Molecular genetics of a biological clock in Drosophila

(peri 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 HindIII 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 6.9 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 per1, while the locomotor rhythms of per1 mutants have a 19-hr period, and no rhythms can be detected in per0 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 per1 mutant sings a 40-sec song and a per0 mutant sings a song with an 80-sec period. A rhythm cannot be detected in the song of a per1 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 of 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 corresponding to 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)w511, Df(1)62d18, Df(1)64j4, Df(1)w5646, and T(1;4)JC43 were provided by B. Judd. Df(1)TEM202 was provided by J. Lim, and Df(1)N5487 was provided by W. Welshons. Df(1)w511 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 EcoRI restriction fragment is detected in this region in Df(1)62d18/Df(1)N5487 heterozygotes. Df(1)N5487 removes Notch and the chromosomal region distal to it (ref. 9; unpublished observation), so the 12.5-kb EcoRI fragment must be derived from the Df(1)62d18 chromosome. DNA corresponding to this new fragment was isolated from an EcoRI 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 CanGen-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 phases, 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. 1b). 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-

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

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moves per and the chromosomal interval proximal to it (to the right in Fig. 1), up to and including the white locus (5, 7). Df(1)64j4 removes per and two lethal complementation groups distal (left) to it (6). Because some of the deficiencies genetically overlap only for per, viable aneuploids can be constructed. Df(1)TEM202/Df(1)64j4 heterozygotes and Df(1)62d18/Df(1)64j4 females are both viable and arrhythmic (6, 7). Fig. 1 shows that the latter aneuploids are homozigously deficient for a 16-kb interval (coordinates +1 to +17), while the former females are homozigously deficient for about 10 kb (coordinates +7 to +17).

The effects of a chromosomal translocation breakpoint on the per locus have been studied in special detail (6, 7). In contrast to all other chromosome aberrations of this region, T(1;4)JC43 is associated with a novel per phenotype. When the eclosion behavior of populations of T(1;4)JC43 flies is examined, T(1;4)JC43 behaves as an arrhythmic mutant (6, 7). In contrast, locomotor activity studies of individual T(1;4)JC43 flies have shown that they often express rhythms, but these are rhythms with a long (35 hr) period (7). Occasionally, T(1;4)JC43 flies express rhythms with a wild-type period in these tests (7). It has been shown in locomotor activity tests involving aneuploids homozigously deficient for the interval separating the T(1;4)JC43 and Df(1)64j4 rearrangement breakpoints that the X-distal element of this translocation (which carries the tip of the X chromosome up to the 3B breakpoint) provides chromosomal sequences capable of generating both of these rhythmic phenotypes (7). Fig. 1 shows that the breakpoint of this translocation lies about 7 kb proximal (right) to the Df(1)TEM202 breakpoint. Because Df(1)TEM202 is per	extsuperscript{Δ}, some of the DNA required for rhythmic behavior seen in T(1;4)JC43 flies should be found in the 7-kb interval separating the breaks of these two rearrangements.

One last deficiency, Df(1)w	extsuperscript{−64d}, complements per	extsuperscript{Δ} (6, 7). Genetically it deletes the chromosomal region proximal to per extending through the white locus. Fig. 1 indicates that this deficiency removes DNA sequences proximal to coordinate +25.

The positions of Df(1)TEM202, Df(1)64j4, and Df(1)w	extsuperscript{−64d} were established by hybridizing cloned DNA to restriction fragments of mutant genomic DNA. Representative hybridizations are shown in Fig. 2. The positions of breakpoints associated with Df(1)62d18 and T(1;4)JC43 were determined by analysis of restriction fragments contained in DNA cloned from these mutants. The positions of breakpoints not cloned were verified by in situ hybridization of cloned Canton S DNA to polytene chromosomes carrying the chromosomal rearrangements (data not shown). The physical location of Df(1)64j4 indicated in Figs. 1 and 2 agrees with that previously determined (11).
Locations of Transcripts. The physical studies described in the preceding section indicate that DNA sequences affecting rhythmic behavior can be found in a 7-kb region separating the rearrangement breakpoints of Df(1)TEM202 and T(1;4)JC43 (+10 region). To establish the relationship of DNA sequences found in this interval to transcribed regions, restriction fragments covering the entire 25-kb region presented in Fig. 1 were hybridized to poly(A)+ RNA isolated from embryos, larvae, pupae, and adults. Representative hybridizations to poly(A)+ RNA from wild-type (Canton S) adults are shown in Fig. 1c. For three of the transcribed regions, hybridizations are also shown for RNA extracted from per flies (Fig. 1c and Fig. 1 legend).

