Review

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Evolutionary constraints in variable environments, from proteins to networks

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Environmental changes can not only trigger a regulatory response, but also impose evolutionary pressures that can modify the underlying regulatory network. Here, we review recent approaches that are beginning to disentangle this complex interplay between regulatory and evolutionary responses. Systematic genetic reconstructions have shown how evolutionary constraints arise from epistatic interactions between mutations in fixed environments. This approach is now being extended to more complex environments and systems. The first results suggest that epistasis is affected dramatically by environmental changes and, hence, can profoundly affect the course of evolution. Thus, external environments not only define the selection of favored phenotypes, but also affect the internal constraints that can limit the evolution of these phenotypes. These findings also raise new questions relating to the conditions for evolutionary transitions and the evolutionary potential of regulatory networks.

Epistasis in variable environments

Evolutionary adaptation is commonly thought of in terms of two distinct factors. On the one hand, external selective environments drive evolution to particular favored phenotypes, whereas, on the other hand, internal organismal constraints limit access to these phenotypes. Generally, evolution may be limited by physicochemical constraints [1] or by genetic exigencies [2], for instance when rare combinations of mutations are required for a functional change. In laboratory experiments, selection and constraint have been quantified for environments and phenotypes that are constant in time [3–7]. In comparison, little is known about selection and constraint in variable environments. The effects of environmental variability could be significant: different environments may not only favor different phenotypes, but also give rise to different evolutionary constraints and, hence, blur the line between the external and internal factors that determine evolutionary adaptation.

These issues are of general relevance given the variable character of natural environments. They are important for regulatory systems in particular. Regulatory systems may well experience selection and evolve in constant conditions, but their ability to respond to environmental changes is logically considered to be shaped by a history of selection in changing environments [8]. However, the mechanisms of regulatory evolution in variable environments remain incompletely understood, despite detailed insights into function [9–11] and sequence evolution [12–16]. Elucidating these questions will be central to understanding how the complex regulatory circuitries of cells have evolved, may offer routes to engineer synthetic regulatory functions, and provide new perspectives on the function of regulatory networks.

At the most elementary level, genetic constraints in constant environments can be expressed in terms of the interaction between two mutations, which is commonly referred to as epistasis (Table 1). For instance, a reconstruction of neighboring genotypes of the protein β-lactamase revealed that mutating a particular residue could increase resistance to antibiotics, but only if a second residue was mutated first, otherwise the resistance decreased [4,17]. Such sign–epistatic interactions [5–7] can result from the highly integrated nature of molecular structures [18] and the interplay between protein stability and catalytic activity [19]. Sign epistasis affects selection, because fitness-increasing mutations are more readily fixed than neutral or fitness-decreasing mutations. In particular, the mutations will then be fixed in a specific order. Thus, sign–epistatic interactions between functionally important mutations constrain the number of mutational pathways accessible by positive selection. By contrast, forms of epistasis without changes in the sign of the effect, such as positive or negative epistasis (Table 1), do not have such drastic effects on selection, although they do provide important insights into functional relations.

The number of paths accessible by positive selection may also reduce to zero. Such a lack of available positively selected mutations could underlie cases of prolonged evolutionary stasis, and can be visualized as entrapment on a suboptimal fitness peak in genotype space [2]. Escape from such evolutionary stasis does remain possible in principle, for instance when multiple mutations are jointly fixed [4,20], or when population expansion limits selection and maintains less fit phenotypes [6], although at much reduced

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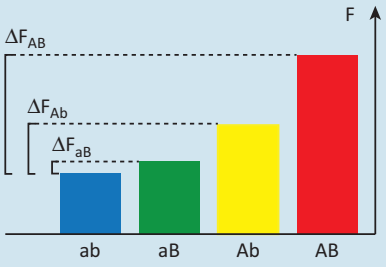
Table 1. Types of epistasis

