Received: 12 July 2022 | Accepted: 16 July 2022

DOI: 10.1111/1462-2920.16140

CRYSTAL BALL

environmental microbiology sfam

SynBio 2.0, a new era for synthetic life: Neglected essential functions for resilience

Antoine Danchin | **Jian Dong Huang**

School of Biomedical Sciences, Li KaShing Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong

Correspondence
School of Biomedical Sciences, Li KaShing Faculty of Medicine, University of Hong Kong, Pokfulam, Hong Kong.
Email: antoine.danchin@normalesup.org; adanchin@hku.hk

Synthetic biology (SynBio) covers two main areas: application engineering, exemplified by metabolic engineering, and the design of life from artificial building blocks. As the general public is often reluctant to embrace synthetic approaches, preferring nature to artifice, its immediate future will depend very much on the public's reaction to the unmet needs created by the pervasive demands of sustainability. On the other hand, this reluctance should not have a negative impact on research that will now take into account the existence of the multiple transitions that cells face over time. The ages of life—birth, development, maturation, senescence and death—reflect specific key transitions and have, until now, rarely been taken into account in SynBio designs. This results in a mismatch between novelty and the very long evolutionary time that led to the existing chassis [remember that this is the common term used to describe the cellular framework as seen by synthetic biologists (de Lorenzo & Danchin, 2008)]. It also implies that, in general, transitions between growth stages will be taken into account with the appropriate embodiment of highly specific processes. This is particularly important when adapting new models to their host, whether natural or artificial. Adaptation follows two stages, short-term accommodation of new entities and long-term assimilation into the cell as a whole. This requires discrimination between classes of entities. We suggest that proteins playing the essential role of Maxwell' demons, here named Maxwell's discriminators (MxDs) to refer to their original function, will permit the assimilation and accommodation of artificial constructs. As a consequence, we can foresee that class discrimination, beyond mere recognition, will be implemented in SynBio 2.0, leading to an authentic paradigm shift (Kuhn, 1996), based on conceptions that embody information as a genuine currency of reality.

The prevailing view of SynBio restricts the field to the implementation of the de novo writing or rewriting of genetic programs in model chassis, with two major aims: metabolic engineering and conceptual design of synthetic life. In practice, this takes the form of controlled circuits that manage the expression of genes of interest for pure knowledge exploration or, most often, for industrial purposes. These circuits are designed in a manner somewhat similar to that of microprocessors (Buecherl & Myers, 2022) and implemented in model organisms selected for their well-known physiology (Bernhards et al., 2022). The most common are bacteria such as *Escherichia coli* (Lynch et al., 2022), *Bacillus subtilis* (Zhao et al., 2022) and more recently *Pseudomonas putida* (Duque et al., 2022). In a minor but conceptually important trend, intended to illustrate how we can access the minimal set of functions that could (should) be assembled de novo to make a cell alive, Mycoplasmas' chassis have also been used (Talenton et al., 2022). In these organisms, very elaborate experiments have been developed despite their restricted translation machinery based on a limited capacity to modify transfer RNAs and a somewhat limited use of the genetic code (UGA being translated into tryptophan, rather than signifying the end of translation). These engineering processes have been the source of two major standardization efforts: BioBricks (see for a recent illustration (Nguyen et al., 2022)) or SEVA (see (Nikel et al., 2022)), collecting essential pieces for the construction of new pathways. However, they have idiosyncratic properties that require better interoperability, a feature that is gradually being addressed (Damalas et al., 2020).

In addition to this general trend, we can easily understand that the current way of building new life forms is still far from the complete synthesis of an

artificial cell. Certainly, this would require combining the synthesis of an artificial molecular carrier for a modified genetic program with a relevant chassis. This goes far beyond application engineering as SynBio is also the paradigmatic way to understand what life is, by building living organisms from scratch. The next most obvious line of research was therefore to change the very nature of the nucleic acid polymers, replacing nucleotides with building blocks of an entirely new design (Chaput, 2021). In this context, the whole chassis must be approached synthetically, without simply using existing living forms: the (re)construction of life cannot do without the de novo generation of artificial host chassis (Danchin, 2012). We expect the current, somewhat limited landscape to change in the coming years, with the gradual introduction of new chassis based on building blocks very different from natural ones.

Many efforts are being made in this direction. Yet they remain limited in scope. Conspicuously, the ages of life—birth, development, maturation, senescence and death—are hardly ever explicitly considered in SynBio's efforts, which deal mainly with growth. We can assume that remarkable innovations will now make it possible to place all these stages and the transitions from one stage to a subsequent one in an appropriate context. As we shall see, this will require a new understanding of how life manages cell growth, metabolic processes and the repair of the inevitable errors associated with any physico-chemical construct. It will require the implementation in new designs of a key neglected function of life, that which allows cells to distinguish between categories of entities, locations or processes. It will also be necessary to implement concrete solutions to the question of growth homeostasis, which must control metabolism in such a way that the growth of a three-dimensional (3D) cytoplasm is consistent with that of two-dimensional membranes, and finally of one-dimensional genomes.

SUSTAINABILITY-DRIVEN METABOLIC ENGINEERING

Engineering is often understood by the general public as the ultimate solution to difficult problems. It provides the societal justification for SynBio. At the present time, this requires placing the field in a context where metabolic engineering will progressively encompass more and more sustainability requirements (Timmis et al., 2017). Today, carbon dioxide management is key. Many studies developed a few years ago are based on the use of gases (see, e.g., Liew et al., 2016; Okano et al., 2018). After the standard reconstruction of chemical pathways, it is expected that the improvement of canonical pathways, e.g. in one-carbon metabolism with carbon dioxide, methane or carbon monoxide as carbon sources (Irla & Wendisch, 2022), will now expand into the production of multiple sources of biofuels (Bouzon et al., 2017) or of important chemicals of industrial interest such as ethylene glycol (Bourgade et al., 2022). This trend is already implemented in a large number of processes, such as those illustrated in He et al. (2020), and will continue to progress. Cyanobacteria are fixing carbon dioxide, but still at a fairly slow rate, and SynBio approaches can do much to improve performance (Treece et al., 2022). All this work is advanced with the help of computational discovery of new pathways and their prospects for expanding metabolic capabilities by combining sources of enzymes encoded by genes spread across the tree of life (Sveshnikova et al., 2022). As an incident, it may be worth noting that research in the domain of carbon-negative bioproduction is now developed at a significant level in China, and because much relevant work is developed locally and published in Chinese (Hu et al., 2022), it becomes important to use the available automatic translation tools to access the corresponding knowledge.

A most promising direction, expected to develop at a rapid pace, is the invention of truly novel metabolism, with pathways and chemical reactions that do not exist in living organisms. This trend is illustrated by the domain of organometallic chemistry, which has maturated at a slow and steady pace for decades. In biology, the field started to grow in success 20 years ago. It seems, as seen in the citations in PubMed, for example, to have reached its peak 10 years ago—contemporary science is very sensitive to fashion—but we can expect that it will hit the headlines again now. As an illustration, in a radically innovative approach based on metal catalysts, bio-orthogonal catalysis—that is, catalysis that does not rest on catalytic processes that emerged during the development of life—is beginning to gain momentum (Liu & Bai, 2020; Vidal et al., 2018).

This success is driven by the fact that many of the obstacles that arise when implementing unusual chemistry in living systems are likely to be mitigated by new biochemical applications based on the extension of concepts such as those related to compartmentalization (Jolly et al., 2022). Indeed, a second major feature of local implementation of SynBio constructs is the recognition that many metabolites or precursors of important products of industrial interest are not compatible with each other, as they will have an inevitable tendency to react together. The solution is to implement compartmentalization, omnipresent in existing living organisms (de Lorenzo et al., 2015; Quinlivan et al., 2003). From this perspective, organelle compartmentalization for terpenoid synthesis has been the subject of much research attention, due to the diversity of the original physico-chemical characteristics of organelles (Jin et al., 2022). A rather different, but certainly

effective, way of compartmentalizing metabolic reactions is to set up co-cultures involving different organisms that develop sub-pathways assembled together via their import and export of intermediates. This approach has been explored in numerous experiments (Thuan et al., 2022). It is illustrated by the role of microbial communities in selectively stabilizing conditions for crops to become resilient, for example, see Abdelfadil et al. (2022) and Tosi et al. (2022). We can expect that, building on the success of biodiverse microbiomes for sustainable agriculture, new artificial microbial communities will be developed (Singh & Ramakrishna, 2021). It should be noted that the understanding of mycorrhizal associations is making considerable progress that will soon lend itself to SynBio designs (Pandit et al., 2022).

However, the main question remains whether and how all these designs could be implemented under natural conditions, as the public is still very reluctant to accept genetically modified organisms (Carter & Mankad, 2020). In this context, the immediate future of SynBio-based metabolic engineering will depend very much on the public's reaction to the unmet needs created by the demands of sustainability in a world dominated by the explosion of the human population. This makes the anticipation of innovation in this field quite hazardous. Nevertheless, synthetic microbiota developed in the laboratory is destined to be invented, at least as proof of innovative concepts (Chialva et al., 2022; Rafieenia et al., 2022).

