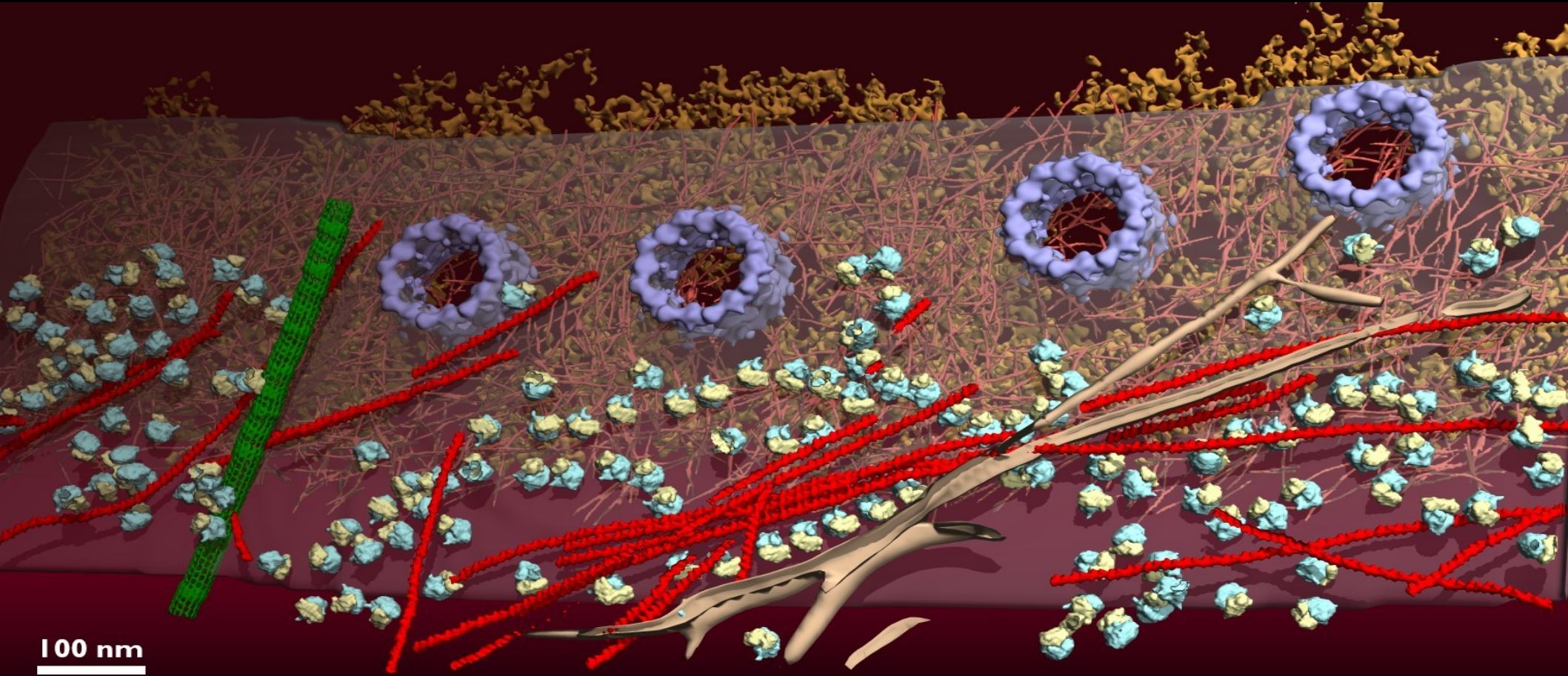


High-resolution structures of the human ribosome to decipher the molecular basis of snoRNAs dysfunction in disease

