ELSEVIER

Rewiring cell signaling: the logic and plasticity of eukaryotic protein circuitry

John E Dueber, Brian J Yeh, Roby P Bhattacharyya and Wendell A Lim*

Living cells rival computers in their ability to process external information and make complex behavioral decisions. Many of these decisions are made by networks of interacting signaling proteins. Ongoing structural, biochemical and cell-based studies have begun to reveal several common principles by which protein components are used to specifically transmit and process information. Recent engineering studies demonstrate that these relatively simple principles can be used to rewire signaling behavior in a process that mimics the evolution of new phenotypic responses.

Addresses
Department of Cellular and Molecular Pharmacology, California Institute for Quantitative Biomedical Research, University of California San Francisco, 600 16th Street, San Francisco, California 94143-2240, USA
*e-mail: wlim@itsa.ucsf.edu

Current Opinion in Structural Biology 2004, **14**:690–699

This review comes from a themed issue on
Proteins
Edited by Wim GJ Hol and Natalie C Strynadka

Available online 28th October 2004

0959-440X/$ – see front matter

DOI 10.1016/j.sbi.2004.10.004

Abbreviations

Arp2/3	actin-related protein 2/3
DED	death effector domain
EGF	epidermal growth factor
EGFR	epidermal growth factor receptor
GAP	GTPase-activating protein
GBD	GTPase-binding domain
GEF	guanine nucleotide exchange factor
GTPase	guanine nucleotide triphosphatase
MAPK	mitogen-activated protein kinase
N-WASP	neuronal WASP
PDZ	PSD95, Dlg and ZO-1
PIP2	phosphoinositol 4,5 bisphosphate
SH	Src homology
WASP	Wiskott–Aldrich syndrome protein

Introduction

Eukaryotic cells use complex networks of signal transduction proteins to make decisions about whether to grow, differentiate, move or die. These cellular networks have information-processing capabilities that rival computers: they can perform complex signal integration, switch states in a manner that retains memory or generate complex temporal behaviors, such as oscillations [1–3]. Just as electronic circuits are built of simpler components, cellular signaling circuits are composed from a modular toolkit of components, including kinases, phosphatases, GTPases (guanine nucleotide triphosphatases) and interaction domains. However, unlike electronic components, which can be linked simply through physical wiring, protein signaling components operate in the complex environment of the cell.

Over the past decade, two major principles have emerged with respect to how signaling networks and their information flow are organized. First, many individual signaling proteins act as gated nodes: their output function (catalysis or binding) is tightly regulated in response to specific input stimuli. In turn, the resulting output activity serves as a stimulus for further downstream proteins. Second, signaling proteins that function in the same pathway often co-assemble into physical complexes. Such co-localization, either through direct interaction or mediated by organizing factors known as scaffolds, is thought to promote specificity [4,5].

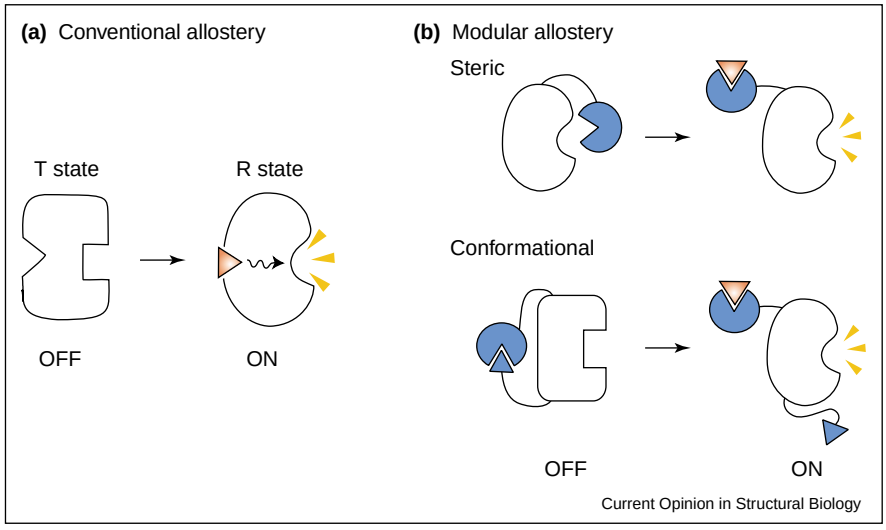
Modular protein interaction domains play a central role in mediating both signaling protein regulation (gating) and signaling complex assembly. This review will focus on two fundamental issues concerning protein circuits. First, we will review how gating and complex assembly allow the myriad signaling proteins in a cell to form complex and robust circuits. Second, we will discuss how these mechanisms lend themselves to the evolution of new responses, a view supported by exciting new experiments demonstrating that these principles can be used to rewire cell signaling and behavior.

Gating by signaling proteins: conventional versus modular allostery

Gating elements are the fundamental components of any information-processing system. In electronic circuits, transistors and related devices are gates in which an output — current flow — is regulated in response to an input — applied voltage. However, in cells, there is no single currency of output (e.g. electron flow in electronics): cellular information currencies include covalent modification (e.g. by kinases/phosphatases), ligand binding and changes in localization. Thus, protein gates must be able to detect this diverse array of inputs and use this information to regulate an equally diverse array of output functions.

It has long been appreciated that the fundamental gating units of cells are allosteric molecules [6]. Many signaling proteins display allosteric behavior: they can exist in two

Figure 1



Comparison of conventional and modular allosteric protein gates. **(a)** Conventional allostery. A single folding unit contains both a catalytic site and secondary regulatory site(s), and can adopt an active or inactive conformation. Binding of an input ligand to the regulatory site preferentially stabilizes the active conformation. The input/output information is propagated by conformational coupling between the sites. **(b)** Modular allostery. The catalytic (output) domain is a physically separable folding unit from the regulatory domains. When isolated, these output domains display constitutive activity, but are often autoinhibited by the regulatory domains. Most proteins that are regulated by modular allostery fall into one of two general categories of autoinhibition: steric and conformational. Steric autoinhibition occurs when the output active site is directly blocked. Conformational autoinhibition occurs when autoinhibitory interactions perturb the conformation of the output domain, reducing its activity. Certain gates can display elements of both steric and conformational autoinhibition. In all of these modular gates, activation occurs through disruption of the autoinhibitory interactions by binding of competing exogenous ligands or by covalent modifications.

or more conformations (active and inactive) that are differentially stabilized by inputs such as ligand binding or covalent modification. However, in recent years, it has become clear that there are at least two general ways to achieve allostery — what we will define as 'conventional allostery' and 'modular allostery' (Figure 1).

