

Bi9690en Synthetic Biology – Lecture 10

Karel Říha, CEITEC MU, A26/209
e-mail: karel.riha@ceitec.muni.cz

 @karlriha



- **Synthetic organelles**
- **Minimal cells**



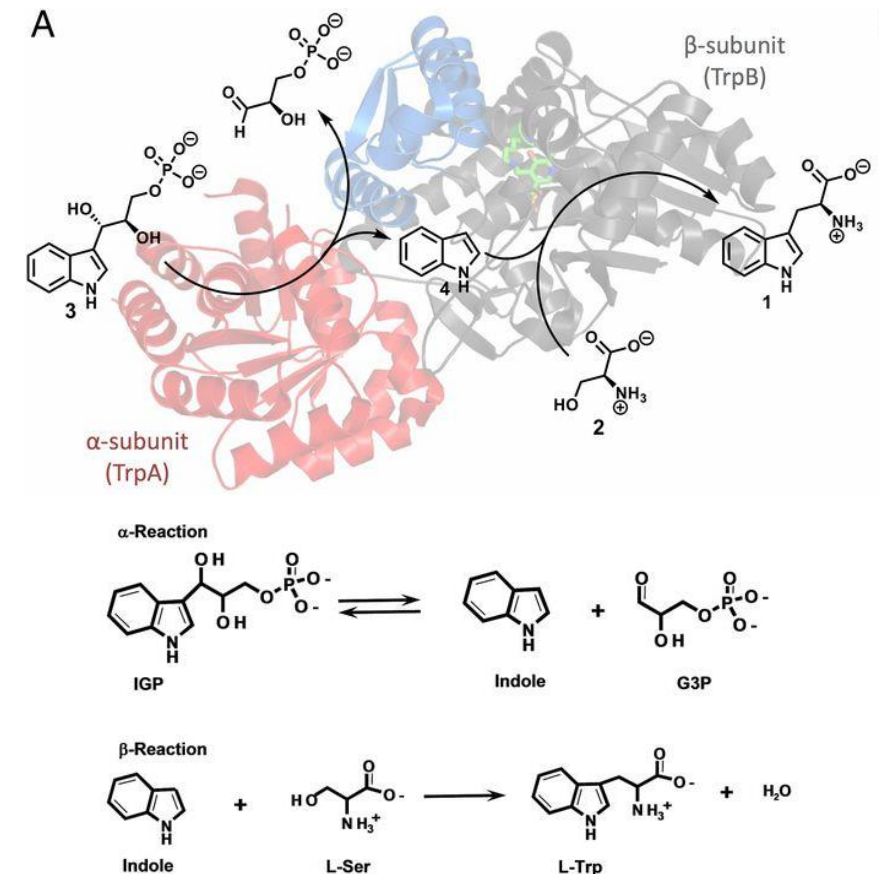
Design and construction of cellular synthetic compartments

An important area of synthetic biology is production of valuable chemicals, such as pharmaceuticals, materials and biofuels from cheap and sustainable biomass. This requires high productivity and yields of engineered pathways. One promising strategy is to repurpose organelles or protein complexes as cellular factories for improving the performance of engineered pathways.

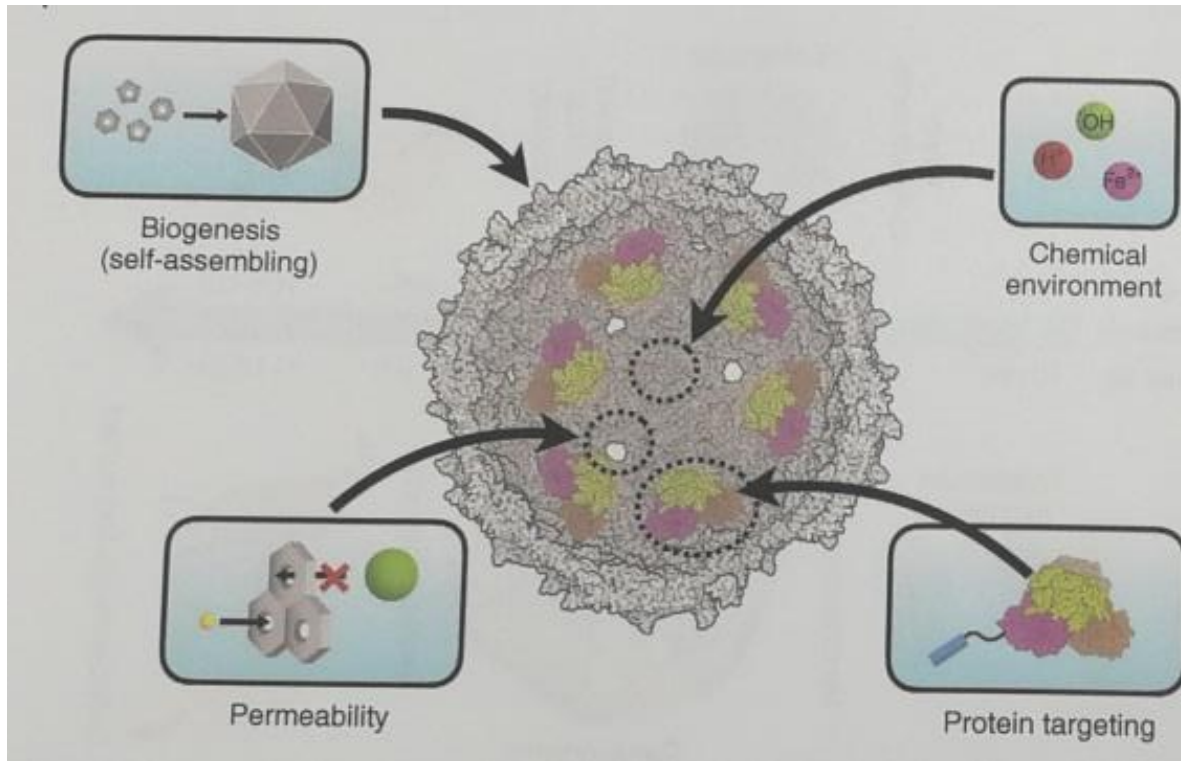
Compartmentalization in biocatalysis:

- Strategy for enabling competing pathways
- Selective regulation of enzymes by localization
- Substrate channeling of intermediates between enzymatic steps
- Sequestering volatile and toxic compounds
- Formation of specific microenvironments

Substrate channelling in tryptophan synthase



Core design principles for synthetic organelles



Biogenesis – process of organelle self-assembly. It will determine organelle size, shape and copy number.

