BIOCHEMISTRY

Loop Grafting and the Origins of Enzyme Species

An enzyme with completely new function can be created in the lab by mimicking natural evolutionary processes that both alter and preserve protein architecture.

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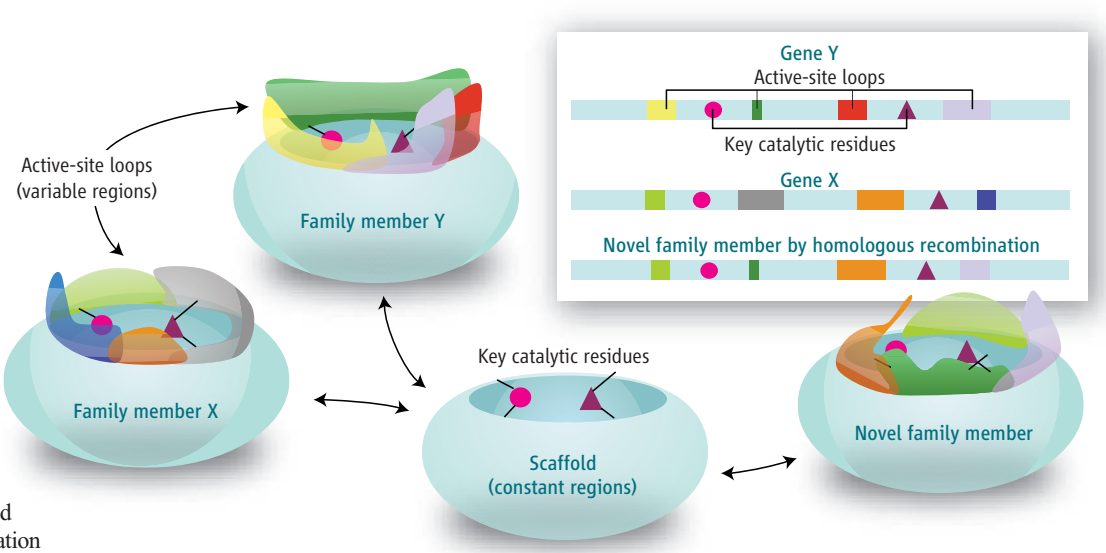
Understanding the emergence of new protein functions is no less a challenge than unraveling the origins of the organisms in which they occur. Proteins, enzymes in particular, exhibit spectacular performance unequaled by any human-made catalyst. Highly proficient and robust apparatuses are usually neither versatile nor easily modifiable. Enzymes, however, exhibit a remarkable evolutionary adaptability. New enzymes have emerged throughout the natural history of this planet. These did not simply turn up, nor were they the subject of "intelligent design." They evolved through Darwinian processes of mutation and selection. In fact, new functions can evolve in a matter of a few decades or even months, as with enzymes that degrade synthetic chemicals (nonexistent on this planet until the 20th century) and the alarming evolution of drug resistance (in which an enzyme evolves to avoid a drug designed to block it).



Loop grafting yields new enzymes. Park *et al.* (*1*) demonstrate that replacing several active-site loops (variable regions) of an enzyme, while retaining its scaffold and key catalytic residues (constant regions; red circle and pink triangle), can yield a family member with new reaction specificity. In nature, the conserved scaffold and key catalytic residues constitute obvious crossover points for homologous gene recombination and may enable the shuffling of active-site loops between family members and the emergence of new enzymatic functions.

On page 535 of this issue, Park *et al.* (*1*) provide insights regarding the emergence of new enzyme functions. They mimicked, in the laboratory and in real time, the divergence of a new, human-made member of a large family of enzymes that has diverged in nature time and again. The work demonstrates that a switch in enzyme function that involves a dramatic change in amino acid sequence and active-site architecture boils down to replacing several of the enzyme's surface loop structures.

