NORCIA,
The search for
the minimal cell

QUESTIONI SULLA ORIGINE DELLA VITA, DAL FILM

1. PASTEUR
2. IL MONDO RNA
3. L‘IMPOSSIBILITA‘ DI CREARE LA VITA CON IL BOTTOM-UP APPROACH

4.LA CONTRAPPOSIZIONE TRA MONDO RNA E APPROCCIO CELLULARE

TWO SCHOOLS OF THOUGHT

....AND TWO DIFFERENT EXPERIMENTAL APPROACHES

1. THE CELLULAR VIEW:

YOU NEED A BOUNDARY (SEMIPERMEABLE MEMBRANE)

IN ORDER TO ACHIEVE THE NECESSARY SPATIAL ORGANIZATION,

LOCAL CONCENTRATIONS, , PROTECTION AND NUTRIENT SELECTION

2. THE MOLECULAR REPLICATION VIEW:

ALL WHAT YOU NEED IS A MOLECULAR SPECIES

(E:G: A RNA QUASI-SPECIES) WHICH IS ABLE TO SELF-REPLICATE

AND MUTATE (EVOLVE) IN THE PROCESS

– – – – – – – – – – – – – – – – – – – -

THESE TWO VIEWS ARE OF COURSE NOT CONTRACDITORY,
ACUALLY THEY ARE COMPLEMENTARY TO EACH OTHER

THE RNA-WORLD

IN THE ORIGIN OF LIFE

Mg-TRANSFER-RNA

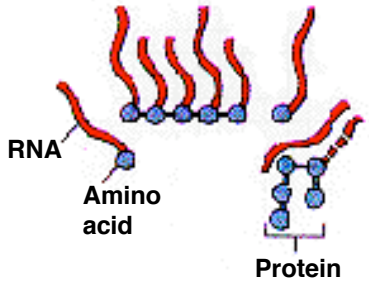
THE BASIS OF THE RNA-WORLD

RNA IS THE PRIME MOLECULE
CAME INTO EXISTENCE
BEFORE PROTEINS AND DNA
AND ORIGINATED THE WHOLE
THING

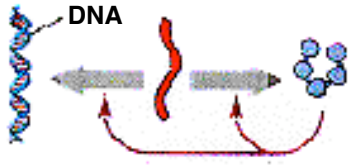
RNA → RIBOZYMES → PROTEIC ENZYMES → DNA

[A] RNA forms

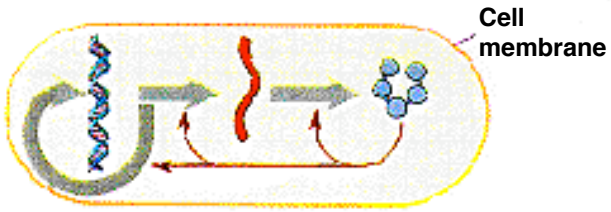
[B] Ribozymes catalyze RNA replication



[C] RNA catalyzes protein synthesis



[D] RNA encodes both DNA and protein



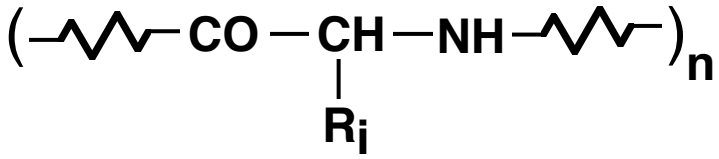
[E] Proteins catalyze cell activities

The Origins of life in a Proposed RNA World

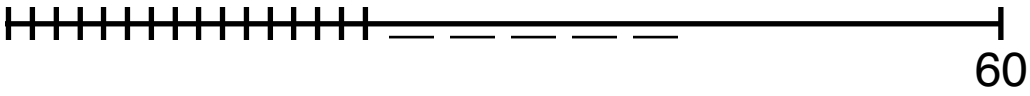
[**A**] Organic subunits could have combined and formed RNA molecules. [**B**] RNA molecules could have acted as ribozymes, catalyzing their own replication. [**C**] RNA molecules could also have catalyzed the synthesis of protein, which in turn stabilized RNA molecules and catalyzed RNA replication. [**D**] DNAs could have been copied from RNA molecules, and at some point, proteins may have begun to catalyze the synthesis of more proteins from information in RNA. [**E**] DNA assumed an information storage role, while RNA continued to be involved in protein synthesis; a cell membrane also appeared.

" The Nature of Life "
Postlethwait J.H., Hopson J.L. (1995)
Mc Graw-Hill Inc. New York

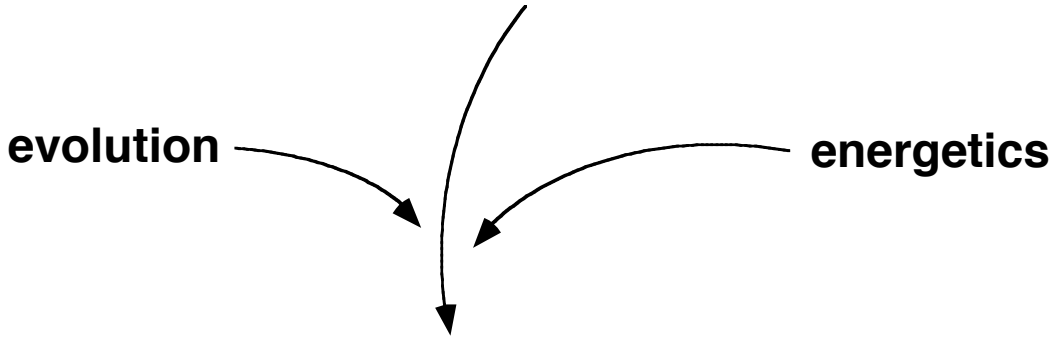
On the importance of being a copolymer



Calculate: How many different macromolecules can you build, when n = 60 and i = 1 - 20



$20 \times 20 \times 20 \times \ldots\ldots \quad N = 20^{60} \simeq 10^{70}$!!!



In nature there are only $10^{12} - 10^{14}$ proteins

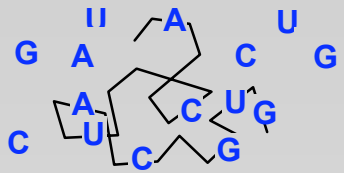
ORIGIN OF LIFE & ORIGIN FROM A SINGLE SELF-REPLICATING MOLECULE

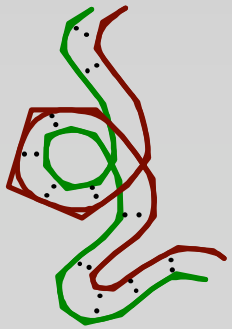
the chemical reality:

- - **the self-replicator must be a sequence long enough to be folded and to display enzymatic activity.**

- - **it must also recognize its own sequence in order to reproduce it.**

- - **this implies a formidable <u>specific</u> multiple (or sequential) binding process.**





- - **problem of strand separation ?**

TWO IMPORTANT LESSONS FROM THE RNA-WORLD

1. INFORMATION AND CATALYSIS MAY BE PRESENT IN THE SAME MOLECULAR STRUCTURE (Ribozymes)

2. SELF-REPLICATION MAY COME BEFORE FUNCTIONAL CATALYSIS (SELF-REPLICATION FIRST)

SELF-REPLICATION + MUTATION → FUNCTIONAL MACROMOLECULES

a personal bias

RNA is the product of primordial cellular life

rather than the origin of life

is the origin of biological evolution

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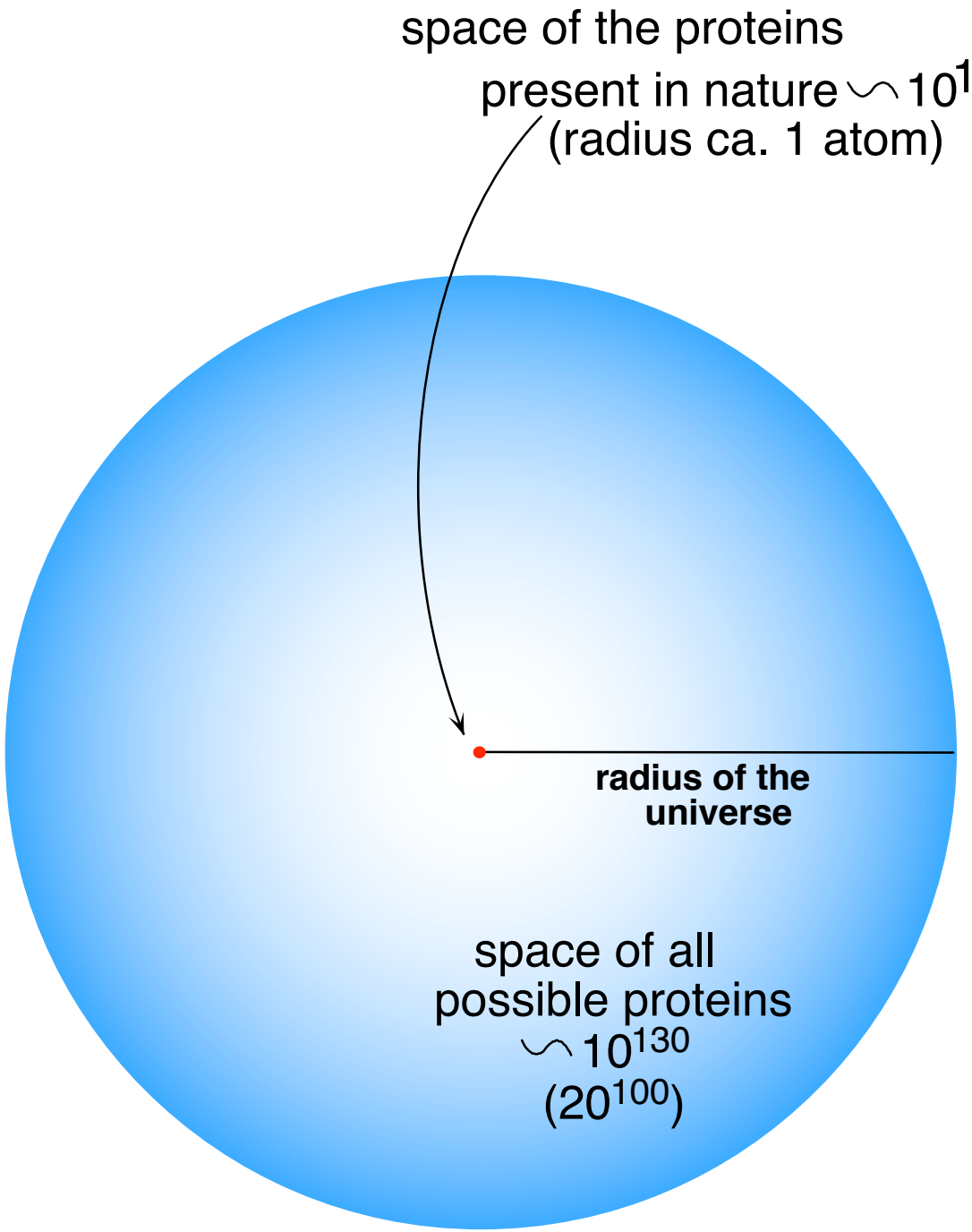
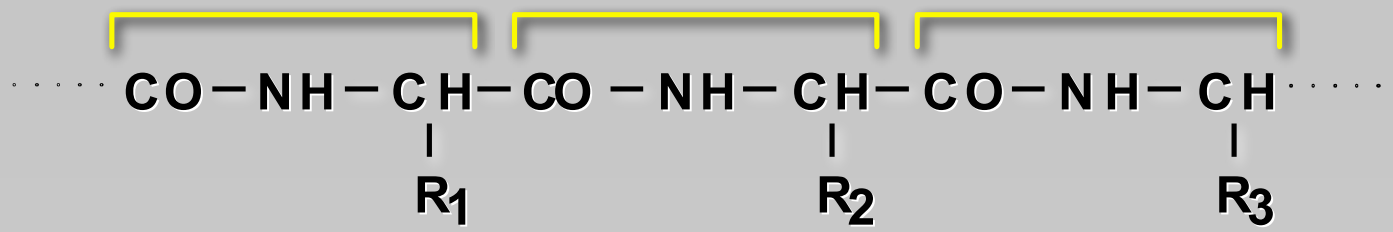
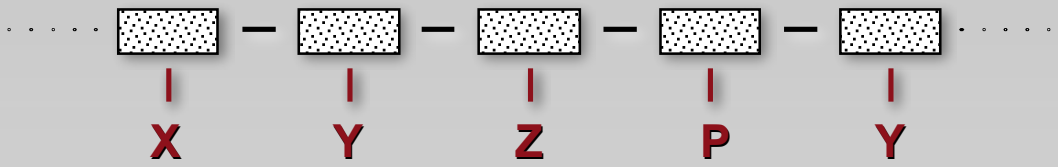
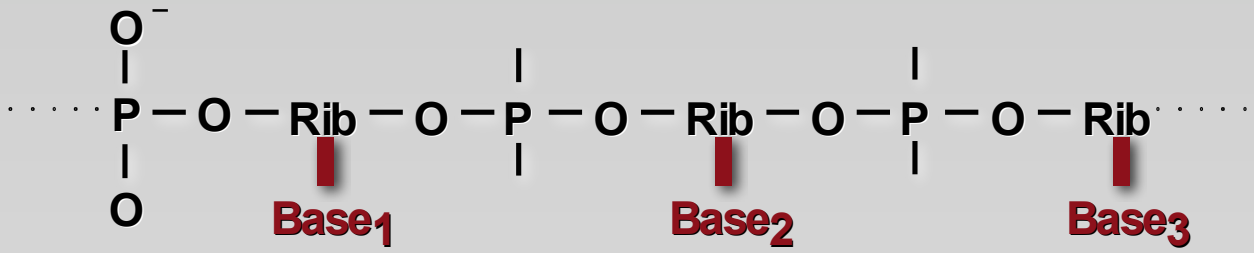
start from the edge of a membrane compartment


space of the proteins
present in nature ~ 10^{12}
(radius ca. 1 atom)
radius of the
universe
space of all
possible proteins
~ 10^{130}
(20^{100})


CO – NH – CH – CO – NH – CH – CO – NH – CH
R1
R2
R3


X
Y
Z
P
Y


O⁻
P – O – Rib – O – P – O – Rib – O – P – O – Rib
O
Base1
Base2
Base3

THE BASIS OF THE RNA-WORLD

RNA IS THE PRIME MOLECULE
CAME INTO EXISTENCE BEFORE PROTEINS AND DNA
AND ORIGINATED THE WHOLE THING

RNA ⟶ RIBOZYMES ⟶ PROTEIC ENZYMES ⟶ DNA

BUT
WHO (WHAT) MADE RNA ?

