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Crystal structure of the 80S yeast ribosome

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The first X-ray structure of the eukaryotic ribosome at 3.0 Å resolution was determined using ribosomes isolated and crystallized from the yeast *Saccharomyces cerevisiae* (Ben-Shem A, Garreau de Loubresse N, Melnikov S, Jenner L, Yusupova G, Yusupov M: The structure of the eukaryotic ribosome at 3.0 A resolution. *Science* 2011, 334:1524–1529). This accomplishment was possible due to progress in yeast ribosome biochemistry as well as recent advances in crystallographic methods developed for structure determination of prokaryotic ribosomes isolated from *Thermus thermophilus* and *Escherichia coli*.In this review we will focus on the development of isolation procedures that allowed structure determination (both cryo-EM and X-ray crystallography) to be successful for the yeast *S. cerevisiae*. Additionally we will introduce a new nomenclature that facilitates comparison of ribosomes from different species and kingdoms of life. Finally we will discuss the impact of the yeast 80S ribosome crystal structure on perspectives for future investigations.

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Introduction

Ribosomes are the cellular molecules responsible for the synthesis of proteins in all kingdoms of life. They consist of a universally conserved core upon which domain-specific additions, mainly on the surface of the ribosomes, are added [1••,2••]. The core was first described in detail from crystal structures of prokaryotic ribosomes [3–8]. It contains the peptidyl transferase center (PTC) that catalyzes peptide bond formation, the polypeptide tunnel where the nascent peptides exit the ribosome and the decoding center that decodes mRNA (for a recent review see [9]). These are all universally conserved properties of the core. However, eukaryotes and prokaryotes differ markedly in other translation processes, especially in initiation of translation (reviewed in [10,11]). Furthermore, eukaryotic ribosomes play central roles in many eukaryote-specific cellular processes [12–15]. Eukaryotic ribosomes are at least 40% larger than their bacterial counterparts due to additional rRNA elements called expansion segments (ESs), extra proteins and insertions into existing proteins [1••,2••,16,17•,18•]. All ribosomes are composed of two subunits. The large 60S subunit of the eukaryotic *S. cerevisiae* ribosome (50S in bacteria) consists of three rRNA molecules (25S, 5.8S, and 5S) and 46 proteins, whereas the small 40S subunit (30S in bacteria) contains one rRNA chain (18S) and 33 proteins. 32 of the 79 proteins in *S. cerevisiae* have no homologues in bacterial or archaeal ribosomes and those that do have homologues can still harbor large eukaryote-specific extensions [19]. Within Eukarya, the most noticeable variability is in certain rRNA expansion segments, whereas the protein components of the ribosomes, from yeast to human, are very similar.

The first structural insights into the overall architecture of the yeast ribosomes came from three-dimensional cryo-EM reconstructions. These studies described different ribosome conformations and provided information about eukaryote-specific rRNA segments as well as the interactions of the ribosome with several translation factors [16,20–22]. Recently, detailed atomic models of the 80S eukaryotic ribosome from *S. cerevisiae* [1••,2••] and structures of the individual 40S and 60S subunits from *Tetrahymena thermophila* [17•,18•] were determined by X-ray crystallography.

Isolation of ribosomes from *S. cerevisiae* for cryo-EM studies

Structural biology methods such as cryo-EM and X-ray crystallography require very homogenous materials for investigation. Although an isolation procedure might be good for production of ribosomes for biochemical studies,

for example, in cell-free systems, it may not produce materials appropriate for cryo-EM or crystallization studies.

Two strategies have been used for isolation of ribosomes for cryo-EM structural studies: The first approach, isolation of vacant ribosomes, is based on a purification using conditions where the ribosomes are homogenized by washing out of functional ligands (tRNA, mRNA and protein factors). This approach has been used for several cryo-EM studies. Frank and co-workers compared the structure of empty yeast ribosomes with empty ribosomes complexed with elongation factor EF2 at 17.5 Å resolution. This revealed that the vacant yeast ribosome prefers to adopt a rotated conformation, that is, where the 40S subunit is rotated relative to the 60S subunit [23]. This conformation was previously characterized in prokaryotic ribosomes as a rotated state corresponding to the pre-translocation state of translation [7,24•,25]. Later Spahn and co-workers studied vacant ribosomes complexed with the CrPV-IRES mRNA at a resolution of 7.3 Å [20].

