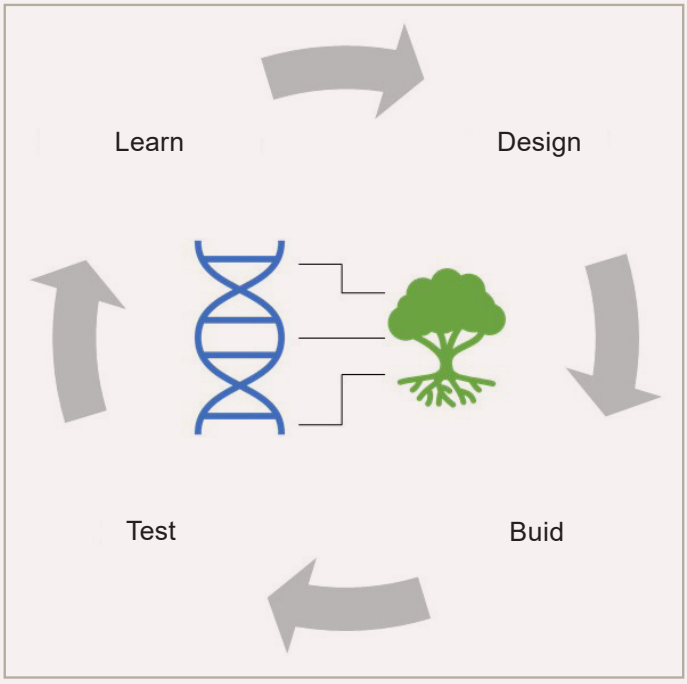
PREMIS DE LA SECCIÓ DE CIÈNCIES BIOLÒGIQUES, III

New Synthetic Biological Functions and their Implications for the Present and Future of Society

Marc Güell Cargol

Premi IEC de la Secció de Ciències Biològiques
August Pi i Sunyer de Ciències de la Salut 2021



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At the proposal of the working group formed by the Messrs. and the Mmes. Joandomènec Ros i Aragonès (president of the Institut d'Estudis Catalans), Josep Enric Llebot Rabagliati (general-secretary of the Institut d'Estudis Catalans) and Montserrat Aguadé i Porres, M. Teresa Estrach i Panella, Xavier Turon Barrera, Dolors Vaqué Vidal and Miquel Vilardell i Tarrés, members of the Secció de Ciències Biològiques. The Permanent Council of the Institut d'Estudis Catalans, at its meeting held on 25 March, 2021, resolved to award the Premi IEC de la Secció de Ciències Biològiques August Pi i Sunyer de Ciències de la Salut a Marc Güell Cargol for his project *New Synthetic Biological Functions and their Implications for the Present and Future of Society*.

Moreover, the Governing Board of the Secció de Ciències Biològiques, at the meeting held on 30 Mach, 2021, passed the resolution to publish the review which, under the title of *New Synthetic Biological Functions and their Implications for the Present and Future of Society*, was edited by Mr. Ramon Bartrons i Bach, a member of the Institut d'Estudis Catalans.

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Summary

Synthetic biology finds itself in the midst of an era characterized by veritable exponential growth. The rapid evolution undergone by genomic technologies enables us to interact with life by employing the language of life itself – deoxyribonucleic acid (DNA). The advances made in sequencing have greatly expanded our understanding of the biosphere while the quantum leap in writing technologies have greatly boosted our ability to engineer living systems. Indeed, the feedback achieved between DNA reading and writing has served to further accelerate this technological revolution.

Basic science has, as a result, received a major boost, providing it with a much better understanding of the fundamentals of biology and enabling it to propose new solutions to the challenges faced by humanity today. The engineering of living systems is providing new therapeutic solutions that are set to impact a growing number of patients. However, the revolution extends beyond the provision of such therapies as it sets in motion the reinvention of many key sectors, including information storage, food production, the development of new biomaterials and the generation of more environmentally friendly industri al processes.

Synthetic biology offers multiple possibilities to address the greatest challenges that the world faces today. The advances being recorded constitute fundamental components of what is being called the 'fourth industrial revolution' and which is driving progress in guaranteeing the future health of humanity and of our planet.

1. Synthetic Biology: The Revolution in Reading and Writing

A fundamental element underpinning the vertiginous progress recorded by the biosciences has been the discipline's acquisition of the ability to communicate with life using its own genuine language – i.e. DNA. As bioscientists, our ability to understand life has been greatly boosted by the reading or sequencing of DNA, while the possibilities of engineering living systems have been revolutionized by our ability to write by means of DNA synthesis and editing. All this has been facilitated by a number of advances that include the marked drop in the costs of DNA sequencing (see the NHGRI report, "DNA Sequencing Costs: Data" n.d.), the fall in prices of synthetic DNA (see the SynBioBeta report, "Time for New DNA Synthesis and Sequencing Cost Curves" n.d.) and the emergence of new techniques, most notably CRISPR for genome editing (Mali et al. 2013). The progress witnessed has led to an acceleration in basic research and its transfer into revolutionary new therapies.

