Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 2142–2146, April 1984
Genetics

Molecular genetics of a biological clock in *Drosophila*

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

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Communicated by Norton D. Zinder, December 16, 1983

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 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 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.

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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-

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

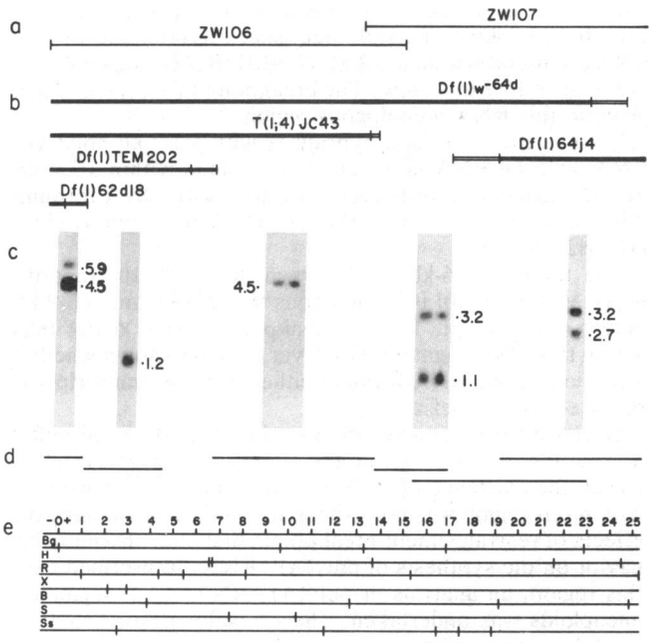

FIG. 1. A physical map of DNA sequences and location of transcripts lying within a subinterval of the 3B region. The centromere is to the right (proximal), and the telomere is to the left (distal). (*a*) Locations of two overlapping phage spanning the breakpoints of chromosomal deficiencies, *Df(1)62d18* and *Df(1)64j4*. Additional phage were isolated covering regions −37 to +56. (*b*) The locations of five chromosomal rearrangements that define the 3B1-2 nonvital region. Bars represent DNA sequences still present and in wild-type configuration in the rearrangement. For *T(1;4)JC43* only the tip of the X chromosome up to the breakpoint is shown. Bracketed areas at the ends of the bars represent region in which the breakpoint is located. These are: $Df(1)w^{-64d}$, +22.9 (*Bgl* II) to +24.5 (*Hin*dIII); *T(1;4)JC43*, +13.3 (*Bgl* II) to +13.7 (*Hin*dIII); *Df(1)64j4*, +16.9 (*Bgl* II) to +19.2 (*Bam*HI); *Df(1)TEM202*, +5.5 (*Eco*RI) to +6.6 (*Hin*dIII); *Df(1)62d18*, 0.0 (*Bgl* II) to +1.0 (*Eco*RI). (*c*) Autoradiographs formed by hybridizing ^{32}P-labeled DNA to 20 μg of poly(A)$^+$ RNA isolated from adult Canton S *Drosophila*. Panels (left to right) were hybridized with the following DNA fragments: 1.6-kb *Eco*RI (−0.6 to +1.0); 3.4-kb *Eco*RI (+1.0 to +4.4); 2.7-kb *Eco*RI (+5.5 to +8.2); 3.6-kb *Bgl* II (+13.3 to +16.9); 6.0-kb *Bgl* II (+16.9 to +22.9). In panels where two tracks are shown, the left track contains 20 μg of poly(A)$^+$ RNA from ethyl methanesulfonate-induced *per*^{0} flies. RNA was separated by electrophoresis on 1% agarose/2.2 M formaldehyde gels and transferred to GeneScreen membrane (New England Nuclear). The size of the RNA was determined by comparison to ^{32}P end-labeled restriction fragments derived from wild-type λ DNA. (*d*) The extent of DNA sequences homologous to each poly(A)$^+$ RNA (see also *Results*). (*e*) Restriction map and 1-kb coordinate scale for cloned Canton S DNA. Restriction enzyme abbreviations are: *Bgl* II (Bg), *Hin*dIII (H), *Eco*RI (R), *Xho* I (X), *Bam*HI (B), *Sal* I (S), and *Sst* I (Ss). Positive numbers increase proximally (toward the centromere) and decrease distally (toward the telomere).

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 homozygously deficient for a 16-kb interval (coordinates +1 to +17), while the former females are homozygously 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 homozygously 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*$^-$, 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, $Df(1)w^{-64d}$, complements *per*^{0} (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 $Df(1)w^{-64d}$ 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).

