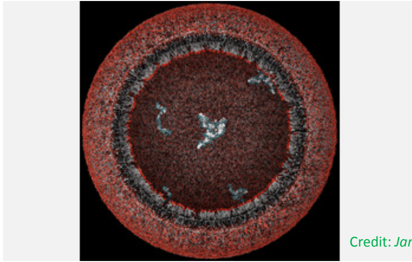
## Encapsulation – essential for life

### Assembly of amphiphilic monomers into protocellular compartments

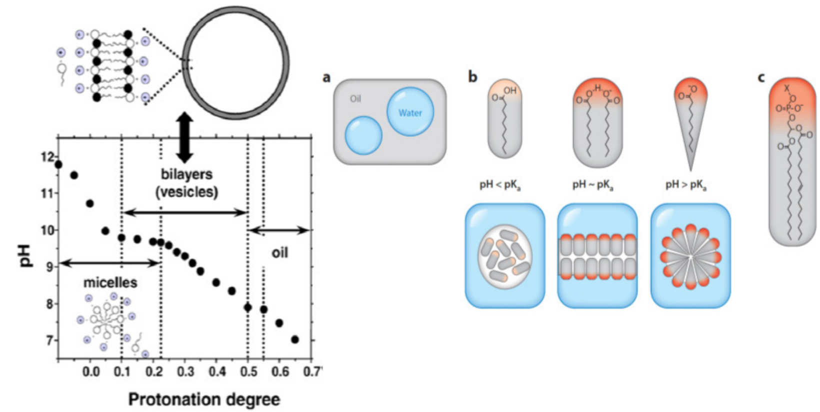


Credit: *Janet Iwasa*

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

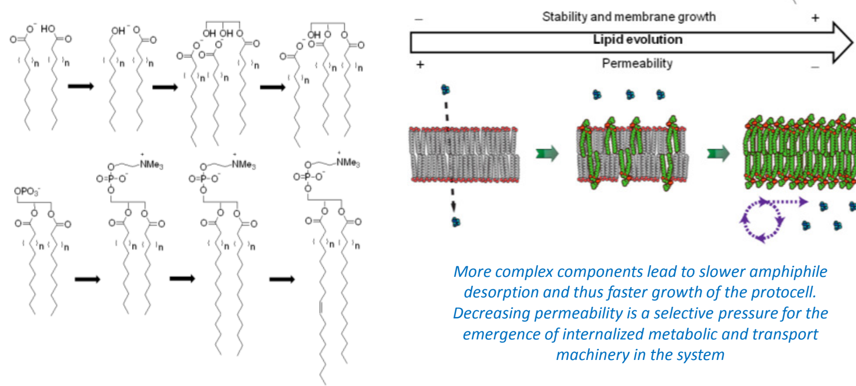
The protocell's fatty acid membrane allows nutrients and DNA building blocks to enter the cell and participate in non-enzymatic copying of the cell's DNA. The newly formed strands of DNA remain in the protocell

## pH-dependent phase behavior of fatty acids in water




*80 mM oleic acid/ sodium oleate in water*

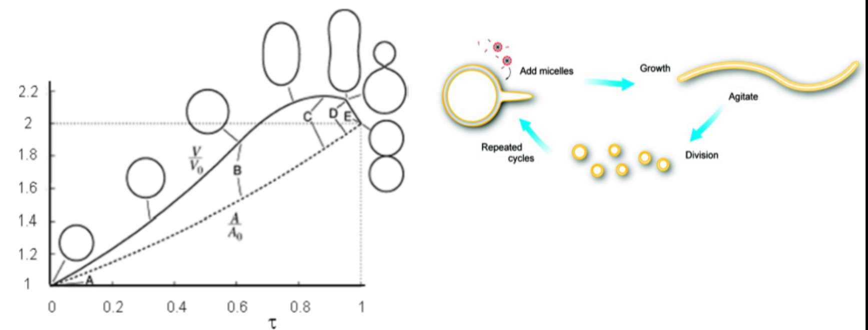
## Scheme of the membrane evolution




*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*

*Chemical evolution of membrane components*

## Growth and division of vesicles




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

## Noncovalent nucleotide association with membranes

a) Amphipathic, cationic molecules

cationic non polar

undecane

Phe, Ile, Trp

Imidazole

Arg, His

lipophilic imidazole

peptide

+POPC SUVs with undecylimidazole

DIC

green channel

red channel

Oleic acid

POPC

% change in FRET

140, 70, 50, 30, 10, 0, -10

RF, RFF, RRRFF, RRRRRFF, RRRFFF, RFFF, RRRRIII, RRRIII, RRRRRWW, RRRWWW, HHWWW, HWW

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

OA / POPC

POPC

no peptide

+R3F3

+R3W3

no peptide

+R3F3

+R3W3

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

a)

