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RNA world

by Alexander Theis and Michael Frischmann

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» Darwinian life

... requires the ability to replicate genotypes and express phenotypes. Although all extant life relies on protein enzymes to accomplish these tasks, life in the ancestral RNA world would have used only RNA enzymes. «

- David P. Horning and Gerald F. Joyce -

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CONTENT

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01

Motivation

Why thinking on an RNA world?

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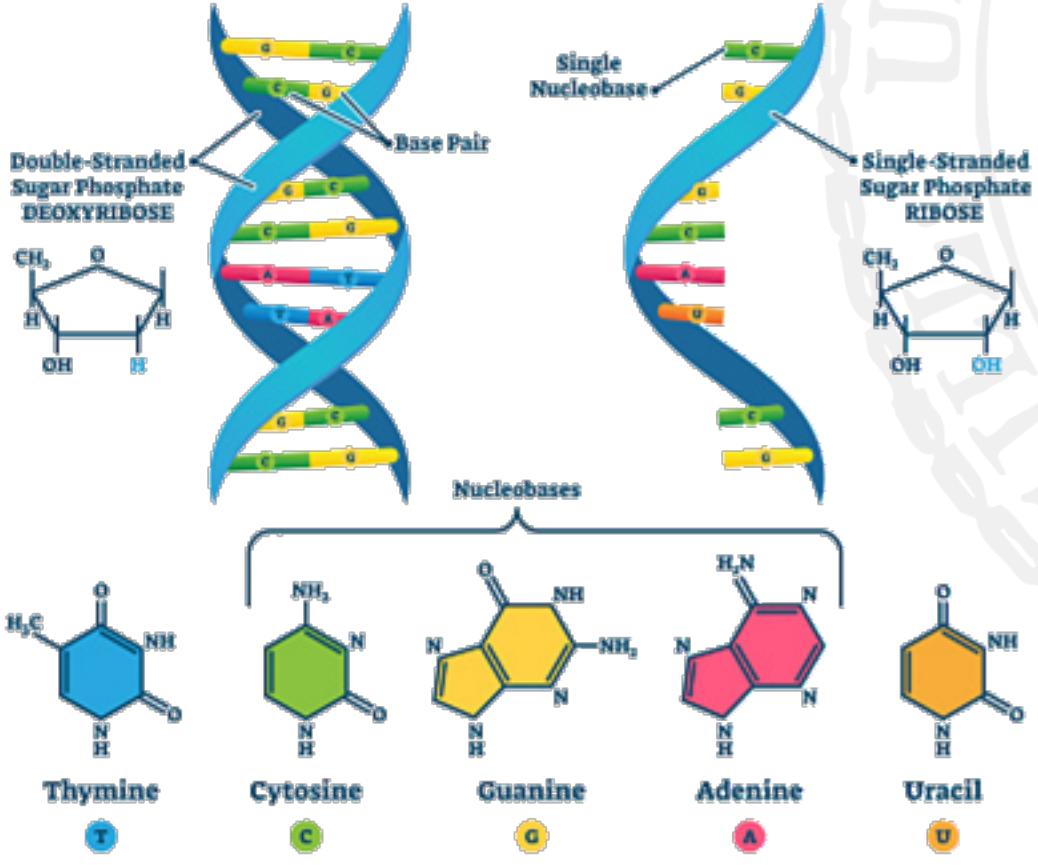
DNA vs. RNA

Desoxyribonucleic Acid

- Storage of the genome



Ribonucleic Acid

- transmission of genetic information
- transcription and translation
- regulation of genes
- catalytic function (ribozyme)

illustration source: iStockphoto.com

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RNA-dependent RNA-Polymerase (RdRP)

- catalyzes synthesis of RNA on basis of RNA
- essential for RNA-viruses
- also in eukaryotic cells (virus protection)
- a protein made from amino acids -> too complicated

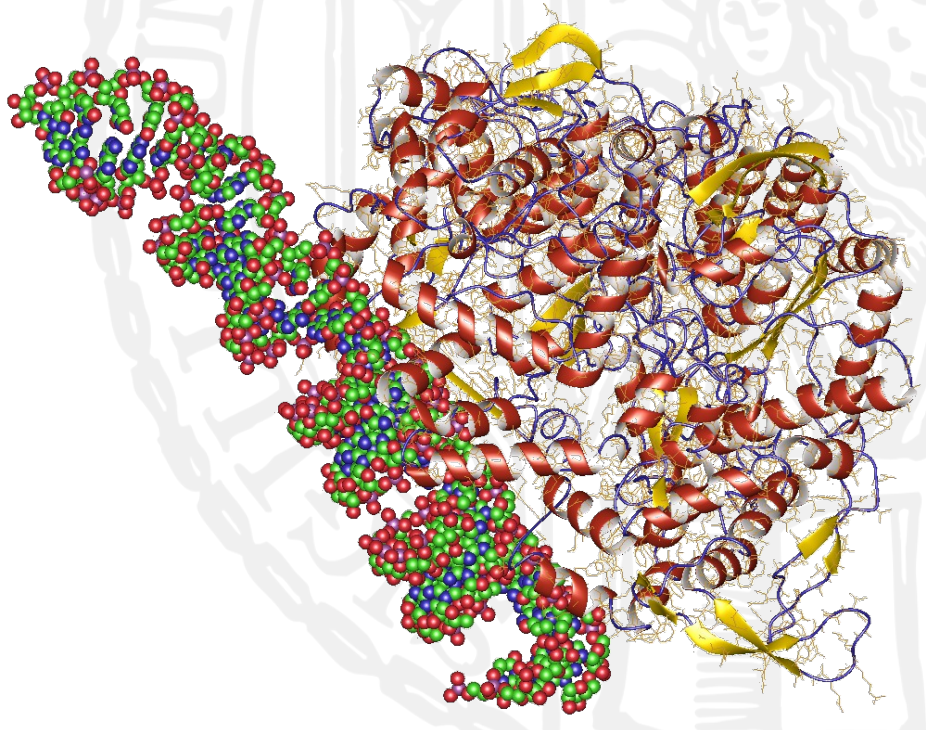

illustration source: Astrojan @wikipedia.org

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Ribozyme

- catalytic active RNA-molecules (act like enzymes)
- important for ribosomes and spliceosomes in pro- and eucaryotic cells
- some of them work without protein parts e.g., Hammerhead-Ribozyme used by some viruses
  -> basis for RNA-world hypothesis
  as *RNA polymerase ribozymes*

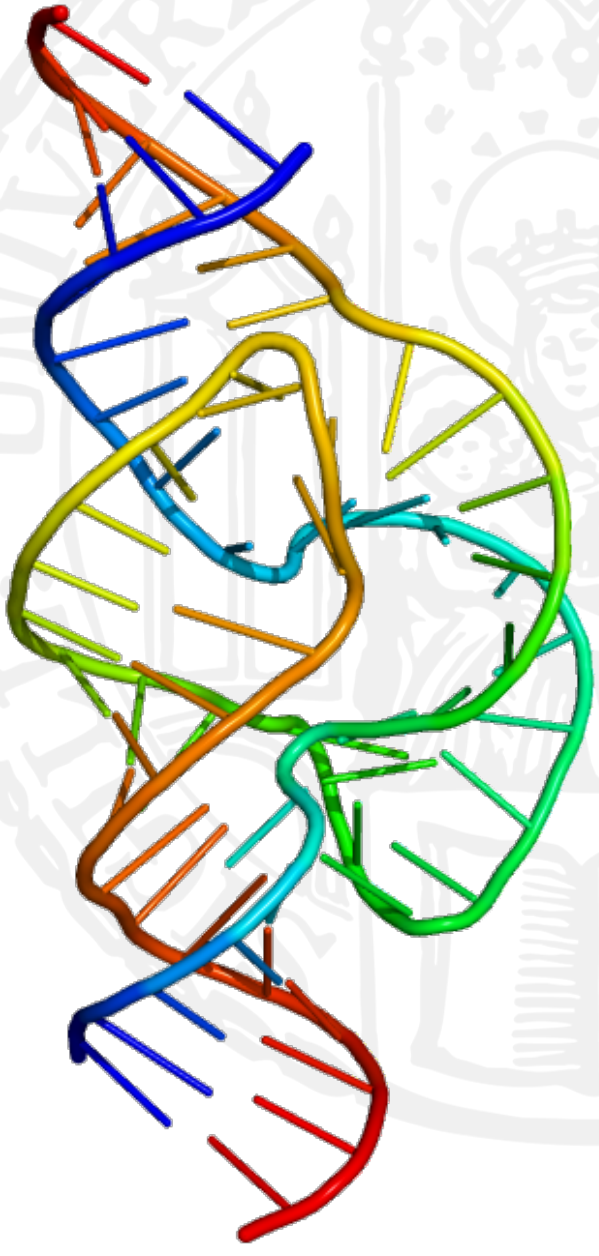

illustration source: Wgscott @wikipedia.org

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RNA-aptamers

- short single stranded (ss) RNA-oligonucleotides that bind to specific target molecules
- ability of inhibiting the function of specific proteins -> molecular tools
- used as therapeutics and in medical diagnosis