The second approach is based on selective 'fishing' for translating ribosomes stabilized at identical functional states that contain specific ligands. This method was used for the initial cryo-EM structural studies of translating ribosomes performed by Frank and co-workers, first at 26 Å and later at 15.4 Å resolution [26,27]. Beckmann and co-workers subsequently improved this isolation approach and the most recent yeast ribosome structures have now been resolved to 5.5 Å resolution. Cryo-EM maps at this resolution allowed interpretation of the structural features of the majority of the rRNAs and ribosomal proteins [28••,29••].

Isolation of ribosomes from *S. cerevisiae* for X-ray analysis

Development of isolation protocols for X-ray analysis can be very challenging. Among the several additional criteria that must be met is final yield. Initially, crystallographic studies of the ribosome required significant amounts of homogenous material to enable systematic searches of crystallization conditions, and later for a continuous production of high-quality crystals for data collection. Additionally, the sample purity generally has to be even higher for X-ray analysis since there is nothing similar to the particle selection and splitting into several conformational classes possible in cryo-EM [30].

Several general procedures for ribosome purification were employed, tested and optimized to develop a suitable isolation protocol. A significant fraction of ribosomes in cell extracts is in the form of polysomes [31]. A traditional approach for isolation of mono-ribosomes from polysomes in bacteria is based on salt-washing in 0.5 M of NH_4Cl or KCl at 5–10 mM of $MgCl_2$ or even to dissociate ribosomes to subunits followed by re-association, thus removing ligands from the ribosomes. While this is a rather harsh approach, it has been successfully used for several structural studies of bacterial ribosomes [7,8,32]. An alternative approach involves binding ribosomes to chromatographic resins (e.g. the Butyl-Toyo-Perl hydrophobic resin used in purification of *Thermus thermophilus* 70S [33]) and washing off ligands on the column. However, the extremely high salt conditions used in hydrophobic chromatography limit this method to ribosomes isolated from extremophiles.

The ability of the sample to crystallize was used as the final test of the ribosome quality. After procedures including treatments based on protocols described above for bacterial systems, the *S. cerevisiae* 80S ribosome failed to crystallize. Preliminary biochemical studies revealed that yeast ribosomes were much more sensitive to changes in salt concentrations, suggesting that they are much less robust than bacterial ribosomes. It was then decided to focus on a radically new approach employing very mild manipulations based on previous experiences with other fragile systems [34]. The final isolation method that yielded 80S ribosomes of sufficient quantity and quality for crystallization is based on a fast but gentle purification of ribosomes [1••,2••]. The critical elements are highlighted here. First, glucose starvation [35] was employed to ensure that most of the ribosomes were in the form of 80S monomers before initiation of the isolation procedure, thus obviating the requirement for harsh salt-wash steps. Gentle cell lysis was accomplished by shaking with glass beads to prevent mechanical damage to the 80S ribosomes and to avoid contamination with 70S ribosomes from organelles. This was followed by PEG fractionation of cell extracts and subsequent sedimentation of ribosomes through a sucrose gradient. Importantly, we also found that L-A, an endogenous dsRNA virus of yeast, co-purified with ribosomes through these procedures. Thus, we developed a yeast strain (JD1370) lacking the virus. This strain also harbors gene deletions of key proteases and nucleases that are typically liberated upon cell lysis. Together, these modifications produced ribosome samples suitable for crystallization and X-ray studies. Following isolation, the 80S ribosomes were crystallized in hanging drops in 4.5% PEG 20K, 20% glycerol, 100 mM KSCN, 5 mM Spermidine, 3 mM Mg-Acetate, 100 mM Tris-Ac pH 7.0, 1.4 mM Deoxy Big CHAP [1••,2••]. More recently, a considerably milder sulfolink column purification method initially developed for purification of bacterial ribosomes [36] was successfully adopted for high yield yeast ribosome isolation. This method results in significant increases in ribosome purity while simultaneously minimizing their degradation during the early steps of the purification procedure [37•]. However, this approach has not yet been tested in terms of crystallization.

