As Time Flew By

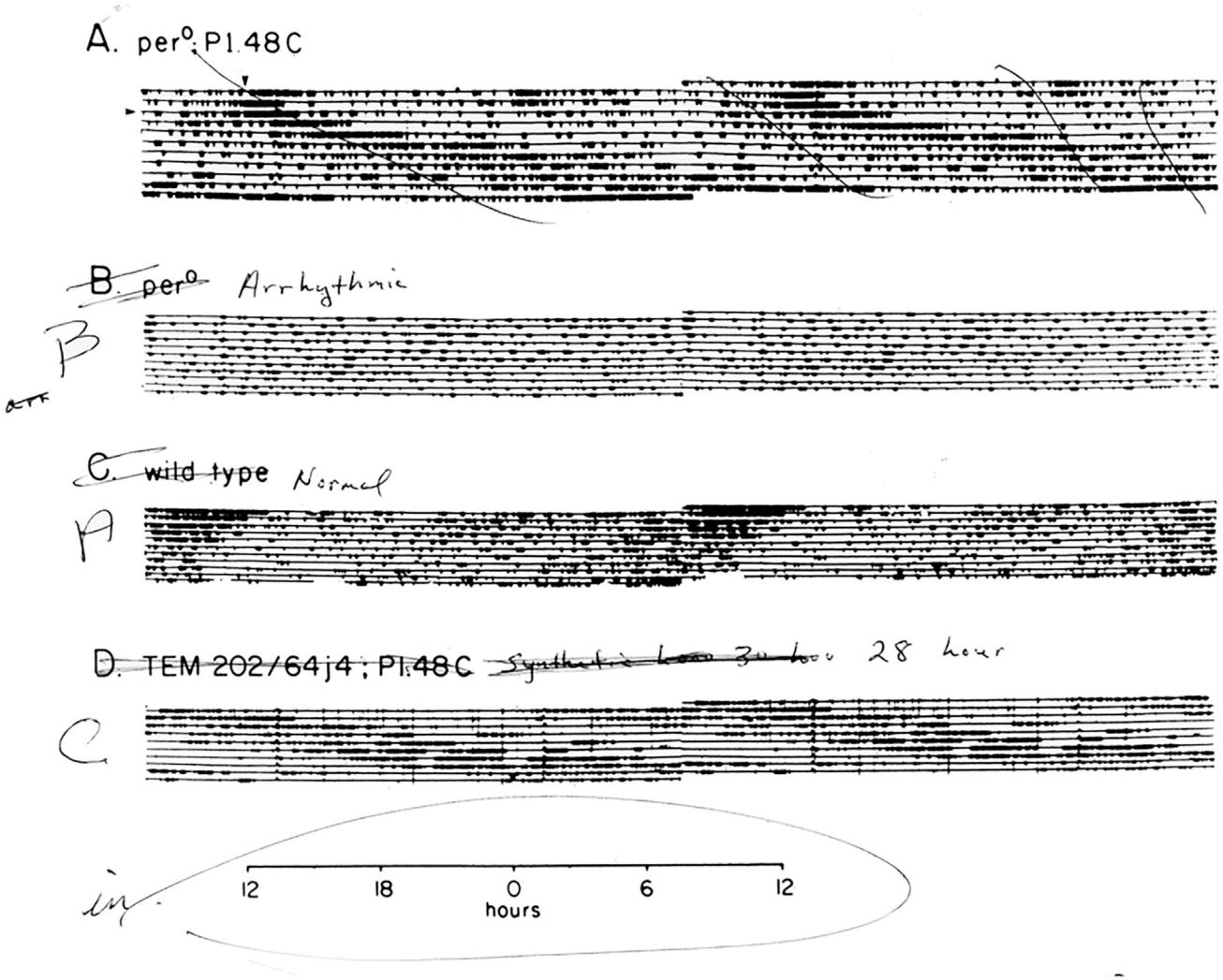
Michael W. Young

My earliest work on circadian rhythms came by chance, when I was a graduate student with Burke Judd at the University of Texas in the early '70s. Burke had offered me a place in his lab for summer undergraduate research, and when that went well, I asked to stay. The lab had been trying to use saturation mutagenesis to identify every gene in a region of the X chromosome that represented ~0.5 percent of the *Drosophila* genome. The average gene density was turning out to be only about 1/25,000 base pairs. We all wanted to know what that implied about eukaryote gene structure.

