Found in Translation: Evolution (of Structural RNAs) in 3D

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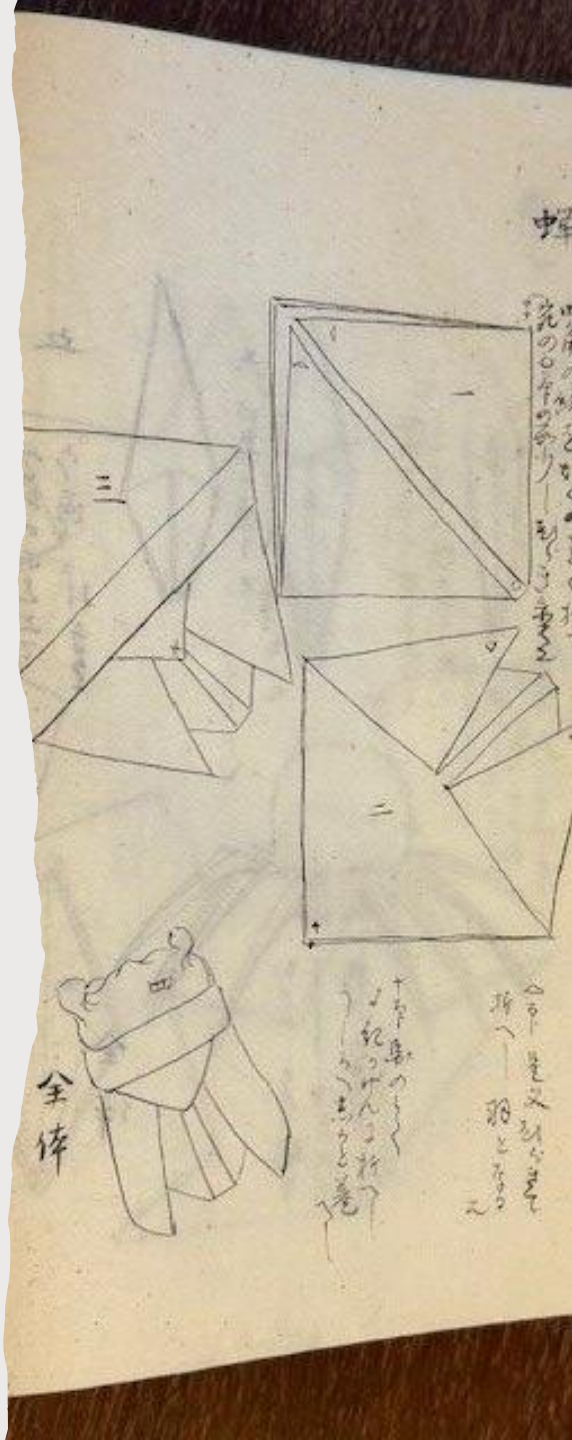
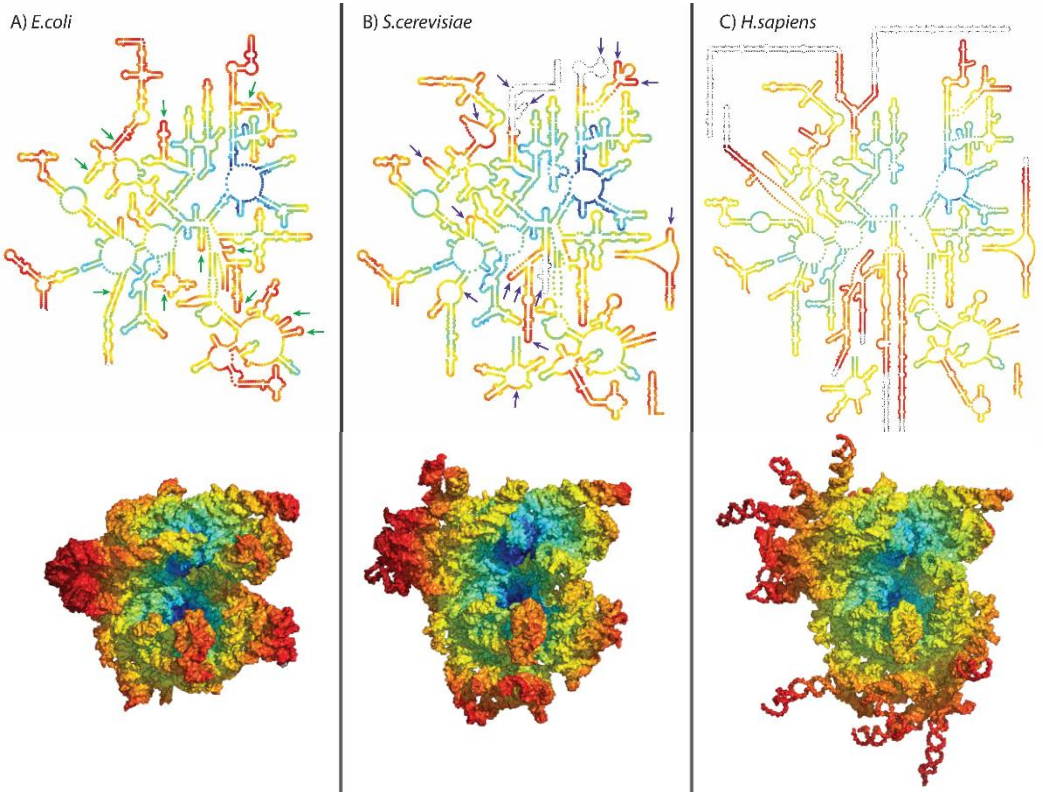
Can (Molecular) Origami Reveal Our Past?

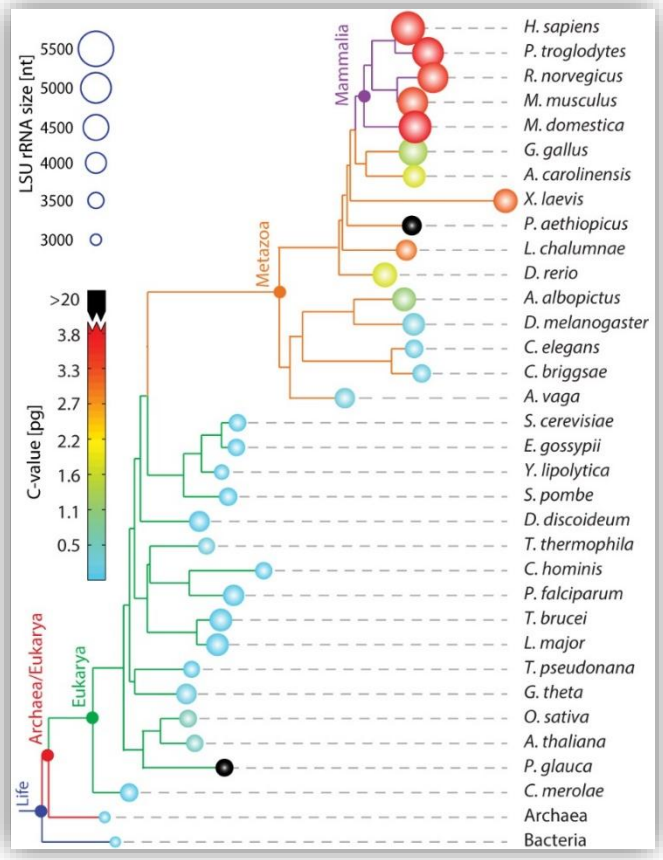
- *https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0240226*
- *https://catalog.loc.gov/vwebv/search?searchCode=LCCN&searchArg=2002536297&searchType=1&permalink=y?loclr=blogfam*
- *https://www.pinterest.com/pin/origami-crane-tree--195414071314337812/*



Eukaryotic Expansion Segments

- Eukaryotic expansion segments grow from the surface of the ribosomal core
- rRNA can be used to reconstruct the Tree of Life (no or little HGT)

