Concerted modification of nucleotides at functional centers of the ribosome revealed by single-molecule RNA modification profiling

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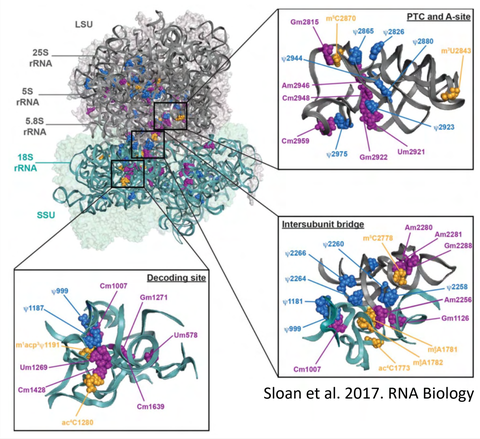
Summary and Conclusions

- A method enabling single-molecule profiling of RNA modifications is developed and reveals heterogeneous classes of modified ribosomes.
- Most rRNA 2´O methylation and pseudouridylation modifications are independent of each other.
- Nucleotides in functional centers of the ribosome are modified in a concerted fashion.
- Loss of function for RNA helicases Dbp3 and Prp43 produce discrete overlapping subpopulations of incompletely modified ribosomes.
- RNA modification profiles are resilient to rapidly changing nutrient conditions and perturbation of translation

1. rRNA is heavily modified at functional sites of the ribosome



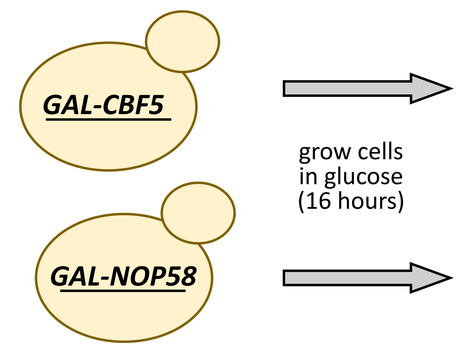
Sloan et al. 2017. RNA Biology

pseudoU
2'Ome
other

- 110 annotated modifications in 18S and 25S rRNA
- Majority of modifications are pseudouridylation and 2'-O-methylation.
- Modifications are guided by box-H/ACA and box-C/D snoRNPs, respectively

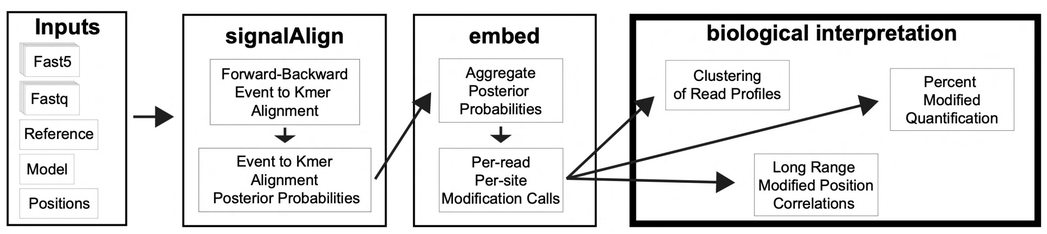
2. A yeast genetic system to turn off rRNA modifications

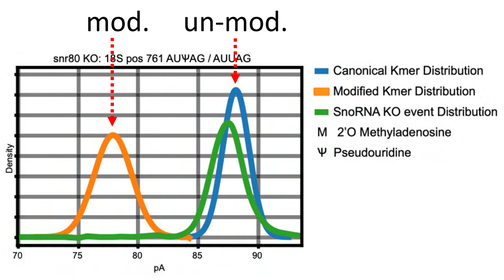
snoRNP components under control of a glucose-repressible promoter



- depletion of pseudouridine modifications.
- depletion of 2'-O-methylation modifications.

