

LETTERS

Rewiring cellular morphology pathways with synthetic guanine nucleotide exchange factors

Brian J. Yeh^{1,2*}, Robert J. Rutigliano^{3*}, Anrica Deb², Dafna Bar-Sagi^{3,4} & Wendell A. Lim²

Eukaryotic cells mobilize the actin cytoskeleton to generate a remarkable diversity of morphological behaviours, including motility, phagocytosis and cytokinesis. Much of this diversity is mediated by guanine nucleotide exchange factors (GEFs) that activate Rho family GTPases—the master regulators of the actin cytoskeleton^{1–3}. There are over 80 Rho GEFs in the human genome (compared to only 22 genes for the Rho GTPases themselves), and the evolution of new and diverse GEFs is thought to provide a mechanism for linking the core cytoskeletal machinery to a wide range of new control inputs. Here we test this hypothesis and ask if we can systematically reprogramme cellular morphology by engineering synthetic GEF proteins. We focused on Dbl family Rho GEFs, which have a highly modular structure common to many signalling proteins^{4,5}: they contain a catalytic Dbl homology (DH) domain linked to diverse regulatory domains, many of which autoinhibit GEF activity^{2,3}. Here we show that by recombining catalytic GEF domains with new regulatory modules, we can generate synthetic GEFs that are activated by non-native inputs. We have used these synthetic GEFs to reprogramme cellular behaviour in diverse ways. The GEFs can be used to link specific cytoskeletal responses to normally unrelated upstream signalling pathways. In addition, multiple synthetic GEFs can be linked as components in series to form an artificial cascade with improved signal processing behaviour. These results show the high degree of evolutionary plasticity of this important family of modular signalling proteins, and indicate that it may be possible to use synthetic biology approaches to manipulate the complex spatio-temporal control of cell morphology.

Rho family GTPases are central signalling molecules in the regulation of the actin cytoskeleton¹ (Fig. 1a). These proteins are conformational switches that exist in GDP- and GTP-bound states; however, only the GTP-bound state actively transduces signal to downstream effectors. Cycling between states is primarily controlled by opposing enzymes: GTPase activating proteins promote hydrolysis of bound GTP to GDP (inactivation), whereas GEFs promote exchange of bound GDP for GTP (activation). The three canonical members of the Rho family—Cdc42, Rac1 and RhoA—stimulate the distinct morphological outputs of protrusive filopodia (thin actin microspikes), protrusive lamellipodia (broad membrane ruffles) and contractile actin:myosin filaments, respectively.

As an initial target for rewiring GTPase signalling, we attempted to reprogramme Dbl family GEFs (Fig. 1b) so that their activity was controlled by protein kinase A (PKA), a well-characterized prototypical kinase⁶ (Fig. 2a). We first designed a PKA-sensitive autoinhibitory module, inspired by natural examples⁷, that consisted of a PDZ (PSD95, Dlg, ZO-1) domain–peptide interaction pair that

could be disrupted by PKA phosphorylation. The syntrophin PDZ domain recognizes short carboxy-terminal peptide motifs (consensus sequence (R/K)E(S/T)xψ-COOH; ψ denotes aliphatic residues)⁸, which are close in sequence to the ideal PKA substrate (RRRRSIIIF)⁹. A hybrid sequence (RRRESIV-COOH) could serve both as an interaction ligand for the syntrophin PDZ domain and

