Insights to the Early Evolution of Earth Life From Studies of the Ribosome

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Seven Pines XV-Origins of Life
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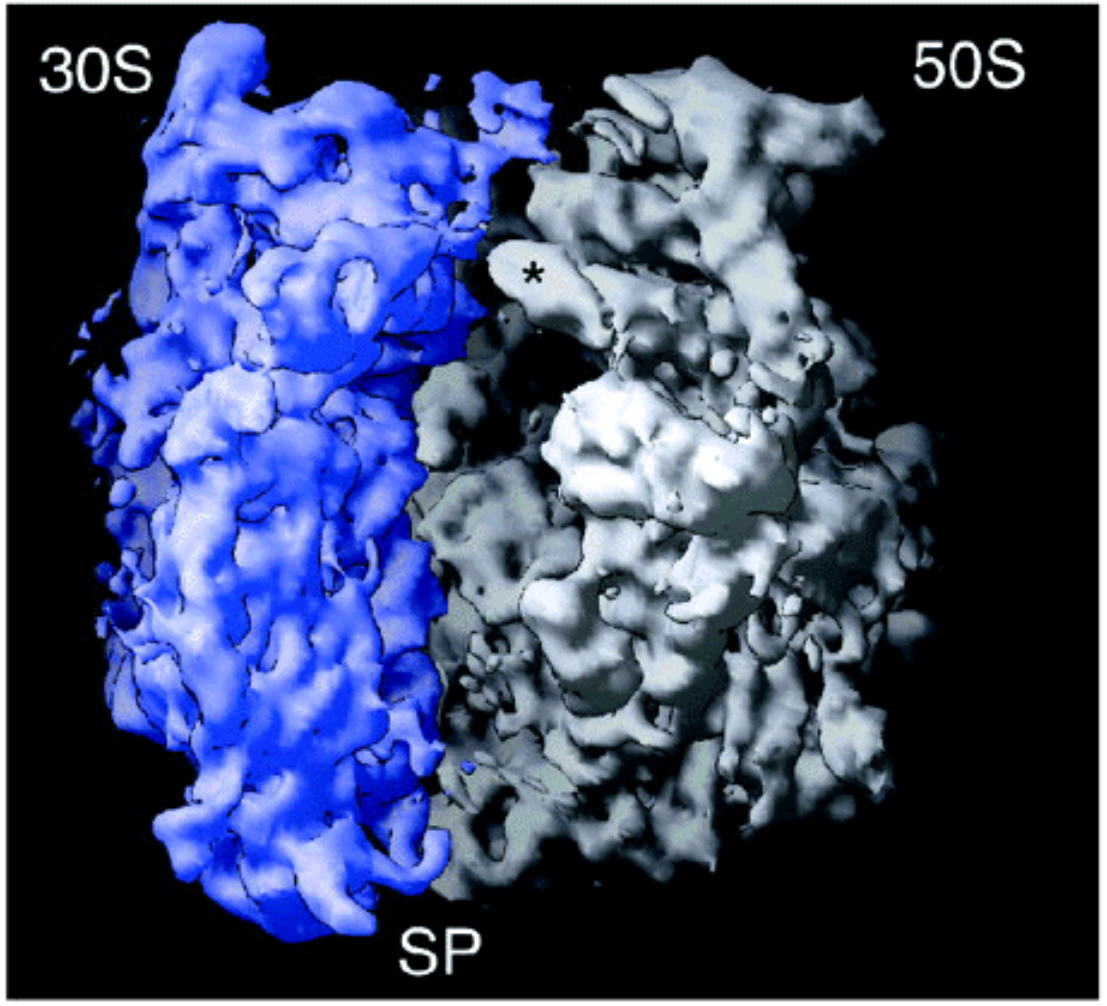
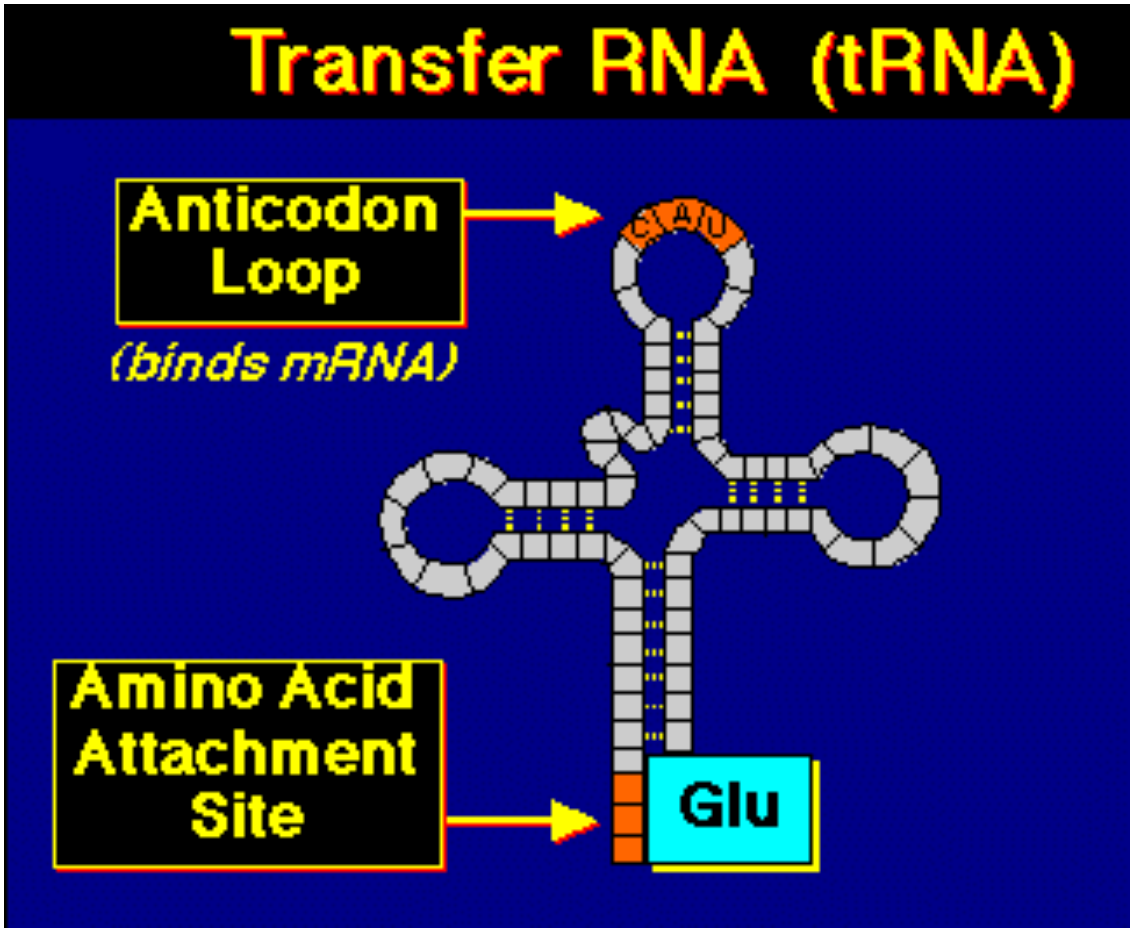
Modern Ribosomes Appear Very Complex

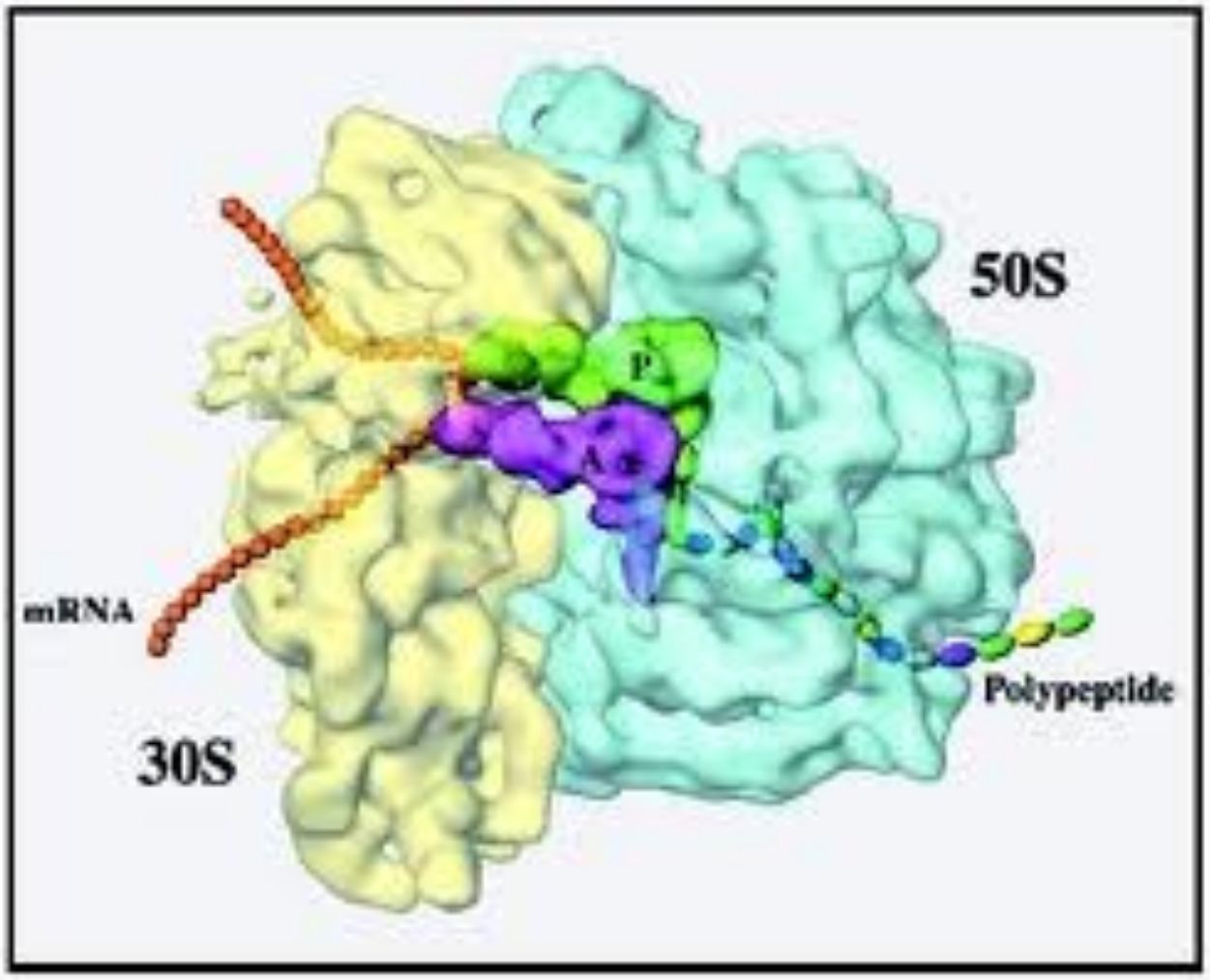
Cate et al., Science 285: 2095-2104 (1999)

