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Reflections on contributing to "big discoveries" about the fly clock: Our fortunate paths as post-docs with 2017 Nobel laureates Jeff Hall, Michael Rosbash, and Mike Young

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ABSTRACT

In the early 1980s Jeff Hall and Michael Rosbash at Brandeis University and Mike Young at Rockefeller University set out to isolate the *period* (*per*) gene, which was recovered in a revolutionary genetic screen by Ron Konopka and Seymour Benzer for mutants that altered circadian behavioral rhythms. Over the next 15 years the Hall, Rosbash and Young labs made a series of groundbreaking discoveries that defined the molecular time-keeping mechanism and formed the basis for them being awarded the 2017 Nobel Prize in Physiology or Medicine. Here the authors recount their experiences as post-docs in the Hall, Rosbash and Young labs from the mid-1980s to the mid-1990s, and provide a perspective of how basic research conducted on a simple model system during that era profoundly influenced the direction of the clocks field and established novel approaches that are now standard operating procedure for studying complex behavior.

1. Introduction

On Oct. 2, 2017, the three of us awakened to the incredible news that the 2017 Nobel Prize in Physiology or Medicine was awarded to our postdoctoral mentors, Drs. Jeff Hall, Michael Rosbash and Mike Young, for "their discoveries of molecular mechanisms controlling the circadian rhythm" (Fig. 1). Our elation was amplified as each of us learned that the Nobel Assembly had included one of our papers on a list of key publications in the body of research that was being recognized. In this article, we reflect on our personal and scientific journeys as we were attracted to join these labs, and as we engaged with the challenges and joys of contributing to work that led to the Nobel Prize. We conclude with some perspectives on how this work in the Hall, Rosbash and Young labs laid the foundation for deciphering the molecular basis of clock function in all animals, pioneered approaches that are now common strategies for studying the neural mechanisms of complex behaviors and represents a powerful example of the value of basic research on model organisms.

2. Kathy Siwicki

As I was finishing my Ph.D. in Neurobiology in the mid-1980s, studying lobster neuropeptides with Ed Kravitz at Harvard, I was attracted to *Drosophila* by the enormous potential of using flies to decipher the mechanisms of complex processes in animal biology. Major technical breakthroughs around that time allowed us, for the first time, to manipulate the fly genome, so biologists suddenly had unprecedented experimental power for studying how genes specify animal development and regulate physiology and behavior. As I looked for postdoc opportunities in labs where I could learn to use *Drosophila* to study how brain circuits are wired to produce complex behaviors, I discovered Konopka's three remarkable *period* mutants (Konopka and Benzer, 1971). As is now well known, the per^{Short} (per^{S}) and per^{Long} (per^{L}) alleles changed the period of the fly's daily rhythms in opposite directions, and the per^{Zero} (per^{0}) mutant rendered flies completely arrhythmic. Even with no formal training in genetics, it was clear to me that the distinct phenotypes of these *per* mutant alleles were strong evidence that this gene was a key that could potentially unlock the mechanism of endogenous biological clocks. Since the gene had just been cloned and sequenced by groups at Brandeis and Rockefeller (Bargiello et al., 1984; Zehring et al., 1984), it seemed like a perfect time for a neurobiologist to begin investigating the cellular functions of the *per* gene product. I was particularly drawn to work with Jeff Hall because of his encyclopedic knowledge of neurogenetics, and the fact

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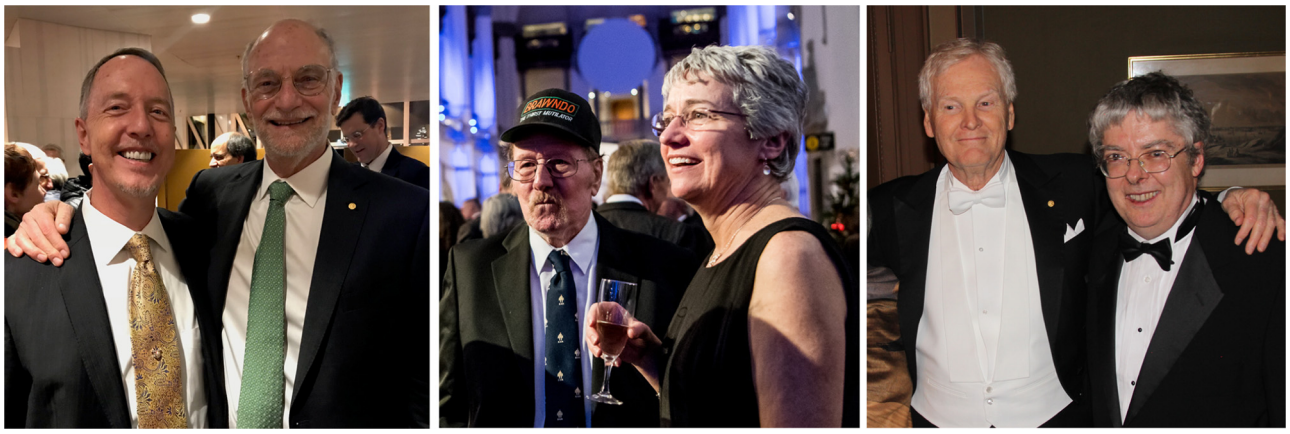

Fig. 1. The authors celebrating in Stockholm with their postdoctoral advisors, the 2017 Nobel laureates in Physiology or Medicine. *Left,* Paul Hardin and Michael Rosbash at the reception following the Nobel Lectures in Physiology or Medicine at the Karolinska Institute. *Center,* Jeff Hall and Kathy Siwicki at the reception honoring the 2017 Nobel Laureates at the Nordic Museum. *Right,* Mike Young and Jeff Price at the reception for Mike Young following the Nobel Prize ceremony at the Grand Hotel.

that his broad interests in fly brains and complex behavior were closely aligned with my own interests.