POPC

5'-FAM-$U_{15}$

no undecylimidazole

DIC

green channel

red channel

b)

POPC SUVs

+POPC SUVs

DIC

green channel

red channel

c)

+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

## Oligonucleotide interactions with lipid membranes

> 500 nm

ΔT or 410 nm

trans-1

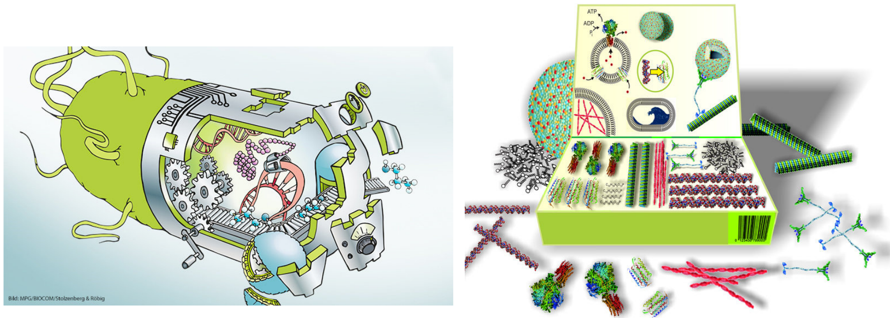
cis-1

**AMPHIPHATIC**

**POLAR**

J. Karcher, Z. Pianowski *Chem. Eur. J.* **2018**, *24*, 11605-11610

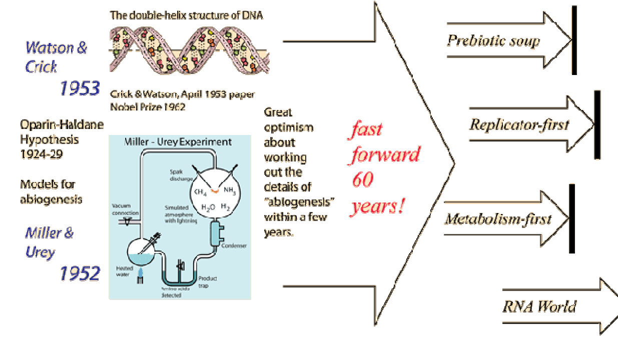
# CHAPTER 3



www.mpg.de/themenportal/synthetische-biologie

## *SYNTHETIC BIOLOGY*

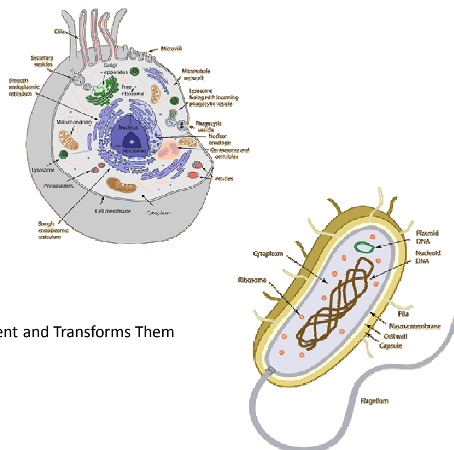
## *The great optimism of the 1950's*



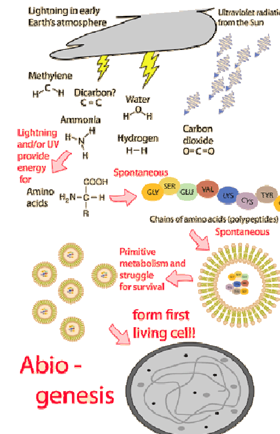

*Life obeys the laws of chemistry and physics*

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

Spontaneous

Primitive metabolism and struggle for survival

form first living cell!

Abio - genesis

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

Venter's synthetic life

Segments of 50 base pairs are assembled, one bp at a time, in a column reactor.

The entire genome of M. genitalium was reproduced synthetically. This reproduction of the 482 gene genome was in preparation for producing the 382 gene minimum genome determined by knockout experiments. If the synthetic genome can be implanted and survive, it will be called M. laboratorium and used as a base for bioengineering.

