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Comparison of Fungal 80S Ribosomes by Cryo-EM Reveals Diversity in Structure and Conformation of rRNA Expansion Segments

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Summary

Compared to the prokaryotic 70S ribosome, the eukaryotic 80S ribosome contains additional ribosomal proteins and extra segments of rRNA, referred to as rRNA expansion segments (ES). These eukaryotic-specific rRNA ES are mainly on the periphery of the 80S ribosome, as revealed by cryo-EM studies, but their precise function is not known. To address the question of whether the rRNA ES are structurally conserved among 80S ribosomes of different fungi we performed cryo-electron microscopy on 80S ribosomes from the thermophilic fungus *Thermomyces lanuginosus* and compared it to *Saccharomyces cerevisiae* 80S ribosome. Our analysis reveals general structural conservation of the rRNA expansion segments but also changes in ES27 and ES7/39, as well as the absence of a tertiary interaction between ES3 and ES6 in *T. lanuginosus*. The differences provide a hint on the role of rRNA ES in regulating translation. Furthermore, we show that the stalk region and interactions with elongation factor 2 (eEF2) are different in *T. lanuginosus*, exhibiting a more extensive contact with domain I of eEF2.

Keywords

Elongation factor 2; *Thermomyces lanuginosus*; 80S; expansion segments

Introduction

The eukaryotic 80S ribosome, when compared to the prokaryotic 70S ribosome, contains several more proteins and additional features in the ribosomal RNA (rRNA) molecules ¹; ²;

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³. The 40S subunit of *S. cerevisiae* contains 33 ribosomal proteins and an 18S rRNA of 1798 nucleotides (nt), as compared to the *E. coli* 30S subunit with 21 proteins and a 16S rRNA of 1542 nt. The yeast 60S subunit consists of 45 ribosomal proteins and three rRNA molecules, known as 25S rRNA (3392 nt), 5S rRNA (121 nt), and the additional 5.8S rRNA (158 nt). Compared to the *E. coli* 50S subunit, the yeast 60S subunit thus has an additional set of twelve proteins, and the combined 25S/5.8S rRNA is 646 nt longer in total than the 23S rRNA. While X-ray structures now exist for ribosomes and ribosomal subunits from several prokaryotic species, three-dimensional structures of eukaryotic ribosomes are only available at lower (~8 Ångström at best) resolution, contributed by cryo-EM.

As evident from a comparison of cryo-EM structures of eukaryotic ribosomes ^{2; 3} with X-ray structures of bacterial ^{4; 5} and archaeal ⁶ ribosomal subunits, the core of the ribosome is conserved among the kingdoms. Extra densities due to eukaryotic-specific proteins and ES in rRNA appear mainly at the periphery of the ribosome ^{2; 3; 7; 8; 9; 10}. Specific functions have been ascribed to some of the unique eukaryotic proteins; for instance, RACK1 appears to provide a platform for signaling proteins on the ribosome ^{11; 12; 13}.

Although the precise function of ES in rRNA is uncertain, evidence is accumulating that some might play an important role in translation. The essential component ES27 ¹⁴ of the large subunit has been proposed to regulate access of factors to the peptide exit channel, based on the observation of the dramatic change in its conformation upon binding of Sec61 ¹⁵. It has also been suggested that the ES6/ES7 in the protozoan *Trypanosoma cruzi* might assist in the recruitment of mRNAs to the ribosome ¹⁶. An interaction between ES24 and Sec61, observed in 80S-Sec61 complex ¹⁵, shows that expansion segments are also involved in recruiting components of the protein-conducting apparatus to the ribosome.

The *S. cerevisiae* 80S ribosome has been subjected to comprehensive cryo-EM studies ^{3; 9; 17}. To investigate the degree of conservation of rRNA ES structure in fungi, we have selected the thermophilic species *T. lanuginosus* for our studies. This filamentous fungus grows optimally at 50–55 °C, which is close to the upper limit for eukaryotes. We generated a cryo-EM map of the 80S ribosome from the *T. lanuginosus* (here referred to as *Tlan80S*), and compared it to the cryo-EM map of the 80S ribosome from *S. cerevisiae* (*Scer80S*) obtained previously at similar resolution.