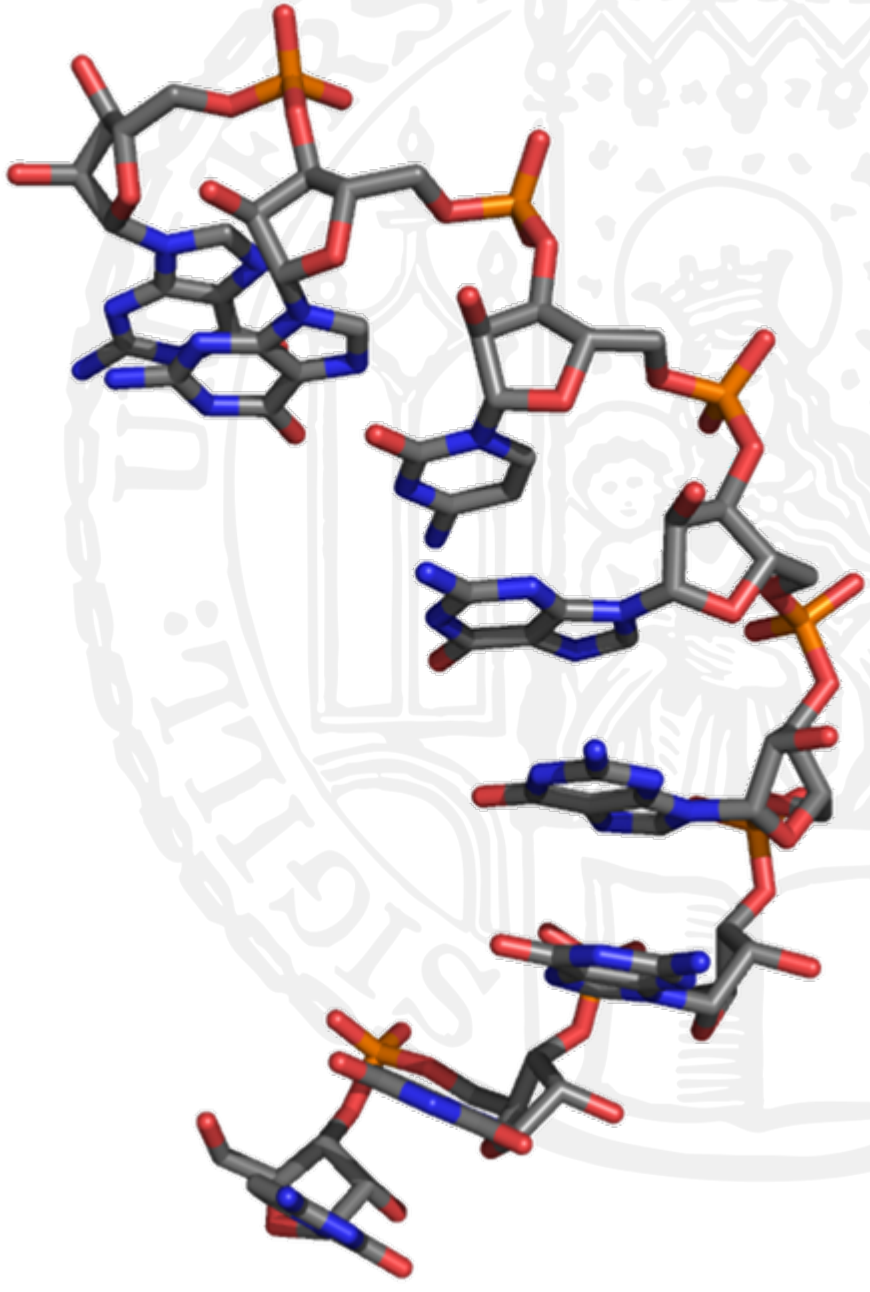

illustration source: S. Jähnichen @wikipedia.org

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RNA world hypothesis

- 120 million years ago -> dinosaurs
- 500 million years ago -> trilobites
- 3,4 billion years ago -> first living cells

-> but what was before?

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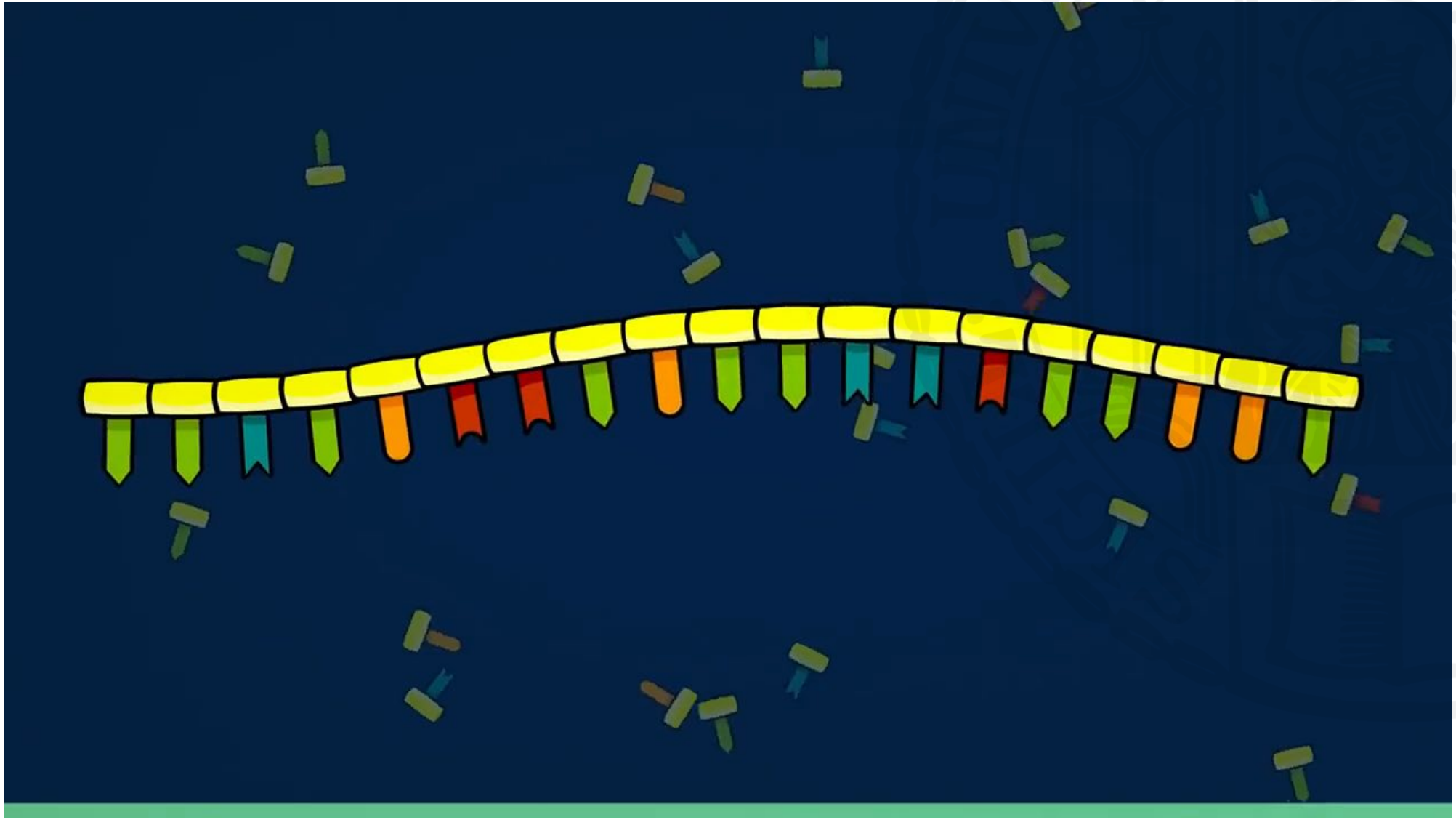
01



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02

Amplification of RNA

by an RNA polymerase ribozyme

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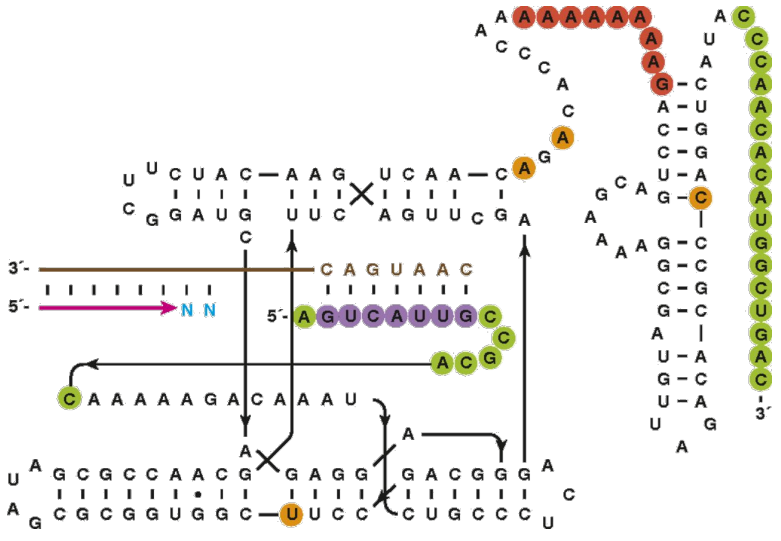
Class I polymerase ribozyme

Engineered wild type (WT)



set of activity-enhancing mutations

24-3 polymerase

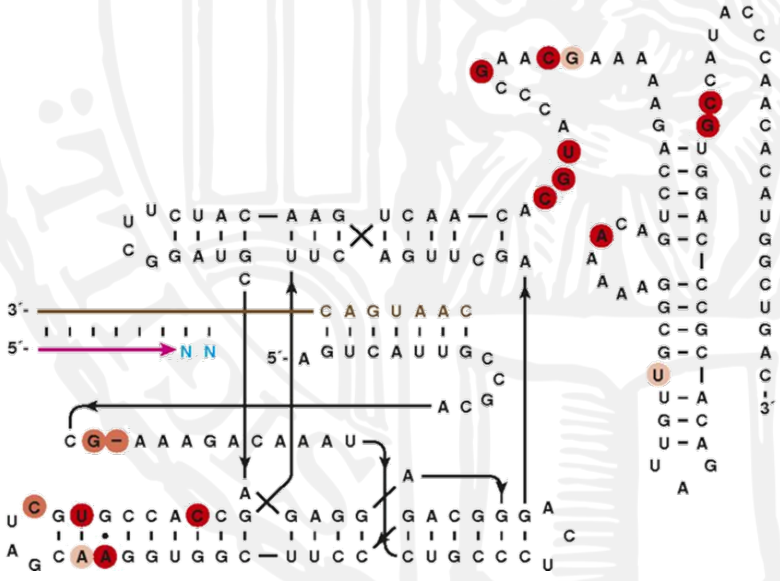


illustration source: Amplification of RNA by an RNA polymerase ribozyme by David P. Horning and Gerald F. Joyce @PNAS.org