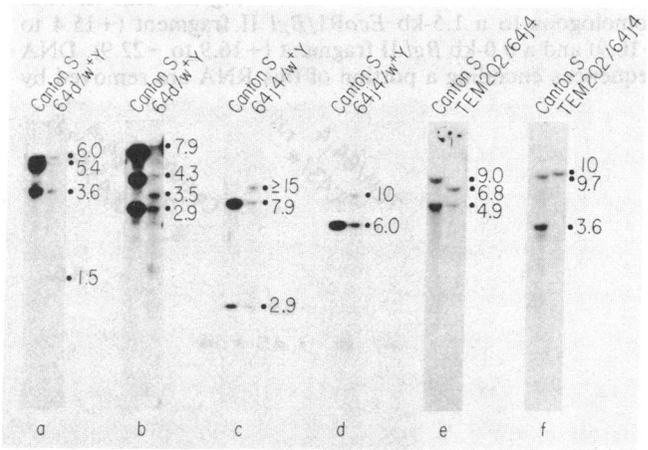

FIG. 2. Mapping the breakpoints of chromosomal rearrangements within the 3B region. DNA from flies of the indicated genotype was digested to completion with the restriction enzymes *Bgl* II (*a*, *d*, and *f*), *Hin*dIII (*b* and *c*), and *Sst* I (*e*), fractionated through 0.8% agarose gels, transferred to a membrane, and hybridized with the following probes: ZW107 to *a* and *b*, 6.0-kb *Bgl* II fragment coordinates +16.9 to +22.9 to *c* and *d*, and ZW106 to *e* and *f*. Male flies of the genotype $Df(1)w^{-64d}/w^+Y$ carry both wild-type and rearranged DNA sequences, whereas females *Df(1)TEM202/Df(1)64j4* are aneuploid and carry only rearranged chromosomes. For example, the 3.5-kb *Hin*dIII fragment in *b* is unique to flies carrying the $Df(1)w^{-64d}$ chromosome. The w^+Y chromosome is indistinguishable from wild-type DNA sequences. A further description of the w^+Y duplication can be found in ref. 4. (*f*) Female flies of the genotype *Df(1)TEM202/Df(1)64j4* lack both wild-type *Bgl* II fragments (9.7 kb and 3.6 kb) but contain a novel 10-kb *Bgl* II fragment, which can be shown to contain DNA sequences homologous to the wild-type 9.7-kb *Bgl* II fragment.

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. 1*c*. For three of the transcribed regions, hybridizations are also shown for RNA extracted from *per*^{0} flies (Fig. 1*c* and Fig. 1 legend).

A 4.5-kb poly(A)$^+$ RNA is detected in the +10 region (Fig. 1 *c* and *d*). DNA hybridizing to this RNA is contained within a 7.1-kb *Hin*dIII fragment extending from coordinate +6.6 to +13.7. The chromosomal rearrangement breakpoint of *T(1;4)JC43* falls within the *Hin*dIII 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)62d18*, and *Df(1)64j4*].

DNA sequences neighboring the 7.1-kb *Hin*dIII fragment are homologous to six additional poly(A)$^+$ RNAs. The locations of restriction fragments homologous to each of these transcripts are indicated in Fig. 1*d*. 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 (*Bam*HI) and +25.3 (*Eco*RI). The DNA encoding this RNA is located proximal to the breakpoint of *Df(1)64j4* and the RNA is homologous to restriction fragments containing the rearrangement breakpoint of *Df(1)w*$^{-64d}$. 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)64j4* 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 *Eco*RI/*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)64j4* (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 *Hin*dIII/*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 *Eco*RI fragment (coordinates +1.0 to +4.4). DNA forming this transcript lies distal to *Df(1)TEM202* but is removed by *Df(1)62d18*.

The terminal 1.6-kb *Eco*RI 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)62d18* 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)62d18* to *Df(1)64j4* 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)64j4* and *T(1;4)JC43/Df(1)w*^{rJ1}. The former females are arrhythmic and are homozygously deficient for the chromosomal region +6.6 to +16.9. Because *Df(1)w*^{rJ1} 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. 3*a* demonstrates that the 4.5-kb transcript and the 5.9-kb transcript homologous to the terminal 1.6-kb *Eco*RI fragment of ZW106 (coordinates −0.6 to +1.0) are present in both of the aneuploid females. Fig. 3*b* shows that the 4.5-kb poly(A)$^+$ transcript from the +10 region is absent in *Df(1)TEM202/Df(1)64j4* females and

