

Molecular insights into the biogenesis of box H/ACA snoRNPs

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Box H/ACA snoRNPs are protein-RNA complexes composed of NOP10, GAR1, and the catalytically active DKC1. The latter is known to interact with small non-coding RNAs containing the Box H/ACA motif. Guided by the RNA, the complex finds its target RNAs and converts the nuclear base uridine to pseudouridine, resulting in increased stability of the target RNA due to an additional hydrogen bond. Pseudouridines are vital for various cellular functions, such as protein translation, as they undergo frequent post-transcriptional modifications in ribosomal RNAs.

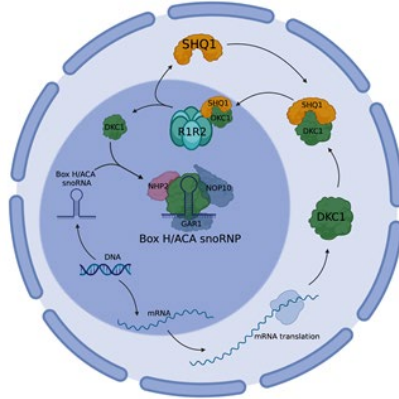
However, it is still poorly understood how the Box H/ACA snoRNP complexes and their individual protein precursors are assembled. SHQ1, a chaperone-like protein, is proposed to prevent DKC1 from binding to nonspecific ribonucleotides during its assembly in the cytosol and subsequently guide the complex to the nucleus, where it interacts with the AAA-ATPase complex RuvBL1/2. These ATPases are known to interact with PIH1D1 and RPAP3 proteins to form the R2TP complex, which is described to play a role in the assembly of multiple macromolecular complexes. Thus, the R2TP complex could be responsible for sequestering SHQ1 from DKC1, allowing DKC1 to interact with Box H/ACA snoRNAs and ultimately assemble the mature snoRNP.

The aim of this work is to elucidate the maturation of the Box H/ACA snoRNP by characterizing its precursor complexes composed of SHQ1, DKC1, and RuvBL1/2. This was achieved using size exclusion chromatography, dynamic light scattering, differential scanning fluorimetry, surface plasmon resonance, mass photometry, cross-linking mass spectrometry, and cryo-EM.

We were able to confirm the interaction between RuvBL1/2 and DKC1 or SHQ1. Additionally, we purified and stabilized the SHQ1:RuvBL1/2 complex and identified multiple complex species. Revealing the interaction site between the AAA ATPases and SHQ1 helped to reconstruct an initial 3D model of the complex's structure processing the movies collected in Cryo-EM experiments.

The biochemical and biophysical analysis of the interaction between R1R2 and SHQ1 allowed us to identify an additional electron density below the unique and regulatory domain II of the hexameric R1R2 complexes. However, our Cryo-EM reconstruction shows high flexibility for the complex, which is why mild cross-linking using GraFix might be a valid option to determine a high resolution 3D structure.

Graphic:



Keywords:

snoRNPs, PAQosome, Cryo-EM, SPR, Chaperones