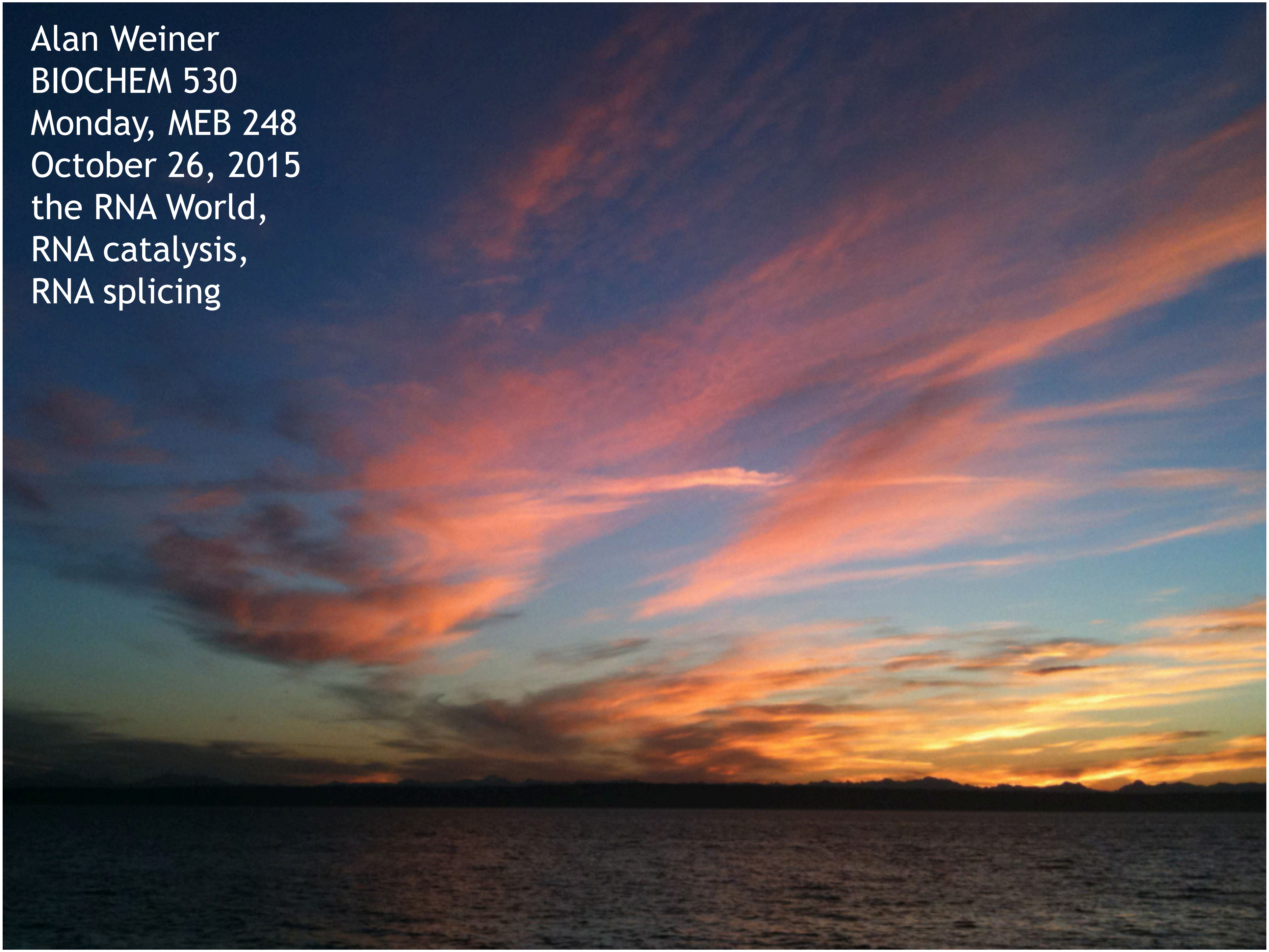
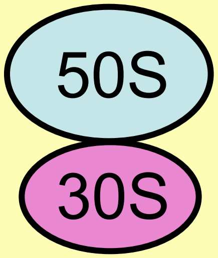

Alan Weiner
BIOCHEM 530
Monday, MEB 248
October 26, 2015
the RNA World,
RNA catalysis,
RNA splicing

The ribosome is a giant ribozyme!



= 34 proteins + 2904 base ribosomal RNA

= 21 proteins + 1542 base ribosomal RNA

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

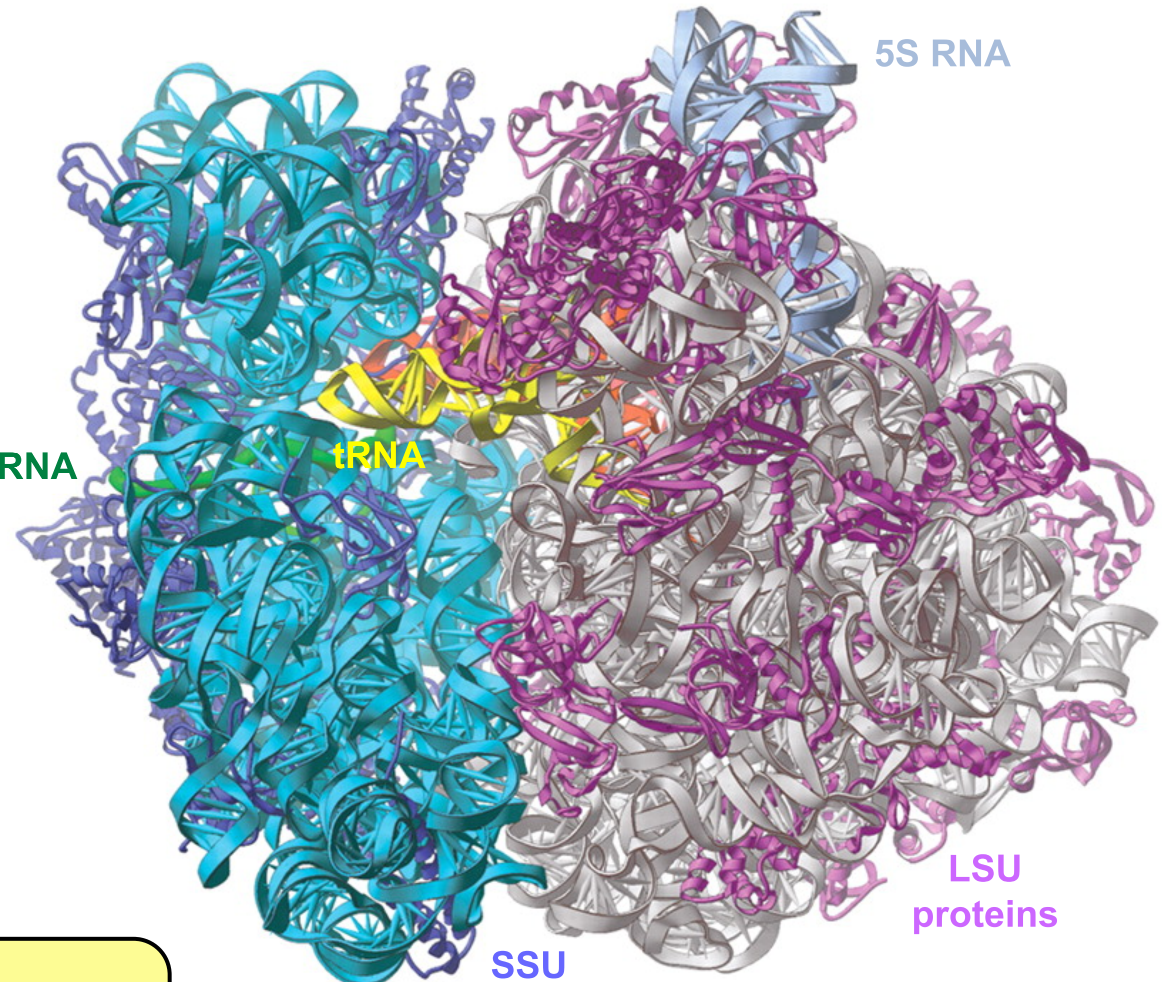
1. Why is ribosome made half of protein and half of RNA?
2. What is job of ribosome? To make protein.
3. Therefore, the first ribosome had NO proteins to help out.
4. Therefore, the RNA itself must have been catalytic.
5. If RNA can be catalytic, RNA could have copied itself, functioning both as genome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

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

tRNA
(76 nt)

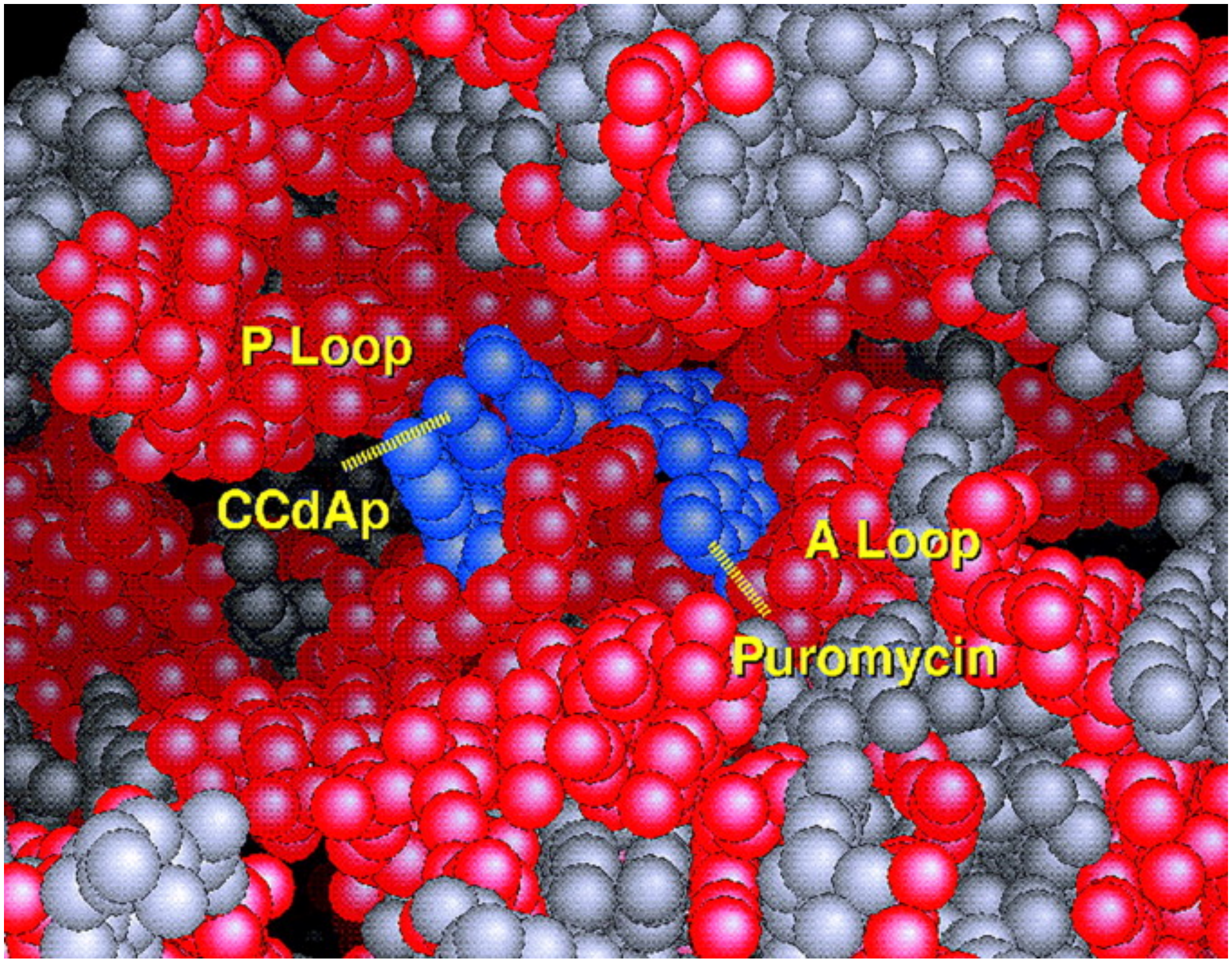
Group I ribozyme
P4-P6 domain
(160 nt)



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.

