follow each particle as it races through the plasma. The computational savings allow team members William Dorland and Michael Kotschenreuther of IFS and Hammett and Michael Beer of PPPL to check the heat transport over a wide range of plasma temperatures, pressures, and other conditions, and compare the results to those obtained by experiments. But the approach neglects some effects, such as the trapping of individual particles in the troughs of turbulent waves, which could affect the transport.

In an attempt to improve on the fluid approximation, researchers such as Andris Dimits of Lawrence Livermore National Laboratory in California and his co-workers, Parker, and others have now built simulations that follow millions of particles directly. From a physics standpoint, says ITER physicist Marshall Rosenbluth, that approach promises "a more fundamental description" of a real plasma. But Parker notes that the large amounts of computer time required mean that these codes "haven't been compared with experiments the way the IFS-PPPL model has," nor have they modeled the heat transport over as wide a range of plasma temperature and pressure profiles.

The session highlighted a handful of comparisons between the Dimits group's gyrokinetic model and the IFS-PPPL results. There is "perfect agreement" in some comparisons, says Dimits. But other experimentally relevant cases, he says, show that heat "conductivity" is lower in the kinetic simulations by as much as a factor of 3. Combine the best of those numbers with optimistic assumptions about the temperature at the edge of the plasma, says Hammett, and the plasma's center could be hot enough for ITER's output to push into the range that its designers hope for.

But why the two sets of models differ is a mystery. "There's a clear difference, and nobody knows why," says Glenn Bateman of Lehigh University in Bethlehem, Pennsylvania. Dorland says the possibilities include bugs or inadequate numerical resolution in the various codes, something in the detailed physics of wave-particle interactions, or even mundane issues such as the different coordinate systems used by the different groups.

The continuing uncertainty about the outlook for ITER led to jousting at the session between proponents of the project and researchers seen as critical of its prospects. But what's clear, says Drake, is that the past year of jockeying between models has rejuvenated the theory of turbulent heat transport. Converging on a theory everyone accepts is no longer out of the question, he says: "I would not phrase this as a right-wrong issue. We're making tremendous progress; it's very exciting."

–James Glanz

CELL BIOLOGY

Multiple Clocks Keep Time in Fruit Fly Tissues

Anyone who has ever flown across two or more time zones doesn't have to be convinced of the importance of the body's internal rhythms. They are all too apparent—say when the East Coaster visiting California pops awake at 4 a.m. and then has to struggle to keep from falling asleep after dinner. For 25 years, neuroscientists have focused on the brain as the master timekeeper for biological rhythms, controlling everything from normal fluctuations in body temperature to midafternoon slumps. But that view is about to change, at least for fruit flies and perhaps for higher species as well.

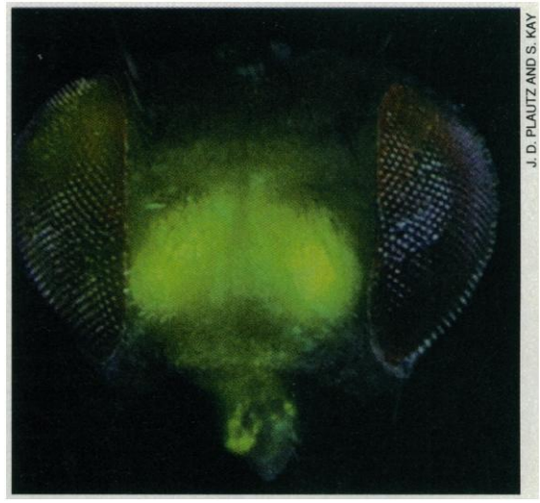
On page 1632, a multidisciplinary team led by geneticist Steve Kay of The Scripps Research Institute in La Jolla, California, reports new evidence indicating that fruit flies have independent clocks throughout their bodies. By harnessing recently developed techniques for imaging proteins in living cells, Kay and his colleagues tracked the production of a timekeeping protein, called PER. Previous work had shown that *per*, the gene that makes the protein, cycles on and off in the fruit fly brain to establish the body's daily rhythms (*Science*, 22 March 1996, p. 1671). Kay and his colleagues now find that this cycling is widespread in fruit fly tissues.

They saw PER appear, disappear, and reappear over and over—in the legs, wings, thorax, head, and abdomen of the insect. "This paper shows clocks all over the place, all at once, in a very graphic fashion," marvels Martin Zatz, a physiologist at the National Institute of Mental Health. "Wherever they look, they find clocks."

