dengue viruses. These candidate vaccines seem promising, but studies are still underway to determine whether they can confer a balanced immune response against all four dengue viruses and avoid immunological interference. For West Nile virus, the lead candidate vaccines—a DNA vaccine and a chimeric yellow fever 17D virus—also appear promising, but there is still no consensus on what constitutes a long-term protective immune response.

Chang *et al.*[5] build on earlier work by Kofler *et al.*[9,10], who showed that the tick-borne encephalitis flavivirus still forms immunogenic virus particles even when much of the capsid gene sequence has been deleted. Using Kunjin, a subtype of West Nile virus found in Australia, Chang *et al.*[5] have developed a 'split-genome' vaccine that generates two RNA species, one encoding the entire Kunjin virus genome except the capsid gene and the other encoding only the capsid gene. As both RNAs are encoded on the same DNA plasmid under the control of two cytomegalovirus promoters configured in a back-to-back orientation, transfected cells transcribe and translate all the viral genes. The capsid protein acts as a helper to assemble virus particles containing the viral genomic RNA lacking the capsid gene. These so-called single-round infectious particles (SRIPs) then infect adjacent cells (**Fig. 1**), in contrast to DNA vaccines that produce viral antigens only in the cells initially infected. Because the viral genome transmitted to neighboring cells does not encode capsid protein, no further viral replication can occur.

Chang *et al.*[5] compare the immunogenicity of SRIPs in mice to a live virus, a traditional DNA vaccine (encoding the viral genome, with the exception of functional capsid) and a DNA vaccine that produces virus-like particles composed of the pre-membrane and envelope proteins. SRIPs confer a superior antibody-mediated immune response in mice and horses, as well as protective immunity in mice, at lower doses of DNA compared with the traditional DNA vaccine. $CD8^+$ T-cell responses elicited by SRIPs in mice were also significantly greater than those produced by the virus-like particle vaccine, although smaller than those following immunization with live virus. Neutralizing antibodies are considered critical for achieving protective immunity, but it is clear that a vaccine must elicit both antibody- and cell-mediated immunity to ensure long-term protection.

Although these results represent an important proof of concept of a technology that should in theory be applicable to any flavivirus, a couple of important points should be considered. First, comparison of DNA-based vaccine strategies is very difficult given the many variables involved (e.g., viral strain, viral gene(s) selected, different parental virus strains and codon optimization). Second, it remains to be seen whether the present findings translate to primates. Several candidate DNA vaccines have performed impressively in lower animals only to disappoint in clinical trials. Prospects for using a SRIP-based approach in veterinary vaccines, such as those against Japanese encephalitis and West Nile virus infections of horses, seem more promising in the short term, especially as killed vaccines do not induce long-term protective immunity and booster doses are required to maintain immunity.

The major issues surrounding new candidate vaccines always concern efficacy and safety. With regard to efficacy, we know that current licensed flavivirus vaccines have neutralizing antibody as the correlate of protection and that only low levels of neutralizing antibodies are required for protective immunity. We do not know whether this will be true for vaccines against dengue and West Nile viruses—and even if it is, as most investigators believe, it is unclear what level of neutralizing antibodies will be required. This question is particularly complicated for a dengue vaccine, as the disease is caused by four genetically and serologically related viruses. For a tetravalent vaccine, higher levels of neutralizing antibodies might be needed to control four viruses simultaneously. Candidate vaccines, such as those involving SRIPs, may help achieve this goal, possibly through a prime-boost regimen, although it remains to be shown that SRIP-based vaccines are effective over the long term.

In the 21st century, safety has become the paramount attribute of a vaccine, even more so then efficacy, as society will not accept any adverse events associated with a vaccine. In this study, Chang *et al.*[5] have boosted efficacy using viral particles that have clear safety advantages over live attenuated vaccines.

COMPETING INTERESTS STATEMENT
The author declares competing financial interests: details accompany the full-text HTML version of the paper at http://www.nature.com/naturebiotechnology/.

