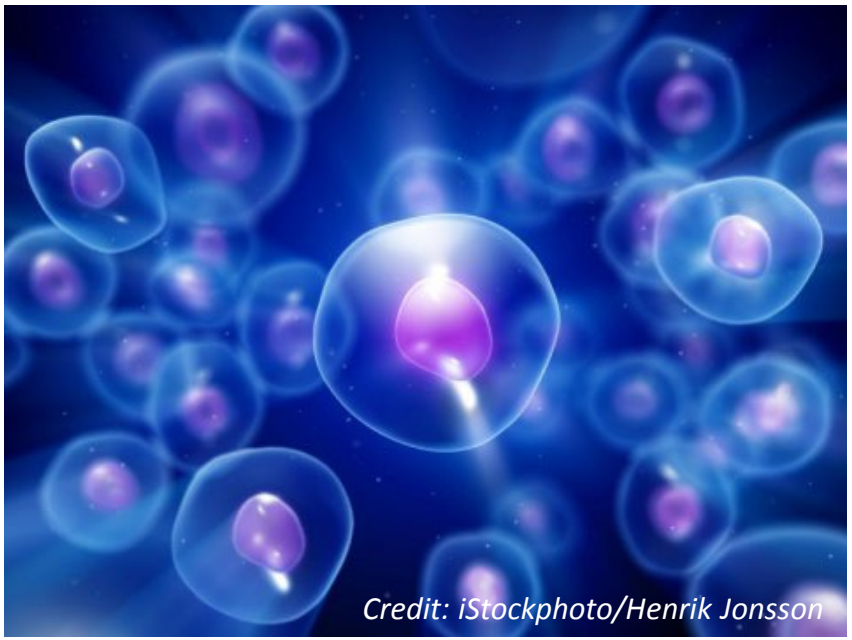
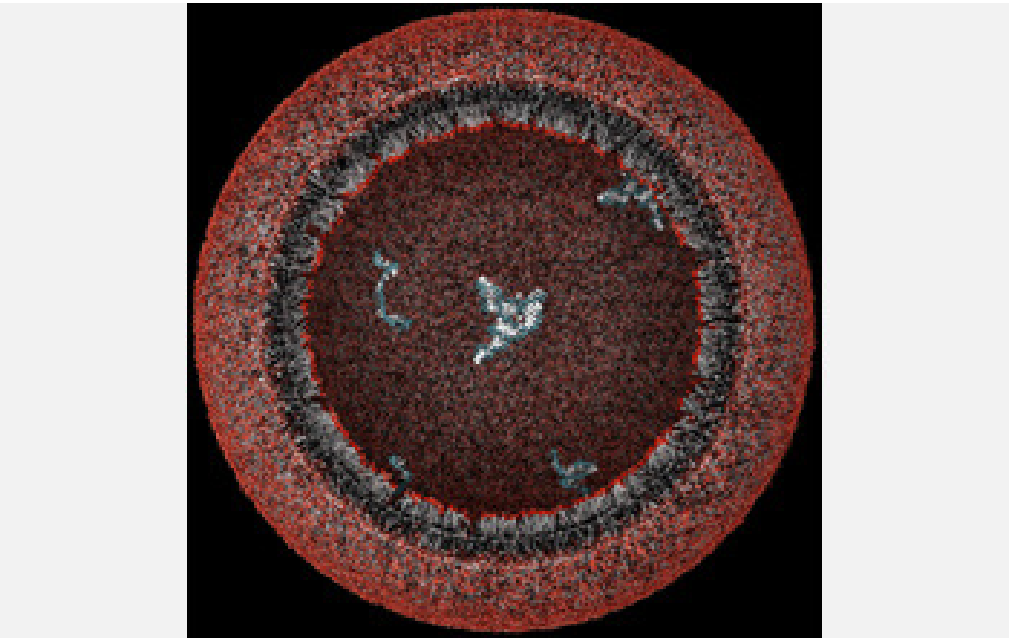
Encapsulation – essential for life



Membrane compartments

Assembly of amphiphilic monomers into protocellular compartments



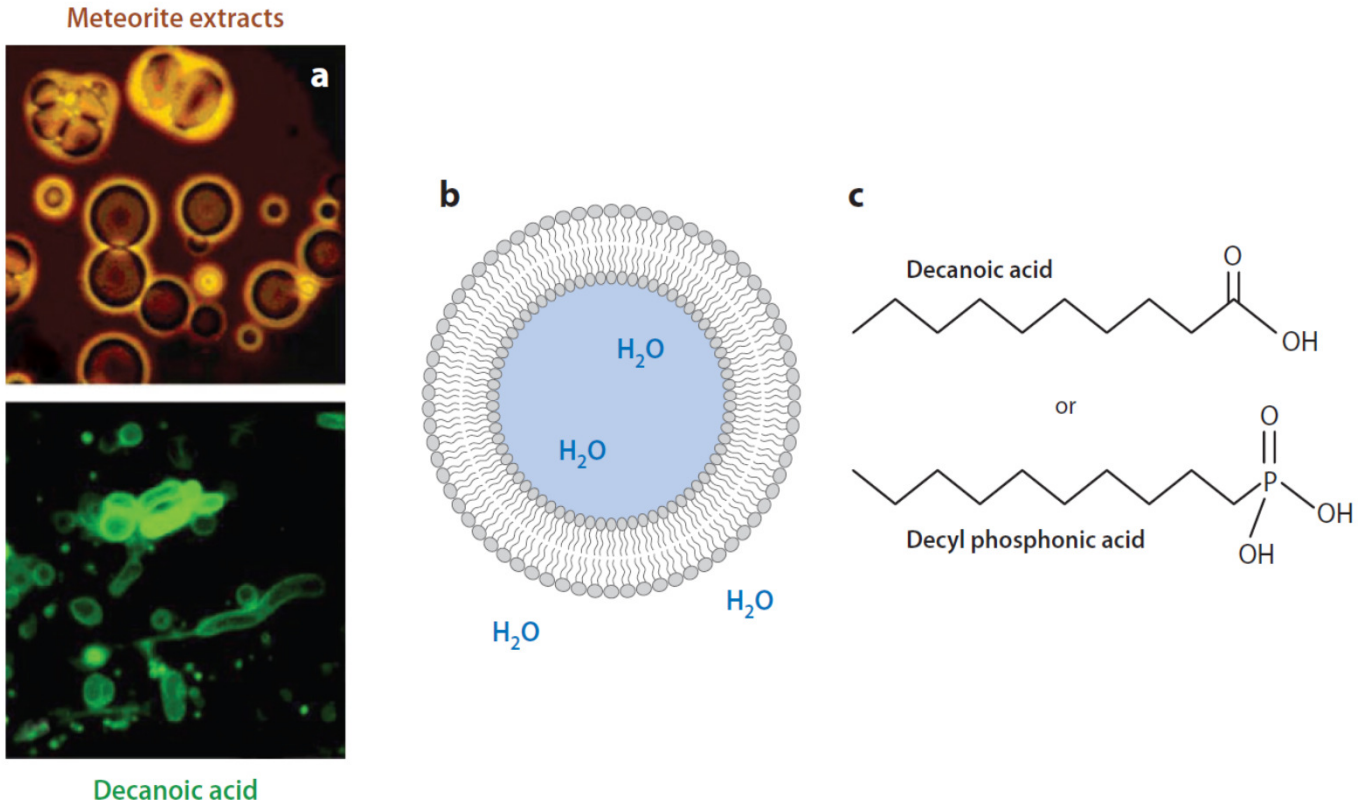
Credit: *Janet Iwasa*

A three-dimensional view of a model protocell (a primitive cell) approximately 100 nanometers in diameter.

The protocell's fatty acid membrane allows nutrients and DNA building blocks to enter the cell and participate in non-enzymatic copying of the cell's DNA. The newly formed strands of DNA remain in the protocell

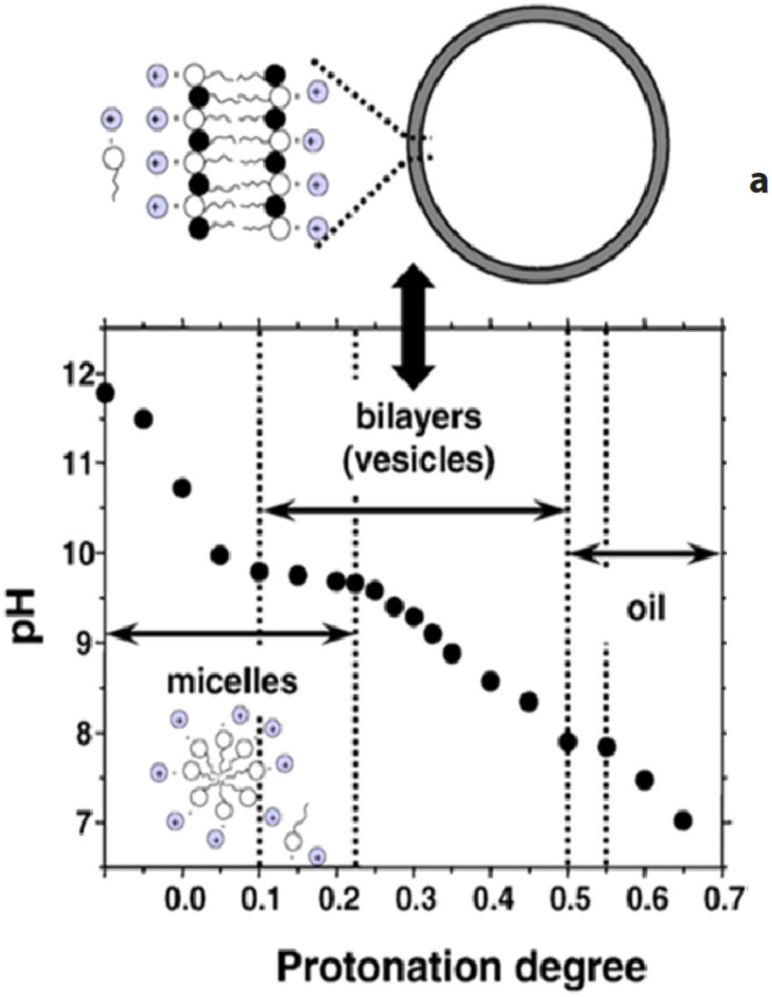
Encapsulation – essential for life

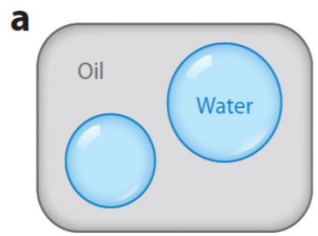
Fatty acids have been found in meteorites – plausible prebiotic synthesis pathways existed in the early Solar System

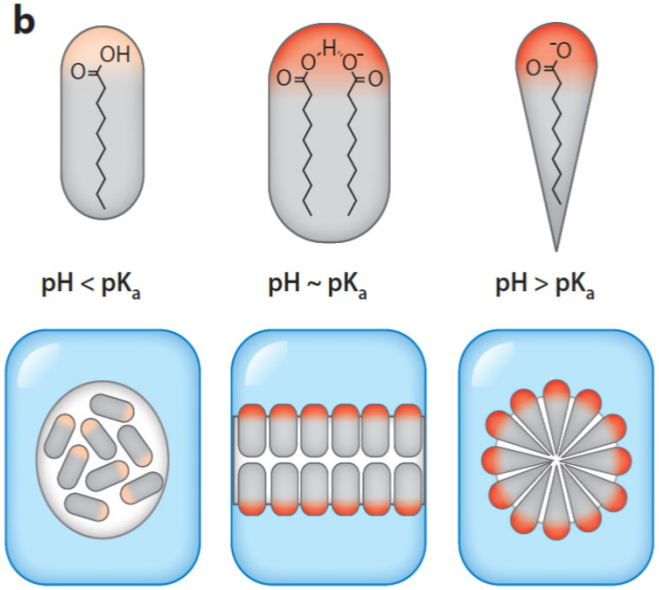


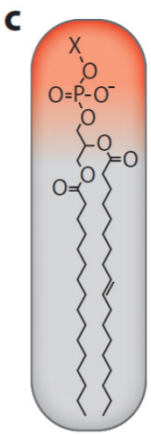
Extracts of meteorites containing these compounds spontaneously form vescicles when hydrated