In conventional allosteric proteins, an output activity and input-binding sites are contained within a single cooperatively folding structural unit [7] (Figure 1a). The protein can adopt at least two conformations; binding of an activating input stabilizes the active conformation. The functional coupling between the active site and the input-binding site is mediated by the dynamic linkage of the amino acids that make up the fold. An example of a conventional allosteric protein found repeatedly in signaling systems is the Ras family of small GTPases [8]. These conserved folds have an effector-binding site (output) that is properly configured only when the protein is bound to the input ligand GTP. Certain classes of kinases also display conventional allosteric activation, adopting a fully active catalytic conformation only in response to conserved phosphorylation and binding inputs [9–11].

By contrast, signaling proteins that utilize modular allostery to achieve gating have structurally separable output and regulatory domains (reviewed previously in [12,13]) (Figure 1b). Such proteins usually contain an output domain that, when isolated, displays constitutive (unregulated) activity. However, other regions of the intact protein autoinhibit this output activity through one of two general mechanisms: sterically blocking access to the output active site [12,14,15] or conformationally distorting the output domain [12]. These modular gates can then be activated by inputs that relieve repression by disrupting the autoinhibitory interaction (e.g. a competitive ligand).

Modularity and evolvability

Although modular allostery may seem like an inelegant and inefficient way to build a molecular gate, many examples of this type of signaling protein are emerging, suggesting that this may be the prevalent solution chosen by evolution when faced with the challenge of generating diverse new regulatory linkages. It may be easier to rapidly evolve radically new input/output relationships using a type of modular framework via relatively simple recombination events. Moreover, because multiple regulatory domains can cooperate to autoinhibit a single output domain, this framework may more readily yield gates capable of integrating multiple inputs.

The notion that modular gates are more evolutionarily flexible is generally supported by the higher level of

Table 1

Examples of signaling proteins gated by autoinhibition.

Protein	Input(s)	Output	Mechanism of autoinhibition	References
			Steric	
EGFR	EGF	Receptor dimerization	Cysteine-rich domain occludes receptor dimerization surface (another cysteine-rich domain)	[25••,27••]
SH2-containing phosphatase 2 (SHP2)	SH2-binding motifs (p-Tyr)	Phosphatase	N-terminal SH2 domain sterically blocks phosphatase catalytic site	[45]
p21-activated kinase (PAK1)	Rac or Cdc42	Ser/Thr kinase	GBD blocks catalytic site, preventing autophosphorylation	[46]
Twitchin	Ca^2/S100 complex	Ser/Thr kinase	Pseudo-substrate motif occupies kinase active site; locked into position by adjacent IgG domain	[47]
p47phox	Phosphorylation by PKC	NADPH oxidase	Intramolecular peptide blocks tandem SH3 domains from interacting with membrane-associated partner, thereby blocking formation of functional oxidase complex	[48]
Vav	Phosphorylation by Src family kinases	Rho, Rac, Cdc42 GEF (DH–PH module)	N-terminal extension blocks GTPase interaction site	[49]
			Conformational	
Src kinases	SH2- and SH3-binding motifs	Tyr kinase	Binding of the SH2 and SH3 domains to intramolecular ligands locks kinase in inactive conformation	[10]
c-Abl	SH2- and SH3-binding motifs; possibly membrane targeting of myristoyl group	Tyr kinase	Binding of N-terminal myristoyl group and SH2 and SH3 domains to sites on or adjacent to kinase domain locks kinase in inactive conformation remarkably similar to the autoinhibited structure of Src	[17••,18••]
N-WASP	Cdc42 and PIP2	Arp2/3 stimulation (actin polymerization)	GBD and a polybasic motif (B) form cooperative intracomplex interactions that conformationally inactivate the N-WASP output domain, blocking its ability to activate the Arp2/3 actin-nucleating complex	[19,20]
			Unknown (evidence of autoinhibition given)	
Polo-like kinase (PLK)	Phosphorylated Cdc25	Ser/Thr kinase	Polo-box domain reduces activity of kinase domain. This repressive interaction can be dissociated by phosphorylation of the kinase domain at Tyr210	[50]
Dbl	PIP2 and PIP3	Rho, Cdc42 GEF (DH–PH module)	N-terminal region binds PH domain and is required for autoinhibition of exchange activity of adjacent DH domain	[51]
Intersectin	Proline-rich region from N-WASP	Cdc42 GEF (DH–PH module)	SH3 domains inhibit the catalytic DH domain. Binding proline-rich region of N-WASP to SH3 domains stimulates DH exchange activity	[52,53]
Cdc24	Rsr1p/Bud1p and Bem1p binding	Cdc42 GEF (DH–PH module)	Phox-Bem1 homology (PB1) domain involved in autoinhibition. Rsr1p/Bud1p binds calponin homology (CH) domain, which is thought to cause a conformational change that dissociates the PB1 autoinhibitory interaction. Bem1 binds the PB1 domain and is thought to trap this active state	[54]
Chimaerin	Phosphatidylserine and phosphatidic acid	Rac GAP	C1 domain required for regulation	[55]

Table 1 Continued

Protein	Input(s)	Output	Mechanism of autoinhibition	References
P-Rex1	PIP3 and $G_{\beta\gamma}$	Rac GEF (DH–PH module)	Coincidence detector for PIP3 and Gβγ, but mechanism of autoinhibition is unknown	[56]
Rho-associated kinase (ROCK)	Rho and arachidonic acid	Ser/Thr kinase	Overexpression of a fragment containing the Rho-binding domain (RB) and PH domain inhibits activity of the kinase. Point mutations disrupting binding of RB to Rho have similar effects. RhoA binding to RB is thought to activate kinase	[57]

DH, Dbl homology; PH, pleckstrin homology; PIP3, phosphoinositol 3,4,5 trisphosphate; PKC, protein kinase C.

evolutionary diversification observed for these proteins. For example, the Rho family GTPases, which play an important role in regulating cell growth and motility, are conventional allosteric proteins [8]. Although there are 24 total isotypes, they can be classified into only three general classes (Rho, Rac and Cdc42). By contrast, there are over 80 Rho GTPase-activating proteins (GAPs) and over 70 Rho guanine nucleotide exchange factors (GEFs), the upstream molecules that negatively and positively regulate these GTPases, respectively [16]. Most of these GEFs and GAPs appear to be regulated through modular mechanisms — each has a conserved catalytic domain within the context of a larger multidomain protein. The diversity of modular architecture found in GEFs and GAPs suggests that evolution of Rho signaling has occurred primarily through modification of GEFs and GAPs, rather than through modification of the GTPases themselves.