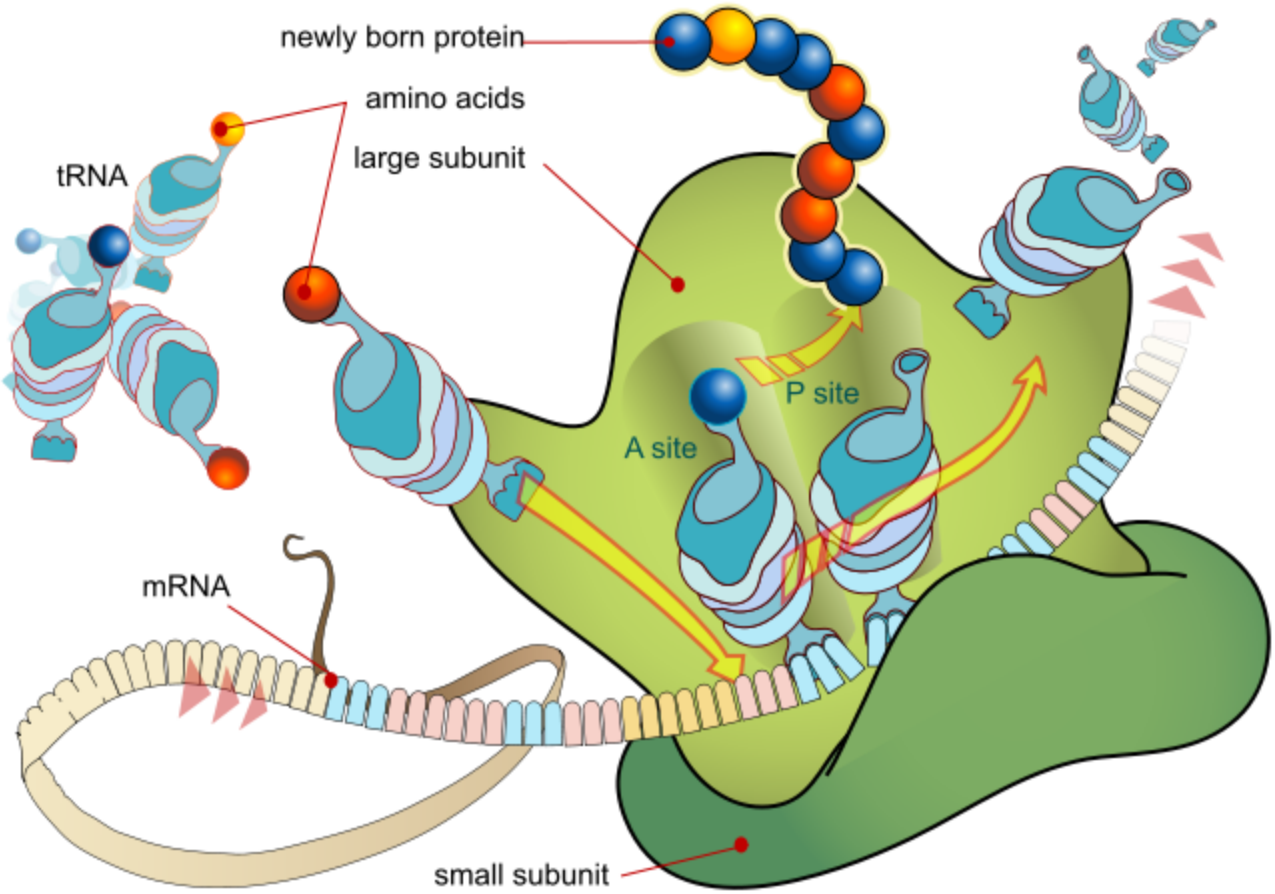


The Translation Machinery Has Numerous Components

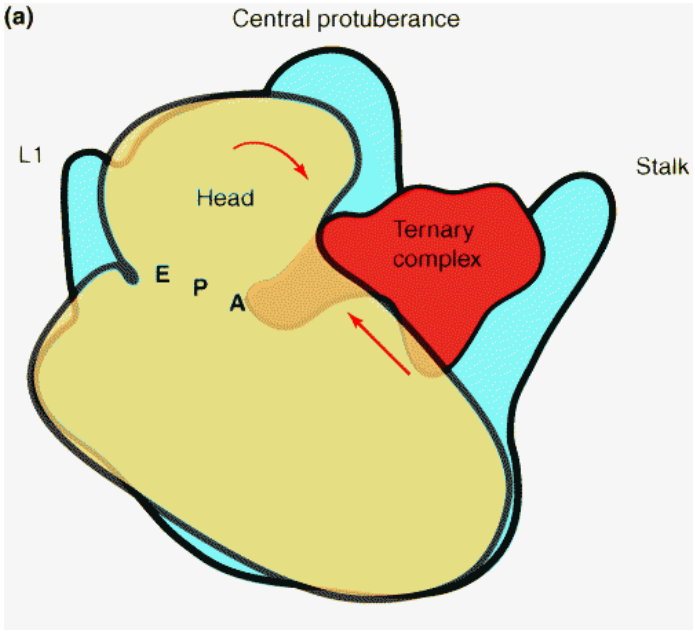
- 16S rRNA, 23S rRNA, 5S rRNA
- tRNAs, Aminoacyl tRNA synthetases
- mRNA
- Ribosomal proteins (more than 50)
- Factors- EF-G, EF-Tu, IF-2, etc.
- rRNA processing- RNAse P, III, E, etc.
- Modification enzymes, pseudouridine, etc.

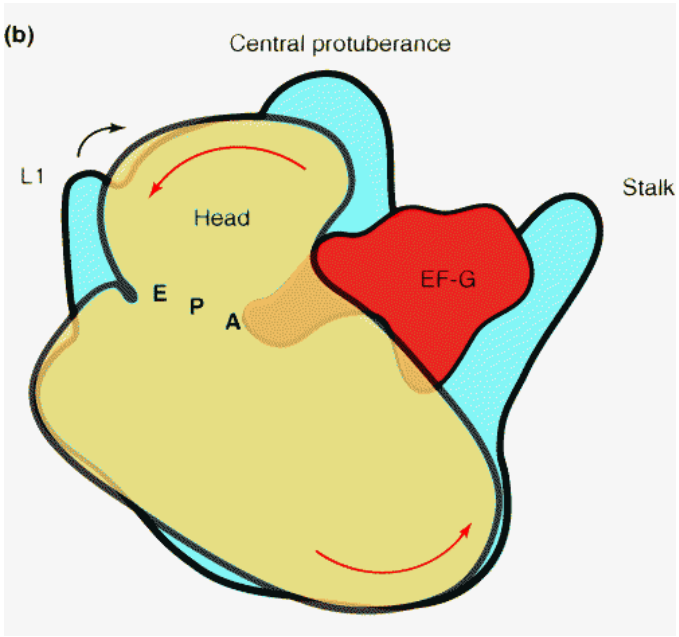

Transfer RNA (tRNA)
Anticodon Loop
(binds mRNA)
CUU
Amino Acid Attachment Site
Glu


50S
P
A
mRNA
Polypeptide
30S


newly born protein
amino acids
large subunit
tRNA
P site
A site
mRNA
small subunit

Ratcheting Motion





30S subunit ratchets relative to the 50S with the result that the mRNA moves relative to the ribosome

When did Translation Evolve?

- Numerous (but not all) components are conserved in all three major lines of descent.

- Implication-1: Translation machinery was operational before LUCA.

- Implication-2: Translation still actively evolving when LUCA appeared.

Ribosome History Relates to Key Questions In Origins

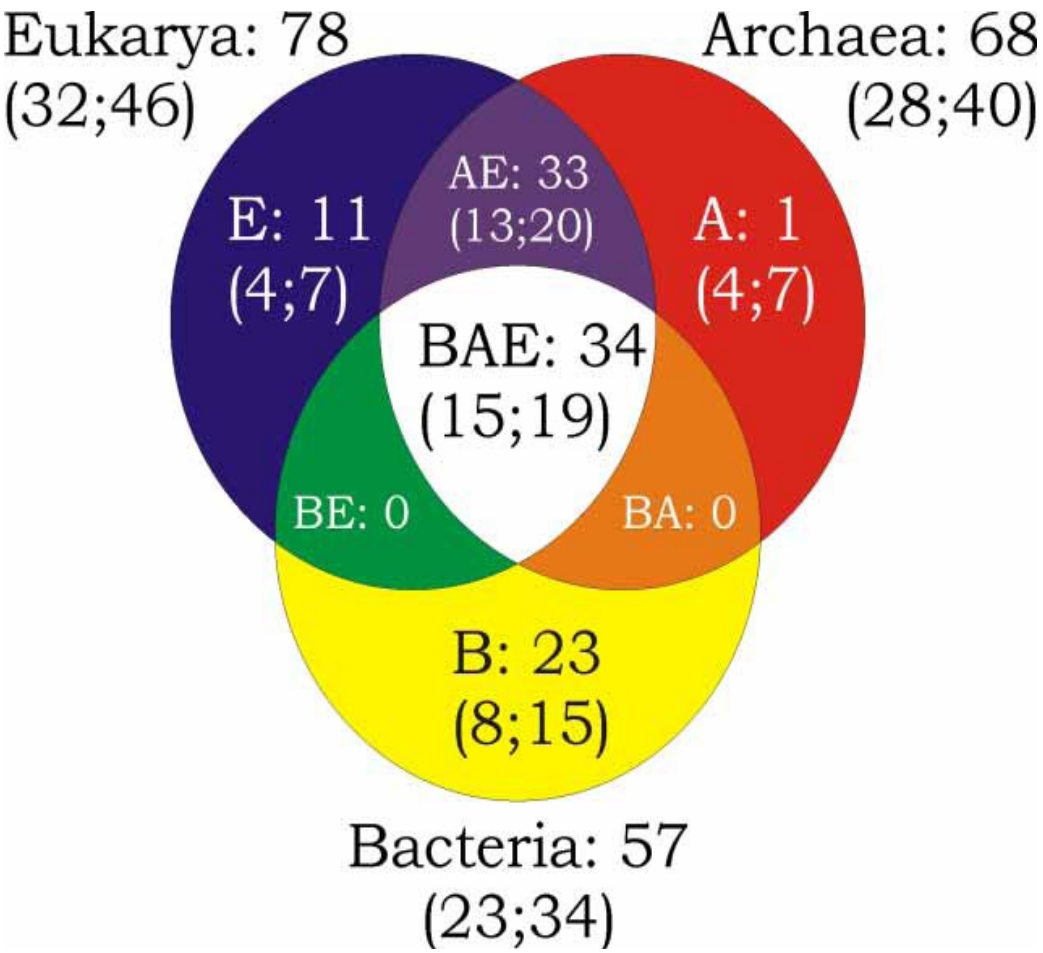
- When did the code arise?
- How did peptide synthesis begin?
- How did chiral peptide synthesis begin?
- Why did LUCA emerge when it did?

How Can We Gain Insight To Ribosome Evolutionary History

Timing Events provide insight to relative age of various components

About 34 Ribosomal Proteins Are Universal

The universal proteins are likely to be the oldest



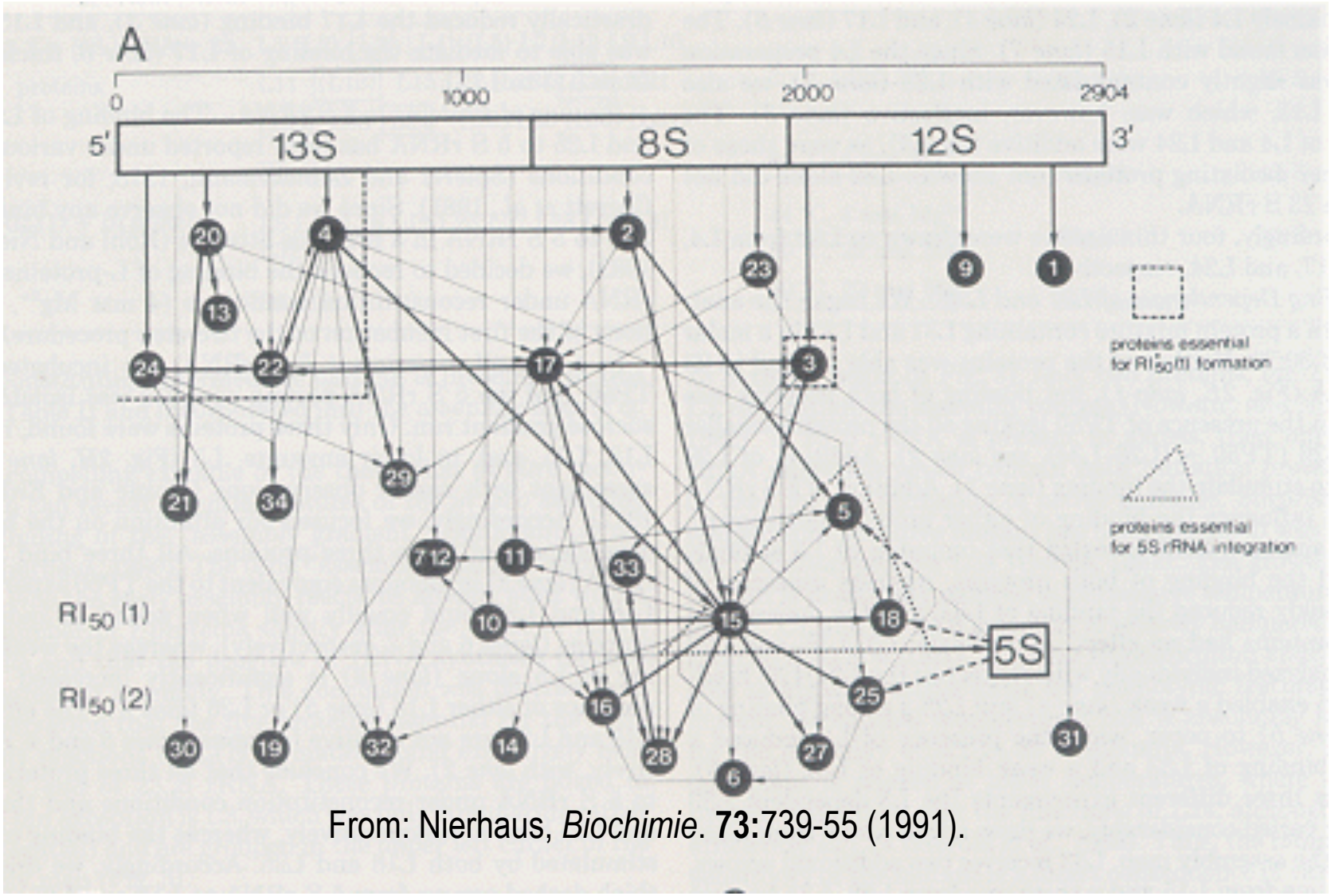
Can we tell which of the universal ribosomal proteins are the oldest among the old??

