Genes and Biological Clocks

Studies of genetic mutations in fruit flies reveal that a single gene may control circadian rhythms

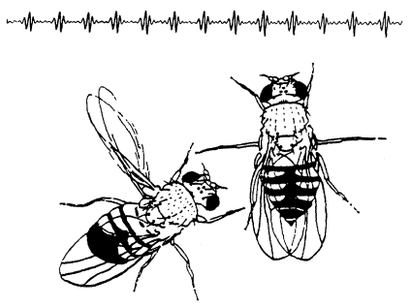
"What we are trying to do," says Michael Rosbash of Brandeis University, "is study circadian rhythms in the same way as oncogenes are being studied. We have a piece of DNA with biological activity. But what is it doing?" He and his colleague Jeffrey Hall are among a small group of investigators who are beginning to get a glimpse, on a molecular level, of the complex genetic controls that determine how animals generate daily rhythms. Genes that control circadian rhythms in fruit flies and, possibly, in vertebrates, appear to code for a class of proteins that are poorly understood and certainly do not fit easily into previous hypothetical models of how biological clocks work.

The story of the search for a molecular basis of biological clocks began 15 years ago when Ronald Konopka of Clarkson University in Pottsdam, New York, was still a graduate student with Seymour Benzer at the California Institute of Technology. Konopka embarked on what seemed like a daring project, and one whose chances of success sounded questionable. He decided to try to find a mutation that affects biological rhythms in fruit flies. At the time, no one had ever created mutations in any clock genes in any organisms, and it was not at all clear that clock genes even existed. "Everybody told me I was crazy," Konopka recalls.

But Konopka, who began looking for flies that emerged from their pupas at night rather than in the day when the flies customarily emerge, was fortunate. "When I finally found one, I couldn't believe it," he says. He isolated three mutations at the same locus, which he called *per* for period. All of the *per* mutations mapped to the same region of the X chromosome, and all altered the biological rhythms of *Drosophila*, although in different ways.

The first mutation appeared to knock out the organism's clock altogether. The flies had no discernible sleep-wake cycles and, in fact, appeared to be insomniacs. Male fruit flies ordinarily sing a mating song by beating their wings together in a pattern that repeats at 60-second intervals. Flies with this "knockout" mutation sing mating songs but the songs have no rhythms.

The other *per* mutations affected the timing of the clock. *Per* s, for short periods, caused the flies to have 18- to 20-hour days and 40 second periods in the male mating songs. *Per* l, for long periods, resulted in 28 to 30 hour days and 80-second mating song periods. Konopka and, independently, F. Robert Jackson of the Worchester Foundation have since isolated mutations at four other loci that also affect *Drosophila*'s rhythms—three of the mutations lengthen the periods by 1 to 2 hours and the other shortens them by 1½ hours. But the *per* mutations have been the most intensively studied and seem to have the most varied effects on the clocks.



Courting fruit flies

The male fly beats his wings to produce a song that repeats every 60 seconds. Per *mutations can speed up or slow down the song or can destroy its rhythm altogether. [Source: Barrie Burnet (fruit flies); J. Hall and S. Kulkarni (songs)]*

Because the *per* mutations affect so many different aspects of the clock's timing, says Michael Young of Rockefeller University, "it seemed that the *per* mutations were close to the heart of the clock itself." Young was a graduate student at the University of Texas at Austin shortly after Konopka discovered the *per* mutations. He and, independently, Konopka, decided to cytologically map the *per* gene, using a series of chromosomal rearrangements in the general location of the gene. As a consequence of this work, the two investigators learned not only that the *per* genes are located between two large polytene chromosome bands but also that when the *per* genes are deleted, the flies have no rhythms. It seemed that a protein or proteins coded by the *per* genes determines the rhythms of *Drosophila*.

The story stopped for awhile because the researchers did not have the technology to carry the work much further. Then 4 years ago, with the advent of improved techniques for isolating DNA segments, Young and his associates and, independently, Rosbash, Hall, and their associates, isolated a 7-kilobase region from the *Drosophila* X chromosome that contains the *per* gene. Now it was possible to find out what the *per* gene looks like, what sort of protein product it codes for and, if the investigators were lucky, how it works. In all their research over the past 4 years, the two groups have been neck-to-neck. "In some sense, there is a list of very similar things that anybody does with a cloned gene," says Hall. Both groups have done them.

One of the most intriguing things the investigators found was that the more *per* gene product a fly makes, the faster its clocks run. "It's as though the level of RNA determines how fast the clock runs," says Young. But the researchers were left with much more difficult questions: How can an oscillating system like a biological clock be controlled by a product produced in constant amounts? And why does an excess of the *per* gene product speed up the clock and a deficit of the product slow it down or obliterate it? "It makes you scratch your head and wonder if it's even possible," says Young.

The Brandeis group, working with Konopka and with Charalambos Kyriacou of the University of Leichester, has focused in part on understanding how the *per* gene product could affect both the daily 24-hour rhythms of *Drosophila* and the 60-second courtship song of the male flies. They decided to see if there is only one central clock in the organisms or if the daily rhythms and the courtship songs are controlled separately but by the same gene product. Their approach was to make mosaic flies—they would produce, for example, a fly with a mutant *per* gene in its head that might speed up the clock and the normal *per* gene in the rest of its body. Or they would do the converse. They found that the daily rhythms are controlled by a clock in the fly's head and the song rhythms are controlled by a clock in the fly's thorax. Now, Hall asks, "How is the *per* gene product involved in these *very* different kinds of clocks?"