The worldwide sequencing of DNA generates enormous volumes of biological data each year, the reading or sequencing of which provides us with a detailed understanding of the corresponding biological systems. Here, technologies have been developed that massively parallelize the sequencing process. In 2003, mapping the human genome cost in the region of $3 billion dollars but by 2019 the cost had dropped below $1,000. In ten years' time or even sooner, the mapping cost is likely to be no more than a handful of dollars. These technical advances have not only helped cut costs but they have also accelerated the pace of experimentation and generated new forms of data that improve our understanding of biology. Advances at the single-cell level, including imaging tools and ribonucleic acid (RNA) sequencing, facilitate the construction of increasingly higher resolution cell maps, which can serve as the basis for research, diagnosis and treatment. Recent studies using single-cell sequencing data are furnishing new clinical solutions, including the ability to describe tumor microenvironment landscapes, predict treatment response, discover novel biomarkers, and subcategorize diseases and tissues (Hong and Park 2020). Increasingly, our ability to understand and engineer biological processes spans more and more dimensions. But this is by no means the end of the road, as the great visionary George Church recently predicted (Abong et al. 2021): "The revolution in reading and writing biology accelerates and ramifies: reading three-dimensional structures as easily as one-dimensional; bridging X-ray crystallography and cryo-electron microscopy (0.3 nanometer scale) with fluorescence in situ sequencing and oligopaints (10 nanometer resolution to multi-centimeter scale) to replace current 'RNA cell atlases' and conventional histology". In other words, not only should we be seeing methods to read linear sequences but we should also be able to map the elements in their spatial localization in cells.

The great advances being made in reading and writing biology are mutually reinforcing. Advances in omics technologies and molecular sequencing enhance the mapping and measurement of molecules and cells, while engineering boosts our understanding of biological processes, as well as allowing us to improve our design of biology. We need look no further for an example than the CRISPR gene editing tool and related technologies. This has been made eminently clear over the last year in the case of *ex vivo* and *in vivo* human genome editing with CRISPR providing promising efficacy data for the treatment of serious genetic diseases (Frangoul et al. 2021; Gillmore et al. 2021). Various solutions provided by these writing technologies are likely to be widely adopted in the very near future and highlight the growing maturity of the field (Tan et al. 2021). Here, again, visionaries like George Church predict great enhancements in genome writing. Future steps, he forecasts will include the combination of machine learning and array synthesis in the design of millions of novel enzymes, antibodies, etc.; codon-recoding several species to be resistant to all viruses; making cell therapies and organs resistant to pathogens, senescence, and cancer; and reviving ecosystems and sequestering carbon, possibly to pre-industrial levels.

A paradigmatic example of the level of maturity reached by reading and writing technologies has been the response to COVID-19. In the spring of 2020, the rapid global spread of the new coronavirus, SARS-CoV-2, posed a massive health and economic challenge for humanity. Almost immediately, innovations were developed in response. First, and within a matter of just weeks, the whole SARS-CoV-2 genome was sequenced and published. A few years earlier, the mapping of the genetic sequence of SARS-CoV-1, which caused the outbreak of severe acute respiratory syndrome, took months. Moreover, it took just a further few weeks to synthetically reconstruct the SARS-Cov-2 virus from the published sequence (Thi Nhu Thao et al. 2020). In parallel with this, advances in genomic technologies ensure that diagnoses are much more effective (PCR methods, antigen tests, CRISPR technologies, etc.). Yet, despite this, the many diagnostic challenges during the COVID-19 crisis also highlight the fact that much remains to be done to optimize diagnoses. Ultimately, though, the speed and scale at which researchers began to deploy their know-how to develop a COVID-19 vaccine was quite remarkable. This response was driven in large part by the emergency faced by the public healthcare sector, but it also reflected innovations, above all, those made in RNA vaccines. By the end of 2020, two RNA-based vaccines had been developed – the work of Moderna and Pfizer/ BioNTech – with efficacy rates exceeding 90% (Kyriakidis et al. 2021). In this instance, the writing of a viral gene in the form of RNA induces robust immune protection.

2. Synthetic biology and the fourth industrial revolution

The visionary founder of Apple, Steve Jobs, claimed back in 2011: "I believe that the greatest innovations of the 21st century will be at the intersection of biology and technology. A new era is beginning". The advances in the biological sciences, combined with the astounding progress being made in computing, data science and artificial intelligence (AI), are fueling a new wave of innovation that could have a very significant impact on many different sectors of the economy, from health and agriculture to consumer goods and energy. Nature was at the heart of the first two industrial revolutions: the first, when coal was used to power the steam engine and, thus, mechanize production; and, the second, when electricity, gas, and oil became the new sources of energy, thus, facilitating industrial progress. Each of these economic leaps generated new sources of wealth and employment. The third industrial revolution, based on computers and the Internet, ushered in the digital age and, with it, the creation of a number of tech giants whose market capitalization equals the gross domestic product of many advanced economies (Wallach, Neufeld, and Ang 2021). At the gateway to this the fourth industrial revolution, we have the opportunity to abandon a model that exploits nature by brute force and to start taking advantage of nature's design principles as a platform for manufacturing and transformation – a concept that the Boston Consulting Group (BCG) has defined as "nature co-design" (see the BCG report, "Nature Co-Design: A Revolution in the Making - Hello Tomorrow" 2021).