Some ways we might get insight include

1. Where is it in the assembly map?
2. Which operon is it in?
3. Where does it interact with the rRNA?
4. Where is it along the exit tunnel **?**

50S Subunit Assembly Map

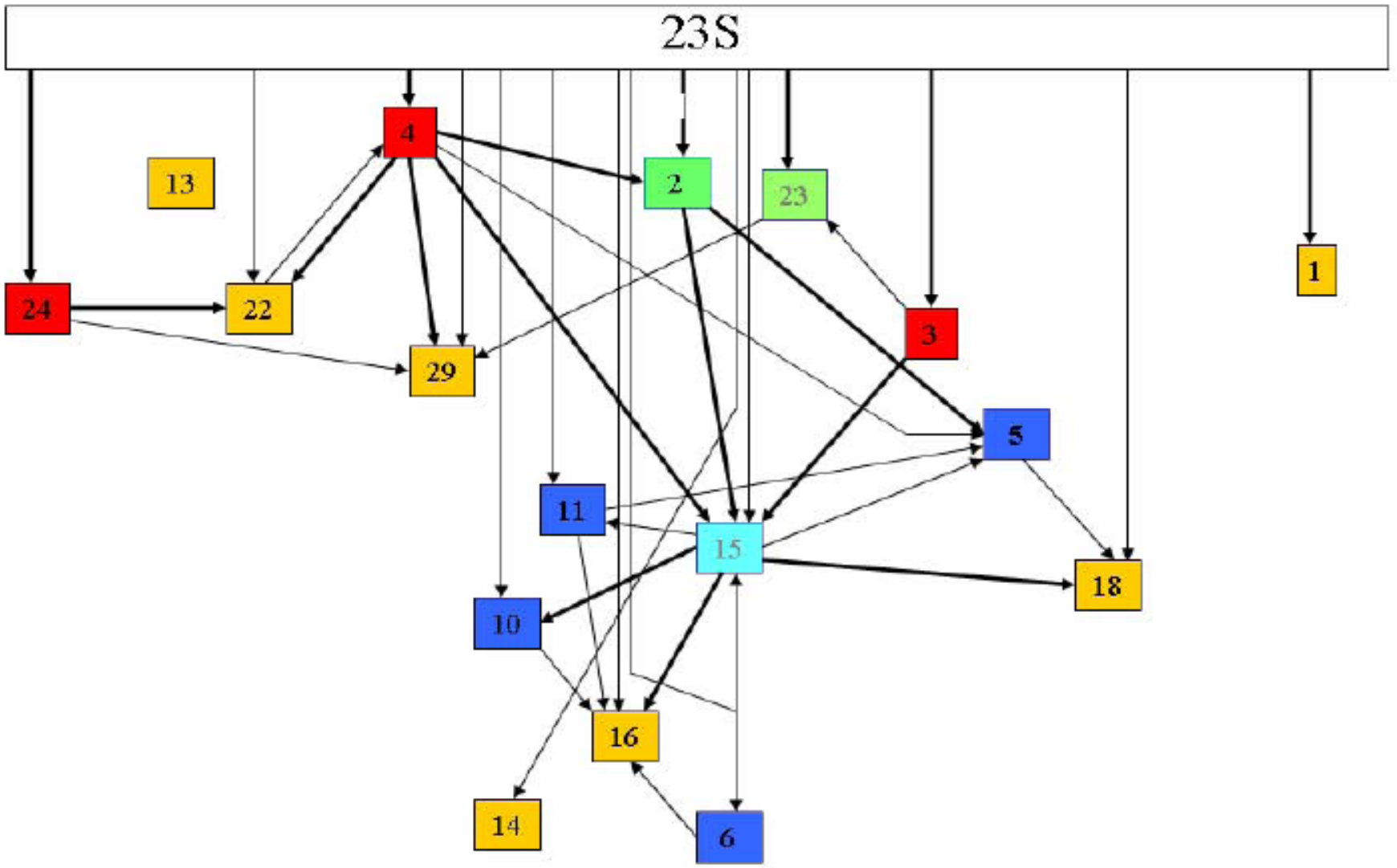
Assembly Map: The last proteins to be incorporated are typically not universal: Maybe the map partially recapitulates ribosome history!?



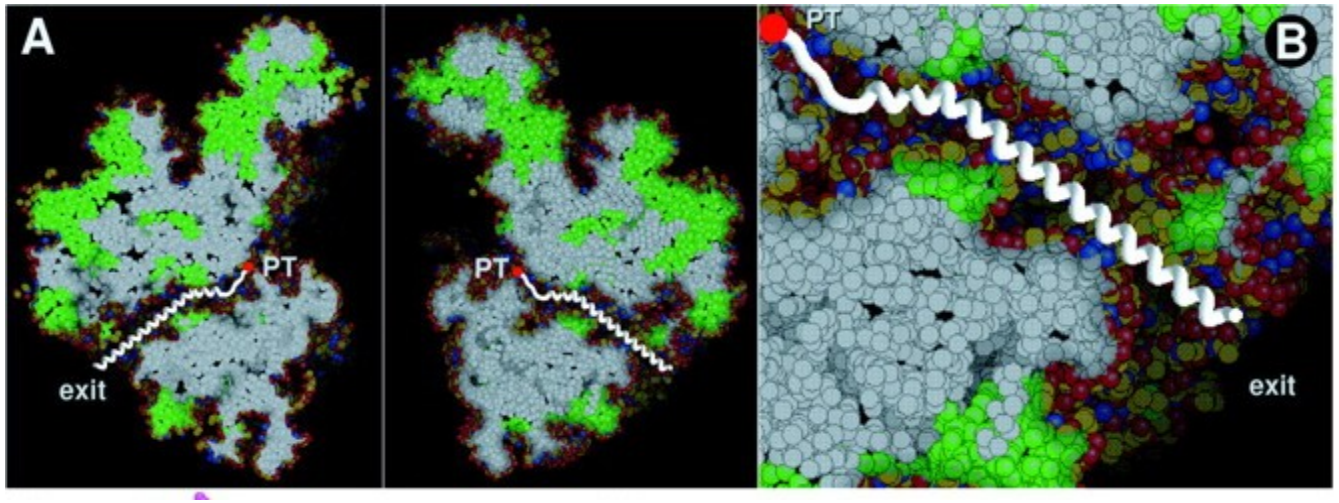
From: Nierhaus, *Biochimie*. **73:**739-55 (1991).

Large Subunit "Universal Protein Only" Assembly Map

Timing Hypothesis: First In & Most Central are Oldest



Nascent Proteins Emerges from the 50S Subunit Via an Exit Tunnel

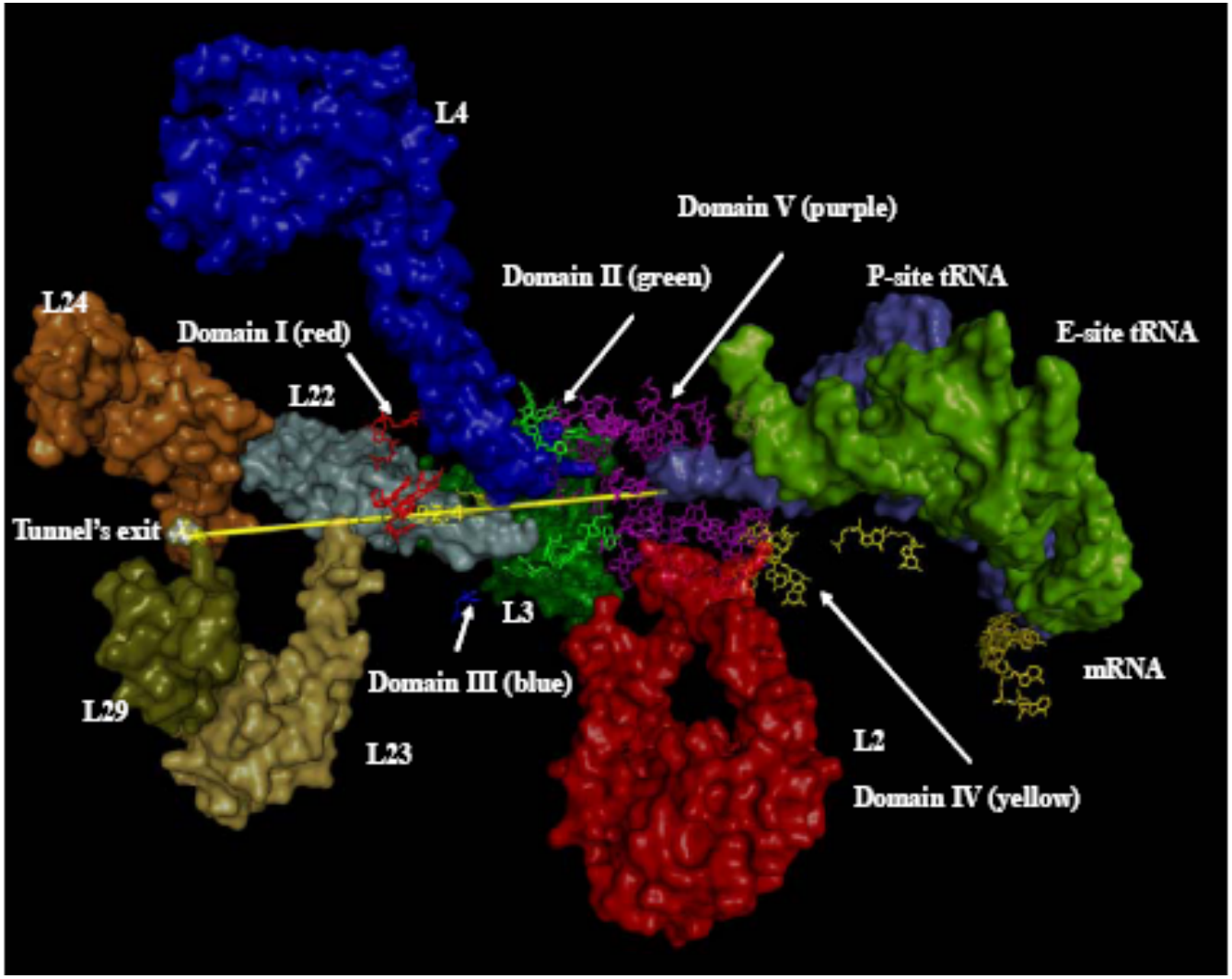


Timing Hypothesis

Ribosome grew larger over time thereby requiring extension of the tunnel

Nissen *et al*., Science **289:** 920-930 (2000).

