Processing of ribosomal RNA

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July 23, 2024

Overview

1. The ribosome
2. To H1 or not to H1
3. Leader-Trailer helices

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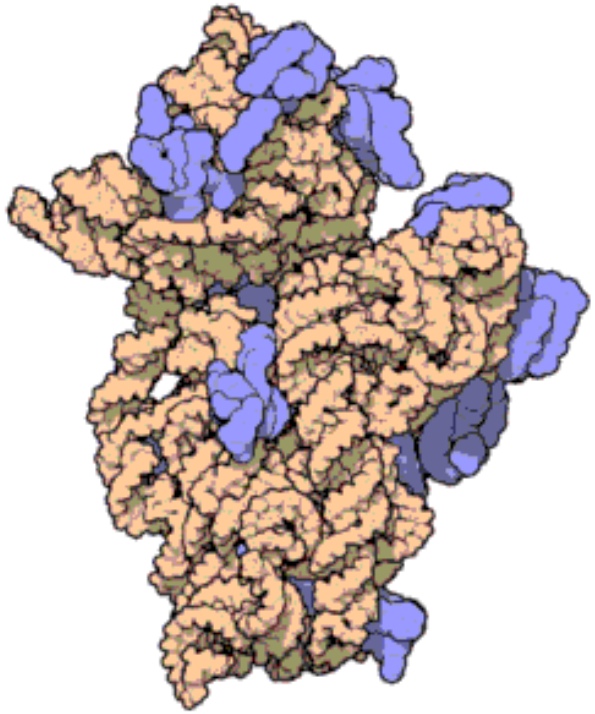
Ribosome background

Background on the ribosome

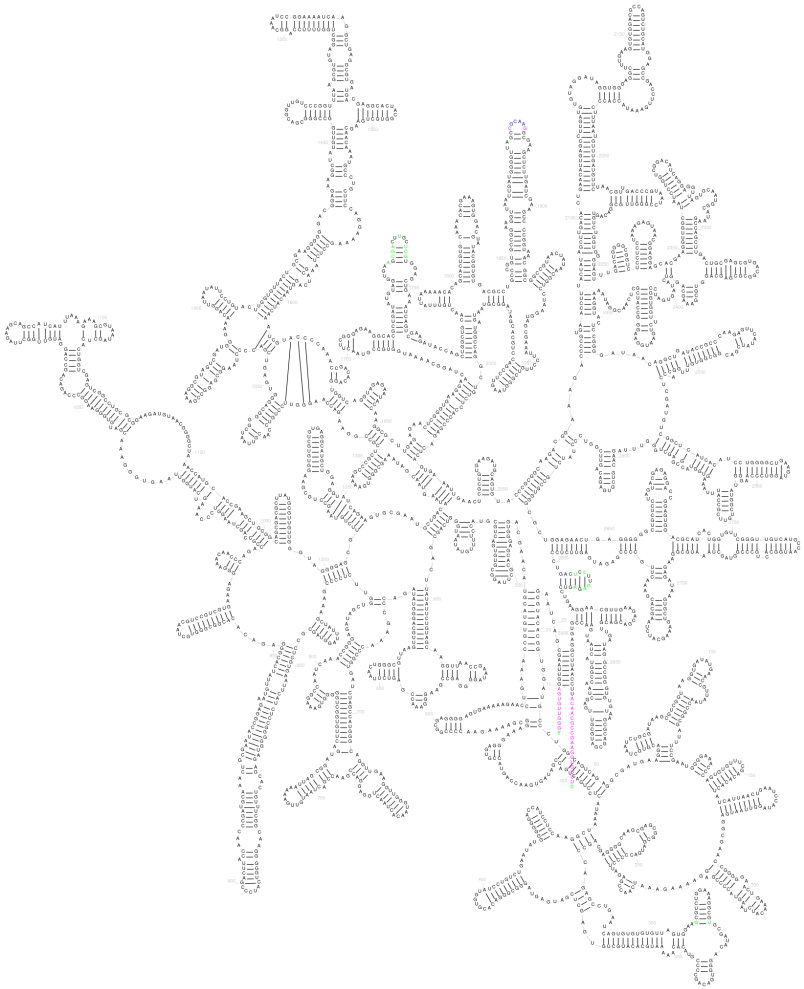
- The ribosome makes all proteins in living systems
- It has two subunits (in bacteria 30S and 50S)
- Both subunits are separately assembled and join on the transcript to be translated
- 30S subunit contains 16S rRNA, 50S subunit 23S and 5S
- All three rRNAs are transcribed together in an operon
- A lot of processing has to happen to make a mature ribosomal subunit