New examples of modular allosteric signaling proteins

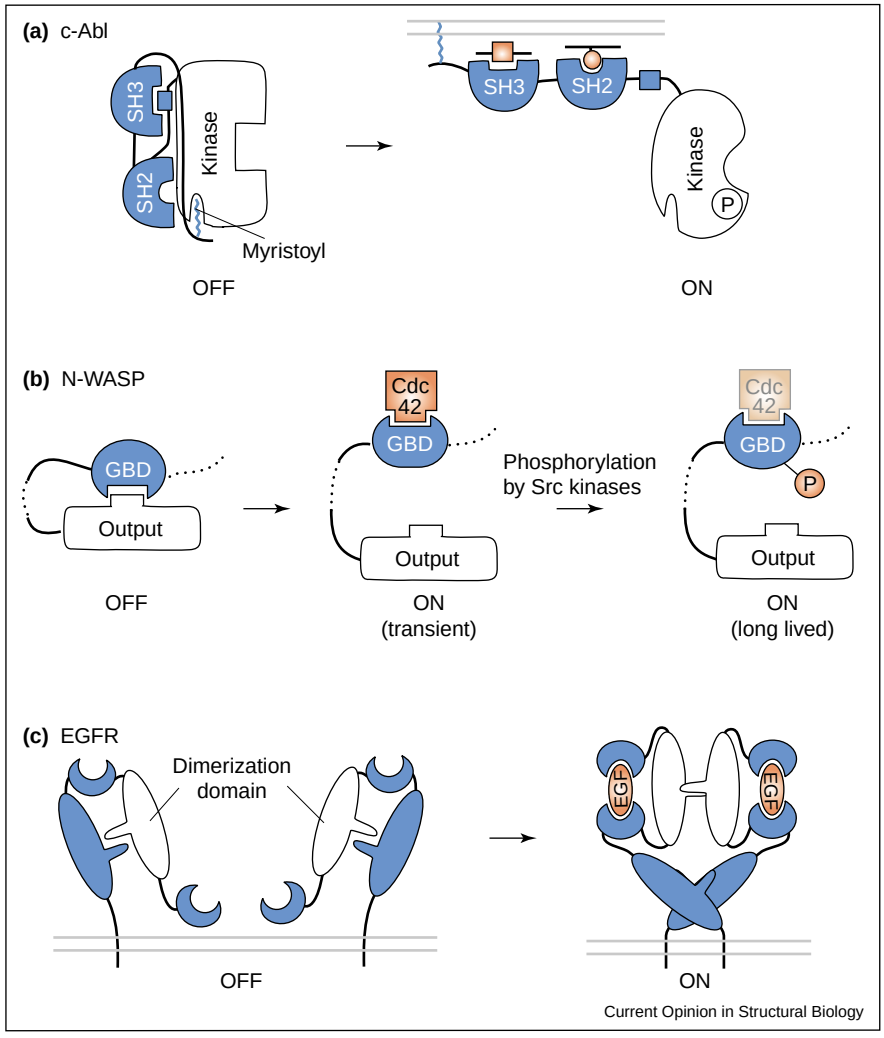
In recent years, the number of signaling proteins that appear to be regulated by modular allosteric mechanisms has exploded. An extensive but not exhaustive list is given in Table 1. The mechanisms of several examples are shown in Figure 2.

It has become clear that signaling proteins that utilize modular autoinhibition can display remarkably complex gating behaviors. For example, the Abl kinase appears to be capable of integrating information from three distinct inputs (Figure 2a). Like the related Src kinase, Abl contains a Src homology (SH) 2 domain and an SH3 domain, which participate in autoinhibitory interactions. However, Abl contains a third interaction required for autoinhibition: an N-terminal myristoyl group binds in a pocket in the kinase [17••]. Myristoyl binding appears to contribute to the nearby SH2-docking site [18••]. These findings are consistent with a model in which Abl acts as a three-input gate: it can be activated by exogenous SH2 or SH3 ligands, as well as by insertion of the myristoyl group into the membrane (alternatively, there may exist an unknown hydrophobic ligand that displaces the myristoyl group from its binding pocket). These three inputs would probably function cooperatively, activating the kinase with high specificity.

The Wiskott–Aldrich syndrome protein (WASP) family of actin-regulatory proteins also appears to be able to coordinate at least three distinct inputs (Figure 2b). Previous work has shown that cooperative autoinhibitory interactions allow neuronal WASP (N-WASP) to respond synergistically to a specific combination of inputs: the GTPase Cdc42 and the phosphoinositide PIP2 (phosphoinositol 4,5 bisphosphate) [19,20]. More recent work has revealed that phosphorylation is a third input and can function intimately with Cdc42 activation [21•,22,23]. The Cdc42-binding module that participates in autoinhibition is referred to as the GTPase-binding domain (GBD). A residue in the GBD (Tyr256) can be specifically phosphorylated in a manner that disrupts its autoinhibitory interaction without perturbing its binding to Cdc42. Thus, both Cdc42 and phosphorylation can function cooperatively to disrupt the same autoinhibitory interaction. This dual activation may provide a type of memory: binding of Cdc42 may transiently disrupt the GBD autoinhibitory interaction, thereby facilitating phosphorylation, which may provide a more long-lived state of activation [21•].

Whereas most examples of autoinhibition involve intracellular signaling proteins, one striking new example is an extracellular signaling protein, the epidermal growth factor receptor (EGFR) (Figure 2c). It had long been thought that, like many other transmembrane hormone receptors, EGFR would be activated by ligand-mediated dimerization [24]. However, a flurry of structural and mechanistic studies revealed that EGFR dimerization is not mediated by typical bridging interactions provided by the ligand [25••]. Instead, the extracellular region has a dimerization domain that is blocked in the unliganded state. Upon ligand binding, autoinhibition is released and the dimerization domain is exposed, allowing receptor monomer association and subsequent activation [26••,27••]. Modular autoinhibition appears to be a general solution for gating utilized by diverse types of signaling machinery.

