alkenes — those with large groups at the R^4 position shown in Figure 1 — are not viable coupling partners. Further fine-tuning of the catalyst structure and reaction conditions might uncover a solution to this problem.

By facilitating the linking of two alkenes through carbon–carbon bond formation, Lo and co-workers' reaction will allow the direct generation of valuable, structurally complex organic molecules from simpler precursors. What is more, the iron catalyst is readily prepared from fairly inexpensive ingredients. This method therefore has the potential to transform the way in which chemists think about constructing complicated molecules. ■

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1. Lo, J. C., Gui, J., Yabe, Y., Pan, C.-M. & Baran, P. S. *Nature* **516,** 343–348 (2014).
2. de Meijere, A. & Diederich, F. (eds) *Metal-Catalyzed Cross-Coupling Reactions* 2nd edn (Wiley, 2004).
3. Nakamura, E. *et al. Org. React.* **83,** Ch. 1, 1–209 (2014).
4. Kato, K. & Mukaiyama, T. *Chem. Lett.* **21,** 1137–1140 (1992).
5. Lo, J. C., Yabe, Y. & Baran, P. S. *J. Am. Chem. Soc.* **136,** 1304–1307 (2014).
6. Rowlands, G. J. *Tetrahedron* **65,** 8603–8655 (2009).
7. Rowlands, G. J. *Tetrahedron* **66,** 1593–1636 (2010).

SYNTHETIC BIOLOGY

Toehold gene switches make big footprints

The development of RNA-based devices called toehold switches that regulate translation might usher in an era in which protein production can be linked to almost any RNA input and provide precise, low-cost diagnostics.

SIMON AUSLÄNDER & MARTIN FUSSENEGGER

A fundamental tool of synthetic biology is a type of genetic device that controls the expression of target genes in a trigger-inducible manner, and so can be used to predictably and robustly program cellular behaviour. The number of such gene switches is growing, and switches have been successfully used in combination with other components, such as enzymes to assemble metabolic pathways that produce biofuels[1] and therapeutic drugs[2], and in designer cells that have the potential to correct metabolic diseases[3–5]. But the design of circuits of interconnecting switches is often complicated by the fact that each switch is made of natural components and is sensitive to its own predetermined trigger compound. A strategy that produces compatible gene switches tailored to desired trigger compounds would enable the switches to be easily assembled in combination, increasing the precision and complexity with which cellular behaviour can be programmed. Writing in *Cell*, Green *et al.*[6] describe a method for generating gene switches that can indeed be tailored to desired RNA inputs.

RNA is gathering momentum as a control device for synthetic biology. RNAs are modular, programmable and versatile. Furthermore, the specific sequence of each RNA dictates which molecules it can interact with and what functions its structure confers. The primary RNA sequence is determined by the sequential arrangement of different nucleotides, and this sequence can be engineered so that it forms secondary RNA structures internally or with complementary DNA or RNA molecules. One such structure is the hairpin loop, which comprises two base-paired sequences ending in an unpaired loop. Secondary structures can affect the translation of messenger RNA, and so can be exploited to regulate protein production from genes of interest.

Translation of mRNA occurs in a complex molecular machine called the ribosome. The ribosome contains a small and a large subunit, both of which are composed of a mixture of ribosomal RNAs and proteins. In bacteria, mRNAs are recruited to the ribosome through their ribosome-binding site (RBS) — a sequence that binds to the small subunit to initiate translation.

The reversible nature of this binding interaction is exploited by a class of engineered RNA-based gene switches called riboregulators, which contain an 'anti-RBS sequence' that binds to the RBS to form a hairpin loop[7], thus preventing the mRNA from accessing the ribosome and lowering the rate of translation[6]. The anti-RBS sequence is located in the target mRNA itself, in a region that will not be translated into protein, upstream of the site where translation begins. Riboregulators are switched by a 'trigger sequence' that interacts with and disrupts the hairpin, forming an alternative RNA structure that permits RBS–ribosome binding. Depending on the presence or absence of the trigger RNA, target gene expression can therefore be switched on or off. However, because typical riboregulators must fit into the upstream mRNA region and bind to the RBS, they can be designed for only a limited number of trigger sequences.