TO CONVALIDATE THE ABOVE VIEW
ONE SHOULD SHOW THAT RNA (IN PARTICULAR RIBOZYMES)
CAN COME TO EXISTENCE SPONTANEOUSLY
WITHOUT THE ASSISTENCE OF ENZYMES AND
OF PRE-ADDED TEMPLATE RNA

.....THIS HAS NOT BEEN ARCHIVED YET

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....AND TWO DIFFERENT EXPERIMENTAL APPROACHES

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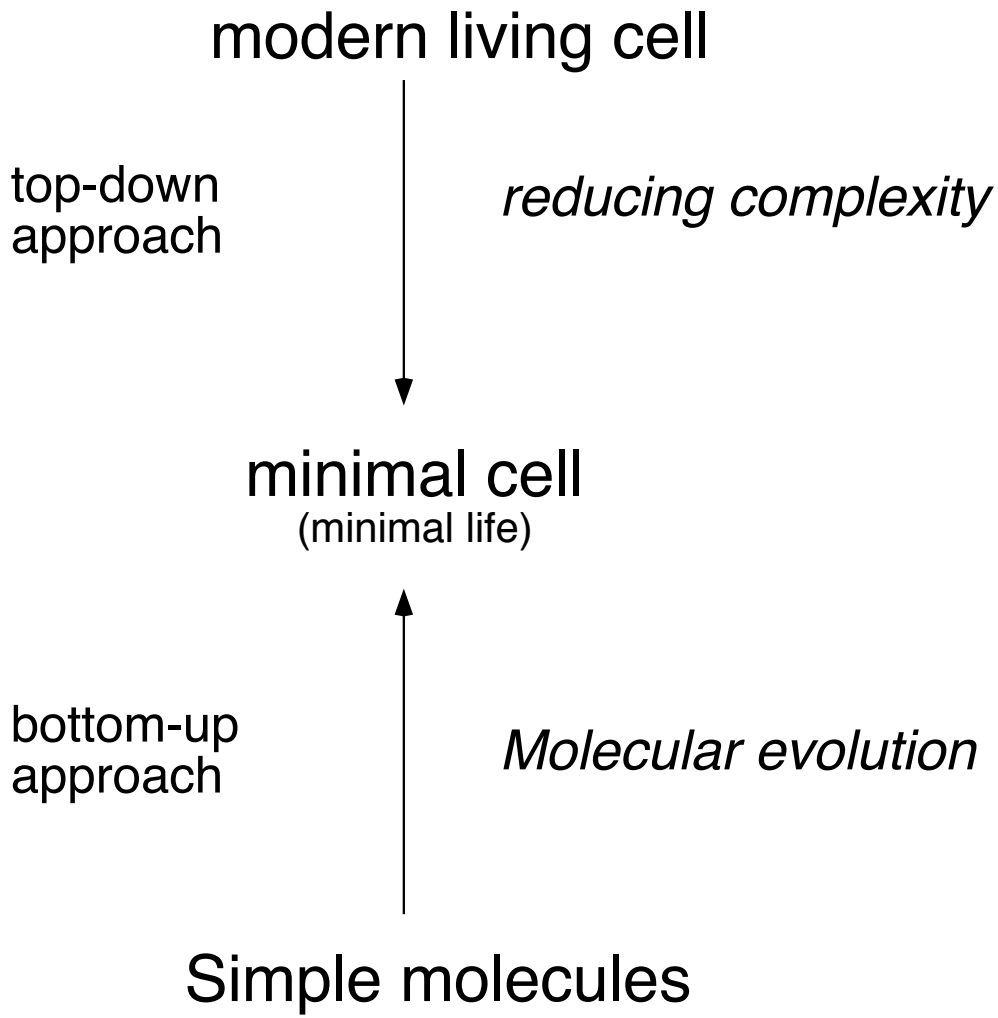
two working directions



CONTINGENCY SEEMS TO MAKE IT IMPOSSIBLE
TO REPRODUCE IN THE LABORATORY THE TRANSITION TO LIFE FROM ITS SIMPLE CONSTITUENTS

SO, WHAT CAN WE DO?

ONE COMMON SCIENTIFIC ATTITUDE:

Even if we cannot reproduce the exact path made by Nature, we can show that this path is, at least in principle,doeble

AND: DO IT THE WAY YOU LIKE - NO STRINGS ATTACHED. JUST SHOW THAT TRANSITION TO LIFE FROM THE INANIMATE IS POSSIBLE

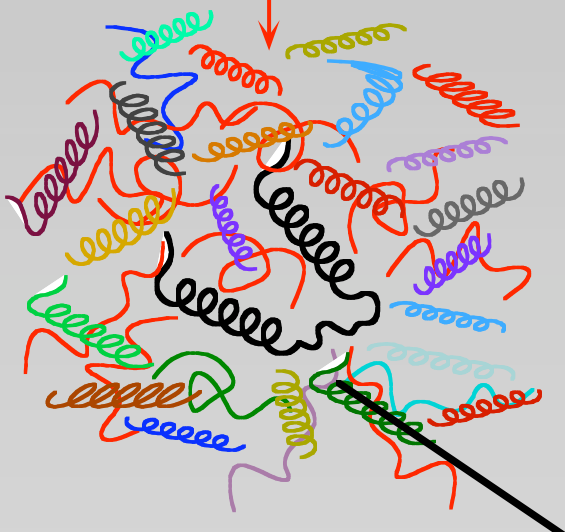
THE ORIGIN OF FUNCTIONAL MACROMOLECULES

(self-replicating, informational or catalytic biopolymers)

all monomers mixture

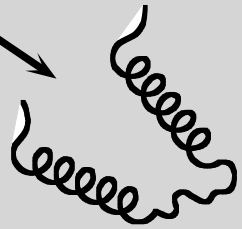
1. SELECTION MECHANISM

random
polymerization



huge library
of polymers
(~ 10^{50})

selection



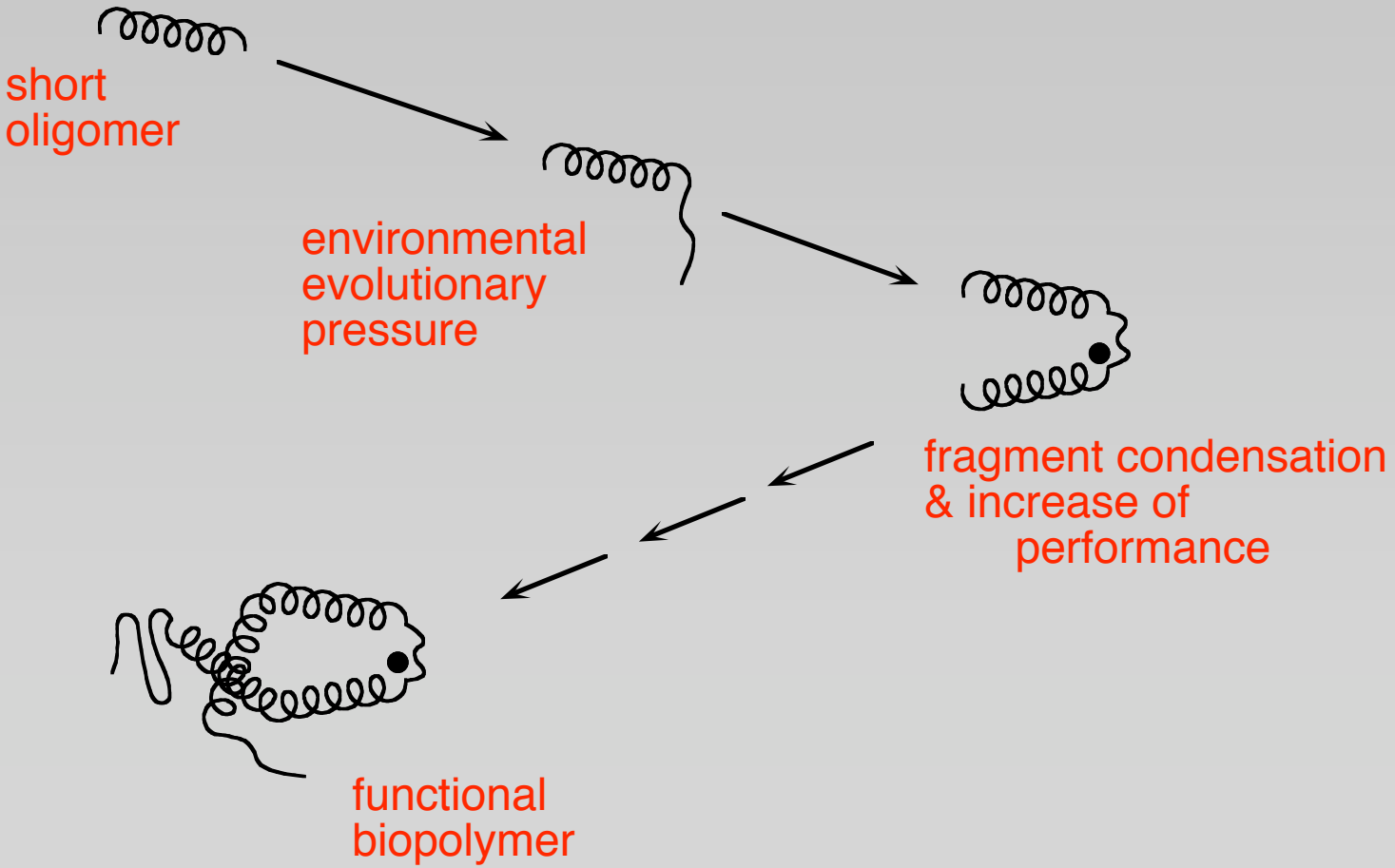
functional
biopolymer

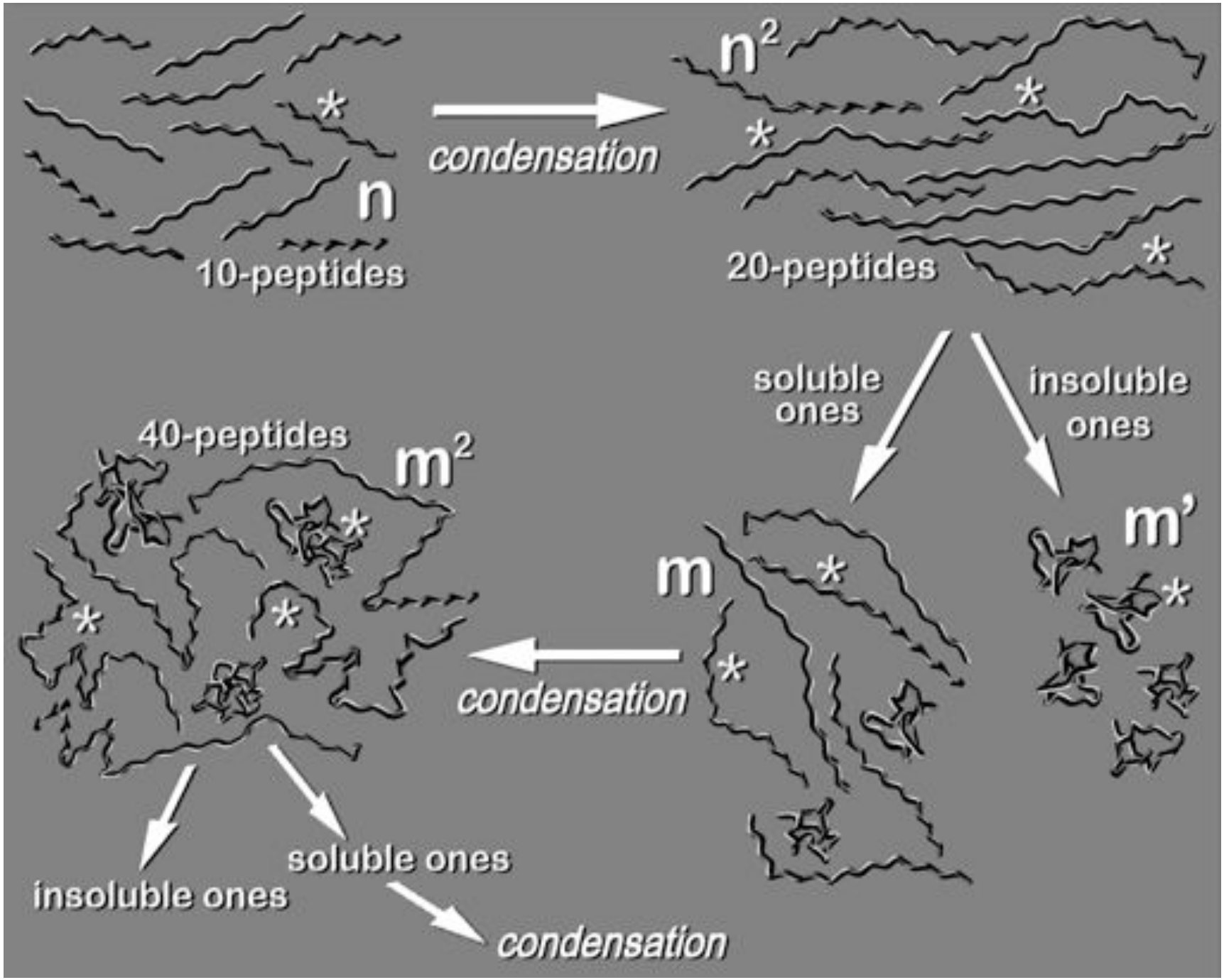
THE ORIGIN OF FUNCTIONAL MACROMOLECULES

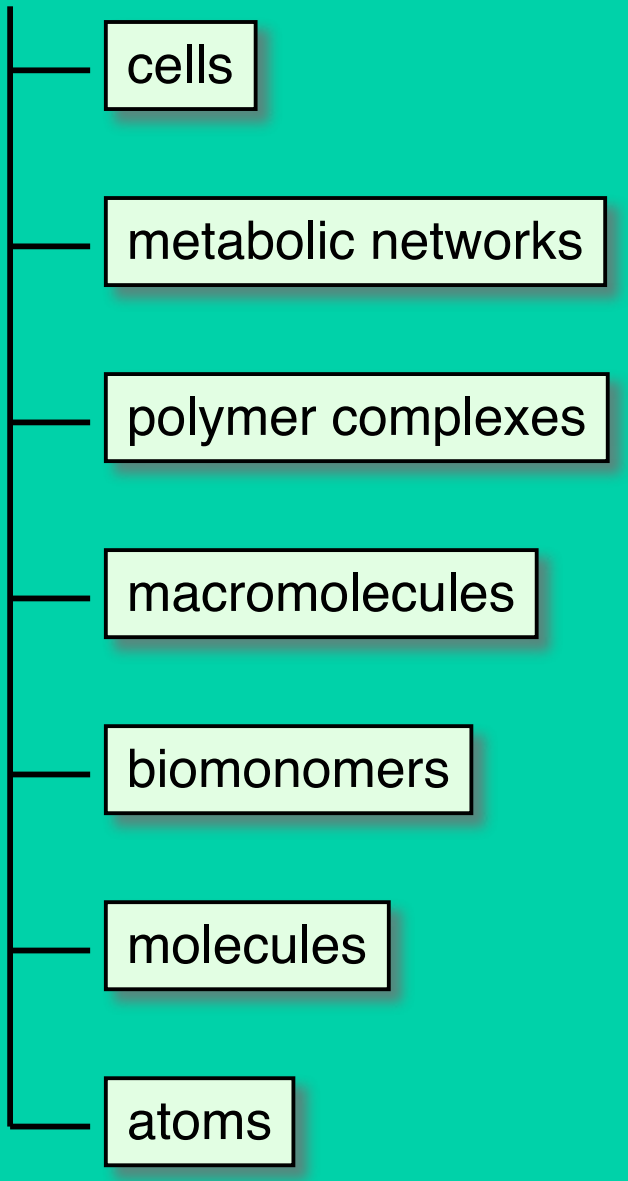
(self-replicating, informational or catalytic biopolymers)