Combine to form segments 5000-7000 bp 50 bp

Enzyme treatments 24,000 bp 72,000 bp 144,000 bp

E. coli E. coli E. coli

The synthetic genome was produced with the help of biological enzymes and the work of the machinery of two existing life forms.

S. cerevisiae yeast

582,970 bp

Mycoplasma genitalium complete genome

## The Minimal Genome Project

Design Synthesis Build Test Outgrowth Transplantation Isolation Cloning Construction

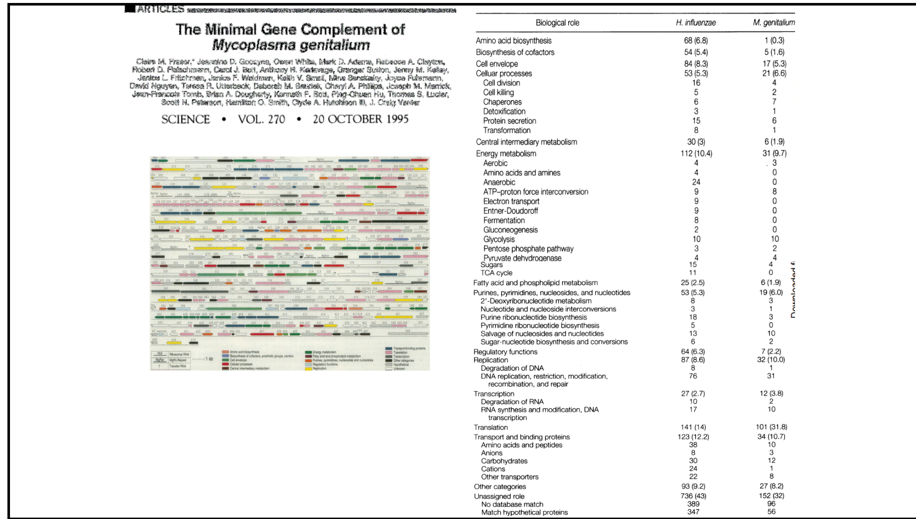
A G C T

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.

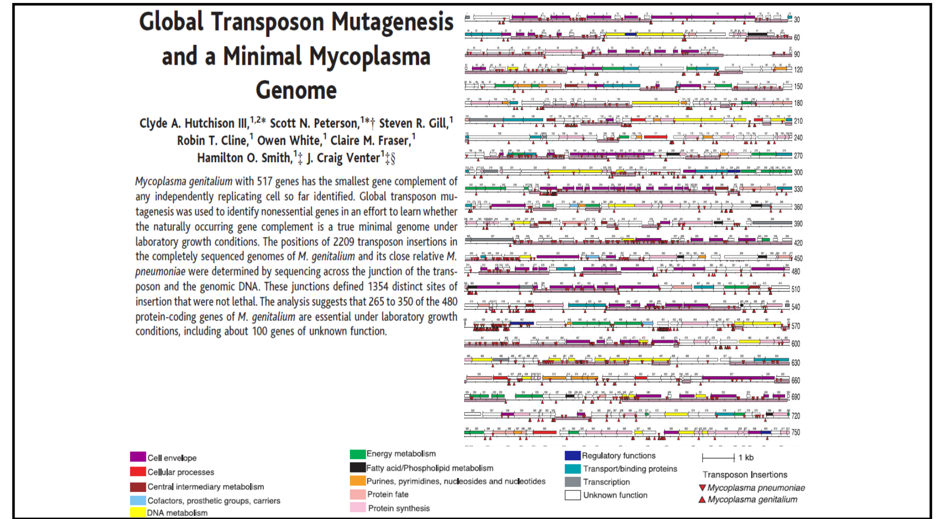
TIME Thursday, Jan. 24, 2008 Scientist Creates Life — Almost By Alice Park

