Volume 8 Number 6 1980 Nucleic Acids Research

Clusters containing different mobile dispersed genes in the genome of Drosophila melanogaster

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Received 11 February 1980

ABSTRACT

Ten clones containing the actively transcribed mobile dispersed gene Dm225 and its flanking sequences were selected from the HindIII bank of the Drosophila melanogaster genome. The Dm225 sequences present in these clones were identical while the flanking sequences were different in all of the clones analysed. Four of them contained, in addition to Dm225, other DNA sequences binding high amounts of cytoplasmic poly(A)$^+$RNA. The properties of these new genes are similar to those of Dm225: they are also actively transcribed, multiple in copies, scattered throughout the genome, and located at varying genome sites which also were scattered throughout the whole genome of D. melanogaster. Thus, different mobile dispersed genes often appear as closely apposing units forming gene clusters in the genome.

INTRODUCTION

It has been found previously that several genes of Drosophila melanogaster responsible for synthesis of abundant mRNAs (poly(A)$^+$RNA from the cytoplasm or purified polysomes) are repetitive and are scattered throughout the genome /1,2/. These genes represent the mobile elements of the genome. Their location in chromosomes varies drastically among different strains of D. melanogaster and even among the individuals of the same stock /1,3,4/. The multigene family Dm225 has been analysed in more detail previously /5/. A 2.9 kb fragment of Dm225 DNA limited by EcoRI restriction sites hybridizes to abundant mRNA. About 250 copies of this gene are found in the genome located at 20-30 sites in polytene chromosomes. Experiments on hybridization of Dm225 DNA to total restricted DNA of D. melanogaster have demonstrated close similarity or rather identity of all these 250 gene copies. In contrast, the

flanking sequences, at least those adjacent to the end of the coding region, were heterogeneous.

To analyse the nature of sequences adjacent to the Dm225 gene, we isolated new clones containing this gene and the flanking sequences. Since the previously isolated EcoRI DNA fragment containing the gene Dm225 has no HindIII restriction sites, we prepared a bank of HindIII Drosophila DNA fragments in pBR322 plasmid. A number of new clones containing the Dm225 gene in DNA fragments up to 20 kb were selected. Four fragments out of ten analysed were found to contain other genes different from Dm225 but possessing many similar properties. They are actively transcribed, repetitive, dispersed, and occupy varying location in the genome. Thus, in the chromosomes of D. melanogaster, there are regions which contain several different mobile dispersed genes in close neighbourhood.

MATERIALS AND METHODS

Cloning. The HindIII bank of D. melanogaster DNA inserted into pBR322 plasmid (a generous gift of Prof. H.Boyer) was prepared as described below. About 3000 colonies were screened for the existence of the Dm225 sequence by colony hybridization /6/ with [^{32}P]labeled Dm225 DNA. Twenty clones bound label efficiently. Sixteen of them were finally selected, grown, and DNA was prepared and restricted by HindIII endonuclease. Six clones containing two HindIII insertion fragments were rejected while ten other clones containing just one HindIII were used for further analysis.

Enzymatic treatment of DNA. The digestion of DNA by all restriction endonucleases used in the work (EcoRI, HindIII, HaeIII, PstI and BamHI) was performed in the same medium containing: 50 mM NaCl; 40 mM Tris-HCl buffer, pH 7.4; 10 mM $MgCl_2$; 10 mM 2-mercaptoethanol and 20-100 μg/ml DNA at 37°C for 20 min. HindIII and PstI endonucleases were a kind gift of Dr. V.Nosikov. The growing and purification of recombinant plasmids were as described previously /5/. Labeling of DNA by nick translation was performed according to Rigby et al. /7/.

To prepare recombinant DNA, the embryonic DNA of D.melano-

gaster and pBR322 DNA were separately digested by an excess of HindIII restriction endonuclease and then fractionated by electrophoresis in 0.8% agarose gel (Calbiochem). The 6–20 kb fragments of Drosophila DNA and a band of linear pBR322 DNA were eluted. About 20 μg of D. melanogaster DNA and 0.1 μg of pBR322 DNA were mixed and ligated in 200 μl of a solution containing: 0.1 M NaCl; 40 mM Tris-HCl buffer, pH 7.5; 10 mM $MgCl_2$; 10 mM 2-mercaptoethanol; 0.08 mM ATP and T4-induced DNA ligase (a kind gift of Dr. G.Dolganov) at 19°C for 1 hr. The reaction was continued for 16 hrs after a 10-fold dilution with the same solution.

