

# A Comparison of the Yeast and Rabbit 80 S Ribosome Reveals the Topology of the Nascent Chain Exit Tunnel, Inter-subunit Bridges and Mammalian rRNA Expansion Segments

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Protein synthesis in eukaryotes is mediated by both cytoplasmic and membrane-bound ribosomes. During the co-translational translocation of secretory and membrane proteins, eukaryotic ribosomes dock with the protein conducting channel of the endoplasmic reticulum. An understanding of these processes will require the detailed structure of a eukaryotic ribosome. To this end, we have compared the three-dimensional structures of yeast and rabbit ribosomes at 24 Å resolution. In general, we find that the active sites for protein synthesis and translocation have been highly conserved. It is interesting that a channel was visualized in the neck of the small subunit whose entrance is formed by a deep groove. By analogy with the prokaryotic small subunit, this channel may provide a conserved portal through which mRNA is threaded into the decoding center. In addition, both the small and large subunits are built around a dense tubular network. Our analysis further suggests that the nascent chain exit tunnel and the docking surface for the endoplasmic reticulum channel are formed by this network. We surmise that many of these features correspond to rRNA, based on biochemical and structural data.

Ribosomal function is critically dependent on the specific association of small and large subunits. Our analysis of eukaryotic ribosomes reveals four conserved inter-subunit bridges with a geometry similar to that found in prokaryotes. In particular, a double-bridge connects the small subunit platform with the interface canyon on the large subunit. Moreover, a novel bridge is formed between the platform and the base of the L1 domain. Finally, size differences between mammalian and yeast large subunit rRNAs have been correlated with five expansion segments that form two large spines and three extended fingers. Overall, we find that expansion segments within the large subunit rRNA have been incorporated at positions distinct from the active sites for protein synthesis and translocation.

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## Introduction

Protein synthesis is a highly conserved process that occurs in all organisms from bacteria to man. This is reflected in the similarity of ribosomal structure and function (Wilson & Noller, 1998). Ribosomes synthesize soluble proteins, as well as

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Abbreviations used: ER, endoplasmic reticulum; PTC, peptidyl transferase center; LSU, large subunit; SSU, small subunit; IF, intermediate filament.

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proteins which are exported or integrated into the membrane. This distinction is highlighted in eukaryotes, in which ribosomes are found free in the cytoplasm and bound to the endoplasmic reticulum (ER) membrane. In the latter case, ribosomes associate with a protein conducting channel formed by the Sec61p complex (reviewed by Matlack *et al.*, 1998). Therefore, two functions of the eukaryotic ribosome are to synthesize proteins and dock to the ER membrane.

A detailed understanding of ribosomal function will require a knowledge of its structure. During recent years, dramatic progress towards a high-resolution structure of the bacterial ribosome has been made. In particular, our knowledge of the bacterial ribosome has been augmented by pioneering studies carried out by electron cryo-microscopy and single particle, 3D image processing (Penczek *et al.*, 1994; Radermacher, 1988, 1994; van Heel 1987). These studies have focused primarily on the 70 S ribosome from *Escherichia coli* (Malhotra *et al.*, 1998; Stark *et al.*, 1995) and in addition, have compared the bacterial ribosome with yeast (Verschoor *et al.*, 1998), rat (Dube *et al.*, 1998a) and rabbit ribosomes (Dube *et al.*, 1998b). This work suggests that the larger eukaryotic ribosomes have a similar topology for the peptidyl transferase center (PTC) and decoding center, relative to the bacterial ribosome. These studies have also revealed a conserved tunnel within the large subunit (LSU), that runs from the PTC to a position on the bottom of the ribosome, where the nascent chain has been localized (Bernabeu *et al.*, 1982, 1983). The yeast Sec61p channel complex was shown to dock on the LSU, with its central pore aligned over the tunnel exit. (Beckmann *et al.*, 1997). A similar juxtaposition of the ER channel with the ribosomal tunnel has been confirmed for mammalian ribosome-Sec61p complexes (Ménétret *et al.*, unpublished results). These observations underscore the universal importance of the LSU exit tunnel in co-translational translocation.

Structural electron microscopy studies of the ribosome are being used to piece together a model of the protein synthesis cycle, in combination with crystallographic data on individual components (Frank, 1998a). For example, the docking positions of the A, P and E-site tRNAs have been determined relative to the decoding center and PTC (Agrawal *et al.*, 1996; Stark *et al.*, 1997a) and a plausible pathway for the movement of tRNAs through the ribosome has been proposed (Frank *et al.*, 1995, Frank & Agrawal, 1998; Wilson & Noller, 1998). In conjunction with these studies, the position of a ternary complex of EF-Tu-GTP Phe-tRNA<sup>Phe</sup> has been mapped on the *E. coli* ribosome. The elongation factor is bound to the L7/L12 stalk and the amino acyl tRNA is parked within the A-site (Stark *et al.*, 1997a,b). In addition, the EF-G-GDP complex has been visualized and shown to interact with the L7/L12 region (Agrawal *et al.*, 1998). These results support the hypothesis that molecular mimicry between the

two elongation factor complexes may reflect an underlying mechanistic similarity in their functions, as deduced from single crystal structures (Wilson & Noller, 1998).

A combined approach using electron cryo-microscopy and X-ray crystallography recently has resulted in medium resolution (9-5 Å) structures of the bacterial ribosome and the LSU (Cate *et al.*, 1999; Ban *et al.*, 1998, 1999). However, the flexibility of the small subunit prevented the use of models derived from electron cryo-microscopy for molecular replacement at low resolution. Nevertheless, the small subunit (SSU) crystals have been solved to a similar resolution (Clemons *et al.*, 1999; Tocilj *et al.*, 1999). These breakthroughs have allowed the positioning of ~12 ribosomal proteins within the maps of the small and large subunits. In addition, a partial identification of the rRNA scaffolding of the SSU has been achieved (Clemons *et al.*, 1999; Tocilj *et al.*, 1999). Moreover, three structures of the *Thermus thermophilus* ribosome at 7.8 Å resolution have now provided a detailed view of the A, P and E-site tRNAs docked within the active-site chamber, and revealed a number of functionally important inter-subunit bridges (Cate *et al.*, 1999). In addition, a channel in the neck of the small subunit was found to be aligned with the 3' end of the mRNA, giving support for the hypothesis that mRNA may use this entrance into the decoding center (Frank *et al.*, 1995; Lata *et al.*, 1996; Cate *et al.*, 1999).

By comparison, our current knowledge of the eukaryotic ribosome is still rudimentary. As the major function of ribosomes is to synthesize proteins, one would expect conservation of the essential features of the ribosome. However, one cannot assume that the structures are identical. For example, the mammalian ribosome is about 50% larger than the *E. coli* ribosome and contains additional proteins and numerous inserted sequences within the rRNAs. These rRNA inserts are termed expansion segments (Schnare *et al.*, 1996; Gerbi, 1996). Moreover, membrane-bound ribosomes exist in eukaryotic cells, whereas it is unclear whether they exist in bacteria. It is therefore possible that prokaryotic and eukaryotic ribosomes differ in certain functional aspects related to membrane docking. Initial structures of eukaryotic ribosomes have now been determined by structural electron microscopy (Dube *et al.*, 1998a,b; Verschoor *et al.*, 1998), and a preliminary assignment of the major expansion segments in the SSU and LSU rRNAs has been made by comparing *E. coli* and rabbit ribosomes (Dube *et al.*, 1998b). However, it is noteworthy that a detailed comparison has not been made of other functional regions of the mammalian ribosome, such as the inter-subunit bridges and the ER membrane docking site.

Here, we compare the 3D structures of frozen-hydrated yeast and rabbit 80 S ribosomes at 24 Å resolution. Particular emphasis has been placed on the similarity of their active sites for protein synthesis and co-translational translocation. It is

interesting that we find a channel within the neck of the yeast and rabbit SSUs, that may serve as the entrance for threading mRNA into the decoding center, lending support to this hypothesis (Frank *et al.*, 1995; Lata *et al.*, 1996; Dube *et al.*, 1998b, Cate *et al.*, 1999). Moreover, we find that the walls of the nascent chain exit tunnel and the Sec61p docking surface are comprised of high-density features, which may correspond in part to rRNA. In addition, the data reveal five prominent inter-subunit bridges that function to hold the ribosome together, including a novel connection between the SSU platform and the LSU.

Finally, rabbit ribosomes are larger than their yeast cousins by ~400-600 kDa (~12%) and this parallels a change in the LSU rRNA from 25 S (~3400 nt) to 28 S (~4700 nt). Indeed, significant size changes in five LSU expansion segments (Gerbi, 1996) may account for most of the major morphological differences between yeast and rabbit ribosomes. In agreement with this analysis, we find five electron dense, tubular features located on the surface of the rabbit LSU, which are present as two long spines and three extended fingers. These expansion segments are likely comprised of double-stranded rRNA that are inserted at positions distinct from the active sites for translation and translocation.

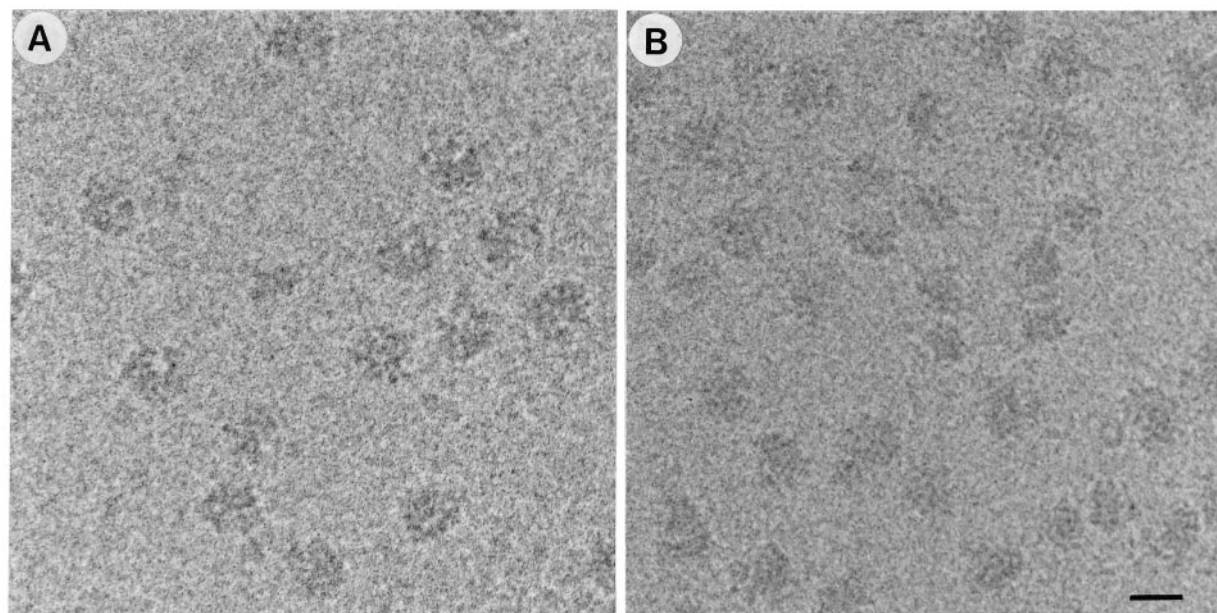
## Results

Yeast and mammalian cells are separated by ~1.5 billion years of divergent evolution from a common ancestral eukaryote. A comparison of their ribosome structures should provide insights

into conserved features utilized in protein synthesis and translocation, while revealing differences due to mammalian expansion segments present in the LSU rRNA.

