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Cryo-Electron Microscopic Study of the Enzymatic Mechanism of the RNA 2'-O-Methyltransferase Box C/D sRNP

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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'-*0*-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.

In 2009, Bleichert *et al.* determined the first structure of a complete box C/D sRNP from *Methanococcus jannaschii* using negative-stain electron microscopy (EM) [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.* [3] 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. This argues that the di-sRNP model is the more physiologically relevant structure.

The structure determination of a complete archaeal box C/D sRNP is a big step forward in our understanding. Unfortunately, because of artifacts of the stain, 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, we decided to use cryo-EM to determine the structure of a box C/D sRNP from *Methanococcus janaschii.* Using this technique, we collected ~200,000 single-particle images. After reference-free 2D alignment and classification by IMAGIC [4] to remove contaminating particles, we obtained a 3D reconstruction at 16-Å resolution using projection-matching refinement by EMAN2 [5]. Interestingly, a new electron density emerges from this map as compared to the previous negative-staining reconstruction. Since all the protein components are accounted for by the other electron densities, this new electron density is very likely the sRNA component of the sRNP. We are currently using biotinylated oligonucleotides complementary to different regions of the sRNA together with streptavidin to label the sRNA in an attempt to verify the identity of this new electron density.

In addition, to fully understand the enzymatic mechanism of the box C/D sRNP, it is essential to study the influence of its RNA substrate on its structure. On this front, we have collected ~20,000 single-particle images of *Sulfolobus solfataricus* box C/D sRNP in the presence of an RNA substrate. 2D classification and 3D projection-matching reconstruction of the images respectively by IMAGIC [4] and EMAN2 [5] reveal that the RNA substrate induces dramatic conformational changes to the structure of the box C/D sRNP complex. From the 2D class averages, there appears to be structural heterogeneity in the sample. To distinguish all the conformations present in the images, we are currently collecting random conical tilt data. Furthermore, to identify which components in the sRNP contribute to the changes in the structure, we are using antibody-labeling techniques to pinpoint the locations of the proteins in the sRNP.

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