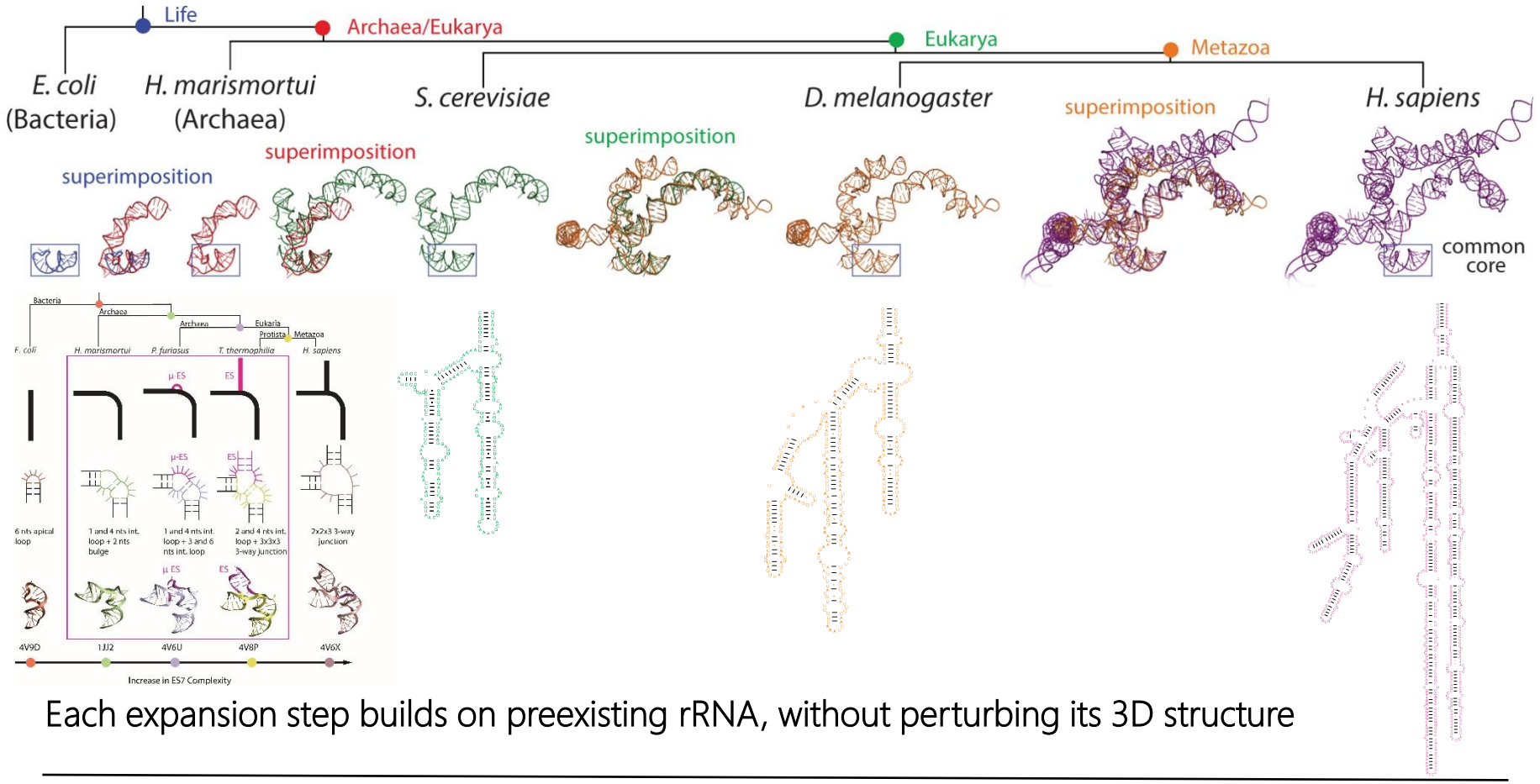




Gerbi S. Expansion segments: Regions of variable size that interrupt the universal core secondary structure of ribosomal RNA. 1996, 71-87.

Petrov A. S. et al. Evolution of the ribosome at atomic resolution. PNAS, 2014, 111, 10251-10256.

Accretion: Eukaryotic Expansion Segment ES7

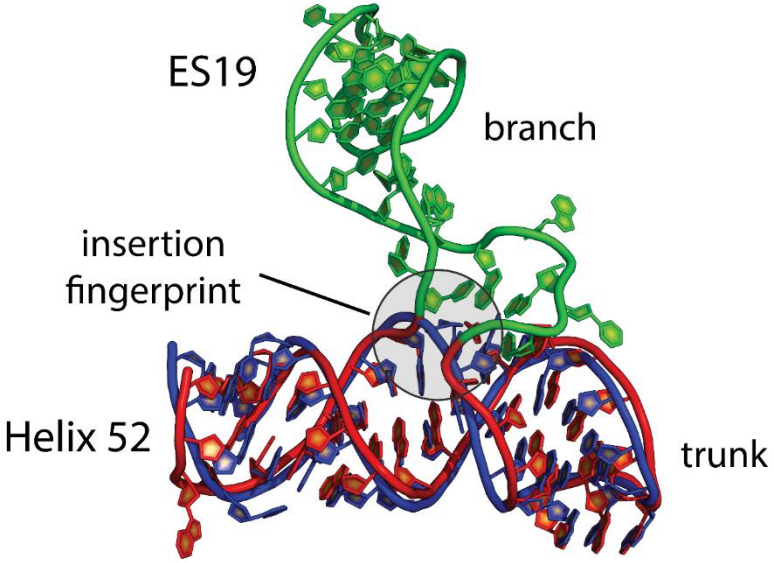


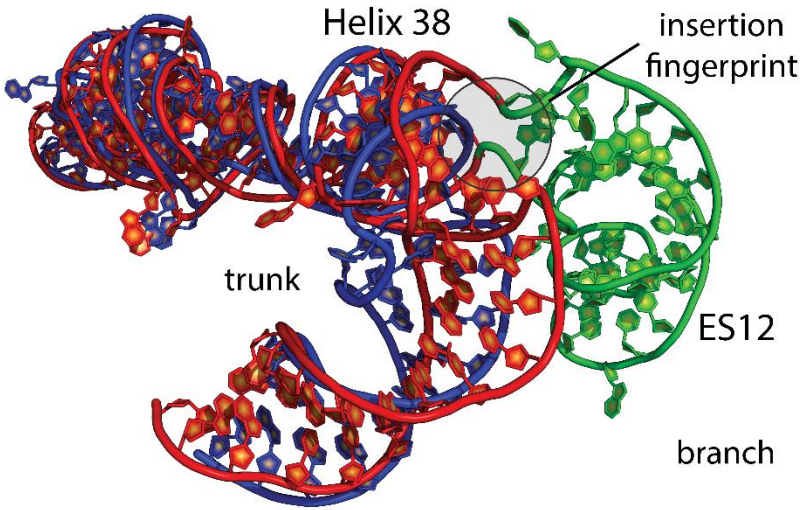
Each expansion step builds on preexisting rRNA, without perturbing its 3D structure

Petrov A. S. et al. Evolution of the ribosome at atomic resolution. PNAS, 2014, 111, 10251-10256.
Biesiada, M. et al. rRNA expansion Segment 7: from Signature Fold to Tentacles, NAR, 2022, 50, 10717-10737.

Eukaryotic Insertion Fingerprints: LSU

Insertion fingerprint is a localized junction between a helical trunk and a branching expansion segment





Expansion Segments may be excised with a minimal perturbation of the common core

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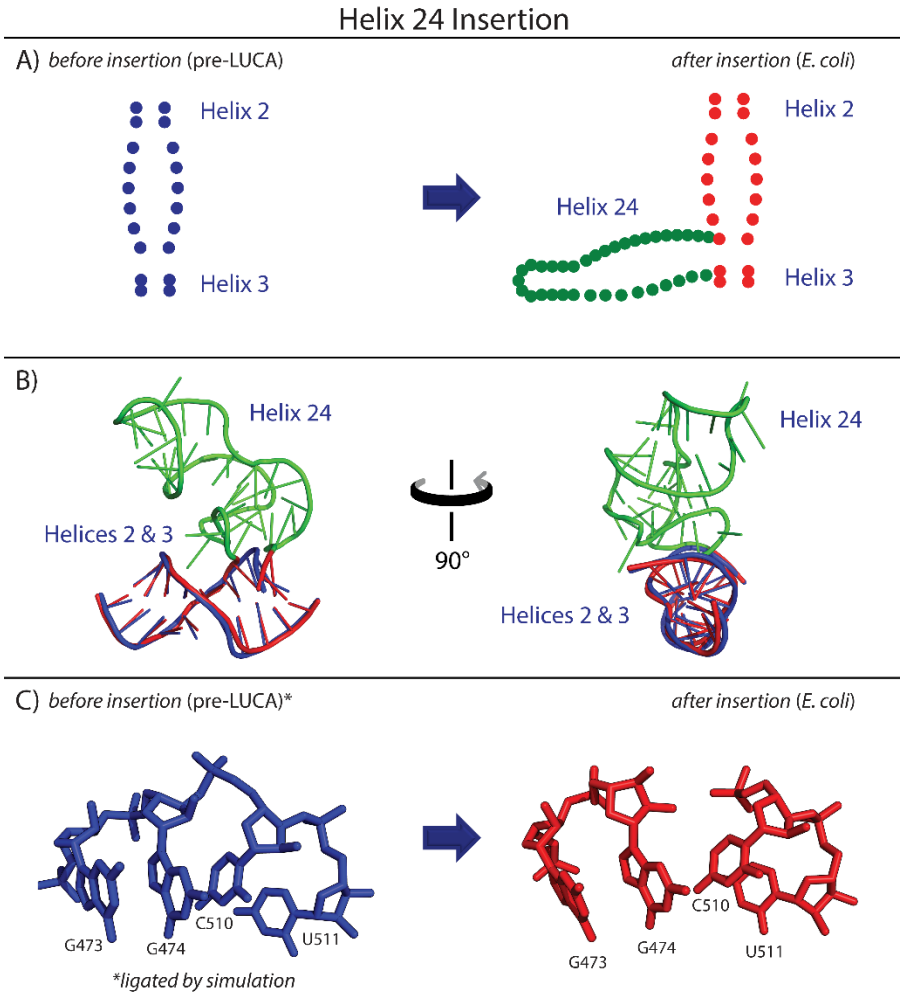
Petrov A. S. et al. Evolution of the ribosome at atomic resolution. PNAS, 2014, 111, 10251-10256.

Ancestral Expansion Segments

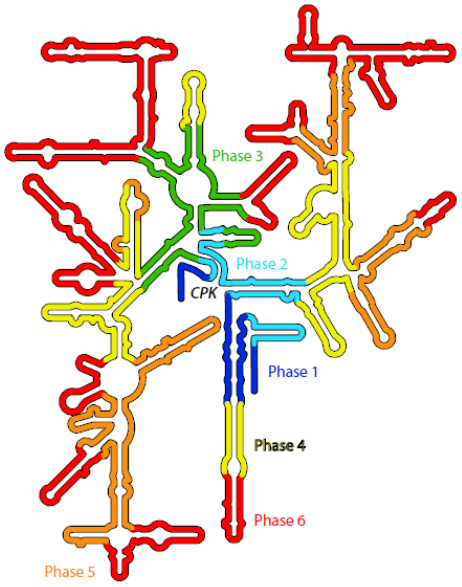


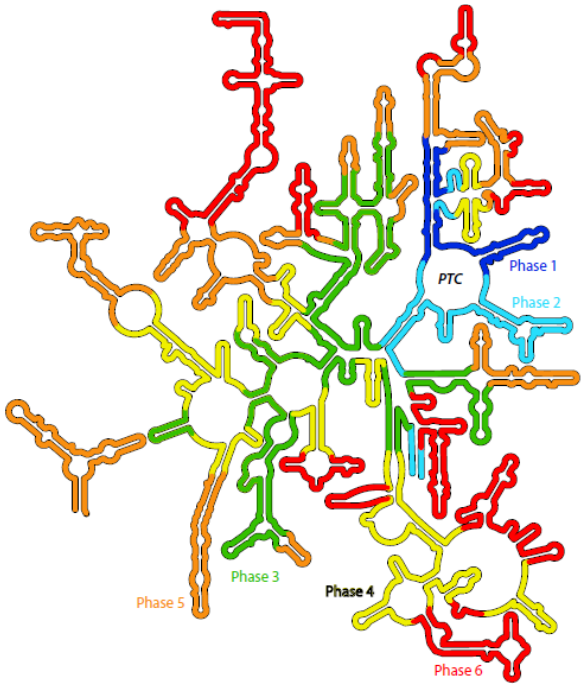
Ancestral insertion fingerprints appear identical in form to modern insertion fingerprints of eukaryotic expansions

