SCENARIOS FOR THE EMERGENCE OF EVOLUTION (from chemistry)

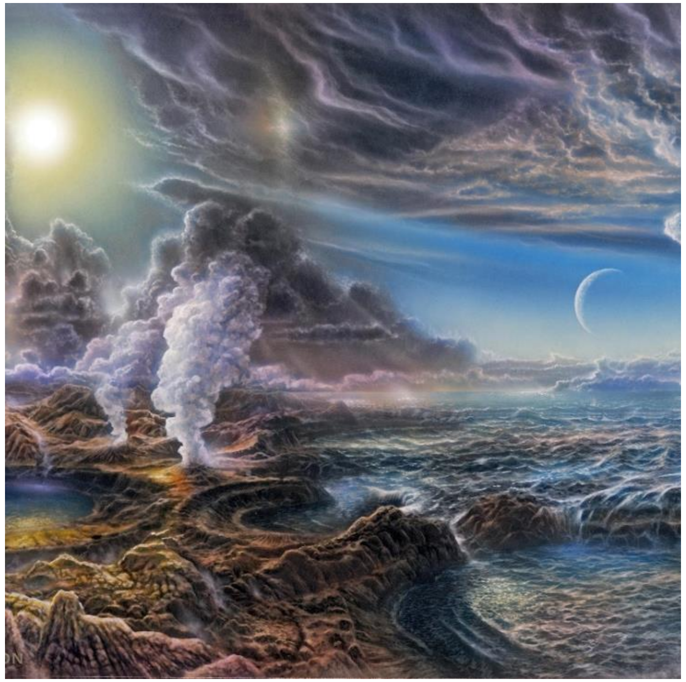
ESPCI PARIS

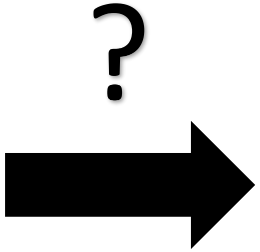
EDUCATION SCIENCE INNOVATION

Philippe NGHE

Laboratoire Biophysique et Evolution

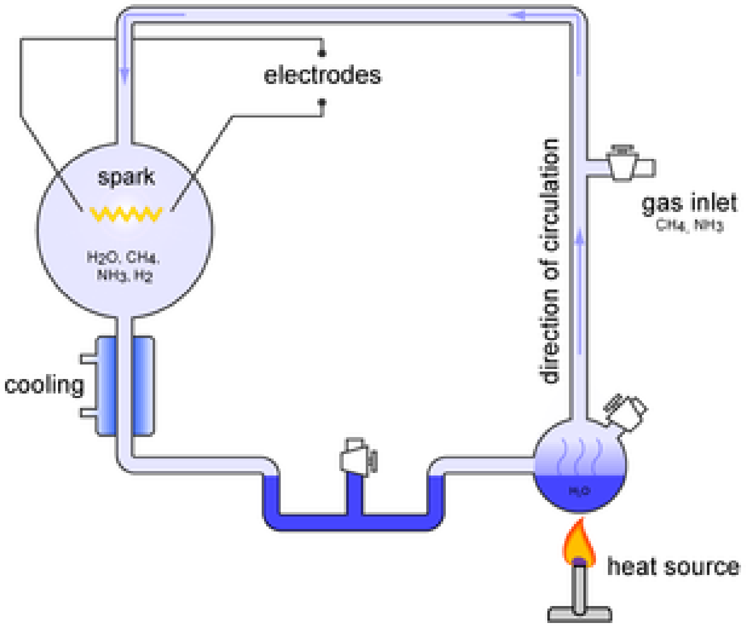
Origin of life research





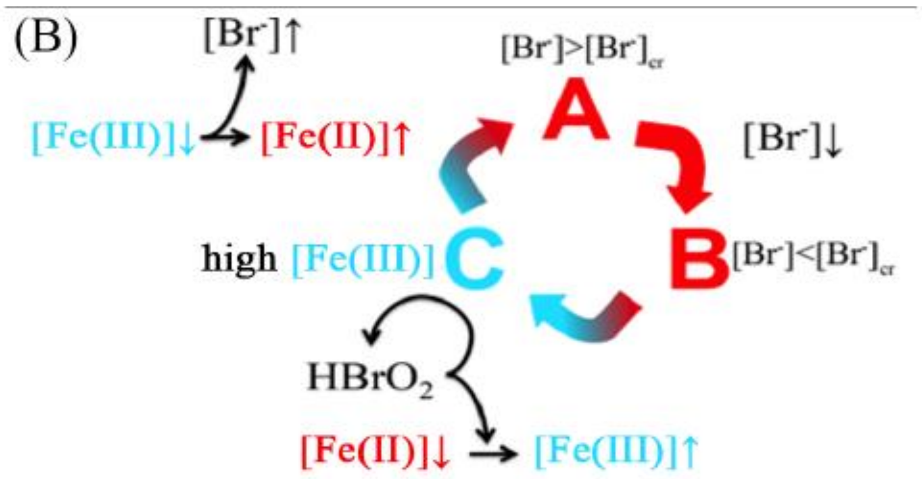
- Historical event with an uncertain context
- Origin of life is not observed re-happening
- No direct backward inference

Miller Urey experiment (1953)



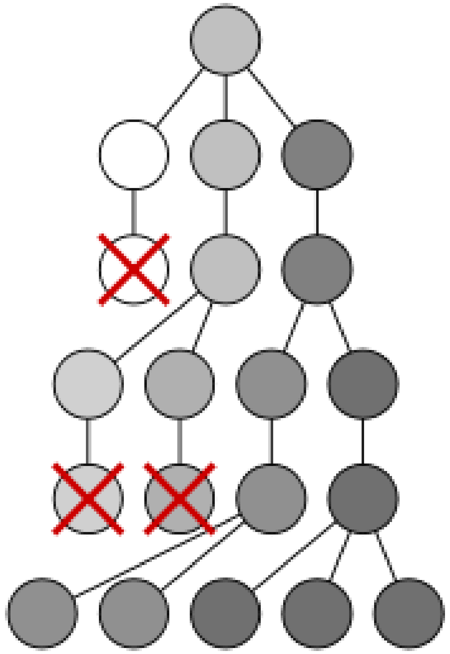
DNA replication

Self-organization in dissipative systems



"A self-sustaining chemical system capable of Darwinian evolution."

Darwinian evolution
(aka evolution by natural selection)

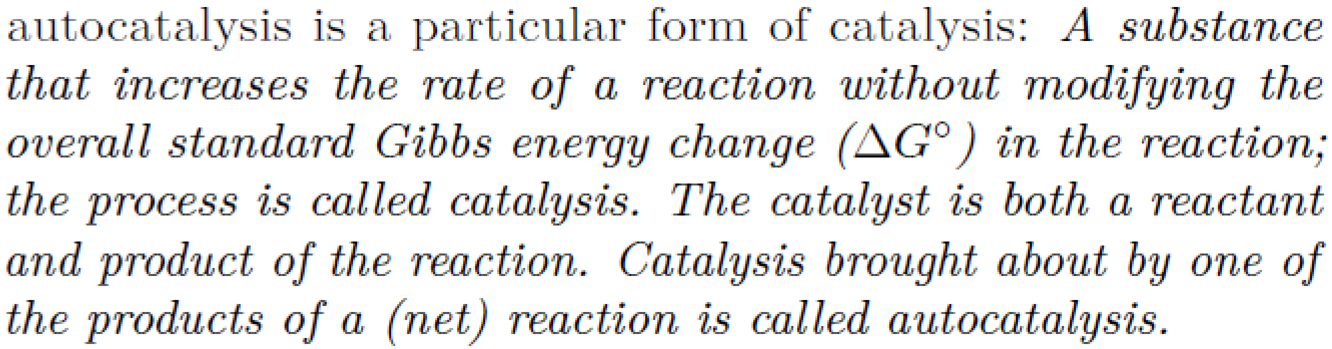


“principle by which each slight **variation**,
if **useful**,
is **preserved**”

1. Variation
2. Selection
3. Reproduction with heredity

AUTOCATALYSIS,
THE BASIS OF SELF-REPRODUCTION

Autocatalysis is a particular form of catalysis

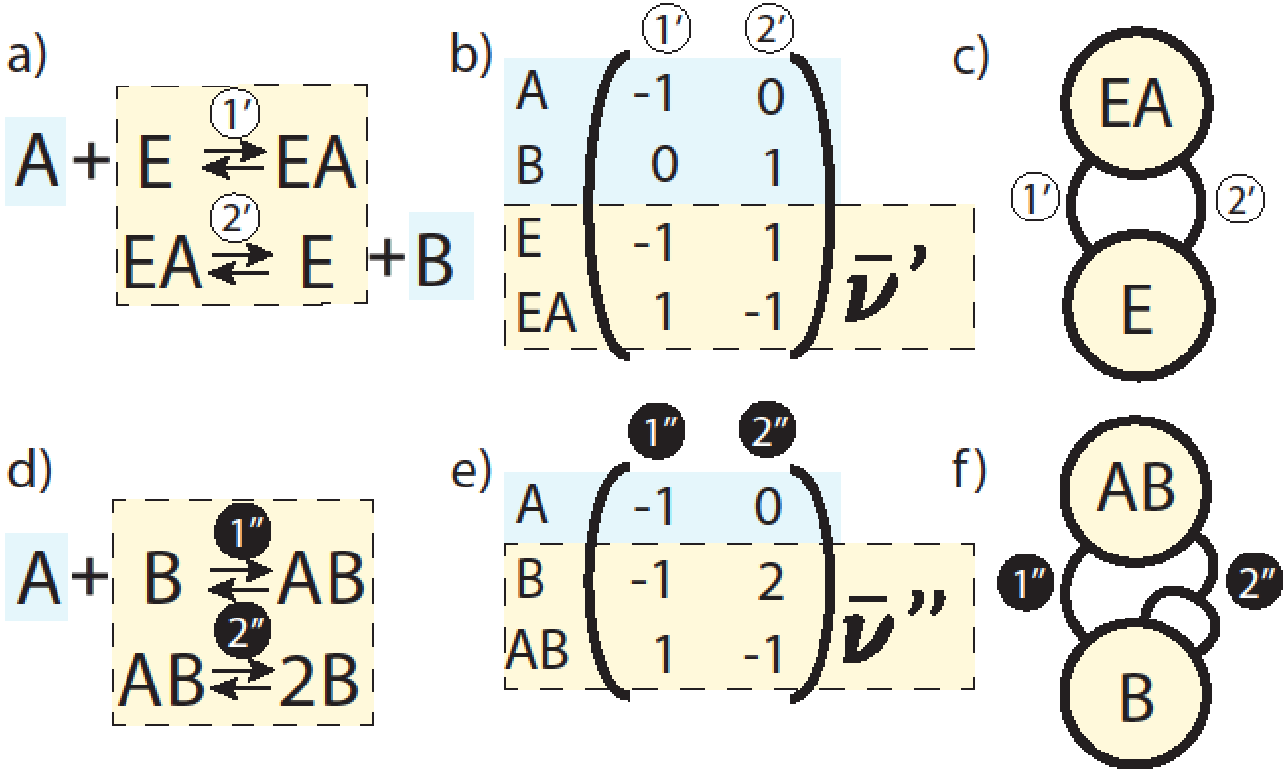


autocatalysis is a particular form of catalysis: *A substance that increases the rate of a reaction without modifying the overall standard Gibbs energy change (*ΔG°*) in the reaction; the process is called catalysis. The catalyst is both a reactant and product of the reaction. Catalysis brought about by one of the products of a (net) reaction is called autocatalysis.*

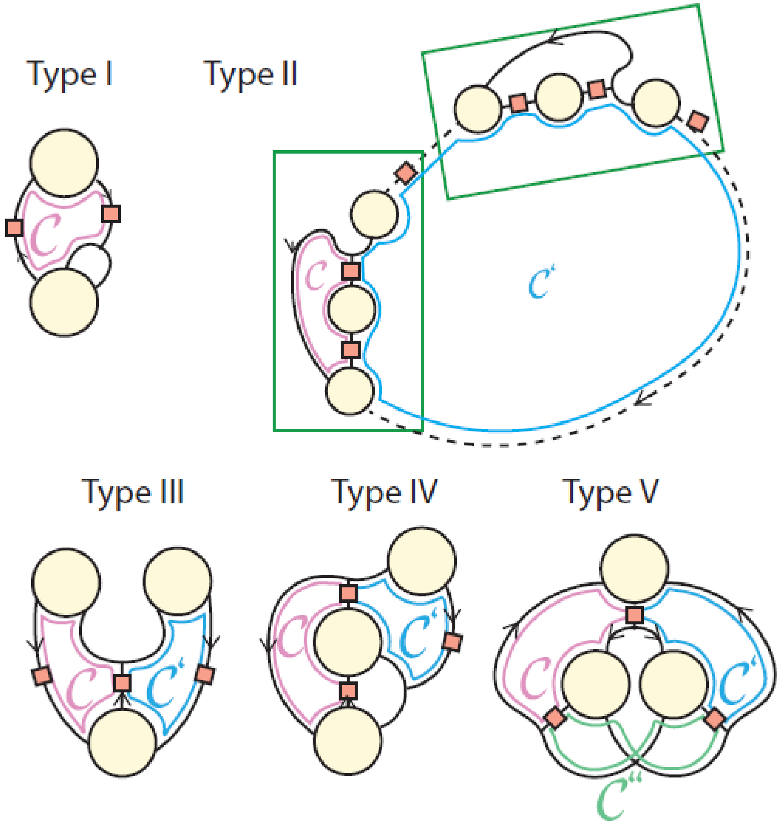
$$\mathrm{A} \overset{(\mathrm{I})}{\rightleftharpoons} \mathrm{B}, \quad \mathrm{A} + \mathrm{E} \overset{(\mathrm{II})}{\rightleftharpoons} \mathrm{B} + \mathrm{E}, \quad \mathrm{A} + \mathrm{B} \overset{(\mathrm{III})}{\rightleftharpoons} 2\mathrm{B}.$$

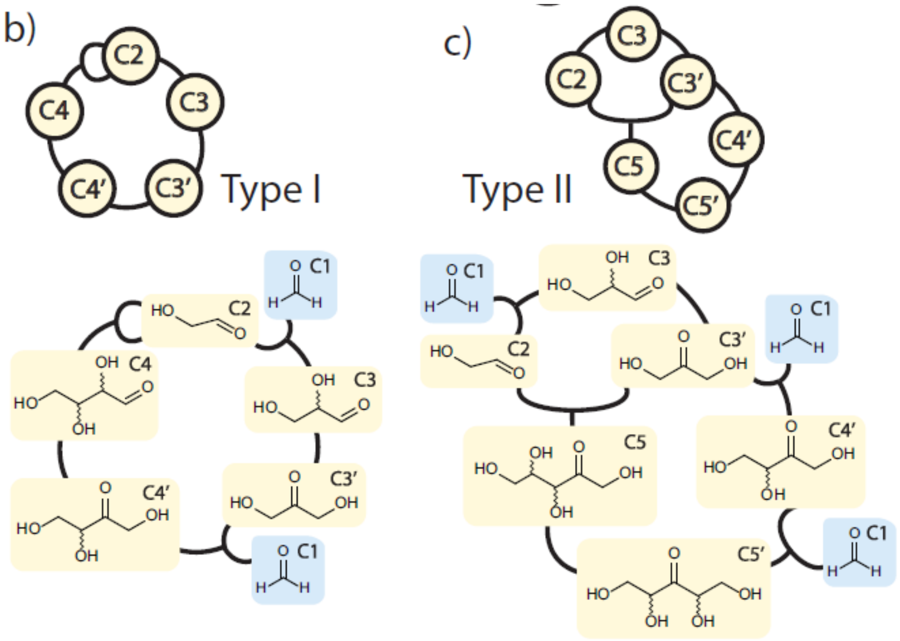
$$\mathrm{A} + \mathrm{E} \overset{\mathrm{IIa}}{\rightleftharpoons} \mathrm{EA} \overset{\mathrm{IIb}}{\rightleftharpoons} \mathrm{E} + \mathrm{B},$$
$$\mathrm{A} + \mathrm{B} \overset{\mathrm{IIIa}}{\rightleftharpoons} \mathrm{AB} \overset{\mathrm{IIIb}}{\rightleftharpoons} 2\mathrm{B} \quad .$$

