ADVANCED BIOLOGY
www.advanced-bio.com

The Lithbea Domain

Jaime Gómez-Márquez

The tree of life is the evolutionary metaphor for the past and present connections of all cellular organisms. Today, to speak of biodiversity is not only to speak of archaea, bacteria, and eukaryotes, but they should also consider the "new biodiversity" that includes viruses and synthetic organisms, which represent the new forms of life created in laboratories. There is even a third group of artificial entities that, although not living systems, pretend to imitate the living. To embrace and organize all this new biodiversity, I propose the creation of a new domain, with the name Lithbea (from life-on-the-border entites) The criteria for inclusion as members of Lithbea are: i) the acellular nature of the living system, ii) its origin in laboratory manipulation, iii) showing new biological traits, iv) the presence of exogenous genetic elements, v) artificial or inorganic nature. Within Lithbea there are two subdomains: Virworld (from virus world) which includes all viruses, regarded as lifeless living systems, and classified according to the International Committee on Taxonomy of Viruses (ICTV), and ii) Humade (from human-made) which includes all synthetic organisms and artificial entities. The relationships of Lithbea members to the three classical woesian domains and their implications are briefly discussed.

1. The Conceptual Framework

Understanding what life is and being able to discriminate between what is a living system and what is not is of great conceptual importance for biology. Clarifying these concepts has implications for research on the origin of life, the search for extraterrestrial life, the resolution of the dilemma of whether viruses are inert entities or living systems, and for establishing whether synthetic organisms or artificial life forms (hereafter A-life) should be considered living beings or not. Without being clear about this, we would never be able to describe in an integrated way all the biodiversity (natural or not) that inhabits our laboratories and ecosystems. Therefore, we need a conceptual framework that allows us, in the first place, to establish whether viruses and all these synthetic organisms and life form A that make up the new biodiversity are living beings or not, to be able to classify them in a coherent way. Unfortunately, the scientific community has not yet established a universally accepted doctrine on the definition of life and living being, hence my reference in this study is my proposal on both concepts.[1]

My definition of life arises from the analysis of the seven traits common to all living systems: i) organic nature (the biochemistry of life is based on the chemistry of carbon), ii) entropy-producing (living beings are open thermodynamic systems that preserve their internal order by exporting entropy to the outside), iii) self-organizing (systems far from thermodynamic equilibrium or dissipative structures can spontaneously self-organize), iv) reworkable pre-program (all living systems contain a mutable molecular program written in their genome), v) capacity to interact and adapt (any living system develops its vital functions through multiple interactions and is capable of adapting to new circumstances), vi) reproduction and vii) evolution (reproduction allows the perpetuation of species and makes the evolutionary process possible).

From the analysis of these traits, I define life as an interactive process occurring in entropy-producing, adaptive, and informative organic systems. Note that this definition of life does not include reproduction and evolution because both traits are not necessary for life; they are optional or facultative traits because not all organisms reproduce and/or evolve (e.g., a sterile animal such as a mule cannot reproduce but is nevertheless a living being). According to this definition of life, a living being is an entropy-producing, adaptive, and informative organic system, i.e., the living system is the "container" where the vital process takes place. Note the importance of the system-process duality, which means that without the living being (the system) there is no life, and without life (the process) the living being ceases to be ordered and decays.[1]

With this definition, it is easy to see why a volcanic rock, a quartz crystal, a complex algorithm, or a metallic robot are not structures that we can identify and classify as living systems. For example, a quartz crystal, although it is a highly ordered structure like any living being, is not a living system because it is neither an organic system, nor does it have genetic information, nor does it can adapt or evolve (evolution is a consequence of the adaptive process) through something like mutation and reproduction.

J. Gómez-Márquez
Department of Biochemistry and Molecular Biology
University of Santiago de Compostela
Santiago de Compostela, Galicia 15782, Spain
E-mail: jaime.gomez.marquez@usc.es

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adbi.202300679

DOI: 10.1002/adbi.202300679

2. Viruses as Lifeless Living Systems

The biological significance of viruses is unquestionable, and they should not be ignored if we are to understand the processes of horizontal gene transmission, the cause of many diseases, the evolution of species, the dynamics of ecosystems, or the biogeochemical cycles.[2–6] Despite this, viruses have been systematically excluded as components of biodiversity because of their acellular nature and because they were not considered living beings.[7]

Are viruses living systems? This question raises one of the most prominent controversies in the field of biology. Viruses are somewhere between living and inert: when they are outside the cell, they are passive structures waiting for the moment to infect their biological host, but when they find it, viruses develop their entire vital strategy to perpetuate themselves. Outside the cell, viruses only have the potential to infect cells, they are just highly organized and informative organic systems that show no signs of life, they are lifeless living systems. On the contrary, when a virus infects a cell, it manifests all the characteristics of a living system (entropy production, expression of its genetic information, self-organization, capacity for interaction and adaptation, reproduction, and evolution) and, as it meets all the requirements to be alive, it should be considered a living system.[1]

In short, viruses are obligate intracellular parasites that do not need to be a cell but, like any parasite, use their host for their own benefit. Viruses pursue the same goals as all living cellular beings: to survive and reproduce or remain in the host cell. The viral duality between the inert organic system (outside the cell) and the living system (during the infection) is what makes viruses to be considered as lifeless living beings and, therefore, as components of the biodiversity that exists on our planet.

3. Synthetic Organisms and A-Life Forms

In the midst of the biological revolution brought about by the great conceptual and technological advances in many areas related to the life sciences, synthetic organisms are playing a major role in the development of our society. They are living entities that are not the result of a natural evolutionary process but have been manufactured through direct cellular or genetic manipulation.[8] Synthetic biology involves redesigning organisms for useful purposes by engineering them to have new abilities, consequently, synthetic organisms, understood as biological entities constructed in the laboratory from natural biological systems, are living systems because they meet all the requirements necessary for the life process.[1] Synthetic biology is already transforming the way we grow food, produce substances of health interest, carry out certain industrial processes, etc., and its importance will increase over time.[9,10]

In a broad sense, A-life could be understood as the synthesis and simulation of living systems, being more closely related to artificial intelligence (hereafter AI) and the design of entities that aim to mimic the living,[11,12] as compared to synthetic biology that aims to build new living systems that are based on organic matter, i.e., carbon biochemistry. Three types of A-life are commonly mentioned in the scientific literature: i) soft A-life (soft from software), which deals with mathematical and computational models; ii) hard A-life (hard from hardware), which refers primarily to mechanical robots; iii) wet A-life, which includes synthetic organisms based on carbon chemistry.[13] Soft and hard A-life cannot be considered as living entities because neither computer programs nor robots or robot-like devices are constructed and function according to the biochemistry of life, they do not meet the requirements to be considered as a living system.[1,14] However, wet A-life is very different from soft and hard A-life, and since it uses organic matter to create new biological entities it falls within the field of synthetic biology[8,15]; I do not consider wet A-life forms as artificial entities but true living systems because they meet all the characteristics to be alive.

In brief, we could define synthetic organisms as authentic living systems that, not being the result of a natural evolutionary process, have all the necessary characteristics to develop the vital process, while A-life (soft and hard) are non-living entities that imitate living ones.

