SYNTHETIC BIOLOGY

Biocircuits in synchrony

Cellular biocircuit design has taken a major step forward. The circuit reuses the cell's own protein-degradation system to synchronize the expression of two synthetic modules throughout an entire bacterial population. SEE LETTER P.387

RICARD SOLÉ & JAVIER MACÍA

A major goal of synthetic biology is to build reliable, predictable networks of molecular and cellular components that can work as new biological devices capable of, for example, sensing chemicals, manufacturing drugs or even fighting disease. However, achieving such goals entails the production of complex synthetic biocircuits, which requires synchronization of multiple components. Although synchronization is well established in electronics[1], synchronizing living cells is a major challenge, because it demands correlation of different phenomena that may be taking place on different temporal and spatial scales. On page 387 of this issue, Prindle *et al.*[2] report that such coupling has been achieved in cells of the bacterium *Escherichia coli*.

In complex circuits, synchronization is required to equilibrate two or more modules that normally work on different timescales — if two components act at different speeds, synchronization ensures that the faster component waits until the slower one has finished its work before progressing, making it possible for the system to operate as a whole[3]. In electrical circuits, synchronization is achieved using an external 'clock signal'. In an electrical oscillator device, for example, when the clock signal is high (or low), the device stops oscillating, starting again only when the clock signal is low (or high). When applied to multiple components, the clock signal can therefore regulate the separate devices so that they work in unison.

This standardized approach to electrical circuits cannot, however, be directly exported to synthetic biocircuits, in which it is difficult to obtain a precise signal that equally affects all modules of the device, because the response of each module to an external stimulus is likely to vary. To solve this problem, Prindle and colleagues have developed an approach for fast and efficient synchronization of oscillators in *E. coli*, based not on an external clock, but instead using the existing machinery of the host cell as a synchronization system.

In general, oscillations arise from the periodic creation and destruction of a signal. In some synthetic bacterial oscillators, this signal is encoded in the concentration of the protein produced by the oscillator module — this protein typically inhibits the promoter that drives the module's own gene expression, a process known as negative feedback. Therefore, to produce oscillations, production of protein by the module must be continuously followed by the protein's degradation by a protease enzyme. Prindle and co-workers simultaneously introduced two previously reported synthetic oscillating modules into *E. coli*, which work on different temporal and spatial scales[4,5].

To produce oscillations at a single-cell level, Prindle *et al.* used an intracellular negative-feedback (NFB) module[5]. The NFB module produces λ-repressor protein, which negatively regulates the module, producing low-amplitude, high-frequency oscillations in each cell (Fig. 1a). The module then drives the expression of an output — in this case, a cyan fluorescent protein — resulting in the same low-amplitude, high-frequency oscillations.

To produce oscillations on a population-wide scale, the authors coupled the NFB module to a second oscillator that works through quorum sensing (QS)[6], the mechanism by which certain bacteria communicate. In QS, each bacterium secretes a signalling molecule into the extracellular medium, so that the concentration of the molecule in the medium depends on the local population density. Once the population reaches a threshold size, the concentration of the QS molecule increases to such a level that it is able to re-enter neighbouring cells, triggering gene transcription. The QS module used by Prindle and colleagues produces not only a QS signalling molecule, but also AiiA, a protein that acts as a repressor of the QS module, generating negative feedback. Expression of the QS module in a sufficiently large bacterial population results in simultaneous oscillation in each cell of the population at high amplitude and low frequency (Fig. 1b). This leads to production of the module's output — yellow fluorescent protein — in oscillations that follow the same pattern.

Prindle *et al.* achieved coupling between the two oscillators by exploiting a natural component of *E. coli*: the ClpXP protease enzyme[6]. The researchers avoided the need to engineer an extra regulatory component to couple the oscillators by tagging the fluorescent and feedback proteins produced by each module with a protein that allows degradation by ClpXP. Thus, protease activity regulates the level of signal available to feed back to each module, and the level of the module's output protein.