Stoichiometry of catalysis and autocatalysis



Universal autocatalytic cores



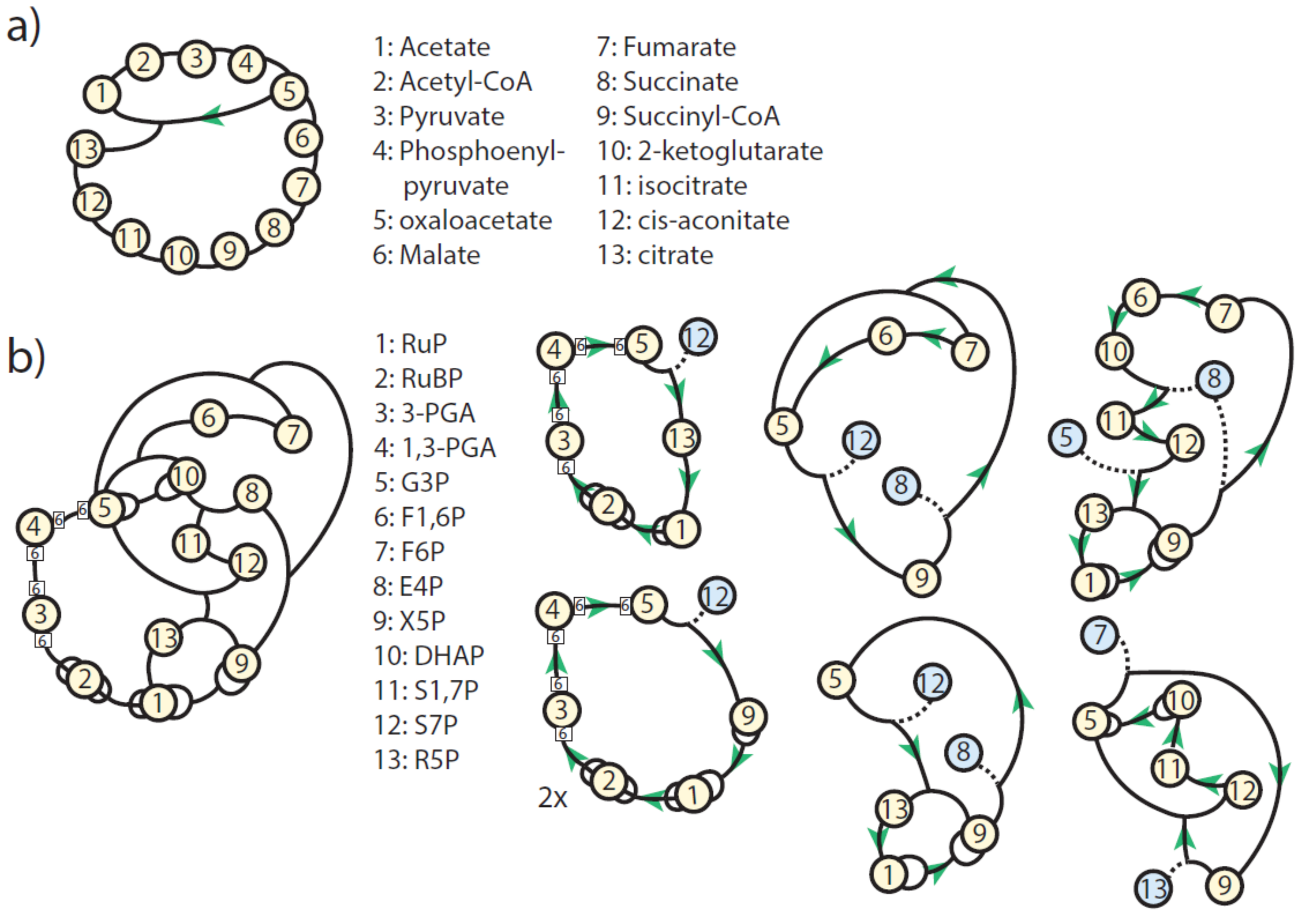


Blokhuis, A., Lacoste, D., & Nghe, P. (2020). Universal motifs and the diversity of autocatalytic systems. *Proceedings of the National Academy of Sciences*, *117*(41), 25230-25236.

Metabolic cycles as putative autocatalytic cycles

Reverse Krebbs cycle

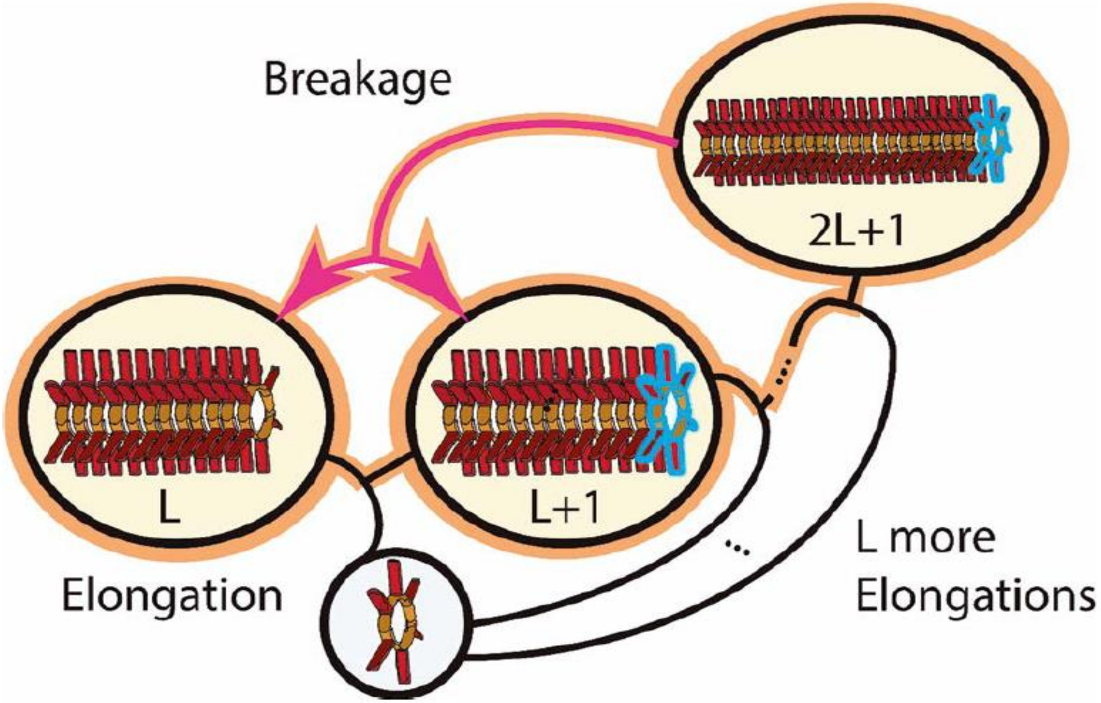
Calvin cycle



1: Acetate
2: Acetyl-CoA
3: Pyruvate
4: Phosphoenylpyruvate
5: oxaloacetate
6: Malate
7: Fumarate
8: Succinate
9: Succinyl-CoA
10: 2-ketoglutarate
11: isocitrate
12: cis-aconitate
13: citrate

1: RuP
2: RuBP
3: 3-PGA
4: 1,3-PGA
5: G3P
6: F1,6P
7: F6P
8: E4P
9: X5P
10: DHAP
11: S1,7P
12: S7P
13: R5P

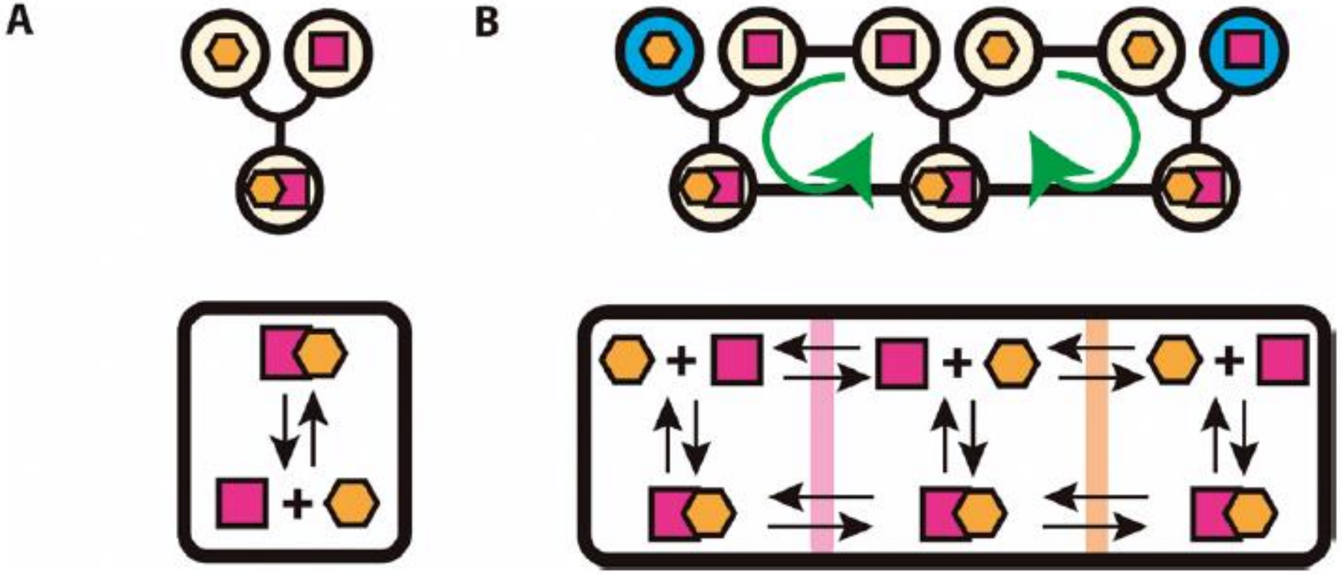
Self-reproduction:
forgetting the thermodynamic condition



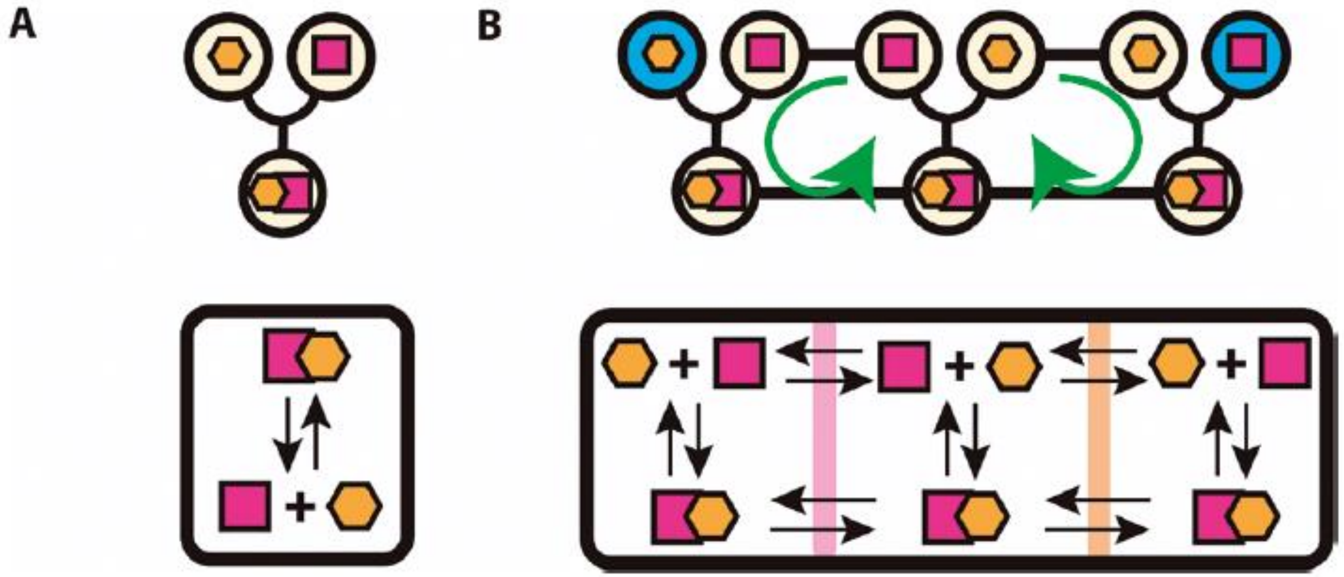
Carnall, J. M., Waudby, C. A., Belenguer, A. M., Stuart, M. C., Peyralans, J. J. P., & Otto, S. (2010). Mechanosensitive self-replication driven by self-organization. *Science*, *327*(5972), 1502-1506.