A 4.5-kb poly(A)+ RNA is detected in the +10 region (Fig. 1c and d). RNA hydridizing to this RNA is contained within a 7.1-kb HindIII fragment extending from coordinate +6.6 to +13.7. The chromosomal rearrangement breakpoint of T(1;4)JC43 falls within the HindIII restriction fragment. DNA sequences coding for the 4.5-kb RNA are entirely removed by all chromosomal deficiencies that delete the per locus [Df(1)TEM202, Df(1)62dl8, and Df(1)64f4].

DNA sequences neighboring the 7.1-kb HindIII fragment are homologous to six additional poly(A)+ RNAs. The locations of restriction fragments homologous to each of these transcripts are indicated in Fig. 1d. A 2.7-kb transcript is detected in the +23 region of Fig. 1. This RNA hybridizes with DNA fragments contained within coordinates +19.2 (BamHI) and +25.3 (EcoRI). The DNA encoding this RNA is located proximal to the breakpoint of Df(1)64f4 and the RNA is homologous to restriction fragments containing the rearrangement breakpoint of Df(1)64f4. The transcribed region may correspond to the l(1)zw6 complementation group (4). A more detailed presentation of the relationship of transcripts and lethal complementation groups in the 3B region proximal to Df(1)64f4 will be presented elsewhere (unpublished data).

A 3.2-kb RNA is found in the +19 region. This RNA is homologous to a 1.5-kb EcoRI/Bgl II fragment (+15.4 to +16.9) and a 6.0-kb Bgl II fragment (+16.9 to +22.9). DNA sequences encoding a portion of this RNA are removed by Df(1)64f4 (Fig. 1). DNA to the left of this region is homologous to a 1.1-kb transcript. Sequences hybridizing to this RNA are found within a 3.2-kb HindIII/Bgl II fragment (coordinates +13.7 to +16.9). The breakpoint of T(1;4)JC43 lies distal to this RNA homologous region.

DNA in the +3 region hybridizes with a 1.2-kb poly(A)+ RNA, and the RNA homology is contained within a 3.4-kb EcoRI fragment (coordinates +1.0 to +4.4). DNA forming this transcript lies distal to Df(1)TEM202 but is removed by Df(1)62dl8.

The terminal 1.6-kb EcoRI fragment of ZW106 (coordinates −0.6 to +1.0) is homologous to a 4.5-kb and a 5.9-kb poly(A)+ transcript. The 3B breakpoint of Df(1)62dl8 falls within this DNA segment. However, it is not known whether a portion of the DNA forming either of these transcripts is removed by this deficiency.

Because earlier genetic analyses had only detected mutations at the per locus within the Df(1)62dl8 to Df(1)64f4 interval, the existence of several distinct poly(A)+ transcripts within this region was not expected. To directly examine the effects of rearrangement breakpoints that alter rhythmic behavior on the synthesis of poly(A)+ RNAs transcribed from this region, an analysis of poly(A)+ RNAs from segmental aneuploids was undertaken. These results are presented in the following section.

Transcripts in Segmental Aneuploids. Two genotypes of mutant flies were constructed, Df(1)TEM202/Df(1)64f4 and T(1;4)JC43/Df(1)w1211. The former females are arrhythmic and are homozygously deficient for the chromosomal region +6.6 to +16.9. Because Df(1)w1211 deletes the entire cloned chromosomal interval (4, 6), the latter females contain only DNA sequences from T(1;4)JC43 in this region. Such flies exhibit either long-period rhythms or are arrhythmic.

Several of the transcripts described above are affected by the chromosomal rearrangements. Fig. 3a demonstrates that the 4.5-kb transcript and the 5.9-kb transcript homologous to the terminal 1.6-kb EcoRI fragment of ZW106 (coordinates −0.6 to +1.0) are present in both of the aneuploid females. Fig. 3b shows that the 4.5-kb poly(A)+ transcript from the +10 region is absent in Df(1)TEM202/Df(1)64f4 females and

![Fig. 3](image-url)

