Opinion piece

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1. Introduction

The Earth appeared out of stardust as a tangible mass approximately 4.5 \times 10^9 years (4.5 Gyr) ago [1]. Life arose relatively rapidly, with recent evidence suggesting that the first biogenic signatures in the geological record may be as old as 4.1 Gyr [2]. Since then, species have evolved to colonize almost every conceivable niche on our planet. Major evolutionary innovations (figure 1) have included the emergence of eukaryotes, of multicellularity (at least 25 times; [3]), and of complex animal body plans during the Cambrian explosion. These organism-level innovations have been enabled by the evolution of molecular processes such as the ability to generate ATP by glycolysis and oxidative phosphorylation, to fix atmospheric CO_2 via oxygenic photosynthesis, and to regulate gene expression with exquisite spatial and temporal control. In many cases, enzyme-catalysed metabolic pathways are likely to have arisen from non-enzymatic reaction sequences [4], as chemistry was harnessed by biology. In the light of such wondrous biochemistry, it is easy to assume that all the major transitions in evolution [5] have already occurred, and that many key metabolic processes are sufficiently fit for purpose that they are therefore essentially immutable.

In this article, we seek to update this view. It will be another 1.75–3.25 Gyr before the expansion of the sun causes the surface temperature of the Earth to soar, the atmosphere to disintegrate and our planet to become sterile [6]. We are only 55–70% of the way through the evolutionary trajectory of life on the Earth (figure 1). With this in mind, our central thesis is that new biochemical innovations, on the scale of photosynthesis or glycolysis, will continue to emerge in the biosphere and to become as central to biology as these processes are currently. Further, we suggest that the field of synthetic biology should be viewed as ‘biology not yet in the databases’. The solutions of synthetic biologists to biochemical challenges may have existed in the past, may exist (but remain undiscovered) in the biosphere today—or of most relevance for our discussion, they may evolve in the future (by natural pathways, rather than via escape of
accepts O₂ as a substrate that competes with CO₂. Circumvent-
Rubisco displays poor kinetic parameters and wastefully
An alternative solution, used by cyanobacteria, is to concentrate
with limited success so far. This is perhaps unsurprising,
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reliance on chlorophylls
length range used by land plants is, in part, due to their
implemented by synthetic biologists. The restricted wave-
have been proposed [8,9], although these are yet to be
strategies that use entirely novel combinations of metabolic
predictions to be more efficient than the predominant natural pathway (the Calvin cycle), have also
been proposed [12]. These design efforts emphasize the numer-
 pathways that use entirely novel combinations of metabolic
enzymes, and that are predicted to be more efficient than the
predominant natural pathway (the Calvin cycle), have also
been proposed [12]. These design efforts emphasize the numerous
routes by which selection may act to effect wholesale changes in the process of CO₂ fixation, particularly if the
phylogenetic distribution of efficient, Thaumarchaeota-type

2. Enhancements to carbon fixation and energy
generation

Energy generation and conservation are central components of
an organism’s evolutionary fitness. The following examples demonstrate ways in which synthetic biology is extending
tatural biochemical processes beyond what evolution has
produced to date.

Carbon enters the biosphere via photosynthesis. Even after
billions of years of evolution (figure 1), aspects of this process
remain woefully inefficient. For example, land plants only
absorb light of wavelengths 400–700 nm, corresponding to
approximately 50% of total incident solar energy. Strategies for engineering plants that use more of the solar spectrum
have been proposed [8,9], although these are yet to be implemented by synthetic biologists. The restricted wave-
length range used by land plants is, in part, due to their
reliance on chlorophylls a and b for light absorption. Diverse
cyanobacteria harvest light over the range 350–1075 nm, due
to photosynthetic reaction centres that contain various bacterio-
chlorophylls [9]. Land plants will effectively double their solar photon capture if they evolve to use hybrid photosynthetic systems that incorporate bacterial reaction centres.