At the same time that the two groups were grappling with the mechanism of

the *per* gene product, Young and his colleagues decided to see whether the *per* gene is present in other species. They identified similar sequences in DNA from humans, mice, and chickens and discovered that it is an unusual repetitive DNA sequence, coding for alternating serines and glycines or threonines and glycines. However, Konopka notes, these similarities are quite limited. It is not as though most of the *per* gene sequence is found intact in vertebrates. For that reason, he remarks, "it's still a possibility that the homologous sequences don't have anything to do with rhythms. We still don't know what part of the *per* locus is responsible for the rhythms."

But the finding of the repetitive sequences "was surprising," says Young, who had suspected, along with others, that the *per* gene might control an ion channel in cell membranes. There were models of how biological clocks might be made to oscillate that involved controlling the opening and closing of ion channels. But these repeating amino acid sequences in the *per* gene product did not look like part of an ion channel protein. On the other hand, says Hall, perhaps ion channels are not what they are assumed to be either. "Two ion channels have been sequenced out of 20 or more—and I emphasize *or more*. God knows what this ultimately means."

But the *per* gene product looked so different from the ion channels that are known that Young and, independently, Rosbash, asked what that kind of amino acid sequence might code for. To their surprise, they found that similar sequences are in proteoglycans. These are mysterious proteins that are poorly understood. They appear outside the cell and have been found in extracellular matrices such as cartilage, for example. Other proteoglycans are believed to be cell adhesion molecules. Proteoglycans are loaded with carbohydrate, "which gets them on the outside of cells," says Young. "We're kind of fuzzy on what to do with something like that. This protein doesn't look like an integral membrane protein so the ion channel model looks less likely. Perhaps we're dealing with a molecule that governs interactions of groups of cells which in turn form a clock."

No one, in all the speculations, had ever suspected that the clock might be controlled by molecules lying outside or between cells. "I expect that whatever we find out now will be all new," says Young. "But we will have to examine the structure and distribution of a rare protein in vivo, so we certainly will have to do some real hard work."

—GINA KOLATA

Additional Reading

1. W. A. Zehring *et al.*, "P-element transformation with *period* locus DNA restores rhythmicity to mutant, arrhythmic *Drosophila melanogaster*," *Cell* **39**, 369 (1984).
2. H. S. Shin, T. A. Bargiello, B. T. Clark, F. R. Jackson, M. W. Young, "An unusual coding sequence from a *Drosophila* clock is conserved in vertebrates," *Nature (London)* **317**, 445 (1985).

Down Syndrome–Alzheimer's Linked

Down syndrome adults get Alzheimer-like changes in their brains and many become demented, leading researchers to ask about the connection

Recent studies of people with Down syndrome and patients with Alzheimer's disease are revealing previously unexpected similarities between the two that may be important to the understanding of each of them. Adults with Down syndrome get a form of dementia that looks very much like Alzheimer's disease, and their brains, on autopsy, have Alzheimer-like lesions. And Down syndrome adults tend to get Alzheimer-type changes in their brains when they are still young—by age 30, the typical plaques and tangles are there.

These studies are possible because, gradually, over the past decade, a population of older Down syndrome patients has accumulated. People with Down syndrome used to die in childhood, but now, as a result of post-World War II antibiotic treatments, the switch to home care rather than institutional care, and heart surgery to correct the congenital defects that afflict 40 to 50 percent of Down syndrome babies, as many as 80 percent of these individuals are living to age 50 or more.

The relationship between Down syndrome and Alzheimer's disease tantalized neurologists. Why should these adults get Alzheimer's disease, and at such young ages? Is there something coded by the genes of the extra chromosome 21 inherited by Down syndrome patients that causes Alzheimer's disease? If so, could that substance be isolated and the seemingly inexorable development of Alzheimer's disease blocked? Within the past few years, several research groups have begun to systematically attack this problem by conducting the sort of longitudinal and morphological studies that are tedious but likely to provide answers. "It's 90 percent perspiration and only 10 percent inspiration," remarks Melvyn Ball, who directs the dementia study of the University of Western Ontario. The results, so far, are surprising.

Krystyna Wisniewski

Down syndrome and Alzheimer's disease

A 55-year old woman who regressed from a 6- or 7-year old mental level to a state in which she cannot speak or comprehend language, is disoriented, and is incontinent.

At a meeting held in New York on 14 and 15 November sponsored by the National Down Syndrome Society, investigators reported that previous anecdotal statements that virtually all Down syndrome adults get Alzheimer's disease if they live long enough are not correct. It is true that all Down syndrome adults over age 30 get brain lesions that are typical of Alzheimer's disease. These are the neuritic plaques, the spherical areas in the gray matter of the brain in which nerve cell ends are degenerating, and neurofibrillary tangles, the strand-like clusters of filamentous protein in the body of nerve cells of the brain. But,