FIG. 3. Effects of chromosomal rearrangements on transcription. Twenty micrograms of poly(A)+ RNA from the indicated genotypes was electrophoresed through a 1.0% agarose/2.2 M formaldehyde gel and transferred to GeneScreen Plus (New England Nuclear). Hybridization of filters was with (a) 1.6-kb EcoRI fragment, −0.6 to +1.0; (b) 2.7-kb EcoRI fragment, +5.5 to +8.2; (c) 6.6-kb EcoRI fragment, +8.2 to +14.8; (d) 3.6-kb Bgl II fragment, +13.3 to +16.9; (e) phage ZW107. All RNAs were isolated from adult females. DNA sequences between coordinates +6.6 and +16.9 are deleted in females of the genotype Df(1)TEM202/Df(1)64f4. Females T(1;4)JC43/Df(1)w1211 have DNA sequences corresponding to only the translocation-bearing chromosomes within the cloned interval. RNA sizes were determined by comparison to 3P-end-labeled λ restriction fragments.
that DNA sequences in this interval are now homologous to a novel transcript of about 11.5 kb in T(1;4)JC43/Df(1)w101 heterozygotes. The probe in Fig. 3b is a 2.7-kb EcoRI fragment (coordinates +5.5 to +8.2). In Fig. 3c, RNA from the mutants is hybridized with the 6.6-kb right terminal EcoRI fragment of 2W106 (coordinates +8.2 to +14.8). Again we see the new 11.5-kb transcript in the T(1;4)JC43/Df(1)w101 aneuploids and no 4.5-kb RNA is detected. This mutant produces the 1.1-kb poly(A)" transcript previously mapped to the +15 region (Fig. 1), but in reduced amounts. It is detected on longer exposures such as that presented in Fig. 3d. Fig. 3c also shows that a new transcript of about 0.9 kb is produced in T(1;4)JC43/Df(1)w101 flies. The novel 0.9-kb transcript hybridizes to a 7.1-kb HindIII fragment (+6.6 to +13.7) (not shown), so this RNA should be transcribed from DNA sequences lying between coordinate +8.2 and the breakpoint of T(1;4)JC43.

Fig. 3c also indicates that the 4.5-kb and 1.1-kb RNAs produced by the +10 and +15 regions in wild-type flies are not expressed in Df(1)TEM202/Df(1)y644 females. Further, the novel 11.5-kb and 0.9-kb transcripts produced by T(1;4)JC43/Df(1)y644 heterozygotes are not detected in the Df(1)TEM202/Df(1)y644 heterozygotes. A 1.3-kb poly(A)" transcript is formed in both strains of aneuploid flies (Fig. 3c) and in wild-type (Canton S) flies (data not shown). This result is surprising because Df(1)TEM202/Df(1)y644 flies are homozygously deficient for the DNA fragment used as a probe in this experiment. We suggest that DNA sequences near the tip of the X chromosome, previously shown to be weakly homologous to 2W106, may be transcribed to form this RNA.

Fig. 3d illustrates that the 3.2-kb RNA from the +19 region (Fig. 1) is absent from Df(1)TEM202/Df(1)y644 heterozygotes but is expressed by T(1;4)JC43/Df(1)w101 aneuploids. Several poly(A)" transcripts are homologous to DNA sequences mapping to the right of the Df(1)644 breakpoint, extending beyond the interval presented in Fig. 1, and their locations will be described in detail elsewhere (unpublished data). Fig. 3e shows that these RNAs are present in both Df(1)TEM202/Df(1)y644 and T(1;4)JC43/Df(1)w101 flies.

Df(1)TEM202/Df(1)y644 and T(1;4)JC43/Df(1)w101 aneuploids have been examined for expression of the 1.2-kb transcript produced by wild-type flies from the +3 region (Fig. 1). This RNA is not detected in either of these strains of mutant females. It is demonstrated in the following section that the 1.2-kb RNA is a male-specific transcript.

In summary, an analysis of transcripts produced by aneuploid flies indicates that each rearrangement breakpoint affects transcription in a limited region of the nonvital chromosomal interval. The structure of the 4.5-kb poly(A)" RNA derived from the +10 region is uniquely affected by all chromosomal rearrangements that produce altered rhythms.

Expression of Poly(A)" RNA During Development. Poly(A)" RNAs from embryos, larvae, pupae, and adults have been hybridized with restriction fragments covering the −0.6 to +16.9 interval shown in Fig. 1. Developmental profiles are presented for all transcripts detected in this region in Fig. 4. The data shown in Fig. 4 indicate that all poly(A)" RNAs found in this region are produced in adults.

The 1.2-kb RNA from the +3 region is unusual in that it is found in male larvae, pupae, and adults but is not produced at any of these developmental stages in females. Data from a mixed population of male and female embryos, larvae, pupae, and adults are presented in Fig. 4. Individual adult male and adult female data are also shown. Longer exposures of Fig. 4 a, c, and d show that the remaining poly(A)" RNAs in the region spanning the Df(1)62d18 and Df(1)y644 breakpoints are produced by both male and female flies.

DISCUSSION

The X-chromosomal region separating the zeste and white loci of D. melanogaster has been the focus of extensive cytogenetic analyses (4–6). It is believed that mutation studies have identified all vital genes within the chromosomal interval. However, several loci that are not essential for viability
have been located here (1, 6, 12). These include genes defined by several female sterile mutations and mutations of the per locus that affect biological rhythms.

