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Myopic selection of novel information drives evolution

Antoine Danchin

Synthetic biology aims at reconstructing life. Besides understanding what life is, its ultimate goal is to design cell factories meant to satisfy pressing needs. The success of genome transplantation demonstrates that the cell is split into a machine and a program. The program codes for processes that reproduce the machine and replicate the program. Reproduction is tightly linked to evolution, because the program codes for information trapping. Degradation processes make room to cope with ageing and inaccurate syntheses. Yet they use energy to prevent degradation of functional entities, thus permitting accumulation of innovation. Synthetic biology faces a dilemma: it will either implement the corresponding genes, and cells will evolve in an unpredictable manner, or omit them, and cells will age and have to be reconstructed periodically.

Address
CNRS URA2171, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

Corresponding author: Danchin, Antoine (antoine.danchin@normalesup.org)

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Introduction

The discovery of restriction enzymes created novel engineering practices. The construction of plasmid pSC101 and derivatives was granted highly profitable patents [1], which played an exemplary role in matters dealing with genetically modified organisms. A number of applications of *in vitro* recombination techniques developed, expressing proteins both in homologous or heterologous hosts. It soon became obvious that proper control of expression required that the foreign genes be expressed under controlled conditions. This substantiated efforts to understand the intricacies of gene regulation. Bacteriophages were used as models: phage lambda's development was analysed in terms of electronic logic gates (in particular the lytic/lysogenic development toggle) [2,3]. Other phages were subsequently submitted to similar analyses, in particular much simpler phages which, like bacteriophage T7, respond to their own (orthogonal) RNA polymerase for gene expression. Endy developed parallel studies of the behaviour of T7 *in vivo* and *in silico* models of its behaviour, based on our knowledge of the phage genome organisation as well as its products and their known or predicted functions [4]. This led him, together with his colleagues at the MIT, to create a lively synthetic biology (SB) based on an engineering view of what life is, illustrated by the famous iGEM competition [5•].

The engineering view and its consequences: human intelligent design

The basic — somewhat naïve [6•] — idea behind the iGEM view of SB is that it will be possible to abstract, decouple, standardise, design and make evolve reproducing machines [7]. In this context, combination of 'biobricks' within a cell's 'chassis' should result in an artificial cell factory both endowed of life and of purposes chosen by the creator of the cell factory [8]. While genetic engineering had already proven that parts of a genome program could be expressed in a particular host, the ultimate goal of the effort implies that it should be possible to create a complete synthetic genome program and make it work. This, in turn, supposes that three proofs of concept are established. Firstly, genome transplantation should be demonstrated: this has been performed in the case of *Mycoplasma* species. However, only one laboratory in the world has been successful in this endeavour [9••], and we are probably still far from a success in other organisms (mycoplasmas are highly degenerate cells, and require a very complex environment to live). Secondly, artificial genomes should be successfully assembled. This has been achieved in the same laboratory, using a combination of *in vitro* syntheses and *in vivo* assembly. Purists will probably ask that the final assembly step which uses *S. cerevisiae* as a recipient be replaced by a purely biochemical step [10]. Thirdly, the synthetic construct must be transplanted and expressed in a proper recipient. This has not been performed yet. We note here that there may be unforeseen difficulties at this point. Going from an abstract view of the cell where the program is separated from the machine requires a material implementation of the program, and, even in computers, the operating system has to be placed in the machine with a material support which is properly recognised by the machine to make it run [11].

As a validation prerequisite before constructing synthetic bacterial genomes, it was of interest to reconstruct simpler pieces of genetic programs such as viruses according to the principles of engineering. Successful refactoring of bacteriophage T7 is now a classic in SB

[12]. A noteworthy observation in this work, however, was that the artificial T7 construct, while producing clear lysis plaques, produced plaques significantly smaller than those of the wild-type parent (see below).

The minimal genome, essential versus non-essential genes

Back in 1988, sequencing the *Bacillus subtilis* genome was commissioned by the European Union as a means to explore how genes are organised in genomes, with a set of some 250 genes proposed to be essential for core life. Deciphered in 1995, the short size of the *Mycoplasma genitalium* genome triggered a reflection on the 'minimal genome', and a similar figure of approximately 250 genes was computed using comparative genomics. Further experiments identified essential genes after gene inactivation, especially in the models *Escherichia coli* [13] and *B. subtilis* [14]. The essential genes number remained of that same order of magnitude, with, as expected, some variations according to the growth context of the cells. Some redundancy had the consequence to hide the essentiality of a few genes [15]. Most genes thus identified code for functions essential to life, as shown by transposon mutagenesis experiments in *M. genitalium* [16]. In a few cases (restriction methylases [17] and toxin/antitoxin systems [18]) essentiality is not directly associated with the processes we wish to introduce in the construction of a synthetic cell, but to processes that have often been labelled 'selfish'. All these genes, together with the cognate annotations are collected in DEG, a database of essential genes [19]. This can make the basis of a conceptual reflection about the functions needed to make a synthetic cell.