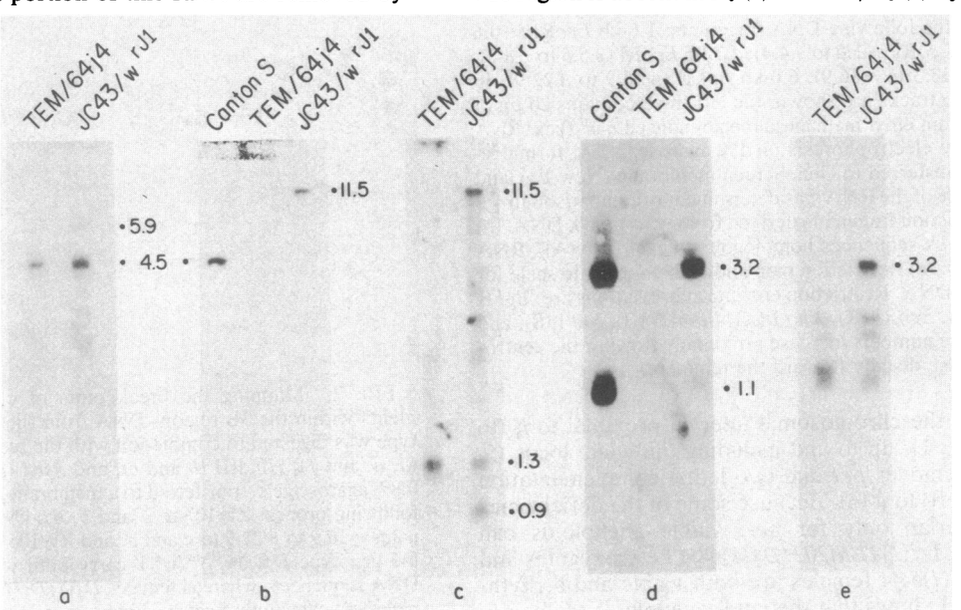

FIG. 3. Effects of chromosomal rearrangements on transcription. Twenty micrograms of poly(A)$^+$ RNA from the indicated genotypes was electrophoresed through a 1.0% agarose/2.2 M formaldehyde gel and transferred to GeneScreen Plus (New England Nuclear). Hybridization of filters was with (*a*) 1.6-kb *Eco*RI fragment, −0.6 to +1.0; (*b*) 2.7-kb *Eco*RI fragment, +5.5 to +8.2; (*c*) 6.6-kb *Eco*RI 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)64j4*. Females *T(1;4)JC43/Df(1)w*^{rJ1} have DNA sequences corresponding to only the translocation-bearing chromosomes within the cloned interval. RNA sizes were determined by comparison to ^{32}P 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)w*^{rJ1} heterozygotes. The probe in Fig. 3*b* is a 2.7-kb *Eco*RI fragment (coordinates +5.5 to +8.2). In Fig. 3*c*, RNA from the mutants is hybridized with the 6.6-kb right terminal *Eco*RI fragment of ZW106 (coordinates +8.2 to +14.8). Again we see the new 11.5-kb transcript in the *T(1;4)JC43/Df(1)w*^{rJ1} 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. 3*d*. Fig. 3*c* also shows that a new transcript of about 0.9 kb is produced in *T(1;4)JC43/Df(1)w*^{rJ1} flies. The novel 0.9-kb transcript hybridizes to a 7.1-kb *Hin*dIII 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. 3*c* 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)64j4* females. Further, the novel 11.5-kb and 0.9-kb transcripts produced by *T(1;4)JC43/Df(1)w*^{rJ1} heterozygotes are not detected in the *Df(1)TEM202/Df(1)64j4* heterozygotes. A 1.3-kb poly(A)$^+$ transcript is formed in both strains of aneuploid flies (Fig. 3*c*) and in wild-type (Canton S) flies (data not shown). This result is surprising because *Df(1)TEM202/Df(1)64j4* 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 ZW106, may be transcribed to form this RNA.

Fig. 3*d* illustrates that the 3.2-kb RNA from the +19 region (Fig. 1) is absent from *Df(1)TEM202/Df(1)64j4* heterozygotes but is expressed by *T(1;4)JC43/Df(1)w*^{rJ1} aneuploids. Several poly(A)$^+$ transcripts are homologous to DNA sequences mapping to the right of the *Df(1)64j4* breakpoint, extending beyond the interval presented in Fig. 1, and their locations will be described in detail elsewhere (unpublished data). Fig. 3*e* shows that these RNAs are present in both *Df(1)TEM202/Df(1)64j4* and *T(1;4)JC43/Df(1)w*^{rJ1} flies.