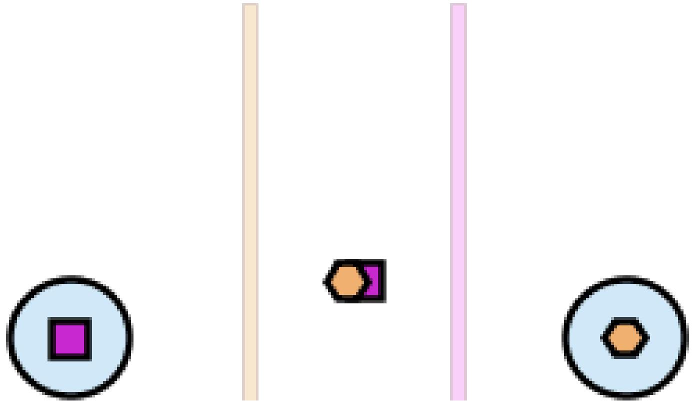
Self-reproduction:
forgetting the stoichiometric condition

Self-reproduction:
Compartments autocatalysis



Self-reproduction:
Compartments autocatalysis





HOW TO COUPLE THE GROWTH OF A COMPARTMENT WITH AN AUTOCATALYTIC CHEMICAL REACTION

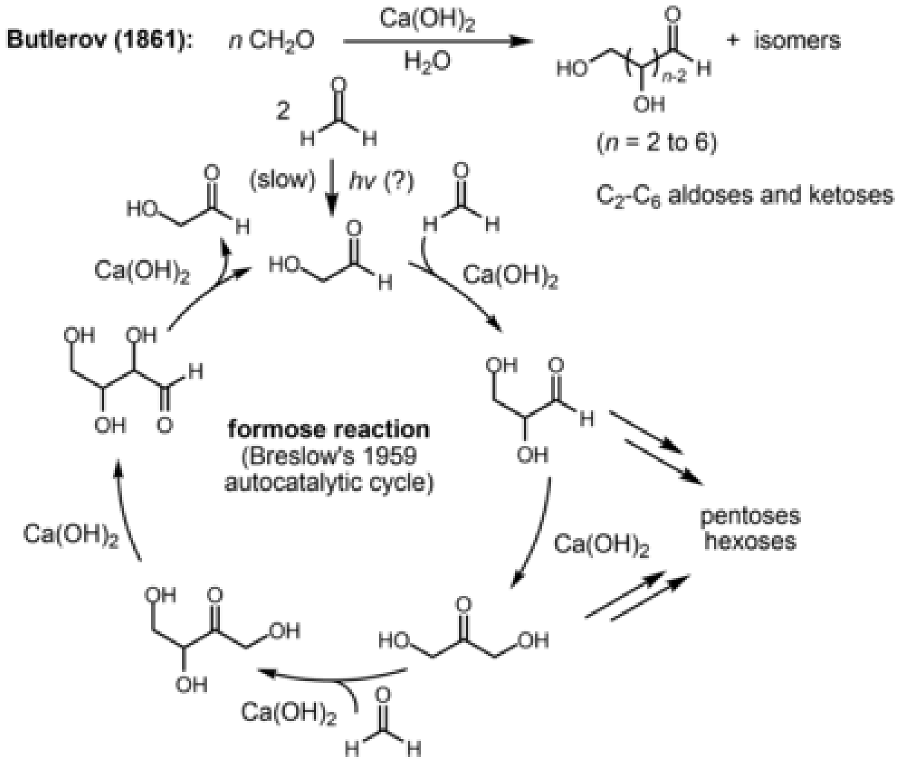
Small-molecule autocatalysis drives compartment growth, competition and reproduction

H Lu†, A Blokhuis†, R Turk-MacLeod, J Karuppusamy, A Franconi, G Woronoff, C Jeancolas, A Abrishamkar, E Loire, F Ferrage, P Pelupessy, L Jullien, E Szathmary*, P Nghe* and A. D. Griffiths*

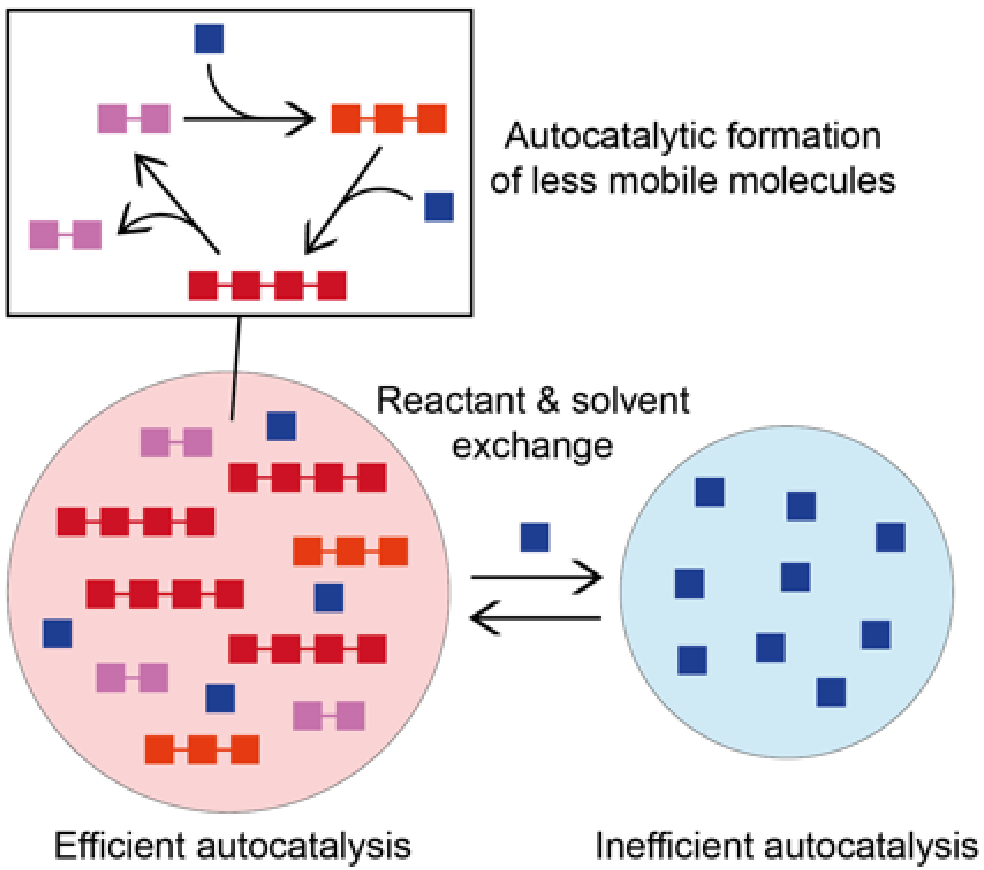
Nature Chemistry (accepted)

What are the simplest mechanisms by which the compartment growth can be coupled to the efficiency of the autocatalytic reaction it contains?

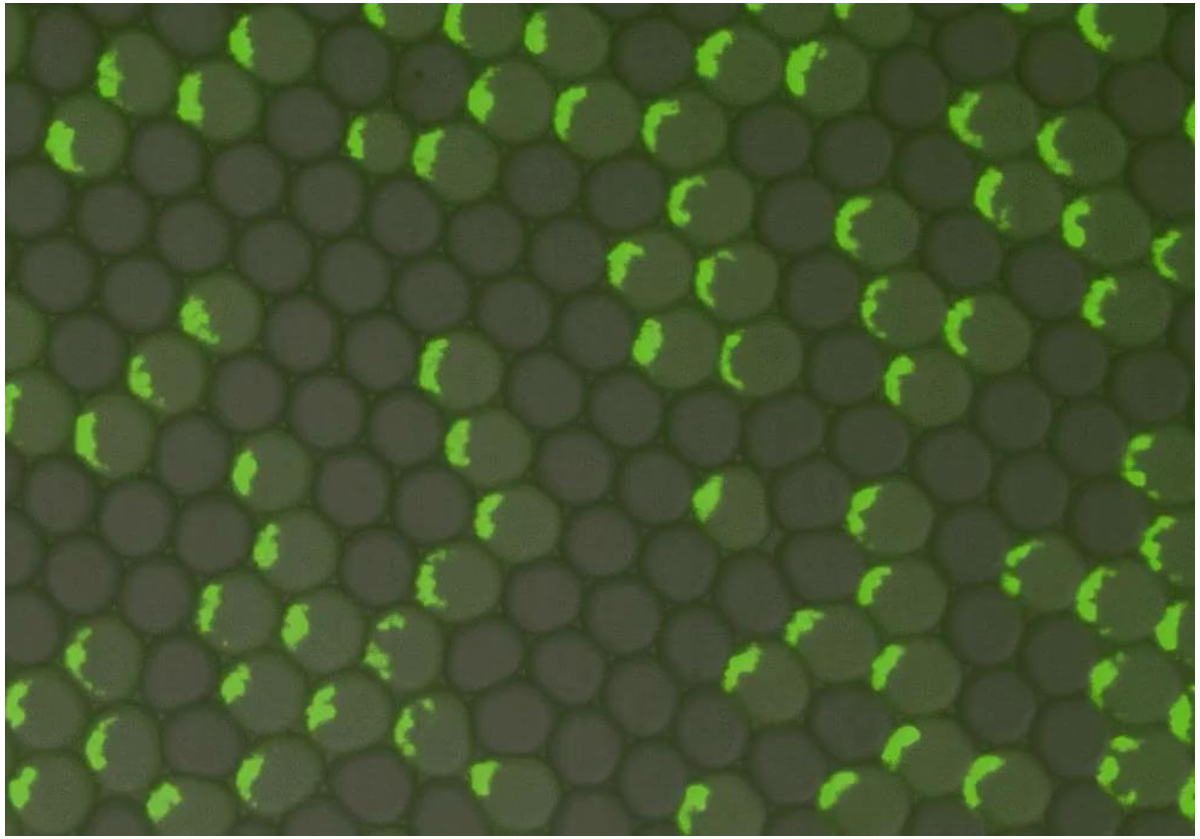
The formose reaction

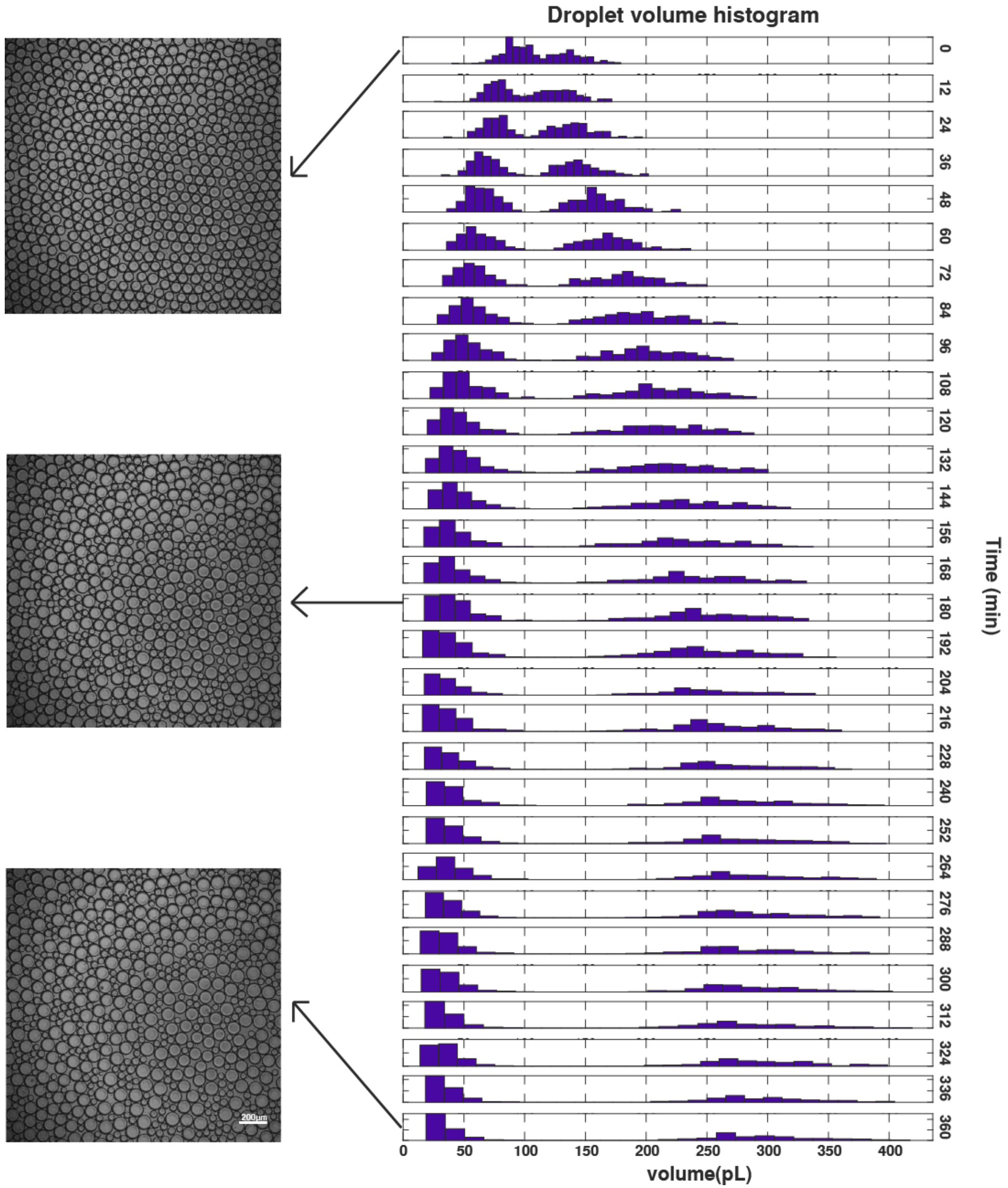


The compartmentalized formose reaction



Growth heterogeneity in 2D





6 hours total

THE RNA WORLD

The RNA world hypothesis

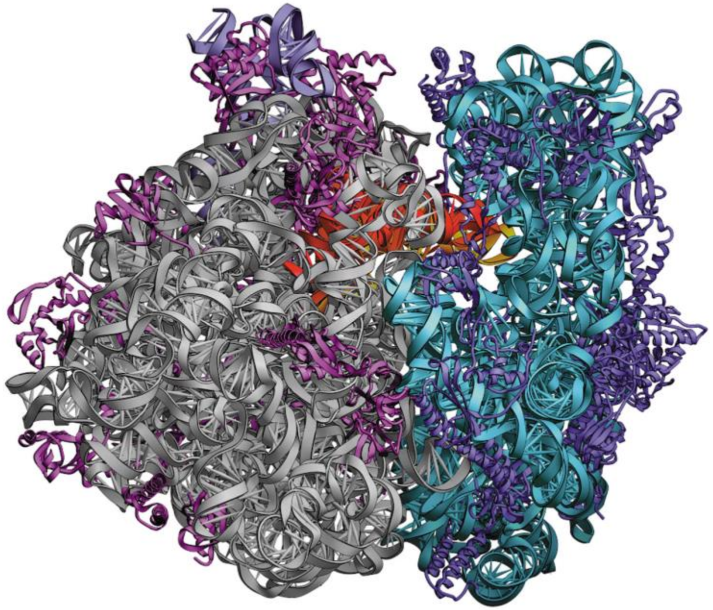
RNA :

- Supports genetic information
- Is able of catalysis

Could resolves the chicken and egg problem of DNA – protein polymerase (Woese 1968).

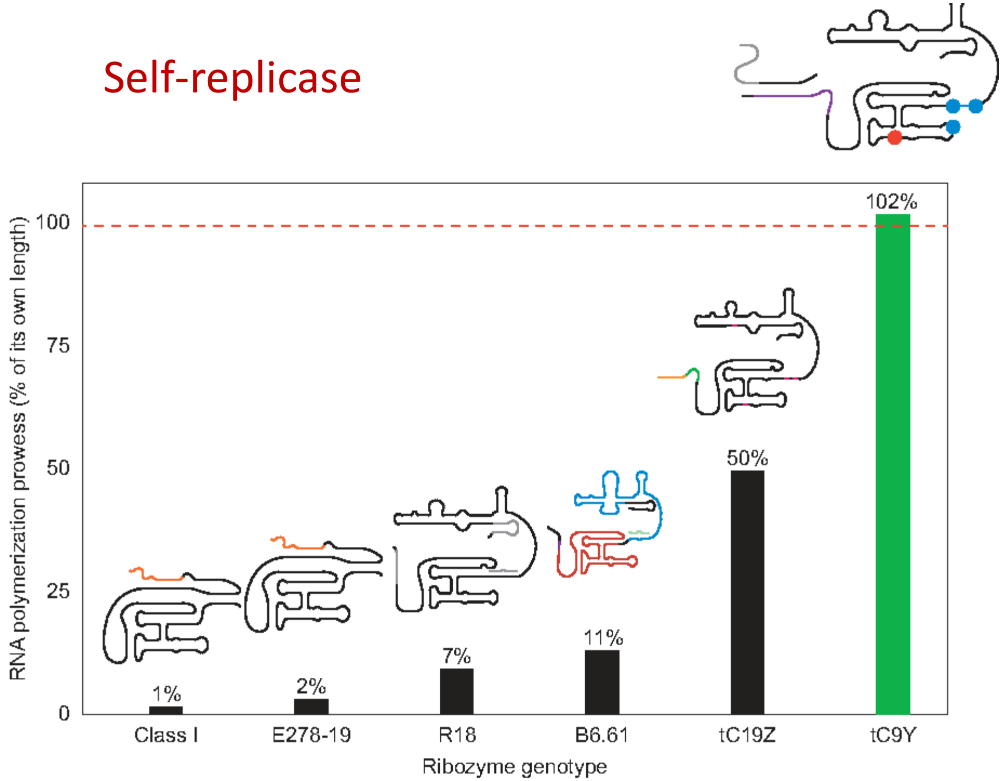
An RNA which cleaves RNA

The ribosome is a ribozyme!