Thus, I joined Jeff Hall's lab in 1985 with the modest aim of figuring out how the PER protein works to endow flies with endogenous daily rhythms. At that point, the basic question of how a specific mutant genotype produces a behavioral phenotype had been pioneered by a few labs studying *Drosophila* learning mutants or ion channel genes, areas of neuroscience where some of the relevant biology had been established through electrophysiological or biochemical approaches. By comparison, the circadian clock was a virtual black box. The sequence of the *per* gene offered no clues about the protein's function – there were no similar sequence domains in the primitive databases of the time, and no other evidence to justify any specific hypothesis about its biochemical function. It was exciting to be joining a team that was forging into uncharted waters, supported by emerging molecular tools and our faith that the compelling phenotypes of Konopka's mutants would lead us to novel discoveries.

I set out to develop an anti-PER antibody that I would use to find the fly's "clock cells." The intense competition between the Brandeis and Rockefeller teams at that time contributed to our sense of urgency. There were pressures to react and respond to rumors about progress in the Young lab by adopting new approaches. Nevertheless, after nearly a year of frustrated efforts, Jeff encouraged me to persist. I was raising and screening rabbit antibodies against a few synthetic peptides that we had selected simply by trying to divine insights about functional domains of the protein while staring at the primary sequence. Several months in, Jeff received an airmail package from Bambos Kyriacou (currently a Professor at the University of Leicester) containing new predictions about possible bends and loops in the protein (Thackeray, 1989). Jeff noticed that one predicted elbow included the just-identified site of the per^S missense mutation (Yu et al., 1987), so he suggested that we add a 14-mer peptide version of this elbow to the pipeline. The first breakthrough occurred when an antibody to this "S-peptide" revealed nuclear staining of the photoreceptors in the compound eyes of wild-type flies and *no staining* in the control genotype (*per* deletion females). I also saw many small nuclei stained throughout the brain and optic lobes that appeared to be glial cells. My most memorable moment of discovery occurred shortly thereafter, upon seeing the famed "lateral neurons" for the first time and recognizing that these were very likely the cellular substrates of the fly's circadian clock.

While it may seem obvious in retrospect that one should look for daily oscillations in the levels of a protein that was hypothesized to have a central function in circadian timekeeping, it was not so obvious at the time. Based on initial reports that *per* transcript levels were constant throughout the day/night cycle (Reddy et al., 1984; Young et al., 1985), we did not expect to find rhythms in PER protein staining, and we failed to fully recognize their significance when the data initially revealed them (Siwicki et al., 1988). Indeed, I recall being frustrated at first by the fact that some flies showed very strong staining for PER protein, while others seemed to have very little! When we recognized that those with the strongest staining had all been done first thing in the morning, I set up temporally controlled experiments and found clear evidence for daily and circadian cycling of PER in photoreceptors (Siwicki et al., 1988). As I was confirming and documenting these protein rhythms, I was also pregnant for the first time, and I was determined to finish experiments for this paper before the baby arrived. Although there were no official maternity leave policies for postdocs in 1987, and in spite of the competitive pressure to publish new findings promptly, Jeff emphatically encouraged me to take a three-month leave with full pay.

Soon thereafter I began working with Danielle Zerr, a talented Brandeis undergraduate (currently a Professor of Pediatrics at the University of Washington), to quantify the temporal features of rhythmic PER protein staining and the effects of *per* mutants on the phase and amplitude of the protein rhythms (Zerr et al., 1990). During this exciting and productive phase of the work, Jeff would often scribble new ideas for experiments on yellow post-it notes that I would find on my desk in the morning (a highly effective mode of communication in the days before email). Our findings that PER protein cycling in per^S mutants was phase-advanced in LD and persisted with a short period in DD were especially important, as they indicated that the mutant PER protein defines the phase and period of its own molecular rhythms in parallel with its effects on behavioral rhythms (Zerr et al., 1990). As Danielle was writing up these results for her senior thesis in the spring of 1989, I had another baby and moved to Pennsylvania to join the faculty of Swarthmore College as a new Assistant Professor. Meanwhile, our evidence for PER protein rhythms in brain cells and photoreceptors led Paul Hardin to question earlier reports of "no cycling" of the *per* transcript, which had been based on RNA extracted from whole flies, and to re-assess the question specifically in head extracts. As he describes below, Paul found compelling evidence for robust daily and circadian cycling of head *per* mRNA (Hardin et al., 1990). Comparing the two sets of data, it was clear that the mRNA and the protein rhythms were nearly opposite in phase, leading to the breakthrough insight that PER protein negatively regulates the transcription of its own gene (Hardin et al., 1990).

In subsequent years, in parallel with advances in understanding the molecular gears of the clock, the details of neural circuits responsible for circadian rhythms emerged from neuroanatomical studies. Distinct clusters of *per*-expressing neurons were counted and described in increasing detail. John Ewer (currently a Professor at the University of Valparaiso) and Brigitte Frisch (who sadly passed away soon after this