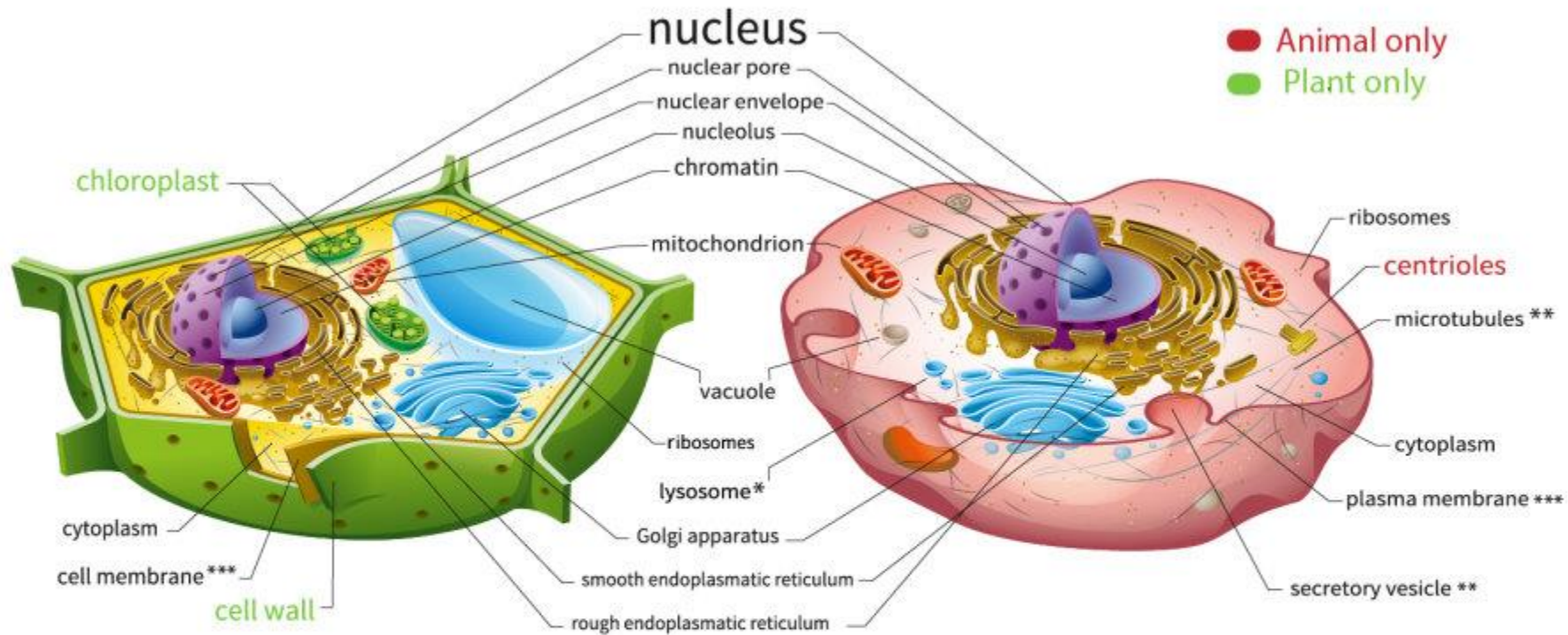
Targeting – selective targeting of desired biochemical activities (enzymes) into the compartment. Often based on protein-protein interactions through the use of signaling sequences.

Permeability – selectivity of surrounding membrane or protein shell that directly affects what can diffuse across or be transported in and out of the compartment.

Chemical environment – result of the interplay between permeability and combined enzymatic activity. It will determine concentration of substrates and products, as well as general properties such as pH.



Lipid membrane-based organelles



* Plants may have lytic vacuoles, which act like lysosomes in animal cells.

** Although they're not labelled here, plant cells have microtubules and secretory vesicles, too.

*** Cell membrane and plasma membrane are just different names for the same structure.

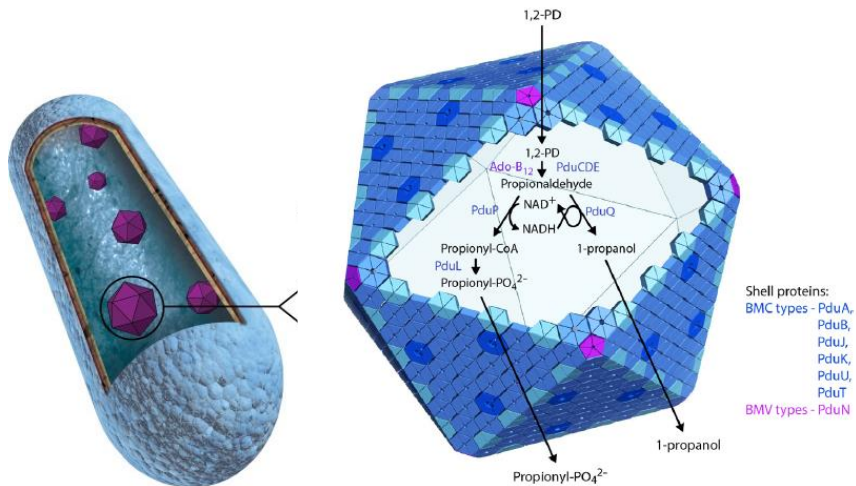


Other means of cellular compartmentalization

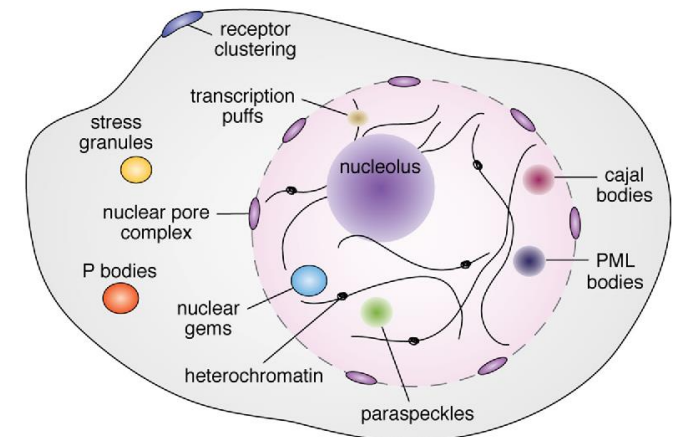
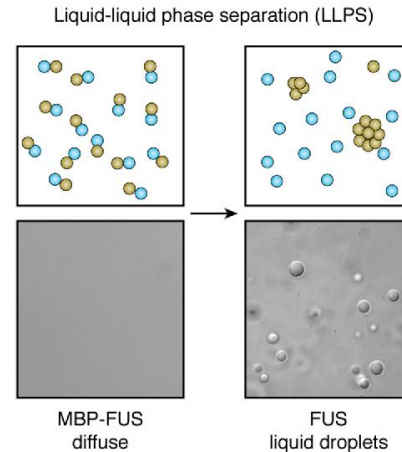
Organelle: physically delimited compartment within a cell