When the levels of protein produced by the QS module are low, enough ClpXP protease is available to degrade the λ-repressor protein, and the intracellular NFB oscillator fluctuates normally. But when the QS module is active, the total amount of protein generated by both oscillators saturates the degradation capacity of the ClpXP protease, and the intracellular

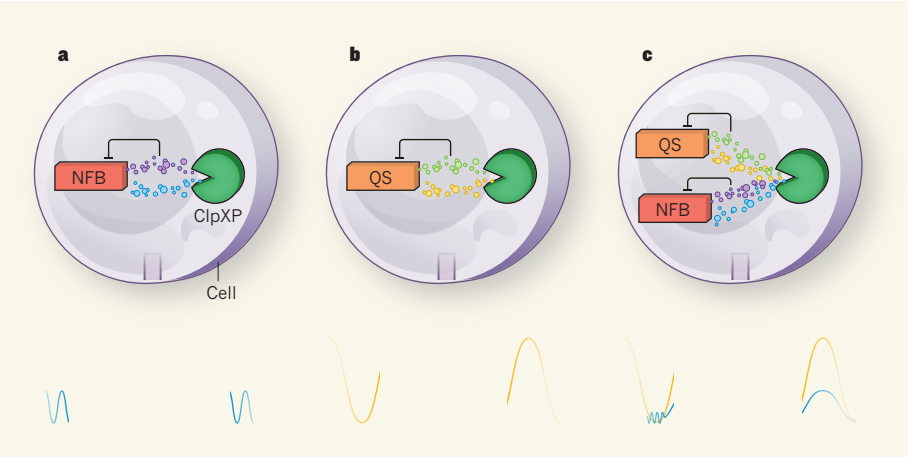

Figure 1 | Climbing up complexity in synthetic biology. **a**, The intracellular negative-feedback (NFB) oscillator is a synthetic module that works at a single-cell level in bacteria. The module produces λ-repressor protein (purple), which inhibits the oscillator's own activity, and a cyan fluorescent protein (blue). These proteins are degraded by the ClpXP protease enzyme, resulting in high-frequency, low-amplitude oscillations, measured by levels of cyan fluorescence (depicted by blue lines). **b**, A quorum-sensing (QS) module produces the negative feedback protein AiiA (green) and a yellow fluorescent protein (yellow), which are also degraded by ClpXP. Oscillation of the QS module is synchronized throughout the microbial population, and oscillations occur at low frequency and high amplitude (depicted by yellow lines). **c**, Prindle *et al.*[2] have generated the first complex synthetic biocircuit by simultaneously introducing these two oscillators into *Escherichia coli* cells. When both compete for degradation by ClpXP, their dynamics are synchronized.

oscillator stalls (Fig. 1c). As a result, the activity of the intracellular module follows that of the QS module. Once the proteins produced by the QS module are degraded to lower levels, sufficient ClpXP is available to degrade λ-repressor protein again, and the intracellular oscillator returns to its normal, high-frequency oscillations.

Prindle and co-workers' approach does not require complex engineering to synchronize the oscillators because the authors have tinkered with the cell's own components. Moreover, whereas genetic devices are often designed to minimize the interactions between different components of the biocircuit, here these interactions have been strengthened. This strategy is appealing because the researchers' data suggest that exploration of the synergistic interactions between genetic devices and host cells will benefit circuit building in synthetic biology.

Prindle and colleagues' results provide an excellent argument for using the cell's natural machinery to integrate multiple synthetic components, because the authors have achieved fast, tunable and robust synchronization of two different modules. Complex decision-making circuits might strongly benefit from the authors' design. However, it is important to remember that scaling up the number of modules in a circuit remains a major issue within synthetic biology[7].

If circuit complexity is to grow in size and diversity, further improvements will be needed. For example, different strategies will be required to synchronize other types of genetic device, such as logic gates. The current work indicates that it may be possible to isolate different parts of a synchronized circuit in different cells, exploiting the cells as natural units of computation to perform basic logic functions such as AND or NOR. In this context, the use of different cell types communicating with one another to perform different functions might be a natural step forward[8–10], expanding on the method designed by Prindle *et al.* to allow generation of more complex, decision-making circuits. ■

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1. Purnick, P. E. & Weiss, R. *Nature Rev. Mol. Cell. Biol.* **10,** 410–422 (2009).
2. Prindle, A. *et al. Nature* **508,** 387–391 (2014).
3. Oklobdzija, V. G. *et al. Digital System Clocking: High-Performance and Low-Power Aspects* (Wiley, 2003).
4. Danino, T., Mondragón-Palomino, O., Tsimring, L. & Hasty, J. *Nature* **463,** 326–330 (2010).
5. Stricker, J. *et al. Nature* **456,** 516–519 (2008).
6. Cookson, N. A. *et al. Mol. Syst. Biol.* **7,** 561 (2011).
7. Kwok, R. *Nature* **463,** 288–290 (2010).
8. Tamsir, A., Tabor, J. J. & Voigt, C. A. *Nature* **469,** 212–215 (2011).
9. Regot, S. *et al. Nature* **469,** 207–211 (2011).
10. Solé, R. V. & Macía, J. *Nat. Comput.* **12,** 485–497 (2013).

