From ancient to modern worlds

RNA back and forth

Looking through viroids and ribozyme motifs

marie-christine.maurel@upmc.fr

http://isyeb.mnhn.fr/Maurel-Marie-Christine

The contribution of Oparin to our understanding of the Origins of life is considerable.

- Oparin opened the way to the modern scientific experimental approach

  Indeed although there are several chemical synthesis of prebiotic interest (synthesis of sugars, urea, uracil, glycine etc) none of them were related to the origins of life.

- Oparin updated the data :

  Sugar synthesis in the primeval hydrosphere
  Pyridine and pyrimidine synthesis from Berthelot
  Uracil synthesis from urea etc.

-Oparin places the problem of the origins in an environmental,

  geological, paleontological and historical setting, initiating nearly 100 years of multidisciplinary researches in this domain.

-Oparin calls on the most contemporary biochemical data available,

  he layed out the problems to be solved to understand the sequence of extraordinary events that were to lead to the origins of life.

Decidedly modern, he still belongs to our times…

In 1957*, Oparin reported the conference of Bernal helded at the University of Moscow (1955) and discuss the early appearance of Nucleic Acids in terms of the chicken-egg problem i.e which arrived the first, RNA or proteins?

*in The Origin of Life on the Earth , 1957
Oliver and Boyd, Edinburgh and London

The leading thread
to imagine the biological past over 4 Gya ago

1 - Continuity

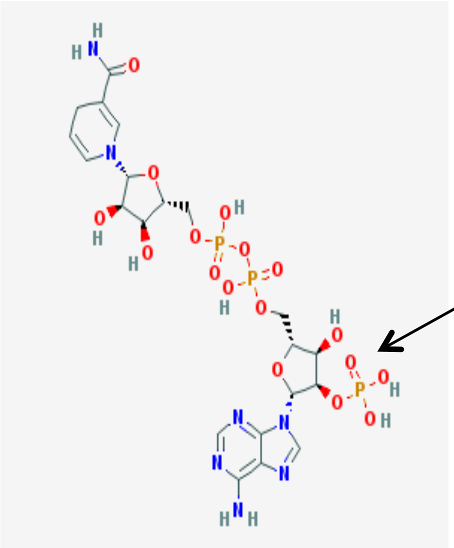
that links predecessors with descendants

- Metallic compounds may have arisen early in evolution, to drive the energetic and growth needs of primitive organisms.

Fe, S, Mg, Na,…, within modern proteins and nucleic acids are an adaptation retained from mineral chemistry.

The leading thread to imagine the biological past over 4 Gya ago

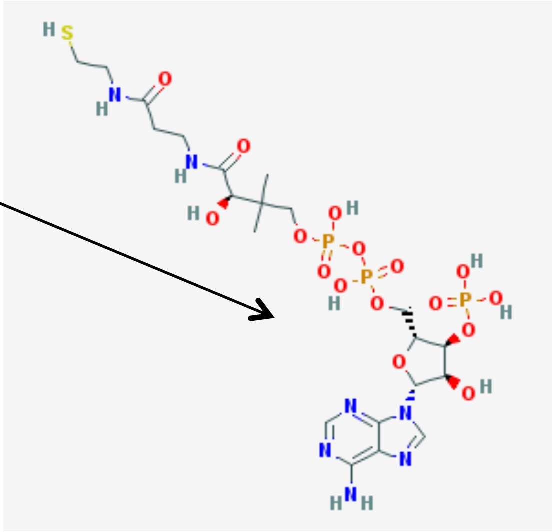
2-Evolutive jump and exaptation



A, NAD, …, some organic cofactors, probably predate the RNA world.

3' P of NADP & 2'P of CoA

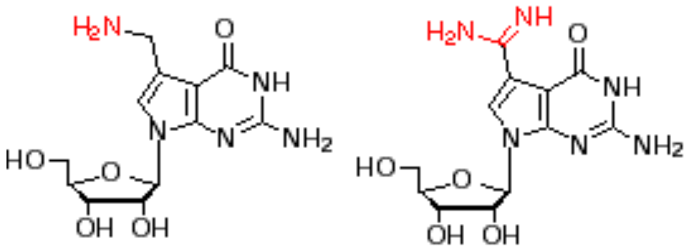
might be the remains of ancient backbone…



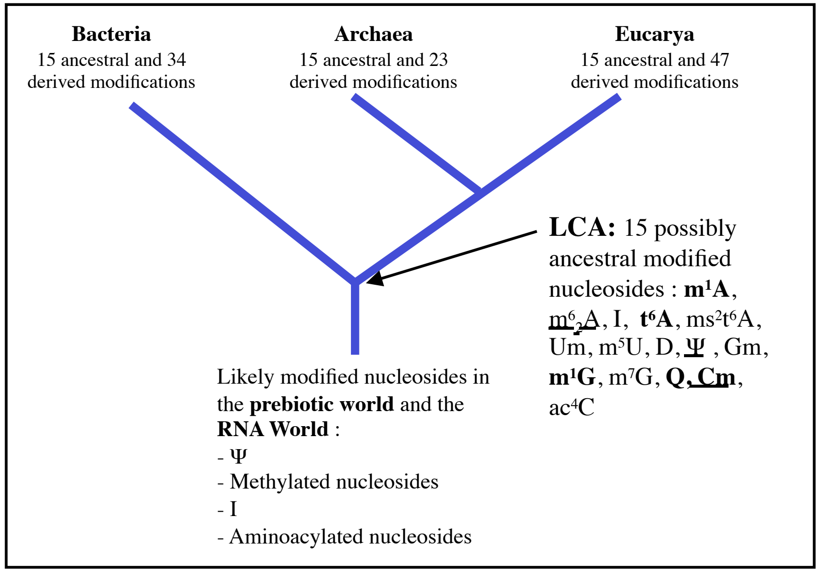
Ribo-nts the first assembly endowed with darwinian evolution (IDA)?

~ 100 modified nucleosides

Phylogenetic analysis of modified nucleosides, structure and chemistry ——> present in great concentrations on the primitive earth.



Nicotinamide (NAD): **prebiotic synthesis** from ethylene and ammonia (Friedmann et al, 1971).



Nucleoside modifications might have occurred in the cenancestor and in the RNA world.

■ Ribonucleotides in cofactors and modified nucleosides could be the **remains of ancient ribozymes**.

Cermakian & Cedregren, Modification and Editing of RNA, 1998, ASM Press.