Proteinaceous bacterial microcompartments

- carboxysomes
- propanediol-utilizing microcompartment
- encapsulins



Molecular condensates

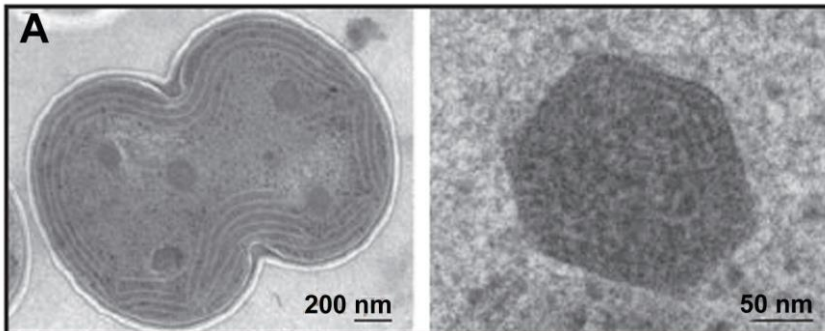
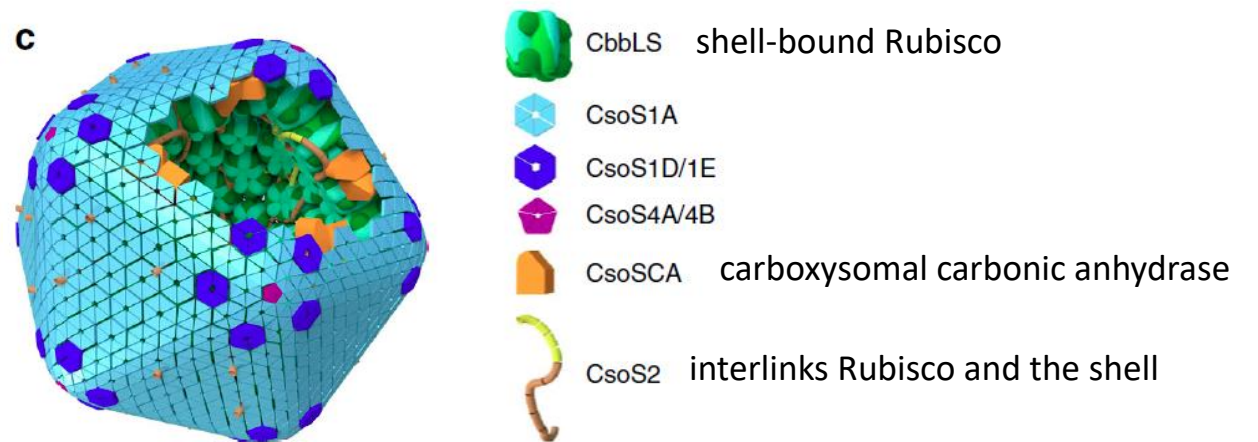
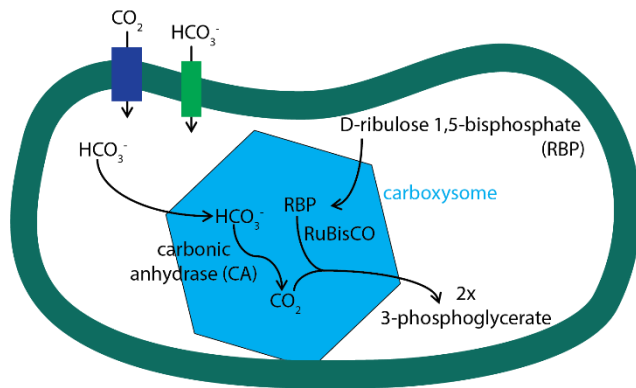


In a mixture of two types of molecules, LLPS leads to the formation of two phases akin to droplets of oil appearing from a mixture of oil and water. Proteins and nucleic acids can undergo a similar phase separation. LLPS is mediated by combination of weak multivalent interactions that may involve intrinsically unstructured protein regions, as well as specific interactions. LLPS underpins the biogenesis of a wide array of membraneless organelles within cells.



Carboxysomes in cyanobacteria

The cyanobacterial carbon concentrating mechanism is a single-cell, bipartite system that first generates a high intracellular bicarbonate (HCO_3^-) pool through action of membrane-bound inorganic carbon (Ci) transporters and CO_2 -converting complexes. This HCO_3^- pool is then utilized by subcellular micro-compartments called **carboxysomes**, which encapsulate the cell's complement of Rubisco. The carboxysome's outer protein shell enables diffusional influx of HCO_3^- and RuBP, where the former is converted to CO_2 by a localized carbonic anhydrase (CA).