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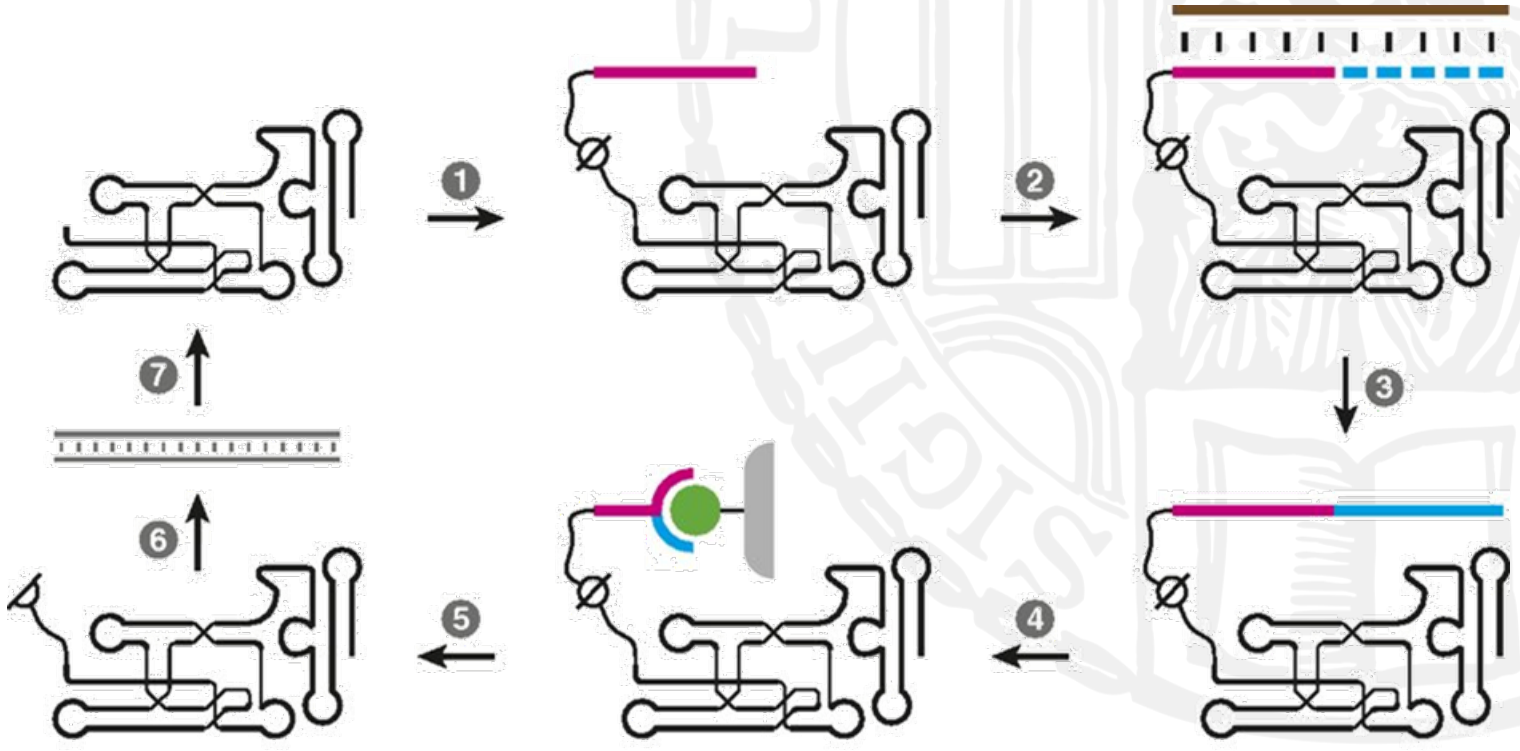
Selective amplification by 24-3 polymerase

- RNA primer
- RNA template
- polymerization of NTPs
- immobilized ligand



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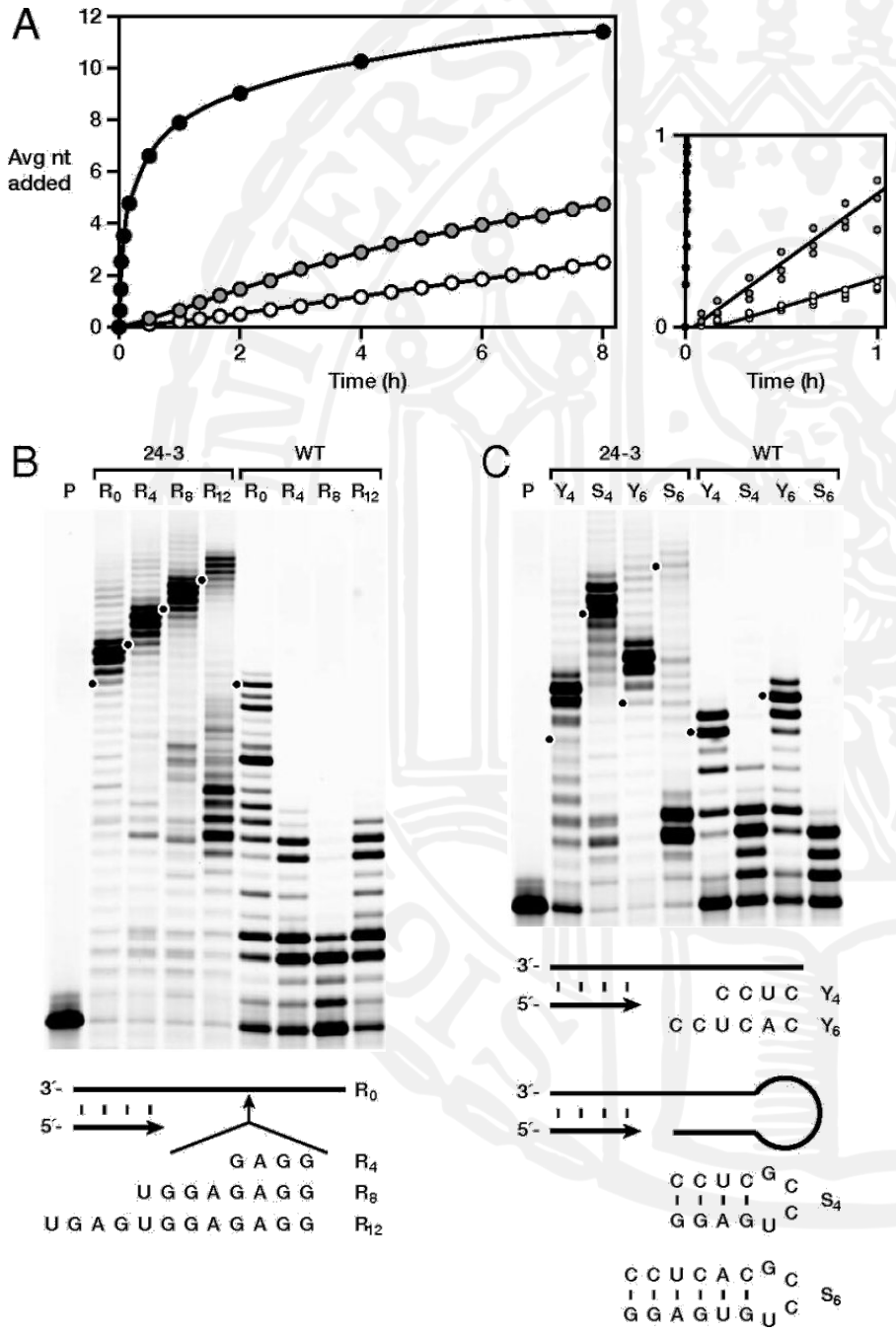
Properties of 24-3 polymerase

- Average rate of primer extension
  - -> 1,2 nt/min (≈100-fold faster than WT)
- Fidelity: 92,0% (WT 96,6%)
- Extends readily purine-rich sequences of 12 nt (WT 4nt)
- Reads through stem-loop up to 8bp long in high yield (WT stops)



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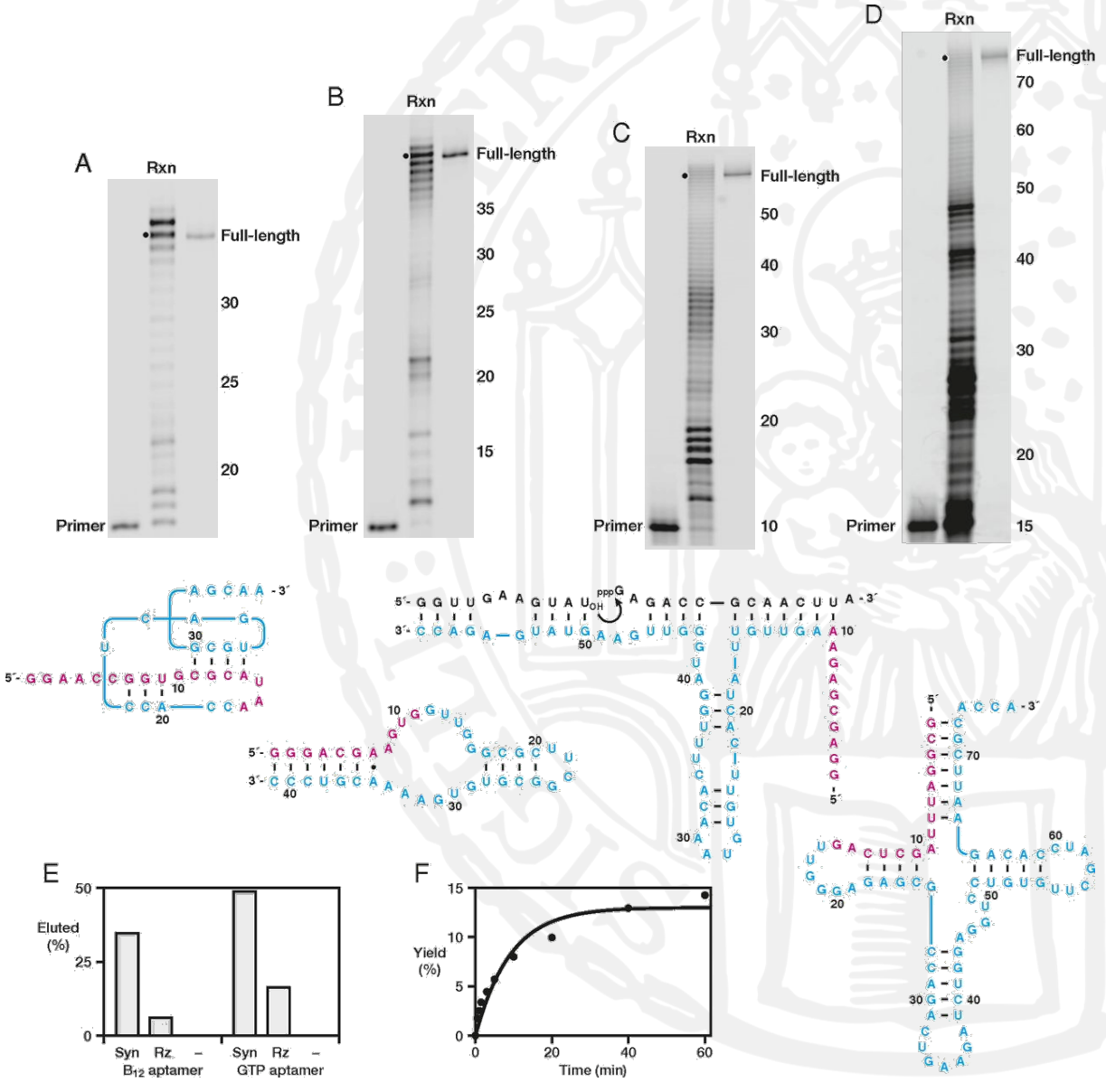
Synthesis of functional RNAs

