Dodeca satellite: A conserved G+C-rich satellite from the centromeric heterochromatin of Drosophila melanogaster

(centromeric satellite/Drosophila yeast artificial chromosome library/in situ hybridization)

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ABSTRACT To identify sequences from the centromeric region, we have constructed a Drosophila melanogaster yeast artificial chromosome (YAC) library and screened it with purified DNA from the minichromosome Dp(1;f)1187 derived from the X chromosome. We describe the structure of one clone isolated in this way. This YAC is structurally unstable and contains tandemly repeated G+C-rich 11-mer and 12-mer units, which we call dodeca satellite. Most of this satellite is located near the centromere of an autosome. Cross-hybridizing sequences are found in the genomes of organisms as distant as Arabidopsis thaliana and Homo sapiens.

The genomes of higher eukaryotes contain large amounts of simple and complex tandemly repeated DNA sequences, classically termed satellite DNA (for review, see ref. 1). It has been known since 1970 that satellite DNAs are located primarily in centromeric heterochromatin (2). However, their role in possible functions of this region of the chromosome, such as chromosome pairing and chromosome segregation, remains largely unknown. Studies on the organization of centromeric heterochromatin have been impeded by the structural properties of satellite DNA. Satellite DNA fragments can be difficult to clone in bacterial vectors (3) and are therefore underrepresented or absent from Escherichia coli libraries. In addition, their very large size and repetitive nature make it difficult to construct complete physical maps of these regions, even by pulsed-field gel electrophoresis (PFG).

Recently, the development of yeast artificial chromosomes (YACs) as vectors for the propagation of exogenous DNA in Saccharomyces cerevisiae (4) has opened up additional possibilities. Libraries have been constructed containing large fragments of DNA from organisms such as humans and Drosophila (5, 6). However, YAC clones containing human DNA showed structural instability for all tandemly repeated sequences examined (7). Little is known about the structural stability of Drosophila satellite sequences in YACs, but, even if they are also unstable, it may at least be possible to use such clones as a source of probes from the centromeric sequences.

The chromosomes of Drosophila melanogaster offer a good system for analysis of the centromeric heterochromatin of higher eukaryotes. Like many eukaryotes, they have a semihemispherical kinetochore with bundles of microtubules attached, and their centromeric heterochromatin makes up a high proportion of each chromosome. In addition, it is possible to generate a panel of chromosomes with centromeric rearrangements by classical genetics, and many mutants in mitosis and meiosis are available.

A total of 11 simple repeated sequences have been cloned from gradient-purified satellite DNA of D. melanogaster and the nucleotide sequence of each repeat conforms to a formula (RRN), where R is A or G and N is any nucleotide (8). In this study, we have developed an approach to the cloning of centromeric heterochromatin sequences from D. melanogaster. Thus, we have discovered a type of tandemly repeated DNA sequence that is located in the centromeric region of some D. melanogaster chromosomes and that cross-hybridizes with DNA from other species including Arabidopsis and humans.**

MATERIALS AND METHODS

Drosophila Stocks and Mammalian Cell Lines. The Dp(1;f)-1187 stock, XY5-yl, Df(1)259, y-w/C(1)RA, I(1)J1In(1)sc2/Dp(1;f)1187, was kindly provided by A. Spradling (Carnegie Institute of Washington). Oregon R was used as a wild-type stock. The mammalian cell lines used were MTL and PEN (human lymphoblastoid male and female cells), 3E7 (mouse Y hybrid), 853 (hamster Y hybrid), Q998-8 (hamster Y hybrid); a gift from Carol Jones). The 3E7 and 853 revertants had lost the human Y chromosomes. The Q998-8 derivative had lost the Y long arm but retained the Y short arm.