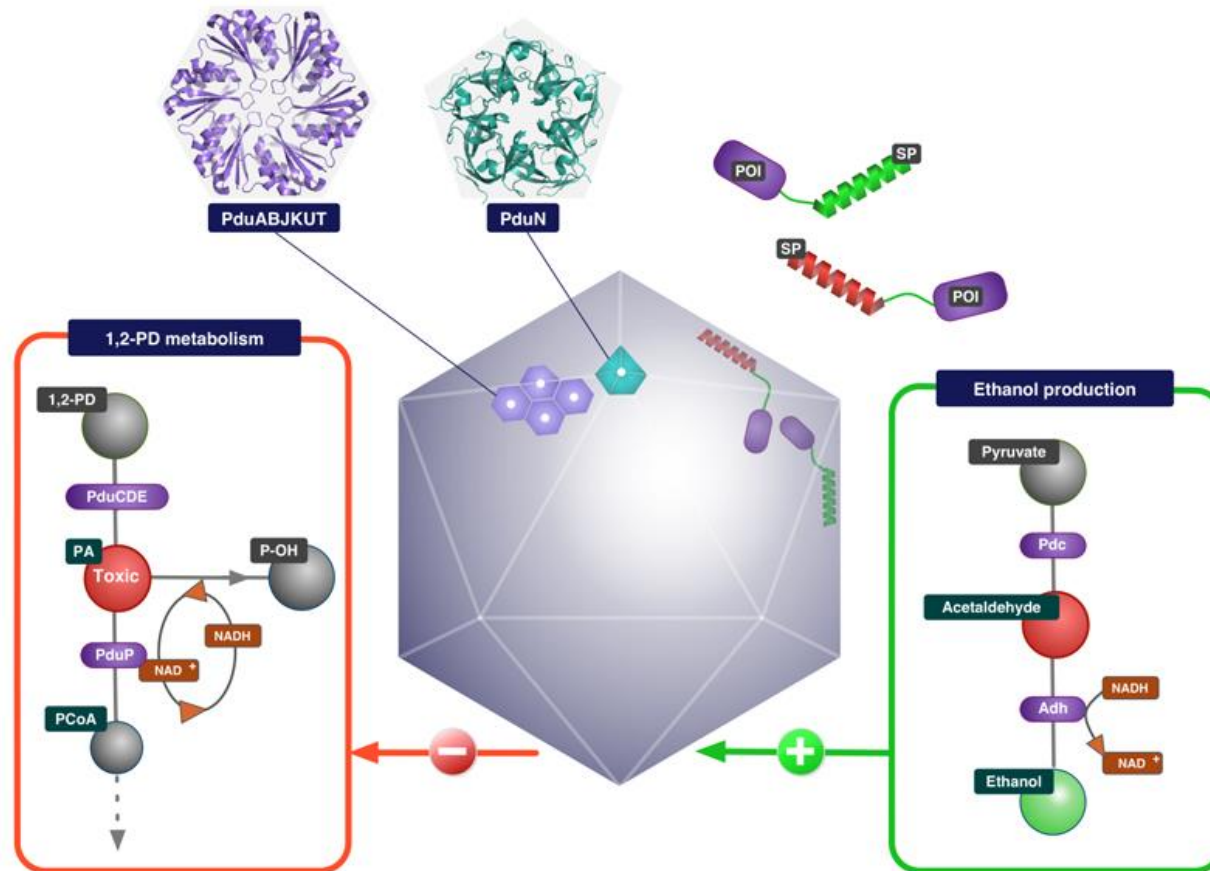


- exclusion of O_2 as the Rubisco competing substrate from the lumen
- CO_2 channelling from carbonic anhydrase to Rubisco via tight clustering
- raising local pH around Rubisco to increase its catalytic activity

Carboxysome of *Cyanobium*



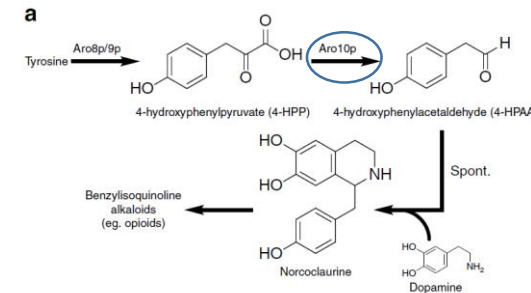
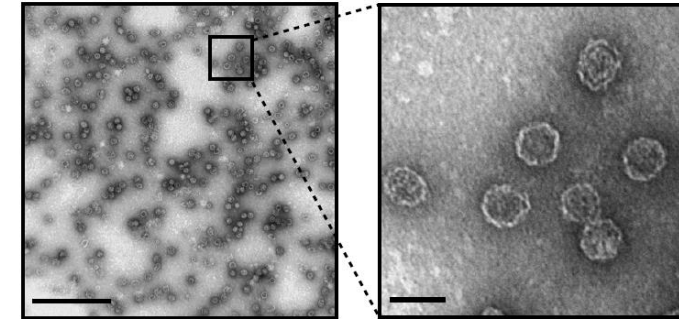
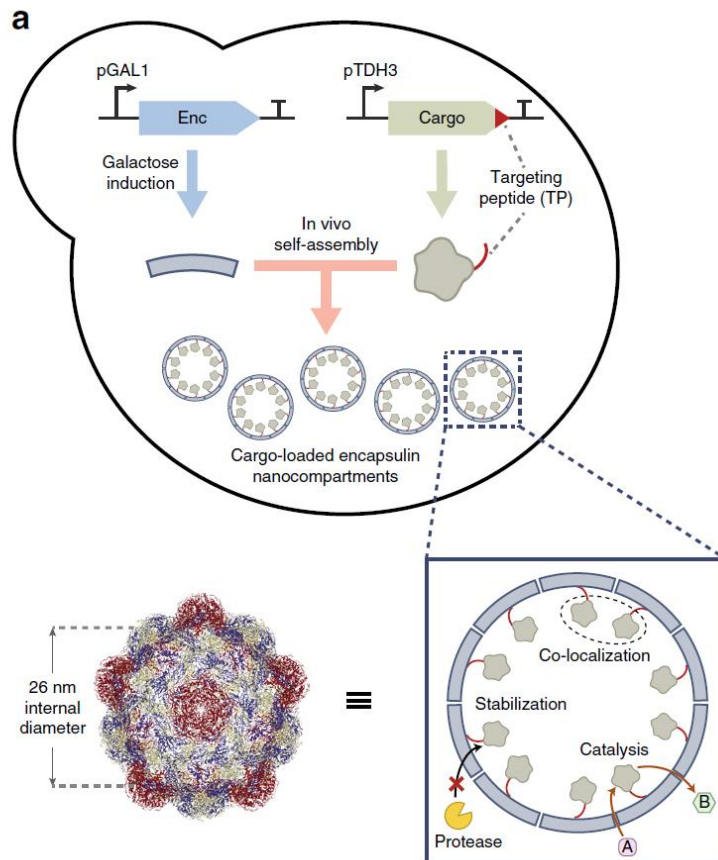
Repurposing propanediol-utilizing microcompartment for ethanol production



The Pdu microcompartment shell is built from hexameric tiles composed of PduA, B, J, K, U and T (purple) that form the facets of the structure whereas pentameric tiles (PduN, cyan) form the vertices. 1,2-Propanediol enters the shell through pores in the shell proteins and is metabolized to propionyl-CoA (red box), which leaves the compartment and is further converted to propionate. Enzymes that are encapsulated within the metabolosome contain short signaling peptides. Changing the specificity of the Pdu microcompartment is achieved by stripping out the Pdu pathway and replacing it with the required pathway *e.g.* ethanol production (green box). Fusion of signaling peptides to the new pathway enzymes – pyruvate decarboxylase (Pdc) and alcohol dehydrogenase (Adh) facilitates internalization of the heterologous proteins an ethanol production. 1,2-PD = 1,2-propanediol, PA = propionaldehyde, P-OH = 1-propanol, PCoA = propionyl-CoA, POI = protein of interest, SP = signalling peptide.