4. Brief Consideration of Artificial Selection

Traditionally, artificial selection techniques have been used to direct the evolution of species of agricultural and livestock or companion animal interest.[16] Thus, the researcher selected the preferred trait and then breed the organism to produce offspring with the desired phenotype. Artificial selection is synonymous with human-directed evolution, and, like natural selection, it works by selecting genotypes and phenotypes, except that in the latter it is nature that makes the decisions about which genetic changes should remain in the species. I consider that the organisms obtained by artificial selection cannot be qualified strictly as synthetic organisms because neither the system used to obtain them (selective breeding that alters gene frequencies) nor the modifications in the genome were obtained by direct genetic manipulation altering the gene content, the genetic code, or whatever it may be.

5. The Tree of Life and the New Biodiversity

When we talk about biodiversity and the evolution of species, we always turn to the “tree of life”, a brilliant idea echoed by Darwin[17] in his seminal book on the origin of species by natural selection when he wrote: “The affinities of all the beings of the same class have sometimes been represented by a great tree. I think this simile largely speaks the truth”. In this line, in the late 20th century, it was proposed a new conception of the “tree of life” that grouped cellular organisms into three domains: Archaea, Bacteria, and Eukarya, as the highest rank of classification.[18] Later on, the discovery of a myriad of diverse archaeal lineages changed our understanding of the evolutionary relationships among the three domains of life and the origin of eukaryotes.[19] At the present time, with the genome revolution underway, the classification of biodiversity is increasingly refined, although a consensus that satisfies the entire scientific community has not yet been reached.[20,21]

In the 21st century, do the current trees of life in their many variants represent all the biodiversity on our planet? And the answer I understand should be negative because viruses are not there, and neither are synthetic organisms (I do not mean to say that these trees are wrong but that they do not include all

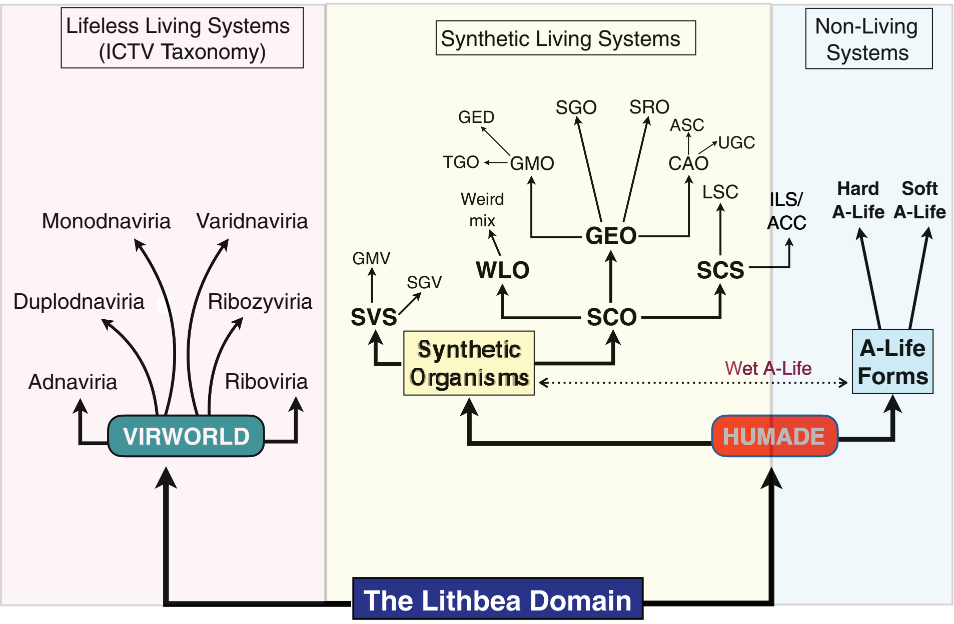

Figure 1. The Lithbea domain. This domain is divided into two evolutionarily unrelated subdomains: Virworld, which includes all realms of viruses following ICTV rules, and Humade that comprises all human-made entities. Synthetic organisms are non-natural living systems (except for ILS), and A-life forms that are non-living systems (the double-arrowed dotted line indicates that wet-life should be included within the A-life forms and synthetic organisms). SVS, synthetic viral systems; GMV, genetically modified viruses; SGV, synthetic genome viruses; SCO, synthetic cellular organisms; WLO, weird living organisms (the expression "weird mix" refers to all these strange organisms such as the xenobots or the system known as brainoware); GEO, genetically engineered organisms; SGO, synthetic genome organisms; GMO, genetically modified organisms; TGO, transgenic organisms; GED, gene-edited organisms; CAO, codon-altered organisms; ASC, altered standard code; UGC, unnatural genetic code; SRO, synthetic replicon organisms; SCS, synthetically cellular systems; LSC, living synthetic cells; ILS/ACC, incomplete living systems/artificially created cells.

biodiversity). Unlike viruses, the apparent exclusion of synthetic organisms from the tree of life is due to the fact that these creatures are not the result of the natural evolutionary process, although some of them could be included within Archaea, Bacteria, or Eukarya because they did not lose their essence as a species, i.e., a transgenic mouse is still a mouse although it is no longer the exclusive fruit of the evolutionary process.[14] There are other synthetic organisms that are very different from any known cellular form and that could not be included or resemble any organism belonging to the Woesian domains.

6. The Lithbea Domain

The Lithbea domain, an acronym for "LIFE-on-THE-Border Entities", was born from the idea of grouping and classifying all those biological (viruses and synthetic organisms) and biological-like (A-life) entities that were not included in the tree of life (it should be noted that lineage and phylogeny have not been determining factors for the classification system presented here). The A-life forms are included because although we cannot consider them as true living systems, they resemble them more and more and help us to understand the phenomenon of life.[14] **Figure 1** shows the complexity of the Lithbea domain.

The criteria for entering Lithbea are: i) the acellular nature of the living system (e.g., viruses), ii) the non-natural origin of the living system (e.g., transgenic organisms), iii) the presence of novel biological traits (e.g., xenobots), iv) the presence of exogenous genetic elements (e.g., plasmids), and v) the artificial or inorganic nature of entities that mimic the life process or the living system (e.g., A-life forms).

In Lithbea there are two subdomains: Humade (a term derived from the contraction of the expression Human-made) which includes all synthetic organisms and A-life forms, and Virworld (a term derived from the contraction of the expression Viral world), which includes all viruses present in nature (obviously viruses could also be created in the laboratory and thus form part of synthetic entities). Unlike the tree of life that shows us the existence of common ancestors, the entities belonging to Lithbea do not have a common ancestor and, consequently, the construction of the tree of life of the Lithbea domain is not based on phylogeny and ancestry, but on the criteria mentioned above. On the other hand, it should be noted that the entities belonging to Humade and Virworld are not isolated entities but can interact with each other and with prokaryotic and eukaryotic organisms. Furthermore, certain synthetic organisms included within Lithbea, such as transgenic organisms, have not lost their species identity and therefore might also form part of the Woesian domains even if their origin is no longer completely natural.

7. The Viral Subdomain (Virworld)

There are several ways to classify viruses considering aspects such as capsid structure, nucleic acid type, physical properties, host species, or the disease caused by their infection.[22] In this respect, it has been recently proposed four principles or recommendations to guide the construction of a coherent and comprehensive virus taxonomy.[23]

Within the Lithbea domain, the viral subdomain includes all-natural viruses classified according to the International Committee on Taxonomy of Viruses (ICTV) taxonomy (Figure 1 shows only the six realms). The ICTV is the official body that classifies viruses and by contrast to the taxonomy of cellular organisms, the taxonomic framework for viruses must adapt to the current view that viruses have multiple origins (polyphyly) and that their diversity cannot be represented in a single tree that encompasses the entire virosphere.[24,25] So far there are six realms within the ICTV taxonomy, each of which is inferred to represent a unique monophyletic evolutionary origin of its component viruses, although recent evidence suggests that the number of independent and ancient evolutionary origins of viruses may be even greater.[25,26]

Trying to figure out the evolutionary history of viruses is fascinating. Viruses may have arisen from mobile genetic elements, may have preceded cellular life, may have originated from free-living forms that became obligate intracellular parasites, or may have arisen by a yet unknown mechanism.[27,28] Biologists have debated how to classify these lifeless living things and how to relate them to the tree of life. The main problem is that we do not know for sure what has been or what their origins have been, but whatever it is, viruses and their hosts have been ecologically and evolutionarily intertwined.[4,29] Be that as it may, a universal taxonomy of viruses is essential for a comprehensive view of the virosphere and their evolutionary relationships.