"In 1978, you could pick any problem in the world and work on it successfully with the new molecular tools."

A problem we faced was that almost all of the genes being counted were vital to the life of the fly or caused a striking morphological change that could not be overlooked. I wanted to know if we were missing significant numbers of genes because they affected more subtle phenotypes. With this in mind, I suggested an approach that would identify regions surrounding the previously identified genes if they were doing something else. It was a trick involving *de novo* production of chromosome translocations that could be recognized, genetically, simply due to breaks in the region under study whether or not they produced any other mutant phenotype. As I was applying this and other methods to look for "non-essential" DNA, Ron Konopka and Seymour

Mike Young at a fishing break, Chronobiology Gordon Conference, 1985.



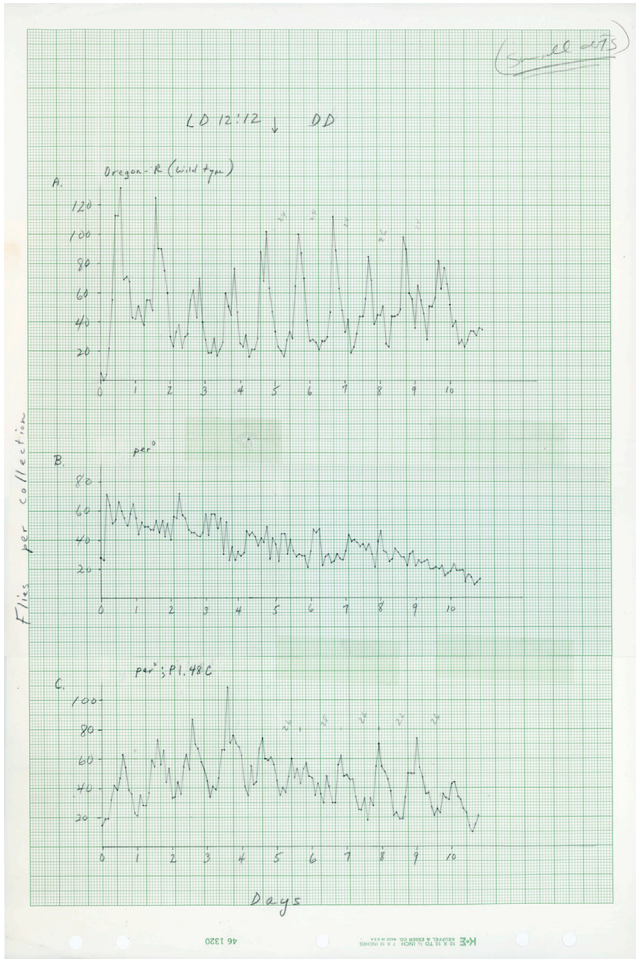
1984 photograph (with hand-written notes) of the assembled locomotor activity records of flies of different *period* genotypes. P1.48C is a wild-type *period* transgene. TEM202/64j4 deletes the *period* gene on both X chromosomes, and *per*-0 is the original arrhythmic point mutation. Rhythms are restored to both strains by adding the transgene. All records obtained from flies in constant darkness.

Benzer published their fascinating paper in *PNAS* describing circadian "clock mutants" that failed to complement each other. They proposed a name for the affected gene: *period* (*per*).

"Ted and I did not get a lot of sleep after loading the first set of flies."

What really caught our attention was that *per* seemed to map close to, and possibly within, the small X chromosome region we had been studying. We asked Ron and Seymour to send the mutants so I could better locate the gene. Michael Menaker, who studied circadian rhythms on another floor, generously provided advice and equipment to monitor behavior. The mutants came right away and all failed to complement a series of overlapping deletions that altered the middle of the region under study. However, none of the *per* mutations were alleles of genes already mapped to the region. My luckiest discovery was that a translocation (T1:4JC43) that I had previously characterized as having no phenotype was mutant for *per*! This work was completed by 1975 and published as part of a longer report three years later in *Genetics*.