The structures of more than 30,000 proteins taught us that nature made use of a rather limited repertoire of core structural platforms, or "scaffolds" (on the order of a few thousand), to mediate an amazingly large diversity of functions (*2*). This diversity had presumably emerged from a small number of progenitor proteins, each with a different basic scaffold, thus creating enzyme families and superfamilies. The vestiges of this process are the scaffold and active-site architecture (or "key catalytic residues") shared by all family members (*3*).

Millions of years of evolutionary drift resulted in sequence changes that largely obscure the precise routes by which these enzymes diverged. Pointing out a route by sequence and structure comparisons is an essential step, but its resolution is inevitably low (imagine the deconvolution of a complex movie plot from a few snapshots). The ultimate test of our understanding, in the view of skeptical experimentalists, is reproducing these routes in the laboratory. In Thomas Edison's words, "Until man duplicates a blade of grass, nature will laugh at his so-called scientific knowledge." Although alteration of enzymatic function through point mutations has become a matter of routine, the reshaping of active sites through insertion and/or deletion of entire polypeptide segments was scarcely exercised (*4*). There is little doubt, however, that major switches in function demand major sequence rearrangements including insertion, deletion, and recombination (*5*).

Through extensive sequence changes, including the deletion and insertion of several structural loops in the active site, Park *et al.* converted one member of the αβ/βα metallohydrolase superfamily (glyoxalase II) into a new family member with a different catalytic function (degradation of a β-lactam antibiotic). Hundreds of natural enzymes belong to the metallohydrolase superfamily, mediating a myriad of different reactions. Despite this diversity of function, they all share the same scaffold and key catalytic residues—a bimetal (typically zinc) active center ligated to the enzyme's scaffold through different residues. These metallo centers activate both the substrate and a water molecule, thereby accelerating the hydrolytic breakdown of the former. Thus, all family members share the same chemistry, although the substrates and the detailed reaction pathways differ. These common themes, which characterize all enzyme superfamilies (*3*), imply that when a need for a new hydrolytic function emerged, nature recruited existing members of hydrolase superfamilies and tinkered with their active sites to fit the new substrate.

In accordance with this scenario, Park *et al.* maintained the basic scaffold and chemistry, whereas the switch in function was triggered by extensive changes in four active-site loops. The resulting protein (evMBL8) bears little resemblance (25% sequence identity) to IMP-1, a natural β-lactamase from the same superfamily, although its active-site architecture is probably close to that of IMP-1 (*1*). Thus, as in nature, sequence diverged much further than structure.

From an engineering perspective, this work extends the powers of protein engineering, one of the first achievements of which (*6*) had been the

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grafting of antigen binding loops from rodent into human antibody scaffolds (*7*). Grafting enzyme loops is far more tricky an exercise. As demonstrated by Park *et al.* (*1*), the combination of rational design (or "rationalized design," i.e., deciding which loops to replace and the simultaneous insertion of several loops containing randomized sequences) and directed evolution has proven useful. However, the engineering of artificial enzymes with catalytic efficiencies that rival those of natural enzymes remains a challenge. The engineered enzyme (evMBL8) is inferior to its natural counterparts by a factor of 1000—and so are other designed enzymes (*8*). Future work may provide additional examples, improved design rules, and computational algorithms that direct the grafting of active-site loops or even replace the scaffold (*9*).