8. The Human-Made Subdomain (Humade)

The Humade subdomain comprises all synthetic organisms and A-life forms and is divided into two major groups: i) Synthetic organisms (non-natural organic living systems) and ii) A-life forms (non-living systems that mimic life). As I mentioned earlier, wet A-life entities would be included in the group of synthetic organisms although some researchers include them as a type of A-life (this duality is shown in Figure 1).

9. Synthetic Organisms

Synthetic biology expands the biodiversity of the planet by constructing new organisms with structural and/or functional elements that are different from natural ones.[8,15] Its development has gone hand in hand with advances in molecular biology, biotechnology, and, more recently, AI, and synthetic organisms will increasingly have a wide range of applications in various sectors, such as medicine, agriculture, energy, manufacturing, and food. The future will bring us hitherto unimaginable creatures that will be increasingly present in our societies and ecosystems.

Within synthetic organisms there are two categories: i) Synthetic Viral Systems (SVS), and ii) Synthetic Cellular Organisms (SCO). The criterion for establishing these two categories is the acellular or cellular nature of biological entities. SVS includes all viruses employed in genetic engineering-related techniques and viruses whose genome has been synthesized in the laboratory. SCO are classified according to the type of genetic manipulation they have undergone and the way they have been created in the laboratory.

SVS is divided into two different classes: i) Genetically Modified Viruses (GMV), and ii) Synthetic Genome Viruses (SGV). SCO is divided into three different classes: i) Genetically Engineered Organisms (GEO), ii) Weird Living Organisms (WLO), and iii) Synthetic Cellular Systems (SCS).

10. The SVS (Synthetic Viral Systems)

SVS comprises all viruses whose genome has been modified for cloning, medical or biotechnological applications (recombinant viruses or GMV), and viruses whose genome has been wholly or partially synthesized in the laboratory (SGV).

GMVs are viruses that have been genetically modified by insertion, deletion, or mutation in the viral genome without losing their ability to infect. GMVs have been used for multiple purposes related to cloning, DNA sequencing, or basic biological research, as well as for medical, agricultural, pharmaceutical, and biotechnological purposes.[30,31] In medicine, GMVs can be used to target and destroy cancer cells, treat various genetic diseases as gene and cell therapy tools, or serve as vaccines or vaccine delivery agents.[32] For example, different viral vectors have been used in preclinical and clinical trials as vaccines against serious diseases caused by infectious agents such as HIV, and *Plasmodium* sp.(Malaria), Ebola and SARS-CoV-2.[33]

The combination of techniques in the field of synthetic biology and computational biology has led to the production of viruses whose genome is synthetic, manufactured in whole or in part in the laboratory. The first virus assembled from synthetic oligonucleotides was a poliovirus.[34] This milestone in synthetic virology was followed by the generation of the first synthetic genome corresponding to bacteriophage phiX174.[35] Subsequently, the construction of an infectious horsepox virus in 2018 from synthesized DNA raised considerable doubts and concerns about the possibility of creating human pathogenic viruses in the laboratory.[36] Synthetic virology has come a long way, and its future looks very promising, with numerous positive applications ranging from medicine to agriculture, although not without risks.[37,38] We now have the technological power to resurrect viruses or create new viruses in the laboratory for multiple purposes. In this sense, scientists, legislators, and society in general should act responsibly by making a reasonable balance between progress and the dangers associated with this powerful technology.

11. SCO (Synthetic Cellular Organisms)

11.1. The GEO (Genetically Engineered Organisms)

Redesigning organisms to produce a certain substance like transgenic rice with beta-carotene or acquire a new capacity like bacteria used for bioremediation, are two examples of the initial goals in synthetic biology. Since then, scientific progress is leading us to advances in genome manipulation that were unthinkable not long ago. GEO includes organisms whose genetic material (the genome or the genetic code) has been modified to give them new properties, organisms whose genome is fully synthetic or semi-synthetic, and organisms that contain extrachromosomal elements. There are four groups of GEO: Genetically Modified Organisms (GMO), Synthetic Genome Organisms (SGO), Codon-Altered Organisms (CAO), and Synthetic Replicon Organisms (SRO).

11.1.1. The GMO (Genetically Modified Organisms)

A GMO is a genetically modified organism whose genomic modifications are not the result of selective mating (artificial selection) or natural recombination, but the result of DNA manipulation in the laboratory. DNA sequences can be introduced, amplified, or deleted within a species, between species, or even between kingdoms. There are two types of GMO: transgenic (TGO) and gene/genome-edited (GED) organisms.

A TGO is a living system whose genetic composition has been modified by introducing a foreign gene by means of biotechnological techniques. TGOs are used for basic biological research as well as for applications related to food supply, human health like the production of substances of therapeutic interest, and the mitigation of environmental pollution. DNA can be transferred within individuals of the same species like a transgenic mouse expressing lactase in the mammary gland to produce low–lactose milk in vivo,[39] between different species like the introduction of rabbit DNA fragments containing the beta-globin gene into mouse germ-line cells[40] or even between kingdoms like the expression of firefly luciferase gene in transgenic tobacco leaves.[41]

In the GED organisms, the alteration of the genome is performed using nucleases that have been engineered to target a specific DNA sequence, where they introduce cuts enabling the removal of existing DNA and the insertion of replacement DNA.[42,43] Gene editing involves the insertion (gene knock-in), silencing (gene knock-out or loss-of-function), deletion, or replacement of DNA to obtain a new function or phenotype.[44] Of particular interest is the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) technology, whose applications have profoundly changed biological research.[45] CRISPR-Cas genome editing systems have transformed our ability to manipulate, detect, and visualize specific DNA and RNA sequences in living cells. The ease, accuracy, affordability, and of this system robustness of this technology have revolutionized genome editing for research ranging from fundamental science to translational medicine.[46,47] For example, the high efficiency and accuracy of the CRISPR/Cas9 technique allow us to explore the functions of cancer-related genes, greatly increasing our understanding of cancer genomics.[48] Although this technique has apparent advantages for gene therapy, CRISPR/Cas9 brings its own set of limitations which must be addressed for safe and efficient clinical translation.[49] Another example of the power of this technology is the edition of genes involved in metabolic pathways to create organisms that produce valuable compounds such as biofuels, drugs, and industrial chemicals.[50] The incorrect use of CRISPR-Cas technology could pose a risk and danger; the debate about the implications of this technology both in the scientific community and in society is important.