Genomic DNA Preparations and PFG. High molecular weight genomic DNA was prepared in two different ways: in solution or in agarose plugs. For Drosophila DNA preparations in agarose plugs, population cages were set up to collect 0- to 12-hr-old embryos. After dechorionation, embryos were disrupted in Ringer's solution supplemented with 50 mM EDTA. For analysis of large DNA fragments, three different PFG apparatuses were used: Pulsaphor system (Pharmacia), CHEF-DR II (Bio-Rad), and a "waltzer" rotational gel electrophoresis apparatus. In the first two systems, 1% agarose (Sigma type 1) gels were run in 0.5× TBE (1× TBE is 90 mM Tris-borate/2 mM EDTA) while in the waltzer apparatus, 1.5% agarose gels were run in 0.5× TAE (1× TAE is 40 mM Tris-acetate/2 mM EDTA).

Construction of Drosophila YAC Library. The cloning vector pYAC-white was constructed by insertion of a white minigene driven by a heat shock protein 70 promoter into the unique Sal I site of pYAC-4 (4). This marker gene was obtained from plasmid pW8 (9) as a 4-kilobase (kb) SpH1/Spe I DNA fragment. The high molecular weight DNA was made from the Dp(1;f)1187 stock. An agarose plug was partially digested with EcoRI. The digested DNA was ligated to

Abbreviations: YAC, yeast artificial chromosome; PFG, pulsed-field gel electrophoresis.

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**The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M86306-M86309).
EcoRI/BamHI-digested and dephosphorylated pYAC-white vector and was then used to transform yeast strain AB1380 as described by Anand et al. (10).

**DNA Probes, Hybridization, and Sequencing.** The 4.4RR probe is a 4.4-kb EcoRI restriction fragment obtained from ysc 94 (11). DNA probes were purified by agarose gel electrophoresis and labeled by the random-primer procedure (12). Filter hybridization conditions were essentially as described by Church and Gilbert (13). Low-stringency hybridizations were performed at 55°C in the same conditions or in 10% dextran sulfate/0.5 M sodium phosphate/1% SDS/5 × Denhardt's solution, and washed in 2× standard saline citrate/0.1% SDS at the same temperature or at 65°C. Sequencing was performed by the method of Sanger et al. (14) with Sequenase 2.0 and the dGTP analogue 7-deaza-dGTP.

**In Situ Hybridization to Metaphase Chromosomes.** In the in situ hybridization to *Drosophila* mitotic chromosomes, the biotinylated probes were detected by an avidin-fluorescein conjugate. A full description of the method will be reported elsewhere (unpublished data). For in situ hybridization to human chromosomes, standard chromosome spreads from lymphocyte cultures were hybridized in situ under low-stringency conditions that allow hybridization between molecules sharing 60–65% homology. Hybridizations were performed essentially as described by Schwarzacher-Robinson et al. (15). The biotinylated probe was detected with streptavidin and biotinylated alkaline phosphatase.

**RESULTS AND DISCUSSION**

**Strategy for Identification of Centromeric Heterochromatin Sequences.** In 1953 Krivshenko and Cooper generated an extensive series of free X chromosome duplications by inducing large interstitial deletions within the inverted X chromosome In(1)sc4. Dp(1)fj1187 is the smallest with a size of <30% of the metaphase length of chromosome 4 (cited in ref. 16; see Fig. 1A). This free duplication is a key element in our cloning of centromeric region sequences. Preliminary characterization shows the size of this minichromosome to be 13 Mb, which includes >1 Mb of heterochromatin (ref. 17 and this paper). Thus, this minichromosome can be seen as a DNA molecule that is highly enriched in centromeric sequences and that can be used as a hybridization probe for identification of centromeric region clones. To isolate the DNA of this minichromosome we have used preparative PFG of intact DNA from embryos. This has been possible because we were able to detect the minichromosome by ethidium bromide staining of the gel (Fig. 1B and C).