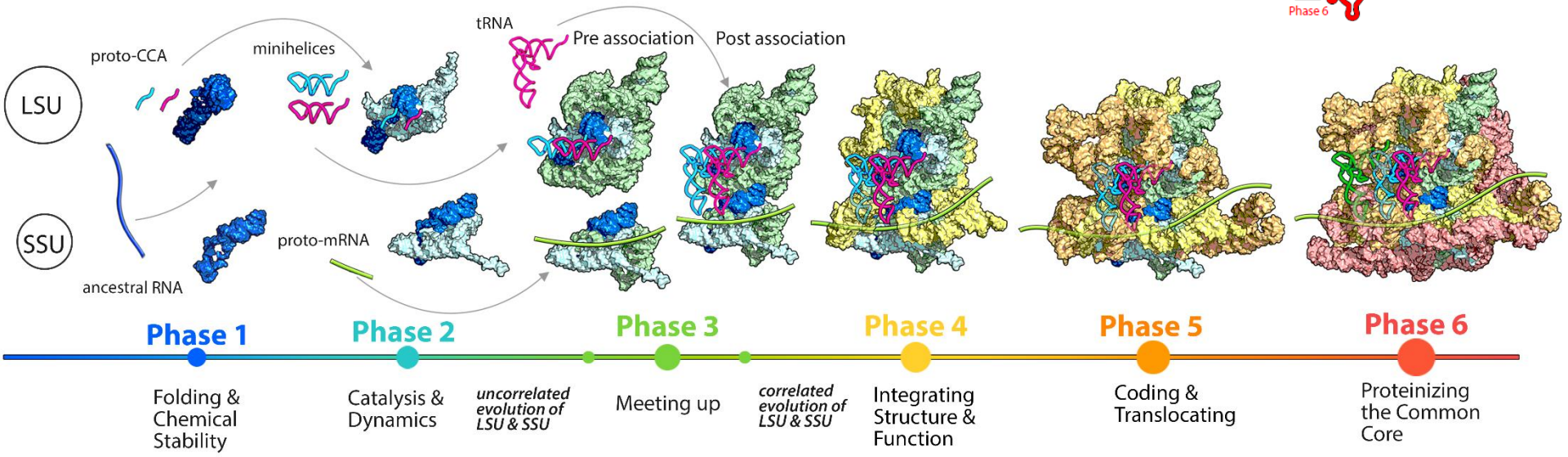
- Ancestral trunk before insertion
- Ancestral trunk after insertion
- Ancestral expansion segment

Petrov A. S. et al. Evolution of the ribosome at atomic resolution. PNAS, 2014, 111, 10251-10256.

Partitioning SSU and LSU into phases







Petrov A. S. et al. History of the ribosome and the origin of translation. PNAS, 2015, 112, 15396-15401

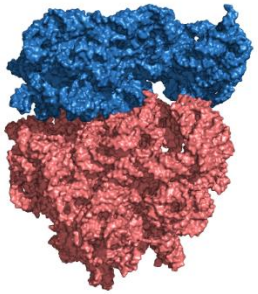
Principles of Evolutionary Accretion

- Folding (helices or stem elbow-stem) fragments
- Dimerization and formation of a minimal catalytic site
- Reinforcement of the catalytic site
- Resinformcent of interactions with the substrate
- Emergence of additional features
- Stabilization by pseudoknots
- Evolutionary diversification

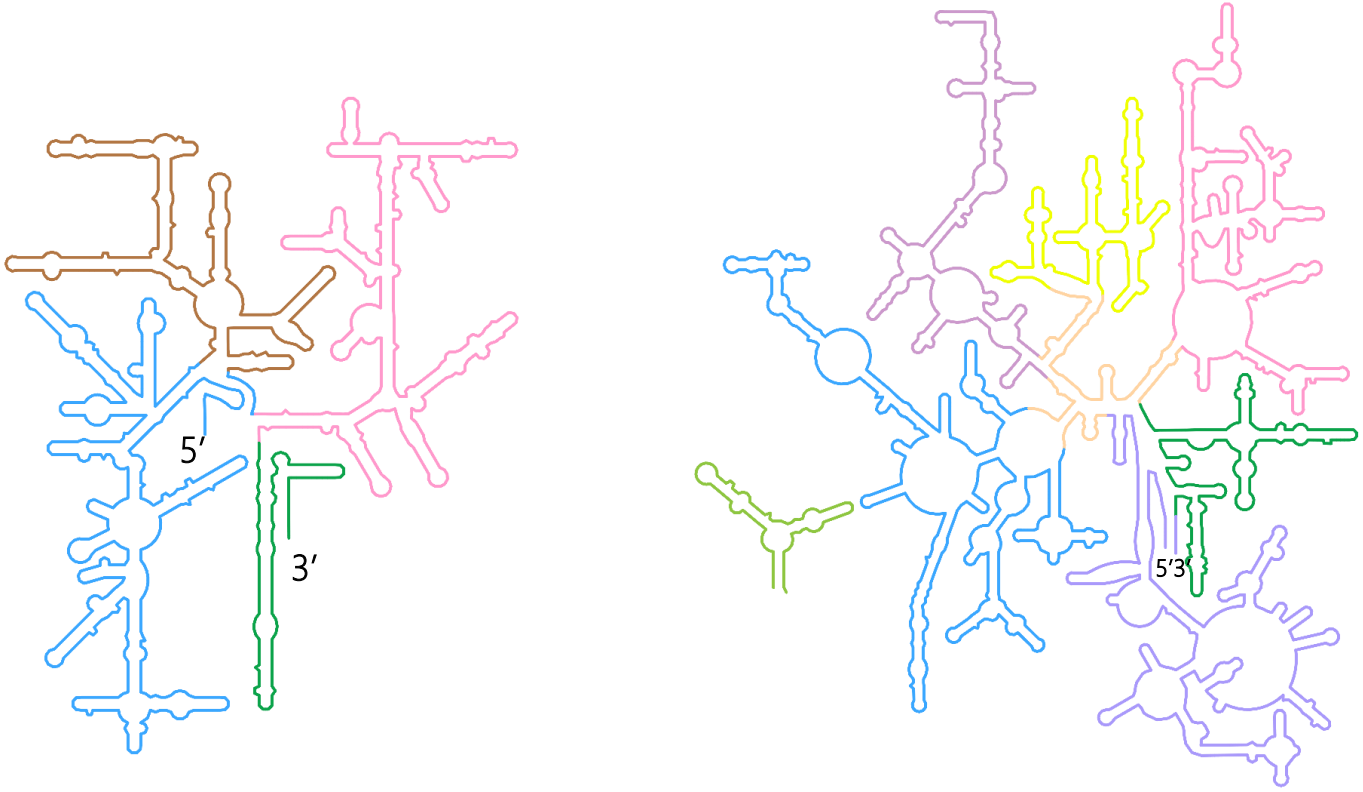
SSU and LSU are really different…

(… SSU came from Mars, LSU came from Venus)

i. Topology of rRNA termini. In the SSU, the termini are dissociated, in the LSU they are associated.
   - CPK (SSU) versus PTC (LSU). A central pseudoknot and the separated strands form the core of the SSU, in comparison to simple stem-loop topology in the core of the LSU (PTC).
ii. Shape. The SSU is oblate spheroid and the LSU is hemispheroid.
iii. Flexibility. The SSU is intrinsically flexible, while the LSU is rigid except on its periphery
iv. Domain Structure. The SSU is dendritic and the LSU is monolithic (built around the exit tunnel).



Topology of rRNA termini

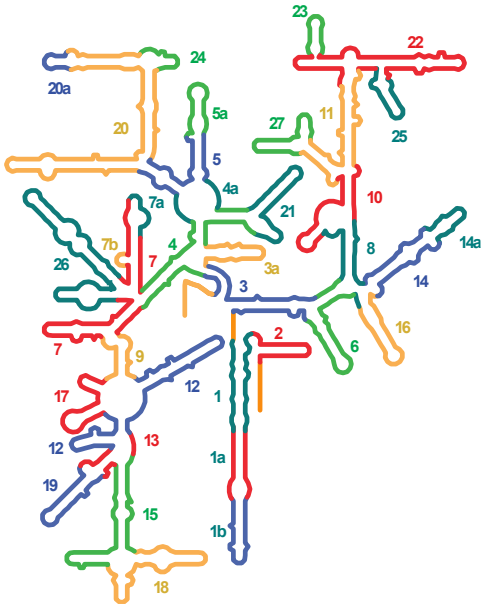


In the SSU, the termini are dissociated, in the LSU they are associated.

Dissociated termini in the SSU and its single stranded 3′-end enables interactions with mRNA

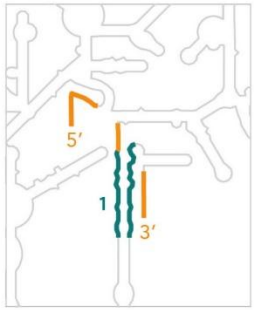
Petrov A. S. et al. Evolution of the ribosome at atomic resolution. PNAS, 2014, 111, 10251-10256.