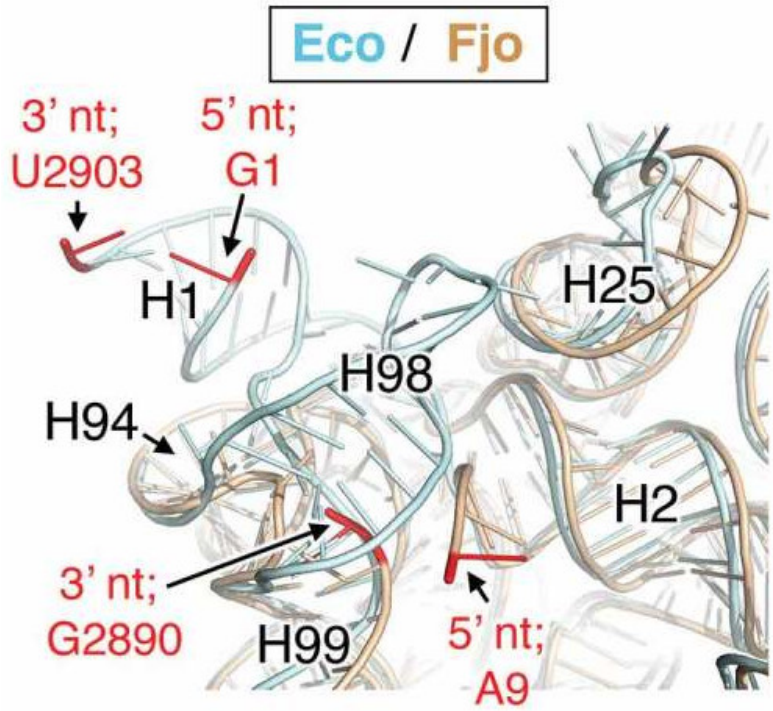


Overview

1. The ribosome
2. To H1 or not to H1
   - The problem
   - Approach #1
   - Approach #2
   - Conclusions on helix H1
3. Leader-Trailer helices

