramagopal, 1991). A classic example in this respect is the P-proteins (eukaryotic L7/L12 orthologs), which are completely lost from stationary phase ribosomes. Furthermore, ribosomes deficient in P-proteins translate a different subset of mRNAs compared to those with P-proteins. A so-called "ribosome filter hypothesis" has been put forward to explain the function of such heterogeneity within ribosome populations (Mauro & Edelman, 2002). Mauro and Edelman (2002) propose that particular sets of ribosomal proteins and/or rRNA could favour the translation of specific mRNAs. In this respect, the ribosome then becomes an important player for the regulation of the cellular environment. Although much work needs to be done to test this model, such a regulation system would add yet another layer to the importance of ribosomal proteins during translation.

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