Figure 2

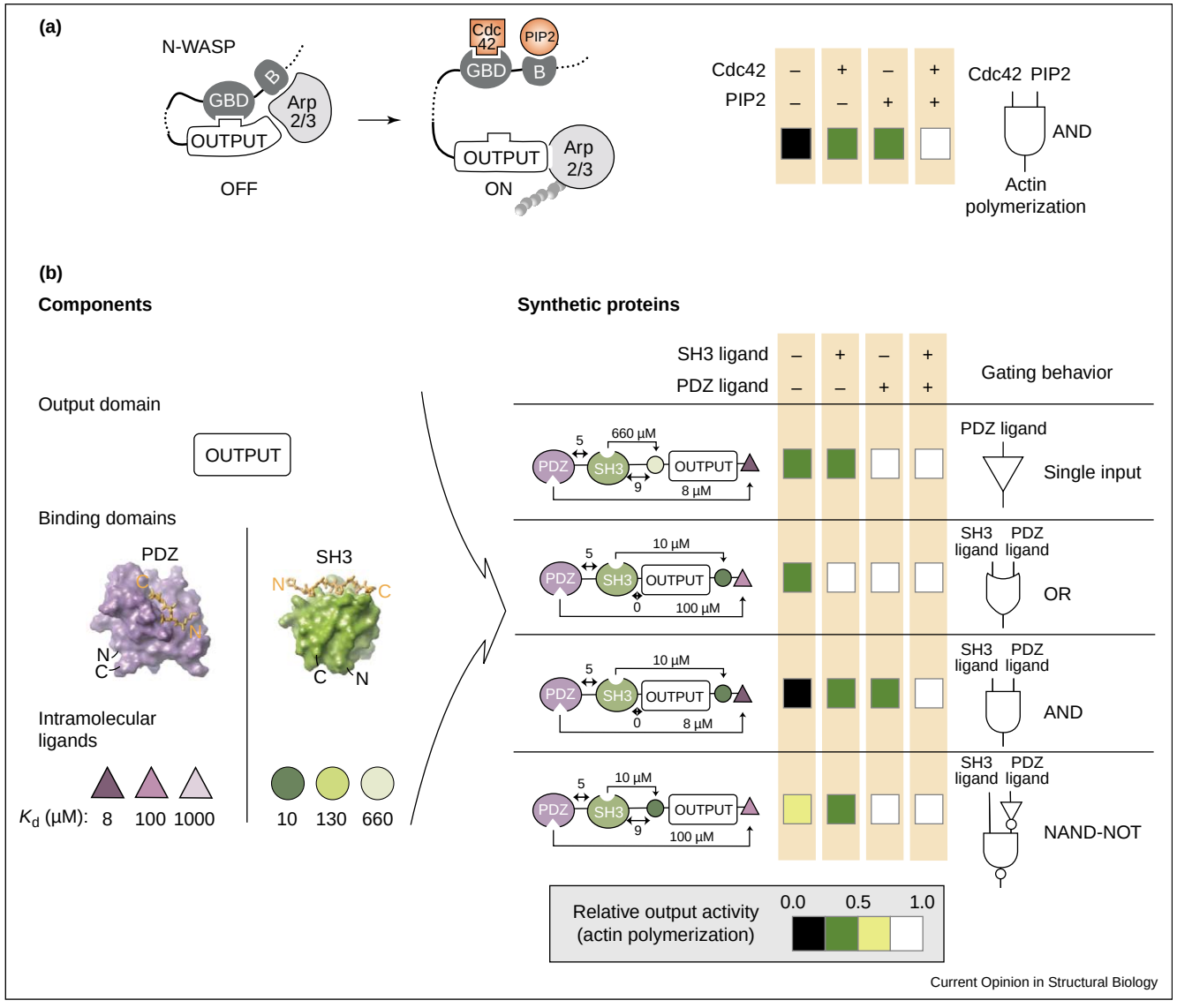


New examples of modular allosteric signaling proteins. **(a)** c-Abl is a modular allosteric switch that may respond to three or more inputs. The kinase domain is conformationally autoinhibited by three coordinated intramolecular interactions: an SH3–peptide interaction, docking of the SH2 domain on the kinase domain and docking of an N-terminal myristoyl group in a hydrophobic pocket on the kinase domain [18••]. The kinase can be activated by ligands that compete with these autoinhibitory interactions, including exogenous SH3 and SH2 ligands, as well as by interactions that might displace the buried myristoyl group (e.g. membrane targeting). Relief of autoinhibition by a combination of these mechanisms allows autophosphorylation and full activation of the kinase. **(b)** WASP family proteins can interact with and activate the Arp2/3 (actin-related protein 2/3) actin-nucleating complex. The output domain, constitutively active in isolation, is autoinhibited by several interactions, including an intramolecular interaction with the GBD. This autoinhibitory interaction can be relieved by binding of the GTPase Cdc42. In addition, recent studies have shown that Src family kinases can phosphorylate Tyr256 on the GBD, destabilizing its ability to participate in the autoinhibitory interaction [21•]. Phosphorylation is only observed when the protein has been activated by Cdc42. Thus, it has been proposed that phosphorylation may provide 'memory' by locking the protein in a longer-lived activated state, even after removal of active Cdc42 as a stimulus. **(c)** EGFR is activated by ligand-mediated dimerization. However, unlike similar receptors, dimerization does not involve any ligand-mediated bridging interactions. Instead, the receptor has a dimerization domain, which, in the inactive state, is occluded by autoinhibitory interactions. EGF ligand relieves this autoinhibition, indirectly promoting dimerization [27••].

Engineering synthetic protein gates: exploiting modularity

The growing number of signaling proteins observed to use modular autoinhibition is consistent with a model in which such mechanisms allow a high degree of evolutionary plasticity. A recent study has attempted to address this hypothesis by mimicking evolution, testing whether domain recombination can be used to reprogram the input control of a modular protein switch [28••] (Figure 3). Specifically, the constitutively active output domain of the actin-regulatory protein N-WASP was fused to combinations of exogenous modular domains (SH3 and PDZ [PSD95, Dlg and ZO-1] domains) and their cognate ligand peptides, in an attempt to generate synthetic

Figure 3



Reprogramming the input control of N-WASP, a modular allosteric switch controlling actin polymerization. **(a)** The output domain of N-WASP can activate actin polymerization via the Arp2/3 complex. Under basal conditions, this domain is conformationally repressed by autoinhibitory interactions involving the GBD and basic (B) domain. These interactions are cooperatively relieved by the binding of Cdc42 and PIP2 to these respective domains. Thus, N-WASP behaves akin to a logical AND gate: the individual inputs are poor activators, but together are highly potent (activity is indicated in truth-table format using color-coded scale) [20]. **(b)** Dueber *et al*. [28••] attempted to reprogram the N-WASP output domain by imposing synthetic autoinhibitory interactions using SH3 and PDZ domains. A library of potential gates was constructed using different domain architectures, and intramolecular SH3 and PDZ domain ligands of varying affinity. The right panel shows examples of diverse gating behaviors generated from this library.

proteins that activated actin polymerization in response to non-native inputs (competing peptide ligands of the domains).

