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DNA sequence adjacent to and specific for the 1.672 g/cm^3 satellite DNA in the *Drosophila* genome

(heterochromatin/genome organization/repetitive DNA/*Drosophila melanogaster*)

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ABSTRACT **The *Drosophila* genome contains nearly 2.8 × 10^4 kilobases of satellite DNA. This simple sequence satellite DNA is contained within transcriptionally inactive heterochromatin that is distributed among all chromosomes with a concentration at the centromeres and along the length of the Y chromosome. To investigate the relationship of the satellite DNA with the surrounding sequences, we have isolated a satellite junction sequence that is repetitive and specifically adjacent to the 1.672 g/cm^3 satellite DNA. It is conserved between strains of *Drosophila melanogaster* and localized to the chromocenter of polytene chromosomes. The characteristics of this sequence suggest a functional role involving the specific organization of large regions of chromosomes.**

All *Drosophila melanogaster* chromosomes contain satellite DNA within regions of transcriptionally inactive heterochromatin. The function of the satellite DNA and heterochromatin is unknown. Satellite DNA represents 20–25% of the *Drosophila* genome and exists predominantly in large blocks greater than 1000 kilobases (kb) long (1). These satellite blocks are restricted to pericentric heterochromatin of the three autosomes, the proximal heterochromatin of the X, and the entire heterochromatic Y chromosome (2–4). The four satellite species found in the *Drosophila* genome represent one complex- and three simple-sequence satellites. The simple sequence satellites have buoyant densities of 1.672, 1.686, and 1.705 g/cm^3 and are composed of 5- to 10-base-pair (bp) repeating units. The distribution of the 1.672 and 1.705 g/cm^3 satellites has been investigated thoroughly by Steffenson *et al.* (4). Each is found on all chromosomes with the greatest amount on the Y chromosome. These two satellites may be adjacent at some chromosomal locations but do not appear to be interspersed. The complex satellite represented by the 1.688 g/cm^3 DNA consists of 254- and 359-bp repeating units (2). Carlson and Brutlag (5) have isolated a repeated sequence, one copy of which interrupts the complex 1.688 g/cm^3 satellite DNA. The function of this sequence is unknown though it is transcribed onto RNA. Additionally, a DNA segment has been isolated that contains a copy of the transposable element *Copia* (6) within the 1.688 g/cm^3 satellite repeating sequence (7). The structure of the naturally occurring junction between satellite and nonsatellite DNA or between heterochromatin and euchromatin has not been determined.

Proteins that complex with satellite DNA have been isolated from *Drosophila* chromatin (8). These proteins appear to bind to a symmetric region in the 359-bp repeat of the 1.688 g/cm^3 satellite sequence and may be involved in the packaging of this satellite sequence into heterochromatin, and could be a factor in functions suggested for heterochromatin such as meiotic pairing (9), dosage compensation (10), or position effect variegation (11).

Position effect variegation is caused by chromosomal translocations that create unusual junctions between heterochromatin and euchromatin. In some cells the nearby euchromatic genes are rendered inactive, apparently due to the condensation of otherwise euchromatic regions into heterochromatin. This phenomenon together with the contrasting regularly occurring fixed delineation of euchromatin and constitutive heterochromatin suggests that the naturally occurring junction region may be important in determining chromatin structure. This hypothesis implies that a sequence in the DNA is involved in signaling the end of a block of heterochromatin that would not occur in a position effect variegation-affected chromosome due to the absence of a normal "heterochromatin stop" signal. If such a signal is contained within the DNA sequence, it could be expected to reside adjacent to satellite DNA thereby determining the borders of the heterochromatic regions.

To examine the junctions that normally bridge the euchromatin and heterochromatin, we have isolated a DNA sequence from the *Drosophila* genome that flanks the highly repetitive 1.672 g/cm^3 satellite DNA species. This satellite-adjacent sequence is clustered at the chromocenter in polytene chromosomes, is specific to the 1.672 g/cm^3 satellite sequence, and is repeated ≈50 times in the genome. These characteristics are consistent with the idea that satellite adjacent sequences have a regulatory role in higher orders of chromatin structure.

