NEWS & VIEWS

SYNTHETIC BIOLOGY

Synchronized bacterial clocks

Martin Fussenegger

By synchronizing clocks, humans make more efficient use of their time and orchestrate their activities in different places. Bacteria have now been engineered that similarly coordinate their molecular timepieces.

The lives of *Escherichia coli* bacteria are hectic — not only are the organisms in constant motion, but they add to the random hurly-burly of their microscopic world by dividing every 20 minutes. It is therefore no small achievement to genetically engineer *E. coli* so that a whole population expresses a fluorescent protein in synchrony, thus producing coordinated pulses of light. Yet Hasty and co-workers[1] report on page 326 that they have done exactly that, in a feat analogous to engineering all the world's traffic lights to blink in unison.

The foundation for this breakthrough was laid by previously engineered biological systems. Synthetic biologists were inspired by natural time-keeping mechanisms that control both short- and long-term processes, ranging from information processing in the brain (which occurs on millisecond to second timescales) to circadian rhythms and the seasonal timescale of hibernation. They analysed the components, assemblies and dynamics that control biological time-keeping, and then assembled functioning synthetic clocks by arranging components that have similar functions into transcriptional circuits — networks of genes and their protein products in which the proteins activate or repress transcription of the genes[2]. These circuits contained negative feedback loops, which drove the clocks like the balance spring in a mechanical watch. The dynamics of the circuits were set up to create time delays that acted as pulse generators, defining a minimal time unit with pendulum-like precision.

This basic design principle made it possible to construct various synthetic oscillators in *E. coli*, beginning with the 'repressilator'[3], a system of three genes connected in a cyclical negative feedback loop so that gene A represses gene B, which represses gene C, which represses gene A. This was followed by the construction of an 'oscillator' — in which a gene activates its own expression and that of another gene, which, in turn, represses the activating gene[4] — and by the 'metabolator', in which signalling molecules in *E. coli* drive oscillating metabolic activity[5]. But these first-generation oscillators lacked robustness and damped rapidly. Furthermore, their oscillatory frequencies and amplitudes could not be adjusted.