pH-dependent phase behavior of fatty acids in water



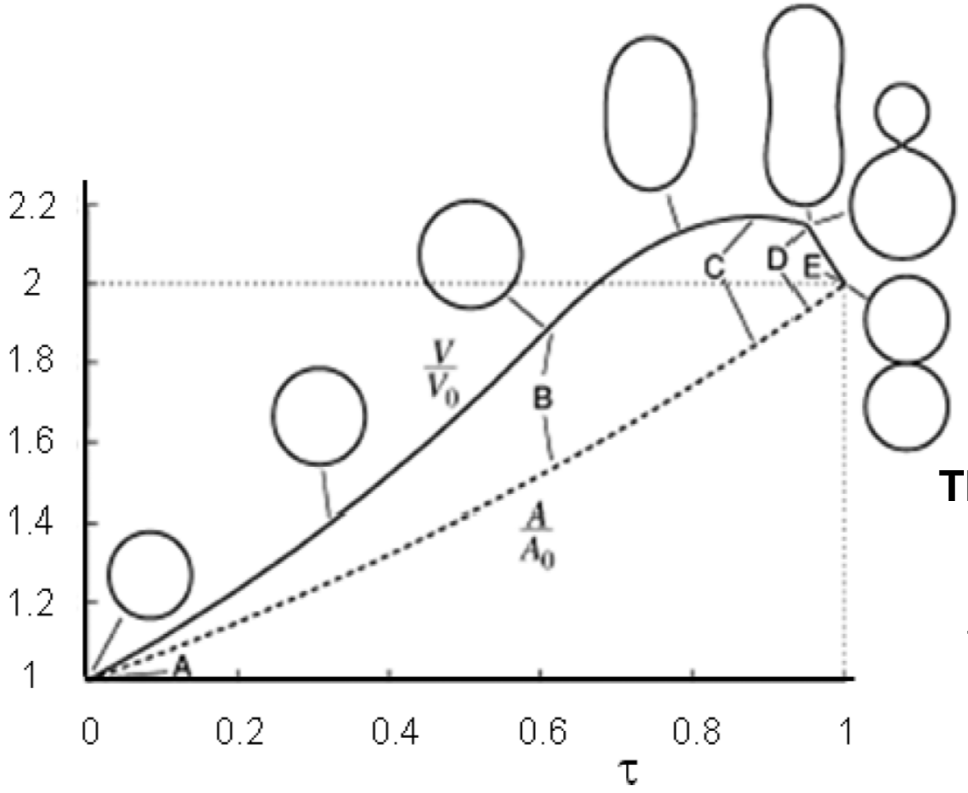


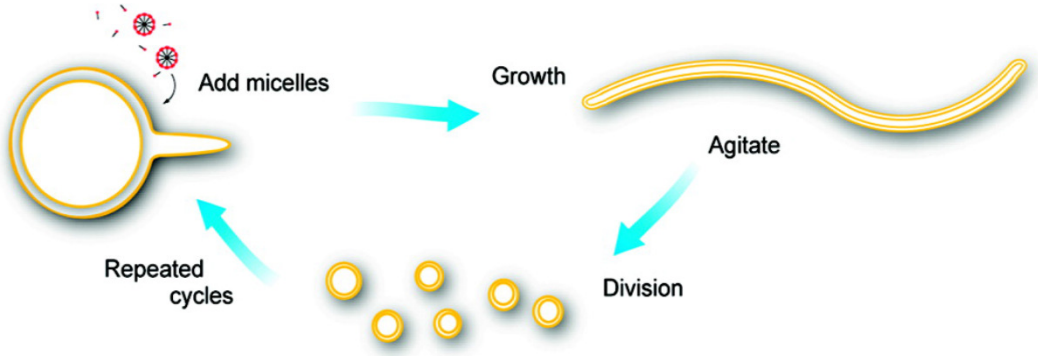




80 mM oleic acid/ sodium oleate in water

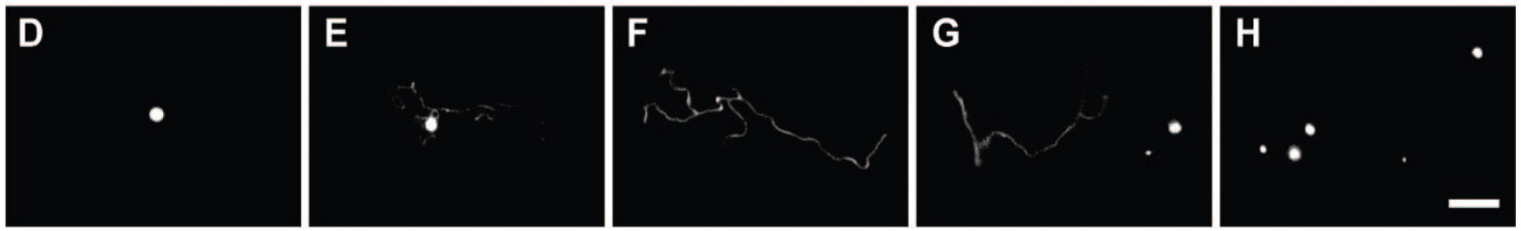
Growth and division of vesicles





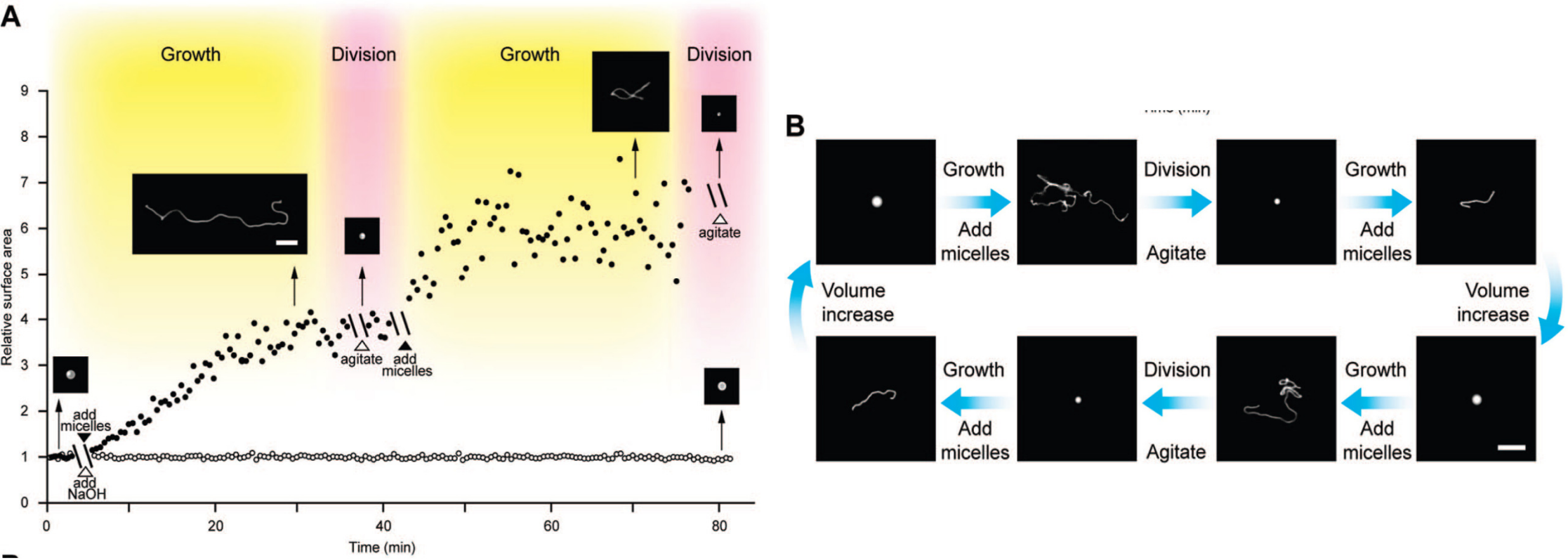
The growth of large multilamellar fatty acid vesicles fed with fatty acid micelles:

when solute permeation across the membranes is slow, the transient imbalance between surface area and volume growth causes formation of long thread-like vesicles. Modest shear forces are then sufficient to divide them into multiple daughter vesicles without loss of internal contents.



Ting F. Zhu, and Jack W. Szostak *J. Am. Chem. Soc.*, **2009**, 131 (15), 5705-5713

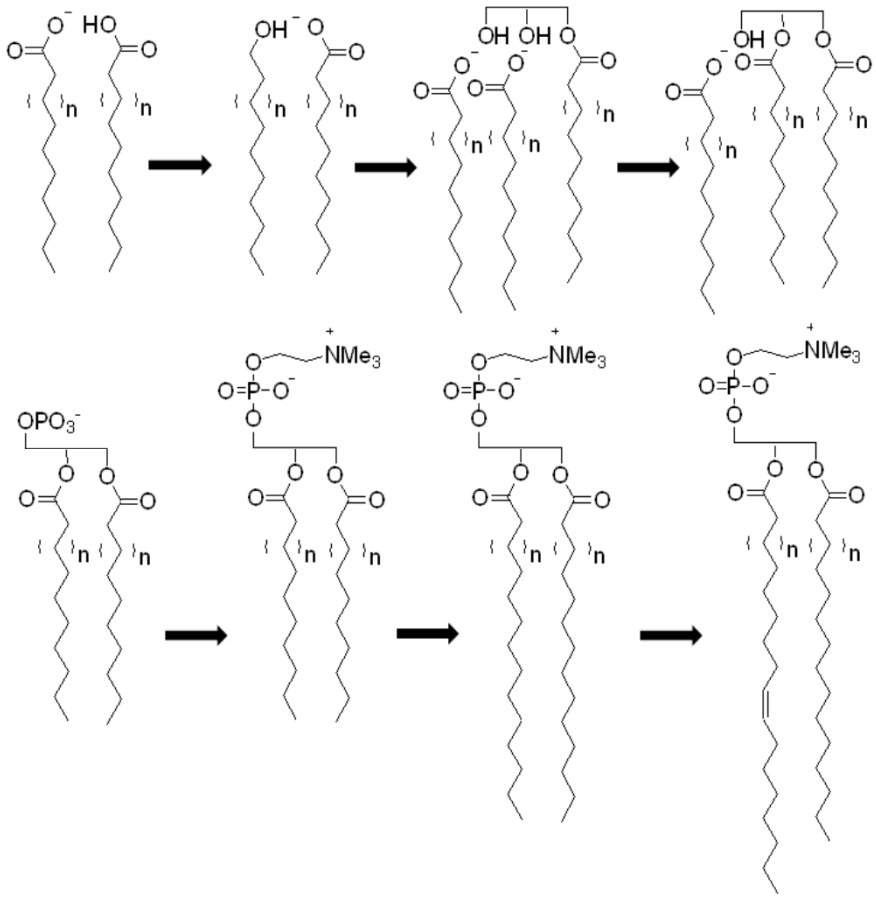
Coupled growth and division of model protocell membranes



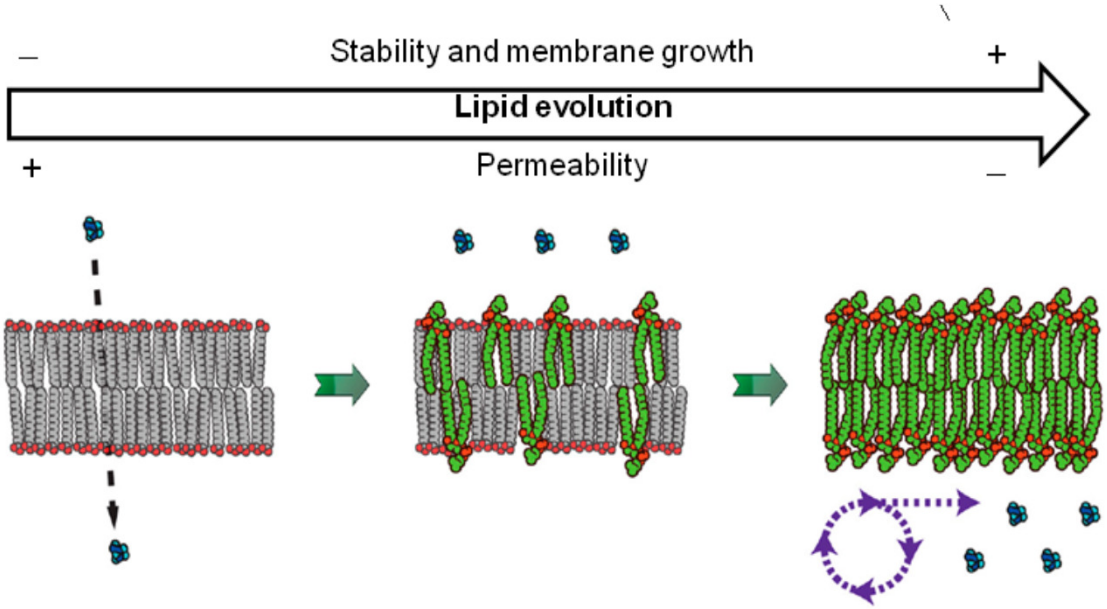
Cycles of vesicle growth and division. (A) Relative surface area after two cycles of addition of 5 equiv of oleate micelles (solid circles) or 5 equiv of NaOH (open circles) to oleate vesicles, each followed by agitation. Inset micrographs show vesicle shapes at indicated times. Scale bar, 10 μm. (B) Vesicle shapes during cycles of growth and division in a model prebiotic buffer (0.2 M Na-glycine, pH 8.5, ~1 mM initial oleic acid, vesicles contain 10 mM HPTS for fluorescence imaging). Scale bar, 20 μm.

Ting F. Zhu, and Jack W. Szostak *J. Am. Chem. Soc.*, **2009**, 131 (15), 5705-5713

Scheme of the membrane evolution

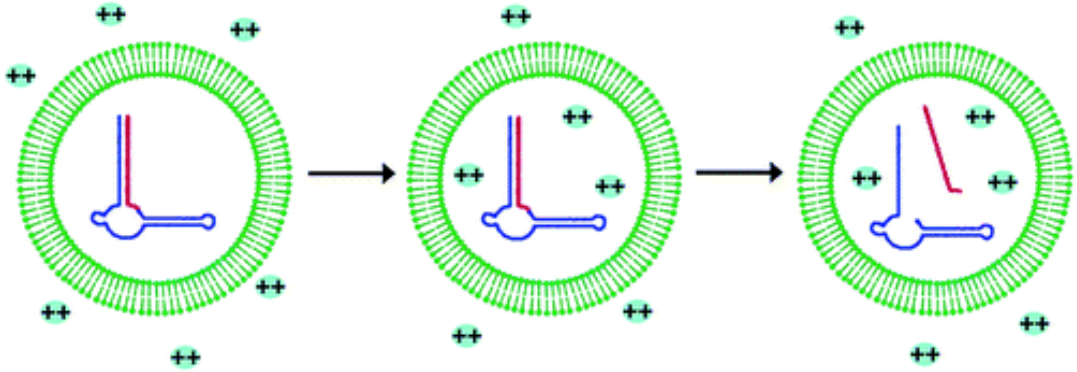


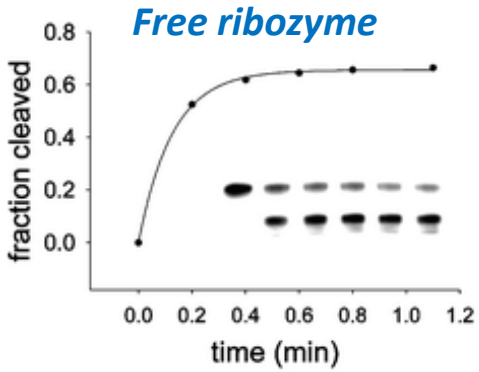
Chemical evolution of membrane components

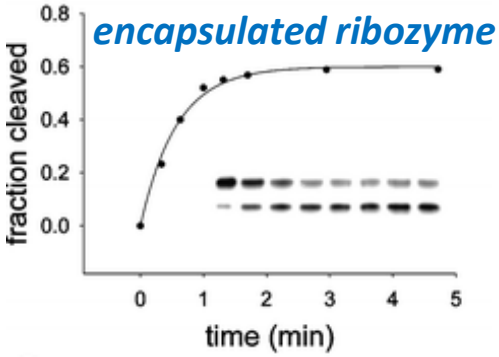


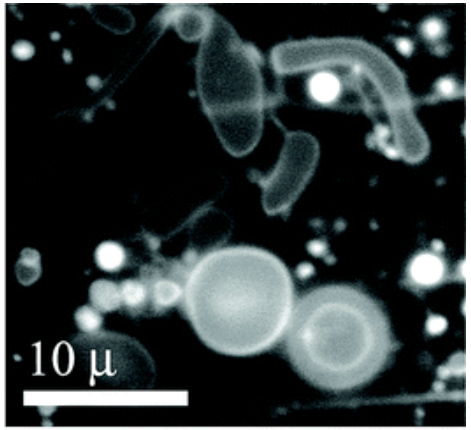
More complex components lead to slower amphiphile desorption and thus faster growth of the protocell. Decreasing permeability is a selective pressure for the emergence of internalized metabolic and transport machinery in the system

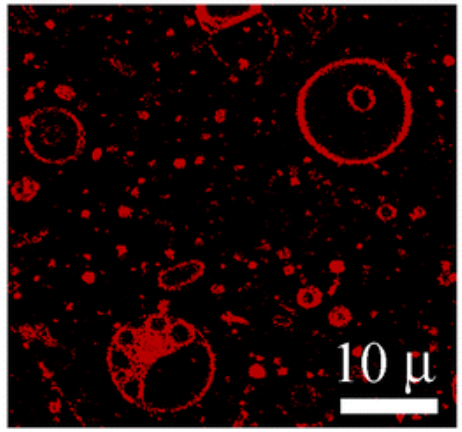
RNA Catalysis in Model Protocell Vesicles











