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Structural Elucidation of Archaeal Box C/D sRNP by Cryo-electron Microscopy

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Ribosome biogenesis is one of the most energetically costly processes that occur in cells. One of the most important processing steps of *in vivo* ribosome synthesis includes the chemical modifications of the pre-ribosomal RNA (pre-rRNA). With ribosomes being one of the most important cellular machines, disrupting these modification steps in the biogenesis of ribosomes is detrimental to the viability of organisms. Therefore, understanding how cells carry out the process of producing a functional ribosome is a biologically relevant problem.

2'-*O*-methylation, which is the addition of a methyl group to the 2' hydroxyl group of the ribose ring of a nucleotide, is one of the two major types of chemical modifications in eukaryotic and archaeal pre-rRNAs. This chemical modification is catalyzed by a ribonucleoprotein (RNP) complex called box C/D small nucleolar RNPs (snoRNPs) in Eukarya or box C/D small RNPs (sRNPs) in Archaea. The core components of archaeal box C/D sRNP have been studied extensively. A functional core archaeal box C/D sRNP contains a box C/D sRNA which allows three core proteins, L7Ae, Nop5 and fibrillarin to assemble onto the sRNA. The biochemical interactions within a core archaeal box C/D sRNP have been determined. However, little was known about its enzymatic mechanism.

Recently, Bleichert *et al.*, for the first time, determined the structure of a complete box C/D sRNP from *Methanococcus jannaschii* using electron microscopy (EM) with the negative staining technique [1]. With the docking of known crystal structures of all the core proteins into the EM volume and the support of biochemical evidence, the structural model suggests the archaeal sRNP to be a dimeric sRNP (di-sRNP), containing two copies of the sRNA and four copies of each of the core proteins. This model was later challenged by an X-ray crystal structure of the sRNP from *Sulfolobus solfataricus* by Lin *et al* [2]. The crystal structure argues for a monomeric sRNP model (mono-sRNP), which contains one copy of the sRNA and two copies of each of the core proteins. A subsequent report by Bower-Phipps *et al.* demonstrated that the mono-sRNP structure was induced by the presence of the synthetic sRNA that did not contain an important internal loop while the di-sRNP structure was reconstituted with a natural sRNA that contains the important loop [3]. This argues that the di-sRNP model is more physiologically relevant structure.

The determination of a complete archaeal box C/D sRNP is a big step forward to understanding the enzymatic mechanism of the complex. Unfortunately, because of the low resolution of the negatively stained EM reconstructions of the sRNP complexes by Bleichert *et al.* and Bower-Phipps *et al.*, the location and the orientation of the sRNA in the structures cannot be determined. Since the sRNA directs the specificity of the enzymatic reaction by base-pairing with its RNA substrate, information about the location and orientation of the sRNA within the structure is important.

Therefore, I decided to use cryo-EM as a structural method to resolve the structure of a box C/D sRNP from *Methanococcus janaschii*. Cryo-EM is a high-resolution structural technique that has been successfully employed to study the structures of numerous relatively large macromolecules. Therefore, cryo-EM should allow the structural reconstruction of the 366kD box C/D sRNP and subsequently the visualization of the sRNA in the complex.

Indeed, I have successfully prepared cryo-specimen of the box C/D sRNP that allows the visualization of the sRNP particles in vitreous ice (Figure 1). I have collected 260 micrographs that are of similar quality and I am currently in the process of image processing to produce 2D class averages. Once I have obtained class averages and the quality of the specimen deemed acceptable, I will continue to begin 3D reconstruction of the sRNP complex. The 3D cryo-EM reconstruction of the box C/D sRNP will be important for understanding its enzymatic mechanism.

References:

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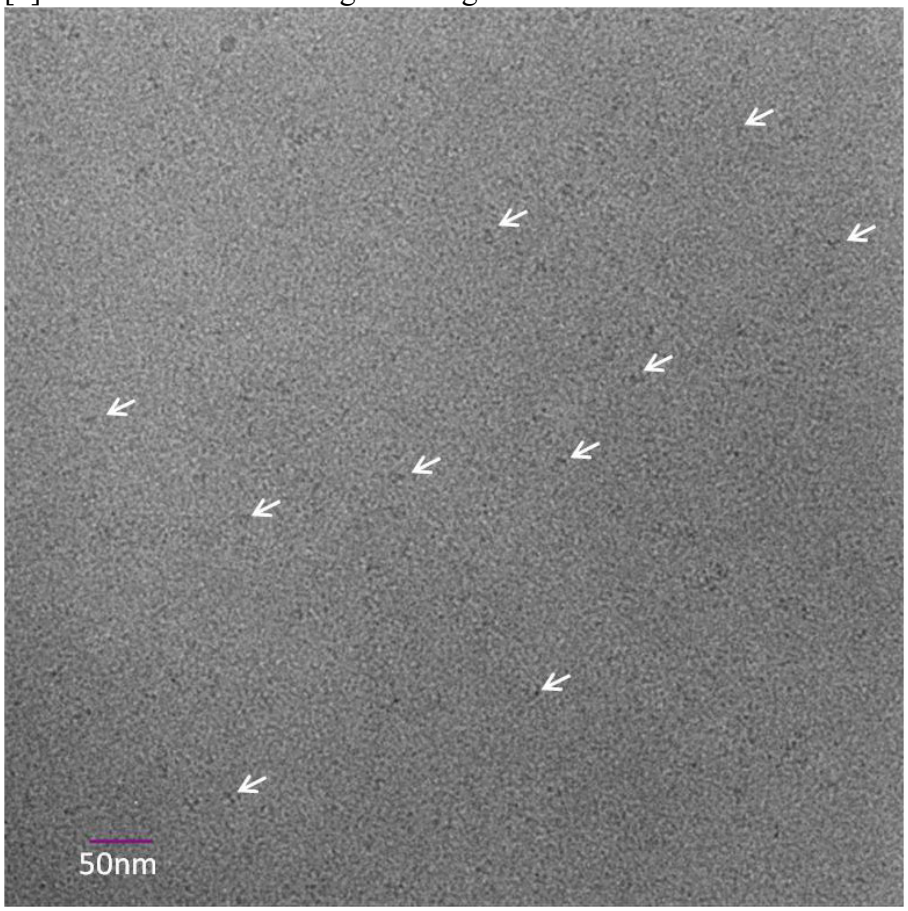

Figure 1. Electron micrograph of box C/D sRNP complex from *Methanococcus jannaschii* in vitreous ice. White arrows indicate some of the representative particles.

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