The enzyme ribulose-1,5-bisphosphate carboxylase/oxyge-
genase (Rubisco) catalyses the incorporation of CO₂ into biomass. Rubisco displays poor kinetic parameters and wastefully
accepts O₂ as a substrate that competes with CO₂. Circumvent-
ing these undesirable properties in the most affected plants
could improve their photosynthetic efficiency [9], although
attempts to achieve this by engineering the enzyme have met
with limited success so far. This is perhaps unsurprising,
given the structural and chemical similarities of CO₂ and O₂.
An alternative solution, used by cyanobacteria, is to concentrate
CO₂ in Rubisco-containing organelles called carboxysomes. A
functional cyanobacterial Rubisco has recently been expressed
within carboxysome-like structures, in the chloroplasts of engi-
eered tobacco plants. While the cyanobacterial enzyme was
expressed at a lower level than the native tobacco Rubisco, it showed higher rates of CO₂ fixation per unit of enzyme [10].
Rather than Rubisco, archaea in the phylum Thaumarchaeota
use a bifunctional acetyl-CoA/propionyl-CoA carboxylase to
fix CO₂ under aerobic conditions, but without a competing oxy-
genase reaction [11]. Plausible, artificial carbon fixation
pathways that use entirely novel combinations of metabolic
enzymes, and that are predicted to be more efficient than the
predominant natural pathway (the Calvin cycle), have also
been proposed [12]. These design efforts emphasize the numerous
routes by which selection may act to effect wholesale changes in the process of CO₂ fixation, particularly if the
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Figure 1. The complete evolutionary trajectory of life on the Earth, with the timeline (in Gyr) drawn to scale. Selected innovations from the past are shown and the yellow shading highlights the time remaining for future innovations to occur.

A number of glycolytic pathways have evolved for the oxida-
tion and splitting of 6-carbon sugars to yield two molecules

| Time Period | Event |
|-------------|-------|
| 4.1 Gyr ago | First life |
| 3.8 Gyr ago | Anoxic photosynthesis |
| 1.85 Gyr ago | Eukaryotic cells |
| 1.75-3.25 Gyr ago | Modern humans |
| Present day | ? |


| Time Period | Event |
|-------------|-------|
| 0.8 Gyr ago | Multicellular organisms |
| 0.0001 Gyr ago | Modern humans |

| Time Period | Event |
|-------------|-------|
| 3.95 Gyr ago | Oxygenic photosynthesis |
| 1.85 Gyr ago | Photosynthesis |
| 1.75-3.25 Gyr ago | Cambrian explosion |
| Present day | ? |


| Time Period | Event |
|-------------|-------|
| 2.95 Gyr ago | Oxygenic photosynthesis |

| Time Period | Event |
|-------------|-------|
| 4.5 Gyr ago | Earth forms |

| Time Period | Event |
|-------------|-------|
| 2.95 Gyr ago | Oxygenic photosynthesis |
| 0.8 Gyr ago | Multicellular organisms |
| 0.0001 Gyr ago | Modern humans |
of 3-carbon pyruvate. For downstream processes such as bio-
synthesis and ATP generation via oxidative phosphorylation,
these pyruvate molecules are converted to acetyl-CoA, with
concomitant loss of CO₂. Thus, only four carbons from the origi-
nal hexose are used by the cell. This fundamental inefficiency
has been circumvented through the design and implementa-
tion of a pathway dubbed non-oxidative glycolysis (NOG)
in *Escherichia coli*, which allows complete conversion of a
hexose to three molecules of acetyl-CoA [16]. In this cyclic path-
way (figure 2), one input molecule of fructose 6-phosphate (F6P)
is combined with two additional F6P molecules, and con-
verted to three molecules of acetyl phosphate (AcP) plus three
molecules of erythrose 4-phosphate (E4P) by phosphoketolase
enzymes. The three E4Ps are rearranged to regenerate the two
F6Ps that were initially invested, while the acetyl phosphate
can enter 2-carbon metabolism. The NOG pathway facilitates
complete carbon conservation but does not generate any redu-
cing equivalents. Essential intermediates from oxidative
glycolysis, such as phosphoenolpyruvate (which drives the
phosphotransferase sugar uptake system), are also absent
from the NOG pathway. Thus, it would not be trivial to replace
glycolysis entirely with NOG. On the other hand, an organism
that evolved to express and regulate both pathways would
have a new ability to economize its resources by managing
carbon flux and minimizing wasteful CO₂ production.

