

Alan Weiner  
BIOCHEM 530  
Friday, MEB 248  
October 23, 2015  
RNA structure,  
the ribosome,  
structure-based drug design

# Longevity Plus RNA

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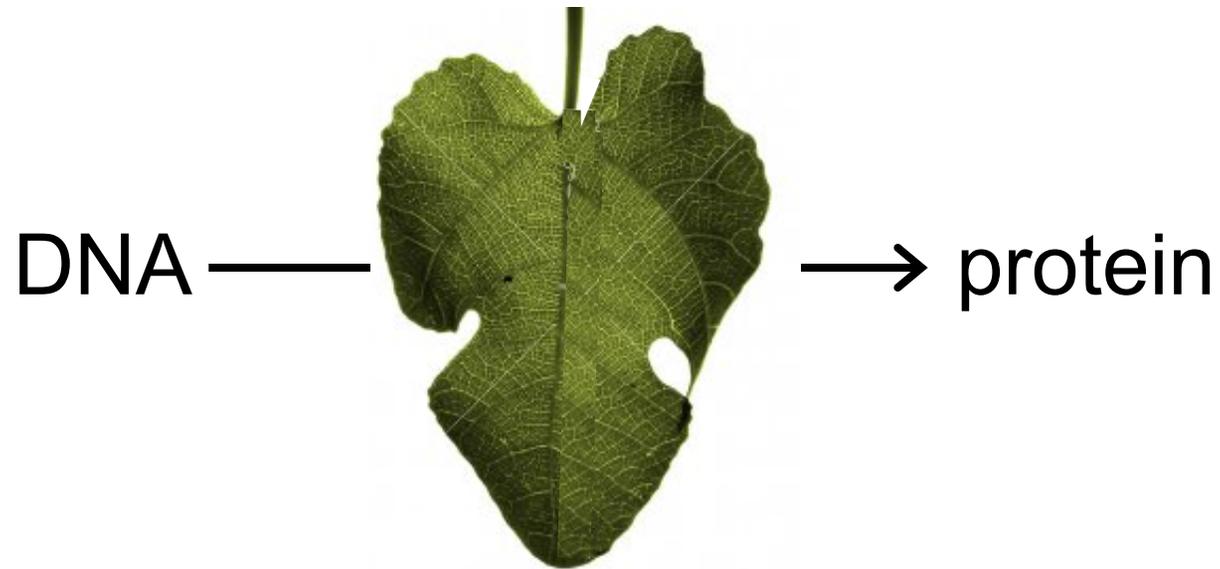
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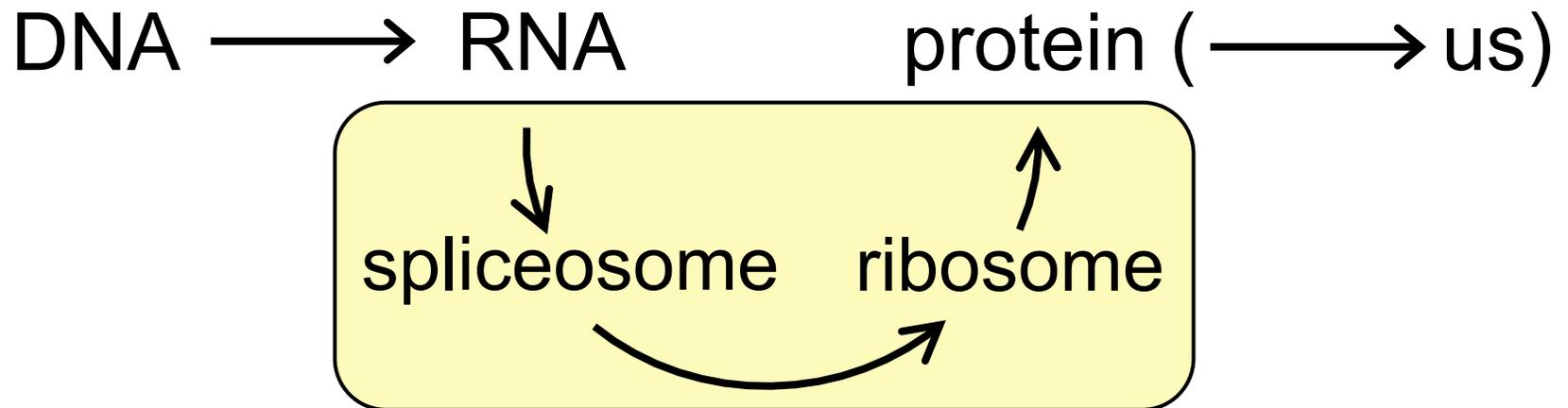
## Crick's "Central Dogma"?

DNA → RNA → protein

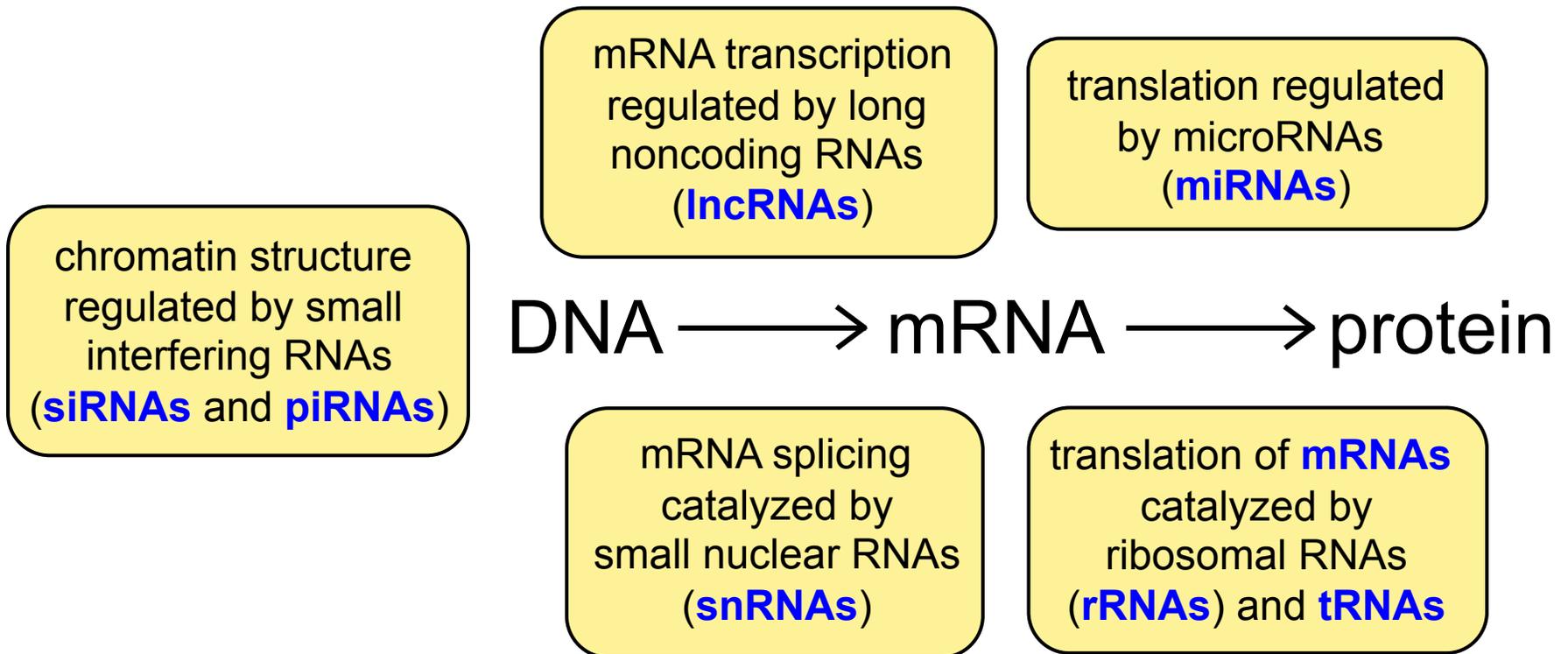
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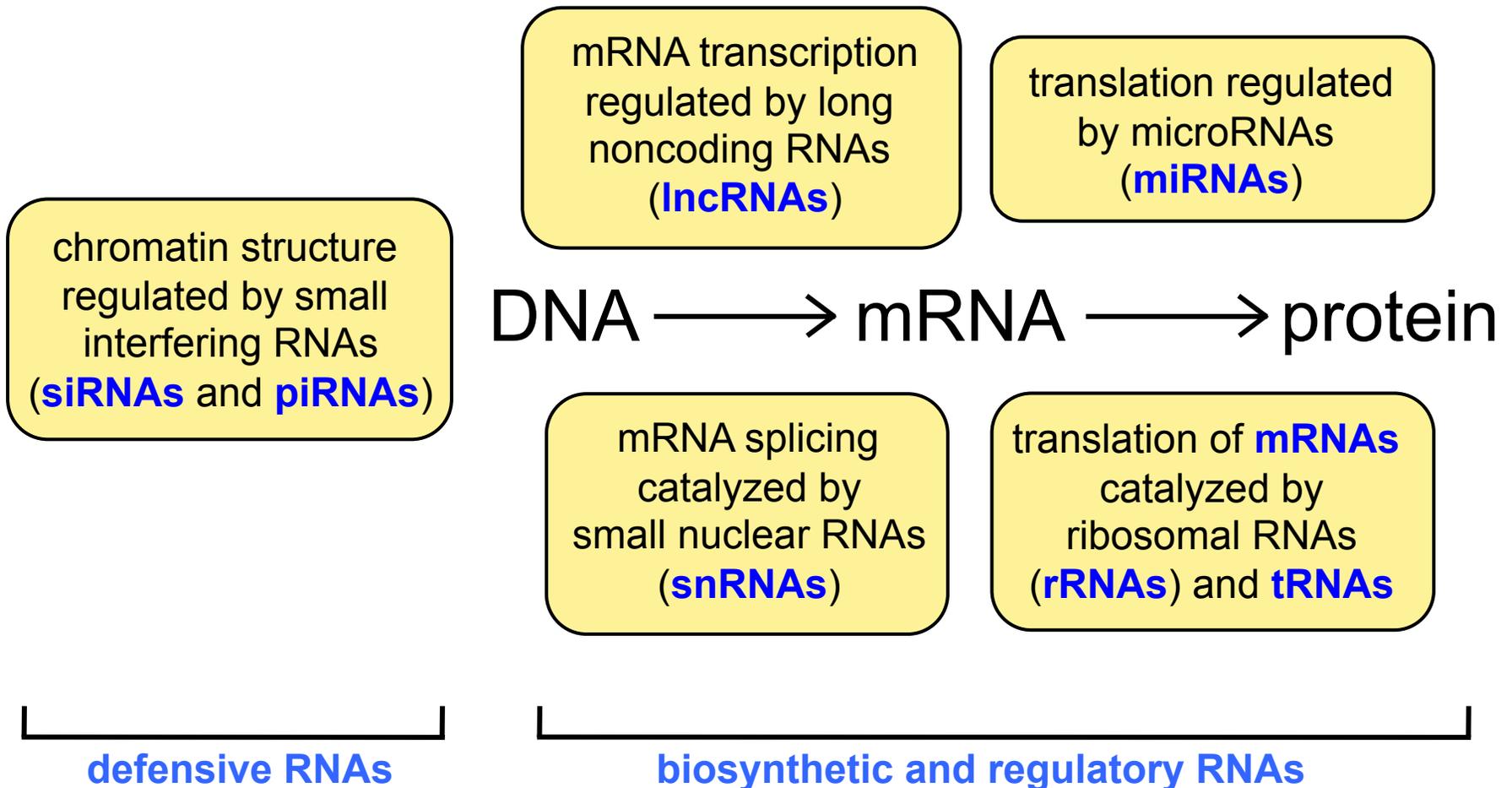
# Crick's "Central Dogma"?