(A) cyanocobalamin aptamer
47% yield after 24 h

(B) GTP aptamer
18% yield after 24 h

(C) F1 ligase ribozyme
2% yield after 24h

(D) yeast phenylalanyl tRNA
0,07% yield after 72h



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RNA-catalyzed exp. ampl. of RNA (riboPCR)

- 1 nM of a 24-nt RNA template resulted in 98 nM newly synthesized templates and 106 nM of its complements
  -> 100-fold amplification
- 20-nt RNA template Indicates exponential amplification with **per-cycle amplification efficiency of 1,3-fold**

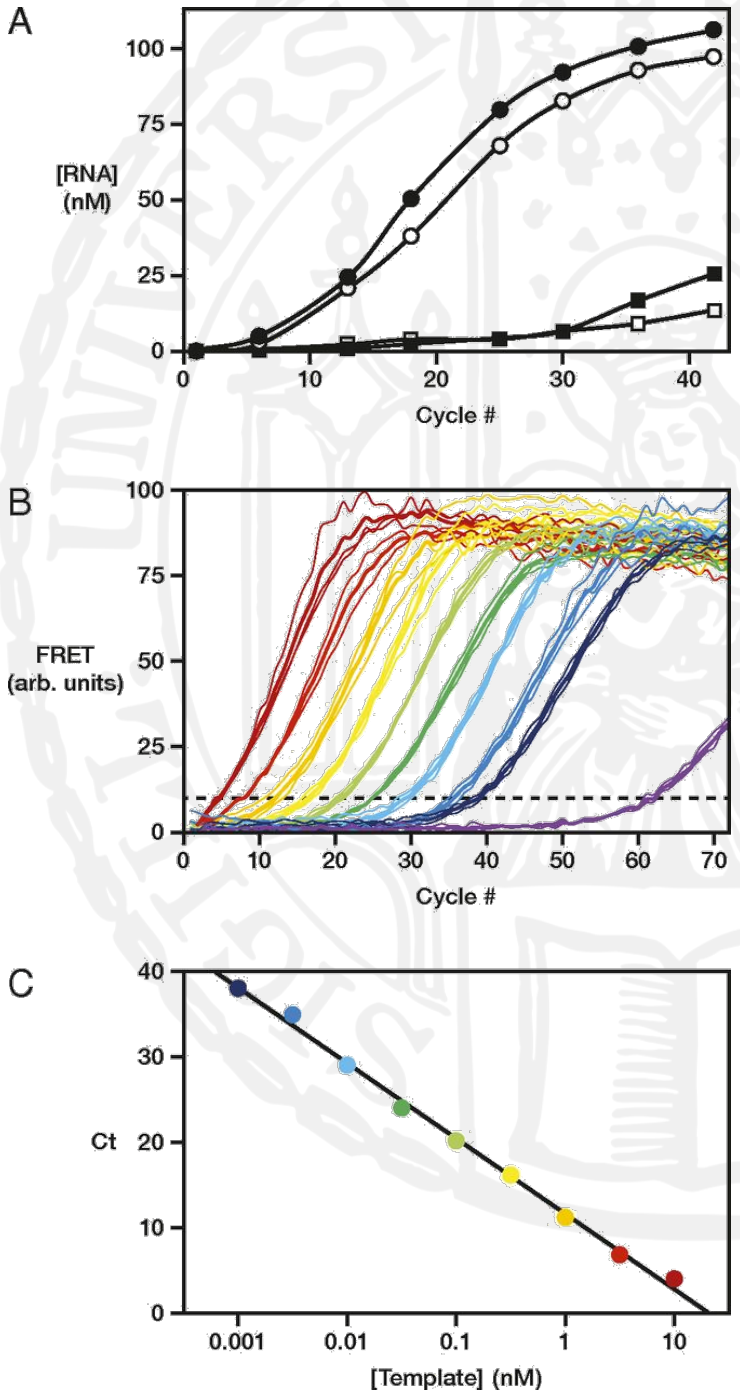

illustration source: Amplification of RNA by an RNA polymerase ribozyme by David P. Horning and Gerald F. Joyce @PNAS.org

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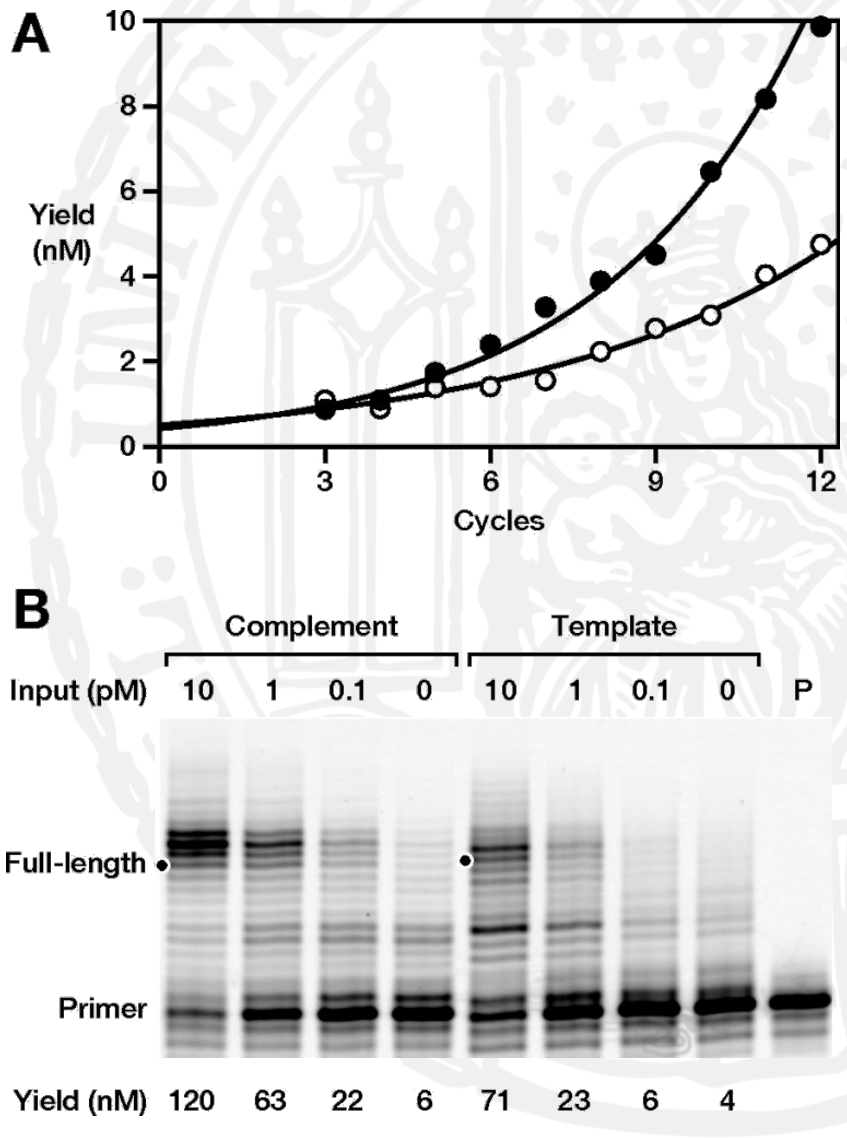
RNA-catalyzed exp. ampl. of RNA (riboPCR)

- 20-nt RNA template Indicates exponential amplification with **per-cycle amplification efficiency of 1,3-fold**
- 1 pM of a 20-nt RNA template amplified to ~40 nM product after 24h

  -> 40.000-fold amplification



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03

Thermal Habitat

for RNA Amplification and Accumulation

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Experimental setup

- Cylindric
- -> laminar gravitational convection
  -> thermophoretic movements
- Max. 80°C
- Min. 17°C

