# Circadian clock in Malpighian tubules

Circadian clocks impose daily rhythms on bodily functions. They have been identified within the central nervous systems in a wide range of animals, from invertebrates to humans, suggesting that circadian time-keeping in animals is achieved by the central nervous system. We have now found that *Drosophila* Malpighian tubules contain a circadian clock that is autonomous from the brain. Temporal integration in *Drosophila* may be regulated by a collection of independent clocks rather than one central pacemaker.

Studies of *Drosophila* have led to the identification of two clock genes, *per* (period) and *tim* (timeless), which produce circadian fluctuations of their messenger RNAs and their encoded proteins (Per and Tim)[1–4]. This time-keeping mechanism involves a Per–Tim heterodimer, which mediates rhythmic transcription of both genes through a negative feedback loop[5]. The clock has been mapped to the brain, because rhythmic and nuclear expression of Per in specific brain regions is essential for the imposition of daily rhythms on behaviour[6]. However, *per* mRNA and protein also occur rhythmically in the abdomen[3,7].

We have investigated the expression of Per and Tim in *Drosophila* Malpighian tubules — non-innervated epithelial tubes comprising large secretory cells involved in urine excretion. By staining the secretory cells of wild-type adults with antibodies to either Per or Tim, we have revealed daily cycles in the expression of clock proteins (the staining was absent in the tubules of null mutants, $per^0$ and $tim^0$; data not shown).

To test the role of the brain in the rhythmic expression of clock proteins in the Malpighian tubules, we removed the heads from adult flies and compared the patterns of expression of the proteins Per and Tim in intact and decapitated flies. In the 12-hour light–12-hour dark cycle (LD 12:12) there was a clear rhythm in the level of clock proteins in both intact and decapitated flies (Fig. 1, upper panel). Per and Tim were abundantly present in the nuclei of all secretory cells towards the end of the dark period, but we did not detect them in the tubules towards the end of the light period. This rhythm closely resembles the rhythm reported for the brain, but the two are independent, because the rhythm in the tubules persisted in headless flies.

To test whether the cycling of clock proteins in the tubules represented a true circadian clock, we subjected intact and decapitated flies to either constant darkness or light–dark phase shifts. The cycling of Per and Tim persisted in complete darkness in both intact and decapitated flies (Fig. 1, middle panels). Furthermore, when decapitated flies were exposed to a 12-h shift in their photoperiod, the Per and Tim rhythms in their tubules were also reset by 12 hours, just as in the intact flies (Fig. 1, lower panels). Thus, tubules exhibit free-running cycling of clock proteins, the rhythm of which can be reset by light, in the absence of both brain and eyes.

We have previously identified a peripheral clock in moth testes[8]. Our results from *Drosophila* show that the peripheral clock uses the same molecules and mechanisms as the brain-located clock. It is not surprising that tissues simpler than the central nervous system have pacemaking ability, as bacteria and simple eukaryotes exhibit circadian rhythms. As complex animals evolved from simple organisms, simple tissues could have retained their rhythm-generating capacity rather than surrender to the central brain clock.

The question remains as to what the function of the Malpighian tubule clock might be. Adult flies have behavioural rhythms that may require periods of higher excretory activity (associated with increased metabolic rates). Fluid excretion in fly tubules is driven by transmembrane ion movements. Ion pumps, such as vacuolar ATPase, operate in this tissue[9], so a circadian clock in the tubules may affect the rates of ion movement across the cell membranes — as in other circadian systems[10]. This clock, which occurs in fly Malpighian tubules and can be manipulated experimentally, may thus provide a model to explore the links between circadian mechanisms and membrane physiology.