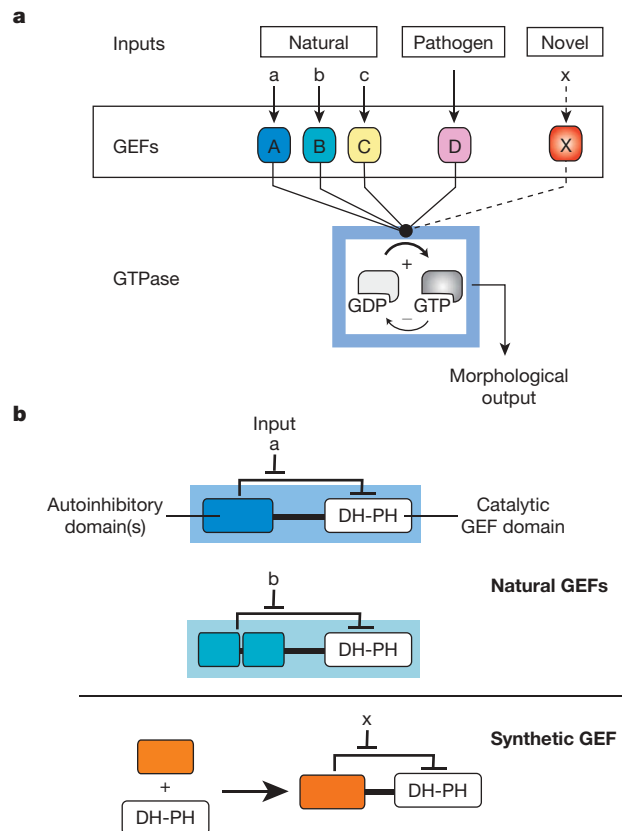


Figure 1 | GEFs link diverse inputs to Rho GTPase modules that control cell morphology. **a**, GEFs functionally connect signalling inputs to activation of Rho GTPases, which regulate morphology of the actin cytoskeleton. Some bacterial pathogens encode GEFs that activate host GTPases³⁰. Synthetic GEFs could, in principle, mediate new connections in living cells. **b**, The largest family of Rho GEFs are Dbl-related proteins, which share a catalytic DH-PH core. In many cases, adjacent modular domains mediate autoinhibitory interactions that can be disrupted by specific inputs. Here we exploit this modular structure to construct synthetic GEFs.

¹Chemistry and Chemical Biology Graduate Program, University of California, San Francisco, San Francisco, California 94158-2517, USA. ²Department of Cellular and Molecular Pharmacology and the Cell Propulsion Lab, UCSF/UCB NIH Nanomedicine Development Center, University of California, San Francisco, San Francisco, California 94158-2517, USA. ³Department of Molecular Genetics and Microbiology, School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794, USA. ⁴Department of Biochemistry, New York University School of Medicine, New York, New York 10016, USA.

*These authors contributed equally to this work.