RNA performs

genetic | catalytic | structural and regulatory roles

RNA world hypothesis (Gilbert, 1986)

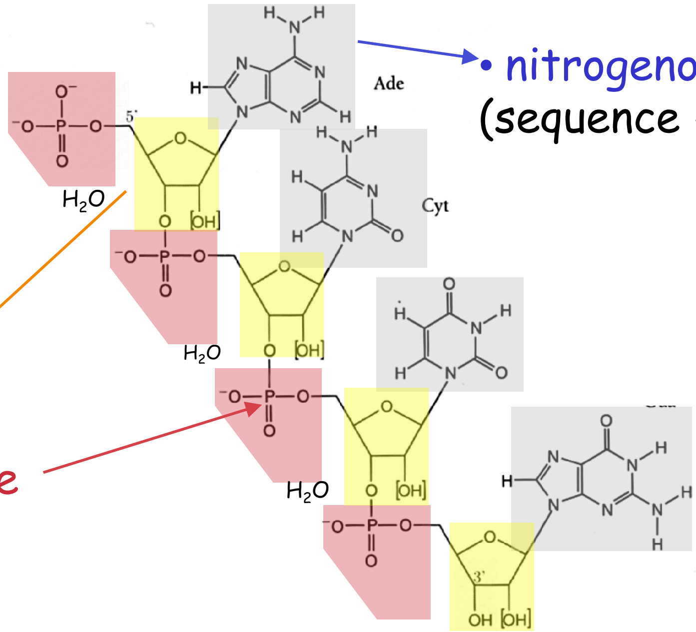
« Thus, the question of the catalytic ctivity of polynucleotides remains open »

Orgel, J. Mol. Biol, 1968

RNA before the DNA, RNA and protein world

RNA

Single strand



• nitrogenous bases in nucleotides
(sequence -->information)

• sugar (ribose)

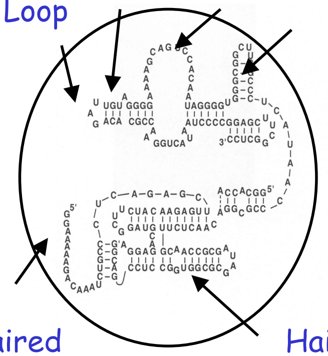
• phosphate

Loop

Bulge

Loop

Hairpin:
ds in helix
(pairings)



Non-paired
segments

Hairpin
with loop

Single strand folds

Double strands by pairing

• robustness
• duplication
•conformational flexibility -->

catalytic & functional properties

RNA scraps

tRNA, mRNA, SnoRNAs...

Responses (T°, Pr) stress

fragmentation

generates classes of RNAs ...

RNA fragments perform functions distinct from their **parents** (exaptation)

RNA

origin of evolutionary innovations

Origins of RNA ?

RNA synthesis on clays

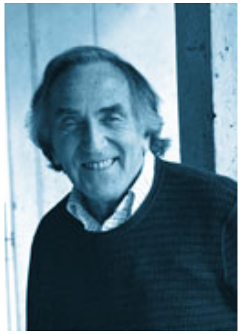
(J. Ferris).

