Chemistry & Biology *13*, 1011–1012, October 2006 DOI 10.1016/j.chembiol.2006.10.003

Innovations

Amber Codon Flashing Ambrx Augments Proteins with Unnatural Amino Acids

Protein-based drugs, especially those that have a natural source, can be a recalcitrant lot. They may trigger severe immune reactions in patients or may survive only a short time in the body, requiring patients to take frequent injections to maintain consistent circulating drug levels. Despite these drawbacks, protein-based drugs such as insulin, interferon, and human growth hormone constitute a $40 billion market that is expected to reach almost $59 billion by 2010. Given their importance and market potential, pharmaceutical companies are looking for methods to optimize the therapeutic properties of these proteins.

A commonly used method for optimization is direct mutagenesis, which generates a sequence of random mutations in the gene that encodes a particular protein. The protein can be either the drug itself or an enzyme involved in the synthesis of the protein drug. The mutation will alter the sequence of amino acids in the target protein, potentially changing its functionality. "Site-directed mutagenesis is the backbone of modern biology," but "we are still horrible at being able to predict what will be the stability of a protein," says Thomas Magliery, assistant professor of chemistry at Ohio State.

Over the past two decades, chemists have started incorporating "unnatural" or synthetic amino acids into proteins to optimize their functionality and to enable scientists to unravel complicated biochemical processes in living organisms. "Unnatural amino acids are in that phase where they will be snowballing in the next five years and everyone will be using them for a variety of uses," said Magliery.

"In contrast to chemists' ability to manipulate small molecules, they really can't manipulate proteins, except … by site-directed mutagenesis," said Peter Schultz, professor of chemistry at The Scripps Research Institute and director of the Genomics Institute of the Novartis Research Foundation (GNF). While his group first used an in vitro approach to proteins, they experienced difficulties in making the aminoacylated tRNAs and in producing any reasonable amount of protein, as it is a stoichiometric process. In the late 80s, Schultz's group spent five years figuring out how to put novel amino acid structures into proteins by genetic engineering.

Ambrx uses its patented technology platform, called ReCode, to substitute artificial amino acids into proteins of bacteria and yeast with efforts in mammalian cells in the works.

"Probably the biggest tool will be drug discovery." Schultz said. "Roughly a third of INDs filed are protein therapeutics. We've traditionally had small molecule therapeutics, and there is going to be an increasing pipeline in biologics. When chemists make small molecule therapeutics, they spend a lot of time optimizing the biological properties, the stability, the chemical properties, the specificity, and everything else. When people make protein therapeutics, you are kind of stuck with what nature gave you."

This method enables you "to chemically modify proteins, but do it genetically." Schultz said. "So everything is absolutely pure, because you are genetically encoding it. You are making protein at the level of the ribosome, and you have tremendous translational fidelity."

A Little Something Extra

San Diego-based Ambrx (www.ambrx.com) and others take that into practice in optimizing proteins to meet the desired properties for biologic drugs. Schultz cofounded Ambrx in 2003 with Richard DiMarchi, former head of biologics at Eli Lilly, where he was a codeveloper of Humalog, an insulin analog, and Troy Wilson. (Schultz is a serial entrepreneur and has cofounded other companies including Affymax Research Institute, Syrrx, Kalypsys, Phenomix, Symyx Therapeutics, and Ilypsa.)

The privately held, 58 person company has been capitalized by three rounds of venture financing and private placement comprising about $90 million, says Martin Mattingly, president and CEO. The company's series C financing in 2005 for $52 million led by Apposite, included Glynn Ventures, the Dow Employees' Pension Plan, and Union Carbide Employees' Pension Plan, as well as other venture funds.

Ambrx uses its patented technology platform, called ReCode, to substitute artificial amino acids into proteins of bacteria and yeast, with efforts in mammalian cells in the works. The company is using their platform to tailor proteins as drugs and to optimize already existing protein-based drugs by reducing their toxicity and/or increasing their half life. "The goal of the process is to incorporate into a biosynthetic protein, in our case therapeutic proteins, chemistry that is not used by natural organisms for the express purpose of making better drugs." says Thomas Daniel, M.D., chief scientific officer.

Ambrx achieves this by using an engineered tRNA so that the coding sequence of the desired protein incorporates a synthetic amino acid at an amber codon rather than letting this codon signal for translation

termination. Influencing one codon will not change the synthesis of the protein in most cases. "That tRNA has been modified so that it reads the amber codon as a coding codon," says Daniel. "It gives you tremendous control because you put the stop anywhere in the recombinant DNA to direct the site on which the amino acid is loaded." The particular enzyme that attaches the synthetic amino acid to the tRNA that is associated with the amber codon, the synthetase, is developed by direct mutagenesis.