Exit Tunnel –Only Proteins Shown



Relative Age of Proteins Suggested by Exit Tunnel Position

L2-> L3/L4 -> L22 -> L23/L24/L29

This is consistent with the assembly map

Candidate Oldest r-Proteins

- Group1: **L2, L3, L4**
- Group 2: **L22, L23, L24**
- Group 3: **L15**
- Group 4: **L5, L6, L10, L11, L13, L14, L16, L17, L18, L29**

23S rRNA: An RNA This Large is Unlikely to Spontaneously Arise in an RNA World

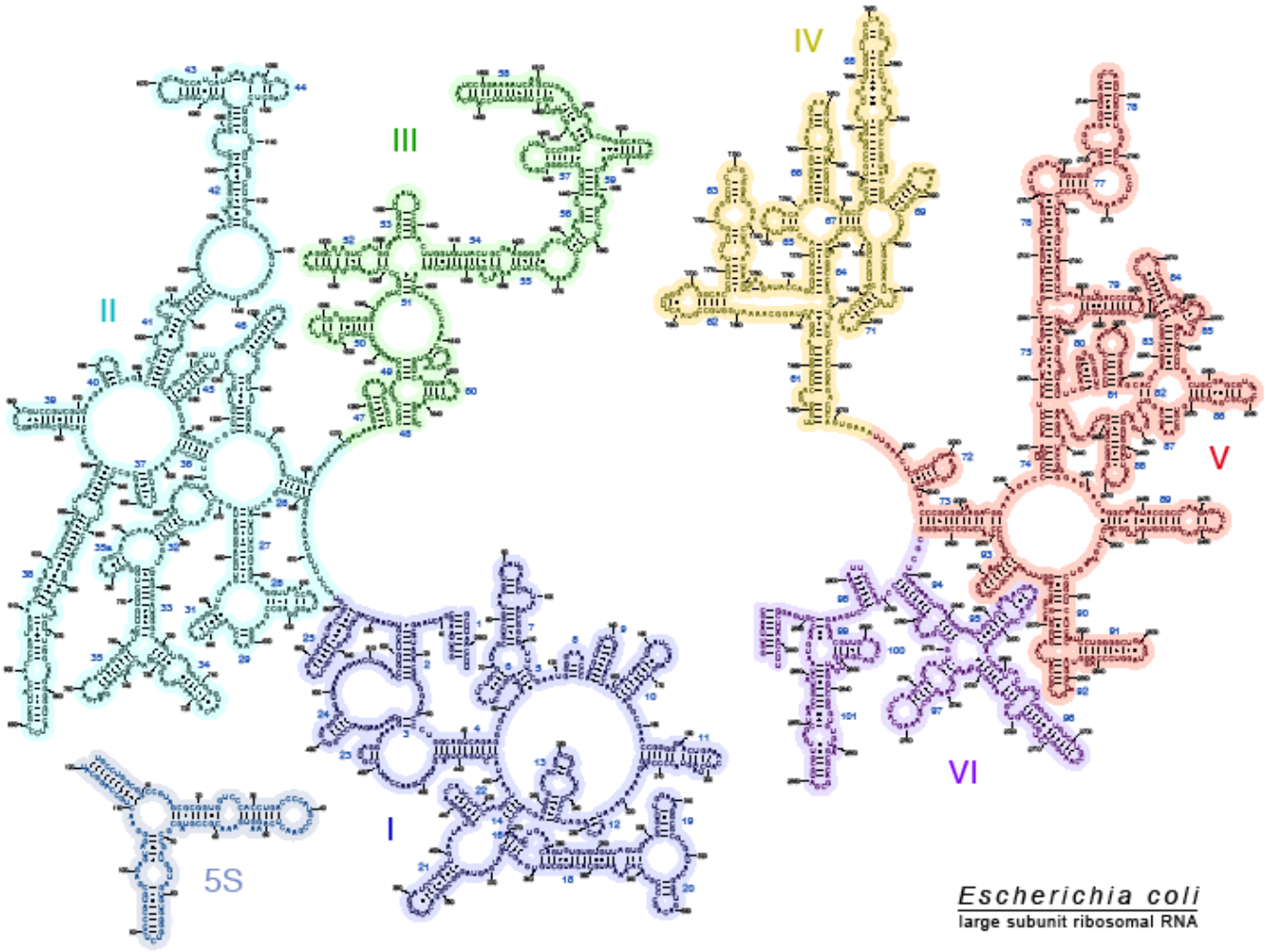


Figure- From Noller Lab
http://rna.ucsc.edu/rnacenter/ribosome_images.html

Some Parts of the RNA are Likely to be Older Than Others!

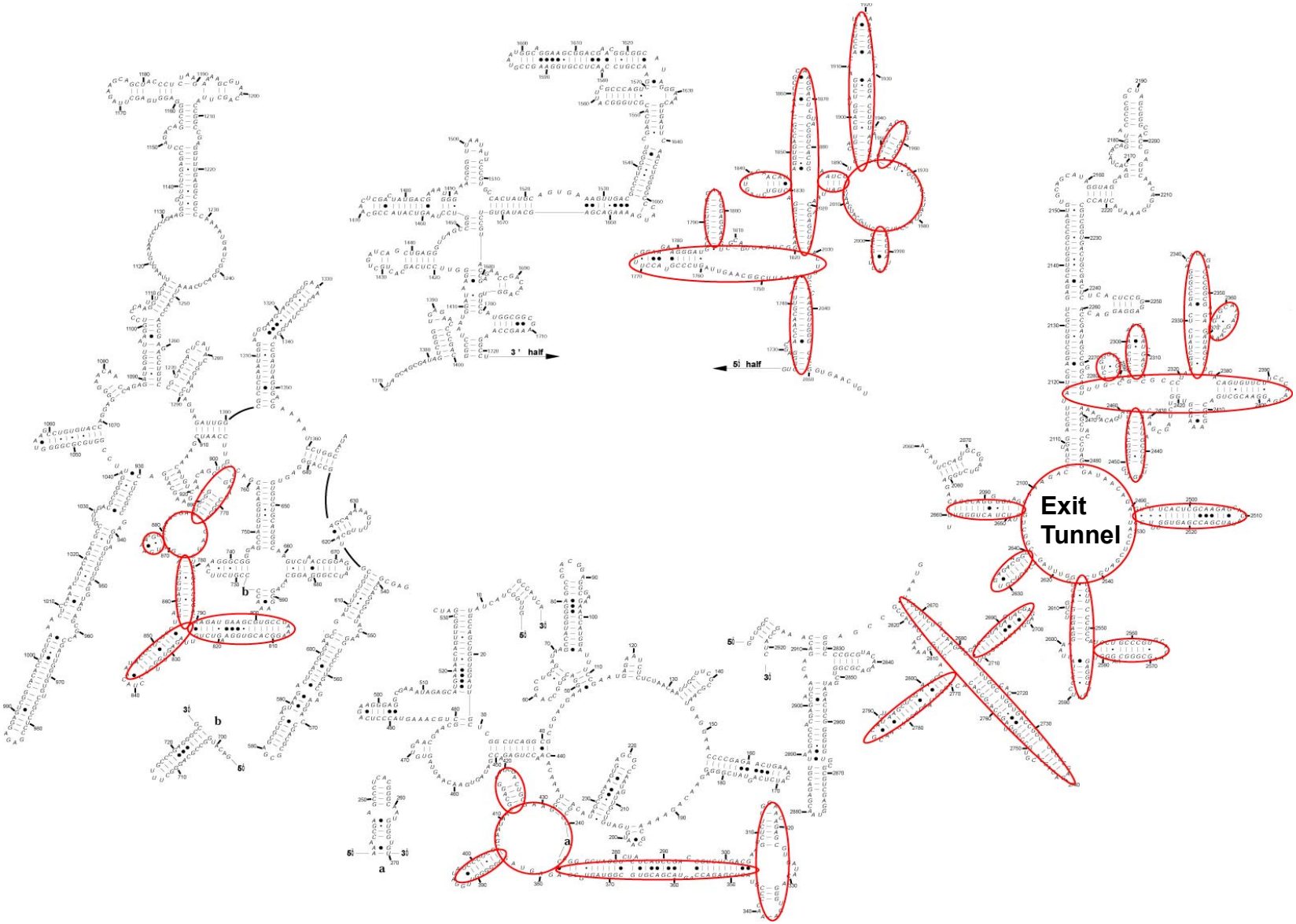
Connectivity Analysis

- **Timing Hypothesis:** The oldest regions of the rRNA are likely to be the most tightly integrated into the structure.

- Implication: Local regions that participate in large numbers of interactions with distant regions are likely to be old.

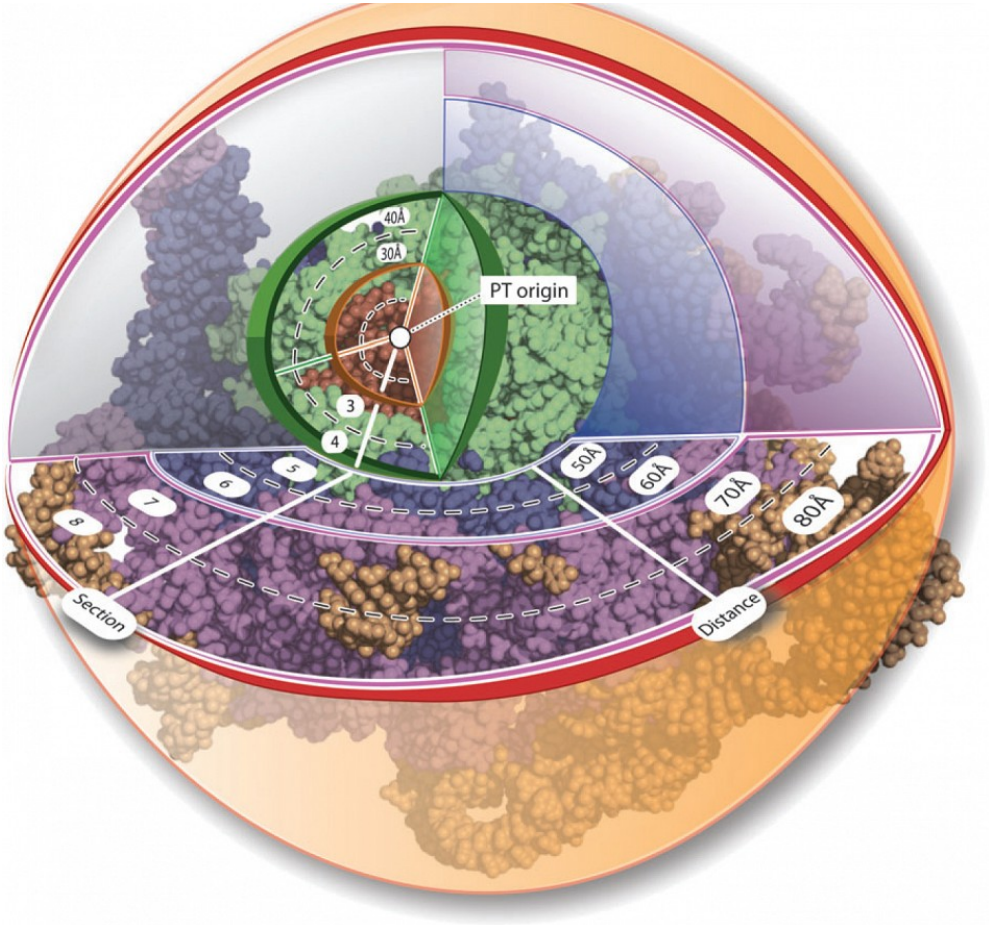
- Hury *et al*., 2006 Counted all base-base interactions between distant regions

Old Regions of 23S rRNA Based on Connectivity Analysis

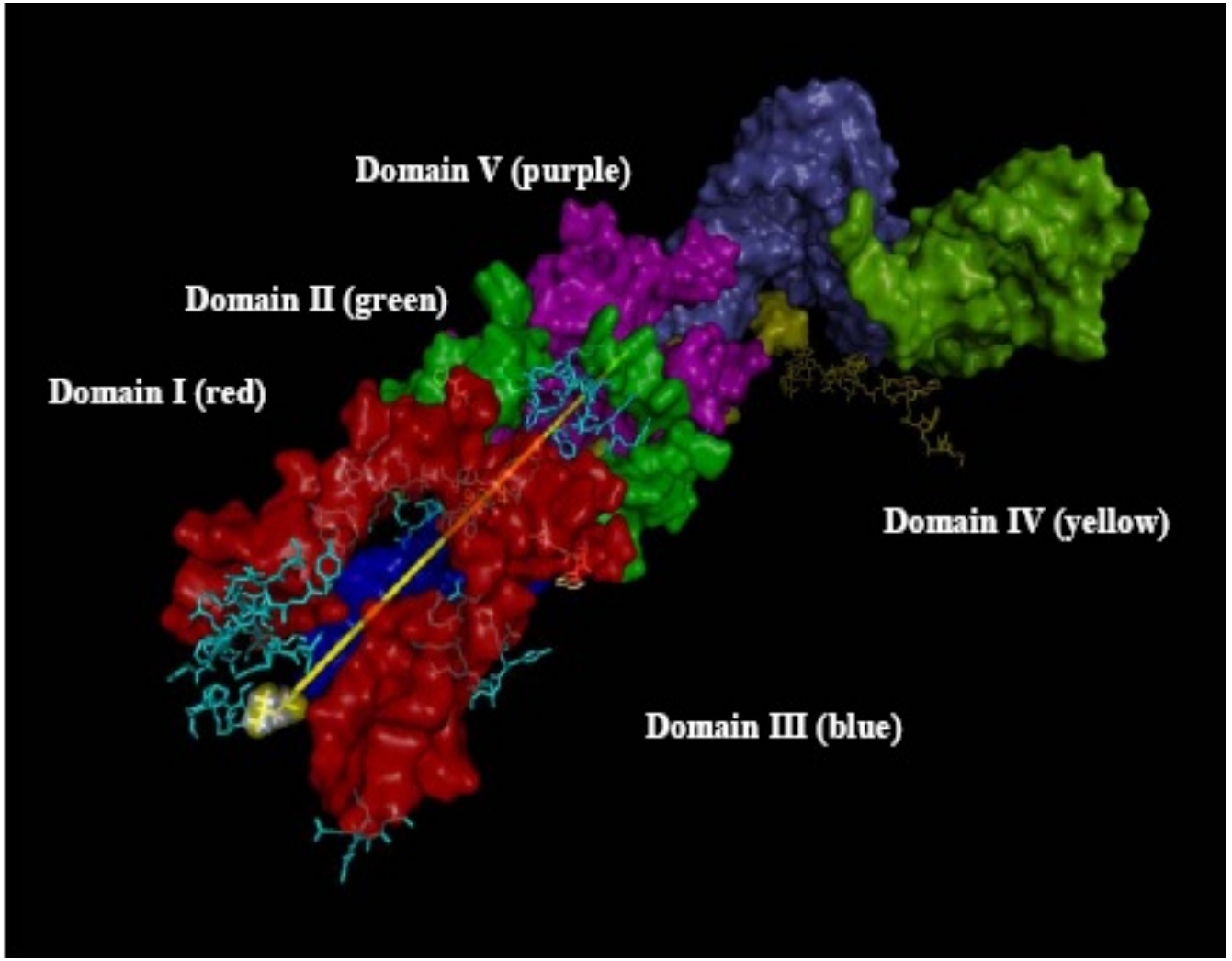


Williams- “Onion Model”