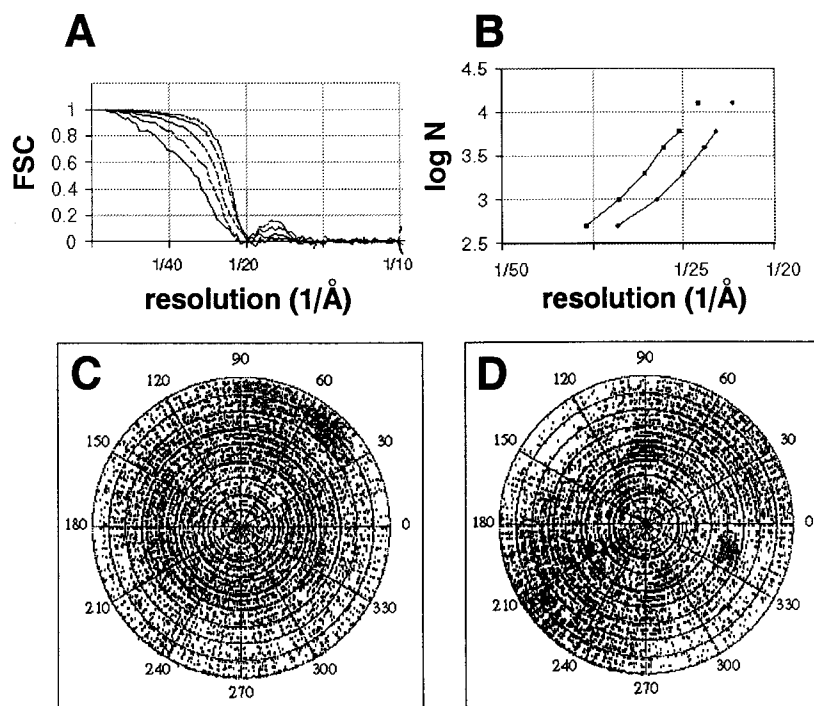
### Structures of the yeast and rabbit 80 S ribosome

We first determined the 3D structure of the frozen-hydrated yeast ribosome using classification (Frank, 1990) and random conical tilt analysis (Radermacher, 1988), to obtain a low-resolution model. Subsequently, 3D Radon alignment methods (Radermacher, 1994; 1997) were used to refine the model and the dataset was expanded to ~12,000 particles. An untilted micrograph of yeast ribosomes in frozen buffer is shown in Figure 1(a). We evaluated the resolution of the yeast ribosome structure by computing a series of pairwise Fourier shell correlation curves (FSC; Saxton & Baumeister, 1982), from final maps containing increasing numbers of randomly chosen particles (Figure 2(a)). The resolution for each comparison, using the FSC<sub>0.5</sub> criteria (Bottcher *et al.*, 1997), was then plotted *versus* the log of the number of particles (Figure 2(b)). Extrapolation suggests that the final 3D volume with ~12,000 particles should have a resolution of ~22 Å. The angular coverage for the projection data is shown in Figure 2(c), as a polar plot of the first two Euler angles. A second maximum at ~1/17 Å in the FSC curves suggests that higher resolution data can be obtained with the Radon method. For our maps, we applied a Fermi low-pass filter at the first zero of the more strongly defocused images which limited the resolution to ~24 Å. However, the major conclusions of this



**Figure 1.** Electron micrographs of the yeast and rabbit ribosome in vitreous ice. (a) A typical field of yeast ribosomes. The protein is shown in black. (b) A typical field of rabbit ribosomes. Scale bar represents 500 Å.





**Figure 2.** Resolution and angular coverage in 3D datasets of the yeast and rabbit ribosome. (a) Fourier shell correlation analysis of yeast 3D datasets. From left to right the correlation curves represent pairwise comparisons between independent datasets, with each set containing: 500, 1000, 2000, 4000 and 6000 particles. (b) The logarithm of particles in pairwise comparisons is plotted *versus* resolution for the yeast (squares) and rabbit (diamonds) ribosome 3D datasets. The resolution was defined as the point at which the FSC curve crosses the 0.5 value. The unconnected data points represent an extrapolation for the full datasets. We used a conservative value of 1/24 Å for the final resolution cutoff. (c) Angular coverage for the yeast ribosome 3D dataset is shown as a polar projection, in which the first two Euler angles are plotted for each image. Some points represent multiple overlaps of aligned images. (d) Angular coverage for the rabbit ribosome 3D dataset.

work would not be changed appreciably upon extension of the data to  $\sim 17$  Å resolution.

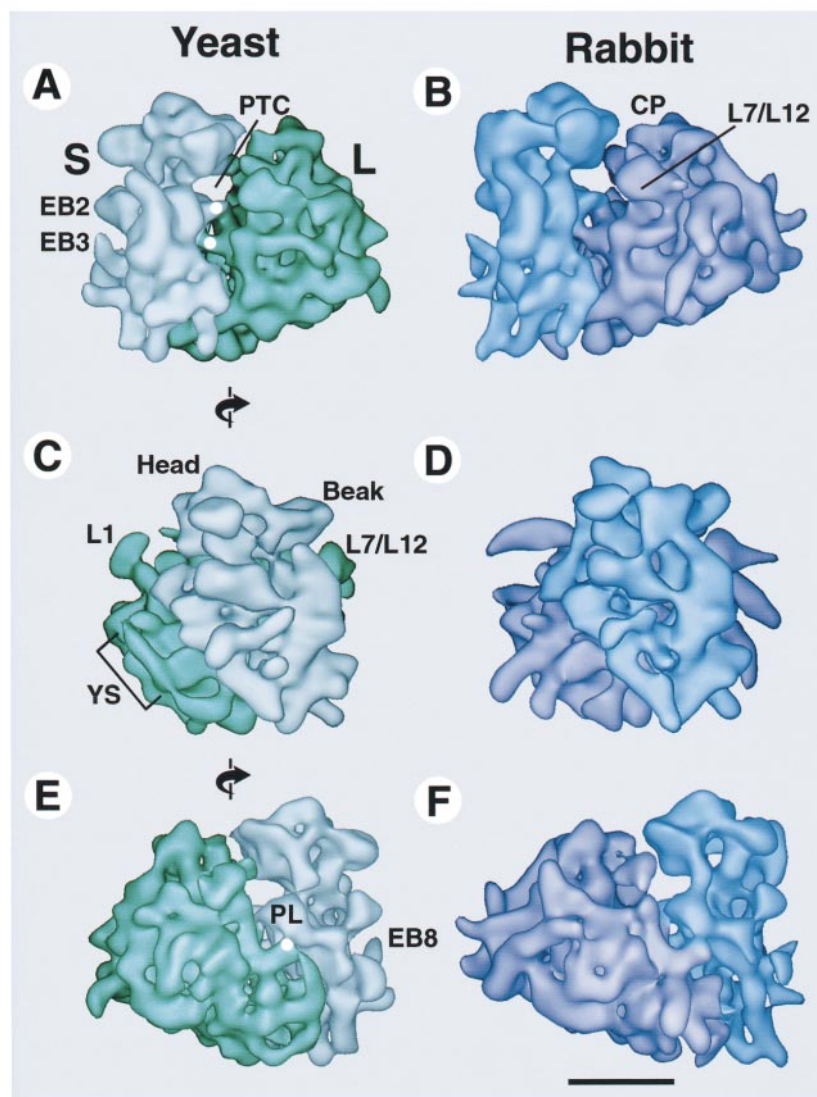
After choosing an appropriate threshold (see Materials and Methods), we separated the yeast ribosome into small and large subunits which allowed them to be studied individually. Color-coded surface maps of the yeast ribosome are shown for the front (Figure 3(a)) and back views (Figure 3(e)). In addition, an intermediate view ( $\sim 90^\circ$  from the front view) is shown in Figure 3(c), in which the LSU is eclipsed by the SSU.

In order to compare yeast and mammalian ribosomes, we next computed a 3D map of the rabbit ribosome from reticulocytes. A representative untitled image of rabbit ribosomes is shown in Figure 1(b). For this structure, the yeast ribosome was filtered to  $\sim 50$  Å resolution and used as the first reference in an iterative 3D Radon alignment. The resolution curve for the final rabbit 3D structure ( $\sim 12,000$  particles) is displaced by  $\sim 2$  Å to the left, relative to the yeast ribosome (Figure 2(b)). The somewhat lower resolution of the rabbit ribosome is likely due to the different defocus values that were used rather than uneven angular coverage (Figure 2(d)). In addition, a second maximum was present at

$\sim 1/19$  Å (not shown), which again suggests that higher resolution will be attainable. The refined rabbit ribosome structure was separated into subunits and Fermi low-pass filtered to 24 Å resolution. Color-coded surface views of the rabbit ribosome are shown in Figure 3(b), (d) and (f), in orientations similar to the yeast ribosome.

Overall, the yeast ribosome appears distinctly smaller and more rounded than its mammalian cousin. This arises in part, from additional features on the rabbit large ribosomal subunit, which contribute to a more triangular profile in the front and back views (compare Figure 3(a), (b) and (e), (f); see Dube *et al.*, 1998a). In this respect, the yeast ribosome is intermediate between the *E. coli* and rat ribosome structures (Dube *et al.*, 1998a). Yeast and rabbit ribosomes share a conserved set of core features, but obvious differences in size and shape suggest that the strategy of using the yeast ribosome as an initial reference was successful.

The basic mechanism of translation has been conserved between bacteria and man, which is reflected in the structural homology between the peptidyl transferase and decoding centers of the bacterial, yeast and rabbit ribosomes. The active site for translation is localized within a large

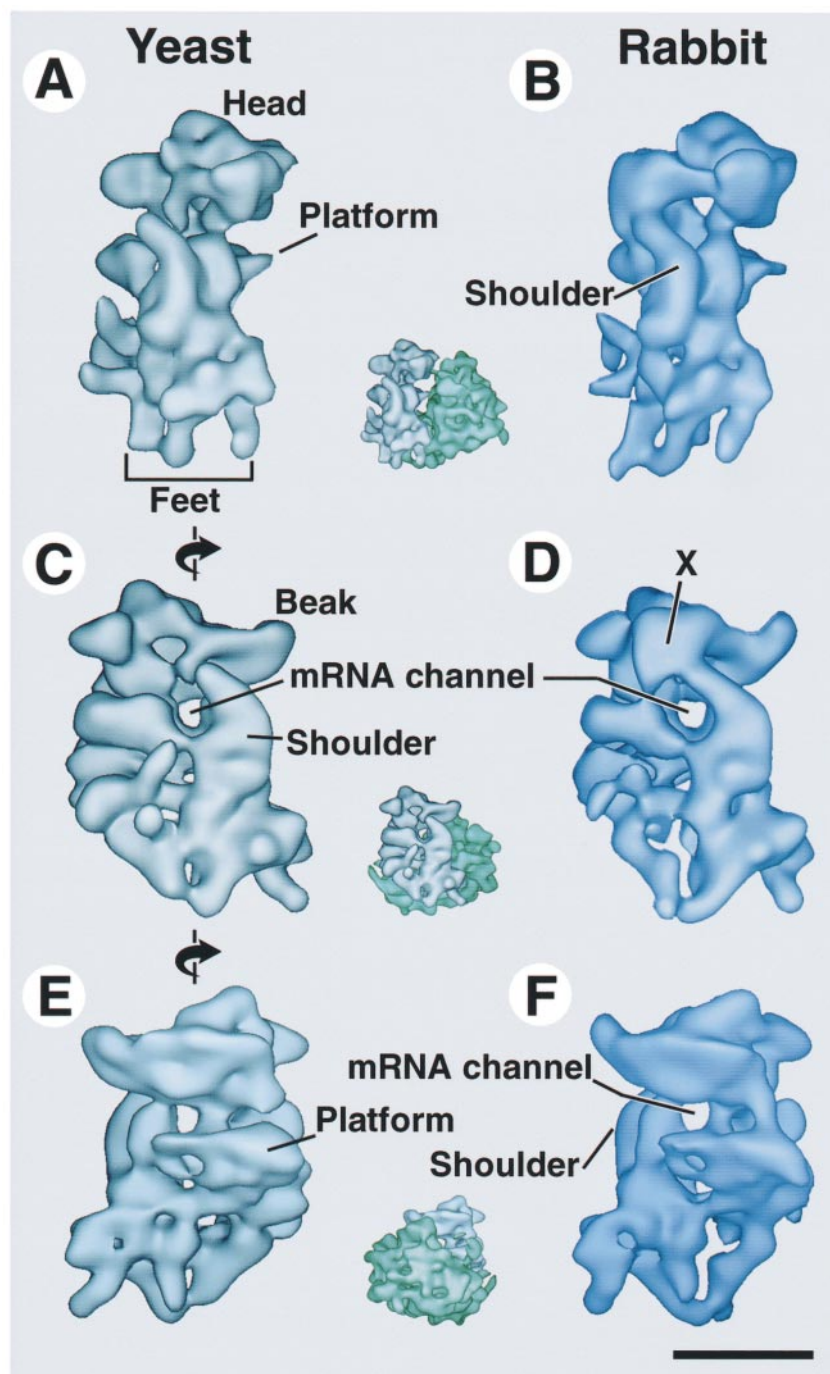


**Figure 3.** A comparison of the yeast and rabbit ribosome. (a) A frontal view of the yeast ribosome is shown in which the small (S) and large (L) subunits have been color-coded a blue-green and teal-green, respectively. The peptidyl transferase center (PTC) is located within a chamber that is formed at the interface of the subunits. Two prominent eukaryotic bridges (EB2 and EB3) are marked by white dots. (b) A frontal view of the rabbit ribosome is shown in which the small and large subunits have been color coded a medium blue and purple, respectively. The central protuberance (CP) and the L7/L12 domains on the large subunit are indicated. (c) The yeast ribosome is shown such that the small subunit eclipses the large subunit. This view is related to the frontal view in (a) by a rotation of  $\sim 90^\circ$ . Hallmarks for the ribosome are indicated, including: the head and beak of the small subunit, the L1 and L7/L12 domains on the large subunit, along with the distinctive yeast spine (YS). (d) The rabbit ribosome is shown in an eclipse view, similar to (c). (e) Back view of the yeast ribosome. This view is related to the eclipsed view in (c) by a rotation of  $\sim 90^\circ$ . The platform (PL) within the small subunit and a prominent bridge (EB8, see white dot) are indicated. (f) The rabbit ribosome is shown in a back view, similar to (e). Scale bar represents 100 Å.

