Synthetic and Systems Biotechnology xxx (2017) 1–6

Contents lists available at ScienceDirect

Synthetic and Systems Biotechnology

journal homepage: http://www.keaipublishing.com/en/journals/synthetic-and-systems-biotechnology/

Experimental evolution and proximate mechanisms in biology

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ARTICLE INFO

Article history:
Received 7 September 2017
Received in revised form
16 October 2017
Accepted 20 October 2017

Keywords:
Experimental evolution
Proximate mechanisms
Ultimate mechanisms
Gene network
Modules

ABSTRACT

Biological functions – studied by molecular, systems and behavioral biology – are referred to as proximate mechanisms. Why and how they have emerged from the course of evolution are referred to as ultimate mechanisms. Despite the conceptual and technical schism between the disciplines that focus on each, studies from one side can benefit the other. Experimental evolution is an emerging field at the crossroads of functional and evolutionary biology. Herein microorganisms and mammalian cell lines evolve in well-controlled laboratory environments over multiple generations. Phenotypic changes arising from the process are then characterized in genetics and function to understand the evolutionary process. While providing empirical tests to evolutionary questions, such studies also offer opportunities of new insights into proximate mechanisms. Experimental evolution optimizes biological systems by means of adaptation; the adapted systems with their mutations present unique perturbed states of the systems that generate new and often unexpected output/performance. Hence, learning about these states not only adds to but also might deepen knowledge on the proximate processes. To demonstrate this point, five examples in experimental evolution are introduced, and their relevance to functional biology explicated. In some examples, from evolution experiments, updates were made to known proximate processes – gene regulation and cell polarization. In some examples, new contexts were found for known proximate processes – cell division and drug resistance of cancer. In one example, a new cellular mechanism was discovered. These cases identify ways the approach of experimental evolution can be used to ask questions in functional biology.

Contents

1. Introduction

As pointed out by ethologist Nikolaas Tinbergen and evolutionary biologist Ernst Mayr, there exist two distinct mechanisms of

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Peer review under responsibility of KeAi Communications Co., Ltd.

https://doi.org/10.1016/j.synbio.2017.10.004

life: proximate and ultimate [1,2]. Proximate mechanisms refer to what organisms are, studied by molecular, systems and behavioral biology; and ultimate mechanisms refer to why organisms have come to be as they are, the subject in evolutionary biology. Fields on the two sides have been developing in parallel with little crosstalk. The question is why should functional biologists care about evolution?

The answer is simply because all biological phenomena with their underlying proximate mechanisms are products of evolution. These mechanisms, one way or another, are representations of the environments organisms have experienced in the long past. Knowing why and how the mechanisms have evolved in history might explain their characteristics presently observed.

Consider ribulose-1,5-bisphosphate carboxylase (RubisCo), the enzyme responsible for fixing atmospheric carbon dioxide into biomass [3]. It is notoriously inefficient in catalysis [4] and constitutes a limiting step in biomass increase on the Earth. This inefficiency is because oxygen competes with carbon dioxide in the active center of Rubisco for a side reaction, inhibiting carbon fixation. However, there exist other carboxylases in nature that fix carbon dioxide while insensitive to oxygen [5]. These differential sensitivities to oxygen are rooted in evolutionary history. RubisCo happened to originate in ancestors to modern photosynthetic organisms – cyanobacteria, algae and most plants – at a time when the atmospheric oxygen was extremely low. Hence, selective pressure against the interference of oxygen to carbon fixation was lacking. On the other hand, the oxygen-insensitive carboxylases, found primarily in bacteria, evolved independently from RubisCo and somehow did not make to the metabolic pathways of photosynthesis [6]. In other words, the oxygen limitation of biomass increase seen in modern photosynthetic organisms might not be due to laws of physics and chemistry but simply an unfortunate accident in history [7].

This example of ancient adaptation requires information about geological record of the Earth and about phylogeny and biochemical properties of RubisCo. The demanding scope of knowledge and the reliance on evolutionary record in this approach [8,9] hinder its application in broad contexts. Indeed, an easier bridge between proximate and ultimate mechanisms is provided by an emerging field, namely experimental evolution. It takes advantage of short generation time of microbes (dozens of minutes) or mammalian cell lines (hours). Organisms are subject to reproduction through hundreds to thousands of generations – the age of *Homo sapiens* thus far is ten thousand generations [10] – under well-defined selective pressure in laboratory environment. Evolved populations and individual organisms are then characterized at both genotypic and phenotypic level. In a nutshell, experimental evolution optimizes a biological system by means of adaptation. The evolutionary dynamics can be precisely resurrected from characterizing organisms archived/frozen across all stages of evolution. This analysis reveals how the system is perturbed by a sequence of mutations to assume functional changes that increase fitness.