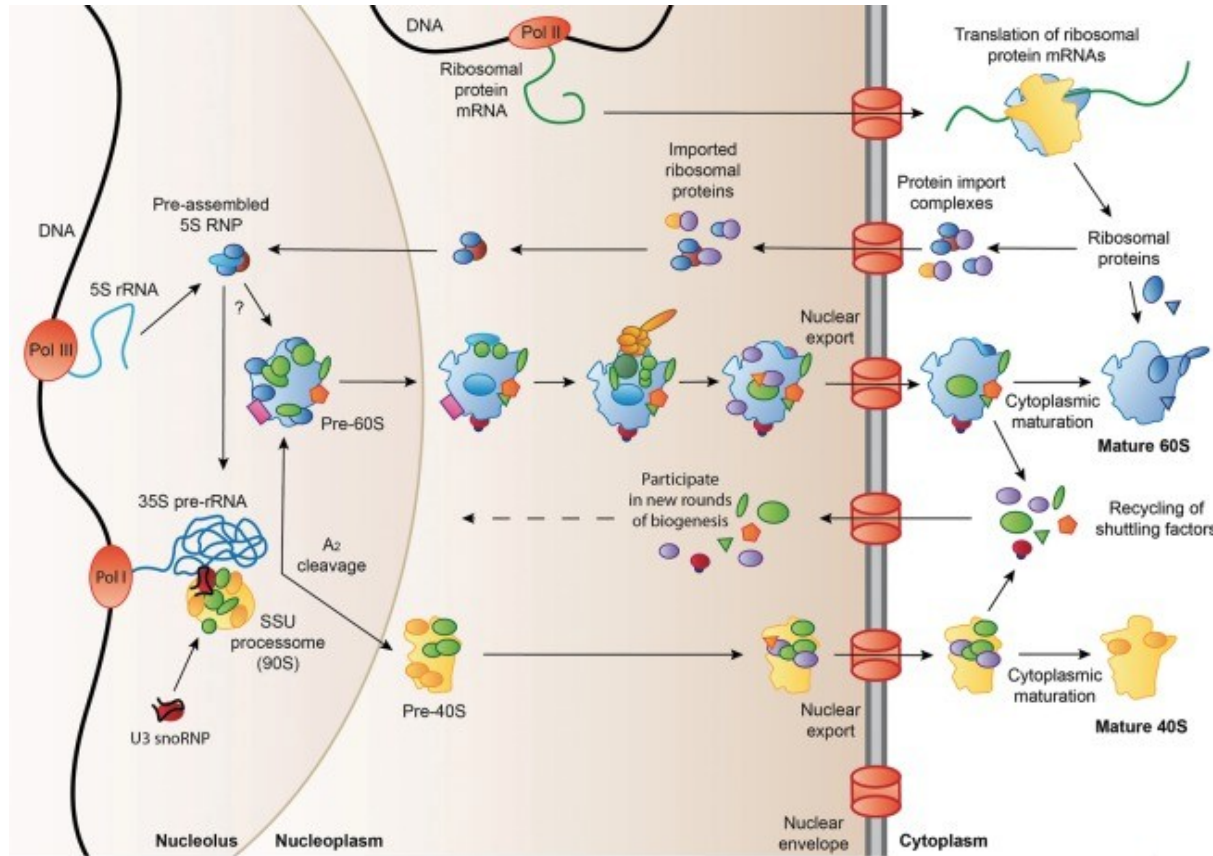
Pellegrino Simone, PhD

**Alan J. Warren's group
Cambridge Institute for Medical Research
University of Cambridge**



100 nm

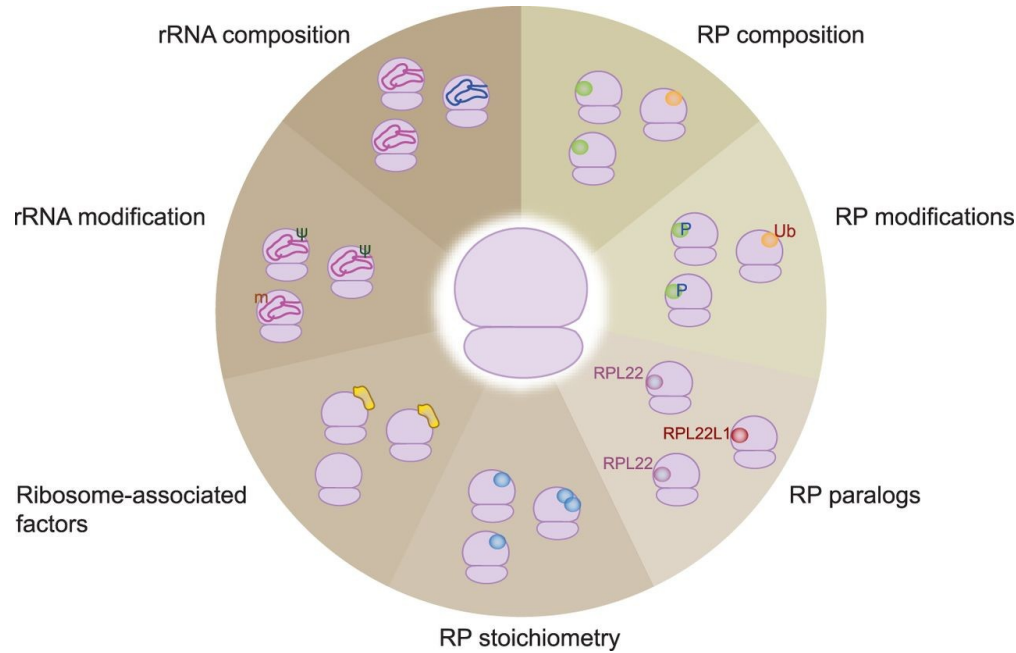
Ribosome assembly in eukaryotes



Process is conserved in humans.

However, > 300 assembly factors and > 400 small nucleolar RNAs (snoRNAs) involved.

Is the ribosome one and only?

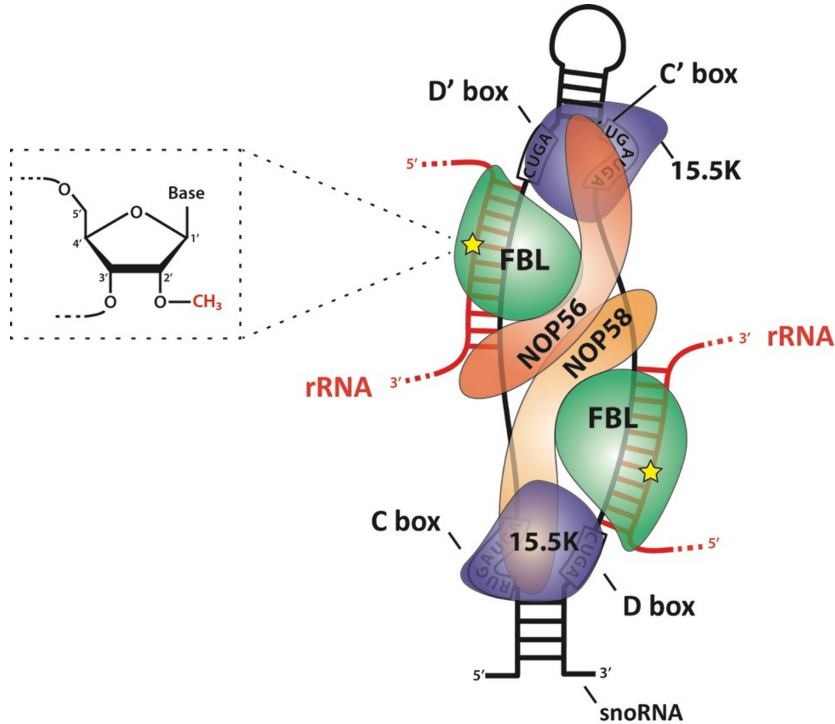


Guo, *Biochem Soc Trans* (2018)

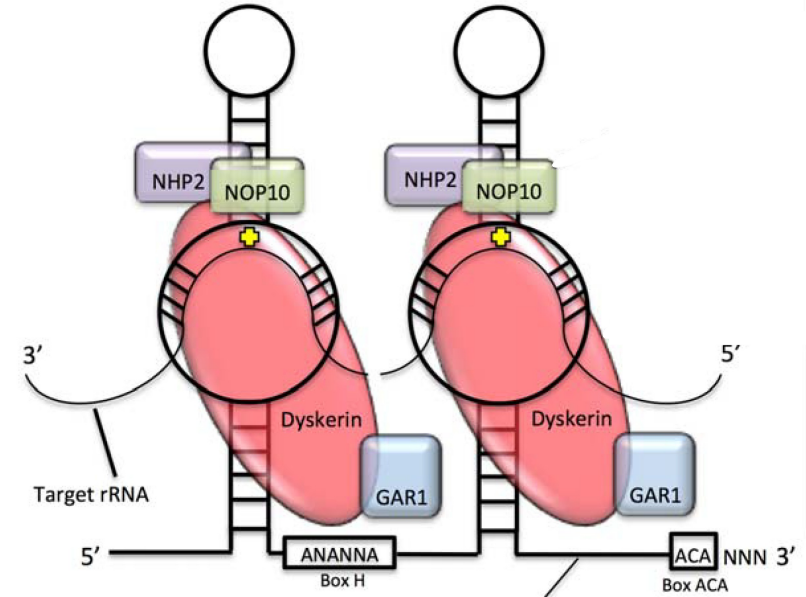
Several sources of ribosome heterogeneity.

Identified during development, response to stress, cell cycle and in disease states.

Small nucleolar ribonucleoproteins (snoRNPs)



Lo Monaco et al., *Biomolecules* (2018).

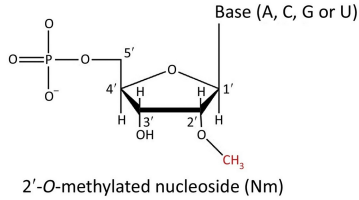


Penzo & Montanaro, *Biomolecules* (2018).