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

Molecular fossils

- A *molecular fossil* is any modern molecule whose structure or function provides a clue to its evolutionary history.

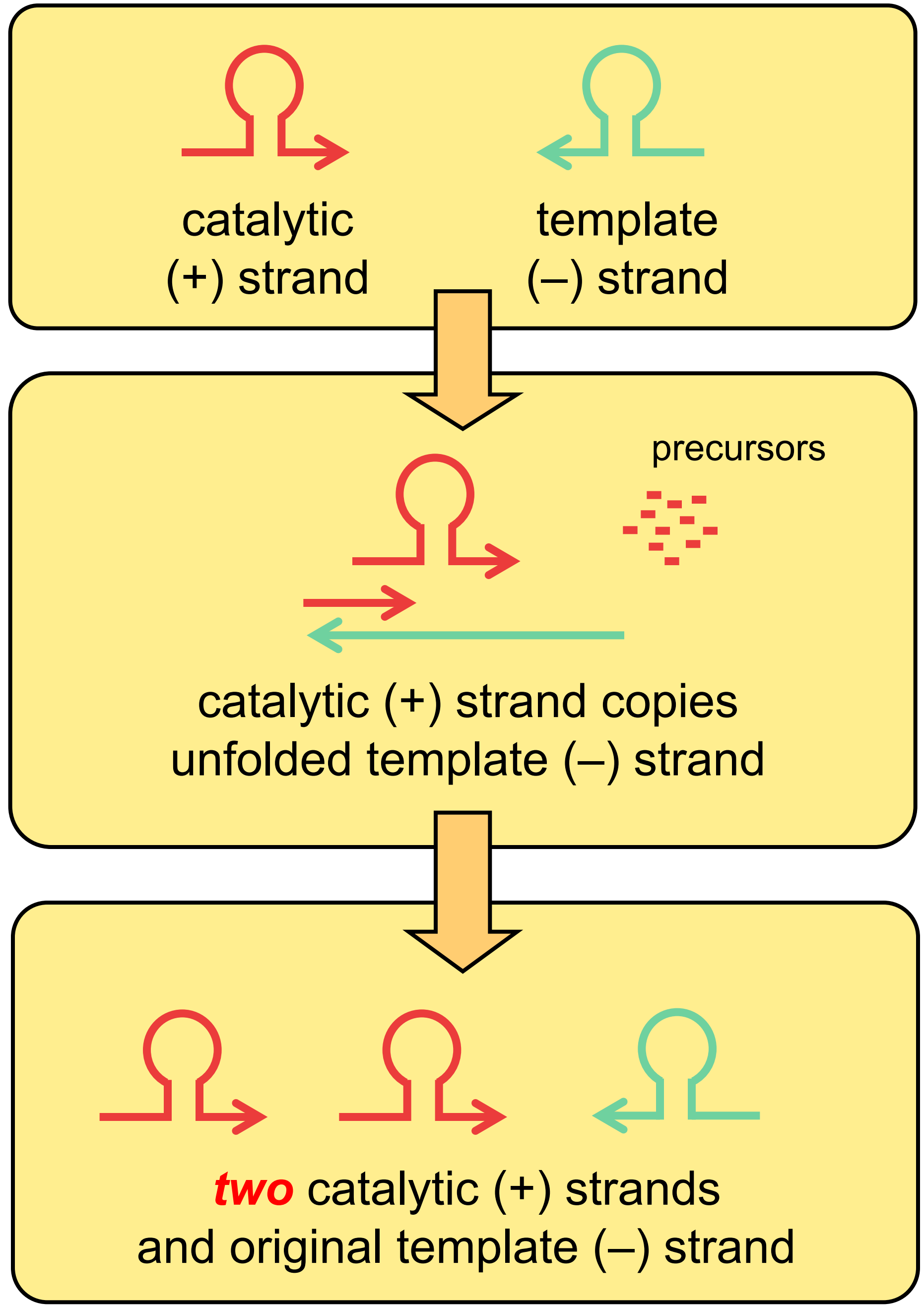
- *Molecular fossils* should not be confused with fossil molecules (DNA or insects preserved in amber) or "living fossils" (a slowly evolving organism like the deep-sea lobe-finned Coelacanth).

Weiner and Maizels (1987) tRNA-like structures tag the 3' ends of genomic RNA molecules for replication: implications for the origin of protein synthesis. *PNAS* 84, 7383-7387; Maizels and Weiner (1999) The genomic tag hypothesis: What molecular fossils tell us about the evolution of tRNA. Chapter 3, in *The RNA World II* (eds. Gesteland, Cech, and Atkins).

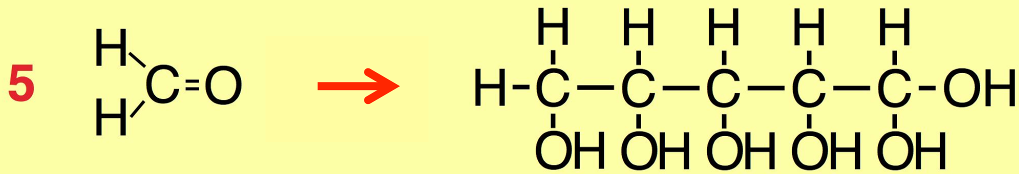
If RNA can be both catalyst and genome, RNA could replicate itself… the first "living" molecule

Crick, 1968

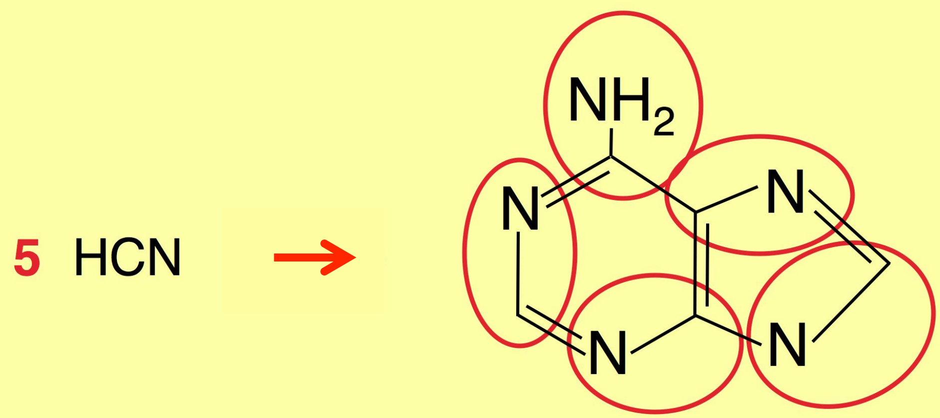
Lawrence and Bartel (2005) New ligase-derived RNA polymerase ribozymes. RNA 11, 1173-1180; Shechner and Bartel (2011) The structural basis of RNA-catalyzed RNA polymerization. Nat Struct Mol Biol 18, 1036-1042; Shechner et al. (2009) Crystal structure of the catalytic core of an RNA-polymerase ribozyme. Science 326, 1271-1275; Attwater et al. (2013) In-ice evolution of RNA polymerase ribozyme activity. Nature Chem 5, 1011-1018; and Lehman (2013) Origin of life: Cold-hearted RNA heats up life. Nature Chem 5, 987-989.



Nucleic acids are plausible prebiotic condensation products

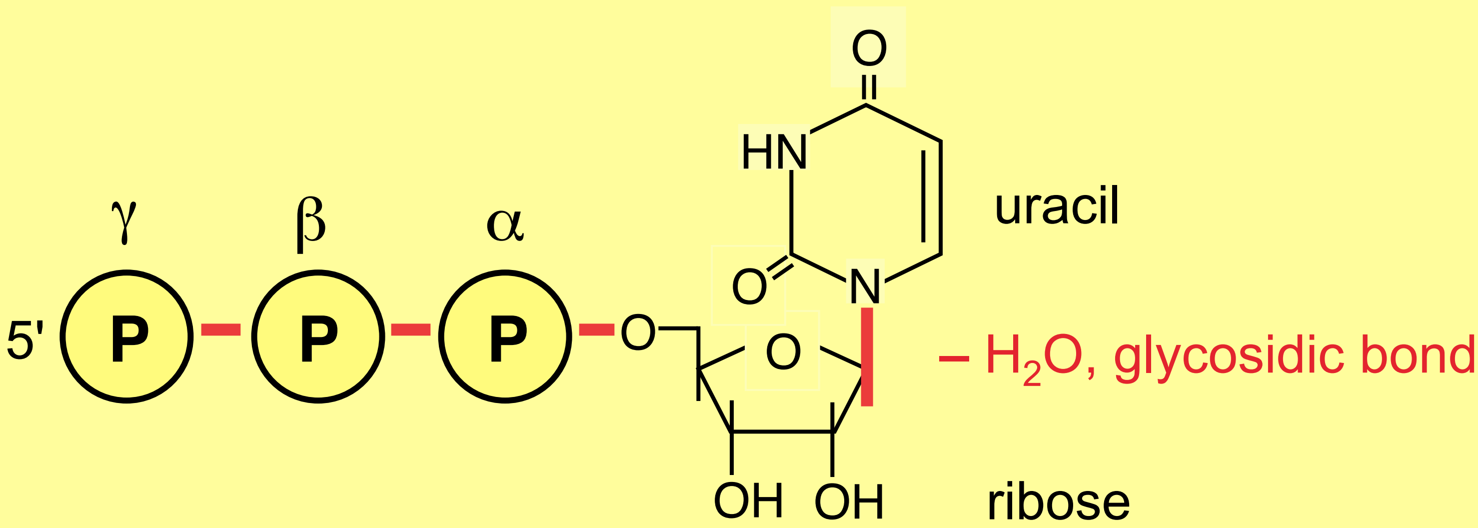


formaldehyde ribose



hydrogen cyanide adenine

Nucleic acids are plausible prebiotic condensation products generated by repeated dehydrations



– H_2O, phosphoester bond (ribose, α phosphate)

– H_2O, phosphoanhydride bonds (α,β and β,γ)

"Systems chemistry on early earth"… proposed by Oparin in 1938, tested by Miller in 1953 as his PhD Thesis!