Montmorillonite clay

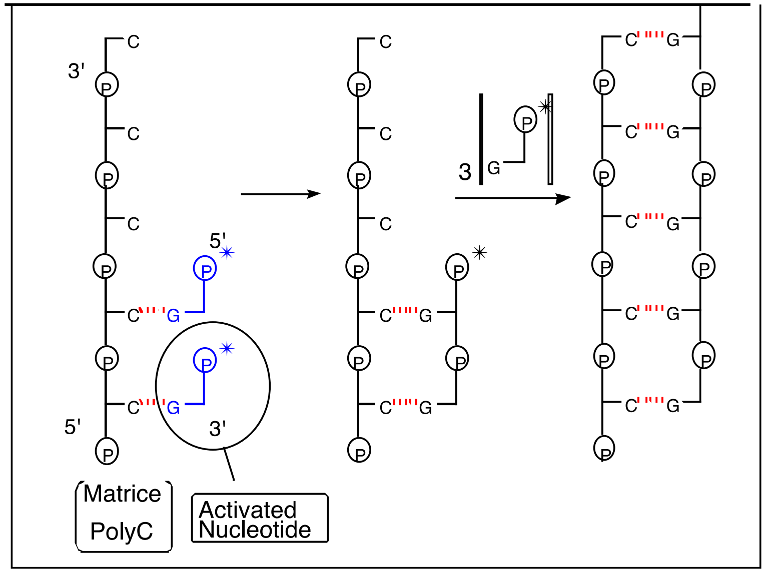
? **Nucleotide =**

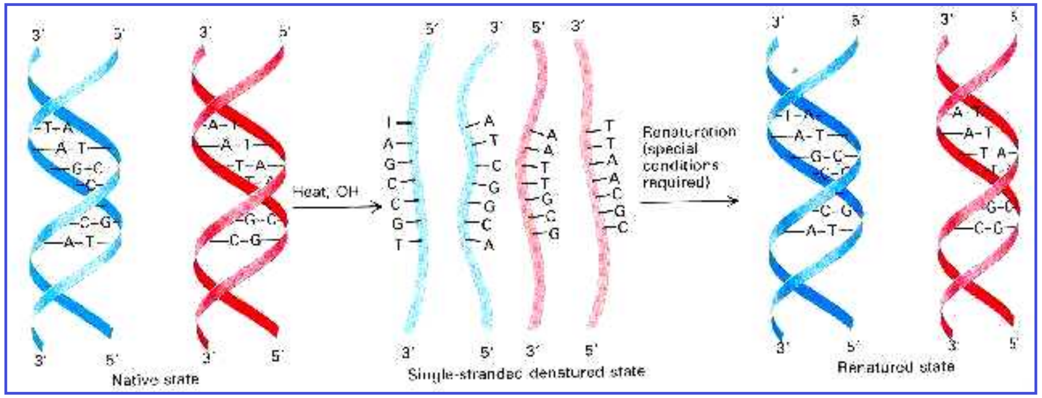
base + Ribose + phosphate

Template-directed synthesis

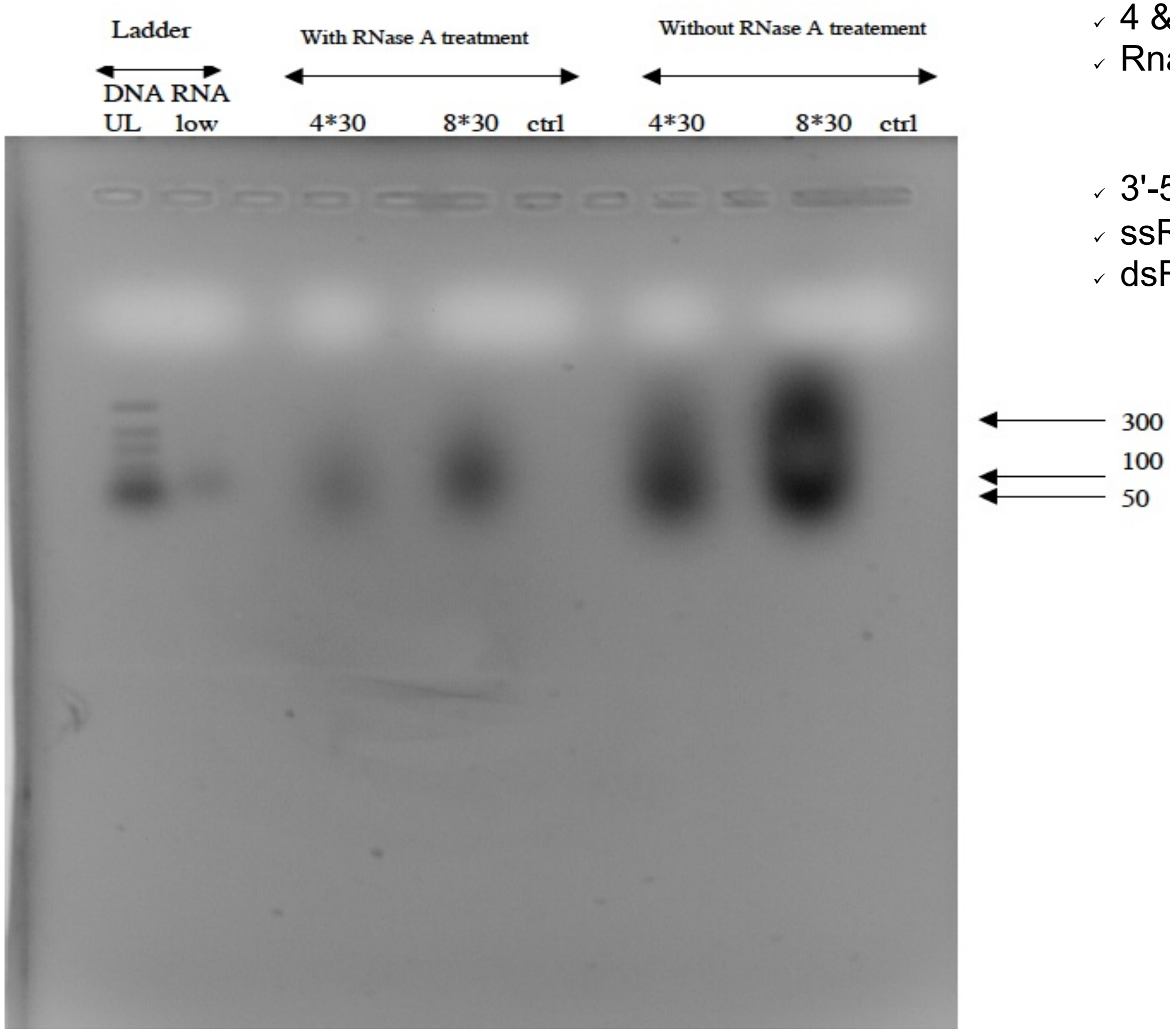


(L. Orgel)





Hydrothermal RNA-like polymers



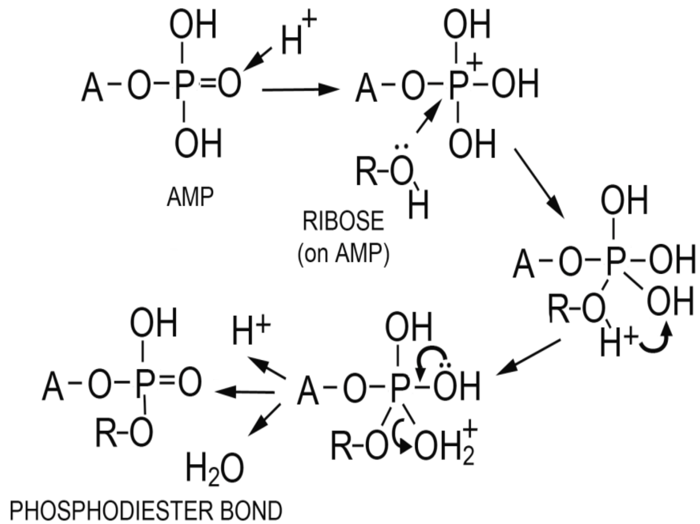
Da Silva, Maurel, Deamer, 2014, submitted

Mix of **AMP** 10mM, **UMP** 10mM, NH_4Cl 0.1M

- 4 & 8 wet-dry cycles of 30min
- Rnase A: ssRNA specific cleaving at U residues

↓

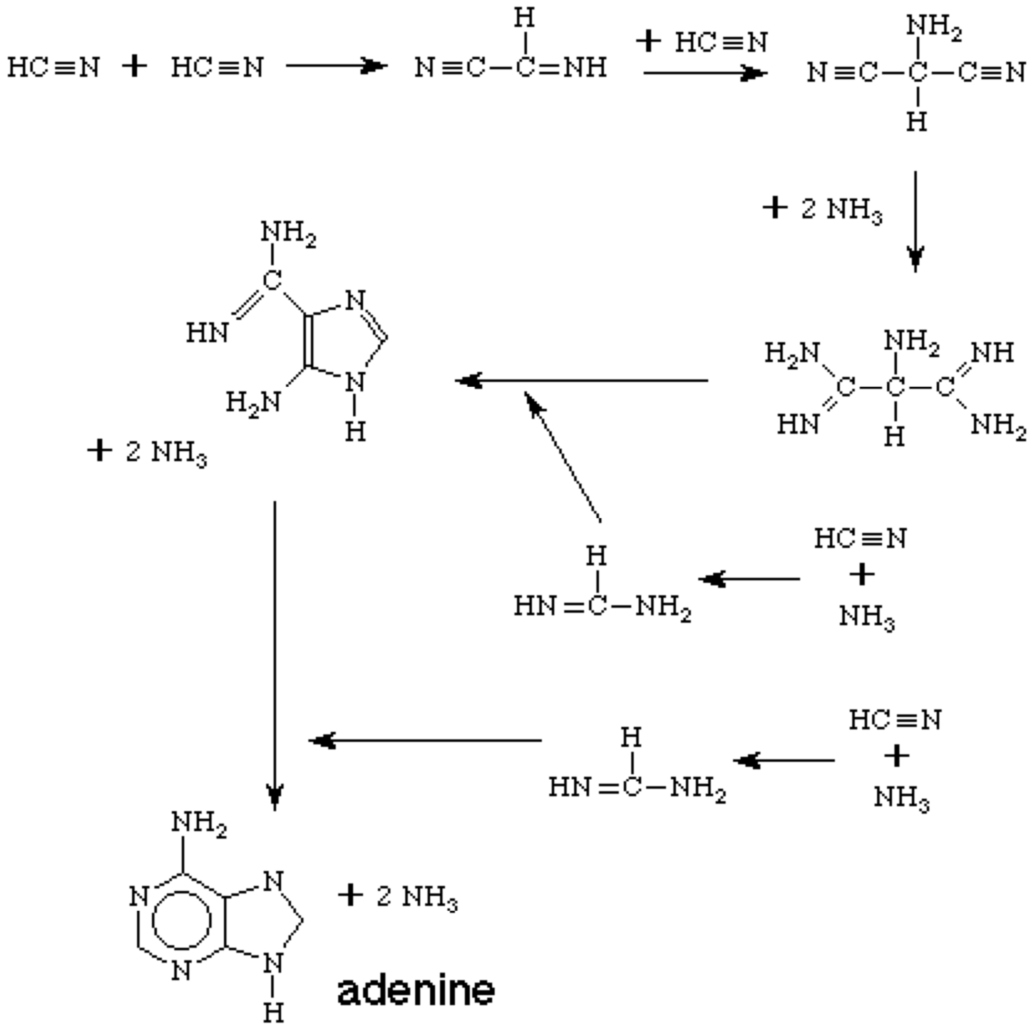
- 3'-5' phosphodiester bonds
- ssRNA
- dsRNA (stain EtBr + Rnases V1, III digestions)