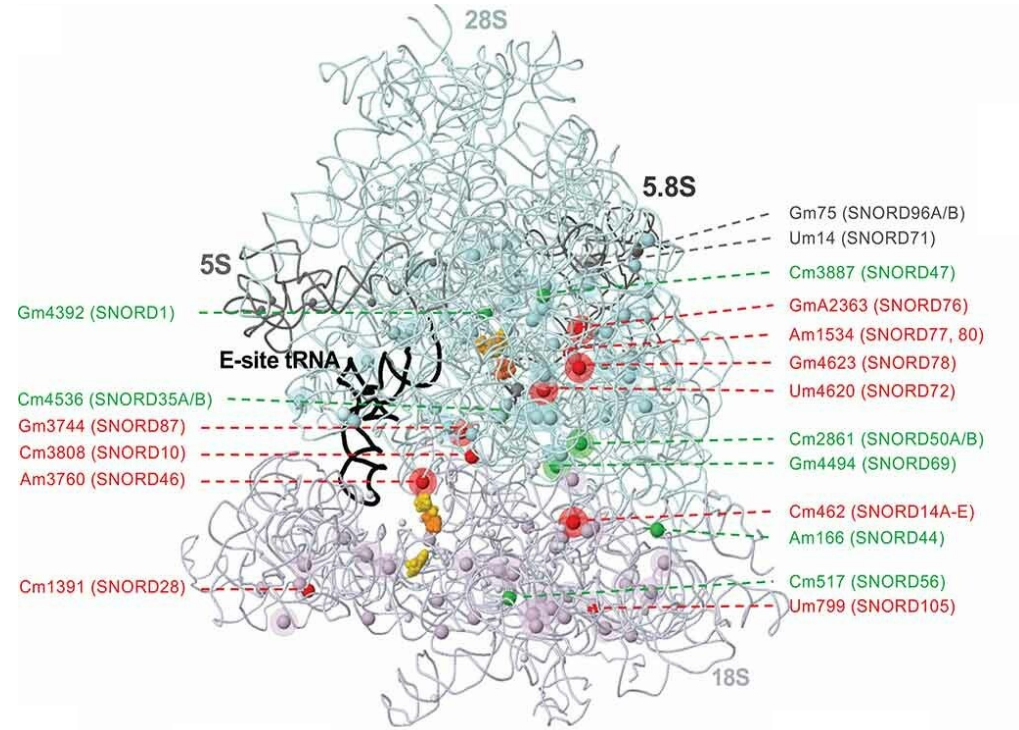
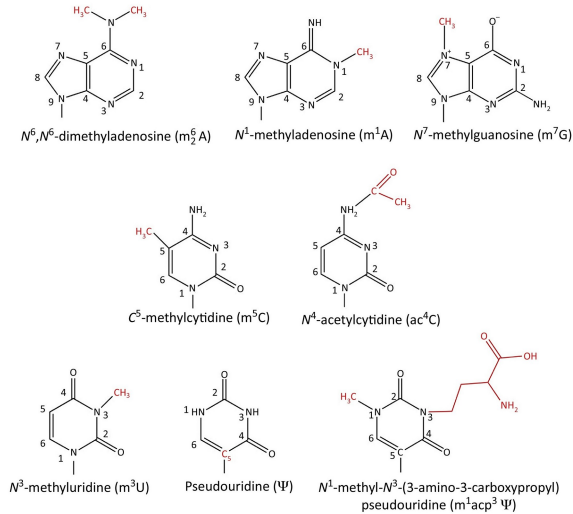
Fibrillarin deregulation and Dyskerin mutations have been associated to disease states.

rRNA modifications: epigenetic control of translation

Sugar moiety



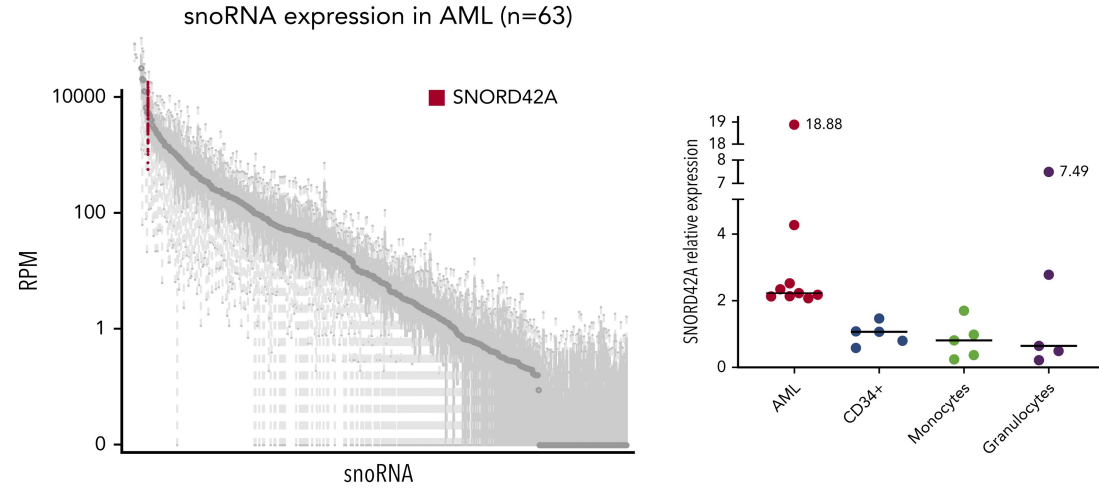
Nucleobase



Barros-Silva, *RNA Biology* (2015)

Hypothesis: differential regulation of translation is dependent on ribosome heterogeneity.

Ribosome specialization in acute myeloid leukaemia

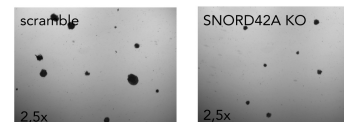
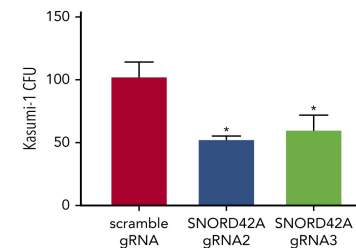
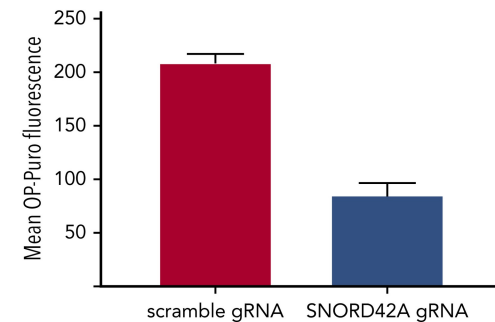
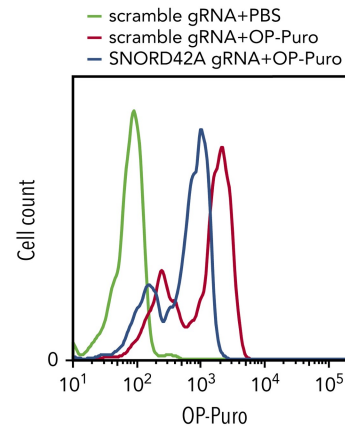
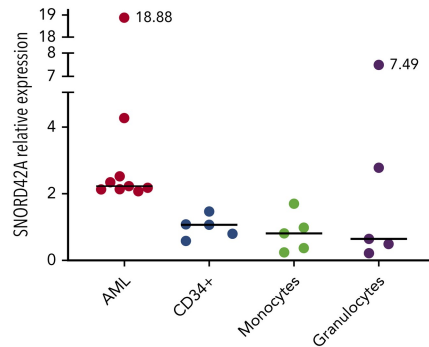
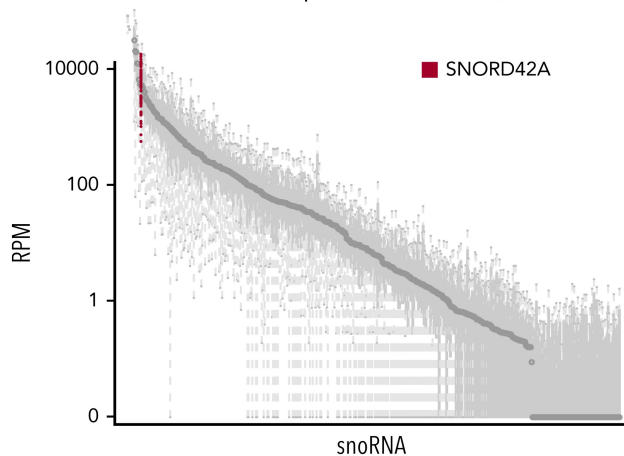