Finally, the key question posed by metabolic engineering, whatever its origins, is whether the constructs will remain stable over time. Indeed, when alien proteins are introduced into an existing chassis—which has evolved over billions of years—one wonders whether they are not recognized as foreign, and progressively selected against. In practice, the remedy to this potentially deleterious situation has been to selectively evolve and stabilize the constructions made in the laboratory (Sandberg et al., 2019). The development of new approaches in this area is expected to change the outcome of difficult designs (Woo et al., 2022; Zhang et al., 2022). The process of evolution in the laboratory results in many mutations. Interestingly, the positive outcome of these experiments in terms of specific mutations is often interpreted as resulting solely from improvement of the catalytic properties of the proteins of interest. However, although generally overlooked, it seems likely that the process of class discrimination—which allows cells to separate classes of entities, such as misfolded or aged proteins, from their functional counterparts—plays a considerable role (Boel et al., 2019). This implies that mutations affecting folding or ageing are certainly critical and belong to the spectrum of mutations collected during evolution. This family of functions should be progressively understood in the coming years and introduced into SynBio designs.

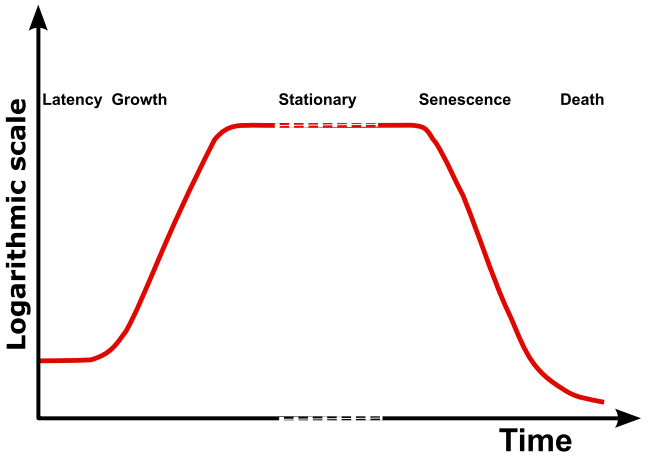

FIGURE 1 The ages of life. The vast majority of laboratory experiments study growing cells and organisms. Yet this is not the most common state of life. Cells and organisms live for a long time in a stable state without growing, and this particular stage of life requires the expression of a limited number of functions that should be understood in priority.

FROM THE PROOF OF CONCEPT TO THE CONSTRUCTION OF LIVING CELLS

Regardless of the public's reluctance to deal with genetically modified organisms unless they are strictly confined to the laboratory, SynBio research continues to advance our understanding of what life is. We can safely anticipate remarkable conceptual and experimental developments in the near future, based on an extension of the way life has been explored so far in the artificial setting of the laboratory environment. Indeed, experiments have long been limited to the analysis of cell growth under highly standardized and uniform conditions that never prevail in natural environments. The next step will be to study in more detail the different phases that cells go through over time. In nature, the ages of life force them to face challenges of very diverse characters. This trend is also consistent with the fact that an organism can be considered alive despite the fact that it does not and will not multiply until it dies: a stationary life is still a life. The ubiquity of the ages of life will also highlight that multiple transitions are the hallmark of life, so that what is interpreted as stress by a common anthropocentric view is in fact the norm, not the exception (Figure 1).

The necessary passage of time that all organisms face has led, during the long course of life's evolution, to a considerable number of solutions to the problems of development, maturation, ageing and death. This evolution has taken place without an ultimate solution, and all individuals are condemned to die at some point. Marking all stages where life is expressed, our anthropocentric position means that we have generally limited the questions raised by the passage of time to the specific case of the macroscopic development of

multicellular organisms. Yet there is no real reason to limit the scope of our exploration to these organisms. Microbes remain key to understand what life is: if we are lucky enough to have universal rules or processes, microbes will be the best organisms to test hypotheses about them. This has an impact on SynBio research. For example, in single-celled organisms, the emergence of processes such as sporulation shows how life has evolved to cope with time over the long term. But we also know that cells that do not sporulate enter a special state in long stationary phase (Feaga & Dworkin, 2021), and rules common to these widely different situations may exist.

Provocatively, the inevitability of ageing and death is sometimes questioned today for various reasons. Apart from the mistaken idea that bacteria do not age (see a discussion in Müller and Nebe-von-Caron (2010)), thus, giving hope to all living organisms, human hubris tends to make us believe that we will be able, in the not too distant future, to overcome all the obstacles we encounter. SynBio is no exception to this fashionable trend. Not only does it seek to understand what life is by reconstructing it from scratch, but it also tries to do better than nature and, why not, to aim for immortality. While this dream is very hopeful, it tends to obscure the challenges that lie ahead, particularly those caused by the inevitability of ageing, which has given rise to an interesting variety of ways of getting rid of old entities (e.g., via proteolysis (Harwood & Kikuchi, 2022)). Everything ages and eventually decays. It is not surprising that living organisms have, in the course of their evolution from inert matter, incorporated a multitude of structures and processes that make the cell, the 'atom' of life, resistant to a considerable variety of challenges, including those that lead to death. Anticipating what will soon make headlines in this field is, as we know, an impossible exercise, but, by limiting our analysis to prokaryotes, we illustrate how the consideration of the age-of-life issue will soon transform SynBio along previously unexpected lines.

Novel chassis

It is often assumed that the polymer hosting the genetic programs, a nucleic acid, is the only part of the cell's chassis that is worth considering for SynBio approaches. Certainly, this has just been illustrated by a most innovative application in the creation of vaccines using a synthetic mimic of protein-coding sequences, a remarkable example of SynBio applications, where uridine is replaced with N1-methyl-pseudouridine (Nance & Meier, 2021), a rare but still natural nucleoside (Pang et al., 1982; Wurm et al., 2010). Due to the undoubted success of this approach, we can safely anticipate that nucleic acid mimics will expand beyond synthetic RNAs used as coding sequences and revive the long-standing exploration of antisense RNA approaches (Moon, 2022). Nucleobase-modified oligonucleotides will be an intermediate step in the search for a generalization of substitution of authentic nucleic acids by analogues (Bartosik et al., 2020). Nucleic acids, seen as *substrates* for chemical reactions and not just as information-carrying *templates*, belong to the chassis of the cell. In this role of a substrate, synthetic constructs will replace natural nucleic acids as aptamers, for example (Alves Ferreira-Bravo & DeStefano, 2021; Mana, Bhattacharya, et al., 2022). At the same time, the second class of informational polymers, proteins, will also be modified in ways that have been developing steadily over the years (Danchin, 2022).

The majority of the experiments and applications where analogues play their informational role, similar to that of a read-only memory in computers, do not modify the whole genome of a cell, but limit their action to one or a few genes. Indeed, one would expect that modifying the whole chassis of a living cell would result in a considerable alteration of its general behaviour, including its ability to generate live offspring, especially after many generations. It may therefore be interesting to first explore how life would react to small changes. This can be done by replacing the canonical isotopes of the basic atoms with others, which is what has been called *isobiology*. In general, with the exception of the change of hydrogen to deuterium—which has measurable but moderate effects—the changes are extremely small. They can, however, help us to understand some of the key processes that "animate" chemical biology, notably by tracking isotope enrichment in mixtures of isotopes of a given atom (Danchin, 2020). More radical changes would use atoms avoided by life but sometimes used, such as fluorine and other halogens (Alves Ferreira-Bravo & DeStefano, 2021; Heine et al., 2018), silicon (Ikeda, 2021) or arsenic (Kabiraj et al., 2022). The narrow scope of the reactivity of these atoms is such that, when used in biological processes, they are and will remain confined to a limited set of metabolic pathways, which can nevertheless be expected to attract attention in the coming years. This exploration is not without its pitfalls: forgetting the true chemical nature of

the atoms in the same column of the Mendeleiev table, supposing that they shared similar properties (Benner, 2011), some believed that arsenic could replace phosphate in nucleic acids. It was soon shown that the experiments believed to have validated this illiterate hypothesis—it is well established that phosphate is unique in that phosphate bonds are metastable in water at the temperature of life (Westheimer, 1987)—were in fact based on phosphate contamination in the media used to prove that bacteria used arsenic in their nucleic acid backbone (Reaves et al., 2012).

Important developments in the field of novel nucleic acid-like polymers have already been in research pipelines for years under different names and are collectively known as *xenonucleic acids* (XNA) of various kinds (see Chaput & Herdewijn, 2019; Alenaizan et al., 2021; Chaput, 2021; Mana, Kundu, et al., 2022, for recent explorations). While this work is currently developing in many interesting avenues, we can be sure that completely different chassis will be used before long. For example, l-nucleotides have rarely been considered ((Dantsu et al., 2021), and only 32 references at PubMed on 30 July, 2022). The chemistry of 4′-thioribonucleosides has been known for a long time (Haeberli et al., 2005), but the interest in these analogues has been revived recently (Betson et al., 2014). In replication, transcription and translation, *E. coli* DNA/RNA polymerases and ribosomes tolerate the replacement of O4′ with S4′ in the nucleotide, despite the fact that sulphur has a larger atomic radius than oxygen. The activity of these polymers for replication, transcription and translation has been established in vitro (Tarashima et al., 2020). Conceptual progress will be made when we understand the limitations faced by organisms when the entire genome has been recoded using these unusual analogues of the standard nucleic acid building blocks (see further discussion below).

