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A gene adjacent to satellite DNA in *Drosophila melanogaster*

(molecular cloning/heterochromatin/transcription/repeated gene family)

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ABSTRACT Several copies of a sequence adjacent to 1.688 g/cm^3 satellite DNA in the *Drosophila melanogaster* genome have been isolated by molecular cloning. This sequence, called the *Dm142* gene, is homologous to a 1.6-kilobase RNA found in both *D. melanogaster* embryos and tissue culture cells. One cloned DNA segment includes two copies of the *Dm142* gene and 1.688 g/cm^3 satellite DNA sequences, which are located between and flanking both gene copies. The *Dm142* gene is repeated many times in the *D. melanogaster* genome, and some copies are not flanked by 1.688 g/cm^3 satellite DNA.

The chromosomes of *Drosophila melanogaster*, like those of other higher eukaryotes, contain centromeric heterochromatin. Although the precise functions of heterochromatin are not known, genetic evidence implicates heterochromatin primarily in germ-line processes, such as meiotic pairing, recombination, and disjunction of chromosomes (1–3). In addition, male fertility factors (J. Kennison and M. Gatti, personal communication) and ribosomal genes (4) have been mapped near constrictions between blocks of heterochromatin.

Physical characterization of the DNA contained in heterochromatin has been previously confined to studies of the highly repeated DNAs, called satellite DNAs, which compose most of the heterochromatic DNA; however, other DNA sequences may have an important role in the functions of heterochromatin. During an analysis of cloned segments of the 1.688 g/cm^3 satellite (1.688 satellite) DNA of *D. melanogaster*, we noted that some segments included nonsatellite DNA sequences adjacent to 1.688 satellite DNA. Since the 1.688 satellite DNA in the genome is located primarily in the heterochromatin of the sex chromosomes (5), it is likely that these cloned adjacent sequences are derived from heterochromatic regions. We report here that one such sequence adjacent to 1.688 satellite DNA is homologous to RNA and has therefore been designated the *Dm142* gene. We have cloned three, possibly four, different copies of *Dm142* that are adjacent to 1.688 satellite DNA. The *Dm142* gene is repeated in the *D. melanogaster* genome, and transcription products from one or more copies are found in both *D. melanogaster* tissue culture cells and embryos.

MATERIALS AND METHODS

Enzymes. Terminal deoxynucleotidyltransferase (6), *Eco*RI endonuclease (7), and polynucleotide kinase (8) were gifts of R. Ratliff, P. Modrich, and J. Chien, respectively. *Hin*dIII, *Xho* I, and *Hae* III restriction endonucleases were purchased from New England BioLabs and *Sst* I from Bethesda Research Laboratories, (Bethesda, MD). *Escherichia coli* DNA polymerase I and *Hin*fI endonuclease were purified as described (9). Bacterial alkaline phosphatase was purchased from Worthington and further purified by DEAE-cellulose (DE52) chromatography.

Preparation and Labeling of DNA. Closed circular plasmid DNAs were isolated as described (9). Phage DNA was prepared from purified phage by adding an equal volume of formamide in the presence of 25 mM EDTA and precipitating with ethanol. Restriction fragments were purified by electrophoresis on agarose gels, followed by electrophoretic elution from the gel into dialysis tubing in Tris/borate/EDTA buffer [89 mM Tris/89 mM boric acid/2.5 mM EDTA at pH 8.3 (10)] diluted 1:20. Uniformly labeled DNA was prepared by nick-translation (11), with [^{32}P]dGTP or [^{32}P]dCTP (300–3000 Ci/mmol; Amersham/Searle) as labeled substrate. Final specific activity of the DNA was 0.5–5 $\times$ 10^8 cpm/μg. For preparation of end-labeled DNA, DNA was first dephosphorylated by incubation with bacterial alkaline phosphatase in 20 mM glycylglycine (pH 9.1) for 1 hr at 60°C. Then the mixture was adjusted to 10 mM potassium phosphate/50 mM glycine-NaOH/10 mM $MgCl_2$/5 mM dithiothreitol at pH 9.5, and 100 pmol of [γ-^{32}P]ATP (1500 Ci/mmol; ICN) was added. DNA was 5′-phosphorylated by incubation with polynucleotide kinase for 30 min at 37°C; then an equal volume of 4 M ammonium acetate was added and DNA was precipitated with ethanol. Subsequent restriction enzyme digestions were carried out in the presence of 10 mM potassium phosphate (pH 7.5).

