Phosphatase specificity and pathway insulation in signaling networks

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***Short Abstract* — Phosphatases play an important role in cellular signaling networks by regulating the phosphorylation state of proteins. Phosphatases are classically considered to be "promiscuous", acting on many different substrates. We recently showed that this could cause crosstalk between pathways due to competitive inhibition. In this work, we demonstrated that phosphatase promiscuity is indeed widespread in eukaryotic genomes. We used mathematical models to characterize a set of possible mechanisms, such as reducing phosphatase saturation or employing adaptor proteins, which cells could use to prevent crosstalk. These mechanisms universally involve evolutionary trade-offs that likely dictate when and where they are deployed within cells.**

***Keywords* — phosphatase crosstalk, regulatory subunits**

I. Introduction

PHOSPHATASES play a critical role in cellular signaling by working with kinases to modulate the phosphorylation state of protein substrates in a signaling network. Using mathematical models, we recently showed that a phosphatase shared between two otherwise independent substrates could couple their responses [1].

While over 500 kinases have been identified in the human genome, there are only about 150 phosphatases [2,3]. There are thus simply not enough phosphatases in the genome to provide a single phosphatase for every kinase, indicating a potential for rampant phosphatase-mediated crosstalk. The goal of this work was to understand the extent of phosphatase promiscuity and to explore a set of possible mechanisms whereby cells could prevent this promiscuity from manifesting itself as unwanted crosstalk between pathways.

II. Results

In order to characterize the generality of the kinase/phosphatase mismatch across different eukaryotic species, we used the UniProt database to determine the number of kinases and phosphatases in 16 complete genomes. We found that the ratio of phosphatases to kinases is on average 4:1 for serine/threonine phosphatases and kinases, but closer to 4:3 for tyrosine phosphatases and kinases. We also investigated the "substrate load" of phosphatases, again using UniProt to find phosphoproteins in each genome. On average each phosphatase would have to act on about 30 substrates, illustrating the potential for phosphatase-mediated crosstalk across a variety of species.

Given the widespread nature of phosphatase promiscuity, we considered several mechanisms whereby phosphatases could act on multiple substrates without coupling their responses. The simplest case is one in which the K_M of the phosphatase for its substrates is very large, since phosphatase crosstalk requires saturation of the phosphatase [1]. While this approach does provide insulation, the phosphatase itself becomes highly inefficient, and the system looses its ability to generate switch-like responses to incoming signals.

We also considered a scenario in which degradation of the phosphorylated substrate could be used in place of a discrete phosphatase. In order for this mechanism to be effective, however, the phosphorylated substrate must have a very short half-life, again reducing the efficiency of the system.

Finally, we considered whether adaptor or regulatory subunits, such as those found as part of the serine/threonine phosphatase PP2A, could insulate substrate responses. We found that this is indeed possible, but only if the regulatory subunit binds the substrate independently of its binding to the catalytically active domain. While adaptor subunits can provide insulation while preserving the capacity for ultrasensitive response, this mechanism requires the evolution of large numbers of adaptor proteins, one for each subset of substrates that must be isolated.

III. Conclusion

Our work demonstrates that, while there is extensive opportunity for phosphatase-mediated crosstalk in eukaryotic cells, a variety of mechanisms can be employed to mitigate this effect. Understanding the influence of phosphatases on the response of specific biological networks will require experimental determination of where and how these mechanisms are actually deployed within cells.

References

[1] Rowland MA, Fontana W, Deeds EJ (2012) Crosstalk and competition in signaling networks. *Biophys J* 103(11):2389-2398.

[2] Arena S, Benvenuti S, Bardelli A (2005) Genetic analysis of the kinome and phosphatome in cancer. *Cell Mol Lif Sci* 62(18):2092-2099.

[3] Manning G, Whyte DB, Martinez R, Hunter T, Sundarsanam S (2002) The protein kinase complement of the human genome. *Science* 298(5600):1912-1934.

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