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CLASSICS OF CHRONOBIOLOGY

Clock Mutants of Drosophila melanogaster

RONALD J. KONOPKA AND SEYMOUR BENZER

In 1971, Ronald J.Konopka and Seymour Benzer discover the first gene affecting the time of circadian phenotypes. By performing a mutagenesis screening, searching for genetics variants affecting flies' behaviour, the two geneticists isolated three novel *Drosophila* mutants, characterized by defects in eclosion (moulting) rhythms and locomotor activity. While population of wild type flies (*CS*) display normal eclosion rhythms (24h) and circadian rest-activity cycles (23.8h), these mutants were characterized by faster rhythms (short-period mutant, about 19.2), slower rhythms (long-period mutant, about 28.5h) or no rhythms at all (arrhythmic mutant). Genetic mapping and complementation tests, performed with the three mutant lines, identified a single gene on the X chromosome which function was drastically affected and resulted in changed properties of rhythmic behaviour. This was the first reported gene affecting the circadian oscillator. Thirteen years later, the gene, that could rescue arrhythmic flies, was identified (T. A. Bargiello & Young, 1984; Thaddeus A. Bargiello et al., 1984; Reddy et al., 1984; Zehring et al., 1984): it was *period*, the core component of the molecular circadian clock.

This seminal paper of Konopka & Benzer has become soon a landmark for all the chronobiology to come. Ten years later the second fly clock gene *timeless* has been identified (Sehgal et al., 1994; Vosshall et al., 1994). The forward genetic approach used to search genetic variants in flies had also been adopted in mammals, leading up to the discovery of the circadian *Clock* gene in mice (Vitaterna et al., 1994). Few years later the homolog *Drosophila clock* gene and its partner *cycle* were also been identified (Allada et al., 1998; Bae et al., 1998; Darlington et al., 1998; Rutila et al., 1998). The work done by Konopka & Benzer thus paved the way for the molecular studies on circadian rhythms. *period, timeless, clock* and *cycle* are indeed part of the same molecular feedback loop: mutations affecting only one of these single genes might result in severe circadian disorders as manifested in the three *period* mutants flies. It turns out that the work done by Konopka & Benzer, not only elected *Drosophila* as a model organism for study behavioural neurogenetics, but also fired

up the molecular chronobiology. This ended up with Nobel-worthy discoveries,on molecular mechanisms controlling the circadian rhythm, leaded by Jeffrey C. Hall, Michael Rosbash and Michael Young (Nobel Prize in Physiology or Medicine in 2017)(Young, 2018).

Clock mechanisms are highly conserved from flies to mammals; the knowledge obtained by studying flies' circadian behaviours might support and enlighten research on mammalian model organisms and *vice versa*. It is noteworthy that a missense mutation on *hper2* gene affects the sleep-wake rhythms in *Homo sapiens* (Toh et al., 2001),miming the period-short mutants discovered by Konopka & Benzer. Individuals carrying this point mutation suffer from "Familial Advance Sleep Phase Syndrome": an autosomal dominant circadian rhythms variant that cause early sleep evening onset as well as early morning awakenings. This results in misalignments between the time dictated by the endogenous clock and the time imposed by the environment. Thus,having a functional circadian timing confer adaptive benefits to an organism.

A lot of work has been done since the discovery of these mutants. For instance, the arrhythmic period flies has been used to target *period* in subsets of clock neurons in the fly brain leading to the discovery of a morning and a evening oscillators that govern the bimodal activity of flies (Grima et al., 2004; Stoleru et al., 2004). Period-short, period-long and arrhythmic mutants then, have been exploited to study disciplines ranging from fundamental genetics to behaviour, for the investigations of clock-controlled genes or processes. As an example, recently it has been demonstrated that also daytime colour preference in *Drosophila* is influenced by the circadian clock (*period*) (Lazopulo et al., 2019).
The Konopka & Benzer period mutants will always be in the limelight.

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