MATERIALS AND METHODS

Isolation of Satellite DNA Containing Phage and Subcloning of the Satellite Fragment. *Drosophila* satellite DNA-containing phage were isolated from the Maniatis *D. melanogaster* Canton S strain recombinant library (constructed by the random shear method, ref. 12) by *in situ* plaque hybridization (13) using a 1.672 g/cm^3 satellite sequence isolated from a plasmid carrying that sequence provided by D. Brutlag (14). The initial isolate from this method was restriction enzyme mapped by sequential enzyme digestion (15). The 3-kb *Bam*HI fragment containing the satellite DNA was subcloned into the plasmid vector pBR322 and restriction enzyme mapped (see Fig. 1). Restriction enzymes were obtained from New England Biolabs; reactions with those enzymes were carried out according to manufacturer's specifications. Phage DNA was isolated by PEG precipitation of a cleared lysate and CsCl equilibrium gradients followed by proteinase K digestion and phenol extraction (15). Plasmid DNA was extracted by the rapid boil method (16). Fragments of the plasmid insert were isolated from low-gelling-temperature agarose (Sigma) by binding the DNA to an "elutip" (Schleicher & Schuell) according to the manufacturer's

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Abbreviations: kb, kilobase(s); bp, base pair(s).

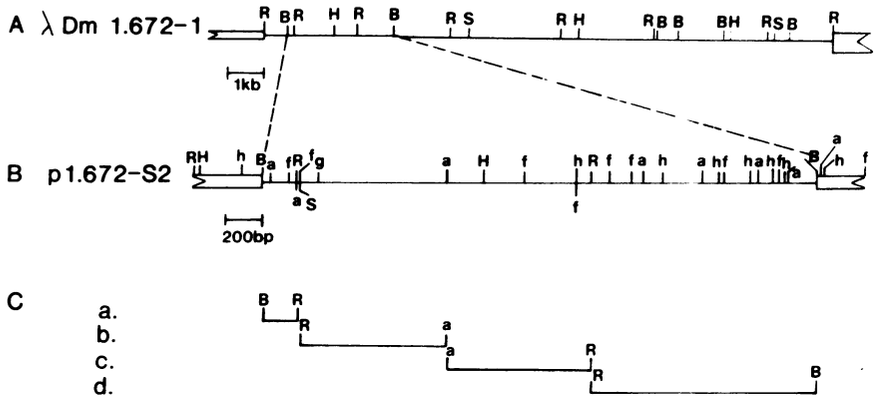

FIG. 1. Partial restriction enzyme map of phage λDm1672-1 and pDm 1672-s2. The recognition sites for the relevant restriction enzymes are shown for λDm1672-1 (*A*) and p1672-s2 (*B*), and the fragments are shown (*C*). The enzymes used for the isolation of the DNA probes used in the experiments are as follows: B, *Bam*HI; H, *Hin*dIII; R, *Eco*RI; f, *Hin*fI; a, *Hae* III; g, *Bgl* II; h, *Hha* I. Thin line, insert DNA; box, vector DNA. Fragment a is the 200-bp *Bam*HI–*Eco*RI fragment, b is the 600-bp *Eco*RI–*Hae* III fragment that contains all of the satellite DNA, c is the 800-bp *Hae* III–*Eco*RI fragment, and d is the 1.2-kb *Eco*RI–*Bam*HI fragment.

protocol. Details of the isolation scheme for all of the probes used in these experiments are shown in Fig. 1.

DNA sequencing on this subcloned fragment was carried out by the Maxam and Gilbert method (17) after 3'-end labeling of the appropriate fragment with Klenow fragment of *Escherichia coli* DNA polymerase as recommended by the manufacturer or by the dideoxy method (18) after subcloning the appropriate fragments into M13mp18 or M13mp19 phage vectors. DNA sequences were analyzed for secondary structure and protein coding capacity with the SEQ-Sequence Analysis System created by Clayton *et al.* (10). Sequences were compared for homology to *Drosophila* and other sequences through the GenBank data base.*

Genomic DNA Isolation and Analysis. *Drosophila* DNA was extracted by the proteinase K/phenol extraction method described by Strausbaugh and Kiefer (19). The resulting high molecular weight DNA was used directly for restriction enzyme analysis. Enzyme-digested DNA was analyzed on 0.8% agarose (Sigma) gels, transferred to nitrocellulose filters (Schleicher & Schuell, ref. 20), and hybridized in 4× SSC (1× SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7.0)/1× Denhardt's buffer (21). The filters were washed in 4× SSC, 1× Denhardt's at 60–65°C for 30 min with one change of buffer, then at room temperature in 2× SSC. The filters were then exposed at −80°C to Kodak XAR-5 film with an intensifying screen (Dupont).