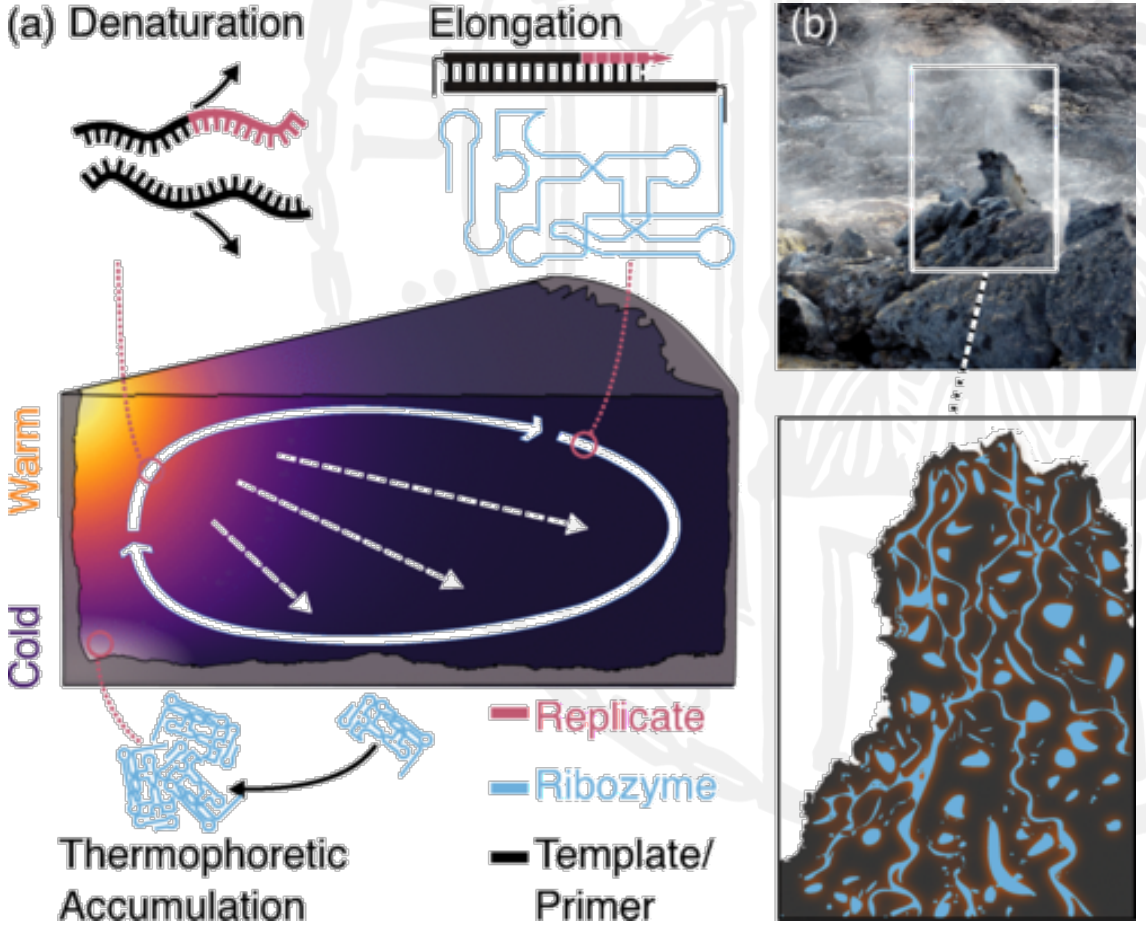

illustration source: bosterbio.com & Thermal Habitat for RNA Amplification and Accumulation by A. Salditt and L. M. R. Keil et al @ APS.org

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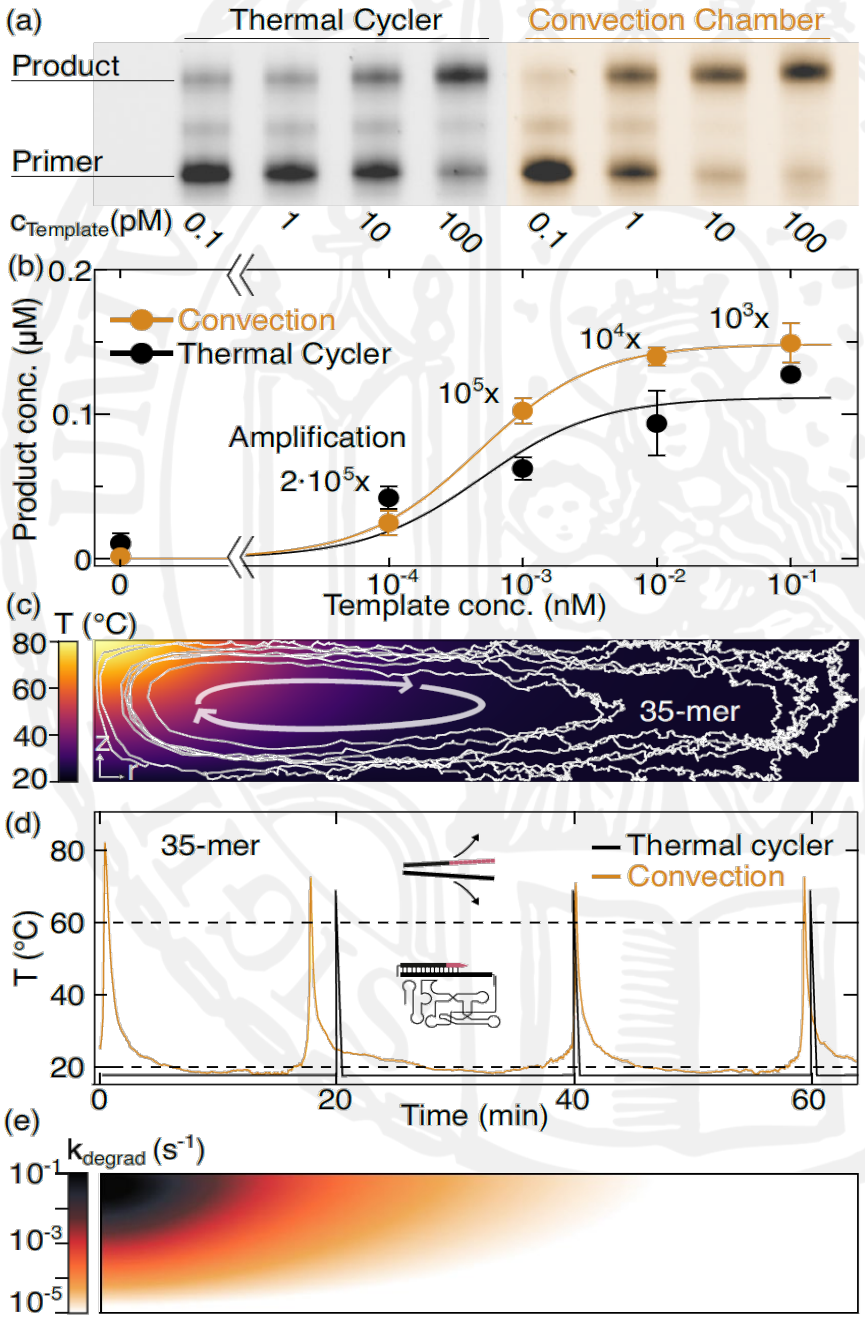
Thermophoretic accumulation of nucleic acids

- system drives accumulation and replication of RNA
- thermal selection bias toward long RNA strands in this system
  -> could guide evolution of longer and more complex strands
- $\sim 10^5$ fold after 24h



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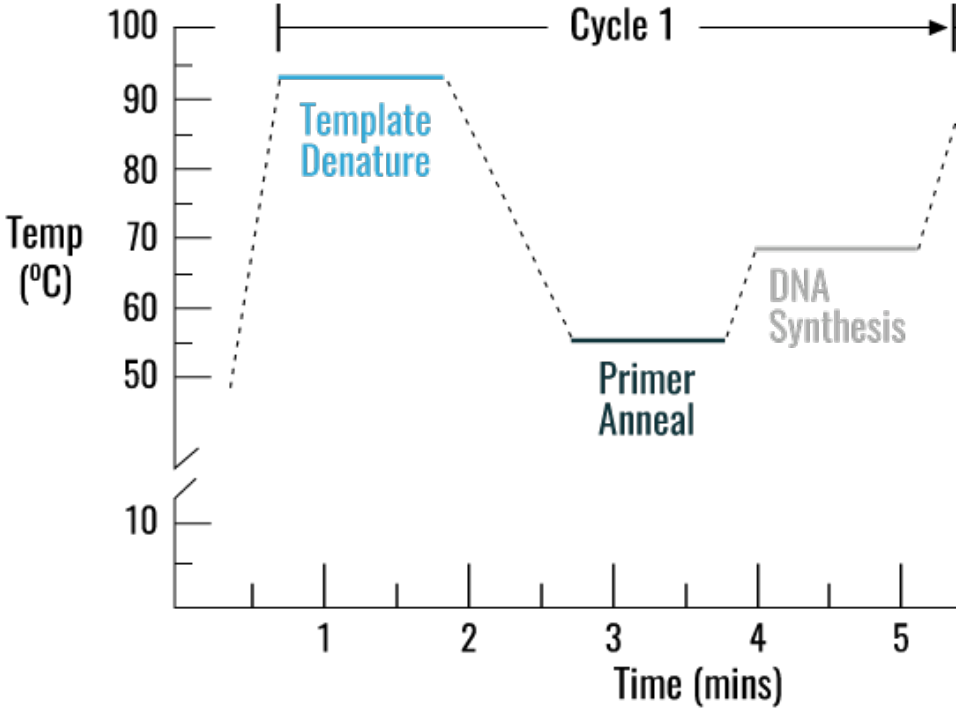
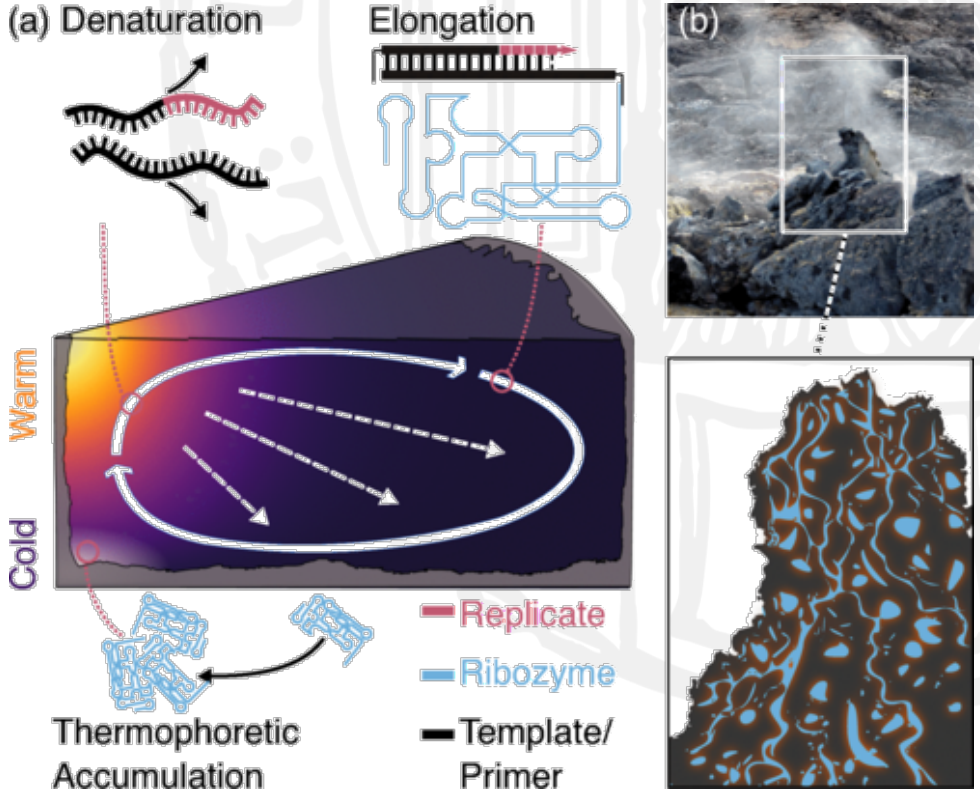
Laboratory conditions vs convection

