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Genetics

Molecular genetics of a biological clock in *Drosophila*

(*per* locus/behavioral rhythms/chromosomal rearrangements/mutant transcripts)

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ABSTRACT **Ninety kilobase pairs (kb) of DNA have been isolated from the 3B region of the X chromosome of *Drosophila melanogaster*. Previous cytogenetic analyses have placed a gene required for rhythmic behavior (*per*) in this chromosomal interval. Physical characterization of a series of chromosomal rearrangements altering *per* locus activity indicates that DNA affecting behavioral rhythms is found in a 7.1-kb *Hin*dIII fragment. A single 4.5-kb poly(A)$^+$ RNA is transcribed from this DNA in wild-type pupae and adult flies. The transcript is eliminated by a *per* mutant that retains some rhythmic activity, but this mutant substitutes two novel transcripts, 11.5 kb and 0.9 kb. It is suggested that the new poly(A)$^+$ transcripts provide residual *per* locus activity.**

Several X-linked mutations have been isolated that alter the rhythmic activities of *Drosophila melanogaster*. Three ethyl methanesulfonate-induced mutations, not associated with chromosomal rearrangements, affect circadian rhythms and appear to be allelic. The 24-hr locomotor activity rhythms of male and female wild-type flies are increased to 29 hr in flies homozygous or hemizygous for the mutation per^l, while the locomoter rhythms of per^s mutants have a 19-hr period, and no rhythms can be detected in per^0 flies (1). Eclosion rhythms are also affected by each of these mutations, according to the expected patterns.

Mutations of the *per* locus modify the courtship song of *Drosophila*. Certain components of the courtship song are repeated with a 55-sec period in wild-type males, but a per^s mutant sings a 40-sec song and a per^l mutant sings a song with an 80-sec period. A rhythm cannot be detected in the song of a per^0 fly (2). Apparently a single gene plays a fundamental role in the construction or maintenance of a biological clock, and this clock governs rhythmic activities of quite different durations.

The *per* locus has been mapped to the 3B region ot the X chromosome (see ref. 3 for nomenclature) by complementation tests involving a series of chromosomal deletions, This is, genetically, an exceptionally well-characterized segment of the *Drosophila* genome (4–6). There appear to be no lethal alleles of the *per* locus and it has been positioned within a nonvital chromosomal interval between two lethal complementation groups, *zw3* and *zw6* (6).

Mutations of the *per* locus are also associated with the breakpoints of several chromosomal rearrangements (6). These have been characterized with respect to their effects on eclosion rhythms (6) and on locomotor activity rhythms (7). Because a number of chromosomal rearrangement breakpoints affect the nonvital region occupied by the *per* locus, and some of these genetically separate *per* from flanking vital genes, it seemed likely that DNA sequences com posing the *per* locus could be identified by physically mapping the locations of rearrangement breakpoints within cloned DNA. In this paper, physical and genetic maps of the interval are correlated, and the transcriptional activity of DNA sequences corresponding to the *per* locus and its chromosomal neighborhood are presented.

MATERIALS AND METHODS

***Drosophila* Stocks.** Mutant chromosomes $Df(1)w^{rJ1}$, *Df(1)62d18*, *Df(1)*64j4, $Df(1)w^{-64d}$, and *T(1;4)JC43* were provided by B. Judd. *Df(1)TEM202* was provided by J. Lim, and $Df(1)N^{5419}$ was provided by W. Welshons. $Df(1)w^{rJ1}$ genetically removes the entire 3B chromosomal interval (4). The extents of the remaining rearrangements are reviewed in *Results*. Ethyl methanesulfonate-induced mutations of the *per* locus were supplied by R. Konopka. Fly stocks were maintained on standard cornmeal agar at 25°C.

Isolation of nucleic acids, cloning of mutant DNA, and hybridization procedures were as described (8).

RESULTS

Isolation of DNA from the 3B1-2 X Chromosomal Region. Cytologically, *Df(1)62d18* is a deletion of polytene chromosome bands 3B2-3C6 (4). The deficiency is mutant for *per* and all genetic loci proximal (toward the centromere) to it, up to but not including *Notch* (6). The proximal breakpoint of this deficiency is located ≈65 kilobase pairs (kb) distal (toward the telomere) to coordinate 0 of the *Notch* locus (see ref. 8 for *Notch* locus coordinates). A novel 12.5-kb *Eco*RI restriction fragment is detected in this region in $Df(1)62d18/Df(1)N^{5419}$ heterozygotes. $Df(1)N^{5419}$ removes *Notch* and the chromosomal region distal to it (ref. 9; unpublished observation), so the 12.5-kb *Eco*RI fragment must be derived from the *Df(1)62d18* chromosome. DNA corresponding to this new fragment was isolated from an *Eco*RI Charon 4 library constructed from mutant genomic DNA. The terminal 1.7 kb of this recombinant phage was demonstrated to be uniquely homologous to the 3B1-2 chromosomal region by *in situ* hybridization (not shown). From this entry point, overlapping clones were retrieved from the Canton S-Charon 4 phage library of Maniatis *et al.* (10).

Using this procedure, ≈90 kb of DNA was isolated from the 3B interval. Figure 1 presents a physical map of ≈25 kb of this region contained in two phages, ZW106 and ZW107. The DNA shown in Fig. 1 appears to be nonrepetitive when hybridized to restriction fragments generated from total genomic *Drosophila* DNA. However, weak hybridization of ZW106 DNA to the tip of the X chromosome has been detected by *in situ* hybridization to polytene chromosomes (not shown).

Physical Maps of Chromosomal Rearrangements. The locations of four additional chromosomal rearrangement breakpoints have been determined within the 25-kb region presented in Fig. 1 (Fig. 1*b*). The coordinates of each breakpoint are given in the Fig. 1 legend. The physical locations of these rearrangements are of interest because most affect *per* locus activity. Like *Df(1)62d18*, two deficiencies, *Df(1)TEM202* and *Df(1)64j4*, genetically delete the *per* locus. *Df(1)TEM202* re-

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Abbreviation: kb, kilobase(s) or kilobase pair(s).