The development paradigm is fairly similar to the principle of innovation in biological systems where diversity is generated and selected and, thus, the properties of biological systems evolve. Academic and industrial manufacturing platforms or *biofoundries* are increasingly implementing an engineering approach centered on the design-build-test-learn (DBTL) cycle, which has long been a central element of product development in traditional engineering (Carbonell et al. 2018; Opgenorth et al. 2019). The full potential of AI is then exploited to make sense of, or to learn from, the data generated during the build and test phases so as to improve the designs, the whole cycle being subject to constant iterations at great velocity until a satisfactory working solution is obtained. This approach has been successfully employed in recent years, evidencing the power of these synergies. I would like to describe in greater detail two cases: AlphaFold and the three-dimensional prediction of proteins and Dyno Therapeutics and the development of new viral capsids for gene therapy.

AlphaFold is an artificial intelligence program, developed by the Google-owned company of DeepMind, which makes predictions about protein structure based on their amino acid chains. Determining their structure is fundamental since the shape a protein folds into largely accounts for its function. In early

2020, DeepMind published its predictions of high-resolution structures (Senior et al. 2020). The program is designed as a deep learning system in which a neural network makes estimates of distances between residues. The system has been trained on a large number of protein structures that have first been determined experimentally by nuclear magnetic resonance, X-ray diffraction or electron microscopy. The scientific community concerned with protein structure prediction organizes a bi-annual event – CASP ("Home - Prediction Center" n.d.) – aimed at critically assessing the prediction tools. AlphaFold software has been ranked first in the last two editions. In 2018, AlphaFold 1 proved especially successful in predicting cases where there was little comparative information for the target proteins. In 2020, AlphaFold 2 achieved a much higher level of accuracy than any other group, scoring above 90 for around two-thirds of the proteins included in CASP's global distance test. This test measures the degree to which a computer program's protein structure prediction coincides with the experimentally determined structure, where 100 represents a complete match.

My second example of the synergies between biological design and AI is provided by the development of new viral capsids. The vector-based adeno-associated virus (AAV) is the main viral vector used for gene transfer *in vivo*; however, the engineering of any new capsids has been limited. Yet, Dyno Therapeutics is pioneering an AI powered approach to gene therapy. Exploiting machine learning and quantitative high-throughput *in vivo* experimentation, the firm has been able to invent new ways to design AAVs (Ogden et al. 2019). Using their CapsidMap platform, they use AI to efficiently optimize AAV capsids. The process involves a DBTL cycle in which millions of capsid sequences are designed simultaneously, the properties of which are at the same time measured in order to train machine learning models to identify the best properties. The cycle is then repeated until the desired properties are obtained ("Dyno's CapsidMap™ Platform - Dyno Therapeutics" 2020).

3. Information storage

In a world awash with data, figuring out where and how to store this information efficiently and economically is becoming more and more of a challenge. One solution that appears to be gaining a certain degree of momentum is archiving information in DNA molecules. Indeed, as Nick Goldman of the European Bioinformatics Institute said, "Because DNA is the basis of life on Earth, the methods for working on it, storing it, and retrieving it will continue to be the subject of continuous technological innovation".

DNA is the language of life. In the same way that our DNA stores information to generate something as complex as a human being, we can use DNA to store

abstract information. Indeed, such is its potential that one kilogram of DNA could hypothetically meet the world's current data storage needs (Extance 2016).

DNA has extraordinary properties for storing information. It has a storage capacity 1,000 times more compact than that of a flash memory and is hundreds of millions of times more energy efficient per unit of information than a hard drive (Panda et al. 2018). Assuming the natural code of four bases (adenine, cytosine, thymine and guanine), the molecular architecture of DNA makes it possible to store 2 bits in each base, that is, 1 gram of DNA can provide storage for up to 455 exabytes. Moreover, DNA is one of nature's most robust biomolecules: The degradation rate of mitochondrial DNA in the bones of Moa (a bird species that lived until 1,300 CE in the forests of New Zealand) has been calculated to be 1 bp every 6,830,000 years (Allentoft et al. 2012). DNA synthesized hundreds of thousands of years ago, for example, has been successfully sequenced (Orlando et al. 2013; Golenberg et al. 1990). DNA data storage combines DNA synthesis, DNA sequencing, and an encoding and decoding algorithm that packs information into the molecule in a more durable and denser way than in conventional silicon-based media. There have been quite a few proofs of concept ranging from the storage and recovery of a 5.27-megabit book (Church, Gao, and Kosuri 2012) to the sheet music from the Mario Bros video game (Lee et al. 2020). Similarly, in 2019, the firm Catalog reported that it had used its DNA writing technology to encode the whole of the English Wikipedia into genetic material, while the firm Twist announced that it had stored an episode of the Netflix show, Biohackers. Looking to the future, improved writing and reading technologies will further facilitate the management of data storage in DNA. However, the synthesis of long-stranded DNA molecules with error rates suitable for data archiving continues to be highly time consuming, while synthesizing these strands with sufficient fidelity and sequencing them to retrieve information with a high degree of accuracy still requires relatively sophisticated laboratories and skilled labor. Yet, with the inevitable advances in technology, levels of automation and sophistication will increase accompanied by a reduction in the cost of both writing and reading DNA.