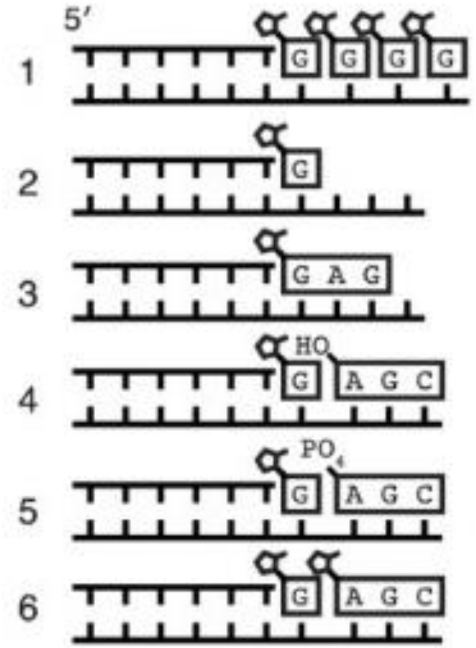


The quest for template-based replication

Self-replicase



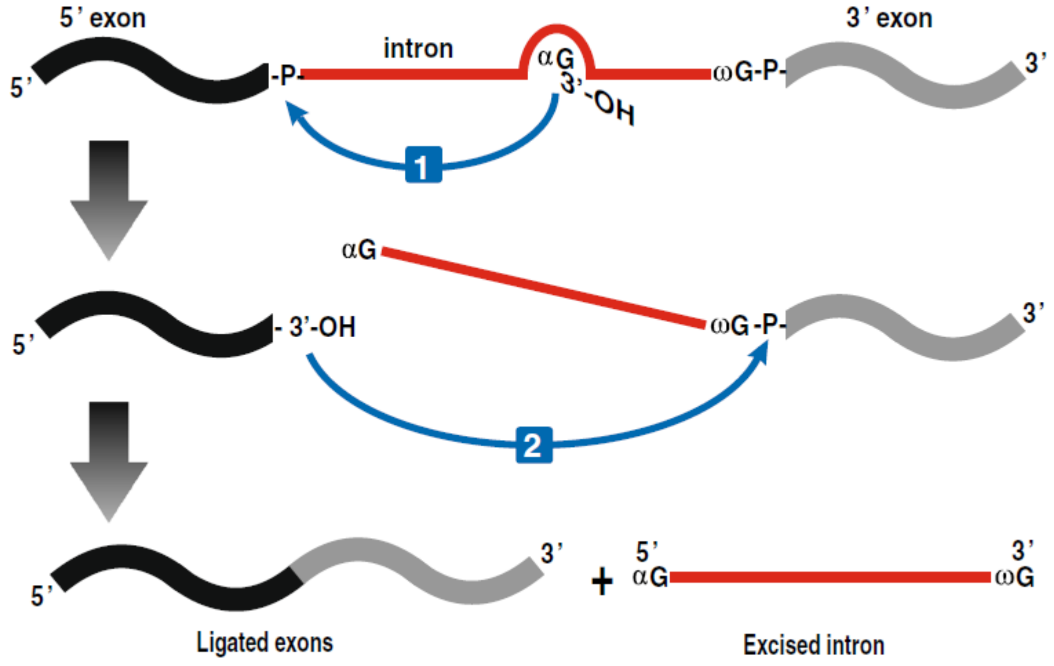
Non-enzymatic template-based replication



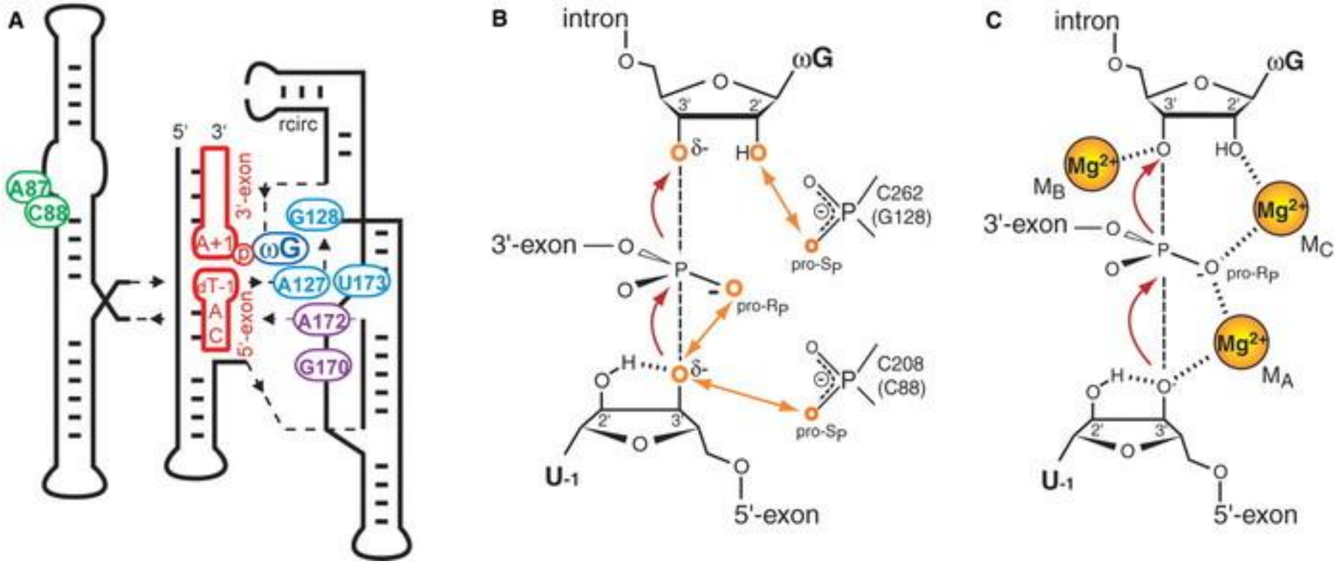
Attwater, J., Wochner, A., & Holliger, P. (2013). In-ice evolution of RNA polymerase ribozyme activity. *Nature chemistry*, *5*(12), 1011.

Prywes, N., Blain, J. C., Del Frate, F., & Szostak, J. W. (2016). Nonenzymatic copying of RNA templates containing all four letters is catalyzed by activated oligonucleotides. *Elife*, *5*, e17756.

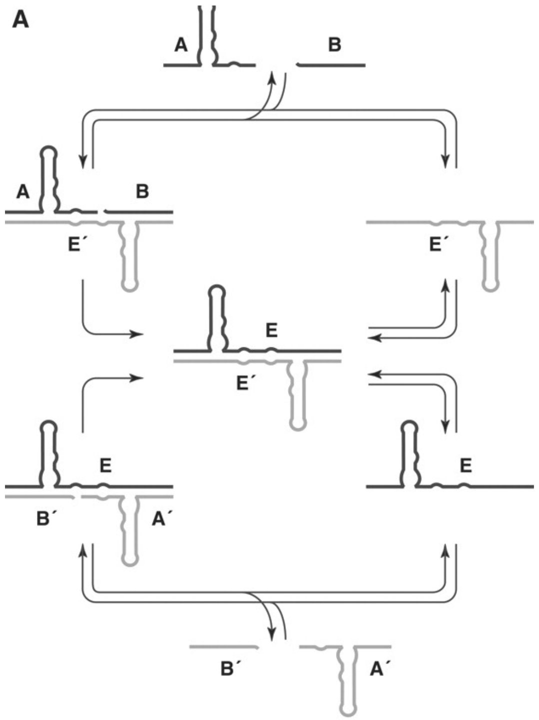
Group I introns



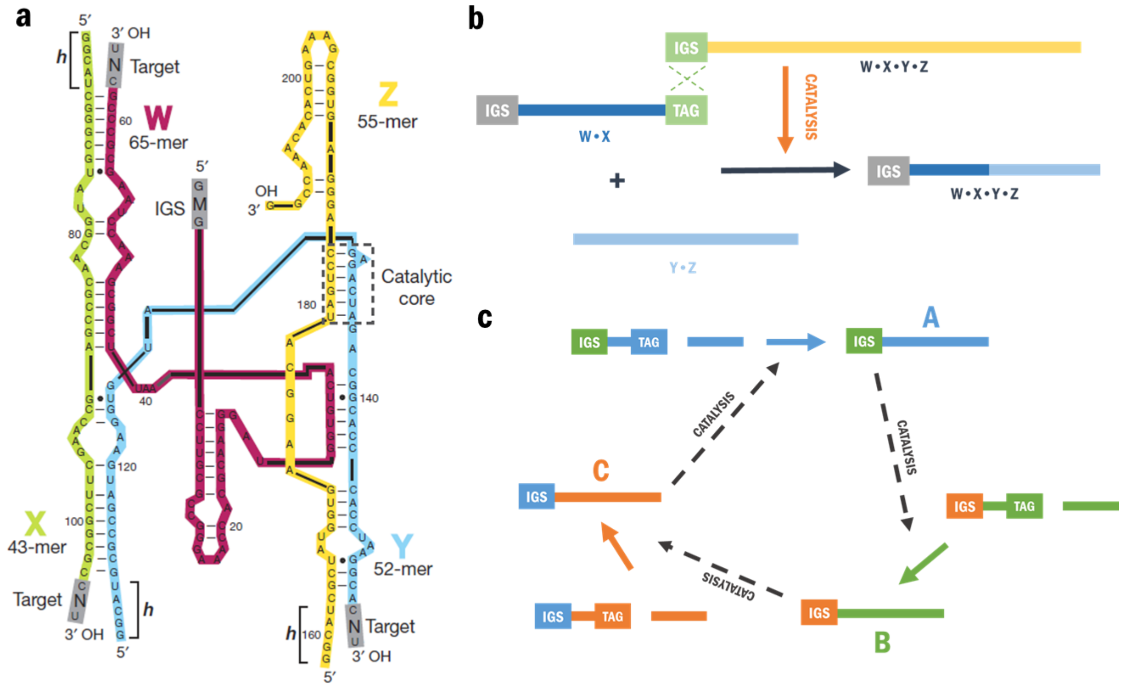
Kruger, K., Grabowski, P. J., Zaug, A. J., Sands, J., Gottschling, D. E., & Cech, T. R. (1982).
Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. *cell*, *31*(1), 147-157.



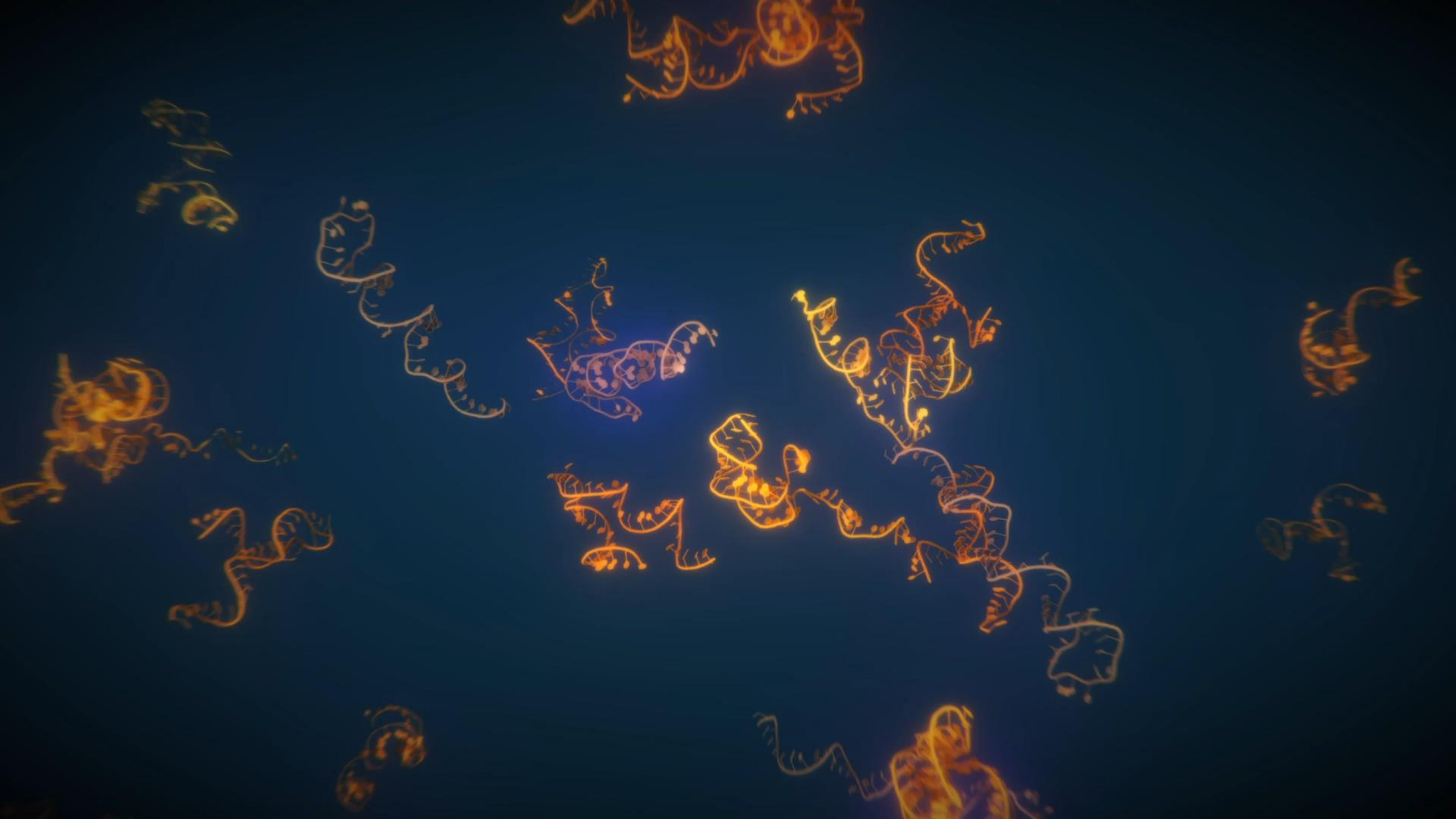
Collectively replicating RNAs

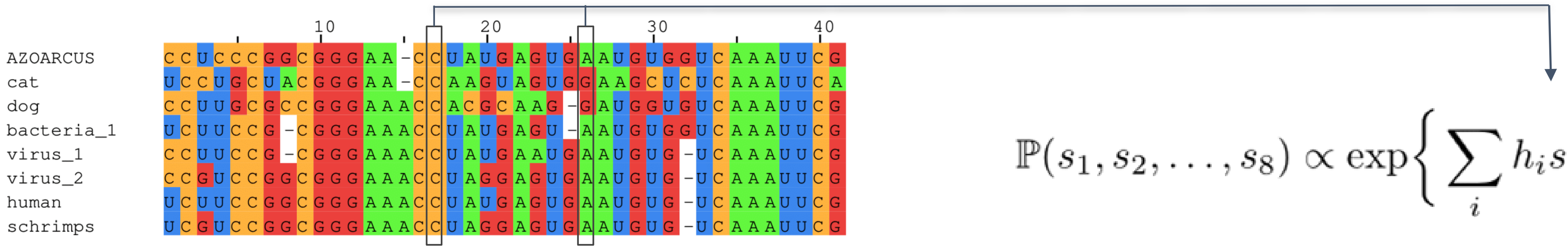


Lincoln, T. A., & Joyce, G. F.
Self-sustained replication of an RNA enzyme
Science 2009



