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NEWS AND VIEWS

The intelligent design of evolution

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The debate between intelligent design and evolution in education may still rage in school boards and classrooms, but intelligent design is making headway in the laboratory. In this case, though, the designer turned out to be just some clever scientist. A recent paper in *Nature* (Yoshikuni *et al*, 2006) presented the iterative evolution of highly specific catalysts from a promiscuous wild-type enzyme via what the authors refer to as designed divergent evolution.

The paper investigated whether catalytic functionality could be rationally engineered into a protein, without recourse to the high-throughput screening techniques necessary for directed evolution. Yoshikuni *et al* (2006) started with a terpene synthase enzyme, γ-humulene synthase, that is promiscuous not in its substrate specificity but in its product selectivity—it catalyzes the formation of 52 different sesquiterpene products from one single substrate, farnesyl diphosphate. (Sesquiterpenes naturally occur in a variety of plants, and their derivatives are used in applications ranging from chemical feedstocks to antifungal compounds.) The predominant product for the wild-type enzyme is γ-humulene, but Yoshikuni *et al* designed seven mutant variants with improved selectivities for eight of the products.

How did they do it? Using prior knowledge of the active sites in the terpene synthase family and the crystal structure of another terpene synthase, the authors identified a set of 19 candidate 'plasticity' residues in γ-humulene synthase that lie along the contour of the active site. ('Plasticity' residues are residues not essential for core catalytic functionality, but that may still interact with the substrate to control product selectivity.) They performed all possible single amino-acid mutations over each of the 19 residue positions and catalogued the resulting single mutants' product selectivities. Finally, they assumed that the mutations were additive—that the effect on selectivity of combining two mutations could be predicted by adding the effect of each mutation done singly. With this assumption, it was straightforward to predict combinations of single mutations identified as controlling selectivity without decreasing the total productivity. The striking result of this design is that the simple additivity assumption was validated—the authors obtained several triple to quintuple mutants with nearly perfect selectivities for the product they targeted. Apparently, intelligent design does not need irreducible complexity after all.

The success of this exercise is particularly relevant to the field of molecular evolution, where the degree to which mutations are nonadditive has been debated at length. Some believe that recent work in developing protein mutant libraries supports the hypothesis that nonadditivity is the rule rather than the exception, and occurs much more often than believed previously (Zaccolo and Gherardi, 1999). Others have put forth both theoretical and experimental evidence suggesting that, though frequent nonadditivity of mutations is still a possibility in these contexts, its existence is not yet supported well (Drummond *et al*, 2005). Since the method of Yoshikuni *et al* identifies only additive mutations, the success of the method implies that the space of well-behaved, additive mutations is big enough for engineering potent changes in activity (see Figure 1). The natural prevalence of nonadditivity in mutations may still be a point of debate, but it might be irrelevant to the protein engineer if the case of γ-humulene synthase is representative of nature as a whole.

While similar approaches will likely find broad applicability in protein design, it may not always be easy to directly apply the method of Yoshikuni *et al* to other enzymatic systems, for a number of reasons. First, the targeted protein must be at least somewhat promiscuous for this technique to be effective, though this restriction is common to some other techniques for evolving protein function (Aharoni *et al*, 2005). Second, the residues responsible for the energetically costly step in catalysis must generally be distinct from the plasticity residues that determine the enzyme's product selectivity. In the case of γ-humulene synthase, all of the 52 possible products are derived from a small set of carbocation intermediates that are energetically costly to form, and the residues responsible for this activity had been identified previously. To control selectivity, the enzyme only has to steer the unstable, reactive intermediate down the proper energetic path toward the desired product. These steps, and the residues responsible for them, are decoupled from the formation of the intermediate and the residues necessary for that step, which allows for mutations that affect selectivity but not overall activity. The designed evolution strategy is likely limited to proteins with such decoupling of steps and residue functionality, otherwise the maintenance of total activity may be extremely difficult and the assumption of independence of mutational effects may no longer be valid.