Several important conclusions emerged from this study. First, two-thirds of the resulting synthetic proteins displayed gating behavior (i.e. output activity altered by addition of input ligands), indicating that, from an evolutionary perspective, it may indeed be relatively easy to generate new input/output relationships through domain recombination. Second, many of these synthetic gates displayed sophisticated multi-input control, including AND, OR and other complex gating. Not only is this finding consistent with the notion that modular gates are evolutionarily flexible, but also it suggests that they represent a highly engineerable platform. Several other

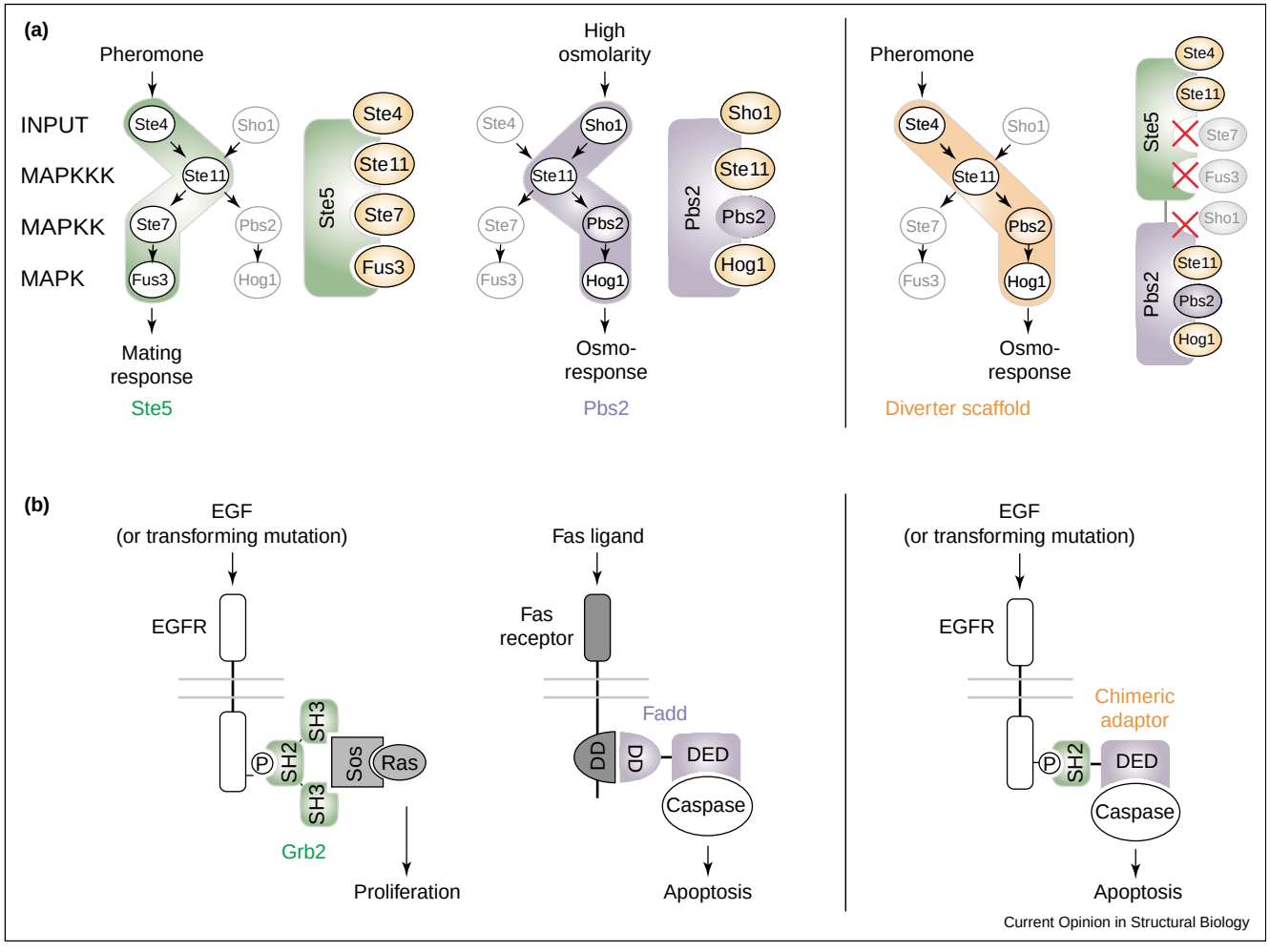
related approaches have been used to engineer protein-based switches with synthetic input/output relationships [29•,30•]. Thus, in the future, it may be possible to engineer a wide variety of protein gates and to link them together into synthetic protein circuits.

Wiring through co-localization: the role of interaction domains, adaptors and scaffolds

Another emerging principle is that co-localization can be a major factor in directing signaling information flow. Consider the seemingly simple problem of how a kinase decides what substrates to phosphorylate. The active site of the kinase may have an intrinsic substrate preference [31]. However, in many cases, other interactions play a major role in recruiting proper substrates. These include docking sites on the kinase domain itself (but distinct from the substrate-binding groove) [9,32] and external modular recruitment domains [4,5,33]. Finally, a growing number of scaffold or adaptor proteins have been shown to contain multiple interaction domains, thereby appearing to guide specificity by tethering the kinase to its substrate. Examples of such scaffold proteins have been reviewed elsewhere [34,35].

One possible advantage of scaffold proteins as wiring devices is their modularity. In principle, scaffolds allow

Figure 4



Synthetic scaffold and adaptor proteins can be used to redirect signaling *in vivo*. **(a)** The yeast mating response and high osmolarity response pathways are both mediated by MAP kinase cascades. Although these pathways share a common MAPKKK, Ste11, the scaffold proteins, Ste5 and Pbs2 (also the MAPKK), organize the pathways into distinct complexes, thereby preventing improper cross-talk. Park *et al*. [36••] showed that a chimeric diverter scaffold could be generated that recruited a novel combination of kinases and yielded a functional pathway in which the mating input (pheromone) stimulated the osmolarity response. **(b)** EGFR and Fas receptor activation is mediated by the conditional recruitment of adaptor proteins, which, in turn, recruit specific downstream effectors. Activation of EGFR leads to receptor autophosphorylation, allowing recruitment of the SH2–SH3 adaptor Grb2. Grb2 in turn recruits the Ras activator Sos, leading to Ras-mediated proliferation. Activation of the Fas receptor leads to the recruitment of the adaptor Fadd, which recruits caspases via its DED. Subsequent activation of these caspases leads to apoptosis. Howard *et al*. [38••] showed that a synthetic SH2–DED adaptor can convert the normal proliferative response of EGFR stimulation into an apoptotic response.

a higher degree of functional flexibility: the same components can be scaffolded into several distinct complexes and therefore can function in different pathways, either in the same cell or in distinct cell types and developmental stages. Furthermore, a scaffold-based platform might be amenable to the evolution of new pathways, as interaction domain recombination and mutation could yield novel scaffolds that recruit novel combinations of components.