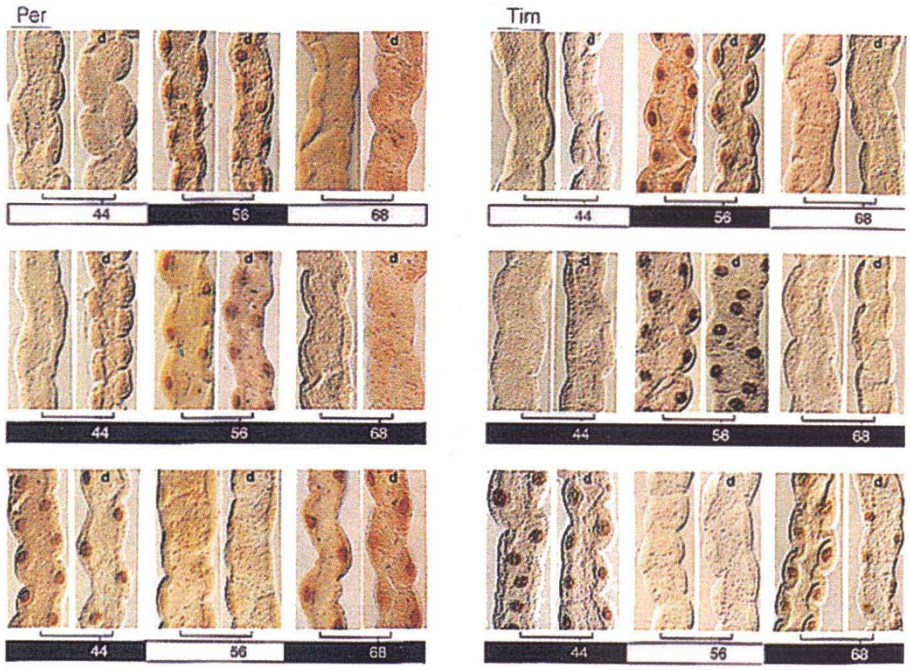


Figure 1 **Immunodetection of Per and Tim proteins in Malpighian tubules of intact or decapitated (d) flies. Flies kept in LD 12:12 were decapitated before the lights were turned off and placed in the following regime: normal LD 12:12 photoperiod (top), constant darkness (middle) and reversed light–dark (bottom). The number of hours in each regime before dissection is indicated on the photoperiod bar. Per and Tim cycling continued in constant darkness and was phase-shifted in reverse light–dark in both control and decapitated flies. Antibodies against Per provided by J. C. Hall and R. Stanewsky, and against Tim by A. Sehgal and M. Hunter-Ensor. Antisera were visualized with the vectastain ABC kit.**

**Jadwiga M. Giebultowicz**
**David M. Hege**
*Department of Entomology,*
*Oregon State University,*
*Corvallis,*
*Oregon 97331, USA*
*e-mail: giebultj@ bcc.orst.edu*

1. Hardin, P. E., Hall, J. C. & Rosbash, M. *Nature* **343,** 536–540 (1990).
2. Sehgal, A. *et al. Science* **270,** 808–810 (1995).
3. Siwicki, K. K., Eastman, C., Petersen, G., Rosbash, M. & Hall, J. C. *Neuron* **1,** 141–150 (1988).
4. Hunter-Ensor, M., Ousley, A. & Sehgal, A. *Cell* **84,** 677–685 (1996).
5. Zeng, H., Qian, Z., Myers, M. P. & Rosbash, M. *Nature* **380,** 129–135 (1996).
6. Ewer, J., Frish, B., Hamblen-Coyle, M. J., Rosbash, M. & Hall, J. C. *J. Neurosci.* **12,** 3321–3349 (1992).
7. Hardin, P. E. *Mol. Cel. Biol.* **14,** 7211–7218 (1994).
8. Giebultowicz, J. M., Riemann, J. G., Raina, A. K. & Ridgway, R. L. *Science* **245,** 1098–1100 (1989).
9. Dow, J. A. T. *et al. J. Exp. Biol.* **202,** 237–245 (1997).
10. D'Souza, T. & Dryer, S. E. *Nature* **382,** 165–167 (1996).