tute for Solid-State Electronics in Berlin. Such studies are of more than academic interest. Communications equipment, televisions, and cellular phones regularly rely on acoustic transducers as filters to exclude unwanted signal around their desired frequencies. Understanding how these devices vibrate can help improve their performance.

Researchers wanting to trace sound waves in a material have generally been limited to coarse images of low-frequency waves. One strategy is to bounce pairs of laser beams off the material's surface and then combine the beams to produce an interference pattern, which indicates how the sound waves are displacing the surface, but the lasers' spot size limits the resolution of the technique. "It is almost impossible to image [high-frequency] waves" that are key to devices such as cellular phones, says Chilla.

But a collection of six European teams known as the Atomic Force Microscopy and Microacoustics consortium, which has been supported by European Union funding, is probing vibrations on a much smaller scale. Under the right conditions, an AFM tip placed near a vibrating surface will stick to it, because of van der Waals and other electrical and viscous forces, causing the tip to follow the surface's oscillation. The tip's motion is read out by bouncing a laser beam off a tiny mirror attached to the cantilever. By pumping ultrasound into a material at different frequencies, then imaging the passing waves, researchers can determine the material's velocity dispersion—the relationship between the waves' velocity and their frequency—which is a clue to its elastic properties on a nanometer scale. This allows the detailed study of how the very small structures found in acoustic filters behave when vibrating and how the different parts influence each other.

Chilla's team is now trying to image the motion of an individual atom as the sound oscillation moves it through an elliptical path. Above frequencies of around several hundred kilohertz, the probe tips just can't keep up. Chilla reported at the Berlin meeting, however, that he and his team can still glean information on high-frequency waves by allowing the tip to skim the surface of the waves, registering their amplitude without following their every up and down. From this information the researchers can derive the local elastic properties of the material at high frequency.

In a variation on this technique, Andrew Kulik and his group at the Swiss Federal Polytechnic Institute in Lausanne eliminated adhesion from the equation. Relying on adhesion to couple the tip and the surface can skew measurements of elasticity, because the adhesive bond between the tip and the material can also stretch and compress. So Kulik's team holds the tip absolutely steady and lets the oscillating surface bump into it. Analyzing how the tip vibrates when it touches the vibrating surface reveals the local elasticity. "We have a depth resolution of about 100 nanometers, and we know that we are imaging elastic properties," says Kulik.

Similarly, a team led by Walter Arnold of the Fraunhofer Institute for Nondestructive Testing in Saarbrücken, Germany, actually pokes the tip into the surface so that it moves with it. "You deform the surface with the tip, and the deformation field contains the stiffness of the tip and the sample," says Arnold. If you know the stiffness of the tip, you can deduce the elasticity of the sample, he explains. The system is so sensitive that it can detect differences in the elasticity in the small areas in magnetic materials in which the magnetic field is oriented in a specific direction. "The various domain orientations have a different contact stiffness," he says.

–ALEXANDER HELLEMANS

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CREDIT: T. JOYCE, M. FREEDMAN, R. J. LUCAS, AND R. G. FOSTER/IMPERIAL COLLEGE

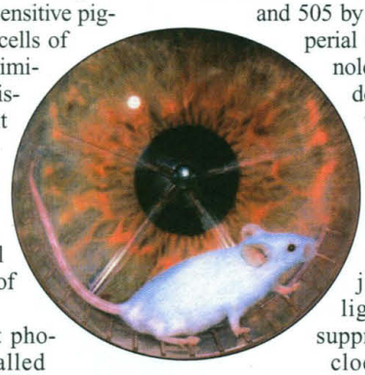
CIRCADIAN RHYTHMS

The Clock Plot Thickens

Researchers prove that a nonvisual light sensor sets our daily clock; a likely candidate for that role appears to fulfill other clock functions, too

One of our most indispensable biological machines is our circadian clock, which acts like a multifunction timer to regulate sleep and activity, hormone levels, appetite, and other bodily functions with 24-hour cycles. The clock generally runs a bit fast or slow and must be reset daily by sunlight. Although many components of the clockwork are known, the crucial photoreceptor that passes light's signal to the clock is still at large.