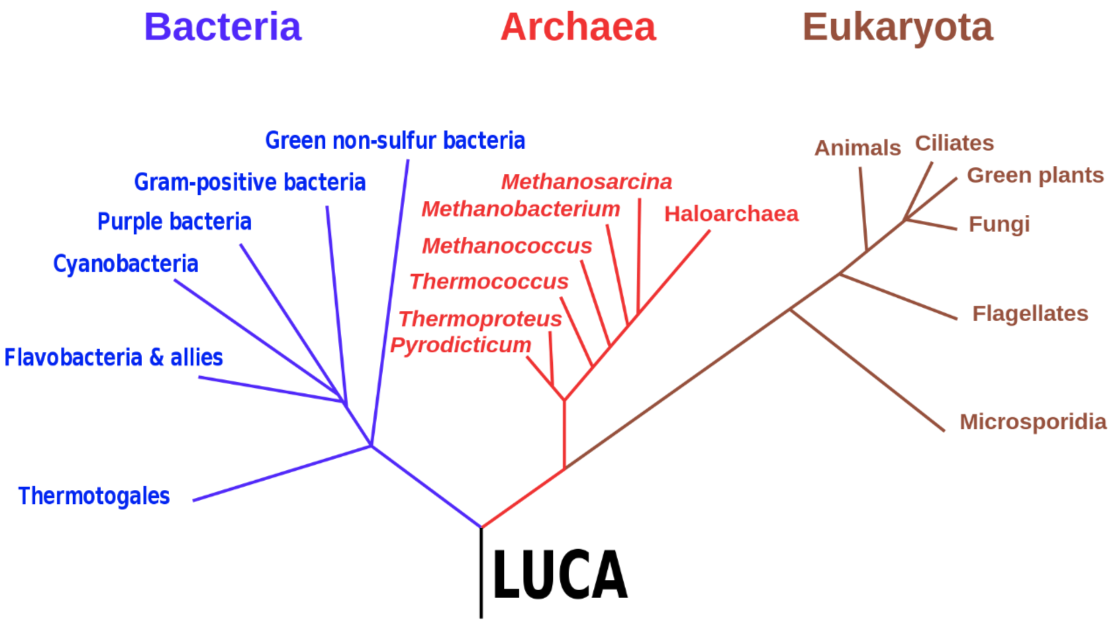
Vaidya, N., Manapat, M. L., Chen, I. A., Xulvi-Brunet, R., Hayden, E. J., & Lehman, N.
Spontaneous network formation among cooperative RNA replicators.
Nature 2012

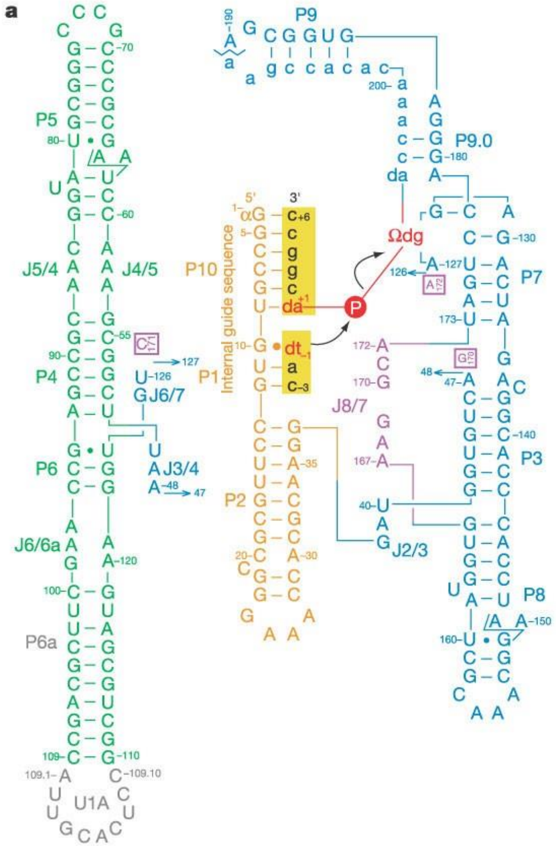




```
                      10        20        30        40
AZOARCUS    C C U C C C G G C G G G A A - C C U A U G A G U G A A U G U G G U C A A A U U C G
cat         U C C U G C U A C G G G A A - C C A A G U A G U G G A A G C U C U C A A A U U C A
dog         C C U U G C G C C G G G A A A C C A C G C A A G - G A U G G U G U C A A A U U C G
bacteria_1  U C U U C C G - C G G G A A A C C U A U G A G U - A A U G U G G U C A A A U U C G
virus_1     C C U U C C G - C G G G A A A C C U A U G A A U G A A U G U G - U C A A A U U C G
virus_2     C C G U C C G G C G G G A A A C C U A G G A G U G A A U G U G - U C A A A U U C G
human       U C U U C C G G C G G G A A A C C U A U G A G U G A A U G U G - U C A A A U U C G
schrimps    U C G U C C G G C G G G A A A C C U A G G A G U G A A U G U G - U C A A A U U C G
```

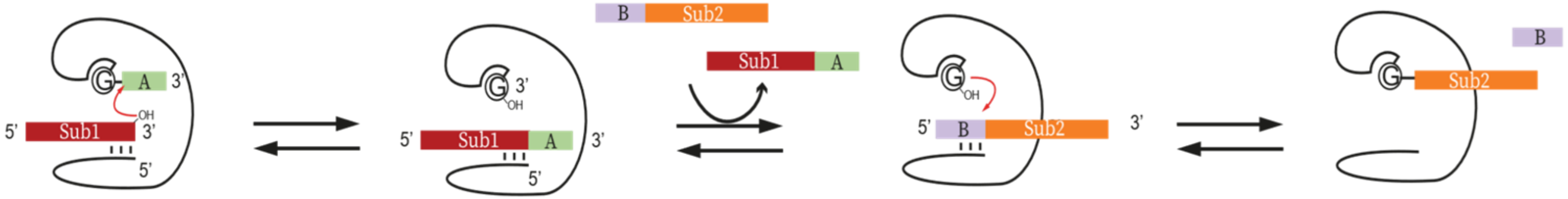
$$\mathbb{P}(s_1, s_2, \dots, s_8) \propto \exp\left\{ \sum_i h_i s_i + \sum_{i<j} J_{ij}(s_i, s_j) \right\}$$





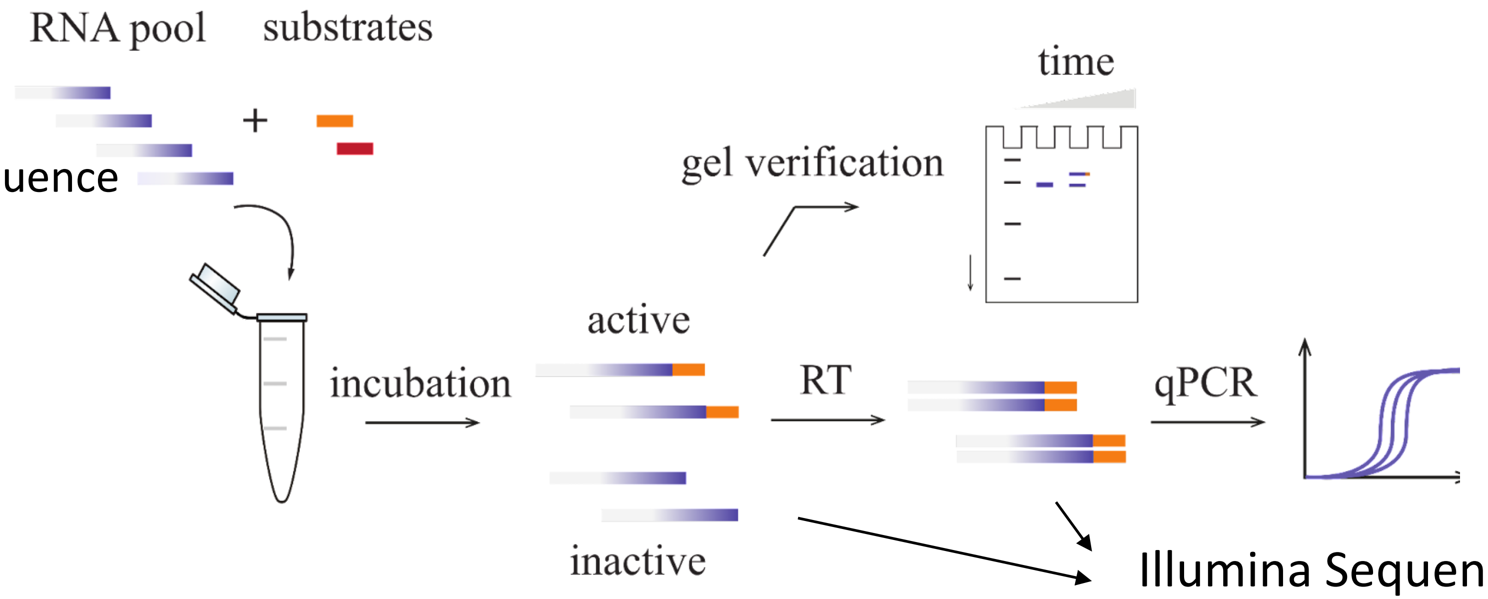
Experimental assay for catalytic activity

Based on self-splicing in two steps



Selection process

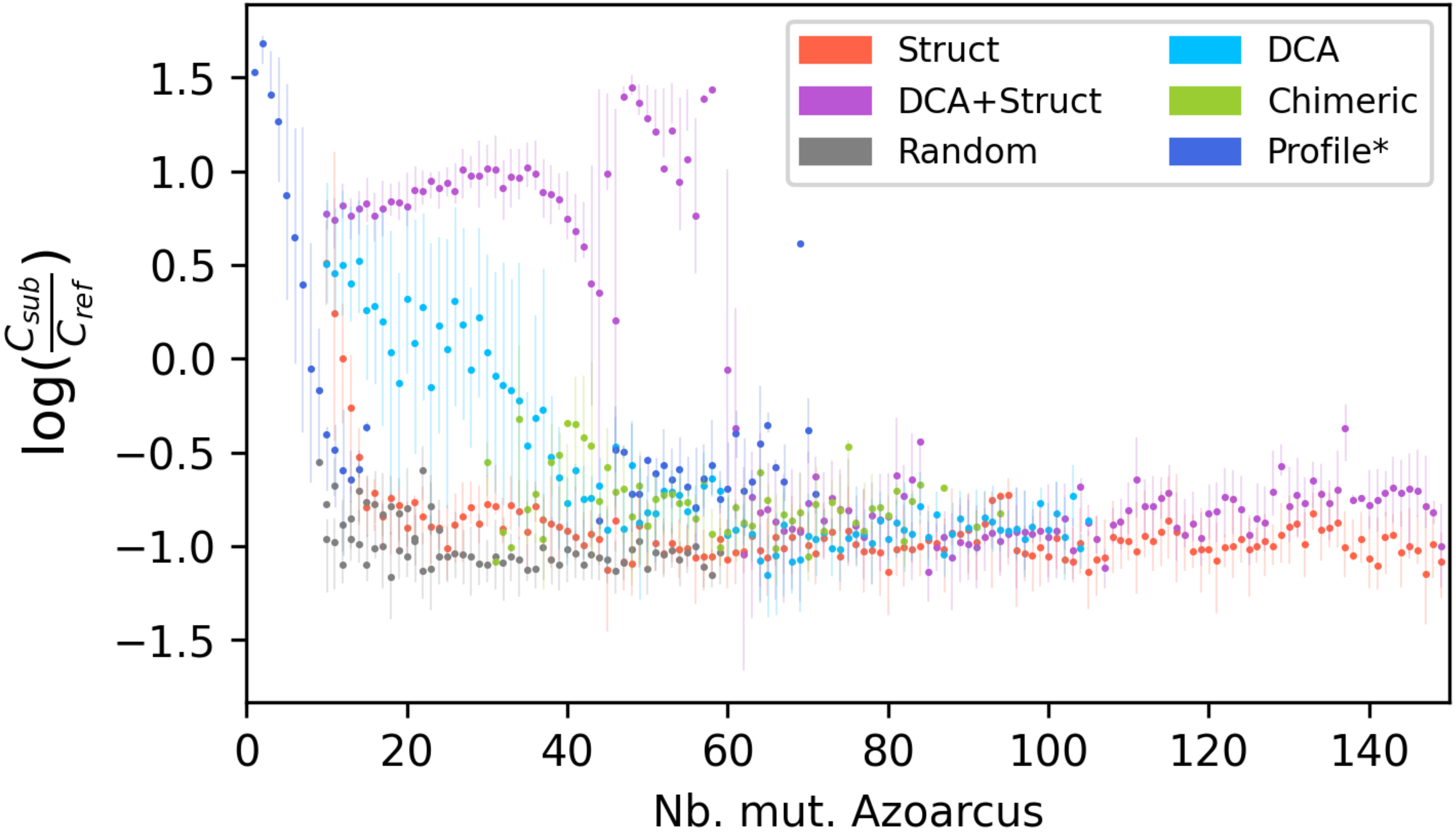
30 cents / sequence



Illumina Sequencing

Score = Log(post assay / pre assay)

Results starting from Azoarcus



COMPARTMENTALIZATION OF REPLICATORS

Matsumura S, Kun Á, Ryckelynck M, Coldren F, Szilágyi A, Jossinet F, Rick C, Nghe P, Szathmáry E, Griffiths AD
Transient compartmentalization maintains catalytically active RNA replicators and prevents functional collapse due to parasites
Science 354 (2016)