While the general architecture of the two 80S ribosomes proves to be very similar, conformational differences in some of the expansion segments are readily identifiable. In addition, our comparison has revealed conformational differences in the dynamic stalk base region, one of the targets of elongation factor binding.

Results and Discussion

Secondary structure of *T. lanuginosus* rRNA

To our knowledge, detailed structural information of rRNA ES from thermophilic fungi has not been presented. As a first step in the characterization of the rRNA as well as the expansion segments from the *Tlan80S* ribosome, we have sequenced the rDNA.

Using the partial sequences of *T. lanuginosus* rRNA available (R. Nazar, unpublished; kindly provided by the author), we were able to amplify the 18S rDNA and 25S rDNA by PCR. Few cycles of amplification were used to minimize errors introduced by the PCR reaction.

Analysis of the rDNA from *T. lanuginosus* revealed a length of 1817 nt of the 18S rRNA and 3375 nt of the 25S rRNA, similar to those of the rRNAs from *S. cerevisiae* (Figure 1) and with 88% and 85% sequence identity, respectively. These findings agree well with what was

previously observed for 5S rRNA and 5.8S rRNA from *T. lanuginosus*^{18; 19}. Several sequence changes are coupled in base pairs of double-stranded regions, thus maintaining the integrity of rRNA helices. Interestingly, the changes cluster in certain regions of the rRNA (Figure 1). Focusing on the expansion segments, several changes are thus present in ES6 and ES12 of 18S rRNA, and in the case of 25S rRNA, three of the larger ES, namely ES7, ES27 and ES39, also show many nucleotide changes (Figure 1).

Analysis of the cryo-EM structure of *Tlan80S*

To characterize the 3D structures of the rRNA expansion segments and to compare them to those of *S. cerevisiae*, we employed cryo-EM and single-particle reconstruction²⁰ of *Tlan80S*. We compared the resulting 13.5 Å density map with that of a previous 15 Å map of the *Scer* 80S ribosome containing a P-site tRNA³. At this resolution the two ribosomes appear very similar (Figure 2). The landmark features of the 40S subunit, such as head, body, and platform, are well defined in *Tlan80S*, as are the “left foot” and “right foot” at the bottom of the subunit. The large subunit of *Tlan80S* clearly shows the central protuberance, the L7/L12 stalk base, and the L1 stalk as prominent features. The L1 stalk adopts the “closed” position as observed by Spahn et al. in the case of *S.cerevisiae*¹⁷.

It should be noted at this point that our comparison reveals an interesting state of the bound tRNA in *Tlan80S*. Previous structures of *Scer*80S have been either with a peptidyl tRNA in the P site or without any tRNA^{3; 17}. Density corresponding to a tRNA is visible at the E site of *Tlan80S* (Figure 3), showing that *Tlan80S* is purified with the E site occupied. A similar observation was made for *E. coli* ribosome complexes, which often showed an occupied E site, attributed to free deacylated-tRNA having a high affinity to the E site²¹. However, in the present map the tRNA is not in the classical E site; rather, it has an intermediate P/E position as identified by comparison with earlier Cryo-EM results²¹ and juxtaposition with classical tRNA positions in the 70S crystal structure⁵. We speculate that this position is occupied due to the effect of ratchet-like subunit rearrangement of the ribosome, and that it might represent, as in *E. coli*, an intermediate in the P to E-site tRNA translocation process. The observation of the ratchet-like subunit rearrangement in *Tlan80S* is consistent with the previous finding that this conformation is favored by *Scer*80S in the absence of a peptidyl-tRNA¹⁷. Bacterial ribosomes, in contrast, prefer the non-ratcheted subunit arrangement both in the presence and absence of peptidyl tRNA²¹.

