Molecular Cell, Vol. 17, 471–477, February 18, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.molcel.2005.02.005

Planetary Systems Biology

Combining paleogenetics, protein engineering, synthetic biology, and metabolic modeling, a planetary biology perspective is brought to bear on adaptive evolutionary events in ancient bacteria.

Darwinian theory holds that natural selection operating on randomly generated chemical structures is the only mechanism to create biomolecules that confer fitness upon their hosts. A considerable gap separates this biological truism from experimental reality, however. As Kreitman and Akashi (1995) noted a decade ago, making the connection between molecular behavior and fitness has proven to be remarkably difficult.

The difficulty arises in large part because of the difference in scale and complexity of molecular behavior (on one hand) and fitness (on the other). In reductionist biology, the behavior of biomolecules is measured on isolated systems removed from their natural environment in virtually every way. Even the buffers commonly used in reductionist biology (such as Tris and HEPES) are not found naturally.

Fitness, in contrast, concerns not only the biomolecule and its contacts within a network inside a cell but also the consequent impact on the cell, organ, and organism, where the value of this impact depends on the prey that the organism eats, the predators that eat it, and the environment, which can change on both a local and planetary scale, even as a consequence of cosmic events (such as a meteorite impact) (Benner at al. 2002). A model for fitness must somehow capture all of these.

At the very least, this requires a polydisciplinary approach to the problem. Polydisciplinary ways to indirectly infer whether a change in molecular behavior is adaptive in a changing environment have become rather clever. These include using paleobiochemical experiments; resurrecting ancestral proteins from extinct organisms so as to recapitulate, in the laboratory, the history of changing biomolecular behavior; and showing how it is rational in the context of the changing ecosystem of the ancient organisms, inferred from paleontology and geology. The adaptive significance of changing behaviors of proteins from eosinophils has been analyzed in this way (Zhang and Rosenberg, 2002). Likewise, a case has been made that the sequence evolution in ribonucleases and lysozymes in ruminants in the Oligocene was adaptive and neutral, respectively, because the changing behavior in resurrected ancestral proteins is sensible in light of changing physiology as ruminant digestion arose (Jermann et al., 1995) (Malcolm et al., 1990).

In a recent publication in *Science*, Zhu et al. (2005) provide a different but equally polydisciplinary approach. Their study targets the protein superfamily that includes isocitrate dehydrogenases (IDHs) and isopropylmalate dehydrogenases (IMDHs). These come in two classes: those that generate NADPH and those that generate NADH. Analysis of the tree interrelating these proteins suggested that their common ancestor generated NADH; in the language of molecular evolution, the production of NADPH is a "derived" trait. The authors then asked whether the emergence of this new phenotype (generating NADPH) was adaptive.

By comparison of the crystal structures of *E. coli* IDH and *Thermus thermophilus* IMDH complexes (Hurley and Dean, 1994), the authors identified amino acid residues involved in selecting the particular nicotinamide cofactor. Through mutation of these residues, they engineered a modified *E. coli*-IDH in which the cofactor specificity is inverted, generating NADH instead of NADPH. The authors then used competition experiments to show that *E. coli* containing the engineered IDH (making the unnatural NADH) is at a selective disadvantage relative to wild-type bacteria in the presence of acetate. From this they inferred that the historical adaptation of IDH to generate NADPH (not NADH) was adaptive in an environment that came to contain acetate.

The observation that an *E. coli* containing an engineered protein is at an adaptive disadvantage relative to native *E. coli* is not surprising and would not make a compelling case for anything in particular. Having evolved for billions of years, natural proteins are expected to be preferred. What makes the case more compelling is that the *E. coli* host that expressed the NADH-generating IDH was at a disadvantage in an environment containing acetate, but not in an environment containing glucose. In addition, the *E. coli* containing the NADH-generating IDH was further engineered to remove other pathways that might biosynthesize NADPH. As alternative sources of NADPH were removed, the selective disadvantage of the strain containing the engineered IDH increased.

Interestingly, the study can be tied to the geological record. The various forms of IDH appear to have diverged at the time when the mitochondria first appeared in eukaryotes (Zhu et al., 2005). As mitochondria use dioxygen, it is not implausible to suggest that the emergence of an NADPH-generating IDH was approximately contemporaneous with the emergence of atmospheric dioxygen. Acetate is not, of course, more highly oxidized than glucose. The arrangement of oxygen atoms in acetate creates a more stable species, however, and acetate may have become more abundant as dioxygen arose as well. Although the timing of the emergence of dioxygen is probably not as old as 3.5 billion years, as Zhu et al. suggest, it was certainly very ancient, with dioxygen almost certainly being present in substantial amounts 2 billion years ago and still larger amounts with the emergence of multicellular animals ca. 600 million years ago (Runnegar, 1991).