In addition to storing information that is transferred to offspring, life naturally exploits the storage of information in its DNA to strengthen immunity and thus 'remember' pathogens that can pose a threat to it. In the case of bacteria, the CRISPR system generates a database of bacteriophages to which the organism has been exposed and, in this way, adaptive immunity is built. When a microbe is invaded by a bacteriophage, the first stage of the immune response is to capture phage DNA and insert it into a CRISPR locus in the form of a spacer (Pourcel, Salvignol, and Vergnaud 2005). Cas1 and Cas2 proteins are found in both types of CRISPR-Cas immune systems and mediate spacer acquisition. These spacers are

used by interference systems such as Cas9 to cleave the bacteriophage genome (van der Oost et al. 2014). It is this system that has been used to generate information acquisition systems. New memories are acquired via the action of a complex of Cas1 and Cas2, which integrates new spacers ahead of the old spaces within the CRISPR array, thus providing a temporal memory of molecular events. This system has been used to record different types of information in cells, including physiological information from the digestive system and even a short digital movie (Shipman et al. 2017; Sheth et al. 2017). A variant of this system uses a reverse transcriptase coupled to the Cas1-Cas2 complex. This allows the genome to record the history of gene expression and, in turn, it facilitates the temporal reconstruction of the evolution of the transcriptional state (Schmidt, Cherepkova, and Platt 2018). The transcriptional histories recorded reflect underlying changes in gene expression and can therefore be used to interrogate biological or disease processes. In the long term, it is envisioned that CRISPR spacer acquisition components could be introduced into other cell types to record the molecular sequences of events and lineage pathways that result in certain cell behaviors, states, and types.

Writing information in DNA molecules allows information to be recorded in a highly unique fashion. In developmental biology, for example, the reconstruction of cell lineages leading to the formation of tissues, organs, and even complete organisms is critical. Clarifying the lineage relationships between the various cell types can provide major insights into the fundamental processes responsible for normal tissue development, as well as valuable information as to what goes wrong in developmental diseases. CRISPR systems have been used to draw the cell lineage map for whole organisms (McKenna et al. 2016). Genome editing has been used to progressively introduce and accumulate mutations in a DNA barcode over multiple rounds of cell division. The barcode, consisting of a set of short palindromic repetitions, marks cells and enables the elucidation of lineage relationships via the patterns of mutations shared between cells.

4. Bioremediation: the environment and sustainability

The transformative power of science-based biotechnology that was first recognized at the end of the last century has been further accelerated in recent years thanks to DNA reading, writing and editing technologies. To date, market forces have meant most research efforts have targeted questions related to agriculture and health. Indeed, spectacular advances have been made in biomedicine and agricultural technologies at a time of a severe global environmental crisis attributable to overpopulation, loss of biodiversity, greenhouse gas emissions and pollution. In this regard, several analyses have identified biology as a transformative element for

meeting the UN's 2015 Sustainable Development Goals (de Lorenzo et al. 2018). But to what extent is synthetic biology the solution to this planetary crisis?

Synthetic biology is reinventing many of the processes of food production, promoting approaches that are more respectful of the planet and animal welfare. The firm Impossible Foods exploits the fact that the essential qualities defining the taste of meat, most notably hemoglobin, can be rebuilt on more sustainable platforms such as yeasts. The firm uses *Pichia pastoris* yeast to produce soy leghemoglobin, which enhances meaty aromas when added to a veggie burger. Compared to the average beef burger, the Impossible Burger requires 96% less land and generates 89% less greenhouse-gas emissions. Yeast is, thus, becoming an extremely flexible engineering platform. Advances in metabolic engineering have simplified the transfer of biological functions from their natural sources to yeast. A number of examples have emerged, including food additives (Amyris's stevia, Perfect Day's vegetable milk and DSM's vitamin E) and molecules with pharmacological properties, such as Taxol and artemisinin. Another interesting example is that of the skin care product, squalene, which traditionally was derived from shark liver oil but can now be produced more sustainably by fermentation of genetically modified yeasts (Han et al. 2018).

A high-impact synthetic biology endeavor seeks to reinvent the process of agricultural fertilization. This need is obviously pressing given that food production will have to be increased if we hope to sustain a world whose population will soon reach 8 billion. Haber-Bosch ammonia synthesis fertilizers have helped increase food production to keep pace with world population growth, but they have many drawbacks, not least the fact that they consume large amounts of energy to convert atmospheric nitrogen into ammonia. It is estimated that these fertilizers consume between 3 and 5% of the world's natural gas supply and are, moreover, responsible for more than 1% of all CO2 emissions. Two firms, Pivot Bio and Joyn Bio, engaged in independent projects, have identified the bacterial strains and engineering needed to generate microbial communities capable of fixing nitrogen directly on the roots of plants with sufficient yield to reduce the need for fertilizers. The adoption of this biological process will greatly facilitate decarbonization and increase access to ammonia, especially if we bear in mind that it costs $3 billion to build a Haber-Bosch facility which, in turn requires a notable natural gas infrastructure. But the disruptive effect of synthetic biology in agriculture is not limited to fertilizers. Other developments include the reduction of the carbon footprint of livestock by increasing the protein contained in plants, the enhancement of a plant's ability to sequester soil carbon, and the creation of insect-based biological control systems to prevent crop destruction (Oxitec).

