Crosstalk and the Evolution of Specificity in Two-Component Systems

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***Short Abstract* — Unlike eukaryotic signaling networks, there is essentially no crosstalk between bacterial Two-Component Signaling (TCS) pathways. It is currently unclear exactly what feature of TCS dynamics necessitates such extreme pathway isolation. In this work we used mathematical models to demonstrate that adding a competing substrate to a TCS pathway will *always* reduce its response. Our findings implied an inherent barrier to the evolution of new pathways. We characterized a set of "near-neutral" evolutionary trajectories that minimize this effect. Evolutionary sequence analysis of TCS genes revealed that the majority of these pathways evolve via the trajectories predicted by our model.**

***Keywords* — Two-Component Signaling, Histidine Kinases, Signaling Crosstalk**

I. Introduction

Two-component signaling represents the primary signaling modality in bacteria [1]. These pathways generally consist of a Histidine Kinase (HK) that senses incoming signals and phosphorylates downstream Response Regulators (RRs). Unlike eukaryotic signaling networks, bacterial TCS networks show essentially no crosstalk between pathways [2-4]. In general, HKs demonstrate a strong "kinetic preference" for their cognate RRs, preferentially phosphorylating them on short timescales [4]. A relatively small number of residues in the protein-protein interface between HKs and RRs is responsible for maintaining this specificity [4, 5]. Recently, Capra *et al.* demonstrated that making just two mutations in this interface could introduce an interaction between an HK (PhoR) and a noncognate RR (NtrX) in *E. coli*, which greatly decreased the growth rate of mutant cells under phosphate-limiting conditions [5]. Despite intense research, however, it is still unclear exactly *why* TCS pathways experience such strong evolutionary pressures that prevent the evolution of significant crosstalk.

In this work we extended a well-studied and validated mathematical model of bifunctional HKs [6] to the case of multiple substrates to reveal the impact of competition on cognate signaling.

II. Results

We found that, since the HK acts as both the kinase and the phosphatase in TCS pathways, the addition of competing interactions with multiple RRs *always decreases* the response of the cognate RR. Our results indicate that the requirement to maintain a strong cognate RR response is sufficient to explain the observed degree of kinetic preference in TCS networks [4].

The pressure to maintain cognate signaling suggests the existence of a barrier in the evolution of new TCS pathways. The duplication of an existing pathway inherently generates crosstalk with the parental pathway. Using our models, we characterized a set of "near-neutral" evolutionary trajectories that minimize the impact of the new pair on the signaling of the parental pathway. All of these trajectories involved insulation the two pathways form one another before establishing new input and output functionalities.

To test this prediction, we separately aligned multiple kinase and input domain sequences taken from HKs in fully sequenced bacterial genomes. Analysis of the K_A/K_S ratios of the most recently diverged domains revealed that the interaction interface of the HK is under very strong positive selection immediately after duplication, introducing mutations that insulate the duplicated pathways from one another. Those changes are then fixed in order to maintain interaction specificity. The pressures on the input domain of the HK are qualitatively different, with a more gradual decrease in selective pressure, consistent with the evolution of new input functionality after the pathways become insulated. Our findings indicate that many recently duplicated HK-RR pairs follow the near-neutral evolutionary trajectories predicted by our model.

III. Conclusion

Our work demonstrates that the bifunctional nature of HKs has likely been a major driving force in the evolution of insulated topologies in bacterial signaling networks.

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