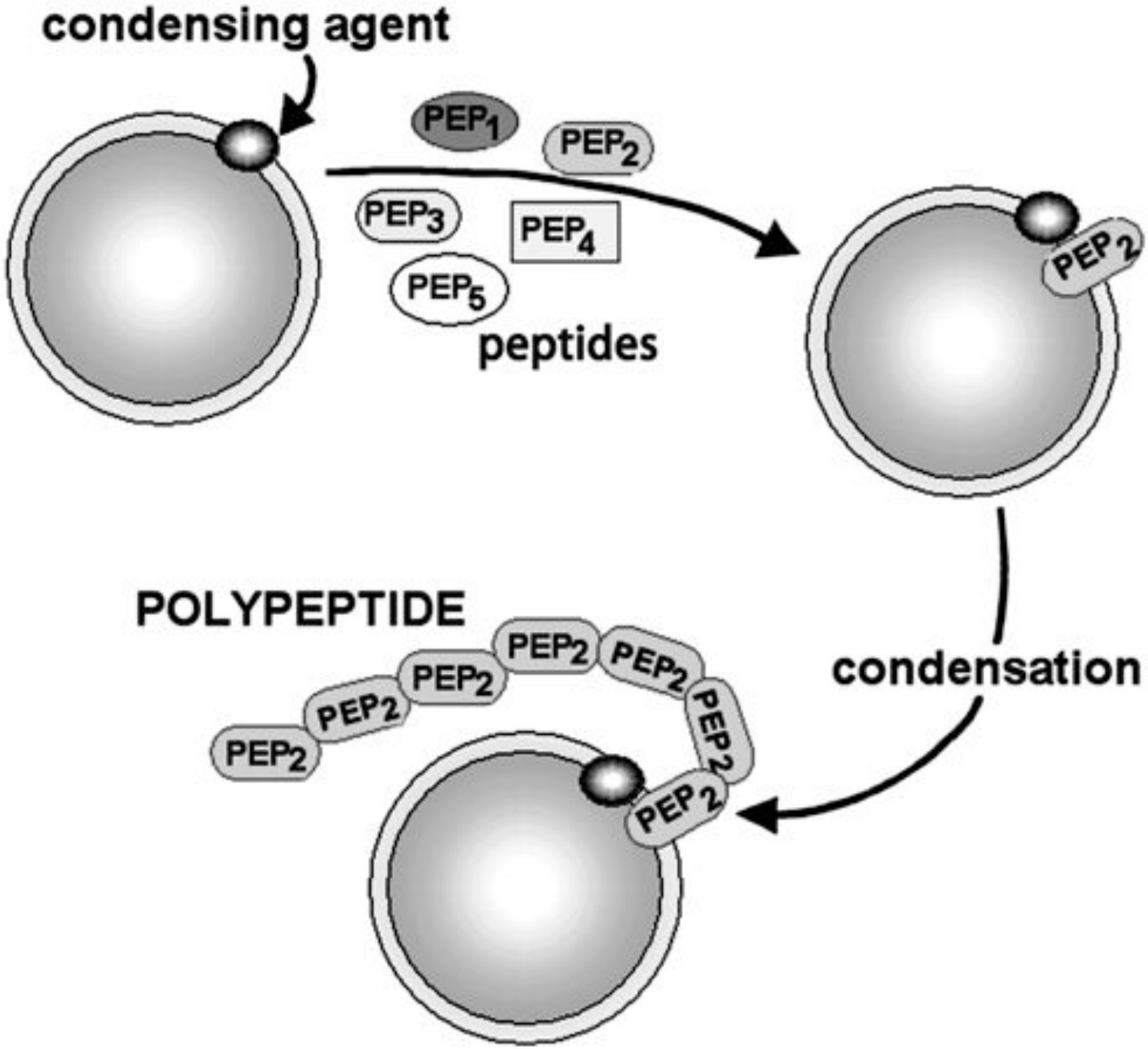
2. EVOLUTION MECHANISM

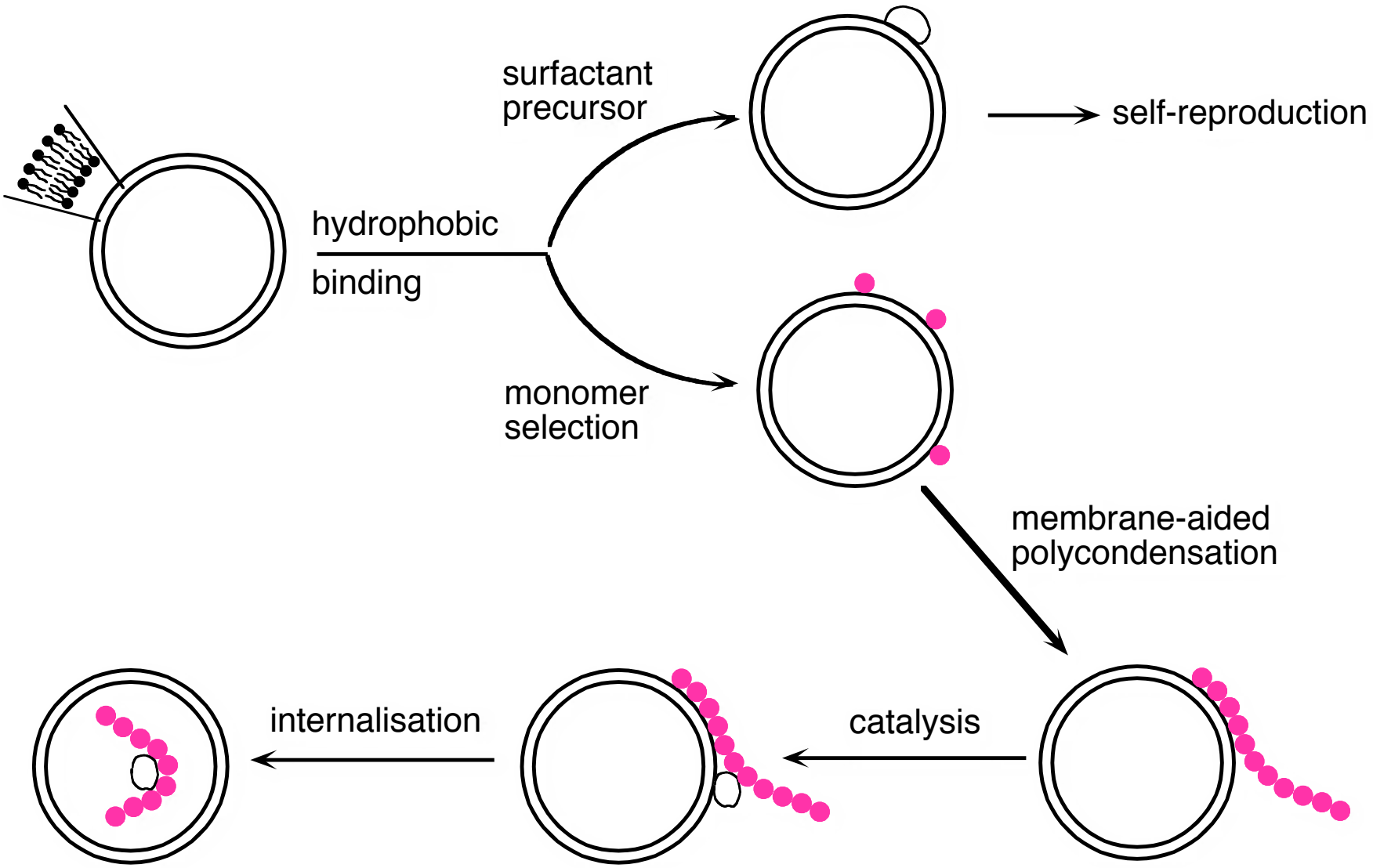
(stepwise increase of size & specificity)

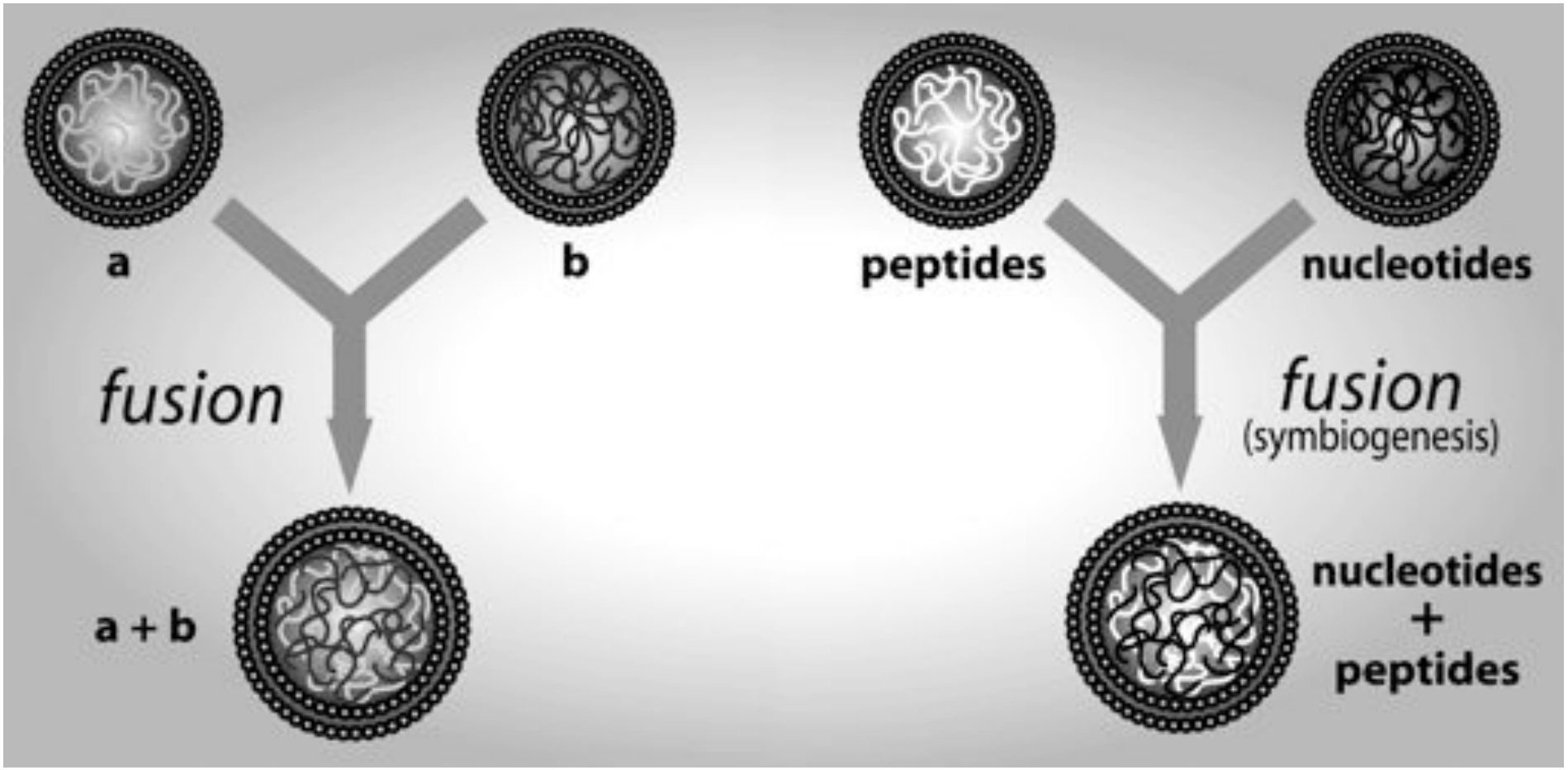



n
10-peptides
condensation
n²
20-peptides
soluble ones
insoluble ones
m'
m
condensation
40-peptides
m²
insoluble ones
soluble ones
condensation


cells
metabolic networks
polymer complexes
macromolecules
biomonomers
molecules
atoms


condensing agent
PEP1
PEP2
PEP3
PEP4
PEP5
peptides
PEP 2
condensation
POLYPEPTIDE
PEP2
PEP2
PEP2
PEP2
PEP2
PEP2
PEP 2


surfactant
precursor
self-reproduction
hydrophobic
binding
monomer
selection
membrane-aided
polycondensation
internalisation
catalysis


a
b
fusion
a + b
peptides
nucleotides
fusion
(symbiogenesis)
nucleotides
+
peptides