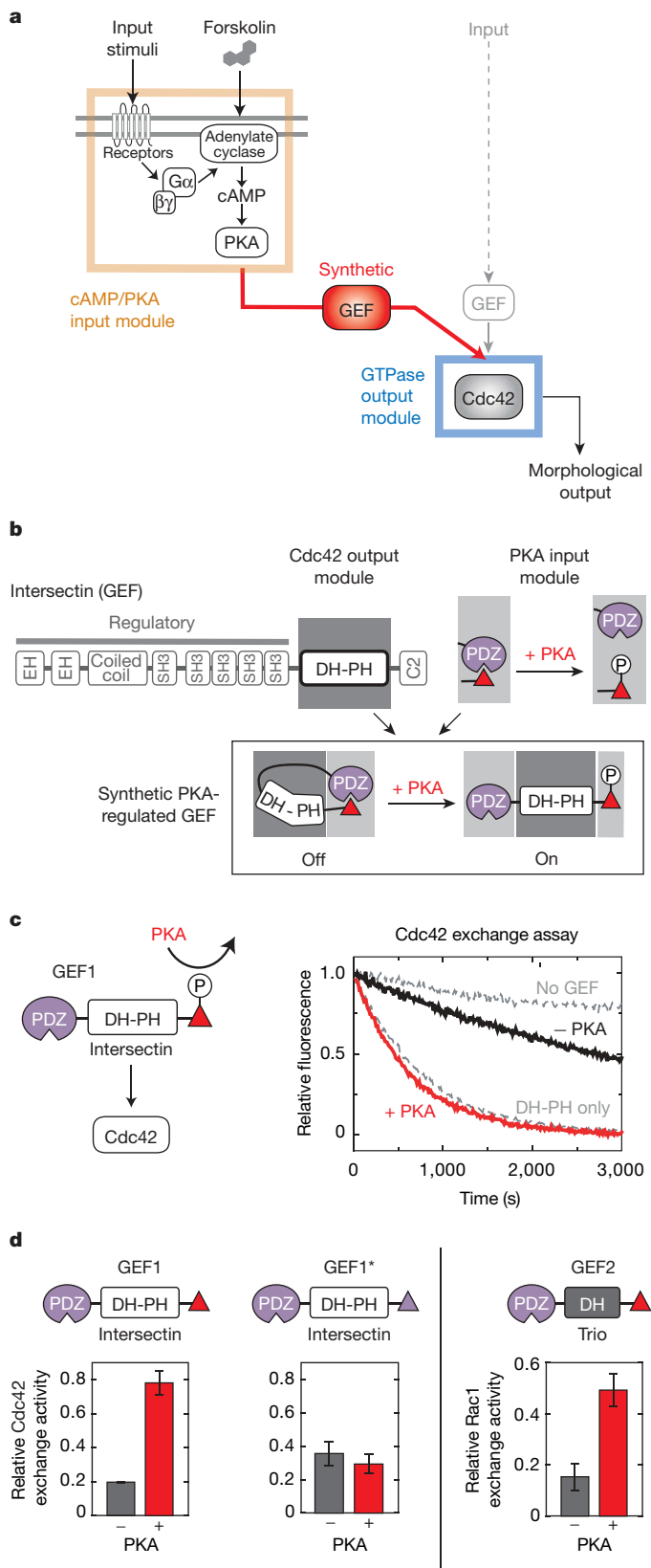


Figure 2 | Modular recombination yields PKA-responsive synthetic GEFs. **a**, We attempted to engineer GEFs that link PKA signalling to specific cytoskeletal changes. **b**, PKA-sensitive GEFs were constructed by fusing Dbl homology (DH) GEF output modules with a PKA input module composed of the syntrophin PDZ domain and a peptide that binds the PDZ domain and is a PKA substrate. **c**, *In vitro* assay of GEF1 showing activation by PKA. Dissociation of fluorescent mant-GDP from Cdc42 was measured in the presence of no GEF or constitutively active intersectin DH-PH (dotted

a PKA substrate. Most importantly, we found that phosphorylation by PKA disrupted binding to the PDZ domain (Supplementary Fig. 1).

To build a Cdc42 GEF that could be activated by PKA, we fused this PKA-sensitive PDZ-peptide interaction module to the Dbl homology-peckstrin homology (DH-PH) catalytic core from intersectin (Itsn1)—a Cdc42-specific Dbl family member, the catalytic activity of which is normally regulated by autoinhibitory SH3 domains^{10,11} (Fig. 2b). We refer to this construct as GEF1 (see Supplementary Tables 1 and 2 for details of all synthetic GEFs). In an *in vitro* Cdc42 nucleotide exchange assay, GEF1 was repressed relative to the constitutively active DH-PH fragment (<20% activity), indicating that the intramolecular PDZ interaction sterically occluded or conformationally disrupted the DH-PH domain (Fig. 2c, d and Supplementary Fig. 2). Phosphorylation of GEF1 by PKA relieved repression, increasing Cdc42 exchange activity (Fig. 2c, d and Supplementary Fig. 2). For a control, we mutated the peptide to a sequence that could still bind the PDZ domain but could not be phosphorylated by PKA. A construct bearing this mutation (GEF1*) was still repressed, but was not activated by PKA (Fig. 2d and Supplementary Fig. 2).

To test if the PKA regulatory module could be transferred to another GEF, we replaced the intersectin DH-PH with the amino-terminal DH domain of Trio, which preferentially activates Rac1 (GEF2)¹². GEF2 was also repressed *in vitro* (relative to the Trio DH domain alone), and could be activated by PKA (Fig. 2d and Supplementary Fig. 2). A control construct bearing a non-phosphorylatable peptide (GEF2*) could not be activated by PKA (Supplementary Fig. 3).

In total, we fused the PDZ-peptide module to the DH and/or DH-PH fragments of five Dbl family members with varying GTPase specificities (including intersectin and Trio), and tested their activity *in vitro*. All seven constructs tested showed some degree of repression under basal conditions, and four out of seven were activated by PKA (Supplementary Table 3). No attempts were made to optimize autoinhibitory affinity, domain orientation or interdomain linker lengths, and it is likely that such efforts would improve activation of the three remaining synthetic GEFs¹³.

To test if these synthetic GEF proteins could create new functional signalling linkages *in vivo*, we introduced GEF1 and GEF2 into cells by microinjection. We first tested the effect of microinjecting the unregulated catalytic GEF modules into the REF52 fibroblast cell line (Fig. 3a). As expected, microinjection of the Trio DH domain led to a constitutive Rac1-associated lamellipodial phenotype. Microinjection of the intersectin DH-PH module yielded a constitutive Cdc42-associated filopodial phenotype in a large fraction of cells; however, a significant but inconsistent fraction of these cells showed an alternative rounded phenotype that is distinct from filopodia and lamellipodia (Supplementary Fig. 4). Co-injection of additional Cdc42 with the intersectin DH-PH resulted only in cells with filopodia, alleviating this dual phenotype problem. Thus, to simplify phenotypic scoring, we co-injected the relevant GTPases in all of the following experiments. We estimate that we are increasing the cellular concentration of the specific GTPases by approximately two-fold, which has no morphological effect in the absence of the GEF domain. This method has previously been used to clarify scoring of GEF-induced phenotypes¹⁴.

lines), GEF1 (solid black line), or GEF1 pre-treated with PKA (red line). **d**, Activities of synthetic GEFs (relative to intersectin DH-PH or Trio DH). GEF1 and GEF2 were basally repressed, but were activated by PKA. GEF1* contains a mutation that abolishes phosphorylation by PKA, but retains binding to the syntrophin PDZ. Error bars represent s.d. of three experiments. Substrate specificities of GEF1 and GEF2 were identical to those of their respective parental DH proteins, intersectin and Trio (Supplementary Table 4).