Difficult to synthesize RNA

? Nucleotide =

base + Ribose + phosphate



In conditions already defined by Oparin, Juan Orò synthesized Adenine

C5H5N5 = (HCN)5

$$5\ HCN \xrightarrow{h\nu} \text{adenine}$$

Cyanhydic acid

A

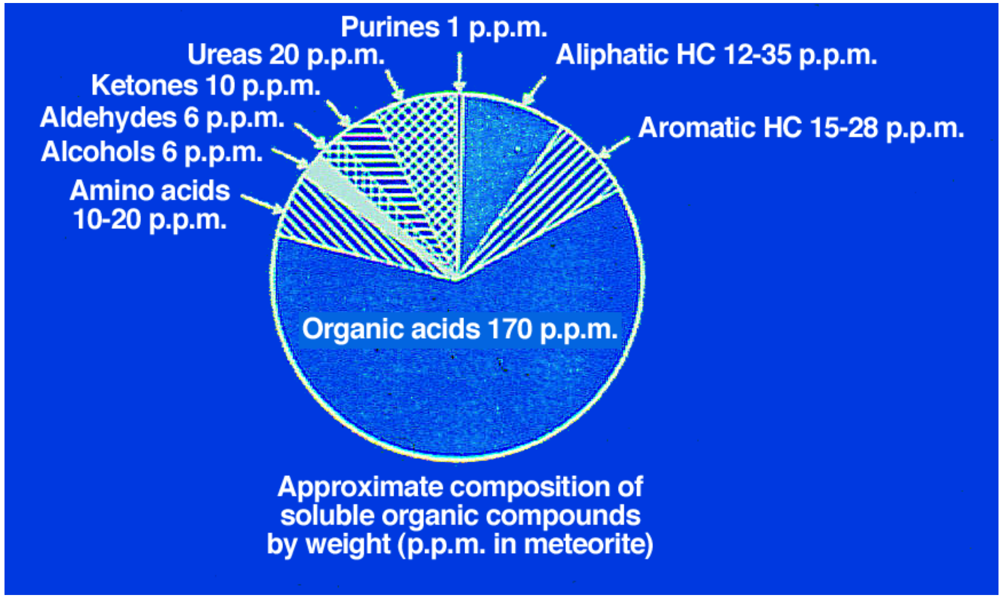
A is a pentamer of hydrogen cyanide.

Smaller amounts of guanine and other purine derivatives are also formed.

Formamide, etc --→NA components (R. Saladino, E. Di Mauro et al)

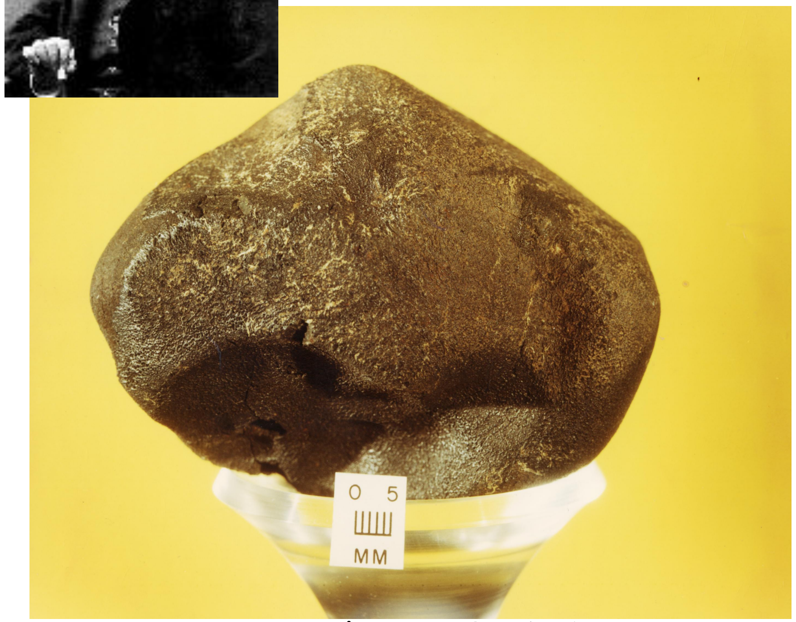
Impact theory :

Synthesis of building blocks from space.
Amino acids, nitrogenous bases, sugars would have been brought by meteorites
—> primitive soup of biological monomers.



From Deamer D.

Svante Arrhenius

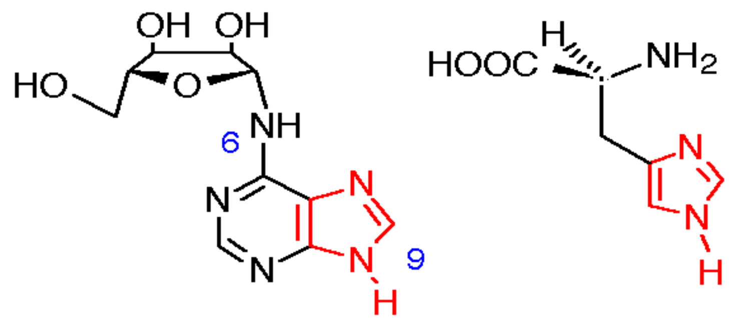


Australia, 28.09.1969

Murchison (4.6 Ga)

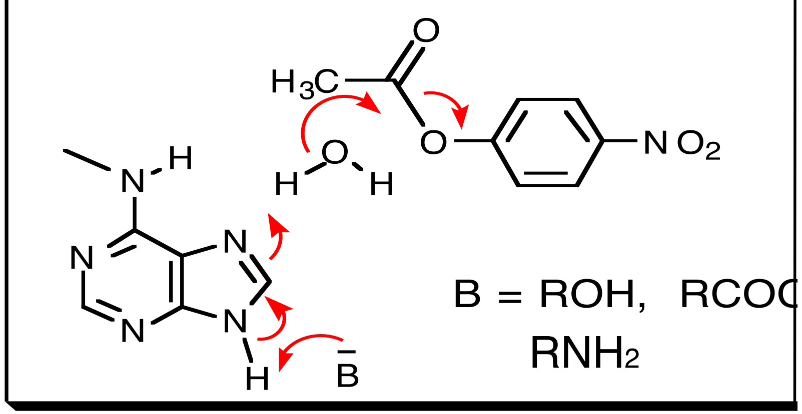
Carbonaceous meteorites contain a wide range of extraterrestrial nucleobases.
Callahan MP, et al. Proc Natl Acad Sci. U S A., 2011

■ **N^6-ribosyl adenine**, condensation of adenine and ribose under prebiotic conditions, exhibits a pronounced catalytic activity in esters hydrolysis as compared to the histidine amino acid.



N^6 -ribosyladenine (Fuller, Sanchez & Orgel, 1972, Maurel et Ninio, 1987)

L-Histidine



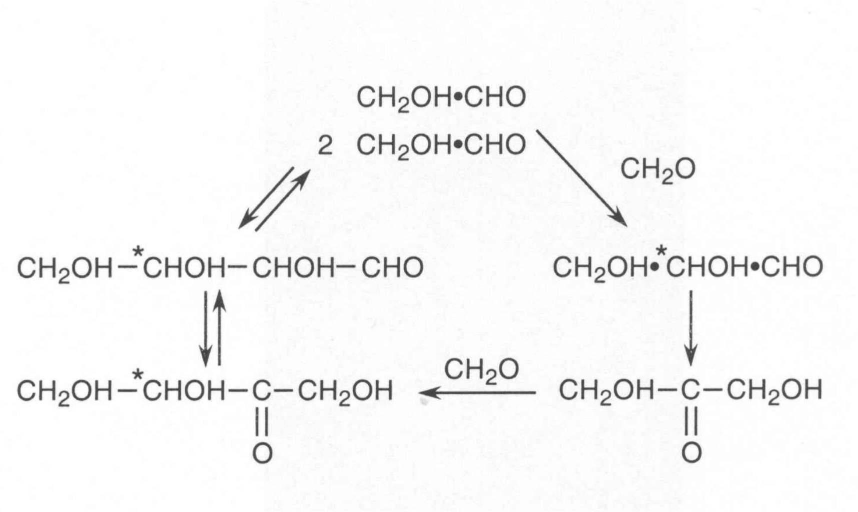
The current role of histidine would have been formerly performed by **adenine**.