Transformation. For transformation of E.coli cells, the strains E.coli K12 802 rK^-mK^+ /9/ and E.coli BH101 rB^-mB^- (a generous gift of Prof. H.Boyer) were used. The first strain gave about 5 times higher transformation rates (up to 10^7 colonies per 1 μg of pBR322 DNA and up to 10^6 colonies per 1 μg of pBR322 used for ligation).

E.coli cells were grown in 100 ml of L broth containing 50 μg/ml of thymidine for 2-2.5 hrs at 37°C. Then the suspension was chilled in an ice-cold water bath for 10-15 min. The cell pellet was suspended after centrifugation at 5000 x g (0°C, 5 min) in 50 ml of a solution containing 50 mM $CaCl_2$ and 50 μg of thymidine /10/. After a 5 min incubation at 0°C and centrifugation, the pellet was collected, suspended in the cold above mentioned Ca^{++} solution (5 ml), and used for transformation. From 50% to 90% of colonies obtained after transformation contained insertions of D. melanogaster DNA. The isolated recombinants could grow on both tetracycline (25 μg/ml) and ampicillin (50–100 μg/ml) plates. The work was done in the P3 conditions.

RNA isolation. The D. melanogaster cell culture /11/ was labeled as previously described /5/. Cytoplasmic poly(A)$^+$RNA was prepared according to McKenzie et al. /12/ after labeling the cells for 9 hrs. Total rapidly-labeled RNA was obtained after the incubation for 2 hrs with [^{32}P]orthophosphate according to Scherrer et al. /13/. Complementary DNA (cDNA) was prepared with the aid of reverse transcriptase using cytoplasmic

poly(A)$^+$RNA as the template /14/. AMV reverse transcriptase was obtained from Prof. L.L.Kisselev.

Hybridization. Molecular hybridization experiments were performed as previously described /5/. Filters for colony hybridization experiments were prepared by method of Grunstein and Hogness /6/ with some modifications. Cell debris was not removed from the filters. This might increase the hybridization rate several times and make the screening more sensitive. The filters, prior to hybridization, were soaked in a 10-fold concentrated Denhardt solution /16/ containing 2xSSC and 5 μg/ml of poly(U) (Reanal), and allowed to dry at room temperature. Then the filters were soaked again in a new portion of the same solution containing about 0.5×10^6 cpm/ml of Dm225 DNA fragment labeled with [^{32}P] by nick-translation. The in situ hybridization was performed as described /4/.

RESULTS

1. Dm225 fragments present in different clones are identical while flanking sequences are different

Ten clones containing the Dm225 sequence were selected and DNA was isolated from them. The DNA was restricted by HindIII and EcoRI endonucleases and fractionated by gel electrophoresis in 1% agarose (Fig. 1). In all cases, a 4.3 kb fragment (the DNA of pBR322 plasmid), a 2.9 kb fragment (corresponding in size to Dm225 DNA), and also a 1.4 kb DNA fragment were present. In 4% agarose gel, an additional 0.25 kb fragment was also present in all clones studied. Moreover, a number of fragments were also observed which differed in DNA from different clones.

The physical map of the cloned DNA fragments is presented in Fig. 2. One can see that the 2.9 kb fragment limited by EcoRI sites is present in all clones. It corresponds to the original Dm225 DNA excised by EcoRI endonuclease from the D. melanogaster genome. It is located in the middle of insertions in agreement with the previous data showing that HindIII sites are absent from the Dm225 sequence /5/. The direction of

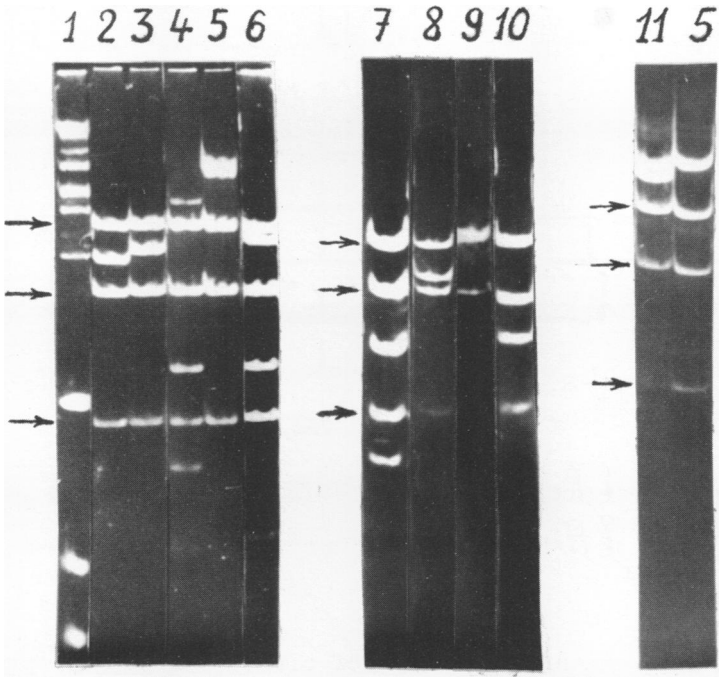

Fig. 1. Electrophoretic separation of DNA fragments obtained by treatment with EcoRI and HindIII endonucleases on 1% agarose slab gels.