From an evolutionary point of view, the key to success seems to be the preservation of scaffold and chemistry. Notably, the latter is mediated by the key catalytic residues that are often associated with the scaffold, whereas the active-site loops vary from one family member to another. This hierarchy of enzyme structure is seen in many enzymes and is probably one of the keys to enzyme evolvability (*10*). Two important questions remain, however. First, can loop swapping be exercised in nature? Homologous recombination of genes encoding different family members seems a most feasible mechanism (see the figure). Second, an essence of Darwinian processes is that they occur gradually while maintaining organism fitness throughout. But the first steps toward evMBL8 led to a complete loss of function. Can a switch in enzyme function that involves multiple and drastic changes in sequence evolve gradually? Well, nature's starting point might have been an enzyme that promiscuously exhibits low levels of the desired function. Indeed, promiscuous activities, or cross-reactivities, are often observed between members of the same superfamily (*11*, *12*). The next step may involve mutations that increase this promiscuous function while maintaining the original function, thereby providing a bifunctional evolutionary intermediate (*10*). Gene duplication could then lead to the divergence of the new gene through recombination with homologous family members and further mutation and selection. Individual steps along these routes have been demonstrated in the laboratory, but reproducing this process in its entirety remains a challenge.

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ATMOSPHERE

Climate Change and Human Evolution

What can we learn about cause and effect relationships between climate and human evolution from the late Cenozoic?

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Climate and biological evolution have interacted throughout Earth's history, together creating many small and a few major transformations in the planet's atmosphere and biota. The role of climate in the origin and adaptations of humans relates not only to our past but also, potentially, to our future (*1*). A number of hypotheses propose that climate-driven environmental changes during the past 7 million years were responsible for hominin speciation, the morphological shift to bipedality, enlarged cranial capacity, behavioral adaptability, cultural innovations, and intercontinental immigration events (*2*–*9*). These hypotheses are based on correlations between global-scale climate shifts documented in oceanic deposits and events in hominin evolution recorded in continental fossil-bearing strata. Establishing cause-effect relationships between climate and human evolution is tantalizing but presents many challenges for paleoanthropology and the geological sciences.

The biggest challenge involves how to relate different types and scales of paleoclimatic evidence between the marine and terrestrial realms. Marine-core records show that a cooler, drier, and more variable global climate regime began about 3.0 million years ago (Ma), gradually intensifying into northern continental glacial cycles by 1.0 Ma (*10*–*12*). The climate shift between ~3.0 and 2.5 Ma thus marks the onset of Northern Hemisphere glaciation (*10*–*13*), and this coincides generally with the timing of the origin of the genus *Homo* [reviewed in (*8*, *14*)] (see the figure). Fluctuations in continent-derived dust and biomarkers in the marine record indicate that climate shifts recorded in the oceans affected the land as well (*12*, *15*). However, in the continental basins that preserve hominin fossils, the record of climate change is much harder to decipher. Paleoclimatic proxy evidence includes stable isotope (*8*, *16*, *17*), pollen (*18*), mammal faunas (*7*), and lake versus land deposits (*9*, *19*, *20*). Although these signals are documented in many vertebrate fossil-bearing localities (*17*, *21*–*23*), each stratigraphic sequence represents only limited portions of the time-space framework of hominin evolution. In addition, the proxy records are subject to local tectonic and climatic processes that often obscure or completely overprint global-scale climate signals. Thus, we must confront the problem of relating a fossil record preserved in strata dominated by local- to regional-scale paleoenvironmental signals to a marine record dominated by continental- to global-scale signals. Long cores from deep African lakes could provide more continuous data and a stronger bridge between oceanic and continental climate records, but these are only beginning to be tapped (*24*).

Another challenge is deciding what constitutes a strong case for a causal link between a climate change and an evolutionary event. We can't step into a laboratory to test the impact of climate change on the human genome, but we do have the results of natural experiments—the proxy evidence for environmental changes in continental rock sequences, as well as many fossils of hominins and other organisms that were evolving on different continents during that same time period. There is a rich body of data to draw upon, but hypotheses are often structured around an assumption that "synchronous" events in the geological and paleontological record constitute evidence for cause and effect. These hypotheses, while seductive in their simple explanation of how our species came to be, do not do justice to the complexity of the climate-evolution problem (see the figure) or to the full range of evidence and scientific methodologies that now can be brought to bear on this problem.

Research into human origins, as well as other fields of science, uses probability-based evidence to test cause-effect hypotheses. Establishing a

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