STRUCTURAL BIOLOGY

Evolutionary Chemistry: Getting There from Here

Gerald F. Joyce

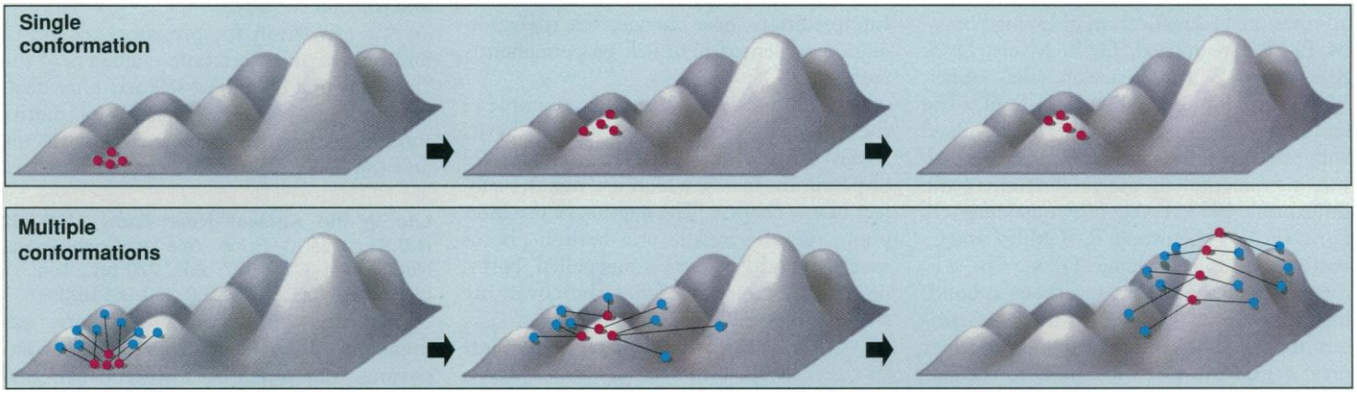
Where do enzymes come from? All of the enzymes that exist in nature are the product of Darwinian evolution, based on natural selection. By comparing an enzyme's sequence to that of related enzymes one can draw inferences about the pathway of its evolutionary development. Rarely, however, does one have the opportunity to witness the evolution of catalytic function firsthand. On page 1665 of this issue, Wedemayer *et al.* (*1*) present high-resolution x-ray crystallographic structures of a catalytic antibody, both before and after the refinement of its catalytic activity. These structures provide new insight into the process of antibody maturation and show how a macromolecule may be preadapted for the acquisition of catalytic function.

The investigators raised antibodies against a phosphonate hapten that is a transition-state analog for the hydrolysis of a nitrophenyl ester (*2*). They isolated a mature antibody that accelerated this hydrolysis reaction by a factor of 10^4 compared to the uncatalyzed reaction. They also isolated the gene for the corresponding germline antibody. The mature antibody contained nine amino acids that were different from the corresponding amino acids in the germline molecule, six in the heavy chain, and three in the light chain. The mature antibody bound the hapten 31,000 times more tightly than did the germline antibody and catalyzed the target reaction 82 times as efficiently (*2*). One might expect that most of the amino acid substitutions in the mature antibody would have occurred near the bound hapten. The crystal structure, however, revealed that this was not the case. On the basis of the nearest approach of their respective van der Waal's radii, two of the substitutions were within 5.5 Å of the hapten, but all of the others were at least 10 Å away.

The results of Wedemayer *et al.* allow structural comparison of the germline and mature antibodies in both the presence and absence of the bound hapten. When the germline antibody binds the hapten, its structure is substantially altered, with a change in the rotation angle between the heavy and light chains of 4.6°. In contrast, there is only a slight alteration of the structure of the mature antibody upon hapten binding, with a change in the rotation angle between the two chains of only 0.4°. Most interestingly, the structure of the mature antibody, in either the bound or unbound state, closely resembles that of the germline antibody in the bound state. It is as if the germline molecule had been imprinted by its contact with the hapten, leading to the maturation of a catalytic antibody that reflects the imprinted conformational state.

Such a Lamarckian mechanism for the immunological evolution of catalytic function makes no sense in light of the well-understood process of antibody maturation, just as it makes no sense for the emergence of phenotypic traits in biological organisms that evolve by natural selection. Yet it is uncanny how well the hapten-bound state of the germline antibody anticipates the structure of the mature antibody. This can be explained without invoking any forward-looking evolutionary mechanisms. It appears that the germline antibody is pluripotential with respect to the conformations it can assume upon contacting various haptens, and that the mutations that become fixed during antibody maturation tend to consolidate a specific conformation that is especially well suited for binding the hapten that drives the immune response. The picture is not that of a simple lock and key, but rather of a blurry lock that comes into focus through repeated contact with a particular key.