Type of epistasis		Evolutionary consequences
No epistasis: $\Delta F_{AB} = \Delta F_{Ab} + \Delta F_{aB}$		Both paths from ab to AB are accessible by positive selection.
Magnitude epistasis: ΔF_{Ab}, $\Delta F_{aB} > 0$	Positive: $\Delta F_{AB} > \Delta F_{Ab} + \Delta F_{aB}$	Both paths from ab to AB are accessible by positive selection; if the effect is strong, multiple mutations are required to confer a large fitness increase.
	Negative: $\Delta F_{AB} < \Delta F_{Ab} + \Delta F_{aB}$	Both paths from ab to AB are accessible by positive selection; the contribution of successive mutations to fitness becomes less and less (diminishing returns), which slows down adaptation.
Sign epistasis: $\Delta F_{Ab} < 0$ XOR $\Delta F_{aB} < 0$		One path is accessible by positive selection, whereas the other is not; hence, a particular order of mutations is favored.
Reciprocal sign epistasis: $\Delta F_{Ab} < 0$ AND $\Delta F_{aB} < 0$		Both paths from ab to AB are inaccessible by positive selection; this is a necessary condition for the existence of multiple local optima.
		Figure T1. Types of epistasis in constant environments and their evolutionary consequences. Between genotypes ab and the fitness optimum AB, two mutational paths are possible: via Ab and via aB. ΔF_{Ab}, ΔF_{aB}, and ΔF_{AB} are the fitness changes relative to the fitness of ab. We note that, because neutral mutations are not positively selected, conditionally neutral mutations ($\Delta F_{Ab} = 0$ OR $\Delta F_{aB} = 0$) can be considered to exhibit (reciprocal) sign epistasis rather than magnitude epistasis.

probability. It has been shown on theoretical grounds that, for systems to display this more severe genetic constraint, they must exhibit reciprocal sign–epistatic interactions (Table 1) [21]. In this case, two mutations are jointly beneficial but each individually deleterious. Such interactions have been observed in the regulator *MSN Three Homolog 1* (MTH1) and transporters hexose transporter 6 and 7 (HXT6 and HXT7) of the yeast glucose utilization pathway [22], among other systems [23].

An emerging question is how epistasis and constraint are affected by environmental variability. Not only is the natural environment intrinsically variable, but the effects of mutations are also often found to depend strongly on the environment. For instance, the change in growth rate for different *Escherichia coli* Tn10 transposon mutants was found to depend on not only the genetic background, but also the type of growth media used [24]. Such interactions between genetic and environmental changes are pervasive in biological systems [25–30]. These observations raise the question of how epistasis itself is impacted by environmental variability. Here, we review recent efforts that aim to address these issues. The approaches are diverse and range from the detailed analysis of interactions between genetic changes and environmental changes in a model transcription factor, to whole-genome investigations of epistatic interactions in complex networks, and exploit ideas from synthetic biology, experimental evolution, and mathematical modeling of cellular networks. These first studies revealed that environmental changes can drastically alter the interaction between two mutations, such that evolutionary paths can switch between being accessible to being inaccessible. At the scale of networks, certain epistatic effects are beginning to be understood mechanistically. The results pave the way to elucidating the evolution of regulatory networks based on a functional understanding of genetic and environmental interactions.

Epistasis within a regulatory protein

Transcriptional regulation is one of the simplest regulatory mechanisms within cells and, therefore, is a good starting point to explore the interplay between genetic and environmental changes. A recent study [31] zoomed in on one of the best-understood model systems for transcriptional regulation, the *E. coli lac*-repressor (Figure 2A). The authors had previously used experimental evolution to produce *inverse* LacI variants [32]. In contrast to the wild type repressor $LacI_{WT}$, these $LacI_{Inv}$ mutants repressed the *lac* genes in the presence of the ligand isopropyl-β-D-thiogalactopyranoside (IPTG), rather than in its absence. The genetic basis of the inverse response could be traced to three amino acid substitutions within the protein. Fixating these mutations involved a variable selection that alternated between favoring expression and repression of the downstream genes.

This scenario contains the basic ingredients for the adaptive evolution of regulatory responses: a succession of genetic and environmental changes in time. An elementary question that then arises is how these changes relate to each other. If these two types of change do not interact (meaning that their effects on phenotype or fitness are independent), then the specific pattern of environmental changes is immaterial to the genetic obstacles to evolution. However, if they do interact, obstacles that exist in one environment could be lifted in another (Figure 1). Hence, insight into the environment × genotype interdependencies as well as the precise patterns of environmental change may be critical to understand the evolutionary adaptation of regulatory systems. Note that, in general, organisms may well fail to show adaptive evolution of regulatory responses to multiple environments and, for instance, rather evolve the same phenotypic change across all environments. To explore these issues, all single and double mutants were constructed for three inverse LacI variants that had been isolated, and their *lac* operon expression was assayed with and without IPTG.

The analysis showed a drastic effect of the environment on the genetic interactions between pairs of mutations. For half of the pairs, an environmental change turned magnitude epistasis into sign epistasis. Take, for instance, the mutations T258A, which is positioned at the dimerization