“chamber” that is formed at the interface between the small and large subunits (Stark *et al.*, 1997a; Agrawal *et al.*, 1996) and houses the A, P and E-sites for tRNAs (see Frank & Agrawal, 1998; Frank, 1998a,b). The decoding center lies underneath the head and beak of the SSU, which together form part of the chamber roof. These features are very similar in the two eukaryotic ribosomes (Figure 3(a) and (b)). Many additional prominent features are located in equivalent environments when comparing the *E. coli*, yeast and rabbit ribosomes (Dube *et al.*, 1998a; Verschoor *et al.*, 1998). Therefore, we will use the bacterial nomenclature for similar features present in the eukaryotic ribosome. For example, the eukaryotic counterparts of L1, the central protuberance (CP) and the L7/L12-stalk are readily identified (Figure 3). A detailed analysis of the eukaryotic ribosomal subunits and their interactions is presented below.

### A comparison of small subunits within the yeast and rabbit ribosome

The small subunit of the ribosome plays a critical role in protein synthesis by interacting with initiation factors and mRNA to form an initiation complex, which precedes formation of the intact translating ribosome. The composition and mass of the yeast and rabbit ribosomes (Table 1), suggests that their SSUs should be similar at low resolution (1123 *versus* 1120 kDa). We were able to separate the small and large subunits after identifying five inter-subunit bridges that are present in the eukaryotic ribosome (see section on inter-subunit bridges). The SSUs of yeast and rabbit are shown as pairs in Figure 4, with yeast ribosome “icons” to show the relative orientation of the small and large subunits. The morphology of the SSU is expected to be highly conserved and this is the case. Overall, the structures of the SSUs reveal a



**Figure 4.** A comparison of small subunits from the yeast and rabbit ribosome. (a) A frontal view of the yeast small subunit is shown, along with a ribosomal icon demonstrating the relative position of the large subunit in this orientation. Characteristic features of the small subunit, including the head, platform and feet are labeled. (b) A frontal view of the rabbit small subunit is shown. The shoulder is labeled. (c) The yeast small subunit is shown in an orientation close to the eclipsed view. The putative mRNA entrance channel can be seen underneath the beak. The channel is aligned with a groove which runs behind the shoulder. (d) The rabbit small subunit is shown in an orientation similar to (c). An unusual domain on the back of the head is labeled (X). (e) The yeast small subunit is shown as viewed from within the ribosomal interface. The mRNA channel passes within the neck of the small subunit and opens onto the leading edge of the platform. (f) The rabbit small subunit is shown in an orientation similar to (e) and the shoulder and mRNA channel are labeled. Scale bar represents 100 Å.

large head and beak (Figure 4(a), (b) and (c), (d)), which contribute to the ceiling and side wall of the active-site chamber, and a body with a pronounced shoulder in the frontal view (Figure 4(a) and (b)). Numerous dense rods are present within the head, body and feet of the SSU, and their diameter suggests they may represent helical, double-stranded rRNA as seen in the *E. coli* subunit (Mueller & Brimacombe, 1997a,b; Mueller *et al.*, 1997; Cate *et al.*, 1999; Clemons *et al.*, 1999). It is surprising that we do not see a prominent 100 Å long rod on the inter-subunit face of the SSU, as described in recent higher resolution structures of

the bacterial ribosome and SSU (Cate *et al.*, 1999; Clemons *et al.*, 1999; Tocilj *et al.*, 1999). This feature should correspond to the penultimate 3' helix in the 18 S rRNA of the SSU (identified as H44 in the bacterial 16 S rRNA). However, a series of discontinuous rod-like features are present in this region, located between the front of the platform and the smaller foot. The lack of a strong rod-like feature for the eukaryotic homolog of H44 is not an artifact of subunit separation, as determined by comparing the subunit maps with unseparated maps of the appropriate ribosome. Overall, the morphology of our rabbit SSU map is similar to an earlier rabbit



**Table 1.** Composition and mass data for the yeast and rabbit ribosome

	Yeast		Rabbit	
	r-Proteins rRNA	Mass (kDa)	r-Proteins rRNA	Mass (kDa)
Large subunit	45 r-proteins	815	38 r-proteins	825
5 S rRNA	121 nt <sup>a,c</sup>	38	120 nt <sup>b,g</sup>	38.6
5.8 S rRNA	158 nt <sup>d</sup>	50.7	158 nt <sup>b,h</sup>	50.7
25 S/28 S rRNA	3392 nt <sup>e</sup>	1091	4712 nt <sup>b,h</sup>	1510
Total for 60 S		1994.7		2424.3
STEM for 60 S		2000		2600
Small subunit	32 r-proteins	550	28 r-proteins	523.8
18 S rRNA	1798 nt <sup>f</sup>	573.2	1863 nt <sup>j</sup>	596
Total for 40 S		1123.2		1119.8
STEM for 40 S		1200		1350
Total r-protein		1365		1348.8
Total rRNAs		1752.9		2195.3
Total for 80 S		3117.9		3544.1
STEM for 80 S		3200		3800

<sup>a</sup> Data compiled from these databases and primary references: genome-www.stanford.edu; www.ncbi.nlm.nih.gov

<sup>b</sup> Values taken from the mouse ribosome because they are not available for rabbit. Primary references for *Saccharomyces cerevisiae*.

<sup>c</sup> Miyazaki, M. (1974).

<sup>d</sup> Rubin, G. M. (1973).

<sup>e</sup> Georgiev *et al.* (1981).

<sup>f</sup> Rubtsov *et al.* (1980).

Primary references for mouse and rabbit rRNAs:

<sup>g</sup> Nendza *et al.* (1987).

<sup>h</sup> Pace *et al.* (1977).

<sup>i</sup> Hassouna *et al.* (1984).

<sup>j</sup> Rairkar *et al.* (1988).

structure (Dube *et al.*, 1998b), but appears somewhat more detailed, perhaps reflecting the additional data in these maps (~12,000 *versus* ~3000 particles).

It is intriguing that a groove between the shoulder and back of the SSU leads into a channel at the base of the head in both species (see Figure 4(c) and (d)). This channel leads into the decoding center and is present above the platform, when viewed from the subunit interface (Figure 4(e) and (f)). The location of the channels, by analogy with the bacterial SSU (Frank *et al.*, 1995; Lata *et al.*, 1996; Cate *et al.*, 1999), suggests that they may function as an entrance for mRNA when it is threaded into the decoding center. The wall of the channel is complete at all reasonable thresholds and is not open laterally, as seen with the isolated SSU in *E. coli* (Lata *et al.*, 1996). In addition, a conserved groove is located between the shoulder and body of the SSU and forms a part of the channel entrance. An additional groove is also present in the neck of the SSU, opposite from the mRNA entrance channel and located above the platform (Figure 4(e) and (f)). In the rabbit SSU, this groove is closed to form a channel. This region could be involved in the egress of mRNA from the decoding center. Additional hollows or channels also are present within the body of the SSU at this threshold (Figure 4(c) and (f)).

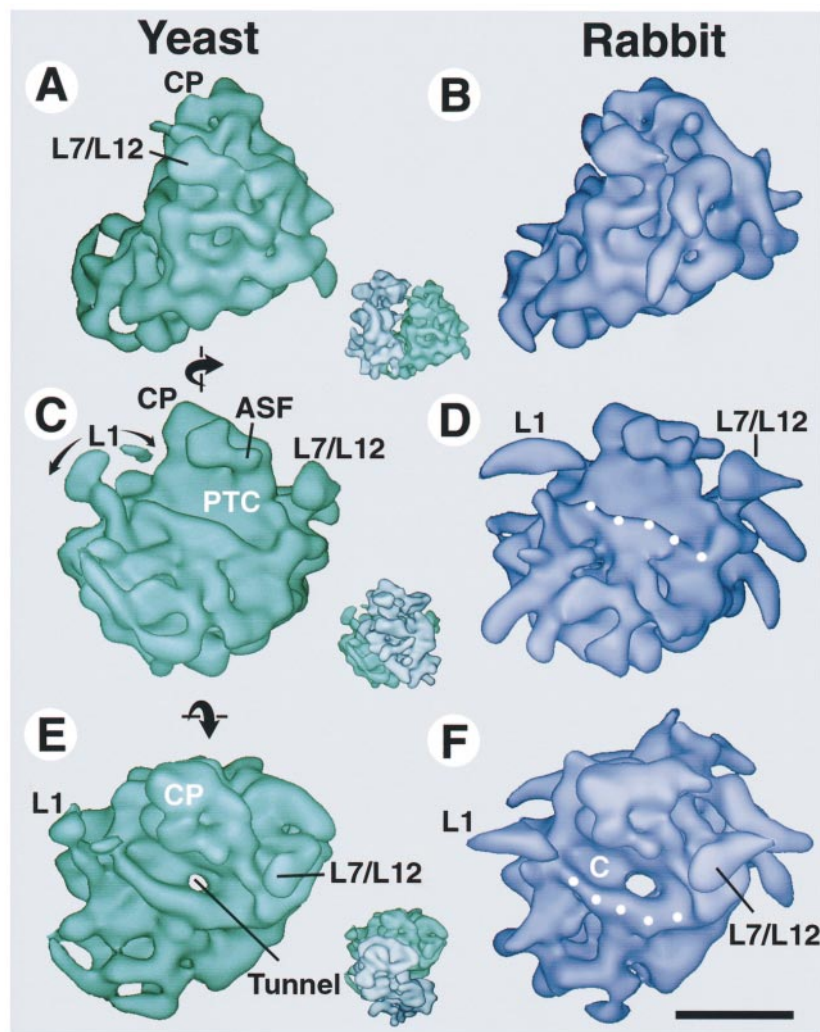
Finally, we observed an unknown density (labeled X in Figure 4(d)) on the back of the head of the rabbit SSU, that is not present in the yeast SSU or in six additional maps of mammalian ribosomes complexed with ER channels (not shown). However, we occasionally observed an additional

bilobed feature attached to the L7/L12 stalk in maps of rabbit ribosomes. It does appear that ribosomes isolated from reticulocyte lysate may carry along additional factors during purification. These factors likely correspond to unidentified initiation, elongation or termination components.