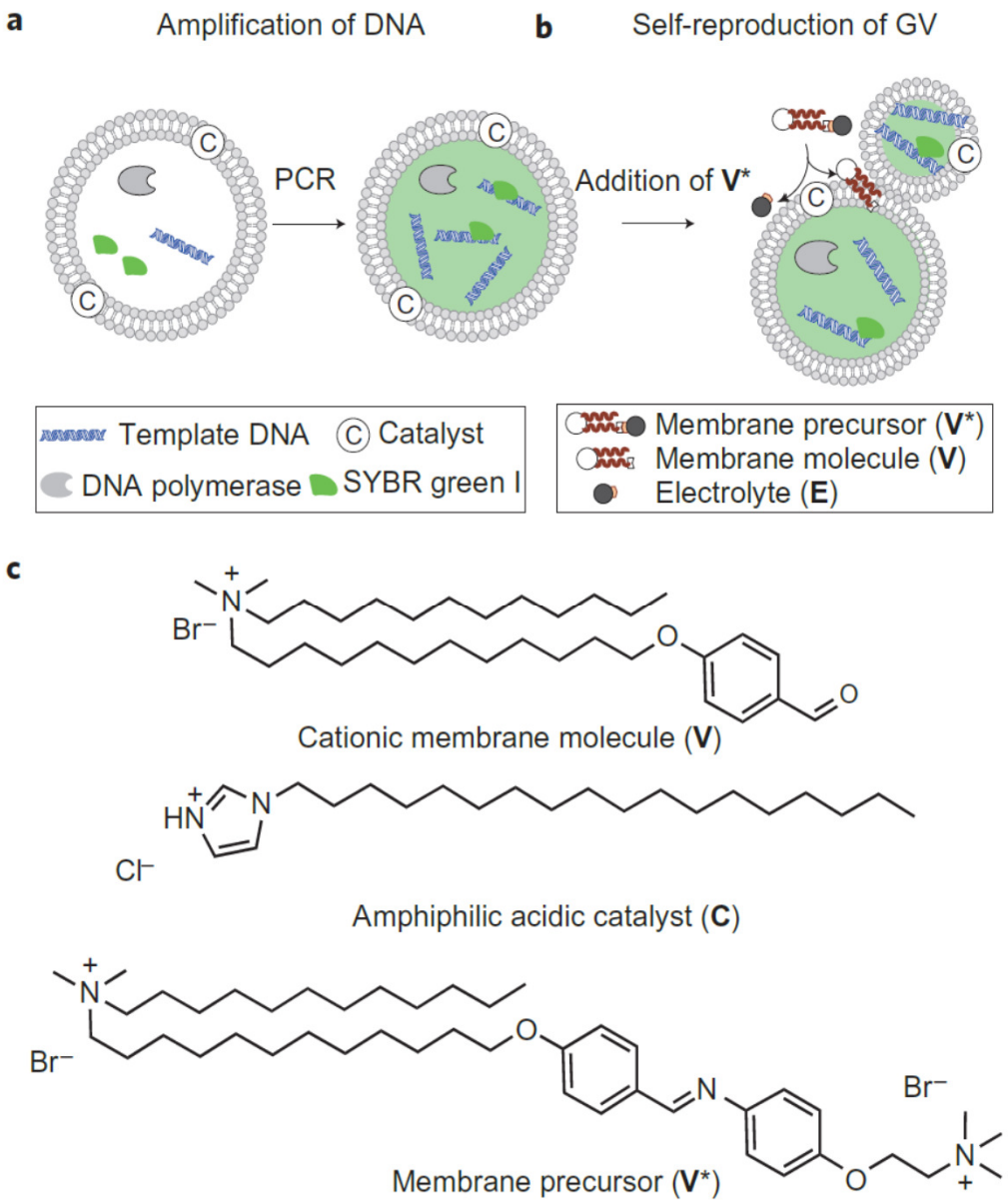
A mixture of myristoleic acid and its glycerol monoester forms vesicles that were Mg^{2+}-tolerant. Mg^{2+} cations can permeate the membrane and equilibrate within a few minutes.

In vesicles encapsulating a hammerhead ribozyme, the addition of external Mg^{2+} led to the activation and self-cleavage of the ribozyme molecules. These vesicles can grow upon addition of micelles. It demonstrates that membranes made from simple amphiphiles can form vesicles that are stable enough to retain encapsulated RNAs in the presence of divalent cations.

Fluorescence microscopy of 2:1:0.3 MA:GMM:dodecane vesicles containing hammerhead ribozyme in the presence of 3 mM $MgCl_2$,

I. A. Chen, K. Salehi-Ashtiani, and J. W. Szostak *J. Am. Chem. Soc.*, **2009**, 127, 13213-13219

Self-reproduction of giant vesicles combined with the amplification of DNA



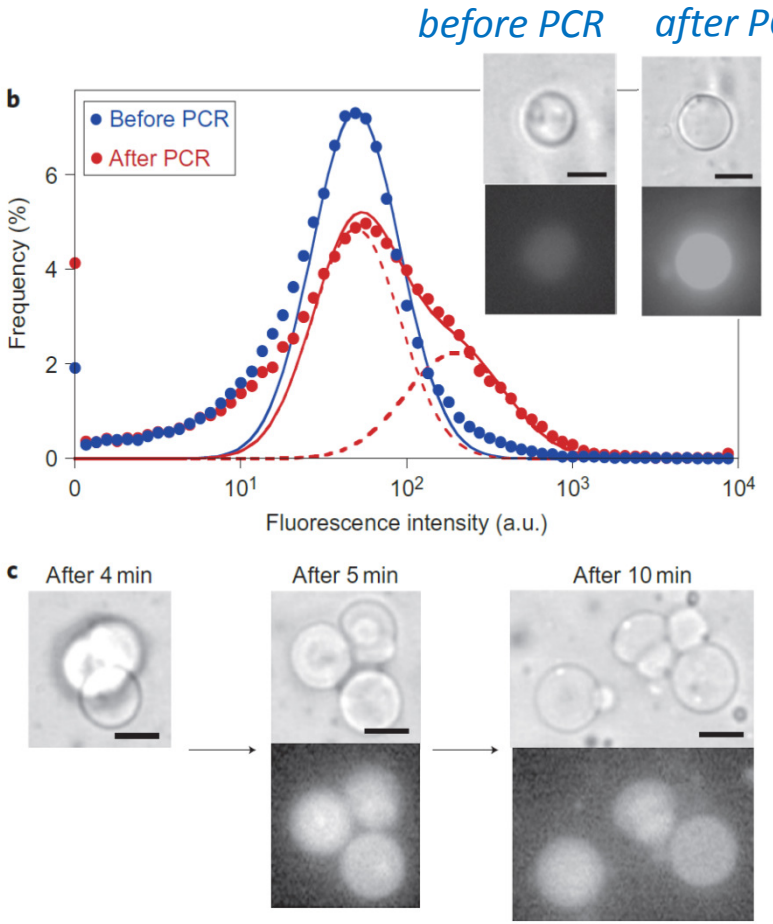
a, ***Amplification of DNA within a GV***. An aqueous dispersion of GVs containing PCR reagents was prepared using a film-swelling method with a buffered solution containing template DNA, primers, fluorescent tag SYBR Green I, deoxynucleoside triphosphates, DNA polymerase and Mg^{2+}.

b, ***Vesicular self-reproduction induced by adding membrane precursor V·.*** Addition of V· produces membrane molecules and electrolytes through hydrolysis assisted by an amphiphilic catalyst. Adhesion of the amplified DNA to the inner leaflet accelerates vesicular growth and division.

c, Chemical structures of membrane molecule V, amphiphile catalyst C and membrane precursor V·.

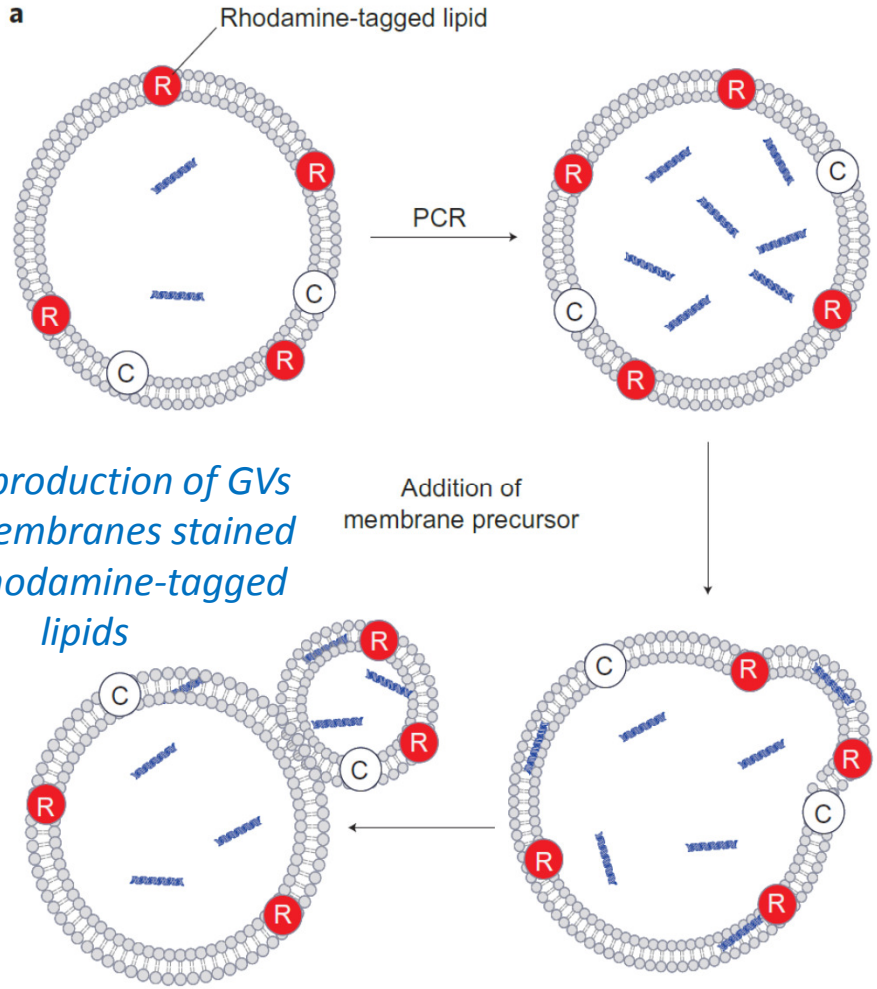
K. Kurihara *et al., Nat. Chem.*, **2011**, 3, 775-781

Self-reproduction of giant vesicles combined with the amplification of DNA



Real-time observation of morphological changes of DNA-amplified GVs after addition of V* .

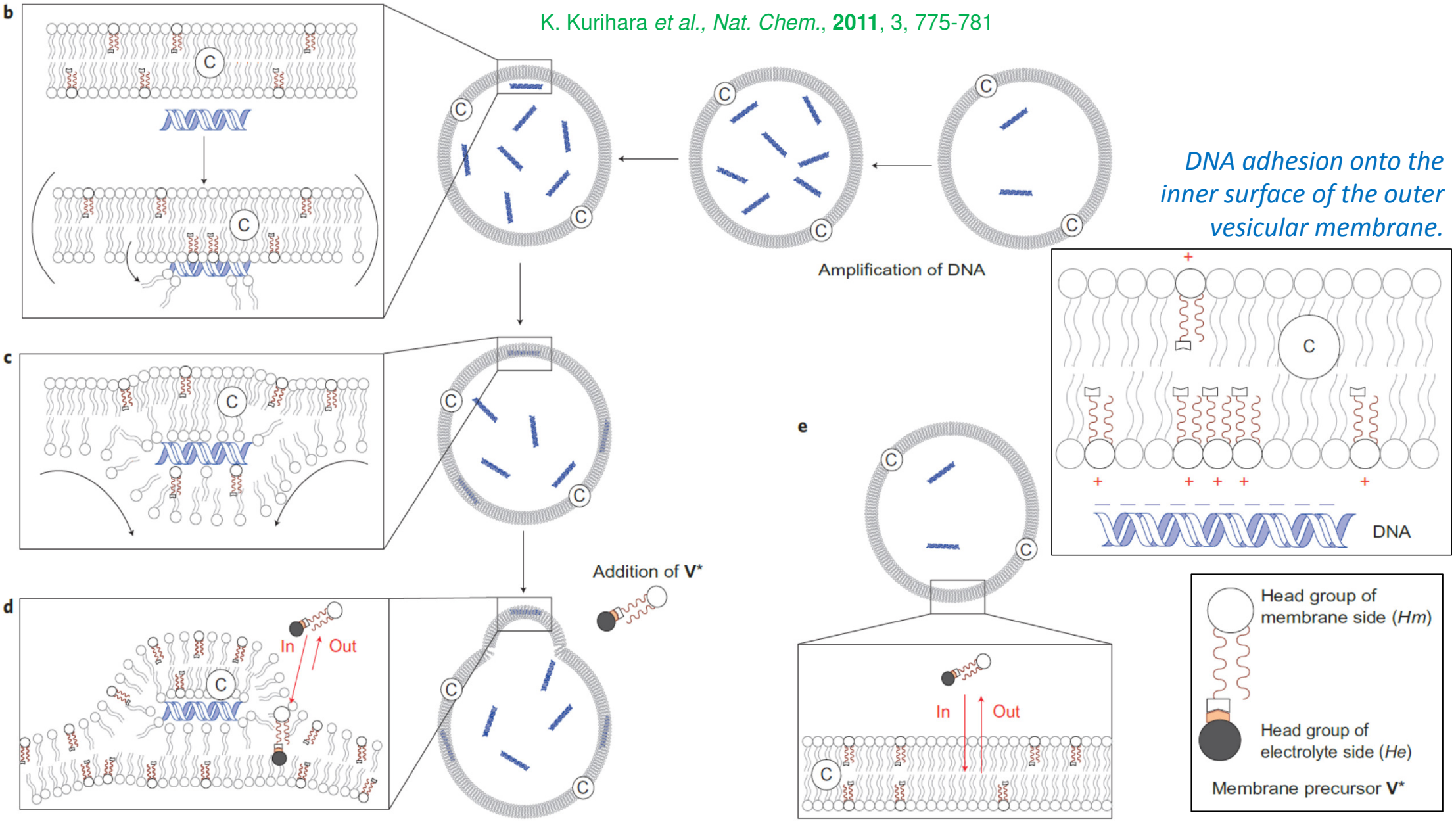
Original GVs began to grow and divide 4 min after adding V. Complete division into four GVs occurred at 5.5 min, and separation occurred at 7 min. Scale bars, 10 µm.*