In addition to other issues unrelated to biology, GMOs could have very serious impacts on biodiversity if they interact with natural species. In this regard, the production of transgenic animals and crops has raised concerns about their potential ecological impact, especially if these organisms escape or are released into the wild and may interbreed with wild flora and fauna. Obviously, the same or even greater concerns arise when we think of genetically modified microorganisms because their ability to mutate and reproduce is much greater. Three different examples of these GMO-nature interactions and the risks associated with them are as follows:

i. Interspecific hybridization between Atlantic salmon (*Salmo salar*) and brown trout (*Salmo trutta*) has been demonstrated through experimental crosses using the growth hormone transgene.[51,52] In this particular case, this interspecific hybridization (which could also occur among many other eukaryotic and prokaryotic species) makes it necessary to assess the ecological and evolutionary consequences of the presence of these growth-accelerated genetically engineered fishes in natural ecosystems.
ii. The introgression of transgenes from genetically modified crops to their wild relatives has been widely reported.[53] More recently, it has been shown the ecological and evolutionary consequences of transgene introgression in a Mexican wild cotton (*Gossypium hirsutum*) population.[54] In general terms, we could say that the biological consequences of the presence of one or more transgenes in a wild species will depend largely on the type of transgene, its insertion site, the plant density, and ecological factors.
iii. The presence of transgenic microbes outside the laboratory could lead to their uncontrolled spread, affecting ecological processes through interaction with native populations and food chains. In addition, there would be the risk of transfer of transgenic DNA by horizontal gene transfer that could give rise to new pathogens. All this makes it important to take all the necessary precautions to avoid unpredictable negative consequences that are very difficult to reverse.[55]

11.1.2. The SGO (Synthetic Genome Organisms)

Synthetic genomics is a nascent field of synthetic biology that involves two basic operations: the synthesis of complete genomes or chromosomes and the utilization of these synthetic nucleic acids to make viruses or living cells. In this context, SGOs are those synthetic organisms whose genome has been entirely or partially synthesized in the laboratory.[56,57] In the near future, the creation of new SGOs will have a strong impact on many areas of our society related to medicine or biotechnology such as, for example, the development of the next generation of vaccines or a new industrial revolution to produce food and chemicals.

The first synthetic bacterial genome was completed in 2008 with the synthesis of the genome of *Mycoplasma genitalium* (named as *M. genitalium* JCVI-1.0), a bacterium that can cause urinary and genital tract infections in humans.[58] This achievement marked an important milestone in synthetic biology because it was the first time a complete genome was synthesized from scratch in a laboratory. Thereafter, the same team constructed *Mycoplasma mycoides (M. mycoides)* JCVI-syn1.0, the first living cell with a fully artificial genome, by first synthesizing the bacterial chromosome sequence and then transferring it into a *Mycoplasma capricolum (M. capricolum)* recipient cell to create new *M. mycoides* cells controlled solely by the synthetic chromosome.[59] In 2016, the genetic information of JCVI-syn1.0 was reduced to produce JCVI-syn3.0, which harbors the smallest genome – 531 kb and 438 genes – of any free-living prokaryote.[60] Later on, it was created JCVI-syn3A for better growth, demonstrating the polygenic nature of cell division and morphology in a minimal cell.[61]

Genome minimization and re-functionalization are very attractive research topics because minimal genomes (the smallest possible number of genes required to support a living cell under a defined set of conditions) may help to find an answer to age-old questions like What is life? and improve the applications of synthetic biology.[62] In this regard, the reduced genome of the synthetic bacterium JVC-syn3A was modified to include the *Spiroplasma* genes involved in motility to create the smallest-ever moving cell.[63] Moreover, natural selection can rapidly increase the fitness of a synthetically constructed minimal cell that has been stripped of all but its essential genes, demonstrating the capacity of a minimal cell to survive and evolve (Morger–Reischer et al. 2023).[64]

In the eukaryotic world, a strain of the yeast *Sacharomyces cerevisiae (S. cerevisiae)* whose genome is more than 50% composed of synthetic DNA has been produced by combining seven synthetic chromosomes into a single yeast cell, resulting in a strain with more than 50% synthetic DNA that survives and replicates similarly to wild yeast strains.[65] This discovery is a milestone in SGO research because, until now, scientists have generated artificial genomes from various viruses and bacteria, but yeast would be the first eukaryotic organism with a viable genome partially generated in the laboratory.[66] Very recently, the in vivo assembly of chromosomal fragments in the moss *Physcomitrium patens* has been published.[67] The researchers produced phenotypically near-wild lines of the moss in which one-third of the coding region of a chromosomal arm is replaced by chemically synthesized, redesigned fragments. We are only at the beginning of this new genomic revolution and these advances with yeast and moss are paving the way for the synthesis of new eukaryotic genomes and opening many windows to study the functioning of genomes and for the creation of new " amazing" organisms.

11.1.3. The CAO (Codon-Altered Organisms)

The genetic code refers to the set of instructions needed to convert the information contained in protein-coding genes into proteins, and it is its universality that allows organisms to exchange genetic information, even between evolutionarily distant species, and to express an eukaryotic protein in bacteria. The technological advances in genetic engineering not only allow us to create synthetic genomes but also to alter the genetic code.[68] Here, we define CAO entities as synthetic organisms in which the genetic code has been altered. In this type of living system, we are modifying the genetic code to change the sequence of codons, to reduce the number of triplets, or to introduce new unnatural codon variants. There is no doubt that CAO organisms could be very useful in the production of synthetic proteins, or in the design of new substances.

There are two main variants of organisms with the natural genetic code altered: i) ASC (from Altered Standard Code) that includes those living systems with variations in the number of standard codons or reassigning the significance of the codons, and ii) UGC (from Unnatural Genetic Code) that includes those organisms with non-natural triplets and/or non-natural amino acids.

To synthesize proteins, the translation machinery of the cells uses 64 triplet codons for the canonical 20 amino acids; 18 of these amino acids are encoded by more than one codon which means that the genetic code is redundant. The first ASC organism was a variant of *Escherichia coli (E. coli)* with the number of codons used to encode canonical amino acids reduced to 61, by genome-wide replacement of target codons with defined synonyms. To achieve this reduction, 18214 codons were recoded to create an organism that uses 59 codons to encode the 20 amino acids and enables the deletion of a previously essential transfer RNA.[69] The generation of organisms with a reduced genetic code, avoiding redundancies without losing information, has been a major scientific achievement that has shown that life can operate with a reduced number of synonymous sense codons. A different example of alterations of the genetic code was the development of *E. coli* cells with an amino acid-swapped genetic code that reassigns two of the six codons from serine to leucine during translation, making the cells resistant to viral infection.[70] This amino acid-swapped genetic code makes cells completely resistant to viral infections by mistranslating viral proteomes and prevents leakage of synthetic genetic information by relying on serine codons to produce proteins that require leucine. As the authors suggest, these findings may provide the basis to make any organism safely resistant to all natural viruses and prevent genetic information flow into and out of genetically modified organisms.

The UGC organisms are synthetic living systems with an expanded nucleotide alphabet by making DNA with unnatural nucleotides.[68] Expansion of the genetic code, in which a single amino acid is replaced by a non-natural amino acid, is also a way to create new proteins or to label proteins without disturbing their structure and thus to better visualize them in living cells.[71] One of the pioneering experiments in this field was the use of engineered tRNAs capable of incorporating non-canonical amino acids using four-letter codons.[72,73] Subsequently, it was created a semisynthetic organism that can stably store genetic information using a six-letter, three-base-pair alphabet[74] and it was demonstrated for the first time that synthetic organisms can use non-natural nucleotides for DNA replication, RNA transcription, and protein translation in vivo.[75] Another major milestone in synthetic biology was the construction of "Hachimoji" DNA which is a genetic system composed of eight letters that can be transcribed by a modified RNA polymerase in the laboratory.[76] The applications of modifying or extending the genetic code are potentially enormous, but we should be aware of the potential risks of these technological advances.