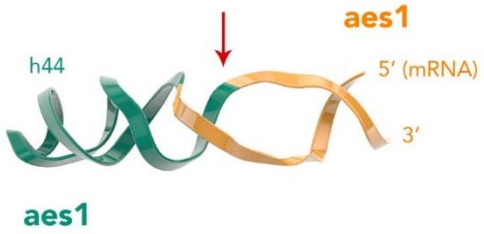
Building up: Phase 1



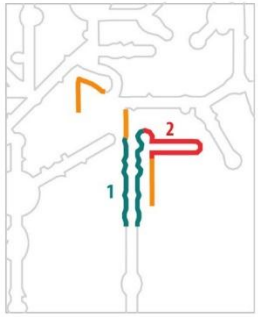
Aes 1 contained a part of helix 44, and a now vanquished helical region composed of the 3′ terminus (the anti SDS) and its complement, modeled by the mRNA

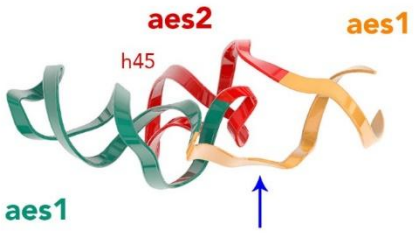
The SSU begins with a stem loop



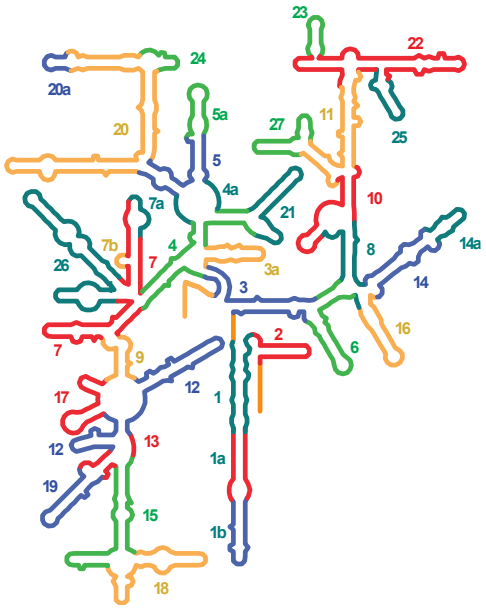


The SSU expands at sites marked by insertion fingerprints



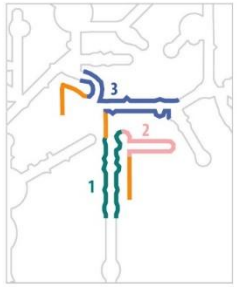


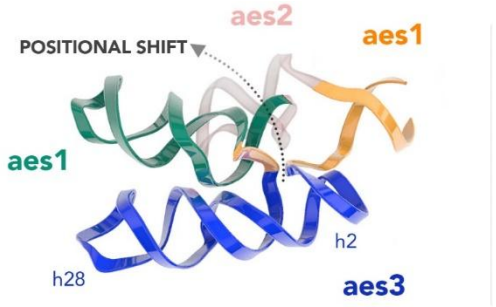
Building up: Phase 2



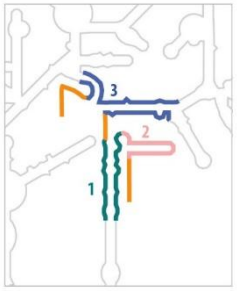
Dissociation of 3′ and 5′ termini is the key event, which results in appearance of single-stranded RNA tails

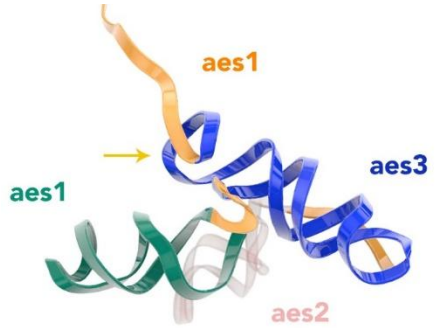
aes 1 acquired aes 2 & 3 as marked by insertion fingerprints





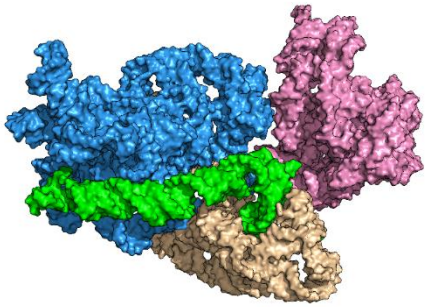
aes 3 shifts as the strand termini dissociate



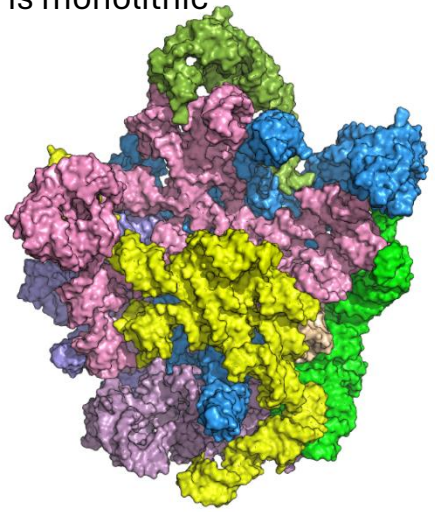


LSU & SSU Domain Structure

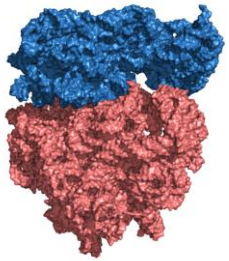
The SSU is dendritic and the LSU is monolithic



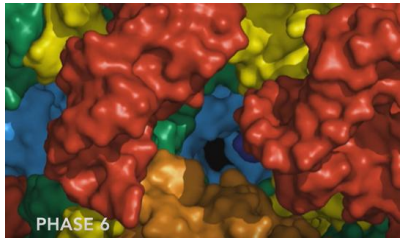
SSU Domains are spatially separated



LSU Domains are entangled



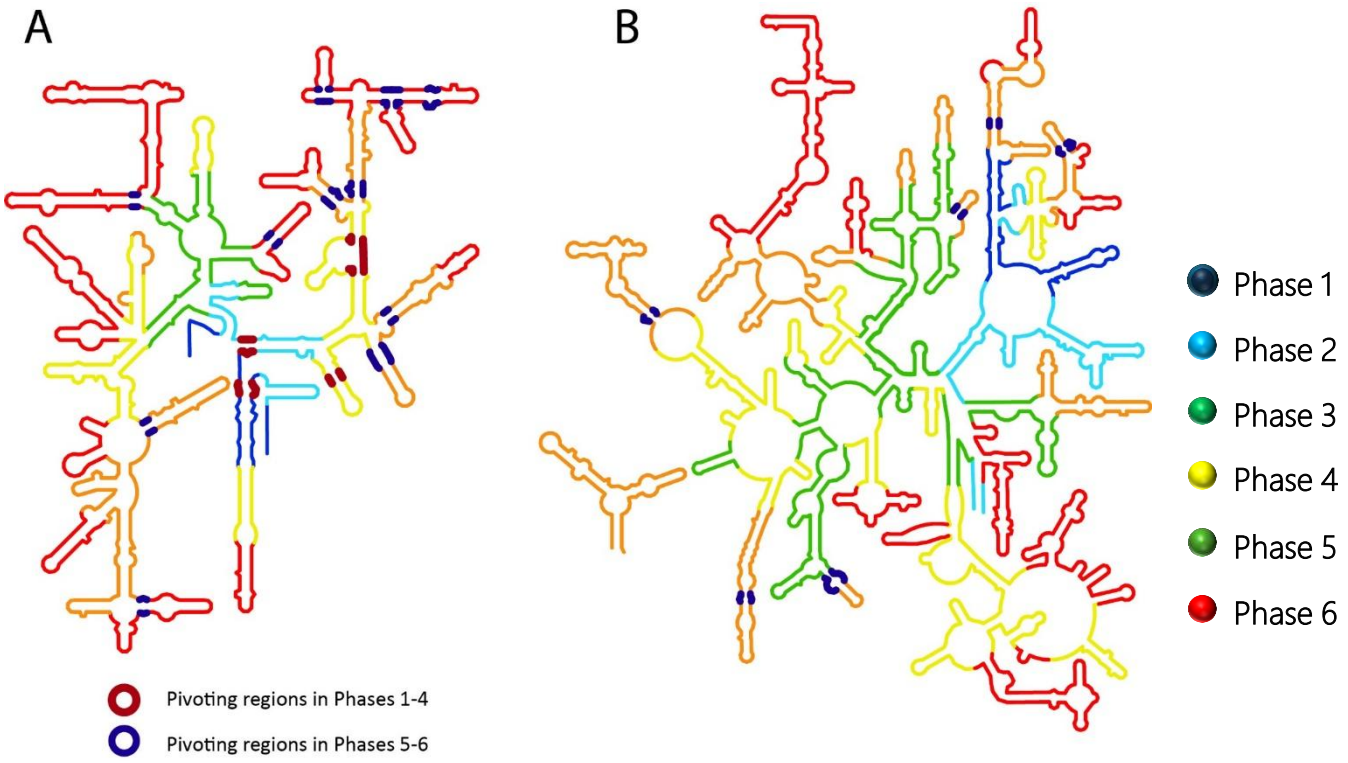
SSU develops at the LSU interface;
Each SSU domain has a specific function



Each phase in the LSU extends the exit tunnel

LSU and SSU Flexibility

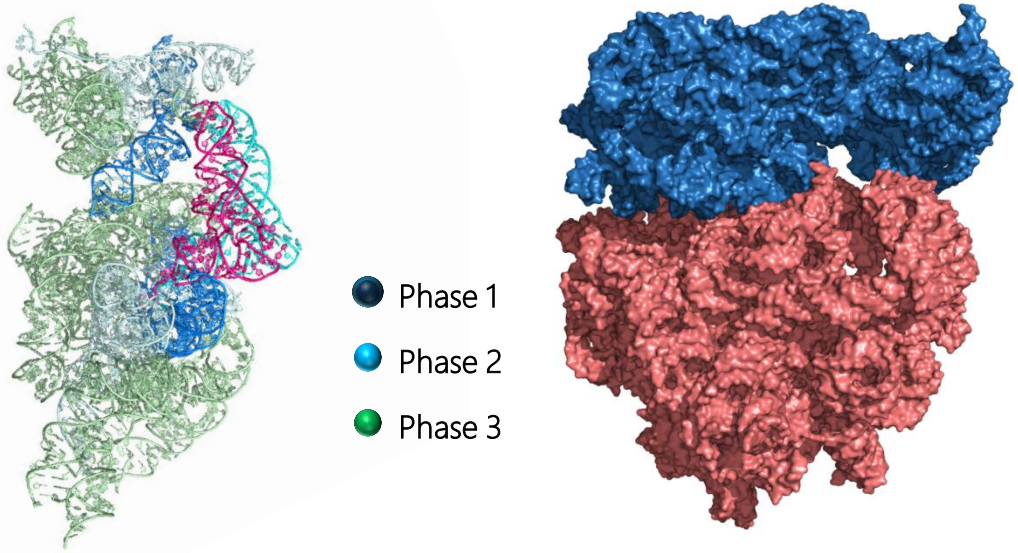
The SSU is intrinsically flexible, while the LSU is rigid except on its periphery