Barring these limitations, the potential applications of Yoshikuni's approach to other proteins and reactions are quite exciting. Many other promiscuous proteins are known and well studied. For example, all enzymes in the enolase superfamily generate a common, high-energy intermediate that is converted by different enzymes into different products. Recent work has shown that these enzymes too can be endowed with altered activity, although doing so required, in

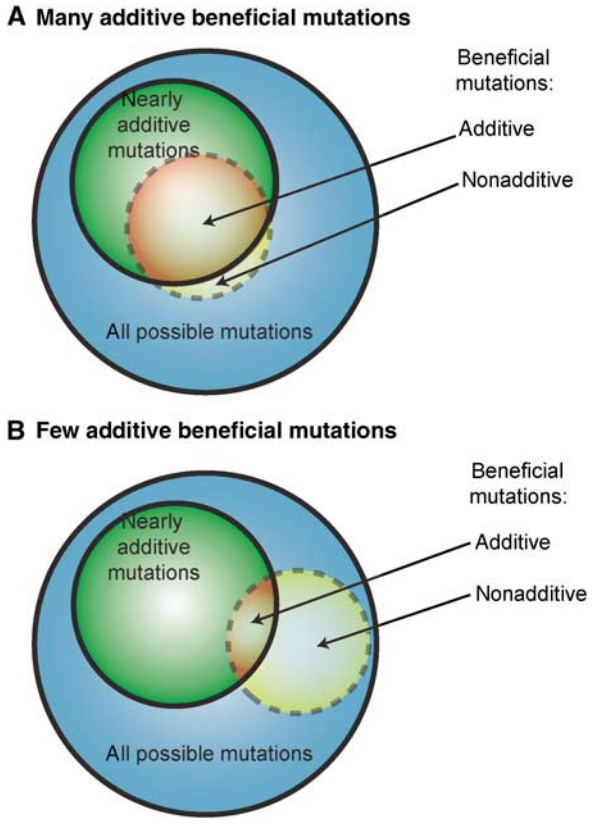

Figure 1 Two possible relationships in mutation space between additive mutations (shown in green) and experimentally identifiable and beneficial sets of mutations (pink and yellow). In (**A**), most of the identifiable, beneficial mutations are nearly additive. In this case, the experimental techniques that focus only on the identification of additive mutations (pink) will reveal most of the identifiable beneficial mutations. In (**B**), only a small subset of all of the identifiable sets of mutations is identified. However, the results in Yoshikuni *et al* show that even if case (B) is the natural reality, the subspace of identifiable, beneficial, and additive mutations (pink) is of sufficient size to effect potent changes in enzymatic activity.

some cases, the identification of nonadditive pairs of mutations (Vick *et al*, 2005). Exhaustive generation and analysis of single-mutant data may nonetheless speed these efforts.

The design strategy presented by Yoshikuni *et al* is particularly exciting in the context of metabolic engineering. This field, focused on the redesign of organisms for the production of valuable chemicals or other ends, could benefit greatly from this novel approach. The expansion of the available repertoire of proteins by rational design could significantly increase our ability to control which products are produced and in what quantities. The ability to design an enzyme's product selectivity raises the intriguing possibility of controlling the flow of metabolism between two competing pathways in the cell by constructing enzyme mutants that bypass native control mechanisms which frequently frustrate our efforts. In addition, with so many of our methods dependent on high-throughput screens for the discovery of viable, productive mutant strains, the designed divergent evolution approach offers a promising reprieve that could open the door to microbial production of compounds that may have been ignored because a good-enough screening technique was not available.

So, scientists everywhere may soon begin their own intelligent designs… and so far, it looks like the best designs are the simplest. At the protein level, at least, it looks like irreducible complexity is out and a rather reducible simplicity is in. Intelligent design, however, may be here to stay.

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