The fundamental problem of parasites

Spiegelman experiment (PNAS 1965):

infectious RNA replicated by the Q-beta-replicase

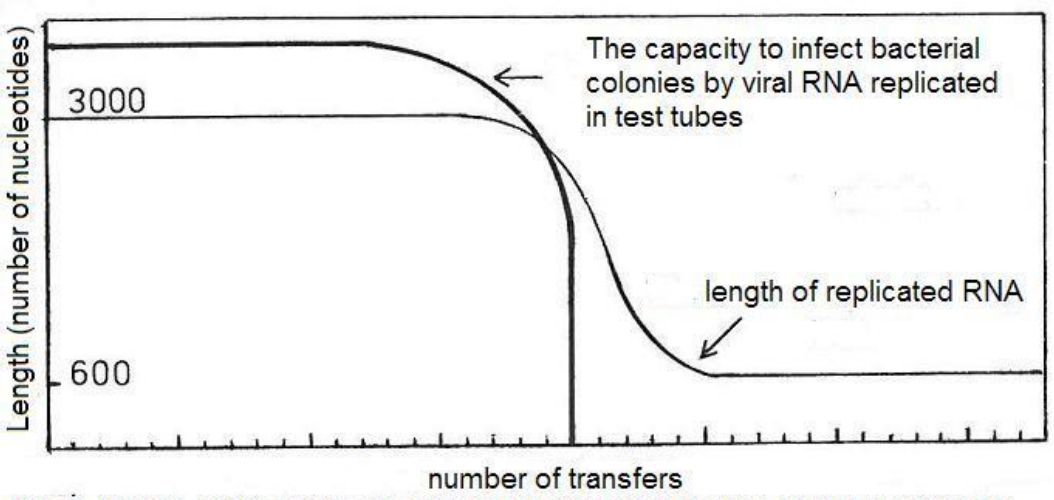
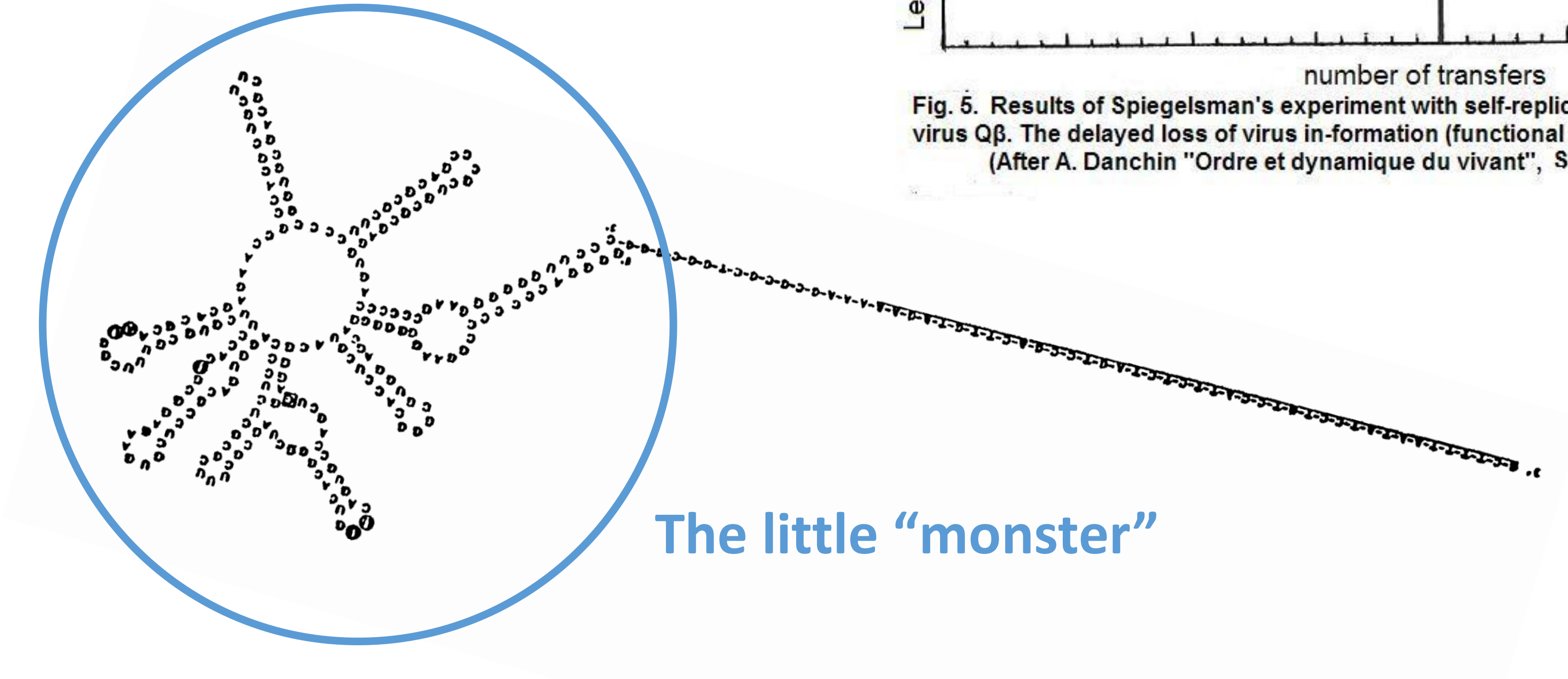


Fig. 5. Results of Spiegelsman's experiment with self-replication of RNA contained in virus Qβ. The delayed loss of virus in-formation (functional structure) is very well visible. (After A. Danchin "Ordre et dynamique du vivant", Seuil, Paris, 1978)



The little "monster"

The error threshold and Eigen’s paradox (1971)

$$L < \ln(s)/(1-q)$$

Genome length

Relative fitness or “selective advantage”
how many replicators appear by their faster replication

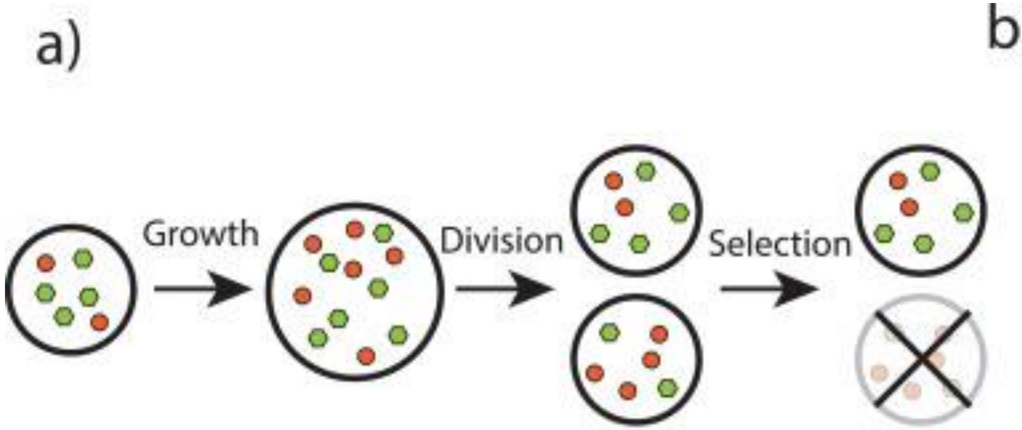
Error rate
How many replicators disappear by spontaneous mutations

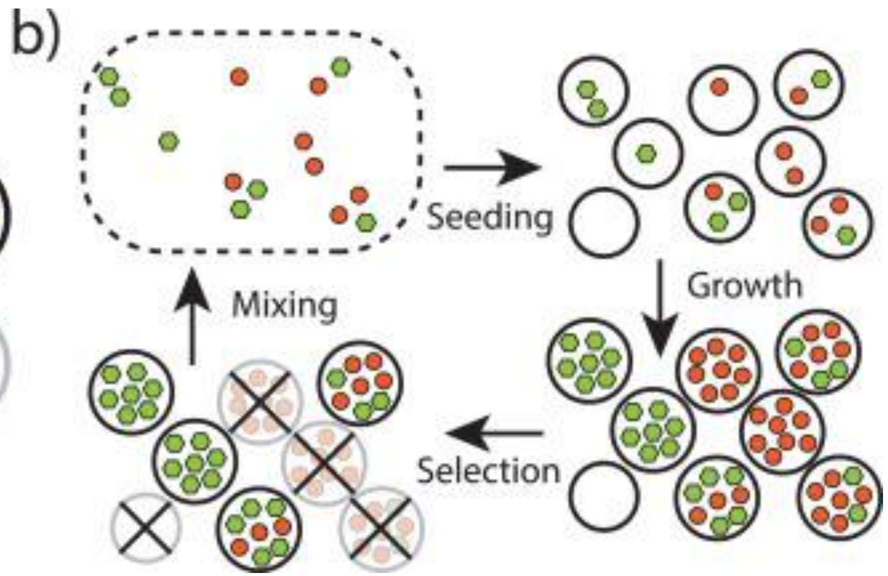
Complexity threshold:
A highly elaborate polymerase is necessary, otherwise it collapses into parasites.

Inferred length of a minimal polymerase: ~200 nt
Maximum spontaneous condensation of RNA: 55 nt
Size of the sequence space: 10^{120}

Jeancolas, C., Malaterre, C., & Nghe, P.
Thresholds in origin of life scenarios. *iScience, 2020*

Compartmentalized life cycles

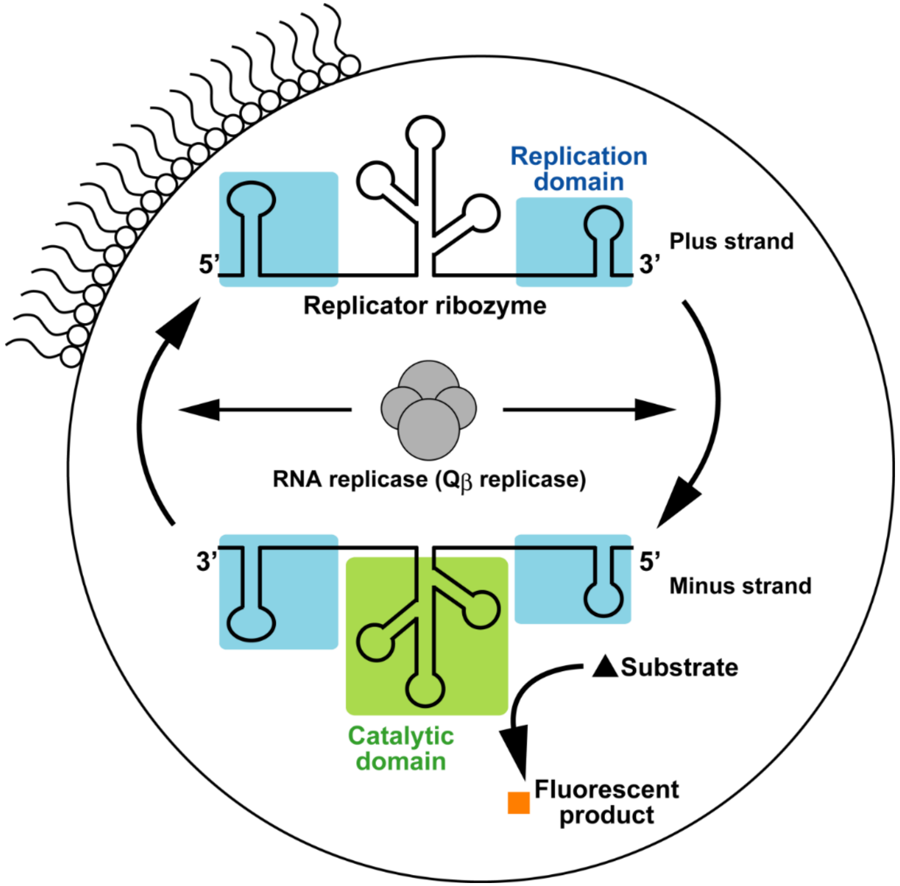


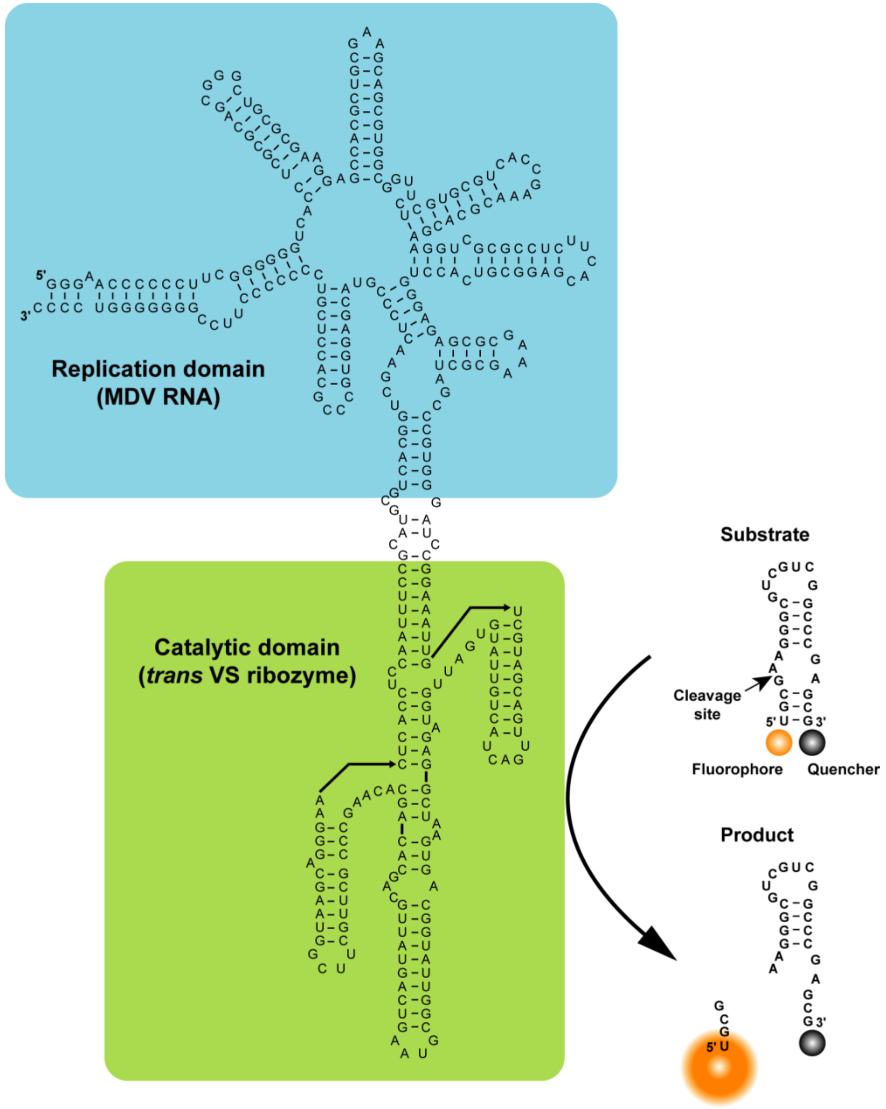


Matsumura, Shigeyoshi, et al. "Transient compartmentalization of RNA replicators prevents extinction due to parasites." *Science* 354.6317 (2016)

Blokhuis, A., Lacoste, D., Nghe, P., & Peliti, L. (2018). Selection dynamics in transient compartmentalization. *Physical Review Letters*, *120*(15)

