



The 27-Hour Mouse

The furry, whiskered creature looks perfectly ordinary, but he harbors a new mutation that makes him the hardest-working mouse in the laboratory.

The mutation, dubbed “after hours,” enables this mouse to extend his day to 27 hours. When other mice have stopped spinning the running wheels in their cages, the after-hours mouse is still furiously spinning away.

Using the techniques of genetics, molecular biology, and biochemistry, Michele Pagano, M.D., the May Ellen and Gerald Jay Ritter Professor of Oncology and Pathology, and Luca Busino, Ph.D., a postdoctoral researcher in Dr. Pagano’s laboratory, found out how the after-hours mutation produces its remarkable effects. They describe a new clock gene called FBXL3 in an article published earlier this year in the journal *Science*. In a second paper published in the same issue, a group led by Patrick M. Nolan at the Medical Research Council in Harwell, U.K., in collaboration with Drs. Pagano and Busino, identifies and characterizes the after-hours mouse

mutation and its effect on daily activity.

Scientists are investigating clock genes because they may play an important role in the development of cancer, infertility, abnormal bone metabolism, impaired memory, and other conditions. Dr. Pagano’s laboratory studies how cancer arises from glitches in the proteins that control the process of cell growth and proliferation.

All animals are governed by an internal biological clock that dictates the daily rhythms of life. Sleep-wake cycles are best known, but body temperature, mental alertness, tolerance to pain, and even the timing of cell proliferation, among other physiological functions, undergo cyclical variations over a 24-hour (circadian) period.

Over the last decade over nine clock genes that control circadian rhythms have been identified. They have names like Clock, Per, and BMal. These genes govern a complex web of cellular interactions involving feedback loops that turn on and off the production of proteins that affect circadian cycles. Clock and BMal, for example, begin a cycle by forming a complex that initiates the production of certain proteins. Over the next several hours, these proteins build up. When they reach a critical level, they switch off the Clock/BMal complex and bring the cycle to an end.

Dr. Pagano became interested in clock genes through his work on the cell cycle, a choreographed sequence of molecular events that leads a cell to create a copy of itself by dividing in two. He and the members of his laboratory study the proteins that control the so-called checkpoints in the cell cycle when a new phase of the cycle begins. When these proteins malfunction, the cell makes runaway copies of itself, giving rise to cancer.

The F-box (FBX) proteins are a huge family of some 46 proteins, but little is known about their biological function. In 1999 Dr.

Pagano’s laboratory identified FBXL3 and found that cells lacking the protein can’t undergo a crucial phase in the cell cycle.

After years of hard work, Dr. Pagano and his laboratory team found a way to probe the biological function of the F-box proteins. They created a system based on an analysis of the mass of purified proteins that identifies the molecules tagged by the F-box proteins. Some F-box proteins link up with other kinds of proteins, forming a complex that tags molecules functioning at checkpoints in the cell cycle. These tags lead to the degradation and disposal of the molecules.

Using this system, they found that FBXL3 tags two proteins, called Cry 1 and Cry 2, which are regulators of the circadian clock. Cry 1 and Cry 2 are part of a complex feedback loop that inhibits the main clock genes Clock and Bmal. Cells with less FBXL3 protein are unhinged

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from their circadian clock. Therefore mice with a mutation in the protein have a circadian clock that runs longer than usual. Hence the 27-hour mouse.

It is unlikely that there would ever be a 27-hour man, says Dr. Pagano. These clock proteins won’t make you more productive or turn a lazy person into a dynamo. But the proteins may eventually yield novel ways to treat cancer. “Nobody understands the cellular rhythms of cancer,” says Dr. Pagano. “Why is the cell cycle in mammals 24 hours? Is it a coincidence or is there a connection to the clock itself? The more we understand about this clock, the more we can think of strategies to prevent and treat cancer.” ●