The structure of the *S. cerevisiae* 80S ribosome

The initial crystal structure of a eukaryotic ribosome determined at 4.2 Å resolution permitted localization

and building of the rRNA structure and conserved proteins [2••]. Soon thereafter, 40S and 60S subunit structures were determined from crystals obtained from isolated ribosomal subunits from the ciliate protozoa *Tetrahymena thermophila* by Ban and co-workers at 3.9 Å and 3.5 Å resolution, respectively [17•,18•].

Only the highest resolution structure of the yeast ribosome determined at 3.0 Å resolution permitted unambiguous determination of the 80 proteins and more than 5500 RNA bases comprising the 80S ribosome at the level of protein sidechains including all eukaryote-specific proteins and protein insertions as well as rRNA expansion segments. Most yeast ribosomal proteins have duplicate genes [38], and the high quality of the X-ray data made it possible to distinguish even the differences in amino-acid sequence between isoforms of ribosomal proteins in several cases [1••]. The structure of the 80S ribosome also provides detailed information of the exact architecture of eukaryote-specific intersubunit bridges and the conformational changes of rRNA and proteins upon movement of ribosomal subunits.

RNA expansion segments

The rRNA expansion elements are located predominantly on the periphery of the solvent-exposed sides of both subunits (Figure 1a–d). The interface between ribosomal subunits as well as the area around the mRNA entrance and the polypeptide exit tunnel is highly conserved and contains only very few expansion segments and eukaryote-specific protein parts [1••]. This observation suggests that the mechanisms behind the elongation phase of translation are similar in both eukaryotic and prokaryotic cells. Perhaps the most impressive example of a eukaryotic expansion segment is the ~200 nucleotide long ES6S in the small ribosomal subunit. This expansion segment emerges at the solvent side of the platform where it is enveloped by several eukaryote-specific proteins including a 60 amino acids — long alpha-helical extension of the C-terminus of protein L19e (Figure 1a,b). ES6S then extends one of its two long arms in the direction of the shoulder where it interacts with protein S8. The second long arm of this expansion segment runs down towards the bottom of the small subunit. The tip of the second arm is thus located roughly 120 Å away from the tip of the first arm. ES6S is in contact with ribosomal components that form part of both the exit and entry sites of the mRNA. Therefore it seems plausible that ES6S is involved in translation initiation, perhaps as a docking surface for factors that participate in activities on both the mRNA exit and entry sites [39].

New nomenclature of ribosomal proteins

The first eukaryotic proteins to be characterized were from rats (reviewed in [40]). Subsequently, the yeast and human proteins were named according to the rat nomenclature, which does not always match the bacterial conventions. To facilitate comparison between ribosomes from different species we adopted a nomenclature that is based on the names of protein families (www.uniprot.org/docs/ribosomp) (Tables 1 and 2). In this convention, the default name for any protein in the universally conserved core (i.e. with bacterial homologues) is the bacterial name because this is the name given to the entire protein family. Eukaryotic proteins lacking bacterial homologues have names that end with 'e'. The following exceptions to those rules were applied: The *S. cerevisiae* name (instead of the protein family name) is used for eukaryote-specific proteins whose protein family names are derived from mammals