A Spoonful of Sugar Makes The Medicine Go Down—and Last Longer

The novel amino acid that Ambrx adds to proteins enables the specific attachment of PEG (polyethylene glycol) to the protein, a process known in the industry as PEGylation. PEGylating proteins such as interferon, G-CSF, and erythropoietin coats the offending bits and helps the compounds to reside longer in the body. Drugs improved by PEGylation include Amgen's Neulasta and Roche's Peginterferon alfa-2a, called Pegasys. However, with current technology, it is impossible to reliably attach PEG chains of consistent molecular weight to proteins. Ambrx can use ReCode to precisely control the PEGs on each molecule. PEGylating proteins is the company's first application, but it has also added antibodies and small molecules to the surface of proteins. The company hopes to cut the cost of PEGylation on an industrial scale as well.

ReCoding Proteins

Currently Ambrx is using ReCode to optimize human growth hormone. According to Mattingly, "we can make scores of versions" of the growth hormone as well as making multiple changes to the protein in multiple places, and by moving the site of PEGylation by an amino acid, "we can get a substantial difference in half life of the drug." Ambrx is expecting to initiate HGH clinical trials in early 2007. The company is also collaborating with Roche, an investor in Ambrx, on a PEGylated version of interferon α.

"If you think of penicillin, a native substance, today you have a second and third generation, from the same core," said Mattingly. "Imagine the future of protein drugs. If you start with active drugs, the potential is enormous." A key hypothesis behind Ambrx's methodology is that adding one synthetic amino acid to a peptide or protein that is made of hundreds if not thousands of amino acids will not significantly alter the 3D structure of the protein. Working within the existing pharmacopeia will serve as a springboard for the company to move on to developing novel proteins as therapeutics.

Getting into the Unnaturals Biz

"A lot of people are doing unnaturals," said Dennis Dougherty, professor of chemistry at Cal Tech. "There is no doubt that Schultz is the pioneer." According to Dougherty, the first method that Schultz developed in 1989 used chemistry to put the amino acid on the tRNA. Then, that reagent was used to incorporate the unnatural amino acid. "Pretty much apart from Pete, nobody in the world used that method because you couldn't make very much protein," said Dougherty. "Because the RNA with the amino acid attached was kind of a difficult reagent to come by, expensive and so on." Now, other groups are taking various approaches to protein design with unnatural amino acids. Uttam RajBhandary, professor of molecular biology at MIT, is trying to insert two or more nonnatural amino acids into proteins, mostly in eukaryotic cells. Shigeyuki Yokoyama of the University of Tokyo is also inserting unnatural amino acids into proteins in mammalian cells. According to Dougherty, Sidney Hecht at the University of Virginia developed the fundamental methodology behind the early Schultz work, but Hecht did not at first incorporate unnatural amino acids, although he is using them now.

Dougherty also commented that the more recent advances that Ambrx utilizes, with which the laboratory of David Tirrell, professor of chemistry at Cal Tech, is also now involved, use an enzymatic method to charge the amino acid onto the tRNA. The result is catalytic turnover, and the system becomes almost like normal protein expression, enabling the expression of much larger quantities of protein.

Dougherty's neuroscience laboratory at Cal Tech, in collaboration with the biology laboratory of Henry Lester, uses Schultz's protocol to study ion channels and neural receptors in vertebrate cells, which requires only small quantities of protein. Dougherty founded a small company, Neurion, to do ion channel drug discovery. According to Dougherty, sixty percent of the targets of drug discovery are membrane proteins, which are not readily amenable to structural biology techniques such as NMR or crystallography. However, the unnatural amino acid technique provides detailed information on drug receptor interactions.

Moving Beyond Proteins

David Tirrell is experimenting with producing fluorinated amino acids as probes. "The really pioneering feature of Tirrell's work is he was certainly the first chemical engineer to recognize that one could engineer proteins in *E. coli* for materials properties and overexpress enough protein to make it reasonable for natural amino acids and, subsequently, with unnatural amino acids," said Dougherty. "The chemical strategy is virtually limitless in terms of amino acids you can use. The catalytic system, if it becomes generally available and in use, could be very powerful. We will see if other labs adopt it."

Ryan Mehl, a former postdoc in Schultz's laboratory, now an assistant professor of chemistry at Franklin and Marshall College, published a paper in 2003 describing an engineered *E. coli* that produces a nonnatural amino acid and readily incorporates it into proteins. Mehl envisions sticky social implications. It is "improving evolution with intelligent design," he quipped.

Wendy Wolfson (wendywolfson@nasw.org) is a science technology writer based in Oakland, CA.