Nature also experiments with variants of the ACGT alphabet. This is the case of the genome of certain bacteriophages in which adenine (A) has been substituted by 2-aminoadenine (Z). These phages contain DNA with an alternative genetic alphabet (ZTGC) that evades the attack of bacterial restriction enzymes allowing the persistence of these viruses in toxic environments.[77] Given that our knowledge of the virosphere is far from complete, one wonders how many surprises its study will bring us in the coming years, and there will probably be many.

11.1.4. The SRO (Synthetic Replicon Organisms)

Replicons are units of DNA that can replicate autonomously within a suitable host. Synthetic Replicon Organisms (SRO) are organisms that have been transformed with a replicon to give them one or more new functions. This is the case of unicellular organisms (bacteria and yeasts) transformed with plasmids, a replicon widely employed in recombinant DNA technology or genetic engineering. Plasmids are extra-chromosomal circular double-stranded DNA molecules that replicate independently of chromosomal DNA. Scientists have harnessed plasmids as cloning vectors to amplify and manipulate genes and DNA fragments, as well as to express recombinant proteins in well-established prokaryotic and eukaryotic hosts, such as *E. coli* or *S. cerevisiae*. These organisms transformed with recombinant plasmids produce important chemicals such as biopolymers or biofuels and proteins like insulin or growth hormone of high therapeutic value.[78] In addition, designing and constructing plasmids from smaller fragments to form complex functional DNA molecules (such as biochemical pathways and genetic circuits) is an excellent synthetic technology for molecular biology studies.[79,80]

An interesting application of the use of SRO is the development of cellular systems for in vivo continuous direct evolution.[81] Thus, using this system, it was established a synthetic orthogonal replication system in *E. coli* that allows for accelerated evolution[82]; to reach this objective, the researchers designed a mutant orthogonal DNA polymerase that does not copy the bacterial genome and selectively increases the mutation rate of the orthogonal replicon.

11.2. The WLO (Weird Living Organisms)

Among all the new synthetic organisms, the WLO represents a heterogeneous group of weird living creatures, which are designed and created by combining non-biological technologies like AI and biological techniques such as embryo manipulation or molecular bioengineering. The first member of this group of bizarre organisms were the "xenobots", a kind of living robot designed using an AI evolutionary algorithm to predict which organisms could deploy useful tasks and build from embryonic cells of the frog *Xenopus laevis*.[83] Xenobots are like miniature machines made from living embryonic frog cells that spend a little over a week crawling or swimming around a Petri dish before disintegrating (as they do not eat, their lifespan is short). The AI program evolves groups of frog cells, based on their shape, to perform any task of interest to the scientists. These strange creatures self-replicate in an unpredicted way never been seen in nature, very different from what we know in other eukaryotic organisms.[84]

Another member of the WLOs completely different from the previous one is a hybrid biocomputer built by combining laboratory-grown human brain tissue with conventional electronic circuitry; this hybrid system, with the name "Brainoware", can complete tasks such as voice recognition.[85] To make the hybrid system, the researchers also used an AI hardware approach, they placed a single organoid onto a plate containing thousands of electrodes, to connect the brain tissue to electric circuits. To test the capabilities of brainoware, the system was employed to do voice recognition by training the system with recordings of people speaking. Among other potential applications, this study opens the possibility of creating biological computers.

Research with xenobots, brainoware system, and other bizarre creatures generated by the combination of AI and biological techniques could be very beneficial for the future, for example, to program these biological entities for decontamination tasks or for regenerative medicine. Xenobots and brainoware are just the beginning of WLO, but I am convinced that there will be more awesome synthetic living systems coming soon.

11.3. The SCS (Synthetically Cellular Systems)

The term SCS includes all synthetic (sometimes referred to by the term artificial) cellular systems created in the laboratory. It consists of two classes: LSC (Living Synthetic Cells), which refers to living cells totally created in the laboratory (there are no members of this category yet), and ACC (Artificially Created Cells) or ILS (Incomplete Living Systems), which are cell-like structures or minimal cells that mimic one or more cellular functions; I keep the name ACC because it appears frequently in the specialized literature although in no case are artificial cells but pseudo-cells that are capable of carrying out some cellular function but cannot be considered living systems.

LSC would be those living cellular systems that have been created from scratch in the laboratory, following a bottom-up approach, i.e., assembling all the fundamental organic and inorganic components of a cell within a membrane, thus generating a living system that has, at a minimum, a genome, a cytoskeleton, and a basic metabolism sufficient to obtain energy and build new molecules to grow.[86,87] Once this goal was achieved, the next step would be to get this primitive living system to reproduce and evolve. From that point on, cellular complexity could be increased at both the subcellular and multicellular levels. However, scientists have not yet succeeded in creating this minimal cell in the laboratory, although good steps are being taken in this direction. A significant advance was the introduction of two types of bacteria in membrane-free droplets, leading to the formation of an artificial cell with a functional and compositional complexity reminiscent of living cells.[88] Despite the technical difficulties in creating a viable cell, it is very likely that in the coming years or decades, scientists will succeed in creating life in the laboratory and for this reason, I have included in the classification of "Synthetic Organisms" the LSC within the SCS. The creation of life from inanimate matter, besides opening a new universe for synthetic biology, will help us to solve the great mystery of biology, which is none other than the origin of life and the beginning of the evolutionary process.[89]

When we refer to ACC or ILS, the first thing to say is that since the concept of artificial cells was proposed in 1957, the terms "artificial cells" and "synthetic cells" have been used interchangeably, without there being a precise definition of them.[90] Here I use the term "artificial" because it is the most used term in the literature on the subject. ACC or ILS entities (also known as minimal cells) are engineered encapsulated particles that can be endowed with various functions by incorporating bioactive materials, such as DNA, RNA, and proteins, within a membrane.[90,91]

An example of this technology is the strategy to assemble biocompatible cell-sized hydrogel-based artificial cells with a variety of different embedded functional sub-compartments, which act as engineered synthetic organelles.[92] The construction of artificial cells has great potential in the study of fundamental biological questions such as example, the origin of life or the organization of cells, and also for use in applications related to disease treatment and biotechnology.[90,93,94]

12. A-Life Forms

As I mentioned earlier, the aim of A-life is to design and analyze systems and processes related to natural life using computer modeling simulations, robotics, and biochemistry.[12] However, biological systems classified within wet A-life could well be considered as belonging to the field of synthetic biology. From this standpoint, there are only two different types of A-life: soft A-life that refers to computational modeling and simulation of lifelike behaviors, and hard A-life, which is related to physical robots or artifacts made of metal and plastic, including medical nanorobots defined as untethered nanostructures that contain an engine or can transform diverse types of energy sources to mechanical forces and perform a medical task (Kong et al. 2023).

Soft and hard A-life forms are not living entities because they do not meet all the requirements to be considered living, just as a flight simulator is not an airplane or a photo of a landscape a tangible reality.[1] The soft and hard A-life forms have nothing equivalent to genetic material, are not based on carbon biochemistry, and cannot adapt and evolve in response to changes in the environment, whereas living systems have a modifiable genetic program, can interact with their environment, and adapt to new circumstances, and consequently can evolve to give rise to new species. These entities always depend on their human designer, they cannot evolve by themselves because of chance and natural selection. A computer program or a sophisticated robot can never be a living system because the moment it becomes a living being it ceases to be a program or a robot. Nonetheless, although soft and hard A-life are not organic entities in which the vital process takes place, they try to imitate the living or design algorithms or systems that could eventually have a biological fit.