23S rRNA secondary structure from RNACentral and helix H1



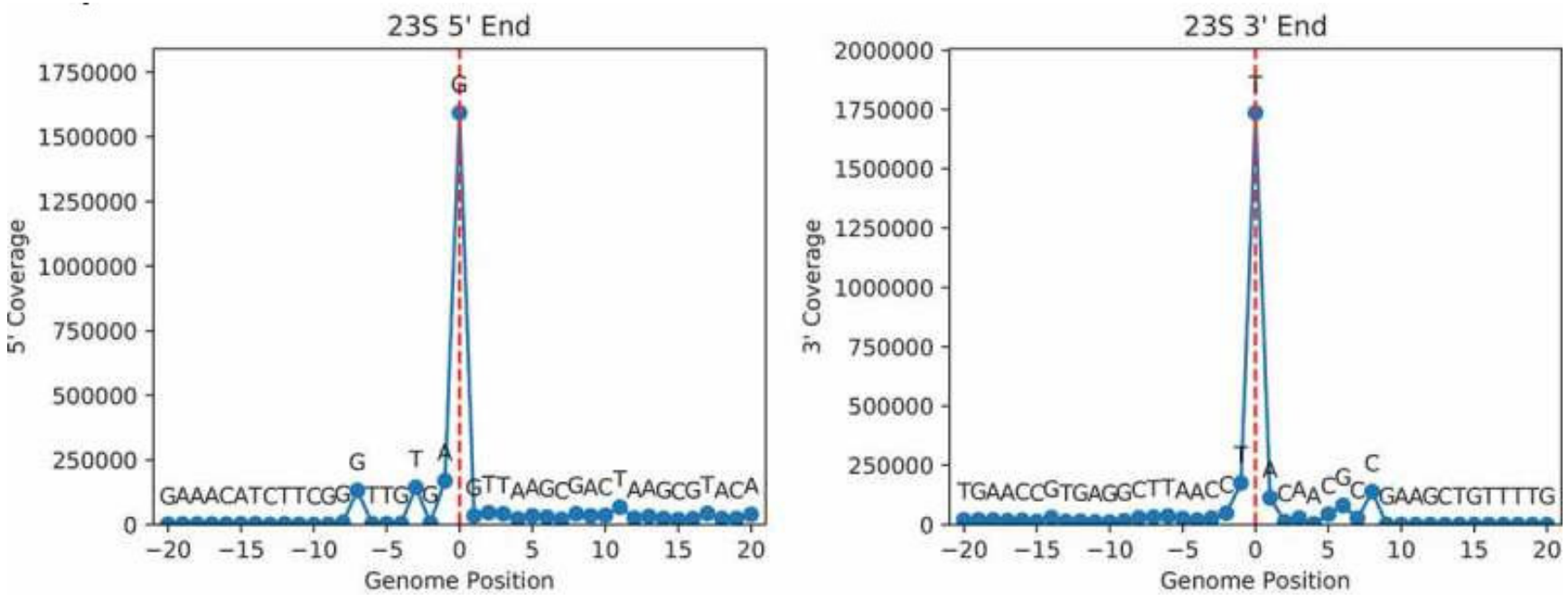


Helix H1 exists in *E. coli* but not in *F. johnsohniae*

Specialized sequencing for a few organisms

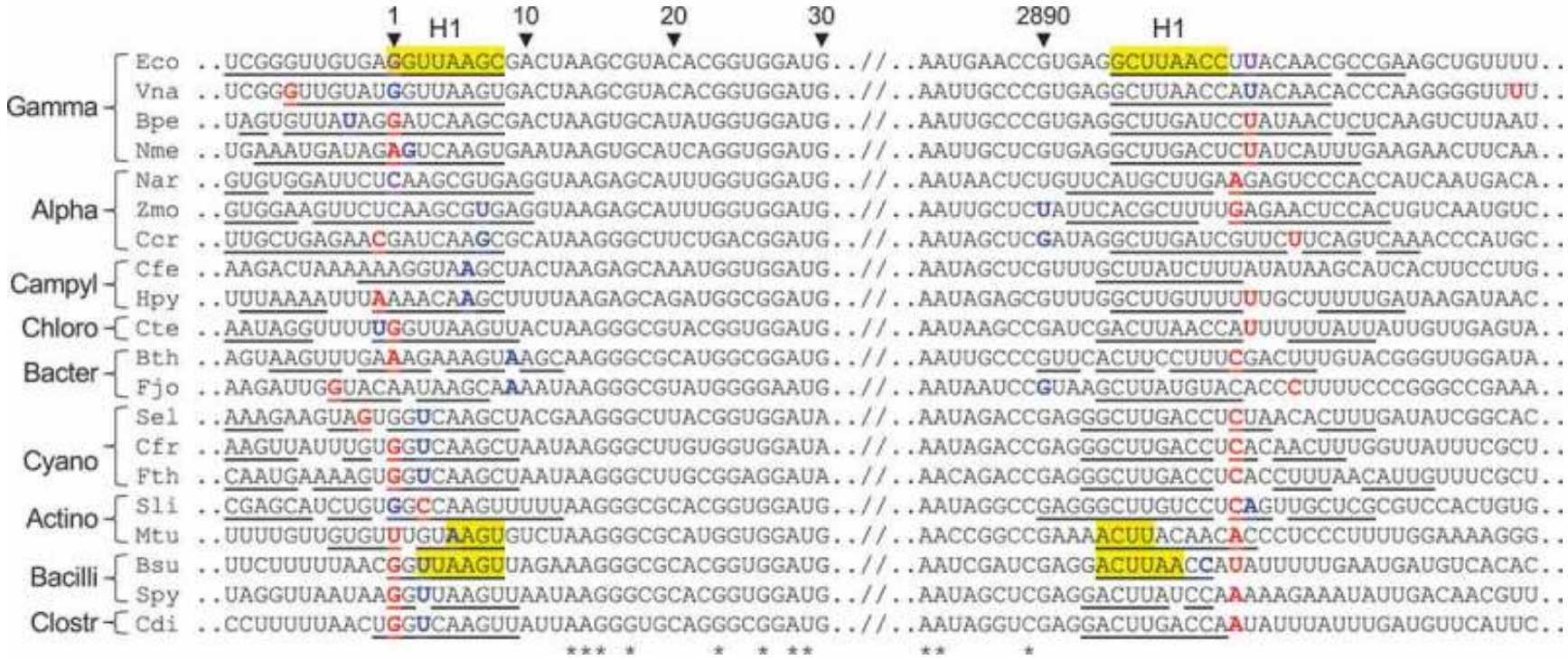
End-specific sequencing

- Find all prokaryotic dRNA-seq, TSS-seq, Term-seq, and Rend-seq data sets
- Yields data sets for 19 prokaryotic species
- Map to 23S rRNA and record ends