snoRD42A is upregulated in AML.

Pauli et al. **Blood** (2020)

Ribosome specialisation in acute myeloid leukaemia

snoRNA expression in AML (n=63)



snoRD42A is upregulated in AML.

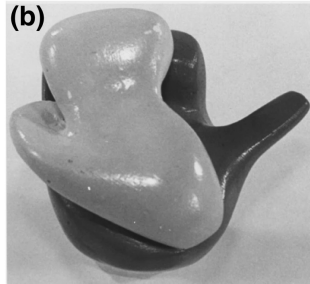
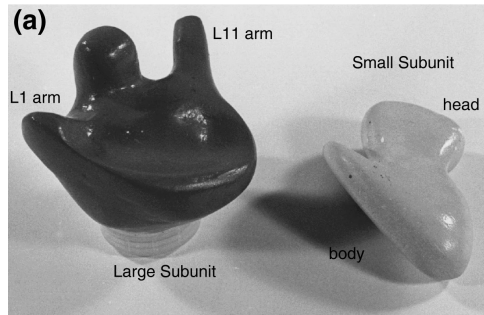
SnoRD42A KO reduces colony forming capacity in AML.

Pauli et al. **Blood** (2020)

How can we spot such a small chemical modification on such a big target (the ribosome)?

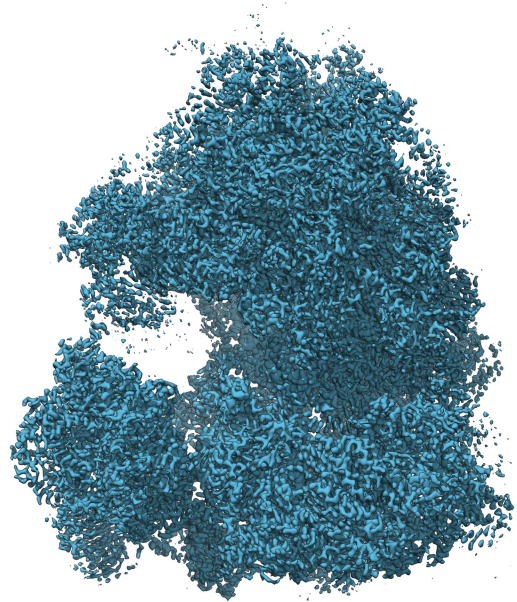
On the way to visualise specialized ribosomes

E. coli 70S at 40 Å resolution



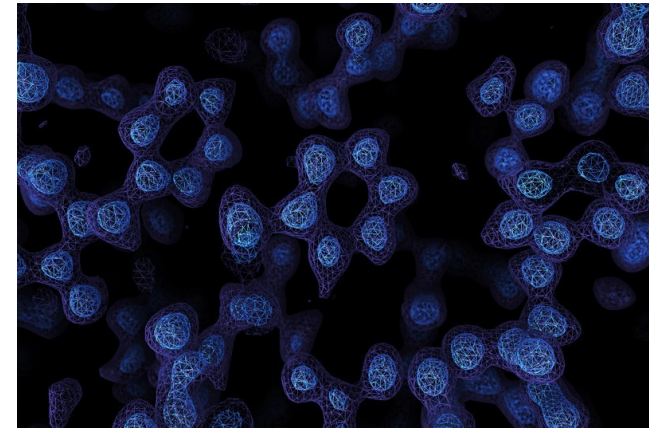
Moore, *J Biol.* (2009)

Human 80S at 2.9 Å resolution



Natchiar et al., *Nature* (2017)

Mouse apoferritin at 1.2 Å resolution



Nakane et al, *Nature* (2020)

Yip et al. *Nature* (2020)

HEK293 in suspension



Run-off to homogenise ribosome population



Dissociation of 80S fractions



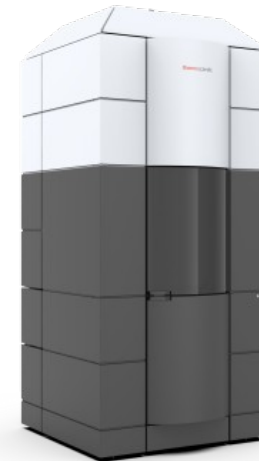
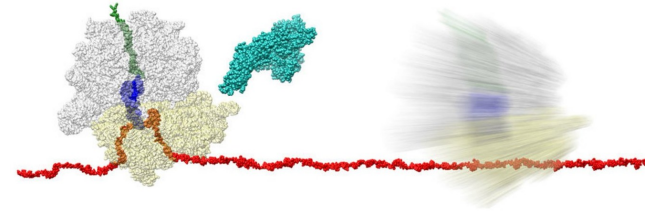
Collect 40S fractions