Self-reproduction of GVs with membranes stained with rhodamine-tagged lipids

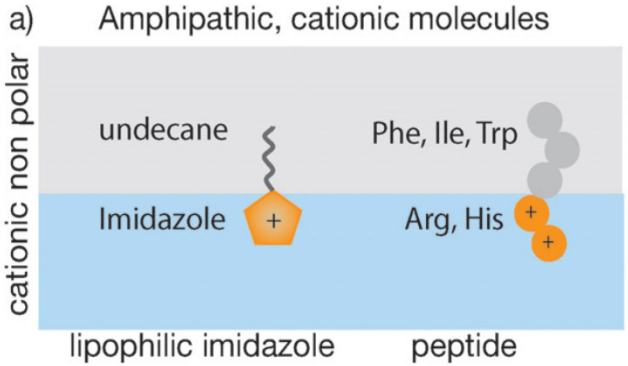
K. Kurihara *et al., Nat. Chem.*, **2011**, 3, 775-781

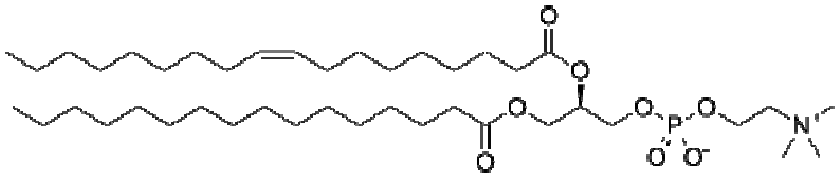
K. Kurihara *et al., Nat. Chem.*, **2011**, 3, 775-781

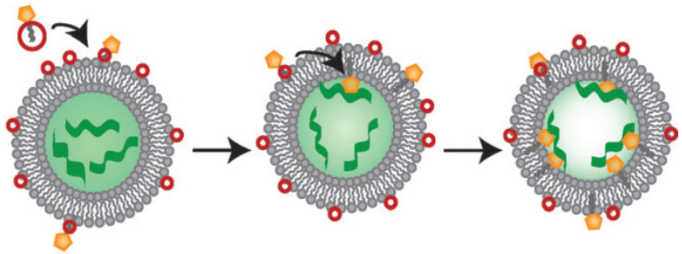


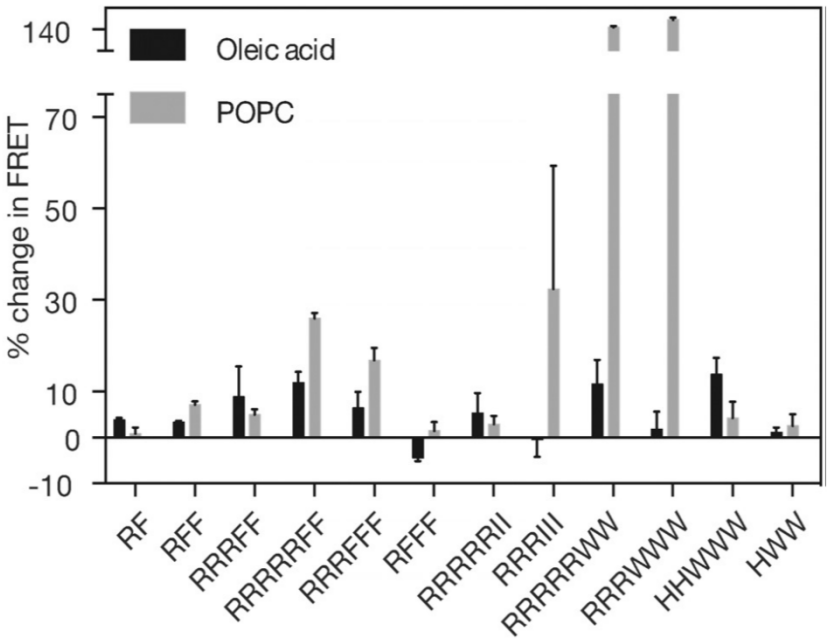
DNA adhesion onto the inner surface of the outer vesicular membrane.

Noncovalent nucleotide association with membranes

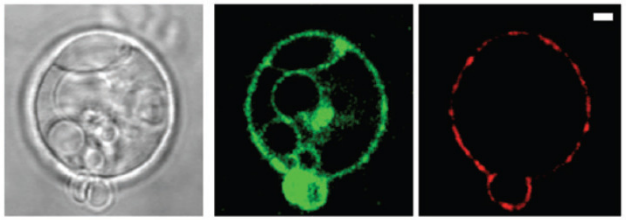








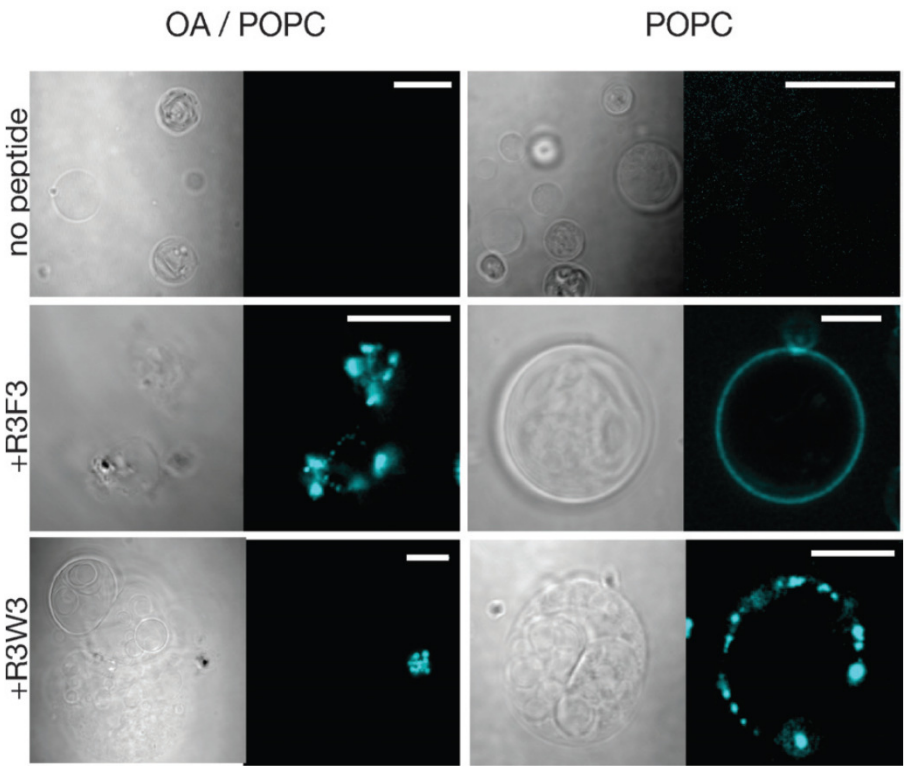
+POPC SUVs with undecylimidazole

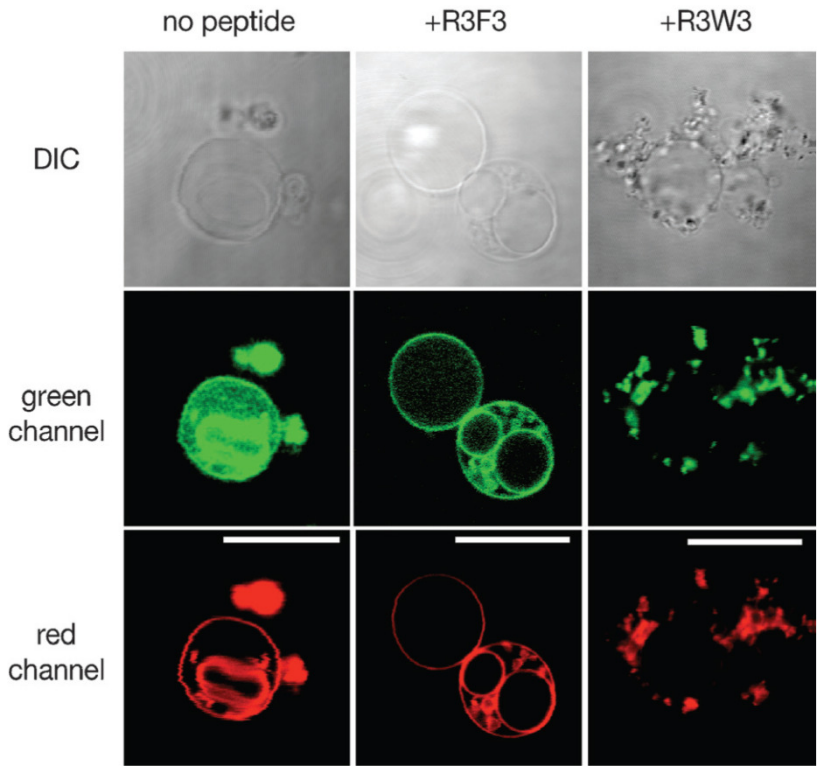


DIC green channel red channel

Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

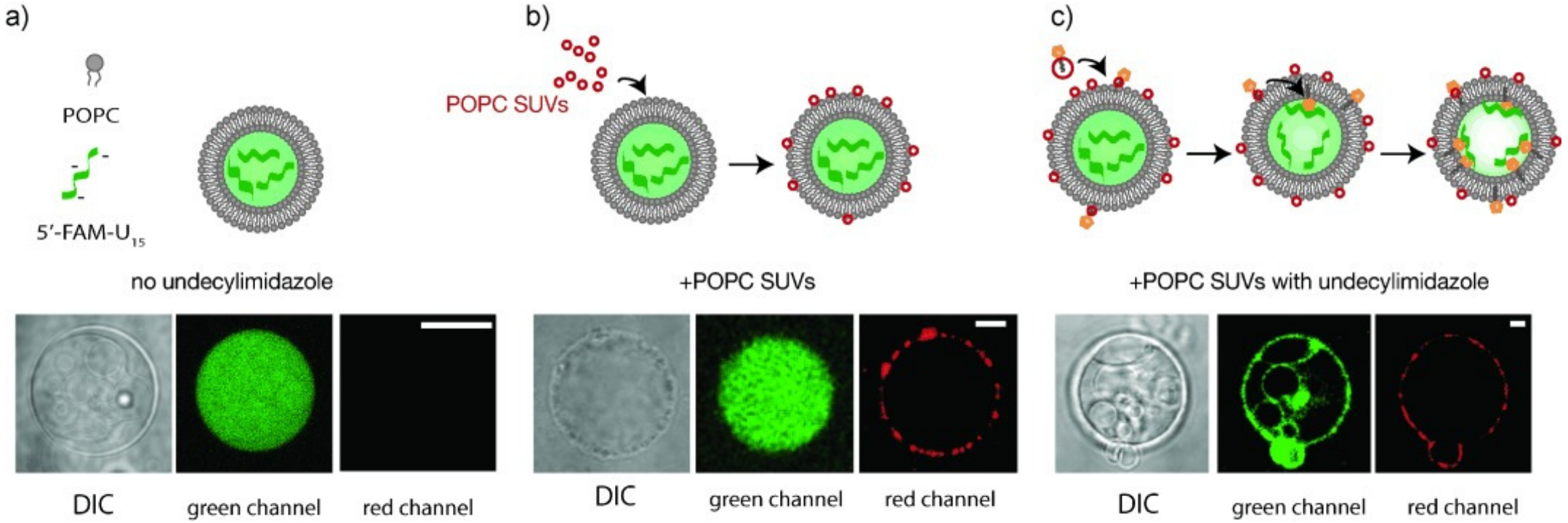
Noncovalent nucleotide association with membranes





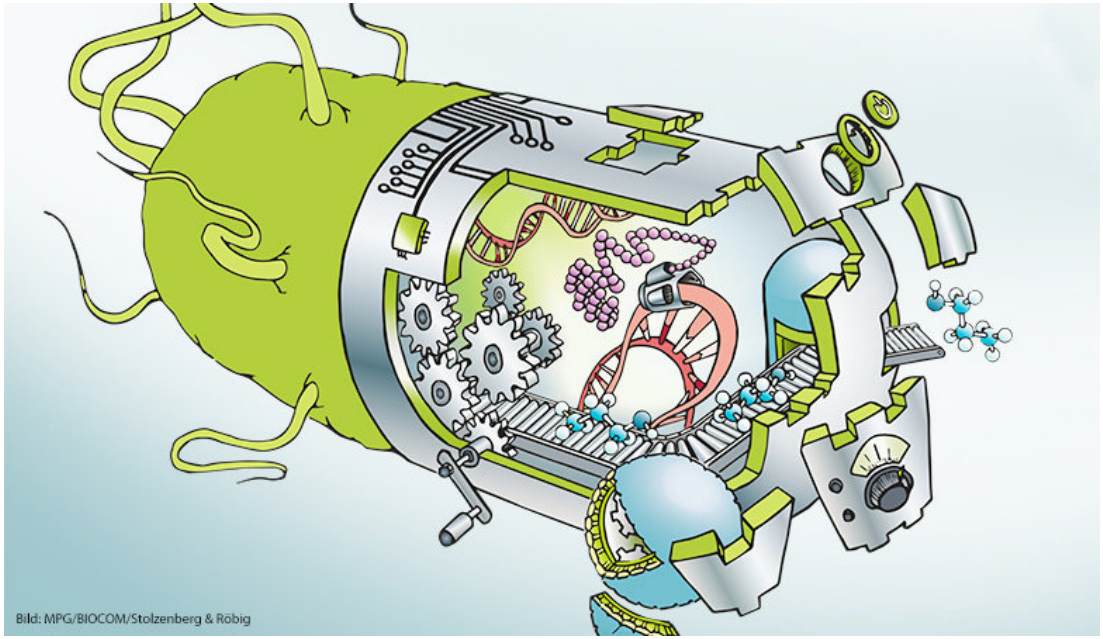
Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

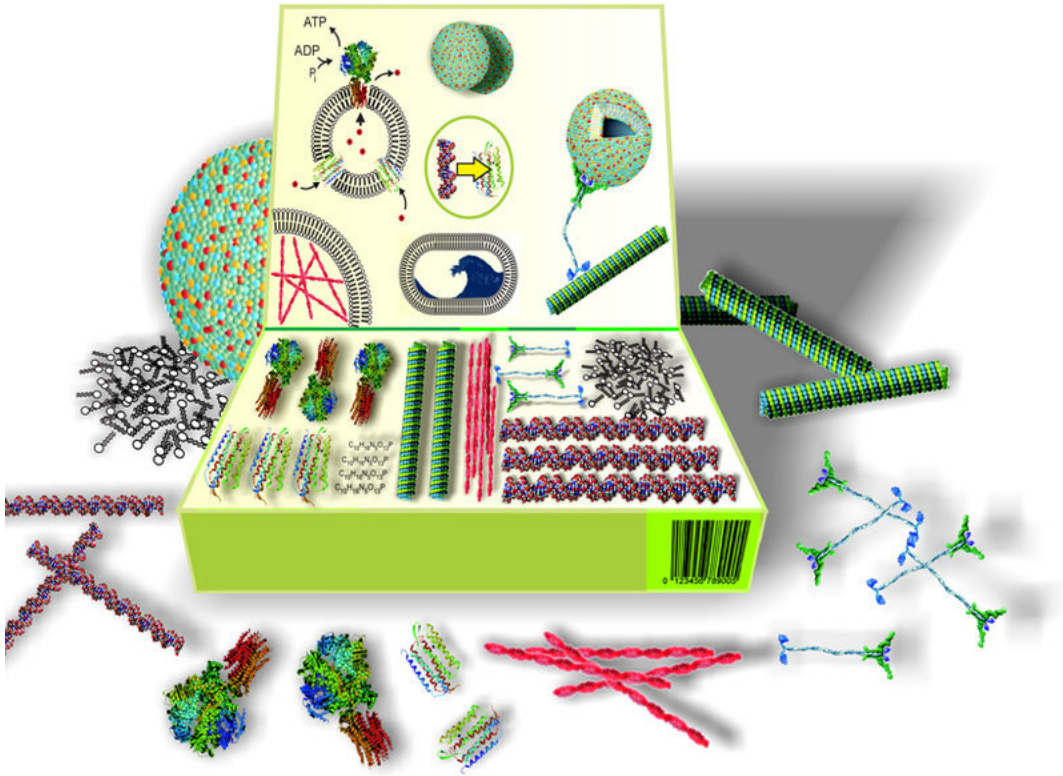
Noncovalent nucleotide association with membranes