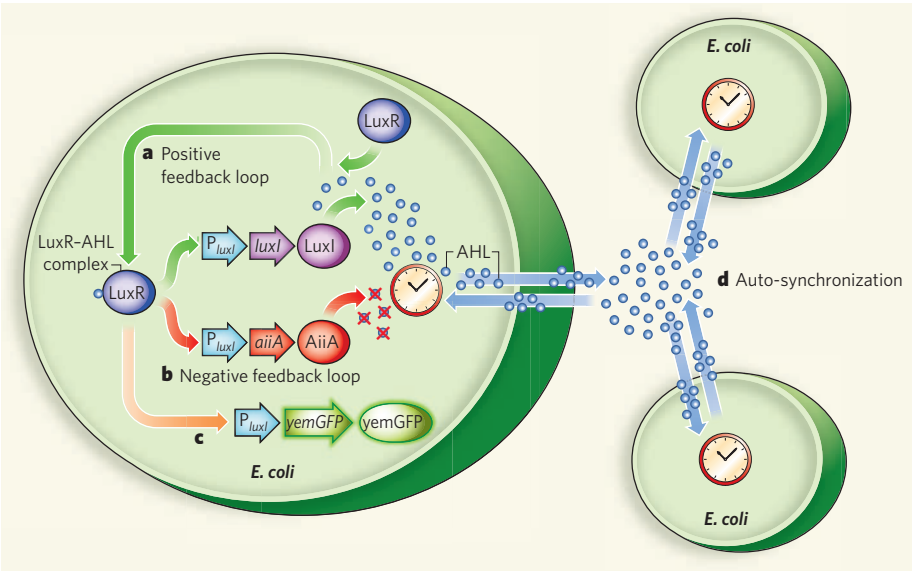

Figure 1 | All together now. Hasty and colleagues[1] have constructed a network of genes and proteins in *Escherichia coli* that acts as a molecular clock and can be synchronized across a population of the bacteria. **a**, When bound to the diffusible molecule acyl-homoserine lactone (AHL), the constitutively expressed LuxR receptor initiates a positive feedback loop by triggering expression of LuxI, the enzyme that synthesizes AHL. The expression of LuxI is driven by P_{luxI}, a DNA promoter sequence. Large arrows indicate DNA promoter sequences or genes. **b**, The LuxR–AHL complex also kicks off a time-delayed negative feedback loop by activating the P_{luxI}-driven production of AiiA, a protein that degrades AHL. **c**, In the final section of the network, the LuxR–AHL complex triggers P_{luxI}-driven expression of a green fluorescent protein (yemGFP). **d**, The dynamic interactions of the positive and negative feedback loops produce regular pulses of AHL, which act as time-keepers in the molecular clock. The AHL molecules diffuse out of each cell and across the membranes of neighbouring bacteria, where they stimulate the expression of yemGFP. All the cells of the *E. coli* population simultaneously send and receive AHL, providing a signal that enables each individual clock to self-adjust and auto-synchronize with the others, as demonstrated by the coordinated pulses of fluorescence produced by the bacteria.

By contrast, the second generation of *E. coli* oscillators — based on the differential activity of two interconnected feedback loops, one positive and one negative — could be adjusted using environmental triggers, such as temperature, growth medium and the concentration of transcription-inducing molecules[6]. What's more, the oscillations were robust, persistent and hereditary (daughter cells produced oscillations just like their parents). The first mammalian oscillators were also robust and had adjustable frequencies[7,8] and amplitudes[8].

Despite the promise of second-generation oscillators, these synthetic clocks couldn't be synchronized across populations of organisms. The problem was that, although the oscillatory frequencies and amplitudes of individual cells could be programmed, each cell was on its own internal time. Without universal time, there can be no coordinated activity. Hasty and colleagues[1] therefore exploited a bacterial communication system known as quorum sensing to achieve the desired synchronicity in a third generation of clocks.

Bacteria that use quorum sensing constantly secrete signalling molecules, which are detected by their comrades. The concentration of the signals in the local environment depends on the population density of the bacteria;

when the concentration reaches a threshold level, it activates the transcription of genes in the bacteria that are associated with some form of collective action (such as aggregating into biofilms). Quorum sensing thus ensures that group behaviour in bacteria occurs only when the population is large enough to make it worthwhile.

Hasty and co-workers[1] assembled their synchronized clock in *E. coli* from various quorum-sensing factors and inhibitors (Fig. 1). In their system, a promoter DNA sequence (P_{luxI}) drives the production of LuxI, an enzyme that synthesizes the quorum-sensing signal acyl-homoserine lactone (AHL). Another copy of P_{luxI} controls the production of AiiA, a protein that catalyses the degradation of AHL, and a third copy triggers the synthesis of a variant of green fluorescent protein called yemGFP. An AHL receptor, LuxR, is constitutively expressed. The authors combined these components to form an autoinducing circuit (AHL activates LuxR, and the AHL–LuxR complex induces P_{luxI}-driven *luxI* transcription and yemGFP production) with a time-delayed negative feedback loop (the AHL–LuxR complex induces P_{luxI}-driven production of AiiA, which degrades AHL). The result was a population of bacteria that produce synchronized pulses of fluorescence, coordinated by quorum sensing (see Supplementary Movie 1).

The design of Hasty and colleagues' transcriptional circuit[1] is similar to those of previously reported synthetic oscillators[6,8]. But the use of the rhythmic synthesis of molecules (such as AHL) as a pacemaker to coordinate the behaviour of individual oscillators in a growing population of cells is a quantum leap in molecular-clock design. The complexity of the resulting system is astonishing. Whereas the central pacemaker in the brain uses one-way synchronization to control oscillators in remote peripheral tissue[9], the authors' bacterial clocks auto-synchronize and self-adjust using continuous two-way crosstalk. This is comparable to the most advanced social networks (such as Twitter) that allow people to share news globally in real time.