The resolution we obtained allows separation of the map into its RNA and protein portions. Using a previously described method²², we separated the *Tlan80S* map into its rRNA and protein portions and compared these respectively to the rRNA and proteins of *Scer*80S³ obtained in the same way (Figure 2). The analysis showed that the ribosomes from the two species have similar protein and rRNA densities, indicating the absence of major changes in *Tlan80S* as a result of elevated growth temperature. However, some of the protein masses, e.g., proteins on the beak (proteins that apparently act as substitutes for helix 33a of 18S rRNA³) and platform (rpS14 (S11p)) regions of the 40S subunit), appear bigger compared to those in *Scer*80S (Figure 2).

In the following we have focused on those ES where the rRNA sequences differ between the two fungi, and where we indeed also find conformational changes. Additionally, our comparison shows changes in the P-protein stalk region. An analysis of each of these features is presented below.

Changes in expansion segments

Among the prominent rRNA regions where large differences in sequences are identified, the most dramatic conformational differences are detected in ES27 and ES7/ES39 of 25S rRNA.

As pointed out before, many of the expansion segments were identified and localized in the *Scer*80S cryo-EM map³. One of them is ES27, an expansion segment essential for viability¹⁴. Part of ES27 is visible in *Scer*80S as a distinct helical structure that runs up at the side of the L1 stalk, with a big mass situated at the base of the helix³. Comparison with the rabbit ribosome revealed that the helical part of the ES27 seen in the back of *S. cerevisiae* 60S subunit (termed “yeast-spine”⁹) is very specific for yeast. In the *Tlan*80S map this helical feature of ES27 is largely absent, with only the base of ES27 being visible (Figure 3), even though the helix is present in the secondary structure of the 25S rRNA of this organism (Figure 1). The 3D structure predicted by MFOLD²³ also indicates that the helices in these segments are maintained. Analysis of the rRNA by denaturing electrophoresis indicated no sign of RNA degradation (data not shown). We therefore conclude that the absence of part of ES27 is due to the positional variability of this rRNA helix. The same conclusion has been derived for 80S ribosomes from other species, where this helix was similarly found missing from the cryo-EM maps^{1; 2; 8}.

Since ES27 is essential for viability of the organism¹⁴, and since its partial absence is noted in all 80S cryo-EM maps studied to date, the dynamic nature of ES27 is most likely important for its function. This structure was seen in two radically different conformations in cryo-EM maps of *Scer*80S. Depending on whether or not Sec61 was attached to the ribosome, the positions of ES27 differed by a 90-degree rotation around an axis normal to the ribosome surface¹⁵. Thus, potentially, this expansion segment could control the binding of factors close to the peptide exit site as part of an allosteric mechanism¹⁵.

A large part of the extra density seen on the solvent side of the 60S subunit, behind the P-protein stalk base of the eukaryotic ribosome, has been proposed to encompass the expansion segments ES7 and ES39 (identified as ES7/39³). Due to the complexity of these expansion segments they cannot be clearly resolved into the individual ES7 and ES39 components at the current resolution, and we will therefore continue to refer to these densities jointly as ES7/ES39. In case of *Scer*80S, one of the rRNA helices of ES7/ES39 protrudes like a finger in the back of the 60S subunit pointing towards the exit of the polypeptide tunnel. Our comparison revealed that in *Tlan*80S this helical part of the ES7/ES39 adopts a different position, characterized by a rotation of 45 degrees away from the polypeptide tunnel exit (Figure 3 and 4), bringing it close to a protein cluster consisting of rpL4, rpL18, rpL32, and an unknown protein density feature previously referred to as ‘VII’³ (Figure 4). More precisely, the tip of the helix appears to contact residues 140–145 in rpL4. This alternative conformation, previously not observed in fungi, will be termed ‘closed’, while the conformation leading to the finger-like protrusion in case of *Scer*80S will be referred to as ‘open’ (Figure 4).

Surveying the large amount of biochemical data available we found reports that rpL18 interacts with rpL36 in *C. elegans*, fruitfly, and human (Stelzl et al., 2005²⁴ and references therein). Therefore, we tentatively assign the protein density VII to rpL36. Indeed, the size of the density VII would be compatible with the size expected for rpL36 (100 amino acids).