The arrows indicate the positions of pBR322 (4.3 kb), Dm225 (2.9 kb), and 1.4 kb fragment bands.

1 - λC1857 DNA cleaved by EcoRI endonuclease and SV40 DNA digested by HaeIII restriction enzyme which were used as markers.

2 -11 The DNAs of clones H512, H115, H52, H34, H27, H251, H22, H110, H130, H47, respectively.

transcription is from the right to the left /5/ (see Fig. 2). The 0.25 kb EcoRI-restricted fragment is located upstream to the coding sequence and the 1.4 kb fragment is found next to it. However, the heterogeneity appears at this point. In some clones, the 1.4 kb fragment is limited by EcoRI sites, in the others, it is limited by EcoRI and HindIII sites. Thus, at least in the distal part of the 1.4 kb fragment, the sequence diversity takes place. Fragments next to the distal EcoRI site of the 1.4 kb fragment are also heterogeneous. Fragments lo-

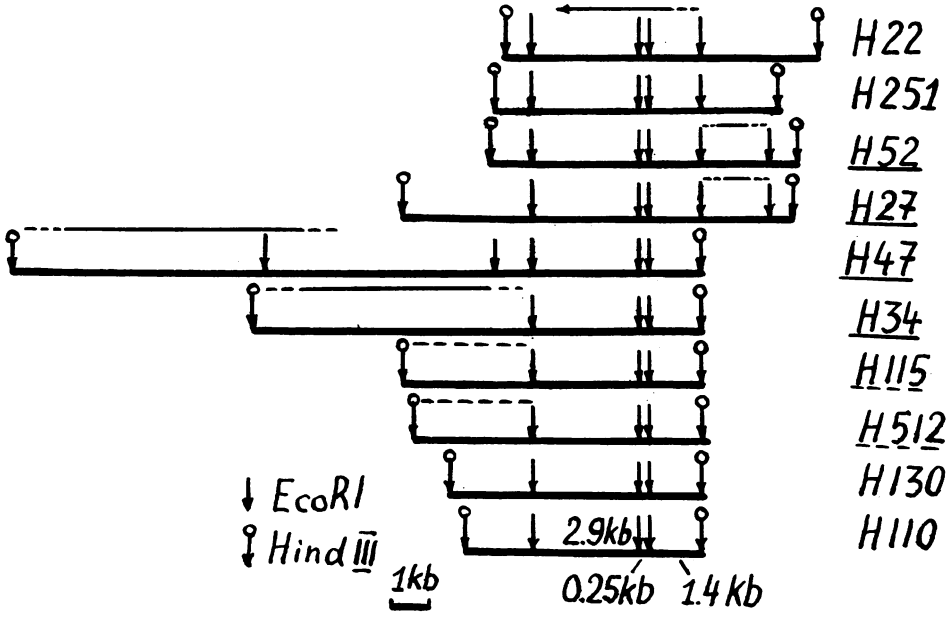

Fig. 2. Restriction enzyme maps of cloned DNA fragments. Location of mRNA transcribed from Dm225 gene sequence and direction of its transcription are shown on top.

calized at the left flank of the Dm225 sequence are different in all clones analysed. This result is in agreement with the previous observation indicating the appearance of differences as high as 100 base pairs after the end of the Dm225 sequence /5/.

The homogeneity of the Dm225 sequence present in ten clones was confirmed by comparing the EcoRI-HaeIII restriction fragments obtained from the 2.9 kb fragments. In all cases, they exactly coincide.