Thermocycler



Convection micro-chamber



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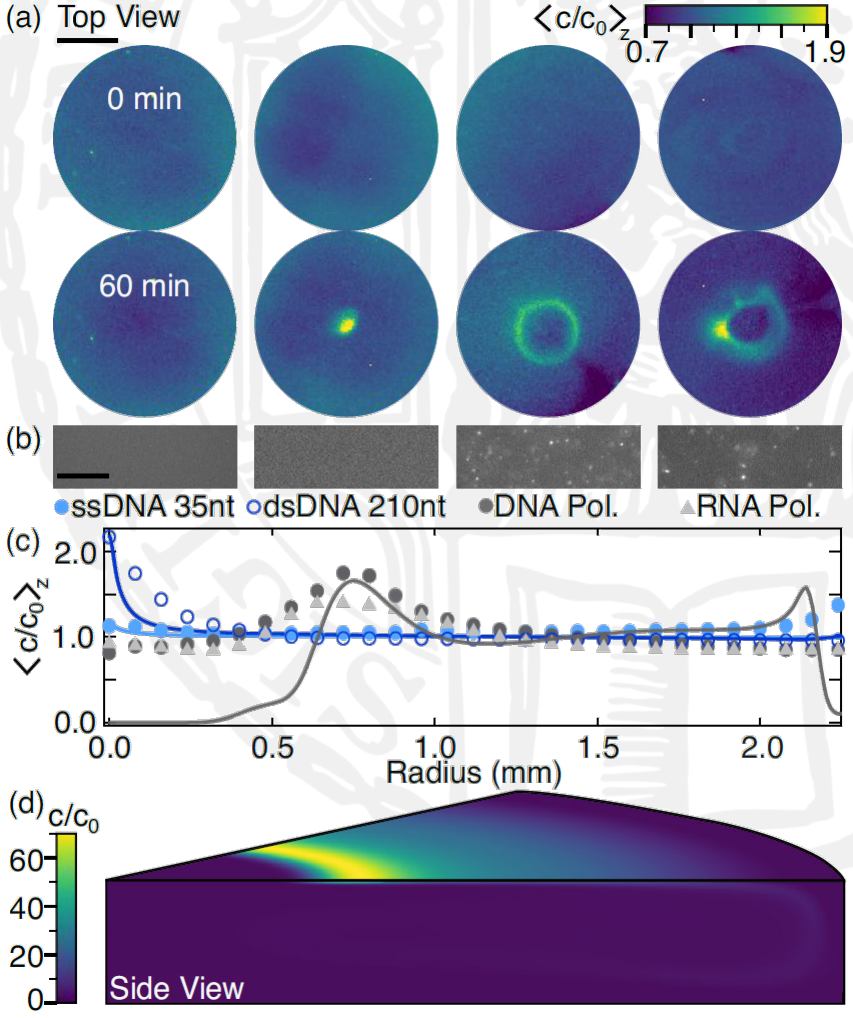
More results

- Add polymerases
  -> ring shaped accumulation?
  -> conglomerates
  -> better environment



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In a nutshell

- Convection -> e.g. rock cavities
  -> early evolution?
- Future outlook:
  -> input of NTPs
  -> adjust T – ≈next topic

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04

In-ice Habitat

for RNA Amplification

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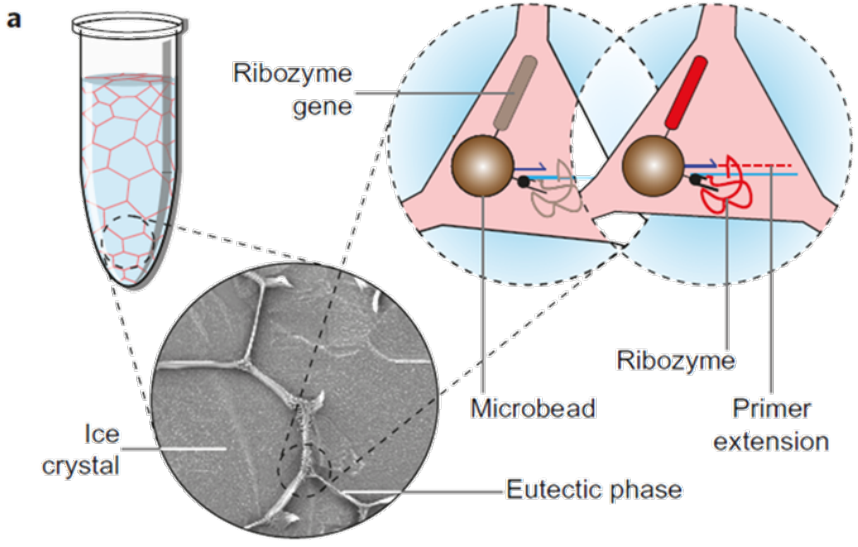
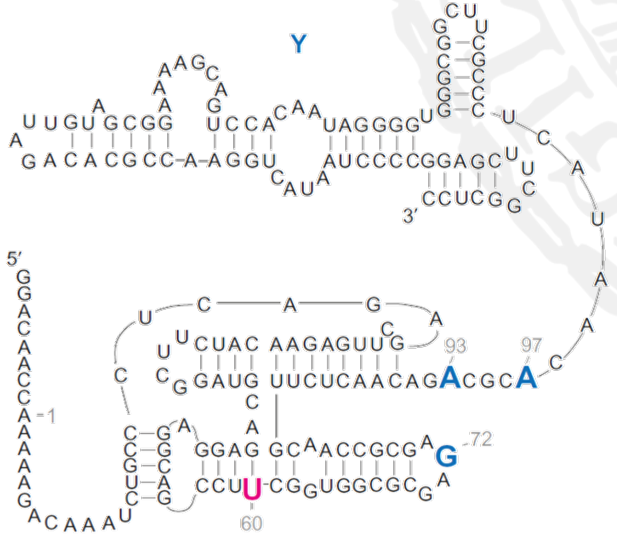
04

Experimental setup

- Compartements -> eutectic phase
- C30 and C8 mutation
- C8: 3 mutations
- Y = modified C8





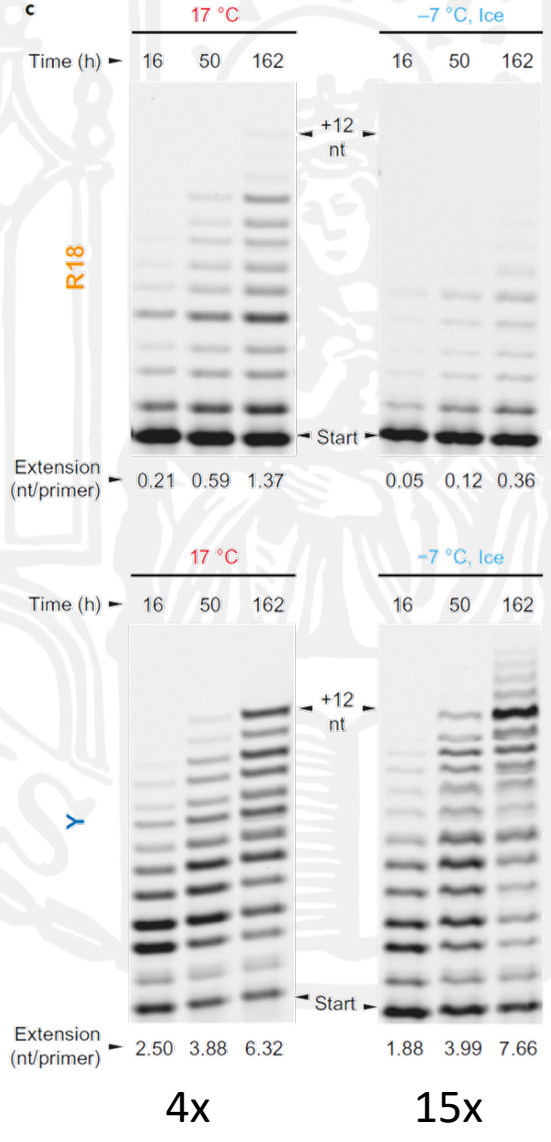


illustration source: In-ice evolution of RNA polymerase ribozyme activity by James Attwater, Aniela Wochner and Philipp Holliger @ nature.com