Global recoding

Some work has already explored the transition from local (usually orthogonal (Costello & Badran, 2021)) to global SynBio designs. The most advanced work is with Mycoplasmas, with entirely recoded genomes (see Talenton et al., 2022, for an example of the most recent trends), and this type of work has also been extended to the unicellular eukaryote, *Saccharomyces cerevisiae* (Zhang et al., 2020), with shuffling genes and merging its chromosomes into a smaller number (Wang et al., 2018). A widely explored line is the follow-up of research into the general exploration of the genetic code alteration (see, e.g., Shakya et al., 2022). This goes hand in hand with orthogonal implementation of specific replication/transcription and translation pathways. Various experiments have modified the (almost) universal codon usage table to allow the introduction of non-canonical amino acids into proteins, limited to a fraction of the genome chassis (de la Torre & Chin, 2021; Lateef et al., 2022; Tang et al., 2022). Exploration of the codon usage table modification has also led to experiments where the table has been consistently reduced throughout a genome, thus representing a global recoding of living cells (Fredens et al., 2019). However, these experiments did not alter the chemical nature of the nucleic acid chassis as a whole, and, at present, there appears to be only one experiment that created an organism with an entirely foreign nucleotide in its entire genome by replacing thymidylate with 5-chloro-uridylate (Marlière et al., 2011). Interestingly, the corresponding chassis has yet to be described in its own physiological and molecular terms, starting with the identification of its explicit genomic sequence. This is despite the fact that the initial construction was carried out more than 10 years ago, which suggests that it is reasonable to anticipate new results in the very near future.

A somewhat similar situation exists in the progeny of a chassis where all proteins have been changed, with 4-fluorotryptophan replacing tryptophan. It was known early on that the fluorinated metabolite could substitute efficiently for its canonical counterpart in *E. coli* (Pratt & Ho, 1975) and a first variant of the bacteria was found to tolerate a high proportion of the analogue (Bacher & Ellington, 2001). It is however only recently, in *Bacillus subtilis*, that full replacement was obtained and mutations characterized (Yu et al., 2014). Again, as in the case of the replacement of thymidine by 5-chloro-uridine, not much has been described about the physiology and stability of the mutants obtained, except for an expected change in tryptophan metabolism control as well as

changes in RNA polymerase. After a decade, we can expect to get more information soon.

EMERGING CORE FUNCTIONS NEGLECTED IN PREVIOUS SYNBIO STUDIES

All these experiments are convincing proofs of concept, monitoring growth over a limited time, but how will the cells cope with the corresponding changes in the long term? It is probably not surprising that the latter experiments, although still ongoing, have not yet been thoroughly exploited. Indeed, the modification of a basic building block, whether throughout the genome chassis or in all proteins, should affect the vast majority of the cell's functions, making the construct particularly unstable. And while many mutations have been described in the mutants, and interpreted as passenger or even neutral mutations, they should be studied in-depth. Indeed, we should not expect the corresponding genomes to stabilize easily, nor should we expect that only a few mutations will generate a stable organism in the long term. We should not forget that existing natural living organisms are far removed from the usual timeline of laboratory experiments. They have witnessed the co-evolution of their components over billions of years. This makes these constructs all the more interesting and gives rise to studies that should soon be available.

Consider, for example, the basic metabolism that ensures the stability of a genome and its nucleotide composition. It can be expected to be strongly coupled to the cell with its environment. Indeed, genomes that differentiate species from one another can be distinguished, among other features, via analysis of their GC vs. AT content. Thousands of studies have examined this fundamental signature across the three domains of life (Li & Du, 2014; Novoa et al., 2019; Reichenberger et al., 2015). We know that there is a fine balance between the different pathways maintaining the intracellular nucleotide concentration. Any alteration of this balance is strongly mutagenic (Kapoor & Varshney, 2020). As a result of natural selection, overall, nucleotide metabolism is organized into two different control systems, with the homeostasis of purine and pyrimidine nucleotide pools organized separately. The critical importance of the corresponding homeostasis is witnessed by the presence, in minimal genomes of autonomous organisms such as *Mycoplasma mycoides* Syn3.0, of conserved enzymes of purine metabolism (*adk*, *apt*, *dgk*, *gmk*, *guaC*), pyrimidine metabolism (*cmk*, *dcd*, *pyrG*, *pyrH*, *tdk*, *tmk*, *udk*, *upp*), and both purine and pyrimidine metabolism (*dck*, *ndk, pnpA*). This is despite the presence in the environment of the cognate metabolites and precursors, associated to relevant transporters, which might have been expected to allow the cell to dispense from these activities (Danchin & Fang, 2016; Hutchison et al., 2016). Remarkably, these same genes have also been deemed dispensable in a complete model of the metabolism of this organism (Rees-Garbutt et al., 2021), demonstrating that key features of SynBio requirements must have been overlooked (Danchin, 2021).

Moreover, cells must differentiate between processes that allow them to control damage and organize repair, whatever their growth status, and those that require specific growth-related syntheses. Indeed, like any process governed by the constraints of physics and the rules of biological chemistry, metabolism, whether on the scale of small molecules or that of the biosynthesis of macromolecules, is subject to error. This is well known for the biosynthesis of nucleic acids and proteins, but it is also true for the synthesis of elementary building blocks. Metabolic accidents are the rule, not the exception (Danchin, 2017; Sun et al., 2017). This feature has rarely been considered in SynBio constructs, except, indirectly, in efforts to make cells encoding the minimal set of essential functions. To gain insight into the nature of the corresponding functions, it is therefore interesting to explore the contents of minimal genomes and, in particular, again that of *M. mycoides* Syn3.0 (Hutchison et al., 2016). A notable observation from this analysis is that about 1 in 10 genes encodes a function that dissipates energy in a role that obviously should not necessitate energy consumption. In particular, many of these functions are related to damage control, via either the degradation of non-functional entities, their export outside of the cell or their repair. Remarkably, the only molecular chaperones that remain in the proteome of this synthetic organism is the DnaJK protein, which appears to allow the cells to dispense from the GroESL complex omnipresent in Firmicutes (Danchin & Fang, 2016). In addition, there is a family of enzymes that deal with possibly toxic variants of standard building blocks. Pyrophosphate hydrolysis drives the degradation of the highly phosphorylated form of modified nucleosides either in a natural pathway or as a result of accidents. Similarly, various metabolites undergo energy-dependent catabolism or export of chemically 'tagged' derivatives to differentiate them from their authentic counterparts (Danchin & Sekowska, 2014; Quinlivan et al., 2003).

Damage control and repair

These observations have led to the search for explicit functions that allow cells to cope with the inevitable errors, accidents and ageing. Of course, the cellular components of a given type are not made up of strictly identical copies. The biosynthetic machinery of macromolecules is prone to errors. Moreover, material things (things with mass) wither away over time. Even small metabolites can differ, for example, in their

stereochemistry. Enantiomers racemize, hence the cellular content consists of a mixture of functional, canonical entities mixed with a multitude of variants, some of which are no longer functional, or worse, perform wrong functions. Gradually, the organized structures or processes mix old and younger entities, and lose their sharpness and precision. If a particular assembly, such as that found in the cytoplasm of cells, has a well-defined structure (i.e. associating things in specific relationships), this structure will become distorted, withered and eventually lost. This opens up a whole category of new functions.

In order to control or repair the resulting damage, the cell must first be able to distinguish the canonical entities from the wide variety of damaged entities, and then subject the class of altered entities to repair, if possible, to degradation, or to expulsion from the cell. This process of discrimination allows a cell or more complex living organism to be given explicit meaning (typically being functional or non-functional). This process is an information management function. Understanding it requires an in-depth analysis of what the functions associated with class discrimination might be (Box 1).

This analysis is often unfamiliar to biologists. It has certainly not yet been included in the family of concepts and understandings that underpin SynBio research (see, for example, the postulates used in modelling (Rees-Garbutt et al., 2021)). As the field progresses, it should become clear that, if only for reasons of reproducibility, these functions will now be part of its most advanced new designs. What follows is an overview of the implications of this new need, first highlighting references to work that developed half a century ago and has been neglected since. For those familiar with the history of science, this should not be surprising if one recalls that Mendel's work was neglected during a similar period.

Based on the pioneering work of physicist Rolf Landauer, it is now understood that the creation of information does not consume energy. It is the process of discriminating between background noise and information that dissipates energy (Landauer, 1961). Importantly, the corresponding reflection was linked to an analysis developed by James Clerk Maxwell in his *Theory of Heat* (Maxwell, 1871), and summarized since then by a metaphor, later named Maxwell's demon and here Maxwell's discriminator (Figure 2, (Binder & Danchin, 2011)).