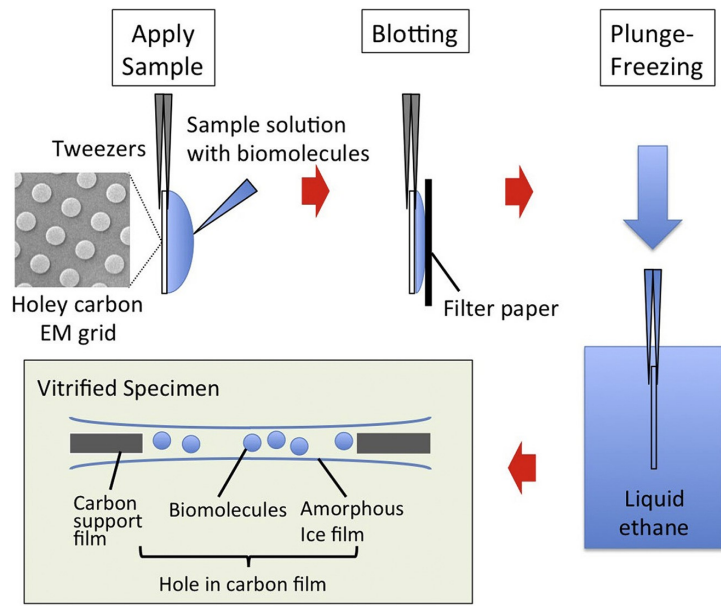


Grid freezing and screen for thin ice

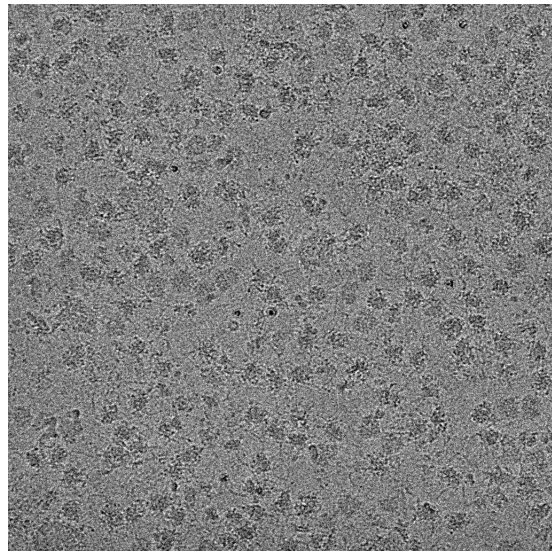


Data collection (less than 24h) and processing

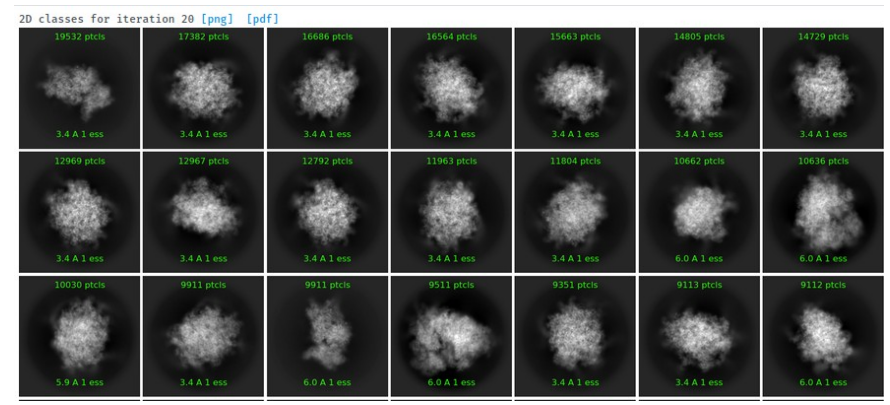




Data collection



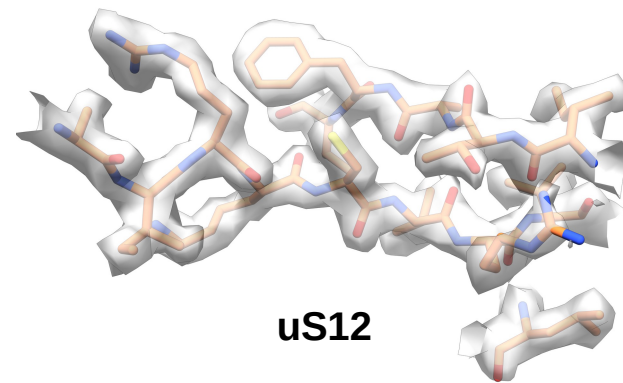
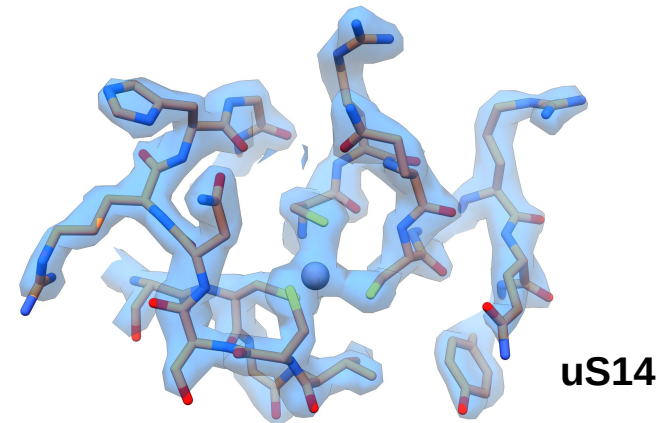
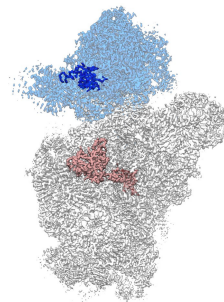
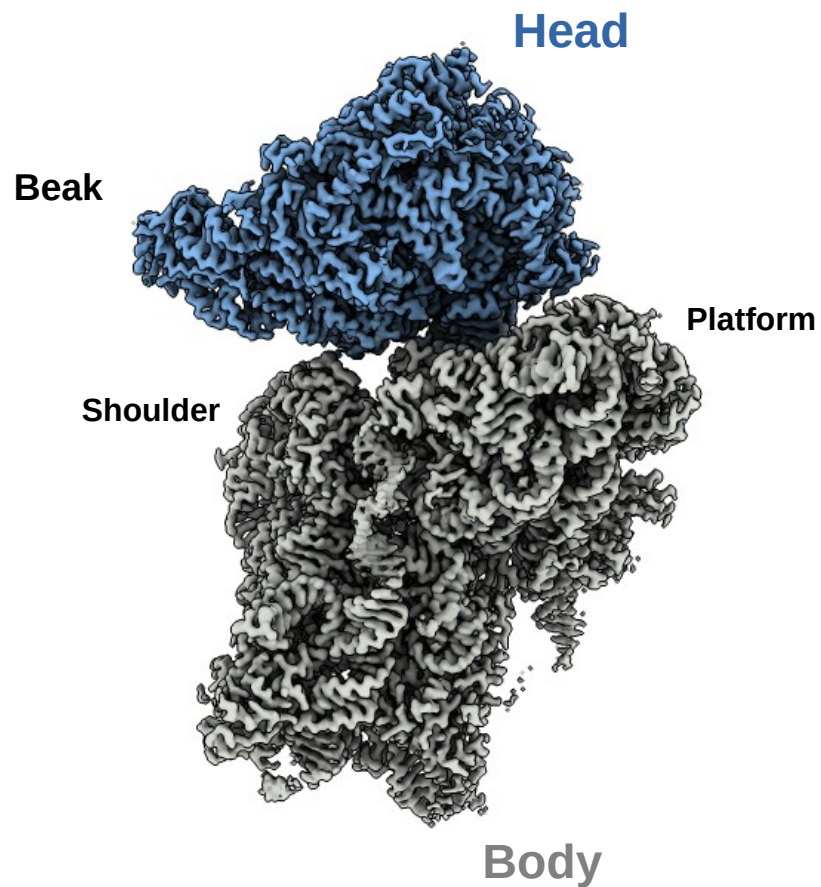
2D