Neha P. Kamat, Sylvia Tobe, Ian T. Hill, and Jack W. Szostak *Angew. Chem. Int. Ed.* **2015**, *54*, 11735 –11739

CHAPTER 3





www.mpg.de/themenportal/synthetische-biologie

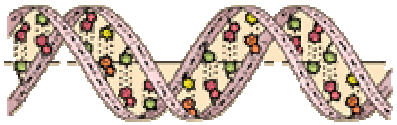
SYNTHETIC BIOLOGY

The great optimism of the 1950's

Watson & Crick

1953

The double-helix structure of DNA



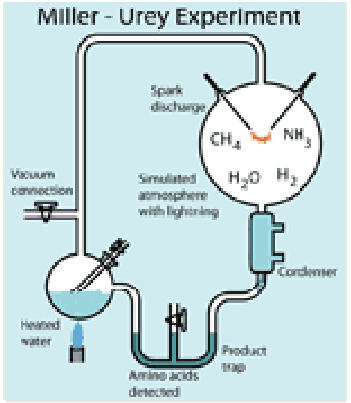
Crick & Watson, April 1953 paper
Nobel Prize 1962

Oparin-Haldane Hypothesis 1924-29

Models for abiogenesis

Miller & Urey

1952



Great optimism about working out the details of "abiogenesis" within a few years.

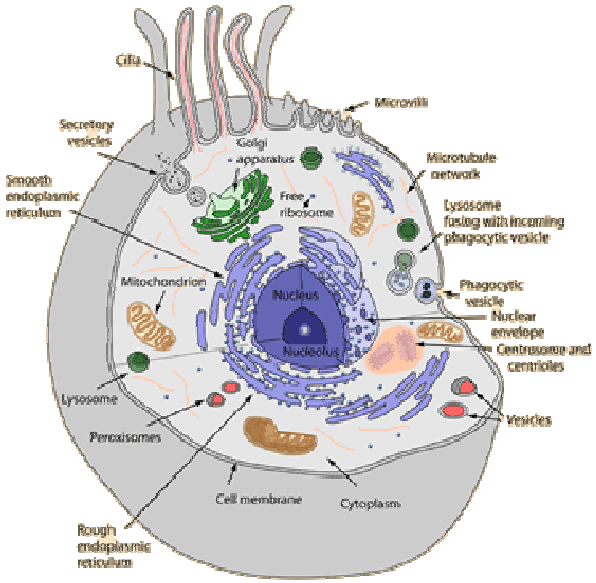
fast forward 60 years!

Prebiotic soup

Replicator-first

Metabolism-first

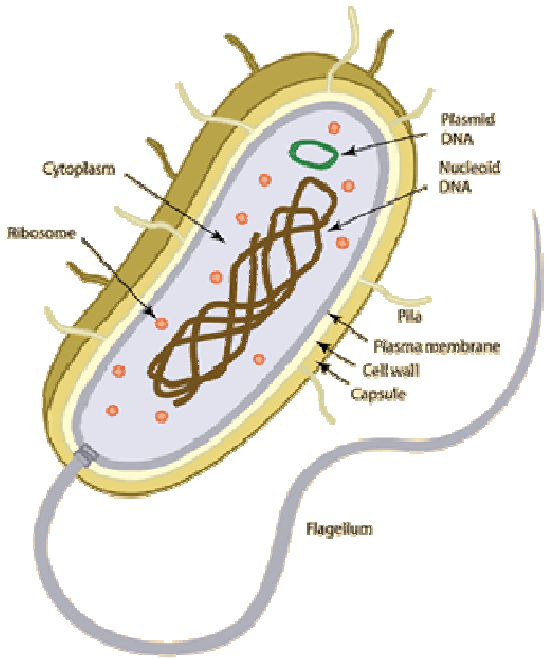
RNA World



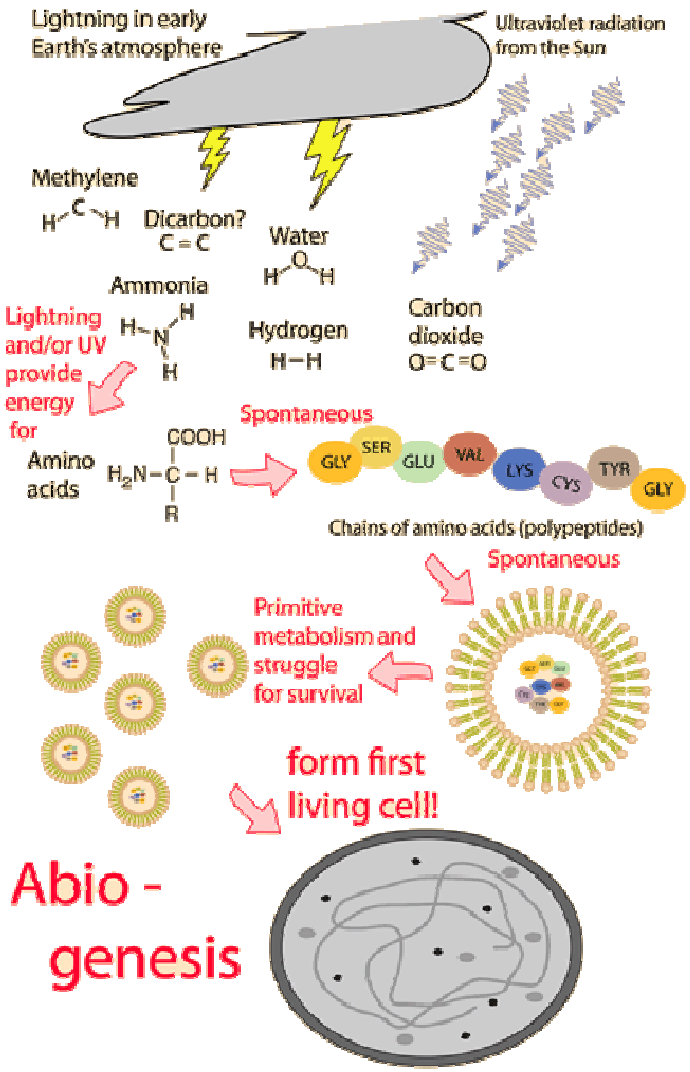
Life obeys the laws of chemistry and physics

Characteristics:

- Life Is Organized
- Life Is Chemically Distinct from Its Environment
- Life Is Homeostatic
- Life Takes Energy and Matter from the Environment and Transforms Them
- Life Responds to Stimuli from the Environment
- Life Reproduces
- Life Is Adapted to Its Environment

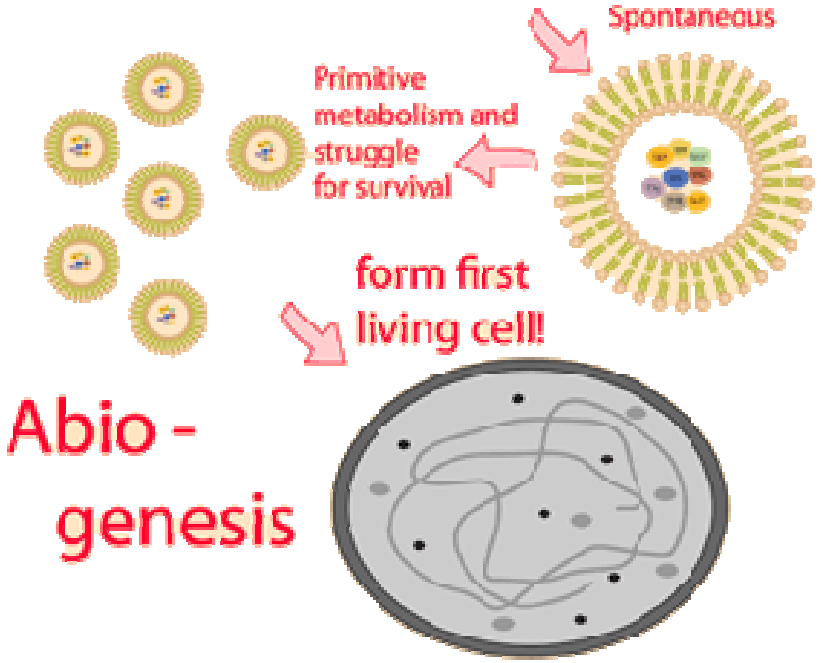


Oparin-Haldane Hypothesis



Oparin (1924) and Haldane (1929) independently hypothesized a scenario for the building of the chemical building blocks of life. Oparin in 1936 discussed further steps that would lead to an origin of life from non-living material, which is popularly called "abiogenesis". The illustration at left summarizes the steps of what has been called the Oparin-Haldane Hypothesis for abiogenesis.

We might need a little more detail on those last steps

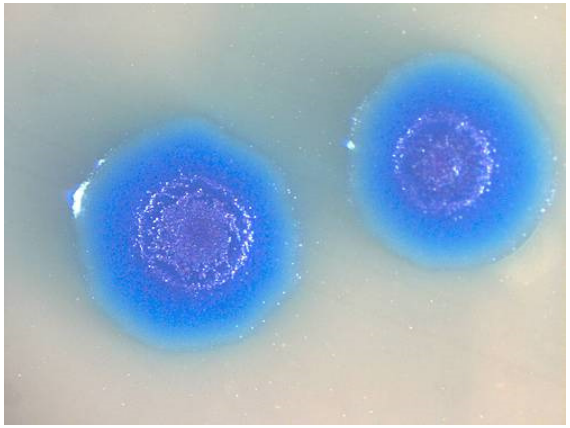


The Oparin-Haldane Hypothesis suggests the action of natural selection in the stages leading from vesicle encapsulation of the biological building blocks to the first living cell.

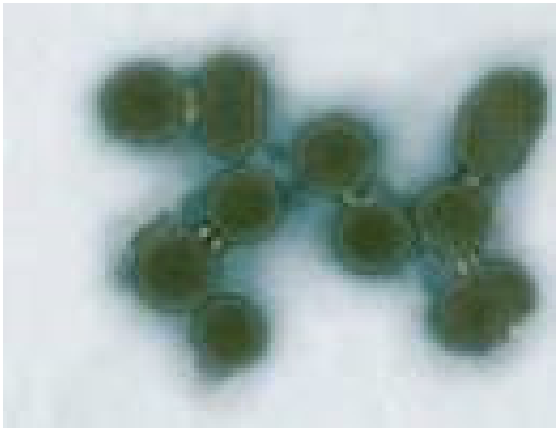
Systems chemistry: bottom-up approach → to build life by self-assembly of biomolecules and biopolymers

Synthetic biology: top-down approach → to simplify currently living organisms and find the lowest limits of „living”

