Circadian rhythms

Period piece for *Drosophila*

from Kevin Moses and Michael Ashburner

NORMAL male fruit flies sing a courtship song to their prospective mates by vibrating their wings. There is a 20-50 ms interval between pulses of sound-and the length of this interval itself oscillates with a period of 50-60 s. This oscillation is under the control[1] of a genetic locus known as *period*, or *per*, which also plays a part in controlling the rhythm of locomoter activity of the flies. It is one of seven known genetic loci that determine the rhythm of eclosion, that is, emergence from the pupa, in a population of flies. The *per* locus was the first of the seven to be discovered, is still the best characterized and has recently yielded to molecular analysis by two groups, with fascinating, if not altogether consistent, results[2,3].

Genetic analysis of circadian rhythms of *Drosophila melanogaster* began with Konopka and Benzer's isolation of mutations affecting the eclosion rhythm[4]. Mutations of *per* can have a variety of effects on rhythm, which can become shorter than the normal 24 h — for example, 19 h in per^S flies; or longer — for example, 29 h in per^L flies; or arrhythmic — for example, in per^0 flies. Similarly, the 50-60 s oscillation in the period between bursts of love song in normal male flies is shortened to 40 s in per^S flies, lengthened to 80 s in per^L flies and absent in per^0 flies.

Thus, mutant alleles of *per* affect both circadian rhythms and much faster rhythmic activities. By transplanting brains between flies that differ in their genotype at the *per* locus, Handler and Konopka have shown that it is the expression of this gene in the brain that determines the fly's phenotype[5]. This suggests that the fly's behaviour is continuously affected by the expression of the *per* gene, a conclusion supported by studies with a temperature-sensitive per^0 allele (ref.6). The nature of the mutations that affect this rhythm is interesting. A complete absence of *per* function (as seen in a homozygous per^- deletion) gives an arrhythmic fly, but a partial loss of function gives a fly with a long cycle. This is the conclusion from the observation that females that are hemizygous for per^+ have a 25-h cycle.

For their molecular analysis of *per*, the groups from Rockefeller University[2] and Brandeis University[3] have used different starting points. The former cloned *per* by jumping from the previously cloned *Notch* gene, crossing the break points of a deletion that brought *Notch* just distal to *per*. From this starting point, they have characterized about 90 kilobases (kb) of contiguous DNA and by mapping the breakpoints of other chromosome aberrations to the DNA, have defined the *per* locus to a 25-kb interval. On the other hand, the group from Brandeis University microdissected polytene chromosomes to isolate DNA from band 3B1.2, which contains *per*, for cloning. Both groups have characterized transcripts in this region and have confirmed the cloning of *per* DNA by transformation.

Within the 25 kb of DNA that must, on genetic criteria, include the *per* locus, several transcripts have been identified. The two groups differ somewhat in their interpretation of the transcription data. Between the limits of two deletions that bracket the *per* gene the Brandeis group have found four transcripts, and the Rockefeller group three. Perhaps the most startling discovery by the Brandeis group is that the amount of the 0.9-kb transcript varies during the day: it is more abundant at 2 pm than 2 am. Furthermore per^0 flies have a greatly reduced amount of this transcript. The translocation *T(1;4)JC43* provides an interesting mutation of *per* because it acts as a per^0 mutation in eclosion assays but as a per^L mutation in locomotor-activity assays. This translocation has a correspondingly complex effect on transcripts from the *per* region, abolishing a 4.5-kb transcript, reducing the level of 1.1-kb transcript and producing two novel transcripts of 11.5 and 0.9 kb from the *per* region, according to the Rockefeller group[2]. The Brandeis group also finds two new transcripts (which they call 10 and 0.5 kb) and a reduction, but not a complete absence, of the 4.5- and 0.9-kb RNAs. Presumably the 0.9-kb Brandeis RNA and 1.1-kb Rockefeller RNA are the same species.

Unfortunately, the transformation data do not help to define the function of the *per* gene transcripts because the DNA used either did not include sequences that code for the cycling 0.9-kb RNA[7] or included the equivalent DNA but also the DNA that codes for the 4.5- and 1.0-kb RNAs[8]. The ambiguity results from the fact that all of the transformants remain mutant, with longer rhythms than normal. Whether this is due to a failure to obtain correct tissue-specific expression of the introduced *per* DNA, or whether the DNA lacks sequences required for normal *per* function, or is due to differences in the assay of the *per* phenotype, is unclear.

The fact that the two groups disagree as to the details of the structure and function of *per* should not detract from their achievements nor from the great interest in this gene. It is clear that we are now on the way to a molecular description of a gene whose function is required for both circadian and other rhythmic behaviours. □

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Archaeology

Using isotope chemistry to detect prehistoric diets.

from Glynn Ll. Isaac

WHEN archaeologists seek to reconstruct the subsistence systems used by prehistoric hunter-gatherers, they face two particularly intractable problems. The first is to quantify the relative importance of plants versus animals in ancient diets, and the second is that of determining patterns of seasonal movements. Studies on bone composition have the potential to solve the second type of problem, as illustrated by the paper of Judith Sealey and Nikolaas van der Merw on page 138 of this issue[1].

In sites where organic remains are relatively well preserved, it is often possible, by patient study of the animal bones and shells, to rank the relative importance of different animal species as a source of food. Sometimes the same can be done for carbonized plant remains. However, it is seldom possible to gauge the proportions of plants and animals relative to one another. Similarly, while particular remains can sometimes allow archaeologists to say that a site was occupied during one season, it is often much harder to be sure that the site was *not* occupied in other seasons. So, having found a few sites with complementary seasons of apparent occupation, is it safe to link up these observations and infer a patterned seasonal round of movements by the occupants?

During the 1960s and 1970s, the seasonality issue was dramatized by the writings of a Cambridge school of economic prehistorians inspired by Grahame Clark and led by the late Eric Higgs. In effect, Higgs suggested that the most efficient use of many landscapes would have involved sweeping seasonal movements of inhabitants and livestock. The model was (and is) attractive, but how was its applicability to particular pieces of prehistory