So how could such a system be used? The ability to synchronize the output of a cell population could provide insight into little-understood phenomena. For example, the interactions of Hasty and colleagues' bacteria seem to replicate the communication between neurons, which forms the basis for the brain's amazing processing capacity. The highly interconnected networks that neurons form can be thought of as weakly coupled oscillators. Large numbers of neurons in these networks sometimes take part in synchronized activity, as revealed by the resulting electromagnetic fields that have been measured using electroencephalography[10]. Neural synchronization can be modulated by attention, neuronal communication and motor coordination. Abnormal patterns of synchronization often have severe consequences for health.

In the long term, an improved knowledge of naturally occurring time-keeping processes at the molecular level might improve our understanding of sleeping, learning or motor-coordination disorders. It could also provide insight into the pathologies of epileptic seizures and of the various symptoms of Parkinson's, Huntington's and Alzheimer's diseases. Another exciting possibility for the future is the development of cell implants that contain synchronized oscillators that are tuned to produce therapeutic proteins at specific times and in precise doses. Such systems could make the taking of pills in specified amounts and in precise doses a thing of the past. ■

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MATERIALS SCIENCE

Membrane magic

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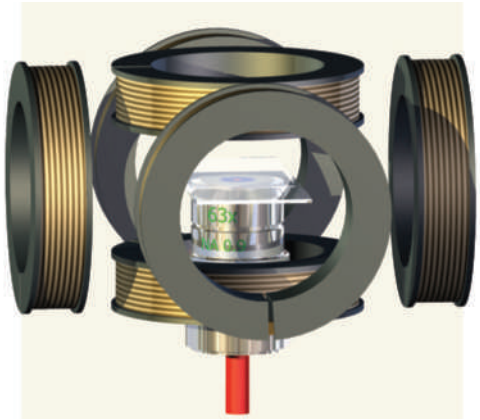
The use of magnetic fields to assemble particles into membranes provides a powerful tool for exploring the physics of self-assembly and a practical method for synthesizing functional materials.

The complex form and functions of living systems are underpinned by molecular self-assembly, and a basic goal of materials science is to create synthetic materials based on particle assembly. Materials scientists and technologists are actively pursuing methods for manipulating and quantifying particle interactions, to obtain precise control over the morphology of self-assembled particles and their stability in response to mechanical perturbations and changes in thermodynamic conditions. Writing in *Physical Review Letters*, Osterman *et al.*[1] investigate how this type of assembly can be achieved by subjecting micrometre-sized, colloidal particles to dynamically modulated magnetic fields. In particular, they observe self-assembled particle membranes that have many characteristics of biological self-assemblies, including structural self-healing when mechanically perturbed.

Membrane organization of particles that is driven by external fields — such as acoustic fields[2] and time-varying, orthogonally oriented magnetic fields[3–5] — has been investigated for some time. The novelty of Osterman and colleagues' work[1] lies in their experimental quantification of the interactions governing particle self-assembly in triaxial magnetic fields (Fig. 1). Furthermore, the authors confirm the prediction that these interactions result in non-trivial topological changes in particle aggregation, as well as in the emergence of characteristic structural scales as the strength and character of the driving magnetic field are varied.

Osterman *et al.* generate their particle membranes using three orthogonal pairs of Helmholtz wire coils: one pair creates a uniform magnetic field along the vertical direction, with the other two producing an oscillating biaxial field in the horizontal plane (Fig. 1). Flat membranes of particles[4] form in the horizontal direction for large biaxial-field strengths (or, equivalently, large negative dipole interactions) — rather than particle chains in the vertical direction, which would form in a uniform field applied vertically. The two fields together create a precessing triaxial[1,3,5] magnetic field that has a tunable angle



JAMES MARTIN

Figure 1 | Set-up of Osterman and colleagues' experiment[1]. Three pairs of Helmholtz wire coils are used to apply a triaxial magnetic field to a cell (centre) containing micrometre-sized, colloidal iron particles. The vertical pair produces a uniform magnetic field in the vertical direction; the two horizontal pairs generate an oscillating biaxial field in the horizontal direction. A laser beam (red) acts as a tweezer for manipulating the particles.