Recombinant DNAs. The construction of plasmids pDm142 and pDm194 will be described elsewhere, as will the construction of cDm688.52, a hybrid plasmid containing 1.688 satellite DNA. Recombinant plasmid aDm142.1 was prepared by inserting a purified *Hin*fI fragment (see *Results*) into the *Pst* I site of pBR322 (12) by the poly(dG)·poly(dC) tailing method of W. Röwekamp and R. Firtel (personal communication). Poly(dG)-terminated pBR322 was a gift of J. Lis. Transformation of *E. coli* HB101 (13) and selection for tetracycline resistance have been described (9). Phages λDm142.1 through λDm142.4 were recovered from a collection of hybrid phages, which contain segments of *D. melanogaster* (Oregon R) DNA inserted into a λ phage vector (Sep 6) by poly(dA)·poly(dT) joining (E. Meyerowitz, D. Kemp, L. Prestidge, and D. Hogness, personal communication). ^{32}P-Labeled *aDm*142.1 DNA was used to screen this collection by plaque hybridization (14). Phage were grown on *E. coli* C600 (*hrs*) (15), obtained from R. W. Davis, and were purified by two successive CsCl density step gradients (16) and an equilibrium CsCl density gradient. All work with recombinant DNA was performed under the EK1, P2 containment conditions specified by the National Institutes of Health Recombinant DNA Research Guidelines (*Federal Register*, July 7, 1976).

Gel Electrophoresis of DNA and Hybridization Analysis. Restriction fragments were analyzed on 3–7% polyacrylamide gradient slab gels (14 $\times$ 14 $\times$ 0.16 cm), prepared in Tris/borate/EDTA buffer from a stock of 20% acrylamide/1%

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Abbreviation: kb, kilobase.

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N,N'-methylenebisacrylamide, by electrophoresis at 100 V for 2–5 hr.

Electrophoresis in agarose gels has been described (9). DNA fragments were transferred from agarose gels to nitrocellulose by the method of Southern (17). The nitrocellulose sheet was preincubated for several hours at 65°C in hybridization buffer [0.6 M NaCl/75 mM sodium citrate/0.1 M sodium phosphate at pH 7, containing 0.02% (wt/vol) each bovine serum albumin, Ficoll (Pharmacia), and polyvinylpyrrolidone (18) and 0.1% (wt/vol) sodium dodecyl sulfate]. Hybridization with ^{32}P-labeled probes was carried out for 24 hr at 65°C in hybridization buffer containing 100 μg of salmon sperm DNA and 100 μg of poly(A) per ml. The nitrocellulose sheets were washed at least four times in hybridization buffer at 65°C for 30 min each.

RESULTS

Isolation of Recombinant Plasmids Containing 1.688 Satellite DNA and Adjacent Sequences. The 1.688 satellite DNA of *D. melanogaster* is primarily composed of 359 base-pair units repeated in tandem. Most of the 1.688 satellite DNA contains one *Hae* III site in almost every 359 base-pair unit; however, some regions of the satellite DNA contain no *Hae* III sites for more than 10,000 base pairs (9). While studying recombinant plasmids containing these regions of satellite DNA that lack *Hae* III sites (unpublished data), we discovered several plasmids that also contain DNA sequences adjacent, but not homologous, to 1.688 satellite DNA.

Briefly, recombinant plasmids were constructed by inserting large *Hae* III-resistant fragments, purified from *Hae* III-cleaved 1.688 satellite DNA, into the plasmid vector pSC101 by the poly(dA)·poly(dT) joining method (19, 20). Two of these recombinant plasmids, pDm688.142 and pDm688.194 (abbreviated pDm142 and pDm194), are analyzed here in detail. Originally, these two plasmids were found to contain *D. melanogaster* sequences adjacent to 1.688 satellite DNA by hybridization analysis (17) of *Hin*fI restriction fragments. Fig. 1 shows that the *Hin*fI fragments from the *D. melanogaster* DNA segment of each plasmid include one fragment that does not hybridize to a cloned 1.688 satellite DNA probe, in addition to fragments containing satellite DNA.