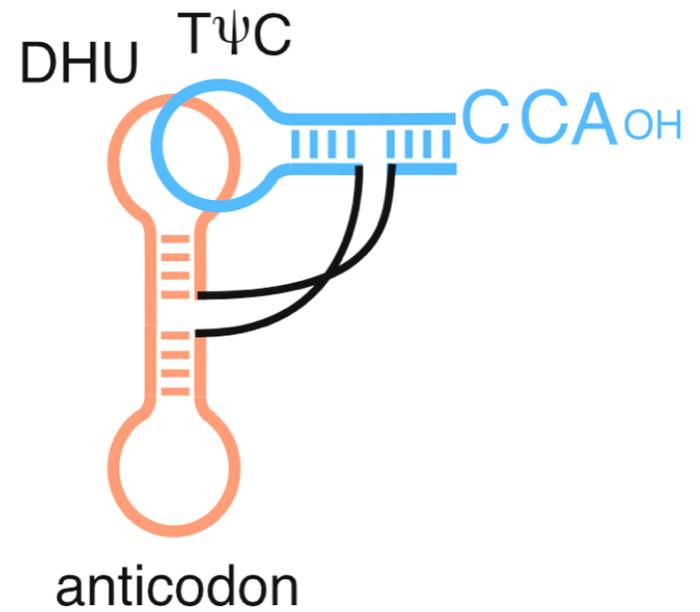
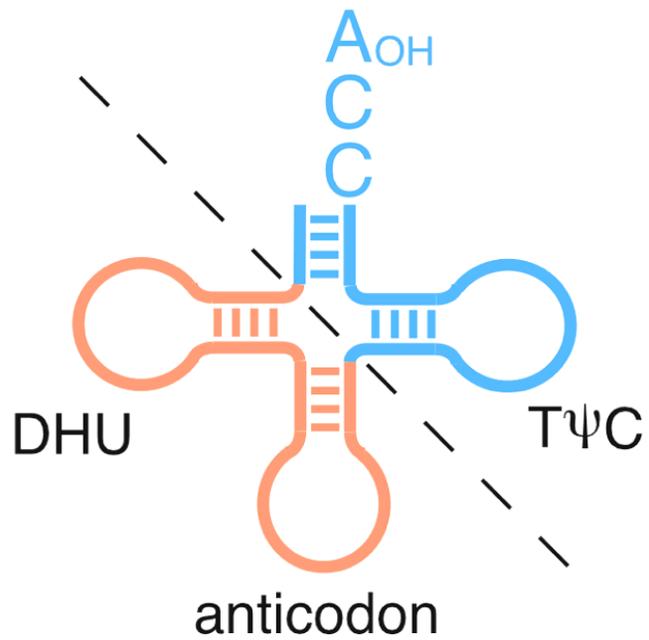
RNA is more than a structure or informational sequence...  
key steps in the "Central Dogma" are *catalyzed* and *regulated* by RNAs



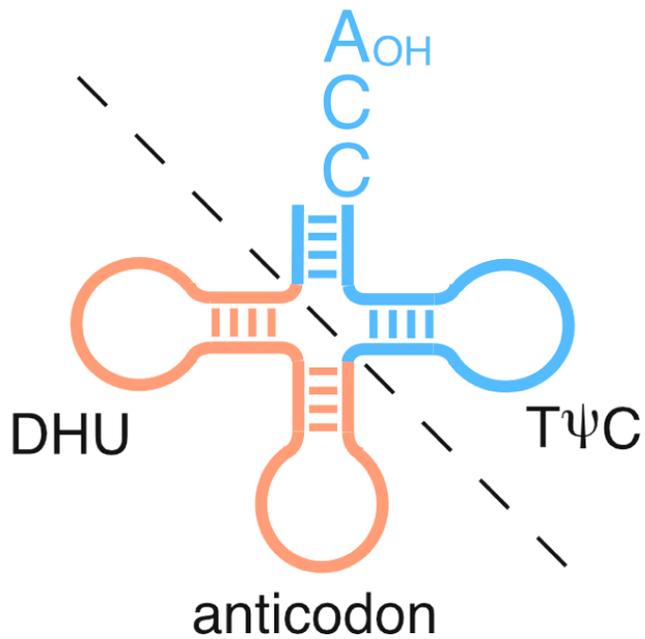
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# The principles of RNA folding



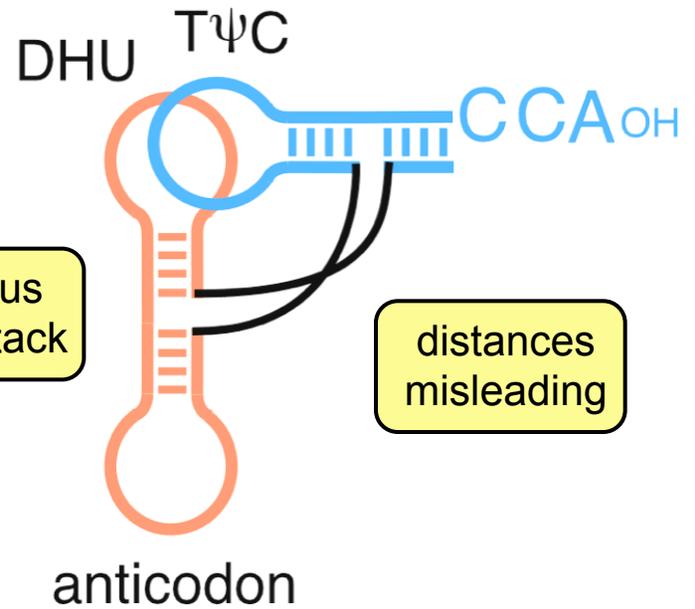
# The principles of RNA folding



tertiary interactions  
very important

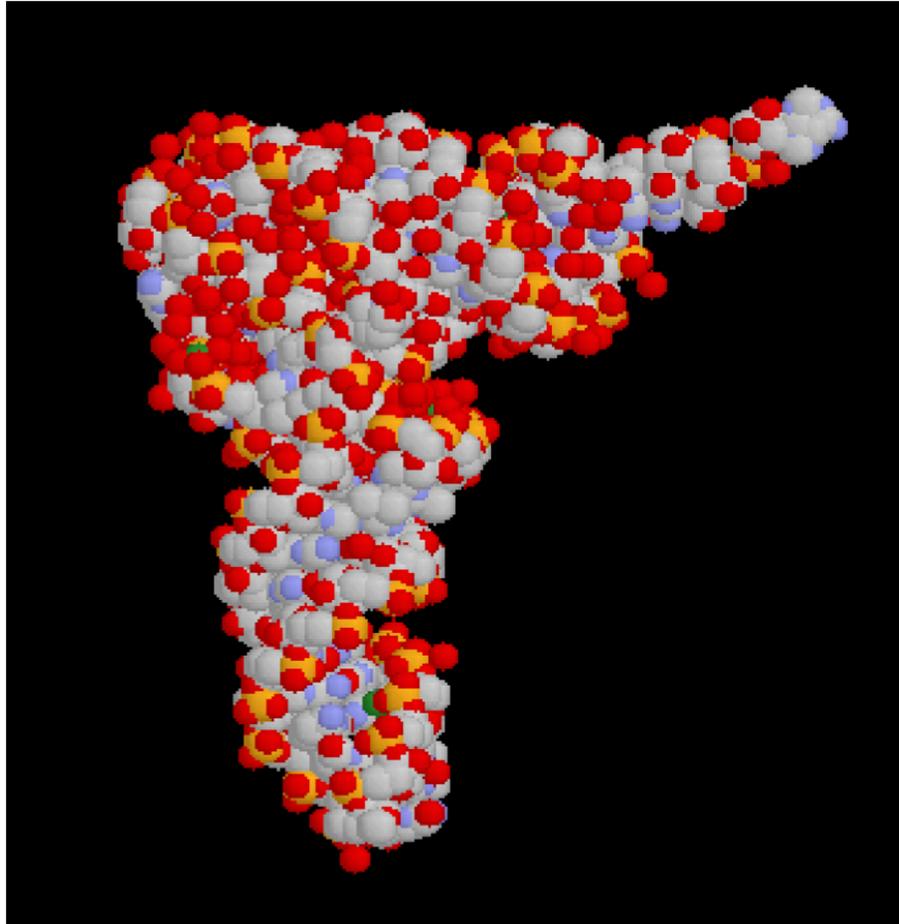
continuous  
coaxial stack

distances  
misleading

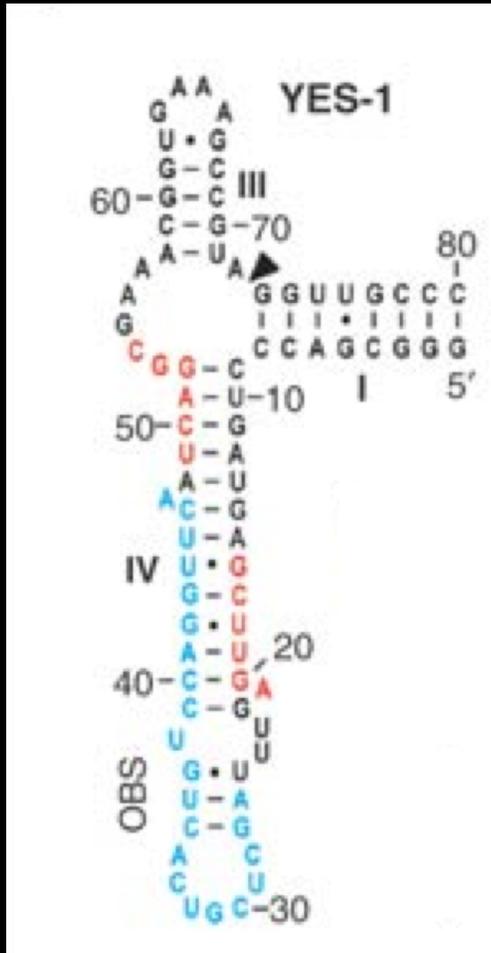


tRNA is built from two structurally  
independent domains tethered together

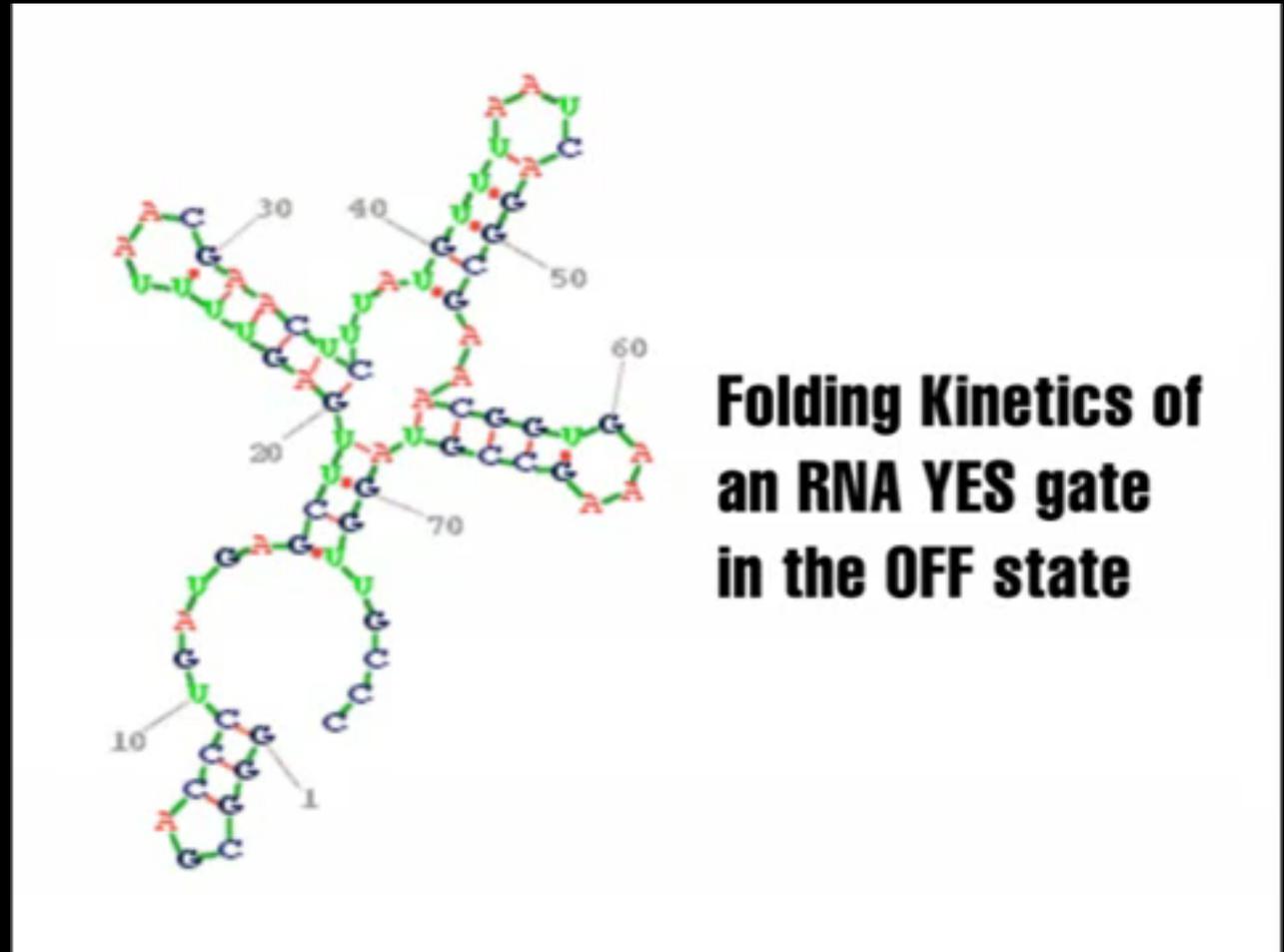
A space-filling model of a folded tRNA looks a lot like a globular protein



# RNA folding (music and colors added)



experimentally determined  
(2D) structure



<http://www.youtube.com/watch?v=PWETG3B6wek>; Penchovsky and Breaker (2005) Computational design and experimental validation of oligonucleotide-sensing allosteric ribozymes. Nat Biotechnol 23, 1424