13. Concluding Remarks

First: The new biodiversity. In general, when talking about biodiversity, reference is always made to the variety of prokaryotic and eukaryotic species that make up ecosystems, but viruses and synthetic organisms are not mentioned. This should not be the case because the evolutionary relationship of viruses with their natural hosts and their role in ecosystems is unquestionable and because synthetic organisms can also colonize ecosystems and even hybridize with their natural "relatives". I have no doubt that future studies on evolution, biodiversity, and ecology will take into account the creatures belonging to the Lithbea domain because their relationship with other living beings on the planet will be increasingly intense.

Second: The unpredictability of the unknown. In the 21st century, we are faced with a growing box of biological surprises that are the result of enormous progress in biology and new technologies. These new biological entities may be very useful in the future for medical, environmental, or industrial purposes, but they also generate new dangers derived from the unpredictability of the unknown.[14] We will never be fully certain of the outcome and consequences of the creation of a new synthetic living being. Even if the unknown could become largely predictable, there will always be a principle of uncertainty, as is the case with the processes of the subatomic world.

Third: Evolution that evolves. S. Newman,[95] in an interesting reflection on the inherent forms and the evolution of evolution, argues that "Once a novel kind of biological matter has been constituted, evolution is channeled in new, preferred directions, ...". In this direction, it is possible to speculate that new rules may appear in the evolution of some of the synthetic living beings because their creation is not the result of a natural process (it has not been tested by natural selection), but of direct changes in the genetic program or the development of new synthetic beings. Perhaps the best evidence of this so far is the xenobots, because frog cells, released from their natural developmental pathway, are organized in distinctly un-frog-like ways, they clustered into spherical clumps that behaved like tiny organisms. Anyway, in my opinion, in order to try to predict the evolution of synthetic organisms, at least four factors must be taken into account: i) the process of creation of the synthetic organism because it will affect both its internal dynamics and its relationship with the environment; ii) the unpredictability of the unknown or, in other words, the emergence of new rules governing the evolution of synthetic organisms; iii) the influence of the laws of physics and chemistry, from which living matter cannot escape; iv) the principle of inexorability that says "things are so because they must be so" and that applied to synthetic organisms tells us that if the synthetic organism wants to fly it will need wings, that if it wants to grow it must feed itself, or that glucose will be converted into pyruvate unless we have introduced changes in the glycolytic enzymes.[96]

Fourth: Playing Gods (the mention of God has no religious component but a metaphorical one). Before the advent of the biological revolution, the ability to design and create new life was the "exclusive power" of the Gods, but now we have the capacity to control our own evolution and that of other species, and to manufacture new living beings with characteristics some of which we cannot even imagine. That is why it is very important at this point to set ethical limits, to establish legal rules that, without mutilating scientific progress, do not allow the creation of biological aberrations or microbial weapons of mass destruction.

Fifth: Questions in the air. The advances in molecular biology, computer technologies, and biological engineering that make this revolution possible raise many questions for which we do not yet have a clear answer. Let me give some examples. If organisms with an altered genetic code or synthetic genome were released into the wild, could they hybridize with their natural relatives and eventually displace them by taking their place in the ecosystem? What would happen if a synthetic pathogenic microorganism could escape from the laboratory and infect human populations? Could large-scale cultivation of genetically modified plants displace wild species and cause irreversible damage to the ecosystem? What would happen if xenobots or other strange living beings invaded our environment? And so on and so forth. We have to find a balance between scientific progress, the safety of people, the maintenance of ecosystems, and the ethical principles that tell us that not everything goes for the sake of scientific success.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

artificial Life Forms, life concept, lithbea, synthetic organisms, tree of life, viruses

Received: December 11, 2023
Revised: February 9, 2024
Published online:

