

more physically accessible to the catalyst. The catalyst can also be attracted to a specific location by binding to a pre-existing functional group within the reactant, thus attacking only a nearby C–H bond. A goal in the area is to understand the relevant selectivity trends from the previous results, in order to predict the outcome in any subsequent case. Predictability is essential for the design of a multistep synthetic route relying on a selective, late-stage C–H

activation, because failure at a later step would vitiate the entire scheme.

This remarkable work is part of an emerging trend, in which different types of selective, catalytic C–H activation reactions are being successfully applied to more complex molecules than previously envisaged (4–6). With the conceptual barrier breached for hydroxylation, further striking applications to complex molecules are likely to emerge in the near future.

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## SYSTEMS BIOLOGY

# A Clock with a Flip Switch

Andy C. Poon and James E. Ferrell Jr.

Two years ago, Takao Kondo's group showed that when a phosphate source (adenosine 5'-triphosphate) and three purified proteins were mixed in a test tube, they spontaneously generated sustained oscillations in the phosphorylation state of one of the proteins (1). The three proteins—KaiA, KaiB, and KaiC—were previously identified as important for the daily patterns of activity and behavior (circadian rhythms) in the cyanobacterium *Synechococcus elongates*. Astonishingly, oscillations of phosphorylation in the reconstituted system were similar to the bacterium's natural circadian rhythm of about 24 hours. Moreover, mutations in one of the proteins, KaiC, that change the circadian period in vivo had nearly identical effects in vitro. Thus, a relatively simple and highly robust timekeeper seemed to set the pace for this complicated organism. The big remaining challenge was to determine how this clock works. Two studies, reported by the Kondo group (2) and by Rust *et al.* on page 809 of this issue (3), now provide a satisfying answer to this question. The oscillations arise from the slow, orderly addition and then subtraction of two phosphates from the KaiC protein. This provides a fascinating example of reductionistic systems biology, where the ability to pick apart a complex system has yielded an understanding of how the whole system works.

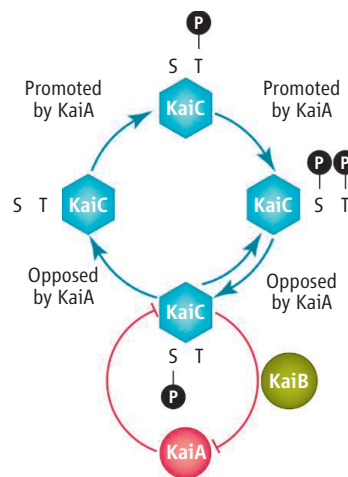
The addition and removal of phosphate can alter a protein's function, and if the protein is part of a network of interacting factors, its phosphorylation status may relay information that impinges on some cell behavior. The dynamics of reversible phosphate addition

and removal in cells are usually rapid—occurring on time scales of seconds or minutes—and so seem poorly suited for slow circadian rhythms. Both studies examine details of the timing of KaiC phosphorylation during oscillations. KaiC is phosphorylated at two sites and in a particular order: first on a threonine residue, then on a serine. Subsequently, the threonine and then serine are dephosphorylated and the KaiC returns to an unphosphorylated state (see the figure). The KaiA protein regulates these transitions by promoting autophosphorylation and inhibiting autodephosphorylation by KaiC.

But a cycle of phosphorylations and dephosphorylations would not necessarily be expected to generate oscillations. Consider, for example, activation and inactivation of the enzyme Erk2 (extracellular signal-regulated protein kinase 2). Like KaiC, Erk2 cycles among four chemical states, modified by enzymes that phosphorylate a tyrosine residue and then a threonine (4), and then by enzymes that dephosphorylate tyrosine first and then the threonine (5). But there is no hint that this system oscillates. What, then, keeps the cyclic phosphorylation and dephosphorylation of KaiC from settling into a static steady-state and allows it to oscillate?

The key insight was the discovery by Rust *et al.* that the serine-phosphorylated form of

The heart of circadian timekeeping in cyanobacteria is a toggle switch that controls the periodic phosphorylation of a key circuit protein.



**The cyanobacterial circadian clock.** Cyclic phosphorylation and dephosphorylation of the clock protein KaiC on serine (S) and threonine (T) becomes oscillatory through a double-negative-feedback loop (red) that toggles between two states with high or low concentration of free KaiA protein. KaiB is the third protein of the oscillator.

KaiC (S-KaiC) binds stoichiometrically to both KaiA and KaiB. The formation of the KaiA-KaiB-KaiC complex prevents KaiA from activating KaiC phosphorylation. Thus, when S-KaiC concentration is high, KaiA is sequestered by S-KaiC and KaiB, and KaiC dephosphorylation predominates; when S-KaiC concentration is low, KaiA is released and KaiC phosphorylation is activated.

Through modeling studies, Rust *et al.* show that the stoichiometric inhibition of KaiA by S-KaiC allows the cyclic phosphorylation-dephosphorylation system to become an oscillator. This inhibition closes a feedback loop and makes KaiA and S-KaiC mutually antagonistic. KaiA is a negative regulator of S-KaiC, because it pushes the balance between S-KaiC and ST-KaiC (phosphorylated on serine and threonine) toward the latter. Conversely, S-KaiC is a negative regulator of KaiA, because it sequesters KaiA (with the help of KaiB). This mutual antagonism, or double-negative-feedback loop (see the figure), allows S-KaiC and KaiA to function as a bistable toggle switch with two alternative stable steady-states; oscillations could then arise from the successive flipping of the KaiA/S-KaiC switch between these two states. Starting with unphosphorylated KaiC and the KaiA/S-KaiC switch in its low-S-KaiC concentration state, phosphorylation

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lation will predominate, and more and more ST-KaiC will build up. As the concentration of ST-KaiC increases, S-KaiC will increase too. The increasing S-KaiC will sequester more and more KaiA, which increases the rate of formation of S-KaiC. This then causes more sequestration of KaiA, and so on. Eventually dephosphorylation dominates, and the system is driven back to unphosphorylated KaiC.