The Minimal Genome Project



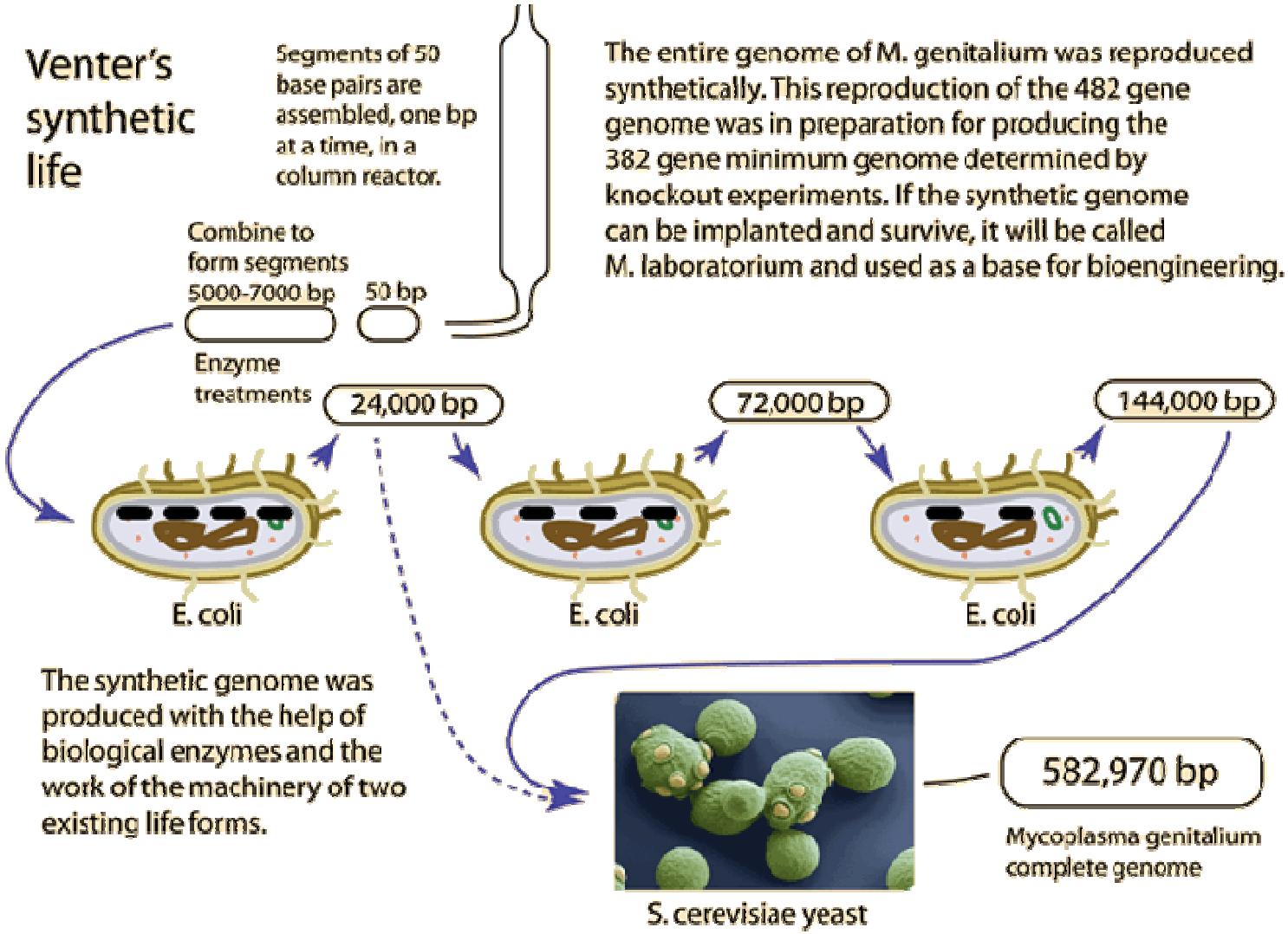
Mycoplasma genitalium

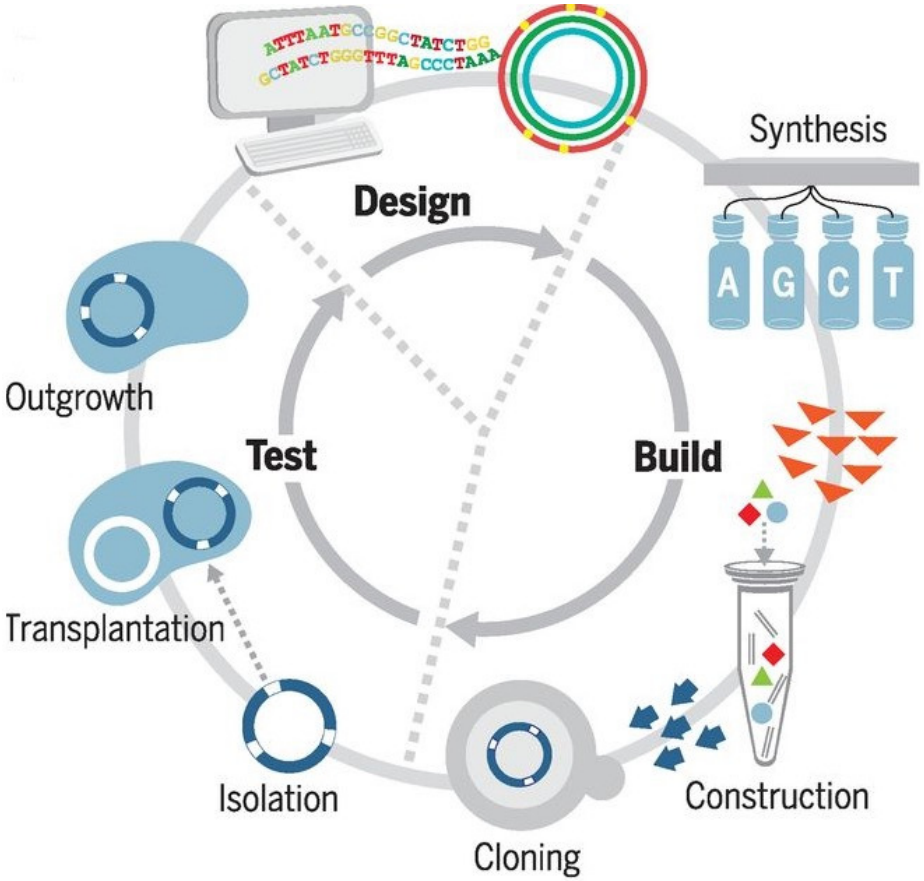


Mycoplasma laboratorium

Mycoplasma laboratorium is a designed, partially synthetic species of bacterium derived from the genome of *Mycoplasma genitalium*. This effort in synthetic biology is being undertaken at the J. Craig Venter Institute by a team of approximately 20 scientists headed by Nobel laureate Hamilton Smith, and including DNA researcher Craig Venter and microbiologist Clyde A. Hutchison III. *Mycoplasma genitalium* was chosen as it was the species with the smallest number of genes known at that time: the genome consists of 482 genes comprising 582,970 base pairs, arranged on one circular chromosome (the smallest genome of any known natural organism that can be grown in free culture). The researchers systematically removed genes to find a minimal set of 382 genes that can sustain life – the synthetic organism *Mycoplasma laboratorium*.

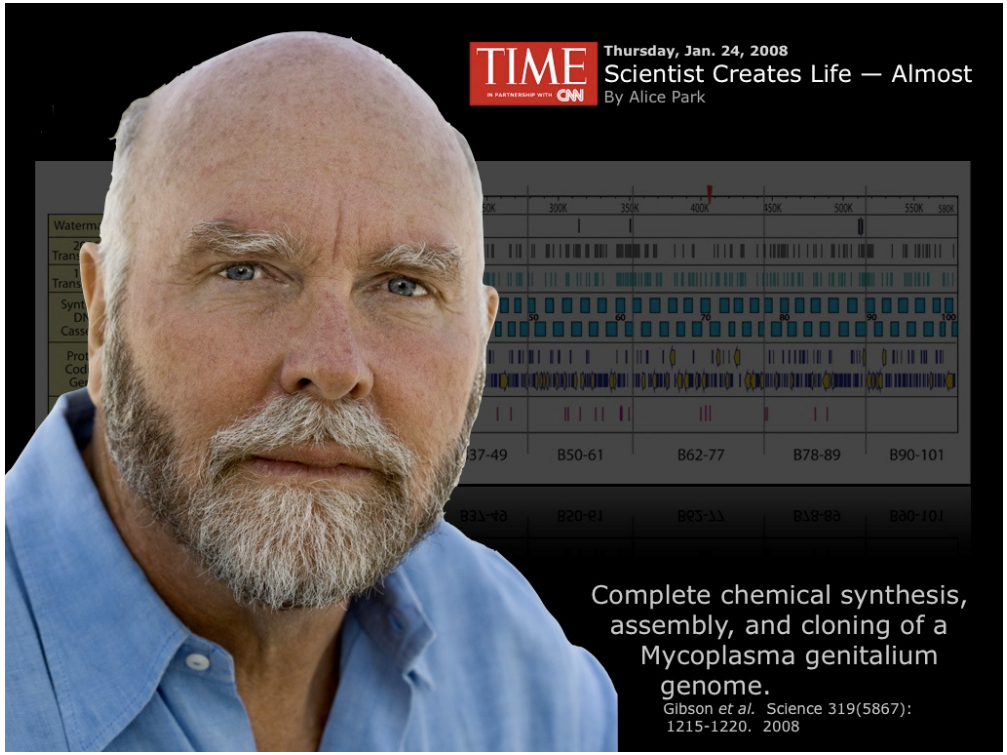
The Minimal Genome Project





The Minimal Genome Project

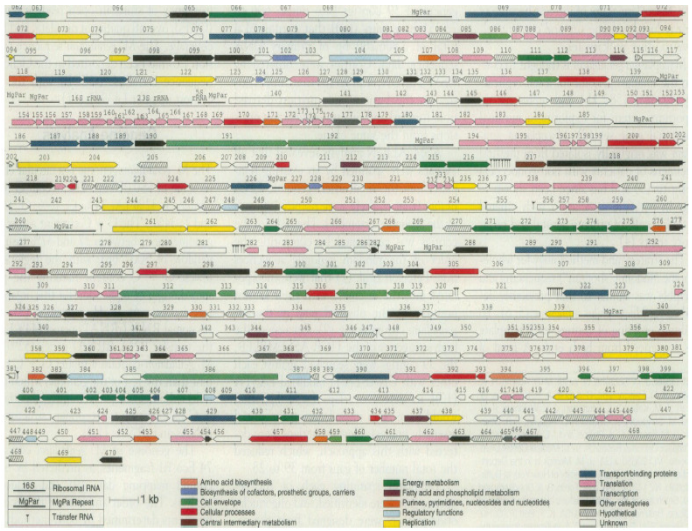
The resulting *Mycoplasma laboratorium* bacterium is expected to be able to replicate itself with its man-made DNA, making it the most synthetic organism to date, although the molecular machinery and chemical environment that would allow it to replicate would not be synthetic. Craig Venter hopes to eventually synthesize bacteria to manufacture hydrogen and biofuels, and also to absorb carbon dioxide and other greenhouse gases.



The Minimal Gene Complement of *Mycoplasma genitalium*

Claire M. Fraser,* Jeannine D. Gocayne, Owen White, Mark D. Adams, Rebecca A. Clayton, Robert D. Fleischmann, Carol J. Bult, Anthony R. Kerlavage, Granger Sutton, Jenny M. Kelley, Janice L. Fritchman, Janice F. Weidman, Keith V. Small, Mina Sandusky, Joyce Fuhrmann, David Nguyen, Teresa R. Utterback, Deborah M. Saudek, Cheryl A. Phillips, Joseph M. Merrick, Jean-Francois Tomb, Brian A. Dougherty, Kenneth F. Bott, Ping-Chuan Hu, Thomas S. Lucier, Scott N. Peterson, Hamilton O. Smith, Clyde A. Hutchison III, J. Craig Venter

SCIENCE • VOL. 270 • 20 OCTOBER 1995



Biological role	*H. influenzae*	*M. genitalium*
Amino acid biosynthesis	68 (6.8)	1 (0.3)
Biosynthesis of cofactors	54 (5.4)	5 (1.6)
Cell envelope	84 (8.3)	17 (5.3)
Celluar processes	53 (5.3)	21 (6.6)
Cell division	16	4
Cell killing	5	2
Chaperones	6	7
Detoxification	3	1
Protein secretion	15	6
Transformation	8	1
Central intermediary metabolism	30 (3)	6 (1.9)
Energy metabolism	112 (10.4)	31 (9.7)
Aerobic	4	3
Amino acids and amines	4	0
Anaerobic	24	0
ATP–proton force interconversion	9	8
Electron transport	9	0
Entner-Doudoroff	9	0
Fermentation	8	0
Gluconeogenesis	2	0
Glycolysis	10	10
Pentose phosphate pathway	3	2
Pyruvate dehydrogenase	4	4
Sugars	15	4
TCA cycle	11	0
Fatty acid and phospholipid metabolism	25 (2.5)	6 (1.9)
Purines, pyrimidines, nucleosides, and nucleotides	53 (5.3)	19 (6.0)
2′-Deoxyribonucleotide metabolism	8	3
Nucleotide and nucleoside interconversions	3	1
Purine ribonucleotide biosynthesis	18	3
Pyrimidine ribonucleotide biosynthesis	5	0
Salvage of nucleosides and nucleotides	13	10
Sugar-nucleotide biosynthesis and conversions	6	2
Regulatory functions	64 (6.3)	7 (2.2)
Replication	87 (8.6)	32 (10.0)
Degradation of DNA	8	1
DNA replication, restriction, modification, recombination, and repair	76	31
Transcription	27 (2.7)	12 (3.8)
Degradation of RNA	10	2
RNA synthesis and modification, DNA transcription	17	10
Translation	141 (14)	101 (31.8)
Transport and binding proteins	123 (12.2)	34 (10.7)
Amino acids and peptides	38	10
Anions	8	3
Carbohydrates	30	12
Cations	24	1
Other transporters	22	8
Other categories	93 (9.2)	27 (8.2)
Unassigned role	736 (43)	152 (32)
No database match	389	96
Match hypothetical proteins	347	56

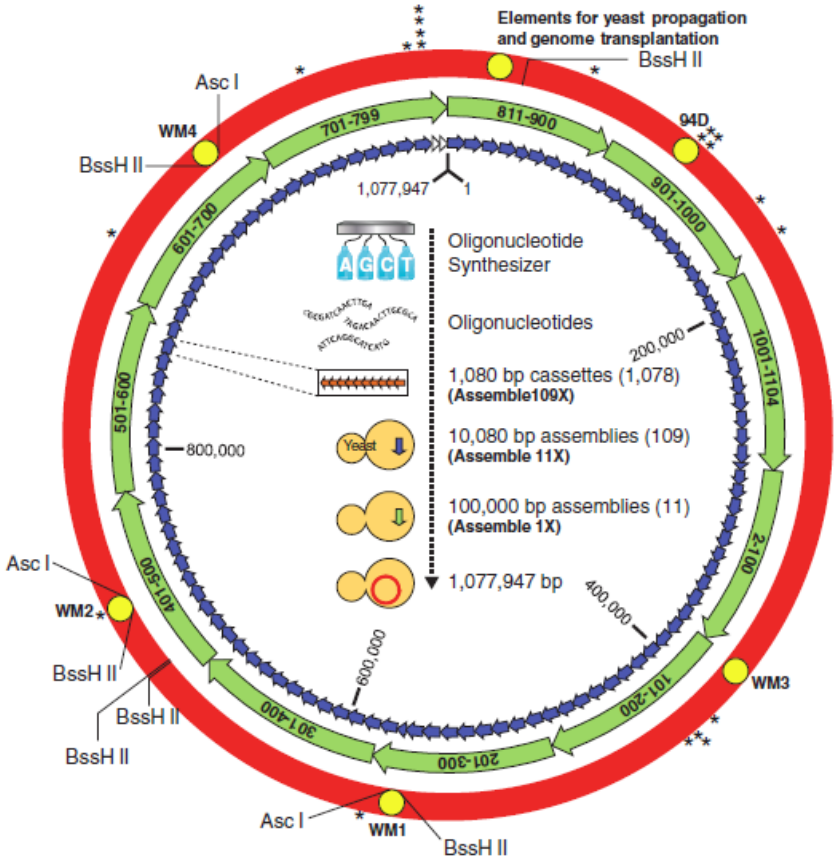
Global Transposon Mutagenesis and a Minimal Mycoplasma Genome

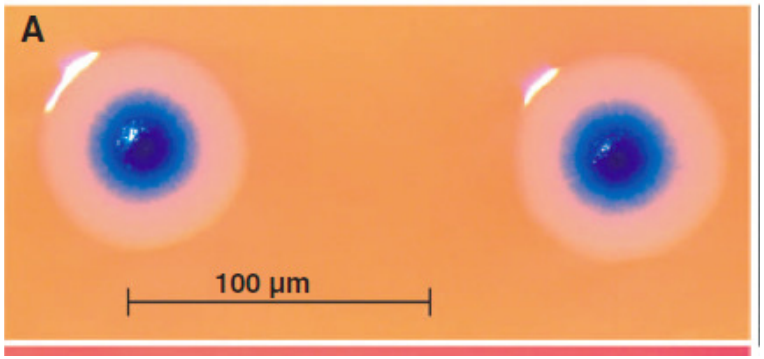
Clyde A. Hutchison III,[1,2*] Scott N. Peterson,[1*†] Steven R. Gill,[1] Robin T. Cline,[1] Owen White,[1] Claire M. Fraser,[1] Hamilton O. Smith,[1‡] J. Craig Venter[1‡§]

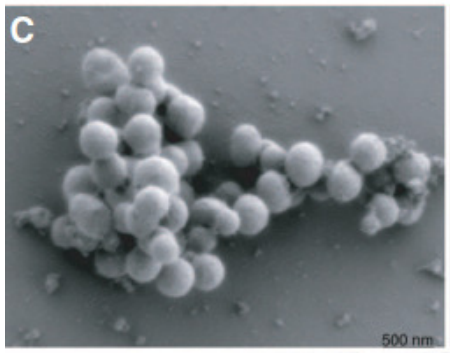
Mycoplasma genitalium with 517 genes has the smallest gene complement of any independently replicating cell so far identified. Global transposon mutagenesis was used to identify nonessential genes in an effort to learn whether the naturally occurring gene complement is a true minimal genome under laboratory growth conditions. The positions of 2209 transposon insertions in the completely sequenced genomes of *M. genitalium* and its close relative *M. pneumoniae* were determined by sequencing across the junction of the transposon and the genomic DNA. These junctions defined 1354 distinct sites of insertion that were not lethal. The analysis suggests that 265 to 350 of the 480 protein-coding genes of *M. genitalium* are essential under laboratory growth conditions, including about 100 genes of unknown function.