3D



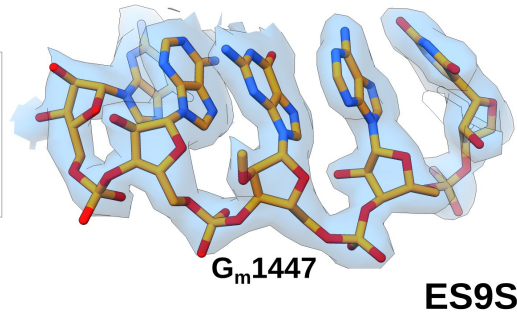
Cryo-EM reconstruction of human 40S



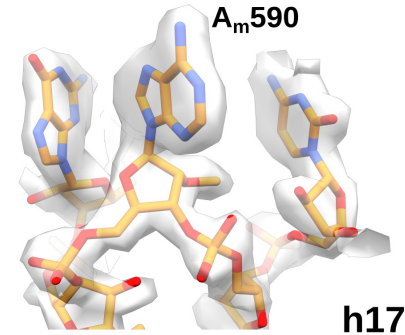
Pellegrino et al., *BioRxiv* (2022)

Visualization of rRNA modifications

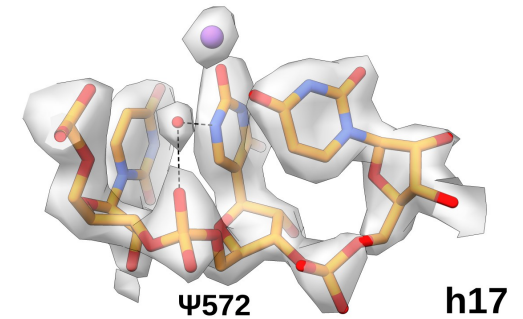
Differentially methylated
in breast cancer (Marcel
et al., 2020).



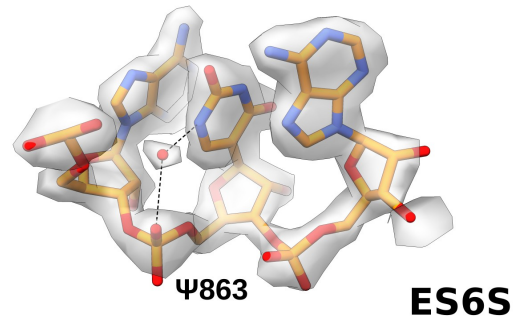
Regulated during
mouse brain
development
(Hebras et al., 2020).



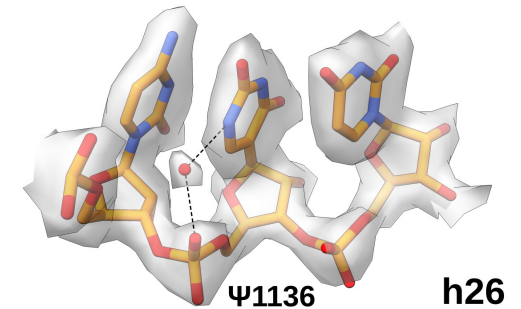
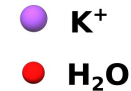
Reduced in Dyskeratosis
congenita patients
(Bellodi et al., 2013).

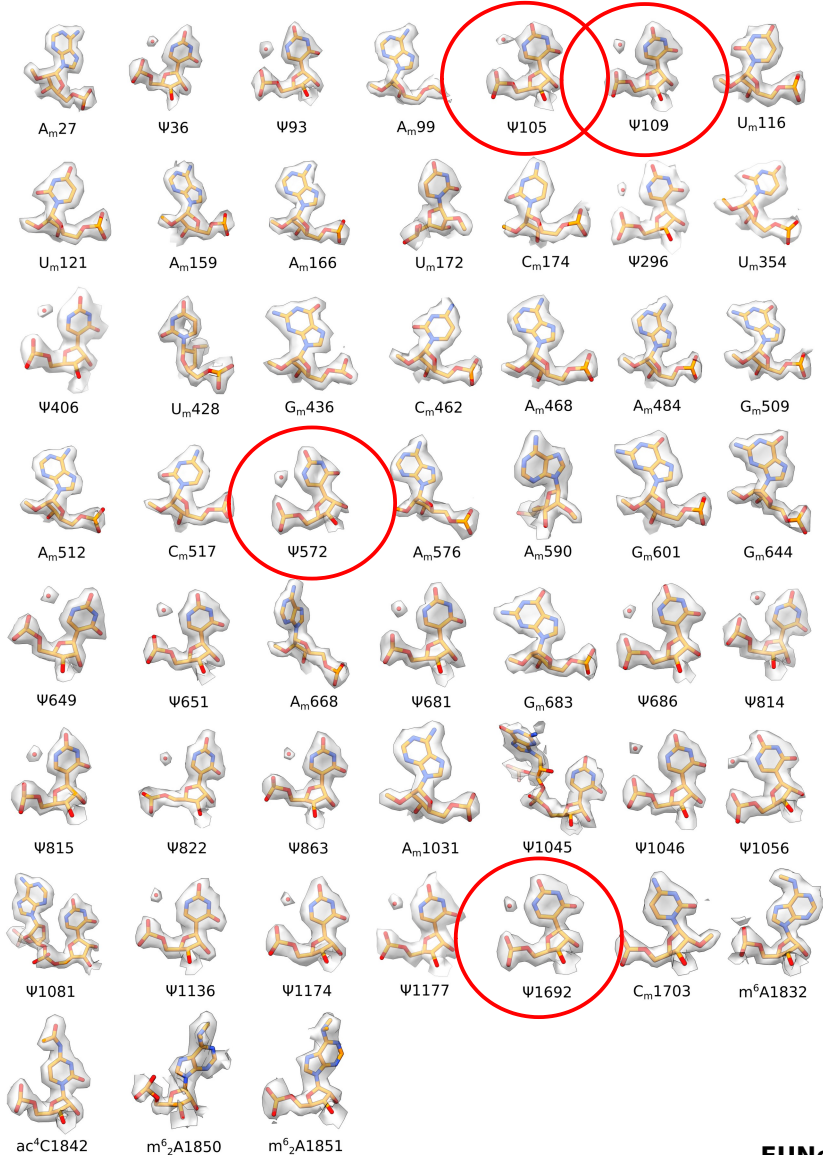


Lack of ψ863 alters
translation fidelity
(McMahon et al., 2019).



