Bait and Switch: Synthetic GEFs Divert an Input Signal to Diverse Cellular Responses

Christopher S. Chen[1,*]
[1] Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA
*Correspondence: chrischen@seas.upenn.edu
DOI 10.1016/j.devcel.2007.06.007

The Rho family GTPases regulate many cellular functions including the organization, mechanics, and dynamics of the actin cytoskeleton. Re-engineering the highly conserved autoinhibitory structure of GEFs, Yeh et al. (2007) generated novel synthetic proteins that regulated Rho GTPase signaling and cell morphology, highlighting the power of protein module engineering.

Synthetic biology promises to build novel proteins, regulatory networks, functions, and even cells by design. That is, to apply engineering principles to understand living systems sufficiently well to be able to predict a priori how a particular manipulation or construction would affect the system. More practically, starting from a desired function, one hopes to be able to predict the proteins, networks, or manipulations necessary to generate that function. The slow transition from tinkering à la the Wright brothers to engineering à la modern computer-aided aircraft design is understanding how cellular signaling really works.

Several simple genetic regulatory networks have been successfully engineered de novo over the past decade by a number of unrelated laboratories, suggesting that at least transcriptional cascades could be usurped by man in the near future. In contrast, re-engineering of protein signaling networks has been less prolific, perhaps due to the complex nature of protein-protein interactions. How does one modify two previously noninteracting proteins to bind or signal? In the few reported cases of redirecting signaling from one pathway to another previously unrelated pathway, the linkage involves the connecting of protein modules between the two pathways in one form or another (Howard et al., 2003; Inoue et al., 2005; Park et al., 2003; Dueber et al., 2003). In one such case, Lim and his team swapped autoinhibitory domains to allow new nonphysiologic signals to regulate N-WASP function (Dueber et al., 2003). In a recent paper published in *Nature*, this same group generalizes this same basic approach to re-engineer signaling in the Rho GTPases (Yeh et al., 2007).

The Rho GTPases, including Rho, Rac, and Cdc42, belong to the Ras superfamily of small G proteins that are largely responsible for regulating actin cytsokeletal structure and dynamics. Classical studies have established that Rho induces contraction of the actin cytoskeleton and formation of stress fibers, Rac is involved in lamellipodia formation, and Cdc42 causes the extension of filopodia (Jaffe and Hall, 2005). Their ability to relay signals is modulated by being in a GTP-bound (active) or GDP-bound (inactive) state. Signaling through the Rho GTPases is largely modulated by two mechanisms: GTPase activating proteins (GAPs) arrest signaling by stimulating GTP hydrolysis to GDP, and guanine nucleotide exchange factors (GEFs) activate signaling by promoting exchange of GDP for GTP. Despite the common motifs in many GEFs, GAPs, and Rho GTPases, this network of proteins relays a surprisingly large number of diverse extracellular signals to many morphological and functional responses, resulting in a complex signaling hub that is at the center of the many links between cellular structure and function.

Many GEFs, while specific for different Rho GTPases, use a common autoinhibitory mechanism for signaling that involves a Dbl-homology domain. Yeh and colleagues engineered one such GEF, intersectin, for Cdc42 using a similar strategy to their prior study reconstructing N-WASP, by swapping out the autoinhibitory domain for a PDZ domain, and then placed a PDZ-domain-binding short peptide sequence on the other end of the molecule (Yeh et al., 2007). Here, however, they introduced an important twist. By slightly modifying the peptide sequence, they were able to engineer it as a phosphorylation target of PKA so that the PDZ domain would not bind (and autoinhibit the molecule) in the phosphorylated state. They showed that the synthetic GEF in vitro derepressed and activated Cdc42 only in the presence of PKA.

Interestingly, when the authors used this same strategy to generate synthetic versions of six other GEFs, all exhibited some autoinhibitory function, and three demonstrated activation via PKA. Microinjecting into cells two of the synthetic GEFs, one for Cdc42 and the other for Rac1, they demonstrated that PKA signaling could be redirected to regulate cellular activities associated with Cdc42 and Rac1. Using the small molecule forskolin to rapidly activate adenylate cyclase and downstream PKA activity, they observed rapid upregulation of filopodia in the presence of the synthetic Cdc42 GEF and lamellipodia with the synthetic Rac1 GEF.