### A comparison between large subunits within the yeast and rabbit ribosome

The large subunit contains peptidyl transferase activity and provides the second half of the active-site chamber, along with an exit tunnel for the nascent chain and a docking site for the Sec61p channel. Composition and mass analyses of the large ribosomal subunits (Table 1) suggest that their size is rather different in yeast and rabbit (1995 kDa *versus* 2424 kDa). This difference can be attributed in part to an expansion of the LSU rRNA from 25 S to 28 S. The separated LSUs from yeast and rabbit are compared in Figure 5(a) and (b) in the frontal view, with an icon of the yeast ribosome to show the relative orientations of the large and small subunits. The major landmarks of the *E. coli* LSU are clearly revealed in the crown view of the eukaryotic subunits (Figure 5(c) and (d)). These include the L1 stalk which is close to the E-site, the central protuberance and associated A-site finger (ASF) which overhang the P and A-sites (Stark *et al.*, 1995; Agrawal *et al.*, 1996) and the L7/L12 stalk, which interacts with elongation factors (Stark *et al.*, 1997b; Agrawal *et al.*, 1998; Ban *et al.*, 1999).

In comparing the eukaryotic L1 regions, we observed indications of mobility which may



**Figure 5.** A comparison of large subunits from the yeast and rabbit ribosome. (a) A frontal view of the yeast large subunit is shown, along with a ribosomal icon demonstrating the relative position of the small subunit. Characteristic features of the large subunit, including the central protuberance (CP) and L7/L12 domain are labeled. (b) Frontal view of the rabbit large subunit. (c) Yeast large subunit is shown in a crown view. This orientation is related to the view in (a) by a rotation of  $\sim 90^\circ$ . The major domains which encircle the PTC are indicated, including the L1 domain, the central protuberance (CP), the A-site finger (ASF) and the L7/L12 stalk. Possible mobility of the L1 stalk is indicated by the fragmented density and the arrows. (d) The rabbit large subunit is shown in a crown view. L1 appears as an elongated feature and the rim of the interface canyon is labeled with white dots. (e) The yeast large subunit is viewed from above the central protuberance, looking down into the interface canyon. This orientation is related to the view in (c) by an out-of-plane rotation of  $\sim 75^\circ$ , towards the reader. The entrance of the nascent chain exit tunnel is marked. (f) The rabbit large subunit is shown in an orientation similar to (e). The canyon (C) and rim (white dots) are indicated. Scale bar represents 100 Å.

involve the linker which attaches L1 to the LSU and indeed, L1 is missing in the 5 Å map of the bacterial LSU (Ban *et al.*, 1999). However, the atomic structure of the L1 protein could be modeled into a map of the *E. coli* 70 S ribosome, determined at 14.9 Å resolution by electron microscopy (Malhotra *et al.*, 1998). It has been suggested that the region adjacent to L1 may provide an additional binding site for exiting tRNAs (the E2 site; Frank, 1998b); hence, the conformation of L1 may reflect the absence or presence of E-site tRNAs. The L7/L12 stalk is also known to be flexible and the physiological basis for this plasticity involves conformational changes during the elongation cycle mediated by interactions with EF-G (Agrawal *et al.*, 1999; Verschoor *et al.*, 1985). An indication of this flexibility is reflected by the appearance of the L7/L12 regions in the yeast and rabbit ribosomes (Figure 5(c) and (d)). The L7/L12 stalk is more elongated in the rabbit map and smears out laterally at lower thresholds (not shown).

The L7/L12 stalk, CP and L1/E-sites frame the PTC and a conserved groove or canyon, with the ASF positioned above the middle of the canyon (Figure 5(c) and (d)). The canyon rim is formed by a long, high-density tubular feature with a diameter of 20–25 Å, suggesting that it may be comprised of rRNA (see white dots in Figure 5(d) and (f)). This feature is consistent with X-ray crystallographic structures determined between 9–5 Å resolution for bacterial LSUs (Ban *et al.*, 1998; 1999; Cate *et al.*, 1999). The floor of the canyon contains the entrance to the nascent chain exit tunnel, as shown in Figure 5(e) and (f), where the LSUs are tilted from the crown view towards the reader about the horizontal axis. In the rabbit LSU additional large finger and spine-like features are observed (Figure 5(b), (d) and (f)), relative to similar views of the yeast LSU. We attribute these features to expansion segments present within the mammalian 28 S rRNA (as discussed below).



## The nascent chain exit tunnel

A large tunnel that leads from the PTC to the lower back of the LSU was previously observed in bacterial, yeast and mammalian ribosomes (Frank *et al.*, 1995; Verschoor *et al.*, 1998; Dube *et al.*, 1998). It was suggested that this tunnel represents the long sought nascent chain pathway through the LSU. In support of this hypothesis, the central pore of the ER channel in yeast and mammalian translocation complexes has been shown to align with the tunnel exit on the LSU (Beckmann *et al.*, 1997; Ménétret *et al.*, unpublished results). In Figure 6(a) and (b), a slab of density from the ribosomal maps is shown in “O” (Jones *et al.*, 1991), which reveals the tunnel and the interface between the small and large subunits. The topology of the tunnel is highly conserved between the yeast and rabbit ribosome, with a length of  $\sim 100$  Å and an average diameter of  $\sim 20$  Å. At thresholds that include  $>150\%$  of the expected volume, a single constriction appears close to the PTC (not shown).

The separated LSU maps were also contoured at a higher threshold to reveal a network of dense features. A cylindrical portion of these maps ( $\sim 90$  Å diameter) was extracted along the tunnel axis and color coded in red. These mini-maps are superimposed on the full ribosome to highlight dense features which line a considerable part of the exit tunnel (Figure 6(a) and (b)). The area containing the tunnel was then sectioned into three cylindrical slabs as indicated in Figure 6(a), which correspond to the tunnel entrance at the PTC (Figure 6(c) and (d), panel 1), the central region (Figure 6(c) and (d), panel 2) and the tunnel exit domain (Figure 6(c) and (d), panel 3), for the yeast and rabbit LSUs. In general, the maps from the two species are very similar and several tubular dense features in these regions are likely comprised of rRNA. For example, one such feature corresponds to the rim of the interface canyon, cut in cross-section at the PTC (see asterisk in Figure 6(a), (c) and (d)). This rim is formed by dsRNA in the prokaryotic ribosome (Ban *et al.*, 1998; 1999; Cate *et al.*, 1999; Mueller *et al.*, 2000). A similar dense tubular feature lies along the back of the interface canyon and has been modeled as rRNA in the prokaryotic LSU by Mueller *et al.* (2000). Although we cannot unambiguously identify internal rRNA at this resolution, it seems reasonable that some of the dense features within the tunnel walls will be comprised of rRNA, given the proportions of rRNA and protein in the ribosome.

Finally, the nascent chain exit tunnel debouches onto a flat surface at the bottom of the LSU for both eukaryotic ribosomes (Figure 6(e) and (f)). This surface forms a roughly pentagonal profile around the tunnel exit (see dots in Figures 6(e) and (f)) with a diameter similar to the Sec61p channel (Hanein *et al.*, 1996). The Sec61p channel has been shown to dock to the LSU in this region (Beckmann *et al.*, 1997; Ménétret *et al.*, unpublished results). A recent biochemical analysis (Prinz *et al.*,

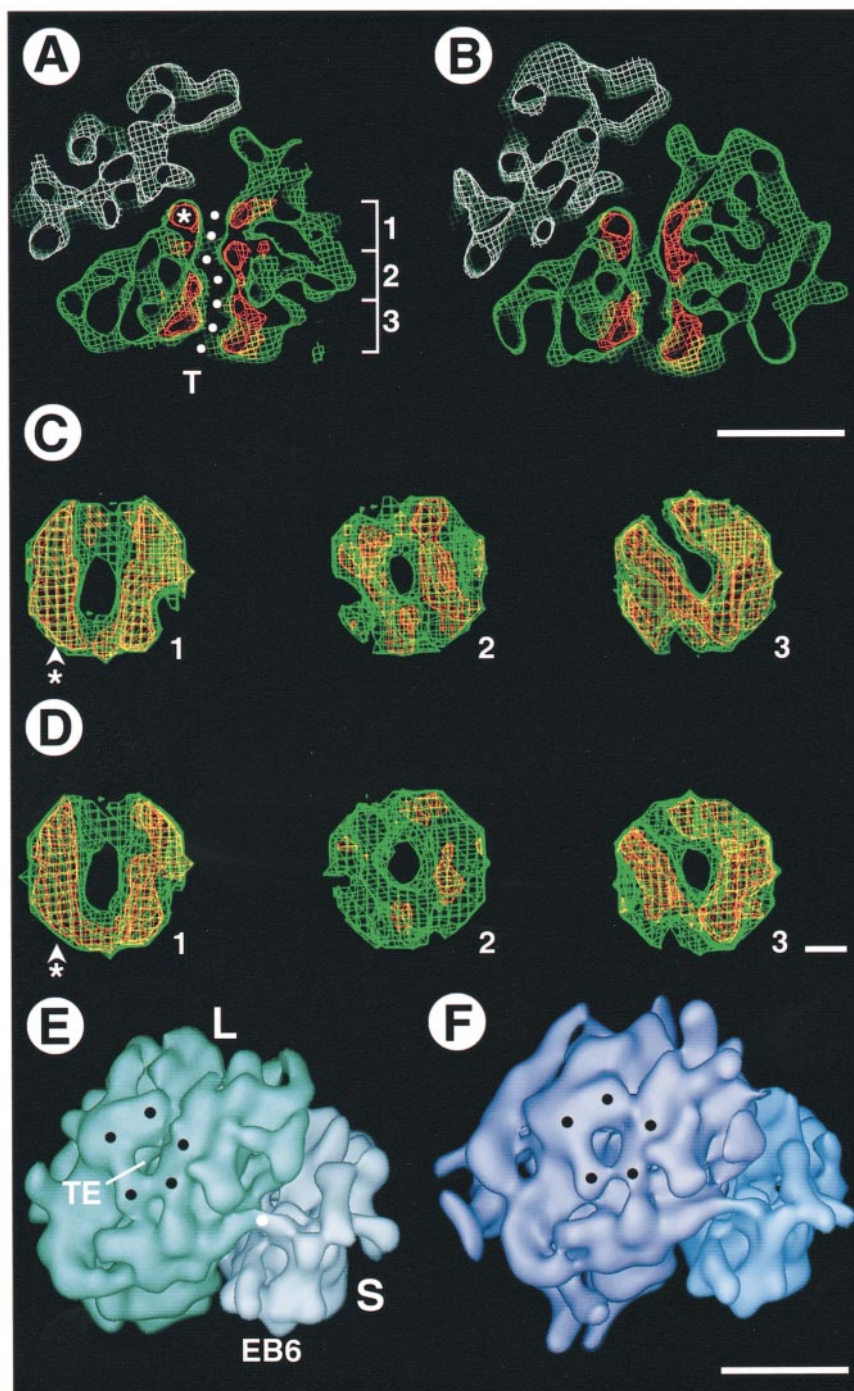
2000) indicates that LSU rRNA mediates ribosome-channel interactions in both prokaryotes and eukaryotes. As the docking region is comprised in part of high-density features (Figure 6(c) and (d), panel 3), it seems likely that they correspond in part to rRNA.

## Inter-subunit bridges

Assembly of the ribosome from its constituent subunits and subsequent disassembly are major events in the translation cycle. The association of small and large subunits is mediated by specific contacts, called bridges in the prokaryotic ribosome (Cate *et al.*, 1999; Lata *et al.*, 1996). Moreover, hybrid ribosomes formed between prokaryotic and eukaryotic subunits are active (Klein & Ochoa, 1972; Boublik *et al.*, 1979), suggesting that key features of the subunit interface between the small and large subunits are conserved. The recent 7.8 Å map of the *T. thermophilus* ribosome (Cate *et al.*, 1999) and lower resolution studies of the *E. coli* ribosome (Gabashvili *et al.*, 1999; Lata *et al.*, 1996) have provided a detailed inventory and nomenclature of the inter-subunit bridges. We wished to locate significant inter-subunit bridges in the eukaryotic ribosome. Therefore, we used the criteria that a bridge must be present in both the yeast and rabbit ribosome structures over a reasonable threshold range.