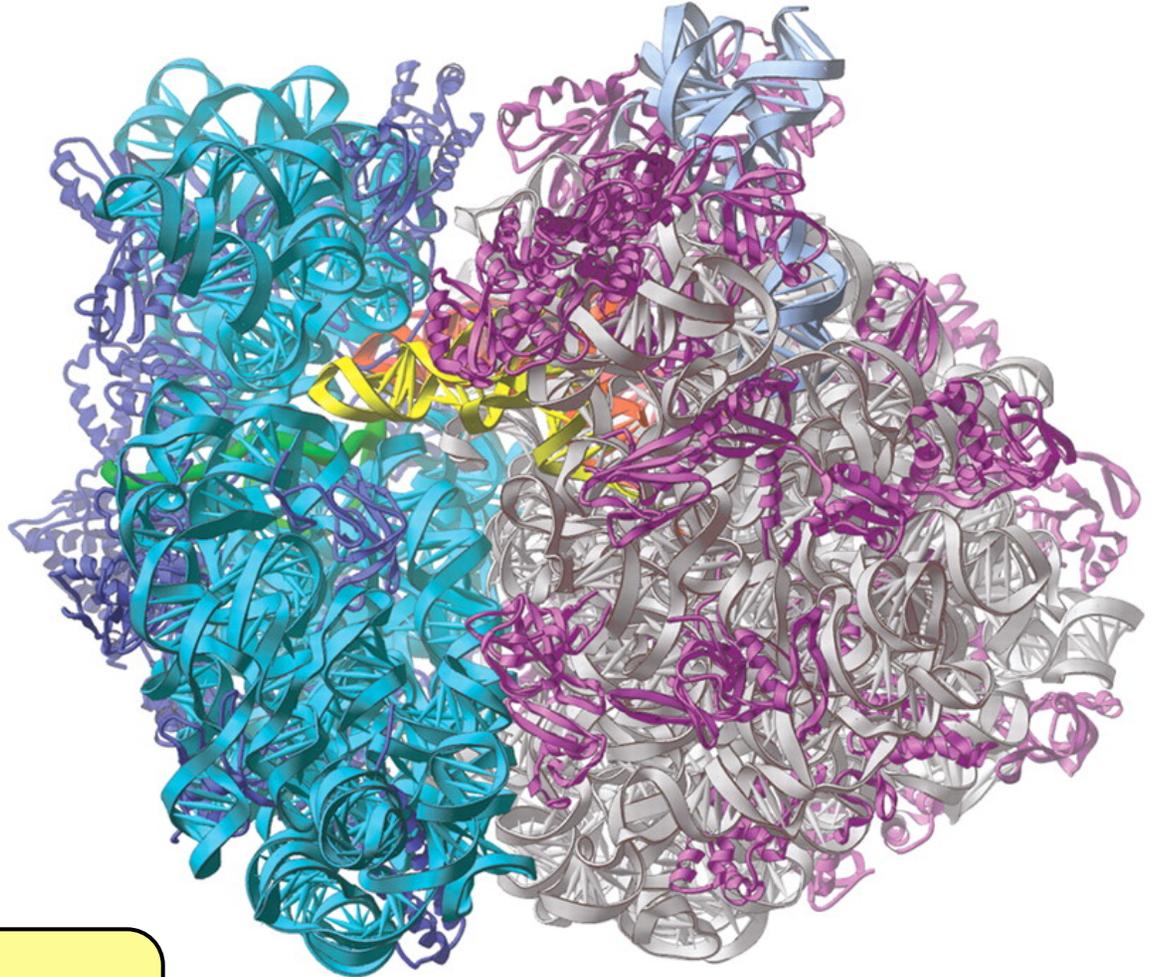
1974 to 2005: tRNA to Group I introns to the ribosome



tRNA  
(76 nt)



Group I ribozyme  
P4-P6 domain  
(160 nt)



70S ribosome  
(4530 nt total plus 50 proteins),  
3 rRNAs, 2 tRNAs, 1 mRNA

HN Noller (2005) RNA Structure: Reading the Ribosome. *Science* 309, 1508-1514.

1974 to 2005: tRNA to Group I introns to the ribosome

coaxial stacking,  
kissing, tethering



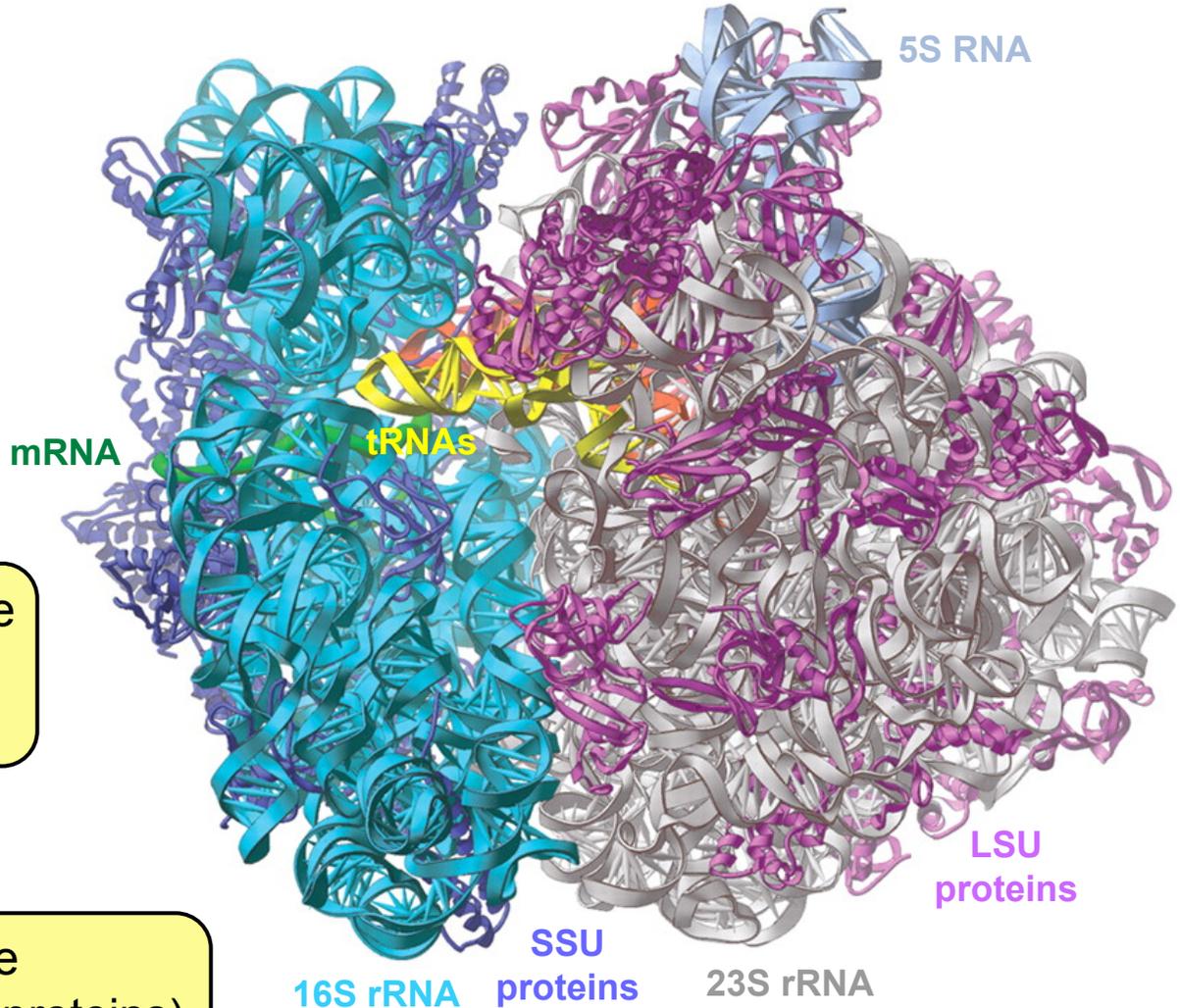
tRNA  
(76 nt)

docking



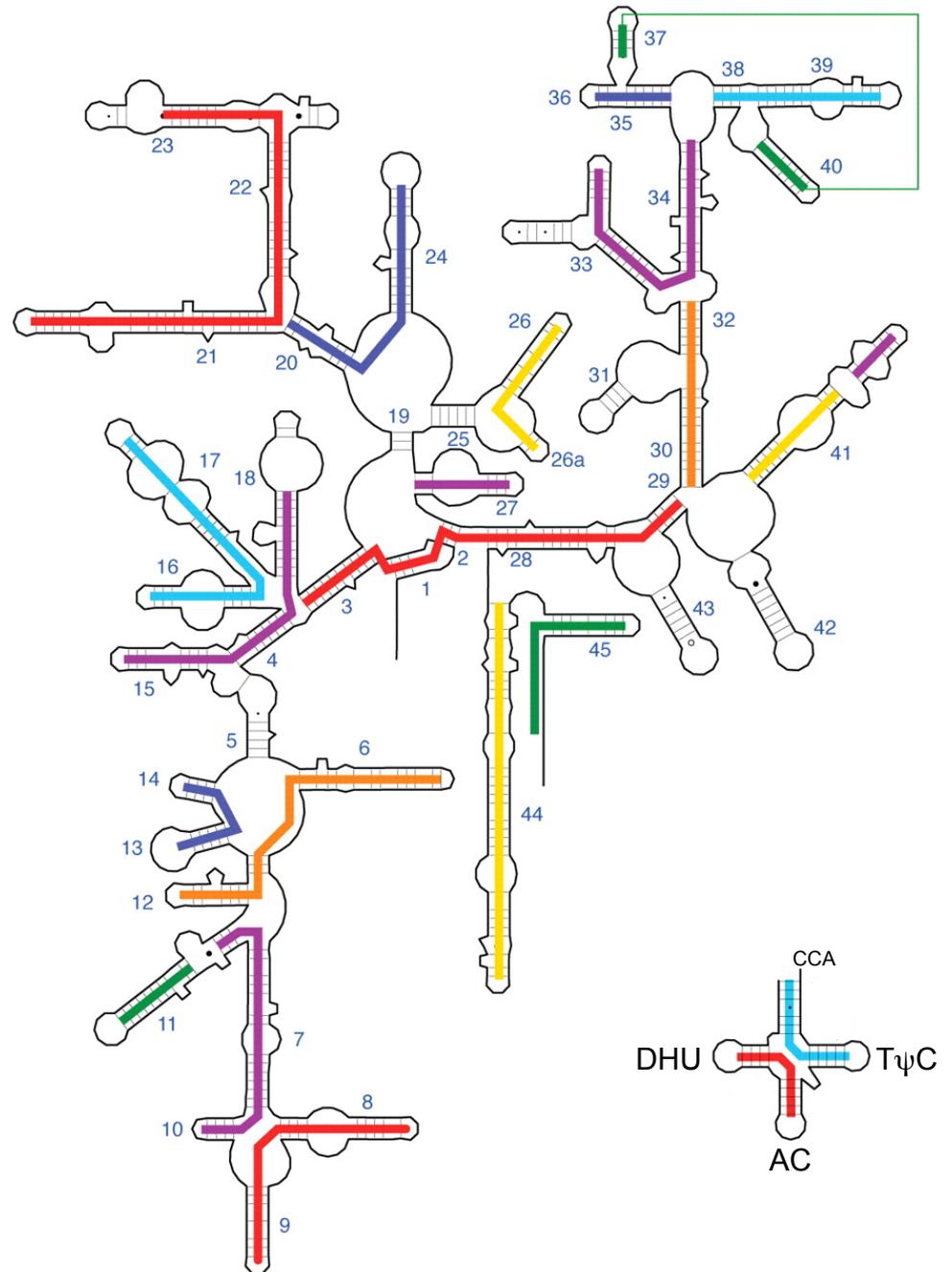
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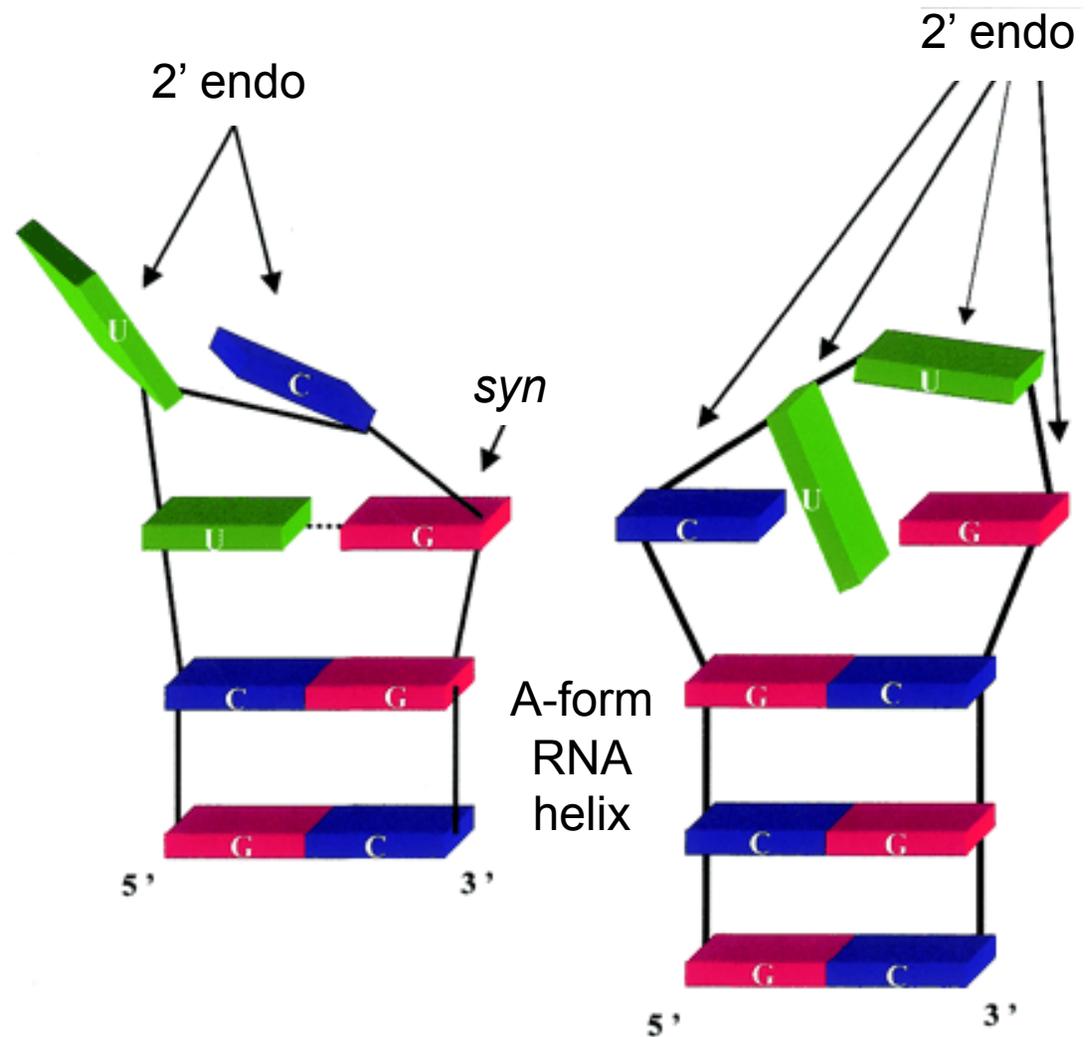
HN Noller (2005) RNA Structure: Reading the Ribosome. Science 309, 1508-1514.

