cut off from the more distant wells by impermeable barriers in the rock. Sophisticated extraction techniques—heating and thinning the viscous oil by pumping steam into the rock or floating the oil upward with injections of water—can also wring unexpected amounts of oil from the rock. Given such extra effort, many fields "just seem to grow and grow," says Gautier, some with no end in sight.

In the 1980s, there was plenty of extra effort because of the "feeding frenzy" among drillers when oil prices skyrocketed in the late 1970s and early '80s. During the boom, the intensive, at times scattershot drilling enlarged some fields more than expected. And fields continued to grow faster than expected even after the boom collapsed, says Gautier, as drillers were forced to become more efficient at finding oil and gas. New technology like 3D subterranean seismic imaging and horizontal drilling helped them capitalize on clues that had come to light during the drilling boom, adds William Fisher of the University of Texas, Austin.

The USGS had based its gloomy 1989 assessment on data collected by industry groups between 1969 and 1979, before the field-expanding boom and bust. This time the assessment team was able to use more recent proprietary data on 46,000 fields, collected from companies by the Department of Energy from 1977 to 1991. By extrapolating from the pace of new oil and gas discoveries in known fields over that period, the team estimated the total oil and gas remaining in the fields.

Gautier and his colleagues caution that their huge new "finds" will only materialize if the drillers can sustain the success rate they achieved in the 1980s. Gas fields, in particular, may not respond so well to greater drilling effort, warns Joseph Riva of the Congressional Research Service; because gas is so much more mobile than oil, a smaller number of wells may be enough to fully exploit a field. And the new estimates ignore how much it would cost to steam, flood, or otherwise coax out the additional oil and gas. Certainly no one would bother with much of these inferred reserves at today's low oil and gas prices, but prices can change.

That was Masters's message to the forum. "In the next couple of decades, there will probably be lots of oil and gas" worldwide from a range of suppliers both large and small, he said. But "in the middle part of the next century," he warned, "the gap is pretty big between what we think we know about [world] supply and what demand may be." The Middle East will come to dominate the oil supply and Russia the gas supply, he said, and the threat of "economic terrorism" will loom. Even pricey inferred reserves could look good then.

–Richard A. Kerr

PLANT GENETICS

Shedding Light on the Ticking Of Internal Timekeepers

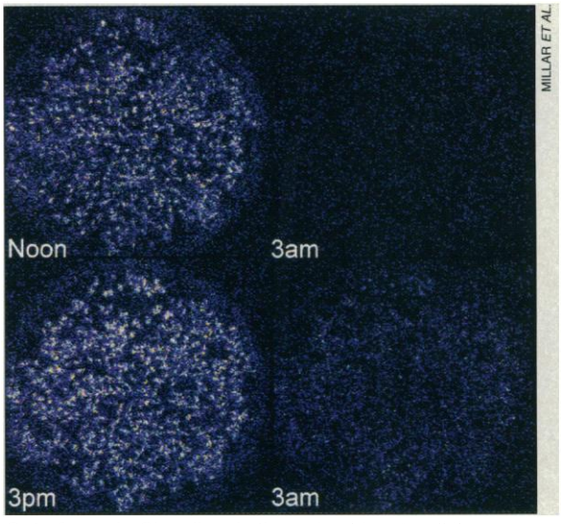
As any jet-lagged traveler knows, our internal clock is a powerful timepiece that can keep a globetrotter wide awake at night and yawning through the day. Experiments over the years have shown that such clocks govern a host of daily physiological events, such as body temperature changes in animals and leaf position in plants, and can be set—and reset—by exposure to light.

But the inner workings of these clocks, as well as the means by which they are set, have remained a mystery, and nowhere was that mystery murkier than in plants. Now, in two papers on pages 1161 and 1163 of this issue, a research team headed by Steve Kay of the University of Virginia has begun to crack the plant clock puzzle. In one paper, they identify genetic mutations that alter the internal clocks of the tiny laboratory plant *Arabidopsis*. And in the other, they show that both red and blue light can influence the clock's rhythms, and do so via two different biochemical pathways.