We have initiated a physical study of a nonvital region that is bordered by two vital genes, zw3 and zw6. Cytologically, this chromosomal segment is defined by the breakpoints of two genetically overlapping deficiencies, Df(1)62d18 and Df(1)64j4. Both deficiencies are clock mutants and fail to complement ethyl methanesulphonate-generated mutations at the per locus (6). Aneuploid females of the genotype Df(1)62d18/Df(1)64j4 are viable, fertile, and arrhythmic (6). In this paper, the physical locations of the 3B breakpoints of these deficiencies have been determined with the result that they are shown to remove a common 16-kb DNA segment.

Four poly(A)⁺ transcripts have been detected in this 16-kb region. These RNAs include a 3.2-kb transcript from the +19 region, a 1.1-kb RNA from the +15 region, a 4.5-kb transcript from the +10 region, and a 1.2-kb transcript from the +3 region. The 1.2-kb RNA detected in the +3 region is male specific. Because the effects of a deletion of the +3 interval have only been assessed in aneuploid females and because earlier genetic screens were designed to detect lethal mutations of loci in the zeaste-white interval in females (4–6), we cannot conclude that this transcript is involved in a nonvital function in males. Therefore, at least three transcripts should have nonvital functions.

Additional chromosomal rearrangements in this region have been shown to affect rhythmic behavior. Df(1)TEM202 fails to complement per⁰ and gives an arrhythmic phenotype in combination with Df(1)64j4 (7). Three of the transcripts described above are removed in Df(1)TEM202/Df(1)64j4 flies. These are the 4.5-kb RNA from region +10, the 1.1-kb RNA from the +15 region, and the 3.2-kb transcript from the +19 region (Fig. 1). Thus, one of these RNA coding regions should correspond to the per locus. As described earlier, the X-distal element of T(1;4)JC43 provides chromosomal sequences capable of generating rhythmic behavior (7). Analysis of cloned DNA spanning the T(1;4)JC43 breakpoint has placed it between coordinates +13.3 (Bgl II) and +13.7 (Hind III). Only one transcript, the 4.5-kb RNA from region +10, is homologous to DNA lying distal (left) to the T(1;4)JC43 breakpoint and is removed by Df(1)TEM202. This transcript is formed by DNA contained within a 7.1-kb HindIII fragment (coordinates +6.6 to +13.7).

Analysis of transcripts isolated from flies of the genotype T(1;4)JC43/Df(1)w⁰ has indicated that the 4.5-kb RNA is not synthesized by these aneuploids, but DNA from this region is used to form a novel 11.5-kb transcript. A second new transcript (0.9 kb) is also homologous to DNA sequences lying distal to the T(1;4)JC43 breakpoint.

The simplest interpretation of these results seems to be that DNA sequences required for rhythmic behavior are found within a 7.1-kb HindIII fragment separating the chromosomal rearrangement breakpoints of Df(1)TEM202 and T(1;4)JC43. The loss of the wild-type (4.5 kb) transcript and concomitant appearance of new RNA species in T(1;4)JC43/Df(1)w⁰ aneuploids from the +10 region suggest that the 4.5-kb poly(A)⁺ RNA is required for the expression of wild-type rhythmic behavior and that the novel RNAs provide the residual per locus activity found in T(1;4)JC43 flies.

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The 4.5-kb poly(A)⁺ RNA that is altered by T(1;4)JC43 is expressed in per⁰ flies. There is no detectable difference in the size of the per⁰ and wild-type 4.5-kb transcript (see Fig. 1 and legend). The remaining RNAs transcribed from the 25 kb of cloned DNA are also indistinguishable from their wild-type counterparts in per⁰ flies (data for +15 and +19 regions are also shown in Fig. 1). Analyses of restriction fragments generated from total genomic per⁰ DNA indicate no obvious changes in the physical map of the 25-kb cloned interval. DNA isolated from per⁰ and per⁰ flies also appears to be unchanged, and the mutants produce the 4.5-kb RNA from the +10 region (data not shown), so that a more detailed analysis will be necessary to determine the relationship of these ethyl methanesulphonate-induced mutations to the structure or regulation of the 4.5-kb RNA.

It is not possible to ascribe functions to the remaining poly(A)⁺ transcripts homologous to the cloned, nonvital interval. Nor can the possibility be excluded that some of these transcripts are involved in the expression of rhythmic behaviors. However, the developmental patterns of expression of certain transcripts indicate that they could not be linked to a biological clock in as fundamental a way as is the per locus.

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