Sequences Adjacent to Satellite DNA in pDm142 Are Homologous to RNA. The *D. melanogaster* sequences adjacent to 1.688 satellite DNA in pDm142 were chosen first for further investigation. A probe specific for these sequences, but not homologous to 1.688 satellite DNA, was prepared by subcloning the 1.1-kilobase (kb) fragment generated from pDm142 by *Hin*fI endonuclease cleavage (see Fig. 1). This fragment, which

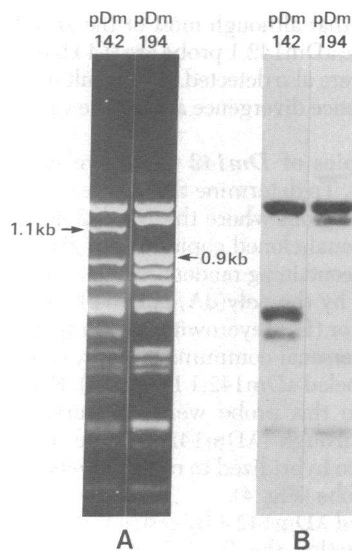

FIG. 1. *D. melanogaster* sequences adjacent to 1.688 satellite DNA in pDm142 and pDm194. Plasmid pDm142 and pDm194 DNAs were cleaved with *Hin*fI endonuclease, and the resulting fragments were electrophoresed on a 1.2% agarose gel (*A*). The fragments were then transferred to nitrocellulose (17) and hybridized with ^{32}P-labeled 1.688 satellite DNA probe, prepared from hybrid plasmid cDm688.52. An autoradiogram of the nitrocellulose (*B*) shows that each plasmid produced several fragments containing 1.688 satellite sequences. In addition, pDm142 produced a 1.1-kb fragment which is derived from the *D. melanogaster* insert but did not hybridize to the probe. Plasmid pDm194 produced an analogous unlabeled fragment, 0.9 kb in size, which migrates close to a vector fragment of similar size. The unlabeled fragments common to the two plasmids are derived from the vector.

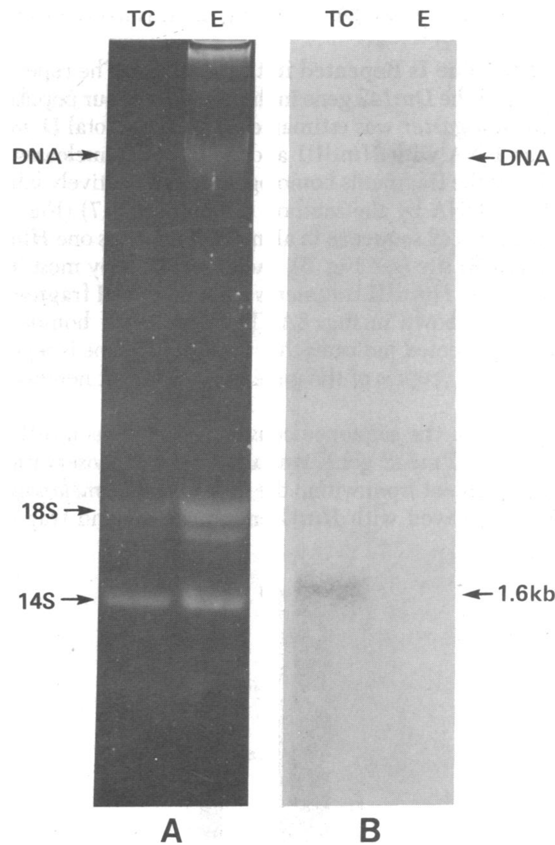

FIG. 2. RNA homologous to aDm142.1. Total nucleic acid was extracted from cultured cells of Eschalier's Kc_0 line (21) and poly(A)-containing RNA was selected by oligo(dT)-cellulose chromatography (gift of R. Lifton). Total nucleic acid extracted from 19-hr *D. melanogaster* embryos was a gift of G. Rubin. Poly(A)-containing RNA was selected from both these samples by chromatography on poly(U)-Sepharose (Pharmacia) (22). Poly(A)-containing RNA from tissue culture cells (TC) and embryos (E) ($\approx$10–30 μg) was denatured by treatment with dimethyl sulfoxide and glyoxal and electrophoresed on a 1.0% agarose gel (23), shown in *A*. *Hin*dIII-cleaved phage λ DNA was also glyoxylated and included on the gel as a size standard. The positions of 18S ribosomal RNA, which migrates close to 28Sα and 28Sβ ribosomal RNAs, and of 14S mitochondrial RNA are indicated. The gel was treated with 50 mM NaOH for 50 min at 25°C and washed twice with 0.1 M sodium phosphate (pH 6.8) and twice with 0.02 M sodium phosphate (pH 6.8). The RNA was then transferred to diazobenzyloxymethylcellulose-paper in such a way as to maintain the relative positions of different RNA species (21) and hybridized with ^{32}P-labeled aDm152.1 DNA. An autoradiogram of the paper (*B*) showed that a 1.6-kb RNA species was detected in both RNA samples. However, so little of this RNA is present in our embryonic RNA that it was not visible after photographic reproduction. DNA in the embryonic RNA sample was also labeled.