Hsiao *et al.*, Mol Biol Evol. 26: 2415-25 2009



Exit Tunnel- 20 Angstrom Radius

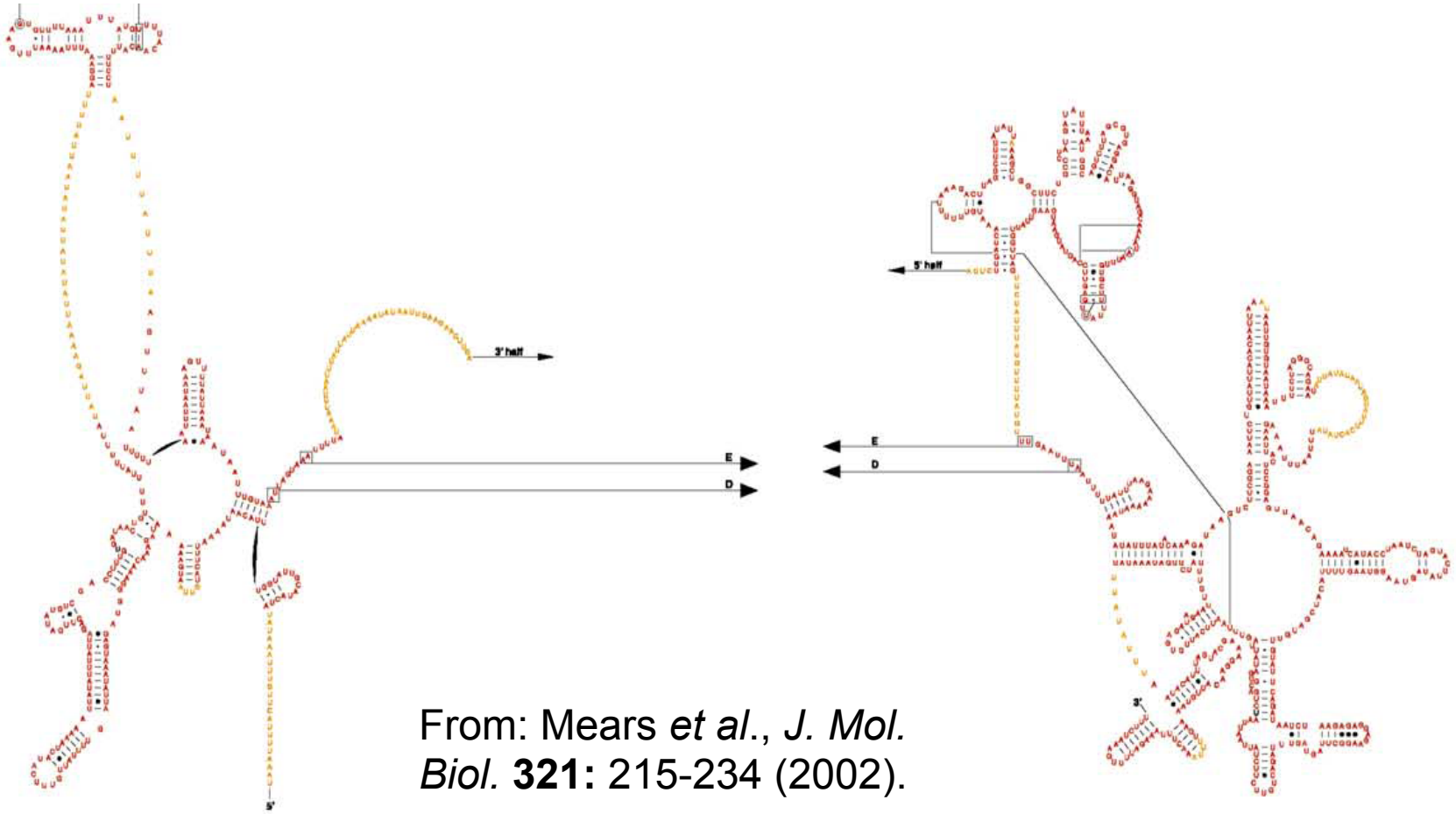


Order of RNA Domains in Exit Tunnel

V-> IV-> II-> I-> III

This agrees with the Connectivity Analysis!

2. Minimal LSU rRNA Based on Comparative Analysis

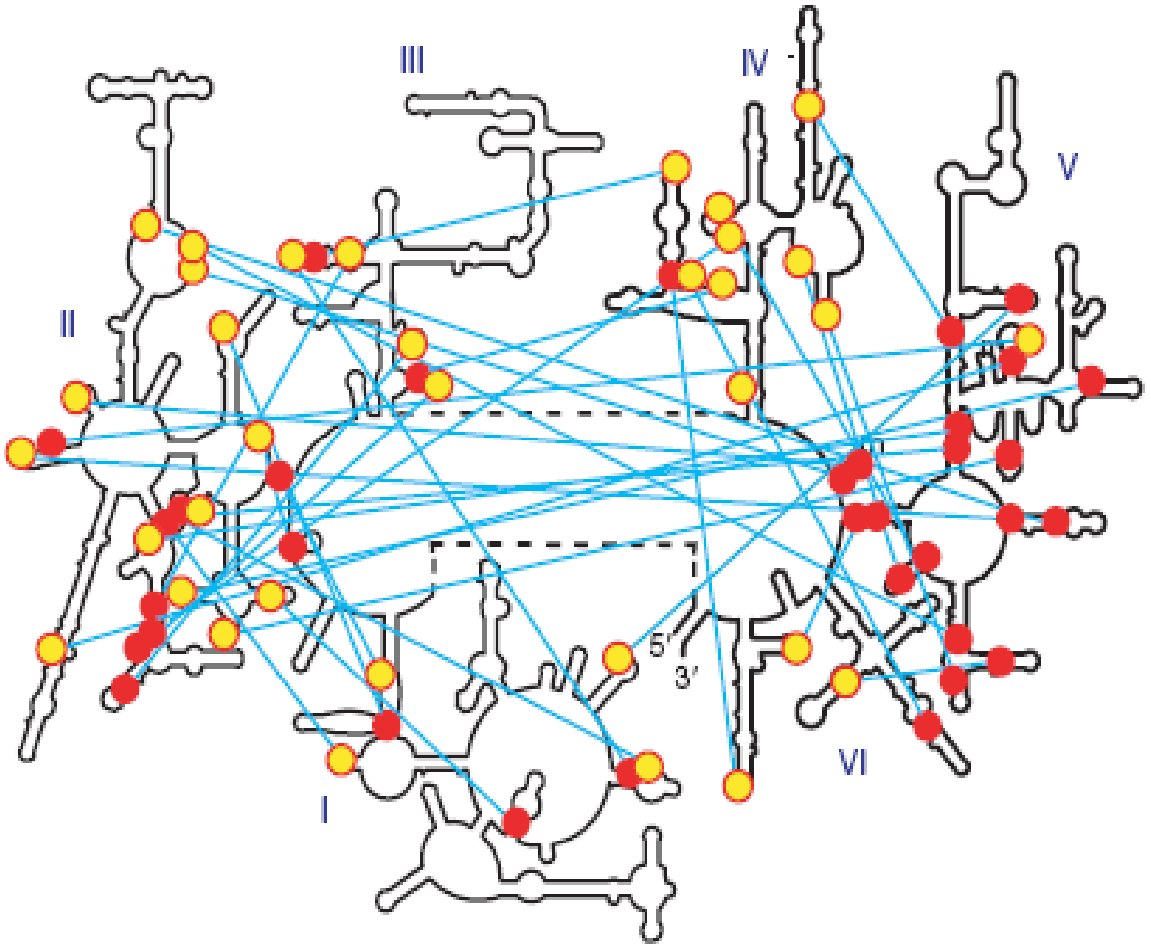


From: Mears *et al*., *J. Mol. Biol.* **321:** 215-234 (2002).

A-Minor Interactions as Timing Events

- Large numbers of A-minor motifs are found in 23S rRNA.
- An A-minor interaction occurs when a group of unpaired residues (usually A's) pack in the minor groove of an RNA duplex region.
- **Bokov & Steinberg** found that A-minor interactions in Domain V always involved a helical element in Domain 5 with an adenosine stack elsewhere. Because Domain 5 is very old this suggested A-minor interactions could be used as timing events.

Location of Inter-Domain A-minor Interactions



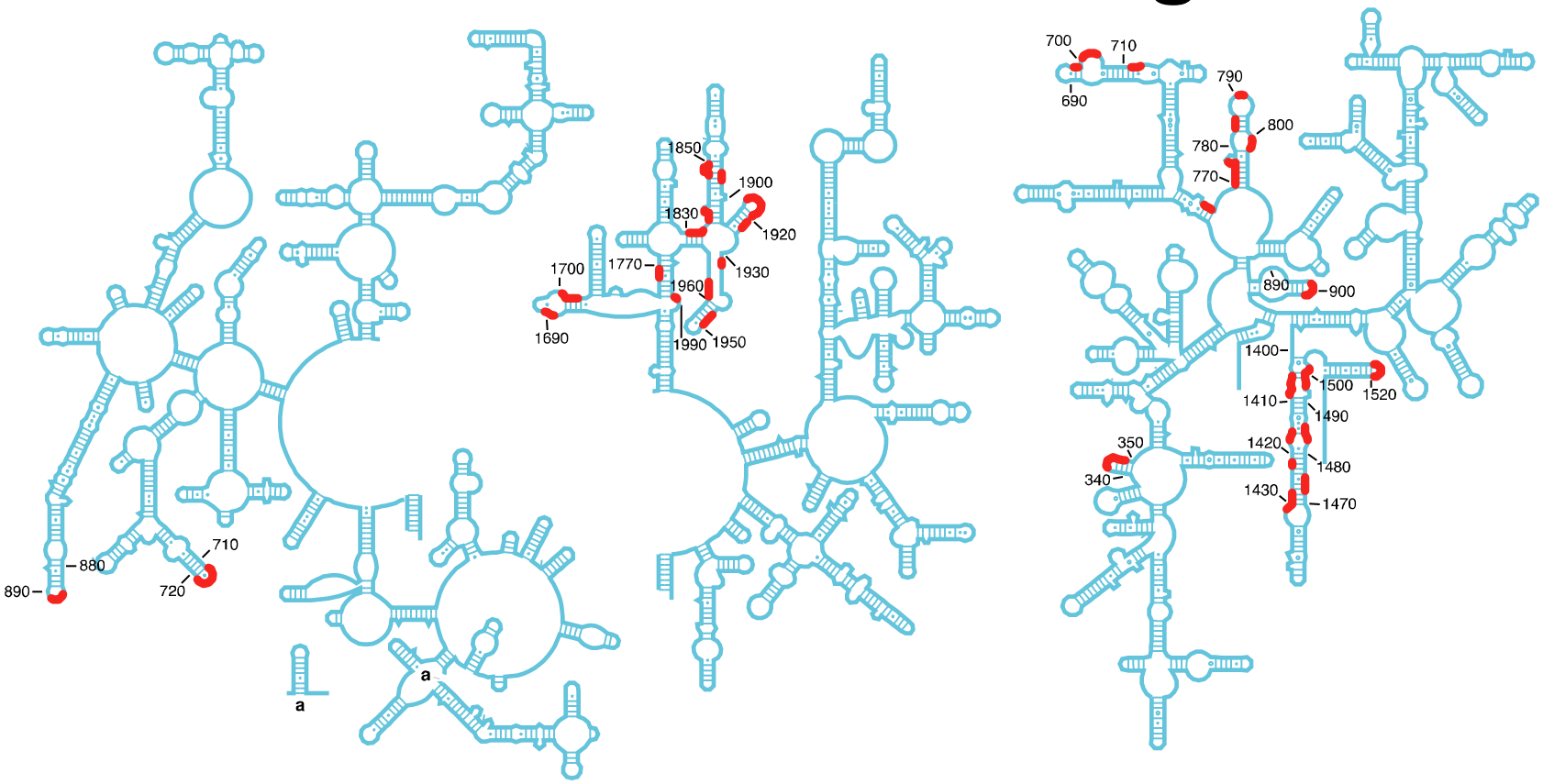
From K Bokov & SV Steinberg *Nature* **457:** 977-980 (2009).