Coaxial stacking of helices  
in 16S rRNA and tRNA  
*and*  
the *RNA* folding problem!



HN Noller (2005)  
RNA Structure: Reading the Ribosome.  
Science 309, 1508-1514.

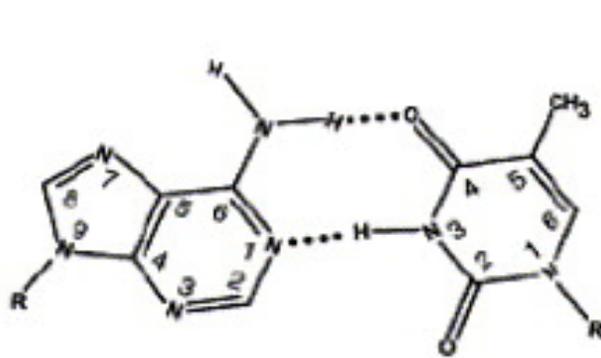
Tetraloops are magical, unexpected, evolutionarily conserved, tight packings of loops containing only four nucleotides



Baumruk et al. (2001) Comparison between UUCG and CUUG tetraloops: thermodynamic stability and structural features analyzed by UV absorption and vibrational spectroscopy. *Nucleic Acids Res* 29, 4089–4096

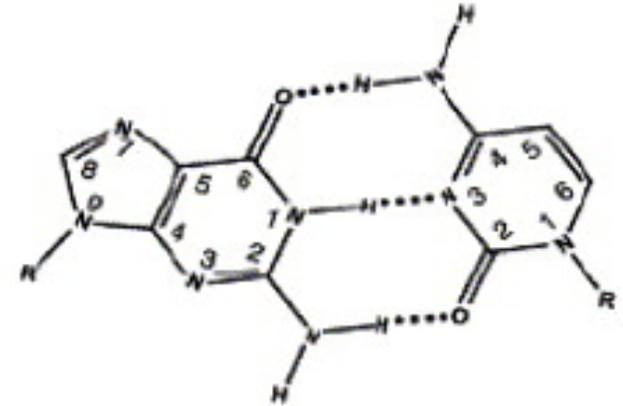
Hoogsteen base pairs use the top edge of the purine base

Watson-Crick  
base pairs



Adenine (*anti*)

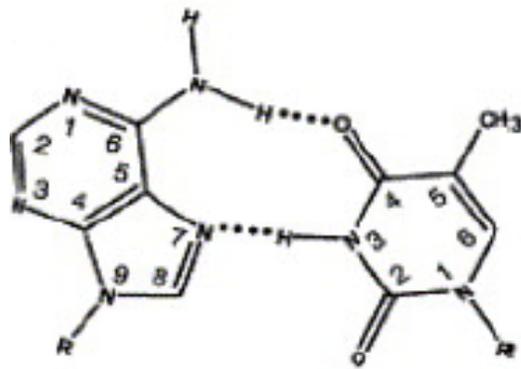
Thymine (*anti*)



Guanine (*anti*)

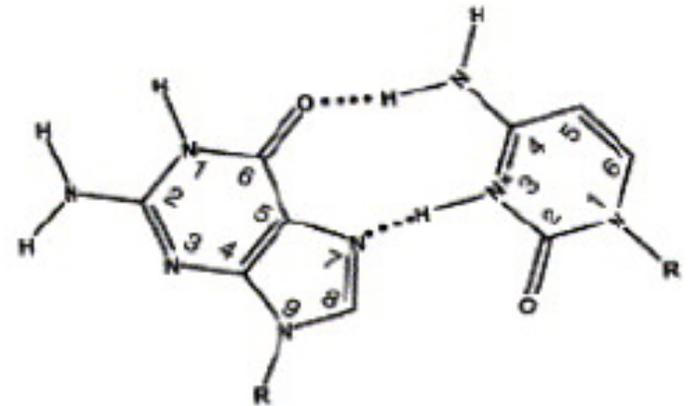
Cytosine (*anti*)

Hoogsteen  
base pairs



Adenine (syn)

Thymine (anti)



Guanine (syn)

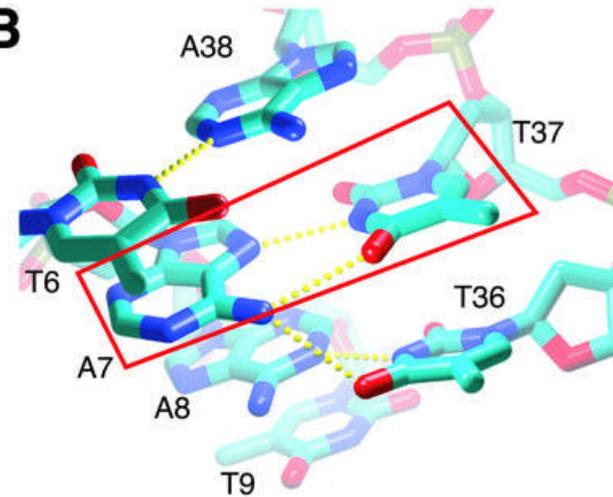
Cytosine (anti)

Yeast MAT $\alpha$ 2 homeodomain bound to DNA:  
a Hoogsteen base pair embedded in (almost) undistorted B-DNA

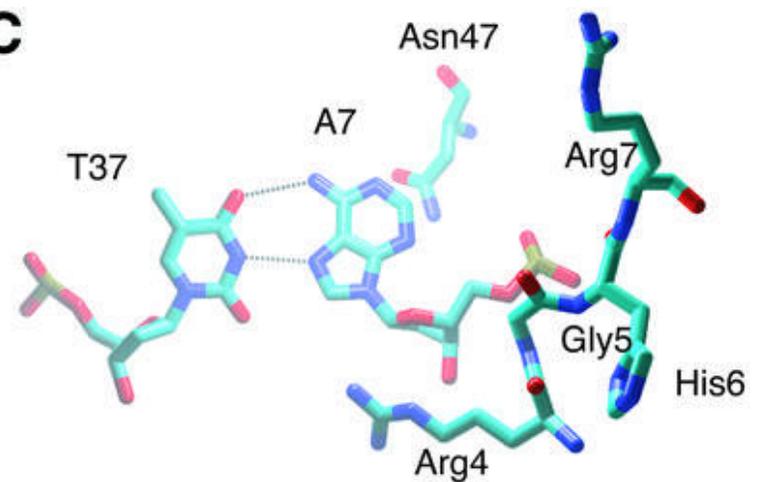
**A**



**B**



**C**

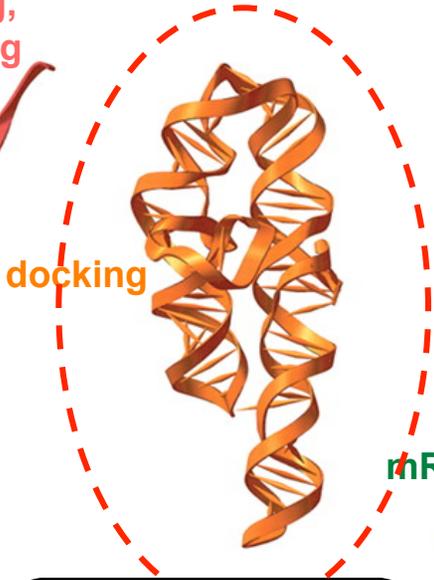


1974 to 2005: tRNA to Group I introns to the ribosome

coaxial stacking,  
kissing, tethering

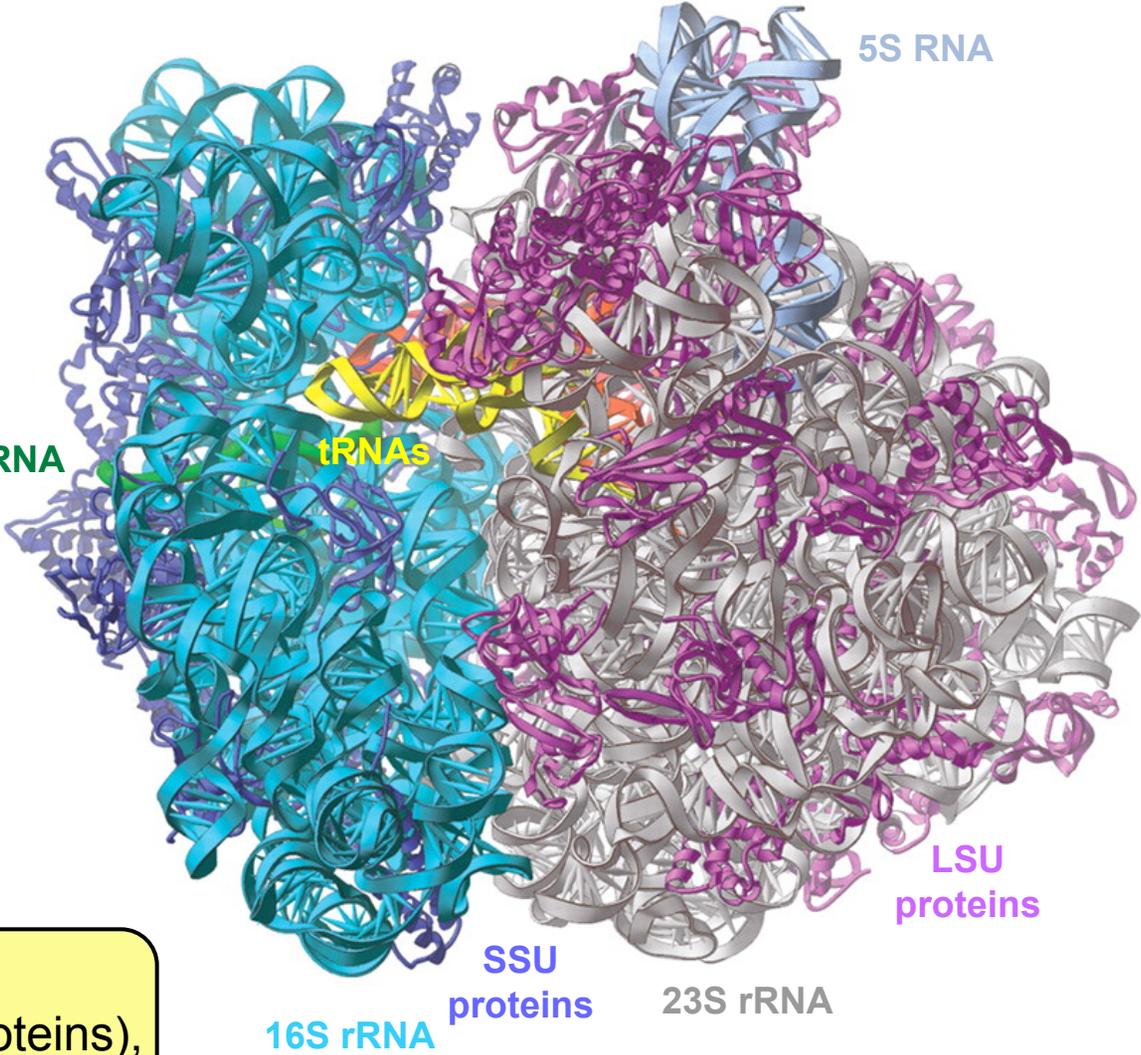


tRNA  
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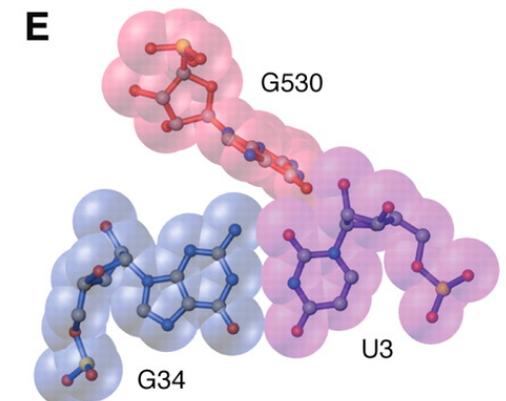
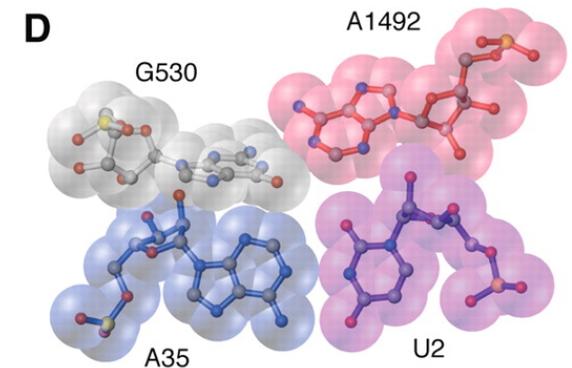
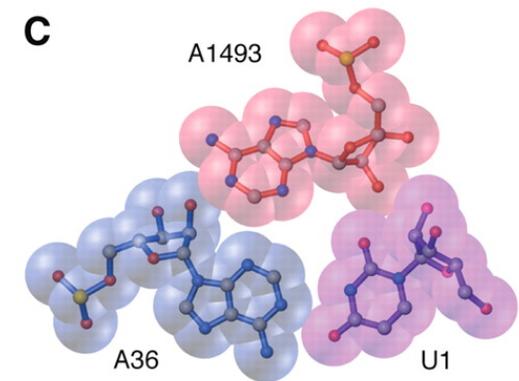
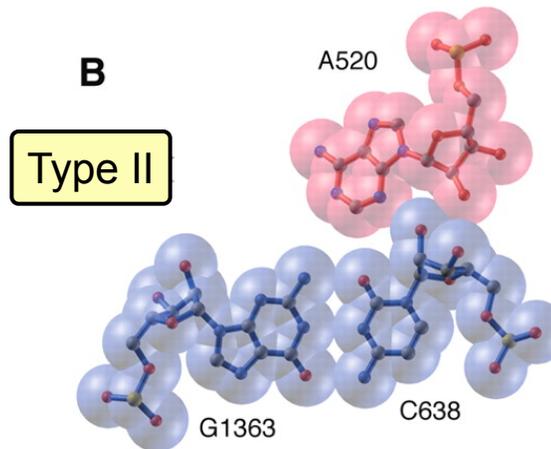
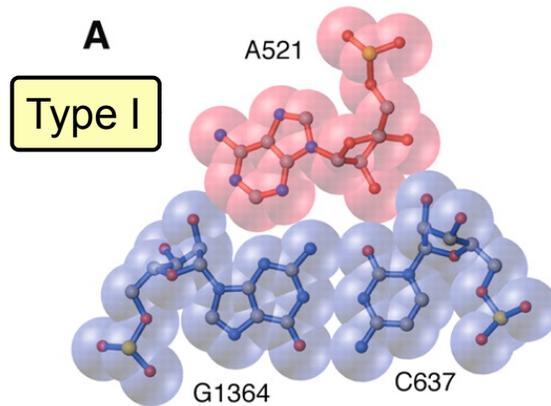
Group I ribozyme  
P4-P6 domain  
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70S ribosome  
(4530 nt total plus 50 proteins),  
3 rRNAs, 2 tRNAs, 1 mRNA