Df(1)TEM202/Df(1)64j4 and *T(1;4)JC43/Df(1)w*^{rJ1} 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)64j4* 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

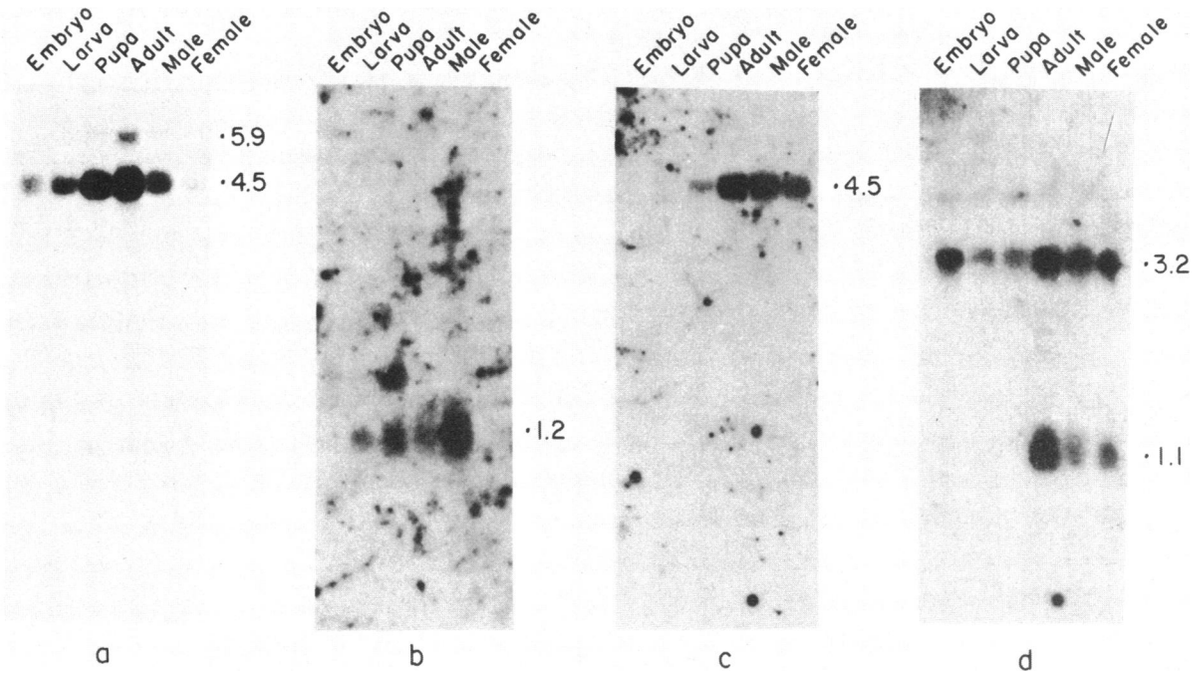

FIG. 4. Developmental profile of poly(A)$^+$ RNA in the 3B subinterval. Ten micrograms of poly(A)$^+$ RNA from the stages indicated was fractionated and transferred to GeneScreen membrane. The filter was successively hybridized with the following probes: (*a*) 1.6-kb *Eco*RI fragment, −0.6 to +1.0; (*b*) 3.4-kb *Eco*RI fragment, +1.0 to +4.4; (*c*) 3.6-kb *Bgl* II, +9.7 to +13.3; and (*d*) 3.6-kb *Bgl* II, +13.3 to +16.9. All tracks, except where indicated, contain a mixture of Canton S male and female RNAs. The tracks labeled male and female are derived from adult stages.

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 methanesulfonate-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 *zeste–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 *per*^{0} 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 (*Hin*dIII). 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 *Hin*dIII fragment (coordinates +6.6 to +13.7).

Analysis of transcripts isolated from flies of the genotype *T(1;4)JC43/Df(1)w*^{rJ1} 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 *Hin*dIII 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)w*^{rJ1} 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.

The 4.5-kb poly(A)$^+$ RNA that is altered by *T(1;4)JC43* is expressed in *per*^{0} flies. There is no detectable difference in the size of the *per*^{0} 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 *per*^{0} flies (data for +15 and +19 regions are also shown in Fig. 1). Analyses of restriction fragments generated from total genomic *per*^{0} DNA indicate no obvious changes in the physical map of the 25-kb cloned interval. DNA isolated from *per*^{l} and *per*^{s} 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 methanesulfonate-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.

We wish to thank Peter Bingham and Julie Pan for technical assistance and Simon Kidd and Johanna Renvall for providing some of the RNA and cloned DNA used in these experiments. We thank Norton D. Zinder for helpful criticism of the manuscript. This work was supported by a fellowship from the Andre and Bella Meyer Foundation and by a grant from the National Institutes of Health to M.W.Y.

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