Last, the authors asked whether this strategy could be used to generate a multistep pathway. One of Cdc42's native activities is the derepression of the autoinhibited N-WASP protein. By using the autoinhibitory cassettes from N-WASP to flank the effector domains of a Rac1 GEF, they were able to engineer a Rac GEF that activated with Cdc42 signaling. Coinjecting both the synthetic PKA-responsive Cdc42

GEF and the Cdc42-responsive Rac1 GEF, they showed that forskolin stimulated lamellipodial formation. Importantly, removal of either GEF or replacing with a nonresponsive synthetic GEF abrogated this activity. Interestingly, they show that the 2-step cascade transforms the Michaelis-Menten kinetics of their system into a nonlinear, ultrasensitive response, consistent with predictions from theoretical studies.

In summary, this paper takes a few steps farther on the road to synthetic biology. The use of a novel autoinhibitory interaction that is kinase responsive (and can be triggered with a small molecule) is a major accomplishment. In so doing, Yeh et al. (2007) have successfully reconnected some of the core currencies of information used in cell signaling, e.g., protein phosphorylation and GTPases. These are elements that can in principle be used both as node inputs and outputs, and which therefore are used extensively in complex circuits in nature. It remains to be seen whether this strategy can be generalized to other kinases or pairwise binding partners. The attempt to transfer the specific domains to numerous GEFs is laudable, and the better than 50% rate of success is remarkable given the number of possible modes of failure for protein re-engineering, and suggests the authors' implication that module swapping in proteins may in fact be more robust than previously thought. Whether this strategy can be truly transferred to all Dbl-containing GEFs cannot be assessed until it is clear how the three nonresponsive synthetic GEFs failed.

While these efforts are encouraging, we are still a long way from the level of control in Rho GTPase signaling exhibited by cells. Cells in vivo rarely produce only filopodia or lamellipodia. Instead, these structures form and disappear on the order of seconds to minutes, and do so with spatially organized patterns across the cell, often with different classes of actin microarchitecture coexisting in different parts of the cell. Thus, understanding how to activate or inactivate certain pathways is only a first step. Knowing how to engineer these signals in space and time, to control their localization and transport, and how to operate multiple pathways simultaneously, remain a mystery. As such, we are currently more tinkerer than designer.

REFERENCES

Dueber, J.E., Yeh, B.J., Chak, K., and Lim, W.A. (2003). Science *301*, 1904.

Howard, P.L., Chia, M.C., Del Rizzo, S., Liu, F.-F., and Pawson, T. (2003). Proc. Natl. Acad. Sci. USA *100*, 11267–11272.

Inoue, T., Heo, W.D., Grimley, J.S., Wandless, T.J., and Meyer, T. (2005). Nat. Methods *2*, 415–418.

Jaffe, A.B., and Hall, A. (2005). Annu. Rev. Cell Dev. Biol. *21*, 247–269.

Park, S.-H., Zarrinpar, A., and Lim, W.A. (2003). Science *299*, 1061.

Yeh, B.J., Rutigliano, R.J., Deb, A., Bar-Sagi, D., and Lim, W.A. (2007). Nature *447*, 596–600.

Wnt/β-Catenin Signaling and Cardiogenesis: Timing Does Matter

Eldad Tzahor[1,*]
[1] Department of Biological Regulation, Weizmann Institute of Science, Rehovot 76100, Israel
*Correspondence: eldad.tzahor@weizmann.ac.il
DOI 10.1016/j.devcel.2007.06.006

Recent findings in mouse and zebrafish embryos, as well as in embryonic stem cells, emphasize the critical importance of the Wnt/β-catenin pathway in the regulation of cardiogenesis, and highlight the exquisite timing and specific cellular responses by which this signaling pathway exerts its influence. These studies clearly demonstrate that the Wnt/β-catenin pathway plays distinct, even opposing, roles during various stages of cardiac development.

The diversity of cardiac progenitor populations in various vertebrate species is an emerging area of intense focus in many laboratories; knowledge of these progenitors has profound implications for our understanding of heart development during embryogenesis, and also highlights the enormous therapeutic potential of these avenues for regenerative medicine. In the vertebrate embryo, cardiac progenitors derive from the lateral plate mesoderm, migrate to form the cardiac crescent, and subsequently contribute to the myocardium and endocardium of the heart. Recent studies indicate that the heart is formed from two distinct mesoderm populations or "heart fields" that arise from a common origin and express both distinct and overlapping molecular markers. The earliest population of cardiac progenitors, the first heart field, corresponds to the anterior lateral mesoderm and subsequently to the cardiac crescent. Ultimately, cells from the first heart field contribute to the left ventricle and