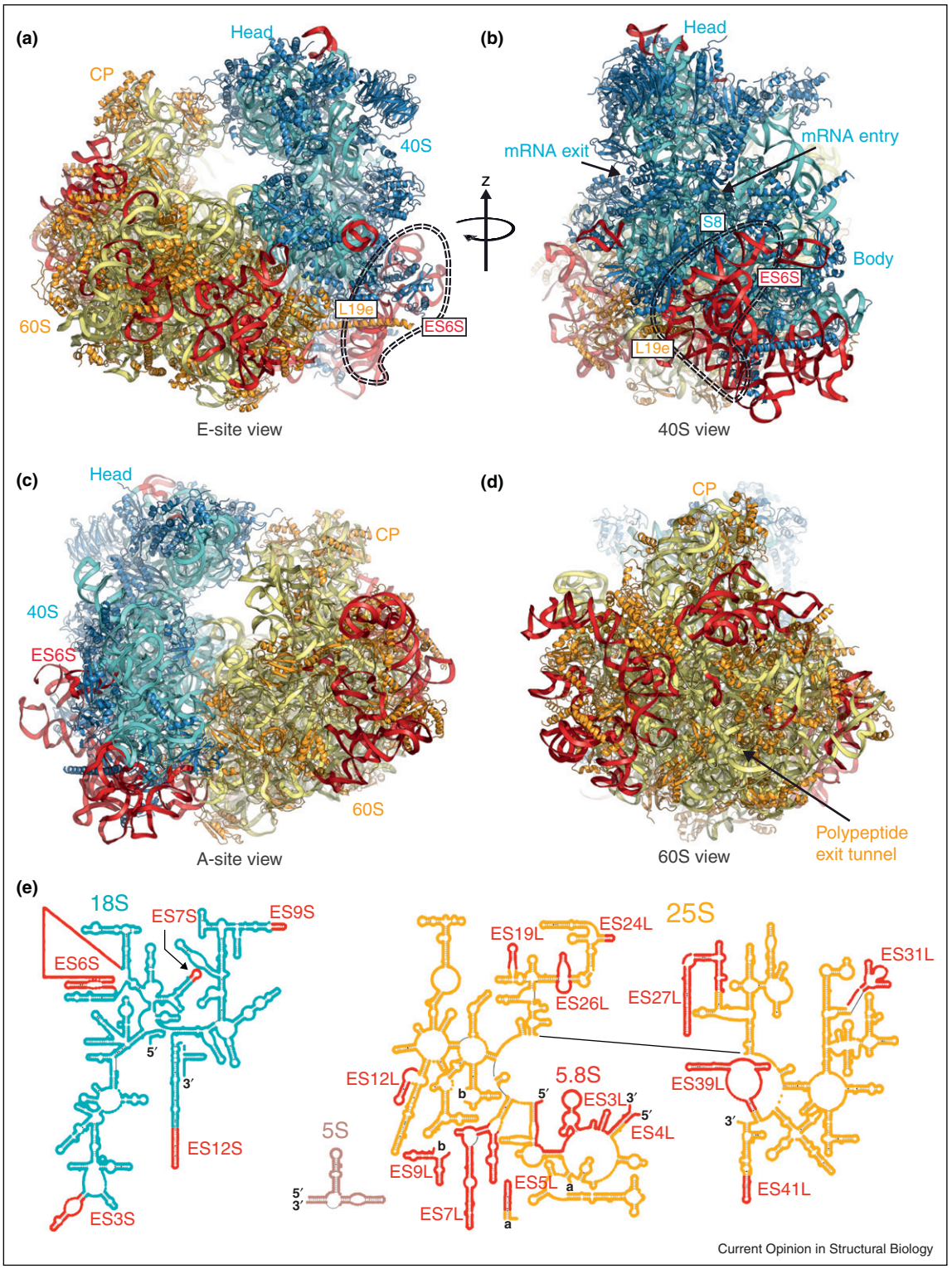
Table 1

New nomenclature for yeast proteins from the small ribosomal subunit.