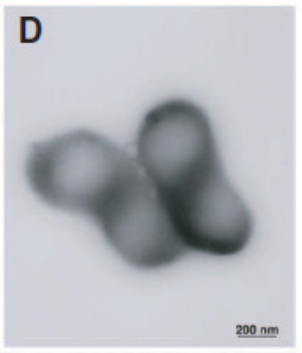
Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome

Daniel G. Gibson,[1] John I. Glass,[1] Carole Lartigue,[1] Vladimir N. Noskov,[1] Ray-Yuan Chuang,[1] Mikkel A. Algire,[1] Gwynedd A. Benders,[2] Michael G. Montague,[1] Li Ma,[1] Monzia M. Moodie,[1] Chuck Merryman,[1] Sanjay Vashee,[1] Radha Krishnakumar,[1] Nacyra Assad-Garcia,[1] Cynthia Andrews-Pfannkoch,[1] Evgeniya A. Denisova,[1] Lei Young,[1] Zhi-Qing Qi,[1] Thomas H. Segall-Shapiro,[1] Christopher H. Calvey,[1] Prashanth P. Parmar,[1] Clyde A. Hutchison III,[2] Hamilton O. Smith,[2] J. Craig Venter[1,2]*









Whole Genome Synthesis

Complete Chemical Synthesis, Assembly, and Cloning of a *Mycoplasma genitalium* Genome

Daniel G. Gibson, Gwynedd A. Benders, Cynthia Andrews-Pfannkoch, Evgeniya A. Denisova, Holly Baden-Tillson, Jayshree Zaveri, Timothy B. Stockwell, Anushka Brownley, David W. Thomas, Mikkel A. Algire, Chuck Merryman, Lei Young, Vladimir N. Noskov, John I. Glass, J. Craig Venter, Clyde A. Hutchison III, Hamilton O. Smith*

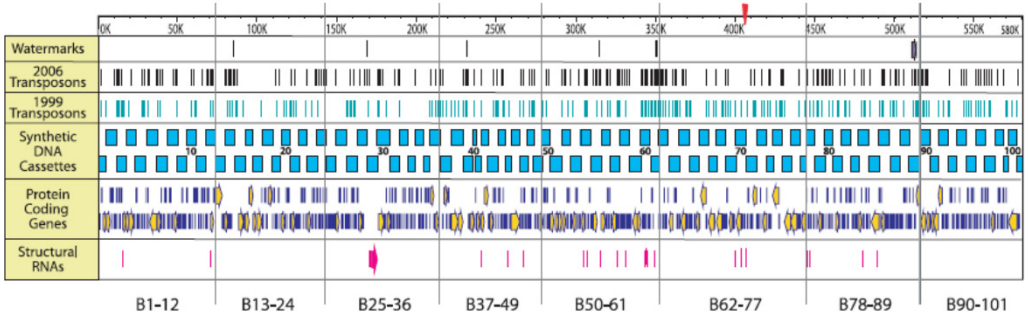


Fig. 1. Linear GenomBench (Invitrogen) representation of the circular 582,970-bp *M. genitalium* JCVI-1.0 genome. Features shown include locations of watermarks and the aminoglycoside resistance marker, viable Tn4001 transposon insertions determined in our 1999 and 2006 studies (*3*, *4*), overlapping synthetic DNA cassettes that comprise the whole genome sequence, 485 *M. genitalium* protein-coding genes, 43 *M. genitalium* rRNA, tRNA, and structural RNA genes, and B-series assemblies (Fig. 2). The red dagger on the genome coordinates line shows the location of the yeast/*E. coli* shuttle vector insertion. Table S1 lists cassette coordinates; table S2 has FASTA files for all 101 cassettes; table S3 lists watermark coordinates; table S4 lists the sequences of the watermarks.

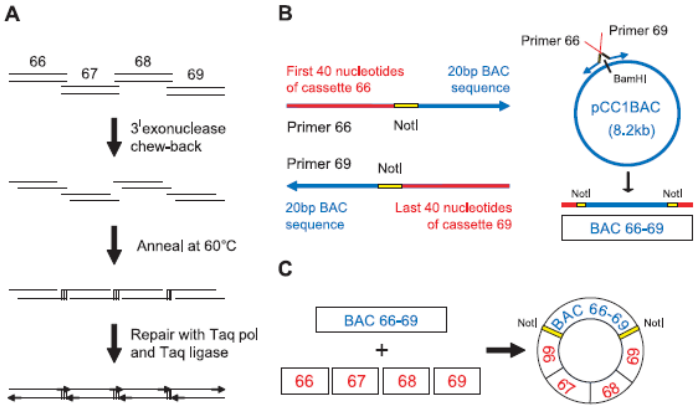
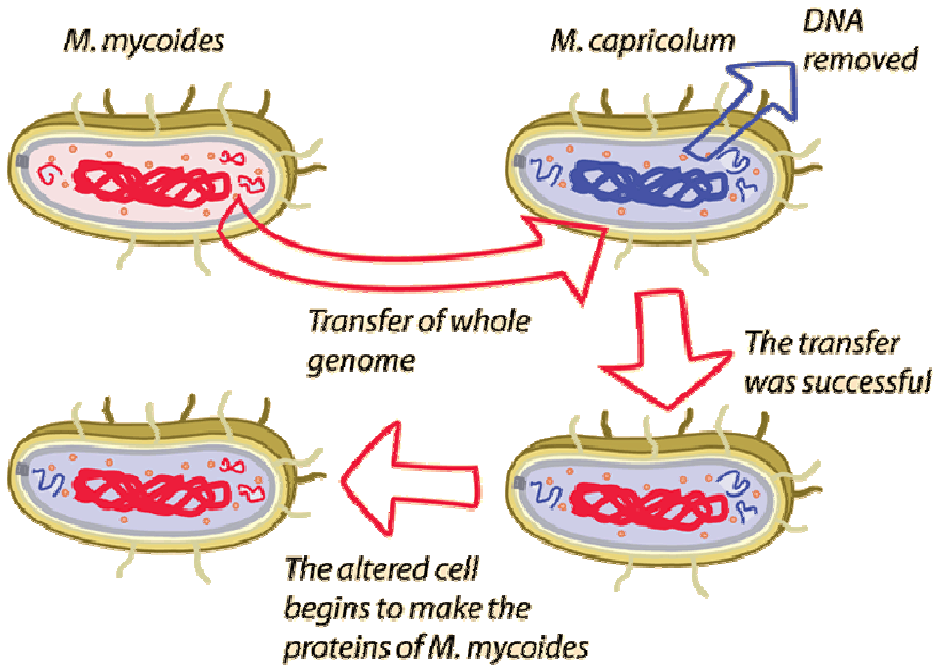


Fig. 3. Assembly of cassettes by in vitro recombination. (**A**) Diagram of steps in the in vitro recombination reaction, using the assembly of cassettes 66 to 69 as an example. (**B**) BAC vector is prepared for the assembly reaction by PCR amplification using primers as illustrated. The linear amplification product, after gel purification, is included in the assembly reaction of (A), such that the desired assembly is circular DNA containing the four cassettes and the BAC DNA as depicted in (**C**).

Synthia

***Synthia*- "the first species.... to have its parents be a computer"**

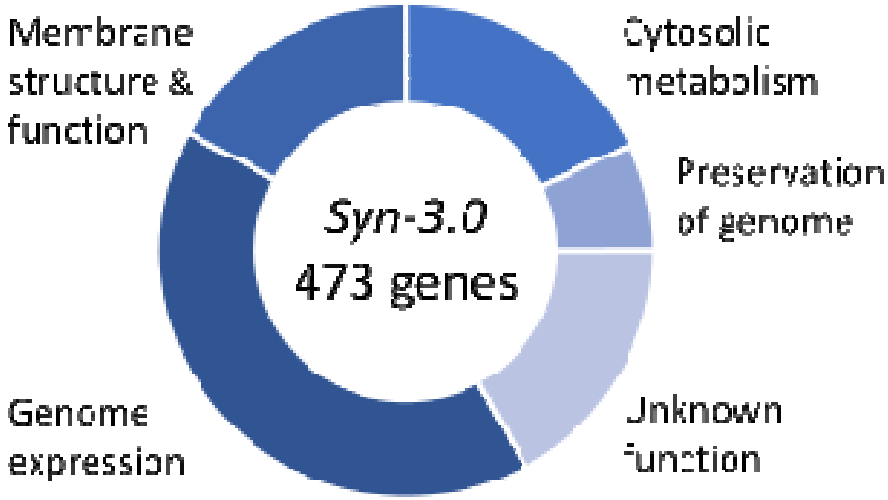


On May 21, 2010, Science reported that the Venter group had successfully synthesized the genome of the bacterium *Mycoplasma mycoides* from a computer record, and transplanted the synthesized genome into the existing cell of a *Mycoplasma capricolum* bacterium that had had its DNA removed. The "synthetic" bacterium was viable, i.e. capable of replicating billions of times. (The team had originally planned to use the *M. genitalium* bacterium they had previously been working with, but switched to *M. mycoides* because the latter bacterium grows much faster, which translated into quicker experiments.) – **JCVI-syn1.0**

Synthia

In 2016, the Venter Institute used genes from **JCVI-syn1.0** to synthesize an even smaller genome they call **JCVI-syn3.0**, that contains 531,560 base pairs and 473 genes.

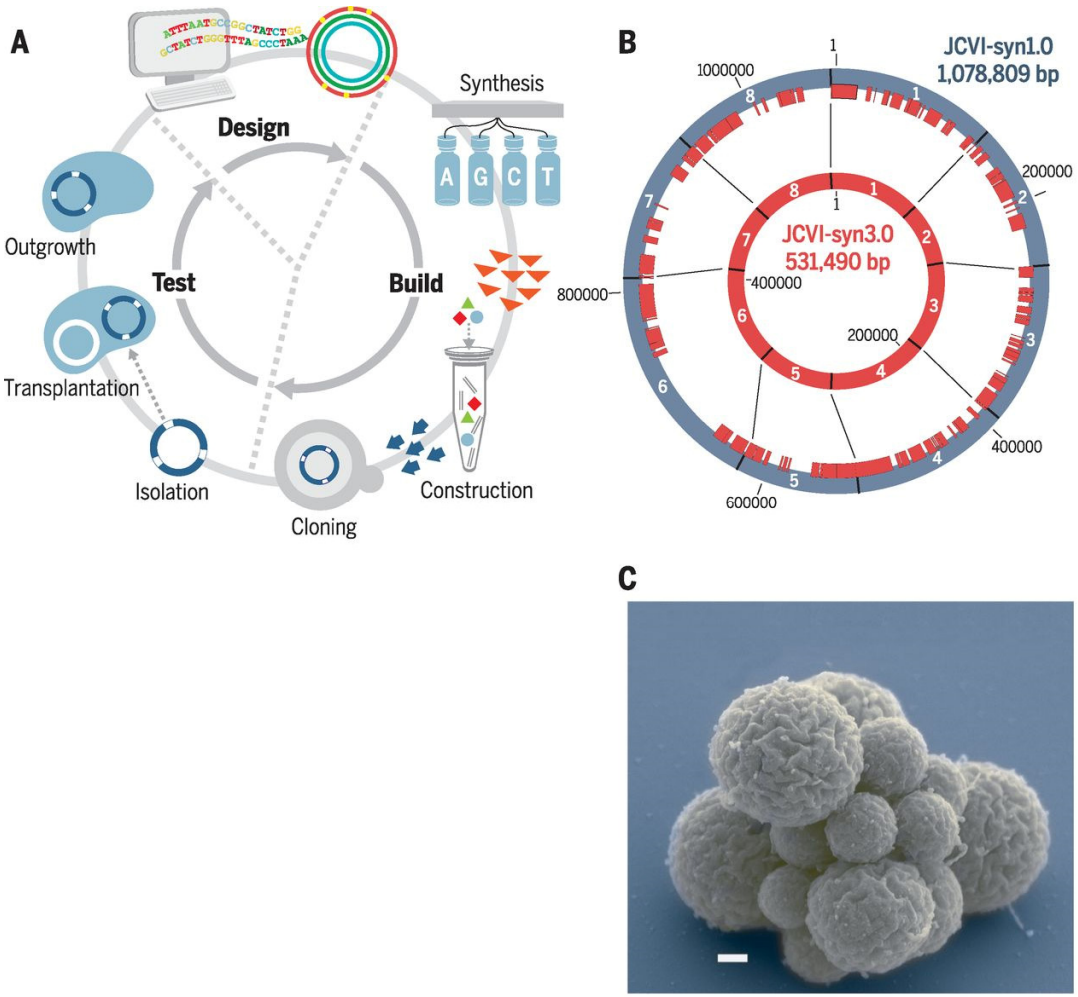
Originally in 1996, after comparing M. genitalium with another small bacterium Haemophilus influenza, Arcady Mushegian and Eugene Koonin had proposed that there might be a common set of 256 genes which could be a minimal set of genes needed for viability. In this new organism, the number of genes can only be pared down to 473, 149 of which whose functions are completely unknown



Clyde A. Hutchison III1,*,†, Ray-Yuan Chuang1,†,‡, Vladimir N. Noskov1, Nacyra Assad-Garcia1, Thomas J. Deerinck2, Mark H. Ellisman2, John Gill3, Krishna Kannan3, Bogumil J. Karas1, Li Ma1, James F. Pelletier4,§, Zhi-Qing Qi3, R. Alexander Richter1, Elizabeth A. Strychalski4, Lijie Sun1,||, Yo Suzuki1, Billyana Tsvetanova3, Kim S. Wise1, Hamilton O. Smith1,3, John I. Glass1, Chuck Merryman1, Daniel G. Gibson1,3, J. Craig Venter