Synthetic biology is also gaining ground in the materials sector. For example, nylon today can be made from genetically engineered microorganisms rather

than petrochemicals. The precursor to nylon, caprolactam, is traditionally refined from oil, with annual emissions of some 60 million tonnes of CO2. Exploiting synthetic biology, Genomatica is driving a microorganism-based project to ferment plant sugars to produce caprolactam and, therefore, nylon in a 100% renewable way (Turk et al. 2016). Other materials synthesized in biological systems include lab-grown leather. The firm Modern Meadow has succeeded in biomanufacturing animal-free leather. It transpires that the essential biological component of leather is not the animal skin, but rather collagen. At first, Modern Meadow grew skin cells to create skin, but the company has since refined its approach and now uses a fermentation process to make collagen directly. The firm's scientists have bioengineered a strain of yeast that, when fed sugar, produces collagen, which is then purified and processed to create a material that is biologically and perceptibly almost indistinguishable from animal skin. An alternative here are mycelium-based skins. These mushroom-derived leather substitutes are an emerging class of environmentally and ethically responsible fabrics that are increasingly meeting consumers' aesthetic and functional expectations and winning favor as an alternative to bovine and synthetic leathers. The firm Bold Threads has developed the Mylo type leather, the appearance and texture of which is similar to leather but made from the fungal mycelium.

The above examples are illustrative of the way in which synthetic biology is redesigning nature. Rather than exploiting natural resources, nature co-design uses natural laws to reinvent nature.

However, these developments still have a very long way to go. Nature allows us to generate patterns in many dimensions, albeit that during these early stages most exploitations have been one dimensional: the production, for example, of stevia and collagen. Yet, nature permits developments with considerably greater dimensional and functional complexity. An exciting, inspirational, example is the bird of paradise, whose appearance can undergo radical color changes, generated by precise micropatterns in its plumage. It has 8 layers of boomerang-shaped melanin that produce spectacular color changes depending on the orientation of the incident light (Stavenga et al. 2011). Likewise, the human skin is a multidimensional, multifunctional structure. The human skin cells, keratinocytes, produce keratin giving it mechanical strength, the skin's fibroblasts produce collagen and elastin that further strengthen the extracellular matrix, its melanocytes produce melanin to impart color, while its Langerhans cells detect and respond to the presence of pathogens.

The advances being made in synthetic biology are providing the foundations for multidimensional and multifunctional design. One material that has proved highly promising for the exploration of its multifunctional qualities is cellulose. In this particular case, the manufacture of living materials based on functional

bacterial cellulose has been successfully achieved and it provides 3-dimensional support via a stable co-culture of *Saccharomyces cerevisiae* yeast and bacterial cellulose-producing *Komagataeibacter rhaeticus* bacteria. In this way, yeast strains can be designed to secrete enzymes into bacterial cellulose and, thus, generate multifunctional materials that incorporate sensors and other functionalities (Gilbert et al. 2021).

5. Therapeutic applications based on genome and epigenome engineering

Advances in gene editing have had a major impact on basic science and new therapies and, indeed, the gene editing toolbox has expanded greatly in recent years. Traditionally, gene editing has been based on the design of artificial endonucleases that induce a double-strand break (DSB) in the genome sequence of interest (Porteus and Carroll 2005). Cells repair the DSB via one of two main pathways: non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Sander and Joung 2014). Repair of DSBs by NHEJ occurs in all cells, both dividing and non-dividing, and is usually more efficient than HDR. To induce NHEJ, all that is needed are a cleavage element, such as a CRISPR interference Cas9 protein, and the gRNA to direct it. This repair pathway, which tends to generate both errors is commonly used to generate *knockouts*. This has been exploited to develop new therapies with considerable success. The firm CRISPR Therapeutics has developed a CRISPR gene editing therapy for patients with β-thalassemia and sickle cell disease. Both diseases are caused by mutations in the hemoglobin subunit β (HBB) gene. Therapy is based on upregulating the expression of fetal hemoglobin (HbF) using Cas9 to disrupt the regulator that keeps it repressed during adulthood. HbF is a form of oxygen-carrying hemoglobin that is naturally present before birth and which is then replaced by the adult form of hemoglobin. The CRISPR system is used *ex vivo* by electroporation of the Cas9 protein and ribonucleic acid complex and the corresponding gRNA in hematopoietic stem cells. The process of induced NHEJ introduces mutations into the natural regulator that keeps HbF expression repressed so that high levels of fetal hemoglobin are produced in red blood cells. Frangoul et al. (2021) report that, a year after such treatment, two patients presented high levels of allelic editing in bone marrow and blood, increased fetal hemoglobin and an improvement in the symptoms of the two monogenic diseases. Another major milestone in gene editing was the first demonstration *in vivo* of CRISPR technology. In this instance, the firm Intellia has developed a treatment for transthyretin (TTR) amyloidosis, where deposits of the TTR protein accumulate in various tissues. Treatment consists of a systemic injection of a lipid nanoparticle encapsulating

messenger RNA for Cas9 protein and a single guide RNA. Administration of this treatment was only associated with mild adverse events and resulted in a very significant decrease in serum TTR protein concentration (Gillmore et al. 2021).

