Amino Acid Changes in Protein Homologs Have Similar Effects on Stability

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Phylogenetic studies of protein evolution assume that changes in a protein at a given amino acid occur independently of other amino acids in the protein. However, the actual process of protein folding depends upon an intricate series of interactions between the constituent amino acids for a polypeptide to assume its appropriate conformation. A recent computational study suggests that some mutations may dramatically impact the stability of a protein in some genetic contexts, but not in others (Pollock *et al*., 2012). In a recent paper published in the *Proceedings of the National Academy of Sciences (U.S.A.),* Dr. Orr Ashenberg, L. Ian Gong, and Dr. Jesse D. Bloom (Division of Basic Sciences and Computational Biology Program) tested this question experimentally, and demonstrate that mutations tend to affect the stability of divergent protein homologs in similar ways. This work suggests that epistasis during protein evolution is unlikely to cause frequent, unpredictable changes that would confound current phylogenetic approaches.

In general, epistasis is an interaction in which a change at one site modifies the phenotype of a different site. For example, previous work from the Bloom laboratory demonstrated that some mutations that evolved during the natural history of influenza nucleoprotein (NP) were only fixed after an earlier, stabilizing mutation occurred in NP. Without the epistatic stabilizing effect of this earlier mutation, these mutations destabilized NP on their own (Gong *et al*., 2013). However, it is unclear whether the stabilizing effect of a mutation in one genetic background would have the same stabilizing effect in other NP ortholog contexts. To test this hypothesis experimentally, the authors analyzed the impact of six naturally occurring mutations on a panel of four influenza A NP homologs ranging in identity from 94% to 72%. These mutations have been experimentally determined to be stabilizing, neutral, or destabilizing in one of the NPs included in this panel (Gong *et al*., 2013). When these mutations were introduced into the other members of the panel, the authors found that the impact of each mutation on protein stability, as measured by melting temperature, was largely conserved across all four homologs, even in the most divergent NP isolated from a bat.

This experimental approach was limited to only four homologs. Therefore, the authors also used a computational approach to introduce random mutations into NP and predict the relative stability of

the parent and mutated proteins over a broader range of simulated homologs. Using two separate mathematical models of intramolecular interactions the impact of mutations on protein stability was predicted to be largely similar across proteins that diverged as much as 50% from each other. Ashenberg *et al*. introduced altered one methodological approach that may account for the difference between this current study and a previous computational study. In the Pollock *et al*. study, destabilizing mutations were retained during the simulated process of evolution, forcing stabilizing mutations to arise at distant sites in the protein. In contrast, Ashenberg, *et al*. permitted destabilizing mutations to revert to more stable residues in their simulation, a method more closely resembling the natural evolutionary process.

The current study demonstrates both experimentally and computationally that the effect of a given mutation on protein stability is largely conserved between different homologs. Although the relative impacts of mutations on protein stability are conserved, the authors emphasize that these results do not suggest that epistasis does not play a role in protein evolution. Rather, this study provides evidence that sudden and dramatic changes in protein stability are unlikely and support the validity of site-independent substitution models as an approximation for phylogenetic analyses. However, the authors do note that certain sites in a protein are biased to particular amino acids. "We are now pursuing a novel approach where the site preferences are measured directly by experiment. We believe this approach will be significantly more accurate than existing approaches, and we will apply it to a better understanding of influenza's evolutionary past," said Dr. Orr Ashenberg.

Ashenberg O, Gong LI, Bloom JD. 2013. Mutational effects on stability are largely conserved during protein evolution. *Proc Natl Acad Sci U S A*. 110(52):21071-6.

See also: Gong LI, Suchard MA, Bloom JD. 2013. Stability-mediated epistasis constrains the evolution of an influenza protein. *Elife*. doi: 10.7554/eLife.00631.

See also: Pollock DD, Thiltgen G, Goldstein RA. 2012. Amino acid coevolution induces an evolutionary Stokes shift. *Proc Natl Acad Sci USA* 109(21):E1352–E1359.

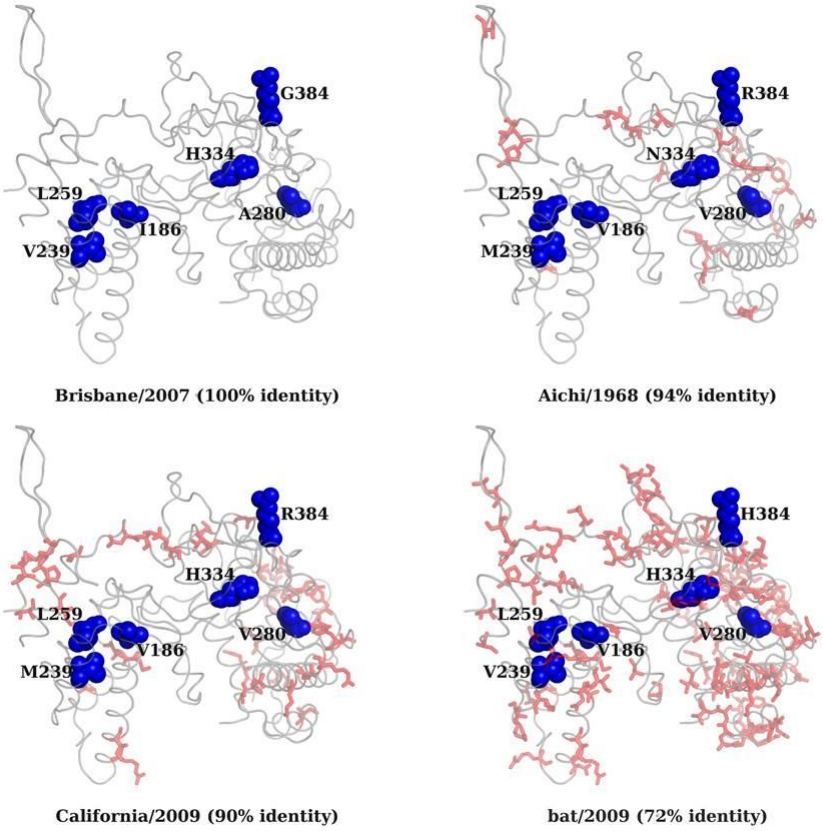

Image courtesy Dr. Orr Ashenberg.

Wire diagrams of the four NPs included in the study. Blue amino acids highlight the residues that were experimentally mutated during this study, while the red residues highlight amino acid differences relative to the Brisbane/2007 NP.