***In Situ* Hybridization.** Salivary glands from Samarkand isogenic third instar larvae were fixed, squashed in 45% (vol/vol) acetic acid, and prepared for hybridization as described by Pardue and Gall (22). The squashes were hybridized to ^{35}S-labeled DNA for 12 hr at 65°C in 4× SSC/1× Denhardt's buffer. The slides were autoradiographed in diluted (1:1) Kodak NTB-2 emulsion exposed at 4°C for the times indicated in the figures. Chromosomes were stained with Giemsa and photographed with a Nikon photomicroscope.

RESULTS

Isolation and Characterization of Satellite-Containing Recombinant Phage. A Canton S *Drosophila* library (12) was probed with a 1.672 g/cm^3 satellite sequence isolated from a plasmid provided to us by D. Brutlag (14). Any phage isolated by this screening method is expected to contain satellite-adjacent sequences due to the elimination of repetitive sequences by the *E. coli* recombination systems that eventually render any phage containing only satellite DNA insufficient for packaging (23). One phage (λDm1672-1) was isolated and found to contain a small segment (<500 bp) of the 1.672 g/cm^3 satellite sequence (Fig. 1). The *Bam*HI fragment containing the satellite DNA was inserted into pBR322 for analysis of the satellite-adjacent sequence portion of the *Drosophila* DNA. This *Bam*HI fragment contains the 1.672 g/cm^3 satellite DNA along with flanking sequences of 400 bp and 2.0 kb to the left and right, respectively (p1672-S2; Fig. 1).

To confirm the existence of satellite sequences within this fragment, the satellite DNA-containing segment was nucleotide sequenced and found to be comprised of repeating units of the 5-bp 1.672 g/cm^3 consensus sequence (TTATA, ref. 5). The 600-bp *Eco*RI–*Hae* III fragment (fragment b in Fig. 1*C*) that contains the satellite DNA is flanked by nonsatellite DNA on both sides. This entire region has been sequenced and analyzed (to be published elsewhere). The region of flanking DNA that extends from the satellite into fragments b and c (Fig. 1*C*) contains no homologous sequences to any of the *Drosophila* sequences or to yeast centromeric sequences recorded in the GenBank sequence system.* The 750-bp directly adjacent to the satellite DNA are A+T rich (63.8% A+T) but contain no obvious regions of potential secondary structure that would indicate that this region of DNA functions through palindromic interactions. However, conformational alterations in the DNA helix has been attributed to A+T richness alone (24, 25) which, in this region, may contribute to the change in packing density between the heterochromatin and euchromatin. One open reading frame exists in this DNA sequence that could code for a small (79 amino acid) protein. The limit of the junction fragment is difficult to define due to the lack of a functional assay, but results of genomic analysis with the junction sequences (see below) suggest that the junction extends at least 2 kb from the satellite DNA.

The existence of a relatively small, internal satellite fragment would not be predicted by the reported organization of the satellite DNA in the genome (1). One possible explanation for this arrangement is that the phage has been modified in the cloning procedure via recombination in the bacterial host that would require the unlikely events of a double infection into a single bacteria and a crossover between those two phage within the satellite sequences. A second possible explanation for this arrangement is that some satellite DNA exists in the genome in small segments in addition to the large blocks. The small satellite segments along with their flanking sequences would be preferentially cloned in the λ library due to the selective elimination of large repetitive sequences in the bacteria (23). Three other phage (not shown) have been isolated by the same methods. Two have been found to contain internal satellite sequences and the other contains a terminal satellite sequence.