1. Whitehead, S.S., Blaney, J.E., Durbin, A.P. & Murphy, B.R. *Nat. Rev. Microbiol.* **5**, 518–528 (2007).
2. Dauphin, G. & Zientara, S. *Vaccine* **25**, 5563–5576 (2007).
3. Petersen, L.R. & Roehrig, J.T. *J. Infect. Dis.* **196**, 1721–1723 (2007).
4. Martin, J.E. *et al. J. Infect. Dis.* **196**, 1732–1740 (2007).
5. Chang, D.C. *et al. Nat. Biotechnol.* **26**, 571–577 (2008).
6. Hayes, E.B. & Gubler, D.J. *Annu. Rev. Med.* **57**, 181–194 (2006).
7. Barrett, A.D.T. *Ann. NY Acad. Sci.* **951**, 262–271 (2001).
8. Edelman, R. *Clin. Infect. Dis.* **45** Suppl 1, S56–S60 (2007).
9. Kofler, R.M., Heinz, F.X. & Mandl, C.W. *J. Virol.* **76**, 3534–3543 (2002).
10. Kofler, R.M. *et al. Proc. Natl. Acad. Sci. USA* **101**, 1951–1956 (2004).

Customized signaling with reconfigurable protein scaffolds

Patrick Guye & Ron Weiss

Engineering protein scaffolds creates signaling networks with novel properties.

The emerging field of synthetic biology aims to design sophisticated biological systems that exploit diverse mechanisms for regulating information flow. New functions have been implemented using engineered transcriptional and translational networks, but little progress has been achieved in constructing protein-protein networks with complex connectivities. A recent report in *Science* by Lim and colleagues[1] addresses this challenge with a general approach for controlling signaling by protein scaffolds, as demonstrated by engineering of the pheromone response of the mitogen-activated protein kinase (MAPK) pathway in yeast.

The ability to design and implement sophisticated information-processing circuits was fundamental to the success of the computer revolution. To obtain a desired behavior, a circuit designer connects well-characterized components and modules into particular topologies that actuate the behavior. Similarly, in biological systems, connections between regulatory components (that is, circuit topology) help determine how cells process and react to information.

Patrick Guye and Ron Weiss are in the Departments of Molecular Biology and Electrical Engineering, Olden Street, Princeton University, Princeton, New Jersey 08544, USA.
e-mail: rweiss@princeton.edu

Until now, synthetic biology researchers have focused on elements of transcriptional regulation, mainly because they are easy to manipulate and connect. Transcription factors can usually be connected by fusing a promoter sequence targeted by one transcription factor to the gene sequence of a second transcription factor. Behaviors such as oscillations[2], bi-stability[3], ultrasensitivity[4] and transient responses[5,6] are engineered by constructing transcriptional networks with connections that include feedback and feed-forward motifs. More recently, RNA-based regulation[7–9] and metabolism[10] have been incorporated as additional useful components in circuit design.

In contrast with these approaches, the use of protein-protein interactions to engineer circuits enables faster processing of information with less consumption of cellular resources. However, establishing a new direct regulatory link between two or more proteins is inherently difficult as it usually requires a profound understanding of the proteins' binding affinities and folding. Nonetheless, the modularity of many signaling pathways suggests that general-purpose protein-protein circuit engineering should be feasible. Proteins involved in such interactions typically contain various combinations of a limited set of well-described domains[11]. This collection of modular domains might be used in a 'plug and play' fashion to develop new signaling topologies and functionalities.

Lim's group has pioneered the design of protein-protein interactions using protein scaffolds. Their latest paper[1] demonstrates how scaffold proteins can be easily reconfigured to create new protein network topologies and therefore serve as general-purpose information-processing nodes. Building on their extensive expertise concerning the role of the Ste5 scaffold protein in MAPK pathway regulation in yeast, they engineered this signaling complex to respond in multiple ways to the same stimulus.