Two suspects, the light-sensitive pigments in the rod and cone cells of the mammalian eye, are eliminated by two papers in this issue. "The really important conclusion from these experiments is that there is another photoreceptor" affecting the clock, says circadian biologist Michael Menaker of the University of Virginia, Charlottesville.



The eyes have it. The mammalian clock receptor, although not in the rods and cones, is in the eye.

One candidate for that photoreceptor is a protein called cryptochrome. But a report in yesterday's issue of *Nature* puts an intriguing wrinkle in that story, fingering cryptochrome as a likely part of the clock itself. In mice that lack cryptochrome, the group found, the clock doesn't run at all. "We have never seen [in mice] a mutant like this, where there is instant arrhythmicity," says clock researcher Steve Kay of The Scripps Research Institute in La Jolla, California. That means cryptochrome is essential for clock function, but leaves open the question of whether it is the long-sought circadian photoreceptor in mammals.

Biologists have known since the 1960s that the clock-setting light signal in mammals normally comes via the eyes, because eyeless rodents and humans with few exceptions are unable to reset their clocks to light. One obvious possibility is that the molecules that capture light for vision—the opsins in the rod and cone cells of the retina—also send light signals to the clock.

Evidence against that has mounted as researchers have found that mice lacking either rods or cones have clocks that respond to light. But the chance remained that rods and cones both can do the job, and either can do it alone. The reports on pages 502 and 505 by Russell Foster of the Imperial College of Science, Technology and Medicine in London and his colleagues rule that out.

The researchers introduced genes that destroy retinal rod and cone cells into mice. They found that in those mice, just as in normal mice, light resets the clock and suppresses production of the clock-controlled nocturnal hormone, melatonin. "That says that you don't need rods and cones" for the light response, says Menaker, and means another photoreceptor must do the job.

Cryptochrome, which is found in the eye, became a hot candidate for the photoreceptor last fall, when three teams reported that it seems to help light reset the clock in plants, fruit flies, and mice (*Science,* 27 November 1998, p. 1628). A group led by Aziz Sancar at the University of North Carolina, Chapel Hill, and Joseph Takahashi at Northwestern University in Chicago mutated *cry2*, one of two mammalian cryptochrome genes, in mice. The animals' clocks lost some light responsiveness, suggesting that Cry2 is a light sensor, but not the only one. Researchers wondered if Cry1 might be the other, and

waited to see if mice missing both cryptochromes could adapt to light.

In *Nature* this week, Jan Hoeijmakers at Erasmus University in Rotterdam, Netherlands, Akira Yasui of Tohoku University in Sendai, Japan, and their co-workers report the first tests on such mice. But instead of providing an answer about light response, the results delivered a surprise: The mice have no clock. Under conditions of 12 hours of light followed by 12 hours of dark, they act like normal mice, running in their exercise wheels in the dark and sleeping when it is light. But in constant darkness, when the clock would normally maintain the alternating cycles, their behavior loses that pattern; they run on and off around the clock.

Those results suggest the animals' clocks fail in constant darkness. But further tests show they actually have no clock at all. When normal animals are subjected to a new light-dark pattern, they begin to adapt their clocks, a slow process as any jet-lagged traveler knows. But the mutant mice instantly adjust to any light pattern; they run when the lights go out and stop when they come on. That, says clock researcher Jeff Hall of Brandeis University in Waltham, Massachusetts, is the kind of behavior observed in clockless animals. Without a clock to control their behavior, it is driven directly by the light. Sancar and Takahashi, working with Takeshi Todo of Kyoto University in Japan, have also made double cryptochrome knockout mice and have preliminary results similar to those of the Dutch-Japanese team.

Slight abnormalities in *cry2* mutant mice that Sancar and Takahashi reported last fall suggested that *cry2* might play a central role in the clock, but most researchers were surprised to learn it is essential for the clock to work. That creates a new mystery: What is the cryptochrome doing in the clock? But it does little to solve the old puzzle of whether cryptochrome transmits light signals.