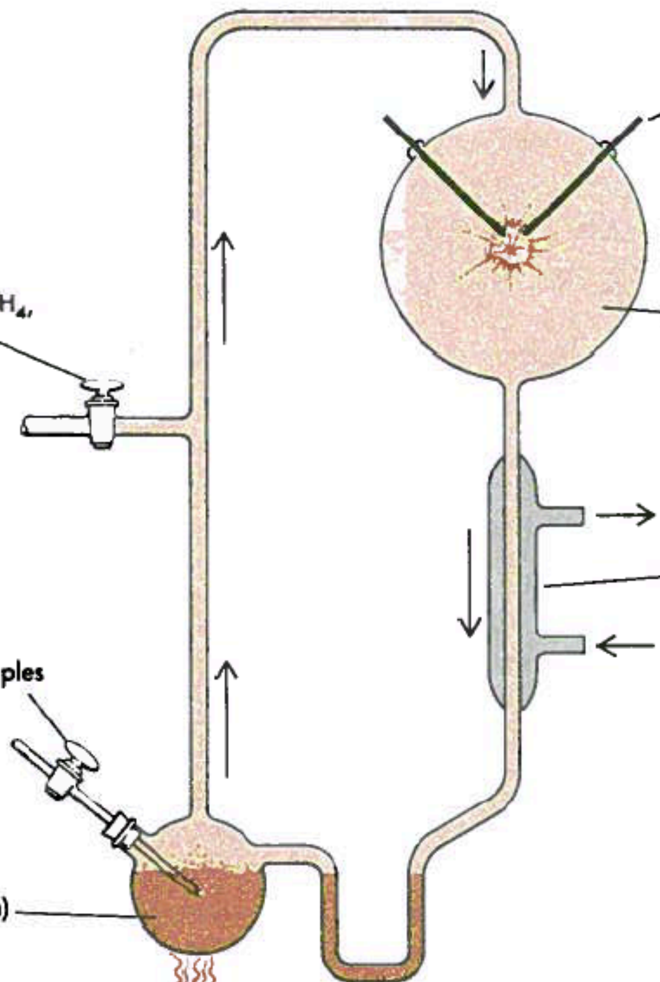
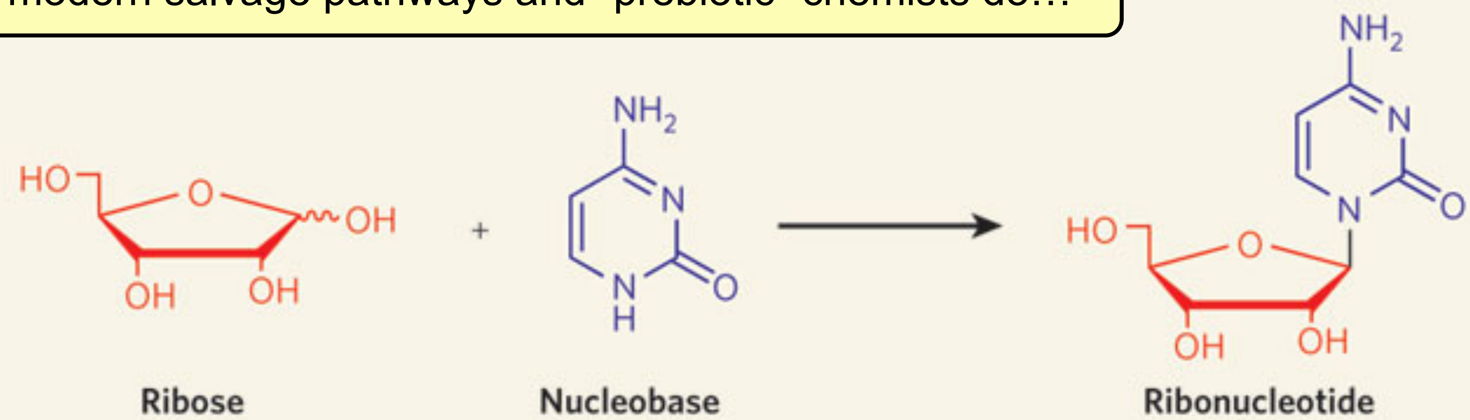


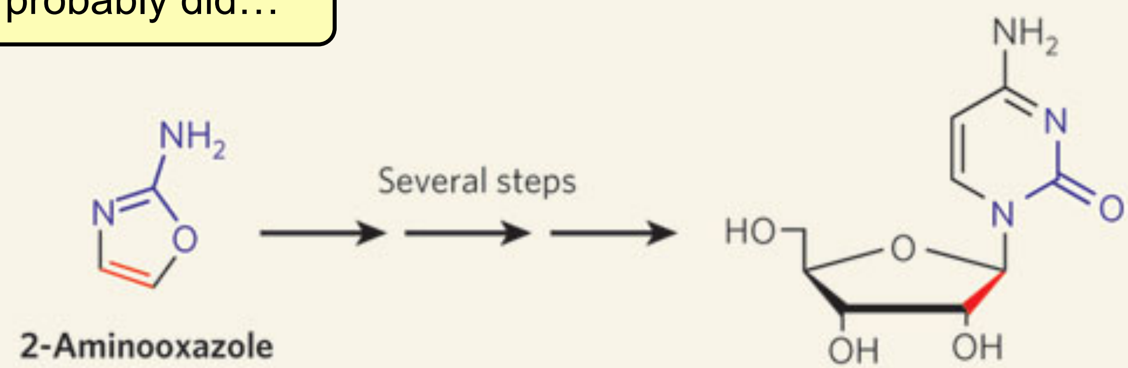
Figure 28-2
A reflux apparatus used to simulate prebiotic synthesis of organic molecules under conditions thought to prevail on the primitive Earth. [After S. L. Miller and L. E. Orgel, *The Origins of Life on Earth* (Englewood Cliffs, N.J.: Prentice-Hall, 1974), Figure 7-1.]

Whoops, right impulse, wrong concept!

What modern salvage pathways and "prebiotic" chemists do…

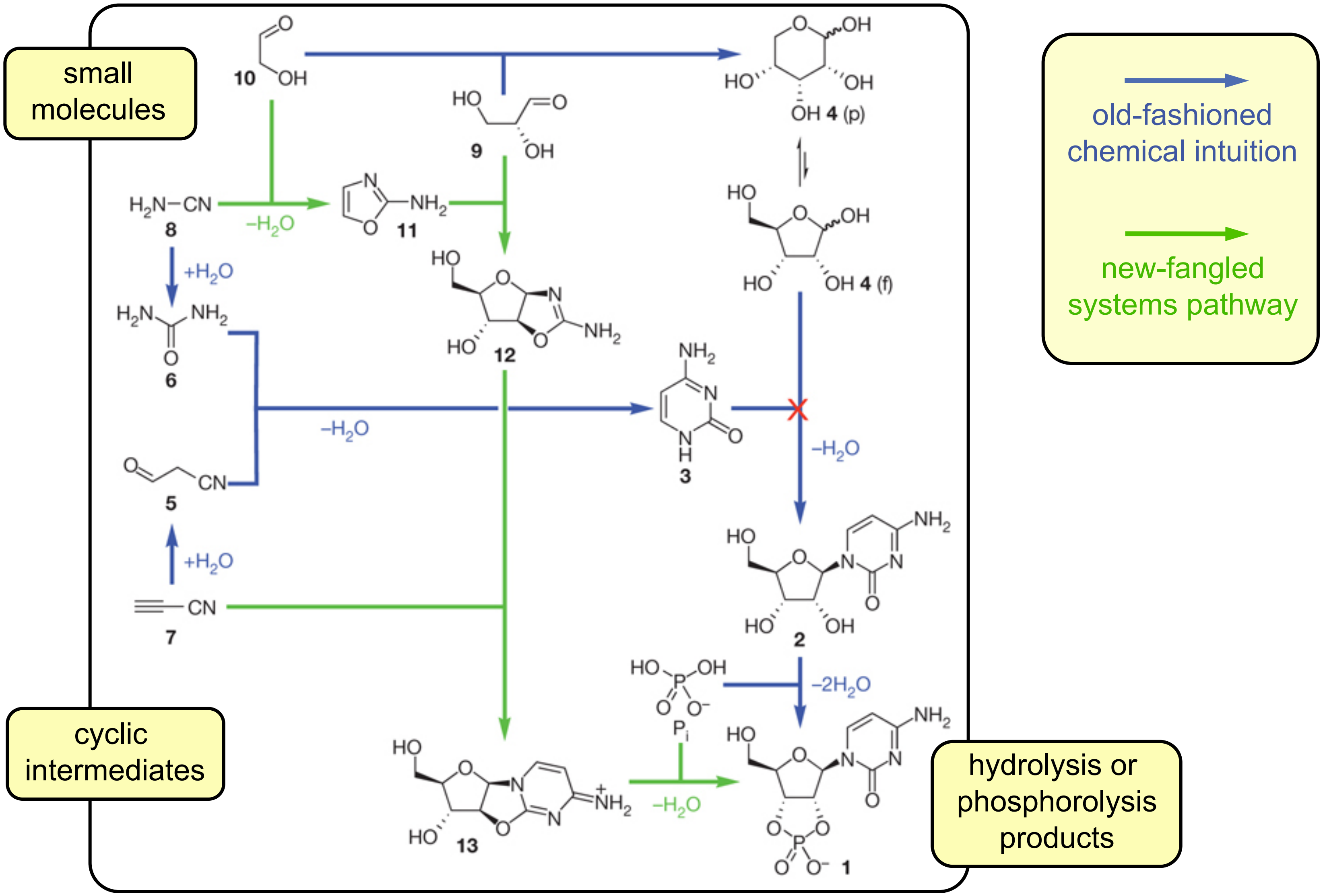


What Nature probably did…



Szostak (2009) Origins of life: Systems chemistry on early Earth. Nature 459, 171-172 [News and Views]

"Systems chemistry on early earth"…
Options for pyrimidine ribonucleotide assembly