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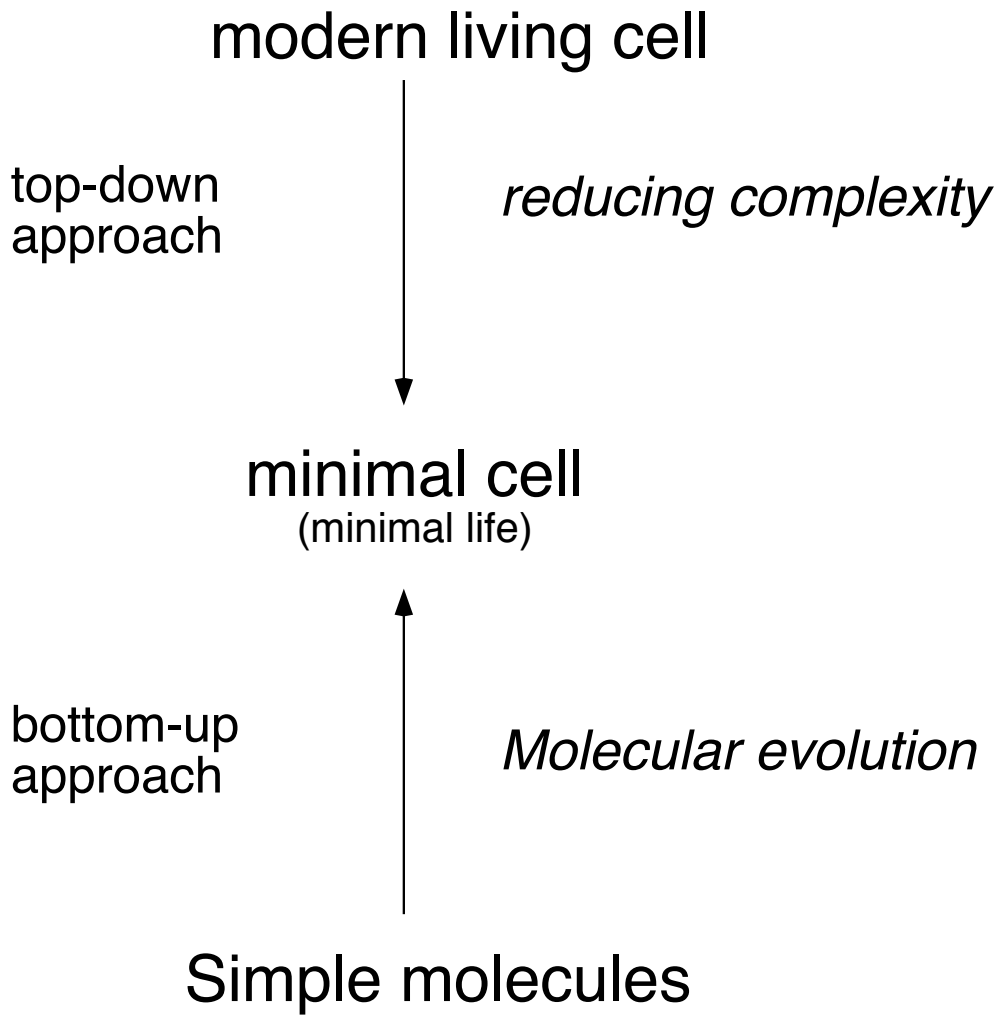
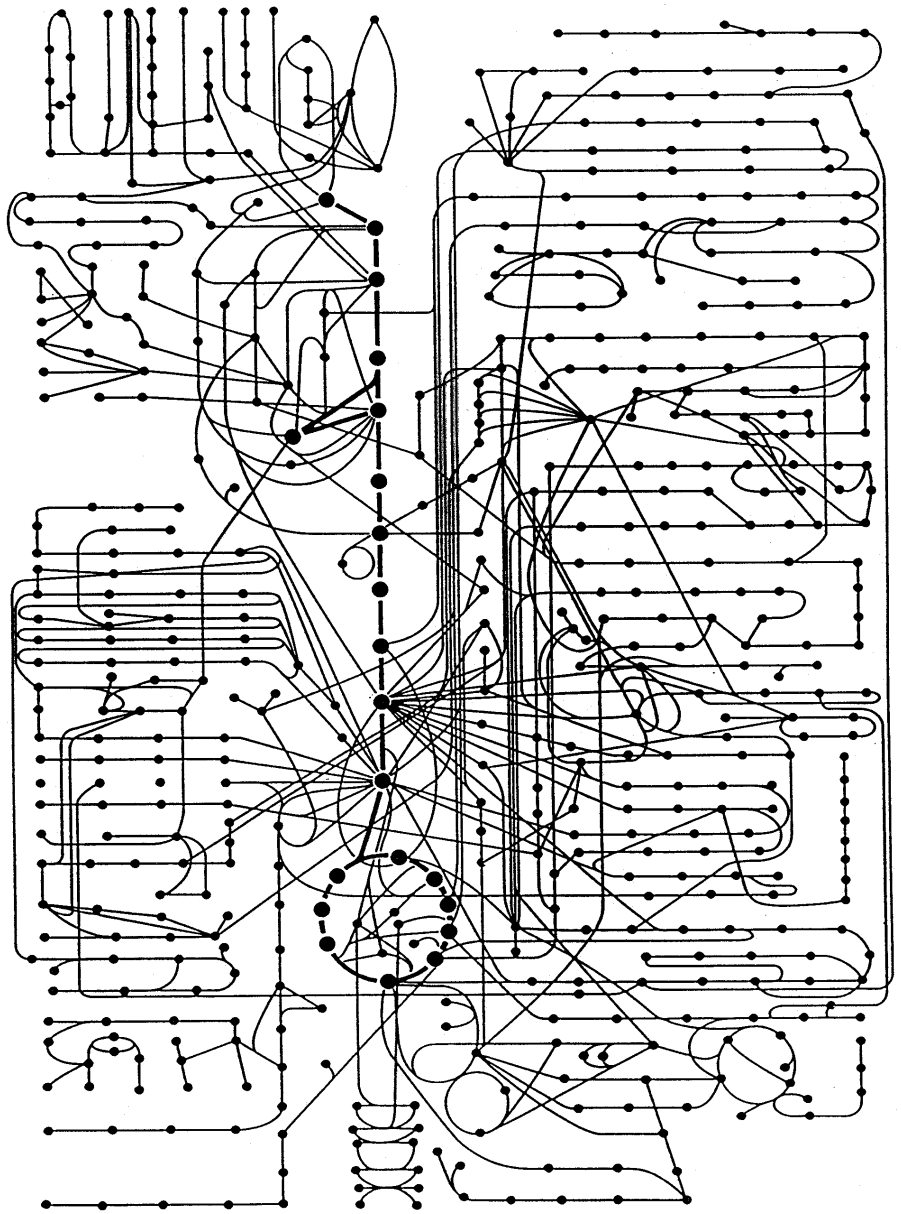


A maze illustrating the chemical reactions that interconvert small molecules in cells.

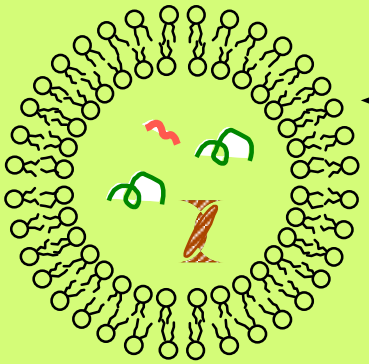


The simplest living cells on our Earth contain at least 500 genes, and more generally a simple microbe has 1000 or more genes,

i.e., it has a few thousand macromolecules (enzymes and nucleic acids) inside, all in -teracting with each other...

IS THIS HUGE COMPLEXITY REALLY ESSENTIAL FOR LIFE?

the notion of the minimal cell:



lipidic bilayer

containing the minimal and sufficient number of components to be "alive"

alive → self-maintenance
alive → reproduction
alive → mutation

towards an operational definition of minimal life

- at which level of molecular complexity does the quality emerge, which one can call life?

- what are the minimal and sufficient conditions for a physical system to have "life"?

- which is the simplest possible synthetic chemical equivalent of a living cell?

The work on the minimal living cell
must be accompanied by studies on the
early cells - namely the protocells at
the time before the advent of ribosomes
and before the high selectivity
of modern times —

This would eliminate the 55 ca. genes of the ribosomal proteins
and would reduce the 20 t-RNA genes to a much lower number

→ DOWN TO a final 20-25 GENES??

1. The study of the minimal genome

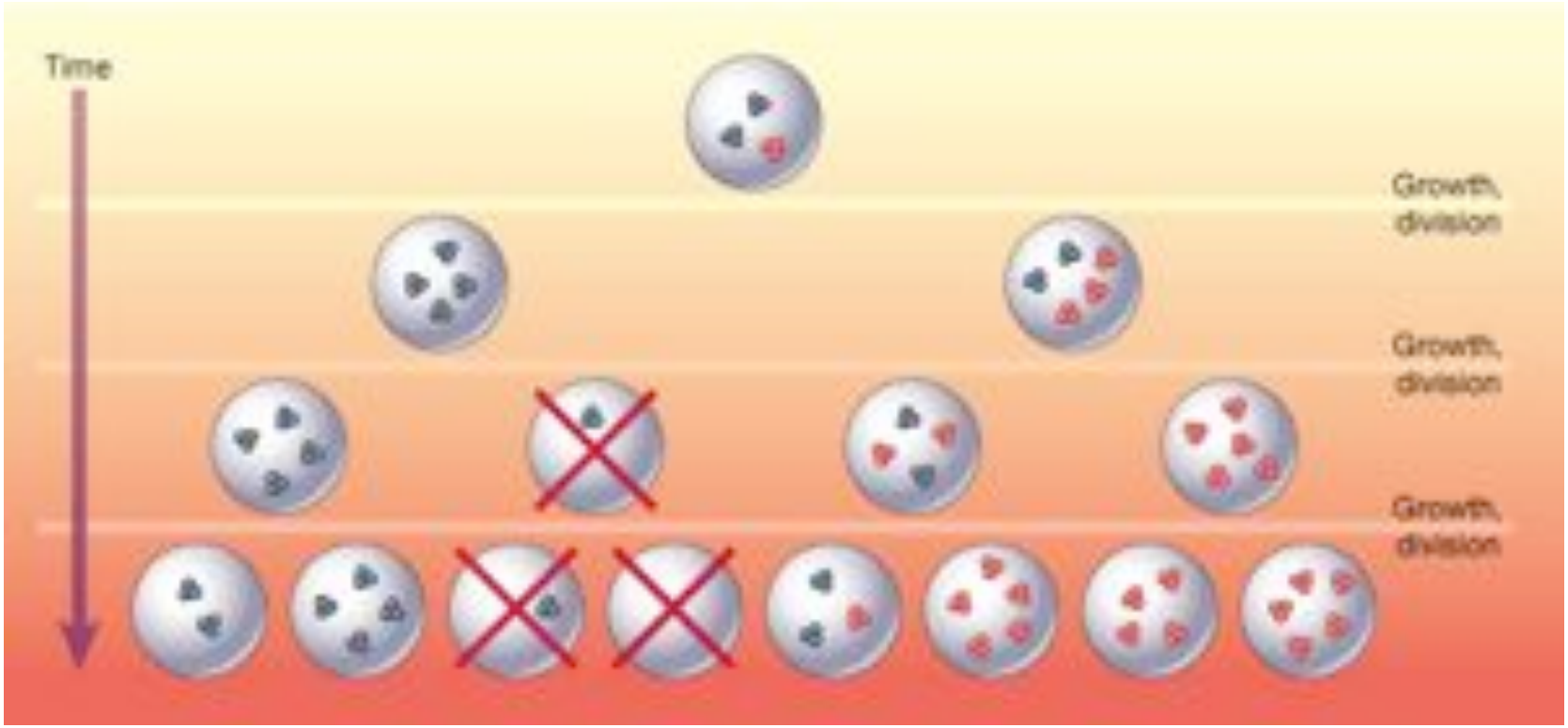
2 Its implementation on a minimal cell

...a difficult project, must be done in various steps

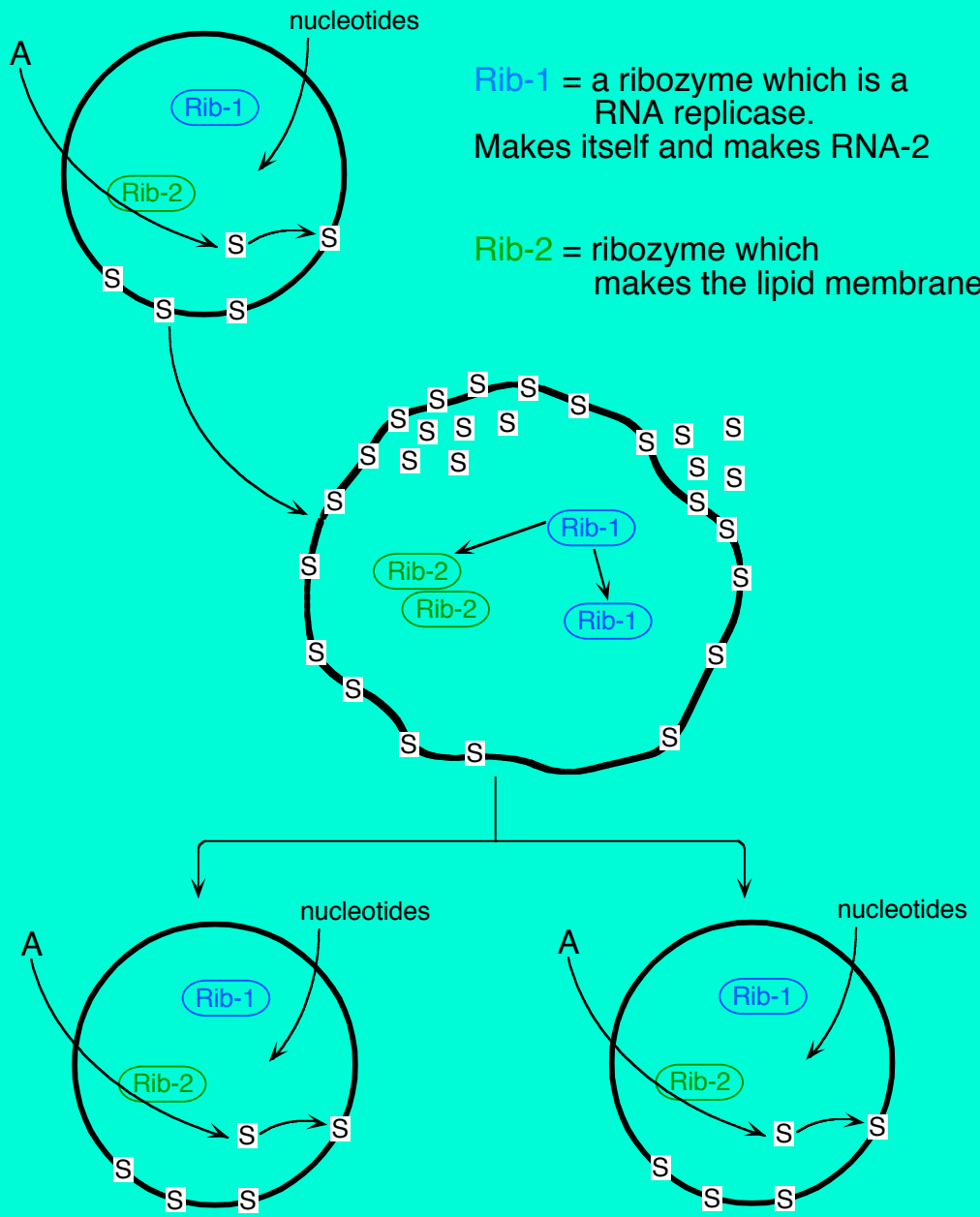
Nature 409, 387 - 390 (2001)

