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Laboratory Experiments Take the Express Route to Evolution

Laboratory experiments have enabled researchers to bypass half a billion years of evolution, giving one protein the ability to function like a distantly related protein with just a few simple changes. The elegant experiments illustrate a powerful way to probe the structure of proteins and may open a way to making more effective pharmaceuticals.

Using a technique known as directed evolution, Howard Hughes Medical Institute researchers randomly altered the structure of a protein that helps newly formed proteins fold into their proper shape. They found that subtle changes were enough to give that protein the ability to protect cells from the toxic element copper - even though the naturally occurring protein that serves that role has a distinct structure, molded by hundreds of millions of years' of evolution.

"If we can understand how *E. coli* creates these bonds, we can build the *E. coli* we want, and this might allow us to create medically important proteins more quickly and easily."

— James C. A. Bardwell

Usually people consider evolution from a retrospective perspective, looking at the sequences of proteins as they now exist and trying to deduce how these proteins might have changed to have the function they now have, said James C.A. Bardwell, a Howard Hughes Medical Institute investigator at the University of Michigan, who led the team that conducted the research. We took one protein that was related to another and tried to see if we could convert the first protein into one having the function of the second protein. The group's work is described in a paper published online in the *Proceedings of the National Academy of Sciences* the week of June 25, 2007.

The proteins Bardwell and his coworkers studied — DsbC and DsbG — are helper proteins that enable newly formed amino acid chains to fold into their proper protein configurations. In particular, DsbC rearranges the bonds between sulfur atoms in proteins, creating a framework of disulfide bonds that act as struts to hold the protein together and allow it to fold and function

properly. The correct arrangement of disulfide bonds is often so important to a protein that without the right arrangement, the protein does not fold correctly and is destroyed, said Annie Hiniker, an MD-PhD student at the University of Michigan who is the first author of the paper.

The two proteins are both descended from a common ancestral protein, just as two distantly related cousins share a common ancestor. But over the last half billion years, they have evolved distinct differences. For example, the sites where they bind newly formed amino acid chains are shaped differently, and they have distinct patterns of charge on their surfaces. In fact, only about 25 percent of the amino acids in their sequences are the same, said Hiniker.

DsbC — but not DsbG — can serve as an antidote to the toxic element copper. When copper gets into a cell, it can cause incorrect sulfur bonds to form in proteins, ultimately killing the cell. But DsbC counteracts this effect by putting the sulfur bonds in the correct places, enabling a cell to grow in copper-containing environments. Could directed evolution give DsbG the protective effects of DsbC? Bardwell's team decided to find out.

First they mutated the *DsbG* gene, creating thousands of DsbG proteins that differed from the original DsbG by one to four amino acids. They then placed the mutated *DsbG* genes into *Escherichia coli* bacteria that lacked the ability to make DsbC and grew the cells in Petri dishes containing copper. If a mutant DsbG protein could protect the cell from the copper, the cell survived and produced a colony in the dish. You don't see the mutants you're not interested in, because they die, explained Bardwell. The ones you want grow as a colony, so you select them and sequence them.

From 110,000 DsbG mutants, Bardwell's group found four that nicely reproduced at least part of DsbC's copper-protecting activity, both in the cell and the test-tube. Surprisingly, each of the four differed from the original DsbG by just a single amino acid. You could change a protein into one having the function of another just by single mutational changes, said Bardwell, even though there have been hundreds of mutations that have occurred over evolutionary time.

Structural analysis of the mutated proteins revealed that the mutants had not undergone a massive change of shape — the alterations were more subtle. Several of the mutations altered the position of a particular part of the protein. Several also changed a positively charged region near the binding site of the protein to a negative charge, sometimes in completely unexpected ways. One mutation, Bardwell said, moved a positively charged part of the protein out of the way, revealing a glutamic acid below — like a magic trick, so that now you have a positively charged patch. We would never have come up with that as a modeler or structural biologist. That was the bacterium coming up with a different way of producing the structure required for the function.

Besides offering valuable insights into how evolution tinkers with the structure and function of proteins, the experiment has important practical implications, Bardwell said. Most proteins produced as pharmaceutical agents have sulfur bonds that are critically important to the functioning of the

protein. But when bioengineered bacteria are put to work making pharmaceutical agents, they are less efficient than human cells at making those bonds, Bardwell said.

E. coli is a pretty good alchemist, Bardwell said. It can take commonly occurring substances like sugars, air, and salts and make very valuable pharmacological proteins out of them. But Bardwell and his colleagues would like to make *E. coli* a better alchemist by manipulating its ability to form sulfur bonds in the correct places.

If you can understand something, you can build it, said Bardwell. If we can understand how *E. coli* creates these bonds, we can build the *E. coli* we want, and this might allow us to create medically important proteins more quickly and easily.