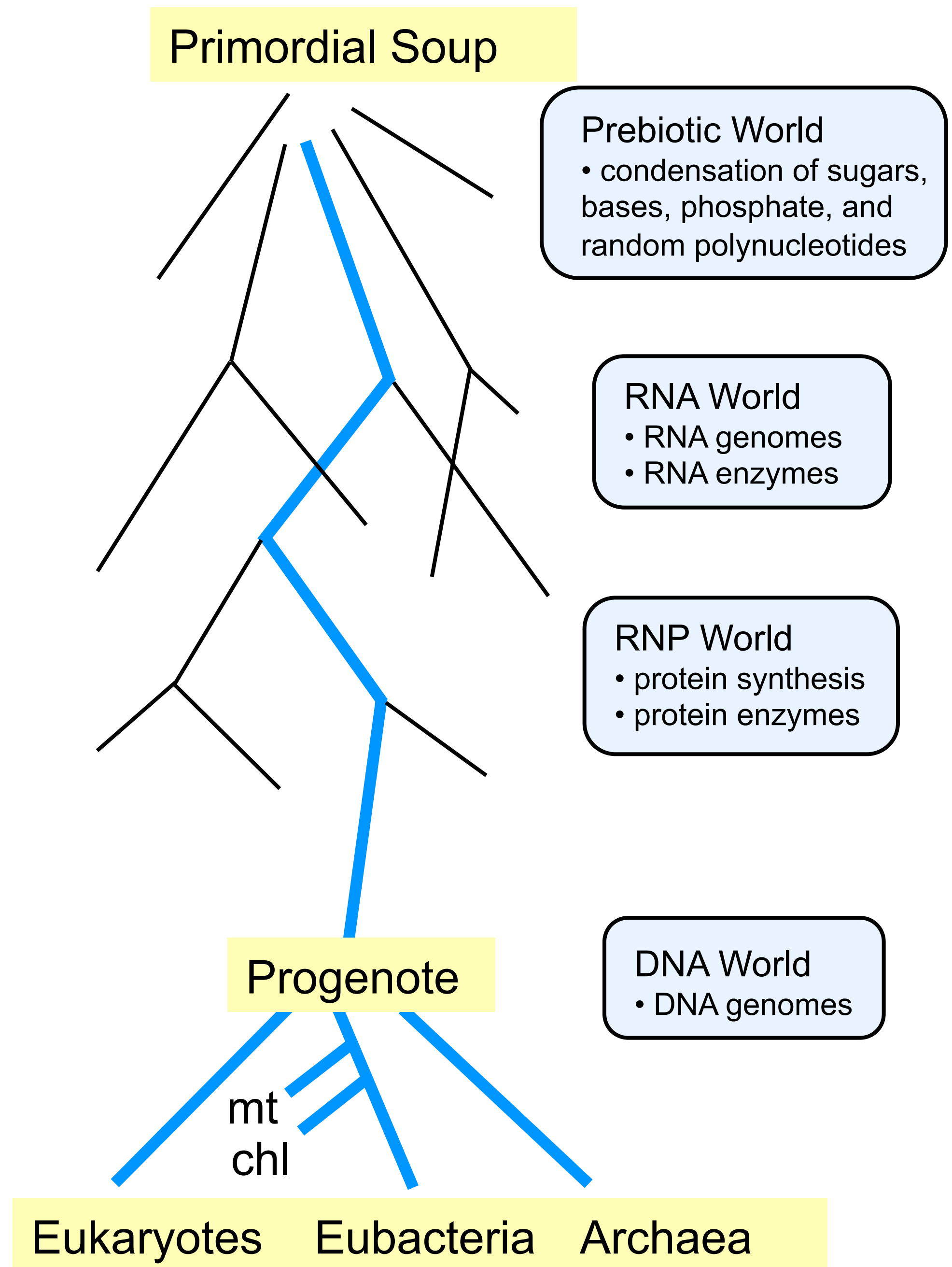
Powner et al. (2009) Nature 459, 239-242; Sutherland et al. (2015) Nature Chem 7, 301-307

The RNA World

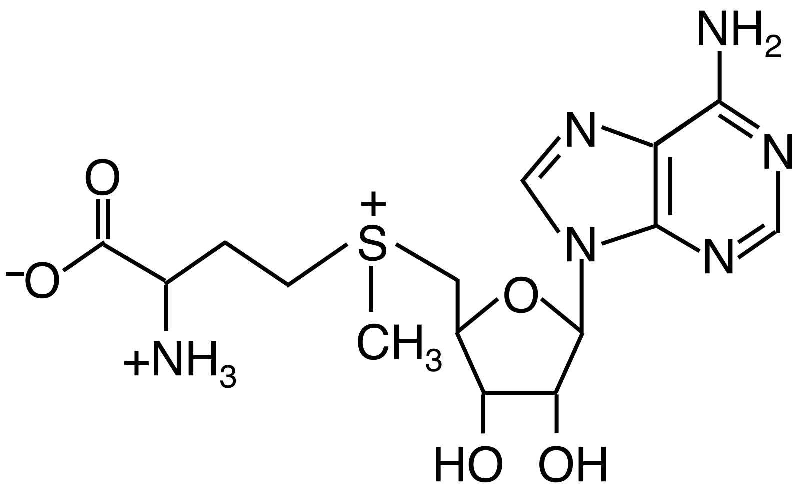
If RNA is catalytic, it could function as both genome and replicase, replicating itself, and perhaps also encoding ribozymes that would carry out intermediary metabolism to make more RNA precursors.

We can debate whether an RNA World existed, or how complex it might have been, and whether RNA may have been preceded by a simpler RNA-like polymer that could also function as genome and replicase.

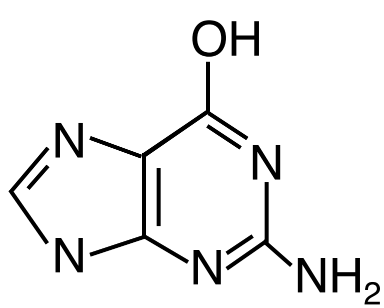
However, if life did begin in an RNA-like World, it may *still* be an RNA World today only slightly disguised by a veil of DNA!



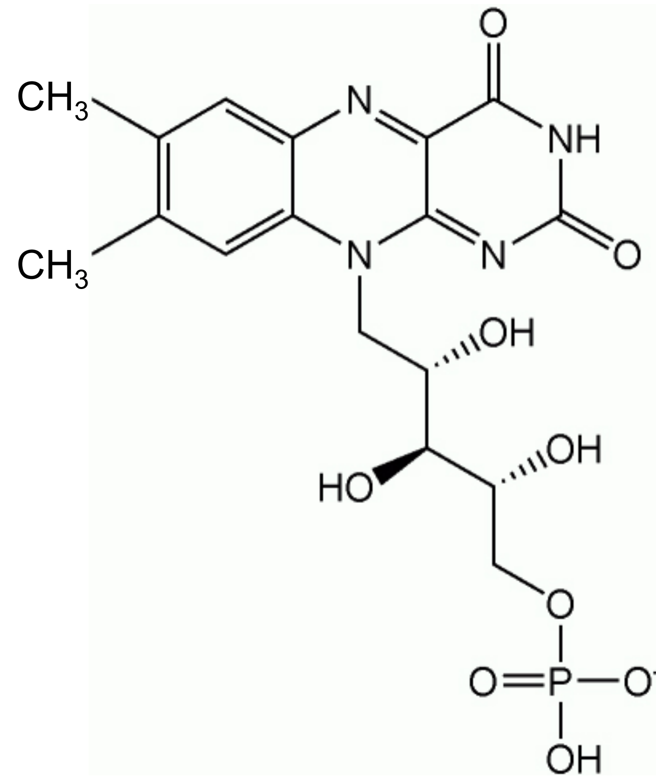
Nucleotide cofactors could be *molecular fossils* of metabolism in an RNA World



S-adenosylmethionine (SAM)



guanine



riboflavin-5'-phosphate
flavin mononucleotide (FMN)

White HB (1976) Coenzymes as fossils of an earlier metabolic state. J Mol Evol 7, 101-104

The Genomic Tag Hypothesis for the Origin of Protein Synthesis

Weiner and Maizels (1987) tRNA-like structures tag the 3' ends of genomic RNA molecules for replication: implications for the origin of protein synthesis. PNAS 84, 7383-7387

Maizels, N. and A. M. Weiner (1993) The genomic tag hypothesis: modern viruses as molecular fossils of ancient strategies for genomic replication. In *The RNA World*, R.F. Gesteland and J.F. Atkins (eds.), Cold Spring Harbor Press, Cold Spring Harbor, pp. 577-602.

Maizels, N. and Weiner, A. M. (1999) The genomic tag hypothesis: what molecular fossils tell us about the evolution of tRNA. Chapter 3, in *The RNA World II*, R. F. Gesteland, T. R. Cech, and J. F. Atkins, eds. Cold Spring Harbor Press, Cold Spring Harbor, NY.

Riboswitches can regulate mRNA expression without help from proteins or transcription, and are promising drug targets

