CLASSIC PERSPECTIVE

The 50th anniversary of the Konopka and Benzer 1971 paper in PNAS: "Clock Mutants of *Drosophila melanogaster*"

Joseph S. Takahashi[a,b,1]

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On September 1, 1971, unknowingly to most, the world changed for the fields of behavioral genetics and circadian clocks. Ronald Konopka, a graduate student with Seymour Benzer at Caltech, published a paper (1) that I would argue is the most important discovery ultimately leading to our current molecular understanding of the circadian clock in animals. In this classic paper, Ron and Seymour reported the isolation of three single-gene mutants in *Drosophila* that dramatically altered circadian rhythms in pupal eclosion and locomotor activity. One mutant exhibited no rhythmicity, another had a short 19-h period, and a third had a long 28-h period. Remarkably, all three mutants mapped to the same locus on the X chromosome. They named this gene *period*.

Seymour Benzer had recently joined the faculty at Caltech in 1967, after two previously successful careers in physics and molecular biology, and spurred on by Max Delbruck to do something more interesting, launched his third career in behavioral biology (2–5). Having done a sabbatical at Caltech with Roger Sperry, Seymour interacted with Ed Lewis, a giant in *Drosophila* genetics who trained with Alfred Sturtevant (descendent of Thomas Hunt Morgan) (6). Seymour chose *Drosophila* as a model system because its nervous system was intermediate in complexity "between a single neuron and the human brain" yet exhibited complex behavior and was amenable to genetic analysis (7). That same year, Seymour published his first paper (8) using mutagenesis and countercurrent technology to isolate phototaxis mutants in *Drosophila*. The opening sentence of this paper reads, "Complex as it is, much of the vast network of cellular functions has been successfully dissected, on a microscopic scale, by the use of mutants in which one element is altered at a time. A similar approach may be fruitful in tackling the complex structures and events underlying behavior, using behavioral mutations to indicate modifications of the nervous system."

Shortly afterward, Ron Konopka joined Seymour's laboratory as his first graduate student at Caltech (9, 10) (Fig. 1). Ron was previously interested in circadian rhythms, and with the tutelage of Ed Lewis, decided to do a screen for ethyl methanesulfonate–induced mutants on the X chromosome. Using the timing of eclosion of flies as a screen, Ron found an arrhythmic mutant in the first 200 lines that he screened. This led to what is known as Konopka's First Law: "If you don't find it among the first 200, quit" (9, 11). He went on to screen 1,900 lines and found two more mutants: the short period and long period mutants. Ron then went on to map the mutants using recombination and found them to be left of the *white* locus on X. To test for whether the three mutants were alleles of the same gene, he performed complementation tests for all combinations of the three mutants with each other and with wild type. None of the complementation tests produced a normal rhythm, suggesting the three mutants were alleles of the same gene. The arrhythmic and long mutants were recessive to wild type, and the short period mutant was semidominant. Crossing either the short or the long mutant over the arrhythmic mutant showed that

[a]Howard Hughes Medical Institute, University of Texas Southwestern Medical Center, Dallas, TX 75390; and [b]Department of Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75390

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[1]Email: joseph.takahashi@utsouthwestern.edu.

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Fig. 1. Photograph of Ron Konopka (*Left*) and Seymour Benzer (*Right*) taken in October 2000 in Pasadena, California. Photo courtesy of Howard Hughes Medical Institute, Holiday Lectures on Science, December 2000, "Clockwork Genes: Discoveries in Biological Time." Reprinted from ref. 14 by permission of SAGE Publications, Inc. copyright © 2004.

the arrhythmic mutant behaved as a null. Complementation tests with deficiencies of the X chromosome localized the gene within bands 3A6 to 3C2. Thus, Konopka and Benzer were able to infer that the arrhythmic mutant was a null mutant and that the short and long mutants were likely missense mutations. This prediction was confirmed many years later when the mutant alleles were sequenced (12, 13).

At the time that Konopka and Benzer published their paper, there was great skepticism that something as complex as behavior could be strongly influenced by single genes (4, 11). When Seymour reported the results to Max Delbruck, he told him it was impossible. To which Seymour responded, "But, Max, we found the gene, we've already done it!" Still Delbruck insisted that it was impossible and told Seymour, "I don't believe a word of it" (4, 11).

The Konopka and Benzer paper (1) completely refuted the notion that single genes could not influence behavior. Not only did the three *period* mutants affect the timing of eclosion, a developmental event, but the mutants affected the circadian locomotor rhythms of individual adult flies in the same way. Importantly, the phenotypes of the three alleles were so different: One could speed up the clock, one could slow it down, and one led to the abolition of circadian rhythms. These features emphasize the importance of choosing the best phenotypes to screen: For circadian rhythms, the period or rate of the underlying oscillator is most informative because there are few ways to change the rate of the oscillator in a nonspecific or irrelevant manner (14, 15).

