# Structural investigation of the 40S hnRNP particles

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Heterogeneous nuclear ribonucleoproteins (hnRNPs) constitute a prominent family of RNA-binding proteins abundant within the nucleus. They play pivotal roles in various aspects of nucleic acid metabolism, encompassing mRNA stabilization, alternative splicing, transcriptional and translational regulation, RNA export, and degradation [1]. Early investigations in the 1970s revealed that upon lysing nuclei without RNase inhibitors, a significant portion of pre-mRNA formed a distinct protein-RNA complex, sedimenting at 40S [2]. Notably, the core constituents of this complex were identified as hnRNP C1/C2, hnRNP A1/B2, and hnRNP A2/B1. This observation brought up the intriguing proposition that the 40S hnRNP particle might serve as an analogue to the DNA nucleosome [3].
Our objective is to describe the biogenesis of the 40S particle, provide a structural description of the 40S particle using cryo-electron microscopy (cryo-EM) and visualize it in its native context. We have generated TRex-293 cell lines expressing FLAG-tagged hnRNPC1/C2 proteins, isolated the 40S particles and analyzed them by negative staining EM. Furthermore, to investigate the ribonucleosome within intact cells, we have immunostained the key ribonucleosome components and generated thin lamellae using cryo-focused ion beam scanning electron microscopy (cryo-FIB/SEM). Using cryo-electron tomography we have acquired data from FIB-milled lamellae.

**Keywords:**

40S particles, immunostaining, cryo-FIB, cryo-ET

**Reference:**

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