a virulence-activating transcription factor in *Listeria monocytogenes*

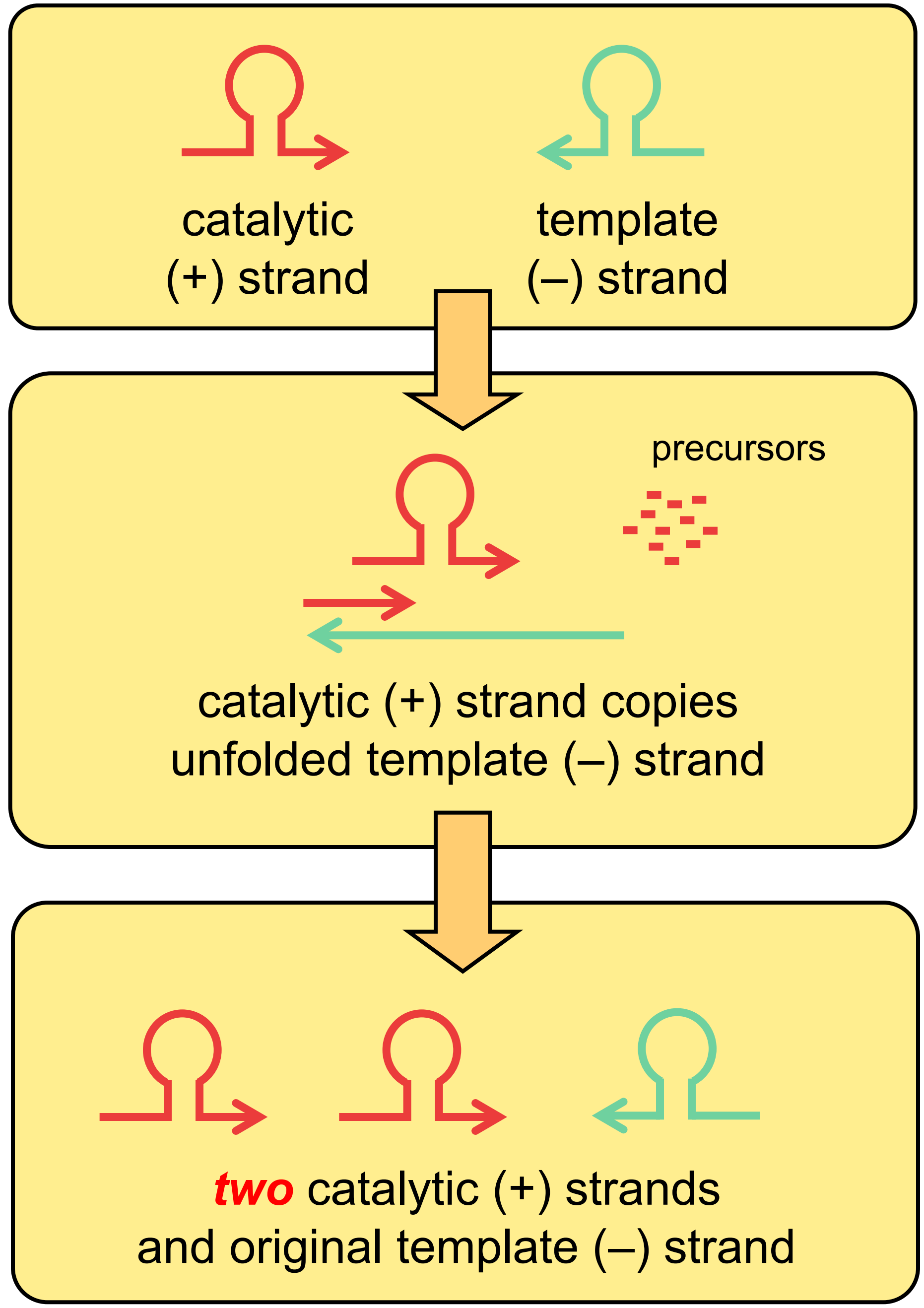
RNA secondary structure in 5’ untranslated region (5’ UTR) is a thermosensor: stable at 30°C, melts at 37°C [Johansson et al. (2002) Cell 110, 551–561]

proteins involved in methyl metabolism (SAM), purine metabolism and transport (guanine), and flavin mononucleotide biosynthesis (FMN) in *Bacillus subtilis*

RNA secondary structures can bind small metabolites such as SAM, guanine, and FMN, coordinately controlling genes involved in methyl, purine, and cofactor metabolism [Winkler et al. (2003) Nat Struct Biol 10, 701–707]

Breaker (2004) Nature 432, 838; (2007) Nature 447, 497; (2012) Cold Spring Harb Perspect Biol 4(2).

If RNA can be both catalyst and genome, RNA could replicate itself… the first "living" molecule!



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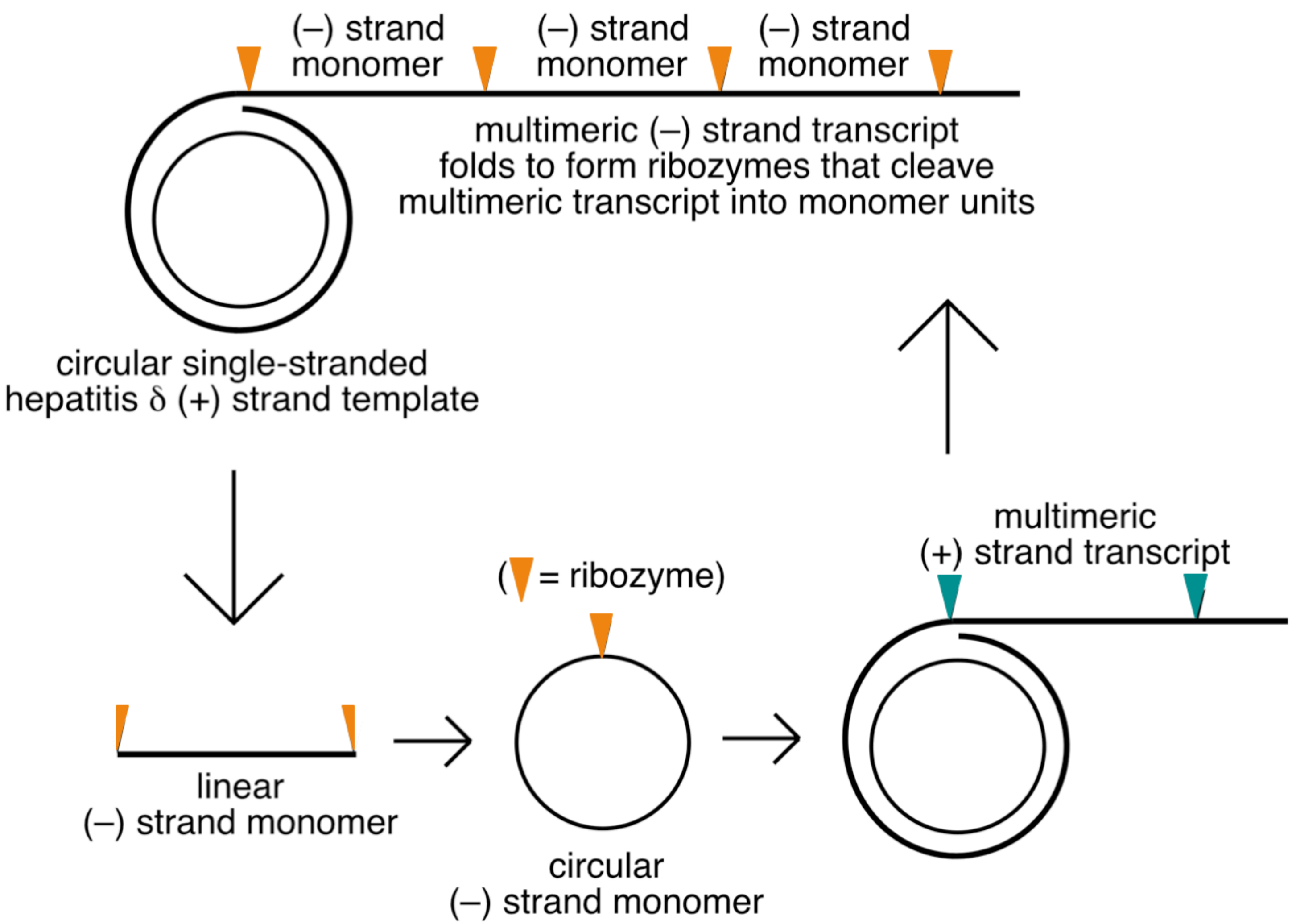
The smallest natural ribozymes are the hepatitis δ ribozyme and the hammerhead ribozyme found in plant viroids



Australia battled citrus exocortis virus (oranges, lemons, and etrogs on susceptible rootstocks) in the 1940s and 1950s

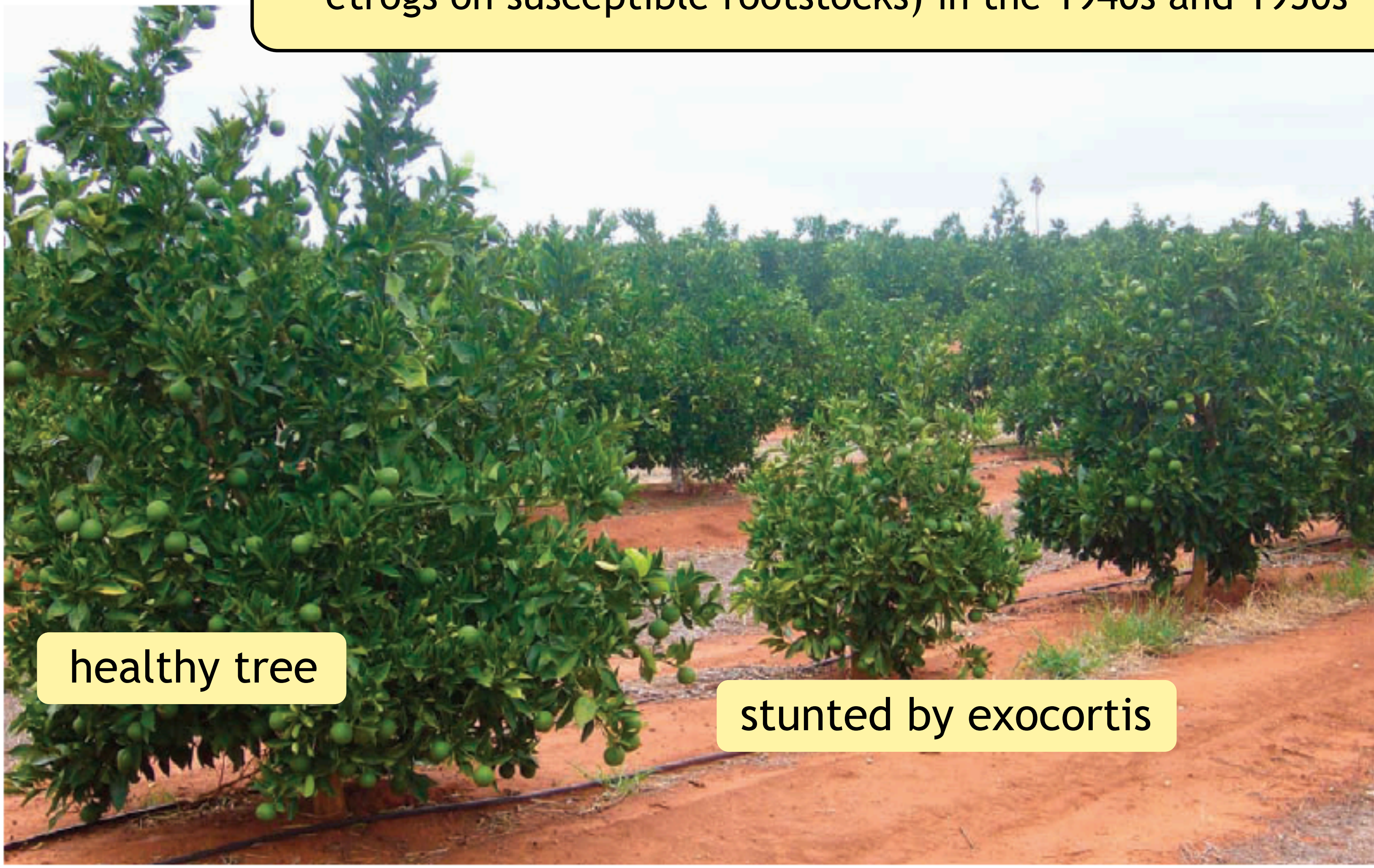
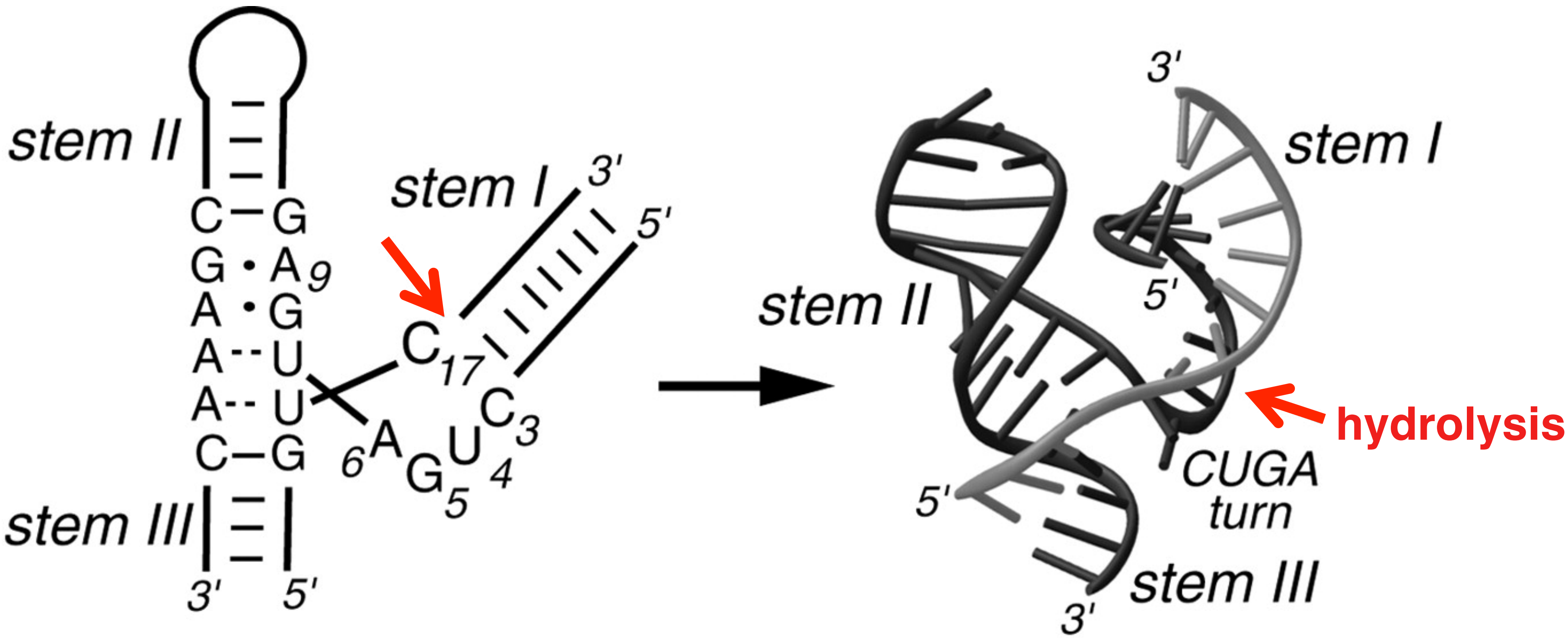