Protein rpL4 is a conserved ribosomal protein with an extension that comes close to the peptidyl-transferase center⁶. Together with rpL22 it produces a point of constriction within the peptide exit tunnel of the large subunit²⁵. The rpL4 protein has been suggested to relay information from the peptidyl-transferase center to the surface of the ribosome, in the vicinity of the binding sites for the protein-conducting translocon¹⁵ and the SRP²⁶. We speculate that conformational changes in rpL4 in response to the presence of peptide could lead to a change in the ES7/ES39 conformation that in turn would influence the binding of factors, such as SRP, that are close to this rRNA element. Following this line of thought we investigated the position of this helix of the ES7/ES39 in the cryo-EM maps of human², wheat germ²⁶, and *T. cruzi*¹⁶ 80S ribosomes. Inspection of these cryo-EM maps shows that in both humans and

wheat germ this helix of ES7/ES39 remains in the ‘closed’ position, similar as in *Tlan80S* and the contact to rpL4 is maintained (Figure 4c,d). Although the *T. cruzi* ribosome looks quite different compared to other eukaryotic ribosomes¹⁶, this particular helix appears to be in the ‘closed’ position as well (Figure 4e).

This part of ES7/ES39 is close to the contact region of the SRP and 80S ribosome²⁶. It appears from the results for wheat germ by Halic et al.²⁶ that the ‘open’ position of the helix still allows SRP binding, provided the helix is short. In human, by contrast, this RNA helix is extremely long (Figure 4), making the ‘open’ conformation incompatible with SRP binding.

We believe that the observed conformational difference in ES7/ES39 between *Tlan80S* and *Scer80S* might not merely be a result of the species-related differences in primary structures, but rather manifestations of a conformational switching mechanism required for functional dynamics. This switching mechanism could potentially regulate SRP binding in species with large ES7/ES39 helices.

Besides these major differences in ES27 and ES7/39 of 25S rRNA, we observe another difference for ES6 of 18S rRNA. While for *Tlan80S* the ‘right foot’ component of the 40S subunit³, constituted by the ES12 of 18S rRNA (extension of helix 44), is similar to that in *Scer80S* (Figure 2) the ‘left foot’ (bottom part of ES6) no longer interacts with ES3 as seen in *Scer80S*³ (Figure 2). It has been suggested that ES6 and ES3 form a 7–9 nucleotide helix through base pairing of their single-stranded loop regions²⁷. In contrast to the results of the study of Alkemar and Nygaard²⁷, we find that the sequence of ES3 is not completely single-stranded; instead, part of it is now folded into a helical structure. This conformation could prevent helix formation between ES6 and ES3, and thus could provide a plausible explanation for the absence of a tertiary interaction between ES3 and ES6 evident in our *Tlan80S* map.

Changes in the P-protein stalk region

In addition to the changes in ES, a conformational difference was also identified in the P-protein stalk region, a region that plays a crucial role in elongation factor binding and in inducing GTP hydrolysis in the bound factors²⁸. This region can be separated into the stalk base, composed of part of 25S rRNA rpL12 and P0 (L10 in prokaryotes), and an extended stalk structure, composed of several copies of P1 and P2 (L7/L12 in prokaryotes).

The extended part of the stalk is not visible in the vacant *Tlan80S* examined here (Figure 2 and 5), presumably because this part is fixed only by factor binding, as indicated by previous studies^{29; 30}. The appearance of the stalk base is different compared to that observed in *Scer80S* (Figure 5). The special feature of the acidic P-proteins that appear as a “side-lobe” close to the N-terminal domain of rpL12 (L11 in prokaryotes) in *Scer80S*³ is not visible in *Tlan80S*. Instead, an extra long thread of density hangs off the top of the rpL12 mass (Figure 5). This extra thread of density could either be due to a protein extension of rpL12 specific for *T. lanuginosus* or be part of the extended stalk structure. As the sequences of *T. lanuginosus* ribosomal proteins are not available we cannot distinguish between these two possibilities at this time.