Ste5 lacks any catalytic activity; it binds and brings together three kinases that might otherwise not interact with each other. Upon sensing an external signal relayed to this complex, the three kinases activate and phosphorylate each another sequentially to relay and amplify the signal. By appending a leucine zipper to the C terminus of Ste5, the authors added a docking site for recruitment of a fourth binding partner. Binding of a positive modulator to this additional site amplifies the response of the cascade, whereas a negative modulator dampens the response. Feedback loops were engineered by placing the additional binding partner under the control of a promoter whose activity depends on the cascade's output (**Fig. 1a**,**b**). Feedback was tuned either by changing the promoter strength or by using leucine zippers with different binding affinities. The expression of decoy leucine zippers as sinks and co-expression of positive and negative regulators with different affinities to the scaffold provide diverse behaviors, including acceleration, pulse generation, delayed regulation and ultrasensitive switching (**Fig. 1c**,**d**).

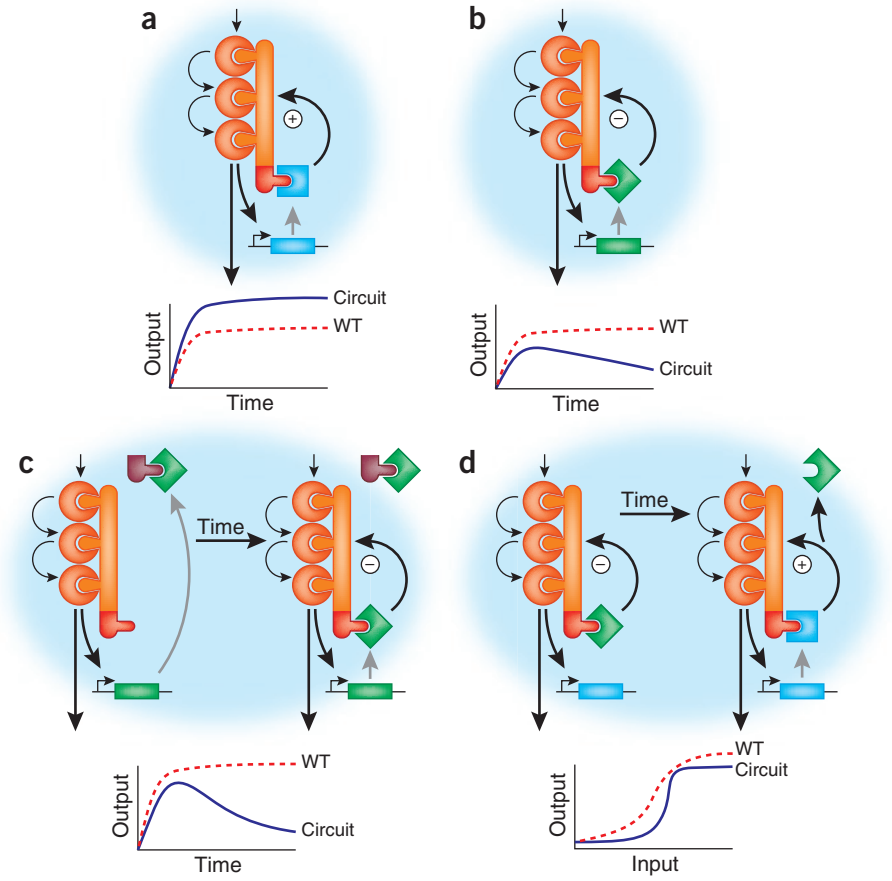

Figure 1 Examples of signaling topologies using the engineered scaffold protein. (**a**) Positive feedback. Signaling through the scaffold induces expression of a positive regulator, which binds the scaffold's leucine zipper to amplify the signal. (**b**) Negative feedback. Signaling through the scaffold induces expression of a negative regulator, which binds the scaffold's leucine zipper to dampen the signal. (**c**) Pulse generator. The induced negative regulator first binds a constitutively expressed high-affinity leucine zipper (decoy). Upon saturation of the decoy, the negative regulator binds the scaffold's leucine zipper and represses output. (**d**) Hypersensitivity. A constitutively expressed negative regulator binds the scaffold and represses it. Signaling through the scaffold induces expression of a positive regulator that displaces the negative one and activates scaffold signaling in an autocatalytic fashion.