"Perhaps both functions, the clock and the light input, are being taken out" in the double mutants, says Takahashi. Ironically, the lack of a clock in the mutant mice makes it hard to test that hypothesis; one can't measure the effect of light on a nonexistent clock. But Kay notes that even if the clock is disabled, some of its molecular parts remain and should be able to respond to light. He suggests the authors check the behavior of those proteins to see if a light signal is getting through, something both groups plan to do. Wherever the search for the circadian photopigment leads as it moves beyond the rods and cones, one thing is for sure: Cryptochrome has guaranteed itself a place in the story of the circadian clock. **–MARCIA BARINAGA**

TISSUE ENGINEERING

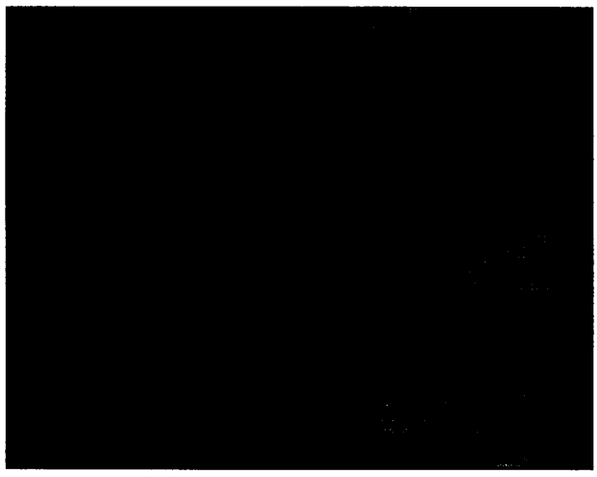
Lab-Grown Organs Begin To Take Shape

With the need for transplant organs growing, researchers are making progress toward developing them, using cultured cells and special polymers

Call it the seaweed that's changing medicine. On a balmy summer afternoon in 1986, surgeon Joseph Vacanti of Harvard Medical School in Boston was sitting on a stone breakwater near his Cape Cod vacation house watching his four children play on the beach. He and biomedical engineer Robert Langer of the Massachusetts Institute of Technology (MIT) had been trying for more than a year to devise new ways to grow thick layers of tissues in the laboratory—a first step toward their long-term goal of growing replacements for damaged tissues and organs.

But even though they were using the latest in cell-friendly, biodegradable polymers as scaffolds to support the growing tissue, the thickest slices they could grow were thinner than a dime—not much use for building complex three-dimensional organs like livers, kidneys, or hearts. The problem, Vacanti realized, was that as the tissues thickened, the interior cells couldn't take in enough nutrients and oxygen or get rid of sufficient carbon dioxide to continue growing.

Then, as Vacanti gazed into the water, inspiration struck. He spotted a seaweed waving its branches, silently soaking up nutrients from the water around it. He immediately made the connection: Branching is nature's way of maximizing surface area to supply thick tissues with nutrients, and polymer materials that branch, rather than being completely solid, would be porous enough to support growing tissue in the lab. Vacanti raced up the road to a pay phone to call Langer. "He asked if we could design [biodegradable] polymers that had a branching structure," Langer recalls. "I said, 'Well, we could probably do that,' and we tried and we did."



Web site for cells. The micrograph shows smooth muscle cells growing in a porous polymer used for tissue engineering.

CREDIT: NIKLASON *ET AL.*

Thirteen years later, branched biodegradable plastics and related sponge-shaped plastics undergird tissues growing in dozens of laboratories around the world. Some of the simpler of these tissues, including skin and cartilage, have already made it to the clinic or are on their way (see sidebar). But, fueled by recent advances in polymer chemistry, in the design of the bioreactors that incubate the tissues, and in the understanding of basic cell and tissue biology, researchers are also beginning to grow organs with more complex architectures.

Two months ago, for example, a team at Harvard Medical School reported in *Nature Biotechnology* that it had used tissue engineering to produce new urinary bladders that appeared to work normally in dogs. And on page 489, Langer, anesthesiologist and biomedical engineer Laura Niklason of Duke University in Durham, North Carolina, and their colleagues report growing functioning pig arteries. Less advanced, but showing progress, are efforts to engineer tissues to fill in for failing hearts, livers, and kidneys—organs for which the demand for transplants far outstrips supply.

"I'm very excited about it all," says biomedical engineer Michael Sefton of the University of Toronto. "Things are going much better than I expected." Indeed, he has organized more than 25 leading tissue engineers into an informal interna-