Injection of GEF1 into REF52 cells resulted in a new PKA-activated filopodial response. After microinjecting the purified proteins, we tested the cellular response to stimulation with increasing doses of forskolin, a pharmacological activator of PKA¹⁵ (Fig. 3b). When GEF1 was injected into cells, even in the absence of PKA stimulation, there was a weak background activity; 14% of the cells showed filopodia, probably owing to somewhat leaky repression of GEF activity. However, this phenotype was much weaker than that observed with injection of an equivalent amount of the unregulated DH-PH module (>95% of cells with filopodia). Most importantly, filopodia were stimulated in a dose-dependent fashion as a function of forskolin concentration such that >60% of the cells had filopodia at the highest forskolin concentrations tested (Fig. 3c). Furthermore, induction of filopodia was observed within minutes of forskolin addition to cells pre-injected with GEF1 (Supplementary Movie), demonstrating the rapid timescale of response with protein-based networks that do not require transcription and translation. Forskolin treatment of cells lacking GEF1 (injected only with Cdc42) led to a small background stimulation of filopodia (~20%), indicating that there is only a weak endogenous linkage between PKA and filopodia formation in REF52 cells. As an important control, we observed no significant stimulation of filopodia in cells microinjected with GEF1*, which is autoinhibited but cannot be activated by PKA. These results imply that the strong stimulation of filopodia is the result of a new, functional signalling connection mediated directly by the engineered GEF1 protein.

Similarly, injection of GEF2 into REF52 cells resulted in a PKA-inducible lamellipodial response. Injection of GEF2 and Rac1 had little basal effect on the cells (4% of cells with lamellipodia); however, treatment with forskolin resulted in a dose-dependent increase in the number of cells with lamellipodia (to >60%) (Fig. 3d). Activation of lamellipodia also occurred within minutes of stimulation (data not shown). Cells injected with GEF2* showed no significant lamellipodial response to forskolin (Supplementary Fig. 3). Thus, both synthetic GEFs are capable of mediating linkages between

the endogenous PKA signalling pathway and Rho GTPase-mediated morphological rearrangements in live cells.

Many complex behaviours observed in living cells are mediated by multiple signalling proteins that do not function alone, but instead are linked into more complex multistep pathways¹⁶. For example, the canonical GTPase Ras can activate multiple effectors, including the Rac1 GEF, Tiam1 (ref. 17). Thus, we asked whether we could link synthetic GEFs with specifically engineered input–output linkages into a two GTPase cascade in which PKA would activate Cdc42, and Cdc42 would in turn activate Rac1 (Fig. 4a). GEF1 could provide the connection between PKA and Cdc42; however, the second step required a Cdc42-responsive autoinhibitory module, which we extracted from the signalling protein N-WASP as a GTPase-binding domain (GBD) that recognizes a short central domain (C). The GBD–C interaction is normally involved in autoinhibition of N-WASP, and can be disrupted by activated Cdc42 (refs 18–21). We fused the GBD–C module to the Trio DH domain, producing a Rac1-specific GEF that is activated by Cdc42 (GEF3). *In vitro* analysis of GEF3 showed that its Rac1 exchange activity was regulated by Cdc42, as expected (data not shown), providing further evidence for the flexibility of this overall framework for engineering diverse signalling linkages.

Co-injection of GEF1 and GEF3 (along with Cdc42 and Rac1) into REF52 cells resulted in a new signalling cascade: PKA stimulation by forskolin ultimately led to Rac1 activation and a lamellipodial phenotype (Fig. 4b). Almost no filopodial response was observed, perhaps because lamellipodia tend to be dominant over filopodia, and because much of the activated Cdc42 may be sequestered by binding to GEF3 instead of other effectors. To confirm that signal is passing through both synthetic GEFs, we disrupted each individual component. GEF3 was selectively disrupted by a small deletion in the GBD that blocks binding to Cdc42(GTP) but does not affect autoinhibition (GEF3*)^{20,21}. When GEF1 and GEF3* were injected into REF52 cells, forskolin treatment led to only the activation of Cdc42, resulting in robust formation of filopodia (Fig. 4b). Similarly, GEF1* is a