Rewiring cells with synthetic scaffolds and adaptors: testing the power of assembly

Although scaffold and adaptor proteins are thought to impose signaling specificity, it is difficult to evaluate their role in the wiring of new pathways. Recently, several studies have addressed this question by attempting to rewire cellular pathways using synthetic scaffold proteins.

In one case, Park *et al.* [36••] examined the ability to rewire yeast mitogen-activated protein kinase (MAPK) pathways using synthetic scaffolds (Figure 4a). Yeast mating and osmoresponse pathways both use scaffold proteins to organize their three-kinase cascades. Strains containing a mutant version of the mating scaffold (Ste5) cannot recruit one pathway member and cannot mate. However, pathway flux can be restored by re-recruiting the missing component through heterologous recruitment domains. Moreover, they showed that a synthetic 'diverter' scaffold that assembled a hybrid combination of kinases could rewire the cell: when stimulated by the mating input (pheromone), the cell responded with the output program normally observed upon osmostress. Similarly, Harris *et al.* [37] found that co-localization by covalent tethering imparts specificity to a kinase that can normally function in multiple pathways.

In another example, Howard *et al.* [38••] showed that synthetic adaptor proteins could be used to redirect growth inputs into a cell death response (Figure 4b). Normally, EGF signaling is propagated by receptor autophosphorylation, followed by recruitment of the SH2–SH3 adaptor Grb2, which in turn activates Ras signaling by recruiting the Ras GEF Sos. Similarly, tumor necrosis factor (TNF) signaling is propagated by adaptor proteins that recruit caspases via death effector domains (DEDs). Howard *et al.* showed that expression of a synthetic adaptor composed of SH2 and DED domains could convert a normally proliferative EGF signal into an apoptotic response. Such rewiring could, in principle, be used as a therapeutic strategy to selectively kill oncogenically transformed cells.

Interestingly, some pathogens seem to have utilized non-native recruitment to rewire host signaling. For example, the *Yersinia* YopM protein appears to act as a synthetic scaffold that directs RSK1 to phosphorylate PRK2, two kinases that are normally not functionally linked [39]. The relevance of this rewiring event in virulence is unclear, but it illustrates the potential for pathogens to exploit scaffolds to their advantage.

These studies highlight the functional modularity of scaffolds that direct the connectivities of signaling proteins. The power of simple co-localization in determining cellular signaling linkages supports the idea that evolution could use similar mechanisms to create new pathways. Nonetheless, it is also clear from studies of natural scaffolds that far more complex mechanisms of regulation are often layered on top of simple co-localization to achieve very precise and controlled signaling responses [34,40•,41•,42•].

Conclusions

Over the past decade, a few simple design principles have emerged concerning how cells guide and evolve specific cellular circuits. Modular domains can often be used to achieve diverse input/output functions, either through allosteric gating or through component co-localization. A major recent advance has been experimental confirmation of the functional plasticity that results from such a modular framework: domain recombination has been used to generate synthetic switches and scaffolds that can specifically alter input/output relationships and generate novel cell signaling behavior. This work supports long-standing ideas about how signaling proteins work and sheds light on how they may have evolved. Perhaps most exciting, however, is that this work opens the door to the possibility of rationally rewiring cell signaling circuits, much in the way that one can readily rewire transcriptional circuits [43,44]. The ability to create new signaling pathways and circuits with precise connectivities and quantitative input/output behaviors would be an invaluable tool in trying to dissect the systems behavior of complex, higher order circuits. Moreover, such tools might provide new therapeutic strategies based on detecting, repairing or rewiring intrinsic cellular defects.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Xiong W, Ferrell JE Jr: **A positive-feedback-based bistable 'memory module' that governs a cell fate decision**. *Nature* 2003, **426**:460-465.
2. Bhalla US, Ram PT, Iyengar R: **MAP kinase phosphatase as a locus of flexibility in a mitogen-activated protein kinase signaling network**. *Science* 2002, **297**:1018-1023.
3. Tyson JJ, Chen KC, Novak B: **Sniffers, buzzers, toggles and blinkers: dynamics of regulatory and signaling pathways in the cell**. *Curr Opin Cell Biol* 2003, **15**:221-231.
4. Pawson T, Nash P: **Assembly of cell regulatory systems through protein interaction domains**. *Science* 2003, **300**:445-452.
5. Smith FD, Scott JD: **Signaling complexes: junctions on the intracellular information super highway**. *Curr Biol* 2002, **12**:R32-R40.

6. Monod J, Changeux JP, Jacob F: **Allosteric proteins and cellular control systems**. *J Mol Biol* 1963, **6**:306-329.

7. Perutz MF: **Hemoglobin structure and respiratory transport**. *Sci Am* 1978, **239**:92-125.

8. Boguski MS, McCormick F: **Proteins regulating Ras and its relatives**. *Nature* 1993, **366**:643-654.

9. Biondi RM, Nebreda AR: **Signalling specificity of Ser/Thr protein kinases through docking-site-mediated interactions**. *Biochem J* 2003, **372**:1-13.

10. Sicheri F, Kuriyan J: **Structures of Src-family tyrosine kinases**. *Curr Opin Struct Biol* 1997, **7**:777-785.

11. Huse M, Kuriyan J: **The conformational plasticity of protein kinases**. *Cell* 2002, **109**:275-282.

12. Lim WA: **The modular logic of signaling proteins: building allosteric switches from simple binding domains**. *Curr Opin Struct Biol* 2002, **12**:61-68.

13. Pufall MA, Graves BJ: **Autoinhibitory domains: modular effectors of cellular regulation**. *Annu Rev Cell Dev Biol* 2002, **18**:421-462.

14. Taylor SS, Radzio-Andzelm E: **Protein kinase inhibition: natural and synthetic variations on a theme**. *Curr Opin Chem Biol* 1997, **1**:219-226.

15. Kobe B, Kemp BE: **Active site-directed protein regulation**. *Nature* 1999, **402**:373-376.

16. Karnoub AE, Symons M, Campbell SL, Der CJ: **Molecular basis for Rho GTPase signaling specificity**. *Breast Cancer Res Treat* 2004, **84**:61-71.

