In search of the limits of evolution

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Negative trade-offs are thought to be a pervasive phenomenon and to inhibit evolution at all levels. New evidence shows that at the molecular level, there may be no trade-offs preventing the emergence of an enzyme with multiple functions.

Evolution, like politics, is an art of the possible. The second law of thermodynamics prohibits the evolution of a membrane transporter that works as Maxwell's Demon. Such fundamental limitations, however, may constitute only a small fraction of the Scylla and Charybdis of genotype space around which natural selection must navigate to create functional molecules, cells and organisms.

We have a good understanding of extrinsic 'evolvability'[1] (*i.e.*, how natural selection shapes genetic variation provided by mutation[2,3]). In contrast, little is known about the intrinsic limits of evolution[1] imposed by the geometry of fitness surfaces in genotype space (**Fig. 1**). The main obstacle to understanding intrinsic evolvability is that only a small fraction of genotype space is populated by existing genotypes, and most possible genotypes will never exist. Yet it is necessary to know the phenotypes and fitnesses of all genotypes, existent and nonexistent, to understand how evolution works. Thus far, no radically new organisms have been created in the laboratory, but data on artificially evolved molecules are accumulating[4].

Modifying enzyme function

On page 73 of this issue, Aharoni *et al.*[5] address a simple question: is it possible to endow a protein (enzyme) with new, additional functions without compromising its ability to carry out the original, native function? Their answer is a resounding 'yes'. The enzymes chosen for their study catalyze reactions of specific substrates but can also catalyze reactions of non-native or 'promiscuous' substrates at very low rates. Random mutations were introduced into these enzymes and then screened for increased efficiency of promiscuous functions. Some mutant enzymes showed markedly higher rates of promiscuous catalysis and virtually unaffected native functions[5]. No pressure to maintain the native function was applied; therefore, its preservation cannot be explained by selection for compensatory substitutions.

The evolutionary uncoupling of native and promiscuous functions is unexpected because negative trade-offs between different functions are assumed to be pervasive[6]. For example, aging has been proposed to be an optimal strategy because maintaining high performance late in life comes at the expense of early performance that directly affects fitness[7]. At the molecular level, however, we now know that such trade-offs between different enzyme functions may be absent.

The ability of one enzyme to carry out multiple functions may be due to the evolution of a new active site. This does not seem to be the case in this study, however, because the mutations that improve promiscuous function localize to the walls of the native active site[5]. The basic model of enzyme function assumes a specific interaction between the substrate and the enzyme, commonly described as a lock-and-key interaction. This model may therefore be an oversimplification, because it seems that multiple substrates can fit the active site of one enzyme. Alternatively, the newly evolved enzyme may fold into a variety of structural isoforms such that different isoforms catalyze different reactions[8].

Evolution of gene function revisited

There are many examples of proteins that have multiple functions: crystallins, antifreeze proteins, the p53 tumor-suppressor protein and a broad class of enzymes that recognize different substrates[9] all come to mind. On the other hand, many other proteins can be described with the broad generalization of one gene–one function. Why do proteins not take advantage of the opportunity to carry out multiple functions more often? It is true that evolution cannot produce the optimal solution, especially if doing so requires radical changes; think of a bilaterally

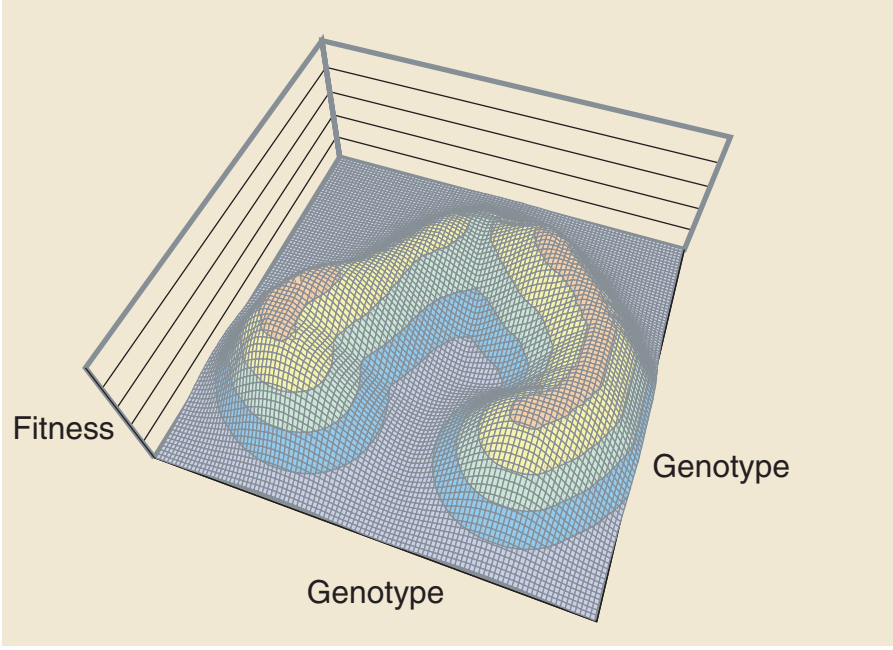

Figure 1 A three-dimensional representation of the concept of fitness surfaces in genotype space. On the *x-y* plane, each position represents a unique genotype, and the *z* axis represents the fitness of these genotypes. Evolution can proceed only along ridges of high fitness and must avoid fitness valleys. Therefore, the ruggedness of fitness ridges defines how evolution must proceed in genotype space[15].