A remarkable consequence of this view is that it extends the current way of quantifying information, via the minimum energy dissipated on each relevant entity to create classes. This highlights the critical need to identify the concrete biological processes that enable discrimination. Although rarely known, this view is not new in biology. Yet it has long been overlooked and, as it is not introduced explicitly in the standard theory of information, we can safely anticipate its revival in the coming years. Fifty years ago, for example, Horton Johnson—who was not aware of Landauer's work—noted that

BOX 1 Recognition and discrimination

A recognition process associates a recognizing entity with what is recognized. It is a highly specific process, which guarantees a one-to-one match, based on the key and lock complementarity rule that was proposed by Emil Fisher in 1905. This has the consequence that, in terms of the genetic content of a particular genome, recognition is extremely costly, as it requires at least one recognition entity for each element that makes up the cell. The alternative, well known in artificial intelligence, is discrimination between classes of things. In this case, the discriminating entity only needs to sample a sufficient number of features of a thing possibly belonging to a particular family to tell whether that thing belongs or not to the class. It is important to recall, as did Félix Vicq d'Azyr in the XVIIIth century, that one can construct relevant classes where any individual entity does not have to display simultaneously all the characters used to define a given class and yet belong to that class because it displays a sufficient number of them (Félix Vicq d'Azyr, 1792). This allows a discriminating agent to be able to identify any member of a class, without needing, as in the recognition process, to identify simultaneously all the characters defining that class. As a consequence, two individual entities may differ significantly while still remaining members of a particular class. In terms of genetic requirements, this is a gene sparing process. As discussed in the main text, this works at a cost: the discrimination process will have to dissipate energy.

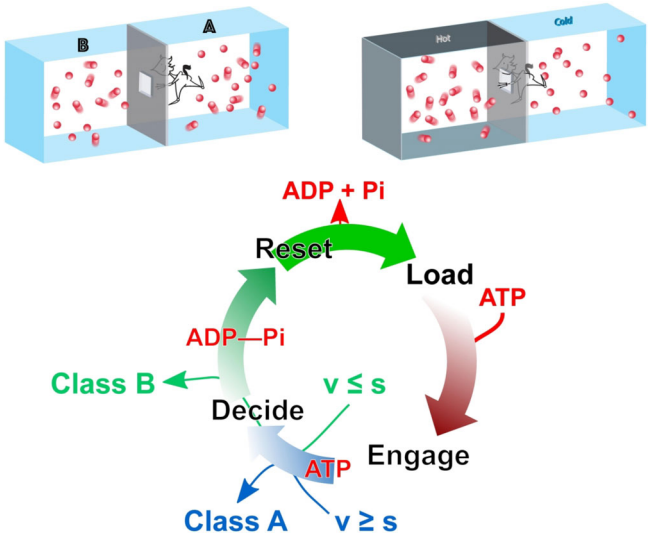

FIGURE 2 An agent that uses discrimination to make classes, the Maxwell's demon, modified from figure 2 of Boel et al. (2019). Although it is basic knowledge in thermodynamics, the metaphor proposed by Maxwell to suggest how perpetual motion could be created against the second law of thermodynamics is often overlooked nowadays. Consider a closed container filled with a gas, with no heat exchange with the outside, and made of two chambers, A and B. The chambers are separated by a wall with a small hole in it, closed by a movable trap door. This door can be opened or closed at will by an agent capable of making a decision, depending on the speed of the approaching gas atoms. Let us suppose that the agent opens and closes this hole, so that only the fastest molecules pass from A to B, and only the slowest ones pass from B to A. This will increase the temperature of B and decrease that of A, in contradiction with the second law of thermodynamics, and allow the formation of the thermal machine. Remarkably, this type of operation, which manipulates information, is very reminiscent of the way young proteins are separated from old ones in yeast or during gamete formation. When cells grow and form a bud as future offspring, the old proteins remain in the mother cell while the neosynthesized bud contains only young proteins (Aguilaniu et al., 2003; Koch-Bojalad et al., 2021). The discrimination between both classes is performed by a protein complex that dissipates energy from ATP or GTP in a process that loads energy in the first step but does not dissipate it until the complex has interacted with the class meant to be discriminated against.

discrimination is exactly the function described by Maxwell's demon allegory (Johnson, 1970). It is the low thermodynamic efficiency of the kidney, a long-standing paradox in animal physiology, that triggered Horton's reflection. He proposed that the paradox resulted from the considerable dissipation of energy due to the main function of the kidney, discrimination between ions of diverse nature (Johnson & Knudsen, 1965).

In his work, Johnson also emphasized the role of thermal noise and that of structural information, particularly via the spatial organization of relevant entities, showing, for example, that the dilution of a particular configuration would tend to make meaningful information gradually disappear. This vision is very relevant because it can explain the difficulty of modelling in vitro what happens in vivo (Danchin, 2022). It is certain that work on modelling the minimal functions necessary to enable life results in a significantly lower number of genes than that found in the original minimal autonomous synthetic organism. In particular, the presence of the *dnaK* gene is deemed dispensable (Rees-Garbutt et al., 2021). This completely overlooks the critical function of discrimination to build up classes. We can safely predict that experiments will soon refute this claim and pave the way for understanding the importance of Maxwell's discriminators in SynBio. Finally, it is also important to distinguish between general thermal noise (a direct consequence of the temperature of life) and the effects of temperature on living structures and processes, which is related to meaningful information (Johnson & Pavelec, 1972). In short, we can expect to see experiments soon that measure the way energy is dissipated in SynBio constructs, not only in the chemical processes involved per se, but also in the general maintenance of the constructs. Typically, at the temperature of life, the energy dissipated in each implementation of a discrimination event corresponds to the hydrolysis of a phosphate bond, which is about 20–30 times more than that of the amount actually needed. This may be considered as a waste, but in fact this allows the accuracy of the system to be only slightly affected by thermal noise (Boel et al., 2019). The future of stable designs of SynBio organisms with modified building blocks will require the identification of processes where the energy involved is at least an order of magnitude greater than that of the local thermal noise, so that the process can stand out from the background.

Assimilation and accommodation

As we saw earlier, SynBio manipulates different types of chassis, inserting artificial constructs for metabolic engineering and even changing their chemical nature. All the elements thus combined together have no reason to harmonize well with the different classes of things that have co-evolved

over billions of years in natural cells. In short, we must expect them to be perceived as foreign, grouped into a specific class destined to be repaired, degraded or expelled from the cell. In a field remote from SynBio but highly relevant to understanding the general process of adaptation, psychology, Jean Piaget proposed an interesting dichotomy: when challenged by changes, the adaptation of the organization of a living entity follows processes forming two categories, assimilation and accommodation (Block, 1982). Generally speaking, assimilation refers to the reintegration of new external elements into a pre-existing internal structure; accommodation refers to the adaptation of the organism to external variations that it fails to assimilate. Accommodation can be implemented relatively quickly, while assimilation is a long-term process.

This can be transposed to cells facing the challenge brought about by SynBio constructs, for example, for the degradation of plastic things (Brandenberg et al., 2022). A proliferation of similar studies can be expected in the coming years, if not months. They will immediately be faced with the need to accommodate the new designs within the host chassis as they will have to integrate them into their existing structure. This will result in an evolutionary phase of the type usually explored immediately after the implementation of a particular metabolic design. The subsequent assimilation stage is more important and, conceivably, impossible, as it will require the cell to completely remodel its progeny in the course of its evolution. This situation should soon be illustrated by the adaptation to a new nucleotide, such as 5-chlorouridylate.

Identifying agents for class discrimination

However, this view remains descriptive. We need to identify the actual cellular entities and processes that drive assimilation and accommodation. While the process of assimilation can be envisaged for small sets of genes—and the existence of widespread horizontal gene transfer supports this—it seems difficult to accept that it can work on the laboratory time scale, when entire genomes are modified. In this case, the process is likely to take much longer, being understood as going beyond accommodation, requiring much deeper changes in cellular organization. This is where the discrimination function of Maxwell's discriminators comes in. How can we identify, experimentally, that agents functioning like this fictitious wizard are actually involved? At least two families of experiments, which can be expected to be reconsidered very soon, illustrate new developments in this direction. Standard chassis, such as *E. coli* strains, are limited in their temperature range, with normal functioning stopping below 15°C because RNA and protein folding is known to be more or less 'frozen' at lower temperatures. Manuel Ferrer and his colleagues had the bold idea of considering whether a molecular chaperone from an Antarctic bacterium, *Oleispira antarctica*, transformed into *E. coli*, could alleviate this constraint. Unexpectedly, they found that this was indeed the case (Ferrer et al., 2004). Temperature is a statistical property that reflects the degree of agitation of all entities present in an environment of sufficient size. This involves, in particular, water molecules and their associated hydrogen bonds. Temperature influences all chemical processes simultaneously to varying degrees, so it seems difficult to understand how a single protein could alleviate its global effect (Strocchi et al., 2006). This is unless the local temperature differs from that of the environment, however. Remarkably, an experiment that measured the local temperature of active mitochondria may solve the riddle. In experiments using thermosensitive colour markers, it was suggested that mitochondria were much warmer than the cell in which they live [(Chrétien et al., 2018), Box 2].