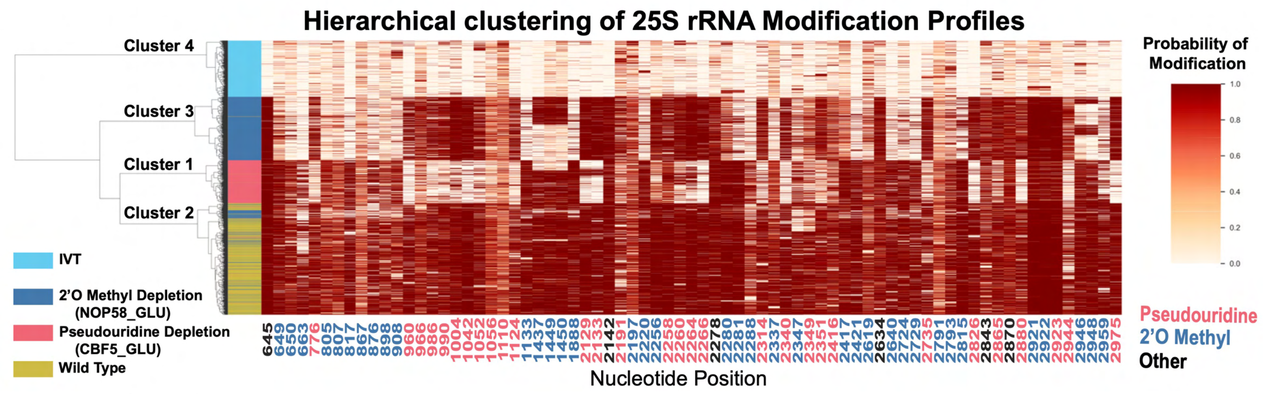
3. Training a model to distinguish modified and unmodified rRNA nucleotides (modification profiling)





- Trained signalAlign on current distributions of WT (modified) vs in vitro transcribed (unmodified) rRNA reads.
- **Model estimates the probability of modification for all 110 modified nucleotides for each individual rRNA molecule.**
- Clustering and correlation analysis provides meaningful biological information about modification regulation.

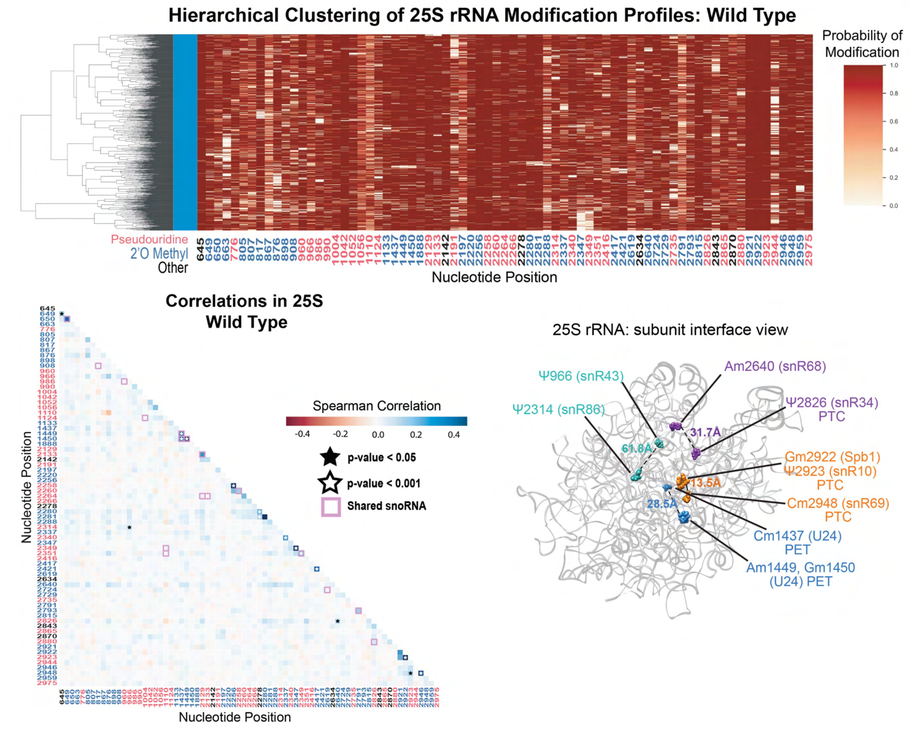
4. rRNA 2'-O-methylation and pseudouridylation are largely independent of each other