Synthesizing life

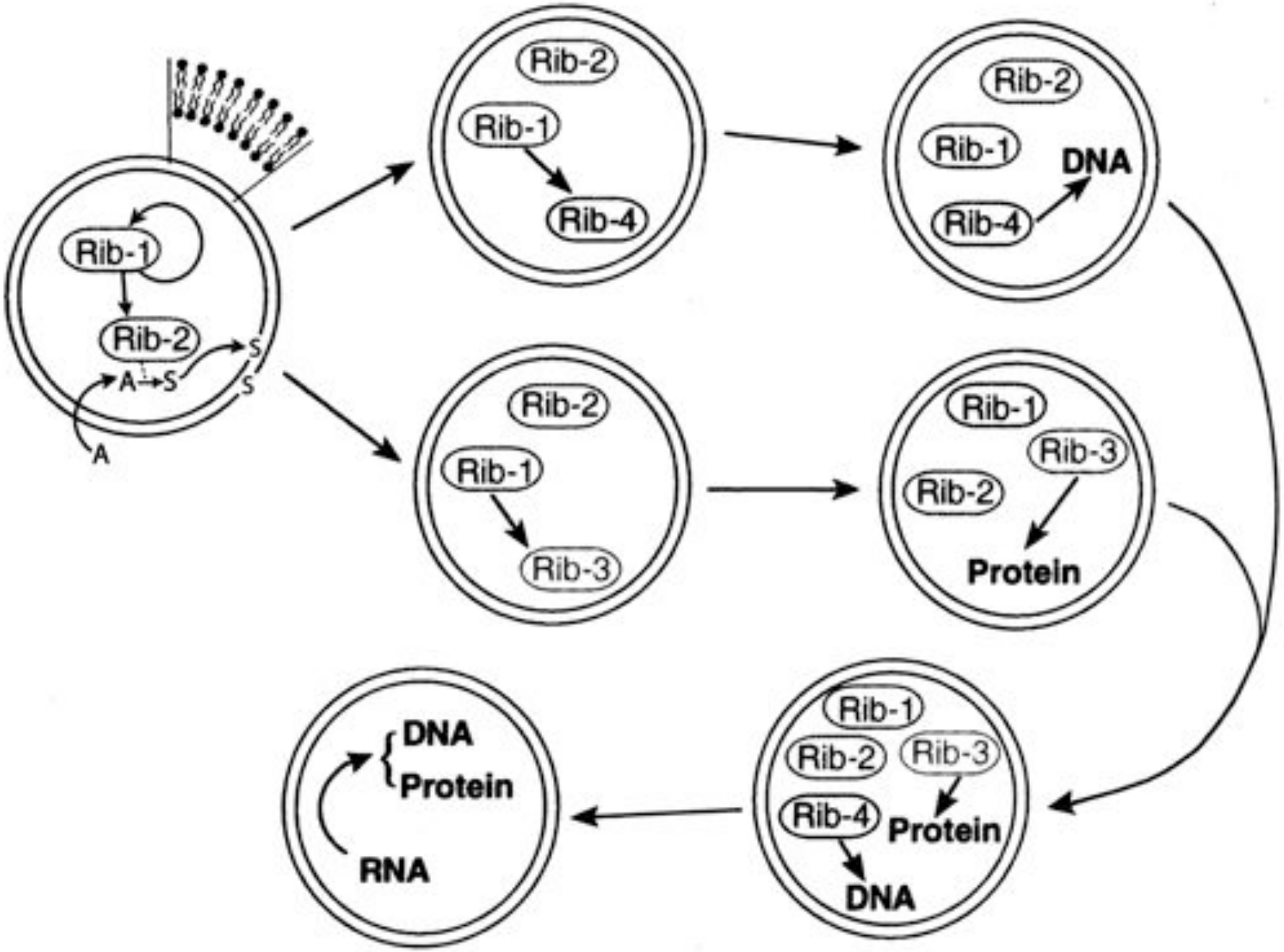
JACK W. SZOSTAK, DAVID P. BARTEL & P. LUIGI LUISI



A self-reproducing RNA cell with only two genes



This cell can replicate evolve. It does not make proteins.


Rib-1
Rib-2
A→S
S
S
A
Rib-2
Rib-1
Rib-4
Rib-2
Rib-1
DNA
Rib-4
Rib-2
Rib-1
Rib-3
Rib-1
Rib-3
Rib-2
Protein
Rib-1
Rib-2
Rib-3
Rib-4
Protein
DNA
DNA
Protein
RNA

THE RNA MINIMAL CELL IS A THEORETICAL CONSTRUCT.
RNA REPLICATING RIBOSOMESDO NOT EXIST

MORE REALISTIC, EVEN IF MORE COMPLEX.
IS THE WORK WITH DANN/PROTEIN MINIMAL CELLS

HOW SMALL IS A MINIMAL CELL CAPABLE OF DISPLAYING FULL-FLEDGED SELF-REPRODUCTION (AND MUTATION)?

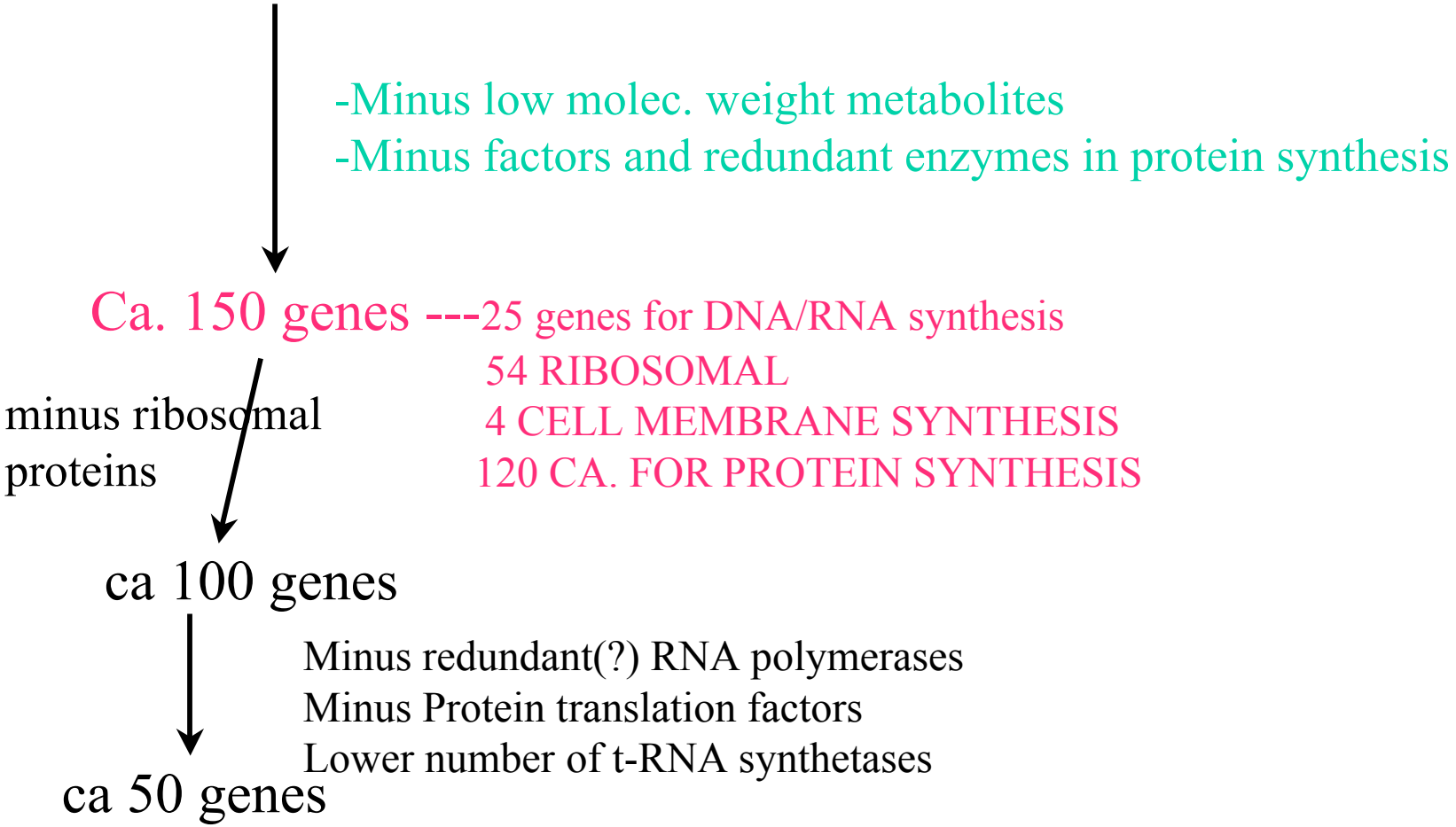
..**HOW MANY GENES?**

THE MINIMAL DNA/PROTEIN CELL

Luisi et al., Helv. Chim. Acta 2002

Micoplasma genitalium

(470 coded regions)



First step:

find a suitable compartment as cell model

And show that this is capable of hosting the Complex molecular biology reactions of life

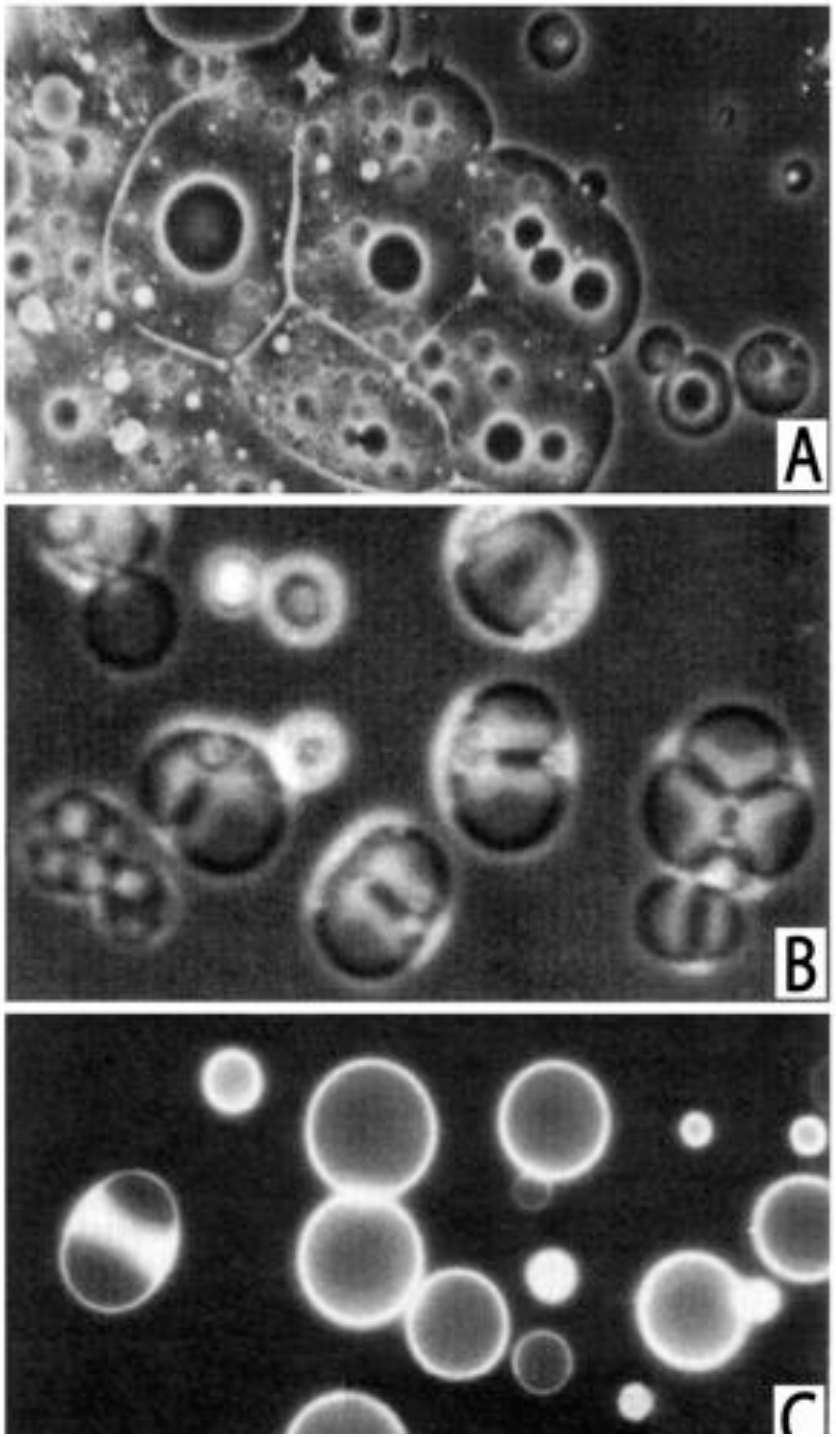
HOW DO YOU PROCEED EXPERIMENTALLY

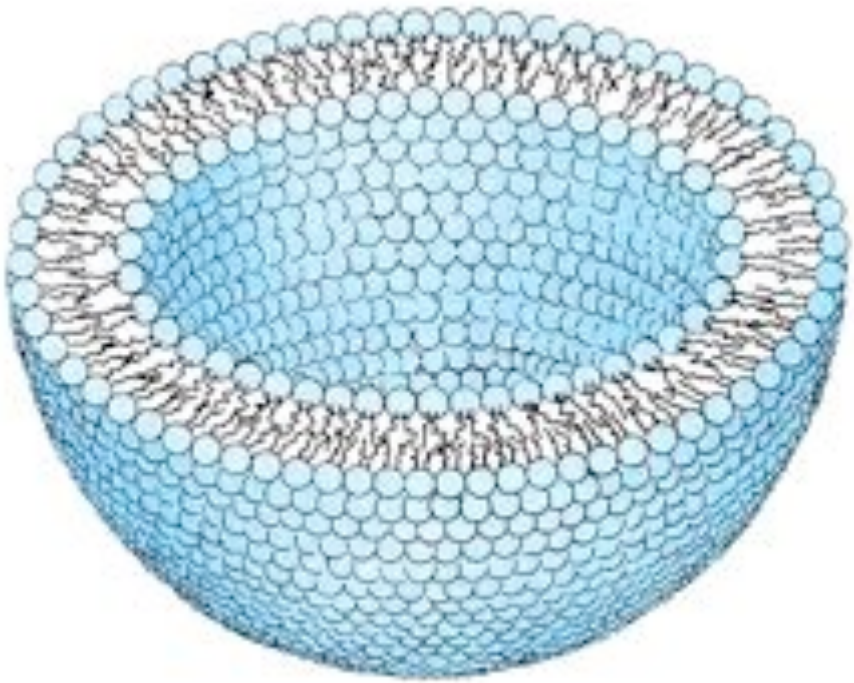
FOR CONSTRUCTING IN THE LABORATORY

A LIVING CELL MODEL?

Liposomes, as closed spherical bilayers, are considered the most likely precursors of early living cells (protocells)

LIPOSOMES ARE JUST TINY SOAP BUBBLES


A
B
C

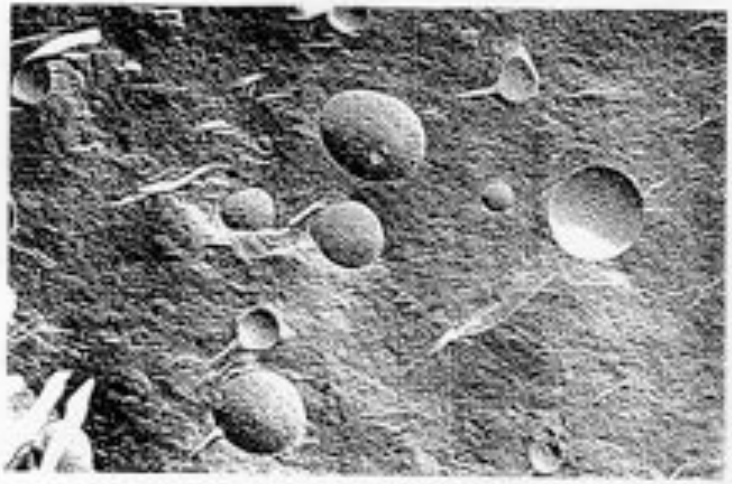


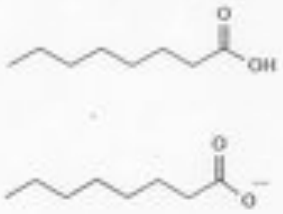
MOLECULAR ARCHITECTURE of the animal-cell membrane is determined primarily by the interactions of phospholipid molecules in water. Phospholipids can minimize their energy in water by forming a bilayer about 40 angstrom units thick. The hydrophobic tails of the molecules sequester themselves on the inside of the bilayer and the hydrophilic heads (*blue*) face the water on both sides of the bilayer. If any edge of the bilayer were open to the water, hydrophobic tails along the edge would be exposed; hence the bilayer closes to form a vesicle, effectively segregating fluid inside the vesicle from fluid surrounding it.