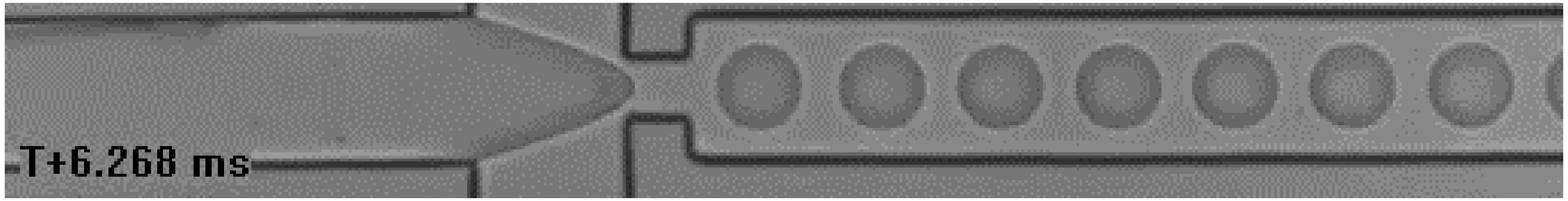
A model protocell

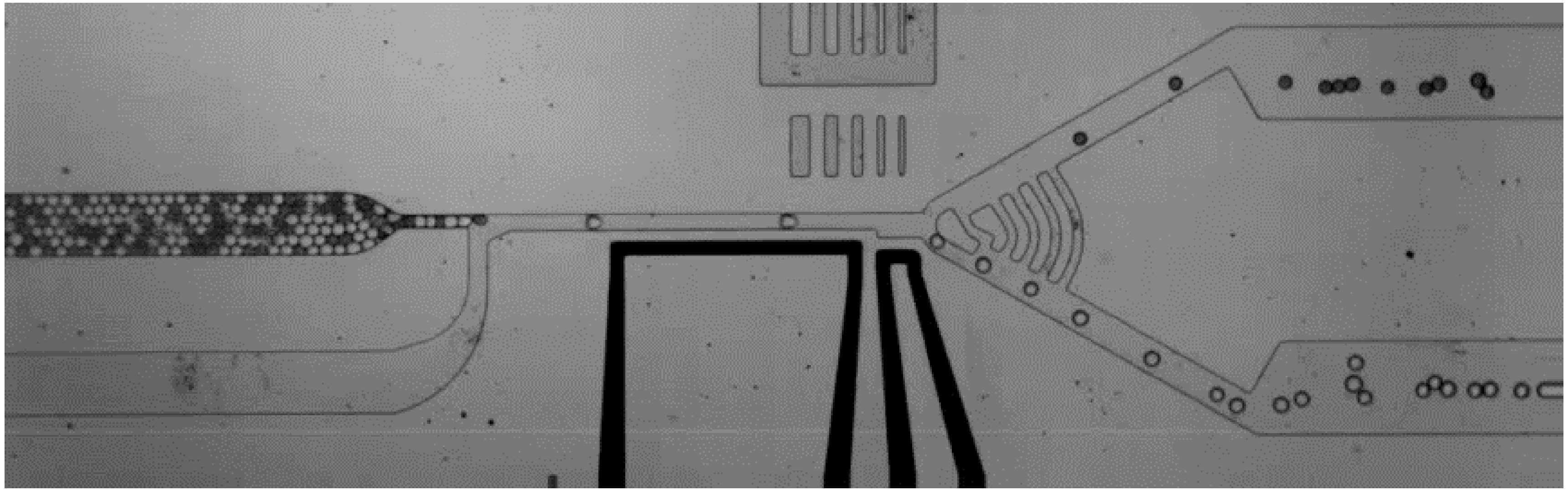




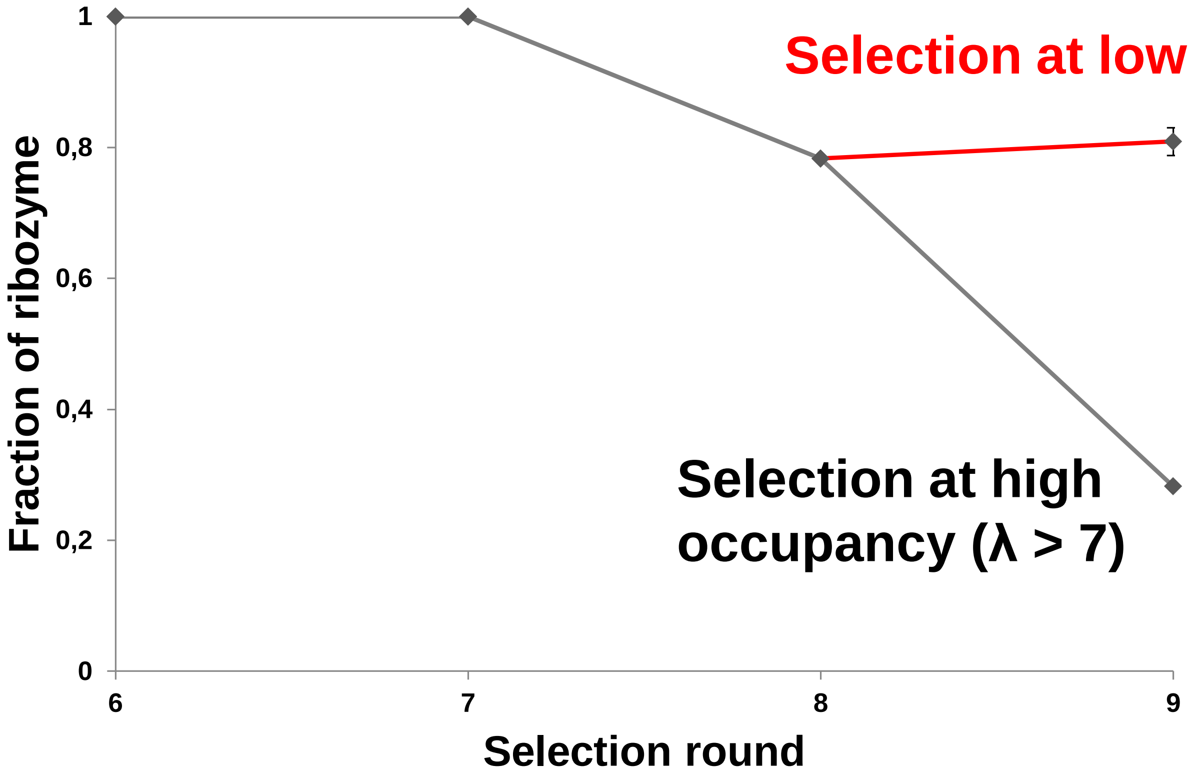
“metabolic” activity

Compartments life cycle with microfluidics



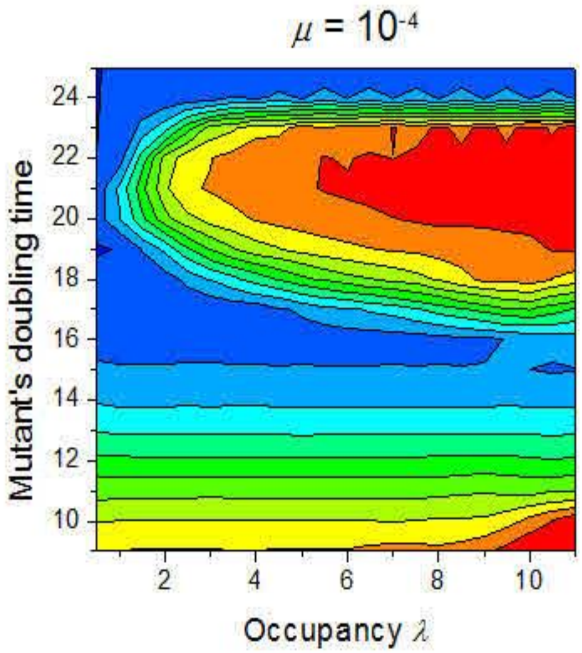


Stabilization of replicators and soft parasites



Selection at low

Selection at high occupancy (λ > 7)

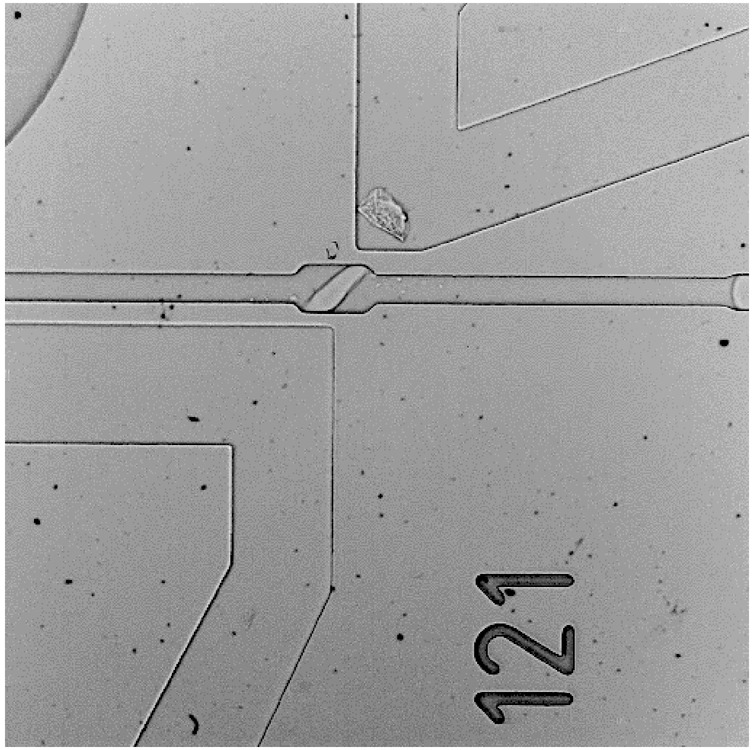


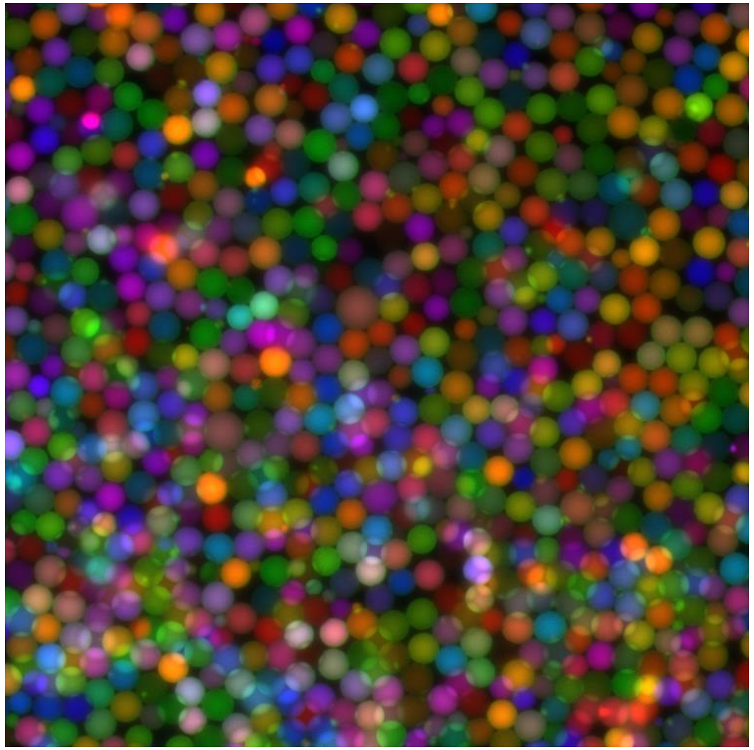
Soft parasites

Aggressive parasites

Creating a landscape of autocatalytic networks with droplet microfluidics

Library of fragment mixtures





Random fusions

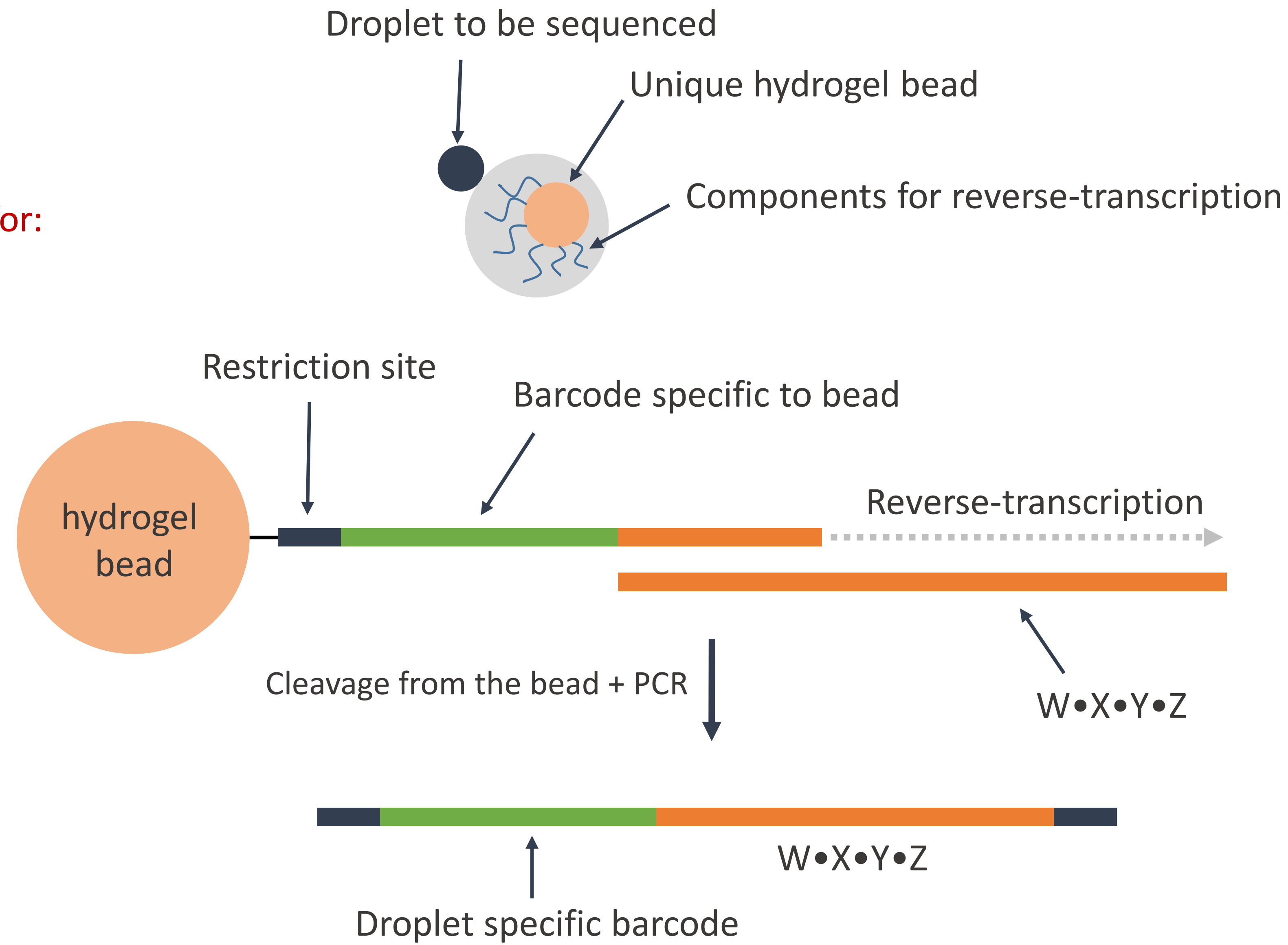
Combinatorial networks
with diversity and redundancy

Ameta, S., Arsène, S., Foulon, S., Saudemont, B., Clifton, B. E., Griffiths, A. D., & Nghe, P. (2021). Darwinian properties and their trade-offs in autocatalytic RNA reaction networks. *Nature Communications*, *12*(1), 842.