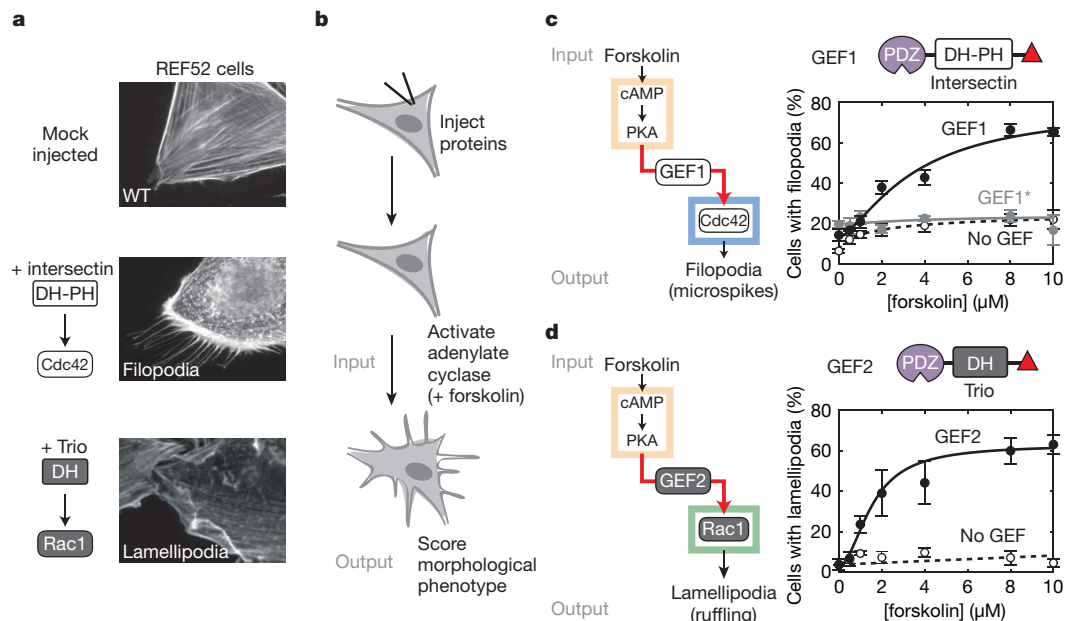


Figure 3 | Synthetic GEFs generate new PKA-dependent morphological changes in cells. **a**, Microinjection of constitutively active intersectin DH-PH (and Cdc42) induced filopodia in REF52 cells. Constitutively active Trio DH (and Rac1) induced lamellipodia. **b**, After microinjecting synthetic GEFs, cells were treated with forskolin, which activates endogenous PKA. Morphological response was scored by counting cells exhibiting filopodia or lamellipodia. **c**, Filopodia were stimulated by forskolin treatment of cells injected with GEF1 (solid black line). Forskolin treatment of cells lacking

GEF1 led only to a weak background stimulation of filopodia (dashed line). Filopodia were not significantly stimulated in cells injected with GEF1* (grey line), which is autoinhibited but cannot be activated by PKA. Data points represent mean \pm s.d. of three experiments (>50 cells scored per experiment), and were fit to a conventional Hill equation. **d**, Injection with GEF2 allowed stimulation of lamellipodia by forskolin (solid line). Little or no response was observed in cells lacking GEF2 (dashed line). Error bars as in **c**.

