RNA Modification

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Structural Basis for Regulation of Ribosomal RNA 2′-O-Methylation**

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2′-O-methylation · NMR spectroscopy · ribosome biogenesis · RNA modification · small-angle neutron scattering

Ribosomes, among the most complex macromolecular machineries in cells, catalyze peptide bond formation and perform cellular protein synthesis. In eukaryotes, they consist of four RNAs and about 80 proteins, that is, they are ribonucleoproteins (RNPs), and numerous additional proteins, including initiation, elongation, and recycling factors are important to ensure proper function.[1] Regulation of ribosome biogenesis is an essential prerequisite for cellular homeostasis. The intricate nature and the large number of individual components that assemble along the ribosomal RNA (rRNA) scaffold render orchestrated ribosome biogenesis a difficult task.

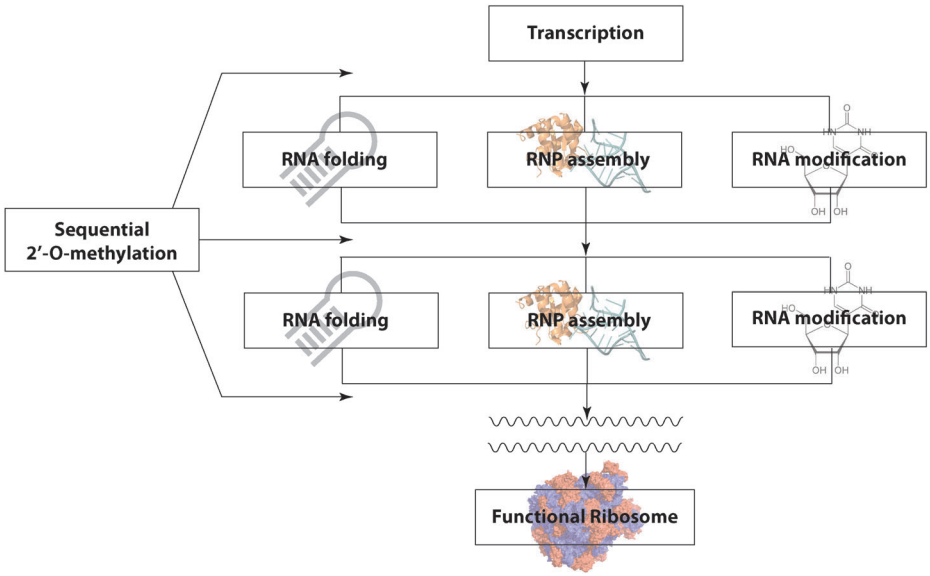

Figure 1. From transcription to incorporation into the functional ribosome, rRNA undergoes a multitude of folding and assembly steps. Each of these may be possibly influenced or altered by the orchestrated introduction of 2′-O-methylation.

After transcription, ribosomal RNA (rRNA) undergoes extensive maturation. Maturation of rRNA involves cleavage and trimming to the correct length and termini.[2] It is not common textbook knowledge that rRNAs are also purposefully modified posttranscriptionally (Figure 1). The most common among roughly 100 different modifications are methylations and pseudouridinylations,[3] which are—together with recently identified DNA modifications—assumed to constitute an important additional layer of cellular information transfer.

Eukaryotic ribosome biogenesis requires RNA-guided RNA modification as an essential mechanism in the maturation of rRNA. For example, in eukaryotes the *S*-adenosylmethionine (SAM)-dependent methylation of the 2′-hydroxy moiety of RNA nucleotides is catalyzed by the box C/D RNP complex, which serves as a molecular platform for recognition and catalysis of the chemical modification.[4] The recruited small nucleolar RNA (snoRNA) provides this complex with two seemingly independent guide sequences (D and D′) that target their substrate RNA by canonical Watson–Crick base pairing. In addition to the RNA, three proteins constitute the archaeal core of this enzyme: L7Ae, Nop5, and the methyltransferase fibrillarin.

Despite a number of studies on structural aspects of this complex,[5] both the precise orientation of the subunits and the subunit stoichiometry have remained elusive up to now. In fact, the reported data have been controversial, depending on method of analysis, the type of guide RNA used, and the absence or presence of substrate RNA.