Sequencing the genome of several strains of a given species permits identification of the minimal gene set defining the species. Tettelin and co-workers named *pan-genome* the set of genes present in strains of *Streptococcus agalactiae*, remarking that it was much larger than the genome of any individual strain [20]. This view was later extended to a set of 573 bacterial genomes, suggesting that the pan-genome of the domain Bacteria remains of unlimited size [21•], while approximately 250 genes belonged to an extended bacterial *core-genome*. In the case of *E. coli*, approximately 2000 genes form its core-genome, showing that the definition of the species extends far beyond essential genes. The corresponding pan-genome is still of unlimited size, comprising already some 20 000 genes [22••]. Thus, approximately 2500 non-core-genome genes — forming the *cenome* (the word biocenose was created by Karl Möbius in 1877 to represent the ecological niche of a species [23]) — are selected from a pool of 18 000 genes (the size of which still increases whenever a new *E. coli* strain genome is sequenced) to make a given strain, depending on its niche preferences.

While some functions must be ubiquitous in autonomous bacteria, the recruitment of various genes/structures for any function implies that functional ubiquity cannot be matched by gene orthology. The number of conserved orthologues keeps going down as new genome sequences are available. This is impractical, but because living organisms evolve by descent, an organism tends to transmit what is functional to its progeny. Gene persistence can be used as a surrogate approach to investigate functional ubiquity [24] and to explore the role and organisation of the corresponding genes. In parallel, except for bacteria living in restricted niches, genomes are invaded by foreign genes in a process of horizontal gene transfer [25], systematically shuffling their gene distribution. However — remember operons — genes are not randomly distributed. In metabolic maps, essential genes reveal an interesting overall organisation, which must be taken into account when trying to understand how the core metabolism is evolving [26].

Persistent genes are not randomly distributed in the genome. Whether essential or non-essential, persistent genes are mainly transcribed with the same orientation as that of the replication fork movement [24]. Despite their ancient origin, they tend to remain clustered together. However, their clustering is not uniform, it is made of three layers where genes which can be ascribed to a function form a consistent pattern: genes coding for ancient core metabolic functions [26], retaining very poor clustering; genes clustered with tRNA synthetases and finally genes involved in the translation and transcription machineries. Remarkably, this organisation is reminiscent of a scenario for a mineral origin of life, suggesting *paleome* as a name for the corresponding gene set [23]. Should all persistent genes be placed in a synthetic genome? What would be the effect of the choice of particular genes on the spontaneous stability and evolution of the cell's progeny?

Myopic selection and directed evolution

Extremely conserved genes are likely to be functionally very important, explaining the universal but wrong perception of slower evolution of more important genes [27••]. This makes interesting to explore further the structure of the paleome, as it codes for the functions needed both to construct life and to make it perennial. On the one hand, we have essential genes, which make half of the paleome of all presently available autonomous bacterial species. On the other hand, we have a set of non-essential persistent genes of similar size. How can we put together these observations, and use them for the construction of a synthetic bacterium?

Living organisms have been seen as factories. However, they have a remarkable feature, not shared with man-made artefacts: they *replicate* their construction program, while as an ageing machine, they *reproduce* into a young progeny. This implies that the machine evolves in a positive way [28]. These facts need to be taken into account in any attempt to engineer biological systems,

either from existing ones, or *de novo*. Reproduction is not associated to perennisation of the parent organism, but to its ability to make a young, that is a progeny where novel information has been recruited from an existing pool or even created entirely *de novo*. During this process, growth, mostly dependent on the essential genes in the paleome, is combined with accumulation of information, mostly dependent on the non-essential part of the paleome. Accumulation of information needs making room, that is, erasing some existing entities [29], while avoiding destruction of novel information. Briefly, cells behave as information traps by using degradation processes that use energy to *prevent* degradation of functional entities [30••]. This process cannot have any global picture of the organism. Myopic, it measures *locally* what is functional. There is no in-built intelligent design, and because this implies recruitment of novel information, *whatever its source*, this explains the 'tinkering' behaviour of evolution. As it is inventive by construction, this essential property of life goes very much against the most straightforward goals of SB.

An illustration of this evolution pattern is the robustness of natural bacteriophage T7 [31]. While a synthetic construct with all the properties of T7 development was designed rationally [12], adaptation to specific environmental conditions chosen to select quantitative phenotypic outcomes resulted in unexpected outcomes, with only a fraction of the compensatory nucleotide changes observed during the adaptation being rationalised based on our understanding of T7 biology [32].