In our maps, the small and large subunits are connected by five strong eukaryotic bridges (EB) and two close contacts along their interface (although additional contacts may be revealed at higher resolution or lower thresholds; see Figure 7). In Figure 7(a), a central slab of density is shown in “O” which contains the subunit interface from the yeast ribosome in a frontal orientation. A similar view from the rabbit ribosome is shown in Figure 7(b). Starting at the top of the subunit interface, a “weak” contact is observed in both the yeast and rabbit ribosomes, located between the head of the SSU and the CP region of the LSU. However, this contact is only visible at lower thresholds ( $\sim 150\%$ ; see open circles labeled EB1 in Figure 7(e) and (f)). This contact is markedly weaker than its *E. coli* counterpart (Lata *et al.*, 1996; Cate *et al.*, 1999).

Next, a prominent double bridge is present within the active-site chamber (see the pair of white dots in Figure 3(a)), which we have labeled EB2 and EB3. These bridges may correspond to previously identified bacterial bridges B2a and B3, for the top and lower bridge, respectively. Eukaryotic bridge-2 connects the upper rim of the LSU canyon with the front of the platform on the SSU (Figure 7(a)). Additional details of the double bridge are shown in Figure 7(c) and (d), which correspond to density slabs viewed from the back of the LSU canyon, looking into the subunit interface (Figure 7(c)) and a view into the canyon from above (Figure 7(d)). In all three views, EB2 clearly connects a high density tubular feature in the can-



**Figure 6.** Architecture of the nascent chain exit tunnel in the yeast and rabbit ribosome. (a) A slab of the yeast ribosome 3D map is shown as a wiremesh in "O", and is centered on the nascent chain exit tunnel (T). This view is similar to the frontal orientation of the ribosome. The small (S) and large (L) subunits are light blue and green, respectively. The tunnel is delineated by a string of dots, labeled T. The region that encircles the tunnel is further highlighted by the superposition of a high threshold map (red mesh, radius from the tunnel axis = 45 Å). The asterisk indicates the position of the interface canyon rim. (b) View of the rabbit ribosome, similar to that in (a). Scale bar represents 100 Å. (c) A cylindrical core (radius of 45 Å) which contains the nascent chain exit tunnel was extracted from the yeast ribosome map. This mini-map was sliced into three consecutive stacks (see guide bars in (a)) and displayed at two thresholds: 100% (green) and 50% (red). The maps are viewed from the PTC and include: the tunnel entrance (panel 1), the intermediate region (panel 2) and the tunnel exit domain (panel 3). In (a), an asterisk marks a dense feature that forms the interface canyon rim (also indicated in (d)). (d) Sequential cylindrical mini-maps of the tunnel region from the rabbit ribosome are shown. These panels should be compared with their yeast counterparts in (c). Scale bar represents 50 Å. (e) Bottom view of the yeast ribosome. It reveals the surface topology of the tunnel exit domain. Individual subunits (S and L), the tunnel exit (TE), the Sec61p docking surface (five black dots) and an inter-subunit bridge (EB6/white dot) are labeled. (f) View of the rabbit ribosome, in an orientation similar to that in (e). The raised, quasi-pentagonal docking surface for the Sec61p channel is indicated by black dots. Scale bar represents 100 Å.

yon rim with a similar recurved feature within the platform of the SSU. The bacterial counterpart (B2a) is formed by a short double helical rRNA stem from the LSU, which makes contact with the top of the penultimate 3' stem in the 16 S rRNA within the SSU (Cate *et al.*, 1999). This stem, denoted H44 (Cate *et al.*, 1999), is conserved between bacterial and eukaryotic SSU rRNAs and plays a prominent role in mediating inter-subunit contacts. In the eukaryotic ribosome, the second part of the double bridge (denoted EB3) connects the canyon wall on the LSU, with a point just below and in front of EB2, in the platform region of the SSU. This connection in the bacterial ribosome also involves the H44 stem (Cate *et al.*, 1999).

A third strong bridge (EB6), connects the subunits at the bottom of the subunit interface, as shown in a surface view (white dot in Figure 6(e)) and in density slabs (Figure 7(a) and (c)). An analogous contact in the bacterial ribosome (B6) involves the H44 helix on the 16 S rRNA (Cate *et al.*, 1999). Based on the similarity of these three eukaryotic and bacterial bridges, we suggest that the penultimate stem helix in the 18 S rRNA is located in this region of the SSU although H44 may be somewhat disordered in our specimens.

A close contact (EB5) occurs between EB3 and EB6 (see Figure 7(e) and (f)) and may correspond to B5 in the bacterial ribosome. However, this bridge is only connected at much lower thresholds. Bridge 5 in the bacterial ribosome is mediated by H44 on the SSU (Cate *et al.*, 1999); therefore, this feature of the 18 S rRNA is expected to participate in the eukaryotic ribosomal interface. Although there is a rod-like feature which encompasses EB3/EB6 on the eukaryotic SSUs, its density is lower than other high-density rods, and is either discontinuous or missing. Although H44 has been observed in a recent 3D map of the *E. coli* ribosome at 11.5 Å resolution (Gabashvili *et al.*, 2000), it has not been identified in earlier structures.

An additional bridge (denoted EB7; see appropriate white dots with black centers in Figure 7(c) and (d)) occurs adjacent to EB2 and appears to be an analog of a bacterial bridge (B7), because it connects the middle of the platform with the canyon rim, about 50 Å distant from the L1 stalk (Cate *et al.*, 1999; see Figure 7(e) and (f)). Finally, a strong eukaryotic bridge connects the back of the platform to a protrusion directly underneath L1 (see Figures 3(e) and (f) and 7(c) and (d)). We have designated this novel feature EB8 because it occurs in a region of the platform that does not exist in the smaller prokaryotic SSU. Indeed, a large expansion segment (ES6) exists between H21 and H22 in the eukaryotic SSU rRNA (see Gutell's Web page at <http://www.rna.icmb.utexas.edu>; Gerbi, 1996). Since the H21 region in the SSU joins the body and back of the bacterial platform (Clemons *et al.*, 1999), it seems likely that ES6 may contribute additional mass to the eukaryotic platform.

The positions of the five prominent and two weak eukaryotic bridges have been mapped onto

surface views of the small and large subunit, and are shown in Figure 7(e) and (f), respectively. Overall, our data suggest that the geometry of major inter-subunit bridges is conserved between bacterial and eukaryotic ribosomes.

### Visualization of expansion segments within the rabbit large subunit

To analyze in more detail the observed size difference between yeast and mammalian ribosomes (Figure 3), we compared the composition and mass of yeast and rabbit ribosomes (Table 1). The values for the yeast ribosome should be accurate, as the entire genome has been sequenced for *Saccharomyces cerevisiae* (note that we have used *Saccharomyces uvarum*, a subspecies of *S. cerevisiae*). Mass values for the 5 S, 5.8 S and 28 S rRNAs from rabbit were not available, so we used values from the mouse. However, the size of the 5 S and 5.8 S rRNAs should be unchanged. This analysis suggests that the rabbit ribosome is at least 400 kDa larger than its yeast counterpart, with the major mass difference arising from the 25 S to 28 S transition in the LSU rRNA. Indeed, it is well documented that 41 hyper-variable expansion segments are present within the LSU rRNA (Schnare *et al.*, 1996; Michot *et al.*, 1984; reviewed by Gerbi, 1996).

Since we wanted to localize a subset of the LSU expansion segments, we needed to know if the actual size difference between yeast and rabbit ribosomes is comparable to that based on our calculations. Therefore, we determined the mass of yeast and rabbit ribosomes in parallel experiments, with darkfield images taken on the Brookhaven STEM I (Wall & Hainfeld, 1986). During sample adsorption and subsequent freeze drying, some 80 S ribosomes were converted to subunits. Mass histograms for the eukaryotic ribosomes and their subunits were determined and the peak values are given in Table 1. The observed mass values for the yeast and rabbit ribosomes were 3.2 MDa and 3.8 MDa, respectively. This corresponds to a size difference of ~600 kDa rather than the calculated value of ~400 kDa. Overall, the experimentally measured size difference suggests that the mouse 28 S rRNA is an adequate, although imperfect substitute for the rabbit 28 S rRNA.

A sequence comparison between yeast and mouse 25 S/28 S rRNAs reveals five expansion segments with size differences greater than ~50 nt (Table 2). Together with the additional 35 nt in ES9, they account for >95 % of the total differences in LSU rRNAs between these species. A similar comparison between yeast and rat LSU rRNAs reveals four expansion segments larger than ~50 nt (Table 2), which again accounts for >95 % of the nucleotide differences. These large expansion segments are spread throughout the LSU rRNA and in the mouse occur in five of six major sequence domains.



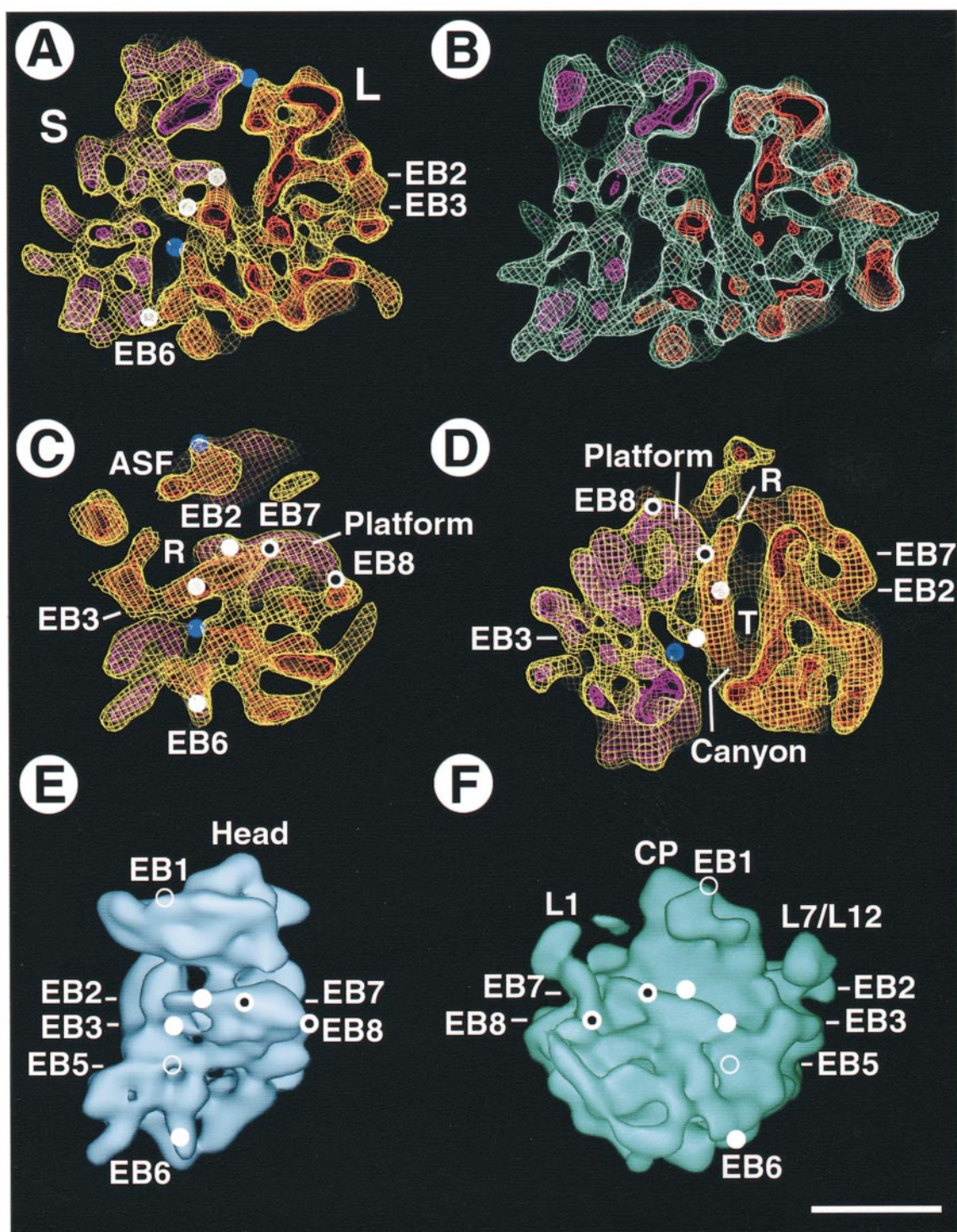


Figure 7 (legend opposite)

We next identified features on the rabbit LSU that were not present in the yeast subunit. Surface views of the yeast LSU are shown for the front, crown and bottom views in Figure 8(a), (c) and (e). For comparison, similar views of the rabbit ribosome are shown in Figure 8(b), (d) and (f). Major differences arise on the back and sides of the rabbit

LSU (red) and correspond to tubular features with a diameter of  $\sim 25$  Å. The diagnostic rod-shape, diameter and high density of these features suggests that they may originate from duplex rRNA. The two largest features are shaped like elongated spines and based on connectivity they likely represent independent extensions of double-

**Table 2.** Expansion segments in the mouse/rat 28 S rRNA, relative to yeast, mapped within the universal core of the bacterial 23 S rRNA

Expansion segment <sup>a</sup>	<i>E. coli</i> 23 S coordinates <sup>b</sup>	Nucleotide differences <sup>c</sup>	Domain in 23 S rRNA
ES 7	533-560	~479/566	I
ES 9	630-650	~34/37	II
ES 15	1170-1180	~125/128	II
ES 27	1720-1740	~489/472	IV
ES 30	2130-2160	~52/55	V
ES 39	2790-2810	~87/24	VI

<sup>a</sup> Expansion segment code from Gerbi (1996), based on Gutell's maps of the universal core of the 23 S rRNA. See Gutell's web-site at (<http://www.rna.icmb.utexas.edu>).