17. •• Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J, Superti-Furga G: **A myristoyl/phosphotyrosine switch regulates c-Abl**. *Cell* 2003, **112**:845-857.
See annotation to [18••].

18. •• Nagar B, Hantschel O, Young MA, Scheffzek K, Veach D, Bornmann W, Clarkson B, Superti-Furga G, Kuriyan J: **Structural basis for the autoinhibition of c-Abl tyrosine kinase**. *Cell* 2003, **112**:859-871.
Two papers [17••,18••] describe the mechanism of autoinhibition of the kinase c-Abl. Like the Src family kinases, intramolecular SH2 and SH3 domain interactions participate in autoinhibition. In addition, an N-terminal myristoyl group docks against the kinase domain to form a third autoinhibitory interaction. Thus, inputs that disrupt any of these three autoinhibitory interactions may serve as activating stimuli.

19. Rohatgi R, Ma L, Miki H, Lopez M, Kirchhausen T, Takenawa T, Kirschner MW: **The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly**. *Cell* 1999, **97**:221-231.

20. Prehoda KE, Scott JA, Mullins RD, Lim WA: **Integration of multiple signals through cooperative regulation of the N-WASP-Arp2/3 complex**. *Science* 2000, **290**:801-806.

21. • Torres E, Rosen MK: **Contingent phosphorylation/dephosphorylation provides a mechanism of molecular memory in WASP**. *Mol Cell* 2003, **11**:1215-1227.
This study, together with [22,23], shows that tyrosine phosphorylation of the autoinhibitory GBD can serve as an activating input for the actin-regulatory protein WASP. Torres *et al.* examined the relationship between Cdc42 and phosphorylation-dependent activation. Their findings indicate that phosphorylation of Tyr256 is unfavorable unless activated Cdc42 is present. They suggest that transient activation of N-WASP by Cdc42 may allow long-term activation via phosphorylation, a form of memory.

22. Cory GO, Garg R, Cramer R, Ridley AJ: **Phosphorylation of tyrosine 291 enhances the ability of WASp to stimulate actin polymerization and filopodium formation. Wiskott-Aldrich Syndrome protein**. *J Biol Chem* 2002, **277**:45115-45121.

23. Badour K, Zhang J, Shi F, Leng Y, Collins M, Siminovitch KA: **Fyn and PTP-PEST-mediated regulation of Wiskott-Aldrich syndrome protein (WASp) tyrosine phosphorylation is required for coupling T cell antigen receptor engagement to WASp effector function and T cell activation**. *J Exp Med* 2004, **199**:99-112.

24. Kossiakoff AA, De Vos AM: **Structural basis for cytokine hormone-receptor recognition and receptor activation**. *Adv Protein Chem* 1998, **52**:67-108.

25. •• Burgess AW, Cho HS, Eigenbrot C, Ferguson KM, Garrett TP, Leahy DJ, Lemmon MA, Sliwkowski MX, Ward CW, Yokoyama S: **An open-and-shut case? Recent insights into the activation of EGF/ErbB receptors**. *Mol Cell* 2003, **12**:541-552.
See annotation to [27••].

26. •• Garrett TP, McKern NM, Lou M, Elleman TC, Adams TE, Lovrecz GO, Zhu HJ, Walker F, Frenkel MJ, Hoyne PA *et al.*: **Crystal structure of a truncated epidermal growth factor receptor extracellular domain bound to transforming growth factor alpha**. *Cell* 2002, **110**:763-773.
See annotation to [27••].

27. •• Ferguson KM, Berger MB, Mendrola JM, Cho HS, Leahy DJ, Lemmon MA: **EGF activates its receptor by removing interactions that autoinhibit ectodomain dimerization**. *Mol Cell* 2003, **11**:507-517.
These three papers [25••–27••] describe recent structural studies that have elucidated the mechanism of activation of the EGF/ErbB receptor family. Contrary to expectations, the EGF ligand induces receptor dimerization by relieving autoinhibitory interactions that occlude a dimerization domain. These studies are excellently reviewed in [25••].

28. •• Dueber JE, Yeh BJ, Chak K, Lim WA: **Reprogramming control of an allosteric signaling switch through modular recombination**. *Science* 2003, **301**:1904-1908.
Inspired by the modular architecture of many signaling proteins, heterologous protein–protein interaction domains were recombined with the output domain of the actin polymerization switch N-WASP. The resulting synthetic proteins displayed diverse and complex gating behaviors analogous to those observed in natural signaling proteins. These findings support the hypothesis that such a modular framework may facilitate the evolution of novel gates and, therefore, novel signaling networks.

29. • Guntas G, Ostermeier M: **Creation of an allosteric enzyme by domain insertion**. *J Mol Biol* 2004, **336**:263-273.
See annotation to [30•].

30. • Radley TL, Markowska AI, Bettinger BT, Ha JH, Loh SN: **Allosteric switching by mutually exclusive folding of protein domains**. *J Mol Biol* 2003, **332**:529-536.
Two papers [29•,30•] describe another approach to generating synthetic proteins with gating behavior. In both of these studies, two distinct protein folds are energetically linked by inserting one within the sequence of the other. This thermodynamic coupling allows ligands of one fold to modulate the activity of the other fold.

31. Songyang Z, Carraway KL III, Eck MJ, Harrison SC, Feldman RA, Mohammadi M, Schlessinger J, Hubbard SR, Smith DP, Eng C *et al.*: **Catalytic specificity of protein-tyrosine kinases is critical for selective signalling**. *Nature* 1995, **373**:536-539.

32. Bardwell AJ, Abdollahi M, Bardwell L: **Docking sites on mitogen-activated protein kinase (MAPK) kinases. MAPK phosphatases and the Elk-1 transcription factor compete for MAPK binding and are crucial for enzymic activity**. *Biochem J* 2003, **370**:1077-1085.

33. Miller WT: **Determinants of substrate recognition in nonreceptor tyrosine kinases**. *Acc Chem Res* 2003, **36**:393-400.

34. Morrison DK, Davis RJ: **Regulation of MAP kinase signaling modules by scaffold proteins in mammals**. *Annu Rev Cell Dev Biol* 2003, **19**:91-118.

35. Elion EA: **The Ste5p scaffold**. *J Cell Sci* 2001, **114**:3967-3978.

36. •• Park SH, Zarrinpar A, Lim WA: **Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms**. *Science* 2003, **299**:1061-1064.
In this study, alternative scaffolding interactions were used to rewire MAPK signaling in yeast. Interaction-defective scaffolds could be rescued by re-recruiting components via heterologous protein–protein interactions. Moreover, chimeric scaffolds that recruit a novel set of kinases could redirect signaling, resulting in cells that display a novel, non-physiological input/output signaling response. This paper and the following one [37] highlight the importance of co-recruitment in determining signaling specificity.