rows = single reads
columns = position

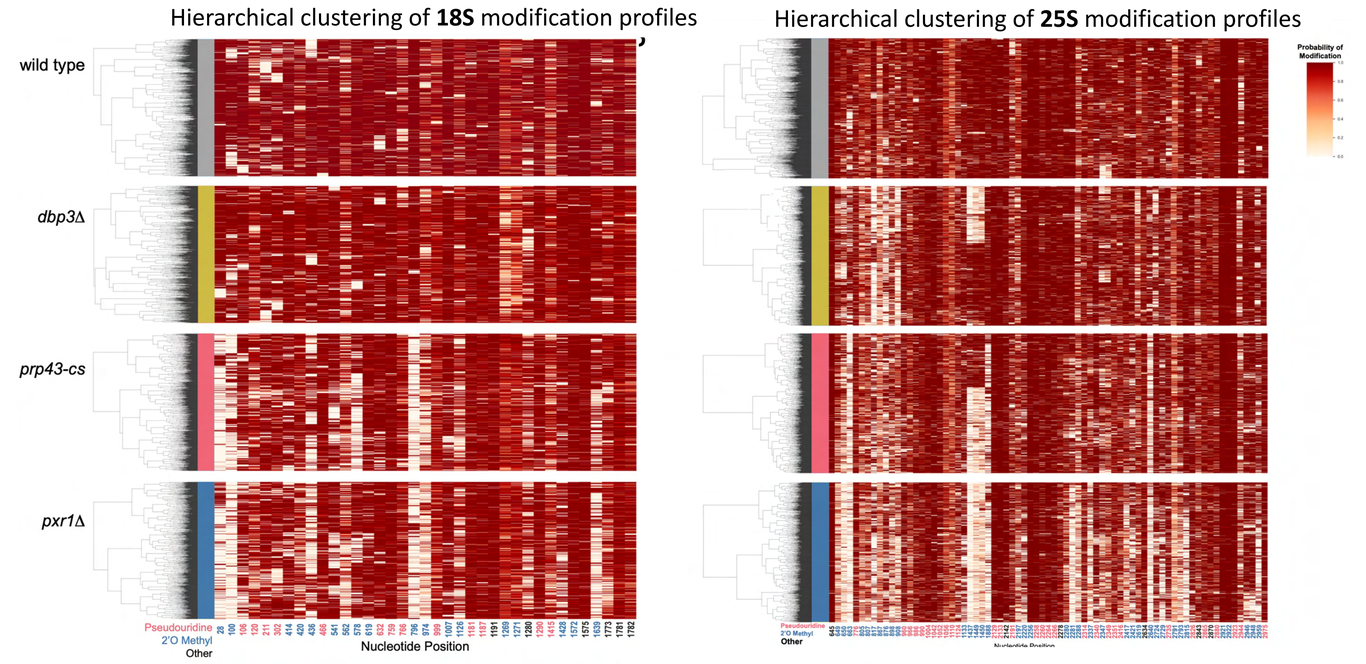
- Modification profile heatmap of clustered reads from WT, IVT, and rRNAs depleted of 2'-O-methyl and pseudouridine modifications (shown: 1000 reads/experiment).