<sup>b</sup> Coordinates from the alignment of eukaryotic expansion segments within the 23 S rRNA universal core by Gutell and co-workers (Schnare *et al.*, 1996). Aligned sequences in this work obtained from the Ribosome Data Project at (<http://www.cme.msu.edu/RDP>).

<sup>c</sup> Nucleotide differences between yeast and mouse followed by yeast and rat.

stranded rRNA. In addition, there are two smaller protrusions (labeled 2a and 2b in Figure 8(b)) which extend from the base of spine 2.

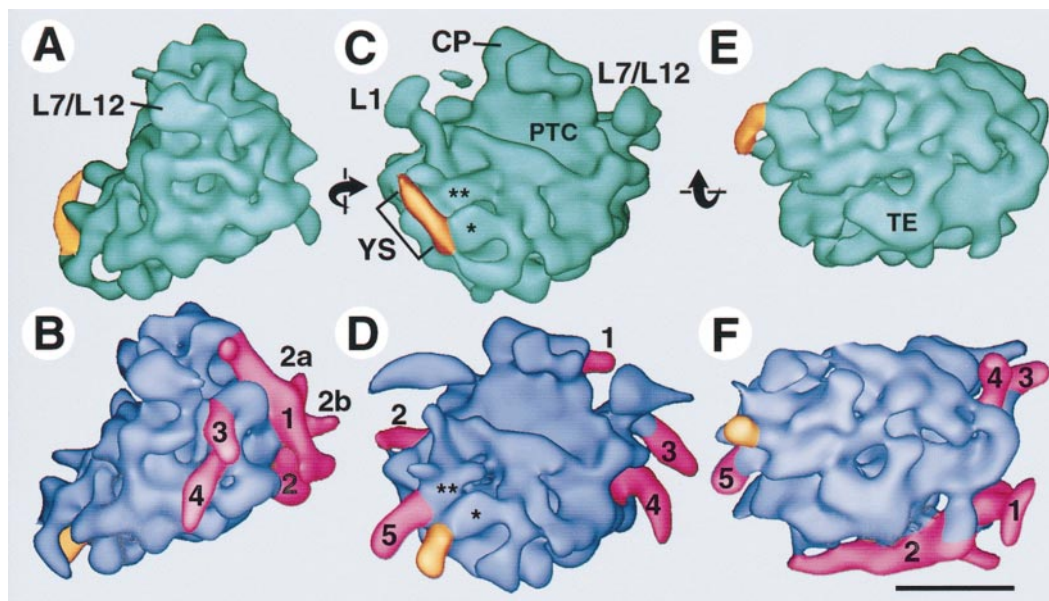
A pair of finger-like protrusions (labeled 3 and 4) are located on the LSU, behind and below the L7/L12 stalk (Figure 8(b) and (d)). A second pair of fingers, one labeled 5 and the other color coded in gold, originate near the bottom of the rabbit LSU (see crown view, Figure 8(d)). However, a comparison of yeast and rabbit LSUs in this region is complicated by the appearance of an additional extended dense spine on the yeast LSU (color coded in gold; Figure 8(c)). This spine is absent in the rabbit LSU. Both the yeast spine and the finger colored in gold originate from equivalent positions on their respective LSUs (see single asterisk in Figure 8(c) and (d)). We believe that the yeast spine is comprised of rRNA which has been reorganized to form the finger colored in gold in the rabbit LSU. With this caveat, we observe a total of five major surface extensions which likely corre-

spond to the five largest expansion segments identified in Table 2. It is important that the LSU expansion segment differences within the rabbit ribosome do not encroach upon either the subunit interface or the Sec61p docking site (compare Figure 8 with Figures 3(c) and (d), 6(e) and (f)). These data further suggest that the LSU rRNA has a complicated topological fold in which at least five sequence domains have surface exposed expansion segments.

The expansion segments in the mouse and rat 28 S rRNA fall into three groups based on size. Expansion segments 7 and 27 contain >400 additional nt relative to the yeast 25 S rRNA; ES15 and ES39 contain ~100 additional nt in the mouse (note that ES39 in the rat is much smaller); ES9 and ES30 contain ~30-50 nt. In comparing the rabbit and yeast LSUs, the two largest new features are spines 1 and 2 (each >100 Å long), whilst the next two largest features are ~50-70 Å long (fingers 3 and 4). The smallest finger (number 5) is less than

**Figure 7.** Inter-subunit bridges in the yeast and rabbit ribosome. (a) A slab of the yeast ribosome 3D map is shown as a wiremesh in "O", centered on three prominent bridges (EB2, EB3 and EB6, positions indicated with white dots). Much weaker contacts are indicated with blue spheres. The small (S) and large (L) subunits are indicated. The intact ribosome is contoured at the 100% threshold in yellow; the 50% threshold is color coded magenta and red for the separated small and large subunits, respectively. (b) Slab of the rabbit ribosome 3D map as a wiremesh in "O", in an orientation similar to that in (a). The 100% threshold is blue-green whilst the high-threshold maps are color coded as in (a). (c) Slab of the yeast ribosome 3D map as a wiremesh in "O", which is centered on the subunit interface and contains the five prominent bridges (white dots) and the two weaker contacts (blue dots). This view was obtained by slicing into the ribosome from the back of the LSU, with the SSU located in a similar orientation as in Figures 4(e) and 7(e). The A-site finger (ASF) and canyon rim (R) on the large subunit are visible, as are the head and platform of the small subunit, which are located behind the LSU in this view. EB7 and EB8 are marked with white dots containing black centers, to help in delineating them from the other bridges. (d) A slab of the yeast ribosome is shown, looking from the head of the small subunit down into the PTC/canyon (analogous to the view in Figure 5(e) with the head and CP removed). Two of the stronger bridges (EB2 and EB7) make contacts between the canyon rim (R) and the recurved platform, while EB3 makes a contact between the rim and a point below the platform (also see Figure 7(a)). Bridge 8 makes a contact on the underside of the platform, at the position indicated (EB8). The entrance to the nascent chain tunnel (T) is marked on the canyon floor. (e) The positions of the five strong and two weak bridges are shown on the interface view of the small subunit as white and empty dots, respectively. This view is equivalent to the view of the bacterial SSU shown in Figure 3(b) of Cate *et al.* (1999). (f) The positions of the five strong and two weak bridges are shown on the interface view of the large subunit as white and empty dots, respectively. Note that three of the bridges lie along the canyon rim (EB7, EB2, EB3). Three prominent landmarks on the LSU are indicated: L1, central protuberance (CP) and the L7/L12 stalk. This view is equivalent to the view of the bacterial LSU shown in Figure 3(a) of Cate *et al.* (1999).





**Figure 8.** Enlarged 28 S expansion segments on the large subunit of the rabbit ribosome (bottom panels), relative to the yeast ribosome (top panels). (a) Frontal view of the yeast LSU. The L7/L12 stalk is labeled and the yeast spine (YS) is colored in gold. (b) Frontal view of the rabbit LSU. Enlarged expansion segments are highlighted in red. They include a large spine (1), a pair of fingers (3,4) adjacent to the L7/L12 stalk and two small extensions from spine 2, labeled 2a and 2b. (c) Crown view of the yeast LSU. Prominent features which frame the PTC are labeled (L1, CP and L7/L12). The yeast spine (YS) originates on a prominent ridge (\*) and may rearrange to form the golden finger-like projection shown in Figure 8(d). This view corresponds to a rotation of panel A by  $\sim 90^\circ$ . (d) Crown view of the rabbit LSU. In addition, to the pair of fingers (labeled 3 and 4) adjacent to the L7/L12 stalk, a second pair of fingers, labeled 5 and colored gold, are present at the lower left. It is possible that sequences within the yeast spine are reorganized to form the golden finger as they originate from the same ridge (\*), whilst finger 5 may represent an enlarged expansion segment which originates from a tubular feature (\*\*) that is present in both LSUs. (e) Bottom view of the yeast LSU. The tunnel exit is marked (TE). This view corresponds to a rotation of panel C by  $\sim 90^\circ$ . (f) Bottom view of the rabbit LSU. By comparison with the yeast LSU in (e), a second large spine (marked 2) is clearly visible as it runs across the back of the subunit, below the tunnel exit domain. Scale bar represents 100 Å.

50 Å long. Based solely on size, we tentatively assign spines 1 or 2 to either ES7 or ES27, whilst fingers 3 and 4 correspond to ES39 and ES15, respectively. In this scenario, finger 5 would be formed by ES30. Some of these assignments differ from those proposed by Dube *et al.* (1998b), although the independently derived 3D maps of the rabbit ribosome are comparable. Importantly, we are analyzing ribosomes that are more similar in size ( $\sim 3.2$  and  $3.8$  MDa), whereas Dube *et al.* (1998b) compared *E. coli* ( $\sim 2.4$  MDa) and rabbit ribosomes ( $3.8$  MDa). Indeed, a comparison of our yeast ribosome map with any recent *E. coli* map reveals major additions at the periphery/back of the LSU, while the yeast and rabbit ribosomes are essentially identical in regions that are distinct from the five expansion segments (colored red in Figure 8).

Dube *et al.* (1998b) have identified spine 1 (our nomenclature) with ES7. This agrees with our data, although we are unable to distinguish ES7 and ES27 based solely on their sizes. The pair of fingers adjacent to the L7/L12 stalk were previously equated with ES15 and ES39 (Dube *et al.*, 1998b), again in agreement with our data. However, based on relative size we suggest that finger 4 may correspond to ES15 which is  $\sim 50$ - $100$  nt larger than

ES39, as finger 4 is markedly larger than finger 3 (see Figure 8(d)). It is interesting that ES39 was recently localized in the *E. coli* ribosome to a region well below and behind the L7/L12 stalk (Spahn *et al.*, 1999). This site is fairly distant from fingers 3 and 4 in our map. As mentioned above, the yeast and rabbit ribosomes are dramatically enlarged in this region relative to the *E. coli* LSU. Hence, we suggest that the localization of ES39 by Spahn *et al.* (1999) may not necessarily conflict with our assignments (this work, Dube *et al.*, 1998b).

The region of our rabbit LSU map containing finger 5 also reveals an additional finger that may have arisen from a reorganization of the yeast spine (as discussed above). Dube *et al.*, (1998b) have assigned ES27 to this region of their rabbit ribosome map and a direct localization of this expansion segment has been reported in this vicinity of the *E. coli* ribosome (Spahn *et al.*, 1999). However, the tubular features in our maps which differ between yeast and rabbit (even including the gold colored finger) are far too small to account for the expected difference of  $\sim 450$  nt due to ES27 (Table 2). We therefore assigned finger 5 to ES30 ( $\sim 50$  nt). The reason for this apparent discrepancy is not known, but again may reflect large differences in the size of the LSUs in this region.



