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Function : “Principal Investigator, Charité, Berlin”

RESEARCH ARTICLE

RIBOSOME

Nucleolar maturation of the human small subunit processome

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The human small subunit processome mediates early maturation of the small ribosomal subunit by coupling RNA folding to subsequent RNA cleavage and processing steps. We report the high-resolution cryo–electron microscopy structures of maturing human small subunit (SSU) processomes at resolutions of 2.7 to 3.9 angstroms. These structures reveal the molecular mechanisms that enable crucial progressions during SSU processome maturation. RNA folding states within these particles are communicated to and coordinated with key enzymes that drive irreversible steps such as targeted exosome-mediated RNA degradation, protein-guided site-specific endonucleolytic RNA cleavage, and tightly controlled RNA unwinding. These conserved mechanisms highlight the SSU processome's impressive structural plasticity, which endows this 4.5-megadalton nucleolar assembly with the distinctive ability to mature the small ribosomal subunit from within.

The biogenesis of human ribosomes requires more than 200 ribosome assembly factors, which facilitate transcription, RNA folding, and processing and modification of preribosomal RNA as well as the incorporation of 80 ribosomal proteins to form the small (40*S*) and large (60*S*) ribosomal subunits (*1*). This process starts in the human nucleolus, a tripartite biomolecular condensate formed through multivalent protein–nucleic acid interactions (*2*). Here, a 47*S* precursor transcript is formed, which contains coding segments for the small ribosomal subunit (18*S*) and large ribosomal subunit (28*S* and 5.8*S*) as well as key regulatory regions including the 5′ external transcribed spacer (5′ ETS) (*3*). The 5′ ETS plays a pivotal role during the initial stages of ribosome assembly, where it coordinates the recruitment of assembly factors and provides a structural blueprint for early assembly intermediates of the small subunit (SSU) (*4*). In contrast to lower eukaryotes such as *Saccharomyces cerevisiae*, human preribosomal RNA contains drastically larger spacer regions, so that the 5′ ETS is almost twice the size of the entire 18*S* rRNA. The trend toward larger 5′ ETS segments in mammals suggests that in addition to an immediate function in ribosome assembly, these 5′ ETS segments may have acquired additional macroscale functions, such as participation in biomolecular condensate formation in mammalian nucleoli (*5*).

After the transcription of preribosomal RNA, the first stable nucleolar ribosome assembly intermediate is the SSU processome, a giant precursor of the small ribosomal subunit (*6*). In yeast, the temporal order in which more than 50 ribosome assembly factors form the SSU processome has been elucidated (*7*, *8*). Cryo–electron microscopy (cryo-EM) structures of yeast SSU processomes have been determined before cleavage at site A1, which separates the 5′ ETS and 18*S* (*9*–*12*), and during transition states toward a pre-40*S* particle that involve the RNA degradation machinery, the RNA exosome (*13*–*15*). However, despite these advances, limited resolution near 4 Å has so far only allowed a partial interpretation of these molecular snapshots. As a result, the molecular logic that drives SSU processome maturation at a mechanistic level has remained obscure, and the mechanisms and control of key enzymes, including the yeast RNA helicase Dhr1 (DHX37 in humans) within the SSU processome, or substrate recognition by the RNA exosome are still poorly understood.

Insights into human small ribosomal subunit assembly are even more modest and currently limited to late nuclear and cytoplasmic stages (*16*, *17*), as the human nucleolus exhibits distinctive biochemical properties in the form of a biomolecular condensate whose contents have thus far proved impenetrable for biochemical and structural studies. An inability to purify intact human nucleolar ribosome assembly intermediates such as the human SSU processome has therefore limited our knowledge of their composition to known homologs of yeast ribosome assembly factors and large-scale genetic screens (*18*–*20*). The lack of structural information has further hindered our understanding of the roles and functions of about one-quarter of human ribosome assembly factors, precluding a mechanistic understanding of severe human diseases in which mutations of ribosomal proteins result in dysfunctional ribosome assembly at the level of the SSU processome (*21*).

In this study, we developed a human genome editing platform and biochemical methodologies to permeabilize the biomolecular condensate of the human nucleolus and determined the high-resolution cryo-EM structures of maturing human SSU processomes at resolutions of 2.7 to 3.9 Å. These structures reveal the exquisite levels of control that the SSU processome provides by enforcing a strict chronology and molecular choreography for irreversible steps of ribosome assembly, such as RNA degradation, site-specific RNA cleavage at site A1, and RNA unwinding.

Cryo-EM structures of maturing human SSU processomes

A critical hurdle for the study of endogenous human ribosome assembly intermediates is the ability to access all endogenous components. To overcome this challenge, we developed a human genome editing platform that couples the genome editing activity of the CRISPR RNA-guided endonuclease Cas9 with cell surface display to rapidly isolate biallelically edited cells by flow cytometry. In this platform, two DNA repair templates are used, one for each allele of the same gene. While both DNA repair templates code for an identical C-terminal tag of the gene of interest, they contain two different cell surface epitopes, which are produced as separate polypeptides. Thus, cells in which two alleles of a given gene have been repaired with two different DNA repair templates can be selected using flow cytometry (fig. S1, A to E). Using this editing tool, the endogenous human ribosome assembly factor NOC4L, which is present in human SSU processomes, was biallelically tagged and used as bait for subsequent purifications (fig. S1, F to H). To permeabilize the human nucleolus, we developed a sequential extraction procedure in which human nucleolar assembly intermediates containing tagged NOC4L are released in the last step of the extractions, thereby providing the starting material for subsequent nanobody-based purification and structural analysis by cryo-EM. The purified particles include pre-rRNA species that are uncut (30*S* and 26*S*) or cut (21*S*) at site A1 (fig. S2, B and C). A total of 84,904 cryo–electron micrographs were collected on a Titan Krios microscope equipped with a K3 detector, yielding 9.3 million autopicked particles (fig. S3). Extensive three-dimensional (3D) classification revealed three classes that

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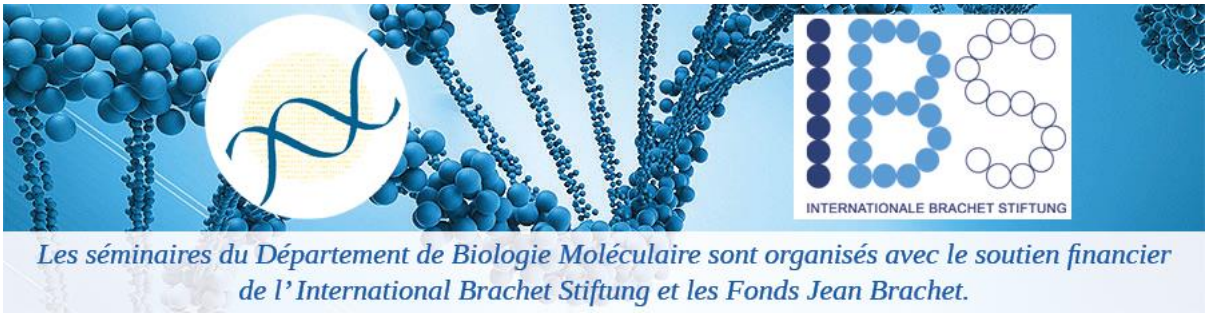

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