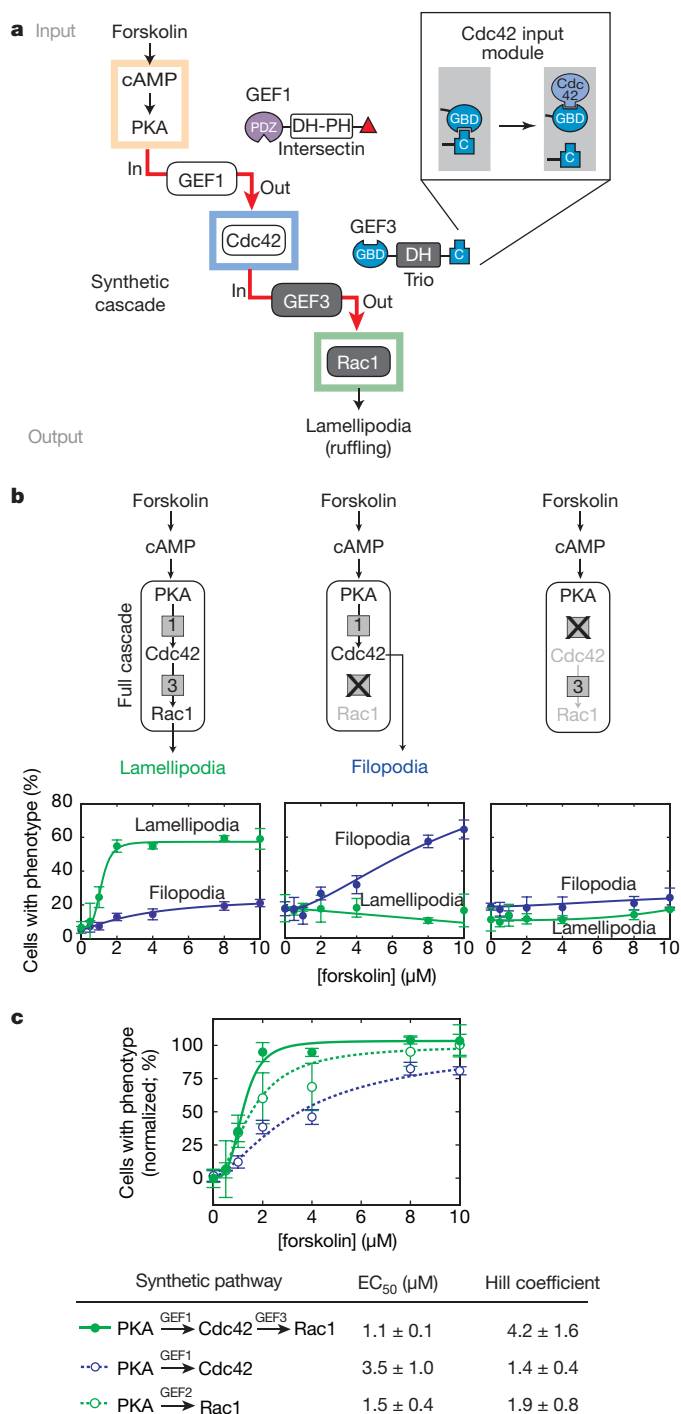


Figure 4 | Two synthetic GEFs can be linked in series to form a higher order cascade. **a**, GEF1 and GEF3 form a cascade in which PKA activates Cdc42, which in turn activates Rac1. GEF3 is composed of the Trio DH domain and a Cdc42 input module extracted from N-WASP. **b**, Co-injection of GEF1 and GEF3 resulted in a functioning cascade (left panel). Forskolin induced lamellipodia (green curve) with very little induction of filopodia (blue curve). Forskolin treatment of cells co-injected with GEF1 and GEF3* (which cannot respond to Cdc42) resulted only in filopodia (middle panel). Cells co-injected with GEF1* and GEF3 showed no significant filopodial or lamellipodial response (right panel). Data points represent mean ± s.d. of three experiments (>50 cells scored per experiment), and were fit to a conventional Hill equation. **c**, Comparison of the GEF1–GEF3 cascade (solid green line) to direct single-GEF circuits mediated by GEF1 (dashed blue line) or GEF2 (dashed green line). Data were normalized to lower and upper baselines obtained from fits to the Hill equation. EC₅₀, effector concentration for half-maximum response. Error bars as in **b**.

variant of GEF1 that cannot be phosphorylated and activated by PKA. As predicted, cells injected with GEF1* and GEF3 showed no activation of either Cdc42 or Rac1 (no significant filopodial or lamellipodial response) on forskolin treatment. Together, these results imply that GEF1 and GEF3 form a functional signalling cascade that links PKA to a lamellipodial response.

The GEF1–GEF3 cascade demonstrated several properties that distinguished it from the direct, single GEF circuits (Fig. 4c and Supplementary Fig. 5). First, the synthetic cascade had dampened noise: there was a ~2-fold reduction in basal response (no forskolin stimulation), both in terms of filopodial and lamellipodial output, when GEF3 was introduced downstream of GEF1. Second, the cascade seemed to amplify response within a certain range of stimulation. In the direct PKA→Cdc42 circuit (GEF1 only), the amount of Cdc42 activated by 1–2 μM forskolin was insufficient to mount a significant filopodial response. However, in the GEF1–GEF3 cascade, this low Cdc42 activation was sufficient to activate GEF3, producing a stronger Rac1-mediated lamellipodial response. Third, the cascade is ultrasensitive—it had a sharp activation threshold with an apparent Hill coefficient (n_H) of >4, despite the fact that its individual components respond in a linear (Michaelian) fashion. The increased ultrasensitivity of the cascade is consistent with theoretical and experimental studies that compared pathways with increasing number of steps^{16,22}. Although individual signalling proteins can exhibit non-linear behaviours¹³, these simple synthetic GEFs can be linked into higher order architectures that begin to show complex emergent properties.

Here we demonstrate that Rho GEFs provide a flexible framework for engineering novel signalling pathways. Modular recombination allows the expansion of GTPase control relationships beyond those generated through evolution. GTPases regulate many biological processes (nuclear trafficking, endocytosis, and so on)²³; thus, such approaches could be applied to manipulate these processes. These findings, along with related studies, demonstrate that modular protein signalling components can be engineered in a relatively facile manner, indicating that it may be possible to apply synthetic biology approaches to generate cells with precisely engineered target behaviours^{5,24–27}. Although there has been significant progress in engineering transcriptional networks in living cells^{28,29}, there are comparatively fewer examples of synthetic signal transduction networks. These protein-based networks are important because they mediate many of the rapid and spatially precise responses in cells, including complex properties such as cell shape and movement. The ability to manipulate these properties will be critical for engineering cells with diverse therapeutic and biotechnological applications.