The Satellite-Adjacent Sequence Is Specific to the 1.672 g/cm^3 Satellite. Both the 1.672 g/cm^3 and 1.705 g/cm^3 satellite species are highly repeated, abundant, similarly distributed within the *Drosophila* genome and, therefore,

*National Biomedical Research Foundation Computer Data Base, National Institutes of Health (1983) *Genetic Sequence Data Bank: GenBank* (Research Systems Div., Bolt, Beranek, and Newman, Inc., Boston), Tape Release 15.0.

could show similarities in structure, sequence, and function of the flanking sequences. The satellite-adjacent sequence contained in the p1672-s2 plasmid was isolated and used to probe the *Drosophila* library. Phage isolated with the fragment c probe (800-bp *Eco*RI–*Hae* III fragment, see Fig. 1), the 1.705 g/cm^3 satellite probe, and the 1.672 g/cm^3 satellite probe were subsequently tested for the presence of the 1.672 g/cm^3 flanking sequence and the satellite sequence. The results (Table 1) show that 70% of the 1.672 g/cm^3 satellite-containing clones (7/10) hybridize to the 1.672 g/cm^3 flanking sequence (fragment c), and none of the 1.705 g/cm^3 satellite-containing clones (0/7) hybridize to the 1.672 g/cm^3 SAS (fragment c). Several of the phage clones hybridize to both the 1.672 g/cm^3 and 1.705 g/cm^3 satellite probes due to the ability of these two sequences to cross-hybridize (23) or the presence of both satellite species in the same clone.

The Satellite-Adjacent Sequence Is Conserved and Repeated in the *Drosophila* Genome. Specific sequences flanking the satellite DNA could be expected at several locations in the genome, namely every junction between satellite DNA and nonsatellite DNA. Fig. 2 shows that fragment c (800-bp *Hae* III–*Eco*RI, see Fig. 1) hybridizes to four major bands on a *Hae* III restriction spectrum of *Drosophila* DNA and two bands in the *Hin*dIII digest, indicating that homologous sequences are repeated in the genome. The *Hin*fI digestion of genomic DNA produces a single homologous fragment of 0.94 kb, suggesting that some areas within this flanking sequence are highly conserved in the genome. Because the restriction map of the cloned flanking sequence does not predict the presence of a 0.94-kb *Hin*fI fragment, the fragment cloned in p1672-s2 may not represent a major type of flanking sequence though the homologous regions are present in both the cloned and genomic fragments. Analyses of 39 independently isolated phage containing sequences homologous to both 1.672 g/cm^3 satellite and fragment c (800-bp *Hae* III–*Eco*RI fragment) show that each phage contains one or more *Hin*fI fragments of the sizes predicted from the genomic data.

Satellite DNA contains no restriction enzyme recognition site and, therefore, bands at the exclusion limit of the gel. It is significant that this high molecular weight band appears in the *Hin*dIII, *Hin*fI, and *Eco*RI autoradiographs, indicating that the probe sequence occurs adjacent to satellite DNA. The *Eco*RI-digested genomic DNA shows hybridization predominantly to this high molecular weight fragment, indicating that in most locations in the genome the first *Eco*RI site downstream from the satellite is beyond the region of homology to the probe, thus leaving the homologous flanking DNA attached to the satellite DNA. Conversely, the *Hae* III endonuclease site within 200 bp of the satellite (see Fig. 1) appears to be conserved due to the absence of hybridization to the high molecular weight satellite band in the genomic digests (Fig. 2*A*) as compared to the presence of that

Table 1. Cross-hybridization analysis of satellite DNA-containing phage clones

Satellite DNA fragment	Number of clones hybridizing/ total clones tested		
	1.705 g/cm^3 clones	800* clones	1.672 g/cm^3 clones
1.705 g/cm^3	7/7	0/10	5/13
800*	0/7	10/10	7/13
1.672 g/cm^3	5/7	7/10	13/13

Phage containing either 1.705 g/cm^3 satellite, fragment C (800), or 1.672 g/cm^3 satellite (top row) were isolated from a *Drosophila* Canton S library. These phage were then hybridized to the same set of probes (left column) to determine the degree of cross homology between satellites and satellite-associated sequences.
*800-bp *Hae* III–*Eco*RI fragment (fragment c, Fig. 1).