Results from approach #1

23S findings from specialized sequencing



- Several organisms are missing H1
- rRNA ends are horrendously misannotated
- Organisms mostly either have the full H1 or no H1 at all

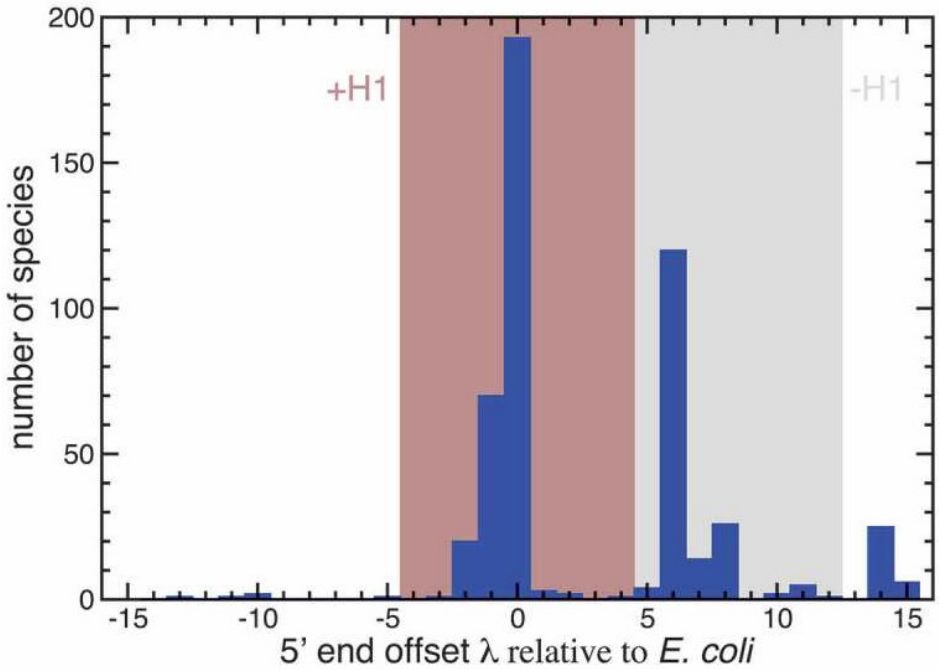
Trash to treasures - more organisms

Large scale 5' end reannotation

- Obtained metatranscriptomic data set from TARA Oceans consortium
- TARA Oceans used Clontech SMART-seq kit following RiboZero
- SMART-seq adds non-encoded GGG to the 5' end of an RNA before adding the template switch oligo
- Identified 10^8 reads that start with GGG and include a conserved motif from the 5' end of 23S rRNA
- Align these reads to a database of prokaryotic 23S rRNA sequences
- Target identifies species; location identifies 5' end
- Obtained 5' annotations for 23S rRNA in 498 species