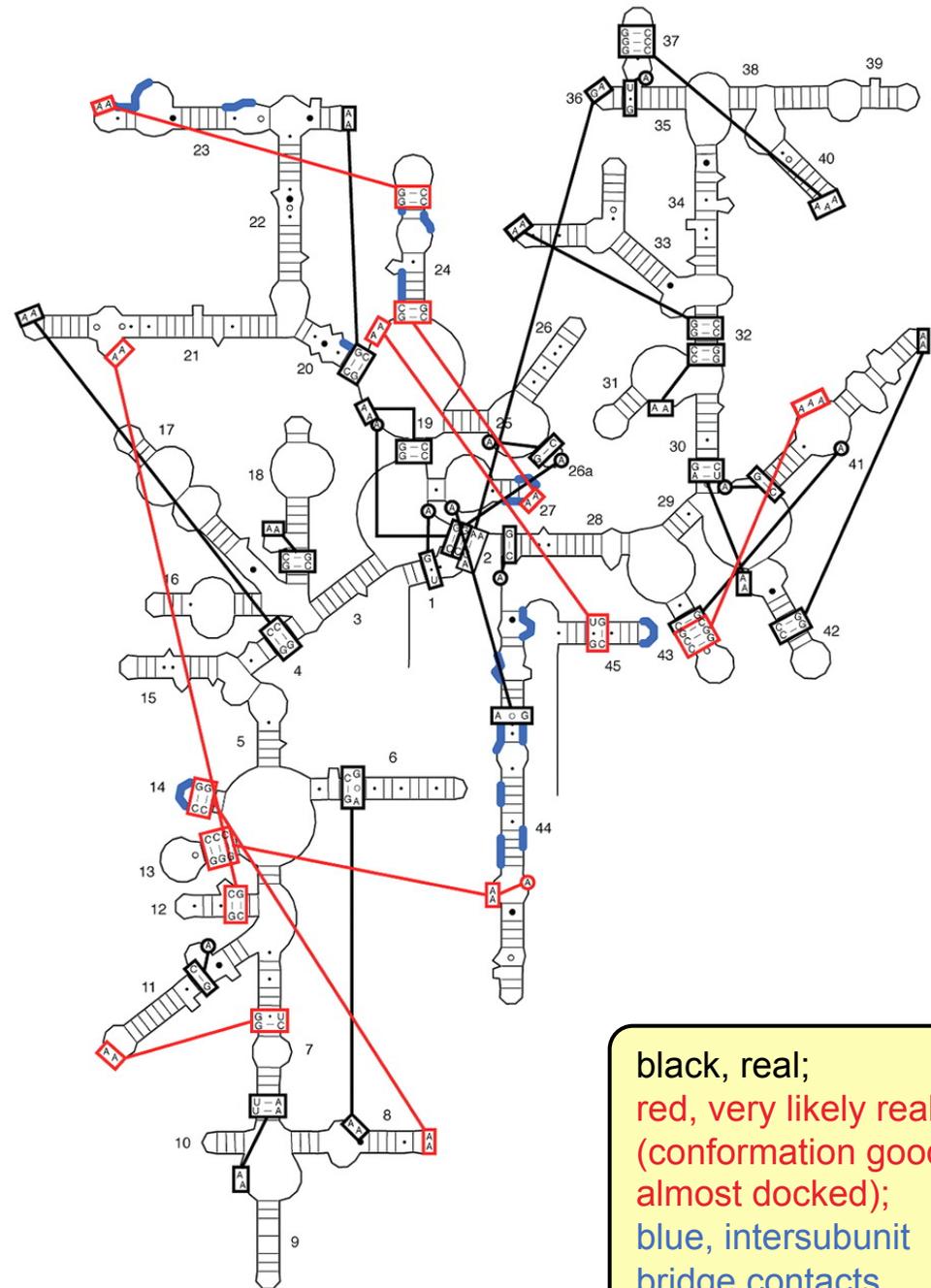
HN Noller (2005) RNA Structure: Reading the Ribosome. Science 309, 1508-1514.

"A-minor" interactions:  
a lock-and-key  
between (usually)  
an adenosine and  
the minor groove  
of a Watson-Crick pair



HN Noller (2005)  
RNA Structure: Reading the Ribosome.  
Science 309, 1508-1514.

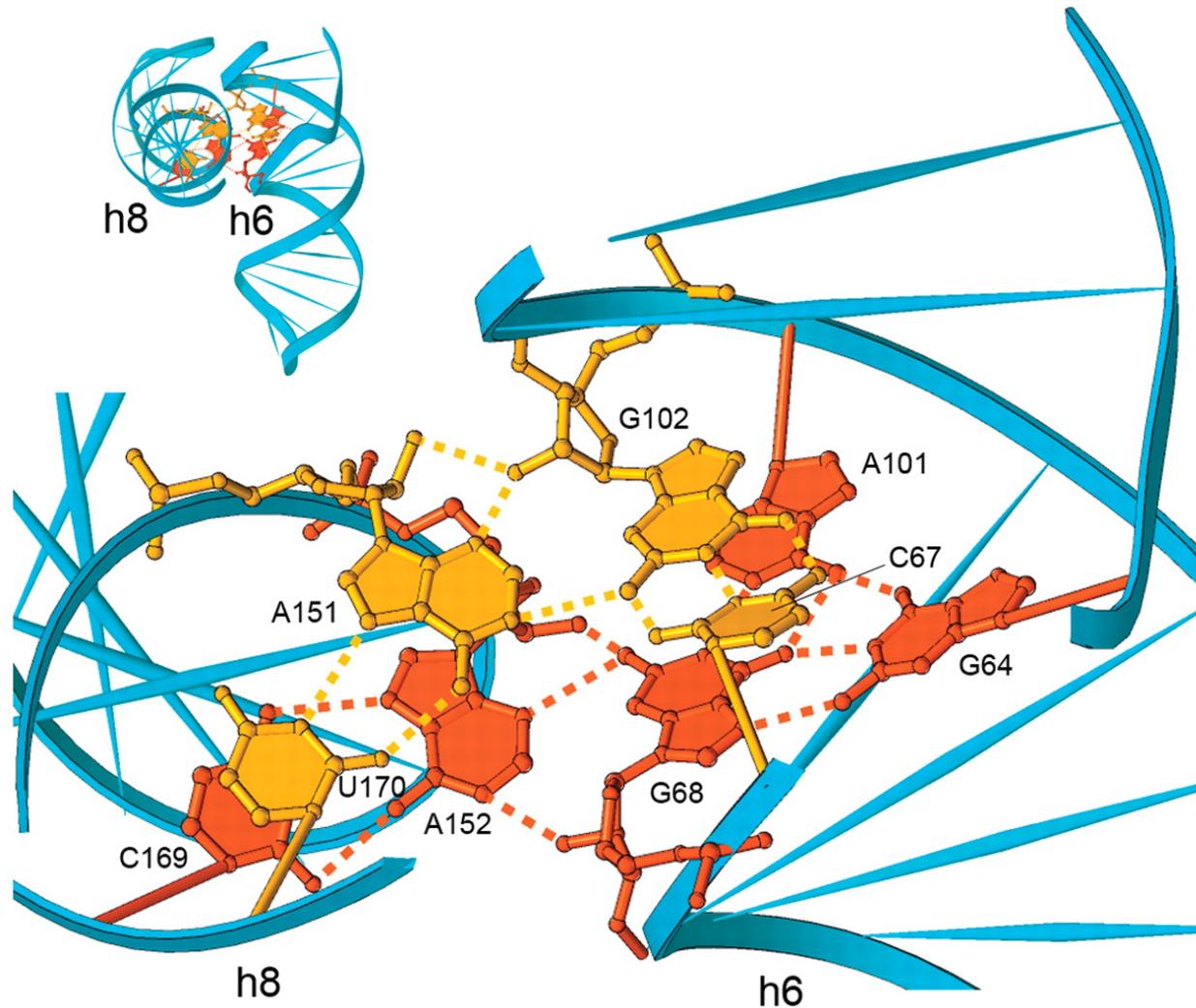
A-minor interactions in 16S rRNA as seen in the 3.0 Å crystal structure of the *Thermus thermophilus* 30S ribosomal subunit



black, real;  
red, very likely real;  
(conformation good,  
almost docked);  
blue, intersubunit  
bridge contacts

HN Noller (2005) RNA Structure: Reading the Ribosome. Science 309, 1508-1514.

How the ribosome packs two helices in 16S rRNA  
at right angles to each other



HN Noller (2005) Science 309, 1508-1514.

# *RNA-Puzzles: A CASP\**-like evaluation of RNA three-dimensional structure prediction

JOSÉ ALMEIDA CRUZ,<sup>1</sup> MARC-FRÉDÉRIC BLANCHET,<sup>2</sup> MICHAL BONIECKI,<sup>3</sup> JANUSZ M. BUJNICKI,<sup>3,4</sup> SHI-JIE CHEN,<sup>5</sup> SONG CAO,<sup>5</sup> RHIJU DAS,<sup>6,7</sup> FENG DING,<sup>8</sup> NIKOLAY V. DOKHOLYAN,<sup>8</sup> SAMUEL COULBOURN FLORES,<sup>9</sup> LILI HUANG,<sup>10</sup> CHRISTOPHER A. LAVENDER,<sup>11</sup> VÉRONIQUE LISI,<sup>2</sup> FRANÇOIS MAJOR,<sup>2</sup> KATARZYNA MIKOLAJCZAK,<sup>3</sup> DINSHAW J. PATEL,<sup>10</sup> ANNA PHILIPS,<sup>3,4</sup> TOMASZ PUTON,<sup>4</sup> JOHN SANTALUCIA,<sup>12,13</sup> FREDRICK SIJENYI,<sup>13</sup> THOMAS HERMANN,<sup>14</sup> KRISTIAN ROTHER,<sup>4</sup> MAGDALENA ROTHER,<sup>4</sup> ALEXANDER SERGANOV,<sup>10</sup> MARCIN SKORUPSKI,<sup>4</sup> TOMASZ SOLTYSINSKI,<sup>3</sup> PARIN SRIPAKDEEVONG,<sup>6,7</sup> IRINA TUSZYNSKA,<sup>3</sup> KEVIN M. WEEKS,<sup>11</sup> CHRISTINA WALDSICH,<sup>15</sup> MICHAEL WILDAUER,<sup>15</sup> NEOCLES B. LEONTIS,<sup>16</sup> and ERIC WESTHOF<sup>1,17</sup>

## ABSTRACT

We report the results of a first, collective, blind experiment in RNA three-dimensional (3D) structure prediction, encompassing three prediction puzzles. The goals are to assess the leading edge of RNA structure prediction techniques; compare existing methods and tools; and evaluate their relative strengths, weaknesses, and limitations in terms of sequence length and structural complexity. The results should give potential users insight into the suitability of available methods for different applications and facilitate efforts in the RNA structure prediction community in ongoing efforts to improve prediction tools. We also report the creation of an automated evaluation pipeline to facilitate the analysis of future RNA structure prediction exercises.