New name	Taxonomic range*	Bacteria name	Yeast name	Human name
–	B	S1	–	–
S1e	A E	–	S1	S3A
S2	B A E	S2	S0	SA
S3	B A E	S3	S3	S3
S4	B A E	S4	S9	S9
S4e	A E	–	S4	S4
S5	B A E	S5	S2	S2
–	B	S6	–	–
S6e	A E	–	S6	S6
S7	B A E	S7	S5	S5
S7e	E	–	S7	S7
S8	B A E	S8	S22	S15A
S8e	A E	–	S8	S8
S9	B A E	S9	S16	S16
S10	B A E	S10	S20	S20
S10e	E	–	S10	S10
S11	B A E	S11	S14	S14
S12	B A E	S12	S23	S23
S12e	E	–	S12	S12
S13	B A E	S13	S18	S18
S14	B A E	S14	S29	S29
S15	B A E	S15	S13	S13
–	B	S16	–	–
S17	B A E	S17	S11	S11
S17e	A E	–	S17	S17
–	B	S18	–	–
S19	B A E	S19	S15	S15
S19e	A E	–	S19	S19
–	B	S20	–	–
–	B	S21	–	–
–	B	THX	–	–
S21e	E	–	S21	S21
S24e	A E	–	S24	S24
S25e	A E	–	S25	S25
S26e	E	–	S26	S26
S27e	A E	–	S27	S27
S28e	A E	–	S28	S28
S30e	A E	–	S30	S30
S31e	A E	–	S31	S27A
RACK1	E	–	Asc1	RACK1

* B: bacteria, A: archaea, and E: eukaryotes.

Figure 1



Crystal structure of the *S. cerevisiae* 80S ribosome. ((**a**)–(**d**)) Views from the E site, small subunit side, A site and large subunit side. B, C and D have been rotated 90°, 180° and 270° around the *z*-axis with respect to A. Polypeptide exit tunnel, mRNA entry and exit tunnels are indicated. The large subunit is shown in yellow with orange proteins and the small subunit in cyan with blue proteins. Eukaryote expansion segments (ES) are shown in red. Clearly, most of the expansion segments are located on the surface of the ribosome and concentrated in two large clusters. (E) Secondary structure of yeast ribosomal rRNA with expansion segments marked. Note that no additional rRNA chains have been added for eukaryotes (5.8S and 25S rRNA together are homologous to 23S rRNA in bacteria) but rather expansion segments are inserted into already existing rRNA chains. As we move to higher eukaryotes the major differences involve enlargement of already exiting rRNA expansion segments.

Table 2

New nomenclature for yeast proteins from the large ribosomal subunit.

New name	Taxonomic range*	Bacteria name	Yeast name	Human name
L1	B A E	L1	L1	L10A
L2	B A E	L2	L2	L2
L3	B A E	L3	L3	L3
L4	B A E	L4	L4	L4
L5	B A E	L5	L11	L11
L6	B A E	L6	L9	L9
L6e	E	–	L6	L6
L8e	A E	–	L8	L7A
–	B	L9	–	–
L11	B A E	L11	L12	L12
–	B	L12/L7	–	–
L13	B A E	L13	L16	L13A
L13e	A E	–	L13	L13
L14	B A E	L14	L23	L23
L14e	A E	–	L14	L14
L15	B A E	L15	L28	L27A
L15e	A E	–	L15	L15
L16	B A E	L16	L10	L10
–	B	L17	–	–
L18	B A E	L18	L5	L5
L18e	A E	–	L18	L18
–	B	L19	–	–
L19e	A E	–	L19	L19
–	B	L20	–	–
L20e	E	–	L20	L18A
–	B	L21	–	–
L21e	A E	–	L21	L21
L22	B A E	L22	L17	L17
L22e	E	–	L22	L22
L23	B A E	L23	L25	L23A
L24	B A E	L24	L26	L26
L24e	A E	–	L24	L24
–	B	L25	–	–
–	B	L27	–	–
L27e	E	–	L27	L27
–	B	L28	–	–
L28e	E	–	–	L28
L29	B A E	L29	L35	L35
L29e	E	–	L29	L29
L30	B A E	L30	L7	L7
L30e	A E	–	L30	L30
–	B	L31	–	–
L31e	A E	–	L31	L31
–	B	L32	–	–
L32e	A E	–	L32	L32
–	B	L33	–	–
L33e	A E	–	L33	L35A
–	B	L34	–	–
L34e	A E	–	L34	L34
–	B	L35	–	–
–	B	L36	–	–
L36e	E	–	L36	L36
L37e	A E	–	L37	L37
L38e	A E	–	L38	L38
L39e	A E	–	L39	L39
L40e	A E	–	L40	L40
L41e	A E	–	L41	L41
L43e	A E	–	L43	L37A
L44e	A E	–	L42	L36A
P1/P2	A E	–	P1/P2 ($\alpha\beta$)	LP1/LP2
P0	B A E	L10	P0	LP0