Figure 1. A 4-year-old orange tree on *P.trifoliata* showing severe stunting as a result of infection by exocortis

http://www.dpi.nsw.gov.au/agriculture/horticulture/citrus/health/diseases/citrus-exocortis

NSW DEPARTMENT OF **PRIMARY INDUSTRIES**

Hammerhead ribozyme structure



cartoon showing stems, loops, and 17 conserved nucleotides

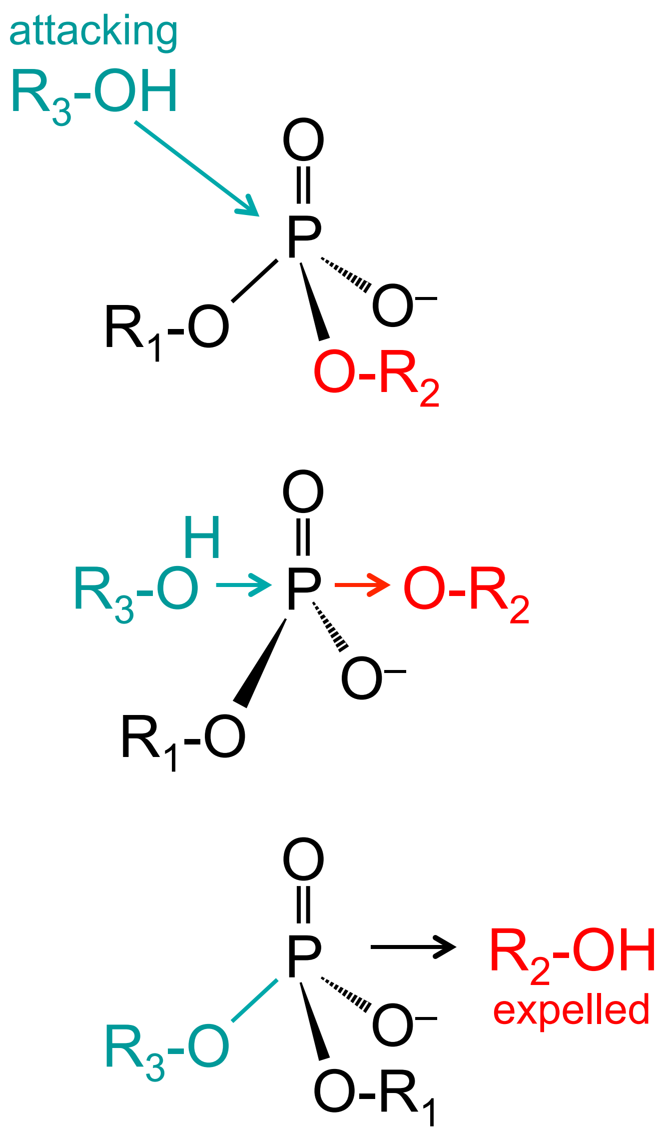
backbone trace of actual structure

Martick et al. (2006) A discontinuous hammerhead ribozyme embedded in a mammalian [lectin] messenger RNA. Nature 454, 899-902; Chi et al. (2008) Capturing hammerhead ribozyme structures in action by modulating general base catalysis. PLoS Biol 6, e234.

All natural ribozymes (*except* the ribosome!) use the same mechanism:
the attacking hydroxyl group expels the leaving hydroxyl group,
making a new phosphoester bond as it breaks the old one

If R_3-OH is RNA-OH,
the result is
RNA isomerization.

If R_3-OH is H-OH
(water), the result is
RNA hydrolysis.



<u>before</u>
tetrahedral phosphate
with two ester bonds
(phosphodiester)

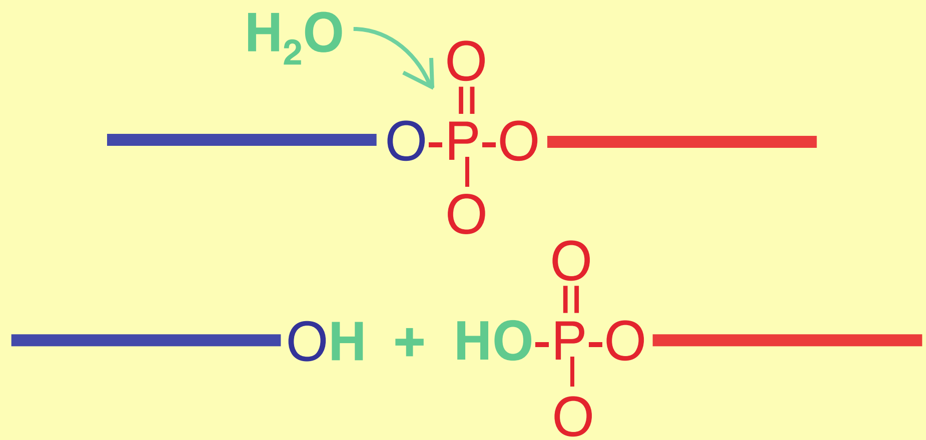
<u>during</u>
attacking hydroxyl
generates a trigonal,
bipyramidal intermediate

<u>after</u>
bipyramidal intermediate
turns "inside out"
like umbrella in high wind
("inversion of configuration")

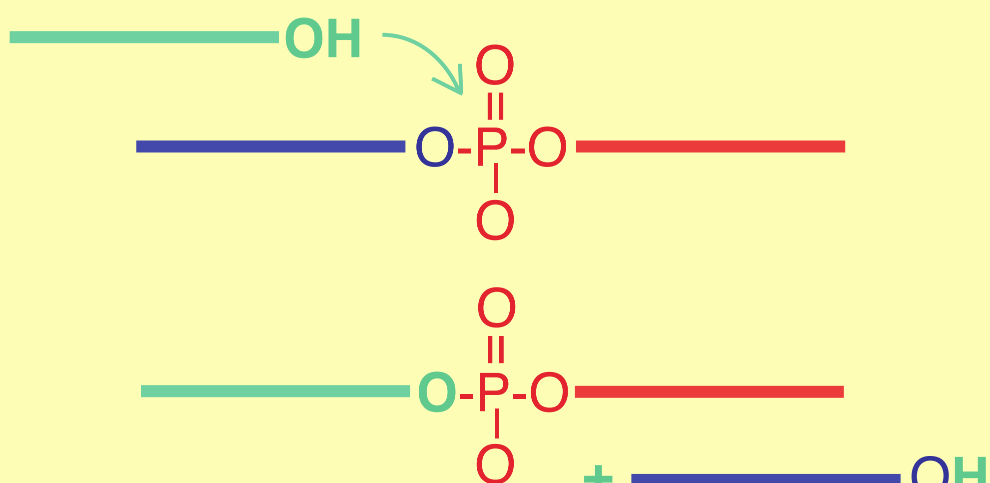
Westheimer (1987)
Why nature chose phosphates.
Science 235, 1173

All natural ribozymes (*except the ribosome!*) use the same mechanism: the attacking hydroxyl group expels the leaving hydroxyl group, making a new phosphoester bond as it breaks the old one

If the attacking hydroxyl belongs to H_2O, the RNA is hydrolyzed (cleaved).