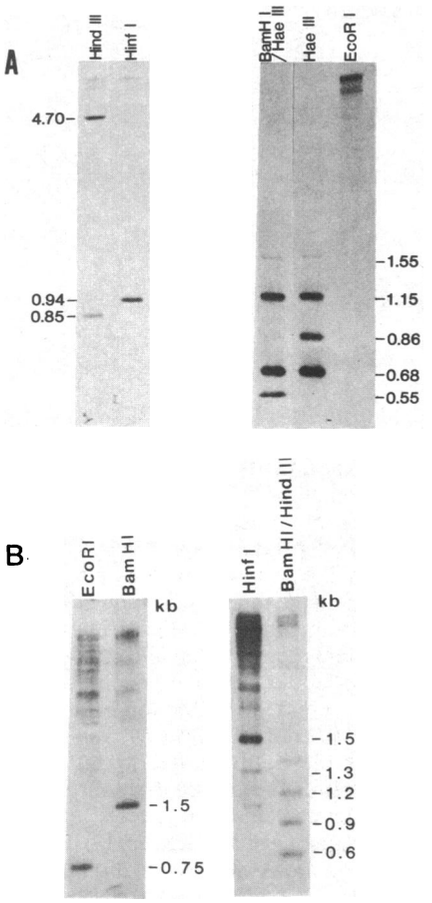

FIG. 2. Genomic DNA analysis of the 1.672 g/cm^3 flanking sequences. Total *Drosophila melanogaster* Samarkand DNA (5 μg) was digested with the indicated restriction enzymes, separated on a 1.0% agarose gel, and transferred to nitrocellulose. The filters were hybridized with the 800-bp *Hae* III–*Eco*RI fragment (*A*, fragment c) and the 1.2-kb *Eco*RI–*Bam*HI fragment (*B*, fragment d). The sizes of the major fragments are given in kb and were determined from the migration of *Hin*dIII-digested λ DNA (New England Biolabs) and *Hae* III-digested φX174 DNA (New England Biolabs). The lane labeled *Eco*RI contains 10 μg of DNA.

hybridization in the *Hin*dIII-, *Hin*fI-, and *Eco*RI-digested genomic DNA. When the same genomic digests are probed with fragment b (600-bp *Hae* III–*Hae* III fragment), the satellite portion of the fragment hybridizes to the high molecular weight band as well as a smear throughout the gel due to the random size and abundance of satellite DNA (data not shown).

Fragment d (1.2-kb *Eco*RI–*Bam*HI fragment, Fig. 1), which lies adjacent to fragment c (800-bp *Hae* III–*Eco*RI fragment), hybridizes to a set of genomic fragments that suggest a tandem arrangement of 0.5-kb variable repeat units in the genome (Fig. 2*B*). The following several results suggest that these repeats contain variability: (*i*) the ladder effect of *Hin*fI fragments that results from adjacent repeats frequently missing a restriction enzyme recognition site, (*ii*) hybridization to several bands of variable molecular weight (i.e., non-unit multiples) in all enzyme digests, and (*iii*) the presence of a prominent 1.5-kb *Bam*HI fragment that is not represented in p1672-s2.

The Satellite-Adjacent Sequence Is Clustered at the Heterochromatic Chromocenter. Since all of the heterochromatin in *Drosophila* salivary gland polytene chromosomes is in the chromocenter, this flanking sequence, if it is exclusively associated with satellite DNA and heterochromatin, should localize to the chromocenter. Indeed, salivary gland

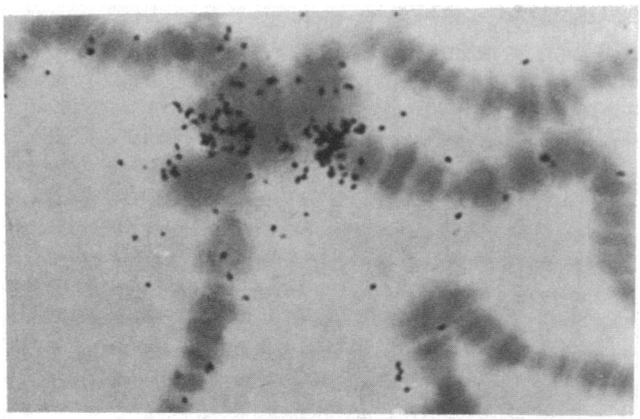

FIG. 3. Chromosomal location of the 1.672 g/cm³ satellite-adjacent sequence. The 800-bp *Hae* III–*Eco*RI fragment (fragment c) was hybridized *in situ* to salivary gland chromosome squashes of Samarkand larvae. Exposure time was 2–6 days.

chromosome squashes from Samarkand wild-type flies show hybridization of the 800-bp *Hae* III–*Eco*RI fragment only to the chromocenter, and in short exposures of appropriate spreads the grains appear to be localized to the region where chromosome arms enter the chromocenter (Fig. 3). This

