The evolution of crosstalk in signaling networks

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***Short Abstract* — The degree of crosstalk observed in signaling networks varies widely across evolution. In eukaryotes, crosstalk is widespread, with some kinases and phosphatases acting on hundreds of downstream targets. In bacteria, however, signaling pathways are essentially completely isolated from one another. We used mathematical models to characterize the evolutionary pressures driving these vastly different topologies. In eukaryotes, crosstalk increases ultrasensitivity and couples signal responses, which could yield phenotypic benefits. In bacteria, which utilize bifunctional Histidine Kinases, crosstalk *always* reduces signal response. Our findings have important consequences for how we understand the function and evolution of information-processing networks within cells.**

***Keywords* — Signaling Networks, Crosstalk, Kinases, Phosphatases, Two-component Signaling**

I. Introduction

EUKARYOTIC signaling networks are notoriously complex. A major driver of this complexity is "pathway crosstalk," the sharing of input signals among multiple downstream response elements. Some eukaryotic kinases, like Cdk1 in yeast, have over 100 substrates, and many phosphatases are similarly promiscuous [1,2]. In contrast, bacterial signaling networks show almost no crosstalk at all, with most bacterial kinases acting on a single substrate [3].

It is unclear what pressures have driven the evolution of such dramatically different topologies. The basic building block of eukaryotic networks is the "Goldbeter-Koshland" (GK) loop, consisting of an enzyme that modifies a substrate (e.g. a kinase), and a separate enzyme that undoes this modification (e.g. a phosphatase). Bacterial networks are dominated by Two-Component Signaling, which involves a single Histidine Kinase (HK) that acts both as kinase *and* phosphatase for its substrate Response Regulator (RR) [3].

In this work, we use mathematical models to demonstrate that the difference in global topology likely derives from differences in the dynamical behavior of these two motifs.

II. Results

GK loops display a classic behavior known as "0^{th}-order ultrasensitivity:" a kinase/phosphatase pair will generate a switch-like response to inputs when a single substrate saturates the two enzymes [4]. We extended models of GK loops to include multiple substrates, and found that ultrasensitivity becomes *transitive*: if a single substrate saturates the enzymes, then all substrates will respond ultrasensitively. A large group of substrates can even generate switch-like behavior when no individual substrate saturates the enzymes on its own [5]. Crosstalk thus provides a natural mechanism for generating ultrasensitivity without having to express any one substrate at high levels, which could be phenotypically beneficial. We found that varying the ways in which kinases and phosphatases interact with substrates can generate a variety of different coupled behaviors in the system [5].

We recently extended the above work to the case of bacterial TCS. We found that adding competing substrates to TCS pathways *always* decreased the response of the system; this decrease is a consequence of the bifunctional kinase-phosphatase role played by HKs. The observed reduction in signaling likely explains the dramatic decreases in fitness observed when TCS crosstalk is engineered into bacterial cells [6]. Indeed, we found that the pressure to maintain signaling responses is sufficient to explain the observed kinetic preferences of HKs for their cognate RRs [3].

New TCS pathways evolve through the duplication and divergence of existing HK/RR pairs. Duplication leads to unavoidable crosstalk, implying the existence of a barrier in the evolution of new pathways [6]. Using our models, we characterized a set of "near-neutral" evolutionary trajectories that minimize this effect. Analysis of HK sequences confirmed that most TCS pathways evolve through the trajectories predicted by our model.

III. Conclusion

Our findings indicate that the different topologies observed for eukaryotic and bacterial signaling networks derive from fundamental differences in the behavior of the basic motifs from which the networks themselves are constructed. These differences have important consequences for both the function and evolutionary dynamics of information processing systems within cells.

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References

[1] Ubersax JA, *et al*. (2003) "Targets of the cyclin-dependent kinase Cdk1." *Nature* **425**(6960):859-864.
[2] Hill SM (1998) "Receptor crosstalk: communication through cell signaling pathways." *Anat Rec* **253**(2):42-48.
[3] Laub MT & Goulian M (2008) "Specificity in two-component signal transduction pathways." *Annu Rev Genet.* **41**:121-145.
[4] Goldbeter A & Koshland DE, Jr (1981) "An amplified sensitivity arising from covalent modification in biological systems." *Proc Natl Acad Sci USA* **78**(11):6840-6844.
[5] Rowland MA, Fontana W & Deeds EJ (2012) "Crosstalk and competition in signaling networks." *Biophys J* **103**(11):2389-2398.
[6] Capra EJ, *et al*. (2012) "Adaptive mutations that prevent crosstalk enable the expansion of paralogous signaling protein families." *Cell* **150**(1):222-232.