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THERMOELECTRICITY

The ugly duckling

Single crystals of tin selenide have been shown to display, along one crystallographic direction of their high-temperature state, the highest thermoelectric efficiency of any bulk material. SEE LETTER P.373

JOSEPH P. HEREMANS

More than 90% of the energy we use comes from thermal processes[1], which produce the bulk of the electricity generated by power plants, as well as powering aeroplanes and most cars. Heat engines have existed since the early eighteenth century, drove the Industrial Revolution and gave rise to the science of thermodynamics. Thermoelectricity was discovered about a century later[2] and is based on the same thermodynamic principles that heat engines depend on, except for the fact that thermoelectric power generators use electrons, rather than steam or air, as the working fluid. A testament to the importance of these fields is the fact that progress in the thermal sciences has been unrelenting: this includes work by Zhao *et al.*[3] on page 373 of this issue.

The second law of thermodynamics dictates that, to deliver work, heat engines must operate between a source of heat at a hot temperature (T_{hot}) and a heat sink at a cooler temperature (T_{cold}). The Carnot efficiency, $\eta_{max} = 1 - (T_{cold}/T_{hot})$, is the upper bound for the efficiency (η) of a heat engine, where η is the ratio between the amount of work an engine does and the amount of heat it uses. A thermoelectric generator works as follows. A temperature gradient, ∇T, across two thermoelectric materials — a semiconductor in which most of the charge carriers are electrons (n-type semiconductor) and a semiconductor that has mostly notional holes created by the absence of electrons (p-type) — creates an electric field, E, between the cold side and the hot side of each material (Fig. 1). The Seebeck coefficient, S, which is given by the ratio $E/\nabla T$ and is negative for the n-type material and positive for the p-type, corresponds[4] to the entropy of the electron divided by its charge. The two materials complete a cycle that converts the heat supplied at the hot side into electrical power. Assuming that S does not vary along the length of each thermoelectric material even though the temperature does, if this cycle were reversible it would have the Carnot efficiency.

Thermodynamically irreversible processes limit the efficiency of the cycle to a value much lower than that of the Carnot efficiency. Examples of such processes are heat conduction through the crystal lattice of atoms that constitute the semiconductors and the Joule heating that arises inside the semiconductors when the voltage produced by the electric field is used to deliver a current to an external electrical load (Fig. 1). The fraction of the Carnot efficiency of a thermoelectric cycle is quantified by the thermoelectric figure of merit ZT

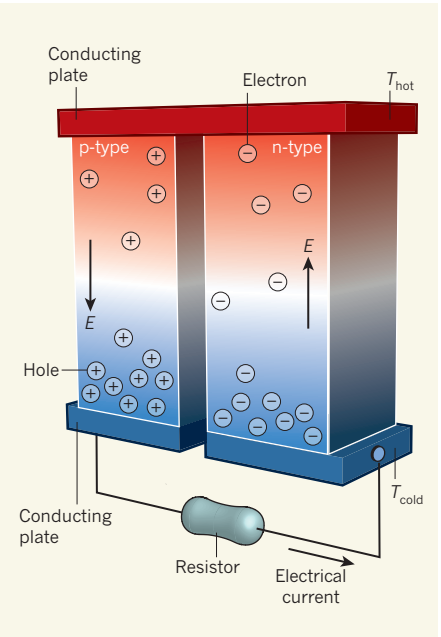

Figure 1 | Working principle of a thermoelectric generator. a, A thermoelectric generator consists of two thermoelectric semiconductors (n-type and p-type) subjected to a temperature difference, $T_{hot} - T_{cold}$, and electrically connected in series through conducting plates on the top and bottom. In the n-type semiconductor, most charge carriers are negatively charged electrons, whereas in the other one most of the carriers are positively charged holes. In a temperature gradient, electrons and holes tend to accumulate on the cold side. An electric field E develops between the cold side and the hot side of each material, which gives a voltage when integrated over the length of each. The voltages of the n- and p-type semiconductors add up and drive an electrical current through an electrical load, here an electrical resistor. The product of the voltage and the current is the electrical power output of the generator.