Genome editing techniques independent of DSB have recently been developed, based on either the direct editing of DNA bases with deaminases, i.e. base editors (BEs) (Rees and Liu, n.d.) or the substitution *in situ* of DNA bases with the help of a reverse transcriptase (RT), i.e. prime editors (PEs)(Anzalone et al. 2019). BEs are based on the combination of CRISPR technology with a deaminase-type enzyme. The CRISPR system drives the chimeric protein to the editing point and the deaminase-type reaction modifies the bases. Cytosine BEs or CBEs change C-> T while the adenine BEs or ABEs change A-> G. Broadly speaking, the BEs require three essential elements: 1) Cas9 nickase (a Cas9 variant that only cuts one of the two strands of DNA) fused with a deaminase that does the editing; 2) a gRNA that targets Cas9 at a specific locus; and, 3) a base or bases of destination to edit within the edit window specified by the Cas9 protein. Base editing systems have been employed in different disease models. A notable application was recently reported in which an ABE was used to reduce blood cholesterol levels of a primate model (Musunuru et al. 2021). The ABE was formulated into a lipid nanoparticle and administered systemically in order to generate a *knockout* of *PCSK9* in the liver. Although some gain-of-function mutations in human *PCSK9* cause familial hypercholesterolemia, naturally occurring *PCSK9* loss-of-function variants (affecting 2-3% of the population) result in lower blood cholesterol levels and a reduced risk of cardiovascular disease (Rao et al. 2018). The single BE treatment introduced loss-of-function artificially and reduced cholesterol levels by 60%, a change that remained stable over time.

More recently, we have seen the development of PE technology, which combines the search capability of Cas9 with the writing capability of an RT. In this instance, the gRNA has also been modified, so that it contains not only the genomic address encoded in the protospacer, but also the message to be written by the RT. The PE system retains the specificity of CRISPR targeting, but carries additional information in the form of an RNA template containing editions as a contiguous extension of the gRNA (known as pegRNA) that can be written by the M-MLV RT fused to the Cas9 nickase. The use of Cas9 nickase prevents the formation of a DSB and simply cuts the non-complementary strand of DNA. This exposes a DNA flap with a 3' OH group that binds to the RNA template's binding site. This serves as a primer for RT, which extends the 3' flap by copying the edit sequence of the pegRNA. Although this 3' flap is thermodynamically less likely than the unedited 5' flap to hybridize to the unedited complementary strand, the inherent preference of the endogenous endonuclease FEN1 to excise 5' flaps leads to the hybridization of the edited 3' flap, resulting in highly efficient editing. It is

expected that this technology will soon be used in different therapeutic applications.

Despite obvious progress, gene editing tools still have their limitations and more technologies are needed in particular for conducting small and large edits. Likewise, base and prime editors, while extremely promising, are not without certain constraints: BEs are limited to A-> G or C-> T transitions within the editing window (Rees and Liu, n.d.) while PEs present various design constraints, which means edits have to be programmed downstream of the cleavage side of the gRNA and near a PAM sequence (Anzalone et al. 2019). Bearing in mind that pathogenic genetic defects can range from a few bases to large deletions, BEs and PEs can only repair a small number of bases, and HDR-based editing scales poorly in size and is ineffective in postmitotic cells. Certain NHEJ-based methodologies have, however, been developed, including, for example, homology-independent targeted integration (HITI) (Suzuki et al. 2016). This methodology has been shown to be effective for insertions of several kilobases, but not for very large editions. Thus, while HITI might work to insert exons, it may not be effective enough to robustly insert entire coding regions of large genes such as dystrophin (~14 kb) or laminin-α2 (LAMA2, ~9 kb). Likewise, more flexible technologies are needed to complement the BE and PE toolboxes when editing small alleles, while new techniques have to be developed for editing large alleles.

In nature, large gene transfer is mediated by transposases, recombinases, or integrases. These proteins effectively bind the ends of DNA and catalyze controlled and efficient gene transfer. In general, these systems are not programmable, although programmable transposons in bacteria have been described (Klompe et al. 2019; Strecker et al. 2019). Previous attempts to fuse zinc or Cas9 fingers with mammal-compatible transposases such as piggyBac (PB) have resulted in very low-precision systems (Yusa et al. 2011; Hew et al. 2019). We have managed to evolve a programmable transposon in mammals based on a modified fusion protein PB-Cas9 that exceptionally works in human cells (>15% efficiency at the desired insertion site). With this system, we combine the efficiency of classical gene transfer technologies with the precision of modern techniques, such as CRISPR/Cas9 (Pallarès et al, *under review*; patent filed). This programmable insertion technology can be administered with different delivery vehicles and it forms the basis of a newly created spin-off – Integra Therapeutics – of the Pompeu Fabra University.