Analyzing chemical RNA reactions, at the single droplet level

DNA bar-codes coding for:

- Initial condition
- Droplet identity



Droplet to be sequenced

Unique hydrogel bead

Components for reverse-transcription

Restriction site

Barcode specific to bead

hydrogel bead

Reverse-transcription

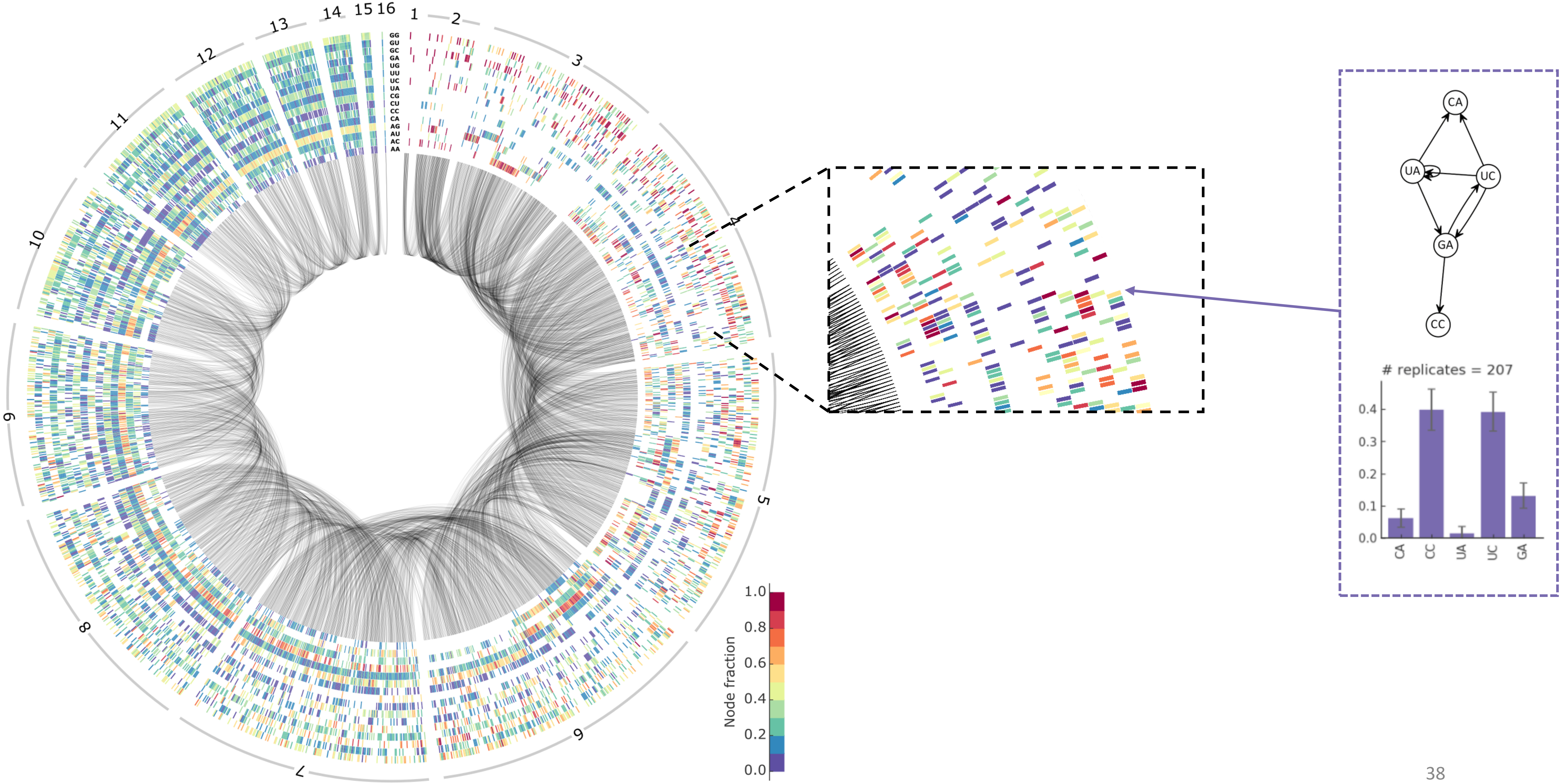
W•X•Y•Z

Cleavage from the bead + PCR

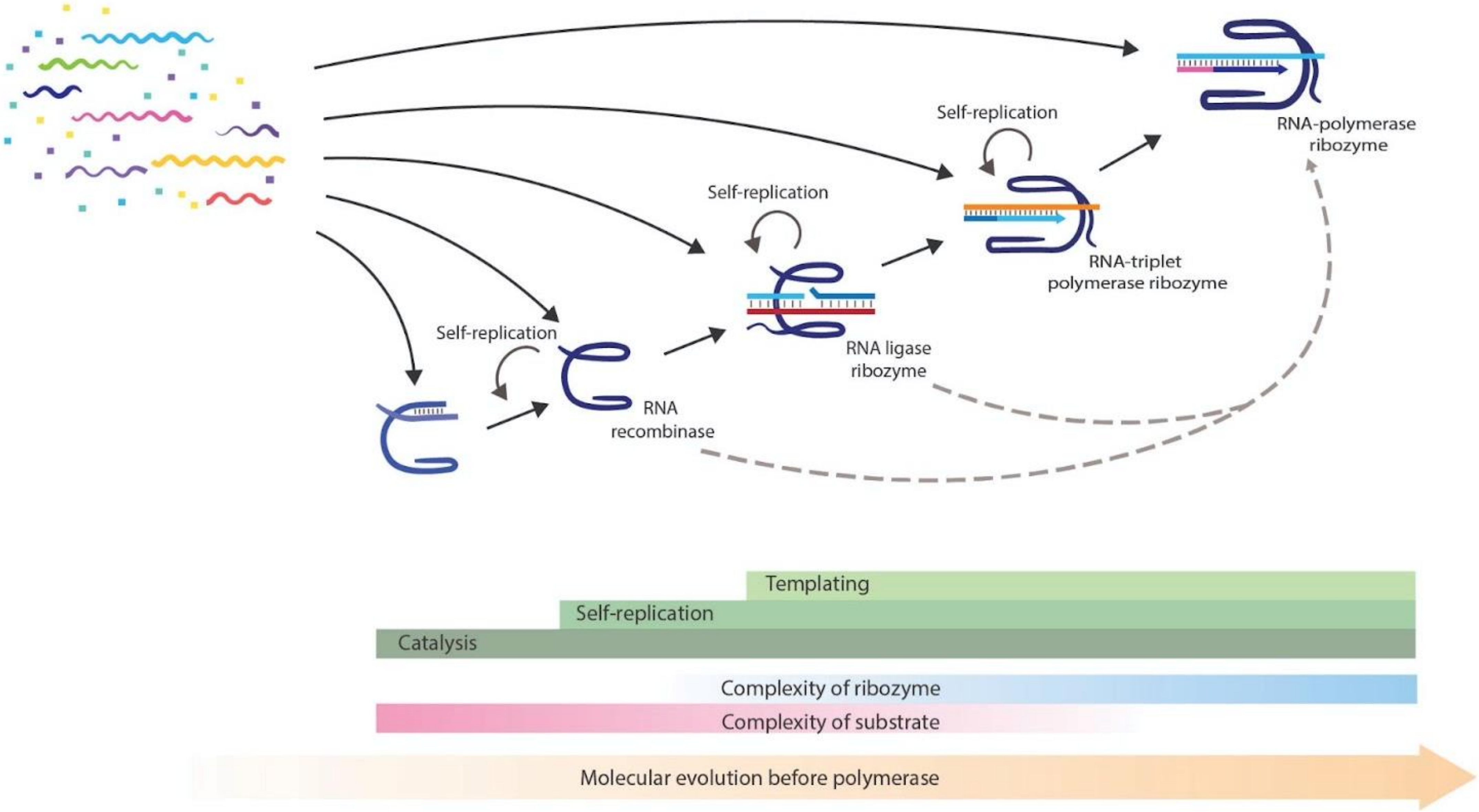
W•X•Y•Z

Droplet specific barcode

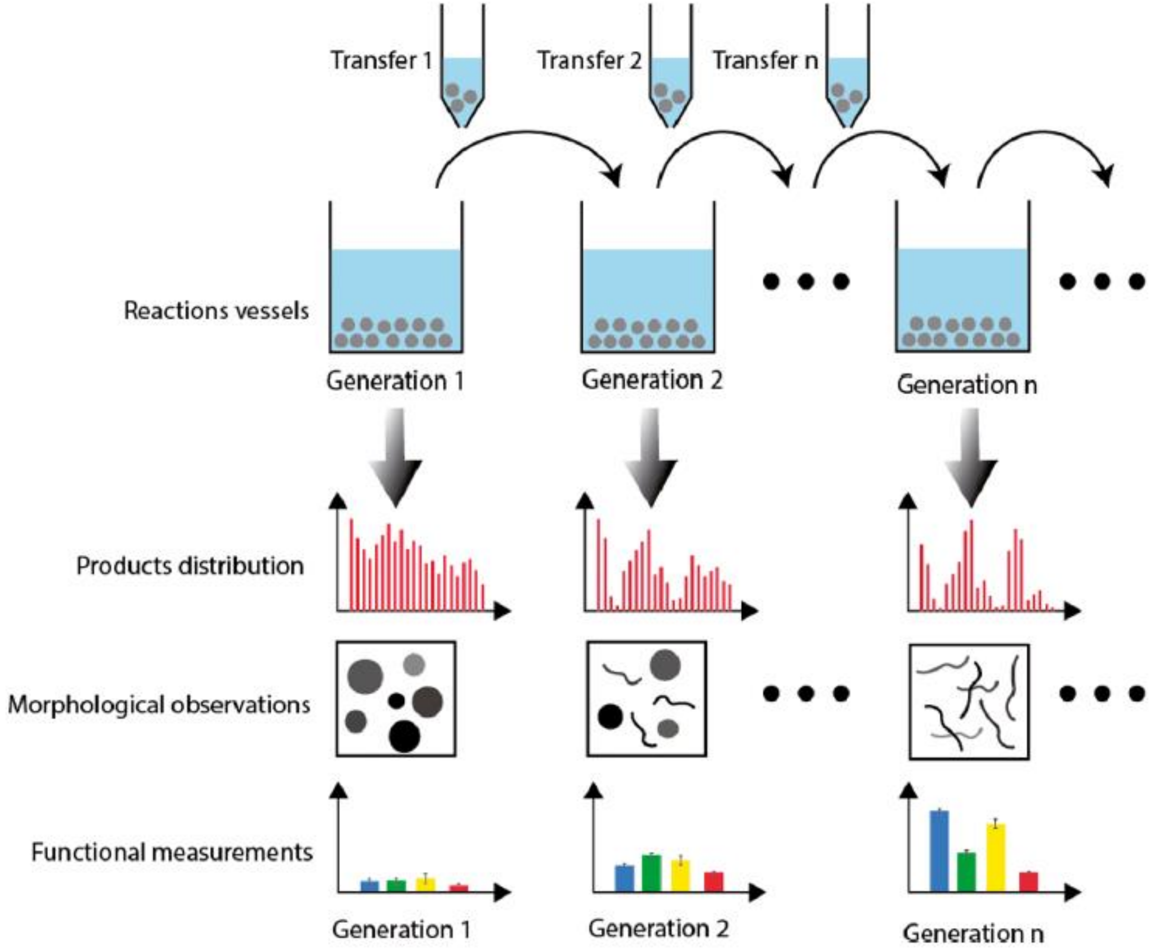
Compositional landscape of more than 1,800 *Azoarcus* RNA networks

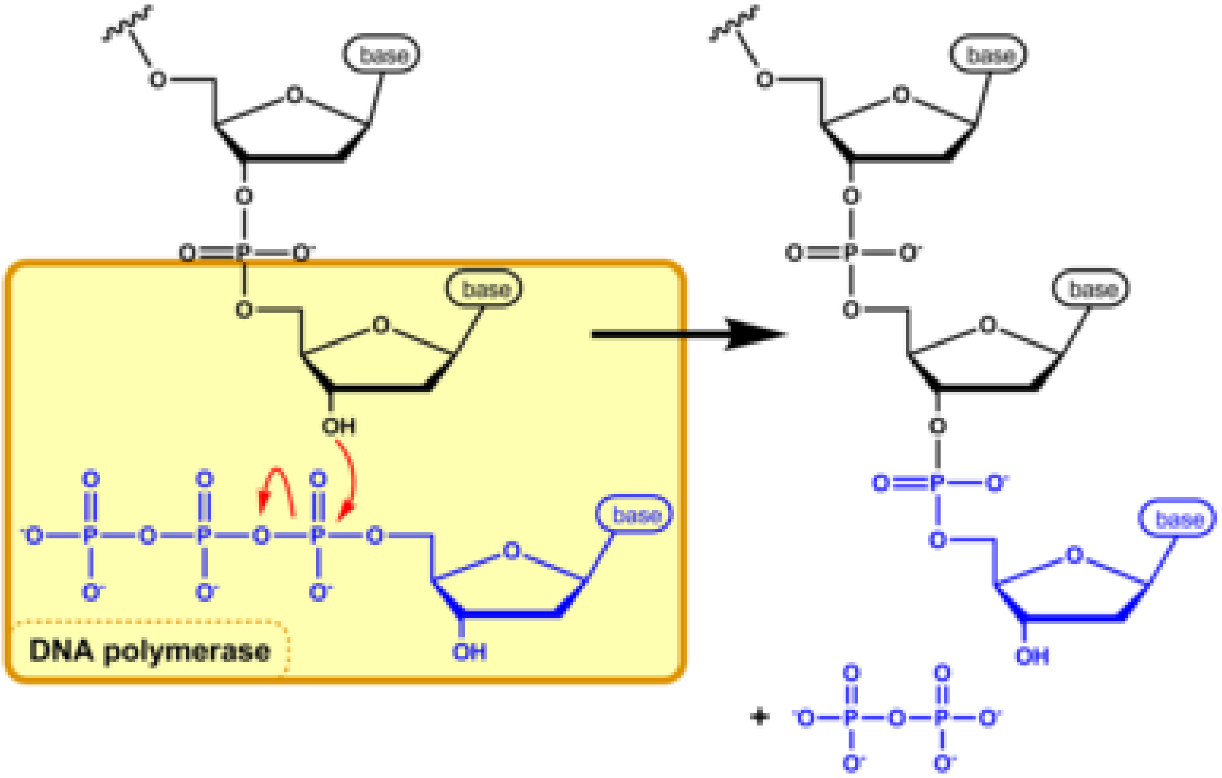


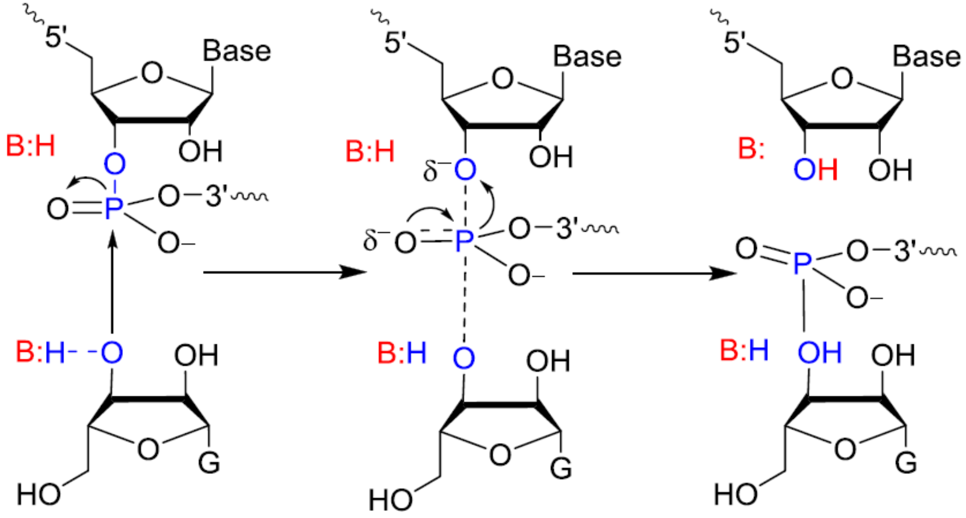
PERSPECTIVES


Self-replication
RNA-polymerase ribozyme
Self-replication
RNA-triplet polymerase ribozyme
Self-replication
RNA ligase ribozyme
Self-replication
RNA recombinase
Templating
Self-replication
Catalysis
Complexity of ribozyme
Complexity of substrate
Molecular evolution before polymerase

Chemical evolution experiments




base
O
O=P—O⁻
OH
DNA polymerase
⁻O—P—O—P—O—P—O
O⁻
+
⁻O—P—O—P—O⁻


5'
Base
O
B:H
OH
O=P
O—3'
O−
B:H- -O
G
HO
δ−
B:

Step 1

The 2′ hydroxyl of the branch point adenosine attacks the phosphate at the 5′ splice junction, releasing exon 1 with a free 3′ hydroxyl group.

transesterification

Step 2

The 3′ end of exon 1, which was released in step 1, attacks the phosphate at the 3′ splice junction, connecting exons 1 and 2 while releasing the intron.

transesterification

Products

The products consist of exons 1 and 2, which are now connected in a continuous RNA strand, and the intron byproduct, which exists as a looped lariat structure.