[1] J. Gómez-Márquez, *Sci. Nat.* **2023**, *110*, 53.
[2] R. K. French, E. C. Holmes, *Trends Microbiol.* **2020**, *28*, 165.
[3] C. Gilbert, R. Cordaux, *Curr. Opin. Virol.* **2017**, *25*, 16.
[4] H. M. B. Harris, C. Hill, *Front. Microbiol.* **2021**, *11*, 604048.
[5] M. A. O'Malley, *Stud. Hist. Phil. Biol. Biomed. Sci.* **2016**, *59*, 71.
[6] C. A. Suttle, *Nat. Rev. Microbiol.* **2007**, *5*, 801.
[7] P. López-García, D. Moreira, *Nat. Rev. Microbiol.* **2009**, *7*, 615.
[8] K. L. Garner, *Essays Biochem* **2021**, *65*, 791.
[9] M. M. Hanczyc, *Artif. Life* **2020**, *26*, 260.
[10] C. A. Voigt, *Nat. Commun.* **2020**, *11*, 6379.
[11] W. Aguilar, G. Santamaría-Bonfil, T. Froese, C. Gershenson, *Front. Robot. AI* **2014**, *1*, 8.
[12] C. Gershenson, V. Trianni, J. Werfel, H. Sayama, *Artif. Life* **2020**, *26*, 391.
[13] M. A. Bedau, J. S. McCaskill, N. H. Packard, S. Rasmussen, *Artif. Life* **2010**, *16*, 89.
[14] J. Gómez-Márquez, *Global Philos.* **2023**, *33*, 4.
[15] S. Fernau, M. Braun, P. Dabrock, *PLoS One* **2020**, *15*, e0235808.
[16] J. K. Conner, *Ecology* **2003**, *84*, 1650.
[17] C. Darwin, *On the Origin of Species by Means of Natural Selection, or, The Preservation of Favoured Races in the Struggle for Life*, J. Murray, London **1859**.
[18] C. R. Woese, O. Kandler, M. L. Wheelis, *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 4576.
[19] L. Eme, A. Spang, J. Lombard, C. W. Stairs, T. J. G. Ettema, *Nat. Rev. Microbiol.* **2017**, *15*, 711; Erratum in: L. Eme, A. Spang, J. Lombard, C. W. Stairs, T. J. G. Ettema, *Nat. Rev. Microbiol.* **2018**, *16*, 120.
[20] L. A. Hug, B. J. Baker, K. Anantharaman, C. T. Brown, A. J. Probst, C. J. Castelle, C. N. Butterfield, A. W. Hernsdorf, Y. Amano, K. Ise, Y. Suzuki, N. Dudek, D. A. Relman, K. M. Finstad, R. Amundson, B. C. Thomas, J. F. Banfield, *Nat. Microbiol.* **2016**, *1*, 16048.
[21] T. Stephan, S. M. Burgess, H. Cheng, C. Danko, C. A. Gill, E. D. Jarvis, K. Koepfli, J. Koltes, E. Lyons, P. Ronald, O. Ryder, L. M. Schriml, P. Soltis, S. VandeWoude, H. Zhou, E. A. Ostrander, E. K. Karlsson, *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2115644119.
[22] J. Louten, *Virus Structure and Classification. Essential Human Virology*, Academic Press, Cambridge US **2022**, Vol. *2*.
[23] P. Simmonds, E. M. Adriaenssens, F. M. Zerbini, N. G. A. Abrescia, P. Aiewsakun, P. Alfenas-Zerbini, Y. Bao, J. Barylski, C. Drosten, S. Duffy, W. P. Duprex, B. E. Dutilh, S. F. Elena, M. L. García, S. Junglen, A. Katzourakis, E. V. Koonin, M. Krupovic, J. H. Kuhn, A. J. Lambert, E. J. Lefkowitz, M. Łobocka, C. Lood, J. Mahony, J. P. Meier-Kolthoff, A. R. Mushegian, H. M. Oksanen, M. M. Poranen, A. Reyes-Muñoz, D. L. Robertson, et al., *PLoS Biol.* **2023**, *21*, e3001922.
[24] International Committee on Taxonomy of Viruses Executive Committee, *Nat. Microbiol.* **2020**, *5*, 668.
[25] S. G. Siddell, D. B. Smith, E. Adriaenssens, P. Alfenas-Zerbini, B. E. Dutilh, M. L. Garcia, S. Junglen, M. Krupovic, J. H. Kuhn, A. J. Lambert, E. J. Lefkowitz, M. Łobocka, A. R. Mushegian, H. M. Oksanen, D. L. Robertson, L. Rubino, S. Sabanadzovic, P. Simmonds, N. Suzuki, K. Van Doorslaer, A. M. Vandamme, A. Varsani, F. M. Zerbini, *J. Gen. Virol.* **2023**, *104*, 001840.
[26] E. V. Koonin, V. V. Dolja, M. Krupovic, *Cell Host Microbe* **2022**, *30*, 917.
[27] M. Krupovic, V. V. Dolja, E. V. Koonin, *Nat. Rev. Microbiol.* **2019**, *17*, 449.
[28] V. G. Wilson, *Viruses: Intimate Invaders*, Springer, Cham **2022**.
[29] D. R. Wessner, *Nat. Edu.* **2010**, *3*, 37.
[30] M. G. Mateu, *Protein Eng. Des. Sel.* **2011**, *24*, 53.
[31] C. Varanda, M. D. R. Félix, M. D. Campos, P. Materatski, *Viruses* **2021**, *13*, 2073.
[32] M. Mietzsch, M. Agbandje-McKenna, *Annu. Rev. Virol.* **2017**, *4*, iii.
[33] T. Travieso, J. Li, S. Mahesh, J. D. F. R. E. Mello, M. Blasi, *npj Vaccines* **2022**, *7*, 75.
[34] J. Cello, A. V. Paul, E. Wimmer, *Science* **2002**, *297*, 1016.
[35] H. O. Smith, C. A. Hutchison, C. Pfannkoch, J. C. Venter, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 15440.
[36] L. DeFrancesco, *Nat. Biotechnol.* **2021**, *39*, 1185.
[37] R. A. WhiteIII, *mSystems* **2021**, *6*, e00770.
[38] V. Thiel, *PLoS Pathog.* **2018**, *14*, e1007019.
[39] B. Jost, J. L. Vilotte, I. Duluc, J. L. Rodeau, J. N. Freund, *Nat. Biotechnol.* **1999**, *17*, 160.
[40] F. Costantini, E. Lacy, *Nature* **1981**, *294*, 92.
[41] W. M. Barnes, *Proc. Natl. Acad. Sci.* **1990**, *87*, 9183.
[42] T. Gaj, S. J. Sirk, S. L. Shui, J. Liu, *Cold Spring Harb. Perspect. Biol.* **2016**, *8*, a023754
[43] J. van der Oost, C. Patinios, *Trends Biotechnol.* **2023**, *4*, 396.
[44] A. M. Khalil, *J. Genet. Eng. Biotechnol.* **2020**, *18*, 68.
[45] J. Y. Wang, J. A. Doudna, *Science* **2023**, *379*, eadd8643.
[46] S. Nidhi, U. Anand, P. Oleksak, P. Tripathi, J. A. Lal, G. Thomas, K. Kuca, V. Tripathi, *Int. J. Mol. Sci.* **2021**, *22*, 3327.
[47] A. Pickar-Oliver, C. A. Gersbach, *Nat. Rev. Mol. Cell. Biol.* **2019**, *20*, 490.
[48] S. W. Wang, C. Gao, Y. M. Zheng, L. Yi, J. C. Lu, X. Y. Huang, J. B. Cai, P. F. Zhang, Y. H. Cui, A. W. Ke, *Mol. Cancer* **2022**, *21*, 57.
[49] F. Uddin, C. M. Rudin, T. Sen, *Front. Oncol.* **2020**, *10*, 1387.
[50] D. Zhao, X. Zhu, H. Zhou, N. Sun, T. Wang, C. Bi, X. Zhang, *Metab. Eng.* **2021**, *63*, 148.
[51] R. H. Devlin, L. F. Sundström, R. A. Leggatt, *BioScience* **2015**, *65*, 685.
[52] K. B. Oke, P. A. Westley, D. T. Moreau, I. A. Fleming, *Proc. R. Soc. B* **2013**, *280*, 20131047.
[53] F. Felber, G. Kozlowski, N. Arrigo, R. Guadagnuolo, *Adv. Biochem. Eng. Biotechnol.* **2007**, *107*, 173.
[54] V. Vázquez-Barrios, K. Boege, T. G. Sosa-Fuentes, P. Rojas, A. Wegier, *Sci. Rep.* **2021**, *11*, 1959.
[55] F. Stirling, P. A. Silver, *Mol. Cell* **2020**, *78*, 614.
[56] J. C. Venter, J. I. Glass, C. A. Hutchison, S. Vashee, *Cell* **2022**, *185*, 2708.
[57] W. Zhang, L. A. Mitchell, J. S. Bader, J. D. Boeke, *Annu. Rev. Biochem.* **2020**, *89*, 77.
[58] D. G. Gibson, G. A. Benders, C. Andrews-Pfannkoch, E. A. Denisova, H. Baden-Tillson, J. Zaveri, T. B. Stockwell, A. Brownley, D. W. Thomas, M. A. Algire, C. Merryman, L. Young, V. N. Noskov, J. I. Glass, J. C. Venter, C. A. Hutchison, H. O. Smith, *Science* **2008**, *319*, 1215.
[59] D. G. Gibson, J. I. Glass, C. Lartigue, V. N. Noskov, R. Y. Chuang, M. A. Algire, G. A. Benders, M. G. Montague, L. Ma, M. M. Moodie, C. Merryman, S. Vashee, R. Krishnakumar, N. Assad-Garcia, C.