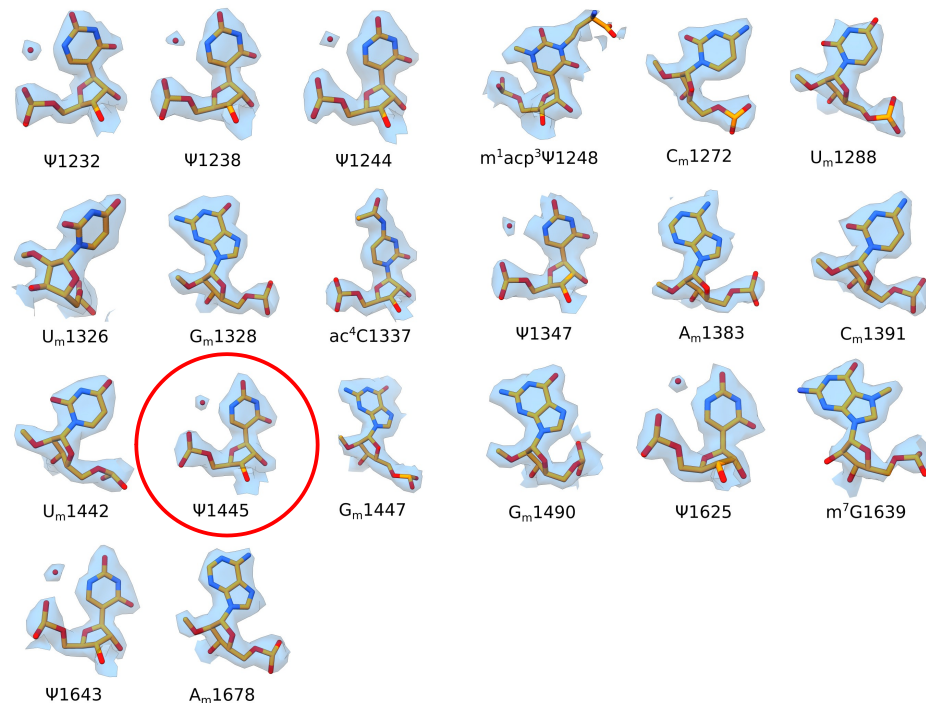
Legend



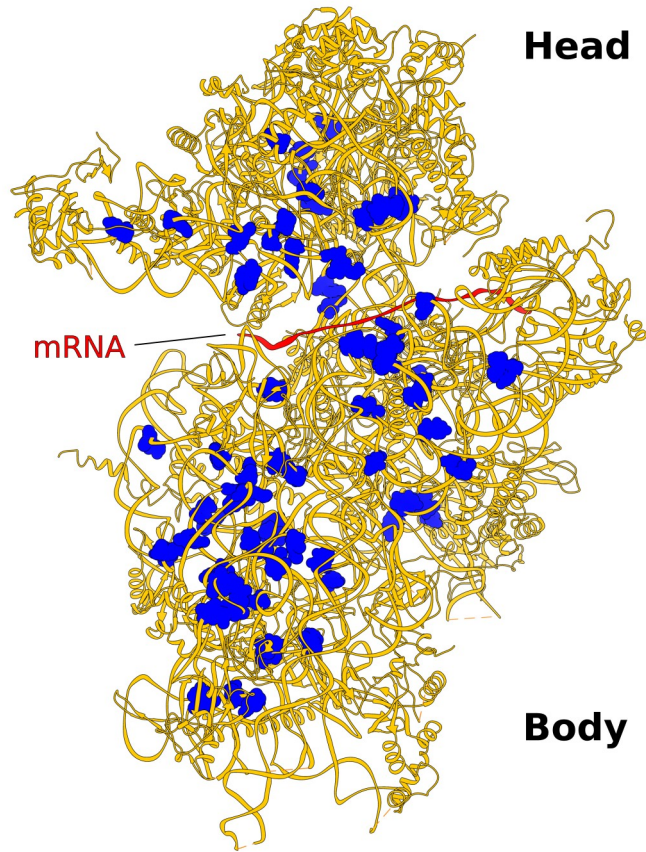


We visualised with great accuracy 73 out of 91 rRNA modifications.

Lay the basis for investigating differential expression of snoRNAs and defects in incorporation.



Chemical modifications shape structure and function of ribosome.

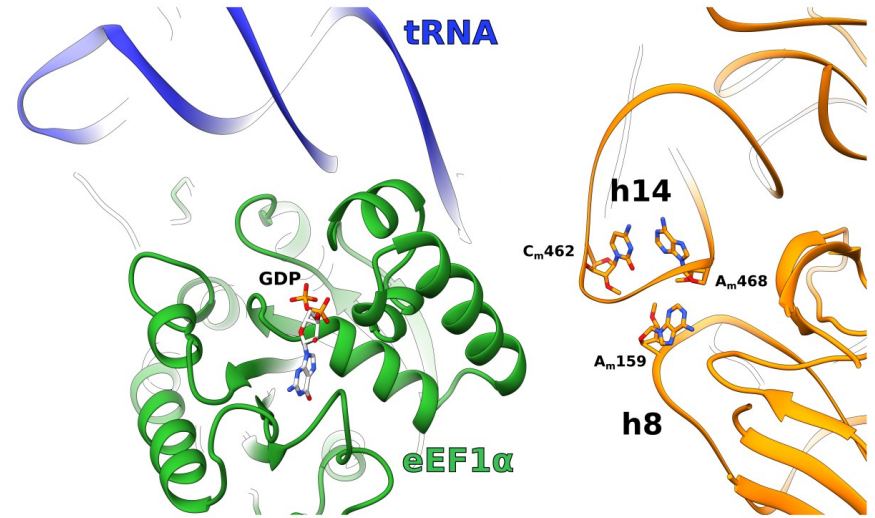
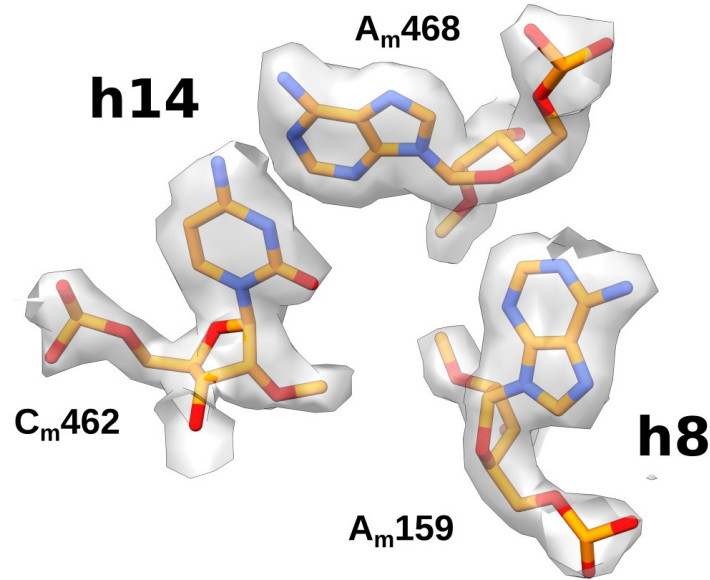


rRNA modifications surrounding the 40S functional sites, such as decoding centre

Several others embedded within the core: function in rRNA maturation and folding.

Several modifications positioned in proximity of binding platform for translation factors.