A major question is the identification not only of the genes involved in accumulating information (many must belong to the non-essential genes of the paleome), but also of the features recognised to measure functionality. Specific features of amino acids related to ageing (such as aspartate or asparagine cyclisation, or proline puckering) could be cases in point [30••]. Another intriguing possibility is that protein sequences, far from being random, permit them to display a significant level of functional promiscuity [33••], so that they may be involved in many metabolic processes at a low level, preventing their degradation and keeping them for eventual use later on. If substantiated, delineating the borders of promiscuity could play a considerable role in the choice of the better genes permitting to evolve synthetic constructs.

Evolution of synthetic constructs

In many instances the engineering goal is straightforward: specific chemicals must be produced, or their production must be enhanced. Identification of metabolic pathways permits investigators to identify bottlenecks, and focus on directed evolution of specific enzymes. Metabolic reconstruction using extant organisms, which, to be efficient, needs to be performed on a multi-dimensional scale [34], is a basis for human intelligent design [35••].

Directed evolution of individual genes

Directed evolution is used to change enzyme physicochemical properties (solubility, temperature of action) or specificity. This can be performed *in silico*, followed by construction of synthetic genes, or *in vivo* using selective procedures (see for a recent example, the *ISce*I new target DNA sequence that differs from the wild-type cleavage sequence by 4 bp [36]). Many studies associated combinatorial gene exchanges with novel techniques for evolving enzymes in the laboratory [37]. Directed evolution can be obtained by neutral drifts that maintain the protein's original function [38]. An interesting track has not yet been fully explored: in a kind of 'Jurassic Park' of genes and proteins, it is possible to reconstruct ancestral proteins, and make inferences about their activity [39••]. This type of paleogenetics could be used as start point for further evolution, in appropriate laboratory set up for continuous growth.

From blind evolution to orthogonality

In summary, selection accumulates information by direct action at the protein or RNA level. However, macromolecules do not exist in isolation, when modified they co-evolve with a variety of traits. A bacterial chassis for genome engineering is therefore important [40]. *Acinetobacter* species, highly versatile and prone to transformation and recombination, have been suggested as a fairly universal recipient for further evolution [41]. When using living cells rather than individual proteins, the phenotype is the feature directly submitted to selection. The environment must be organised along lines meant to favour specific traits. Analysis of spontaneous evolution of populations has been used to help us understand some of the tracks followed by natural populations. The difficulty of this type of approach is that unobtrusive, but a posteriori trivial outcomes may be observed. Indeed, in serial transfers from test tubes of *E. coli* B over many generations the cells tend to adapt in shortening as much as possible their lag phase before regrowth [42•]. In chemostats, bacteria escape the astute human setups meant to drive them in the designed direction by making biofilms and often creeping backwards towards the vessels keeping sources of nutrients. We can certainly expect that cells will evolve even by modifying their shape as bacteria evolved to become *square* in an environment where right angles are ubiquitous [43]. A way out has been proposed, with a cycle of automatic shifts from one growth chamber to a new one and complete chemical cleaning of the chambers [44]. In any case, local functioning is not directly related to phenotypic cost, especially in populations, hence the positive role of horizontal gene transfer despite its apparent load [45].

Finally, a major challenge, when one wishes to evolve new forms of life, is the interference between the newly created processes and those that are already active in the host. The way out is to allow the use of basic building blocks (made by the parent or host cell machine) while

expressing a program in a way which is otherwise invisible to the host. This situation has been named *orthogonality* to express the fact that it develops in a dimension which differs from that of the host. A way towards orthogonality is to divert some specific (usually unused) codons towards a novel function. This already exists in natural systems with the 21st and 22nd amino acids selenocysteine (which is coded by codon UGA, when in a special context) or pyrrolysine [46] and can be used for artificial expansion of the genetic code [47••].

Conclusion

Genetic evolution is constrained by gene function, the structure of genetic networks, and populations [48]. We have suggested how the structure of the paleome indicates that evolution may be predictable to some extent, but not in the direction favoured by SB. Yet, SB has already been quite successful in permitting the design and evolution of various activities in the laboratory. However, as evolution is a basic feature of life, extending these approaches to a stable engineering perspective has to manage the trade-off between reproducibility and inventivity. From the pure engineering point of view inventivity must be avoided. Inventivity is the result of the action of degradative functions that have features of Maxwell's demon: they choose what they will destroy, using energy to protect any functioning entity against degradation. Preventing innovation in synthetic cells will be possible if one does not include genes coding for energy-dependent degradative processes in the synthetic build up. The drawback will be that the cells will age, and have only a limited number of possible divisions [49••]. As mechanical factories, the cell factories will have to be rebuilt periodically. This is a considerable inconvenience for all processes requiring large scaling up, which we already know as a major hurdle for genetic engineering [50]. Evolution towards large-scale productions, such as production of biofuels, would require harnessing inventivity, coded by the genes we have identified as the drivers of evolution [30••], to the production goal. Whether this will be possible remains an open question.

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