Green and colleagues have developed a more diverse type of riboregulator, which they call a toehold switch. Toehold riboregulators are designed to interact with the region around the protein-coding start site of each mRNA instead of the RBS, but are not complementary to the start site itself (Fig. 1). Furthermore,

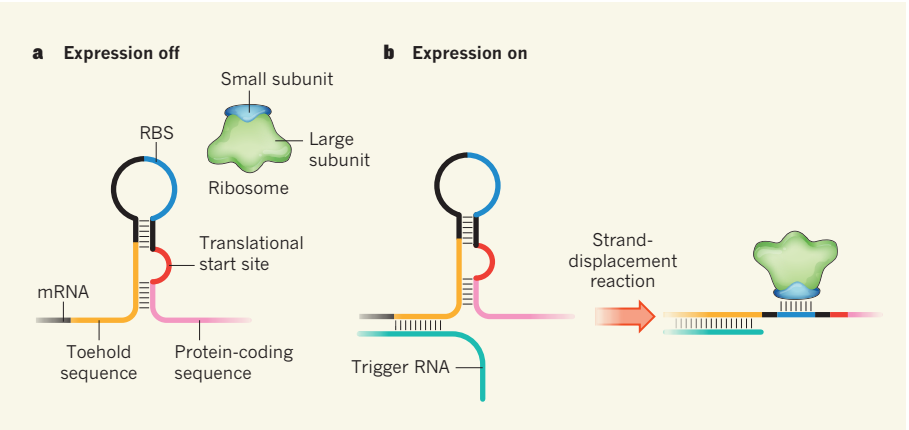

Figure 1 | The design of toehold switches. **a**, Green *et al.*[6] have designed an RNA-based device, called a toehold switch, that can regulate translation of bacterial messenger RNA in response to the presence or absence of any desired 'trigger' RNA. Toehold switches are located upstream of the site at which translation begins. The switch has an exposed single-stranded region called the toehold sequence that is designed to be complementary to the trigger RNA. To be translated, bacterial mRNA must bind to the small ribosomal subunit through a ribosome-binding site (RBS), but, if the trigger RNA is absent, the presence of the toehold switch causes the formation of a hairpin structure that blocks RBS–ribosome binding, thereby preventing translation. **b**, The presence of the trigger RNA causes a strand-displacement reaction that breaks up the hairpin structure, exposes the RBS to the ribosome and induces translation.

each has an exposed, single-stranded 'toehold sequence' adjacent to the mRNA-binding sequence, which facilitates binding of the trigger RNA to the riboregulator. This design strategy enables riboregulators to be programmed at will.

The authors demonstrated the potential of toehold riboregulators by independently controlling 12 toehold switches inside one cell. They experimentally and computationally characterized their first-generation library of 168 switches to identify specific parameters that are crucial for the proper performance of switches. These parameters enabled the computer-aided design of toehold switches with predictable performance, which was validated for 13 second-generation switches.

These second-generation devices modulated translation extremely efficiently — protein production was up to 650-fold higher when the switch was on than when it was off. This performance is unmatched for other RNA-based switches, and is typically reached only by devices that exert control at the transcriptional level. Demonstrating the versatility of their devices, Green *et al.* produced switches that detect and report on the presence of endogenous RNA sequences, and programmed cellular behaviour using synthetic trigger RNAs.

Might the potential flexibility of toehold switches be exploited in diagnostics? A follow-up report[8] examined the diagnostic capability of a toehold switch in which the trigger was Ebola virus RNA, and the mRNA under control encoded an enzymatic 'reporter' protein. The switch was freeze dried in cell-free extracts and stored on paper discs. These paper-based switches could reliably detect the Ebola virus RNA with great sensitivity. Furthermore, the switches worked even after long-term storage at ambient temperature. Although designed for use in bacteria, paper-based toehold switches also worked in combination with mammalian cell extracts as protein-based biosensors that quantified blood glucose levels. In the future, paper-based diagnostics might also be used to detect when RNA molecules such as microRNAs are expressed in patterns that are hallmarks of cancer or metabolic disorders[9].