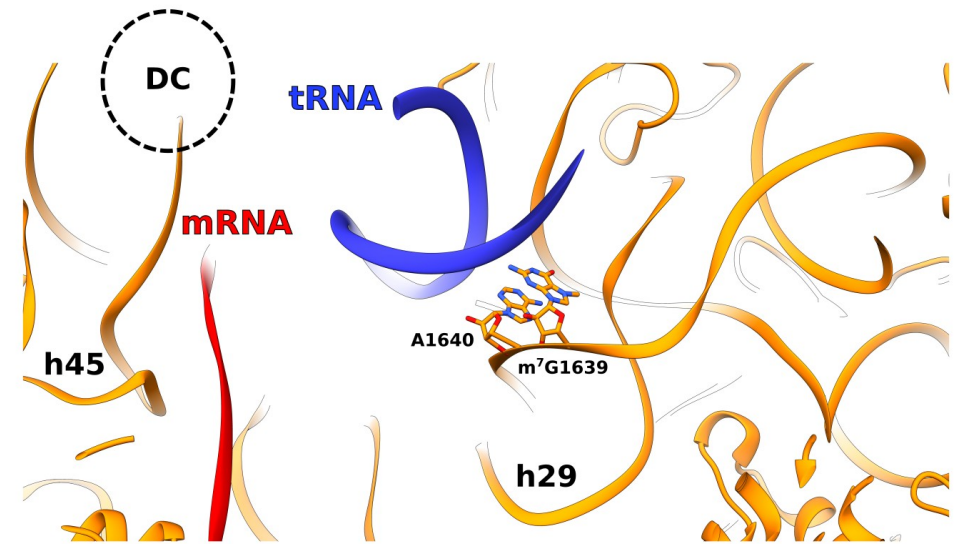
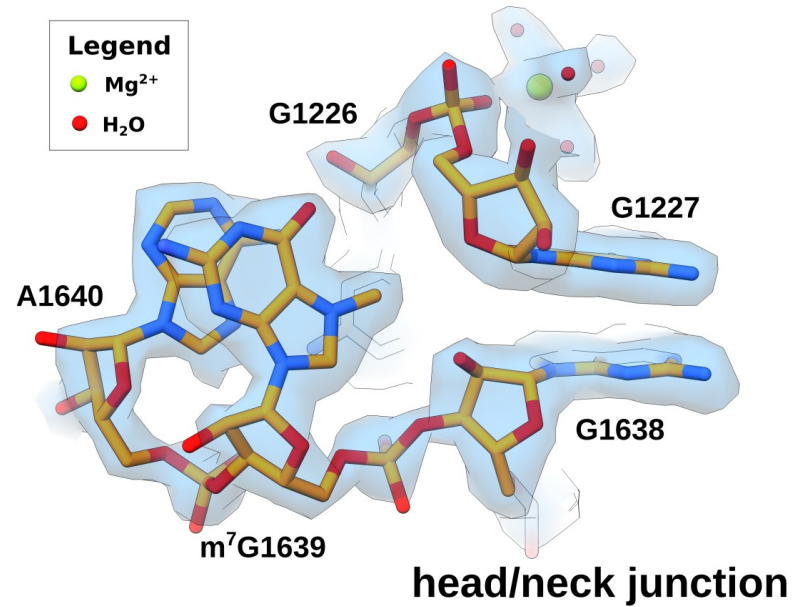
Regulation of translation initiation and elongation



Pellegrino et al., **BioRxiv** (2022)

h14 constitutes an anchoring point for translation factors on the 40S. Deletion of snoRD14 (C_m462) impairs leukaemogenesis *in vivo* (Zhou et al., **Nat Cell Bio** 2017).

Additionally, h14 regulates aa-tRNA selection process (McClory et al., **RNA** 2010)

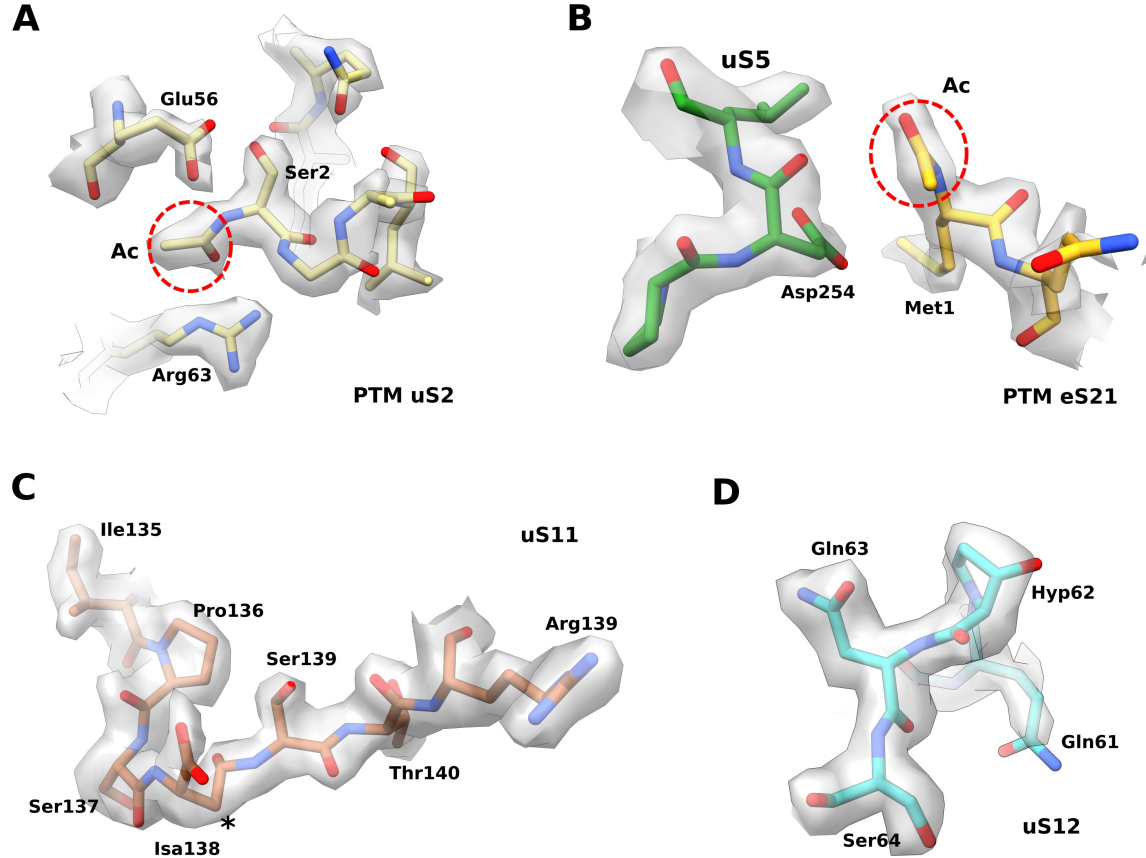


Pellegrino et al., **BioRxiv** (2022)

m⁷G1639 participates in the process of translocation during protein synthesis.

Stand-alone enzyme: WBSCR22. Loss of modification causes pre-rRNA processing defects.

Visualization of PTMs



Post-translational modifications on ribosomal proteins can have structural roles to promote folding of partially unstructured N-term loops.

Others may be driving the formation of particular structural motifs.

Conclusions and perspectives

Developed a pipeline to achieve close to 2 Å resolution reconstructions of human 40S.

High-resolution reconstruction allowed to visualise ~ 80% rRNA modifications.

Four PTMs visualised: establish additional contacts with neighbouring residues.

Paves the way to understand the basis of ribosome specialisation in fine-tuning translation.

Shed new light on the molecular consequences of snoRNAs differential expression in disease states.

Acknowledgements

The Warren lab



Lab members

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Shengjiang Tan
David Traynor

Cryo-EM facility

Dima Chirgadze
Lee Cooper
Steven Hardwick

Former lab members

Kyle Dent



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