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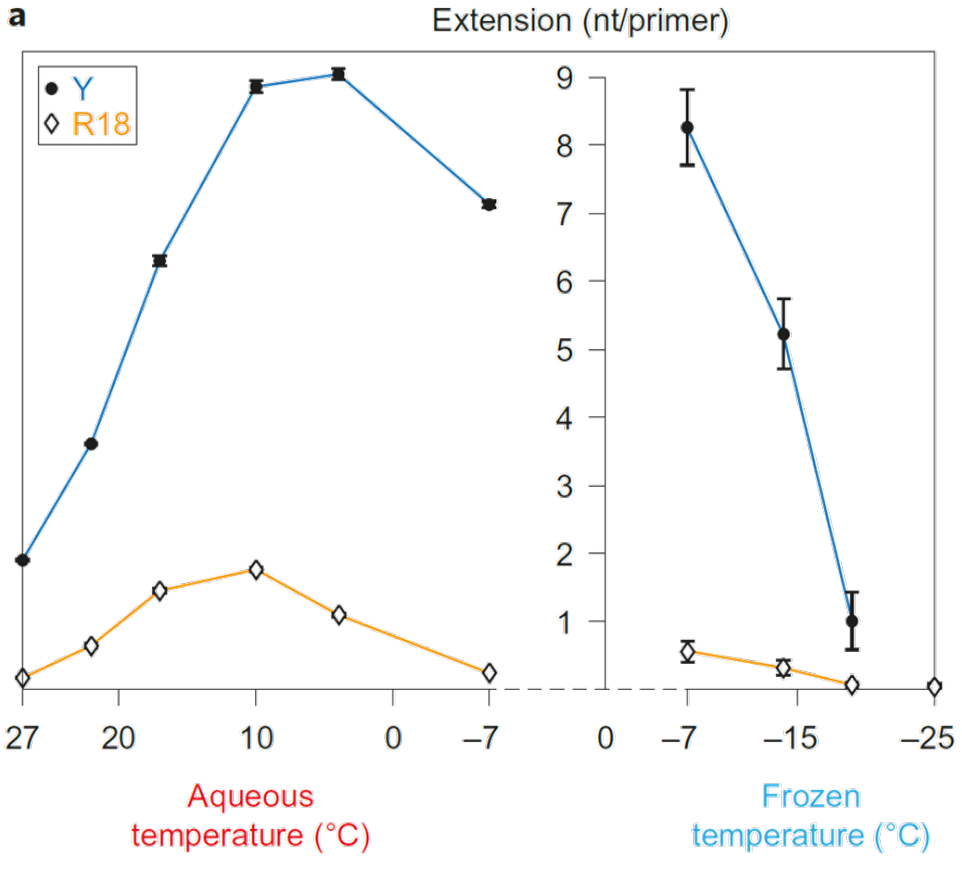
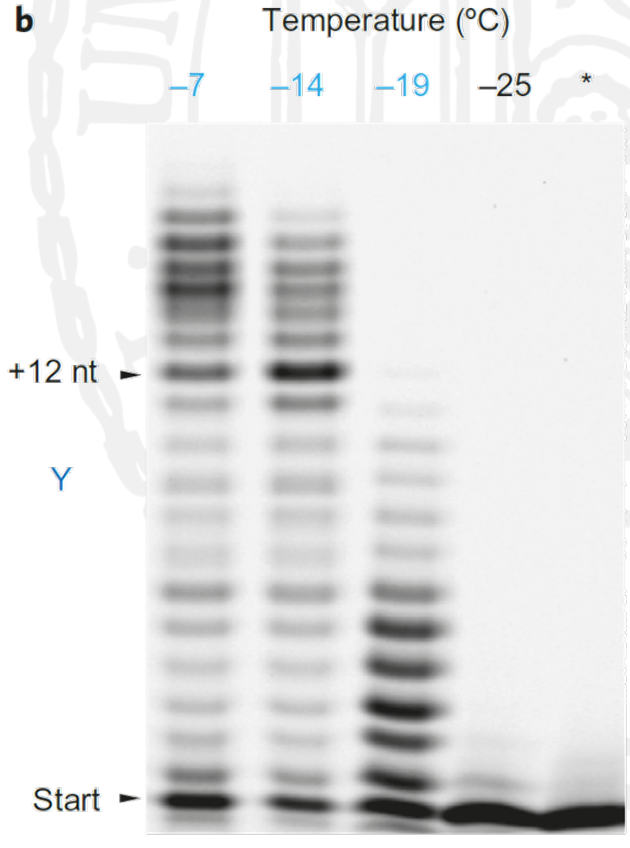
Adaption of ribozyme activity





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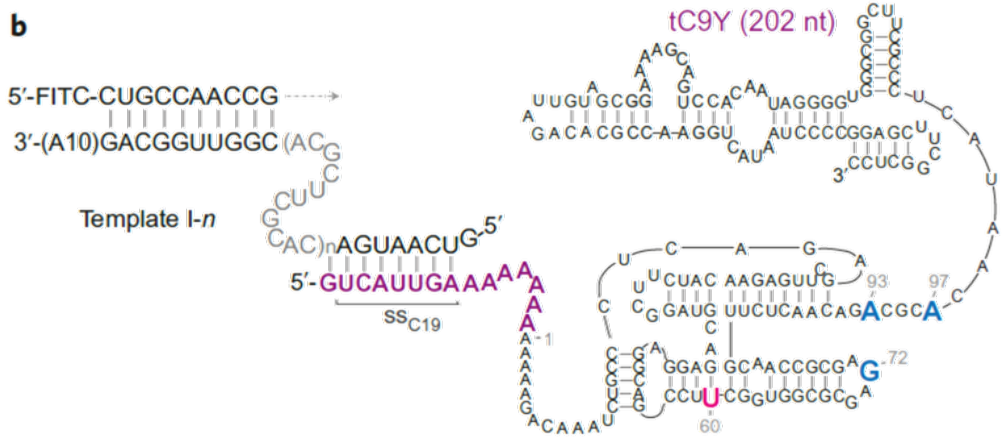
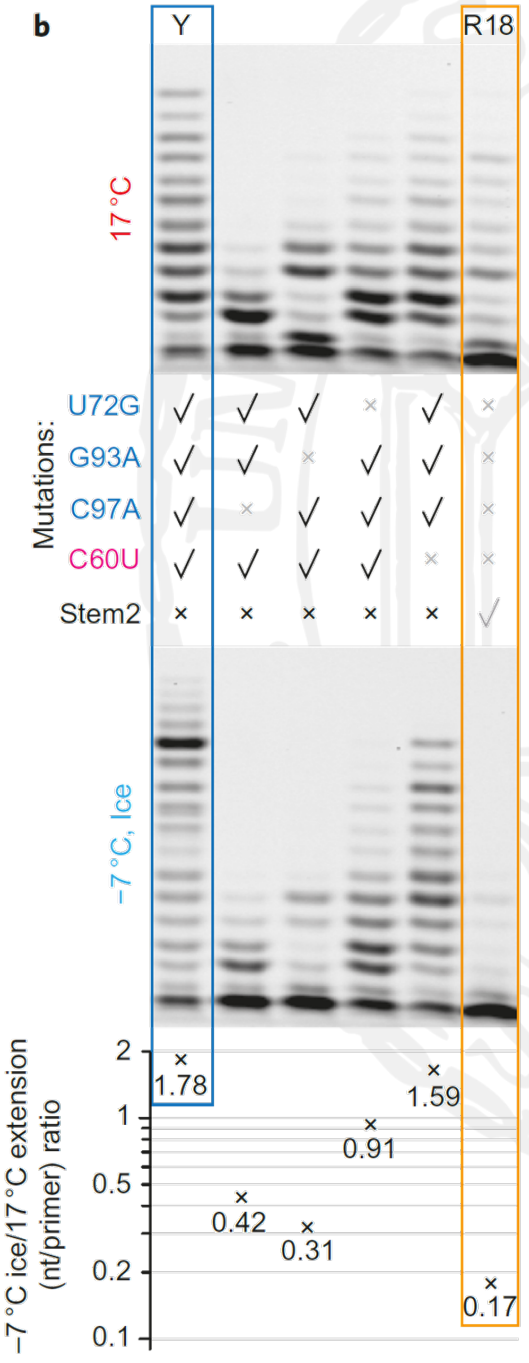
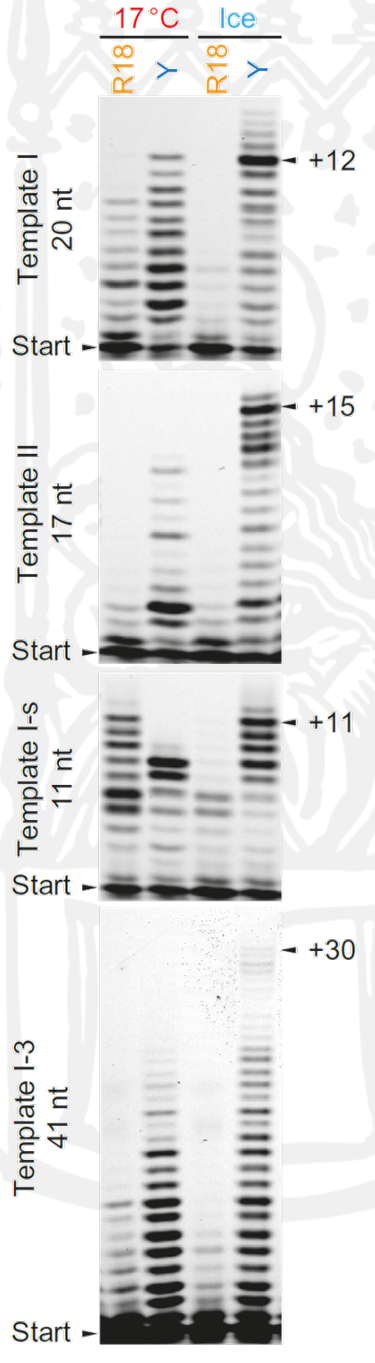
Different mutations

- only two significant
  -> better template binding?

- Add ss_{C19} to 5‘
  -> even better binding
  -> “creation“ of tC9Y







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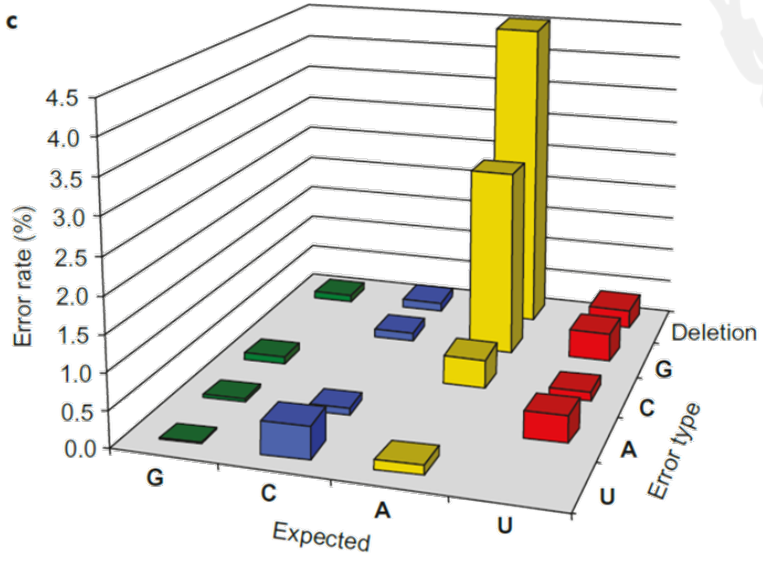
tC9Y performance

- Best termination rate (≈2%; 2013)
- Self-replication possible! -> 206nt > 202nt
- More error towards 3‘? 0,8% -> 7,1%
  -> degradation? -> 1:300