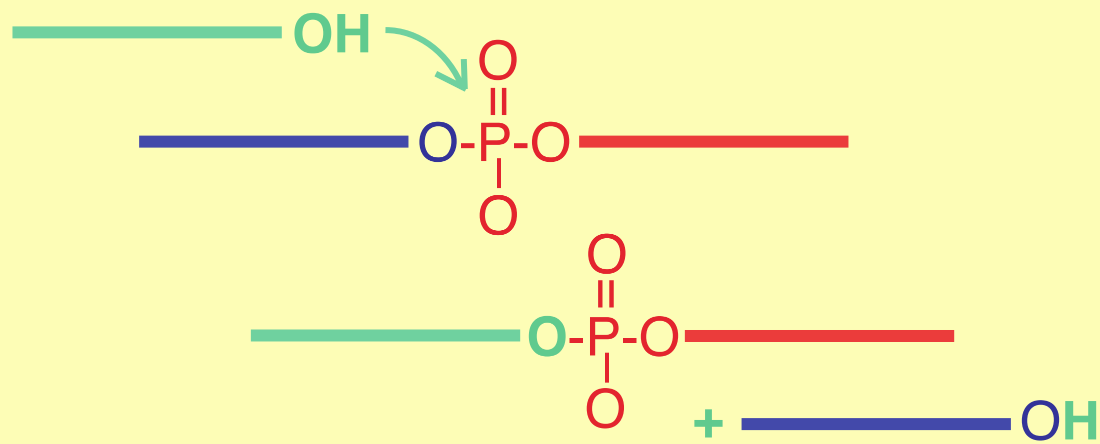


If the attacking hydroxyl belongs to RNA, the RNAs are isomerized.



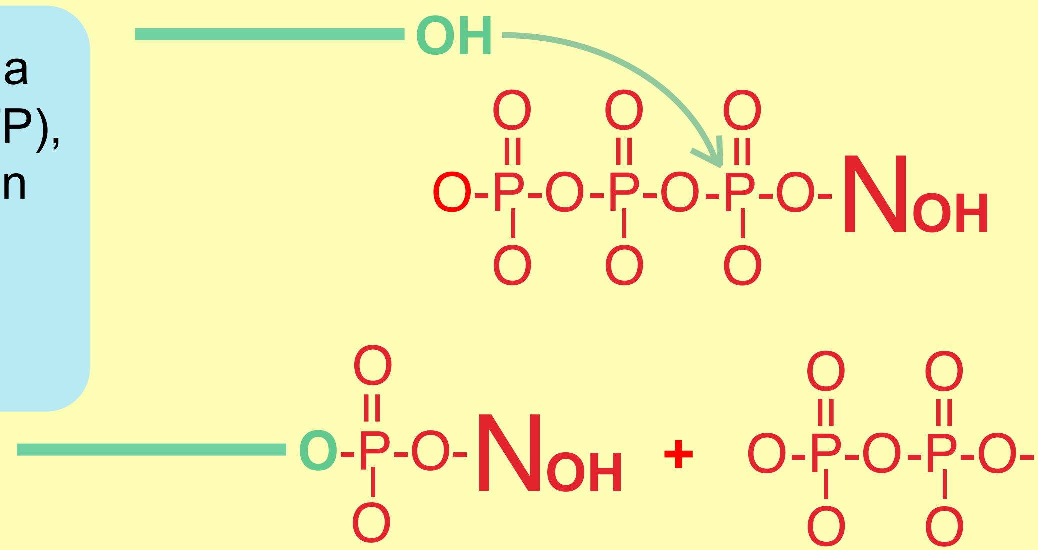
RNA splicing and replication are chemically similar

If the attacking hydroxyl belongs to RNA, the RNAs are isomerized as in splicing.

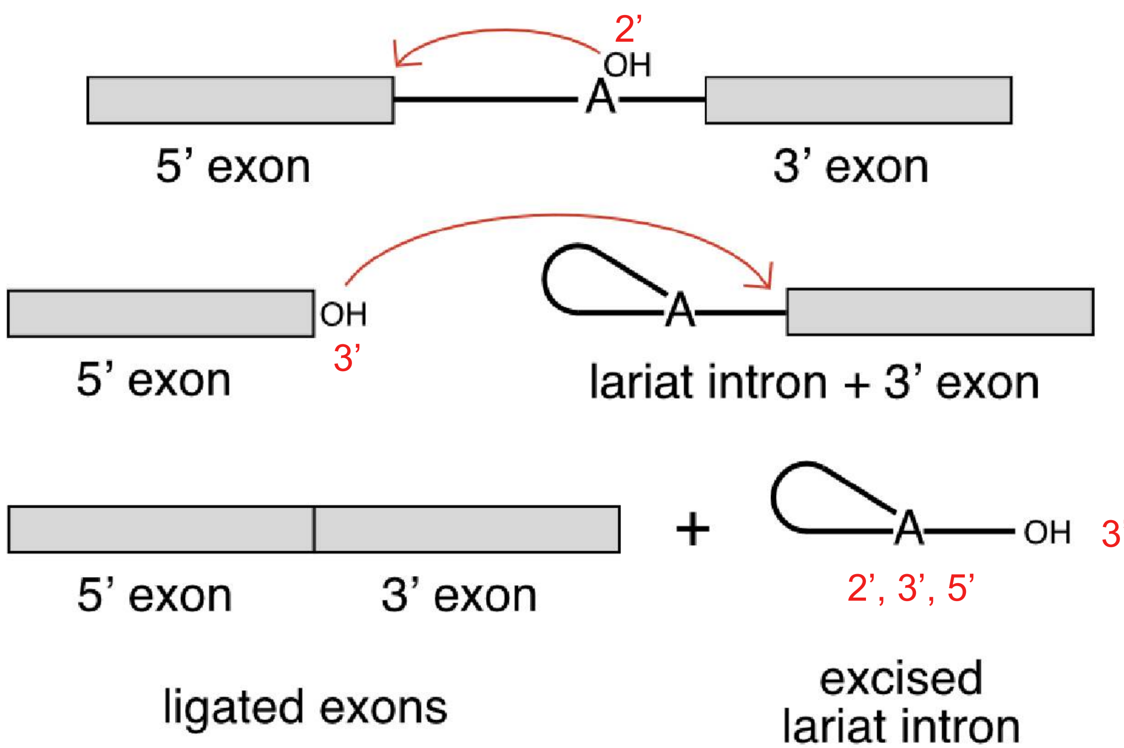


If the RNA 3' hydroxyl attacks a ribonucleotide triphosphate (NTP), the reaction is a polymerization as in replication!

N = nucleotide



mRNA splicing is just an isomerization…
a new bond is made for every bond broken



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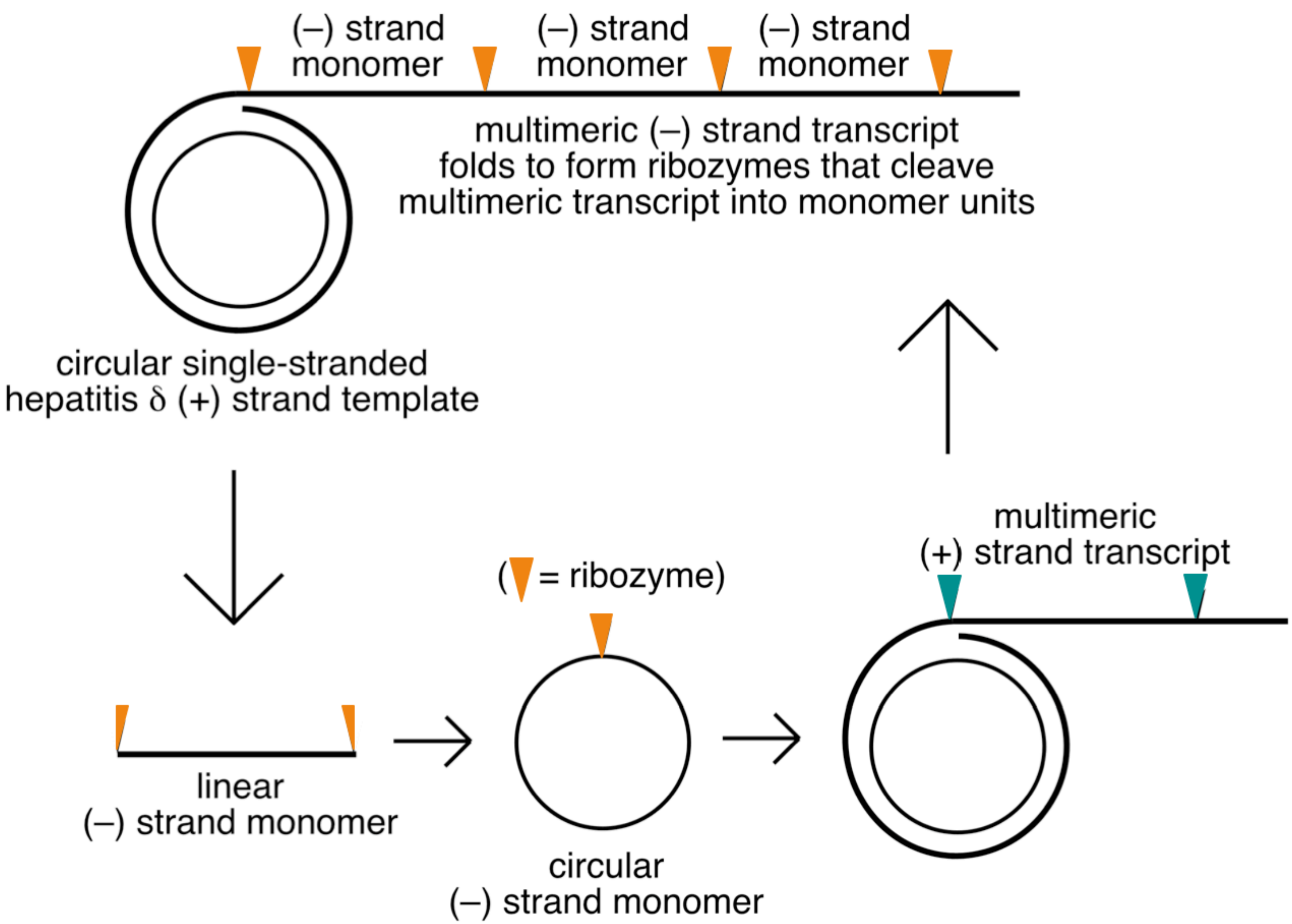
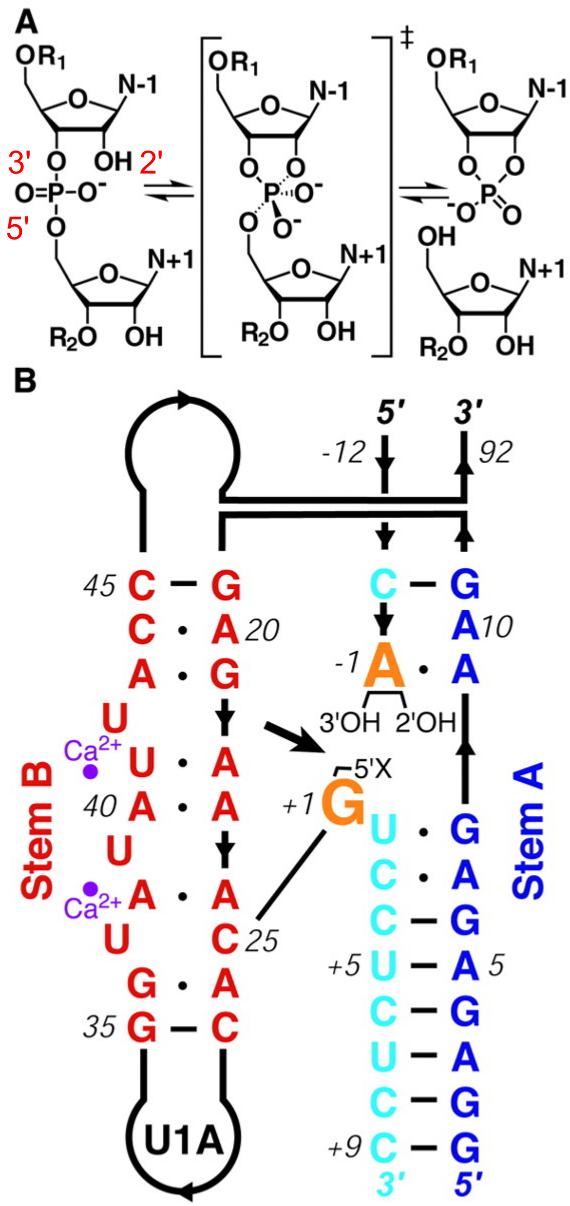




The "hairpin" ribozyme cleaves the negative strand of tobacco ringspot virus satellite RNA

- To crystallize "the" precursor, the attacking 2'-OH was replaced by the methoxy group 2'-OCH_3 which cannot dissociate.