37. Harris K, Lamson RE, Nelson B, Hughes TR, Marton MJ, Roberts CJ, Boone C, Pryciak PM: **Role of scaffolds in MAP**

kinase pathway specificity revealed by custom design of pathway-dedicated signaling proteins. *Curr Biol* 2001, **11**:1815-1824.

38. •• Howard PL, Chia MC, Del Rizzo S, Liu FF, Pawson T: **Redirecting tyrosine kinase signaling to an apoptotic caspase pathway through chimeric adaptor proteins**. *Proc Natl Acad Sci USA* 2003, **100**:11267-11272.

This study illustrates the evolutionary flexibility resulting from the modular design of adaptor proteins. The EGF signaling pathway, which normally stimulates growth and survival following receptor activation, was redirected by a synthetic SH2–DED adaptor to a death response. The studies described in [36••,38••] reveal that modular scaffolds and adaptors are highly flexible organizing factors that can facilitate pathway evolution and engineering.

39. McDonald C, Vacratsis PO, Bliska JB, Dixon JE: **The *Yersinia* virulence factor YopM forms a novel protein complex with two cellular kinases**. *J Biol Chem* 2003, **278**:18514-18523.

40. • Nguyen A, Burack WR, Stock JL, Kortum R, Chaika OV, Afkarian M, Muller WJ, Murphy KM, Morrison DK, Lewis RE *et al.*: **Kinase suppressor of Ras (KSR) is a scaffold which facilitates mitogen-activated protein kinase activation *in vivo***. *Mol Cell Biol* 2002, **22**:3035-3045.

See annotation to [42•].

41. • Ory S, Zhou M, Conrads TP, Veenstra TD, Morrison DK: **Protein phosphatase 2A positively regulates Ras signaling by dephosphorylating KSR1 and Raf-1 on critical 14-3-3 binding sites**. *Curr Biol* 2003, **13**:1356-1364.

See annotation to [42•].

42. • Matheny SA, Chen C, Kortum RL, Razidlo GL, Lewis RE, White MA: **Ras regulates assembly of mitogenic signalling complexes through the effector protein IMP**. *Nature* 2004, **427**:256-260.

These three studies [40•–42•] exemplify new findings indicating that natural scaffold proteins utilize higher order mechanisms of regulation in addition to simple tethering of pathway components. The interactions of the Ras pathway scaffold KSR (kinase suppressor of Ras) are highly regulated. First, signaling activity induces KSR membrane recruitment and interaction with ERK (via displacement of a 14-3-3 protein). Second, signaling activity also relieves repression by the newly identified negative regulator IMP (impedes mitogenic signal propagation), which sequesters KSR in an inactive state. These multiple layers of scaffold regulation are likely to result in a precise, finely tuned Ras pathway response.

43. Elowitz MB, Leibler S: **A synthetic oscillatory network of transcriptional regulators**. *Nature* 2000, **403**:335-338.

44. Kobayashi H, Kaern M, Araki M, Chung K, Gardner TS, Cantor CR, Collins JJ: **Programmable cells: interfacing natural and engineered gene networks**. *Proc Natl Acad Sci USA* 2004, **101**:8414-8419.

45. Hof P, Pluskey S, Dhe-Paganon S, Eck MJ, Shoelson SE: **Crystal structure of the tyrosine phosphatase SHP-2**. *Cell* 1998, **92**:441-450.

46. Lei M, Lu W, Meng W, Parrini MC, Eck MJ, Mayer BJ, Harrison SC: **Structure of PAK1 in an autoinhibited conformation reveals a multistage activation switch**. *Cell* 2000, **102**:387-397.

47. Kobe B, Heierhorst J, Feil SC, Parker MW, Benian GM, Weiss KR, Kemp BE: **Giant protein kinases: domain interactions and structural basis of autoregulation**. *EMBO J* 1996, **15**:6810-6821.

48. Groemping Y, Lapouge K, Smerdon SJ, Rittinger K: **Molecular basis of phosphorylation-induced activation of the NADPH oxidase**. *Cell* 2003, **113**:343-355.

49. Aghazadeh B, Lowry WE, Huang XY, Rosen MK: **Structural basis for relief of autoinhibition of the Dbl homology domain of proto-oncogene Vav by tyrosine phosphorylation**. *Cell* 2000, **102**:625-633.

50. Jang YJ, Lin CY, Ma S, Erikson RL: **Functional studies on the role of the C-terminal domain of mammalian polo-like kinase**. *Proc Natl Acad Sci USA* 2002, **99**:1984-1989.

51. Bi F, Debreceni B, Zhu K, Salani B, Eva A, Zheng Y: **Autoinhibition mechanism of proto-Dbl**. *Mol Cell Biol* 2001, **21**:1463-1474.

52. Hussain NK, Jenna S, Glogauer M, Quinn CC, Wasiak S, Guipponi M, Antonarakis SE, Kay BK, Stossel TP, Lamarche-Vane N *et al.*: **Endocytic protein intersectin-l regulates actin assembly via Cdc42 and N-WASP**. *Nat Cell Biol* 2001, **3**:927-932.

53. Zamanian JL, Kelly RB: **Intersectin 1L guanine nucleotide exchange activity is regulated by adjacent src homology 3 domains that are also involved in endocytosis**. *Mol Biol Cell* 2003, **14**:1624-1637.

54. Shimada Y, Wiget P, Gulli MP, Bi E, Peter M: **The nucleotide exchange factor Cdc24p may be regulated by auto-inhibition**. *EMBO J* 2004, **23**:1051-1062.

55. Ahmed S, Lee J, Kozma R, Best A, Monfries C, Lim L: **A novel functional target for tumor-promoting phorbol esters and lysophosphatidic acid. The p21rac-GTPase activating protein n-chimaerin**. *J Biol Chem* 1993, **268**:10709-10712.

56. Welch HC, Coadwell WJ, Ellson CD, Ferguson GJ, Andrews SR, Erdjument-Bromage H, Tempst P, Hawkins PT, Stephens LR: **P-Rex1, a PtdIns(3,4,5)P3- and Gbetagamma-regulated guanine-nucleotide exchange factor for Rac**. *Cell* 2002, **108**:809-821.

57. Amano M, Chihara K, Nakamura N, Kaneko T, Matsuura Y, Kaibuchi K: **The COOH terminus of Rho-kinase negatively regulates rho-kinase activity**. *J Biol Chem* 1999, **274**:32418-32424.