The observed differences in the stalk region of *Tlan80S* led us to ask whether there might be any difference in the interaction of the ribosomes with elongation factors. To address this question, *Tlan80S* ribosomes were incubated with eEF2 from either *T. lanuginosus* or *S. cerevisiae* and GDP in the absence or presence of the fungicide sordarin, which is known to trap eEF2 on the ribosome¹⁷. Following isolation of ribosomes, the amount of associated eEF2 was measured by a ribosome pelleting assay. We found that *Tlan80S* forms a strong complex with eEF2 from both fungi and that complex formation was moderately stimulated by GDP and strongly stabilized by sordarin (Figure 5). Protein sequencing of peptides from

T. lanuginosus eEF2 by mass spectrometry showed above 80% identity for peptides compared to the corresponding peptides from *S. cerevisiae* (Table 1), indicating that eEF2 from the two species are quite similar. Also, the fact that eEF2 from *S. cerevisiae* is able to bind to *Tlan*80S and form functional complexes indicates a similar mode of binding for elongation factors in the two fungi.

To further confirm the similar mode of interaction of eEF2, we generated a ~16Å cryo-EM map of the *Tlan*80S:eEF2 complex stalled by sordarin, and compared it with a previous cryo-EM map of the sordarin-stalled 80S:eEF2 complex from *S. cerevisiae*¹⁷ (Figure 5d,e). The comparison shows that overall the domain arrangements of eEF2 and its interactions with ribosomal subunits are very similar in both cases. The only difference observed is in the interacting region of the factor (domain I) and the stalk-base, where more extensive contacts are seen in *Tlan*80S (Figure 5d,e). The morphological features of the stalk-base conformation in the complexes appear to be a reflection of the conformation in the respective empty ribosomes (shown with arrows in Figure 5a,b,d,e). The observed structural differences in this region are most likely due to differences either in structural dynamics or, potentially, the number of copies of the P1/P2 proteins between the mesophilic *S. cerevisiae* and the thermophilic *T. lanuginosus*, similar to the observation of a heptameric stalk complex of the *Thermus thermophilus* 70S ribosome³¹.

Conclusion

We have obtained a biochemical and structural characterization of the 80S ribosome of the thermophilic fungus *T. lanuginosus* - a model system that could prove useful in high-resolution studies of eukaryotic ribosomes and their complexes with elongation factors. In addition, our study has provided further insight into the function of ES27 and ES7/ES39. We provide evidence that these two expansion segments exist in a novel “fungal” conformation in *Tlan*80S and we speculate that it could be of functional importance as a means of regulating factor binding to the peptide exit site. Further structural and functional studies are required to address the precise role of these rRNA elements in translation.

Even though the overall mechanism and mode of interaction of eEF2 appear to be very similar between *S. cerevisiae* and *T. lanuginosus* ribosomes, we do find differences in the stalk region and at the level of interaction with domain I, which constitutes the nucleotide binding site of eEF2. The difference in the stalk region could be due to difference in the ribosomal proteins constituting the stalk or differences in the dynamical nature of this region. The difference in the stalk region leads to more extensive contacts with domain I of eEF2 in *T. lanuginosus* 80S. At the current resolution we are however unable to address the question whether this reflects a difference, between the two ribosomes, in the mechanism by which GTP hydrolysis is stimulated in eEF2. We are currently pursuing this question with higher-resolution structures of ribosome complexes.

Materials and Methods

Purification of ribosomes and eEF2 from *T. lanuginosus*

80S ribosomes were purified as described before¹³. eEF2 from *T. lanuginosus* was purified as for eEF2 from *S. cerevisiae* as described³².

Mass spectrometry of eEF2

Protein bands were excised from gel and in-gel digested with trypsin (Promega, Madison, USA). The peptide sequences from *T. lanuginosus* were generated using chemical assisted

fragmentation in a Micromass Q-TOF Ultima Global mass spectrometer (Micromass, Manchester, UK) operated in MALDI Q-TOF mode ³³.