5. Clustering and correlation analysis reveal concerted modifications in WT ribosomes



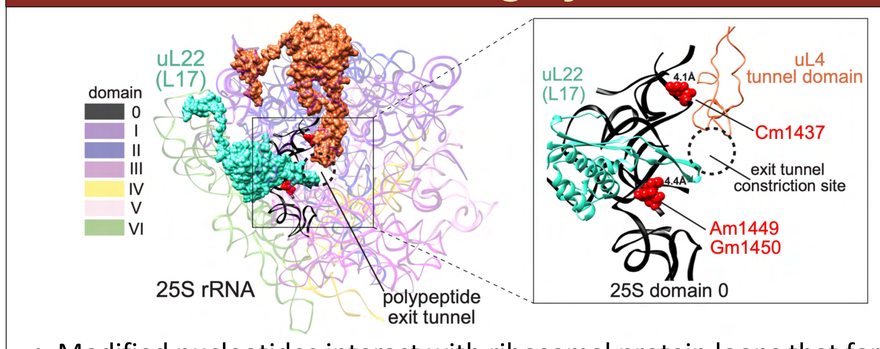
- Correlated positions are located in functional centers of the ribosome such as the peptidyl transferase center (PTC) and the polypeptide exit tunnel (PET)

6. Loss of different RNA helicase-related functions results in distinct subpopulations of differently modified rRNA molecules



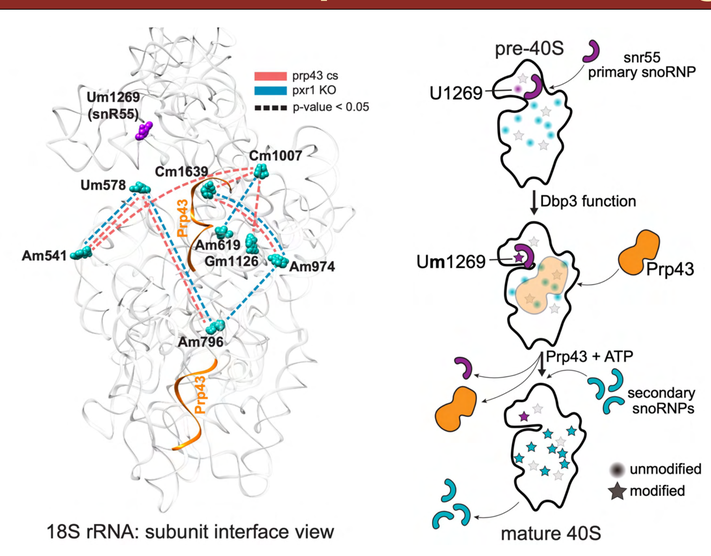
- Dbp3 and Prp43 are proposed to regulate the binding and release of snoRNPs during ribosome biogenesis (Aquino et al. 2021)
- Inhibition of helicases Dbp3 or Prp43 or g-patch protein Pxr1 results in distinct and overlapping modification profiles.
- Correlation analysis (not shown) reveals a concerted set of modification losses and suggests a hierarchy of rRNA modifications regulated by Dbp3 and Prp43 during ribosome biogenesis (see below, Aquino et al. 2021)

7. Modifications surrounding the polypeptide exit tunnel are highly concerted



- Modified nucleotides interact with ribosomal protein loops that form the polypeptide exit tunnel.
- Modifications of Cm1437, Am1449, and Gm1450 occur in a concerted all-or-none manner in WT and helicase compromised yeast.
- Suggests U24 acts through a processive mechanism to modify nucleotides at the PET.

8. Concerted changes in modification suggest a hierarchy of Dbp3 and Prp43 function during ribosome biogenesis



- In the absence of Dbp3, snR55 is retained on the pre-40S subunit, and U1269 is not modified (Aquino et al. 2021).
- A conformational change by Dbp3 function promotes modification of Um1269 by snR55.
- Prp43 binding (Bohnsack et al. 2009) and function promotes release of snR55 and binding and modification by secondary snoRNPs.
- A prp43-cs ATPase mutant binds, but prevents release of snR55 and subsequent secondary snoRNP binding (Leeds et al. 2006)

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Selected references

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