Andrews-Pfannkoch, E. A. Denisova, L. Young, Z. Q. Qi, T. Segall-Shapiro, C. Calvey, P. Parmar, C. A. Hutchison III, H. O. Smith, J. C. Venter, *Science* **2010**, *329*, 52.
[60] C. A. Hutchison, R. Y. Chuang, V. N. Noskov, N. Assad-Garcia, T. J. Deerinck, M. H. Ellisman, J. Gill, K. Kannan, B. J. Karas, L. Ma, J. F. Pelletier, Z. Q. Qi, R. A. Richter, E. A. Strychalski, L. Sun, Y. Suzuki, B. Tsvetanova, K. S. Wise, H. O. Smith, J. I. Glass, C. Merryman, D. G. Gibson, J. C. Venter, *Science* **2016**, *351*, aad6253.
[61] J. F. Pelletier, L. Sun, K. S. Wise, N. Assad-Garcia, B. J. Karas, T. J. Deerinck, M. H. Ellisman, A. Mershin, N. Gershenfeld, R. Y. Chuang, J. I. Glass, E. A. Strychalski, *Cell* **2021**, *184*, 2430.
[62] X. Xu, F. Meier, B. A. Blount, I. S. Pretorius, T. Ellis, I. T. Paulsen, T. C. Williams, *Nat. Commun.* **2023**, *14*, 1984.
[63] H. Kiyama, S. Kakizawa, Y. Sasajima, Y. O. Tahara, M. Miyata, *Sci. Adv.* **2022**, *8*, eabo7490.
[64] R. Z. Moger-Reischer, J. I. Glass, K. S. Wise, L. Sun, D. M. C. Bittencourt, B. K. Lehmkuhl, D. R. Jr Schoolmaster, M. Lynch, J. T. Lennon, *Nature* **2023**, *620*, 122. (Erratum in: Nature 2023, Jul 26).
[65] D. Schindler, R. S. K. Walker, S. Jiang, A. N. Brooks, Y. Wang, C. A. Müller, C. Cockram, Y. Luo, A. García, D. Schraivogel, J. Mozziconacci, N. Pena, M. Assari, M. D. C. Sánchez Olmos, Y. Zhao, A. Ballerini, B. A. Blount, J. Cai, L. Ogunlana, W. Liu, K. Jönsson, D. Abramczyk, E. Garcia-Ruiz, T. W. Turowski, R. Swidah, T. Ellis, T. Pan, F. Antequera, Y. Shen, C. A. Nieduszynski, et al., *Cell* **2023**, *186*, 5237.
[66] Y. Zhao, C. Coelho, A. L. Hughes, L. Lazar-Stefanita, S. Yang, A. N. Brooks, R. S. Walker, W. Zhang, S. Lauer, C. Hernandez, J. Cai, L. A. Mitchell, N. Agmon, Y. Shen, J. Sall, V. Fanfani, A. Jalan, J. Rivera, F. X. Liang, J. S. Bader, G. Stracquadanio, L. M. Steinmetz, Y. Cai, J. D. Boeke, *Cell* **2023**, *186*, 5220.
[67] L. G. Chen, T. Lan, S. Zhang, M. Zhao, G. Luo, Y. Gao, Y. Zhang, Q. Du, H. Lu, B. Li, B. Jiao, Z. Hu, Y. Ma, Q. Zhao, Y. Wang, W. Qian, J. Dai, Y. Jiao, *Nat. Plants* **2024**.
[68] N. Ostrov, A. Nyerges, A. Chiappino-Pepe, A. Rudolph, M. Baas-Thomas, G. M. Church, *Curr. Opin. Syst. Biol.* **2020**, *24*, 32.
[69] J. Fredens, K. Wang, D. de la Torre, L. F. H. Funke, W. E. Robertson, Y. Christova, T. Chia, W. H. Schmied, D. L. Dunkelmann, V. Beránek, C. Uttamapinant, A. G. Llamazares, T. S. Elliott, J. W. Chin, *Nature* **2019**, *569*, 514.
[70] A. Nyerges, S. Vinke, R. Flynn, S. V. Owen, E. A. Rand, B. Budnik, E. Keen, K. Narasimhan, J. A. Marchand, M. Baas-Thomas, M. Liu, K. Chen, A. Chiappino-Pepe, F. Hu, M. Baym, G. M. Church, *Nature* **2023**, *615*, 720.
[71] K. Aphicho, N. Kittipanukul, C. Uttamapinant, *Curr. Opin. Chem. Biol.* **2022**, *66*, 102108.
[72] J. C. Anderson, N. Wu, S. W. Santoro, V. Lakshman, D. S. King, P. G. Schultz, *Proc. Natl. Acad. Sci.* **2004**, *101*, 7566.
[73] T. Ohtsuki, T. Manabe, M. Sisido, *FEBS Lett.* **2005**, *579*, 6769.
[74] Y. Zhang, B. M. Lamb, A. W. Feldman, A. X. Zhou, T. Lavergne, L. Li, F. E. Romesberg, *Proc. Natl. Acad. Sci.* **2017**, *114*, 1317.
[75] E. C. Fischer, K. Hashimoto, Y. Zhang, A. W. Feldman, V. T. Dien, R. J. Karadeema, R. Adhikary, M. P. Ledbetter, R. Krishnamurthy, F. E. Romesberg, *Nat. Chem. Biol.* **2020**, *16*, 570.
[76] S. Hoshika, N. A. Leal, M. J. Kim, M. S. Kim, N. B. Karalkar, H. J. Kim, A. M. Bates, W. N. E. Jr, H. A. SantaLucia, A. J. Meyer, S. DasGupta, J. A. Piccirilli, A. D. Ellington, S. L. J. Jr, M. M. Georgiadis, S. A. Benner, *Science* **2019**, *363*, 884.
[77] M. W. Grome, F. J. Isaacs, *Science* **2021**, *372*, 460.
[78] J. Kroll, S. Klinter, C. Schneider, I. Voss, A. Steinbüchel, *Microb Biotechnol* **2010**, *3*, 634.
[79] B. Enghiad, P. Xue, N. Singh, A. G. Boob, C. Shi, V. A. Petrov, R. Liu, S. S. Peri, S. T. Lane, E. D. Gaither, H. Zhao, *Nat. Commun.* **2022**, *13*, 2697.
[80] R. A. Hughes, A. D. Ellington, *Cold Spring Harb. Perspect. Biol.* **2017**, *9*, a023812.
[81] R. S. Molina, G. Rix, A. A. Mengiste, B. Alvarez, D. Seo, H. Chen, J. Hurtado, Q. Zhang, J. D. García-García, Z. J. Heins, P. J. Almhjell, F. H. Arnold, A. S. Khalil, A. D. Hanson, J. E. Dueber, D. V. Schaffer, F. Chen, S. Kim, L. A. Fernández, M. D. Shoulders, C. C. Liu, *Nat. Rev. Methods Primers* **2022**, *2*, 36.
[82] R. Tian, F. B. H. Rehm, D. Czernecki, Y. Gu, J. F. Zürcher, K. C. Liu, J. W. Chin, *Science* **2024**, *383*, 421.
[83] S. Kriegman, D. Blackiston, M. Levin, J. Bongard, *Proc. Natl. Acad. Sci.* **2020**, *117*, 1853.
[84] S. Kriegman, D. Blackiston, M. Levin, J. Bongard, *Proc. Natl. Acad. Sci.* **2021**, *118*, e2112672118.
[85] H. Cai, Z. Ao, C. Tian, Z. Wu, H. Liu, J. Tchieu, M. Gu, K. Mackie, F. Guo, *Nat. Electron.* **2023**, *6*, 1032.
[86] H. Jia, P. Schwille, *Curr. Opin. Biotechnol.* **2019**, *60*, 179.
[87] C. Wang, J. Yang, Y. Lu, *Front. Mol. Biosci.* **2021**, *8*, 781986.
[88] C. Xu, N. Martin, M. Li, S. Mann, *Nature* **2022**, *609*, 1029.
[89] K. Powell, *Nature* **2018**, *563*, 172.
[90] W. Jiang, Z. Wu, Z. Gao, M. Wan, M. Zhou, C. Mao, J. Shen, *ACS Nano* **2022**, *16*, 15705.
[91] C. Xu, S. Hu, X. Chen, *Mater Today* **2016**, *19*, 516.
[92] M. E. Allen, J. W. Hindley, N. O'Toole, H. S. Cooke, C. Contini, R. V. Law, O. Ces, Y. Elani, *Proc. Natl. Acad. Sci.* **2023**, *120*, e2307772120.
[93] B. Ghosh, *Emerg. Top. Life Sci.* **2022**, *6*, 619.
[94] Y. Lu, G. Allegri, J. Huskens, *Mater. Horiz.* **2022**, *9*, 892.
[95] S. A. Newman, *J. Exp. Zool. Mol. Dev. Evol.* **2019**, *332*, 331.
[96] J. Gómez-Márquez, *Commun. Integr. Biol.* **2020**, *13*, 97.