Review of: "Ribosomal RNA 2’-O-methylations regulate translation by impacting ribosome dynamics"

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During ribosome biogenesis, the ribosomal (r)RNA is subjected to various chemical modifications at distinct nucleotides. 2'-O-methylation of the ribose can take place on all types of nucleotides, and it is among the most prevalent modifications, which typically occur in conserved and functionally important parts of the ribosome. Data from bacteria and yeast suggest that 2′-O-methylation broadly influence ribosome production and function, and a recent study on human cells has revealed how differential 2’-O-methylation of a single nucleotide in 18S rRNA can alter translation of subsets of mRNAs. However, the full functional impact of differential methylation and individual methylated sites on translation is still not understood.
In Eukaryotes, most 2′-O-methylations are added by the methyltransferase Fibrillarin, which is guided to specific sites by different C/D box small nucleolar (sno)RNAs via base-pairing interactions with their target rRNA. Box C/D snoRNAs assemble with four protein components (three in addition to Fibrillarin) to form mature ribonucleoprotein complexes called box C/D snoRNPs. However, in order to reach the mature state, several dedicated snoRNP assembly factors are needed.
In this study, Khoshnev and colleagues construct a viable strain of budding yeast that has a mutation in the snoRNP assembly factor Bcd1 (Bcd1-D72A), which leads to an overall reduction in the levels of box C/D snoRNPs and a concomitant reduction in the fraction of 2’-O-methylation at many nucleotides in rRNA. The authors use RiboMeth-Seq to quantify 2’-O-methylation levels and find an average reduction to approximately half of wildtype levels upon mutation of Bcd1. Analysis of the differential methylation pattern for each individual modified nucleotide (54 in budding yeast rRNA) reveals that some modifications are essential whereas others are dispensable. The authors then move on to use reporter assays and structural probing to describe various functional and structural differences between wildtype and hypomethylated ribosomes. In summary, the strain with hypomethylated ribosomes display lowered levels and altered function of ribosomes with some functional features increased (e.g. frameshifting) and others decreased (e.g. initiation from IRES element). The associated structural probing indicates that these functional changes are due a switch in rotational status of the ribosome structure and changed recruitment of translation initiation factor eIF1.

This study is carefully conducted and it provides a nice addition to our understanding of the importance of 2’-O-methylation for ribosome biogenesis and function. However, it remains unclear whether 2’-O-methylations, box C/D snoRNP-chaperoning or some other feature in Bcd1-D72A directly causes a change in ribosome dynamics or whether this occurs through defects in ribosome biogenesis (see also below).

Major points:

1. Figure 3A: The effect of G913 PGO reactivity is small and not convincing. While comparison to rpl3 mutants represent appropriate controls in the PGO probing experiment, the authors should include a panel with a primer extension reaction directed towards another unaffected yet modified nucleotide within the 18S rRNA, to fully demonstrate the specificity of their rotation model.
2. p.5, Figure 3G-H: The temperature sensitive mutation of Fibrillarin does not change snoRNA levels, but what about snoRNP levels? The experiment to demonstrate that the effect of lowered box C/D snoRNA levels in the Bcd1-D72A strain comes more from lowered 2'-O-methylations than the chaperone activities of box C/D snoRNAs, would benefit from an experiment with a catalytic mutant of Fibrillarin that can still assemble into snoRNPs.
3. It is unclear whether the effect of mutating Bcd1 (and the consequential lowering of box C/D snoRNA levels and 2'-O-methylations) on ribosome dynamics is directly affecting ribosome function or whether it is a consequence of defects in ribosome biogenesis. This could for example be probed by comparing the protein constituents of purified ribosome from wildtype and Bcd1- D72A cells.
4. The manuscript lacks a citation and discussion of a highly relevant paper from the Lund lab (Jansson et al., NSMB, 2021; PMID: 34759377), where it is shown that differential methylation of the ribosome at 18S:C174 regulates translation of specific mRNAs in human cells.

Minor points:

1. p.4: "Because changes in ribosome number and/or composition can affect the accuracy of protein synthesis and impact the ability of ribosomes to initiate from internal ribosome entry sites (IRESs)". Can changes in ribosome number really affect the accuracy of protein synthesis?
2. Figure 4F-G: The DMS reactivity towards A579 is reportedly significantly lowered in the bcd1-D72A mutant. Although the band intensities are normalizaed to all bands within each lane, it seems that all RT stops are somewhat decreased in the bcd1-D72A mutant, leaving the quantified changes subtle and the conclusion somewhat weak. Equal amounts of SSU have been used in the RT reactions, but this should be verified more carefully by e.g. a Northern blot or additional RT primers targeting other regions.
3. p.5: In the description and interpretation of the data presented in Figure 3C-D, it should be explained more carefully why overexpression of eEF2 is expected to affect the Bcd1-D72A strain less than a wildtype strain.
4. p.5: The hypothesis related to Sordarin treatment (Figure 3E-F) could also be elaborated somewhat. Moreover, it would probably be beneficial to also show the relative changes in doubling time to support the description.