Finally, based on our maps the most likely candidate for ES27 is spine 2. This assignment is consistent with the additional nucleotides present in mammals relative to yeast for ES27 (>450 nt) and the fact that spine 2 is a long, continuous high-density feature in the rabbit LSU. Dube *et al.* (1998b) have previously assigned this feature (with some adjoining regions) to ES5, ES9, ES19 and ES20. However, there should be only an additional ~70 nt arising from these four expansion segments, based on the differences between yeast and mammalian LSU rRNAs (see Ribosome Data Project at <http://www.cme.msu.edu/RDP>). This value is clearly too small to account for the size of spine 2.

Given the inconsistencies between our data, the model of Dube *et al.* (1998b) and the recently determined positions of ES27 and ES39 (Spahn *et al.*, 1999), it would appear that a simple visual "mapping" of the bacterial LSU rRNA within the yeast and mammalian ribosomes may not be possible. However, higher quality maps of all three ribosomes should allow a direct side-by-side comparison and reveal the expected common LSU rRNA core with expansion segments.

## Discussion

We have solved 3D structures of the yeast and rabbit ribosome at ~24 Å resolution. Each of the structures contains ~12,000 particles; hence, the maps have a remarkable clarity that has allowed a detailed comparative analysis. We find that maps of the yeast and rabbit ribosome are virtually indistinguishable at the active sites for protein synthesis and co-translational translocation. In agreement with previous work, we suggest that the landmark domains which form or encircle the decoding and peptidyl transferase centers in eukaryotic ribosomes are strongly conserved with their counterparts in *E. coli* (Dube *et al.*, 1998a,b; Agrawal *et al.*, 1996; Stark *et al.*, 1995).

## Channels, tunnels and bridges

We have identified a conserved groove on the surface of the eukaryotic SSU, that leads past the shoulder into a channel located within the neck region. This channel emerges above the platform, within the central chamber formed by the ribosomal subunits. It has been suggested that a similar portal in the prokaryotic ribosome may allow mRNA to be threaded into the decoding center during protein synthesis (Frank *et al.*, 1995; Lata *et al.*, 1996; Cate *et al.*, 1999). In addition, the putative mRNA channel is replaced by a horizontal slot in isolated SSUs from *E. coli* (Lata *et al.*, 1996). As a result, this region of the bacterial SSU may undergo a conformational change when mRNA is bound, thereby allowing the SSU to clamp mRNA within the channel, during formation of the initiation complex. Such a mechanism would help ensure processivity during translation (Lata *et al.*, 1996). Based on our observations, a similar mRNA

clamping mechanism may occur in eukaryotic ribosomes.

Previously, a tunnel was identified that leads from the PTC to an exit site on the bottom of the LSU (Frank *et al.*, 1995; Stark *et al.*, 1995), where emerging nascent chains have been localized (Bernabeu *et al.*, 1982, 1983). More recently, the tunnel exit was shown to align with the central pore of the ER channel complex in yeast and mammals (Beckmann *et al.*, 1997; Ménétret *et al.*, unpublished results), thereby providing a continuous conduit from the PTC to the ER lumen for nascent chains. We find that the topology of the tunnel exit site on the LSU is remarkably similar in yeast and mammals and is formed by a ring of high-density features. Indeed, Prinz *et al.* (2000) have shown that the LSU rRNA is sufficient to mediate direct interactions with the Sec61p channel. We suggest that the high-density features within the docking domain are comprised in part of rRNA, thereby providing a conserved docking site for the Sec61p complex. Moreover, the acceptor end of the P-site tRNA points into the mouth of the LSU tunnel within the PTC (Stark *et al.*, 1997a; Malhotra *et al.*, 1998; Cate *et al.*, 1999). Together, these data suggest that the nascent chain is inserted into the tunnel, to give the characteristic accessibility patterns that are found during protein synthesis on free and membrane bound ribosomes (Tsalkova *et al.*, 1998; Jungnickel & Rapoport, 1995).

Our analysis also suggests that the nascent chain exit tunnel is lined with dense features over much of its surface. Given the composition of the ribosome, it is likely that the nascent chain is exposed to rRNA within the tunnel. Verification of this hypothesis must await a higher resolution analysis of the ribosome (e.g. see the recent model of Mueller *et al.*, 2000). This point is of interest because the chemistry of the tunnel wall may affect the conformation adopted by nascent chains during translocation, especially within the exit domain.

Ribosomes are dynamic machines which undergo cycles of assembly and disassembly during the initiation and termination of protein synthesis. Inter-subunit bridges make important contributions to this process by stabilizing the intact ribosome, even when ribosomes are not programmed for protein synthesis. Early work on ribosomes demonstrated that functional hybrid ribosomes could be formed from prokaryotic and eukaryotic subunits (Klein & Ochoa, 1972; Boublik *et al.*, 1979), which suggests that a minimal subset of inter-subunit bridges must be conserved between prokaryotes and eukaryotes. We have identified five major bridges (EB2, EB3, EB6, EB7 and EB8) which are conserved between yeast and rabbit ribosomes. Four of the bridges (EB2, EB3, EB6, EB7) may have structural homolog in bacterial ribosomes (Cate *et al.*, 1999; Lata *et al.*, 1996). Therefore, our analysis supports the hypothesis that a set of highly conserved inter-subunit bridges are present in all ribosomes. The identification of

conserved inter-subunit bridges also provides anchor points that should allow the high resolution structure of a bacterial ribosome or its subunits, to be mapped within the eukaryotic ribosome in future work.

### The role of expansion segments in eukaryotic large subunits

Our structural data indicate that the rabbit ribosome is significantly larger than its yeast counterpart and a STEM mass analysis confirms that they differ by ~400-600 kDa. During evolution, the transition from bacteria to mammals has resulted in a size expansion of ribosomes from ~2.4 MDa to ~3.6 MDa. The changes between bacterial, yeast, and mammalian ribosomes are spread over ribosomal proteins and rRNAs. A previous comparison of *E. coli* and rabbit ribosomes allowed the tentative identification of numerous expansion segments in both the 18 S and 28 S rRNAs, using the rat LSU rRNA sequence (Dube *et al.*, 1998b). These differences were correlated with models for ribosomal RNA and protein distribution in the *E. coli* ribosome (Mueller & Brimacombe, 1997a,b; Mueller *et al.*, 1997, 2000).

In general, features within the rabbit ribosome as determined in this work and a previous study (Dube *et al.*, 1998b) are quite complementary. However, we have focused on expansion segments that differ significantly in size between the yeast and mammalian LSU rRNAs. Published comparisons of the yeast and mouse sequences for the LSU rRNA have identified five expansion segments with sequences larger than ~50 nt (Schnare *et al.*, 1996; Gerbi, 1996). Together, these sequences account for greater than 95% of the mass differences between the LSU rRNAs. Given their sizes, these expansion segments should be visible in our map of the rabbit ribosome. We then identified five tubular features that are present in the rabbit ribosome, but are absent in yeast. We suggest that these dense tubular features correspond to the five LSU expansion segments. The positions of two of the five major expansion segments in the mammalian LSU rRNA have recently been mapped within the *E. coli* ribosome (Spahn *et al.*, 1999). However, it is not currently possible to completely reconcile our data with either this work or the model by Dube *et al.* (1998b), using a visual comparison of published images. A higher-resolution analysis of the mammalian ribosome by structural EM, in combination with X-ray crystallography on the bacterial ribosome should allow the path of the 28 S molecule to be traced within the LSU. This in turn, should resolve any ambiguities in the physical assignment of the expansion segments within the LSU rRNA.

Currently, the function(s) of the expansion segments are not known. However, the structural data (Dube *et al.*, 1998; Spahn *et al.*, 1999; this work) suggest that 28 S rRNA expansion segments are

located on the surface of the LSU, as might be expected for the insertion of helical rRNA stems within the ribosomal framework. Importantly, the expansion segments are located at sites within the LSU rRNA which do not overlap with the PTC, decoding center, subunit-subunit interface and the exit tunnel. This observation suggests a role for the expansion segments that is distinct from protein synthesis and co-translational translocation. Functional studies of the LSU rRNA expansion segments are limited, but deletion of the largest segment (ES27) in *Tetrahymena* 26 S rRNA was lethal and could be rescued by the substitution of similar sequences from other species (Sweeney *et al.*, 1994). Moreover, it has been shown that monosomes will associate with intermediate filaments (IFs), both *in vitro* (Bauer & Traub, 1995) and in cells treated to disrupt polysomes (Traub *et al.*, 1998). This interaction may require rRNA and is electrostatic in nature, as the binding is sensitive to pancreatic RNase and high salt (Bauer & Traub, 1995). Therefore, surface-exposed expansion segments on both the small and large subunit are ideally positioned to mediate binding to IFs. Perhaps IFs serve as a storage sites for non-translating ribosomes in animal cells as suggested (Traub *et al.*, 1998), or alternatively, expansion segments may provide another as yet unknown targeting function during ribosome biogenesis.

### Three-dimensional analysis using Radon methods

Finally, it must be noted that previous structure determinations of prokaryotic and eukaryotic ribosomes have used 3D projection matching (Penczek *et al.*, 1994) or angular reconstitution (Dube *et al.*, 1998; Stark *et al.*, 1995, Stark *et al.*, 1997a,b). In this work, we have employed Radon alignment methods to refine the three projection angles for each individual image (Radermacher, 1994; 1997), using an initial low-resolution model obtained from a random conical tilt analysis (Radermacher, 1988). Our current data indicate that individual ribosome images were aligned with an accuracy sufficient to retrieve information at 1/17 Å resolution, as judged by the FSC curves. Recently, the 3D analyses of single particles have undergone a dramatic improvement with work on viruses (Bottcher *et al.*, 1997, Conway *et al.*, 1997), the *E. coli* ribosome (Gabashvili *et al.*, 2000) and the LSU (Matadeen *et al.*, 1999) achieving resolutions in the 7.5-11.5 Å range. In the future, we expect that higher resolution will be obtained on eukaryotic ribosomes and ribosome-channel complexes, which is especially critical as 3D crystals are not available for these complexes. This work further suggests that Radon alignment methods can be added to our arsenal for solving the 3D orientation of noisy 2D projections, a problem central to the single particle method.

## Conclusions

The ribosome plays a critical role in all cells by mediating the biosynthesis of proteins. In this work, we have compared the structures of 80 S ribosomes from yeast and rabbit, to reveal a strongly conserved framework at 24 Å resolution. Ribosomes from bacteria, yeast and mammals show structural homology in the functional domains that encircle and define the decoding center, the PTC and the nascent chain exit tunnel. The structures also reveal a conserved channel in the neck of the SSU that may be used to thread mRNA into the decoding center during translation. Interestingly, a deep groove in the shoulder of the SSU forms an entrance to the putative mRNA channel. Our analysis further suggests that rRNA may have two additional functions within the ribosome. First, we propose that sufficient quantities of double-stranded rRNA within the exit tunnel could influence the conformation of the translocating nascent chain. Second, rRNA may form part of the docking surface for Sec61p channels on the LSU and mediate interactions with this complex. In addition, we have identified five strong intersubunit bridges, four of which appear to be conserved between prokaryotes and eukaryotes, and presumably are responsible for allowing the formation of hybrid ribosomes between different species. Finally, there are marked differences in size and morphology when comparing yeast and rabbit ribosomes. We show that expansion segments within the LSU rRNA play a major role in defining the larger morphology of the mammalian ribosome. However, the function of these segments remains a mystery.

This work now sets the stage for future studies of the structure and function of the mammalian ribosome and ribosome-ER channel complexes, by electron cryo-microscopy and single particle 3D analysis. Importantly, the accumulating data suggest that mechanistic and structural insights from the bacterial ribosome (Ban *et al.*, 1999; Cate *et al.*, 1999; Clemons *et al.*, 1999; Tocilj, 1999; Mueller & Brimacombe, 1997a,b; Mueller *et al.*, 1997, 2000) will be applicable to the larger eukaryotic ribosome (Dube *et al.*, 1998b).