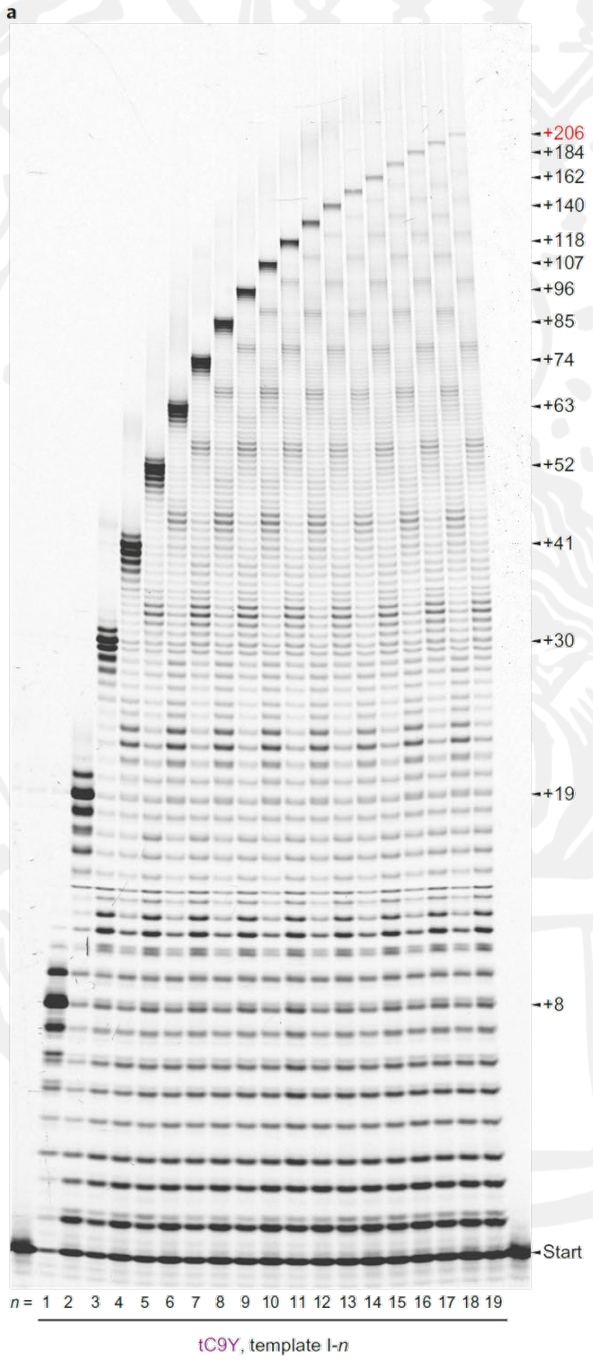


illustration source: In-ice evolution of RNA polymerase ribozyme activity by James Attwater, Aniela Wochner and Philipp Holliger @ nature.com

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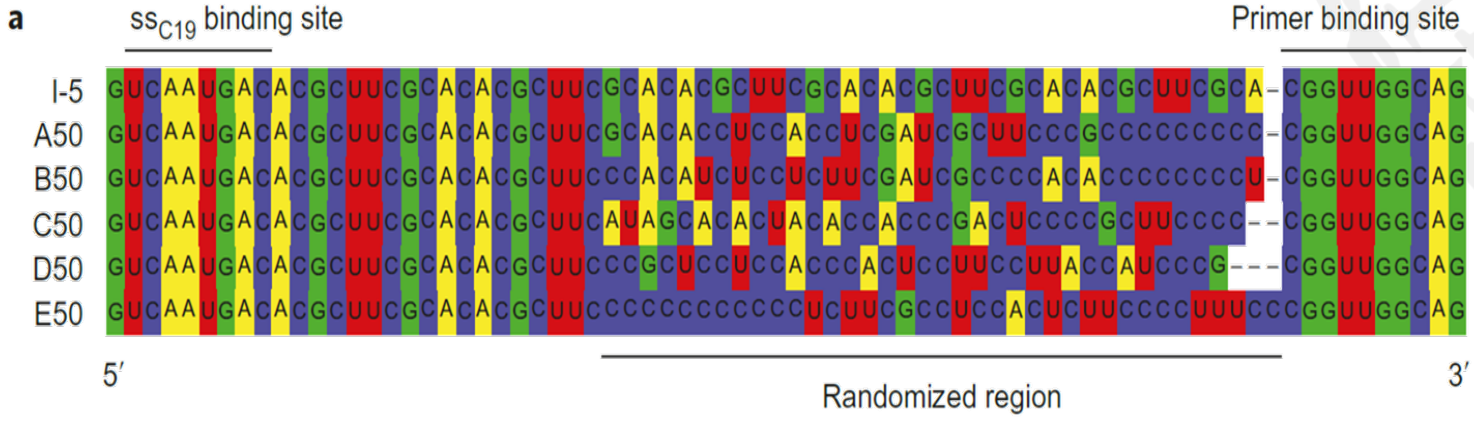
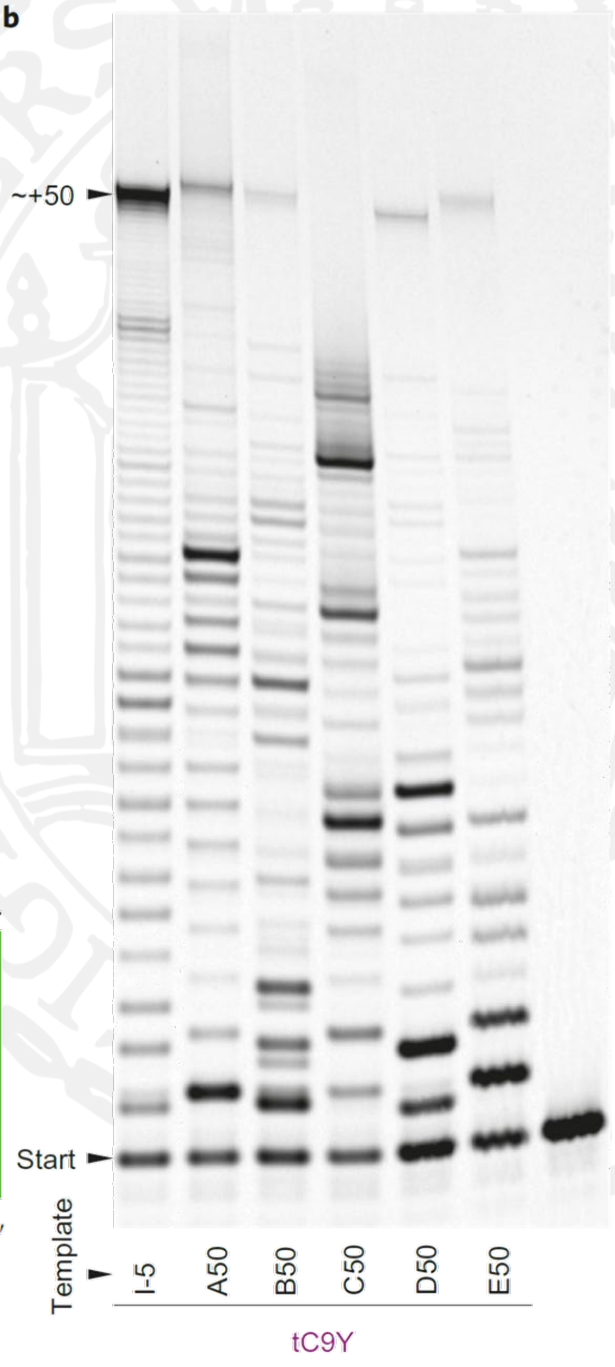
Template selection

- Selection also on templates (n=4)
  ->randomly pick 5 (<50nt)

- Comparison to poor templates:
  -> What do you think?





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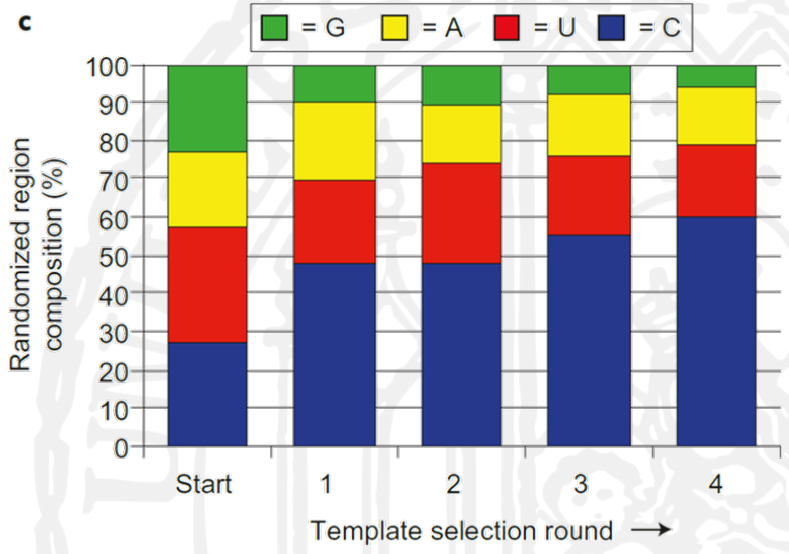
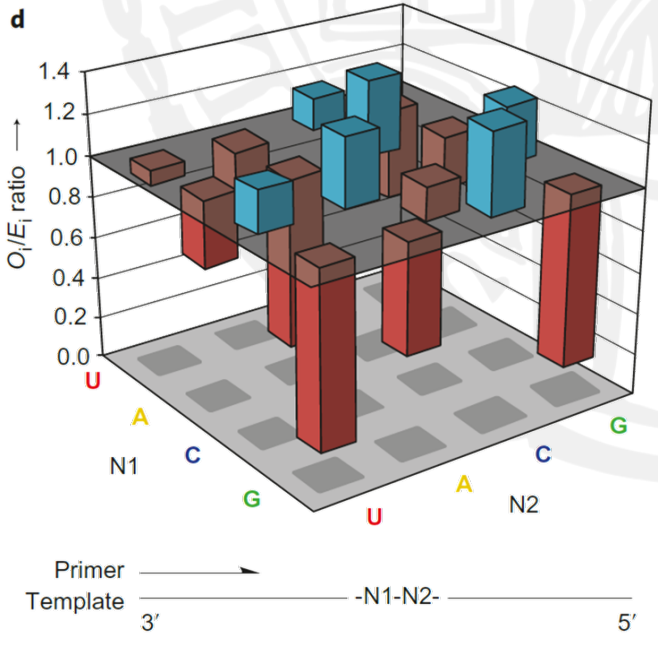
Template selection

- Poor in G and rich in C – Why?
  -> Secondary structure

- Under-representation of NpM ($N, M \in \{U, G, A\}$)
  -> better binding/translokation?





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Summary

- Compartmentalization gives rise to evolution
- Possible -> -19°C
- Self-replication possible
- Next challenge: overcome secondary structure

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Are there questions? RNA world

by Alexander Theis and Michael Frischmann