Paci M. and Fox G.E. Major centers of motion in the large ribosomal RNAs. NAR, 2015, 43(9):4640-9
Mohan S.. and Noller H. Molecular mechanics of 30S subunit head rotation. PNAS 2015, doi: 10.1073/pnas.1413731111

LSU & SSU Shape

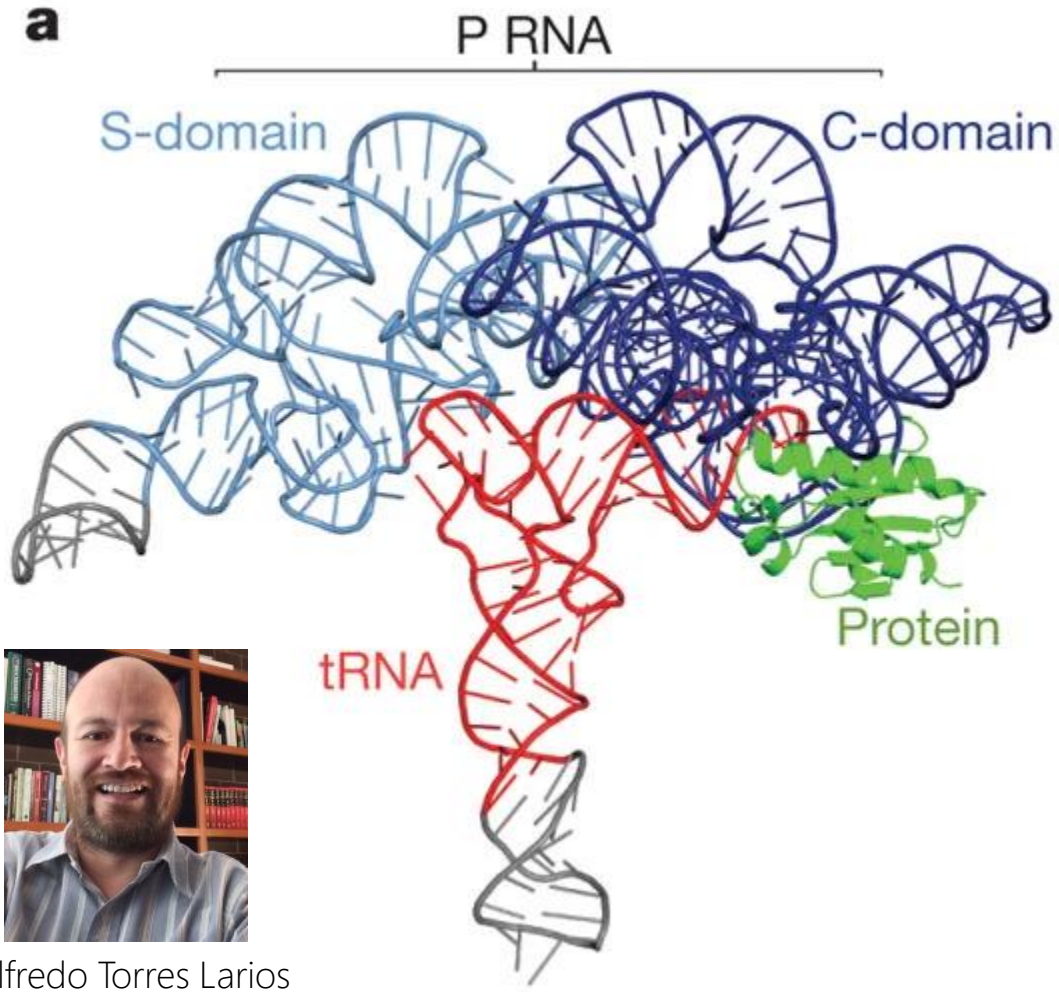
The SSU is oblate spheroid and the LSU is hemispheroid



At the time of acquisition of the interface:

- The LSU was well-established, contains the PTC with a substantial exit tunnel
- The SSU was smaller and less developed.

3D Structure of RNase P

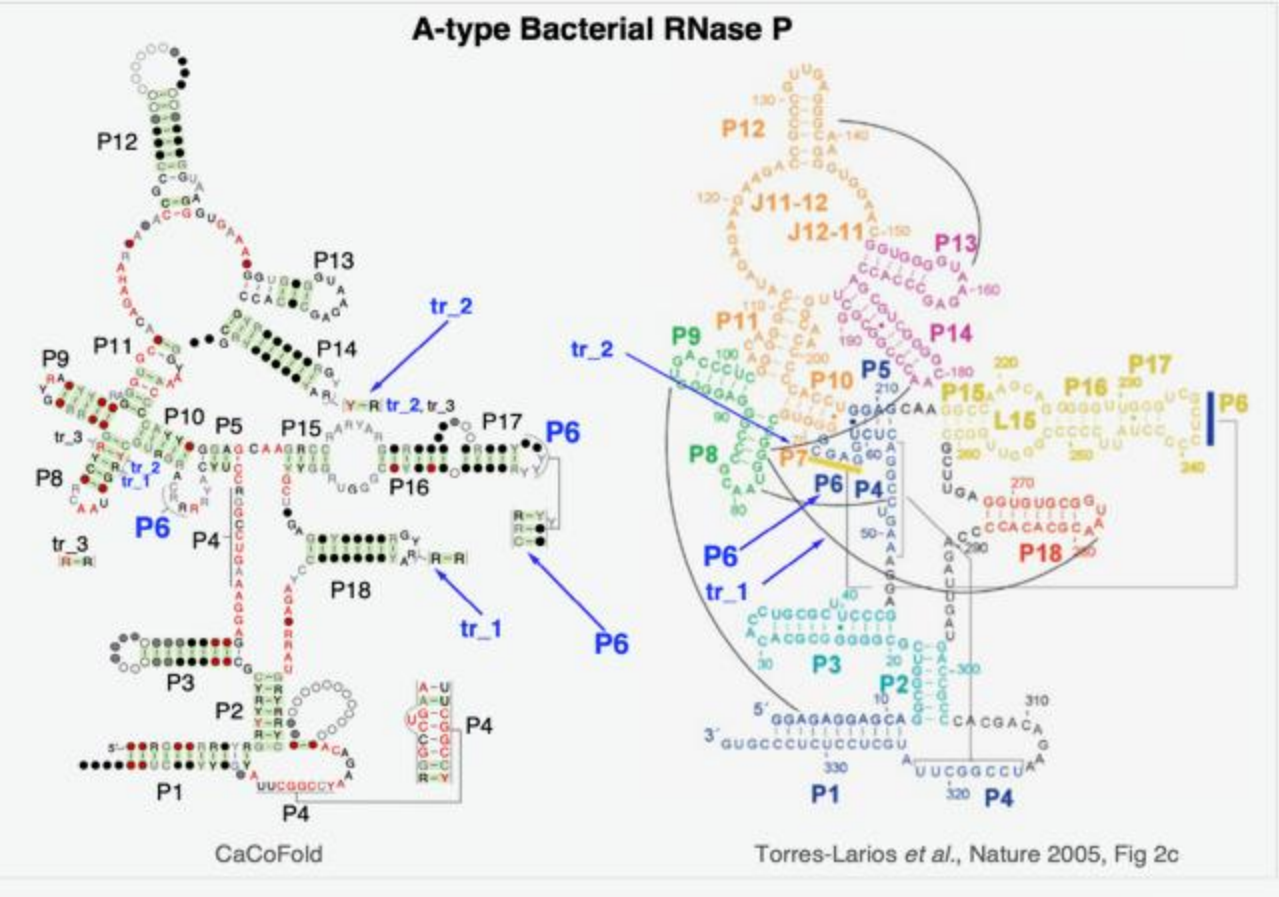


Alfredo Torres Larios

- RNase P is a universally present ribozyme,
- It cleaves the 5'-leader segments of precursor-tRNAs, transforming them into mature tRNAs.
- The atomic-level structures of RNase P are now available for bacterial, archaeal, and eukaryotic species.
- Catalytic RNA contains two distinct regions: the catalytic domain and the specificity domain

Torres-Larias, A., et al. Crystal structure of the RNA component of bacterial ribonuclease P.. Nature, 2005, 437, 584.R
Reiter N. J. et al, Structure of a bacterial ribonuclease P holoenzyme in complex with tRNA, Nature 2010 ,468, 784–789.