Octanoic acid/octanoate vesicles

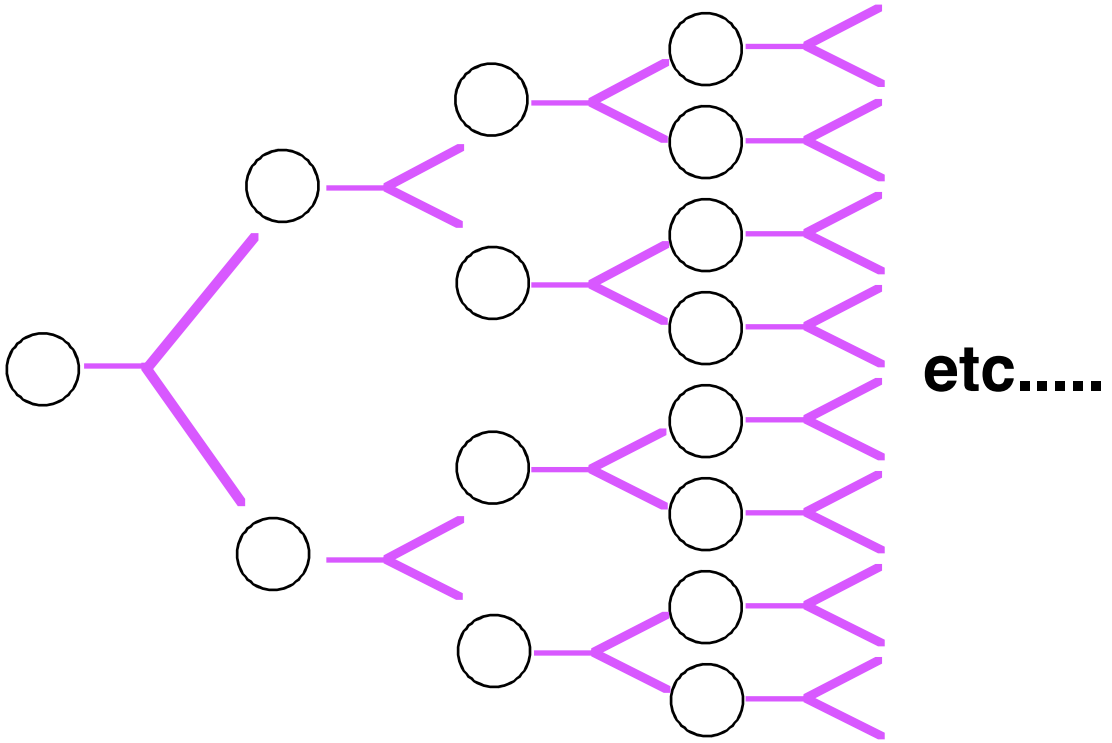
250 mM, pH 7

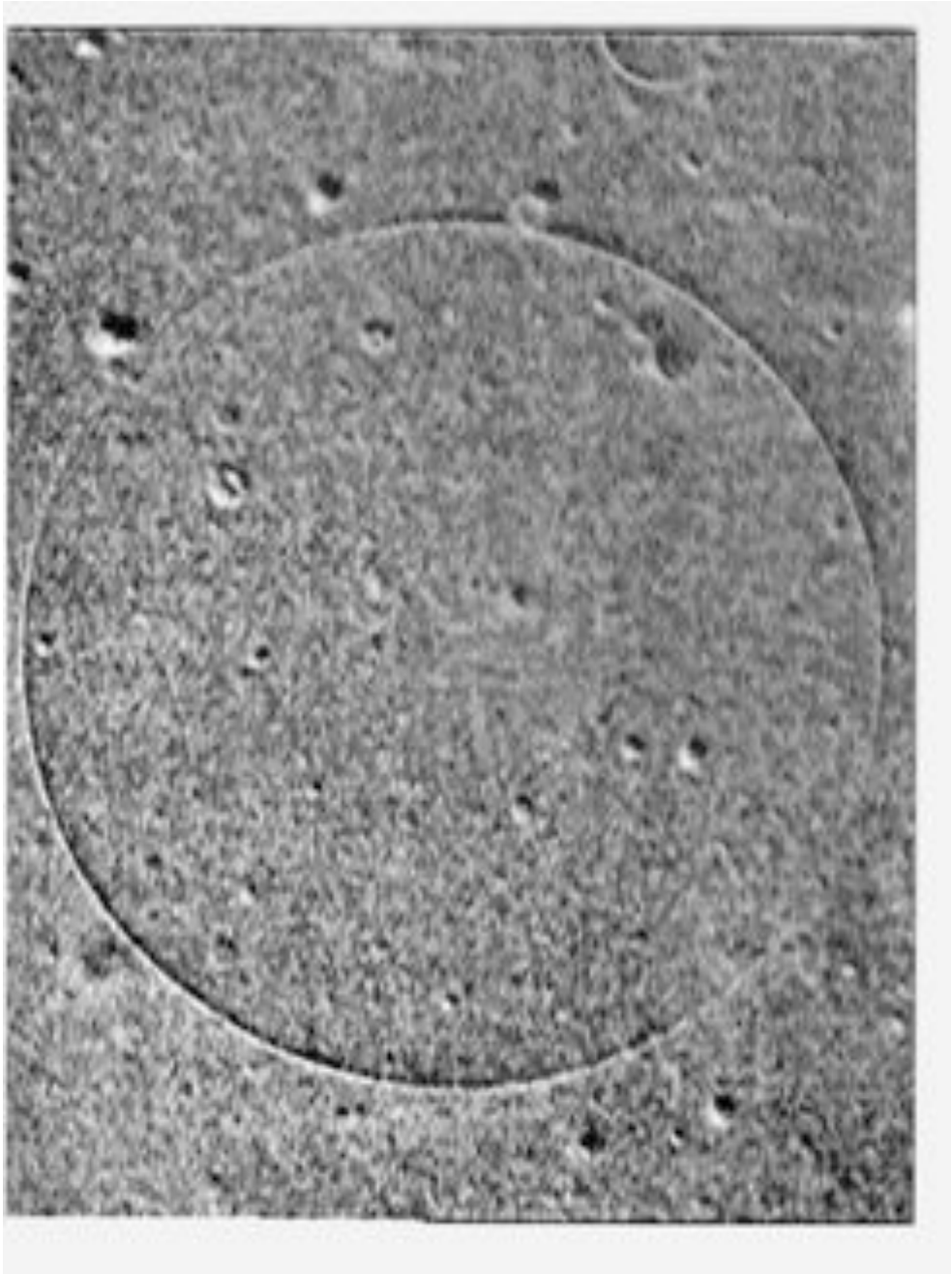
bar = 400 nm

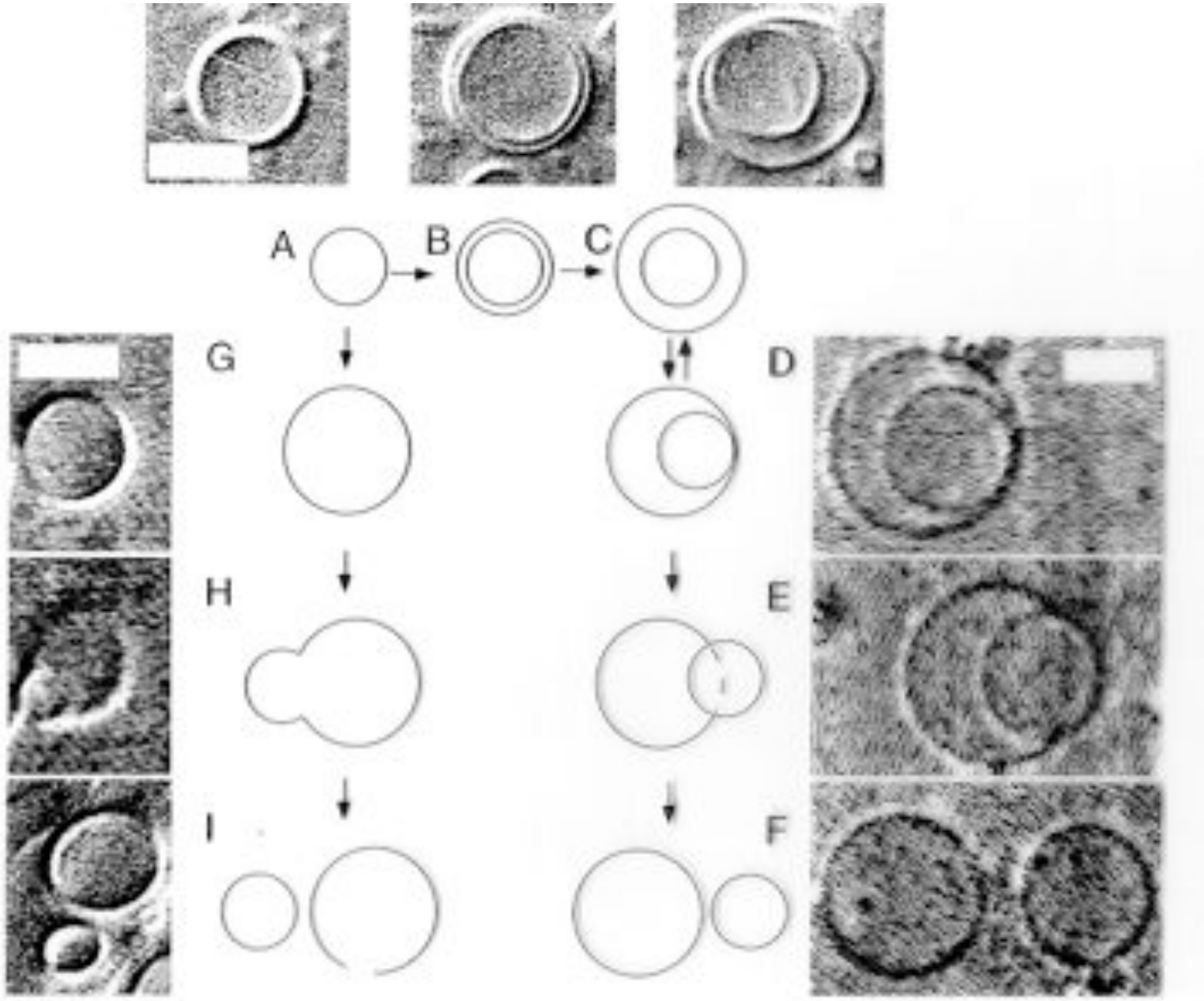




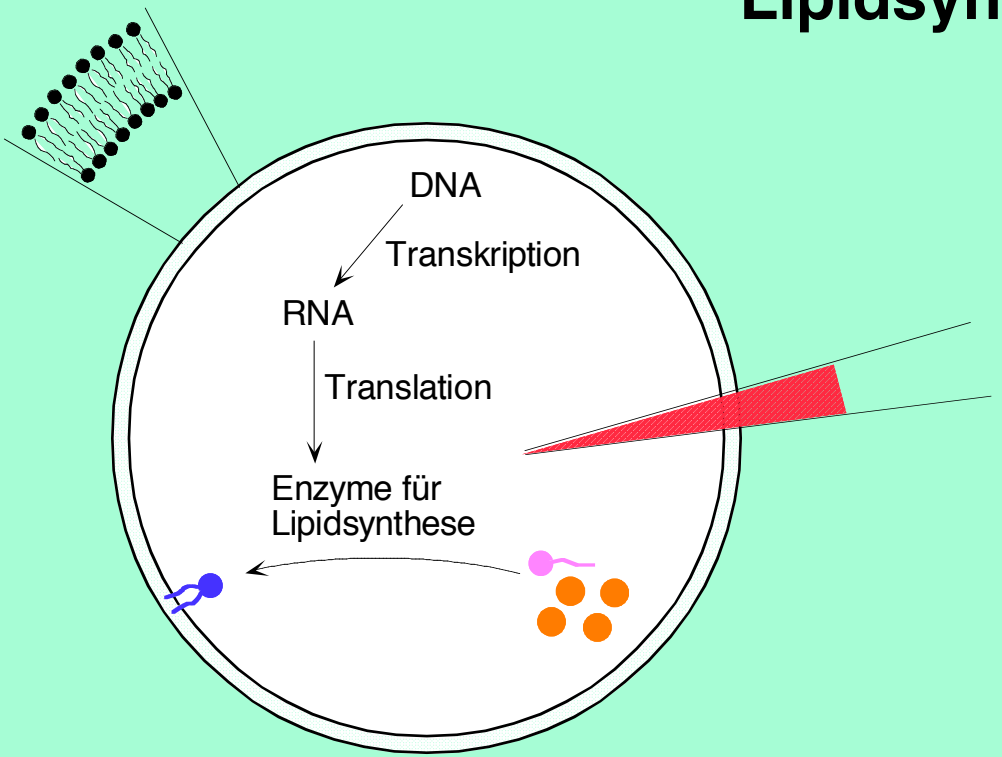
SELF-REPRODUCING MICELLES, LIPOSOMES & CHEMICAL AUTOPOIESIS