METHODS

For detailed information on all methods see Supplementary Information.

Synthetic GEFs. Proteins (sequence details in Supplementary Tables 1, 2 and 3) were expressed as hexahistidine fusions in *Escherichia coli* BL21 (DE3)RIL, and purified by chromatography on Ni-NTA resin (Qiagen).

In vitro nucleotide exchange assays. Dissociation of *N*-methylanthraniloyl (mant)-GDP from Cdc42 (qualitative assays) or association of mant-GDP with GTPases (quantitative assays) were measured using a SpectraMax Gemini XS (Molecular Devices) fluorescence multi-well plate reader (25 °C; excitation, 360 nm; emission, 440 nm).

Microinjection experiments. Rat embryo fibroblasts (REF52) were grown as sub-confluent monolayers overnight and serum-starved for 24 h before injection. Proteins were injected into the cytosol of cells using an Eppendorf 5246 pressure system and an Eppendorf 5171 microinjector. GEFs were injected at the molar equivalent of 2 mg ml⁻¹ intersectin DH-PH, and associated GTPases were co-injected at 0.5 mg ml⁻¹ (concentrations are in the needle). Injected cells were incubated for 30 min at 37 °C, treated with the indicated concentration of forskolin for 30 min, and allowed to recover from any deleterious effects of the drug for 30 min. Cells were then fixed, stained with rhodamine-phalloidin, and mounted onto glass slides. Morphological phenotypes were scored in a blind fashion (without knowledge of the experimental condition). Cells showing at least 5 protrusive spikes were scored positive for filopodia, and cells that had dense peripheral actin staining were scored positive for lamellipodia (no cells

were observed with both filopodia and lamellipodia). The percentage of cells with each phenotype was calculated by dividing the number of cells with the scored phenotype by the total number of cells scored.

Received 19 December 2006; accepted 17 April 2007.

Published online 21 May 2007.