Science **2016**, *351* (*6280)*, aad6253, p. 1414

Synthia



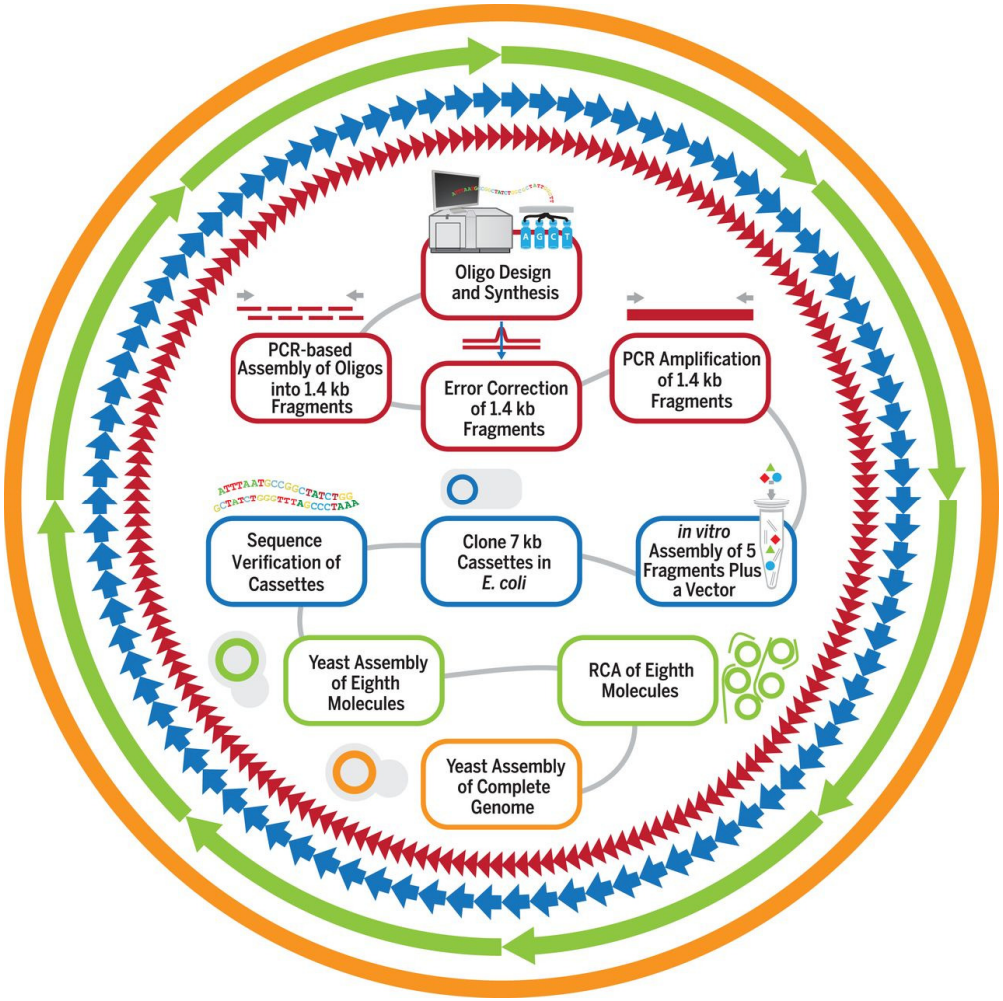
(**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis.

(**B**) Comparison of JCVI-syn1.0 (outer blue circle) with JCVI-syn3.0 (inner red circle), showing the division of each into eight segments. The red bars inside the outer circle indicate regions that are retained in JCVI-syn3.0.

(**C**) A cluster of JCVI-syn3.0 cells, showing spherical structures of varying sizes (scale bar, 200 nm).

Clyde A. Hutchison III et al., *Science* **2016**, *351* (*6280*), aad6253, p. 1414

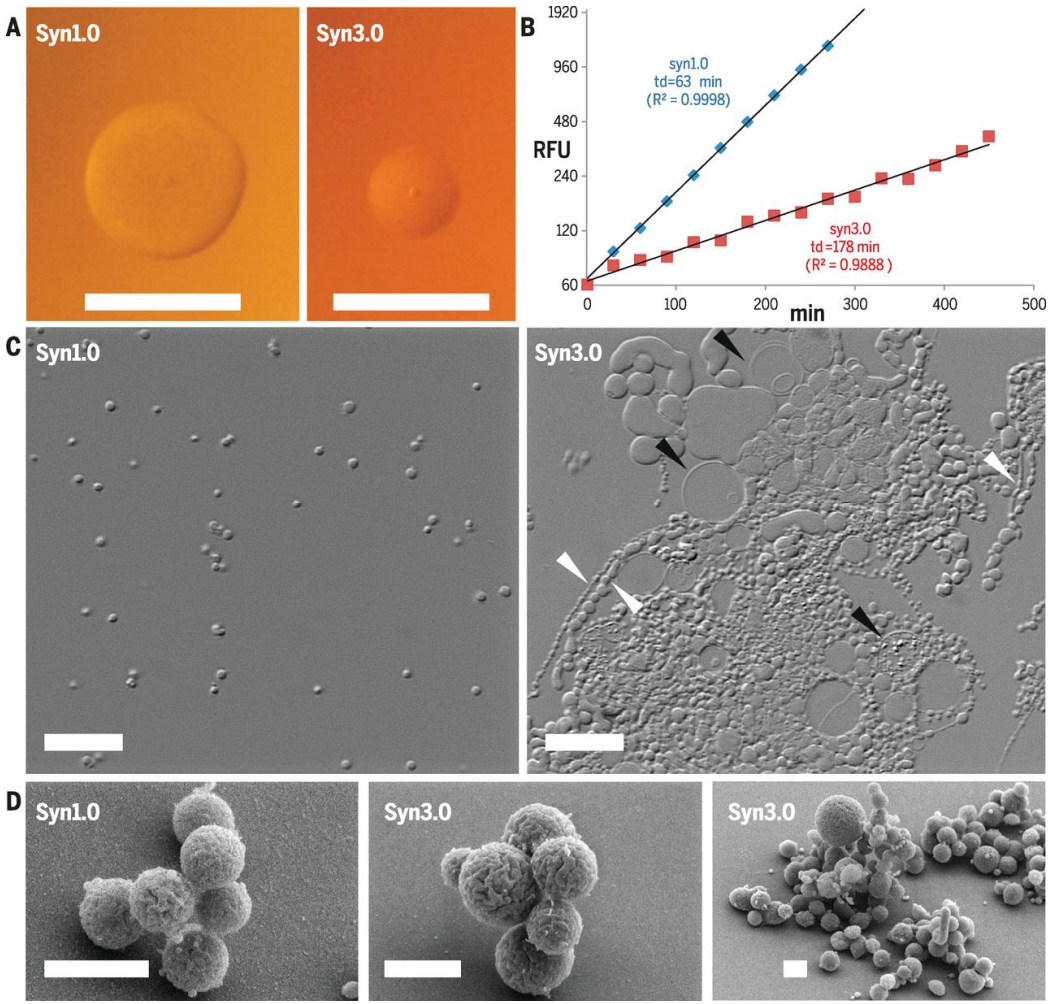
Synthia



Overlapping oligonucleotides (oligos) were designed, chemically synthesized, and assembled into 1.4-kbp fragments (red). After error correction and PCR amplification, five fragments were assembled into 7-kbp cassettes (blue). Cassettes were sequence-verified and then assembled in yeast to generate one-eighth molecules (green). The eight molecules were amplified by RCA and then assembled in yeast to generate the complete genome (orange).

Clyde A. Hutchison III et al., *Science* **2016**, *351* (*6280*), aad6253, p. 1414

Synthia



(**A**) Cells derived from 0.2 µm–filtered liquid cultures were diluted and plated on agar medium to compare colony size and morphology after 96 hours (scale bars, 1.0 mm).

(**B**) Growth rates in liquid static culture were determined using a fluorescent measure (relative fluorescent units, RFU) of double-stranded DNA accumulation over time (minutes) to calculate doubling times (td). Coefficients of determination (R^2) are shown.

(**C**) Native cell morphology in liquid culture was imaged in wet mount preparations by means of differential interference contrast microscopy (scale bars, 10 µm). Arrowheads indicate assorted forms of segmented filaments (white) or large vesicles (black).

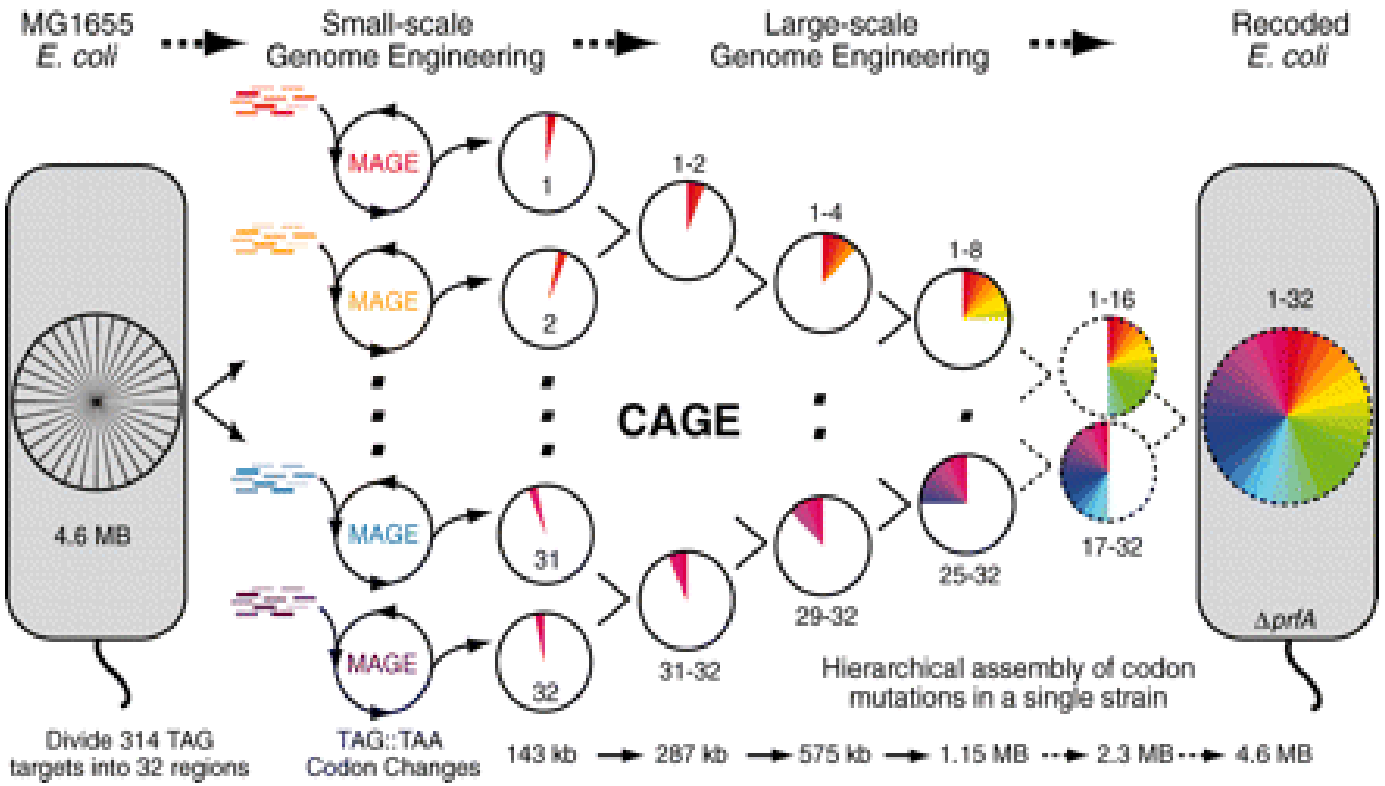
(**D**) Scanning electron microscopy of syn1.0 and syn3.0 (scale bars, 1 µm). The picture on the right shows a variety of the structures observed in syn3.0 cultures.

Clyde A. Hutchison III et al., *Science* **2016**, *351* (*6280*), aad6253, p. 1414

Synthetic biology of E. coli

George Church (Harvard, MIT) - His team is the first to tackle a genome-scale change in the genetic code. This was done in a 4.7 million basepair genome of an industrially useful microbe (*E. coli*) with the goal of making a safer and more productive strain; this strain uses non-proteinogenic amino acids in proteins and is metabolically and genetically isolated from other species.

Engineering The First Organisms with Novel Genetic Codes



Strategy for reassigning all 314 TAG codons to TAA in *E. coli*.

First, the genome was split into 32 regions each containing 10 TAG stop codons. In parallel, MAGE (multiplex automated genome engineering) was used to execute all 10 TAG::TAA codon modifications in a single strain for each genomic region. These partially recoded strains were paired such that a targeted genomic region of one strain (donor) was strategically transferred into a second strain (recipient), permitting the hierarchical consolidation of modified genomic regions using CAGE. Once all TAG codons have been converted to TAA, the *prfA* gene will be deleted to inactivate TAG translational termination.

Precise manipulation of chromosomes in vivo enables genome-wide codon replacement

Farren J. Isaacs, Peter A. Carr, Harris H. Wang,…JM Jacobson, GM Church - *Science*, 2011, 333 (6040), 348-353

Challenges in writing genomes

Key challenges and milestones for synthetic genomes

KEY TECHNOLOGY DEVELOPMENT TARGET	EXAMPLE OF DESIRED MILESTONES	ESTIMATED TIME (YEARS)
Genome design		
Develop tools for genome-scale design, visualization, and quality control.	Design a virus-proof mammalian chromosome.	3
Integrate structural information (2D and 3D) into genome design software	Predict the conformation of a synthetic yeast chromosome.	5
Develop sequence-to-phenotype whole-cell modeling.	Optimize metabolic profile, accurate to within twofold, for 100 key gene products of a synthetic virus-proof chromosome.	10
DNA synthesis		
Increase coupling efficiency for oligonucleotide synthesis.	Synthesize high-fidelity oligonucleotides longer than 500 nucleotides.	3
Increase efficiency of in vitro DNA assembly for fragments >20 kb.	Assemble 20 kb with >50% yield.	4
Develop methods for synthesis of difficult sequences, including homopolymers, high-GC content, and secondary structure.	Synthesize a centromere.	5
Develop enzymatic methods for direct synthesis of multikilobase DNA fragments.	Synthesize a 10-kb fragment (without assembly).	7
Decrease cost of DNA synthesis by 1000-fold.	Synthesize and assemble DNA for one haploid human genome (i.e., 3.2×10^9 bases) for $1000.	10
Genome editing		
Expand multiplexity and precision of DNA editing.	Simultaneously edit 1000 different targets in a single bacterial, mammalian, or plant cell with 1 off-target hit per 10,000 genomes.	2
Increase efficiency of homologous-directed repair (HDR)–mediated editing in mammalian and plant cells.	Perform HDR-mediated editing in nondividing mammalian cells at >90% efficiency.	3
Develop editing enzymes for precise substitution of any nucleotide at any desired genomic locus, with increased efficiency.	Perform allele editing of human cells at sites lacking PAM sequence, with >95% efficiency.	5
Chromosome construction		
Develop methods for temporal and spatial control of single chromosomes, such as chromatin state.	Engineer segregating, stable human artificial chromosomes (HAC).	2
Develop specialized host cells with high efficiency for DNA assembly, particularly for difficult-to-assemble sequences.	Establish in vivo chromosome assembly methods in the host *Streptomyces coelicolor* (72% GC content).	5
Develop efficient, inexpensive methods for routine and automated delivery of entire chromosomes into cells.	Demonstrate routine, device-based chromosome delivery in mammalian cells by cell fusion.	3
Develop methods for assembly and testing of Mb-size chromosomes.	Assemble a synthetic recoded human chromosome 21 from DNA fragments.	10

Nili Ostrov *et al.*, *Science* **2019**, *366* (*6463)*, p. 310-312