2D structure of RNase P

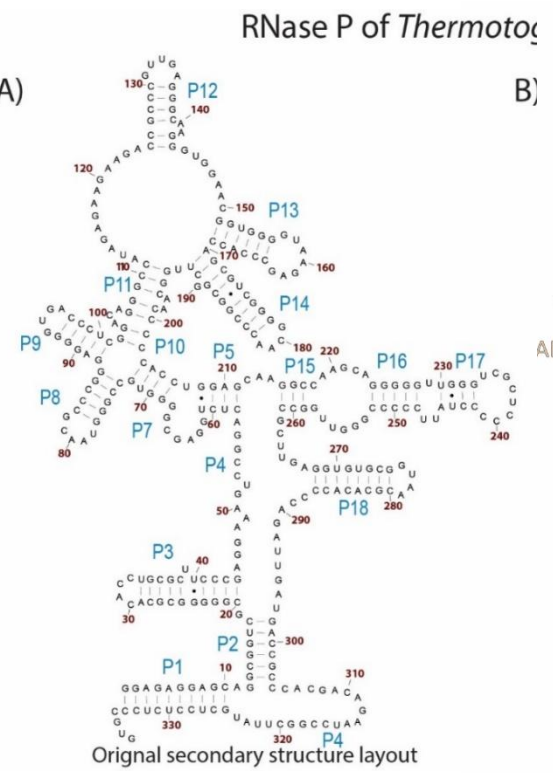


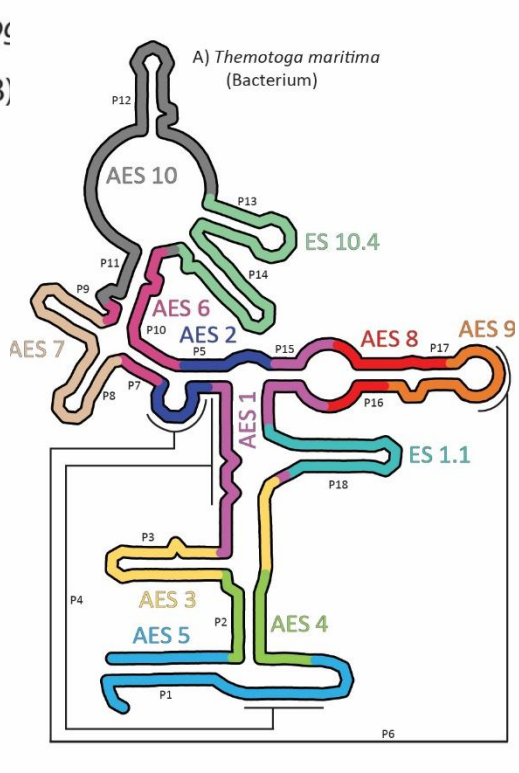
Norman Pace

The secondary structure layouts of RNase P were initially proposed by Norman Pace using covariational methods. It contained P2 as a helix and P4 as a pseudoknot

James B. D. et al. The Secondary Structure of Ribonuclease P RNA, the Catalytic Element of a Ribonucleoprotein Enzyme. Cell, 1988, 52, 19-26.;
Rivas, E. RNA structure prediction using positive and negative evolutionary information. PLOS Computational Biology, 16 e1008387, 2020.

Accretion based on canonical 2D structure of RNase P leads to topological conflicts

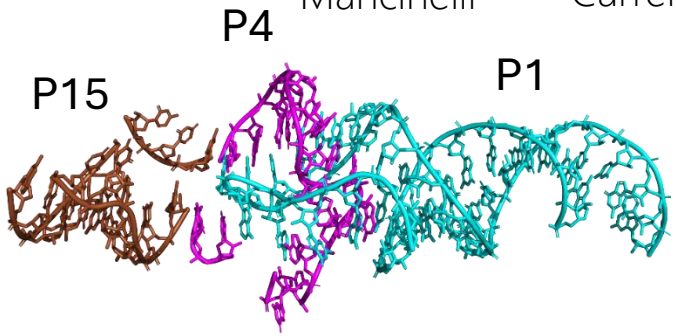




Mark Ditzler

Brooke Rothschild-Mancinelli

Claudia Alvarez-Carreño



If P4 is a pseudoknot, two expansion segments form an entanglement; this violates the continuity of the accretion process

The accretion model would only be possible from topological considerations and continuity of the accretion process, if P4 is a helix, and P2 is a pseudoknot.

BIOINFORMATICS

From knotted to nested RNA structures: A variety of computational methods for pseudoknot removal

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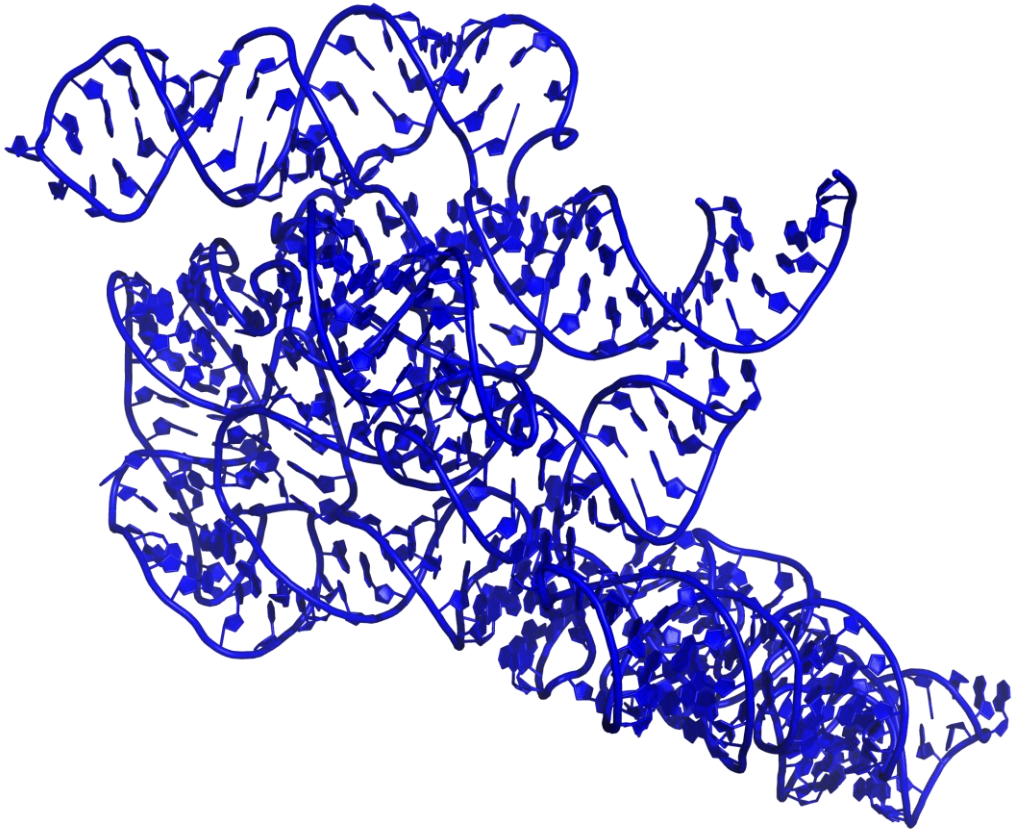
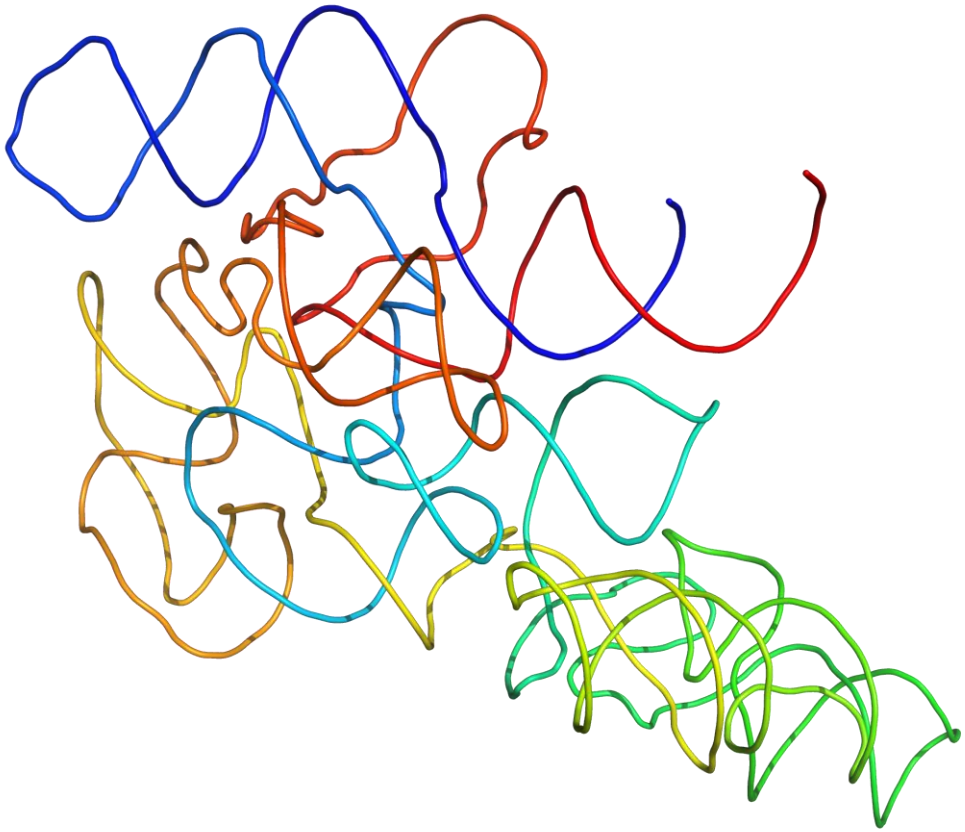
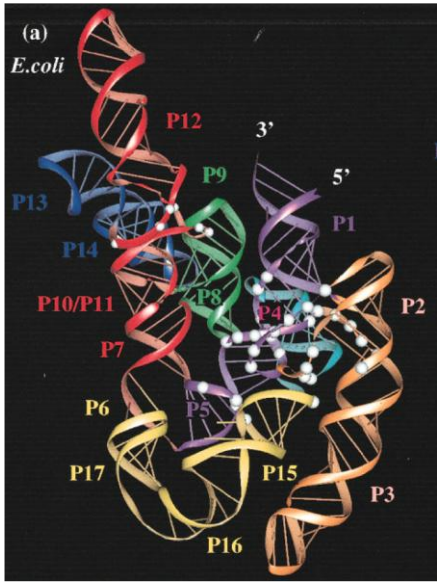
[1]Centre for Integrative Bioinformatics VU (IBIVU), Vrije Universiteit Amsterdam, 1081 HV Amsterdam, The Netherlands
[2]Centre for Medical Systems Biology, 2300 RA Leiden, The Netherlands
[3]Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, 02-109 Warsaw, Poland
[4]Department of Chemistry and Biochemistry, University of Colorado, Boulder, Colorado 80309, USA

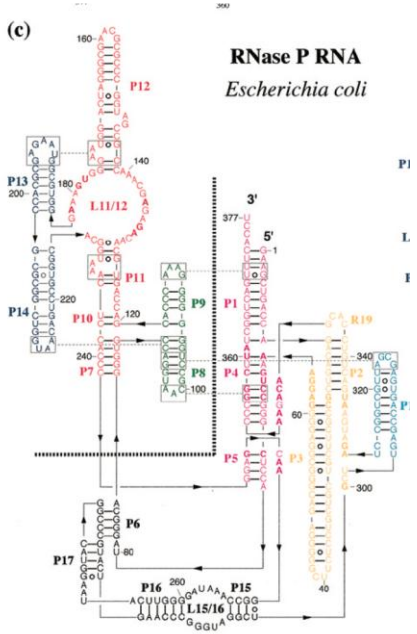
- For example, in the set of canonical base pairs from the RNaseP structure (PDB ID: 2A64) (Kazantsev et al. 2005), the EC and IR methods found a nested structure containing 82 base pairs (helix P2 broken), while the EG, IO, and IL methods reached a solution of 81 base pairs (helix P4 broken). When adding the immediate helix extensions, there were two optimal solutions containing 95 base pairs each, indicating that P2 and P4 were of equal importance in terms of the number of base pairs. The fact that multiple optimal solutions for the same knotted structure might exist is also illustrated in Figure 2. Finally, we show (in Fig. 2) that applying a different scoring function will change the outcome of the optimization approach.
- The optimization method provides a formally optimal solution ..., but this solution is often not unique. Therefore, we recommend that analyses are performed **either by using each optimal solution and averaging the results or by** using additional biologically informed criteria that are applied in a well-described and consistent manner to choose an optimal solution.

