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Rewiring Bacteria, Two Components at a Time

Michael A. Kohanski[1,2] and James J. Collins[1,*]
[1]Department of Biomedical Engineering, Center for BioDynamics, and Center for Advanced Biotechnology, Boston University, 44 Cummington St., Boston, MA 02215, USA
[2]Boston University School of Medicine, 715 Albany St., Boston, MA 02118, USA
*Correspondence: jcollins@bu.edu
DOI 10.1016/j.cell.2008.05.035

In this issue, Skerker et al. (2008) present a rational method for rewiring the protein-protein interactions and output responses of prokaryotic two-component signal transduction systems. This work has important implications for understanding the specificity of protein interactions and for designing protein-based synthetic signaling cascades.

The rational design of biological networks and pathways promises to reveal ways to rewire cells for new biological functions or to gain insights into the behavior of natural systems. Much of the work to date has focused on the manipulation of transcriptional and posttranscriptional elements to create synthetic gene networks with desired functions (Bayer and Smolke, 2005; Becskei and Serrano, 2000; Elowitz and Leibler, 2000; Gardner et al., 2000; Isaacs et al., 2004). In this issue, Skerker et al. (2008) present a method for reprogramming the protein-protein interactions and output responses of prokaryotic two-component signal transduction systems. This work expands the synthetic biology toolbox and represents a significant step forward in the engineering of protein-based synthetic pathways.

Bacterial two-component systems of signal transduction consist of a sensor histidine kinase that detects a specific signal and a cognate response regulator that modulates the signal response, usually through the regulation of gene expression (Hoch, 2000). A homodimer of two sensor histidine kinase monomers detects external signals through the signal recognition domain. Signal recognition leads to activation of the histidine kinase autokinase domain that consists of a catalytic ATP-binding subdomain and a phosphotransfer subdomain. This activation promotes autophosphorylation of the histidine kinase monomers and transfer of the phosphate to the cognate response regulator. Phosphorylation of the regulatory domain of the response regulator typically alleviates inhibition of the regulator output domain and results in changes in target gene expression.

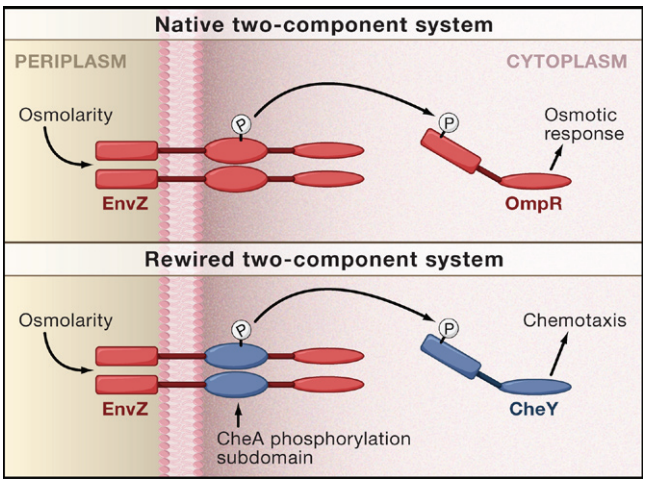

Figure 1. Rewiring a Bacterial Two-Component System
(Top) Shown is the native EnvZ-OmpR two-component signal transduction system (red) from the bacteria *Escherichia coli* for sensing and responding to osmolarity changes. (Bottom) A theoretical EnvZ sensor (red) rewired for chemotaxis pathway (CheA-CheY; blue) specificity, which still senses osmolarity changes but responds by initiating expression of chemotaxis genes.

Skerker and colleagues (Skerker et al., 2008) identified elements required for the specific binding between cog-

nate two-component histidine kinase-response regulator pairs from the bacteria *Escherichia coli* using multiple sequence alignments of 200 bacterial genomes and 1300 two-component pairs. Using a mutual information-based algorithm, the authors examined covariance of amino acids between the unique histidine kinase and response regulator pairs. They found that the amino acids that designate the specificity of histidine kinase binding to the cognate response regulator were predominantly located in the kinase subdomain of the sensor protein.