"This is the first time anybody has been able to get a handle on the molecular basis of the clock in higher plants," says Dartmouth Medical School geneticist Jay Dunlap, who studies these daily clock-driven cycles, known as circadian rhythms, in the bread mold, *Neurospora*. Eventually, understanding plant clocks should enable biologists to compare clocks from widely different species, says Brandeis University biologist Michael Rosbash, who studies the circadian clocks of fruit flies, and thus address a central question: "Will these turn out to be universal mechanisms and universal genes, or is the clock really quite different in different organisms?"

In recent years, researchers have begun to gain some understanding of how clocks work in two model organisms, *Neurospora* and fruit flies (see box on p. 1092), and several groups have recently cloned clock-related genes from mice, hamsters, and cyanobacteria, although little is yet known about how those genes work. But amid this progress in other systems, plant researchers have remained unable to identify any clock genes in plants.

The problem is in the difficulty of pinpointing mutant plants whose clocks are out of synch. To find such mutants in other organisms, biologists have typically used "brute-force" screens, searching through thousands of organisms to find those with abnormal rhythms—for example, mice that run in their exercise wheels while all the other mice are sleeping, or *Neurospora* colonies that put up fruiting bodies at the wrong time of day. But such screens have daunted plant biologists because plant rhythms are so subtle, says Dartmouth's Dunlap. "It would be really tough to do a brute-force screen on thousands of plants, looking for the leaves to be up when they are supposed to be down."



Out of synch. Circadian rhythms should make all these *Arabidopsis* seedlings turn a glowing pigment on and off at the same time. But some, clock mutants, glow out of step.

Kay solved this problem in *Arabidopsis* with a bit of genetic engineering. He and graduate student Andrew Millar began with the DNA regulatory sequences from a clock-regulated *Arabidopsis* gene called *CAB* (chlorophyll-*a*/*b*-binding protein). *CAB* is normally switched on during the day and off at night, and maintains that rhythm—directed by the plant's internal clock—even when the plants are kept in constant light.

The researchers hooked those regulatory sequences up to the *luciferase* gene from fireflies, which produces an enzyme that causes a chemical called luciferin to glow. The experiment worked "like a dream," recalls Kay. When sprayed with luciferin, the plants carrying the engineered *luciferase* gene glowed during daytime hours and didn't glow at night. That made the search for clock mutants simple: Just look for seedlings that glow at the wrong times.

Millar did just that. At various points in a 24-hour period, he took petri dishes filled

Keeping PERfect Time

Biological molecules, and the reactions they undergo, are notoriously sensitive to temperature. Even the cell cycle is slowed when an organism's temperature drops. Yet the circadian clocks that maintain daily rhythms tick on at nearly constant rates, their biological components somehow impervious to heat or cold. On page 1169, Michael Rosbash and his colleagues at Brandeis University suggest that, at least in fruit flies, clocks compensate for temperature changes through a competing set of protein-protein interactions involving a protein known as PER.

For years, evidence has been building that PER (for period), a protein that appears to regulate gene transcription, is at the core of the fruit fly clock. PER levels regularly cycle up and down every day, and PER in fact controls that cycling: As PER levels rise, the protein shuts down the activity of its own gene and thus begins to lower PER levels. Now, Rosbash and grad student Zuoshi Huang have identified a possible mechanism for this self-regulation, one that also explains PER's resistance to temperature changes. Their model involves two competing protein interactions—one activating PER and the other temporarily turning it off—both of which respond similarly to temperature change. The result is a wash: No matter the weather, PER's activity stays constant.

Two years ago, Rosbash and his colleagues got the first evidence that PER interacts with other proteins: They found that it contains an amino acid sequence called PAS, which causes PER to form protein pairs known dimers. Rosbash proposed that dimers of PER (in which PER binds to either another PER molecule or some unknown mate) turn down the *per* gene. Thus, the rate at which dimers form would determine how quickly the gene was turned down and the length of the resulting cycle.

But forming dimers is only one of PER's options. Rosbash's group has now found that PER can apparently fold back on itself, so that a part of the PER protein called the C domain binds to the PAS region, temporarily blocking dimer formation. Rosbash proposes that both the C-PAS reaction and dimer formation might be similarly affected by temperature changes, and if so, that would result in a stable clock. "If you have [two reactions] that are in competition with each other, if they both have the same temperature coefficient, they cancel," he says.