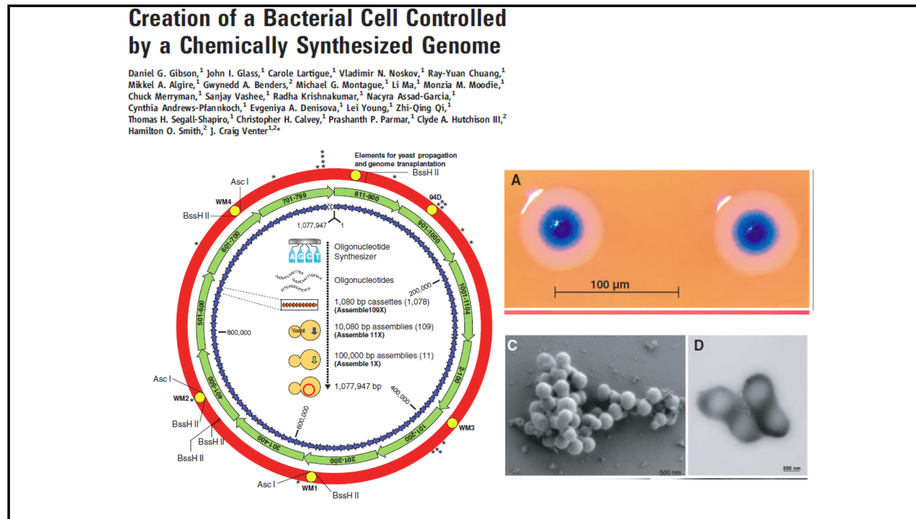
Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome.

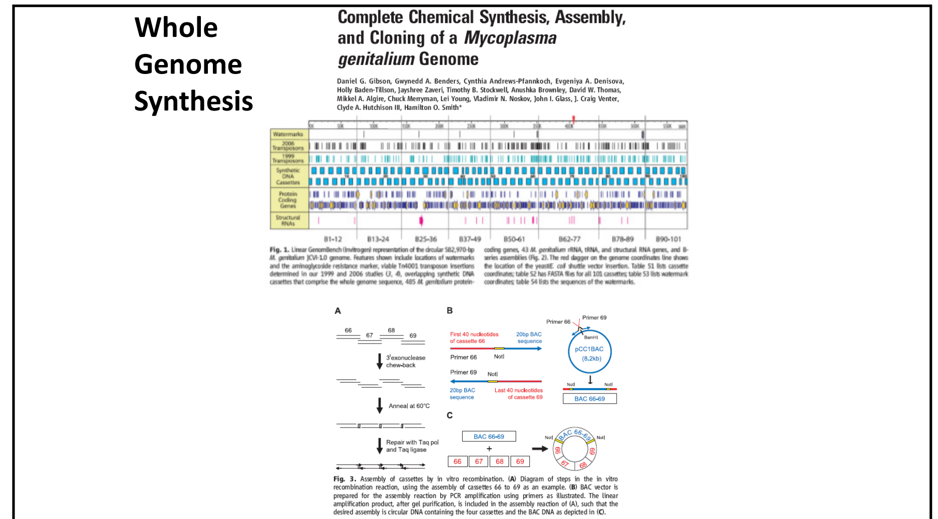
Gibson et al. Science 319(5867): 1215-1220. 2008




| 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) |
| Cellular 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 |

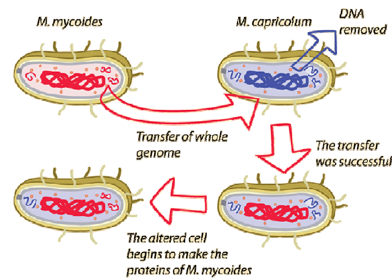


## Synthia

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

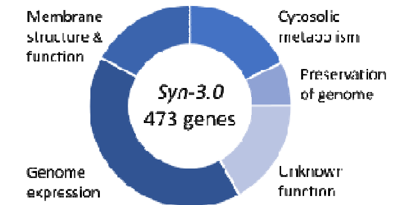



On May 21, 2010, Science reported that the Venter group had successfully synthesized the genome of the bacterium *Mycoplasma mycoides* from a computer record, and transplanted the synthesized genome into the existing cell of a *Mycoplasma capricolum* bacterium that had had its DNA removed. The "synthetic" 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

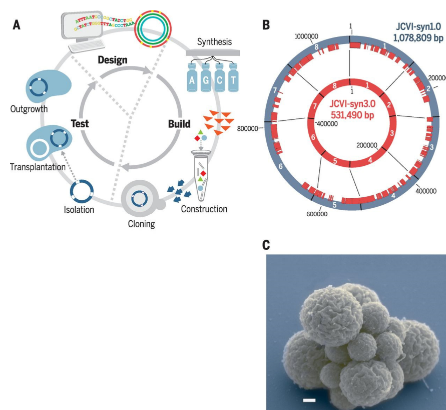
In 2016, the Venter Institute used genes from **JCVI-syn1.0** to synthesize an even smaller genome they call **JCVI-syn3.0**, that contains 531,560 base pairs and 473 genes.