Engineering organisms to argue for adaptive change is, of course, most easily applicable to microorganisms. It is unlikely that we will, any time soon, test adaptive hypotheses in higher organisms by genetically altering a ruminant (for example). The fossil record for microorganisms is very poor compared with the record for verte-

brates and other metazoa, however. This means that the genetic engineering approaches used to argue for adaptation of the type developed by Zhu et al. will be useful for organisms where the paleobiochemical approaches are least useful.

But as macro and microorganisms also interact, the fitness of one determines the fitness of the other. As studies similar to the ones outlined above accumulate, we should be able to combine fitness tests on the two. This will, in turn, lead to a true planetary systems biology, one that spans the biological world from the microorganism to the macroorganism, captures the ecology and planetary history as well, and supports a broad understanding of the role of adaptation, vestigiality, and neutral drift in determining the chemistry of the life that we know on earth today.

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Molecular Cell, Vol. 17, February 18, 2005, Copyright ©2005 by Elsevier Inc. DOI 10.1016/j.molcel.2005.02.001

OS-9: Another Piece in the HIF Complex Story

In this issue of *Molecular Cell*, the Semenza group reports that OS-9, a common protein of unassigned function, promotes the O_2-dependent degradation of hypoxia inducible factor (HIF) via binding to both HIF and the HIF prolyl-hydroxylases, implying that OS-9 is part of a multiprotein complex involved in the hypoxic response (Baek et al., 2005).

The response to decreased oxygen concentration (hypoxia) in the tissues of metozoans is mediated by an α/β-heterodimeric transcription factor, HIF, identified by Semenza and Wang (1992). In hypoxic conditions, HIF binds to response elements and enhances the expression of a set of genes that enables organisms to adapt to reductions in oxygen availability. In humans, hypoxically regulated genes include those encoding for proteins that regulate angiogenesis and erythropoiesis; hence, there is medicinal interest in the HIF signaling pathway and its modulation.

Unlike the β subunit of HIF, which is a constitutive protein, levels of HIF-α are sensitive to oxygen levels. In the presence of sufficient oxygen, HIF-α is degraded via the ubiquitin-proteasome pathway. A separate oxygen-dependent process that blocks the interaction between the C-terminal transactivation domain of HIF-α and the p300 component of the transcription complex also regulates the transcriptional activity of HIF. At the molecular level, the key oxygen-dependent event in both these processes is the posttranslational hydroxylation of HIF-α.

Hydroxylation of conserved prolyl residues in the oxygen-dependent degradation domain of HIF-α enables binding to the von Hippel-Lindau protein (VHL) that acts as a targeting protein for a ubiquitin E3 ligase (Kim and Kaelin, 2003). Three human HIF prolyl hydroxylases (PHDs 1–3) and one asparaginyl hydroxylase have been identified (Bruick, 2003; Schofield and Ratcliffe, 2004). The latter, termed factor inhibiting HIF (FIH), was shown by the Semenza group to associate with and inhibit the transcriptional activity of HIF-α (Mahon et al., 2001). In the light of this work and the discovery that the transcriptional activity of human HIF-α is blocked by hydroxylation of Asn-803, it was subsequently shown that FIH is the HIF asparaginyl hydroxylase (Peet et al., 2004)

Although VHL and the HIF hydroxylases have been identified as central to the HIF-mediated hypoxic response, much remains to be done to complete a molecular description. The apparent multidomain architecture of HIF linked to other problems, including the intrinsically disordered nature of at least part of its sequence, makes this a significant challenge. The Semenza group has now discovered a common partner for HIF-α and the PHDs. In this issue of *Molecular Cell*, Baek et al. (2005) describe elegant work wherein use of a C-terminal fragment of HIF-1α (residues 576–826) in a yeast two-hybrid screen led to the identification of OS-9 as a HIF-1α binding partner.

Immunoprecipitation analyses demonstrated that the OS-9-HIF-1α interaction occurs in both hypoxic and normoxic cells and, significantly, that OS-9 also binds to PHD2 and PHD3. In cells cotransfected with HIF-1α, PHD2, and OS-9, the presence of OS-9 enhanced prolyl