Esterasic activity (Maurel et Ninio, 1987; Maurel, 1990; Maurel et Convert, 1990; Maurel et Décout, 1992; 1993; Décout et al, 1995; Ricard et al, 1996; Maurel et Décout, 1999)

Catalatic activity with copper in presence of peroxyde (Bruston et al, 1999).

Formose reaction

Formaldehyde and glycolaldehyde can react to generate a pentose sugar.



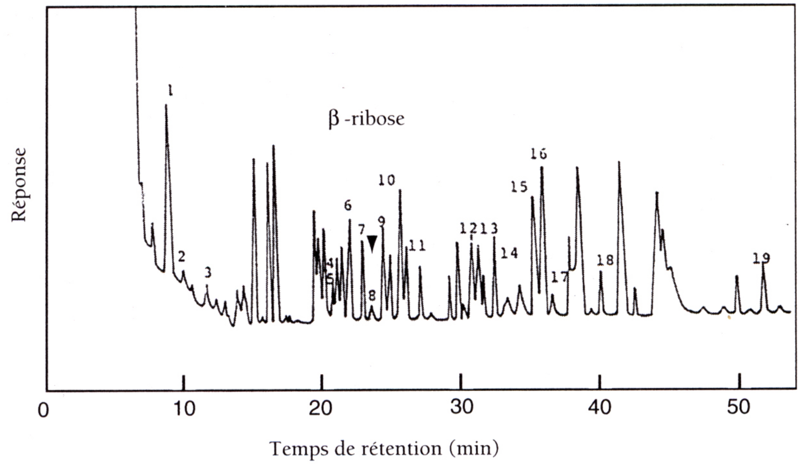


Figure 4.4 Chromatogramme en phase gazeuse de sucres formés par synthèse abiotique.

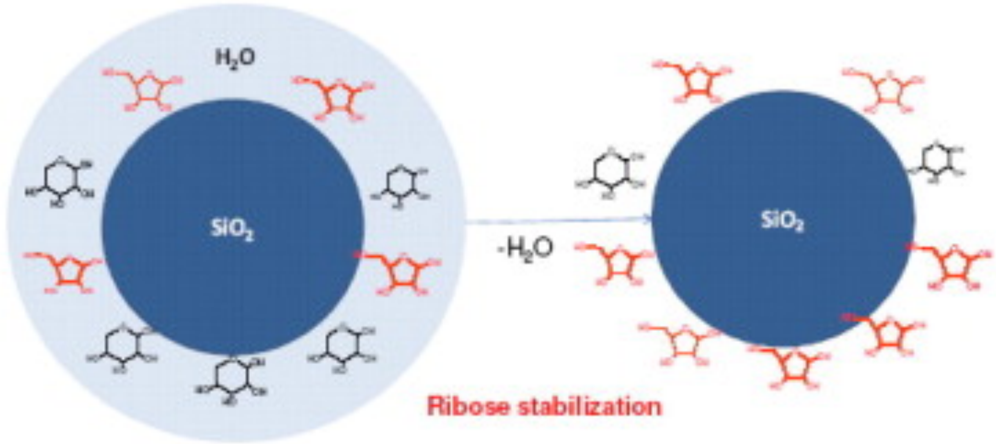
Both formaldehyde and glycolaldehyde are found in interstellar media (Hollis et al 2000, 2001).

Ribose is too unstable under formose conditions to accumulate.

-----------> Stabilizing ribose with Minerals

Benner et al, 2006

Stabilisation of ribofuranose by a mineral surface



High thermal stabilization of D-ribose on silica surface.
(Solid state NMR studies of hybrid composites).

The α-Furanose form of D-Ribose preferentially adsorbed on silica surface.

Georgelin T., Jaber M., Fournier F.,L aurent G., Costa-Torro F., Maurel M-C., Lambert, J-F.
Carbohydrate Research, *in press*, **2014**

http://dx.doi.org/10.101T.,6/j.carres.2014.07.018

Prebiotic world

Biochemical monomers

Macromolecules and pre RNA World

Inside the RNA world

RNA World

Protocells, Ribocytes, RNA/DNA world, others?

- **Could RNA have existed alone** ? Would RNA have been capable of resisting under primeval conditions, such as extreme temperatures or pH, high salts or high pressure? Is it environnement-dependent?

- Through the ages, RNA had not functioned alone. Given what is known today about small ribozymes, what could we learn about the origins of life by studying **RNA interactions with small molecules (**and/or **compartments)?**

- Do we know current **RNA species, vestiges** of the RNA world?

Could RNA have existed alone ?

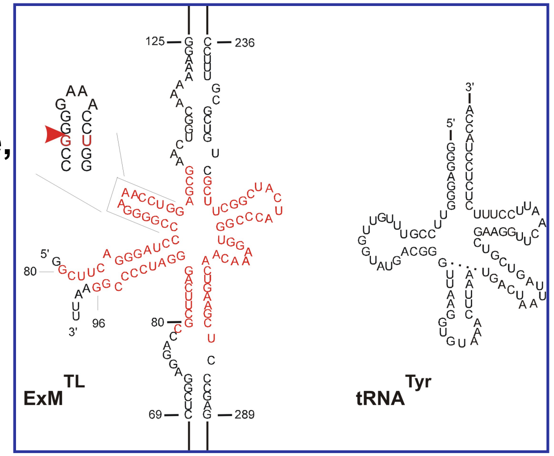
Catalytic motifs

↓

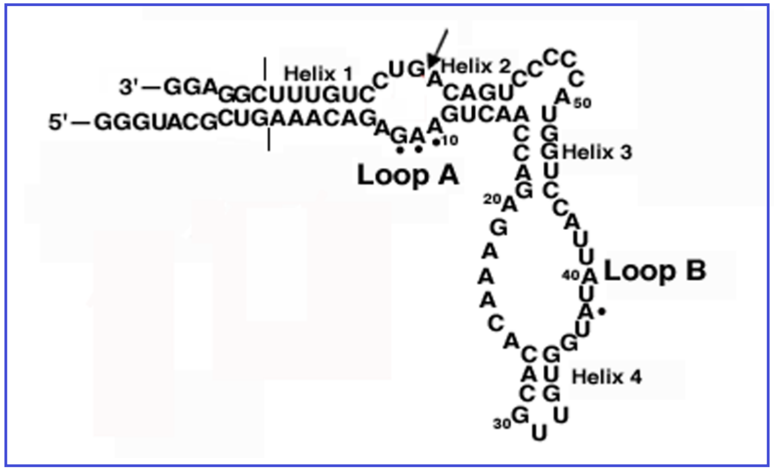
RNA genome perform by itself its own replication

Viroids the smallest pathogens

- compact rod-like II structure (246-574 nts), **cruciform.**
- do not code for any protein, **no envelope,** no capsid
- **ribozymes**
- represent **"living fossils"** of the pre-cellular RNA world, somewhere at the frontier of life (Diener 1989)?



