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NEWS AND VIEWS

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Signaling proteins exhibit a high degree of structural and functional modularity that are thought to enable evolution of new regulatory circuits, using limited protein resources by simple genetic events such as recombination, deletion or insertion. This modularity is manifested by a clear separation between structures that mediate catalytic activity from multiple independent folding motifs that mediate connectivity with other signaling proteins. Examples of these 'connecting' domains include SH3 and PDZ domains that bind to peptide sequences, and the conditional recognition SH2 and PH domains that bind to phosphopeptides and phospholipids, respectively.

Using a synthetic biology approach, in which the signaling machinery that couples cytoskeletal dynamics to extracellular inputs is rewired, Wendell Lim and co-workers (Yeh *et al*, 2007) test the hypothesis that the evolvability of signaling circuits is driven by recombination of protein modules. For this, synthetic proteins were designed that control the activity of Rho family GTPases that are master regulators of the actin cytoskeleton.

The GTPases are conformational switches that exist in GDP- and GTP-bound state, of which the latter actively transduces signals by binding to effectors. The activity of the three canonical members of the Rho GTPases controls the actin machinery-driven morphology of filopodia (Cdc42), lamellipodia (Rac1) and contractile actin:myosin filaments (RhoA). In turn, GTPase activity is controlled by guanine nucleotide exchange factors (GEFs), which exchange bound GDP for GTP, and GTPase activating proteins (GAPs) that promote hydrolysis of GTP to GDP. The authors have rewired cellular morphology pathways to respond to the second messenger cAMP, by generating synthetic GEFs, in which catalytic DH domains from the Dbl family Rho GEFs are combined with newly designed regulatory modules that change their conformation upon phosphorylation by protein kinase A (PKA). Such allosteric regulatory modules can mediate biochemical input connectivity by signal-dependent modulation of the catalytic activity, through steric occlusion or conformational disruption. Alleviation of this autoinhibition can take place by post-translational modification such as phosphorylation that induces a conformational change in the regulatory module.

Based on this principle, Yeh and co-workers designed a PKA-sensitive autoinhibitory module consisting of a PDZ domain–peptide interaction pair that is disrupted by PKA phosphorylation. The PDZ–peptide interaction was made conditional on phosphorylation by PKA by using a hybrid peptide sequence that is both an interaction ligand for the synthropin PDZ domain and a PKA substrate. This PKA responsive module was fused to two types of GEF catalytic DH domains: (1) that of intersectin, which activates Cdc42 and thereby generates filopodia (GEF1), and, (2) that of Trio that activates Rac1, thereby generating lamellipodia (GEF2). The purified synthetic GEFs were shown to respond to PKA activity in an *in vitro* nucleotide exchange assay. Excitingly, after microinjection into cells, the synthetic GEFs induced the expected morphological phenotype (filopodia for GEF1 and lamellipodia for GEF2), in response to the pharmacological activator of PKA, forskolin. In order to obtain a consistent morphological phenotype, the cognate GTPase had to be co-injected with the synthetic GEF, indicating that the balance in the stoichiometries of protein elements plays an important role in the qualitative response properties of signaling networks.

Remarkably, Yeh and co-workers were able to use this protein modularity to engineer an artificial signaling cascade that couples the filopodial (Cdc42) and lamellipodial (Rac1) regulating GTPases in series. For this, they designed a third GEF (GEF3) containing an N-WASP autoinhibitory regulatory module that is activated by Cdc42 and the Trio catalytic DH domain that activates Rac1. Co-injection of GEF1 and GEF3 indeed resulted in a novel PKA responsive signaling cascade, where forskolin treatment led to a lamellipodial phenotype. The authors ascribed three new properties to the cascade that distinguish it from the single GEF circuits: (1) dampened noise, (2) response amplification and (3) ultra-sensitivity. It is however unclear if these are indeed new dynamic properties that emerge solely from the higher-order architecture of the cascade. This is in part due to the course phenotypic readout that was used based on cell population statistics. In this readout, the measured response properties are a convolution of the synthetic cascade with the intrinsic cellular actin cytoskeleton machinery. The difference in steepness of the filopodial versus lamellipodial response to forskolin could therefore be generated by the intrinsic cytoskeletal regulatory networks, rather than solely arising from the properties of the synthetic cascade. Moreover, the apparent response amplification in the coupled GTPase cycles could simply originate from a lower-input threshold of active GEF for the lamellipodial versus filopodial response. In future investigations, the input–response properties of the synthetic cascade could be directly assessed by measurement of the fraction of GTPases in the GTP-bound state by cell-based assays.

The prospects of this study are nevertheless very exciting. Novel connections in signaling networks could be engineered to study the relation between network architecture and

dynamic response. Engineering novel connections in signaling pathways that alter the collective behavior of cells in tissues by responding to new extracellular inputs, could help elucidate the relation between properties of the cellular signaling machinery and developmental processes in organisms.

References

Yeh BJ, Rutigliano RJ, Deb A, Bar-Sagi D, Lim WA (2007) Rewiring cellular morphology pathways with synthetic guanine nucleotide exchange factors. *Nature* **447:** 596–600.