*RNA* 18, 610-625 (2012)

\*Critical Assessment of protein Structure Prediction

THE LATEST NEWS: Cheng et al. (2015) Consistent global structures of complex RNA states through multidimensional chemical mapping. *eLife* 4, e07600

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kissing, tethering



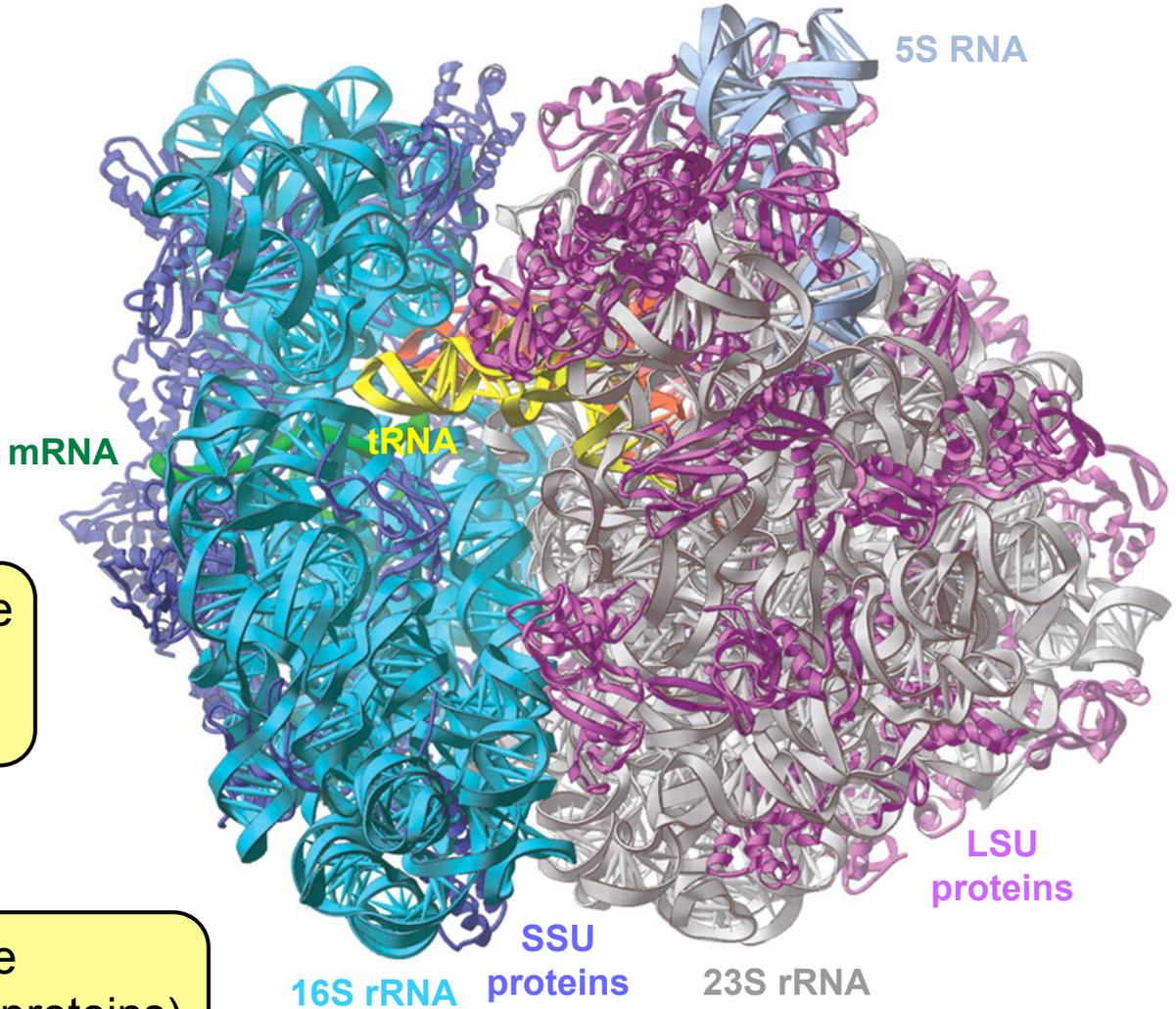
tRNA  
(76 nt)

docking



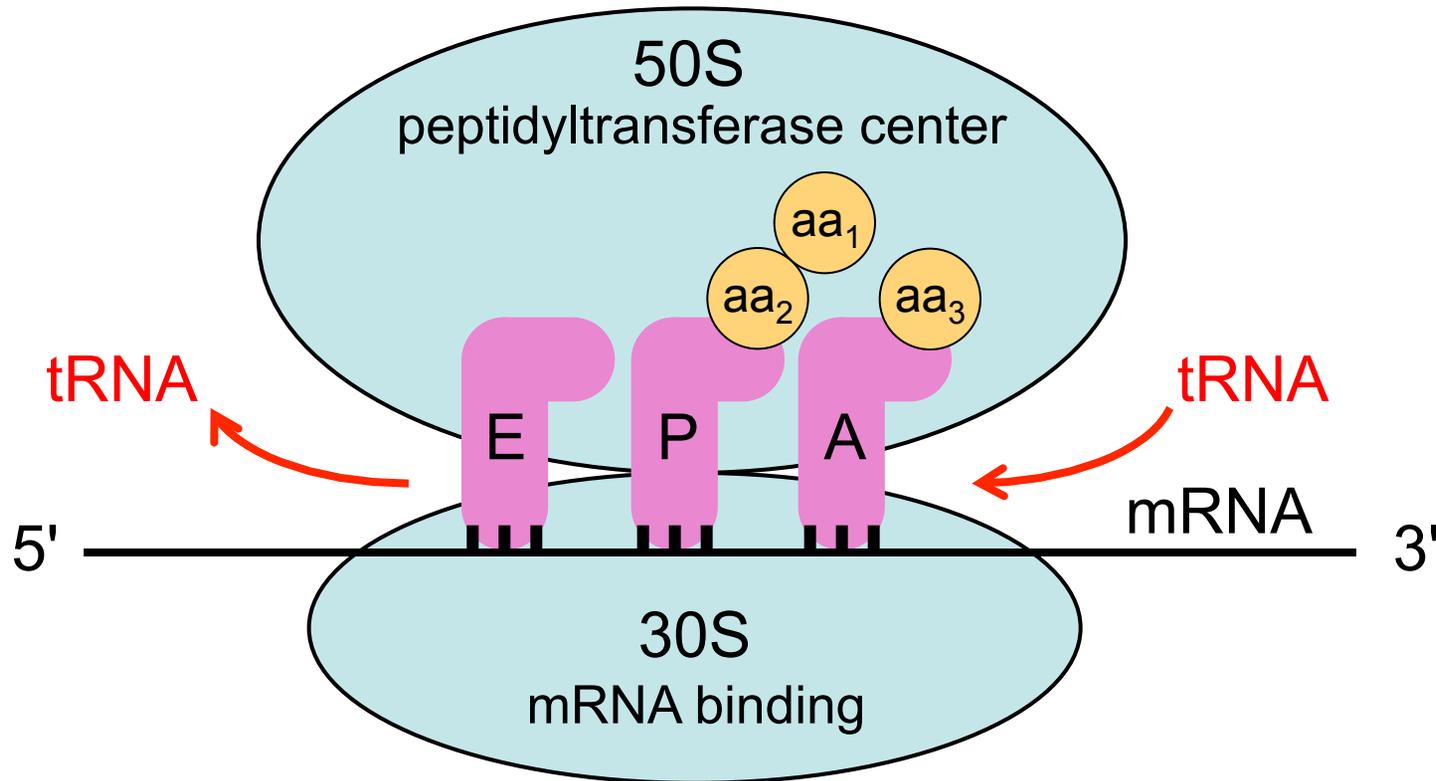
Group I ribozyme  
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70S ribosome  
(4530 nt total plus 50 proteins),  
3 rRNAs, 2 tRNAs, 1 mRNA



HN Noller (2005) RNA Structure: Reading the Ribosome. Science 309, 1508-1514.

All essential parts of the translation apparatus are made of RNA



and translation requires large movements of large molecules in close quarters

Ramakrishnan et al. (2006) Structure of the 70S ribosome complexed with mRNA and tRNA. Science 313, 1935-1942

## RNPs (ribonucleoprotein complexes) you may know...

- ribosome (SSU and LSU)
- signal recognition particle (SRP)
- small nuclear ribonucleoprotein particles (snRNPs)
- small nucleolar ribonucleoprotein particles (snoRNPs)

but long ago, in 1968...

Francis Crick asked a dangerous question using the forbidden **W word** (why?) instead of the publicly acceptable **H word** (how?)

# The ribosome is a giant ribozyme!

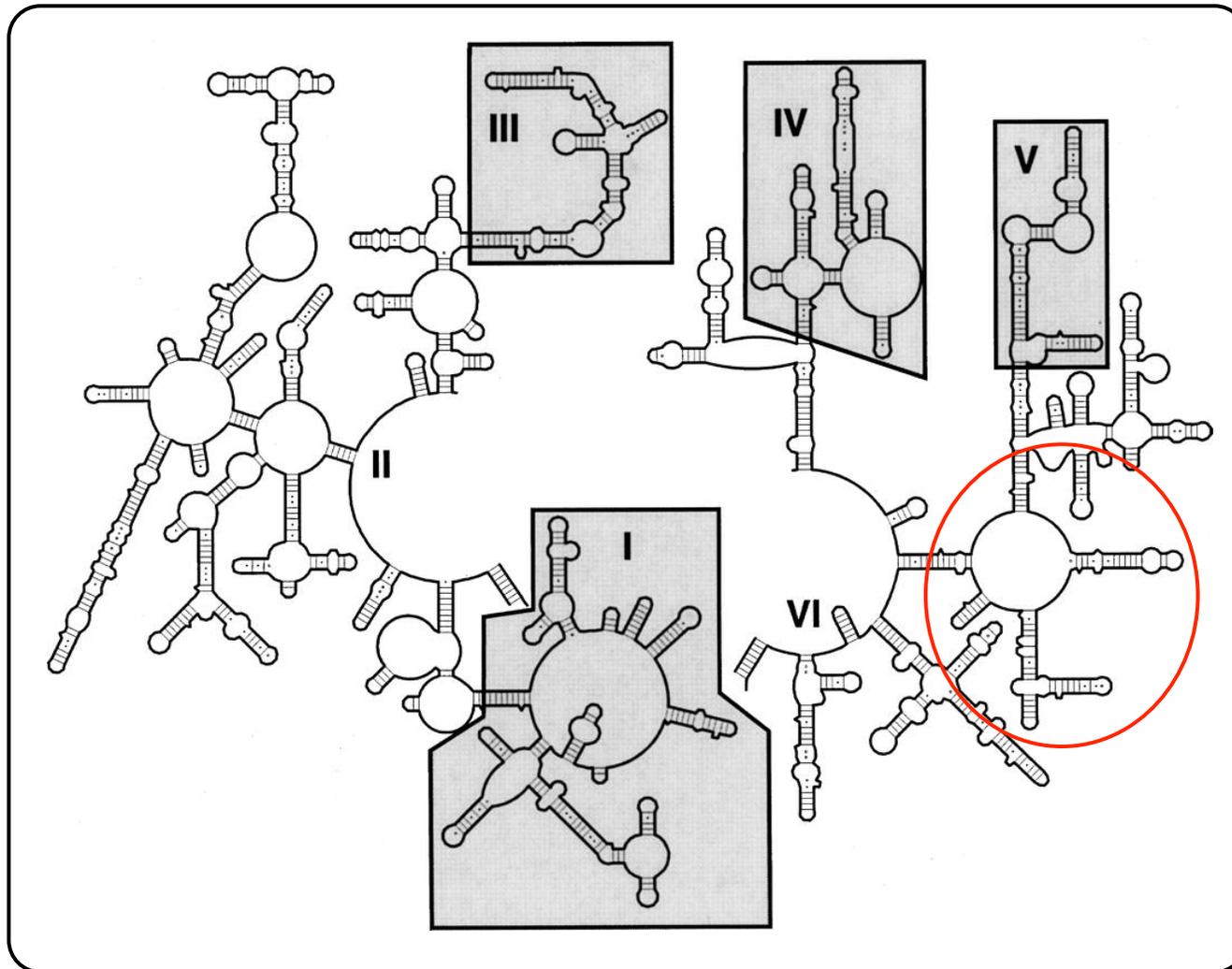
50S	=	34 proteins + 2904 base ribosomal RNA
30S	=	21 proteins + 1542 base ribosomal RNA

Crick argued in 1968 (J Mol Biol 38, 367-379):

1. Why is ribosome made half of protein and half of RNA?
2. What is job of ribosome? To make protein.
3. Therefore, the first ribosome had NO proteins to help out.
4. Therefore, the RNA itself must have been catalytic.
5. If RNA can be catalytic, RNA could have copied itself, functioning both as genome AND as replicase.
6. Therefore, the first "living molecule" could have been RNA!

Gilbert carried this idea further in 1986 (Nature 319, 618)  
by coining a magical phrase, "The RNA World."

