EARLY STAGES IN THE EVOLUTION OF A NEW ENZYME ACTIVITY. Shelley D. Copley, Akhil Khanal and Jamie P. Kershner, University of Colorado Boulder, Department of Molecular, Cellular and Developmental Biology and Cooperative Institute for Research in Environmental Sciences, Boulder, CO 80309

Introduction: Metabolic enzymes are typically prodigious catalysts, catalyzing specific reactions by up to 26 orders of magnitude [1]. These enzymes often have secondary activities that result from binding of atypical substrates in the active site in proximity to catalytic residues, metal ions or cofactors. Secondary activities that do not affect fitness are termed promiscuous activities. Although promiscuous activities can be much less efficient than well-evolved activities, they often enhance reaction rates by orders of magnitude relative to those of uncatalyzed reactions. Thus, promiscuous activities provide a reservoir of novel catalytic activities that can be recruited to serve new functions.

The **I**nnovation–**A**mplification–**D**ivergence (IAD) model [2, 3] posits that new enzymes evolve from promiscuous activities that have become important for fitness. Gene amplification improves fitness by providing more of the inefficient enzyme. Subsequent mutations that improve the inefficient activity allow elimination of extra gene copies. The ultimate result is a pair of genes encoding specialized enzymes. The existence of enzyme superfamilies that have evolved from a common progenitor attests to the importance of this process. However, our understanding of the process itself is limited because most instances played out in the distant past in the context of a population of microbes whose genomic resources are untraceable and that lived in an environment that is unknowable. This talk will focus on two aspects of the early stages of emergence of a new enzyme activity.

The levels and evolvability of promiscuous activities in orthologous enzymes vary: Promiscuous activities in orthologous enzymes are likely to vary in efficiency due to neutral drift over millions or billions of years. An additional consequence of neutral drift is that mutations occur in ever more different structural contexts as time goes on. For both reasons, the potential for evolution of a promiscuous activity in orthologous enzymes may vary. We have shown that the levels of a promiscuous *N*-acetylglutamyl phosphate reductase activity in γ-glutamyl phosphate reductase (ProA) (see Fig. 1) vary substantially among nine bacterial enzymes. Remarkably, a change of the equivalent of Glu383 to Ala allows all of the orthologous enzymes to serve both functions *in vivo*. However, the effects of that single change upon the original and novel activities vary widely due to epistatic effects [4]. These results suggest that promiscuous activities in some organisms will be more evolvable than those in other organisms, and thus the potential for metabolic innovation may vary among organisms.

Evolution of a new activity by the IAD mechanism is complicated by mutations that increase fitness by simply increasing the level of an inefficient enzyme: When an inefficient enzyme limits growth rate, fitness can be enhanced by multiple mechanisms. Often the initial event increases the level of the inefficient enzyme, either by gene amplification or by increasing the level of transcription or translation. When the level of the enzyme is increased by the latter two mechanisms, the selective pressure to maintain multiple copies will be diminished, and progress toward two specialist enzymes will be slowed. The interplay between these factors will be discussed in the context of E383A γ-glutamyl phosphate reductase, whose inefficient N-acetyl glutamyl phosphate reductase activity can replace the function of an enzyme (ArgC) required for synthesis of arginine.

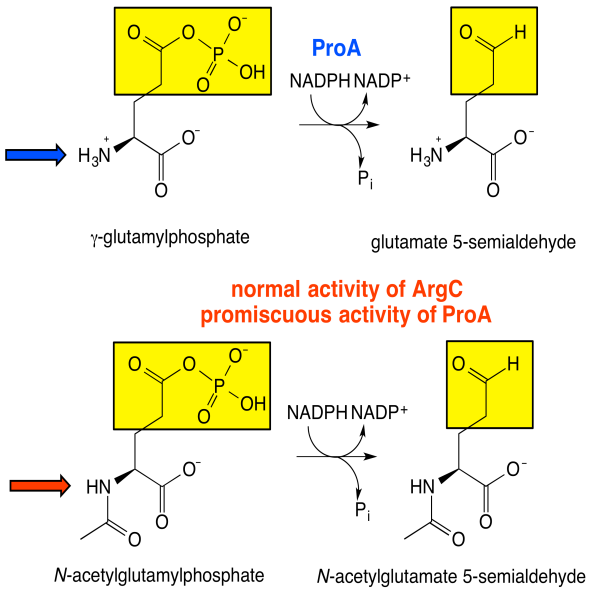

Fig. 1. ProA and ArgC catalyze the same chemical transformation using substrates that vary only at the position indicated by the arrows.

References:

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3. Hughes, A.L. (1994) *Proc R Soc Lond B, 256,* 119-124.
4. Khanal, A. et al. (2015) *Mol Biol Evol, 32,*100-108.