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



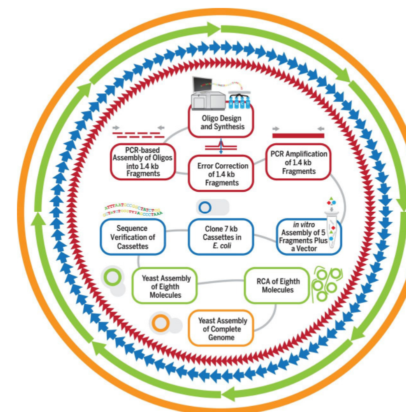

(**A**) The cycle for genome design, building by means of synthesis and cloning in yeast, and testing for viability by means of genome transplantation. After each cycle, gene essentiality is reevaluated by global transposon mutagenesis.

(**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.

The Media Lab | Inventing a Better Future

# rE.coli

The Center for Bits and Atoms

## *Engineering The First Organisms with Novel Genetic Codes*

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

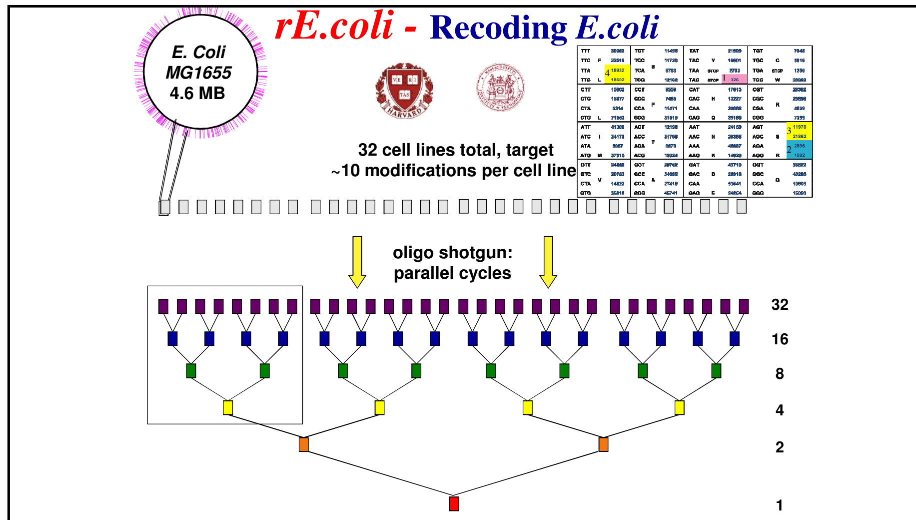
Farren J. Isaacs, Peter A. Carr, Harris H. Wang,...JM Jacobson, GM Church - *Science*, 2011

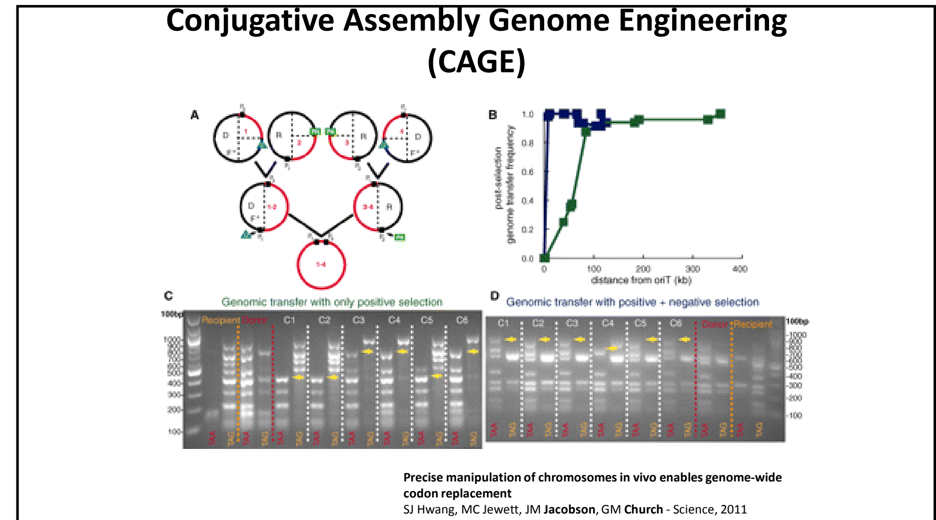
http://www2.le.ac.uk/departments/genetics/vgec/education/post18/topics/dna-genes-chromosomes