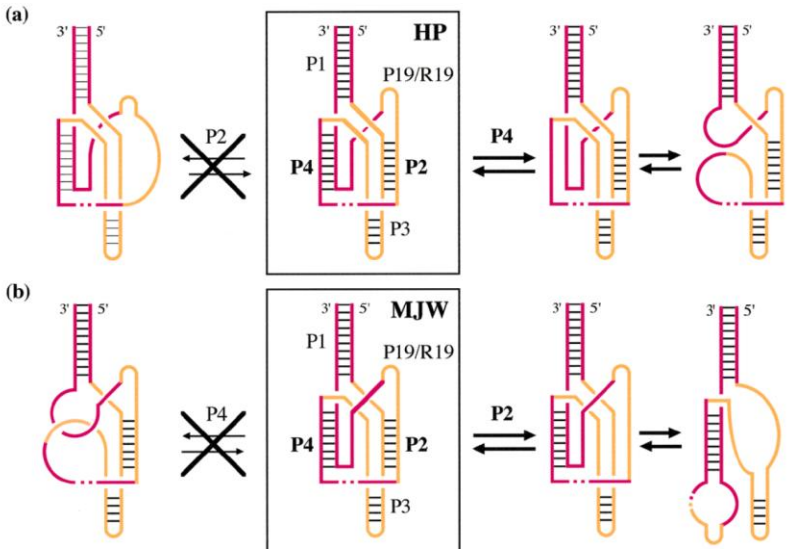




Modeling the unfolding of RNase P.



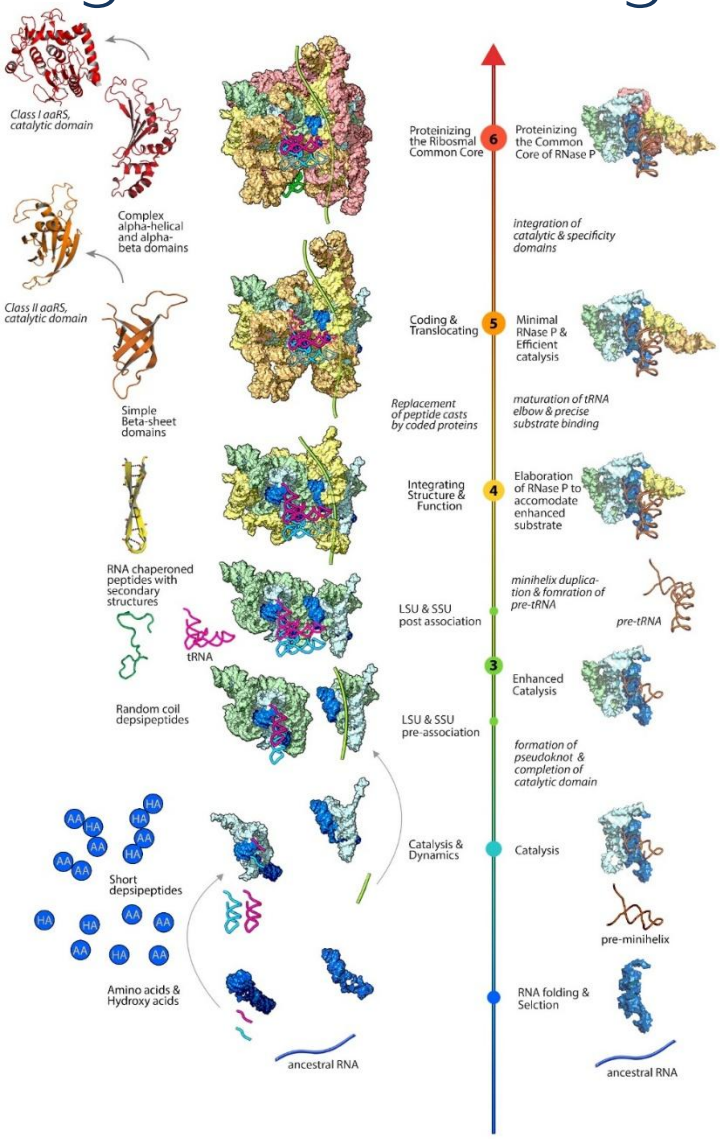


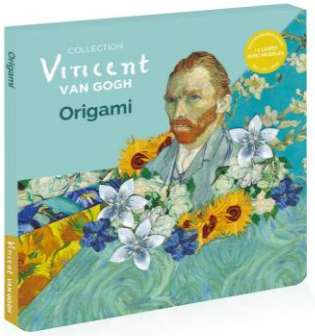


The following facts argue for an alternative folding of the pseudoknot: (i) P4 is indeed longer than P2; (ii) P2 displays greater variation in length than P4; (iii) protections towards the Fe(II)- EDTA hydroxyl radical show that P2 is almost completely exposed to solvent, whereas P4 is deeply buried in the structure; and (iv) experimental evidence for a slower formation of P2 has been reported (Zarrinkar et al.,
1996). These arguments would indicate that P2 is indeed a tertiary interaction with P4 forming the 2D structure.

Massire, C. Jaeger, L. Westhof, E. Derivation of the Three-dimensional Architecture of Bacterial Ribonuclease P RNAs from Comparative Sequence Analysis. 1998, 279, 773.

The Integrated Model for the Origins of Translation (Origami in van Gogh style)



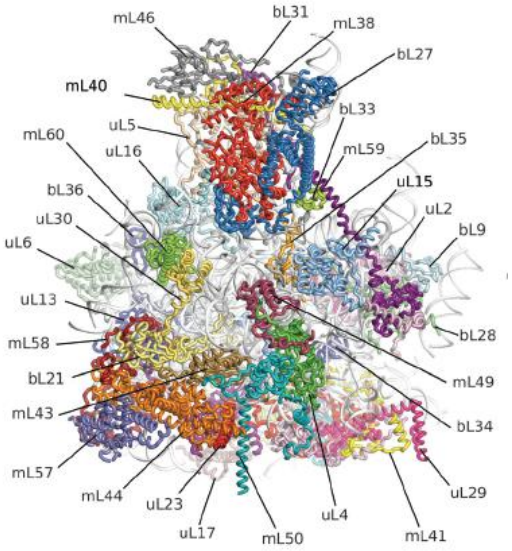


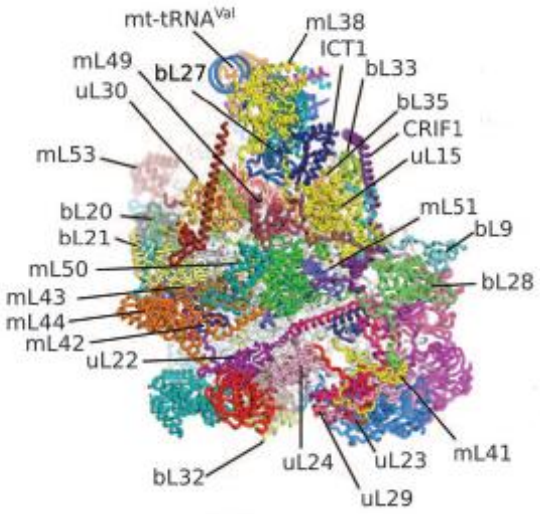
The integrated accretion model reveals **synchrony in the evolution** of ribosome and RNase P by **correlating development of these systems with tRNA** & progressing from small catalytic elements towards large multitasking molecular complexes.

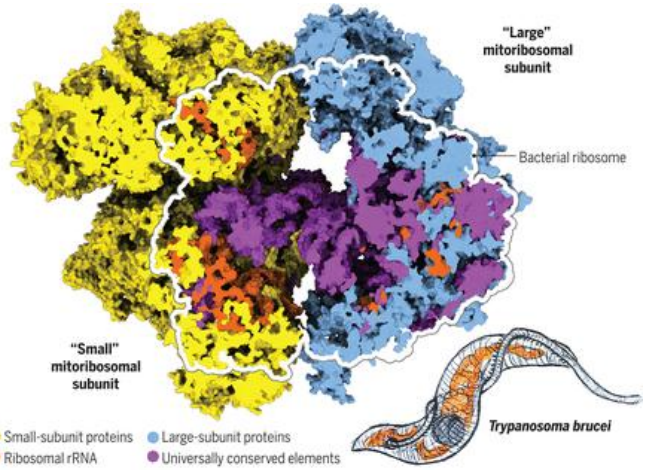
https://shop.clevelandart.org/products/origami-kit-van-gogh

Hyper-variability in Mitoribosomal Structures

3D structures revealed unprecedented remodeling unseen for ribosomes of the same type







Fungal mt-Ribosome:

mt-rRNA expansions;
mt-rProtein accretion (stabilization).

Mammalian mt-Ribosome:

mt-rRNA deletions;
mt-rProtein accretion (substitution).