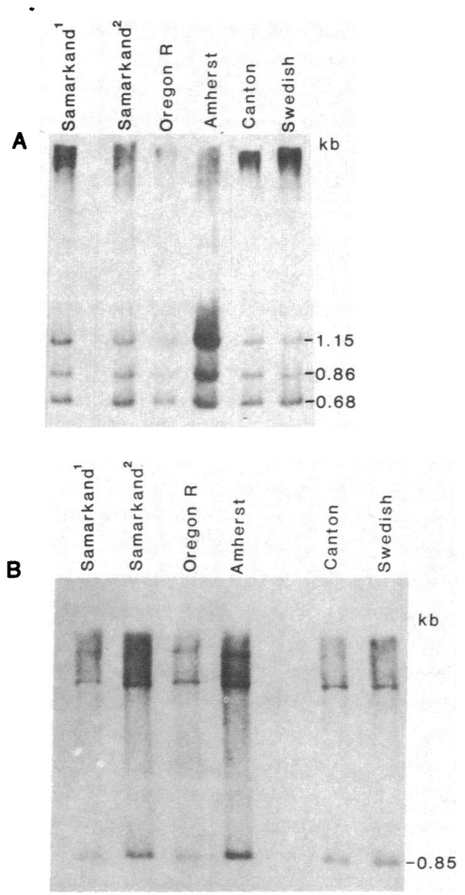

FIG. 4. Comparison of the arrangement of the 1.672 g/cm³ satellite-adjacent sequence between strains of *D. melanogaster*. Genomic DNAs from six strains of *D. melanogaster* were digested with *Hae* III (*A*) or *Hin*fI (*B*), displayed on 1.0% agarose gels, transferred to nitrocellulose, and hybridized to the 800-bp *Hae* III–*Eco*RI fragment. Samarkand[1] represents a Samarkand stock that was isogenized in this laboratory, and Samarkand[2] represents the laboratory stock that has not been isogenized. The results show the sequence to be conserved with respect to the enzyme recognition sites in all six strains.

hybridization at the euchromatin-heterochromatin junction often remains with the arm when it has been removed from the chromocenter as contrasted to the hybridization of satellite DNA remaining with the chromocenter under similar conditions (4). The absence of hybridization to any other location in the genome indicates that this sequence is not a dispersed, middle-repetitive sequence or mobile element (26), and it is not homologous to the repeated sequences found at the telomeres (27), which was also confirmed by direct sequence comparison.

The Satellite-Adjacent Sequence Is Conserved Between Strains of *Drosophila*. The satellite-adjacent sequence was used to probe the DNA from six strains of *D. melanogaster* (including Canton S) to determine if this sequence and the restriction enzyme sites within it are conserved among these strains. Fig. 4 shows the results of *Hae* III and *Hin*fI digestions of genomic DNA hybridized to fragment c (800-bp *Hae* III–*Eco*RI fragment). The band patterns observed in all six strains are identical, demonstrating interstrain conservation of this sequence within the *Drosophila* genome and suggesting a functional requirement for this satellite-adjacent sequence. The *Eco*RI- and *Bam*HI-digested DNA from these same strains shows the same result.

These results contrast with the results of studies on mobile sequences and telomeric sequences that show variation in restriction enzyme patterns between closely related strains of *D. melanogaster* (28, 29, 36). The different restriction enzyme sites in the cloned DNA as compared to the genomic DNA suggest that either the sequence isolated in the clone p1672-s2 does not represent the major repeating unit or that the cloned sequence has been altered during bacterial propagation.

DISCUSSION

To examine the arrangement of the naturally occurring satellite-nonsatellite junctions in the *Drosophila* genome we have isolated a middle-repetitive sequence specific for a single satellite species. This junction sequence is adjacent to and specific to the 1.672 g/cm³ simple sequence satellite. Though it is repeated in the genome, the junction sequence is not a member of a dispersed, repetitive sequence family described by Young (29) or similar to the telomeric sequences that also show homology to the chromocenter (28). Unlike most of the middle-repetitive sequences previously described, it contains a conserved sequence and position as shown by the comparison of restriction enzyme digestion patterns obtained from the total DNA of several *Drosophila* strains (Fig. 3) and by the *in situ* hybridization data (Fig. 4). The data suggest that this junction sequence is only adjacent to satellite DNA as shown in the *Eco*RI digest of total genomic DNA (Fig. 2) in which the junction sequence hybridizes to the restriction enzyme-resistant satellite DNA that bands at the exclusion limit of the gel. At present the exact size of the junction sequence can only be inferred from the data presented here due to the requirement for a functional assay to determine the precise limits. However, we have isolated phage clones from the *Drosophila* library that contain satellite-associated sequences and rDNA sequences. The sequence organization in these rDNA clones suggests that the conserved, satellite-junction sequence may extend as far as 8 kb from the satellite DNA (P. J. Pawlowski, R.J.D., and B.I.K., unpublished results).