contains *D. melanogaster* sequences adjacent to satellite DNA, a poly(dA)·poly(dT) joining sequence, and some vector DNA, was inserted into the plasmid pBR322 (12) by the poly(dG)·poly(dC) joining procedure. The hybrid plasmid aDm142.1 was recovered (see Fig. 5).

Using aDm142.1 as a hybridization probe, we asked whether the sequences adjacent to satellite DNA in pDm142 are homologous to RNA. Poly(A)-containing RNA was prepared from *D. melanogaster* embryos and Eschalier's Kc_0 line of cultured cells, and these RNA samples were analyzed by a technique combining gel electrophoresis and hybridization (21) (Fig. 2). A 1.6-kb RNA species homologous to the probe was detected in both tissue culture cell and embryo RNA. This evidence indicates that the sequences adjacent to satellite DNA in pDm142 are part of a unit, called here the *Dm142* gene, which is homologous to RNA. Since the 1.6-kb RNA species bound to poly(U)-Sepharose (see legend to Fig. 2), it is likely this RNA contains poly(A).

***Dm142* Gene Is Repeated in the Genome.** The repetition frequency of the *Dm142* gene in the genomes of our population of *D. melanogaster* was estimated by cleaving total *D. melanogaster* DNA with *Hin*dIII and *Eco*RI endonucleases and identifying the fragments homologous to radioactively labeled aDm142.1 DNA by the method of Southern (17) (Fig. 3*A*). Since the *Dm142* sequence in aDm142.1 contains one *Hin*dIII and no *Eco*RI site (see Fig. 5), each *Dm142* copy most likely produced two *Hin*dIII fragments and one *Eco*RI fragment in the patterns shown in Fig. 3*A*. The number of homologous fragments detected indicates that the *Dm142* gene is repeated and that many copies of the gene have different neighboring sequences.

To measure the sequence conservation between different copies of the *Dm142* gene, we examined the conservation of a *Hin*fI fragment from within the gene. Total *D. melanogaster* DNA was cleaved with *Hin*fI endonuclease, and fragments

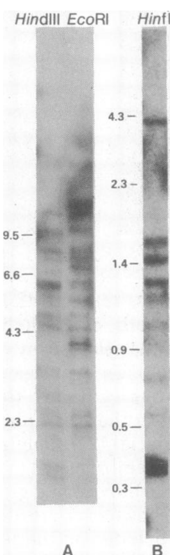

FIG. 3. Repetition of *Dm142* gene in the genome. Total *D. melanogaster* DNA (4 μg), isolated according to Wensink *et al.* (24), was digested with *Hin*dIII and *Eco*RI endonucleases. The digestion products were electrophoresed in a 0.5% agarose gel, transferred to nitrocellulose (17), and hybridized with ^{32}P-labeled aDm142.1 DNA. Fragments homologous to the probe were detected by autoradiography (*A*). Positions of *Hin*dIII-cleaved λ DNA size standards included on the gel are indicated. An identical experiment is shown in *B*, except that cleavage was with *Hin*fI endonuclease and electrophoresis was in a 1.0% agarose gel. The size standards on this gel included those in *A* and also *Hae* III-cleaved PM2 DNA.