Again either full or no H1

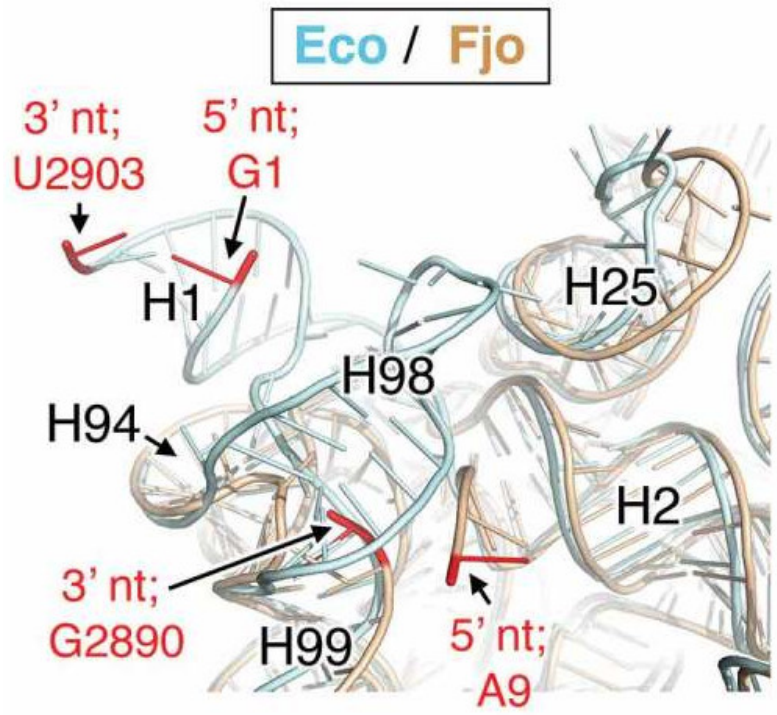
Histogram of 5' end positions



- Species either do or don't have H1
- About half of the species have H1

Keeping H1 aligns with phylogeny

Phylogenetic distribution of H1



- Retention of H1 strongly correlates with phylogeny
- Retention of H1 also strongly correlates with existence of H98

Conclusions

Conclusions

- Helix H1 is removed in about half of bacteria
- Absence of H1 correlates strongly with absence of H98
- Helix H1 is likely excised during subunit maturation
- SMART-seq allows 5' end annotation *en masse*

Future work

- Apply SMART-seq trick more broadly to improve 5' end annotations

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1. The ribosome
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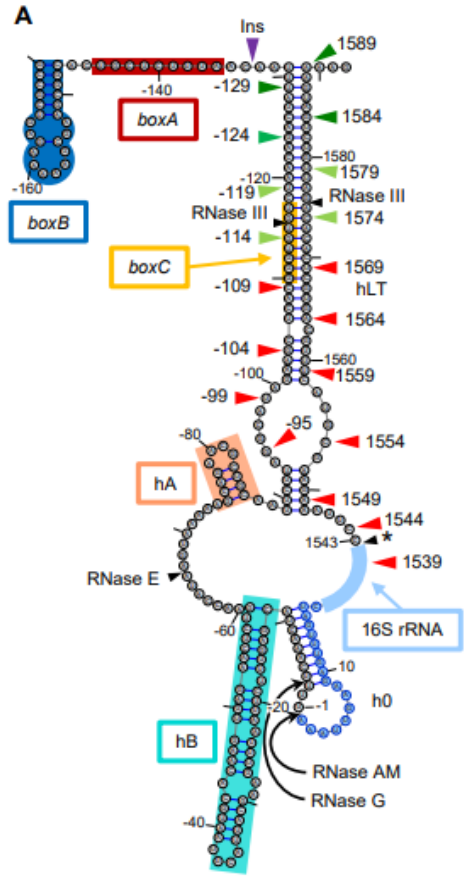
3. Leader-Trailer helices
   - The problem
   - Measuring leader-trailer helices
   - Approach #1
   - Approach #2
   - Results
   - Conclusions on LT helices

Leader-trailer helices

- *E. coli* ribosome subunits assemble from processed rRNAs *in vitro*
- However, this process is slow and requires non-physiological conditions
- In *E. coli*, rRNA are flanked by leader-trailer helices
- In *E. coli*, leader-trailer helices are essential for *in vivo* assembly