Related RNA species	(+) RNA	(-) RNA
VIROIDS		
ASBV	Hammerhead	Hammerhead
PLMV	Hammerhead	Hammerhead
SATELLITE		
sARMV	Hammerhead	**Hairpin**
sCYMV	Hammerhead	**Hairpin**
sTRSV	Hammerhead	**Hairpin**
HDV	Hammerhead	



Hairpin Tobacco Ringspot virus satellite

Why adenine ?

■ Adenine is a **prebiotic** compound

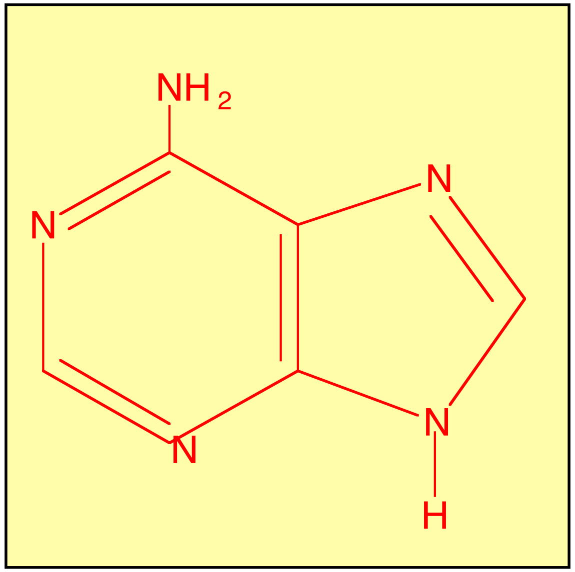
• easily synthezised by HCN pentamerisation
• detected in several **meteorites**

• central in modern cellular metabolism; essential coenzymes (ATP, NAD, NADP, FAD, and coenzyme A)
• one of the four canonic bases in DNA/RNA
• signaling molecules (e.g. cAMP).
• ATP as the energy source
• In addition, in plants, precursors for **purine alkaloids**, and for the adenine moiety of **cytokinin** plant growth regulators.

• exhibiting catalytic properties…

■ **Riboswitch** regulator of gene expression(Mandal et Breaker, 2004).

■ High affinity binding to RNA (Lemay et Lafontaine, 2006). Etc.

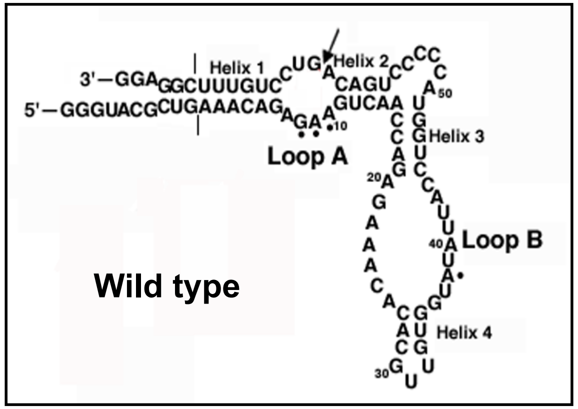


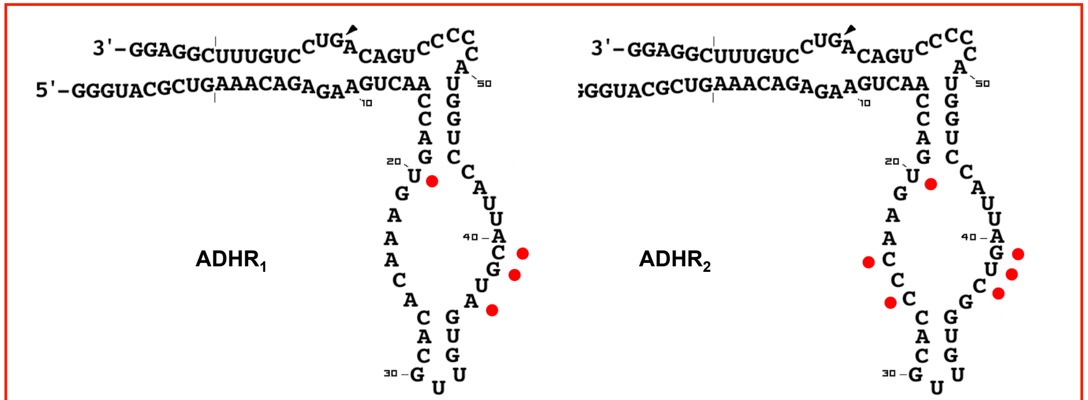
Adenine

Ribozymes could have bound free adenine and handled it as a cofactor to catalyze biochemical reactions.

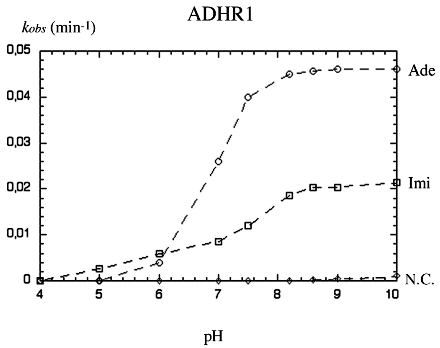
Ribozyme dependent on adenine

- Hairpin ribozyme —> candidate to test hypothesis of adenine as cofactor for early ribozymes.
- From a known hairpin model in which we introduced random nucleotides (20 nt), we selected new active versions (SELEX).
  —> inactive mutants recovered activity with the help of **adenine as extrinsic cofactor**.

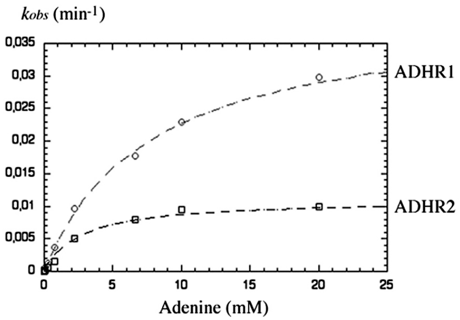




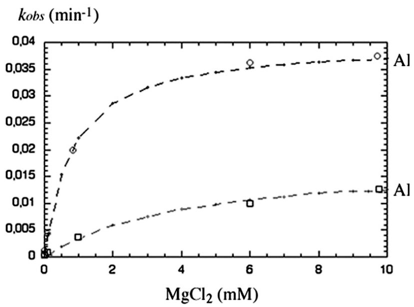
These new variants are selected *in vitro*.



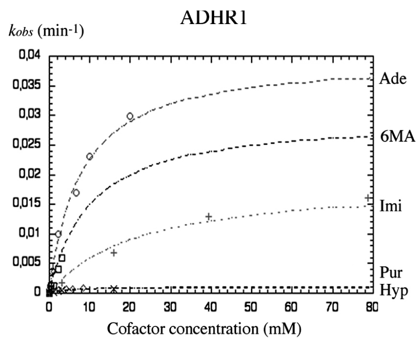
pH Deprotonation events are rate-limiting for catalysis



Adenine ADHR$_1$ is more effective at saturating adenine concentration



$MgCl_2$ mM of Mg^{2+} are required for catalysis



Cofactors Position 6 is involved in H bond formation required for catalysis.

The main protagonists are **nucleobases** and **divalent ions**.

