# Biography of Joseph S. Takahashi

Circadian rhythms keep Joseph Takahashi ticking. The Northwestern University professor and Howard Hughes Medical Institute (HHMI) investigator has devoted his career to understanding biological clocks through many means, from molecular genetics to behavioral analysis. His goal is to decipher the molecular mechanisms that control circadian clocks and uncover how these genes, molecules, and the environment interact to control animal behavior. Takahashi's research has provided some of the most important and groundbreaking discoveries in the field of circadian rhythm research, including the isolation and cloning of the first mammalian circadian rhythm gene, appropriately named the *Clock* gene, in mice in 1997 (1, 2). Through his subsequent research he has continued to elucidate the network of genes and proteins that interact to drive the circadian clock system (3, 4) and the feedback loops that control their expression (5, 6).

In addition to contributing >150 publications to his field, Takahashi has served on the editorial boards for a number of journals, including *Neuron* and *Current Opinion in Neurobiology*. He has been recognized for his academic accomplishments with numerous awards, including the Honma Prize in Biological Rhythms Research in 1986; the Sixth C. U. Ariens Kappers Award from the Netherlands Society for the Advancement of Sciences, Medicine, and Surgery in 1995; and the W. Alden Spencer Award in Neuroscience from the Columbia University College of Physicians and Surgeons in 2001. Takahashi also has been awarded a number of grants, including the MERIT Award from the National Institute of Mental Health in 1987 and the Bristol-Myers Squibb Unrestricted Grant for Neuroscience Research (1995–1999). Takahashi's dedication extends beyond the laboratory and into the classroom, earning him a Faculty Honor Roll award for teaching from the Associated Student Government at Northwestern in 1987. In 2000, Takahashi presented, along with fellow circadian researcher Michael Rosbash, an HHMI lecture series on biological clocks to an international audience of high school students (www.hhmi.org/biointeractive/clocks/index.html). Elected to the National Academy of Sciences in 2003, Takahashi continues to advance the field of biological clocks with his Inaugural Article, "PERIOD2::LUCIFERASE real-time reporting of circadian dynamics reveals persistent circadian oscillations in mouse peripheral tissues," featured in this issue of PNAS (7).

Joseph S. Takahashi

## In the Beginning

Becoming a distinguished research scientist draws on a number of skills not necessarily cultivated through formal education, and Takahashi's youthful interests and experiences provided many of these skills. His father, an economist, took the family along on his many overseas assignments. Takahashi's fascination with the natural world was sparked during the family's residence in the exotic locations to which his father was assigned. From "millipedes the size of cigars" in Burma to the unusual fish he and his father reeled in from the Arabian Sea off the coast of Pakistan, these early experiences were etched in Takahashi's memory and formed the basis of his interest in biology. However, the mechanical world intrigued Takahashi as much as nature. "I was always interested in cars and how things work," he said. He took high school classes in auto mechanics and mechanical drawing at Richard Montgomery High School in Rockville, MD. "In my lab, we build and develop equipment for automated data collection of circadian rhythms of activity, reporter gene luminescence, as well as a wide variety of data acquisition systems for behavioral screens in mice," he said, describing why this technical training is important to his current research. Takahashi credits his management skills to his experience running a kitchen at a local country club during the summers in high school. There he learned the priceless art of multitasking and project management, skills that he draws on daily to run his productive research laboratory.

Takahashi's interest in nature led him to major in biology at Swarthmore College in Swarthmore, PA. Nearly all biology majors, including Takahashi, were pre-med because "that was the default pathway" for a biology major. However, two Swarthmore professors, Norman Meinkoth and Kenneth Rawson, were instrumental in Takahashi's career path, exposing him to the possibility of a career in research. It was Rawson's laboratory course on the physiological basis of animal behavior and his research on circadian rhythms that sold Takahashi on research. Rawson also introduced Takahashi to Patricia DeCoursey at the University of South Carolina, Columbia, with whom Takahashi worked for a year after graduating from Swarthmore in 1974. DeCoursey, the first scientist to demonstrate that light pulses could reset the mammalian circadian clock, was a source of much career advice. Having been accepted to several graduate programs at prestigious universities, Takahashi took DeCoursey's counsel about graduate school to heart. "I chose a person rather than an institution," says Takahashi. That choice was prominent circadian rhythm researcher Michael Menaker, then at the University of Texas, Austin, and later at the University of Oregon, Eugene.