The large (23S) and small (16S) ribosomal RNAs have highly conserved secondary and tertiary structures

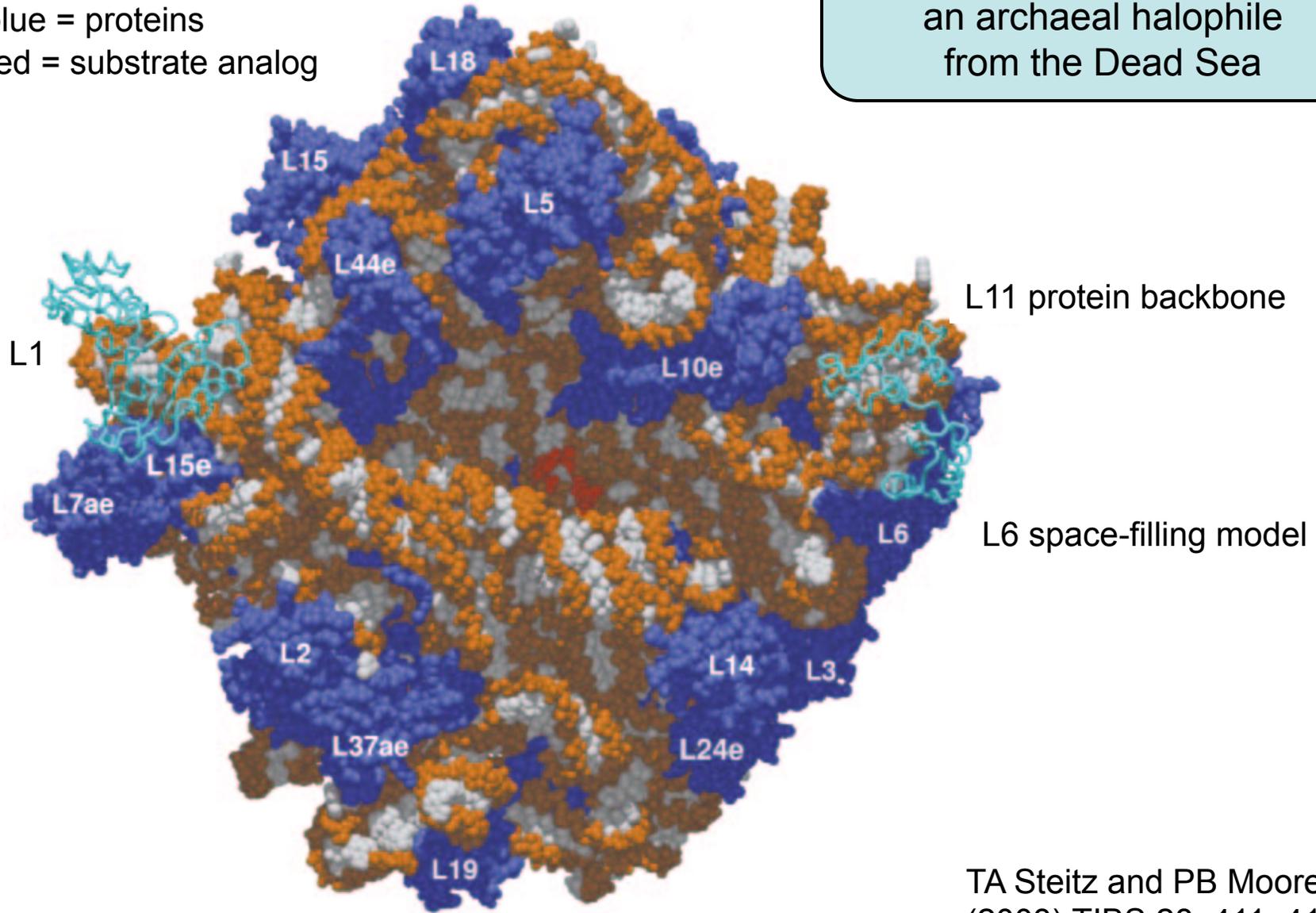


the peptidyltransferase center on 23S rRNA is especially highly conserved; it is the locus of many antibiotic resistance mutations; and it is preferentially inactivated by RNA-modifying reagents

Lieberman et al. (2000)  
J Mol Biol 297, 1129

orange = sugar phosphate backbone  
white = nucleotide bases  
blue = proteins  
red = substrate analog

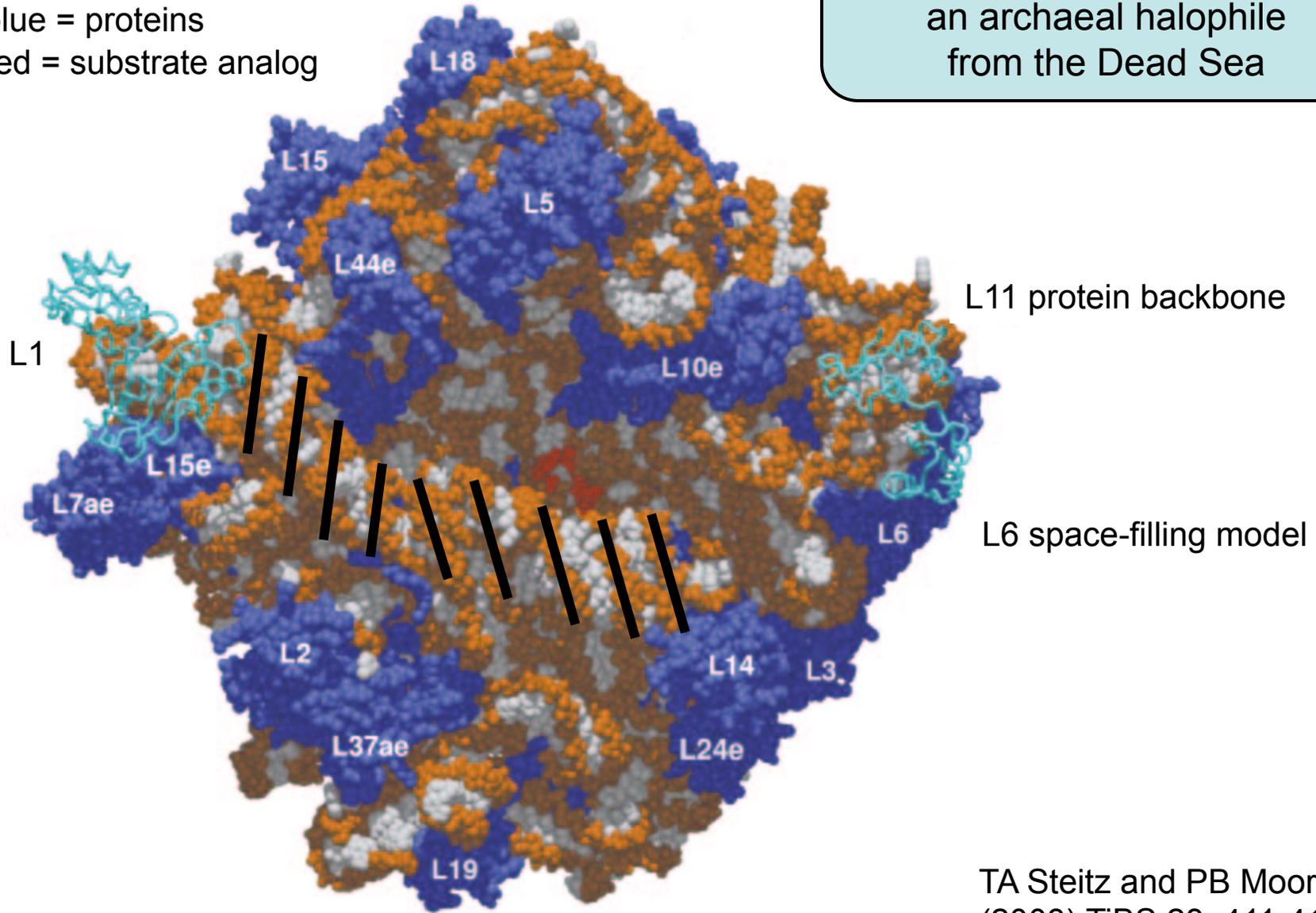
Large (50S) ribosome subunit  
from *Haloarcula marismortui*,  
an archaeal halophile  
from the Dead Sea



TA Steitz and PB Moore  
(2003) TIBS 28, 411–418

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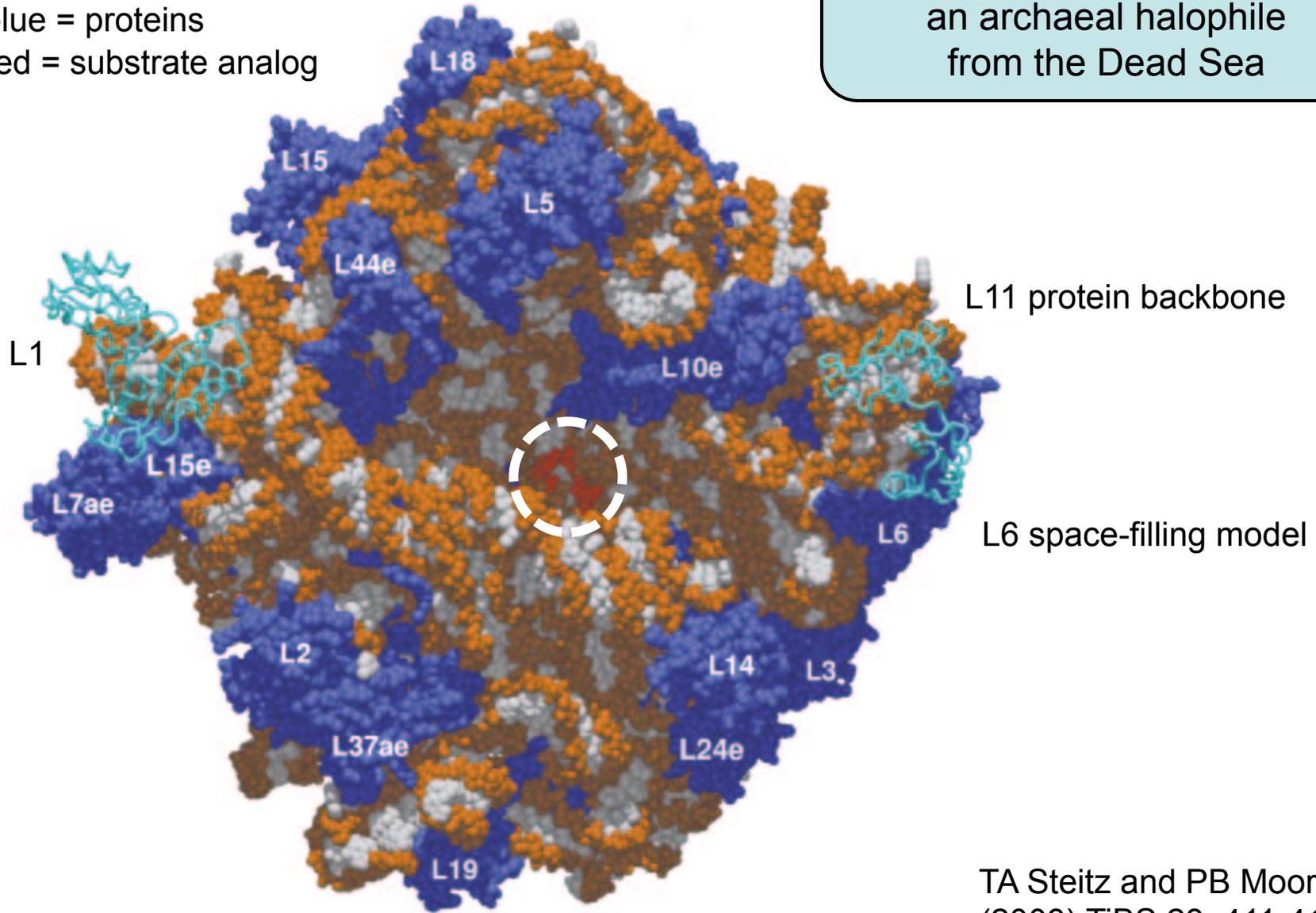
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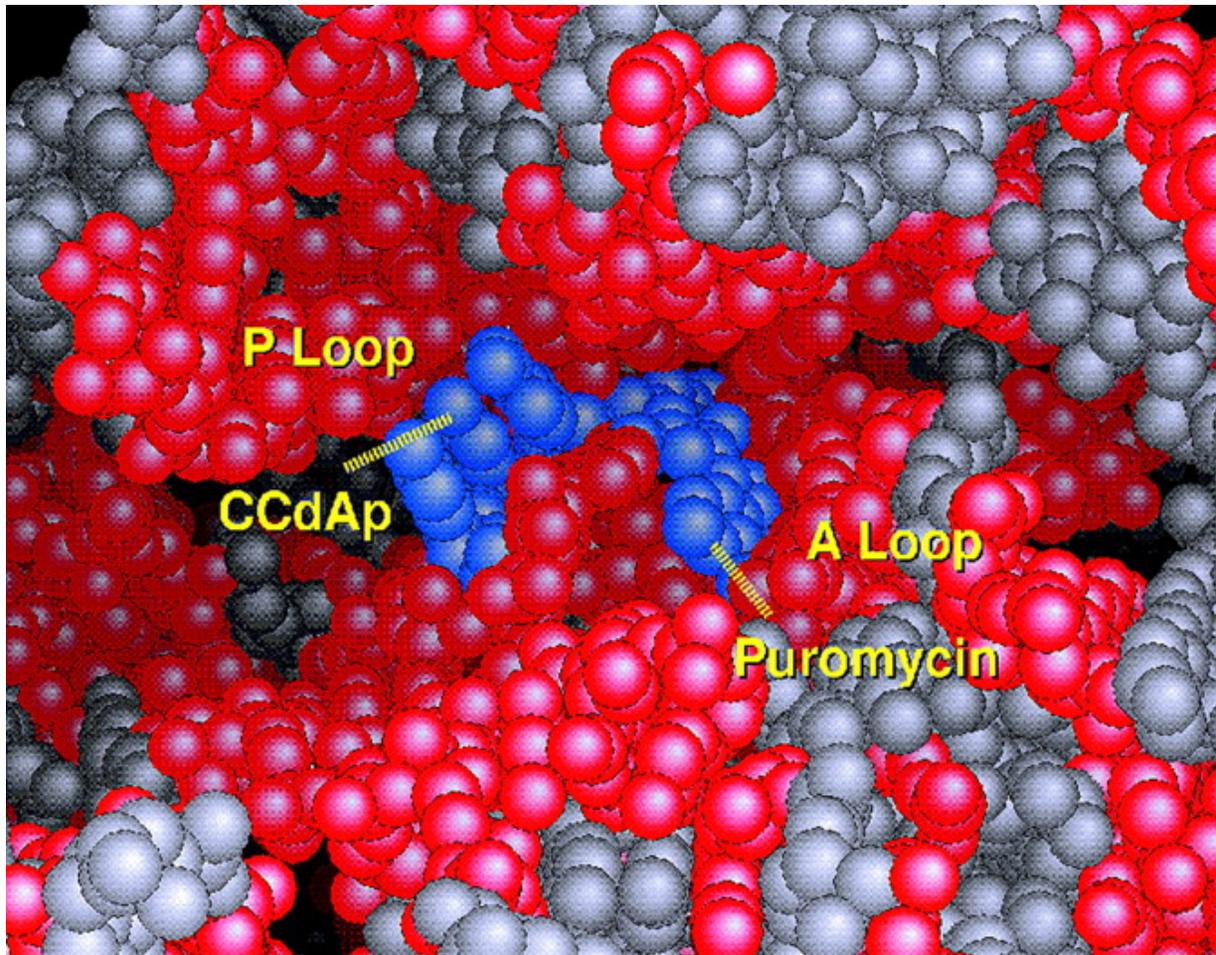
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Large (50S) ribosome subunit  
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TA Steitz and PB Moore  
(2003) *TiBS* 28, 411-418

