MEETING REPORT

GENETIC APPROACH APPLIED TO PROTEIN DESIGN

STONY BROOK, NY—In the tradition of the molecular biologists who deciphered the genetic code twenty years ago, scientists at this year's Stony Brook Symposium aimed at elucidating the more complex rules relating protein conformation to amino acid sequence.

John Abelson (California Institute of Technology), Robert Sauer (Massachusetts Institute of Technology), David Shortle (Johns Hopkins School of Medicine) and Jonathan King (MIT) demonstrated that the genetic approach characteristic of "Classical Period Molecular Biology" is still a powerful tool for revealing structure-function relationships.

Abelson's group has constructed a totally synthetic collection of novel suppressor transfer RNA (tRNA) genes whose products insert, in turn, each of the twenty amino acids in response to a UAG termination codon. Using this collection in conjunction with oligonucleotide site-directed mutagenesis will make it feasible to perform comprehensive amino acid substitutions at any position in a protein. Abelson says that after the collection is fully characterized, he will make it available (at no charge) to academic researchers and (for a nominal fee) to companies.

As powerful as they are, amino acid substitution studies are designed to answer only specific questions about protein design. (For example, what is the effect of changing amino acid X to amino acid Y at position 123 in protein Z?) A way of getting information concerning more general aspects of protein structure is to use genetic selection to provide variants with desired phenotypes. Second-site reversions were used first to establish the colinearity of gene and protein, and later to define the basic features of the genetic code. In the hands of Robert Sauer and David Shortle, second-site reversions are a means of obtaining mutant proteins with increased activity and stability.

Sauer described work from his laboratory—by Michael Hecht and Hillary Nelson—aimed at determining the roles of amino acid side chains in mediating the activity of procaryotic DNA binding proteins. Beginning with a mutant λ repressor that has reduced binding to operator DNA *in vivo*, these scientists isolate revertants of the mutant with restored binding activity. Some of these revertants have amino acid substitutions at positions other than the site of the original mutation (second-site revertants). Further, many of the second-site revertants have global effects—that is, they restore activity to a variety of primary mutants. And several repressors bearing *only* the second-site substitutions have enhanced operator affinity. One amino acid substitution that improves the affinity of the wild-type repressor for the operator is in a residue that does not contact the DNA helix (see figure), showing the importance of long-range interactions in protein conformation. These interactions were not predictable even though detailed X-ray crystallographic data are available.

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Computer-generated representation: Pabo and Lewis's model of the λ repressor-operator complex. The view is down the end of the DNA helix (shown in green). The two chains of the repressor dimer are in blue, and the red corresponds to amino acid substitutions that confer enhanced operator binding affinity to the repressor.

Shortle has used a similar approach to isolate variants of staphylococcal nuclease with increased stability. Again, beginning with a collection of nuclease-defective stability mutants, Shortle isolated a set of revertants. A number of second-site revertants (suppressors of the original mutation) display global suppression patterns. Preliminary characterization of purified proteins bearing only these substitutions indicates enhanced stability.

Jonathan King proposes a complementary strategy. He is investigating the protein folding "code" by analyzing amino acid substitution mutations that influence the folding *pathway* rather than by examining changes that affect properties of the completed protein. Just as one could not infer the presence of signal peptides by examining the mature secreted protein, King argues that residues not important once a protein has assumed its native configuration may nonetheless be critical in the pathway that leads to the mature protein. King has isolated mutants of the tail-spike protein of phage P22 that show a temperature sensitivity for their folding (TSF mutants). These mutants are indistinguishable from their wild-type counterparts in a variety of functional assays—even at the non-permissive temperature—if they are synthesized at permissive temperatures. Analysis of this collection has already produced some intriguing results. Two sequences (residues 232–236 and 365–369) containing threonine-to-isoleucine mutations show direct homology. King, somewhat tongue-in-cheek, offers the term "foldon" to describe such sequences, and hypothesizes that in this particular case the "foldon" Tyr-Gln-Pro-Thr-Val may signal a β-sheet bend. **—Harvey Bialy**