- To crystallize "the" transition state, the scissile phosphate was deleted, and the ribozyme was complexed with vanadate (Na_3VO_4) to mimic the pentacovalent bipyramidal intermediate.

- The product crystallizes naturally (and apparently does *not* undergo the reverse reaction!).

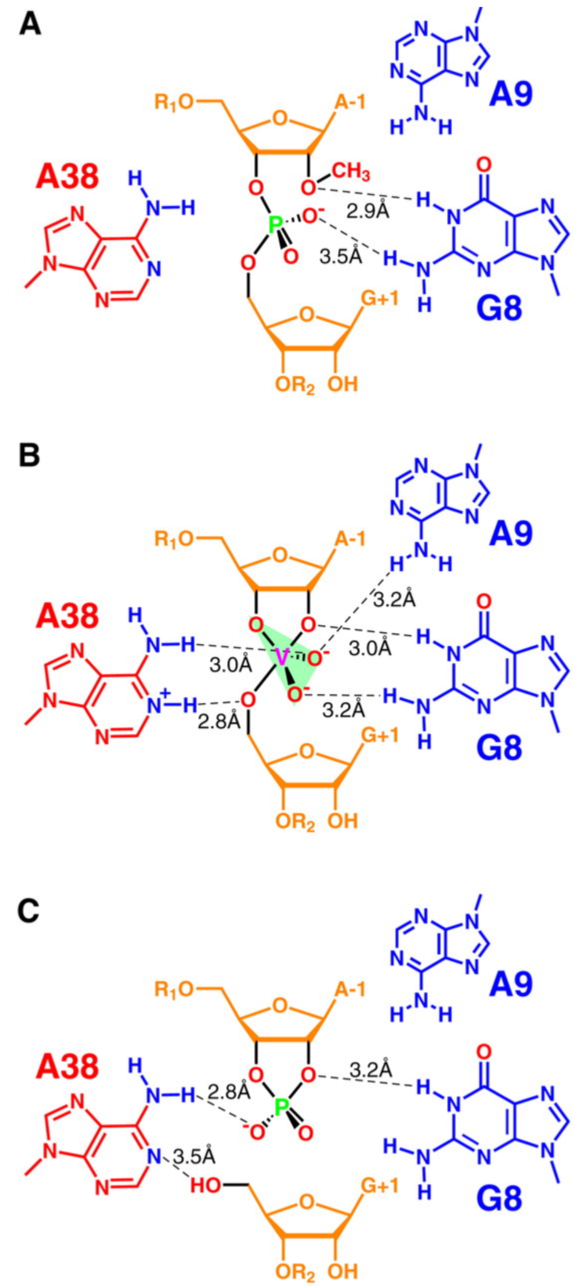
Rupert, Massey, Sigurdsson, and Ferré-D'Amaré, 2002

The transition state is stabilized by more hydrogen bonds than either precursor or product

(A) Precursor complex stabilized by **two** hydrogen bonds (dashed lines) from G8.

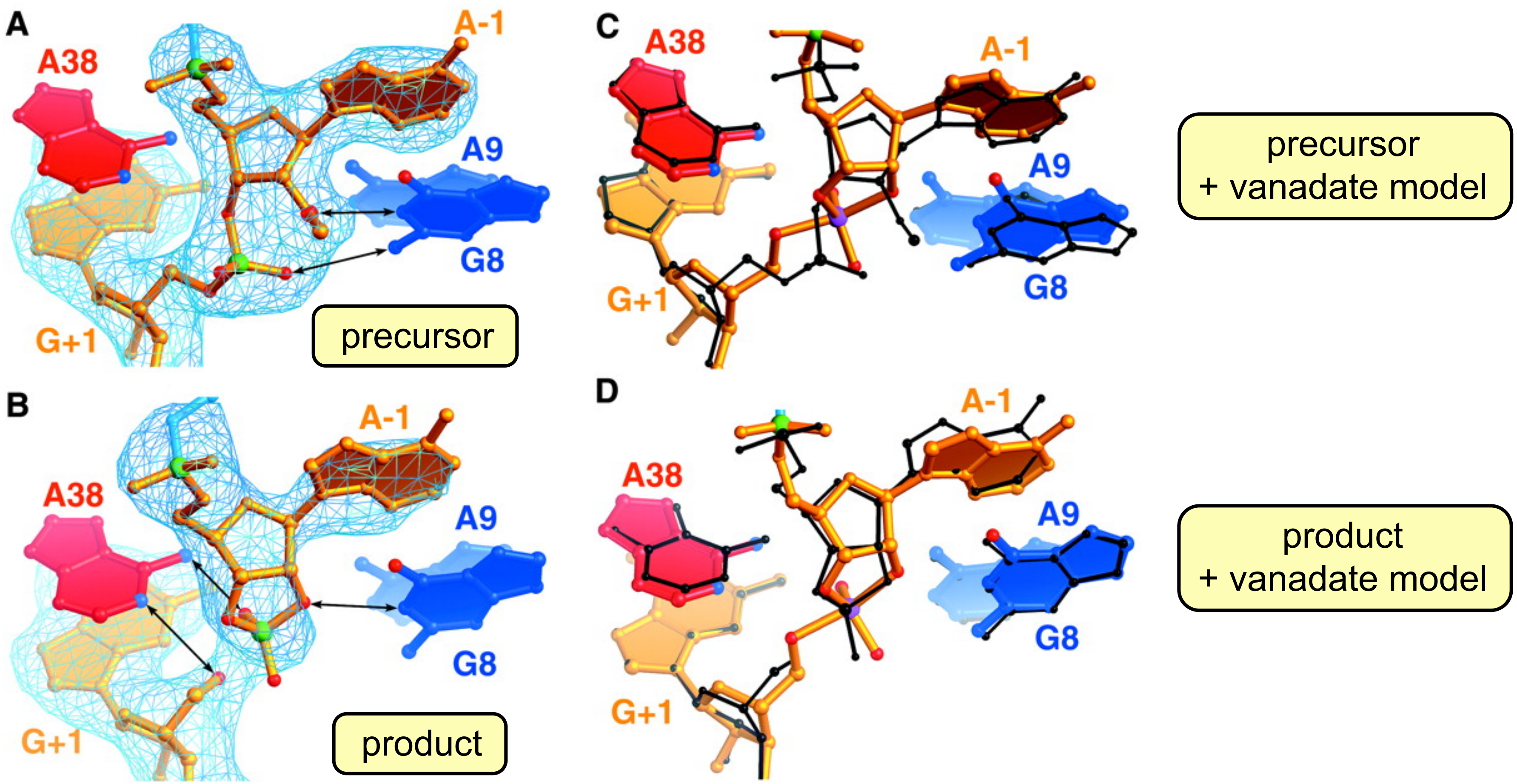
(B) Transition state mimic complex stabilized by **five** hydrogen bonds. (The green triangle connects the equatorial oxygens of the vanadate.) Protonation is inferred from the distance between N1 of A38 and the 5'-oxygen.

(C) Product complex stabilized by **three** hydrogen bonds. The hydrogen bond between N1 of A38 and the 5'-OH of G+1 orients the latter for subsequent ligation.



Rupert, Massey, Sigurdsson, and Ferré-D'Amaré, 2002

Transition State Stabilization by a Catalytic RNA
(Rupert, Massey, Sigurdsson, and Ferré-D'Amaré, 2002)
Precursor and product differ "infinitesimally" from the transition state…



REVIEW

nature
structural &
molecular biology

Ribozyme catalysis: not different, just worse

Jennifer A Doudna[1] & Jon R Lorsch[2]

Evolution has resoundingly favored protein enzymes over RNA-based catalysts, yet ribozymes occupy important niches in modern cell biology that include the starring role in catalysis of protein synthesis on the ribosome. Recent results from structural and biochemical studies show that natural ribozymes use an impressive range of catalytic mechanisms, beyond metalloenzyme chemistry and analogous to more chemically diverse protein enzymes. These findings make it increasingly possible to compare details of RNA- and protein-based catalysis.

With apologies to Jeremy Knowles and his seminal review, "Enzyme catalysis: not different, just better".

(*Nature* **350**, 121–124, 1991).

The emergence of efficient and highly specific catalysts for biochemical reactions was key to the evolution of living systems. Although protein enzymes dominate modern cell biology, discoveries of catalytic RNA molecules, called ribozymes, fueled the suspicion that nucleic acids were key to the origin of biocatalysts, in part because RNA plays central roles in the fundamental process of protein biosynthesis in all cells. According to the 'RNA world' hypothesis, RNA once served as both the genetic material and the principal biocatalyst in living systems. As this primitive RNA-based metabolism evolved, requirements for more sophisticated enzymes with superior catalytic powers are thought to have stimulated the transition to protein-mediated catalysis. It seems possible that naturally occurring ribozymes present in organisms ranging from bacteria to humans are in fact remnants from this envisioned RNA-dominated era. If this is true, at least some of the catalytic functions of modern enzymes were originally carried out by ribozymes.

Doudna and Lorsch (2005)
Nat Struct Mol Biol 12, 395-402.

REVIEW

nature structural & molecular biology

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(*Nature* **350**, 121–124, 1991).

*think of ribosomes and spliceosomes which require large motions of large complexes with many RNA molecules coming and going!

Doudna and Lorsch (2005)
Nat Struct Mol Biol 12, 395-402.

to be continued...