The appearance of heterogeneity within the 1.4 kb fragment, in addition to the above mentioned data, is proved by the following experiment. The [^{32}P]labeled 1.4 kb EcoRI-fragment was hybridized to the Southern filters containing EcoRI restricted total DNA of D. melanogaster. On the radioautograph (Fig. 3), one can see, in addition to the major 1.4 kb labeled band, also 0.9, 1.7 and 3.0 kb EcoRI-bands as well

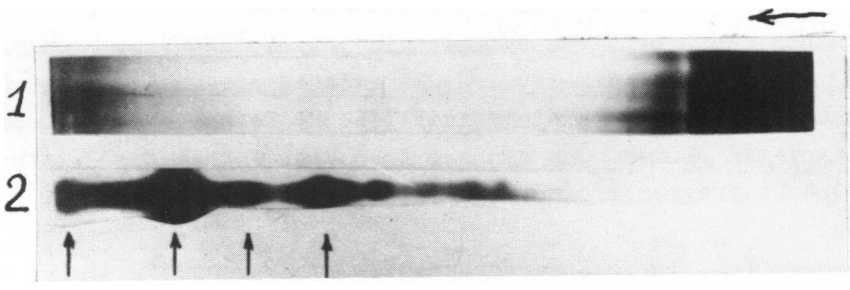

Fig. 3. Heterogeneity of EcoRI fragments containing sequences which are present in the 1.4 kb DNA fragment excised from clone H47 by EcoRI endonuclease.

1 - Electrophoretic separation in 1% agarose gel of total D. melanogaster cell culture DNA digested by EcoRI endonuclease.
2 - Radioautograph of the strip containing EcoRI cleaved DNA hybridized to the [^{32}P]labeled 1.4 kb fragment.

The arrows indicate the positions of 3.0, 1.7, 1.4, and 0.9 kb fragments.

as several minor bands in the range from 3 to 10 kb. However, this heterogeneity probably begins closely to the right EcoRI site as two HaeIII subfragments in the 1.4 kb fragment from all clones analysed were of the same size.

To prove the sequence differences among the fragments differing in size, their restriction maps obtained with the aid of PstI, BamHI, and HaeIII endonucleases were compared. No similarity was found between the long left-arms fragments in the clones H34 and H47 or between the two 1.8 kb fragments and the 0.8 and 0.75 kb fragments present in clones H27 and H52 (Fig. 2).

The conclusion can be drawn that the Dm225 sequence is homogeneous in all of the ten clones and that this homogeneity continues upstream for 1.6 kb. After this point and immediately downstream from the end of the Dm225 sequence, the sequence heterogeneity takes place.

2. Other genes encoding abundant poly(A)$^+$RNAs are located close to Dm225 sequence

To analyse the transcription events within the cloned DNA fragments, we hybridized cytoplasmic poly(A)$^+$RNA (presumably mRNA) or total rapidly-labeled RNA (mostly pre-mRNA) to the Southern filters containing HindIII-EcoRI restricted DNA from the isolated clones. One can see from Fig. 4 that the 2.9 kb and 1.4 kb fragments bind high amounts of the label in all cases.

Moreover, three other fragments, each present in only one of the clones, namely the 7.5 kb fragment of H34 DNA and 1.8 kb fragments of H27 and H52 DNA, hybridized to mRNA. Especially high binding was observed in the case of the 7.5 kb fragment. The same is true of the 7.0 kb fragment in the clone

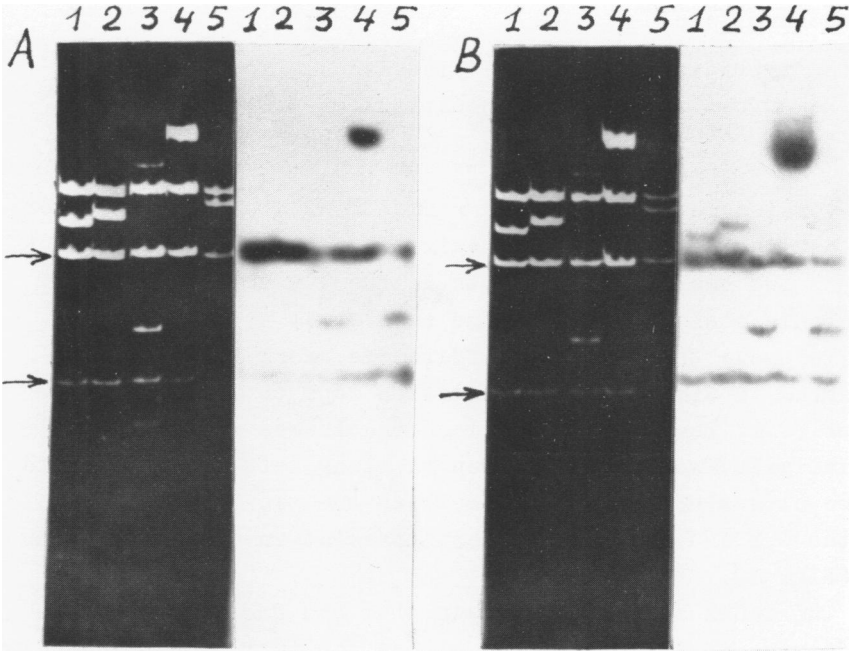

Fig. 4. Hybridization of [^{32}P]mRNA(A) and [^{32}P]pre-mRNA (B) to EcoRI-HindIII fragments of some selected clones.