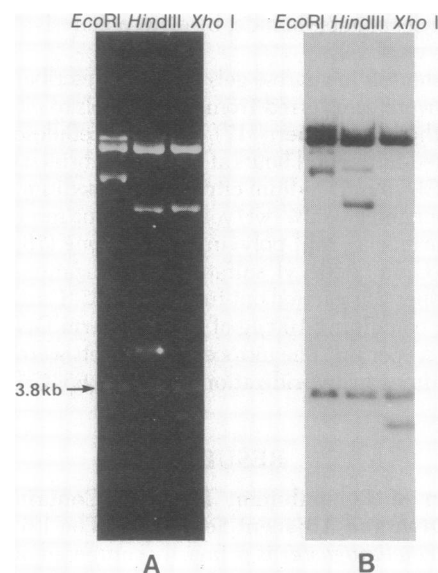

FIG. 4. Restriction fragments of λDm142.4. Phage λDm142.4 DNA was cleaved with *Eco*RI, *Hin*dIII, and *Xho* I endonucleases, and the fragments were electrophoresed on a 0.5% agarose gel (*A*). Cleavage with each of these enzymes generates a 3.8-kb fragment. The DNA fragments were transferred from the gel to nitrocellulose and hybridized with ^{32}P-labeled 1.688 satellite DNA probe prepared from hybrid plasmid cDm688.52. An autoradiogram of the filter (*B*) showed that the 3.8-kb fragments in each digest were labeled by the probe, as were other fragments containing 1.688 satellite sequences. These data and data from analogous experiments with other enzymes allowed us to map regions of λDm142.4, pDm142, and pDm194 that contain satellite DNA (see Fig. 5).

homologous to aDm142.1, which contains sequences internal to the *Dm142* gene (see Fig. 5), were detected by hybridization. Fig. 3*B* shows that although most of the *Hinf* fragments homologous to the aDm142.1 probe are 0.4 kb in size, fragments of other sizes were also detected. This result indicates that there is limited sequence divergence among the various copies of the *Dm142* gene.

Several Copies of *Dm142* Gene Are Adjacent to 1.688 Satellite DNA. To determine the nature of some of the other chromosomal regions where the *Dm142* gene is located, we isolated additional cloned copies of the gene. A collection of hybrid phages containing random segments of *D. melanogaster* DNA inserted by the poly(dA)·poly(dT) joining method into a λ phage vector (E. Meyerowitz, D. Kemp, L. Prestidge, and D. Hogness, personal communication) was screened with radioactively labeled aDm142.1 DNA (14). Four hybrid phages homologous to this probe were recovered and designated λDm142.1 through λDm142.4. One of these phages, λDm142.4, also hybridized to radioactively labeled 1.688 satellite DNA probe (Fig. 4).

We analyzed λDm142.4 by restriction enzyme cleavage to determine whether the *Dm142* sequences are immediately adjacent to satellite DNA, as in pDm142. Fig. 4*A* shows that *Xho* I, *Eco*RI, and *Hin*dIII endonucleases each cleaves λDm142.4 at two sites 3.8 kb apart, and a similar result was obtained with *Hae* III endonuclease. The repetition of a set of four restriction sites at two positions 3.8 kb apart indicated that two copies of a sequence, which is shown below to be the *Dm142* gene, are present in this cloned DNA segment. Fig. 5 shows a map of the *Xho* I, *Eco*RI, *Hin*dIII, and *Hae* III endonuclease sites in λDm142.4. To assess the similarity between the two gene copies, we also mapped the *Hin*fI endonuclease sites within and near both copies (Fig. 5). Previous data had shown that *Hin*fI endonuclease sites are not completely con-