Although diagnosis is fundamental to any preventive care strategy, therapeutics are also vital, and future treatment strategies could combine the two. Synthetic gene networks that operate inside designer cell implants can monitor, process and score molecular indicators of disease, and can also coordinate the production of protein-based therapies within the engineered cell. Designer networks have been used for the treatment of gouty arthritis[3], obesity[4] and diabetes[5] in animal models. So far, therapeutic gene networks have used natural sensor components that might be compatible with the human physiological range, but the design of tailor-made biosensors for specific molecular indicators of disease remains challenging. Toehold switches may be a good starting point to design biosensors specific for any disease-relevant compound — first for microRNAs[9], and eventually for mutated mRNA sequences. The integration of synthetically engineered biosensors into synthetic gene networks that diagnose and treat disease could dramatically shape cell-based treatment strategies in this century. ■

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1. Peralta-Yahya, P. P., Zhang, F., del Cardayre, S. B. & Keasling, J. D. *Nature* **488,** 320–328 (2012).
2. Paddon, C. J. *et al. Nature* **496,** 528–532 (2013).
3. Kemmer, C. *et al. Nature Biotechnol.* **28,** 355–360 (2010).
4. Rössger, K., Charpin-El-Hamri, G. & Fussenegger, M. *Nature Commun.* **4,** 2825; http://dx.doi.org/10.1038/ncomms3825 (2013).
5. Ausländer, D. *et al. Mol. Cell* **55,** 397–408 (2014).
6. Green, A. A., Silver, P. A., Collins, J. J. & Yin, P. *Cell* **159,** 925–939 (2014).
7. Ausländer, S. *et al. Nature Methods* **11,** 1154–1160 (2014).
8. Pardee, K. *et al. Cell* **159,** 940–954 (2014).
9. Xie, Z., Wroblewska, L., Prochazka, L., Weiss, R. & Benenson, Y. *Science* **333,** 1307–1311 (2011).

MALARIA

How vector mosquitoes beat the heat

Intensive longitudinal sampling of malaria mosquitoes in the African semi-desert reveals that three morphologically indistinguishable species have distinctive strategies for surviving the dry season. SEE LETTER P.387

NORA J. BESANSKY

The scale-up of interventions against malaria in the past decade has reduced the global death rate of this disease by an impressive 42%. However, more than 600,000 malaria-related deaths still occur each year[1] — 90% of them in sub-Saharan Africa — meaning that malaria remains one of the most significant sources of infectious-disease mortality. Africa has long been recognized as a crucible for malaria-control efforts, owing to its particular blend of widespread and dominant mosquito species that transmit malaria. One of the great mysteries of malariology has been how these vector populations survive the dry season, when there is little water in which the mosquitoes can lay their eggs. In this issue, Dao *et al.*[2] (page 387) report that they have solved this mystery, but the answer is surprisingly complex, like the vectors themselves.

Three closely related sibling mosquito species belonging to the *Anopheles gambiae* complex are among the most efficient vectors of malaria[3] (there are at least seven species in the complex, collectively referred to as *A. gambiae sensu lato* (*s.l.*)). This status is owed to their strong association with humans and their success at exploiting a variety of ecological conditions across tropical Africa, from humid rainforests to the fringes of the Sahara Desert, as long as humans are nearby. However, there is an Achilles heel in the relay of malaria parasites between these vectors and humans — all mosquitoes have an obligate

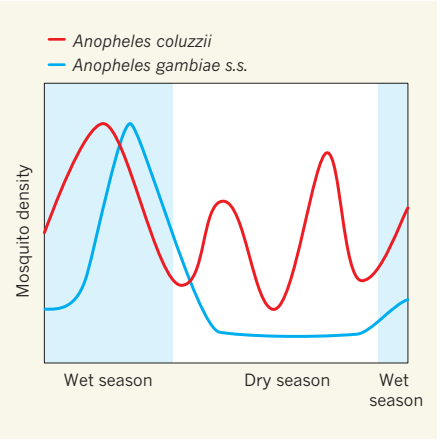

Figure 1 | Species-specific population dynamics. Dao *et al.*[2] find that average population densities of *Anopheles coluzzii* and *Anopheles gambiae sensu stricto* (*s.s.*) mosquitoes fluctuate seasonally in predictable but distinct patterns. In the wet season, when mosquito breeding sites are abundant and the climate is favourable, densities of both species are high, although *A. coluzzii* achieves its peak population density substantially earlier than *A. gambiae s.s.* does. In the dry season, the *A. gambiae s.s.* population disappears and is not found again until the next wet season, with a slow increase in population density that lags behind that of *A. coluzzii*. By contrast, the *A. coluzzii* population remains in the area during the dry season, but cannot be sampled while the insects are hidden in unknown shelters, leading to apparent troughs. Their emergence from those shelters for two short periods during the dry season is reflected by two peaks in the data.