Consensus History of Large Subunit Regions

Domain 5 (PTC)→

Part of Domain 2→Domain 4

Location of 50S – 30S Bridge Sites



50S portion of bridge sites are almost all associated with Domain 4, which is likely newer than PTC (See Sergey Steinberg A-minor interactions)

Implies- 30S & hence coding were added after PTC was functional

Abbreviated Timeline of Major Events in Ribosome History

- Peptidyl transferase center-RNA World-makes non-coded peptides
- Addition of primitive 30S & proto mRNA
- Coded synthesis possible
- 30S Ratcheting driven by Brownian Motion
- GTPase-drives ratcheting motion
- LUCA
- 5S rRNA- Coordination of events

What is Evolution Like in a Progenotic/Prebiotic World?

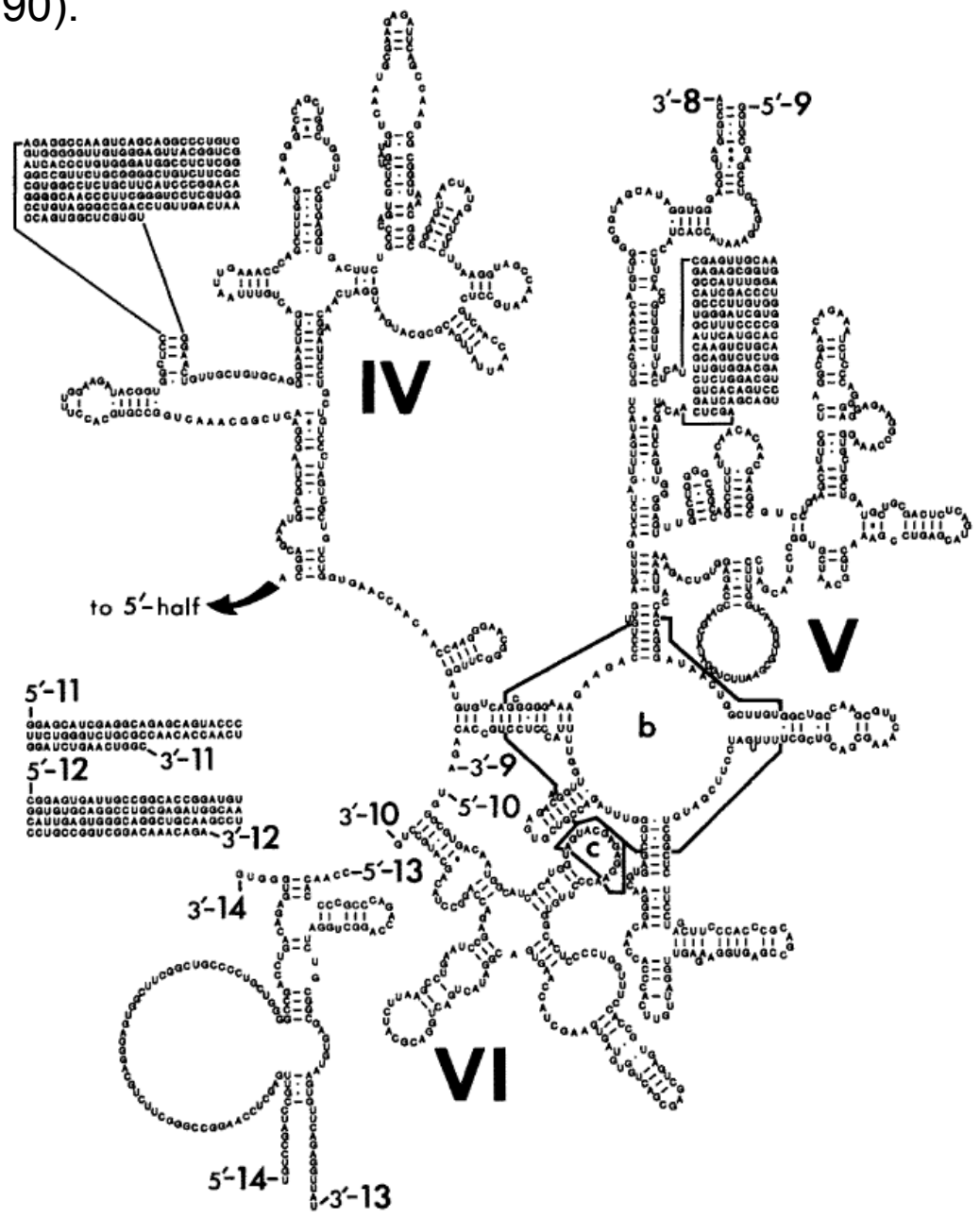
- No selection for biological function.
- To reach the biological world -complexity must increase.
- If an assemblage has a longer lifetime than an alternative then its continued existence will likely be favored.

Unique Properties of RNA and other Nucleic Acids

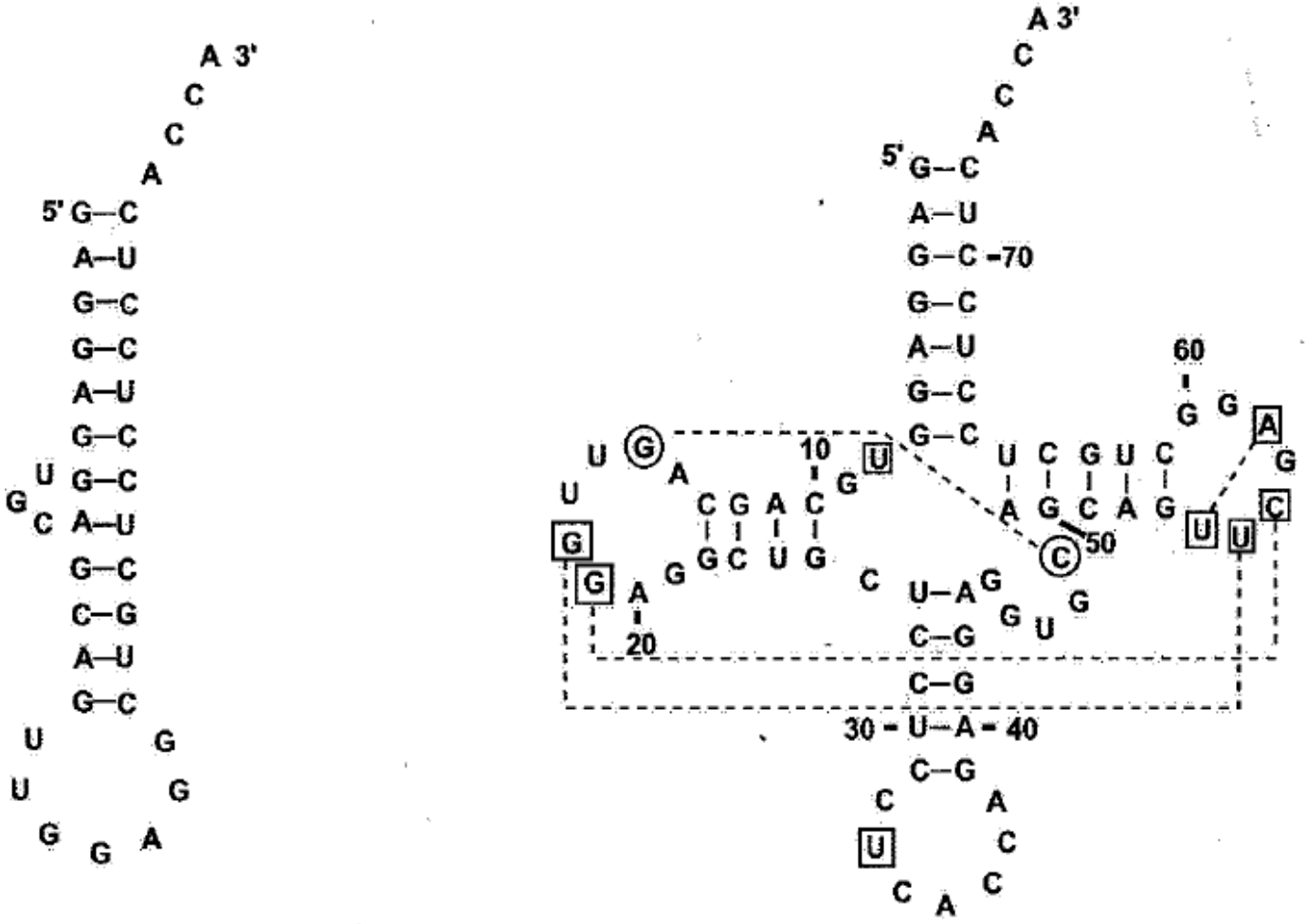
- Self Aminoacylating RNAs potentially common in an RNA World(Turk *et al.,* 2011)
- Complexity is readily increased by hybridization
- Relatively easy to obtain RNA aptamers that catalyze ligation
- Ligation can produce or stabilize larger structures

MN Schnare & MW Gray
J. Mol. Biol. **215:** 73-83 (1990).

Large RNA of *Euglena gracilis* is created by hybridization between 14 Fragments



Ligation Could in Principle Create a Two Domain tRNA



Nagaswamy & Fox, *Org. Life Evol. Biosphere* **33:**199-209 (2003).

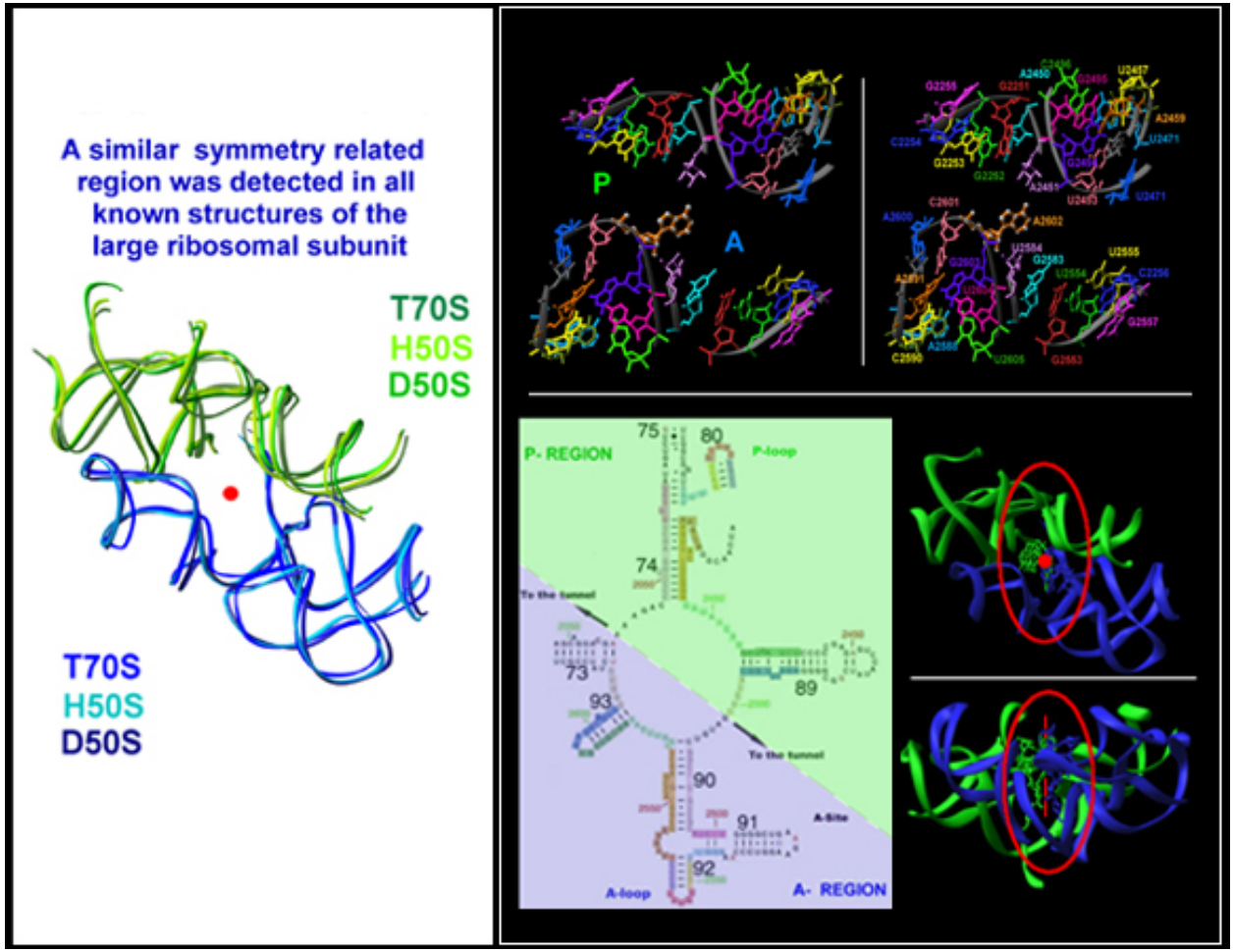
How Did Peptide Synthesis Begin?