This surprising result—of course disputed (Lane, 2018)—was attributed to energy dissipation by ATP synthase, acting as a Maxwell's discriminator (Vigneau et al., 2022). A similar explanation would fit with the function of molecular chaperones. Indeed, hydrolysis of phosphate bonds liberates energy, with consequences in terms of local temperature, reflected by changes in the movements of local water molecules and hydrogen bonds. Any change in the efficacy of molecular chaperones, which act as Maxwell's discriminators to recognize the proteins that they will fold or refold (Boel et al., 2019), will increase the energy they dissipate, and this will change temperature locally. With this understanding, replacing a mesophilic chaperonin with a psychrophilic is likely to increase its error

BOX 2 The 'hot mitochondrion'

As large-scale fermentation units show, heat is released as a product of metabolism. In particular, the oxidation of respiratory substrates drives ATP synthesis and metabolite transport, with a substantial proportion released as heat. In Eukarya, mitochondria are major actors in this thermogenic process. Mitochondrial temperature in situ has been measured under different physiological conditions. A thermosensitive colour assay showed that apparently mitochondria were more than 10°C warmer when the respiratory chain was fully functional, both in human embryonic kidney cells and primary skin fibroblasts. This differential was abolished in cells depleted of mitochondrial DNA or treated with respiratory inhibitors. Note that a difference of 10°C, which seems considerable at first sight, is only very marginal at the temperature of life, 300 K, which is the only quantitative parameter in terms of the physics of the process that interests us. Yet, this observation is controversial (Lane, 2018), but if substantiated, it would validate the idea that Maxwell's discriminators expressed in heterologous systems dissipate more energy than their adapted counterparts, locally rising the temperature.

rate, because it did not co-evolve with its new environment. In turn, this increases the local temperature so that it mimics the mesophilic environment. It should be noted here that the optimal growth temperature of the donor organism is in fact anecdotal. It is the phylogenetic distance between the host and the donor that should be significant. Is this hypothesis far-fetched? We can certainly anticipate that these enigmatic observations, highly relevant for SynBio, will presently come to the fore.

Interestingly, further analysis has shown that temperature sensitivity was associated with a small number of essential proteins, including molecular chaperones as expected, but also metabolic enzymes, notably related to cytosine metabolism, cytidylate kinase and cytidine triphosphate (CTP) synthetase [(de Lorenzo, 2011; Strocchi et al., 2006) and see Figure 3]. These experiments measured growth, not just stationary survival. It is therefore highly significant that these enzymes have been selected as critical agents for the maintenance of homeostasis during growth (Ou et al., 2020), a process we are now addressing.

Understanding non-homothetic growth

Living organisms do not multiply constantly, as in chemostats. They have to manage

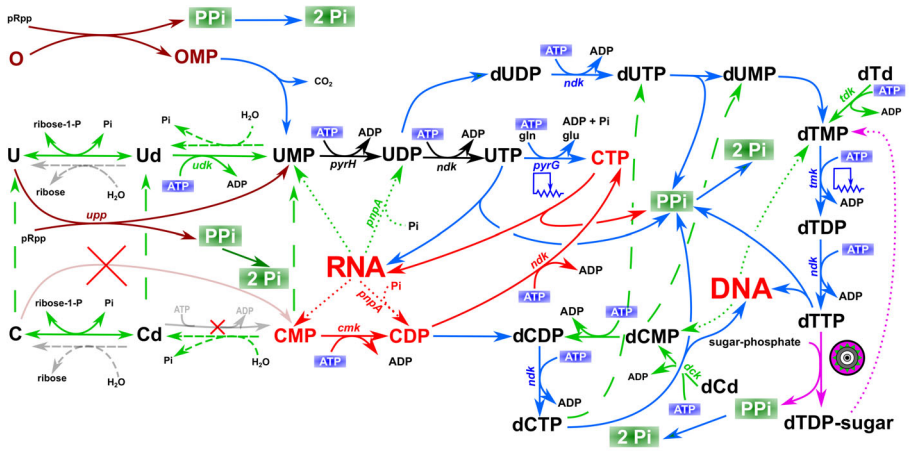

FIGURE 3 A subset of pyrimidine metabolism. Pyrimidine metabolism channels metabolites through CTP, and CTP synthetase acts as a rheostat to maintain the correct level of pyrimidine precursors for RNA and DNA synthesis. The core of the metabolism is represented by black arrows. DNA requires nucleoside diphosphates as precursors to triphosphates, and, in the case of CDP, this requires the degradation of RNA to CMP by nucleases or to CDP by polynucleotide phosphorylase, as shown by red and dotted arrows. Anabolic steps are represented by blue arrows. Salvage pathways are represented by green arrows. The arrows with long dashes show the deamination steps that convert cytosine derivatives into uracil derivatives. The short dashes indicate the steps to dispose of the corresponding elements. Key phosphoribosyltransferases (acting on orotate for de novo synthesis and on uracil for salvage) are represented by brown arrows. The missing cytosine homologue (Ou et al., 2020) is crossed out and shown in faded brown, as are the frequently missing steps (in light grey). Finally, a likely step for storing thymidine derivatives, possibly acting as a flywheel and coupling DNA synthesis to envelope synthesis, is shown with pink arrows.

transitions between survival and multiplication. When they resume growth after a stationary phase, specific metabolic constraints associated with growth must be implemented. Metabolism models are generally calibrated on the idea that the content of all the constituent elements will double with each generation. The necessary non-homothetic distribution of metabolites in the cytoplasm, in a 3D volume, compared with membranes, which are two-dimensional, and genomes, which are linear polymers growing in one dimension, is almost never taken into account. Yet growth implies that, unless a particular metabolic control has emerged during the evolution of life, there will always be "too many" precursors to synthesize genes and genomes and thus pressure to lengthen genomes, not shorten them. Remarkably, natural selection has retained a ubiquitous specificity of metabolic organization, centred on pyrimidine metabolism, to enable the corresponding homeostasis. This is ensured in bacteria by the existence of 'rheostats' such as thymidylate kinase or CTP synthetase, as well as reserves playing the role of "flywheel" such as TDP-sugars, allowing the cell to finely adjust its growth stage. The ultimate goal is achieved by channelling de novo synthesis and salvage of pyrimidines through uracil derivatives. The key metabolite in this process is CTP, synthesized from UTP, and required for at least three essential metabolic steps. It is a key component of (1) the synthesis of host RNAs; (2) the synthesis of cytosine-based liponucleotide precursors; and (3) the synthesis or repair of the 3′CCA end of all transfer RNAs required for translation (Ou et al., 2020). Many other processes also require CTP instead of ATP, such as peptide bond formation for coenzyme A synthesis (Chan et al., 2019) or synthesis of dolichyl-phosphate for glycosylation in Eukarya and Archaea (Makarova et al., 2015). Moreover, intermediary metabolism is built on mandatory steps of synthesis and salvage of cytosine-based metabolites leading to uridine triphosphate, channelling all pyrimidine metabolism through CTP (Figure 3).

In the case of new synthetic genomes, particularly when changing the chemical nature of pyrimidine nucleotides—such as the replacement of thymidylate by 5-chlorouridylate—it can be expected that, while a preliminary step of evolution by accommodation is likely (and indeed seen in published experiments with this new component of the genomic chassis), it is questionable whether true assimilation will be possible using engineering approaches. Yet, it can be expected that this will be explored in the coming years. This should soon be known as well as in the case of similar experiments involving programmed ageing or other substitutes for canonical building blocks.

PERSPECTIVES

Synthetic biology is at a turning point. The number of published works in the field is increasing at a rapid pace. The field is facing a considerable demand for critical experiments that would enable the design of new engineering models to meet the now universally recognized goal of sustainability. This requires a radical change in our understanding of metabolism and its constraints and is deeply linked to the second major objective of SynBio, namely to understand what life is. Until now, research has not seriously addressed the conditions for adequate adaptation of artificial constructs to the constraints of their engineered creation, forgetting that it took life over 3 billion years to reach its current state. We believe here that we will see in the coming years that understanding the processes that develop accommodation and subsequently assimilate new constructs will uncover the neglected functions that have been suggested in this overview of the present situation. We anticipate that these will include agents that behave like Maxwell demons, named here discriminators, and also embody the constraints created by the need for homeostasis to ensure non-homothetic growth when new living cells are faced with the necessary transitions that accompany all types of life.

ACKNOWLEDGEMENTS

This work benefited from conceptual ideas developed in the program I2Cell from the Fondation Fourmentin-Guilbert, and from comments of the audience of the Stanislas Noria weekly seminar. We thank Éric Fourmentin for pointing out the works of Horton Johnson and Chérif Matta.

CONFLICT OF INTEREST

Antoine Danchin is the chairman of the Scientific Council of EureKare, a company supporting industrial developments in Synthetic Biology.