* B: bacteria, A: archaea, and E: eukaryote.

and have an additional 'A' [41]. Thus, for example, the yeast protein S1 that belongs to the protein family S3Ae is here called S1e. Similarly, the P-stalk proteins retain their yeast names (P1, P2). The positions of all ribosomal proteins on the surface of the ribosome are shown with their new nomenclature in Figure 2a–d.

Prospectives for crystallographic studies of *S. cerevisiae* ribosome complexes

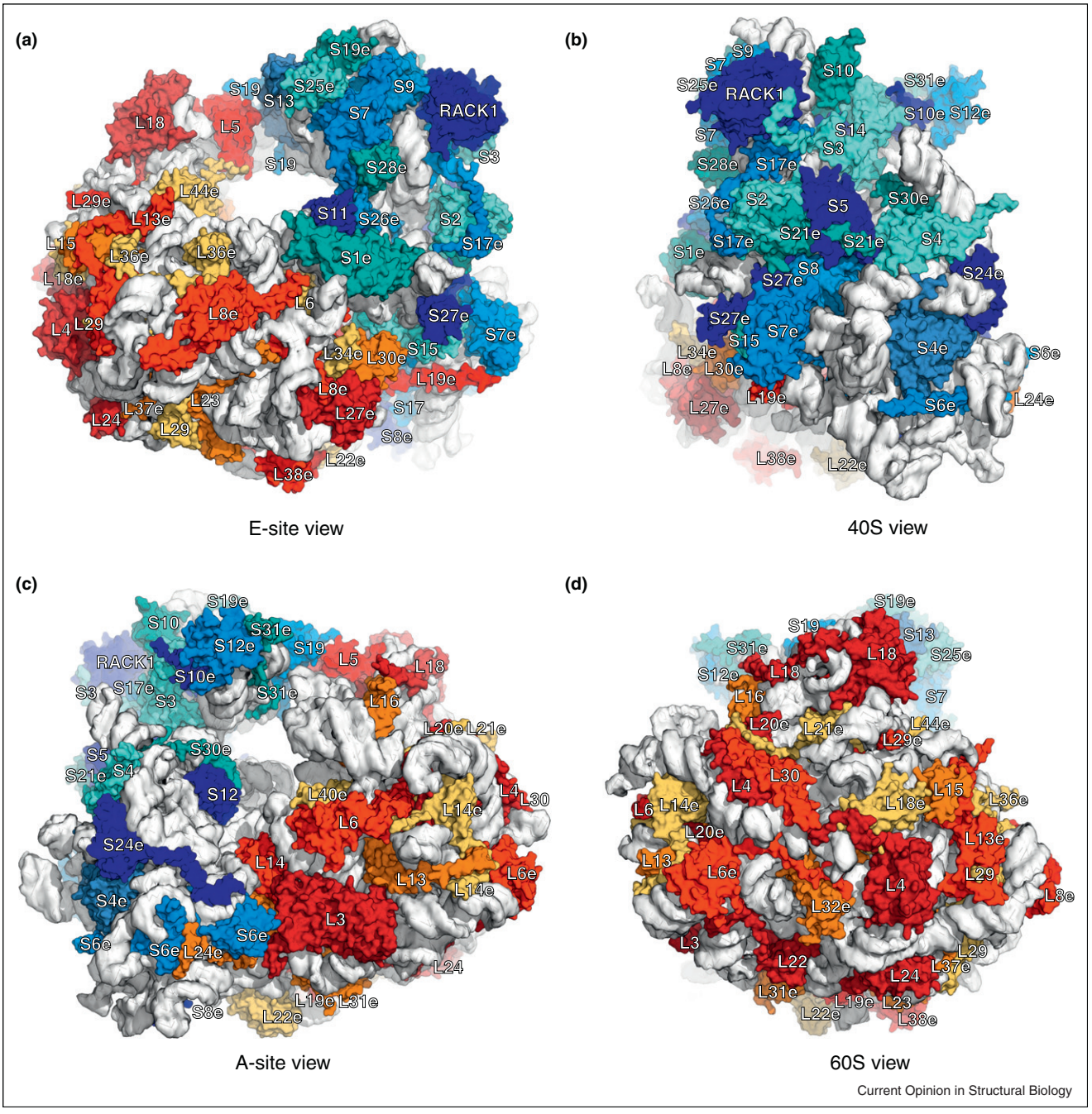
Ribosome complexes and the Stm1 protein

Biochemical studies have shown that stress related protein Stm1 can associate with 80S ribosomes and inhibit translation, but the nature and role of these interactions were unknown [42,43]. In the crystal structure of the vacant 80S ribosome we found that Stm1 binds to the head domain of 40S and prevents mRNA access by inserting an alpha-helix through the mRNA entry tunnel. Furthermore, Stm1 is bound inside the mRNA tunnel from the mRNA entry funnel through the P-site where it effectively blocks for binding of tRNA in the A-sites and P-sites in addition to mRNA, thereby preventing formation of any functional ribosome complexes [1••]. The protein then crosses to the 60S subunit between the 5S rRNA and protein L5. By interacting with the ribosome in this way Stm1 prevents subunit dissociation and stabilizes the 80S particle. For preparation of functional ribosome complexes containing mRNA and tRNA ligands, the first challenge will be to remove Stm1 from the ribosome. This can be done either by elaborating upon the existing isolation procedure for Stm1-containing 80S ribosomes, or genetically by deleting it from the yeast genome, which has been shown not to be lethal [44].

Translocation and the rotated states

The 80S crystal structure consists of two ribosomes molecules in the asymmetric unit, termed ribosome A and ribosome B. They differ markedly in the degree of rotation of 40S relative to 60S (4° in A, 9° in B) and the extent of 40S head swiveling (15.5° in A, 10.5° in B) (Figure 3a,b) relative to the un-rotated 70S state [45]. From previous cryo-EM and crystallographic studies [46–48] it is known that the two subunits rotate and swivel relative to each other during protein synthesis to allow translocation of tRNAs and mRNA along the subunit interface. The two conformations that are observed in the 80S crystal structures seem to be related to two states that occur before (molecule A) and immediately after (molecule B) translocation of tRNA and mRNA across the interface of the ribosome as deduced from recent cryo-EM and crystal structures of the ribosome in different states [24•,49•]. The crystal structure of the 80S *S. cerevisiae* ribosome provides a unique possibility for further studies of translocation events involved in the conformational changes that happen during translation.