Takahashi thrived in the research environment provided by Menaker. "It was a large lab with lots of facilities," Takahashi said, that included satellite laboratories in an abandoned factory. Here Takahashi could apply his technical expertise, building testing apparatuses for use in his research. In 1980, Takahashi and Menaker published a paper in PNAS (8) describing the development of a bird pineal gland culture system to study circadian oscillations *in vitro*. Later, the researchers demonstrated that the suprachiasmatic nucleus (SCN) of the hypothalamus, which had been identified as the control center for circadian rhythms in mammals, played the same role in birds (9). Takahashi also pro-

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This is a Biography of a recently elected member of the National Academy of Sciences to accompany the member's Inaugural Article on page 5339.

duced one of his most highly cited papers during graduate school, in collaboration with DeCoursey, showing that the photoreceptor system responsible for entrainment of circadian rhythms is different from that of the visual system (10).

After graduating with his Ph.D. in neuroscience in 1981, Takahashi continued his work on the pineal gland as a postdoctoral research fellow in the laboratory of Martin Zatz at the National Institute of Mental Health in Bethesda, MD. There Takahashi investigated the role of cyclic nucleotides (i.e., cAMP and cGMP) in circadian rhythm regulation (11, 12). After 2 years as a postdoctoral fellow, Takahashi joined the faculty at Northwestern University in Evanston, IL, as an assistant professor in the department of neurobiology and physiology. There his *in vitro* research on circadian oscillations in the pineal gland and retina continued for several years. In an important collaboration, he and fellow Northwestern professor Margarita Dubocovich provided the first clear documentation of melatonin receptors in chick retina (13). In those early years at Northwestern, he "scaled up and automated" the dissociated cell culture system he and Menaker had developed earlier. "Eventually, we hit a brick wall," recounts Takahashi of his efforts to understand circadian rhythms through pharmacology and cell biology. "We couldn't use the system to find genes and proteins involved, so it became apparent that we had to move to genetics and molecular biology to continue the search."

### Fast "Forward"

The first circadian rhythm gene, called *period* (*per*), had been discovered in *Drosophila* in the early 1970s by Ron Konopka and Seymour Benzer (14), but its cloning had to await the advent of new molecular technologies in the 1980s. Ultimately, the *Drosophila per* gene was cloned in 1984 by two research laboratories (15, 16), paving the way for the blitz of clock research that followed, including Takahashi's.

In his quest to understand the molecular and genetic bases of circadian clocks, Takahashi adopted an approach called "forward genetics." The technique involved creating mutant mouse strains by exposing them to a mutation-inducing chemical (*N*-ethyl-*N*-nitrosourea, or ENU), isolating those strains with altered circadian behavior, and then identifying genes associated with the behavioral defect and their function. The advantage of forward genetics was that it required no advance knowledge of the underlying mechanism.

In collaboration with Northwestern professors Larry Pinto and Fred Turek, and William Dove of the University of Wisconsin at Madison, Takahashi used forward genetics to identify and clone the first mammalian circadian clock gene (2). "It was the most important collaboration of my career," Takahashi says of the project. Dove's laboratory was performing mutant mouse screens for an unrelated project but would ship those mutant mice that failed his screens to Takahashi at Northwestern. Takahashi's laboratory screened hundreds of mice provided by Dove in a behavioral screen of circadian locomotor activity. Mouse no. 25, which had a 25-hour clock, proved to be the goldmine for which Takahashi was searching.

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The heterozygous Clock mutation increased the circadian period by 1 hour, whereas homozygous mutants had periods that were ≈4 hours longer than those in normal mice (a 28-hour clock). Under constant dark conditions, the homozygous mutant lost circadian rhythmicity completely (17). After identifying the mutant, Takahashi, an admittedly "fledgling mouse geneticist," now had to find the gene, despite a skeptical research environment. "You'll never clone the gene and if even you do, it could be a housekeeping gene," seemed to be the predominant opinion Takahashi was working against. However, with the help of a supportive community of mouse geneticists, including Jeff Friedman at The Rockefeller University in New York and Eric Lander at the Massachusetts Institute of Technology in Cambridge, Takahashi proved the skeptics wrong.

At the time of Takahashi's discovery, only three clock genes had been cloned: the *per* and *tim* genes in *Drosophila* and the frequency (*frq*) gene in the fungus *Neurospora*. However, no mammalian circadian genes had yet been identified. Takahashi's research team pinpointed the *Clock* locus to a region of mouse chromosome 5 (18) and used positional cloning to identify the gene in mice in 1997 (1, 2). Their characterization of this gene showed that *Clock* was a novel member of the basic helix–loop–helix (bHLH)-PAS family of transcription factors, similar in sequence to one other known gene: NPAS2 or MOP4. The similarities between *Clock* and bHLH-PAS genes in other organisms suggested that the *Clock* gene might be an evolutionarily conserved gene, which Takahashi's laboratory confirmed by finding similar DNA sequences in a number of vertebrate species from fish to human. In addition, the researchers were able to rescue the *Clock* mutation in mice by transgenic expression of a 140-kb bacterial artificial chromosome (BAC) clone containing an intact *Clock* gene, providing "functional proof that (*Clock*) was the gene"(1). The following year, Takahashi and Steve Kay from The Scripps Research Institute in La Jolla cloned the *Drosophila* ortholog of *Clock* (5).

