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.

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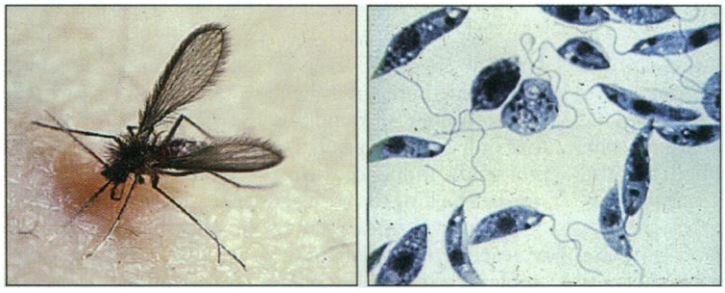
MICROBIOLOGY

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)

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residues and one glutamate (E) residue located at strategic positions in these transposases bind a divalent cation necessary for DNA strand scission in the transposition reaction. The D residues are typically separated by more than 90 amino acids and the final D and E residues by 34 or 35 amino acids. The D, D(35)E superfamily also includes retrovirus transposases such as those of the human immunodeficiency virus (HIV) and Rous sarcoma virus (RSV), retrotransposons of the *copia* and *gypsy* class, *Tc1*-like transposable elements, and various bacterial insertion sequences (6). The MLEs are a unique lineage in having a D, D(34)D signature in the transposase. The final D is essential for MLE activity and cannot be replaced by E (7). Transposition in the superfamily occurs by a cut-and-paste mechanism: A staggered double-strand scission at each end of the element releases it from the donor molecule, and the element is ligated into a staggered cut at the target site (8).

The widespread phylogenetic distribution of *mariner* elements reflects an evolutionary history in which the elements have been transmitted horizontally between diverse hosts repeatedly. Many examples of horizontal transmission have been inferred from the close sequence similarity between *mariner* elements in otherwise highly divergent species. Horizontal transmission has occurred between insect families within the Diptera (9), between orders within Insecta (10), and between phyla within Animalia (11), including at least two horizontal transmissions into the human genome (12).

The extraordinary host range of MLEs implies that few host functions are required for transposition. In their *Leishmania* experiments, Gueiros-Filho and Beverley (1) used a *mariner* element called *Mos1*, known to encode a functional transposase in *Drosophila* (13, 14). They transfected *L. major* cells with two types of plasmid, one containing an intact *Mos1* element and the other containing the *Mos1* transposase-coding region fused with DNA sequences allowing trans-RNA spicing and gene expression in *Leishmania*. (The organism is unusual in that a mini-exon of 39 nucleotides must be trans-spliced onto the 5′ end of every messenger RNA.) Among transfected cells chosen at random, without selection for *Mos1* integration, 23% of the cells had *Mos1* inserted into the genome. Further studies showed that *Mos1* insertions could be used to obtain insertional inactivation of the gene for dihydrofolate reductase–thymidylate synthase—and that a modified *Mos1* element could be used to identify *Leishmania* genes by insertions that fuse *Mos1* and the hygromycin-resistance gene to an RNA-splice acceptor, allowing expression of antibiotic resistance.

The demonstration that *mariner* functions in *Leishmania* adds insertional mutagenesis and transposon tagging to an already impressive set of tools developed for genetic analysis in this organism, including expression vectors, gene knockouts, artificial chromosomes, and inducible expression systems. Of wider significance is that these experiments will inevitably encourage attempts to use *mariner* in developing genetic tools for use with other pathogens, pest species, and organisms of genetic interest. (Judging from the volume of my e-mail requesting *Mos1* vectors, there is already considerable interest.) There is no obvious reason why such experiments should be restricted to lower eukaryotes and invertebrates, because *mariner* can evidently function in at least some vertebrate genomes. Preliminary evidence indicates that the *Mos1* element can integrate into the genome of *Danio rerio*, the zebrafish (15). For *mariner* researchers, the *Leishmania* findings are exceptionally exciting because they emphasize the importance of understanding the evolution, molecular genetics, and self-regulation (16) of this remarkable traveler among animal genomes. Although we did not know it at the time (17), *mariner* was an apt name for this wide-ranging transposable element.

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CHEMISTRY

Ultrafast Reaction Dynamics in Molecular Cluster Ions

Robert Parson and James Faeder

In most chemical reactions, the reactants and products are not isolated but are in contact with their surroundings. Even a solvent that only weakly perturbs the reactants can profoundly influence the course of the reaction by blocking the path of a departing atom or by removing excess energy from products or reactive intermediates. A celebrated example is the photodissociation and recombination of the iodine molecule in nonpolar solvents (1), clusters (2), and solid matrices (3). Solvent effects become still more important when the reaction involves charged species, because the forces between an ionic solute and a polar or polarizable solvent can be as strong as the chemical binding forces between the reactants themselves. The solvent then does not merely interrupt and redirect motion on the potential energy surface of the isolated solute, it actually changes the topography of that surface. This is not news to chemists, who have long appreciated that a solvent will stabilize the transition state of a reaction differently than it does the reactants or products. Understanding the full impact of strong solvent-solute interactions on reaction dynamics has nevertheless proved to be a demanding task.

New experimental techniques, capable of probing molecular dynamics in well-characterized solvent environments on extremely short time scales, are now being brought to bear on these problems. By stimulating molecules with laser pulses having a duration of a few tens of femtoseconds, physical chemists can watch chemical bonds break and reform in real time. By studying reactions in small gas-phase clusters, they can examine the effect of the solvent on a molecular scale. Clusters are particularly well suited for the study of ionic systems because systems of a desired size can be selected with a mass spectrometer.

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