In parallel, we have prepared a YAC library of *Drosophila* DNA partially digested with EcoRI. The library was constructed by using a modified YAC vector, pYAC-white, that contains a *Drosophila* visible marker. The DNA source for the library was the strain carrying Dp(1)fj1187. The library contains 2250 clones with an average size of 125 kb, >1.8 times coverage of the *Drosophila* genome. Screening this library with DNA from the minichromosome as a colony hybridization probe revealed 27 positive clones. DNA mini-preparations of these clones were run on pulsed-field gels, transferred to nylon membranes, and hybridized again with minichromosome DNA. As a result of this second screening, clone yW6E2 gave one of the stronger hybridization signals.

**Molecular Characterization of yW6E2.** Analysis of the undigested YAC clone would be expected to show a single band. However, yW6E2 shows a major band of 60 kb and a minor band of 100 kb, and these are reduced in size by 15 kb (the size of the YAC vector arms) when digested by EcoRI. To investigate whether these two bands correspond to two unrelated clones or reflect instability of the sequences

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**Fig. 1.** Origin and size of Dp(1)fj1187 minichromosome. (A) Dp(1)fj1187 is the product of a deletion within the inverted X chromosome In(1)sc4. Heterochromatin is shown as an open box and euchromatin is shown as a stippled box (the scheme is not drawn to scale). (B) Ethidium bromide staining of a pulsed-field gel. (C) Filter transfer hybridized with 4.4RR. Lanes: 1, yeast chromosomes from strain YPI48; 2, DNA from Dp(1)fj1187 stock. The gel was run in a waltzer apparatus for 43 hr with a 200-sec pulse time at 90 V.

**Fig. 2.** Dodeca satellite sequence. The sequences of the dodeca satellite inserts in pBK6E201, pBK6E209, pBK6E215, and pBK6E218 are displayed to emphasize the 11- and 12-bp repeats.
pBK6E218—were sequenced (Fig. 2). All the subclones are made up of a tandemly repeated sequence based on the pseudopalindromic 11- and 12-bp CCCGTA/CTG/TG and CCCGTA/CTG/TG consensus sequences. We call this repetitive sequence dodeca satellite. These sequences show the following features: (i) they are the same except for the presence of a C in position 9 of the 12-mer, (ii) the 12-mers are usually found in groups of 3 or 4 monomers, (iii) the 11-mer following each of these groups is usually a variant in the consensus with a change in position 7 from a C to a T. These features suggest the existence of a less well defined higher-order structure. The search with the dodeca satellite consensus sequences in the GenBank and EMBL data bases did not detect any other related sequences. The dodeca satellite does not conform to the general formula (RRN)m(RN)n for *Drosophila* satellites and represents a newly discovered class of G+C-rich satellite in *Drosophila*.

**Organization of Dodeca Satellite DNA in the Genome.** To localize dodeca satellite sequences in *Dp(1f)1187*, this minichromosome was purified by PFG, digested with the restriction enzymes *EcoRI* and *Mbo II*, separated by PFG, blotted, and sequentially hybridized with different probes (Fig. 3). The hybridization of *Dp(1f)1187* DNA to itself showed several large fragments (50–180 kb) for both enzymes. Since *EcoRI* and *Mbo II* digest on average every 4 and 0.7 kb, respectively, in euchromatin DNA, these fragments are likely to be derived from heterochromatic regions. Together they account for >300 kb of minichromosome heterochromatin.

4.4RR, a unique sequence immediately adjacent to the distal breakpoint of *In(l)sc8*, hybridizes to a 90-kb *EcoRI* fragment; thus, this 90-kb fragment probably spans the boundary between euchromatin and heterochromatin of the minichromosome, in agreement with the data shown by Karpen and Spradling (17). Hybridization with yW6E2 DNA or its subclone pBK6E218 detected a 55-kb *Mbo II* fragment. No large *EcoRI* fragment was detected, although yW6E2 itself contains large *EcoRI* fragments. The lack of large hybridizing *EcoRI* fragments, together with the low signal shown by the *Mbo II* fragment indicates that clone yW6E2 did not come from the minichromosome. Thus, this clone was detected in the library because of cross-hybridization of the minichromosome to related sequences elsewhere in the genome.

