

Late Stages of Ribosome Assembly Revealed by Cryo-Electron Microscopy

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Four decades after early assembly studies demonstrated that ribosome assembly is a controlled process [1], our understanding of ribosome assembly is still in its infancy. Just as structure determination has been so important to understanding ribosome function [2] so too will it be critical to sorting out the assembly process [3]. However, the fast pace of assembly of ribosomes in bacteria prevents accumulation of assembly intermediates and makes any attempt of structural characterization of the assembly process *in vivo* unfeasible. Here, we show an experimental system that causes the accumulation of specific ribosomal subunit intermediates *in vivo* to allow their structural characterization. The obtained structures provide a three-dimensional view of the ribosome assembly process.

We have made use of a viable deletion in the *yjeQ* gene in *E. coli*, a putative ribosome assembly factor [4], to isolate and structurally characterize immature 30S subunits assembled *in vivo*. We showed by Northern blot analysis that the 16S rRNA of these small subunits was incompletely processed. Using isotope tagging proteomic methodology, we demonstrated that they also had an incomplete protein complement. Cryo-electron microscopy reconstructions revealed that the presence of precursor sequences in the rRNA induces a severe distortion in the 3' minor domain of the subunit involved in the decoding of mRNA and interaction with the large ribosome subunit (FIG. 1) [5]. This cryo-EM reconstruction constitutes the first structure of an *in vivo* assembled immature ribosomal subunit.

This study establishes that the YjeQ protein is a *bona fide* ribosome assembly factor and provides new guiding principles of the ribosomal assembly process, such as that RNA processing events induce local conformations directing the structure toward the mature assembly. We also concluded that rRNA processing, folding and the entry of tertiary r-proteins are interdependent events in the late stages of 30S subunit assembly. These findings demonstrate how studies of emerging assembly factors in ribosome biogenesis can help to elucidate the path of subunit assembly *in vivo*.

References

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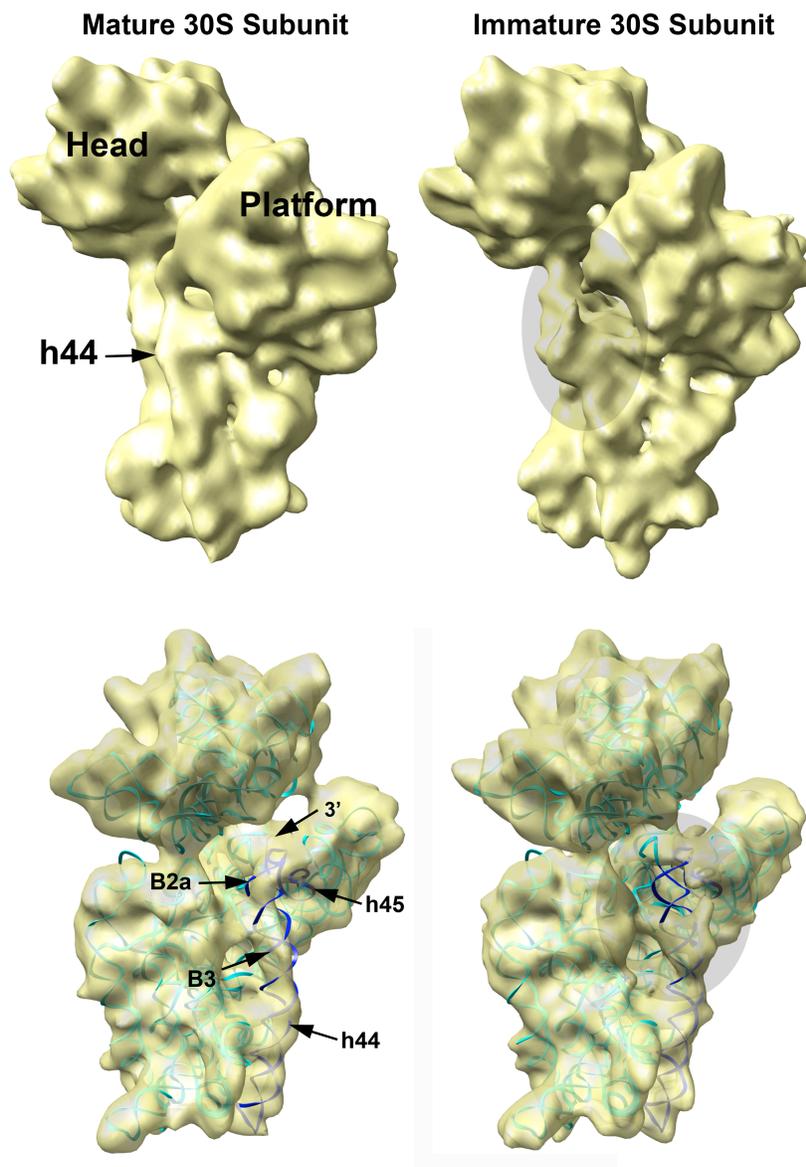


FIG. 1. 3D Reconstruction of the immature 30S ribosomal subunits purified from $\Delta yjeQ$ *E.coli* cells. Platform-view of the cryo-EM maps of the immature (upper right panel) and mature (upper left panel) 30S subunits. Main landmarks of the 30S subunit are indicated. In the panels below, the X-ray structure of the wild type 30S subunit, shown as a ribbon representation, was fitted into the cryo-EM maps of both the mature (left) and immature (right) 30S subunits to illustrate their divergences. In the X-ray structure the rRNA is shown in cyan except helix 44 and 45 that are colored in dark blue. The r-proteins are not shown. Main landmarks of the 30S subunit indicated include helix 44 (h44), helix 45 (h45), the intersubunit bridges B2a (B2a) and B3 (B3) important for the interaction with the 50S subunit and the 3' terminus of the 16S rRNA molecule (3'). The shadowed ovals in the right panels show the area in the helix 44 and decoding center distorted in the EM map of the immature 30S subunit.