Engineering prokaryotic encapsulins as synthetic organelles in yeast

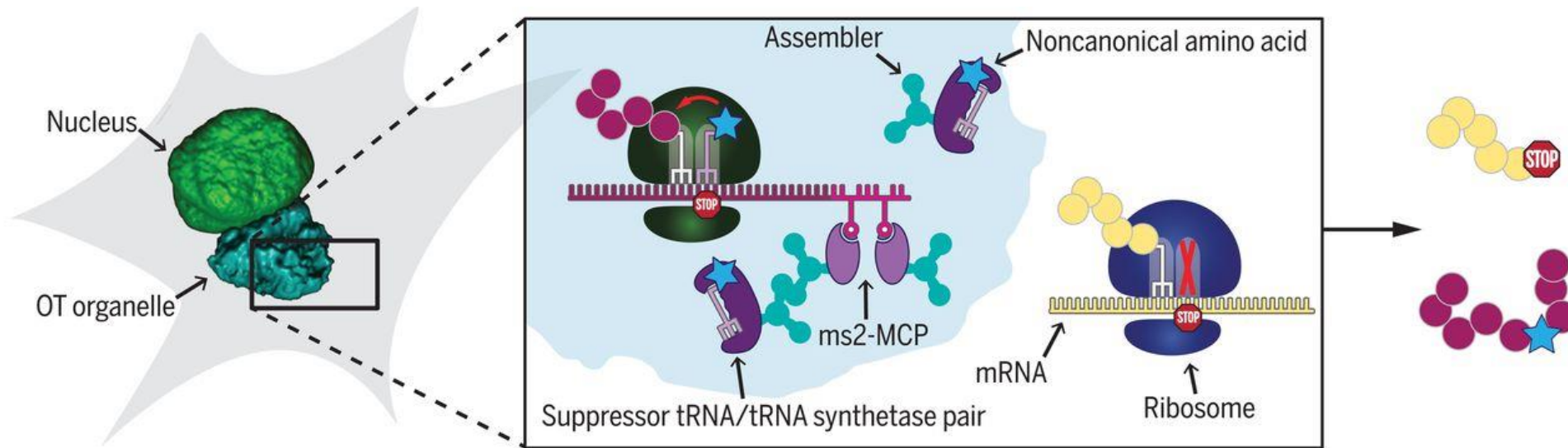
Encapsulins – prokaryotic proteins capable of assembling into a 20-30 nm icosahedral nanocompartments. They represent minimal versions of microcompartments – they consist of a single shell protein and can be targeted with different cargos. However, their small size limits their targeting to one or two enzymes, so they can be used for engineering only very short pathways.



Encapsulin compartments represent a modular platform, orthogonal to existing organelles, for programming synthetic compartmentalization in eukaryotes.