What, then, flips the KaiA/S-KaiC switch back to release KaiA and favor phosphorylation? With KaiA sequestered, S-KaiC formation cannot be maintained indefinitely; the rate of S-KaiC production from its immediate precursor ST-KaiC will eventually slow down as ST-KaiC becomes depleted. This effect is the equivalent of a slow negative-feedback loop—an increase in S-KaiC concentration decreases the amount of ST-KaiC, which

decreases the rate of formation of S-KaiC.

Thus, the circadian oscillator system can be thought of as a bistable switch, toggled first by the slow accumulation of S-KaiC and then by a slow negative-feedback loop. This type of circuit can oscillate, as Rust *et al.* demonstrate through a simple differential equation model whose parameters are constrained by their experimental observations.

At first glance, the circadian oscillator of eukaryotes does not seem to work in the same way. It is composed mainly of transcriptional regulators and directed protein degradation (6), rather than a stoichiometrically controlled autophosphorylating adenosine triphosphatase like KaiC, and none of the eukaryotic components have any sequence homology to KaiA, KaiB, or KaiC. On the other hand, the design principles of the two oscillators

may be quite similar. Both circuits include double-negative-feedback loops that might function as bistable triggers, and both include slow negative-feedback loops (8). In terms of systems-level logic, these oscillators appear more similar than different. Maybe this is how a successful circadian oscillator has to be built.

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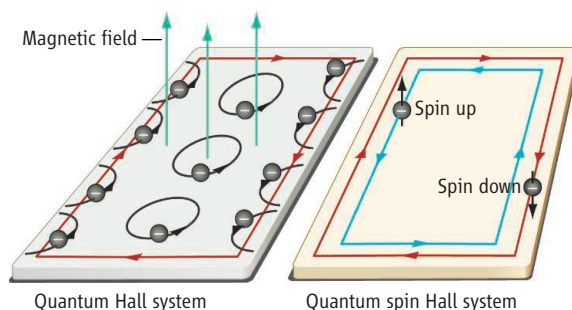
## PHYSICS

# A New State of Quantum Matter

Naoto Nagaosa

Electrons have a property known as spin, and these spins can be controlled and directed by applied electric and magnetic fields. In recent years, researchers in the relatively young field of spintronics have explored this effect for applications in microelectronics [reviewed in (1)]. The goal is to control and use spins much as today's integrated circuits use the property of electric charge for computing operations. On page 766 of this issue, König *et al.* (2) report experimental results that show the existence of a new state of matter that may take spintronics even further. Not only does this work offer us a look at fundamentally new physical phenomena, it may also allow the development of novel spintronics devices.

Many researchers have explored the so-called spin Hall effect as a possible route to spintronic applications. The original Hall effect goes back to the late 1800s, when Edwin Hall noticed that a voltage would form perpendicularly to a current flowing in a conductor in a magnetic field. In the quantum Hall effects, which were discovered in the 1980 and 1982, the electrical conductance takes on quantized values. In the spin Hall effect, the direction of the flow of electrons can be controlled, depending on whether the spin is up or down, by an



**Spin control.** In the conventional quantum Hall system (left), the applied magnetic field causes electrons to bounce off the edge of the sample in circular orbits, forming a net flow of charge around the boundary of the material. No magnetic field is needed, however, in the quantum spin Hall system (right), where spin-up and spin-down carriers flow in opposite directions in edge channel states.

applied electric field. The spin Hall effect was proposed theoretically long ago based on an “extrinsic” mechanism (3), in which impurities in a material deflect the spin-up and spin-down electrons in opposite ways. However, recent interest has centered on an intrinsic form of spin Hall effect and the possibility of spin flow without energy dissipation.

Instead of impurities, the intrinsic mechanism relies on the interplay of the spin and orbital motion of the electrons in the perfect periodic background of the crystal lattice, leading to different paths for the up and down spins. The theoretical proposals for this effect (4, 5) were followed by the experimental discovery of the spin Hall effect in GaAs (6, 7)

Experiments show that electron spins can flow without dissipation in a novel electrical insulator.

and also in metallic systems (8–10). However, the detailed mechanism in these cases still needs to be scrutinized; the flow of charges can still be distorted by impurity scattering and thus contribute to the spin current. Therefore, we need to obtain clear observations of a truly dissipationless spin current.

Dissipationless flow is known to occur in the conventional quantum Hall effect, where the electrons are deflected into circular paths by a magnetic field (see the left panel of the figure).

This motion is not random among electrons but is coherently organized, leading to a collective state. This state is stable because a finite amount of energy (the energy gap) is required to disturb it. In a real sample, the electrons bounce back from the edges, causing a net one-dimensional motion, which corresponds to a flow of current around the edge. Because the direction of this motion is one-way, it cannot be scattered backward, and so the dissipation of the flow of charge is suppressed in the quantum Hall system.

A crucial question is whether a similar state is possible for the spin current. My colleagues and I studied this question theoretically in 2004 (11), and we considered the spin

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