### Partners in Time

The initial cloning of fly and mouse *Clock* proved to be the tip of the iceberg. In 1998, Takahashi and colleague Charles Weitz of Harvard University in Boston found CLOCK's partner in mice: a protein called BMAL1 that colocalized with *Clock* and *Per1* in brain and retinal tissue and formed a heterodimer with CLOCK. This CLOCK–BMAL1 heterodimer was able to drive transcription of the *per1* gene (3). "It was one of those things we fantasized might be true," said Takahashi. The researchers also demonstrated the same interactions among the *Drosophila Clock* orthologs (5) and, importantly, showed that the *timeless* and *per* genes could inhibit the CLOCK–BMAL1 complex in both flies and mice, thus identifying the site of negative feedback (5, 6). By using these findings, the scientists began assembling the known factors into an autoregulatory feedback pathway. "Everything started to fall into place," Takahashi said. Today, about nine mammalian circadian genes have been identified, and their roles within the circadian mechanism have been assigned. However, it remains clear that more factors in this pathway are yet be discovered (19).

### Revisiting the Past

In 2000, Takahashi revisited a problem that had haunted him for years: the cloning of the *tau* mutation in hamsters. After Martin Ralph and Menaker's identification of the mutation in 1988, much information about its role in circadian rhythms had been gained. However, cloning of the gene faced an enormous roadblock, the lack of genetic and genomic resources in hamsters. The problem required a two-pronged approach. First, Phil Lowrey, a student in Takahashi's laboratory, identified fragments of DNA that differed between

homozygous mutant and homozygous wild-type hamsters by using genetically directed representational difference analysis (GDRDA). With those pieces of genomic material identified, Takahashi was able to find regions of synteny in the mouse and human genome by using a method called positional syntenic cloning. His genomic detective work determined that the *tau* mutation was a product of a mutation in the *casein kinase 1 epsilon* (*CKIe*) gene (4). *CKIe* turned out to be an ortholog of the circadian gene *doubletime* (*dbt*), a gene that regulates PER levels in *Drosophila*. Indeed, Takahashi's *CKIe* interacted with mammalian PER, confirming its identity. Because of the unique difficulty of the problem, Takahashi considers the cloning of the *tau* gene to be one of his most significant contributions.

### Here and Now

In his Inaugural Article (7), Takahashi demonstrates that circadian oscillators in peripheral tissues, such as the liver, are as robust as those found in the master pacemaker in the SCN. He and his colleagues show that peripheral tissues are capable of sustaining their own circadian rhythms, without any input from the SCN. By fusing one of the mammalian *per* genes, *mPer2*, with the gene for luciferase, Takahashi was able to monitor the "real-time" activity of the gene in various tissues by assaying the luminescence of the tissue. He found that cells from both the SCN and peripheral organs showed long-lasting, self-sustained circadian rhythms for at least 20 days in culture. Interestingly, when he lesioned the SCN, thereby removing any central circadian control, the rhythmic luminescence observed in the explanted peripheral tissues persisted but eventually became desynchronized. These results suggest that, counter to current dogma, the SCN's role is not to drive rhythms in peripheral tissues but rather to act as a synchronizer of the multitude of oscillators in our bodies.

### Into the Future

"Most of the core components of the clock have been discovered, and we can connect the genes in pathways, but we don't understand the kinetics," said Takahashi. His laboratory continues to test the molecular model in mice by producing conditional mutations, attempting to address remaining questions such as "Why is the cycle 24 hours?" and "What are the important delays and checkpoints in the cycle?"

Always ready for new challenges, Takahashi has recently begun branching out from the circadian rhythm research for which he is known. Because the forward genetics approach was so successful in finding circadian genes, Takahashi plans on using the approach to find genes involved in a number of other complex behaviors. With support from the National Institutes of Health, Takahashi has begun a large-scale mutagenesis screen to find genes important in learning and memory, stress, vision, and responses to drugs of abuse.

Although his study of circadian rhythms has obvious clinical implications for human sleep disorders, Takahashi notes that new research suggests that circadian control might be important in "a lot of things we don't realize yet." Approximately 10% of the genes in any given tissue are under circadian control, according to Takahashi (20). These genes appear to be involved in many of the basic metabolic pathways and the cell cycle, having implications for many physiological processes and conditions such as cancer. Surely, with such an important role attributed to circadian rhythms, Takahashi has more than enough material to keep him ticking for many years to come.

Melissa Marino, *Freelance Science Writer*


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