ORCID

Antoine Danchin https://orcid.org/0000-0002-6350-5001

REFERENCES

Abdelfadil, M.R., Taha, M.H., el-Hadidi, M., Hamza, M.A., Youssef, H. H., Khalil, M. et al. (2022) Clay chips and beads capture in situ barley root microbiota and facilitate in vitro long-term preservation of microbial strains. *FEMS microbiology immunology*, 98(7), fiac064. https://doi.org/10.1093/femsec/fiac064

Aguilaniu, H., Gustafsson, L., Rigoulet, M. & Nystrom, T. (2003) Asymmetric inheritance of oxidatively damaged proteins during cytokinesis. *Science*, 299, 1751–1753. https://doi.org/10.1126/science.1080418

Alenaizan, A., Barnett, J.L., Hud, N.V., Sherrill, C.D. & Petrov, A.S. (2021) The proto-nucleic acid builder: a software tool for constructing nucleic acid analogs. *Nucleic Acids Research*, 49, 79–89. https://doi.org/10.1093/nar/gkaa1159

Alves Ferreira-Bravo, I. & DeStefano, J.J. (2021) Xeno-nucleic acid (XNA) 2′-fluoro-arabino nucleic acid (FANA) aptamers to the receptor-binding domain of SARS-CoV-2 S protein block ACE2 binding. *Viruses*, 13, 1983. https://doi.org/10.3390/v13101983

Bacher, J.M. & Ellington, A.D. (2001) Selection and characterization of *Escherichia coli* variants capable of growth on an otherwise toxic tryptophan analogue. *Journal of Bacteriology*, 183, 5414–5425.

Bartosik, K., Debiec, K., Czarnecka, A., Sochacka, E. & Leszczynska, G. (2020) Synthesis of nucleobase-modified RNA oligonucleotides by post-synthetic approach. *Molecules*, 25, E3344.

Benner, S.A. (2011) Comment on "a bacterium that can grow by using arsenic instead of phosphorus". *Science*, 332, 1149; author reply 1149. https://doi.org/10.1126/science.1201304

Bernhards, C.B., Liem, A.T., Berk, K.L., Roth, P.A., Gibbons, H.S. & Lux, M.W. (2022) Putative phenotypically neutral genomic insertion points in prokaryotes. *ACS Synthetic Biology*, 11, 1681–1685. https://doi.org/10.1021/acssynbio.1c00531

Betson, M., Allanson, N. & Wainwright, P. (2014) A review of methods to synthesise 4′-substituted nucleosides. *Organic & Biomolecular Chemistry*, 12, 9291–9306. https://doi.org/10.1039/C4OB01449A

Binder, P.M. & Danchin, A. (2011) Life's demons: information and order in biology. What subcellular machines gather and process the information necessary to sustain life? *EMBO Reports*, 12, 495–499. https://doi.org/10.1038/embor.2011.83

Block, J. (1982) Assimilation, accommodation, and the dynamics of personality development. *Child Development*, 53, 281. https://doi.org/10.2307/1128971

Boel, G., Danot, O., de Lorenzo, V. & Danchin, A. (2019) Omnipresent Maxwell's demons orchestrate information management in living cells. *Microbial Biotechnology*, 12, 210–242. https://doi.org/10.1111/1751-7915.13378

Bourgade, B., Humphreys, C.M., Millard, J., Minton, N.P. & Islam, M. A. (2022) Design, analysis, and implementation of a novel biochemical pathway for ethylene glycol production in *Clostridium autoethanogenum*. *ACS Synthetic Biology*, 11, 1790–1800. https://doi.org/10.1021/acssynbio.1c00624

Bouzon, M., Perret, A., Loreau, O., Delmas, V., Perchat, N., Weissenbach, J. et al. (2017) A synthetic alternative to canonical one-carbon metabolism. *ACS Synthetic Biology*, 6, 1520–1533. https://doi.org/10.1021/acssynbio.7b00029

Brandenberg, O.F., Schubert, O.T. & Kruglyak, L. (2022) Towards synthetic PETtrophy: engineering *Pseudomonas putidafor* concurrent polyethylene terephthalate (PET) monomer metabolism and PET hydrolase expression. *Microbial Cell Factories*, 21, 119.

Buecherl, L. & Myers, C.J. (2022) Engineering genetic circuits: advancements in genetic design automation tools and standards for synthetic biology. *Current Opinion in Microbiology*, 68, 102155. https://doi.org/10.1016/j.mib.2022.102155

Carter, L. & Mankad, A. (2020) The promises and realities of integration in synthetic biology: a view from social science. *Frontiers in Bioengineering and Biotechnology*, 8, 622221. https://doi.org/10.3389/fbioe.2020.622221

Chan, D.S.-H., Hess, J., Shaw, E., Spry, C., Starley, R., Dagostin, C. et al. (2019) Structural insights into *Escherichia coli* phosphopantothenoylcysteine synthetase by native ion mobility–mass spectrometry. *Biochemical Journal*, 476, 3125–3139. https://doi.org/10.1042/BCJ20190318

Chaput, J.C. (2021) Redesigning the genetic polymers of life. *Accounts of Chemical Research*, 54, 1056–1065. https://doi.org/10.1021/acs.accounts.0c00886

Chaput, J.C. & Herdewijn, P. (2019) What is XNA? *Angewandte Chemie (International Ed. in English)*, 58, 11570–11572. https://doi.org/10.1002/anie.201905999

Chialva, M., Lanfranco, L. & Bonfante, P. (2022) The plant microbiota: composition, functions, and engineering. *Current Opinion in Biotechnology*, 73, 135–142. https://doi.org/10.1016/j.copbio.2021.07.003

Chrétien, D., Bénit, P., Ha, H.-H., Keipert, S., el-Khoury, R., Chang, Y.-T. et al. (2018) Mitochondria are physiologically maintained at close to 50°C. *PLoS Biology*, 16, e2003992. https://doi.org/10.1371/journal.pbio.2003992

Costello, A. & Badran, A.H. (2021) Synthetic biological circuits within an orthogonal central dogma. *Trends in Biotechnology*, 39, 59–71. https://doi.org/10.1016/j.tibtech.2020.05.013

Damalas, S.G., Batianis, C., Martin-Pascual, M., Lorenzo, V. & Martins dos Santos, V.A.P. (2020) SEVA 3.1: enabling interoperability of DNA assembly among the SEVA, BioBricks and type IIS restriction enzyme standards. *Microbial Biotechnology*, 13, 1793–1806. https://doi.org/10.1111/1751-7915.13609

Danchin, A. (2017) Coping with inevitable accidents in metabolism. *Microbial Biotechnology*, 10, 57–72. https://doi.org/10.1111/1751-7915.12461

Danchin, A. (2022) In vivo, in vitro and in silico: an open space for the development of microbe-based applications of synthetic biology. *Microbial Biotechnology*, 15, 42–64. https://doi.org/10.1111/1751-7915.13937

Danchin, A. (2020) Isobiology: a variational principle for exploring synthetic life. *Chembiochem*, 21, 1781–1792. https://doi.org/10.1002/cbic.202000060

Danchin, A. (2012) Scaling up synthetic biology: do not forget the chassis. *FEBS Letters*, 586, 2129–2137. https://doi.org/10.1016/j.febslet.2011.12.024

Danchin, A. (2021) Three overlooked key functional classes for building up minimal synthetic cells. *Synthetic Biology*, 6, ysab010. https://doi.org/10.1093/synbio/ysab010

Danchin, A. & Fang, G. (2016) Unknown unknowns: essential genes in quest for function. *Microbial Biotechnology*, 9, 530–540. https://doi.org/10.1111/1751-7915.12384

Danchin, A. & Sekowska, A. (2014) The logic of metabolism and its fuzzy consequences. *Environmental Microbiology*, 16, 19–28. https://doi.org/10.1111/1462-2920.12270

Dantsu, Y., Zhang, Y. & Zhang, W. (2021) Advances in therapeutic L-nucleosides and L-nucleic acids with unusual handedness. *Genes (Basel)*, 13, 46.

Duque, E., Udaondo, Z., Molina, L., de la Torre, J., Godoy, P. & Ramos, J.L. (2022) Providing octane degradation capability to *Pseudomonas putida* KT2440 through the horizontal acquisition of oct genes located on an integrative and conjugative element. *Environmental Microbiology Reports*. https://doi.org/10.1111/1758-2229.13097

Feaga, H.A. & Dworkin, J. (2021) Transcription regulates ribosome hibernation. *Molecular Microbiology*, 116, 663–673. https://doi.org/10.1111/mmi.14762

Ferrer, M., Lünsdorf, H., Chernikova, T.N., Yakimov, M., Timmis, K. N. & Golyshin, P.N. (2004) Functional consequences of single: double ring transitions in chaperonins: life in the cold. *Molecular Microbiology*, 53, 167–182. https://doi.org/10.1111/j.1365-2958.2004.04077.x

Fredens, J., Wang, K., de la Torre, D., Funke, L.F.H., Robertson, W. E., Christova, Y. et al. (2019) Total synthesis of *Escherichia coli* with a recoded genome. *Nature*, 569, 514–518. https://doi.org/10.1038/s41586-019-1192-5

Haeberli, P., Berger, I., Pallan, P.S. & Egli, M. (2005) Syntheses of 4′-thioribonucleosides and thermodynamic stability and crystal structure of RNA oligomers with incorporated 4′-thiocytosine. *Nucleic Acids Research*, 33, 3965–3975. https://doi.org/10.1093/nar/gki704

Harwood, C.R. & Kikuchi, Y. (2022) The ins and outs of *Bacillus proteases*: activities, functions and commercial significance. *FEMS Microbiology Reviews*, 46, fuab046.