Support for this model has come from experiments on a mutant form of PER called PER^L, which lengthens the circadian rhythm of flies. PER^L also makes the clock temperature-sensitive, so that its daily cycles grow even longer as the temperature rises. Rosbash showed 2 years ago that PER^L doesn't form dimers as well as normal PER does. And now Huang has found the apparent reason: PER^L slips more easily into the C-PAS configuration, thus preventing dimer formation. Indeed, Huang found that when PER^L and PER had their C domains chopped off, they formed dimers equally well, suggesting that the enhanced attraction between C and PAS is the cause of PER^L's abnormal behavior. Huang also found a likely explanation for PER^L's temperature sensitivity. It favors the C-PAS reaction more as the temperature goes up, and so is even less apt to form dimers.

"I think this is the first molecular proposal for what could be responsible for the temperature compensation phenomenon" in circadian clocks, says Harvard University biochemist Woody Hastings, who 38 years ago suggested that competing reactions could make the clocks temperature-stable. The Rosbash lab's work takes that generic idea much further, he says, by identifying actual molecules that seem to be doing the job.

–M.B.

with mutant *Arabidopsis* seedlings, sprayed them with luciferin, and photographed them with a sensitive camera to capture their dim glow. Each photo "looks like a constellation of stars," says Kay. When the photographs were compared, most of the "stars" brightened and dimmed in unison. But Millar found 26 seedlings that were out of synch, with rhythms ranging from 21 to 28 hours.

The discovery of the mutants is only the beginning: The team has not yet determined the altered genes responsible for the changed rhythms, nor identified any proteins they produce. But the ingenuity of the method, and the potential of the mutants to finally crack the mystery of plant clocks, has the plant community excited. "Brilliant is a good word" to describe the work, says Dartmouth plant geneticist Rob McClung. "The prospect for the future is that this is a system where we can get a lot of [clock] mutations."

And the future prospects of the Kay team's work aren't limited to mutants in the clock mechanism itself. The group also teamed with Joanne Chory of the Salk Institute in La Jolla, California, to ask how light exerts its effect on the clock. Recently, Chory's lab, as well as several others, has identified *Arabidopsis* plants with mutations in the biochemical pathways that sense and respond to light. Researchers knew that light has a profound effect on the clocks of plants—left in the darkness, for example, a plant's clock slows dramatically, while exposure to light will bring it back to a shorter rhythm. Plants have several light-receiving systems which respond to different wavelengths of light, but no one knew which of these pathways were tied in to the clock. Kay and Chory realized that, with Chory's mutants and Millar's *CAB-luciferase* gene, they had just the tools necessary to ask the question. "We could cross our marker into all these different mutant backgrounds," says Kay, "and then very quickly by just measuring luciferase activity look at how that mutant background affects circadian function."

The investigators introduced the *CAB-luciferase* gene into various mutants made in Chory's lab and checked the effect the mutation had on the plants' rhythms. When they put the gene into plants that lack an element of the red-light pathway and then grew those plants in red light, the plants' clocks slowed way down, as if they were growing in the dark, an expected result, because the red-light pathway was not working. Blue light, however, had a very different effect, bringing the plants back to a shorter cycle. Clearly blue light was getting through to the clock, and doing it by a different pathway than that used by red light.

The researchers also put the *CAB-luciferase* gene into mutants in which the red-responsive pathway is continually turned on, even in the dark. Grown in total darkness, these plants' clocks ran as if they were growing in light, showing that the red-light path also affects the clock.

Because Chory's mutants define individual steps in the light-reception pathways, says Dunlap, the research not only distinguishes between the effects of the two pathways, but also has the potential to "put in a lot of the players between photoreceptor and the clock." In most cases the identity of the players is not yet known, because the genes and proteins affected by Chory's mutants haven't been isolated. But even this first step is a big one, Dunlap says, and is "something that has not been done in any other organism, not in *Neurospora*, not in flies."

Researchers still don't know how widely clocks, or the mechanisms that set them, are shared among different organisms. But these developments in plants may mean that we are closer to knowing whether there is indeed a common circadian rhythm machine that keeps the beat in many forms of life.

–Marcia Barinaga