In the fall of 1975, I left for Stanford. I had only contacted one lab about postdoctoral work (there really was no substitute). After chasing down Dave Hogness at a Genetics Society meeting earlier



Original 1984 eclosion (adult hatching) records showing circadian rhythmicity in constant darkness when *per*-0 flies received the wild type *period* transgene.

in the year (he would never answer my letters), we talked briefly, and I somehow convinced him to train this fly geneticist to do molecular biology. Recombinant DNA was brand new, Dave was focusing on *Drosophila*, and when I arrived, everyone in his lab knew that what could be learned about fly biology was about to change forever.

"After more than 7,000 bottles of flies, they found something new and important."

To get a great job coming out of Stanford Biochemistry in those days just required a slide showing a piece of eukaryotic DNA stitched into a plasmid using poly(dA):poly(dT) joints. One of my lab mates, a graduate student Kevin Struhl, who had already famously shown that a yeast gene could restore function in a bacterial mutant, recommended that I stress my plans to map cloned fly DNA in relation to genetically defined chromosome rearrangements through hybridizations to *Drosophila*'s giant salivary chromosomes. I did, and after two years at Stanford, I was heading to The Rockefeller University to start a lab.

In 1978, you could pick any problem in the world and work on it successfully with the new molecular tools. Remembering Ron and Seymour's mutants, I picked up *per* again. The role of a gene in establishing behavior was the most mysterious and riskiest problem I could tackle. I knew a long "chromosomal walk" from a previously cloned DNA segment could eventually get to *per*, but back then, how would you know you had finally gotten to the gene? Ron and Seymour's *per* mutants were EMS induced and likely point mutants, so you had no way to recognize them in a stretch of tens of thousands of base pairs of DNA. I was not worried; I had a *per* mutant that could pinpoint the gene- T(1:4JC43).

In the early 1980s, we crawled along the X chromosome and finally came to a point where there was an abrupt transition in the restriction map when comparing DNA cloned from wild-type flies versus T(1:4JC43). Working with a postdoc in the lab, Ted Bargiello, we began to locate transcribed regions. In wild-type flies, we found a region that produced a 4.5 kb mRNA. T(1:4JC43) flies did not make this RNA; instead, they produced a much longer RNA, ~12 kb, that had sequences from both the X chromosome region and the 4th chromosome—in other words, the new RNA crossed the T(1:4JC43) breakpoint. These results were published in the spring of 1984 in *PNAS*.

P-element-mediated transformation had recently been developed by Allan Spradling and Gerry Rubin, so Ted Bargiello prepared DNA from wild-type flies that encompassed the putative *per* transcription unit, and I micro-injected it into hundreds of *Drosophila* embryos. We developed two kinds of *Drosophila* stocks. One carried our transgene in Konopka and Benzer's *per*-0 (arrhythmic) mutants. The second stock contained a synthetic deletion, removing 10 kb corresponding to the putative *per* gene and a good deal of neighboring DNA. We placed individual flies in a device created from scratch for us by the Rockefeller University Machine Shop. The device was placed in a dark incubator and monitored interruptions in a beam of infrared light caused by a walking fly. It was wired to an Esterline Angus Event Recorder, and deflections of a pen on slowly moving chart paper registered movements of the fly. Our device could measure the activities of five flies separately but simultaneously.

Ted and I did not get a lot of sleep after loading the first set of flies. Every few hours, we would walk over to take a look at the chart paper spilling out of the event recorder. After four days and 10–12 feet of paper, it was clear that we had reestablished circadian behavior in both fly stocks, a conclusion cemented by performing eclosion assays on many hundreds of flies. We published these new findings in the 1984 year-end issue of *Nature*.

While all of this was going on at Rockefeller, Jeff Hall and Michael Rosbash were also trying (successfully) to find *per* in cloned DNA at Brandeis. Ted and I did not know Jeff and Michael were also after the gene until we were well into our work, and vice versa. With two labs hammering away at the same gene, things became pretty competitive, and their findings were published in *Cell* the same year as our *PNAS* and *Nature* papers. The pressure of competition evaporated, however, with the discovery of a new clock mutant.