This study therefore shows how a signaling module can be reprogrammed to perform different computations on the same input signal. Reprogramming is accomplished in a modular fashion by exchanging transcriptional nodes that regulate the feedback control. Just as reusability and modularity have been indispensable throughout evolution, they have emerged as core design principles in synthetic biology. Understanding the interplay of signaling domains and how to rewire them easily will be critical in developing new systems.

As scaffolds enable one to spatially arrange and compartmentalize signaling nodes, it should eventually be possible to build microstructures containing precisely placed interacting nodes, or scaffold-based integrated circuits. Integrating state-dependent binding sites (e.g., phosphotyrosine-containing motifs that bind common protein-protein interaction domains) into these systems may provide reconfigurable signaling pathways based entirely on protein-protein interactions. Depending on the state of one computing node, signaling elements or even other computing nodes could be recruited in a time-dependent and dynamic manner. Specialized nodes (recruiting nodes) may induce assembly or disassembly of signaling complexes (processor nodes), thereby enabling the rewiring of pathways on the fly, similar to the use of field programmable gate arrays in computers.

As we develop more sophisticated systems, one of the greatest challenges will be in deciding when and how to integrate the various types of regulatory nodes (based on protein-protein, transcriptional and translational interactions) into larger circuits. How does a circuit designer select the optimal mechanism in a specific situation, and how would other choices affect the overall system? Although much can be learned from analyzing the interconnections selected in nature, synthetic systems do not face the same evolutionary constraints as their natural counterparts. Computer-aided design tools based on biological system design principles free of evolutionary constraints would significantly accelerate the development of synthetic biology.

1. Bashor, C.B., Helman, N.C., Yan, S. & Lim, W.A. *Science* **319**, 1539–1543 (2008).
2. Elowitz, M.B. & Leibler, S. *Nature* **403**, 335–338 (2000).
3. Gardner, T.S., Cantor, C.R. & Collins, J.J. *Nature* **403**, 339–342 (2000).
4. Hooshangi, S., Thiberge, S. & Weiss, R. *Proc. Natl. Acad. Sci. USA* **102**, 3581–3586 (2005).
5. Basu, S. *et al. Proc. Natl. Acad. Sci. USA* **101**, 6355–6360 (2004).
6. Mangan, S. & Alon, U. *Proc. Natl. Acad. Sci. USA* **100**, 11980–11985 (2003).
7. Rinaudo, K. *et al. Nat. Biotechnol.* **25**, 795–801 (2007).
8. Bayer, T.S. & Smolke, C.D. *Nat. Biotechnol.* **23**, 337–343 (2005).
9. Isaacs, F.J. *et al. Nat. Biotechnol.* **22**, 841–847 (2004).
10. Fung, E. *et al. Nature* **435**, 118–122 (2005).
11. Pawson, T. & Nash, P. *Science* **300**, 445–452 (2003).

Retinal anti-angiogenesis by a new route

Roy Bicknell

A regulator of VEGF signaling shows therapeutic potential for retinal disease.

Retinal damage from vascular leakage associated with pathological angiogenesis presents a widespread clinical challenge in conditions such as age-related macular degeneration, retinopathy of prematurity and diabetic retinopathy. Writing in *Nature Medicine*, Jones *et al.*[1] have proposed a new approach to the treatment of these pathologies based on activation of the roundabout signaling pathway by the ligand Slit2 in the vasculature of the eye.