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symmetrical flatfish larva struggling to mold itself into an adult. But the results of Aharoni *et al.*[5] show that the lack of stepping stones is probably not an obstacle for the evolution of new enzymatic functions, because even a small number of simple amino-acid substitutions are enough to improve promiscuous functions.

It is possible that broadening the range of functions of a molecule may be unnecessary, so that natural selection maintains the native function but never acts to improve the promiscuous one. This is analogous to mutational explanations of aging[10], which argue that aging occurs because natural selection does not act against mutations that impair performance only after reproductive age. This hypothesis, however, cannot explain why the evolution of new functions, when they are actually needed, does not generally proceed through the acquisition of multiple functions by a single enzyme.

Because negative trade-offs do not preclude the evolution of multifunctional molecules, one explanation for their rarity remains: multiple enzymes each with a specialized function are preferred, at the cellular level, over one enzyme that has several functions[11]. For example, in metabolic and genetic networks, an enzyme that has two functions in the same pathway may not achieve independent dosage control, whereas two specialized enzymes may be expressed independently. As the field of systems biology matures, we will be able to ask more specific questions about the constraints imposed on the evolution of molecules by their interactions at a cellular level.

So, how do new protein functions evolve? There is no question that gene duplications are involved, yet the most widely cited model assumes that it is impossible to evolve a new function without losing the previous one[12]. If so, new function evolves through gene duplication when a redundant gene copy is released from functional constraint[12]. Others have pointed out that redundancy cannot release only one copy from selection[13] and hypothesized that a gene duplication event allows evolution to distribute multiple functions of one gene among its copies[13,14]. But these models do not explain how genes evolve the multiple functions in the first place. Ahroni *et al.* add a crucial piece to this puzzle and formulate a new model of evolution of new functions through natural selection: new functions can evolve before duplication, after which specialization of duplicate copies may allow for fine-tuning of these functions and independent patterns of expression.

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Is Rett syndrome a loss-of-imprinting disorder?

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Most cases of Rett syndrome are caused by mutations in *MECP2*. Transcriptional profiling analyses of the brains of individuals with Rett syndrome have not provided consistent data about genes that are silenced by MECP2. A new study finds loss of imprinting of a maternally imprinted gene, *DLX5*, both in *Mecp2*-null mice and in some lymphoblastoid cell lines obtained from individuals with Rett syndrome.

Rett syndrome (RTT) is a severe neurodevelopmental disorder that affects girls almost exclusively[1]. Approximately 80% of classical RTT cases are caused by mutations in *MECP2*, a transcriptional silencer that can bind any DNA sequence containing at least a CpG island[2]. On the basis of this function, it was hypothesized that RTT might be caused by 'global transcriptional noise'. This idea was soon proved wrong by data showing that MECP2 probably regulates only a limited number of genes. Four papers reporting transcriptional profiling analyses of MECP2-deficient brains failed to provide consistent data about genes that are silenced by MECP2 (refs. 3–6). On page 31 of this issue, Shin-ichi Horike and colleagues[7] report loss of imprinting of a maternally imprinted gene, *DLX5*, in both *Mecp2*-null mice and some lymphoblastoid cell lines obtained from individuals with RTT. Other authors have tested and discarded the loss-of-imprinting hypothesis[8]; however, they had limited their analysis to well-established imprinted genes such as *H19*, *IGF2*, *SNRNP*, *IPW* and *NDN*, which Horike *et al.*[7] also show not to lose imprinting.

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New MECP2 targets

Current research on RTT is largely focused on the identification of MECP2 targets to understand the events that result from disruption of MECP2 function. The authors identified *DLX5* as a MECP2 target gene because it has a MECP2-binding sequence 52 kb from its 3′ end. Using a chromatin immunoprecipitation strategy, they identified 33 Mecp2-binding sequences. They checked the expression of flanking genes for only two of them. Of the remaining 31 sequences, three fall in the CpG islands of three genes, contactin 2, forkhead box A3 and sialyltransferase 4A. These findings identify new genes that warrant further research and may lead to the identification of new MECP2 effectors. To date, only *BDNF* has been proven to be a target of *MECP2* in mammals[9].

Characteristic chromatin

The switch of a gene from a silenced state to an active one is a complex and strictly regulated process that involves exact interactions between transcriptional silencers, activator complexes and DNA sequences. One of the main factors in gene activation is chromatin structure and