Parasitic mt-Ribosome:

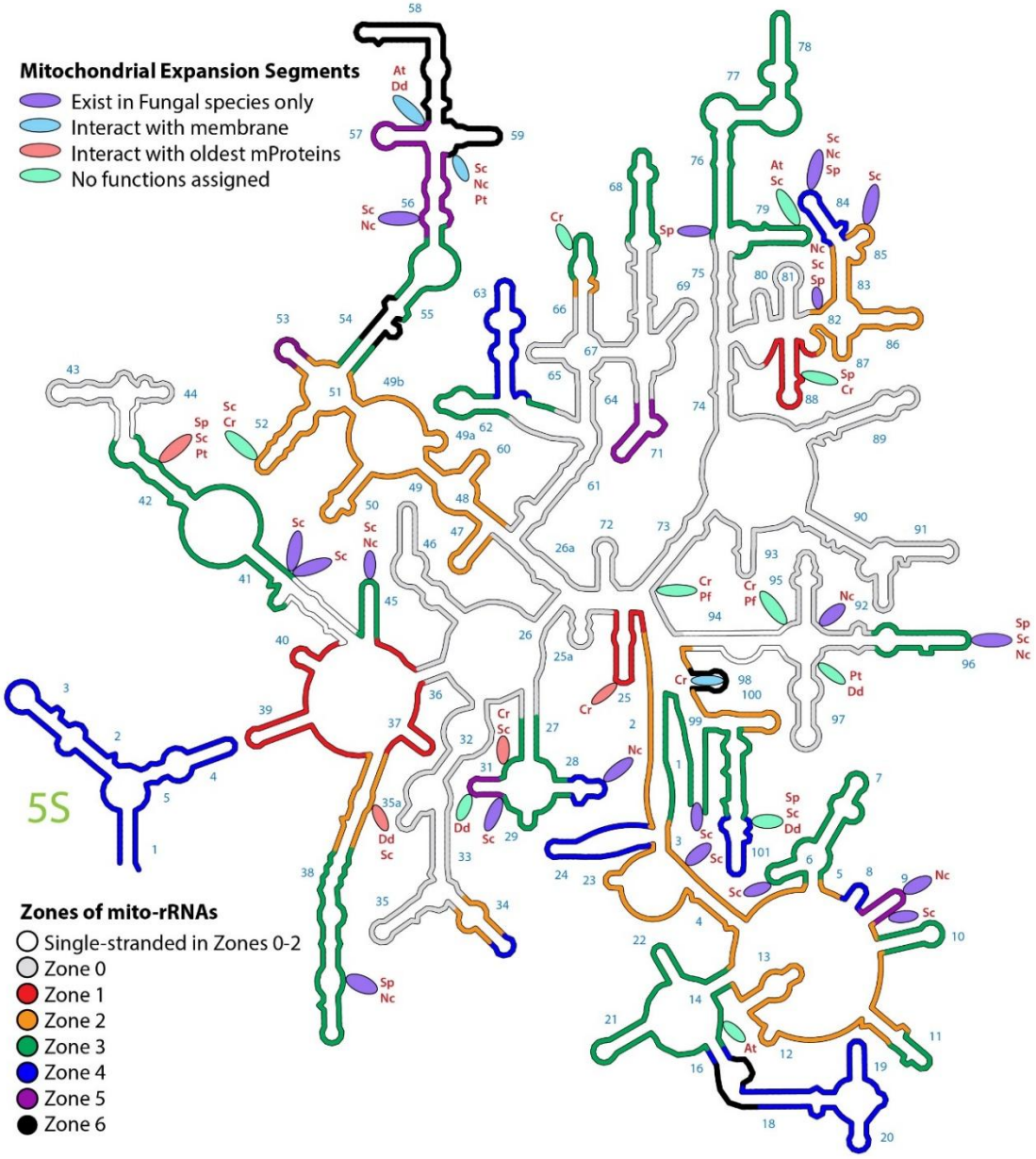
mt-rRNA severe deletions;
mt-rProtein over-accretion (protein expansion)

Science. 2014 Mar 28;343(6178):1485-9

Science. 2014 Nov 7; 346(6210): 718–722.

Science. 2018 Mar 28;362(6413):1485-9

Diversity of mt-ribosomal rRNAs



Reduction of rRNA has occurred in majority of species;

Reduction of rRNA is highest in Metazoans and parasitic protists (Zones 0-2);

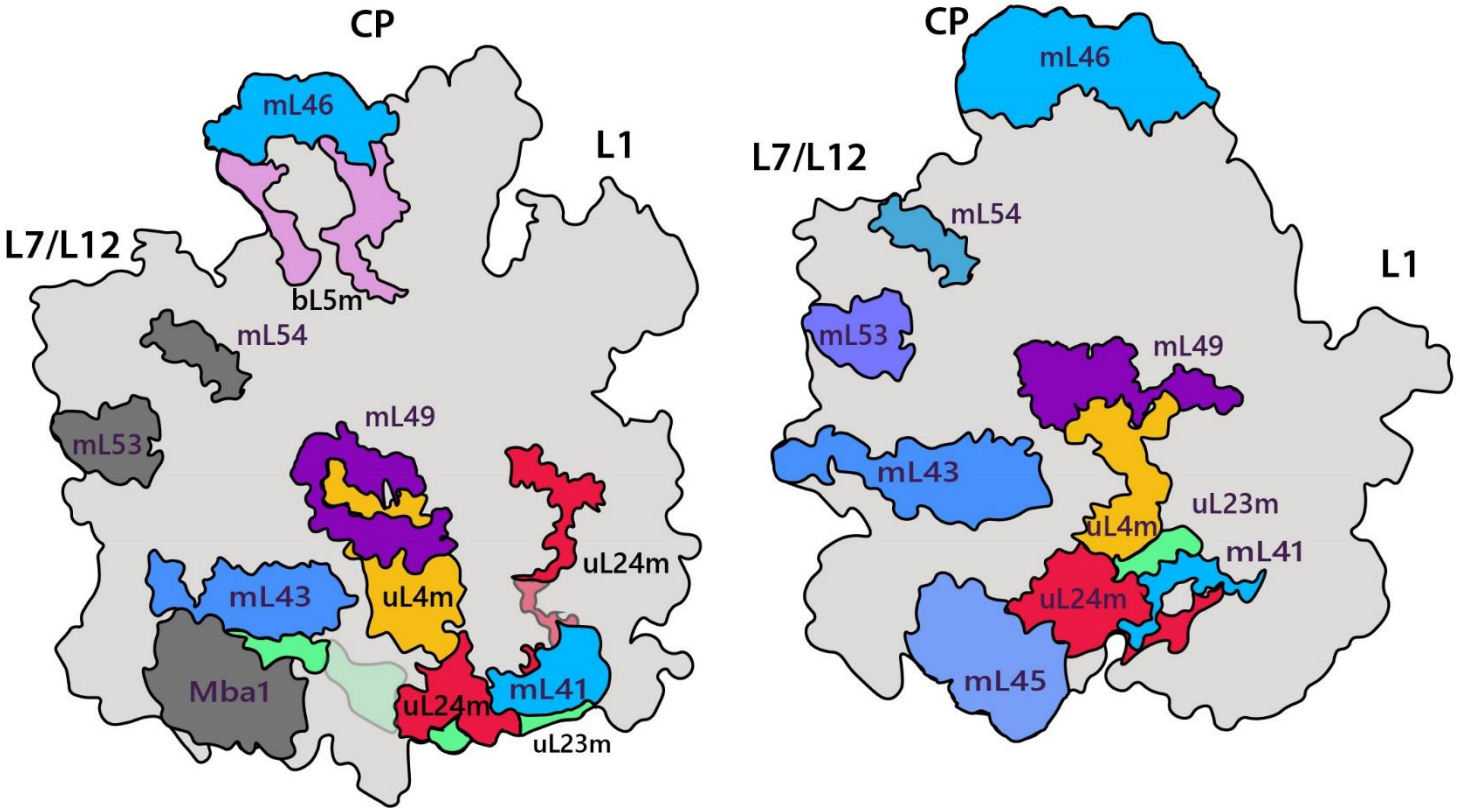
Moderate rRNA deletions occurred in Protists (Zones 3-5);

Most of deletions occurred within LSU domains I and III, which comprise the exit tunnel of the LSU;

Short rRNA expansions observed in plants and some protists;

The rRNA expansions are highly elaborated in some fungal species.

Oldest Proteins in Mito-ribosomes



Mt-ribosome of *S. cerevisiae* Mt-ribosome of *H. sapience*

Structural analysis of the available mito-ribosomal structures revealed that the oldest proteins are clustered in two regions: a) the LSU exit tunnel; b) the Central Protuberance (CP).

Petrov, A.S., Wood, E.C., Bernier, C.R., Norris, A.M., Brown, A. and Amunts, A. (2018) Structural Patching Fosters Divergence of Mitochondrial Ribosomes. Molecular Biology and Evolution, 36, 207-219.

Conclusions

- Ribosomes are molecular fossils
- Ribosomal RNA evolved by accretion
- Ribosomal common core extended by eukaryotic expansions
- Ribosomal extension segments reveal insertion fingerprints
- The same fingerprints observed in the common core
- The evolutionary development of RNase P, crucial for tRNA processing, follows an accretion model where RNA elements gradually accumulated to form its catalytic and specificity domains.
- The accretion model is only possible when helix P4 within the RNase P RNA is treated as part of the secondary structure, and P2 is considered a pseudoknot (tertiary interaction).
- We proposed a revised secondary structure layouts for a family of RNase P RNAs representing major domains on the tree of life.
- The model further offers insights into the gradual assembly of RNase P and its coevolution with tRNA and the ribosome, illuminating the evolutionary dynamics of these essential biological systems.

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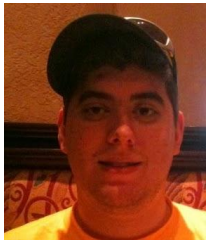
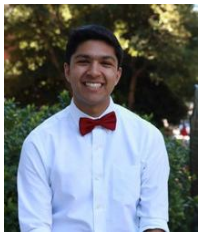


Chad Bernier



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