Ron Konopka found his first clock mutation after screening very few flies. Well before anyone at Rockefeller or Brandeis set out to find *per* physically, Ron jokingly made a statement, which by the 1980s was unfortunately bandied about as Konopka's Law, that, "if you don't find it in the first 200, quit." Prior to cloning *per*, several half-hearted attempts to find additional clock mutants produced nothing more of interest, reinforcing the notion.

In the late 1980s, two very brave postdocs in my lab, Amita Sehgal and Jeff Price, began genetic screening in earnest. The 200th bottle came and went without a hint of success, and by the thousandth bottle, Amita and Jeff were wondering whether to pursue some of the developmental curiousities emerging from the screen; but much later, after more than 7,000 bottles of flies, they found something new and important. In one bottle, there were flies that were arrhythmic, like *per*-0; and when Leslie Vosshall, a graduate student in the lab, looked for the PER protein in these new flies, it was gone. I described all of this to my colleague Jeff Friedman at the time. Jeff's reply: "You have to call it *timeless*."

We cloned *timeless* (*tim*) in a short chromosomal walk and identified a small deletion, unique to the *tim* mutant, that affected a single encoded protein, and published it in *Science* in 1995. In a collaboration with Amita Sehgal (now at the University of Pennsylvania), we learned that *tim* RNA cycles in phase with the RNA cycles originally found for *per* by Jeff Hall and Michael Rosbash. We had also established a collaboration with Chuck Weitz (at Harvard) and Amita aimed at identifying proteins that would physically associate with PER. She generated a fly cDNA library and Chuck used that library and *per* to form a yeast 2-hybrid screen. Dozens of yeast colonies were identified as potentially encoding a PER partner. Chuck hybridized the *tim* DNA that we had recovered from our chromosomal walk to these. Most were positive. PER and TIM were partners. These findings were also published in *Science* in 1995.

The identification of *tim* proved that further genetic screening was worthwhile. More importantly, because PER and TIM, the first two genes to be identified in the fly, worked together, we saw that additional genes could be studied in terms of their relationships to each other and to *per* and *tim* and that with further genetic screening, a complete clock mechanism might emerge. In the early 1990s, with all of this in the air, Gene Block at the University of Virginia brought together the Rockefeller and Brandeis Drosophilists, as well as Joe Takahashi at Northwestern and Steve Kay at Virginia, to plan a multi-institutional Science and Technology Center. The goal, which won NSF support for the next 10 years, was to cooperatively apply saturation genetic screening to flies, mice, and *Arabidopsis*. During that period, clock mutations were identified in all three organisms, and collectively, Rockefeller and Brandeis identified roles in the clockworks for *double-time* (*dbt*/*CK1*), *shaggy* (*sgg*/*GSK-3*), *vrille* (*vri*), *PAR domain protein 1* (*Pdp1*), *dclock* (*Clk*), *cycle* (*cyc*), *casein kinase 2* (*ck2*), and *cryptochrome* (*cry*). As originally imagined, the roles of all came together like puzzle pieces to form something that, to my eyes, remains quite a beautiful machine.

ACKNOWLEDGMENTS

Most of my research support has come from the National Institutes of Health. In fact, NIH has supported me continuously since 1971. I was a pre-doctoral NIH trainee from 1971–1975 and had a postdoctoral fellowship from NIH at Stanford, and my first NIH R01 grant arrived shortly after moving to Rockefeller in 1978. This whole pathway of research opportunities was not uncommon in the 1970s. During my time at Rockefeller, I have had unwavering interest and encouragement from many of my colleagues, but especially Norton Zinder, Jim Darnell, Gunter Blobel, and Torsten Wiesel. I also had the good fortune to marry an immunologist in the 1970s, Laurel Eckhardt, now Hesselbach Professor at Hunter College, who has loved discussing the ''clock problem'' as it unfolds almost as much as I have. This work was supported in part by a grant from Calico Life Sciences.