A
B
C
G
D
H
E
I
F

Lipidsynthese in Riesenliposomen



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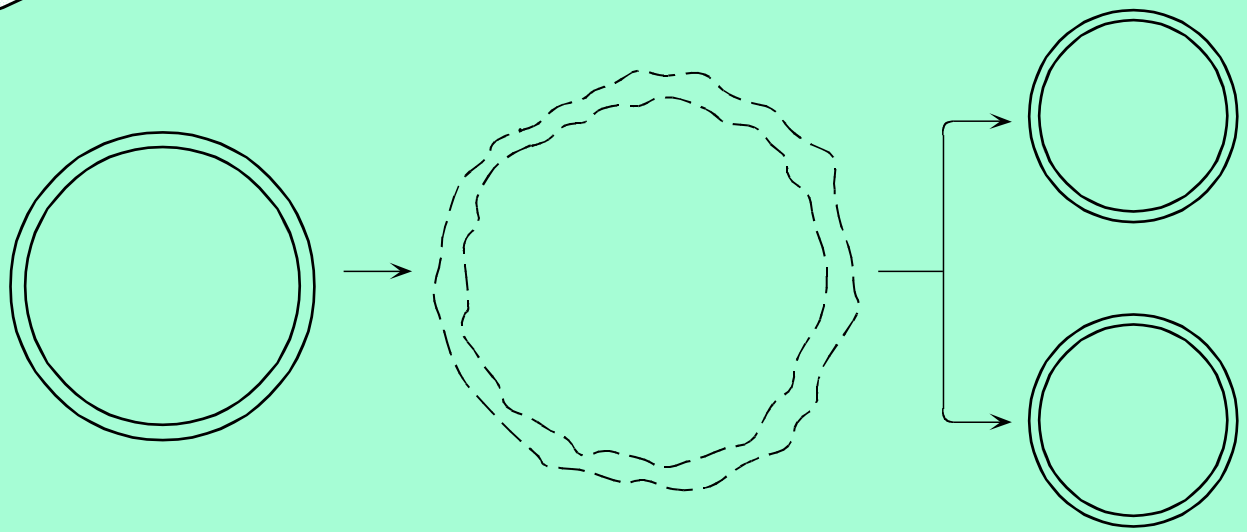
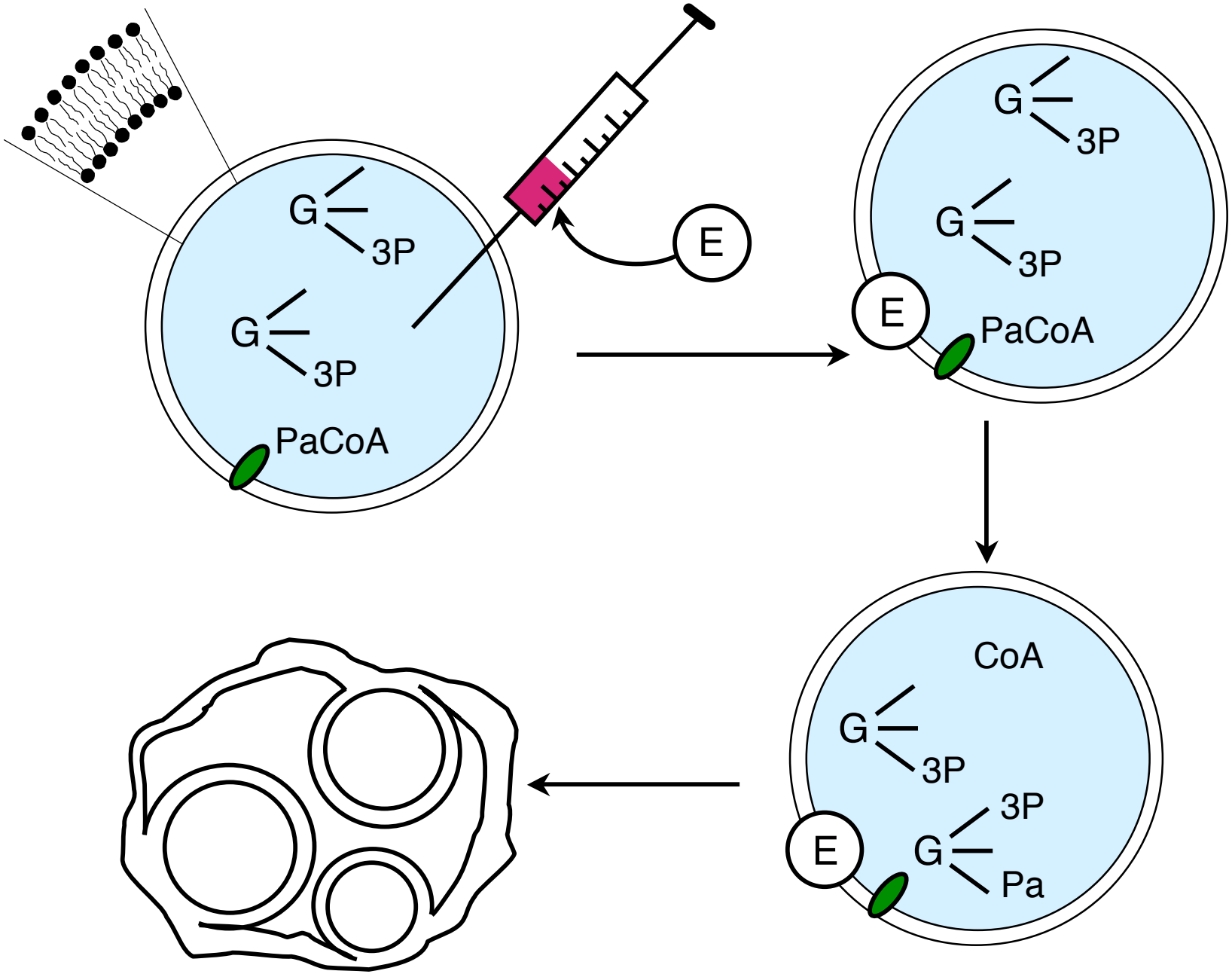
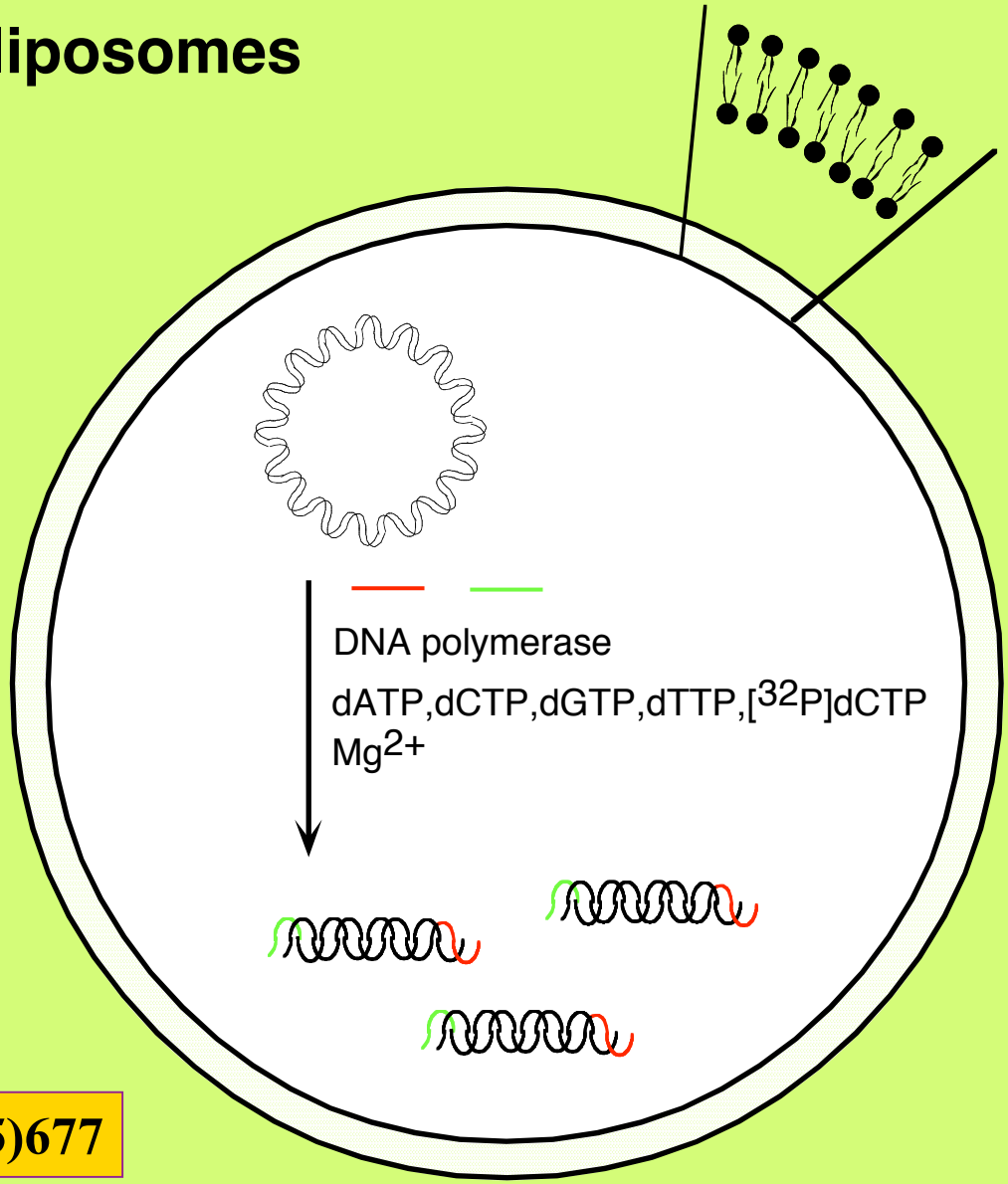


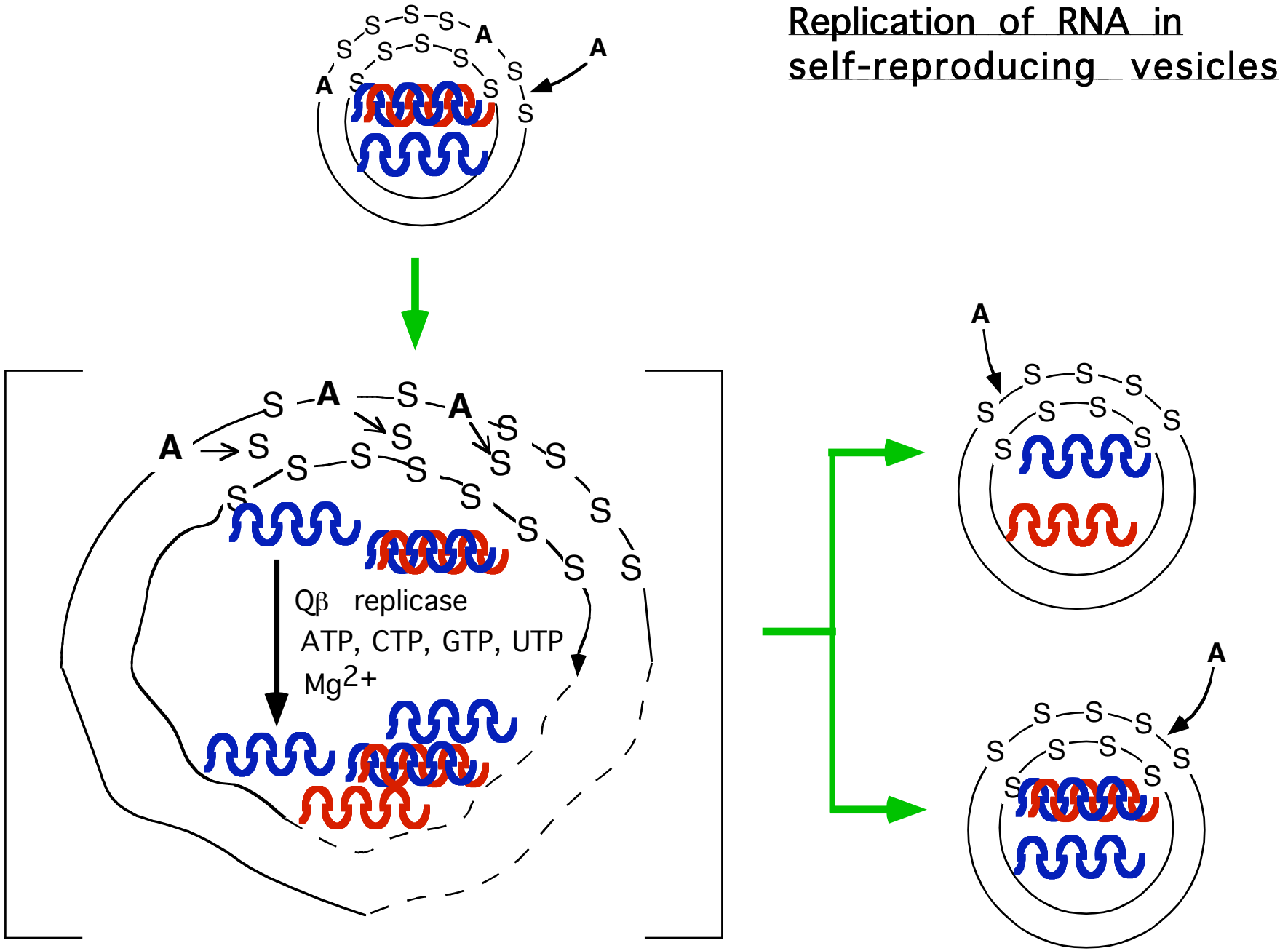

G
3P
G
3P
PaCoA
E
G
3P
G
3P
E
PaCoA
CoA
G
3P
E
3P
G
Pa

PCR in liposomes



Chem &Biol 2(1995)677

Replication of RNA in self-reproducing vesicles



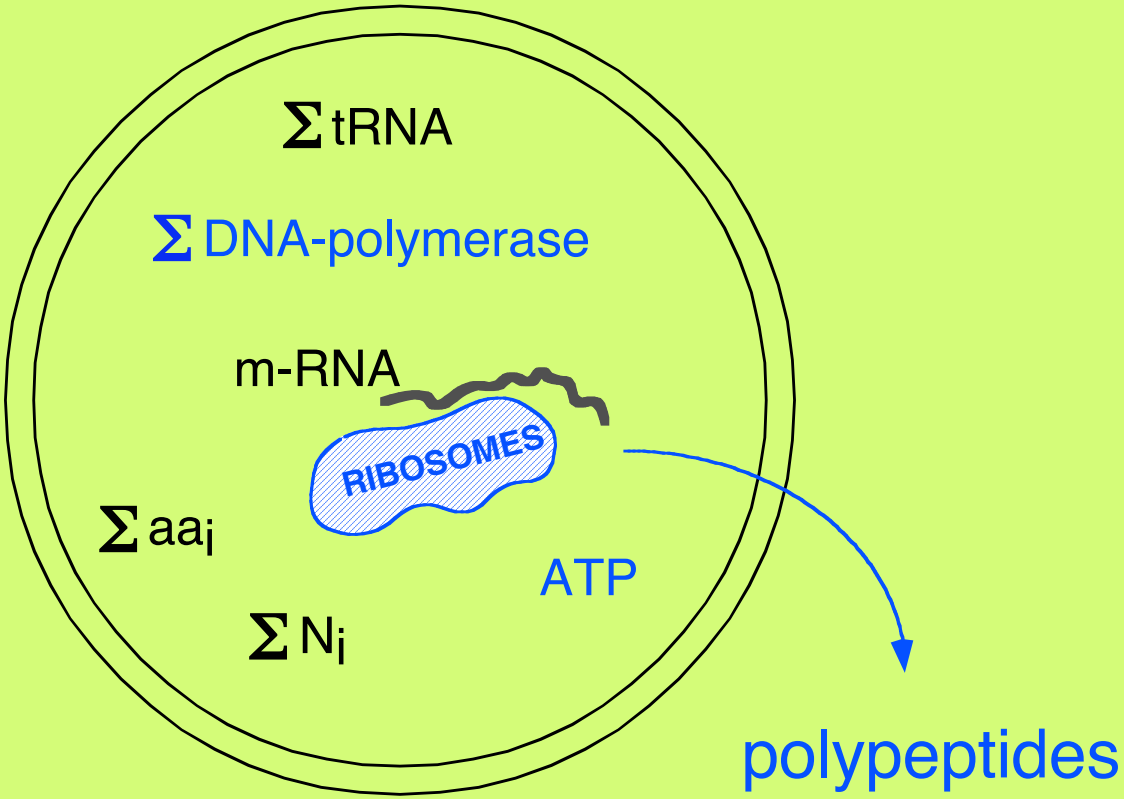
Is this life?