To demonstrate the feasibility of rewiring two-component systems using their data, the authors focused on modifying the specificity of the osmolarity sensor protein from *E. coli*, called EnvZ, that normally targets the response regulator protein OmpR (Figure 1). To confirm their computational prediction that specificity is encoded by the phosphorylation subdomain, Skerker et al. first modified EnvZ by replacing its entire phosphorylation subdomain with either that of another *E. coli* sensor protein called RstB or that of a sensor protein called CC1181 from the bacteria *Caulobacter crescentus*. The authors found that the modified EnvZ sensor protein successfully phosphorylated the correct cognate response regulators of either RstB or CC1181 in vitro depending on the identity of the phosphorylation subdomain. Skerker et al. then further confirmed their prediction of the precise region in the phosphorylation subdomain that contains the residues required for sensor-regulator specificity. They replaced this region in the EnvZ phosphorylation subdomain with corresponding regions from five other *E. coli* two-component sensor kinases. These modified EnvZ proteins containing partial subdomain replacements also demonstrated the correct specificity in cognate response regulator phosphorylation.

Skerker et al. then mutated the specific amino acid residues within the EnvZ phosphorylation subdomain that were predicted to be required for substrate specificity. Surprisingly, the authors found that mutating as few as three residues within the EnvZ phosphorylation subdomain was sufficient to change its in vitro response regulator specificity to that of RstB. However, to change the in vitro substrate specificity of EnvZ to that of other two-component sensor kinases, mutation of all of the predicted specificity residues as well as the replacement of a helix-connecting loop was required. Interestingly, the authors noted that the specificity residues in this loop region, previously thought to be important for substrate selection, may have been missed by their computational analysis due to the difficulty in aligning the sequence of the region. This issue is clearly worthy of further study.

Finally, Skerker et al. put their predictions to the test in *E. coli* cells. The authors used reporter constructs expressing fluorescent proteins to confirm the in vivo functionality and specificity of EnvZ sensors mutated based on their computational predictions to interact with the response regulators CpxR and PhoP. They observed significant increases in fluorescence from a CpxR-regulated promoter construct only in cells expressing the EnvZ sensor rewired with CpxR specificity. Similarly, increased fluorescence from a PhoP-regulated promoter construct was observed only from cells expressing the EnvZ sensor rewired with PhoP specificity.

Importantly, Skerker et al. have demonstrated that they can reprogram the output responses of bacterial two-component systems by rationally modifying kinase-substrate interactions. Furthermore, this work establishes a robust computational approach for studying protein-protein interactions. The authors showed that the analysis of amino-acid covariance in large multiple sequence alignments can be used independent of structural information to identify the specificity determinants in a kinase-substrate interaction. This provides a platform for gaining insight into the biologically relevant roles of two-component signal transduction systems, including those involved in mediating pathogenicity, cell-cycle progression, and bacterial developmental processes such as sporulation (Hoch, 2000). Furthermore, analyses such as the one performed by Skerker et al. should be useful for the study of the evolution of two-component sensor specificity. They can also contribute to understanding how multiple two-component systems have coevolved to avoid crosstalk between pathways. Lastly, this computational framework could prove useful for studying other types of protein-protein interactions such as those between bacterial toxins and antitoxins.

Most intriguingly, the present study establishes a new framework for designing protein-based synthetic signaling cascades. This work complements recent efforts involving the use of engineered two-component systems to reprogram *E. coli* to sense and record visual images (Levskaya et al., 2005). These developments together provide a powerful arsenal for designing and constructing synthetic circuits with de novo cellular behavior. For example, reprogramming a two-component sensor to activate the chemotaxis response regulator could be used to induce the swarming of bacteria toward a stimulus of interest. The bacteria could then be further engineered to execute a specific task once they arrive at the region of interest. For example, they can be programmed to encase the area in a biofilm or to produce a compound to degrade an unwanted substance in the local environment. Clearly, the work by Skerker and colleagues broadly expands our ability to harness and reprogram the behavior of microbes, generating many exciting opportunities in synthetic biology and biotechnology.

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