The question

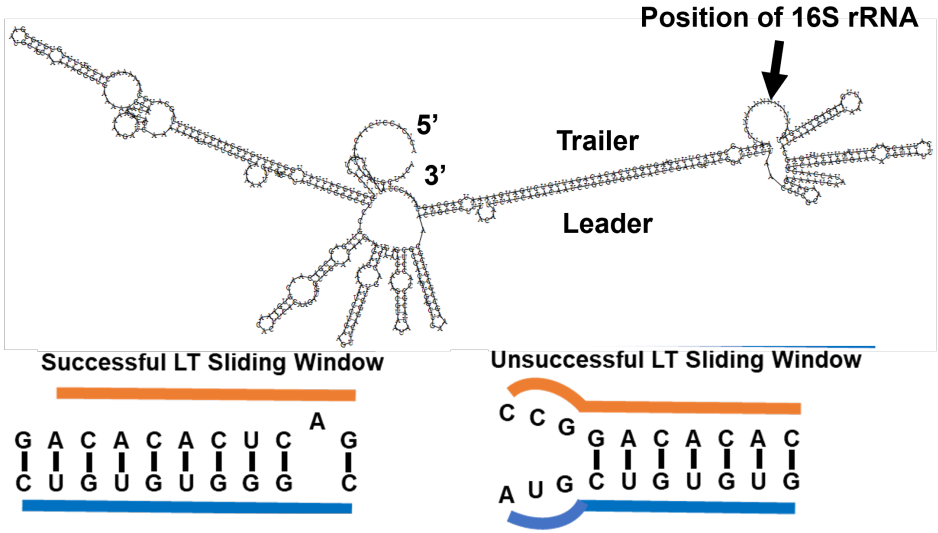
The question

- If LT helices are that important, they should be universal
- But prior work (Saito *et al.*, 2000) found no LT helices in *Deinococcus radiodurans* and some others after analyzing a handfull of species
- Question: Who has leader-trailer helices?

Quantitating LT helices

Counting successful sliding windows (SSWs)

- Concatenate leader and trailer
- Predict structure
- Successful Sliding Window (SSW): A window of length 10 that has at least 8 base pairs with a window of size 10 in the opposite strand

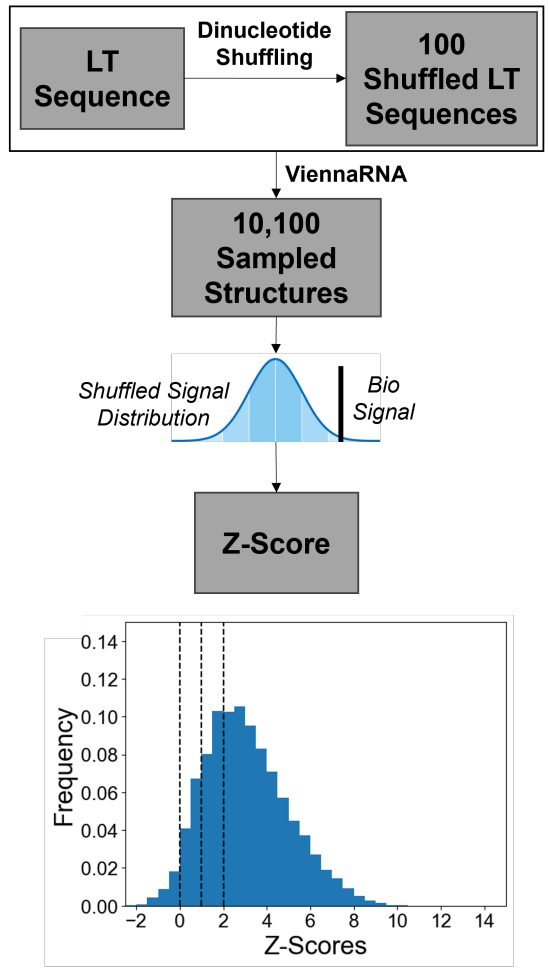


- Quantify LT "helicity" by counting SSWs

Shuffling method

The shuffling method

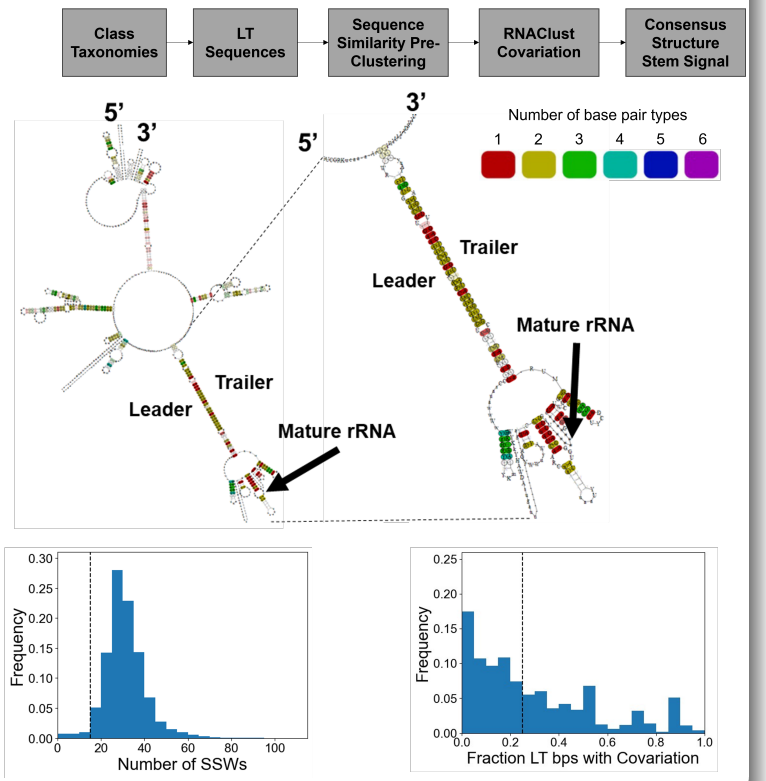
- Take leader and trailer up to next annotated gene (max 500/300nts)
- Average #SSWs for each sequence over 100 randomly sampled structures
- Redo for 100 dinucleotide shuffled sequences
- Calculate z-score
- Distribution skews heavily positive
- Downside: false negatives due not including enough nts