3. The future of gene regulation

Much of synthetic biology is concerned with designing com-
plex genetic circuits that allow logical programming to be
executed in living cells. From an evolutionary perspective,
advances in gene regulation have been critical for innovations
such as multicellularity, the emergence of complex develop-
mental pathways, and the rise of morphological diversity in
animals [17]. The evolution of new regulatory logic is certain
to underpin dramatic phenotypic changes in future lineages.

Natural ligand-responsive transcription factors can be
readily incorporated into new regulatory contexts. For example,
an engineered pathway for biodiesel production was improved
dramatically by introducing a dynamic sensor-regulator
system, which continuously monitored the level of a key inter-
mediate (fatty acyl-CoA) and controlled flux through the
pathway accordingly [18]. Using the LuxR transcriptional acti-
vator, which responds to cell-permeable acyl-homoserine
lactones, it has also been possible to engineer a population of
*E. coli* cells that display synchronized oscillations in their pat-
terns of gene expression [19]. These examples emphasize the
relative ease with which new temporal, spatial and intercellular
behaviours can emerge.

We are currently witnessing an explosion in innovative
biotechnological applications for CRISPR/Cas technology.
Bacteria use CRISPR/Cas to defend against viral infection;
however, it is plausible that they will evolve to expand its
use for processes such as genome maintenance and modifi-
cation. For example, synthetic biologists have shown that
the introduction of multiple guide RNAs (gRNAs) effectively
accelerates genome evolution via simultaneous modification
of multiple loci [20].

In CRISPR interference (CRISPIR), a catalytically inactive
variant of the Cas9 enzyme is used to manipulate gene
expression by mediating repression or activation [21]. It has
rapidly emerged as a versatile system for constructing robust
and orthogonal circuits. A recent example is a set of logic
gates that interfaces with natural regulatory networks to trans-
duce synthetic inputs (e.g. anhydrotetracycline) into global
cellular outputs (e.g. changes to bacteriophage resistance pro-
files) [22]. In human cell lines, there is substantial interest in
engineering cell fate. An intriguing prospect is to reprogramme
differentiated cells into induced pluripotent stem cells, by

Figure 2. A comparison of natural (oxidative) glycolysis and synthetic (non-oxidative) glycolysis. Abbreviations are defined in the text. Redrawn from [16].
controlling expression of the relevant transcription factors. The first step towards this goal—activation of the reprogramming factor OCT4—has recently been achieved [23].

Naturally occurring CRISPR/Cas-based systems that are capable of manipulating and reprogramming cell fates in higher eukaryotes may sound fanciful; however, biotechnology may also be providing insight into what will evolve over the next few billion years, simply by piecing together pre-existing cellular machinery. Indeed, multicellular organisms have evolved from unicellular ancestors in numerous lineages, and they have also reverted to unicellular states [3]. In the time remaining for life on the Earth, it is inevitable that adaptation via variation in genetic circuitry, perhaps mediated by CRISPR/Cas-like machinery, will lead to the emergence of new multicellular lineages, as well as the reversion to unicellularity of others.

4. Concluding remarks

Evolutionary biology seeks to explain the diversity of life on the Earth today, by inferring past events. Here, we have instead sought to focus attention on the future evolution of biochemistry. We have highlighted metabolic inefficiencies, the solutions to which are likely to confer adaptive advantages. We have also conjectured that the nascent field of synthetic biology may be offering glimpses of future evolutionary events. One highlight is the effective utilization of protein-based complexes such as carboxysomes and cellulosomes. Another is the potential for complex regulatory and developmental pathways to be implemented by new mechanisms, such as CRISPRi. We have suggested it is highly probable that these molecular innovations will arise naturally. Ultimately, however, only time will tell whether there are viable evolutionary trajectories for realizing them in ways that increase organismal fitness.