The ribosome is a ribozyme...  
there are no proteins within 18 Angstroms of the active site



A space-filling model of the peptidyltransferase center, with substrate analog (puromycin) in **blue**, RNA in **red**, and proteins in **gray**

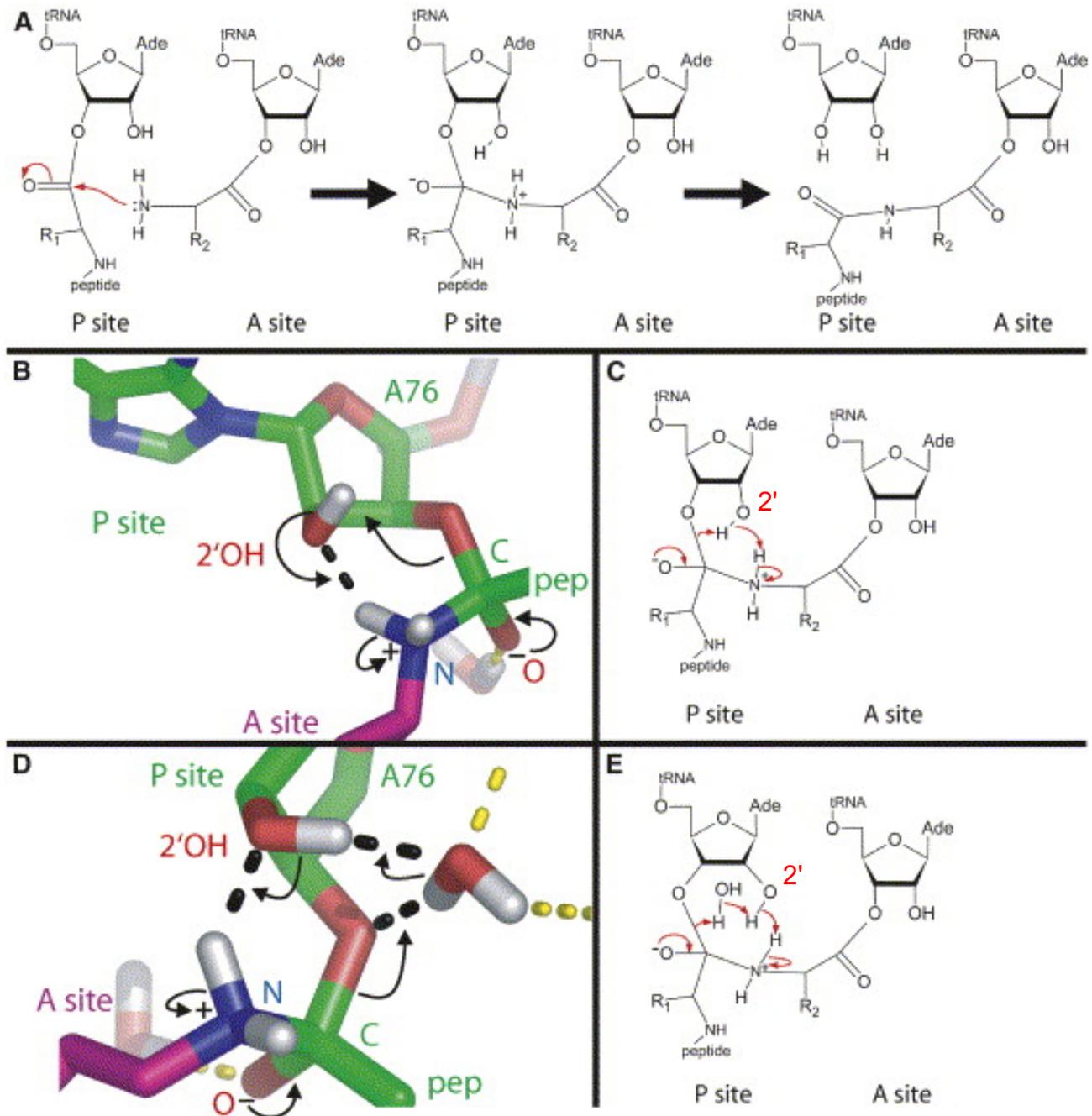
Steitz and Moore (2003)  
TIBS 28, 411-418

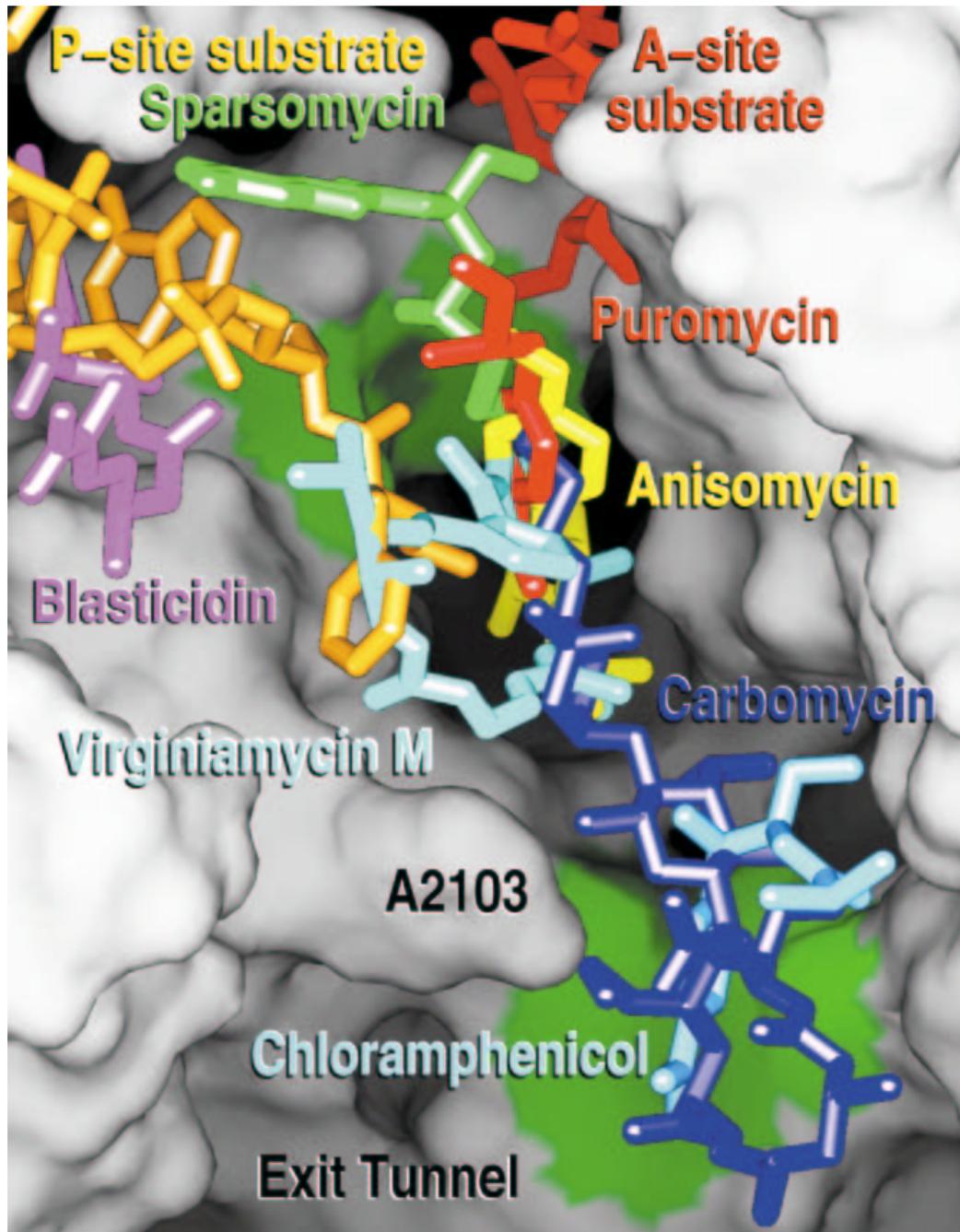


## Substrate-assisted peptidyl transfer

The  $\alpha$ -amino group of the incoming aminoacyl-tRNA attacks the ester carbon of the resident peptidyl-tRNA to yield a tetrahedral intermediate (A). The intermediate breaks down to deacylated tRNA and elongated peptidyl-tRNA through a proton shuttle mechanism involving either the 2' hydroxyl of A76 on the peptidyl-tRNA substrate (B and C) or water molecules that interact with the 2' and 3' hydroxyls of A76 (D and E). Note that converting the 2' hydroxyl of A76 to a 2' deoxy reduces the rate of peptidyl transfer by  $10^6$  consistent with this proton shuttle mechanism.

Schmeing, Strobel,  
Steitz et al. (2005)  
Mol Cell 20, 437-448



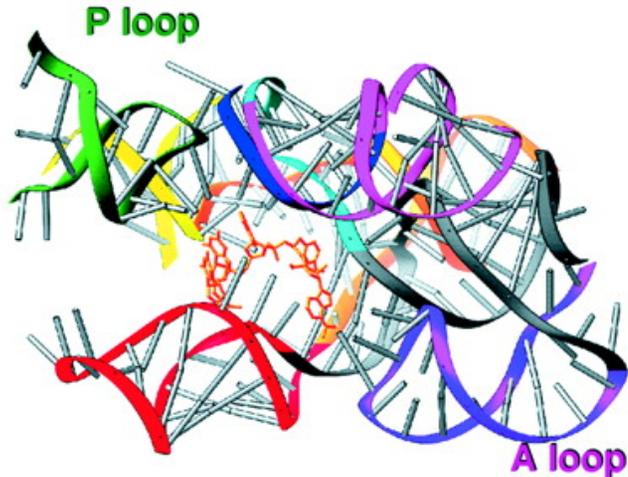


Many antibiotics target overlapping regions of the peptidyltransferase center, or block the exit tunnel for the growing polypeptide.

TA Steitz and PB Moore  
(2003) TIBS 28, 411-418

Many antibiotics interact with the large ribosomal RNA that surrounds the peptidyltransferase center

backside view

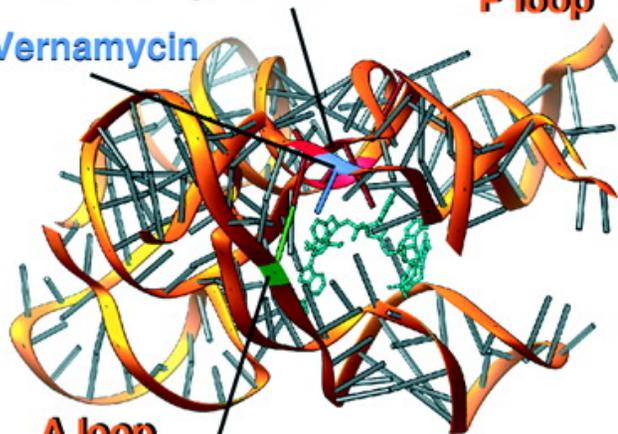


Chloramphenicol

P loop

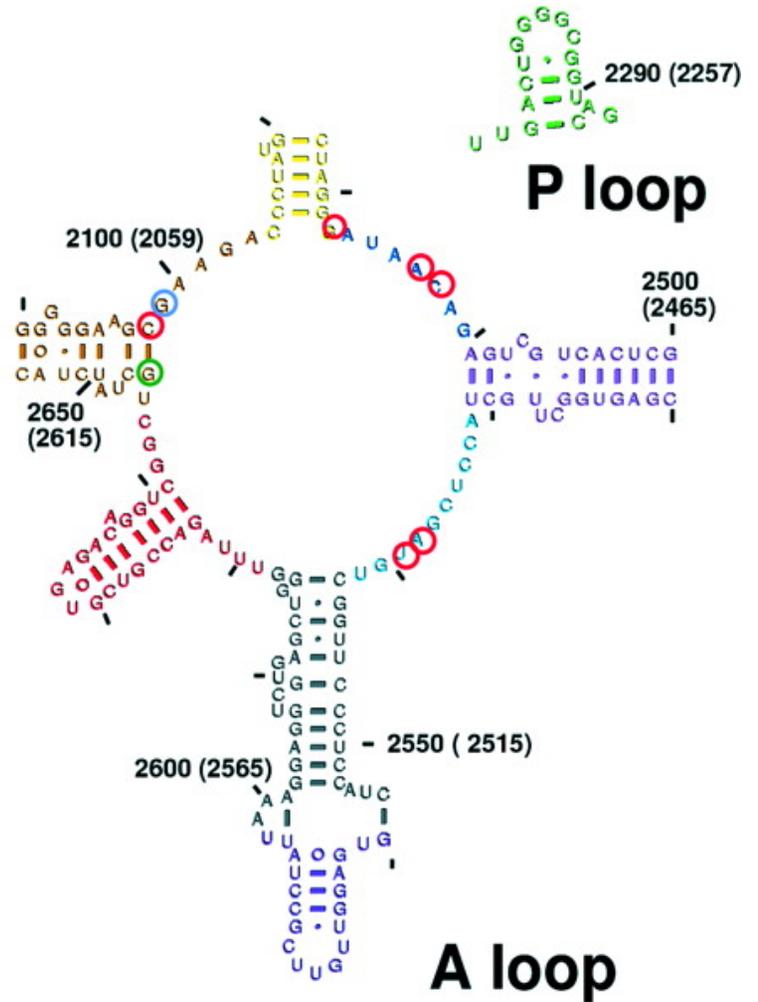
Vernamycin

frontside view



A loop

Erythromycin



to be continued....