Covariation method

The covariation method

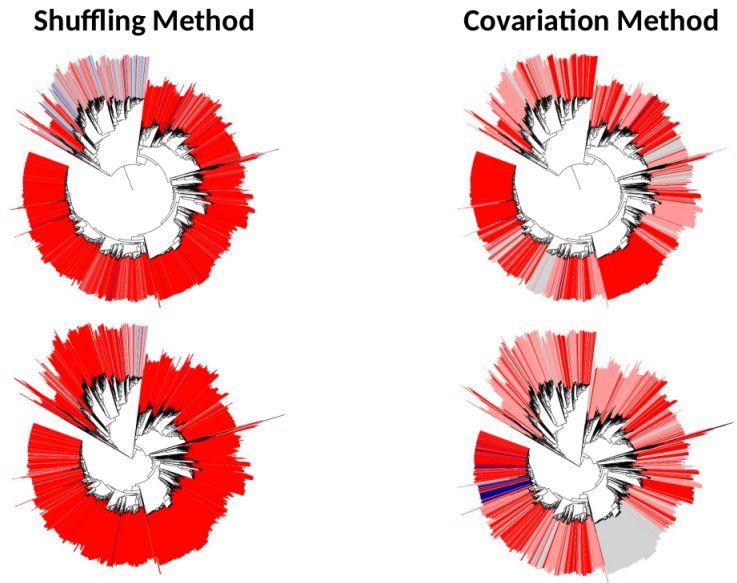
- Take 500nt leader and 300nt trailer
- Divide sequences by phylogenetic class
- Pre-cluster sequences
- Run RNAClust
- Get LocARNA consensus structure
- Count SSWs and look at covariation
- Upside: Irrelevant parts of sequence "disappears"
- Downside: missing predictions due to clusters of one



Approaches complement each other

Comparison of approaches

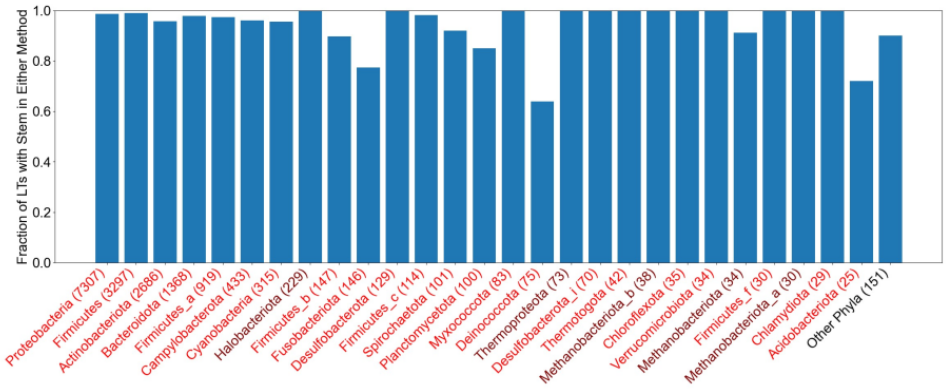
- Show on phylogenetic tree
- Here: Proteobacteria
- Red: has LT helix
  Blue: no LT helix
  Gray: no prediction
- Most species have a LT helix in at least one of the two methods
- Actually, most have one in both