Phase separation sequesters the machinery for repurposing stop codons

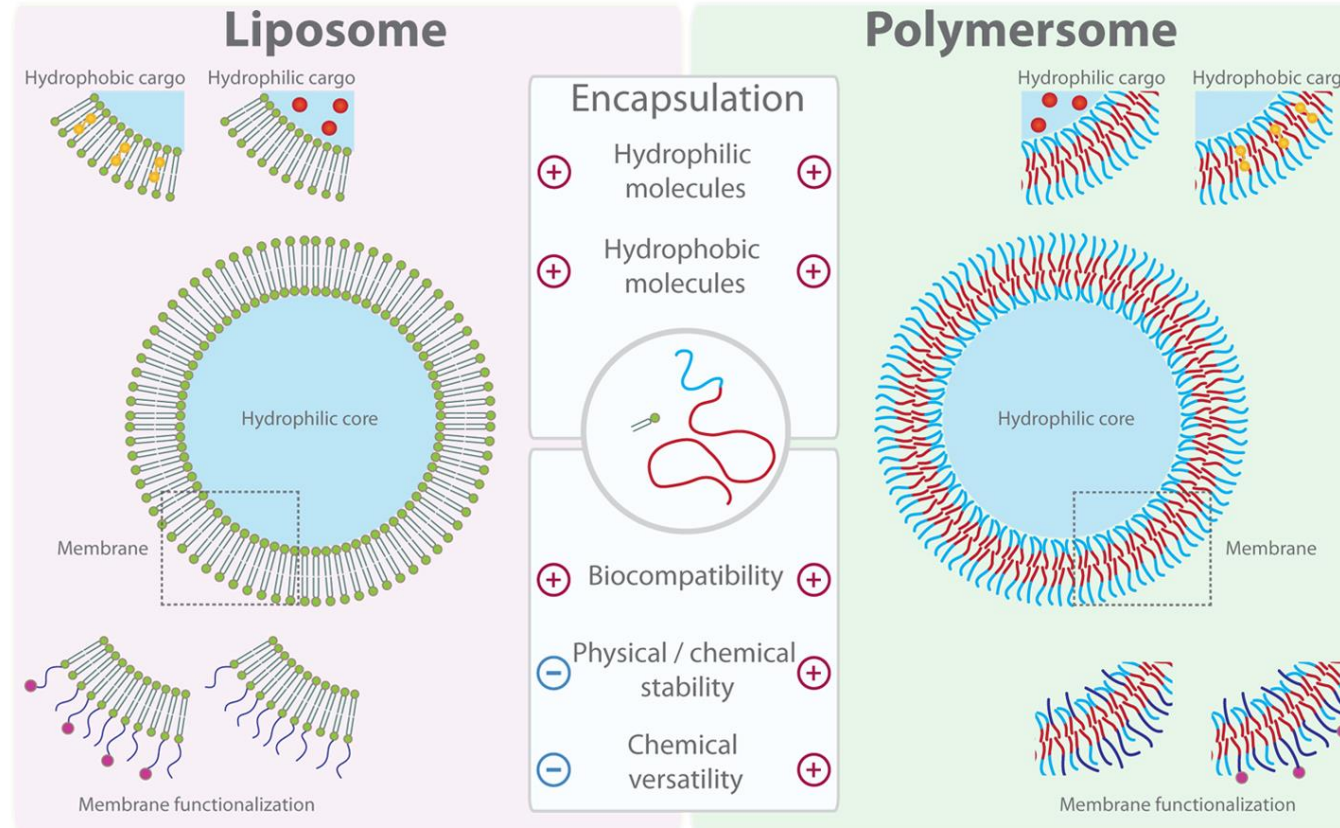


The assemblers: proteins capable of phase separation fused to the assembled components - kinesin motors (localization to microtubules), suppressor tRNA synthase, and MCP proteins (bind to ms2 loops on targeted mRNA)

Inside the designer orthogonal organelle, nonnatural amino acids can be added during protein synthesis at the location of the Amber stop codon. Outside the organelle, the stop codon still halts protein synthesis.



Towards artificial organelles: liposomes and polymersomes



Polymersomes are synthetic analogues of liposomes and are constituted of amphiphilic block copolymer membrane. Whilst most properties are similar for both carriers, polymersomes exhibit a high versatility and an enhanced stability.



Synthetic endosymbiosis: inspired by kleptoplasty

Microinjection of cyanobacteria into zebrafish embryo

Kleptoplasty: a symbiotic phenomenon whereby chloroplasts from algae, are sequestered by host organisms.

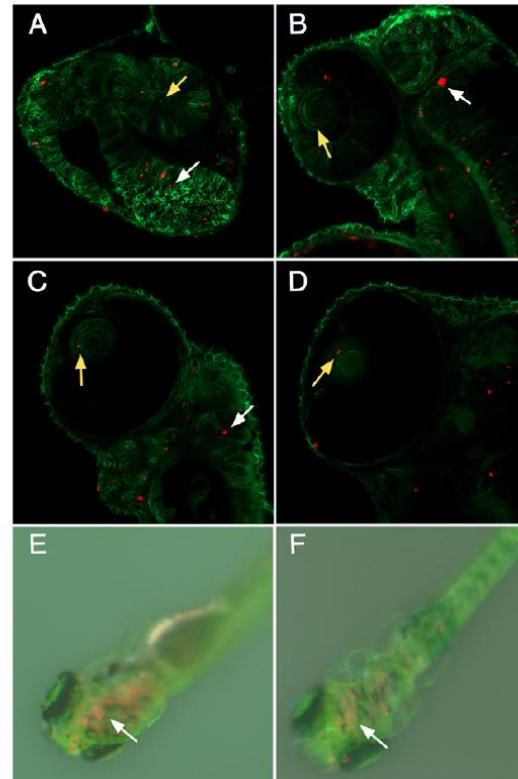


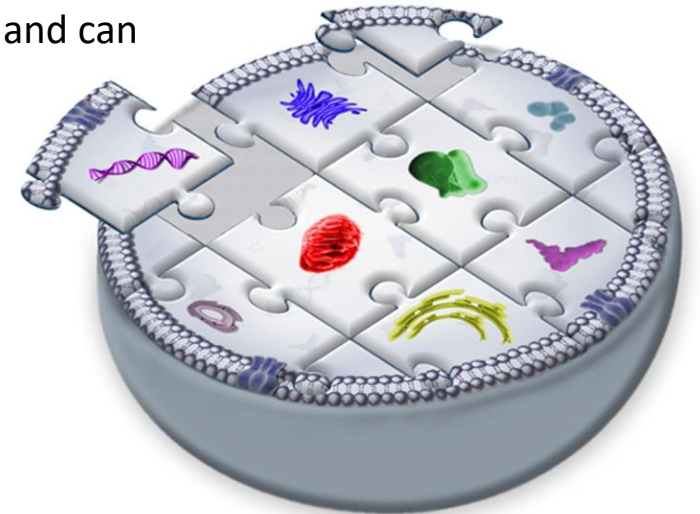
Figure 2. Tracking intracellular *S. elongatus* through zebrafish development. Single optical slice confocal microscopy images of the anterior of the zebrafish embryo at A.) Day 1 post injection, B.) Day 2, C.) Day 3, D.) Day 4, and dissecting microscope images of embryos E.) Day 4, F.) Day 12 post injection. Zebrafish cell membranes are outlined in green, with red autofluorescent bacteria visible in cells throughout the embryo, including the eye (yellow arrows) and brain (white arrows). Red autofluorescence gradually decreased over the course of experimental observations, but remained visible in the brain of the young zebrafish even after 12 days.

- no adverse immune response
- cyanobacteria expressing listeriolysin and invasins to escape lysosome digestion were able to proliferate in macrophages for several days



Minimal cell

- A cell whose genome only encodes the minimal set of genes necessary for the cell to survive
- The genes in minimal cell are, by definition, essential
- A minimal cell has all of the machinery for independent cellular life – there is no redundancy.
- In reductionism approach is a minimal cell key to learn the first principles of cellular biology by mapping function of all genes and components – then it may be possible to achieve a complete understanding of what it takes to be alive.
- With this knowledge, it may be possible to model the minimal cell's behavior on computer. And from there one may be able to build cells that are more complex.
- The minimal cell is the ultimate goal for creating a chassis cell - a cell with reduced complexity that is designed for one or several biotechnological applications and can be modified and controlled with precision and in a predictive manner.