He, H., Höper, R., Dodenhöft, M., Marlière, P. & Bar-Even, A. (2020) An optimized methanol assimilation pathway relying on promiscuous formaldehyde-condensing aldolases in *E. coli*. *Metabolic Engineering*, 60, 1–13. https://doi.org/10.1016/j.ymben.2020.03.002

Heine, T., van Berkel, W.J.H., Gassner, G., van Pée, K.-H. & Tischler, D. (2018) Two-component FAD-dependent monooxygenases: current knowledge and biotechnological opportunities. *Biology (Basel)*, 7, E42.

Hu, G., Song, W., Gao, C., Guo, L., Chen, X. & Liu, L. (2022) Advances in synthetic biology of CO2 fixation by heterotrophic microorganisms. *Sheng Wu Gong Cheng Xue Bao*, 38, 1339–1350. https://doi.org/10.13345/j.cjb.210627

Hutchison, C.A., Chuang, R.-Y., Noskov, V.N., Assad-Garcia, N., Deerinck, T.J., Ellisman, M.H. et al. (2016) Design and synthesis of a minimal bacterial genome. *Science*, 351, aad6253.

Ikeda, T. (2021) Bacterial biosilicification: a new insight into the global silicon cycle. *Bioscience, Biotechnology, and Biochemistry*, 85, 1324–1331. https://doi.org/10.1093/bbb/zbab069

Irla, M. & Wendisch, V.F. (2022) Efficient cell factories for the production of N-methylated amino acids and for methanol-based amino acid production. *Microbial Biotechnology*, 15, 2145–2159. https://doi.org/10.1111/1751-7915.14067

Jin, K., Xia, H., Liu, Y., Li, J., Du, G., Lv, X. et al. (2022) Compartmentalization and transporter engineering strategies for terpenoid synthesis. *Microbial Cell Factories*, 21, 92.

Johnson, H.A. (1970) Information theory in biology after 18 years. *Science*, 168, 1545–1550. https://doi.org/10.1126/science.168.3939.1545

Johnson, H.A. & Knudsen, K.D. (1965) Renal efficiency and information theory. *Nature*, 206, 930–931. https://doi.org/10.1038/206930a0

Johnson, H.A. & Pavelec, M. (1972) Thermal injury due to normal body temperature. *The American Journal of Pathology*, 66, 557–564.

Jolly, B.J., Co, N.H., Davis, A.R., Diaconescu, P.L. & Liu, C. (2022) A generalized kinetic model for compartmentalization of organometallic catalysis. *Chemical Science*, 13, 1101–1110. https://doi.org/10.1039/D1SC04983F

Kabiraj, A., Biswas, R., Halder, U. & Bandopadhyay, R. (2022) Bacterial arsenic metabolism and its role in arsenic bioremediation. *Current Microbiology*, 79, 131.

Kapoor, I. & Varshney, U. (2020) Diverse roles of nucleoside diphosphate kinase in genome stability and growth fitness. *Current Genetics*, 66, 671–682. https://doi.org/10.1007/s00294-020-01073-z

Koch-Bojalad, B.A., Carson, L. & Yu, H.-G. (2021) Forever young: the key to rejuvenation during gametogenesis. *Current Genetics*, 67, 231–235. https://doi.org/10.1007/s00294-020-01133-4

Kuhn, T.S. (1996) *The structure of scientific revolutions*, 3rd edition. Chicago, IL: University of Chicago Press.

Landauer, R. (1961) Irreversibility and heat generation in the computing process. *IBM Journal of Research and Development*, 3, 184–191.

Lane, N. (2018) Hot mitochondria? *PLoS Biology*, 16, e2005113. https://doi.org/10.1371/journal.pbio.2005113

Lateef, O.M., Akintubosun, M.O., Olaoba, O.T., Samson, S.O. & Adamczyk, M. (2022) Making sense of "nonsense" and more: challenges and opportunities in the genetic code expansion, in the world of tRNA modifications. *International Journal of Molecular Sciences*, 23, 938. https://doi.org/10.3390/ijms23020938

Li, X.-Q. & Du, D. (2014) Variation, evolution, and correlation analysis of C+G content and genome or chromosome size in different kingdoms and phyla. *PLoS One*, 9, e88339. https://doi.org/10.1371/journal.pone.0116565

Liew, F., Martin, M.E., Tappel, R.C., Heijstra, B.D., Mihalcea, C. & Köpke, M. (2016) Gas fermentation—a flexible platform for commercial scale production of low-carbon-fuels and chemicals from waste and renewable feedstocks. *Frontiers in Microbiology*, 7, 694. https://doi.org/10.3389/fmicb.2016.00694

Liu, Y. & Bai, Y. (2020) Design and engineering of metal catalysts for bio-orthogonal catalysis in living systems. *ACS Applied Bio Materials*, 3, 4717–4746. https://doi.org/10.1021/acsabm.0c00581

de Lorenzo, V. (2011) Genes that move the window of viability of life: lessons from bacteria thriving at the cold extreme: mesophiles can be turned into extremophiles by substituting essential genes. *BioEssays*, 33, 38–42. https://doi.org/10.1002/bies.201000101

de Lorenzo, V. & Danchin, A. (2008) Synthetic biology: discovering new worlds and new words. *EMBO Reports*, 9, 822–827. https://doi.org/10.1038/embor.2008.159

de Lorenzo, V., Sekowska, A. & Danchin, A. (2015) Chemical reactivity drives spatiotemporal organisation of bacterial metabolism. *FEMS Microbiology Reviews*, 39, 96–119. https://doi.org/10.1111/1574-6976.12089

Lynch, J.P., Goers, L. & Lesser, C.F. (2022) Emerging strategies for engineering *Escherichia coli* Nissle 1917-based therapeutics. *Trends in Pharmacological Sciences*, S0165-6147(22), 00026–00028.

Makarova, K.S., Galperin, M.Y. & Koonin, E.V. (2015) Comparative genomic analysis of evolutionarily conserved but functionally uncharacterized membrane proteins in archaea: prediction of novel components of secretion, membrane remodeling and glycosylation systems. *Biochimie*, 118, 302–312.

Mana, T., Bhattacharya, B., Lahiri, H. & Mukhopadhyay, R. (2022) XNAs: a troubleshooter for nucleic acid sensing. *ACS Omega*, 7, 15296–15307.

Mana, T., Kundu, J., Lahiri, H., Bera, S., Kolay, J., Sinha, S. et al. (2022) Molecularly resolved, label-free nucleic acid sensing at solid-liquid interface using non-ionic DNA analogues. *RSC Advances*, 12, 9263–9274.

Marlière, P., Patrouix, J., Döring, V., Herdewijn, P., Tricot, S., Cruveiller, S. et al. (2011) Chemical evolution of a bacterium's genome. *Angewandte Chemie (International Ed. in English)*, 50, 7109–7114. https://doi.org/10.1002/anie.201100535

Maxwell, J.C. (1871) *Theory of heat*. London: Longmans, Reed and Co.

Moon, T.S. (2022) Model-based design of synthetic antisense RNA for predictable gene repression. *Methods in Molecular Biology*, 2518, 111–124.

Müller, S. & Nebe-von-Caron, G. (2010) Functional single-cell analyses: flow cytometry and cell sorting of microbial populations and communities. *FEMS Microbiology Reviews*, 34, 554–587.

Nance, K.D. & Meier, J.L. (2021) Modifications in an emergency: the role of N1-Methylpseudouridine in COVID-19 vaccines. *ACS Central Science*, 7, 748–756. https://doi.org/10.1021/acscentsci.1c00197

Nguyen, J.T., Riebschleger, K.K., Brown, K.V., Gorgijevska, N.M. & Nybo, S.E. (2022) A BioBricks toolbox for metabolic engineering of the tetracenomycin pathway. *Biotechnology Journal*, 17, e2100371. https://doi.org/10.1002/biot.202100371

Nikel, P.I., Benedetti, I., Wirth, N.T., de Lorenzo, V. & Calles, B. (2022) Standardization of regulatory nodes for engineering heterologous gene expression: a feasibility study. *Microbial Biotechnology*, 5(8), 2250–2265. https://doi.org/10.1111/1751-7915.14063

Novoa, E.M., Jungreis, I., Jaillon, O. & Kellis, M. (2019) Elucidation of codon usage signatures across the domains of life. *Molecular Biology and Evolution*, 36, 2328–2339. https://doi.org/10.1093/molbev/msz124

Okano, K., Honda, K., Taniguchi, H. & Kondo, A. (2018) De novo design of biosynthetic pathways for bacterial production of bulk chemicals and biofuels. *FEMS Microbiology Letters*, 365, 365.