Programming cells by multiplex genome engineering and accelerated evolution

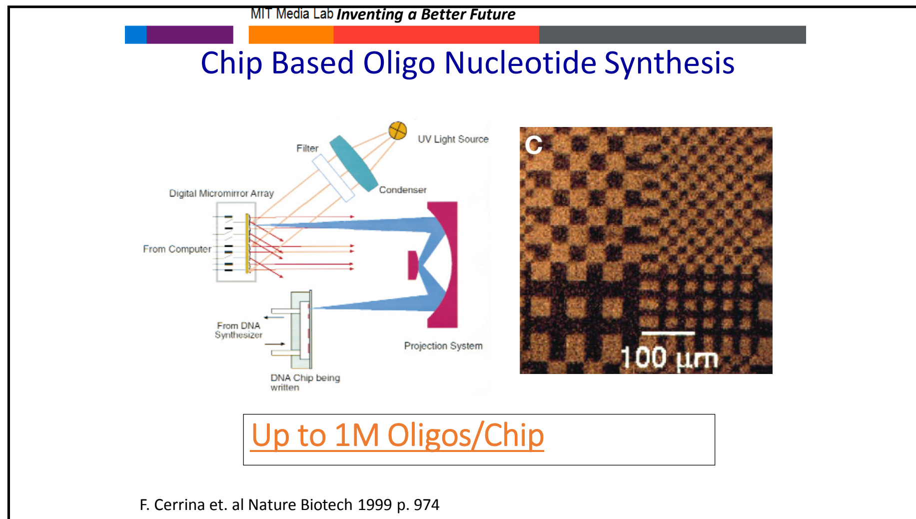
Harris H. Wang, Farren J. Isaacs, Peter A. Carr, Zachary Z. Sun, George Xu, Craig R. Forest & George M. Church *Nature* **460**, 894-898(13 August 2009)

http://profiles.umassmed.edu/profiles/ProfileDetails.aspx?From=SE&Person=240

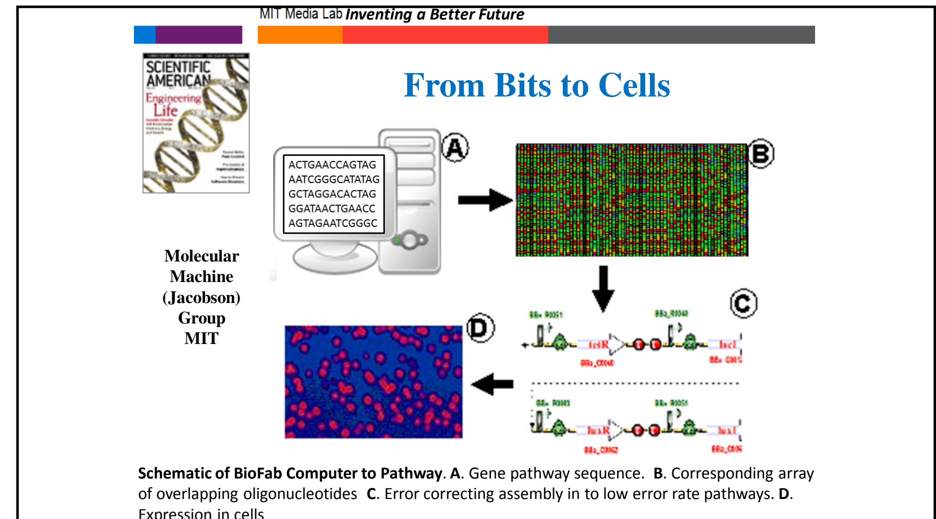


Precise manipulation of chromosomes in vivo enables genome-wide codon replacement
SJ Hwang, MC Jewett, JM **Jacobson**, GM **Church** - Science, 2011



F. Cerrina et. al Nature Biotech 1999 p. 974



**Schematic of BioFab Computer to Pathway**. **A**. Gene pathway sequence. **B**. Corresponding array of overlapping oligonucleotides **C**. Error correcting assembly in to low error rate pathways. **D**. Expression in cells