E. coli and *S. enterica* take different evolutionary trajectories when subjected to the same selective pressure.

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Introduction: When microbes are faced with an environmental challenge or opportunity, pre-existing enzymes with promiscuous secondary activities can be recruited to provide newly important functions. Mutations that increase the efficiency of a new activity often compromise the original activity, resulting in an inefficient bifunctional enzyme. We have investigated the mechanisms by which growth of *Escherichia coli* and *Salmonella enterica* can be improved when fitness is limited by such a weak-link enzyme, E383A ProA (ProA*). ProA* can serve the functions of both ProA (required for synthesis of proline) and ArgC (required for synthesis of arginine) (Fig. 1), albeit poorly [1,2].

Results: A strain of *E. coli* that lacks *argC* and carries the *proA** allele is under strong selection for improvement in the ability to synthesize arginine and proline. We have identified four genetic changes that improve growth rate in this strain by up to 6.2-fold [3]. Either of two point mutations in the promoter of the *proBA** operon increases expression of the entire operon. Massive amplification of a genomic segment around the *proBA** operon up to 50 copies also increases expression of the entire operon. Finally, a synonymous point mutation in the coding region of *proB* creates a new promoter for *proA**. This synonymous mutation increases the level of ProA* by 2-fold, but increases growth rate by 5-fold, an ultrasensitive response likely arising from competition between two substrates for the active site of the inefficient bifunctional ProA*.

We have used the same experimental system to examine the mechanism by which mutations can improve fitness in *S. enterica.* In this case, we found only one point mutation in the promoter for the *proBA** operon, and a synonymous mutation in *proB* that generates a new promoter for *proA** did not occur. Notably, segmental amplification *does not occur* in *S. enterica.* Rather, we found two synonymous mutations and one non-synonymous mutation within *proA** that substantially increase fitness. Both synonymous mutations increase the level of the *proA** mRNA. The non-synonymous mutation may decrease the ability of ProA* to interact with ProB (glutamyl kinase), which delivers the unstable substrate L-γ-glutamyl phosphate directly to the active site, and thereby increase the accessibility of the ProA* active site to the newly important substrate *N*-acetyl-L-γ-glutamyl phosphate.

Conclusions: The marked differences in the adaptive responses to a common selective pressure in *E. coli* and *S. enterica* illustrate that increased expression of a weak-link enzyme can be achieved by multiple mechanisms in each organism, but that those mechanisms differ even among relatively closely related organisms. An additional significant finding is that synonymous mutations resulted in increased levels of the mRNA encoding the weak-link enzyme in both cases, but the locations of the mutations and the mechanisms by which they operate are different in *E. coli* and *S. enterica.*

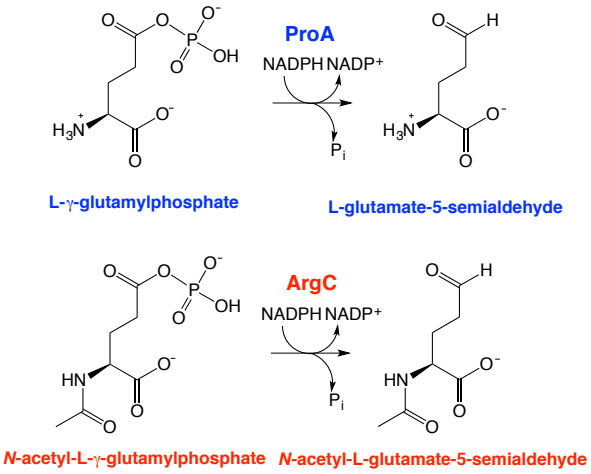

Fig. 1. Reactions catalyzed by ProA and ArgC.

References: [1] Yu McLoughlin, S. and Copley S. D. (2008) *Proc. Natl Acad. Sci. USA 105*, 13497–13502. [2] Khanal A., Yu McLouglin, S., Kershner, J. P. and Copley, S. D. (2015) *Mol. Biol. Evol. 32*, 100-108. [3] Kershner J. P., Yu McLoughlin S., Kim J., Morgenthaler A., Ebmeier C. C., Old W. M., and Copley S. D. (2016) *J Bacteriol., 198*, 2853-2863.