Determining a minimal set of genes: comparative genomics

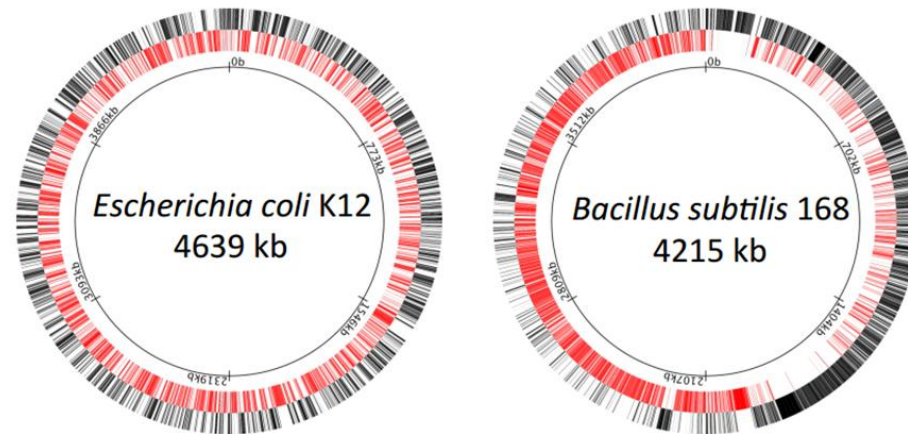
1996: Comparison of first bacterial genomes *H. influenzae* and *M. genitalium* - 256 orthologous genes specify core functions

2003: all sequenced organisms - 65 orthologous genes

2004: 147 prokaryotic genomes available – less than 50 common orthologous genes (mostly translation)

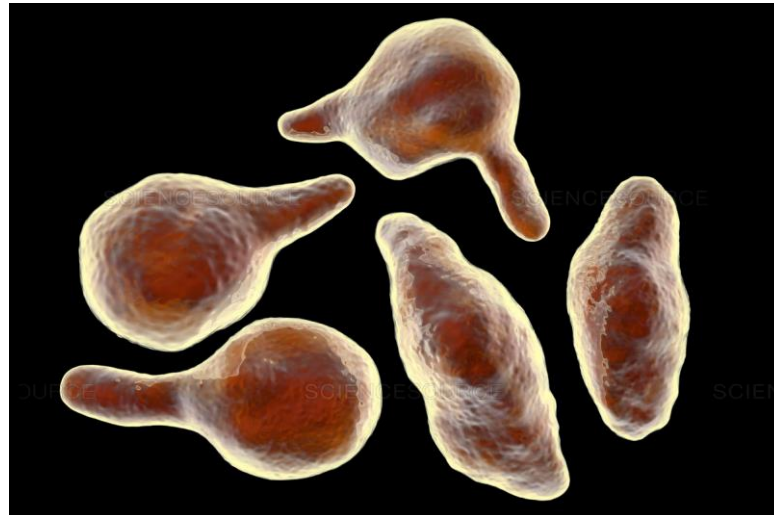
2012: 20 strains of *Mycoplasma* family – core of only 196 orthologs

Nonorthologous gene displacement – orthologs evolved too far to be recognizable as such, or an essential function was originally provided by two redundant genes that separated in the course of evolution.



Mycoplasma as a model for minimal cell

- *Mycoplasmas* are a group of bacteria characterized by the lack of cell wall, obligate parasitic lifestyle, metabolic simplicity, and small genomes.
- *Mycoplasma* did not evolve as the simplest form of cellular life. They descent from a conventional bacteria (like *B. subtilis* or *S. aureus*) through massive gene loss due to adopted parasitic lifestyles in highly nutrient rich and stable environment.
- *Mycoplasma genitalium* has the smallest genome with 580,076 bps encoding 507 genes
- Because nutrients are imported rather than synthesized, all that *Mycoplasma* do is to synthesize DNA, RNA and proteins.



Determining a minimal set of genes: genetic approach

Transposon insertion mutagenesis in *Mycoplasma*

Essential genes (E) – the cell in which essential gene is inactivated cannot be propagated

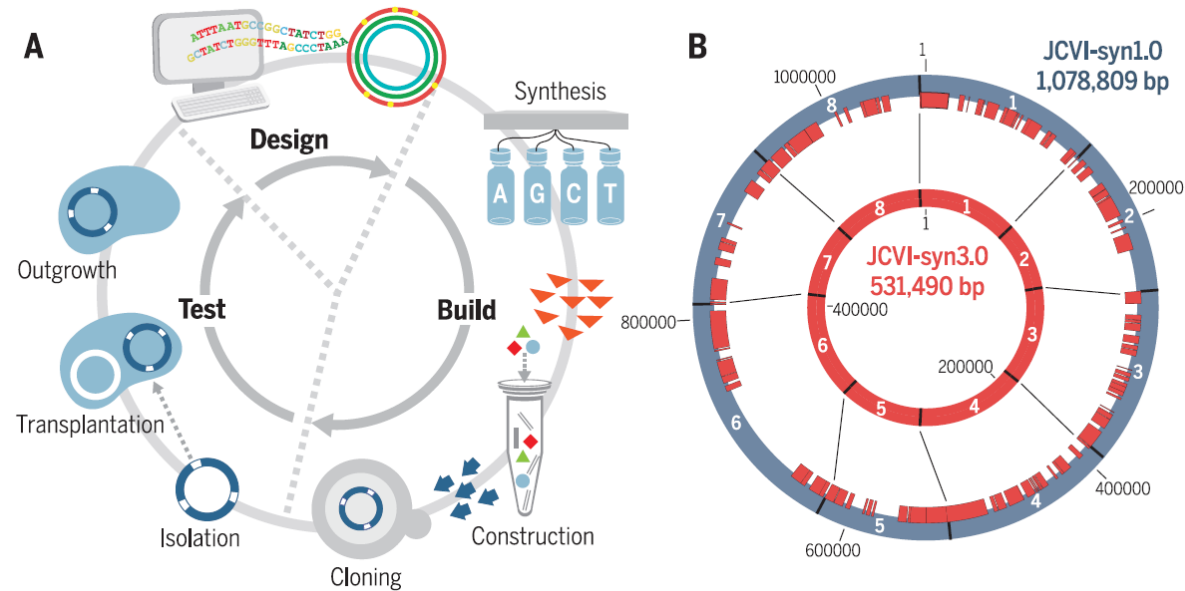
Nonessential genes (NE) – can be inactivated without affecting the viability or growth rate (in a specific environment)

Quasi-essential genes (QE) – their disruption impairs growth. They are important for robust growth, but not strictly essential.