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Competing interests. We have no competing interests.

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References

1. Nisbet EG, Sleep NH. 2001 The habitat and nature of early life. Nature 409, 1083 – 1091. (doi:10.1038/3509210)

2. Bell EA, Boehnke P, Harrison TM, Mao WL. 2015 Potentially biogenic carbon preserved in a 4.1 billion-year-old zircon. Proc. Natl Acad. Sci. USA 112, 14 518 – 14 521. (doi:10.1073/pnas.1517557112)

3. Grosberg RK, Strathmann RR. 2007 The evolution of sponges. Annu. Rev. Ecol. Evol. Syst. 38, 621 – 654. (doi:10.1146/annurev.ecolsys.36.102403.114735)

4. Fani R, Fondi M. 2009 Origin and evolution of unicellular states. Phys. Life Rev. 6, 23 – 52. (doi:10.1016/j.plrev.2008.12.003)

5. Maynard Smith J, Szathmáry E. 1995 The major transitions in evolution. Oxford, UK: Oxford University Press.

6. Rushby AJ, Claire MW, Osborn H, Watson AJ. 2013 Habitable zone lifetimes of exoplanets around main sequence stars. Astrobiology 13, 833 – 849. (doi:10.1089/ast.2012.0938)

7. Ochman H, Lawrence JG, Groisman EA. 2000 Lateral gene transfer and the nature of bacterial innovation. Nature 405, 299 – 304. (doi:10.1038/35025000)

8. Blankenship RE et al. 2011 Comparing photosynthetic and photovoltaic efficiencies and recognizing the potential for improvement. Science 332, 805 – 809. (doi:10.1126/science.1200163)

9. Ort DR et al. 2015 Redesigning photosynthesis to sustainably meet global food and bioenergy demand. Proc. Natl Acad. Sci. USA 112, 8529 – 8536. (doi:10.1073/pnas.1424031112)

10. Lin MT, Occhialini A, Andralojc PJ, PARRY MA, Hanson MR. 2014 A faster Rubisco with potential to increase photosynthesis in crops. Nature 513, 547 – 550. (doi:10.1038/nature13776)

11. Könneke M et al. 2014 Ammonia-oxidizing archaea use the most energy-efficient aerobic pathway for CO2 fixation. Proc. Natl Acad. Sci. USA 111, 8239 – 8244. (doi:10.1073/pnas.1402028111)

12. Bar-Even A, Noor E, Lewis NE, Milo R. 2010 Design and analysis of synthetic carbon fixation pathways. Proc. Natl Acad. Sci. USA 107, 8889 – 8894. (doi:10.1073/pnas.0907176107)

13. Wei N, Oh EJ, Million G, Cate JH, Jin YS. 2015 Simultaneous utilization of celluliose, xylose, and acetic acid from lignocellulosic biomass for biofuel production by an engineered yeast platform. ACS Synth. Biol. 4, 707 – 713. (doi:10.1021/ sb500364q)

14. Blumer-Schuette SE, Brown SD, Sander KB, Bayer EA, Kataeva I, Zurawski JV, Conway JM, Adams MW, Kelly RM. 2014 Thermophilic lignocellulose deconstruction. FEMS Microbiol. Rev. 38, 393 – 448. (doi:10.1111/1574-6976.12044)

15. Wen F, Sun J, Zhao H. 2010 Yeast surface display and acetic acid from lignocellulosic biomass for biofuel production by an engineered yeast platform. ACS Synth. Biol. 4, 707 – 713. (doi:10.1021/sb500364q)

16. Wartiovaara K, Otonkoski T. 2015 Conditionally stabilized dCas9 activator for controlling gene expression in human cell reprogramming and differentiation. Stem Cell Rep. 5, 448 – 459. (doi:10.1016/j.stemcr.2015.08.001)