Experimental evolution has been extensively reviewed elsewhere [11–17]. In this review, five examples will be discussed in-depth to demonstrate how experimental evolution can be utilized to do unique service to functional biology. In two examples, experimental evolution of model systems for gene regulation and cell polarization uncovered unexpected properties of the systems. In another two examples, the classic topics in cell biology – cell division and drug resistance of cancer – found their connection to multicellularity, an unsolved problem in evolution. In the last, evolving organisms in a cyclic environment in laboratory revealed that an existing gene network can be reprogrammed by a single amino acid substitution to generate a new behavior.

2. New insights into known proximate mechanisms

2.1. Reversing mode of regulation in gene expression

A classic model of regulation in gene expression, the *lac* operon is arguably the most understood molecular system with several decades of research [18]. To express the structural genes for the metabolism of lactose, lactose binds to a transcription factor lacI that has been sitting on the promotor region of the operon DNA to repress transcription of the structural genes. This binding changes conformation of the transcription factor, which in turn falls off the promoter DNA, and expression of the operon ensues. This paradigm of inducer-repressor received a surprising update recently. Poelwijk et al. discovered that as few as three amino acid substitutions at lacI were sufficient to convert the inducer molecule (lactose) into co-repressor [19]. That is, the binding of lactose to the mutant lacI facilitates repression of the structural genes, the opposite of what it does to wildtype lacI.

This discovery was made through experimental evolution. A synthetic operon was made where lacI was used to regulate expression of two proteins, one conferring resistance to the antibiotic chloramphenicol; and the other, sensitivity to sucrose. Hence, expression of the operon was beneficial to the host bacterial cell in the presence of chloramphenicol but deleterious in the presence of sucrose. A library of lacI mutants were introduced using error-prone PCR. A population of cells each carrying a different lacI mutant were competed in a cyclic environment. In this environment chloramphenicol and sucrose alternated their presence, and inducer was added with either chloramphenicol or sucrose (Fig. 1a). When inducer was added with chloramphenicol, competition confirmed that wildtype took on optimal fitness. When inducer was added with sucrose, however, wildtype lacI was selected against, and the mutants that converted inducer into co-repressor swept the population after multiple cycles of environmental shift.

This work exposes functional flexibility at the level of a single macromolecule: It takes only a few mutations at a regulatory protein to turn an inducible system into a repressible one. A few distinct genotypes were identified with the reversed mode of regulation but shared a common mutation that substituted serine 97 at the dimer interface with proline (red residue in Fig. 1b), which is known to devastate the allosteric transition, i.e., the inducer-caused conformational change needed for the function of wildtype lacI [20]. Then the allostery required of the reversed regulation has to be mediated through alternative sets of amino acid residues (Fig. 1b), a phenomenon not obvious from existing knowledge. In the future, crystal structures of these unique mutant proteins combined with their molecular dynamics simulations will bear great hope in elucidating the mechanistic basis of allostery, a fundamental problem in biochemistry [21].

2.2. Recovery of damaged cell polarization

Cell polarization defines a spatial axis of the cell that is essential for division, migration, differentiation, etc. [22,23] Its molecular mechanism has been elucidated, and the underlying gene network empirically mapped out in budding yeast [22]. At the center is GTPase Cdc42, a master regulator that concentrates at a specific spot in the cell membrane where a new bud starts to form. Its functioning is regulated dynamically by an ensemble of mechanisms to ensure bud formation with precise space and time (Fig. 2a). This process is an example of self-organization at the molecular level. The question is how a single site of concentrated Cdc42 is specified on the 2D surface of cell membrane in the face of diffusion (green arrows, Fig. 2a). Many mathematical models have been built to account for this localization event as an emergent

Please cite this article in press as: Yi X, Experimental evolution and proximate mechanisms in biology, Synthetic and Systems Biotechnology (2017), https://doi.org/10.1016/j.synbio.2017.10.004