6. Therapeutic applications based on microbiome engineering

The human body hosts a rich, complex microbial community. The human microbiota resides primarily in the skin, oral mucosa, and gastrointestinal tract, and plays a key role in health and many diseases (The Human Microbiome Pro-

ject Consortium 2012). The development of next-generation sequencing technologies (NGS) has made it possible to study these communities in unprecedented depth and resolution ("Nature Special: Human Microbiota" n.d.).

Over the past decade, our understanding of the composition and functions of the intestinal microbiota has increased significantly. This is largely due to the development of high-throughput genomic analyses of microbial communities, which have identified the critical contributions of the microbiome to human health. Consequently, the intestinal microbiota has emerged as an attractive therapeutic target. The vast majority of microbiota-targeted therapies aim to rebalance the intestinal ecosystem using probiotics or prebiotics. The targeted manipulation of the human microbiome can become a potential therapeutic strategy for the treatment and study of diseases. The most prominent example of this therapeutic principle is the treatment of antibiotic-resistant bacteria *Clostridium difficile* within the intestinal microbiome with the help of fecal transplantation. Following this treatment's success, several projects have developed microbiome-based treatments for intestinal diseases (van Nood et al. 2013; Olle 2013).

The intestinal microbiome has been researched extensively and, more recently, the skin microbiome has become another focus of research. Manipulation of the skin microbiome entails the promise of novel therapeutic approaches for skin diseases. The skin is colonized by a large number of different microorganisms, most of which are beneficial or harmless. We have shown that we can modulate the composition of the skin microbiome using *C. acne*s-based probiotic formulation. Indeed, this represented the very first demonstration of long-term microbiome modulation in humans (Paetzold et al. 2019). This article shows that modifying the skin microbiome in a targeted fashion is possible. Most importantly, the modifications presented stability over time. We have also shown how different microbiomes (or dermatotypes) present different levels of resilience. Significant continuations of this work include an initial clinical test (Karoglan et al. 2019) and a more advanced clinical test (in process) in patients with acne vulgaris. This research today serves as the technological foundation of a biotechnology company (www.sbiomedic.com), which is developing therapeutic programs to treat acne and skin aging.

Each bacterium constitutes a fascinating molecular machine with the potential to host advanced functionality and, not surprisingly, microbiome engineering has, in response, established itself as a vibrant field. Pioneering work in microbiome engineering has been successfully demonstrated. For example, in the case of the intestinal microbiome, Synlogic has developed strains of genetically modified *E. coli* to reduce ammonia levels (Isabella, Kotula, and Antipov 2019) and to eliminate phenylalanine and provide therapy for phenylketonuria (Isabella et al. 2018), and Prokarium is building a vaccination platform based on strains of *Sal-*

monella enterica (Tennant and Levine 2015). In the case of the skin microbiome, Azitra has developed genetically modified strains of *Staphylococcus epidermidis* (*S. epidermidis)* aimed at treating Netherton syndrome, eczema, and ichthyosis vulgaris. Multiple microbiome-based therapies, moreover, have entered clinical phases (Synlogic: NCT03516487, NCT03447730, and Azitra: NCT03820076). Although microbiome engineering has opened up promising prospects for the future, a number of obstacles have still to be overcome, including the implementation of advanced synthetic functionalities in the skin microbiome.

A particularly interesting platform for implementing these new features in the skin is *C. acnes*, given the persistence and low replacement rate that this bacterium has on the skin. However, the genetic engineering of this bacterium is far from straightforward. To date, only one laboratory has demonstrated homologous recombination (Sörensen et al. 2010). However, we have recently developed new tools for the efficient design of *C. acnes* (Knödlseder et al., in preparation). Given that the natural flora of the skin interact intensely with the host, our goal is to engineer *C. acnes* so as to create advanced tools that can interact with specific skin cell processes, such as sebum production and immune modulation. In addition, we are creating genetic sensor circuits in bacteria to listen for eukaryotic cell states and to translate this information into real-time reporters or into recordings of this information in DNA. In the future, our hope is that this line will lead to the development of intelligent drugs where the bacterium can detect the pathology and synthesize *in situ* an active principle to correct it.

7. Future perspectives

Evolution is the most advanced innovation machine known to us and, for some time now, biology-inspired engineering has been creating new paradigms in science and medicine. Indeed, many observations made in nature have been the inspiration for the development of new technologies. The repellant leaves of carnivorous plants, for example, inspired the invention of ultra-slippery surfaces (Wong et al. 2011); bat flight led to the development of self-adjusting wings (Lentink 2013; Gill 2014); and CRISPR-type bacterial antiviral mechanisms have provided us with the most advanced genome editing tools (Mali et al. 2013). The advances being made in bioscience are rapidly becoming the most important elements in the progress of humanity.