Binding of eEF2 to 80S ribosome

5 µg of 80S ribosomes were incubated with 2 µg of eEF2 in 100 µl of buffer A (20 mM Hepes-NH₃ pH=7.2, 100 mM KCl, 20 mM MgCl₂). The final nucleotide concentration was 100 µM and that of sordarin 100 µM. The reactants were incubated on ice for 20 minutes and then placed on top of a 50 µl 30% sucrose cushion in buffer A containing the same composition with regard to nucleotide and sordarin as the reactants. The ribosomes were isolated by ultracentrifugation and the ribosome pellet was analyzed by SDS-PAGE followed by quantization of the bound eEF2 by densitometer analysis.

Sequencing of rDNA

Genomic DNA was prepared from *T. lanuginosus* using the DNeasy Plant Maxi Kit (Qiagen, Valencia, USA). Based on partial sequences of the rDNA of 18S rRNA and 23S rRNA (kindly provided by Ross Nazar) primers were designed to amplify the missing sequences of rDNA. A 18S rDNA fragment was amplified with 5'-TATCTGGTTGATTCTGCCAGT'-3' and 5'-CCGCACTCGGTAATGATCCTTC-3' while a 25S rDNA fragment was amplified using 5'-TGGGTTAGTCGATCCTAAG-3' and 5'-CCAAGGATCGGGCTGACG-3' using Hi-Fidelity Polymerase (Boehringer Mannheim) and 12 cycles of PCR. The PCR fragments were purified and sequenced.

Cryo-EM and 3D image reconstruction

The grids for cryo-electron microscopy were prepared following standard procedures. Micrographs were recorded on a Philips FEI (Eindhoven, The Netherlands) Technai F20 field emission gun electron microscope at 200 kV and a magnification of 50,000 (±2%) following standard low-dose procedures. After a check by optical diffraction for the presence of Thon rings, micrographs were digitized with a step size of 14 µm on a Zeiss/Imaging scanner (Z/I Imaging Corporation, Huntsville, AL), the final pixel size corresponded to 2.82 Å on the object scale.

The image processing using SPIDER ³⁴ included a 3D projection alignment procedure with correction of the contrast transfer function and enhancement of the high-resolution Fourier amplitudes based on X-ray solution scattering data. A total number of 27,708, and 38,566 ribosome particles were selected to obtain a refined 3D cryo-EM map of the *Tlan*80S empty (~13 Å) and eEF2 (purified from *T. lanuginosus*) bound (~16 Å) ribosome (stalled with sordarin; 80S·eEF2·sordarin complex) respectively. The resolution was estimated using a cutoff of 0.5 in the Fourier shell correlation ³⁵. Upon application of a supervised classification method ³⁶ in order to improve the homogeneity of the complex, the resolution changed to ~19 Å.

Visualization tools

The graphic visualizations were done with the programs IRIS Explorer (The Numerical Algorithms Group Ltd. Downers Grove, Illinois, USA), Ribbons ³⁷, O ³⁸ and INSIGHT II (Accelrys Inc. San Diego, California, USA).

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Figure 1.

Mutations in *T. lanuginosus* rRNA mapped on the secondary structure from *S. cerevisiae*. Color codes: Red, mutations; green, deletions; dark blue, additional nucleotides. All expansion segments (ESs) of 18S and 25S rRNAs are marked by golden lines, except ES3 and ES6 of the 18S, and ES7, ES27, ES39 of the 25S, which are marked by blue lines.