-------> Influence of hydrostatic pressure/NMR studies

Meli, Vergne and Maurel, *J. Biol. Chem*. 278, 11, 9835-9842, **2003**

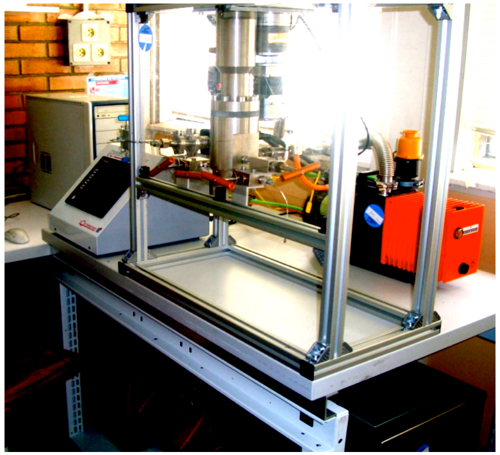
Baro-biochemistry applied to RNA

- High pressures (up to 200 MPa) are of interest :
  - for the behavior of macromolecules at the origins of life.
  - to study life under extreme conditions.

Pressure near **hydrothermal vents**: 40 MPa and can go to 120 MPa (Philippine Pits).

- Pressure modifies hydrophobic, hydrophilic and ionic interactions and **alters the solvatation around macromolecules.**

➡ the volume changes in turn reflect the **conformational dynamics.**



High pressure & High temperature

PV=RT Law of Le Châtelier

Equilibrated reaction :

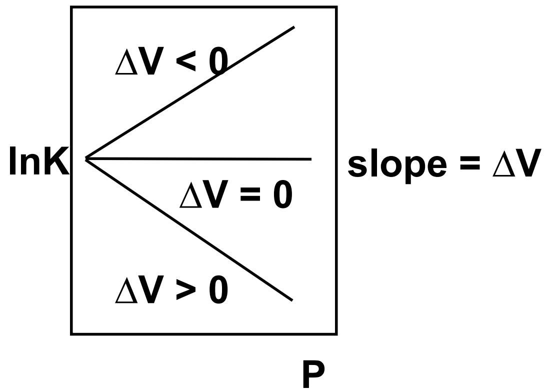
$$A \underset{k_2}{\overset{k_1}{<=>}} B \qquad A+B \underset{k_2}{\overset{k_1}{<=>}} C+D \qquad K=k_1/k_2$$

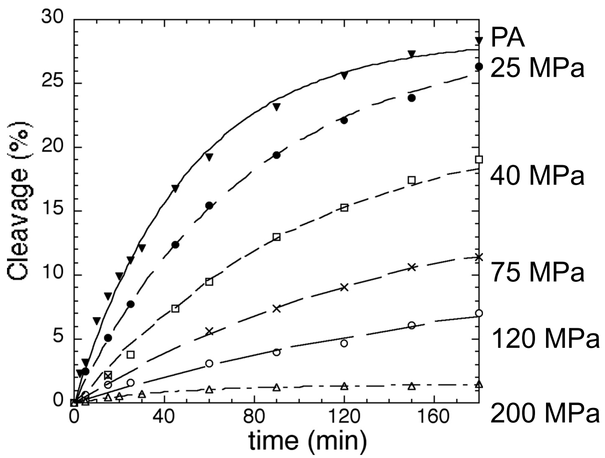
$$\ln K = -\Delta V \left(P/RT \right)$$

$$\left(d\ln K/dP \right)_T = -\Delta V/RT$$

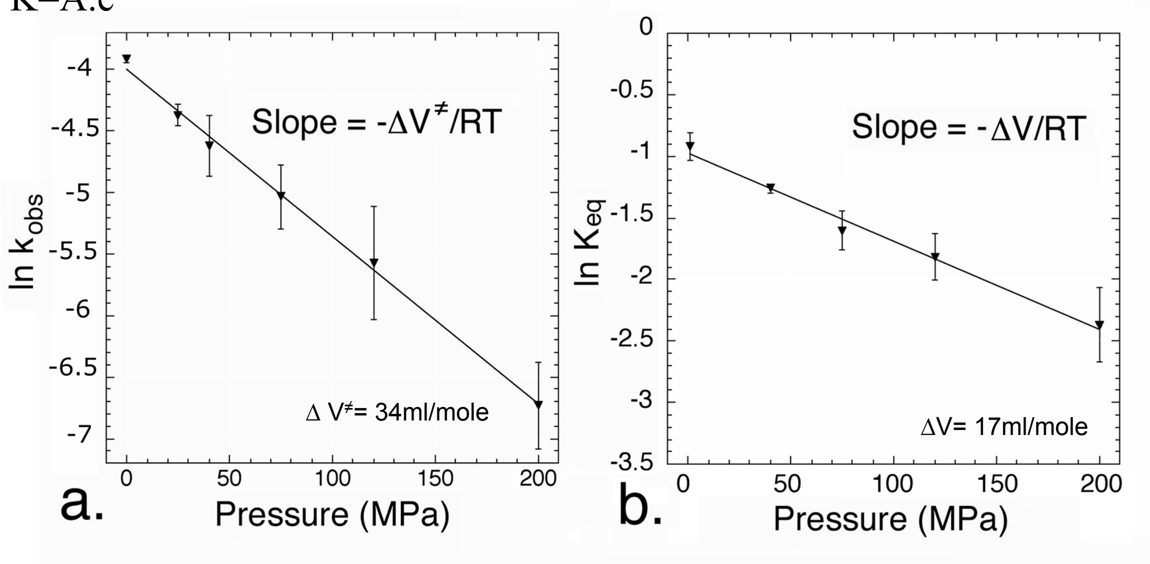
Pressure modifies equilibrium constant if accompanied by a significant volume change (**ΔV)**

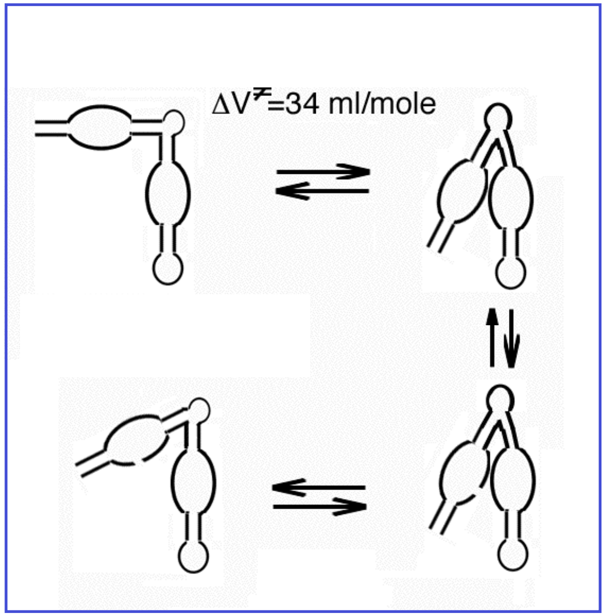
ΔV (reactants + solvent) negative or positive





$K=A.e^{-P\Delta V/RT}$





Model : consistent with cristallographic studies.

The Δ V# of the reaction reflects an important **compaction** of the RNA during catalysis, associated to **water release**.