The fixed set of proteins that constitute the germline antibody repertoire must recognize an almost limitless array of potential antigens. If each of these proteins can adopt a variety of conformations, then the effective diversity of the germline repertoire is greatly expanded. This increases the likelihood that an evolutionary pathway will be found from one of the germline antibodies in one of its conformations to a mature antibody that binds the antigen with high affinity. During antibody maturation, and macromolecular evolution in general, the evolving population must pass through a succession of functional intermediates that lead from an initial set of naïve molecules to an evolved set that is optimized with respect to the selection constraints. Immunological evolution has the added constraint that it must proceed very rapidly, before the pathogen has time to damage the host. Thus, the capacity for rapid discovery and traversal of an evolutionary pathway during antibody maturation is itself



Evolution. An evolving population of macromolecules traverses a "fitness landscape," moving toward positions of enhanced fitness (represented by peaks). (**Top**) Individual molecules with a single conformation (red) move toward nearby peaks, but may not be able to reach higher peaks that lie across distant valleys. (**Bottom**) Individuals with alternative conformations (blue) have an expanded reach, making distant peaks more accessible.

The author is in the Departments of Chemistry and Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA. E-mail: gjoyce@scripps.edu

a trait that is selectively advantageous. The notion that "evolvability" is a beneficial trait that can be optimized through Darwinian evolution has been discussed previously (*3*, *4*). The results of Wedemeyer *et al.* provide an example, at atomic resolution, of how this can come about.

An evolutionary search is a parallel process in which every individual in the population has the opportunity to give rise to novel variants with increased fitness. This parallelism makes it less likely that the population as a whole will become trapped in an evolutionary blind alley from which further improvements in fitness are precluded. Conformational versatility increases the degree of parallelism of the evolutionary search. Even if the population were to become trapped in a local fitness optimum when the molecules are in their dominant conformation, they can assume alternative conformations that provide an opportunity for escape (see the figure).

Gene-duplication events are the primary source of new enzymes in biology. One gene copy maintains the original function, while the other is free to evolve a new function. Alternative conformations of a germline antibody, and perhaps other functional macromolecules, can be viewed similarly. One conformation maintains the fitness of the molecule, while the others are free to vary in search of improvements in fitness. For this behavior to be relevant to the process of antibody maturation there must be the possibility of mutations that preserve hapten binding while perturbing one or more of the alternative conformations. Mutations of this type are expected to occur more frequently at positions that lie distant from the site of hapten binding. Although it is difficult to generalize from the single example of a maturing antibody presented by Wedemeyer *et al.*, their structural data show how remote mutations can refine an alternative conformation in a way that leads to a substantial improvement in fitness.

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Why did the mature catalytic antibody exhibit marked improvement in hapten binding relative to the germline molecule but only modest improvement in catalytic rate? Because you get what you select for. The antibody maturation process optimized binding of the phosphonate hapten, and to the extent that the hapten was a faithful analog of the transition state of the target reaction, that enhanced binding translated into enhanced catalysis. If one were to select directly for catalysis (*5*), then a more substantial improvement in catalytic rate would be expected. It remains to be seen whether this improvement would occur primarily by successive amino acid substitutions that act independently or by conformational changes that affect several amino acids in concert. In either case, the conformational versatility of an antibody is likely to enhance its evolvability.

References

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MICROBIOLOGY

Mariner Sails into *Leishmania*

Daniel L. Hartl

Leishmaniasis—a scourge of the Old and New World tropics—is caused by a number of species of the parasitic flagellate protozoan *Leishmania* (see figure). Spread by bloodsucking sand flies of either the genus *Phlebotomus* (Old World) (see figure) or *Lutzomyia* (New World), leishmaniasis manifests as an often-fatal disease that attacks the internal organs or as a disfiguring cutaneous disease that can destroy the mucosa of the mouth, nose, and throat. No satisfactory vaccines or chemotherapies are available, so genetic approaches to understanding virulence and pathogenicity have been emphasized in the search for more effective treatment. Nevertheless, genetic analysis of parasitic protozoa has been hindered because the organisms are diploid and either have no known sexual cycle or have one that is experimentally intractable. But now on page 1716 of this issue, Gueiros-Filho and Beverley (*1*) report a welcome result. They demonstrate the feasibility of genetic studies in *Leishmania major* with the use of the transposable element *mariner*.

A nasty bite. The phlebotomus sand fly can infect its victim with the flagellated promastigote form of *L. major* (right).

Of transposable elements (pieces of DNA that can move from place to place in the genome), *mariner* is phylogenetically the most widely distributed in animals. It occurs in planaria (*Dugesia tigrina*), nematodes (*Caenorhabditis elegans*), and centipedes (*Scutigera coleoptrata*) and is widespread among insects (*2*). *mariner* elements are also present in multiple copies in the human genome (*3*, *4*). In fact, one pair of *mariner* elements in chromosome 17p has been implicated as a hotspot of unequal crossing-over. The resulting products are either duplicated or deficient for the gene for peripheral myelin protein 22. The duplication causes Charcot-Marie-Tooth disease type 1A, and the deficiency causes hereditary neuropathy with susceptibility to pressure palsies (*5*). The *mariner* elements in animals, referred to collectively as *mariner*-like elements (MLEs), are diverse in nucleotide sequence and have been grouped into several subfamilies; elements in different subfamilies are typically 40 to 56% identical at the nucleotide level. Most MLEs that have been sequenced contain one or more chain-terminating nucleotide substitutions or frameshift insertions or deletions that render them incapable of encoding a functional transposase (*2*).

The functional MLE transposase is a member of a large superfamily of transposase and integrase proteins known as the D, D(35)E superfamily. The two aspartate (D)

The author is with the Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA 02138, USA. E-mail: dhartl@oeb.harvard.edu