Not really.
With progressive
generations, the active components
are diluted out
because they are not fabricated
by the compartment itself

Step two:

Protein synthesis inside liposomes

protein biosynthesis in liposomes

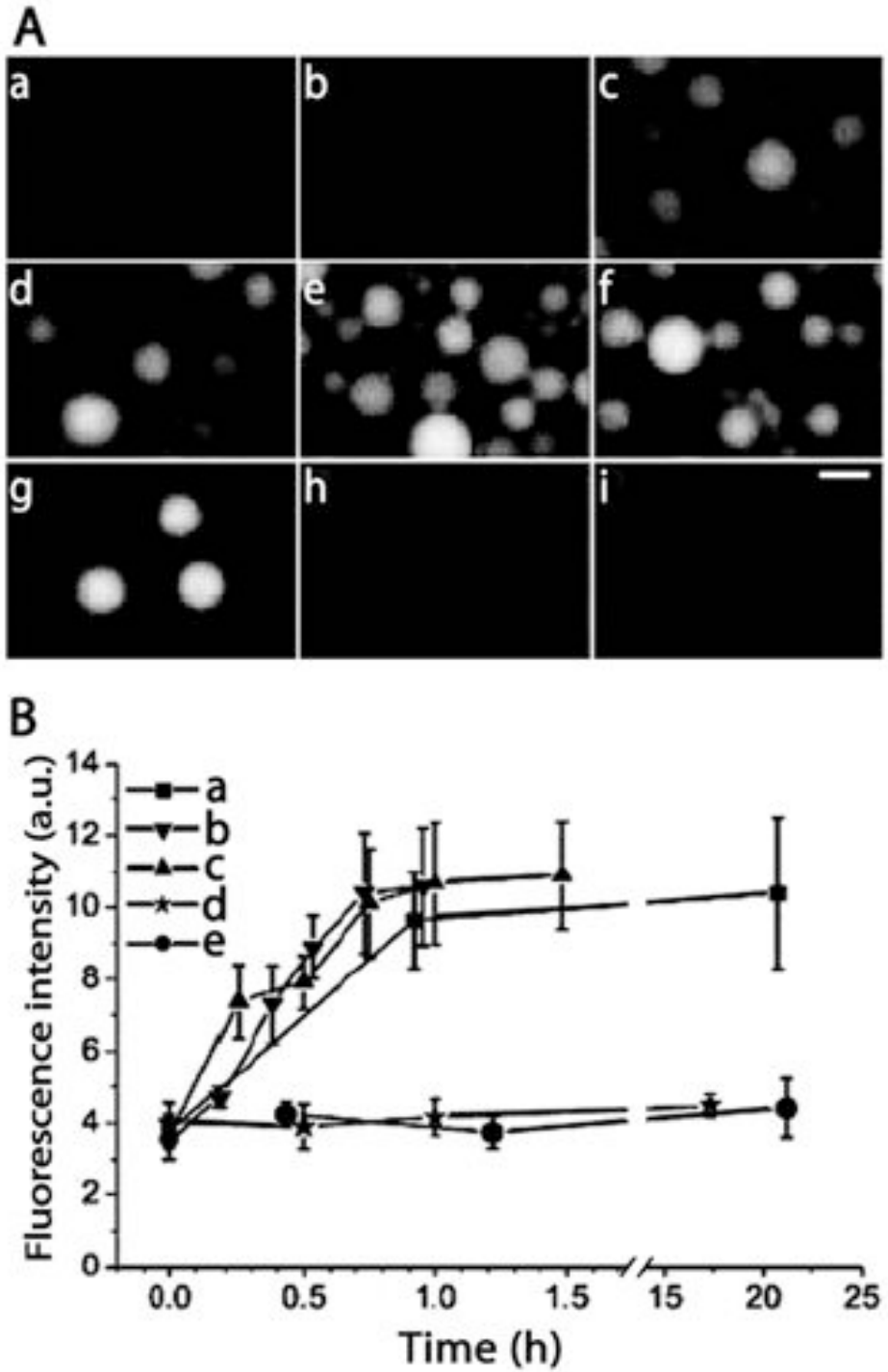


Oberholzer et al., 1999
(only poly-phe)

Bioch.bioph.Res.comm 261(1999)238

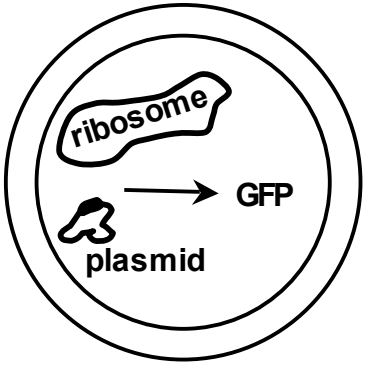
PROTEIN EXPRESSSION IN LIPOSOMES (MOSTLY GREEN FLUORESCENCE PROTEIN) HAS BEEN DESCRIBED BY SEVERAL GROUPS:

Oberholzer et al., 1999, 2001
Yomo et al., 2001
Tsumoto et al., 2001
Fischer et al, 2002
Nomura et al., 2003,
Pietrini et al., 2004
Noireaux et al, 2004
Ishikawa et al, 2004

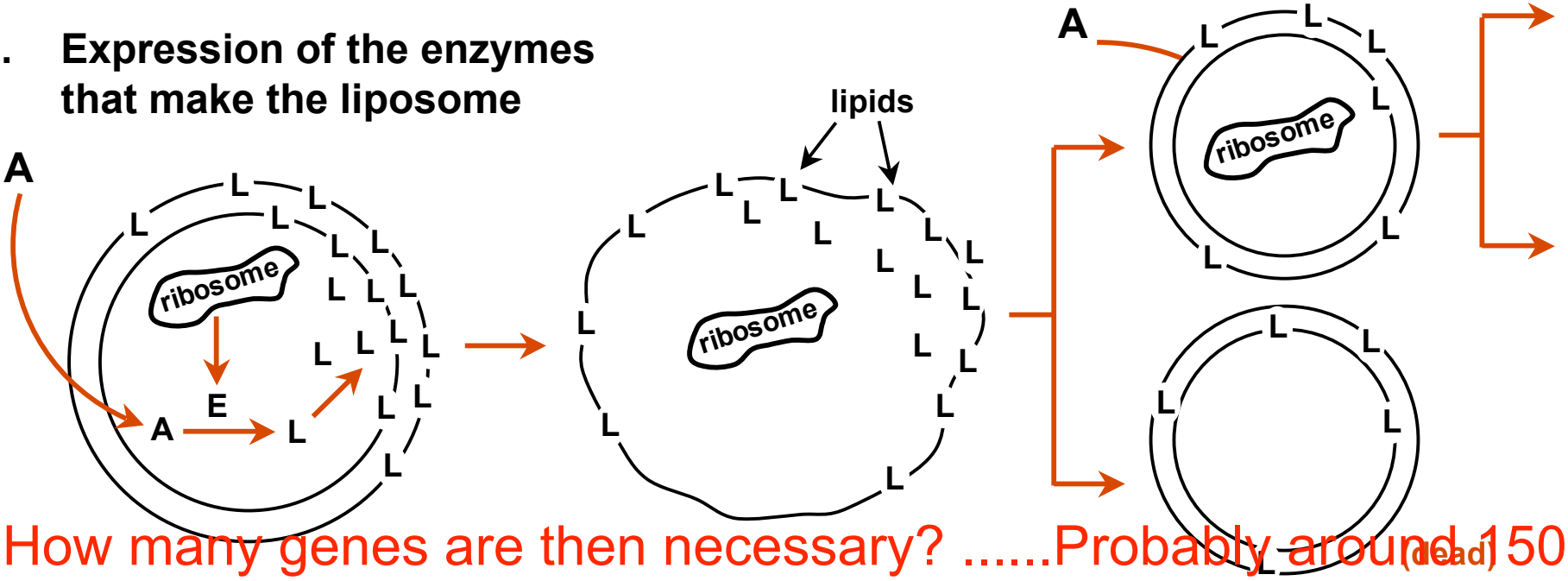

A
a
b
c
d
e
f
g
h
i
B
Fluorescence intensity (a.u.)
0
2
4
6
8
10
12
14
a
b
c
d
e
0.0
0.5
1.0
1.5
15
20
25
Time (h)

Protein expression inside the liposomes
Working plan

1. **Expression of green fluorescent protein (GFP) or any other simple model protein**



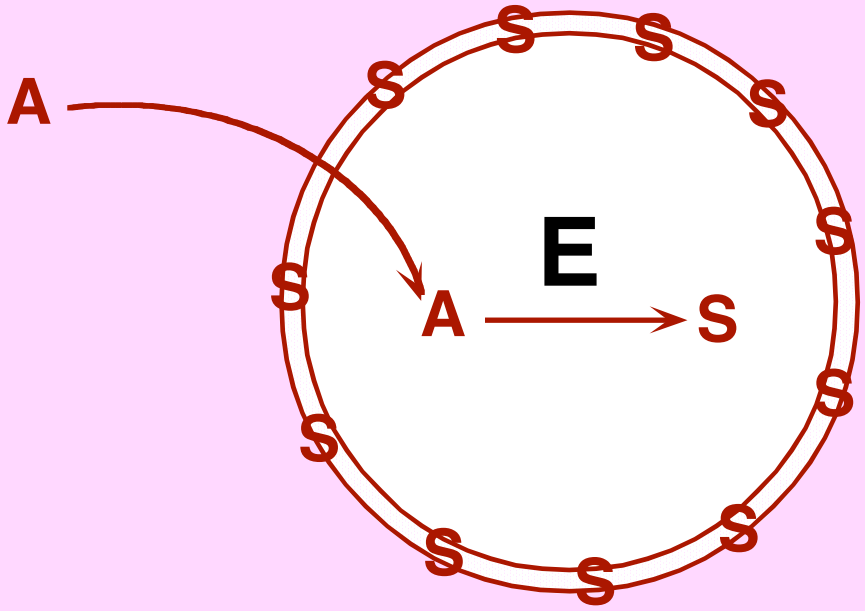
2. **Expression of the enzymes that make the liposome**



How many genes are then necessary? .....Probably around 150 (dead)

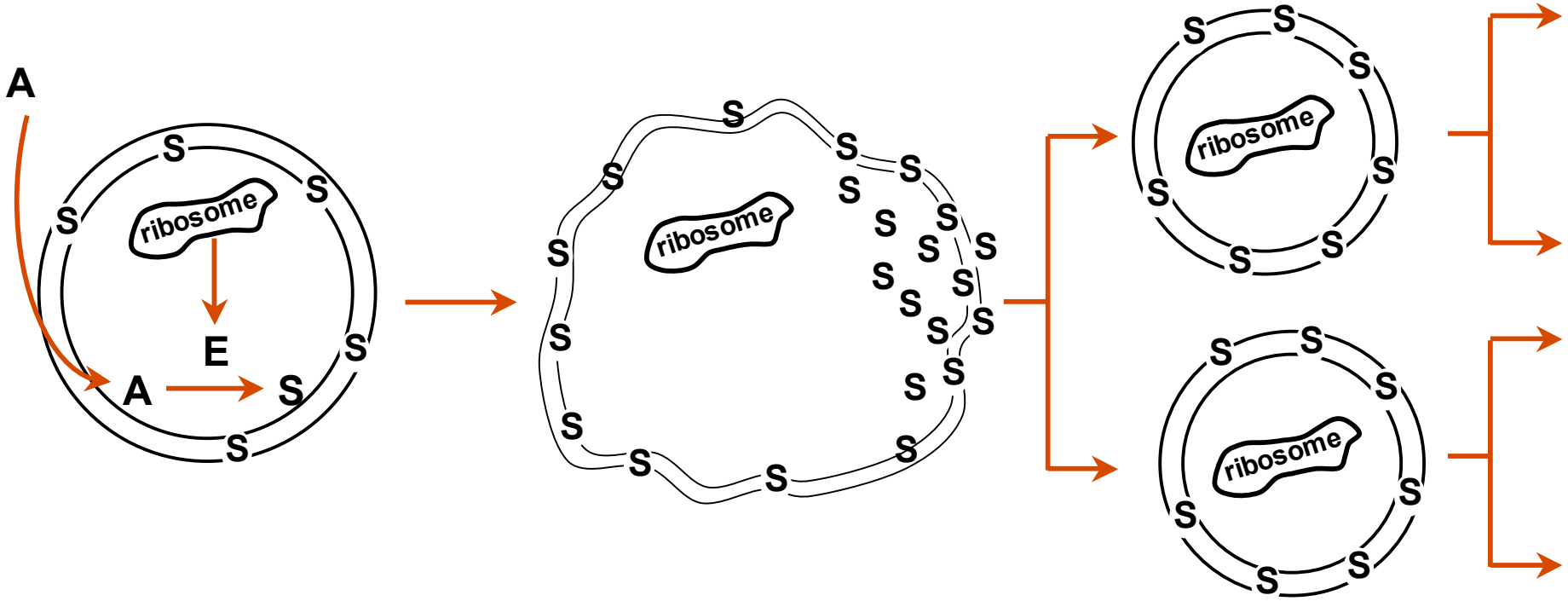
an example of minimal cell :

a cell which creates its boundary



Protein expression inside the liposomes and next step (minimal cell) Working plan

3. **Production of the boundary as in 2., and with self-reproduction of the ribosomes and of the DNA-machinery**



How many genes are then necessary? .....Probably around 200

The work on the minimal living cell
must be accompanied by studies on the
early cells - namely the protocells at
the time before the advent of ribosomes
and before the high selectivity
of modern times —

This would eliminate the 55 ca. genes of the ribosomal proteins
and would reduce the 20 t-RNA genes to a much lower number

→ DOWN TO a final 20-25 GENES??

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Noireaux et al, 2004
Ishikawa et al, 2004

AS A WAY OF CONCLUSION.1

CONCERNING THE TRANSITION TO LIFE FROM THE INANIMATE MATTER:

1. IT HAS NOT BEEN IMPLEMENTED IN THE LAB YET. THEREFORE, IT REMAINS AN HYPOTHESIS.
AND THE BOTTOM UP APPROACH SEEMS TO BE MADE IMPOSSIBLE BY THE LAWS OF CONTINGENCY- CONCEPTUALLY AND EXPERIMENTALLY

2.THE CONSTRUCTION OF SYNTHETIC LIVING CELLS APPEARS POSSIBLE USING EXTANT MACROMOLECULES.
MOST SCIENTISTS BELIEVE, THAT „SOON“,
THIS WILL BE REALIZED.

THE NEW RESEARCH AREA ON THE MINIMAL CELLS INTERESTS NOW ABOUT ONE DOZEN GROUPS AROUND THE WORLD.

WHY IS THIS RESEARCH RELEVANT?

1. UNDERSTANDING THE CHEMICAL ESSENCE OF LIFE BY RECONSTRUCTING IT IN THE LAB

2. UNDERSTANDING OF THE EARLY CELLS

3.BIOTECHNOLOGICAL RELEVANCE (E.G., PROTEINS SYNTHESIS WITH SIMPLE LIPOSOME SYSTEMS

- How far is it master?

- That is irrelevant. Hold your tongue and walk!