Each of these clocks can be set independently, by light, and they keep ticking on their own schedule even when they are isolated from the brain, indicating that they don't need input from a master clock to keep time. Other recent work suggests that mammals, too, have multiple clocks. "It is conceivable that individual cells undergo daily cycles of activity and rest just like whole organisms do," suggests Jadwiga Giebultowicz, an insect physiologist at Oregon State University in Corvallis.

If that proves to be the case, the implications are "quite provocative," says Joseph Takahashi, a clock biologist at Northwestern University in Evanston, Illinois. No one questions a role for the brain's clock in overseeing overall rhythms, such as body temperature or behaviors like waking up which involve coordinating several muscle groups and hormonal changes. But these apparently independent clocks may help various parts of the body tailor their protein production to suit the needs of the hour, Takahashi says. Eyes, for example, may produce different mixtures of photoreceptor proteins at different times of day to make adjustments for night vision, while muscles might rev up their metabolism in anticipation of daytime activities.

The idea of the brain's overriding importance in controlling daily rhythms dates back to 1972 experiments on the effects of damaging or destroying a brain structure called the suprachiasmatic nucleus. Doing so changes or eliminates daily cycles in rats, including the rise and fall of the adrenal hormone corticosterone and daily drinking behavior and locomotor activity. While the fruit fly is not advanced enough to have a suprachiasmatic nucleus, its brain also seemed to be required for the insects to keep their daily schedules. Developing fruit flies with damaged brains, for example, emerged from their pupal cases at random times in the day instead of in the morning, as they normally do, and were no longer active primarily in the morning.



Lighting up. The luminescence (false-colored green) indicates activity of the clock gene **per** in the proboscis and antennae of the fruit fly.

As researchers began discovering the molecular components of this clock, the focus remained on the brain. By tracking down the genes at fault in mutant flies with odd daily rhythms, geneticists discovered clock compo-

nents including *per*. This gene's expression in the brain fluctuates in a predictable pattern over 24 hours. And when a *per* equivalent turned up 2 months ago in humans and mice, researchers found its expression also followed a daily cycle in the suprachiasmatic nucleus (*Science*, 19 September, p. 1762).

But neurobiologist Jeffrey Hall of Brandeis University in Waltham, Massachusetts, and others had found PER in various parts of the fruit fly besides the brain, implying that molecular clocks might not be confined to the brain. To follow up on those hints, they needed a technique that could monitor gene expression in a single living animal over time so they could be sure changes in gene expression were not simply due to individual variation. And that's where a technique Kay had developed 5 years ago for monitoring gene expression in living cells came in.

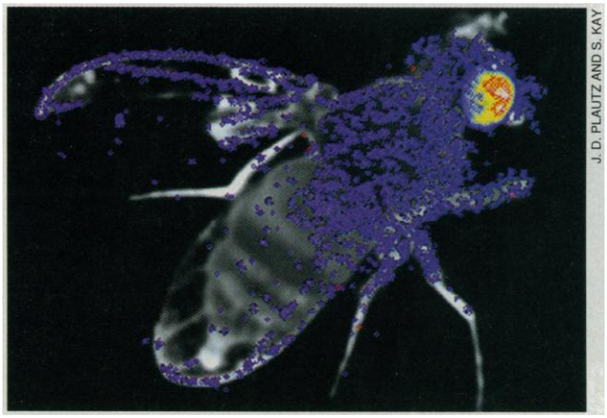
The technique involves fusing the DNA that regulates the expression of whatever gene a researcher wants to study to the gene for luciferase, the enzyme that generates a firefly's light. Kay introduced one such fused gene into plants, then sprayed them with luciferin, the firefly chemical that luminesces when acted upon by luciferase. By simply watching for the glow, he could tell when the regulatory sequence had turned on the target gene.

When Hall learned of that result in 1992, he persuaded Kay to collaborate with his team in trying the same approach for studying *per* expression in fruit fly tissues. In one set of experiments, Kay's graduate student Jeffrey Plautz engineered fruit flies with the *luciferase* gene fused to the DNA that triggers *per* expression. In these fruit flies, Plautz could watch the expression of *per*'s stand-in, the *luciferase* gene, change over time within the fly body, although the signal was too weak to see exactly where the expression was taking place.