Satellite DNA in many other species have large clusters of highly repeated sequences (reviewed in refs. 30–32). Limited information is available on the DNA sequences at the junction between satellite DNA and other DNA. In the African green monkey genome, the DNA sequence of the α satellite at the end of the clusters has been shown to contain a degenerate repeating sequence. This α satellite frequently

abuts another satellite sequence—the deca satellite—that contains a polymorphic 10-bp repeating unit that is found only at the centromere in half of the monkey chromosomes (33). This arrangement may represent a satellite-satellite junction similar to the junctions suggested for the 1.672 g/cm^3 and 1.705 g/cm^3 satellite sequences in *Drosophila*. Linkage of the 1.672 g/cm^3 and 1.705 g/cm^3 satellites was inferred from the chromosomal location of these satellites (2) and from results of density gradient and hydroxylapatite fractionation of total genomic DNA (4). Distinct, intermediate-density peaks and the presence of both satellite species in the same fractions was interpreted to show linkage of the two. However, the presence of conserved, repeated, nonsatellite sequences at the euchromatic end of each cluster of satellite such as the one described in this report could produce the same shift in density or hydroxylapatite binding ability.

The ubiquity of clusters of satellite DNA in nature suggests a functional role for this highly repetitive DNA in the eukaryotic genome. The relationship between satellite DNA and heterochromatin is, at present, only a coincidental one. In *Drosophila* satellite sequences appear to be restricted to regions that are known to be heterochromatic. Whether a causal relationship exists is not yet known (for review, see ref. 31). While the simple sequence of most of the known satellites seems to preclude a strictly sequence-dependent function, the DNA immediately adjacent to the satellite DNA could provide information related to the organization and function of large regions of chromatin. The junction sequence described here is repetitive in the genome, clustered at the chromocenter, specific for a single type of highly repeated satellite sequence, and apparently flanks that sequence at all or most locations in the genome. The conservation of restriction enzyme sites in this flanking sequence between strains suggests that this sequence has remained stable and is under a selective pressure. These characteristics make it a candidate for a signal sequence that may be involved in determining higher orders of chromosome structure. This view is compatible with the data of Tartof *et al.* (34) that shows that satellite sequences are not directly involved in position effect variegation and predicts the need for a nonsatellite sequence to signal the termination of the heterochromatic domain. This function may be facilitated by the high A+T content (63.8%) of the satellite-adjacent sequence that could be a site of DNA conformational changes (24) or specific sites for chromosomal protein binding (25).

In addition to the sequences reported here, we have isolated and characterized a sequence with similar characteristics that is associated with the 1.705 g/cm^3 satellite species. Comparison of the 1.705 g/cm^3 satellite-adjacent sequence with the 1.672 g/cm^3 satellite-adjacent sequence indicates that they contain no regions of homology (unpublished results).

The results reported here suggest the possibility of the natural occurrence of relatively short stretches of satellite DNA within the *Drosophila* genome. These short segments of satellite could have been undetected previously due to the methods used to isolate and characterize the satellite DNA. All studies of satellite DNA reported to date have involved the isolation of satellite DNA on density gradients that could eliminate the types of sequences described in this work from the satellite population. Polytene chromosome *in situ* hybridization studies could not distinguish between short segments and large blocks of satellite sequences. The data presented here suggest that these short satellite sequences are clustered at the chromocenter. Among the heterochromatic elements that are condensed at the chromocenter in *D. melanogaster* (35), the Y chromosome is known to contain nonsatellite, non-rDNA sequences: the fertility genes. Since this chromosome also contains approximately 50% of the total 1.672 g/cm^3 satellite, the λDm1672-1 DNA may reflect a Y-specific organization. If true, this could explain the observed differences between the cloned flanking sequences and the prevalent homologous sequences in genomic DNA.

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