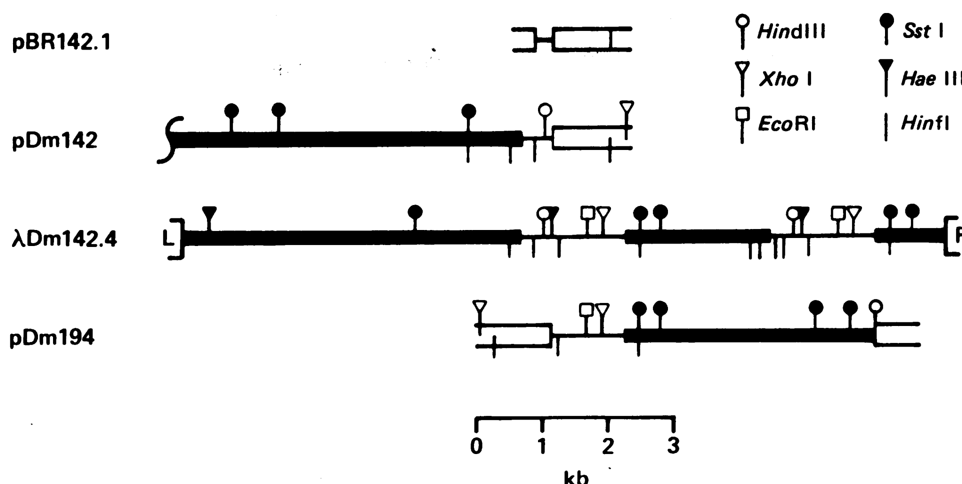

FIG. 5. Restriction maps of cloned *Dm142* sequences adjacent to 1.688 satellite DNA. Dark bars represent 1.688 satellite DNA; the thin lines, *Dm142* sequences. Open blocks represent vector DNA; L and R indicate the left and right arms of the λ vector, respectively. Positions of restriction sites were in most cases determined by digestion with restriction enzymes singly and in pairs, followed by electrophoretic analysis of the products on agarose gels. *Sst* I and *Hin*fI sites were also mapped by analysis of the partial digestion products of end-labeled fragments on agarose or 3–7% gradient polyacrylamide gels (25). For pDm142, a fragment end-labeled with ^{32}P at the *Xho* I site was used. For λDm142.4, two fragments were prepared, each extending leftward on the map from an *Eco*RI site, which was labeled. *Sst* I sites in pDm194 were mapped by labeling the plasmid at the *Eco*RI site. Only those *Hin*fI sites close to or within a *Dm142* sequence are shown on the maps. Regions containing 1.688 satellite DNA or *Dm142* sequences were distinguished by hybridization analysis of restriction fragments, using as probes ^{32}P-labeled aDm142.1, λDm142.3, or satellite DNA (see Fig. 4); this method does not allow us to state with certainty that the regions indicated by dark bars are entirely composed of satellite DNA. The junction between the *Dm142* and satellite DNA regions in pDm142 was mapped between two *Hin*fI sites, and the analogous junctions in λDm142.4 are drawn similarly; however, the exact position of the junction is unknown. The junction at the rightward side of the gene has been mapped between the *Xho* I and *Sst* I sites in λDm142.4 and pDm194, but again the exact position is unknown. From these data the size of the gene is between 1.1 and 1.9 kb. Plasmid pDm142 contains an additional 3.2 kb of satellite DNA not shown on the map. In both pDm142 and pDm194 the *Dm142* gene is truncated by the join to vector DNA exactly at the position of a *Hae* III endonuclease site. Such a structure would be expected because both plasmids were constructed from fragments of 1.688 satellite DNA produced by *Hae* III endonuclease digestion.

served among different copies of *Dm142* (Fig. 3*B*). Although the two gene copies of λDm142.4 would have produced fragments of identical size in the experiment shown in Fig. 3*B*, two *Hin*fI sites are present near one copy but not near the other. One of these *Hin*fI sites may be within the gene; however, the endpoint of the gene has not been mapped precisely.

The locations of 1.688 satellite DNA in λDm142.4 were determined by hybridization analysis of restriction fragments by the technique of Southern (17). Fig. 4*B* shows that fragments derived from either side of the *Dm142* genes and also from between the two genes hybridize to a 1.688 satellite DNA probe. Thus, 1.688 satellite DNA sequences are located both between and flanking the two gene copies. In similar experiments the endpoints of the 1.688 satellite DNA have been mapped within the fragments indicated in Fig. 5. The region assigned to the *Dm142* gene is between 1.1 and 1.9 kb, which corresponds to the size of the RNA species homologous to *Dm142* (Fig. 2).

Next we compared λDm142.4 with pDm142, both to confirm the identification of the two repeated units in λDm142.4 as *Dm142* genes and also to determine whether these two clones were derived from the same or different regions of the *D. melanogaster* genome. Fig. 5 shows a map of pDm142 that has been aligned with the map of λDm142.4 on the basis of restriction site positions. This alignment was confirmed by the finding that radioactively labeled aDm142.1 DNA hybridizes to a 0.4-kb fragment generated by *Hin*fI endonuclease digestion of λDm142.4, as would be predicted (data not shown). Comparison of the maps indicates that the two repeated units in λDm142.4 can be identified as *Dm142* genes by their similarity to the region of pDm142 defined as *Dm142* sequences. This comparison also reveals that the 1.688 satellite DNA sequences adjacent to *Dm142* in the two clones are not identical, as judged by the distribution of restriction sites. λDm142.4 and pDm142 must therefore correspond to different genomic copies of *Dm142*.