To tackle these questions, the research group led by Teresa Carlomagno elegantly combined two structural methods: NMR spectroscopy and small-angle X-ray and neutron scattering (SAXS and SANS).[6] With the atomic resolution

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information obtained by various NMR techniques, and the size and shape restraints from SAXS and SANS, they put forward a model for an apo as well as a holo form of the near-native *Pyrococcus furiosus* box C/D RNP complex (Figure 2).[7]

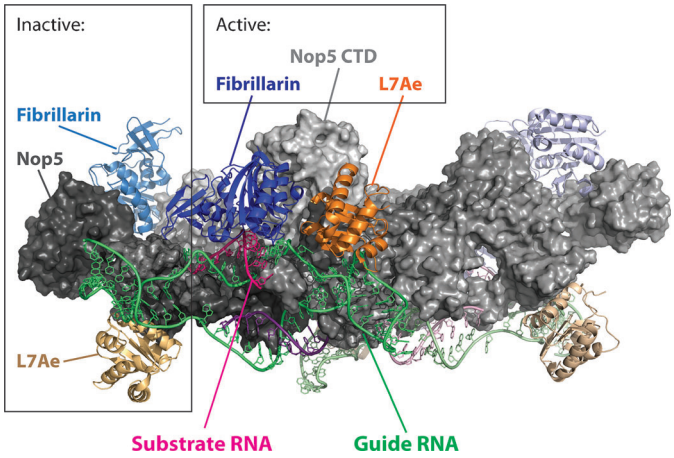

Figure 2. Structure of the holo complex of the Box C/D RNP. The active subunits are engaged in RNA–protein interactions, whereas the inactive group of proteins is located far from its substrate RNA.

The expected size of this complex ranges between 250 and 500 kDa (depending on the assembly model); the reconstitution of such a complex imposes significant technological challenges. Using only very minor alterations to the RNP constituent proteins and RNA, the complex in this study was successfully reconstituted from recombinantly expressed proteins around the sR26 RNA. An initial characterization using size-exclusion chromatography (SEC), gel electrophoresis, and SAXS provided first insights into the composition of the complex: the detected molecular weight of 390 kDa and the radius of gyration support the notion that this complex is indeed a di-RNP, consisting of four protein trimers arranged around two sRNAs.

However, there is a significant gap between the "small-scale" information obtained also by other crystal structures, and the architecture of an asymmetric complex from 14 monomers (protein and RNA). To restrain the positions of each of these within the complex, a combination of two NMR methods gave crucial insight: First, $^{13}CH_3$-Ile, $^{13}CH_3$-Leu, and $^{13}CH_3$-Val were introduced into each of the proteins individually, and the effects on the induced chemical shifts between different subcomplexes were compared.[8]

This information was complemented with analysis of complexes that contain engineered tetramethylpiperidinoxyl (TEMPO) spin labels to gain long-range information from paramagnetic relaxation enhancements (PRE).[9] As an alternative to SAXS, which provides shape restraints of the total complex analyzed, SANS can yield this kind of information on subunits within the complex if these are selectively deuterated. In the present study, these experiments gave further restraints on the shape as well as the stoichiometry of each of the individual subunits. This information was then used in structure calculations to generate a model structure at—with respect to the size of the complex—a quite impressive resolution of 4.8 Å (Figure 2).

This model structure integrated several previous isolated findings for the first time. Previously, for example, it was known that Nop5 adopts a coiled-coil structure, and it was postulated that this structure provides the platform for interaction of all other components.[5a] The Carlomagno group shows that there is no strict assignment of one set of proteins for one guide sequence, but instead the sets collaborate to position the methyltransferases for specific targeting. The way fibrillarins are "sandwiched" between the C-terminal domain of Nop5 and L7Ae is structurally only possible in this tetrameric complex arrangement.

What are the functional implications of this detailed structural model? Surprisingly, in the apo state, none of the four active methyltransferase centers are positioned to reach the substrate RNA. Additionally, the structure suggests that only two diagonally opposing fibrillarins can perform methylations at the same time. This insight from structural studies was confirmed by a titration of substrate RNA, upon which a major conformational rearrangement of the complex results in contacts between two fibrillarin molecules and the substrate RNA. These results show that only two of the four substrates (either both D or both D′ RNAs) within one holo complex can be methylated at the same time. Since the D and D′ guide sequences are asymmetric a question arises: do the modifications occur in a sequential manner?

By following resonances of formation of ^{13}C-methylated free product RNA (using ^{13}C-labeled SAM as a methyl donor), it became apparent that partial methylation of D′ occurs indeed already in the absence of D substrate RNA. By contrast, turnover of D is possible only in the presence of D′, which in turn shows a significantly enhanced methylation in the heterosubstrate complex. The authors conclude that D′ methylation presumably has to precede D methylation. This strictly sequential modification offers an interesting way for regulation of this type of modification, and it remains to be seen whether this mechanistic finding can be functionally linked to the maturation of rRNA. It can be speculated that sequential methylation not only governs the order of folding of structural elements within the rRNA, but also serves as a quality control checkpoint. It will also be of interest to see how the dynamic structure of the holo complex follows the sequential modification steps.

In summary, the work by the Carlomagno group is a remarkable example how methods that interrogate different, yet overlapping structural size regimen can be integrated to gain high-resolution information of large macromolecular complexes. It is also one of the first to show—with backup from structural insights—how the regulation of RNA modification can be achieved.

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