To get around this, Maki Kaneko from Hall's group adapted the technique to use a more vivid optical signal, from the green fluorescent protein (GFP) of jellyfish. She did this by breeding two transgenic strains, one with the GFP gene linked to a yeast promoter and one with the *per* promoter linked to a yeast gene that would trigger expression of the hybrid GFP gene. By watching for the vivid glow of GFP in the crossbred flies, the researchers could see which tissues, and sometimes even what specific cells, make the PER protein.

They first looked at whole fruit flies, confirming in the GFP insects that *per* is indeed active all over. The *luciferase* transgenic fly indicated that *per* also cycles on and off just as it does in the brain. To determine whether the individual tissue clocks are controlled by the brain clock, they cut up the transgenic flies and incubated the heads, thoraxes, and abdomens separately in culture dishes. They found that the tissue *per* genes could cycle even in the absence of a brain.

When exposed to alternating periods of 12 hours of light and 12 hours of darkness, the gene turned on and off in the isolated tissues every 24 hours. And as with brain clocks, the cycling continued when the tissues were kept in complete darkness, although they did not keep time as faithfully as tissues experiencing both light and darkness. Turning the lights on, however, reset these clocks in the various body parts, even those lacking the head. "You don't have to go through the known eyes to get light to this tissue," Hall points out.



J. D. PLAUTZ AND S. KAY

Clocks galore. The blue in this false-color image points to the existence of independent clocks throughout the fruit fly body.

What's more, the researchers could see clock activity not just in whole body sections, but in smaller organs such as the antennae and proboscis, or feeding tube. Indeed, the technique enabled them to identify specific chemosensory cells with periodic *per* expression, which suggests that even individual cells can keep time on their own. This many clocks "was a real surprise," Kay notes.

It's not completely unprecedented, however. In 1989, for example, Oregon's Giebultowicz had found a clock located in the testis of the gypsy moth that governs daily, predictable fluctuations in the release of sperm. And just last April, her team came across another tissue clock. The fruit fly's malpighian tubules, which act like a kidney, have a daily rhythm, evidenced by cyclical expression of *per* and another clock gene called *tim*. This gene activity pattern occurs independently of the brain and can be reset by light, she reported. Also that month, another research team led by Katherine Siwicki of Swarthmore College in Pennsylvania showed that the fruit fly's ring gland, which secretes hormones, also operates on its own time.

But by and large, Giebultowicz says, "those [clocks] were considered exceptions to the rule" that the brain clock was primary. Now, the Kay team's experiments really drive home how widespread clocks are in the body. "[The technique] is spectacular," says Michael Menaker, a neurobiologist at the University of Virginia, Charlottesville.

Much less is known about clock operation in mammals, because the genes involved are only now being discovered. But already there are hints that higher organisms may also have multiple clocks. The mouse *per* gene, for example, is active in many tissues, including the heart, lung, liver, and kidney, and is particularly active in the testis and skeletal muscle. And another mammalian timekeeper gene called *CLOCK*, identified by the Takahashi group, is also expressed in lots of tissues. "This is making us think that there are clocks in other tissues," says Takahashi. To see if that is in fact the case, Kay is now using his *luciferase* technique in mouse tissue to test whether the expression of these mammalian genes outside the brain follows a daily schedule.

To understand the tissue clocks, however, chronobiologists will need to figure out how they sense light. For the brain clock, this job is performed by the retina, although not by the light-sensitive cells responsible for vision. The optic nerve then transmits the information to the brain. But cells outside the retina lack the photosensitive pigments found in the eye. Instead, there are hints that these tissues may use recently discovered proteins that are sensitive to blue light.

Some of these help repair DNA damaged by ultraviolet light, but both plants and mammals have blue light–sensitive proteins that have nothing to do with DNA repair. Aziz Sancar, a biochemist at the University of North Carolina, Chapel Hill, has found two such proteins in humans, both of which are located in the same tissues as the clock proteins. "We think there's an 80% probability that this will turn out to be a blue-light photoreceptor for activating circadian clocks," says Sancar.

He and Japanese researchers are making mice that lack these proteins to test out this prediction. They are convinced light can reach deep into the body where these sensors reside. "We're talking about a different light perception and a different time scale [than in vision]," Giebultowicz agrees. Rather than relying solely on a master clock in the brain to coordinate all body rhythms, for these many other clocks, Kay proposes, "the true master switch is just sunlight."

–Elizabeth Pennisi