Consider the Symmetric PTC Model of Yonath –

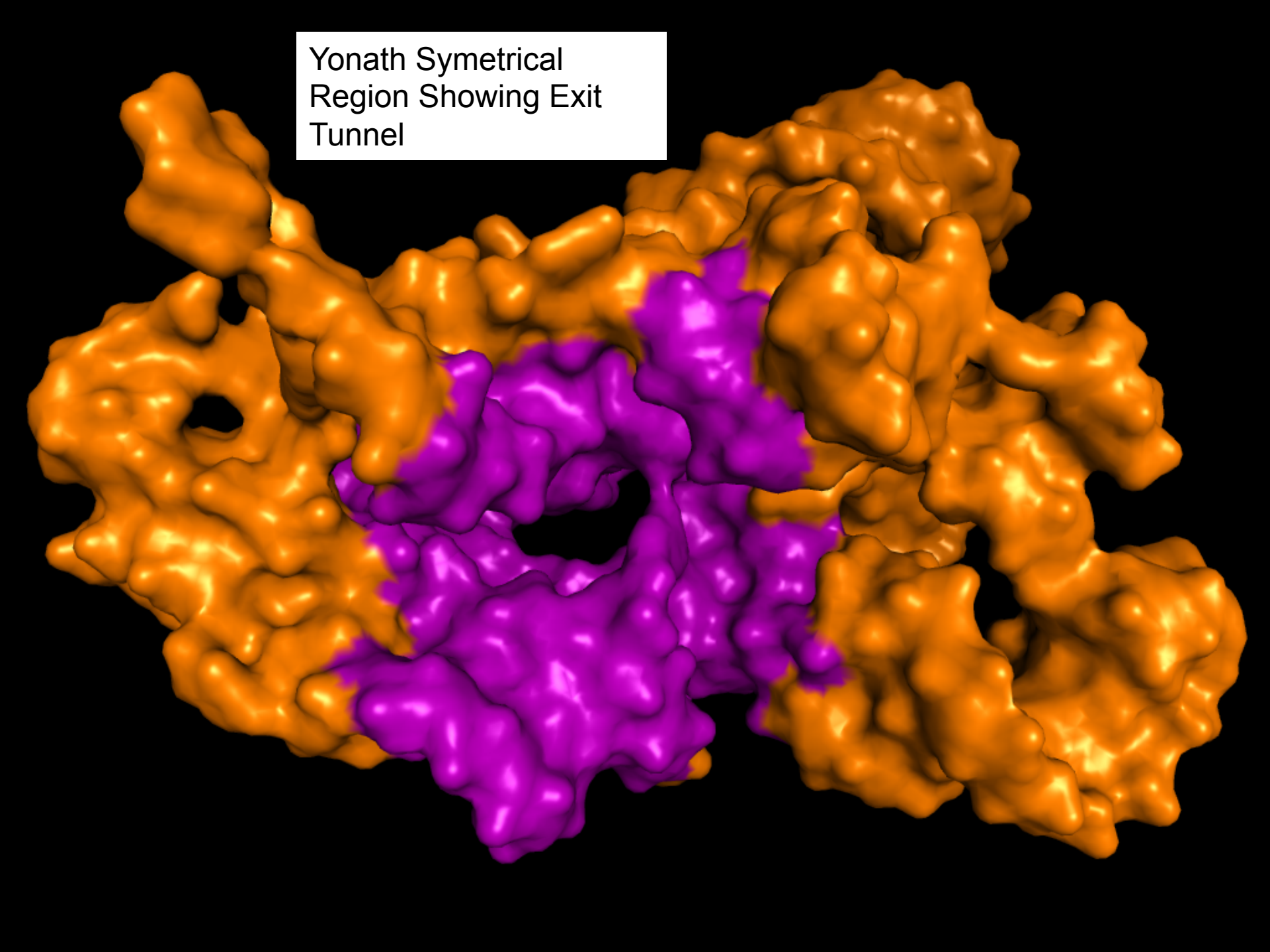
Its not just the PTC!

Symmetry at the PTC (peptidyl transferase center)

First observed by Ada Yonath & Colleagues at Weizmann Institute



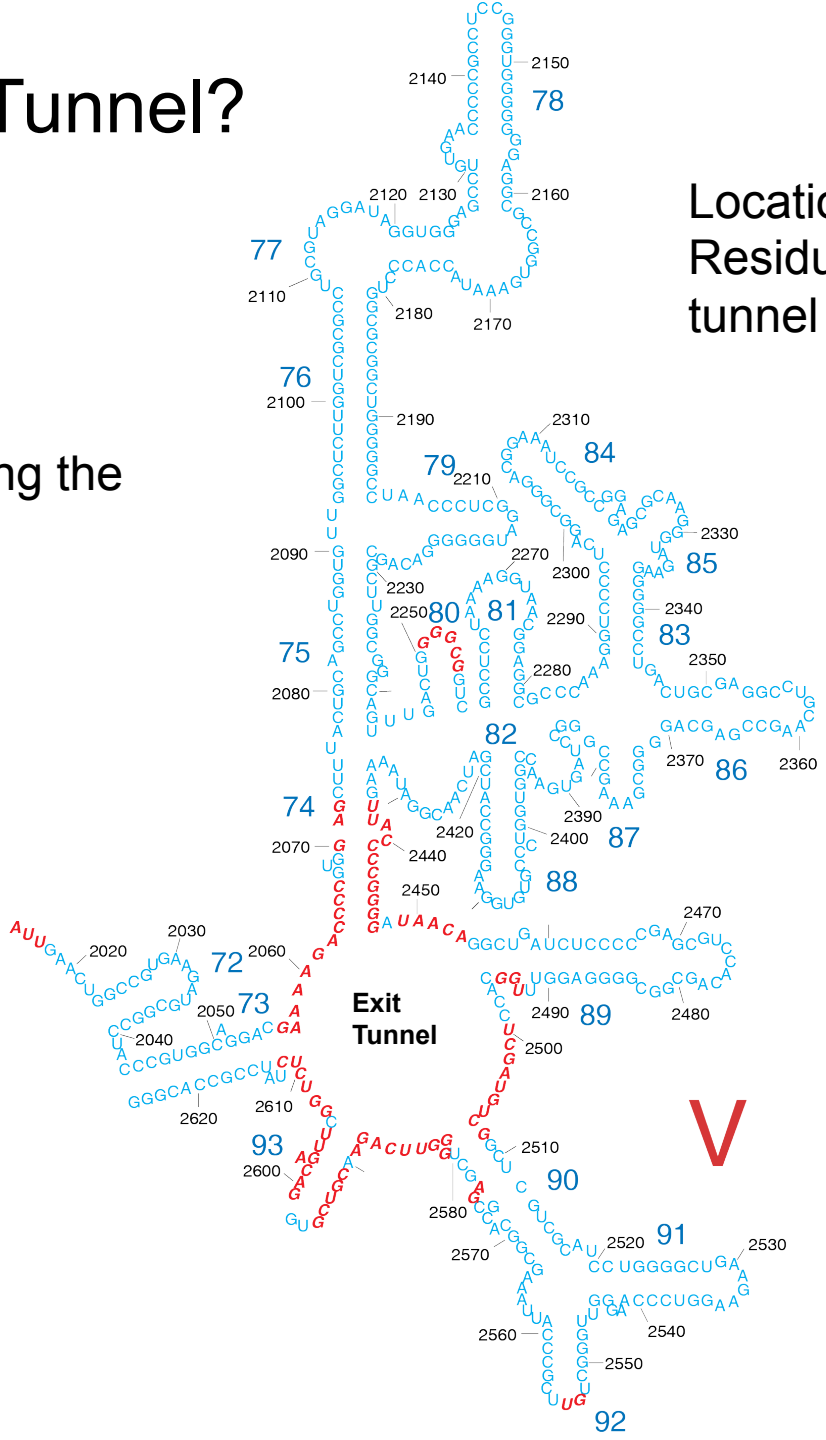
http://www.weizmann.ac.il/sb/faculty_pages/Yonath/00Sc_activities.html


Yonath Symetrical
Region Showing Exit
Tunnel

Where is Exit Tunnel?

Red- Residues Lining the Exit Tunnel at 20 Angstroms

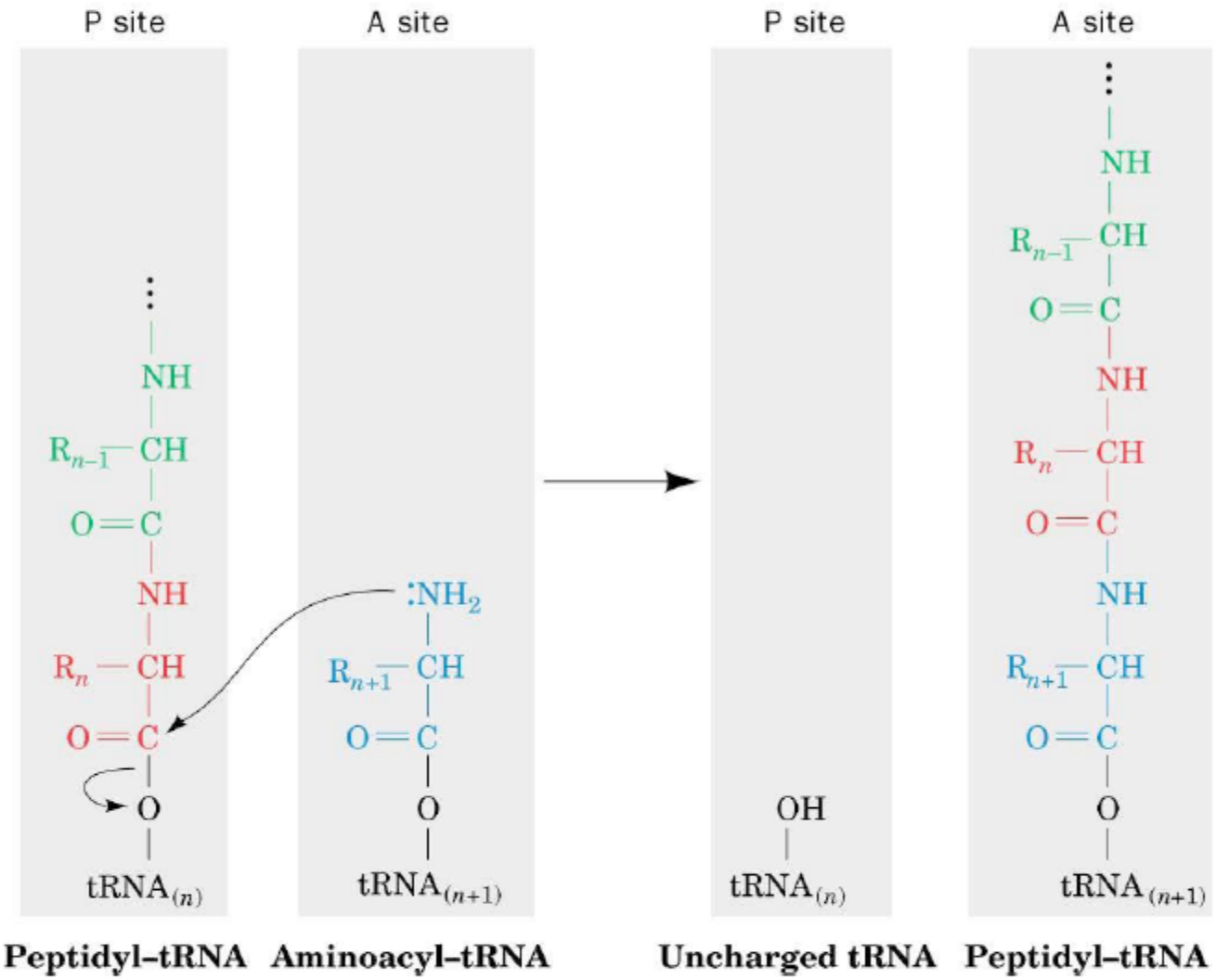
Location of Domain V Residues that line the tunnel



How did Peptide Synthesis Begin?

