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Heterogeneity in the pre-40S ribosomal subunit reveals two distinct regions of variation at functionally important structural elements.

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Ribosomes represent a significant portion of the dry mass of all cells and actively dividing cells assemble about 2,000 ribosomes every minute [1]. This represents a massive investment by the cell in a complex RNA:protein assembly that will go on to translate mRNAs into protein, an essential process for all of life. Much is known about how a ribosome faithfully performs protein synthesis; less is understood about how they are faithfully assembled. Further, several high profile diseases like Diamond Blackfan Anemia (DBA), 5q- syndrome, and isolated congenital asplenia derive from defects in the erythropoetic lineage caused by haploinsufficiency of some ribosomal proteins (r-proteins) [2].

Ribosomes are composed of the small and large subunit, which are assembled independently in the nucleolus and nucleus before being joined after export to the cytoplasm for initiation of mRNA translation into protein (Figure 1). In eukaryotes, over 200 assembly factors (AFs) direct this maturation, chaperoning the structural transitions to ensure quality control as the subunits move through from the nucleolus to the cytoplasm [3].

The small subunit, 40S in yeast, of the ribosome directs mRNA decoding. In particular, the beak, (a structural element of the head), and the platform form the entrance and exit of the mRNA binding channel (Figure 2). As such, correct assembly of these elements is of particular importance and seven AFs have been associated specifically with beak and are critical for blocking premature translation initiation by the pre-40S ribosome [4].

Three AFs at the beak, Enp1/Ltv1/Rps3, have been shown to be critical in holding the beak in a position that sterically blocks subunit assembly [5]. Ltv1 release facilitates Rps3 repositioning that ultimately allows beak repositioning and subunit joining. Movement of the beak is related to nuclease events at the platform that finalize 18S rRNA maturation. How this happens remains undefined. We seek to use 3DEM to dissect the relationship between the beak and the platform.

Recent advances in 3DEM imaging using a direct electron detector and computational image processing using 3D multivariate statistical analysis [6] have facilitated increasingly high-resolution (<4 Å) structure determination of well-ordered complexes. The pre-40S ribosome is a heterogeneous specimen, which inherently limits the achievable resolution; nonetheless these same technical advances also allow precise analysis of structural heterogeneity. We have imaged pre-40 S ribosomes on a direct detector using a Titan Krios (FEI, Hillsboro, OR) microscope (Figure 3). Our analysis of the resulting 3D pre-40S ribosome structures points to heterogeneity at the beak and the platform (Figure 3, inset), suggesting a structural and functional link between these two regions. Here, we present a method for localized 3D classification of heterogeneous specimens that vary at more than one spatially-distinct position on the pre-40S ribosome.

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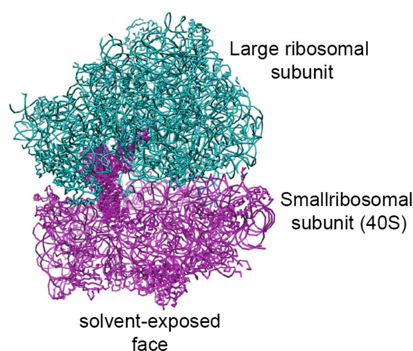

Figure 1. Model of the mature 70S ribosome [6].

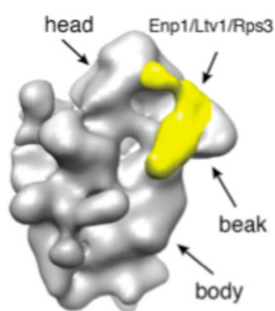

Figure 2. Low-resolution model of the pre-40S ribosome with Enp1/Ltv1/Rps3 and showing the solvent exposed face [5].

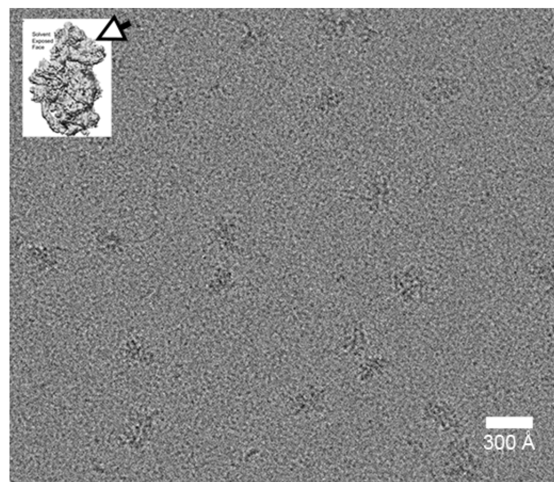

Figure 3. Representative micrograph showing the high quality of the pre-40S ribosomes on FSU's DE20 detector (Direct Electron, San Diego, CA) and lower resolution of the map due to heterogeneity at the beak on the solvent exposed face (arrow).