## Materials and Methods

### Preparation of yeast and rabbit ribosomes

For the preparation of yeast ribosomes, *S. uvarum* (NCYC 74) was grown in Wickerham's media at 25°C to a concentration of  $1.35 \times 10^{10}$  cells/l. Unless otherwise indicated, all procedures were carried out at 4°C using ice-cold solutions. Spheroplasts were made by the method by Rout & Kilmartin (1998). After the final wash to remove the lytic enzymes, the ficoll cushion was aspirated from the pelleted spheroplasts, 10 ml of Solution A (8% PVP, 10 mM BisTris (pH 6.5), 2 mM magnesium acetate, 100 mM potassium acetate, 2 mM DTT with 1:500 Solution P (2 mg pepstatin A, 90 mg PMSF/5 ml

absolute ethanol)) was added, and the pellet resuspended using a Brinkman Instruments polytron on setting number 4 for 60 seconds and 30 seconds, respectively; then centrifuged in an SS34 rotor at 11,000 rpm for 15 minutes. An equal volume of Solution B (10 mM BisTris (pH 6.5), 2 mM magnesium acetate, 100 mM potassium acetate, 2 mM DTT, 1:500 Solution P) was added to the supernatant. Then 18 ml of sample was layered onto an 8 ml cushion of Solution C (30% sucrose, 10 mM BisTris (pH 6.5), 2 mM magnesium acetate, 100 mM potassium acetate, 2 mM DTT, 1:500 Solution P), overlaid with 12 ml of Solution B and spun in a Beckman SW28 rotor at 25,000 rpm for 15 hours. The pellets were resuspended in a total volume of 1 ml of Solution B and homogenized. The sample was then applied to a 10%-30% linear sucrose gradient (10%-30% (w/v) sucrose, 10 mM BisTris (pH 6.5), 2 mM magnesium acetate, 100 mM potassium acetate, 2 mM DTT, 1:500 Solution P) and spun in an SW28 rotor at 25,000 rpm for three hours. Ribosomal fractions were determined by reading the absorbance at 260 nm. Peak fractions were combined, flash frozen in LN<sub>2</sub> and stored at -80°C.

Rabbit reticulocyte ribosomes were prepared as follows. After pre-clearing for ten minutes at 14,000 rpm and 4°C in a Eppendorf 5417R centrifuge, 100 µl of rabbit reticulocyte lysate (Promega) was layered onto a 20%-40% (w/v) linear sucrose gradient (volume of 2.1 ml) in 50 mM Hepes/KOH (pH 7.8), 150 mM potassium acetate, 2 mM magnesium acetate, 2 mM DTT, and protease inhibitors (10 µg/ml leupeptin, 5 µg/ml chymostatin, 3 µg/ml elastatinal, 1 µg/ml pepstatin). The gradient was centrifuged in a TLS55 rotor (Beckman) for 90 minutes at 55,000 rpm at 2°C. Fractions of 100 µl were collected manually. One-fifth of the material was precipitated with trichloroacetic acid and separated in a 7.5%-17.5% gradient polyacrylamide gel followed by Coomassie staining. Fractions containing purified ribosomes were pooled, diluted 1:1 with 50 mM Hepes/KOH (pH 7.8), 150 mM potassium acetate, 2 mM magnesium acetate, 2 mM DTT and centrifuged in a TLA 120.2 rotor (Beckman) for one hour at 120,000 rpm at 2°C. The resulting ribosomal pellet was resuspended in 50 µl of buffer containing 50 mM Hepes/KOH (pH 7.8), 150 mM potassium acetate, 2 mM magnesium acetate and 250 mM sucrose.

### Specimen preparation and electron cryo-microscopy

Frozen-hydrated specimens were prepared from solutions of yeast and rabbit ribosomes by placing 2 µl drops on air-glow discharged holey carbon films (Dubochet *et al.*, 1984; Fukami & Adachi, 1965) coated with a thin continuous carbon film. After ~30 seconds, the grids were rapidly frozen by plunging into liquid ethane, within a humid chamber (>85% RH) at 4°C. Specimens were mounted on a Gatan cold holder (626-DH) cooled to liquid nitrogen temperature, and inserted into a Philips CM12 with stage relocation system and cryo-blade type anti-contaminator. Images were recorded with low-dose methods at 28,000×, as 0/50° pairs (yeast) or individually at 0 and 30° (yeast and rabbit ribosomes). The tilted images were collected using dynamic defocus spot scan software implemented on the CM12 with the help of Dr I. Tews. All micrographs were taken at 1-1.5 µm defocus on Kodak SO163 film and developed in full strength D19 for 12 minutes.



### 3D image processing

Micrographs were scanned with a 7  $\mu\text{m}$  raster on a Zeiss SCAI scanner at either Purdue University or Harvard Medical School. After binning over  $2 \times 2$  pixels, image data were converted into absorbance values using a program obtained from Dr S. Ludtke, Baylor School of Medicine, but modified to generate SPIDER formatted files. The final step size of these images was 14  $\mu\text{m}$  corresponding to 5  $\text{\AA}/\text{pixel}$ .

For the initial yeast ribosome model, ~6000 pairs of individual ribosomes were manually selected from 42 micrograph pairs, taken at  $0/50^\circ$ , using the split screen option in WEB (Frank *et al.*, 1996). The untilted images were centered, aligned to a frontal view and classified using correspondence analysis, dynamic clouds and hierarchical ascendant classification (Frank, 1990). The tilted images corresponding to the largest class were processed to generate an initial low-resolution 3D volume using the random conical tilt procedure implemented in SPIDER (Radermacher, 1988; Akey & Radermacher, 1993). This initial volume was used as a starting model for an extensive series of refinements based on the 3D Radon Fourier transform alignment procedures of Radermacher (1994). The final resolution of this initial model was  $\sim 1/28 \text{\AA}$ .

The structure of the yeast ribosome was refined using additional particles from untilted micrographs. To select particles, the scanned images were cross-correlated against a cylindrically averaged frontal view of the yeast ribosome and individual ribosome images were selected using the peak positions. Bad particles were manually eliminated from further analysis after rescanning the original tilted images. This resulted in ~8000 particles from  $50^\circ$  tilted micrographs and ~4000 particles from untilted images. The particles were processed using 3D Radon Fourier transform alignment. This procedure involved multiple cycles of alignment, where the initial ~12,000 particle images were Radon Fourier transformed and aligned to the 3D Radon Fourier transform of a reference. For the first cycle of alignment, we Fermi low-pass filtered the initial yeast reference to a resolution of  $\sim 1/50 \text{\AA}$  and each 2D image to a resolution of  $\sim 1/25 \text{\AA}$ . Each refinement cycle started with a global search over all non-redundant Euler space in  $20^\circ$  intervals. Such a search generates three angular parameters, ( $\phi$ ,  $\theta$  and  $\psi$ , where  $\psi$  describes a final "in-plane" rotation of the 2D image) along with  $x$  and  $y$ -translation parameters. Subsequently, the angular alignment was refined using a narrower search range centered on the result of the previous search while ignoring the  $x$  and  $y$ -shifts. We repeated this process twice, ending with a final search interval of  $3^\circ$ . At this point, the 2D Radon Fourier transforms were phase shifted to account for the final  $x$  and  $y$ -shifts and a new reference volume was generated using a 3D Radon Fourier inversion. A new cycle of global alignment was initiated in  $20^\circ$  intervals using this updated reference. For each cycle of alignment, we extended the Fermi low-pass filter limits of either the 3D reference, the 2D images or both.

After several additional cycles of alignment, we modified the procedure such that the ribosome images were  $x$  and  $y$ -shifted using the most recent alignment parameters, before calculating their 2D Radon Fourier transforms. After three such cycles of alignment (starting with Fermi low-pass filtering of the reference and the 2D images to  $1/50 \text{\AA}$  and  $1/25 \text{\AA}$  resolution, respectively) most of the  $x$  and  $y$ -shifts were zero and the angular differences between the last two alignments were small.

We summed the  $x$  and  $y$ -shifts generated from the modified alignment procedure, applied the summed shifts to the initial 2D data and masked the resulting images using a circular mask with a 200  $\text{\AA}$  radius. We then generated the final 3D volume using the general weighting and back-projection algorithms for arbitrary geometry (Radermacher, 1988). After determining the resolution of both the yeast and the rabbit ribosomes (see below), both final volumes were Fermi low-pass filtered to  $1/24 \text{\AA}$ .

We used essentially the same procedures to refine the rabbit ribosome. The 2D images used in this work were obtained from both untilted and  $30^\circ$  tilted micrographs and the final data set contained ~12,000 images. The starting reference for the 3D alignment was a model of the yeast ribosome generated from the random conical tilt and the initial 3D Radon alignment, which was low-pass filtered. The largest difference in the two alignment procedures was that changes in the Fermi low-pass filter limits were made more slowly with the rabbit ribosome data. This was done to minimize any alignment effects due to the initial yeast ribosome model. Even after the first cycle of alignment, the rabbit ribosome had the larger size expected of the mammalian ribosome, and its overall shape quickly converged.

### Resolution estimates

The resolution of the final data sets was determined using the  $\text{FSC}_{0.5}$  criterium (Bottcher, 1997). These determinations were performed after splitting the final data sets into two equal portions, generating "independent" 3D volumes and determining the  $\text{FSC}_{0.5}$  resolution. Since this procedure actually determines the  $\text{FSC}_{0.5}$  for half the number of images in the final data set, we measured the trend in the  $\text{FSC}_{0.5}$  with the addition of more data. For each data set, pairs of 3D volumes were generated from randomly chosen collections of images, starting with 500 images in each half pair, and ending with 6000 images per comparison. The  $\text{FSC}_{0.5}$  resolutions were plotted against  $\log(N)$ , where  $N$  is the number of images included in a given 3D volume, and extrapolation of this curve allowed an estimation of the final resolution with the total number of images in the 3D datasets.

### Visualization of 3D volumes

The yeast and rabbit ribosome volumes were examined using both the WEB viewer (Frank *et al.*, 1996) and "O" (Jones *et al.*, 1991). We selected the contour level referred to in the text as "100%" based on multiple criteria. First, the ribosome's mass and the enclosed volumes are commensurate with both calculated and experimentally derived values for the partial specific volume of various ribosomes. Second, all expected features of the ribosome, including the mRNA entrance channel and the nascent chain exit tunnel were clearly identifiable at the chosen contour level. The contour level chosen to represent the yeast ribosome at the "100% level" encloses a volume of  $\sim 3.75 \times 10^6 \text{\AA}^3$  while the rabbit ribosome contour level encloses  $\sim 4.0 \times 10^6 \text{\AA}^3$ .

### Separation of the small and large subunits

At all contour levels examined above, there is clear separation between the small and large subunits over most of the subunit-subunit interface. We separated the subunits using the programs map-art and map-art-comp-

lement (Owen *et al.*, 1996). Map-art works by displaying a 3D volume as a series of xy 2D slices and allows the user to selectively remove regions from each slice, using either a polygon to surround and remove areas, or by "nibbling away" individual pixels along the edge of any area. As a guide, a given contour level is displayed in all sections. Map-art-complement works by generating the "complementary" map to a map-art generated volume: regions which were eliminated using map-art are the only features present in the map-art-complement volume. We oriented the ribosome volumes such that the subunit-subunit interface was perpendicular to the xy plane of the slices displayed in map-art and chose a contour level for display which was significantly lower than the 100% level. By choosing a lower threshold, we were able to over-estimate the subunit boundaries, which in turn allowed us to separate the subunits more accurately. Except for several small regions, the interface between the two subunits was easily separable. In regions where the separation required that contour lines be cut, we placed the cuts through the regions of lowest density such that high density features were connected. Since the use of map-art and map-art-complement involved rotations to orient the ribosome volumes properly (followed by a second rotation to return the volumes to our common orientation), all such operations were performed before final Fermi low-pass filtering of the volumes. Our observations concerning the separated subunits were confirmed by examining the separated and intact maps simultaneously in "O".

### Mass analysis

Samples of yeast and rabbit ribosomes were freeze-dried and imaged on the Brookhaven STEM I in dark field mode (Wall & Hainfeld, 1986). Individual particle images were selected and analyzed interactively with the Brandeis package, using TMV as an internal standard. The resulting mass data were histogrammed and compared to values obtained by searching on-line ribosomal databases and are summarized in Table 1.

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