Figure 2. Comparison of the cryo-EM maps of *Scer80S* (15 Å; a,c), with *Tlan80S* (13.5 Å; b,d). Factor-binding-side view is shown (a,b). Landmarks for 40S (yellow): bk, beak; lf, left foot; rf, right foot; sh, shoulder; pt, platform; for 60S (blue): CP, central protuberance; sb, stalk base; st, extended stalk; asterisk (*) in a marks interaction between ES6 and ES3 in *Scer80S*, which is absent in *Tlan80S* (*) in b). Comparison of the RNA-Protein compositions of 40S (c–d; proteins: green, rRNA: yellow) and 60S (e–f; proteins: golden, rRNA: blue) from *Scer80S* (c: 40S; e: 60S) and *Tlan80S* (d: 40S; f: 60S), all shown in solvent side view. Some of the proteins and RNA helices for each subunit, identified in a previous study³, are labeled. Missing extended

stalk is marked with asterisk (* in f) in *Tlan*80S. We show the 15Å map from Spahn et al.³ because this study provided the most extensive analysis of the *Scer*80S ribosome.

Figure 3. Conformation of ES27 (yellow) and ES7/39 (red) in *Scer80S*³ (a,c) and in *Tlan80S* (b,d). 60S subunits are viewed from intersubunit side (a,b), and solvent side (c,d). Landmarks are as defined in Figure 2. Densities of occupied tRNAs are shown in (a) (P-site) and in (b) (P/E site). Position of the peptide exit channel is indicated in (c) and (d). The arrow (←) in (d) indicates the 'closed' position of the rpL1 stalk, compared to its position in (c).

Figure 4. Solvent-side view of the 60S subunit from *Tlan80S*, with helix of ES7/ES39 colored in red (a). The positions of rpL4, rpL18, rpL32 and unknown protein mass VII are indicated. Homology models³ of rpL4, rpL18 and rpL32 docked into the density are shown in stereo (b). Densities equivalent to the ‘closed’ ES7/39 helix in *Tlan80S* (identified by the comparison of the features) are highlighted in red on the solvent side views of human (c), wheat germ (d), and trypanosome (*T. cruzi*) (e) ribosomes. The most likely position of rpL4 is marked by ‘*’.

Figure 5.

Comparison of close-up views of the stalk-base region for *Scer*80S (a) and *Tlan*80S (b). Left-most side shows thumbnail version of 80S ribosomes (40S: yellow; 60S: blue) for orientation. The ‘side-lobe’ of the *Scer*80S stalk-base is marked, and a curved arrow in (b) marks the extra density in the stalk-base of the *Tlan*80S. (c) Binding of eEF2 from *S. cerevisiae* to *Tlan*80S. Factor eEF2 was incubated with *Tlan*80S in the presence of GDP or GDP and sordarin, and the amount of eEF2 bound following pelleting of ribosomes was quantified. The eEF2 band is indicated on the gel. The faster migrating bands correspond to ribosomal proteins. (d, e) Close-up views of the factor-binding site of eEF2-GDP-sordarin-80S cryo-EM maps. (d) Both factor and the ribosome purified from *S. cerevisiae*. (e) Both factor and the ribosome purified from *T. lanuginosus*. We used the 17.5 Å map³⁰ in (d) to match the resolution of the map shown in (e). The higher-resolution map of the same complex shows identical features¹⁷. The density for eEF2 is colored in red.

Sequences of *T. lanuginosus* eEF2 peptides obtained by mass spectrometry and their corresponding position in eEF2 from *S. cerevisiae*. The degree of identity between the sequences is indicated in the last column.

Table 1

Peptide from <i>T.lanuginosus</i> eEF2	Corresponding residues in <i>S. cerevisiae</i> eEF2	% identity
VNFTIEIR	2-10	44,4
VTGGALVVVDIVEGPAVQTETVLR	121-144	87,5
ALLELQVTK	163-171	88,9
EDIYQSFRR	172-180	77,8
TIESVNVILSTYLDK	181-195	73,3
AETLYEGPTDDEACISIR	353-370	66,7
SGTLTTSETAHNIK	466-479	85,7
DLEEDHAGVPLR	544-555	83,3
VSDPVVQYR	558-564	66,7
ILADYGWDVTDAR	618-631	85,7
DTFVSGFQWATR	661-672	58,3
AYMPVNESFGFDGLR	770-785	81,2