This robust example of a single gene affecting circadian behavior reinforced Benzer's grand scheme that complex behavior could be dissected by Mendelian genetics and led to a groundswell of additional mutants in flies (7, 11). The *Shaker* mutant defined the first potassium channel at the molecular level (16, 17). The *dunce* mutant provided an entrée into the genetics of learning and memory (18, 19). The *sevenless* mutant defined a developmental pathway for photoreceptor determination (20–22). The *bubblegum* and *methuselah* mutants uncovered genes affecting neurodegeneration and life span (23, 24).

Despite the remarkable phenotypes of the *period* mutants, progress on the molecular nature of the *period* gene was slow. This required the development of recombinant DNA technology in the late 1970s and early 1980s in order to clone the gene. Thus, it would be another 13 y before the *period* gene would be identified at the molecular level independently by the laboratories of Jeff Hall and Michael Rosbash at Brandeis (25, 26) and by Mike Young at Rockefeller (27, 28). The cloning of *per* was not very informative at the time. There were few obvious protein motifs to infer function and a number of red herrings and dead ends were reported for the PER protein including its being a proteoglycan or a gap junction regulator (29–31). These side trips were eventually self-corrected by the Brandeis and Rockefeller laboratories (32, 33), and the focus reoriented toward gene expression and nuclear localization. It was shown that PER was actually a nuclear protein and initial antibody staining suggested that the PER protein expressed a circadian rhythm in the CNS and visual system of flies (34). Importantly, Hardin et al. (35) then showed that the *per* mRNA cycled in the brain of flies and that the *per* gene product could feedback on its own gene expression. This discovery led to the hypothesis of an

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autoregulatory loop involving PER negative feedback as the core of the circadian clock mechanism in *Drosophila*.

The magnitude of the *period* mutant discovery can measured in another way: The second core circadian gene to be discovered, *timeless*, did not occur until 1994, 23 y after *per* (36). Soon, it became clear that PER and TIM both cycled and formed a heterodimeric complex (37–40). The loop was finally closed with the discovery of the upstream regulators of *per* and *tim* by the transcription factors, CLOCK and CYCLE in 1998 (41–43).

From a personal perspective, the Konopka and Benzer (1971) paper was life changing for me. It was the exemplar for how to find circadian clock genes, and during midcareer, I switched from neuroscience to genetics because it became clear that in order to find clock genes in mammals, forward genetics was the only feasible path for discovering something that we knew nothing about (44). In 1994, we reported the isolation of the *Clock* mutant mouse, which was found in an *N*-ethyl-*N*-nitrosourea mutagenesis phenotypic screen for circadian locomotor rhythms (45). This mutant had an extreme circadian phenotype: a 28-h period and an eventual loss of sustained circadian rhythms in constant conditions. The mutant was semidominant and mapped to chromosome 5. Three years later, my laboratory was able to identify the gene using positional cloning and transgenic rescue approaches after 30 person years of effort (46, 47). The CLOCK protein was a bHLH–PAS transcription factor, and it acted in concert with another bHLH–PAS protein, BMAL1. Incredibly at the time, we found that CLOCK–BMAL1 could transactivate the *per* gene from *Drosophila* as well as the mouse *Per1* gene (48). Thus, CLOCK–BMAL1 linked directly to the original *period* gene and formed the basis for the previously missing activators of *per*. Identification of the *Drosophila* orthologs of *Clock* and *Bmal1*, *dClock*, and *cycle* (*dBmal1*), completed the description of the conserved core mechanism of the circadian clock in *Drosophila* and mammals (49). In the following two decades, remarkable progress was made in understanding the mechanism of circadian clocks in animals (50) and their relevance to health and medicine (51). Circadian clock proteins have direct molecular interactions with metabolism (52), immune function (53), cancer (54, 55), and neurodegeneration (56).

In 2017, Jeff Hall, Michael Rosbash, and Michael Young were awarded the Nobel Prize in Physiology or Medicine "for their discoveries of molecular mechanisms controlling the circadian rhythm" (33, 57). This would not have been possible without the truly groundbreaking paper by Konopka and Benzer (1) and the discovery of *period*. Sadly, neither Seymour nor Ron would witness the fruits of this paper leading to the Nobel Prize since they passed away in 2007 (2–4) and 2015 (9, 10), respectively. We owe a great debt to these two pioneering scientists who led the way in opening up the many black boxes underlying complex behavior.

See Movies S1 and S2 for interviews of Ronald Konopka and Seymour Benzer.

Data Availability. There are no data underlying this work.

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