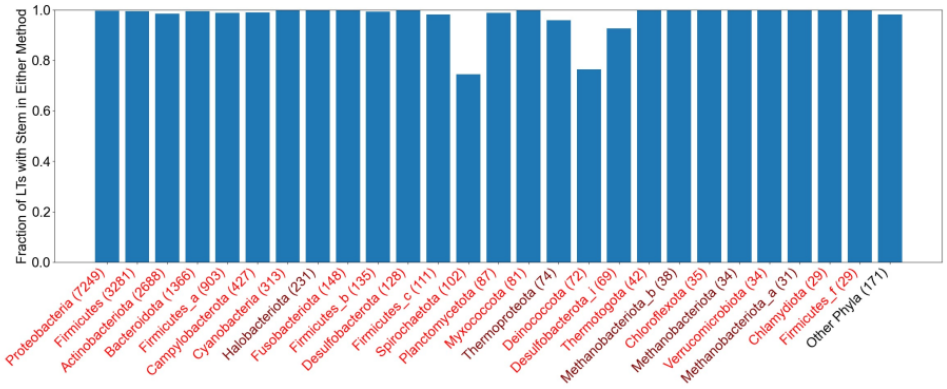
LT helices are ubiquitous

Statistics for all bacterial and archaeal phyla

16S



23S

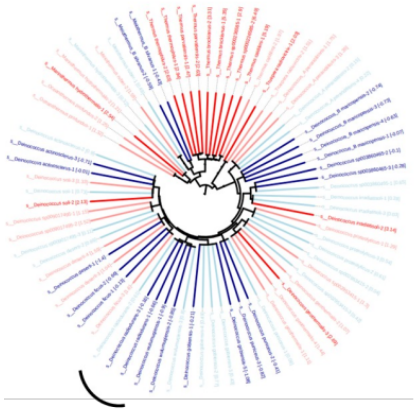


- LT helices are ubiquitous
- Manual inspection of "missing" cases still finds LT helices

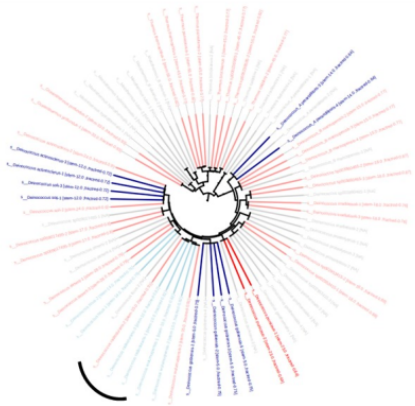
Deinoccocota

Situation in Deinoccocota

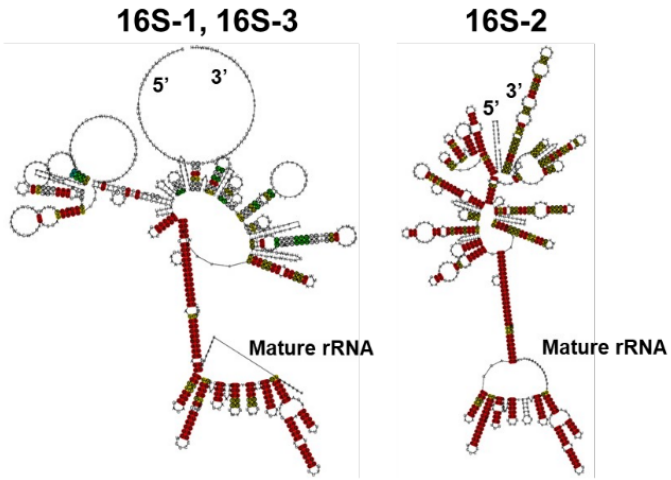
Shuffling Method



Covariation Method



D. radiodurans Consensus Structures



- Even Deinococcota have clear LT helices
- They are interrupted by bigger bulges/internal loops

Conclusions

Conclusions

- LT helices are ubiquitous in bacteria and archaea
- Quantifying "helicity" is actually not trivial
- One always has to calculate ensemble quantities

Future work

- Look at TT and LT structures systematically, which are more diverse
- Look at operon-to-operon differences within a species

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The people

Bryan Gemler

Elan Shatoff

Ben Warner

Kurt Fredrick

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- National Science foundation
  - MCB-2029502, MCB-2344534
- National Institutes of Health
  - R01 GM072528