Human health is one of the most significant domains in which applied biological advances are being made. Today, biology is helping to save lives thanks to innovative treatments fully tailored to our genomes and metagenomes. In the future, we should be able to address a very high percentage of health problems employing scientific principles that are conceivable today. The speed with which

we are reducing the number of incurable pathologies is quite remarkable. A highly illustrative example of the maturity of biotechnology as applied to health is provided by the rapid development of SARS-CoV-2 vaccines, which are offering a solution to one of the greatest challenges faced by this generation. Clearly, much progress is yet to come: Science holds the key to fighting climate change and will underpin the fourth industrial revolution.

The biosciences are spreading to key sectors of the economy. Today, consumers are increasingly demanding products that reflect their more sustainable values and lifestyles. Chemistry is giving way to synthetic biology and organisms made with the same type of fermentation used in making beer, bread and kombucha are set to become the chemical components of clothing, cars and toys. According to McKinsey estimates, up to 60% of the physical inputs that the global economy may need can, in principle, be produced biologically (Sneader and Singhal 2021). McKinsey suggests that about a third of these inputs are biological materials, such as wood, cotton, skins, and animals bred for food, and that multiple innovations based on biological processes should be capable of substantially improving their existing production processes. The remaining two-thirds are non-biological materials, such as plastics and aviation fuels. However, the potential is also there for these inputs to be produced via biological processes as demonstrated already in the case of biofuels and bioplastics.

Yet, to be clear, achieving the full potential to produce these biological inputs remains some way off, but even moderate progress in this direction could be truly transformative. What is certain is that biology has the potential in the future to determine what we eat, what we wear, the products we put on our skin and how we build our physical world. But, at the same time, the level of sophistication that biology should allow us to achieve is likely to make us rethink our relationship with consumption itself. Will the clothes of the future change color according to the environment or our mood? Recently, molecular technology has been successfully exploited and anti-covid masks developed that can actually detect the presence of the virus. Indeed, in this particular instance similar sensitivities to qPCRs have been achieved (Nguyen et al. 2021).

Although we are looking forward to a green future with considerable excitement, we should not ignore concerns related to biosecurity and dual-use research. In these times of pandemic, the destructive power of biology is very apparent. For example, despite the obvious value in our ability to design viruses to generate more effective gene therapies, synthetic viruses can lead to the creation of even more deadly pathogens if they fall into the wrong hands. The synthetic biology community needs to be aware of these risks and to respond to them by analyzing all possible outcomes, while maintaining an open dialogue with regulatory bodies and the media.

The biosphere offers us the most advanced engineering space. Animals made with the same essential components (DNA, proteins, etc.) as us are able to resist cosmic radiation (*Milnesium tardigradum* – see Jönsson 2019), not develop cancer (*Heterocephalus glaber* – see Gorbunova et al. 2012) and live perpetually (*Turritopsis nutricula* – see Carlà et al. 2003). Nature's molecular architecture is an ongoing source of inspiration for learning new scientific and engineering principles. Despite the quite remarkable advances in synthetic biology to date, we still have much to learn and are some distance still from fully mastering the ability of natural evolution to create advanced molecular systems.

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Curriculum vitae of Marc Güell Cargol

Marc Güell Cargol (Olot, 1982) heads the Translational Synthetic Biology Research Group and is a Professor at Pompeu Fabra University. A graduate in both Chemistry and Telecommunications Engineering, he completed his PhD in Biomedicine before taking up a position as Postdoctoral Researcher at Harvard University (2010–2016).

His current research is conducted within the emerging discipline of Applied Synthetic Biology where he is developing research lines in novel genome editing technologies and skin microbiome engineering. His work has resulted in various advances in Synthetic Biology, including new CRISPR methods, a bacterial genome with only 57 codons, PERV-free pigs, and methods for modifying the skin microbiome. His research has made a significant impact, with some 20,000 citations, three spin-off firms developing therapies, and various patents licensed to leading companies. Part of his career has been dedicated to technology transfer in his role as founder and scientific advisor to the following companies: eGenesis Bio (xenotransplantation), S-Biomedic (therapies targeting the skin microbiome) and Integra Therapeutics (gene therapy).

In 2018 he received Catalonia's National Young Talent Research Award and, in 2019, the Web of Science Group named him as one of their Highly Cited Researchers. More recently, he was selected as an EMBO Young Investigator (2020) and this year he was awarded the August Pi and Sunyer Prize by the Institute for Catalan Studies.

Premis de la Secció de Ciències Biològiques

Títols publicats

1 Clara Ruiz-González, *Metacomunitats microbianes: la dispersió i la connectivitat com a factors determinants de la diversitat i la funció dels microorganismes aquàtics = Microbial metacommunities: Dispersal and connectivity as key drivers of the diversity and function of aquatic microorganisms* (2020)

2 Marc Güell, *Noves funcions biològiques sintètiques i implicacions per al present i el futur de la societat* (2021)

3 Marc Güell, *New Synthetic Biological Functions and their Implications for the Present and Future of Society* (2021)


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