Dynamics of docking interactions: **molecular motions** interconnect distal segments of the RNA

Tobé, Heams, Vergne, Hervé, Maurel, *Nucl. Acid Research*, 2005,
Hervé, Tobé, Heams, Vergne, Maurel, *Biochem.Biophys. Acta*, 2006

Pr. & NMR

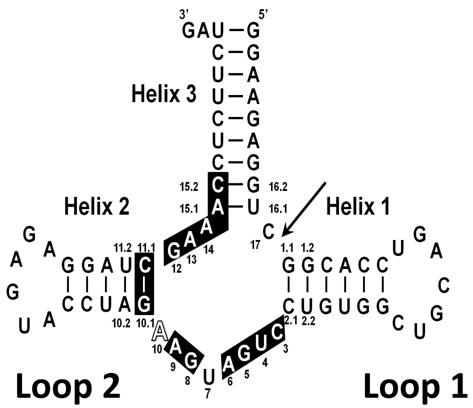
• Mg^{2+} is not active in acid/base chemistry. It induces the **correct folding** of the RNA that is crucial to an active **RNA conformation**.

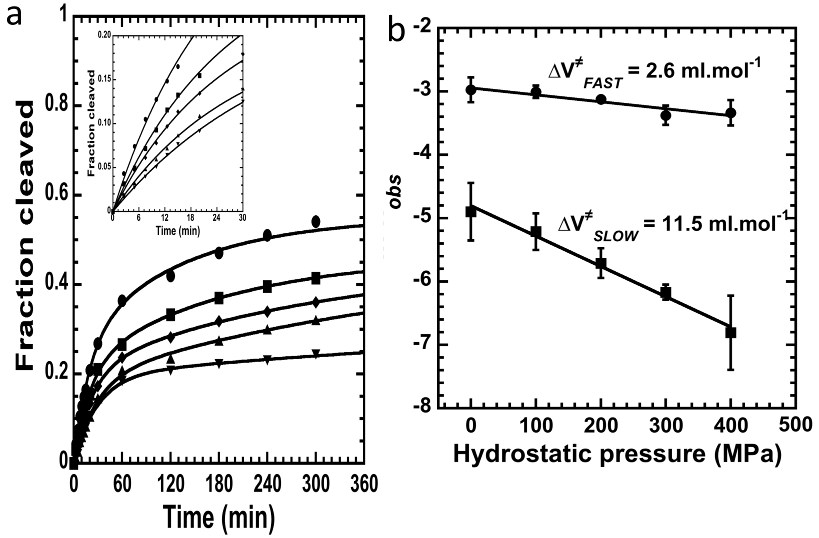
• addition of the **cofactor (A base) transiently aligns the active-site** residues in the ribozyme and participate in transition-state stabilization.

Conformational dynamics is the rate-limiting step in catalysis of the adenine-dependent hairpin ribozyme.

Buck, Li, Richter, Vergne, Maurel, Schwalbe. **ChemBioChem 2009**,10, 2100-2110.
Ztouti, Kaddour, Miralles, Simian, Vergne, Hervé, Maurel. **Febs, 2009**, 2574-2588.

Effect of Pressure on the cis-cleavage of CChMVd-HHR





(a) Cleavage kinetics at AP (l), 75 MPa (o), 100 MPa (u), 175 MPa (D), 225MPa (+) and 300 MPa (x). 0,2mM Mg++
(b) Linear decrease of the log of cleavage rate constants as fonction of pressure.
2 **V≠ fast and slow**

The **hammerhead ribozyme** requires tertiary interactions between its distal loops leading to the closure of the molecule and its stabilization in the active conformation.

---> less important folding than hairpin

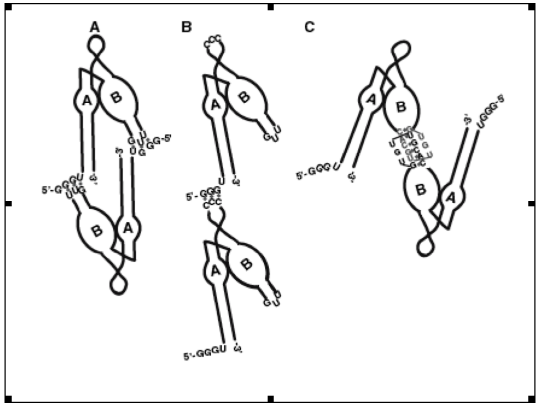
----> **Biphasic kinetic**: **2 different populations** corresponding to **fast** and **slow** cleaving ribozymes.

population fast : initial folded population with tertiary interactions between distal loops (small **V≠**). In agreement with cristallography studies (Scott).

population slow: the folding required to reach the active conformation is more important. Thus **V≠** is more important (pressure has more effect).

Kaddour, Vergne, Hervé & Maurel, 2011

Plasticity of the ribozymes → the diversity of their mechanisms



SANS

A) **Linear/loop** GUU replaced by ‘AAA’ for exp Mutagenesis1.

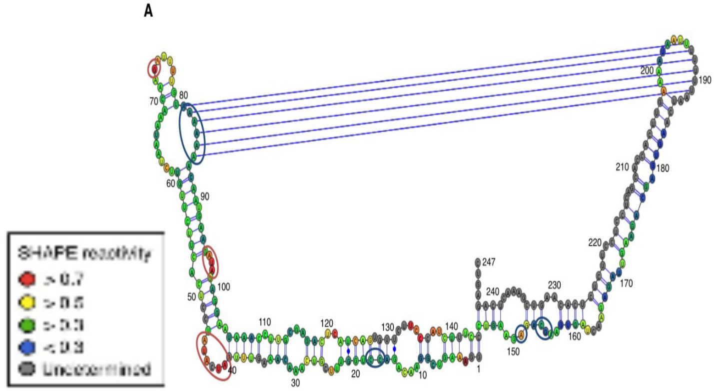
B) **Linear/bulge** CCC replaced by ‘AAA’ for exp, Mutagenesis2.

C) **Kissing** type interaction of ribozyme.

→ Dimerization regulates the hairpin ribozyme function

Li et al. ***E. Biophys. J., 2008***.

SHAPE & TGGE



Kissing-loop interaction in **HHR** of ASBVd (−) (Blue lines)

→ Role in viroid life cycle.

Delan-Forino et al, ***Viruses*, 2014**

Plasticity

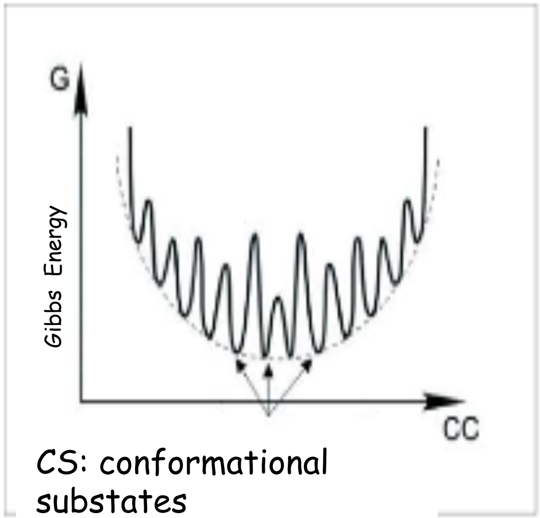
Ability of **small RNAs** to assume different **conformational** and therefore functional states.

Dynamics of subconformations might have been of importance to catalyze various reactions during the early life

From ancient to modern RNA world

The history of life is a history of evolutionary innovations

The activity of a biological system at the molecular level depends on its **movements** rather than on **the structure** only.



This suggest an ***evolutionary selection*** of specific ***dynamics,*** as well as *structure* with relation to *function,* in order to maintain structure and flexibility within the narrow limits required by biological activity

Several **conformational substates**.
At a given T°, the RNA can sample both wells moving from one conformation to another one.

The frontier of life??