A notable recent development in the angiogenesis field is the realization that homologs of neuronal molecules that regulate axonal guidance are present in endothelium[2]. There are four recognized signaling pathways for axonal guidance: delta/notch, Eph/Ephrin receptor, netrin/Unc and slit/roundabout. Our discovery of an endothelial cell–restricted expressed sequence tag with homology to roundabout, and subsequent cloning of Robo4 (ref. 3), indicated that the slit/roundabout pathway, like the other three neuronal guidance pathways, also operates in endothelium.

Roy Bicknell is at the Cancer Research UK Angiogenesis Group, Institute for Biomedical Research, University of Birmingham Medical School, Edgbaston, Birmingham B15 2TT, UK. e-mail: r.bicknell@bham.ac.uk

Earlier work by Gerhardt, Betsholtz and colleagues[4] had shown that new vessels arise from existing ones by the emergence of a nonproliferating tip cell, which is thought to perform a guidance role, followed by the proliferation of stalk cells. By analogy to the role of roundabouts in the developing axonal growth cone, one might expect Robo4 to be expressed on the endothelial tip cell. However, Jones *et al.*[1] found that Robo4 is expressed in the stalk and not on tip cells. This observation, together with other work showing that Robo4 activation by Slit2 abrogates the proliferative and migratory activity of vascular endothelial growth factor (VEGF) and, more importantly, VEGF-induced permeability, led the authors to propose that Robo4 is involved in stabilization of the endothelium (**Fig. 1**).

The authors compared the response of normal and Robo4 knockout mice to treatment with Slit2 in two models of eye disease. The first model employed oxygen-induced retinopathy, which mimics the ischemia-induced angiogenesis observed in both diabetic retinopathy and retinopathy of prematurity. The increase in vascularization in response to oxygen in Robo4 knockout mice was almost twice that in control mice; more interestingly, it was significantly blocked in control mice that had received an intraocular injection of Slit2. In contrast, Slit2 had no effect on oxygen-induced vascularization in Robo4 knockout mice.

The second model involved laser-induced choriodal revascularization, which mimics age-related macular degeneration. A laser is used to disrupt the Bruch's membrane, allowing the underlying choriodal vasculature to penetrate the subretinal pigmented epithelium. In this case, the neovascular response to injury was similar in the control and Robo4 knockout mice, but as in the previous model, administration of Slit2 significantly blocked the vascular response to injury in control but not Robo4 knockout mice. The authors concluded that activation of the Robo4 pathway reduces the angiogenic response to injury and that this response might be of clinical utility.

Earlier work had shown that VEGF is a critical mediator of the angiogenesis associated with retinal pathologies, and, indeed, VEGF-blocking agents, such as the antibody bevacizumab (Avastin), have shown efficacy in the clinic. Although the current study identifies an exciting alternative therapeutic opportunity, VEGF-blocking agents remain the gold standard against which future therapies will be assessed.

Given our limited understanding of the function of Robo4 in endothelial biology and angiogenesis, some caution is warranted in the mechanistic interpretation of Jones *et al.*'s[1] results. A major concern is the lack of data demonstrating a direct molecular interaction between Slit2 and Robo4. Immunoprecipitation and Biacore studies have detected binding of Slit2 to Robo1 but not to Robo4 (ref. 5). The X-ray crystal structure of Slit2 bound to the extracellular domain of Robo1 has permitted modeling of the interaction between Slit2 and other

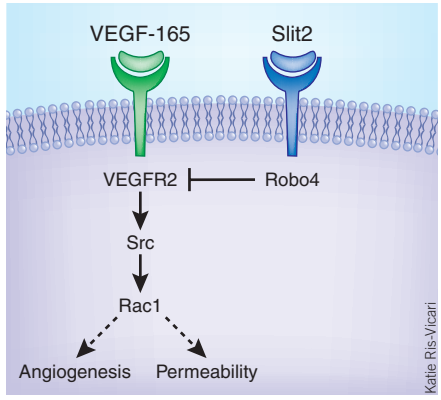

Figure 1 Activation of Robo4 by Slit2 blocks VEGF-stimulated angiogenesis and vascular permeability associated with retinopathies.