It was a surprise to discover that pDm194, an independent hybrid plasmid containing a *Hae* III-resistant fragment of 1.688 satellite DNA, hybridized to radioactively labeled DNA from phage λDm142.3, a clone containing *Dm142* sequences. A map of pDm194 was determined by both restriction enzyme cleavage and hybridization analysis of restriction fragments, and has been aligned with that of λDm142.4 in Fig. 5. Plasmid pDm194 contains part of the *Dm142* gene immediately adjacent to 1.688 satellite DNA. Whether pDm194 represents yet a fourth independent copy of *Dm142* is not clear; the data cannot rule out the possibility that pDm194 corresponds to either the missing half of the *Dm142* gene cloned in pDm142 or the right-most gene copy in λDm142.4.

Heteroduplex analysis (26) of pDm142 and pDm194 has revealed that the orientation of the *Dm142* gene relative to 1.688 satellite sequences is the same in both plasmids. Heteroduplex structures were observed which exhibited base pairing between vector sequences, but not between regions of 1.688 satellite DNA (data not shown). This result shows that the satellite DNA segments have opposite orientations within the two plasmids. Since the orientations of the *Dm142* sequences are also opposite (Fig. 5), it follows that the orientation of *Dm142* relative to satellite DNA is the same in pDm142 and pDm194.

DISCUSSION

We have shown here that three, possibly four, independent copies of the repeated gene *Dm142* are located adjacent to 1.688 satellite DNA in the genomes of our population of *D. melanogaster*. Two copies were recovered in recombinant plasmids constructed from a minor component of this satellite DNA that is depleted in *Hae* III endonuclease sites; indeed, of six recombinant plasmids analyzed, two contained *Dm142* sequences (unpublished observation). The satellite DNA next to

the two genes in λDm142.4 is also depleted in *Hae* III sites, although it was not selected as *Hae* III-resistant DNA. These data suggest that *Dm142* genes are preferentially located next to those regions of 1.688 satellite DNA that lack *Hae* III sites. However, the recovery of three hybrid phages, λDm142.1 through λDm142.3, which are homologous to *Dm142* but do not contain any 1.688 satellite DNA, suggests that not all copies of *Dm142* in the genome are adjacent to 1.688 satellite DNA.

Studies of cloned DNA segments must always confront the question: does this cloned segment faithfully represent sequences in the genome of the organism of origin? We have previously shown that 1.688 satellite DNA cloned in the plasmid pSC101 is stable during transformation and propagation of the DNA in *E. coli* (Rec^+) (9). However, because tandem duplications are known to arise spontaneously in phage λ (27), the possibility remains that the presence of two copies of *Dm142* in λDm142.4 resulted from a duplication event that occurred in *E. coli* rather than in *D. melanogaster*. The finding that the DNA sequences near or within the two gene copies do not contain identical *Hin*fI endonuclease sites makes this possibility less likely, in our view.

One or more copies of the *Dm142* gene must be transcribed to produce the 1.6-kb RNA found in *D. melanogaster* embryos and tissue culture cells, but whether any copies flanked by satellite DNA are transcribed is not known. Since the bulk of the 1.688 satellite DNA has been localized in the heterochromatin of the sex chromosomes of *D. melanogaster* (5), it is likely that the *Dm142* genes adjacent to 1.688 satellite DNA are there also. Heterochromatin and the highly repeated satellite DNA sequences contained within it have long been thought to be transcriptionally inactive. This conclusion appears to be true in particular for 1.688 satellite DNA, as we have been unable to detect sequences homologous to cloned 1.688 satellite DNA probe in RNA from *D. melanogaster* in the prepupal stage of development by using reassociation kinetics (28). However, transcription proceeds from some genes located near constrictions between heterochromatic regions of the chromosomes; for example, the ribosomal genes and the fertility genes on the Y chromosome which are active during spermatogenesis (29). Whether any of these transcribed genes are located immediately adjacent to satellite DNA is not known. In any case, existing evidence does not preclude fine interspersion of transcriptionally active and inactive regions of chromatin, and the possibility remains that *Dm142* genes adjacent to satellite DNA are transcribed.

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