Ou, Z., Ouzounis, C., Wang, D., Sun, W., Li, J., Chen, W. et al. (2020) A path towards SARS-CoV-2 attenuation: metabolic pressure on CTP synthesis rules the virus evolution. *Genome Biology and Evolution*, 12, 2467–2485. https://doi.org/10.1093/gbe/evaa229

Pandit, A., Johny, L., Srivastava, S., Adholeya, A., Cahill, D., Brau, L. et al. (2022) Recreating in vitro tripartite mycorrhizal associations through functional bacterial biofilms. *Applied Microbiology and Biotechnology*, 106, 4237–4250. https://doi.org/10.1007/s00253-022-11996-x

Pang, H., Ihara, M., Kuchino, Y., Nishimura, S., Gupta, R., Woese, C.R. et al. (1982) Structure of a modified nucleoside in archaebacterial tRNA which replaces ribosylthymine. 1-Methylpseudouridine. *The Journal of Biological Chemistry*, 257, 3589–3592. https://doi.org/10.1016/S0021-9258(18)34820-8

Pratt, E.A. & Ho, C. (1975) Incorporation of fluorotryptophans into proteins of *Escherichia coli*. *Biochemistry*, 14, 3035–3040.

Quinlivan, E.P., Roje, S., Basset, G., Shachar-Hill, Y., Gregory, J.F. & Hanson, A.D. (2003) The folate precursor p-aminobenzoate is reversibly converted to its glucose ester in the plant cytosol. *The Journal of Biological Chemistry*, 278, 20731–20737. https://doi.org/10.1074/jbc.M302894200

Rafieenia, R., Atkinson, E. & Ledesma-Amaro, R. (2022) Division of labor for substrate utilization in natural and synthetic microbial communities. *Current Opinion in Biotechnology*, 75, 102706. https://doi.org/10.1016/j.copbio.2022.102706

Reaves, M.L., Sinha, S., Rabinowitz, J.D., Kruglyak, L. & Redfield, R. J. (2012) Absence of detectable arsenate in DNA from arsenate-grown GFAJ-1 cells. *Science*, 337, 470–473. https://doi.org/10.1126/science.1219861

Rees-Garbutt, J., Rightmyer, J., Chalkley, O., Marucci, L. & Grierson, C. (2021) Testing theoretical minimal genomes using whole-cell models. *ACS Synthetic Biology*, 10, 1598–1604. https://doi.org/10.1021/acssynbio.0c00515

Reichenberger, E.R., Rosen, G., Hershberg, U. & Hershberg, R. (2015) Prokaryotic nucleotide composition is shaped by both phylogeny and the environment. *Genome Biology and Evolution*, 7, 1380–1389. https://doi.org/10.1093/gbe/evv063

Sandberg, T.E., Salazar, M.J., Weng, L.L., Palsson, B.O. & Feist, A. M. (2019) The emergence of adaptive laboratory evolution as an efficient tool for biological discovery and industrial biotechnology. *Metabolic Engineering*, 56, 1–16. https://doi.org/10.1016/j.ymben.2019.08.004

Shakya, B., Joyner, O.G. & Hartman, M.C.T. (2022) Hyperaccurate ribosomes for improved genetic code reprogramming. *ACS Synthetic Biology*, 11, 2193–2201. https://doi.org/10.1021/acssynbio.2c00150

Singh, S. & Ramakrishna, W. (2021) Application of CRISPR-Cas9 in plant-plant growth-promoting rhizobacteria interactions for next green revolution. *3 Biotech*, 11, 492.

Strocchi, M., Ferrer, M., Timmis, K.N. & Golyshin, P.N. (2006) Low temperature-induced systems failure in *Escherichia coli*: insights from rescue by cold-adapted chaperones. *Proteomics*, 6, 193–206. https://doi.org/10.1002/pmic.200500031

Sun, J., Jeffryes, J.G., Henry, C.S., Bruner, S.D. & Hanson, A.D. (2017) Metabolite damage and repair in metabolic engineering design. *Metabolic Engineering*, 44, 150–159. https://doi.org/10.1016/j.ymben.2017.10.006

Sveshnikova, A., MohammadiPeyhani, H. & Hatzimanikatis, V. (2022) Computational tools and resources for designing new pathways to small molecules. *Current Opinion in Biotechnology*, 76, 102722. https://doi.org/10.1016/j.copbio.2022.102722

Talenton, V., Baby, V., Gourgues, G., Mouden, C., Claverol, S., Vashee, S. et al. (2022) Genome engineering of the fast-growing *Mycoplasma feriruminatoris* toward a live vaccine chassis. *ACS Synthetic Biology*, 11, 1919–1930. https://doi.org/10.1021/acssynbio.2c00062

Tang, H., Zhang, P. & Luo, X. (2022) Recent technologies for genetic code expansion and their implications on synthetic biology applications. *Journal of Molecular Biology*, 434, 167382. https://doi.org/10.1016/j.jmb.2021.167382

Tarashima, N.S., Matsuo, A. & Minakawa, N. (2020) Gene expression of 4′-thioguanine DNA via 4′-thiocytosine RNA. *Journal of the American Chemical Society*, 142, 17255–17259. https://doi.org/10.1021/jacs.0c07145

Thuan, N.H., Tatipamula, V.B., Canh, N.X. & Van Giang, N. (2022) Recent advances in microbial co-culture for production of value-added compounds. *3 Biotech*, 12, 115.

Timmis, K., de Vos, W.M., Ramos, J.L., Vlaeminck, S.E., Prieto, A., Danchin, A. et al. (2017) The contribution of microbial biotechnology to sustainable development goals. *Microbial Biotechnology*, 10, 984–987. https://doi.org/10.1111/1751-7915.12818

de la Torre, D. & Chin, J.W. (2021) Reprogramming the genetic code. *Nature Reviews. Genetics*, 22, 169–184. https://doi.org/10.1038/s41576-020-00307-7

Tosi, M., Drummelsmith, J., Obregón, D., Chahal, I., Van Eerd, L.L. & Dunfield, K.E. (2022) Cover crop-driven shifts in soil microbial communities could modulate early tomato biomass via plant-soil feedbacks. *Scientific Reports*, 12, 9140.

Treece, T.R., Gonzales, J.N., Pressley, J.R. & Atsumi, S. (2022) Synthetic biology approaches for improving chemical production in cyanobacteria. *Frontiers in Bioengineering and Biotechnology*, 10, 869195. https://doi.org/10.3389/fbioe.2022.869195

Vicq d'Azyr, F. (1792) Discours sur l'anatomie simple et comparée, avec l'exposition du plan que j'ai suivi dans la rédaction de cet ouvrage. *Encyclopédie Méthodique Quadrupèdes*, XXV.

Vidal, C., Tomás-Gamasa, M., Destito, P., López, F. & Mascareñas, J.L. (2018) Concurrent and orthogonal gold(I) and ruthenium(II) catalysis inside living cells. *Nature Communications*, 9, 1913.

Vigneau, J.-N., Fahimi, P., Ebert, M., Cheng, Y., Tannahill, C., Muir, P. et al. (2022) ATP synthase: a moonlighting enzyme with unprecedented functions. *Chemical Communications (Cambridge, England)*, 58, 2650–2653. https://doi.org/10.1039/D1CC06793A

Wang, L., Jiang, S., Chen, C., He, W., Wu, X., Wang, F. et al. (2018) Synthetic genomics: from DNA synthesis to genome design. *Angewandte Chemie, International Edition*, 57, 1748–1756. https://doi.org/10.1002/anie.201708741

Westheimer, F.H. (1987) Why nature chose phosphates. *Science*, 235, 1173–1178. https://doi.org/10.1126/science.2434996

Woo, S., Lim, H.G., Han, Y.H., Park, S., Noh, M.H., Baek, D. et al. (2022) A vibrio-based microbial platform for accelerated lignocellulosic sugar conversion. *Biotechnology for Biofuels and Bioproducts*, 15, 58.

Wurm, J.P., Meyer, B., Bahr, U., Held, M., Frolow, O., Kötter, P. et al. (2010) The ribosome assembly factor Nep1 responsible for Bowen-Conradi syndrome is a pseudouridine-N1-specific

methyltransferase. *Nucleic Acids Research*, 38, 2387–2398. https://doi.org/10.1093/nar/gkp1189

Yu, A.C.-S., Yim, A.K.-Y., Mat, W.-K., Tong, A.H.-Y., Lok, S., Xue, H. et al. (2014) Mutations enabling displacement of tryptophan by 4-fluorotryptophan as a canonical amino acid of the genetic code. *Genome Biology and Evolution*, 6, 629–641. https://doi.org/10.1093/gbe/evu044

Zhang, J., Jin, B., Fu, J., Wang, Z. & Chen, T. (2022) Adaptive laboratory evolution of *Halomonas bluephagenesis* enhances acetate otlerance and utilization to produce poly(3-hydroxybutyrate). *Molecules*, 27, 3022. https://doi.org/10.3390/molecules27093022

Zhang, W., Mitchell, L.A., Bader, J.S. & Boeke, J.D. (2020) Synthetic genomes. *Annual Review of Biochemistry*, 89, 77–101. https://doi.org/10.1146/annurev-biochem-013118-110704

Zhao, X., Chen, X., Xue, Y. & Wang, X. (2022) Development of an efficient iterative genome editing method in *Bacillus subtilis* using the CRISPR-AsCpf1 system. *Journal of Basic Microbiology*, 62, 824–832. https://doi.org/10.1002/jobm.202200134

How to cite this article: Danchin, A. & Huang, J. D. (2022) SynBio 2.0, a new era for synthetic life: Neglected essential functions for resilience. *Environmental Microbiology*, 1–15. Available from: https://doi.org/10.1111/1462-2920.16140