Exit tunnel may have played an unexpected key role- Lets look at Exit Tunnel.

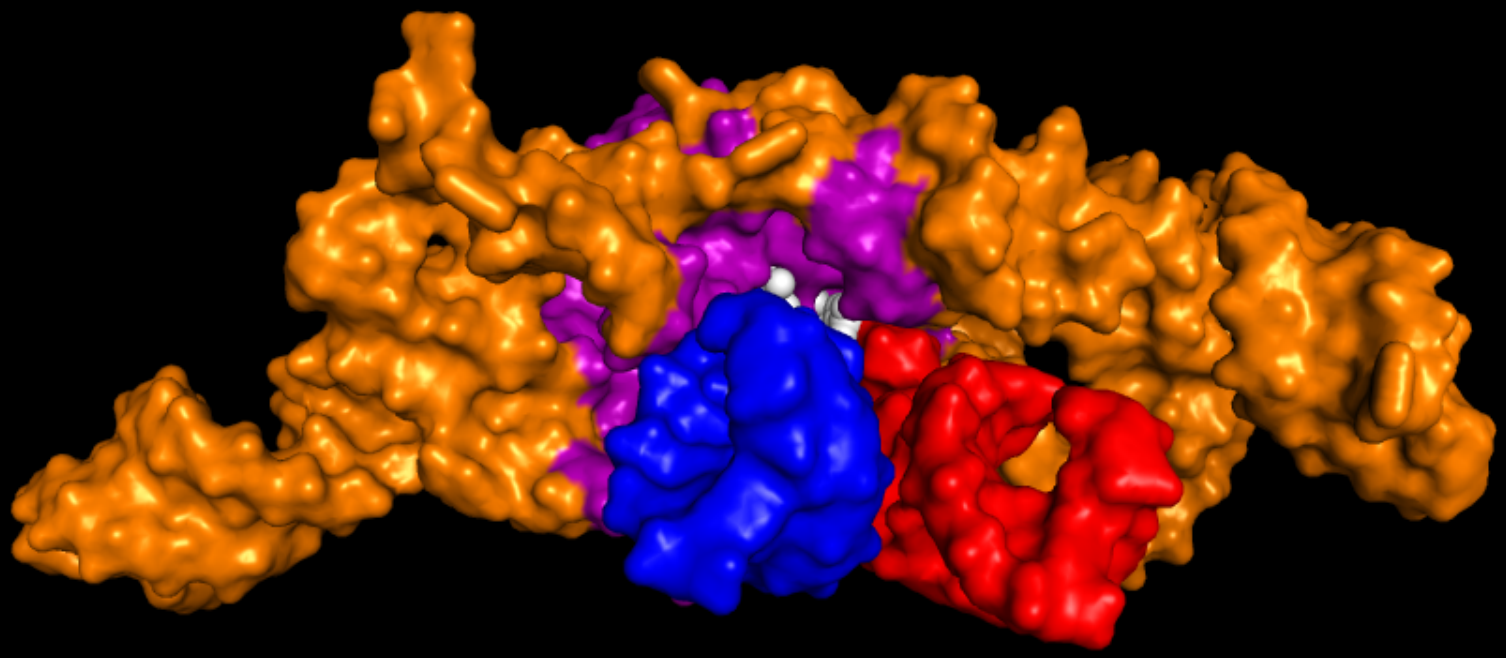
7.4) Peptidyl transferase reaction



Modern tRNAs are too big!

tRNAs Associated with A-site (red); P-site (Blue) and E-site (Green) in “minimal LSU rRNA”

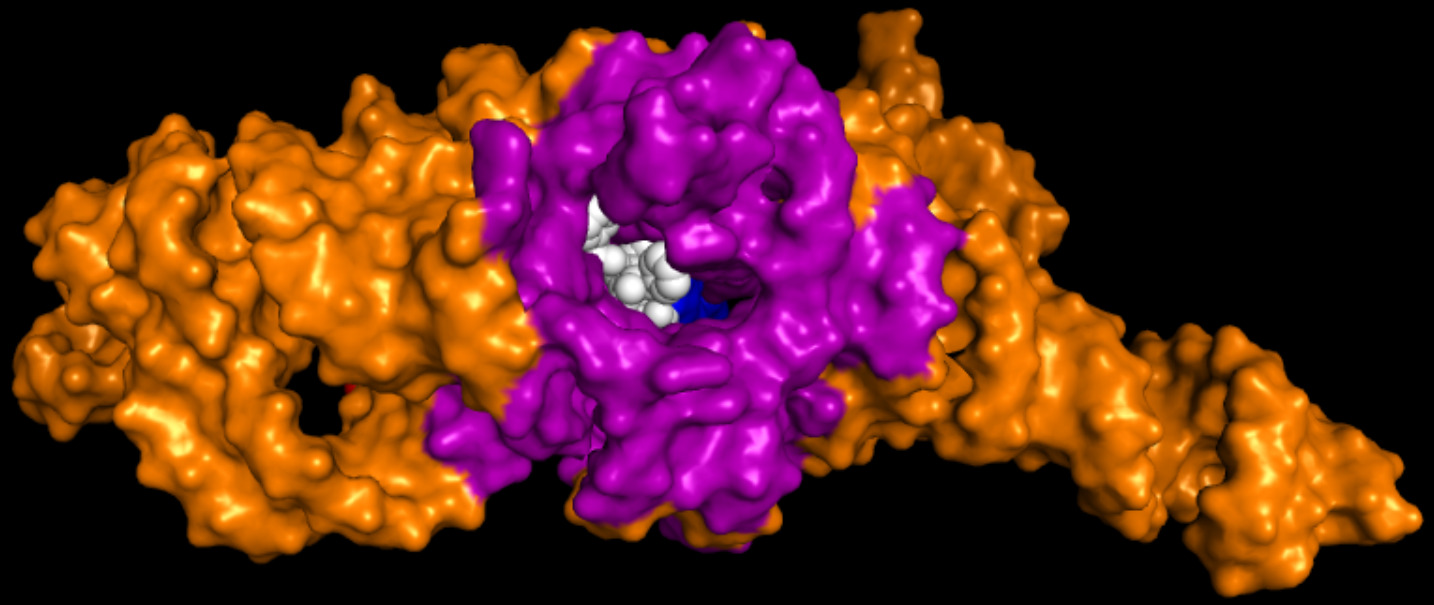
Yonath Symmetrical Region with micro-helix tRNAs
in A-site (Red) and P-site (Blue)



Exit Tunnel Hypothesis for Beginnings of Peptide Synthesis

- Small aminoacylated RNAs may attach to PTC RNA
- If two RNAs happen to be there at same time dipeptide might form.
- Di-peptide will enter exit tunnel. The RNA carrying the dipeptide to be more likely to stay than the other RNA.
- Another small RNA may bring a new amino acid before the RNA carrying di-peptide has chance to leave.
- Tripeptide may be formed.
- Peptides may assist in stabilizing PTC RNA (or on a really good day catalyze RNA replication)

Yonath Symmetrical Region From Exit side- peptide entering exit tunnel in white with portion of P-site tRNA showing in blue



The Beginning of the RNA/Protein World!?

When Did the Ribosome Become Chiral?

Wasn't It Always Chiral?

Protein Synthesis is a Two Tier Process

(1) tRNA is charged with the appropriate amino acid which it delivers to the ribosome

(2) The ribosome makes the peptide bond

Modern Ribosomes Can Not Effectively Use tRNAs Carrying D-amino Acids

- Peptide synthesis can be initiated by D-amino acids (Goto et al., RNA 14: 1390-98, 2009).

- tRNAs carrying D-amino acids are at best poorly incorporated into proteins by ribosomes *in vitro* (Bhuta et al., Biochemistry 20: 8-15, 1981).

- **Modern ribosomes are strongly chiral in their preference for tRNAs carrying L-amino acids**

Ribosomes Can be Mutated to Incorporate D-Amino Acids *in vitro*

(Dedkova *et al*., 45: 15541-15551, 2006)

If ribosomes can be mutated to accept D-amino acids maybe they have evolved to strongly prefer L-amino acids!

Originally the preference for L-amino acids may have been weaker

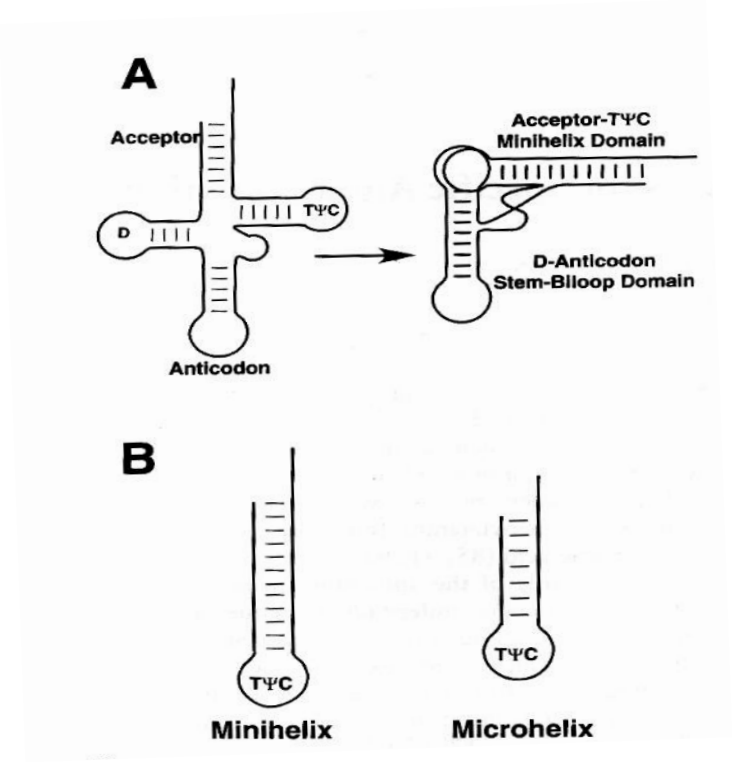
Modern tRNAs Are Charged with D-amino acids with Some Frequency

- Tyrosyl-tRNA synthetase is able to charge tRNAs with both D and L (preferred) Tyrosine (Sheoran et al., J. Biol Chem. 283: 12971-80, 2009)

- Many tRNA synthetases have editing domains that remove D-amino acids .

- Deacylases remove D-amino acids from tRNAs (Soutourina *et al., J Biol Chem* **275:** 32535-42, 2000).

Reminder-Paul Schimmel & Colleagues have shown tRNA minihelices can be charged with amino acids



A Strong Chiral Preference Exists in "Abiotic" Minihelix Charging

(Tamura & Schimmal, *Proc Natl Acad Sci.* **103:** 13750-52, 2006; Tamura *Biosystems* **92:** 91-8, 2008).

A 4 to 1 Preference Was Found

Illangasekare *et al* RNA 16: 2370-83 (2010) have shown that D-ribose RNA preferentially interacts with L-histidine

A 4-1 Preference At Both Steps Would Give Peptides that were 96% Chiral

- **A two tier partially chiral selection may have given the emerging ribosome an advantage over other peptide synthesis systems in the absence of coding**

- It might be useful to study the effects of different levels of random D-amino acid content on the properties of peptides

- Peptides with occasional D-amino acids would likely at the least still be useful in stabilizing early ribosomes

Summary

- Timing information from diverse sources can contribute to developing a timeline of ribosomal development.
- Coded peptide synthesis by ribosomes likely came after the addition of the 30S subunit. Coded synthesis unlikely to be a key advantage of the first proto-ribosomes.
- The exit tunnel may be a key feature of the ancient PTC that facilities synthesis of extended peptides.
- The ability to make peptides approaching homochirality may have been a significant advantage offered by early ribosomes.

CO-WORKERS

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