	Genome size	Total genes	NE genes	Total - NE genes
<i>M. genitalium</i>	580 kb	507	101	406
<i>M. pneumoniae</i>	816 kb	739	259	480
<i>M. pulmonis</i>	963 kb	589	321	468
<i>M. mycoides</i> JCVI-Syn1.0	1080 kb	901	432	469



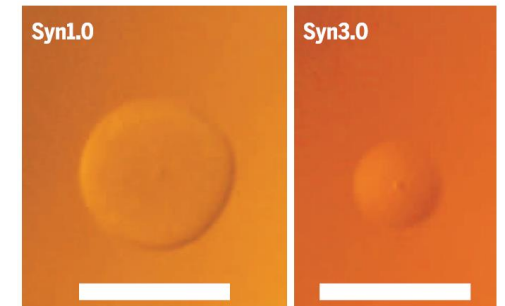
Design and synthesis of a minimal bacterial genome



M. mycoides JCVI-Syn1.0
(1078 kb, 863 protein and 38 RNA-coding genes)
doubling time 60 min

3x reiterative cycles

M. mycoides JCVI-Syn3.0
(531 kb, 438 protein and 35 RNA-coding genes)
doubling time 180 min



Insertional mutagenesis identified additional 53 NE genes – extrapolation to NE equals to 0 predicts 413 essential genes.

Genes retained in the Syn3.0 genome

Fig. 6. Partition of genes into four major functional groups.

Syn3.0 has 473 genes. Of these, 79 have no assigned functional category (Table 1). The remainder can be assigned to four major functional groups: (i) expression of genome information (195 genes); (ii) preservation of genome information (34 genes); (iii) cell membrane structure and function (84 genes); and (iv) cytosolic metabolism (81 genes). The percentage of genes in each group is indicated.

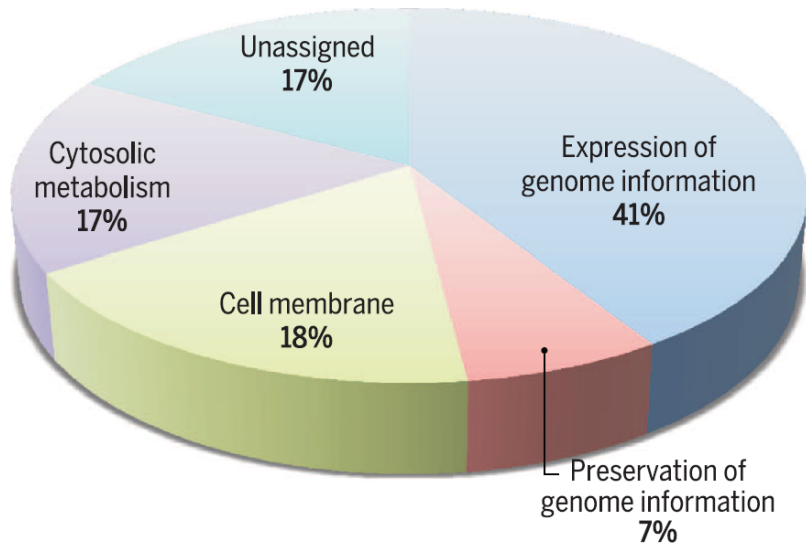


Table 1. Syn1.0 genes listed by functional category and whether they were kept or deleted in syn3.0. Categories with asterisks are mostly kept in syn3.0, whereas those without are depleted in syn3.0. Vector sequences, for selection of the genome and for propagation in other hosts, are not included in these gene tallies.

Functional category	Kept	Deleted
Glucose transport and glycolysis*	15	0
Ribosome biogenesis*	14	1
Protein export*	10	0
Transcription*	9	0
RNA metabolism*	7	0
DNA topology*	5	0
Chromosome segregation*	3	0
DNA metabolism*	3	0
Protein folding*	3	0
Translation*	89	2
RNA (rRNAs, tRNAs, small RNAs)*	35	4
DNA replication*	16	2
Lipid salvage and biogenesis*	21	4
Cofactor transport and salvage*	21	4
rRNA modification*	12	3
tRNA modification*	17	2
Efflux*	7	3
Nucleotide salvage	19	8
DNA repair	6	8
Metabolic processes	10	10
Membrane transport	31	32
Redox homeostasis	4	4
Proteolysis	10	11
Regulation	9	10
Unassigned	79	134
Cell division	1	3
Lipoprotein	15	72
Transport and catabolism of nonglucose carbon sources	2	34
Acylglycerol breakdown	0	4
Mobile elements and DNA restriction	0	73
Total	473	428



Functional classes of protein coding genes

Table 1. Breakdown of protein coding genes in JCVI-syn3A into functional classes.

Functional hierarchy		Protein		Genes		Essentiality			
		%	# unique	%	# unique	# E	# Q	# N	# model
Cellular processes	Cell Growth	1.02	4	0.88	4	1	0	3	0
	Defense	0.23	2	0.44	2	1	0	1	1
	<i>Subtotal</i>	1.25	6	1.33	6	2	0	4	1
Genetic information processing	DNA Maintenance	5.07	38	8.41	38	25	9	4	3
	Folding, Sorting and Degradation	9.58	25	5.53	25	18	7	0	7
	Transcription	3.92	14	3.32	15	8	5	2	0
	Translation	39.5	129	29.7	134	95	28	11	25
	<i>Subtotal</i>	58.1	206	46.9	212	146	49	17	35
Metabolism	Biosynthesis	4.27	29	6.86	31	26	4	1	27
	Central Carbon Metabolism	16.4	46	10.4	47	26	10	11	44
	Energy Metabolism	0.47	4	0.88	4	2	1	1	1
	Membrane Transport	9.37	54	12.6	57	37	16	4	46
	Other Enzymes	1.12	4	0.88	4	2	1	1	1
	<i>Subtotal</i>	31.6	137	31.6	143	93	32	18	119
Unclear	Kegg ortholog defined	1.04	8	1.77	8	3	2	3	0
	No Kegg ortholog	7.98	71	18.4	83	27	30	26	0
	<i>Subtotal</i>	9.02	79	20.1	91	30	32	29	0
<i>Total</i>		100.	428	100.	452	271	113	68	155

Of the 91 genes of unclear function, 30 are essential, 32 are quasi-essential, and 29 are non-essential. Those 30 essential genes could represent new biological mechanisms not yet defined and should motivate the search to discover their function.



Recommended reading:

Lau Y.H. et al. (2018) Prokaryotic nanocompartments form synthetic organelles in a eukaryote. Nat Comm 9:1311

Hutchison C. A. et al. (2019) Design and synthesis of a minimal bacterial genome. Nature 351:1414

