PER and move into the nucleus. Consistent with this, Edery's student, Choogon Lee, found that a light pulse in the early evening delays events in the molecular clock that depend on TIM, such as PER's arrival in the nucleus and the shutting down of the *per* and *tim* genes. The effect of these changes is to set the hands of the clock back a few hours.

Later, in the hours before dawn, things are different. TIM and PER are paired up and in the nucleus, where they have shut down their genes. If light destroys TIM then, there is no mRNA around to make more TIM. And indeed, Young's group showed that light exposure at this time causes a rapid loss of TIM that is not replenished. The early demise of the TIM-PER complex allows the *tim* and *per* genes to come on a few hours earlier in the morning, advancing the clock.

Together, the findings show how light

destruction of TIM can reset the clock in either direction, depending on when the light comes on. Says Takahashi: "It really is a very simple and direct mechanism."

But it's not the only way in which light can reset a clock. Last June, Jay Dunlap and his colleagues at Dartmouth Medical School reported that light resets the *Neurospora* clock not by destroying a protein, but by turning on the gene for the essential clock protein called FRQ (for frequency). But in that paper, Dunlap even predicted that light would reset the fly clock via protein suppression or destruction. The reason, Dunlap says, has to do with the time of day when the proteins are expressed.

FRQ, like TIM and PER, cycles every 24 hours and controls its cycling by shutting down its own gene. But while PER and TIM peak at night, FRQ reaches its highest level

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that via a day protein, Dunlap says, it must increase protein levels, which is easily done by turning up the gene. In contrast, resetting a clock via a night protein requires the opposite—removal of that protein—which is just what happens with TIM. "Evolution chose two internally consistent variations on a theme, the theme being that light will act rapidly to change the level of a clock component," Dunlap concludes. The next question for the fly researchers is how light destroys TIM. The present work

during the day. Light resets a clock by making the night situation more like day. To do

is how light destroys TIM. The present work, Takahashi says, will start geneticists scrambling to devise new ways to screen for mutations in that biochemical pathway, whose identity is now the latest in the long line of unsolved mysteries of the fruit fly clock.

-Marcia Barinaga

Gene Perplexes Epilepsy Researchers

One of the benefits of using genetic linkage analysis to find the faulty gene that causes a disease is that you do not need to know anything about the function of the gene you are looking for. But the other side of the coin is that the results can be perplexing. You may finally pinpoint a gene and find that the protein it produces seems quite unrelated to the disease concerned.

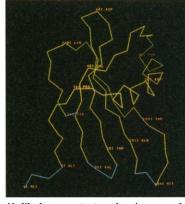
That is exactly what happened to the teams led by Richard Myers of Stanford University and Anna-Elina Lehesjoki at the University of Helsinki in Finland. As they report on page 1731 of this issue, they were searching on chromosome 21 for a gene that, when mutated, causes a rare form of epilepsy known as EPM1. When they pinpointed the culprit gene, they found that it had already been described: It codes for a protein called cystatin B, which acts within cells to block the action of certain cathepsins, protease enzymes that degrade other cell proteins.

"Boy, what a surprising thing," says developmental neurobiologist Chris Walsh of Beth Israel Hospital in Boston, who is tracking down a different epilepsy gene on the X chromosome. The surprise stems from the fact that cystatin B appears to have no connection to the known mechanisms for epilepsy. Epileptic seizures occur when neurons stimulate one another to fire excessively. So the focus of epilepsy research has been on neurotransmitters and their cell-surface receptors—far from cystatin B and the regulation of protease activity inside cells.

The Stanford and Helsinki teams, however, conducted a search designed to pick up the gene at fault in EPM1 whatever its function. They chose EPM1, which causes a gradual neurodegeneration leading to mild dementia, as well as seizures and muscular spasms, because commoner forms of epilepsy can be hard nuts for geneticists to crack: Environmental factors ranging from alcoholism to head injury can contribute to them, the onset of the disease can occur at any age, and they can exhibit many different symptoms. This complexity makes diagnosis difficult, hindering efforts to construct pedigrees of the disease for genetic linkage analysis.

In contrast, the distinct characteristics of

EPM1 make its inheritance easier to track, although it is very rare. Finns, however, show a higher than average incidence of many genetic diseases because they are relatively inbred from a small population dating back 2000 years. "We haven't exchanged genes with our neighbors," says Lehesjoki. The country has enough afflicted families so that the Finnish team could collect tissue samples, build pedigrees, and map the gene with a very fine resolution to a 175,000-base region of chromosome 21.



Unlikely perpetrator. An absence of the protein cystatin B somehow leads to a rare form of epilepsy.

Homing in on that target region, both teams sequenced short pieces from different genes within it and compared them with known genes in sequence databases. When one of the Stanford team's sequences scored a match—with the gene for cystatin B—they went back to the pedigree samples. Using cell lines made from the white blood cells of each patient, Myers's team isolated all the RNA

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molecules transcribed from the DNA in each sample and then fished out just those copied from the cystatin B gene. "What really was the trigger," Myers recalls, was when "we saw that the [cystatin B] messenger RNA was missing from the patients."

To find the underlying genetic defect, Len Pennacchio, Myers's graduate student, continued sequencing the DNA. "We found two different mutations," he says, that could account for the lack of mature RNA copies of the gene and that probably lead to an absence of the protein itself.

Now the researchers are trying to work

out why a lack of this protease inhibitor could lead to epilepsy. "This protein is in every cell [type] that we've seen so far" in normal individuals, says Myers, making it difficult to explain why only the brain should be affected by its absence. But Walsh sees one clue: The cathepsins that are cystatin B's targets are packaged within lysosomes, intracellular compartments resembling the synaptic vesicles that release neurotransmitters into the synapses be-

tween neurons. The resemblance leads Walsh to speculate that "a functional analogy between synaptic vesicles and lysosomes could be a very interesting avenue" for finding a link between cystatin B and epilepsy. But for now, he says he is simply "very intrigued."

-Claire O'Brien

Claire O'Brien is a writer in Cambridge, U.K.