To investigate the structure of these sequences, genomic DNA was digested with restriction enzymes and hybridized with the dodeca satellite (Fig. 4). *BamHI*, *EcoRI*, and *Xho I*...
cleave the bulk of dodeca satellite into very large fragments, but each enzyme also yields distinctive bands between 1 and 20 kb (Fig. 4). The addition of the sizes of the bands produced by each enzyme indicates that, if each band is a single fragment composed entirely of the satellite, the dodeca satellite is distributed over ~1 Mb. In contrast, Hae III and Kpn I produce mainly fragments that are <50 kb, while most Mbo II restriction fragments are ~50 kb (Fig. 4). There is no genomic EcoRI restriction fragment similar in size to the largest insert detected in yW6E2 preparations (Fig. 4B). It is most likely that this insert comes from an even larger EcoRI fragment, which must be at least 350 kb. A less likely possibility is that yW6E2 is derived from a smaller genomic EcoRI fragment by recombination in yeast. To obtain clones of other members of this sequence family, we probed the YAC library using the insert of clone pBK6E218. Three additional positive clones—yW14G7, yW19B7, and yW21F8—were obtained.

To determine the chromosomal location of this new satellite, purified YACs were used for in situ hybridization to Drosophila metaphase chromosomes. Hybridization with yW6E2 shows that dodeca satellite sequences are present in the centromeric region of an autosome (Fig. 5A). The same result was obtained by using a subcloned fragment of this YAC, pBK6E218, containing just the dodeca satellite sequences (data not shown). We were unable to detect any hybridization of either of these probes to the free duplication Dp(1)f1187 in situ. The blotting experiments presented in Fig. 3 do, however, indicate that these sequences are present on this minichromosome, and so we are led to conclude that on this chromosome they are present at levels beyond their limits of detection by in situ hybridization. In situ hybridization with purified DNA from clone yW14G7 gave a strong signal on an autosome, as expected from its known dodeca satellite component. In addition, it showed a moderately strong signal close to the centromeres of a wild-type Y chromosome (data not shown) and the attached XYS-YL chromosome of the strain carrying the free duplication (Fig. 5B). These results suggest either the presence of a dodeca satellite in the centromeric region of the Y chromosome or the occurrence of the dodeca satellite in association with sequences found close to the centromere of this chromosome.

Conservation of Dodeca Satellite. With few exceptions, the major satellite DNAs are labile during evolution. It is not generally expected that different species will share similar satellite DNA sequences. However, the (TTAGGG), repeat is found in all vertebrate telomeric regions and in the pericentric regions of many vertebrate chromosomes (18). To determine whether sequences related to the dodeca satellite are present in other species, hybridizations were performed at lower levels of stringency. We have found cross-hybridization to DNA of five Drosophila species—D. simul-
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were produced Q998-8 derivative, which has lost Yq sequences (lane 8). These cross-hybridizing sequences could represent a newly discovered human sequence family but are more likely to correspond to the satellite 3 family DYZ1, which consists of tandemly repeated 3.5-kb EcoRI fragments located on Yq. Weak homology between the dodeca satellite consensus sequence and the satellite 3 sequence data of Prosser et al. (19) can be found: CCCG-TACTGTT and CAAACCATT(GGAAT).

In conclusion, we have found a G+C-rich satellite in the centromeric region of D. melanogaster chromosomes, which has similarity to repeated sequences in widely different species. We can suggest two kinds of explanation for this similarity. Dodeca satellite and cross-hybridizing satellites might arise independently, for example by similar pathways of divergence from widespread sequence such as the telomeric TTAGGG (animals) or TTAGGG (plants). Alternatively, dodeca satellite might be conserved, for example because of an important role in centromeric function. If these satellites have a function in segregation, they should be present at all centromeres in each species. However, they were only detected in a subset of chromosomes. Smaller amounts or diverged sequences could be present on other chromosomes. Testing these ideas will require further structural analysis and development of appropriate assays for centromere function.

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