Figure 2

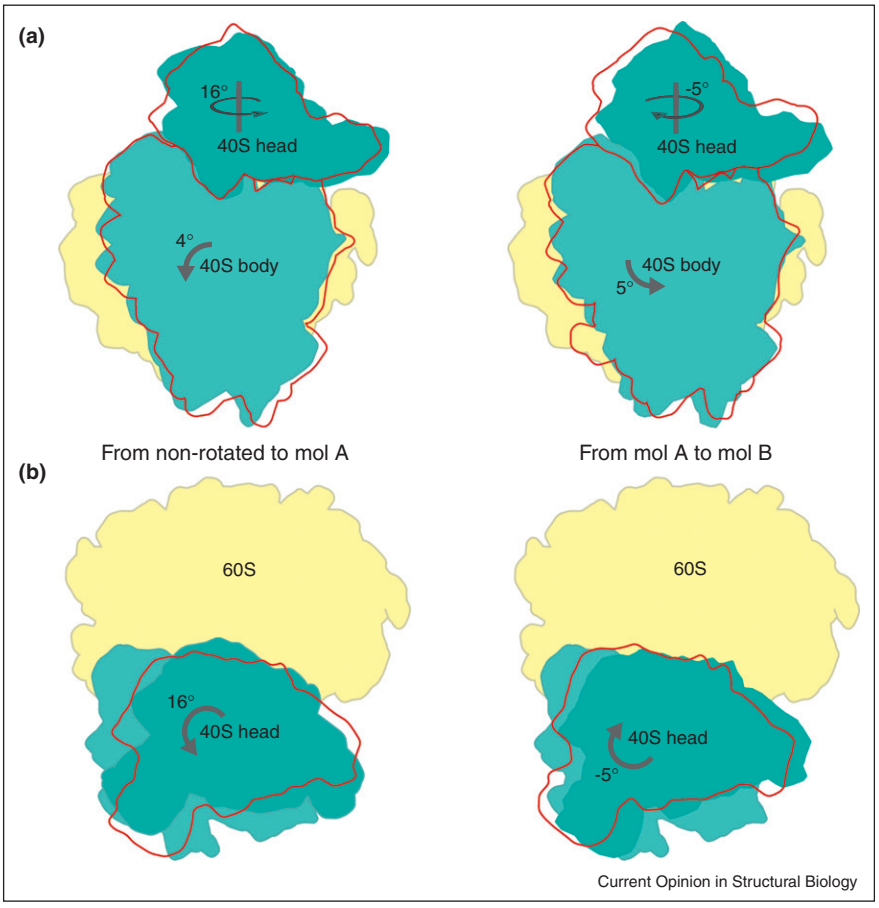


Architecture of the 80S ribosome with ribosomal proteins labeled according to the new nomenclature. ((**a**)–(**d**)) The views are as in Figure 1. Proteins in red, orange and yellow belong to the large subunit whereas proteins in blue, cyan and teal belong to the small subunit. rRNA is represented in white. If a protein is partly buried, the figure might contain labels for each part even though all the ribosomal proteins depicted on the figure occur in only a single copy.

Structural studies of inhibitors of yeast translation

Having an accurate atomic model of the yeast ribosome opens up for structural investigations of the ribosome binding sites for not only translational inhibitors specific for fungi but also for other eukaryotes [50]. These studies can help understand and develop new pharmaceutical compounds against, for example, eukaryotic parasites and fungi.

Figure 3



Rotated states of the eukaryotic 80S ribosome. (**a**, **b**) Schematic representation of the conformational movement from non-rotated to molecule A and from molecule A to molecule B. View from the solvent side of 40S (a) and from the top of the 80S (b). The arrows indicate the trajectory of the motion.

Conclusion

In order to obtain eukaryotic ribosome material suitable for crystallization a radically new approach had to be introduced. This approach, based on very gentle handling of the ribosomes and using glucose starvation as a means to homogenize the ribosome population before purification, leads to the first well-diffracting crystals of yeast 80S ribosomes.

Crystallization and structure determination of the *S. cerevisiae* 80S ribosome has demonstrated a principal similarity across all kingdoms of life in the structural organization of the ribosome subunit interface and other areas responsible for basic ribosome functions involved in protein synthesis. Other areas carry kingdom-specific moieties, bestowing new abilities and means of regulation and control on the particular ribosomes. On the basis of the universally conserved core of the ribosome a new nomenclature for the ribosomal proteins has been introduced. This nomenclature makes it easier to directly compare ribosomes from different species.

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- •• of outstanding interest

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