- Jaffe, A. B. & Hall, A. Rho GTPases: biochemistry and biology. *Annu. Rev. Cell Dev. Biol.* **21**, 247–269 (2005).
- Hoffman, G. R. & Cerione, R. A. Signaling to the Rho GTPases: networking with the DH domain. *FEBS Lett.* **513**, 85–91 (2002).
- Rossman, K. L., Der, C. J. & Sondek, J. GEF means go: turning on RHO GTPases with guanine nucleotide-exchange factors. *Nature Rev. Mol. Cell Biol.* **6**, 167–180 (2005).
- Pawson, T. & Nash, P. Assembly of cell regulatory systems through protein interaction domains. *Science* **300**, 445–452 (2003).
- Bhattacharyya, R. P., Remenyi, A., Yeh, B. J. & Lim, W. A. Domains, motifs, and scaffolds: the role of modular interactions in the evolution and wiring of cell signaling circuits. *Annu. Rev. Biochem.* **75**, 655–680 (2006).
- Walsh, D. A. & Van Patten, S. M. Multiple pathway signal transduction by the cAMP-dependent protein kinase. *FASEB J.* **8**, 1227–1236 (1994).
- Kim, E. & Sheng, M. PDZ domain proteins of synapses. *Nature Rev. Neurosci.* **5**, 771–781 (2004).
- Wiedemann, U. *et al.* Quantification of PDZ domain specificity, prediction of ligand affinity and rational design of super-binding peptides. *J. Mol. Biol.* **343**, 703–718 (2004).
- Songyang, Z. *et al.* Use of an oriented peptide library to determine the optimal substrates of protein kinases. *Curr. Biol.* **4**, 973–982 (1994).
- Snyder, J. T. *et al.* Structural basis for the selective activation of Rho GTPases by Dbl exchange factors. *Nature Struct. Biol.* **9**, 468–475 (2002).
- Zamanian, J. L. & Kelly, R. B. Intersectin 1L guanine nucleotide exchange activity is regulated by adjacent src homology 3 domains that are also involved in endocytosis. *Mol. Biol. Cell* **14**, 1624–1637 (2003).
- Debant, A. *et al.* The multidomain protein Trio binds the LAR transmembrane tyrosine phosphatase, contains a protein kinase domain, and has separate rac-specific and rho-specific guanine nucleotide exchange factor domains. *Proc. Natl Acad. Sci. USA* **93**, 5466–5471 (1996).
- Dueber, J. E., Mirsky, E. A. & Lim, W. A. Engineering synthetic signaling proteins with ultrasensitive input/output control. *Nature Biotech.* (in the press) (2007).
- Nimnual, A. S., Yatsula, B. A. & Bar-Sagi, D. Coupling of Ras and Rac guanine triphosphatases through the Ras exchanger Sos. *Science* **279**, 560–563 (1998).
- Seamon, K. B. & Daly, J. W. Forskolin: a unique diterpene activator of cyclic AMP-generating systems. *J. Cyclic Nucleotide Res.* **7**, 201–224 (1981).
- Ferrell, J. E. Jr. Tripping the switch fantastic: how a protein kinase cascade can convert graded inputs into switch-like outputs. *Trends Biochem. Sci.* **21**, 460–466 (1996).
- Yamauchi, J., Miyamoto, Y., Tanoue, A., Shooter, E. M. & Chan, J. R. Ras activation of a Rac1 exchange factor, Tiam1, mediates neurotrophin-3-induced Schwann cell migration. *Proc. Natl Acad. Sci. USA* **102**, 14889–14894 (2005).
- Miki, H., Sasaki, T., Takai, Y. & Takenawa, T. Induction of filopodium formation by a WASP-related actin-depolymerizing protein N-WASP. *Nature* **391**, 93–96 (1998).
- Rohatgi, R. *et al.* The interaction between N-WASP and the Arp2/3 complex links Cdc42-dependent signals to actin assembly. *Cell* **97**, 221–231 (1999).
- Kim, A. S., Kakalis, L. T., Abdul-Manan, N., Liu, G. A. & Rosen, M. K. Autoinhibition and activation mechanisms of the Wiskott–Aldrich syndrome protein. *Nature* **404**, 151–158 (2000).
- Prehoda, K. E., Scott, J. A., Mullins, R. D. & Lim, W. A. Integration of multiple signals through cooperative regulation of the N-WASP–Arp2/3 complex. *Science* **290**, 801–806 (2000).
- Hooshangi, S., Thiberge, S. & Weiss, R. Ultrasensitivity and noise propagation in a synthetic transcriptional cascade. *Proc. Natl Acad. Sci. USA* **102**, 3581–3586 (2005).
- Colicelli, J. Human RAS superfamily proteins and related GTPases. *Sci. STKE* **2004**, RE13 (2004).
- Dueber, J. E., Yeh, B. J., Chak, K. & Lim, W. A. Reprogramming control of an allosteric signaling switch through modular recombination. *Science* **301**, 1904–1908 (2003).
- Howard, P. L., Chia, M. C., Del Rizzo, S., Liu, F. F. & Pawson, T. Redirecting tyrosine kinase signaling to an apoptotic caspase pathway through chimeric adaptor proteins. *Proc. Natl Acad. Sci. USA* **100**, 11267–11272 (2003).
- Park, S. H., Zarrinpar, A. & Lim, W. A. Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms. *Science* **299**, 1061–1064 (2003).
- Inoue, T., Heo, W. D., Grimley, J. S., Wandless, T. J. & Meyer, T. An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. *Nature Methods* **2**, 415–418 (2005).
- Sprinzak, D. & Elowitz, M. B. Reconstruction of genetic circuits. *Nature* **438**, 443–448 (2005).
- Voigt, C. A. Genetic parts to program bacteria. *Curr. Opin. Biotechnol.* **17**, 548–557 (2006).
- Patel, J. C. & Galan, J. E. Manipulation of the host actin cytoskeleton by Salmonella—all in the name of entry. *Curr. Opin. Microbiol.* **8**, 10–15 (2005).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank J. C. Anderson, A. Arkin, H. Bourne, J. Dueber, G. Kapp, J. Kardon, M. Nyako, and members of the Lim and Bar-Sagi laboratories for assistance and discussion. This work was supported by grants from the NIH (D.B.-S. and W.A.L.), the Packard Foundation (W.A.L.), and the Rogers Family Foundation (W.A.L.). B.J.Y. was supported by a Post-Graduate Scholarship from NSERC and R.J.R. was supported by an NIH-NCI Cancer Biochemistry and Cell Biology training grant.

Author Contributions B.J.Y., R.J.R., D.B.-S. and W.A.L. conceived the experiments. B.J.Y. and A.D. designed and purified the constructs and performed the *in vitro* experiments. R.J.R. performed the *in vivo* experiments.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Correspondence and requests for materials should be addressed to W.A.L. (lim@cmp.ucsf.edu) or D.B.-S. (dafna.bar-sagi@med.nyu.edu).