The electrophoretic patterns and radioautograms are presented. The arrows indicate the positions of 2.9 kb and 1.4 kb bands.

1-5 - The DNA of clones H512, H115, H52, H34, and H27, respectively.

H47 (Fig. 5A). In general, pre-mRNA hybridizes to the same fragments as cytoplasmic poly(A)$^+$RNA does. However, in addition it binds also to the 3 kb, 3.5 kb fragments of H512 and H115 DNAs (Fig. 4).

Experiments were also performed with [^{32}P]cDNA transcrib-

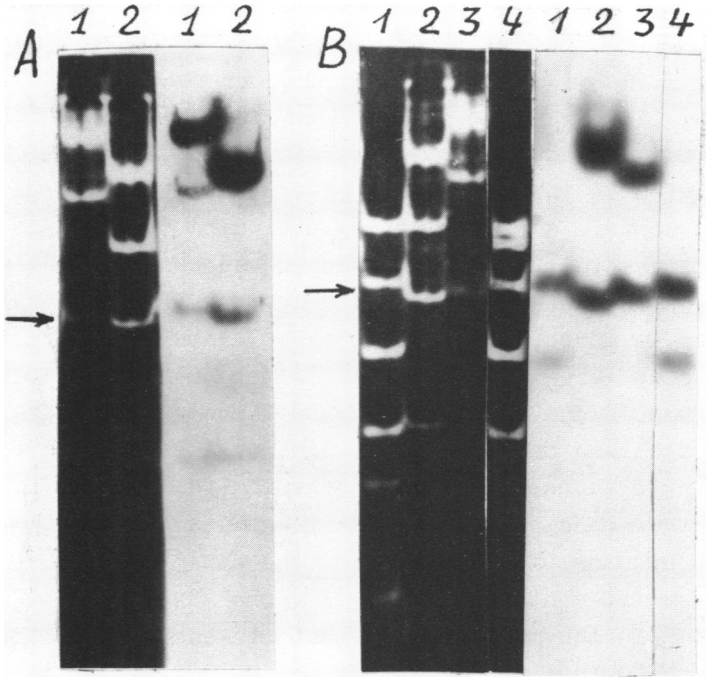

Fig. 5. Hybridization of [^{32}P]labeled mRNA(A) and cDNA(B) to cleaved DNA fragments of some selected clones.

The 1% agarose slab gel was used for electrophoresis and blotting procedure. The electrophoretic patterns and radioautographs are presented.

The arrows indicate the position of the 2.9 kb DNA fragment. The DNA of clone H47 was digested by EcoRI restriction enzyme in order to obtain better separation of the 7.0 and 6.3 kb fragments (the first fragment is attached to the vector and present in 11.9 kb band).All other DNAs were digested by both EcoRI and HindIII restriction enzymes.

A. 1-2 - The DNA fragments of clones H47 and H34, respectively.
B. 1-4 - The DNA fragments of clones H52, H34, H47 and H27, respectively.

ed from cytoplasmic poly(A)$^+$RNA (Fig. 5B). Since cDNA was rather short and transcribed from the region of mRNA just attached to the polyadenylated 3'-end, it did not hybridize to fragments located far from it. Therefore, if two fragments bind cDNA, it means that one deals with two independent transcriptional units.

One can see (Fig. 5B) that the 1.4 kb fragment, in contrast to the 2.9 kb one, does not bind cDNA. Thus, they both seem to encode just one mRNA. On the other hand, the 1.8 kb fragments (clones H27 and H52) hybridize to cDNA and thus they contain independent 3'-ends. The DNA of clone H47 which was not analysed in the experiment presented in Fig. 4 was also found to contain a region strongly binding cDNA. It is located downstream to the Dm225 DNA coding sequence.

To check whether different fragments do really hybridize to different mRNAs, competition experiments were performed. mRNA samples were incubated with filters comprising a certain DNA fragment alone or together with filters containing an excess of the same or other DNA fragment. A strong competition was observed in both filters containing the same DNA fragment. On the other hand, different DNA fragments did not compete for mRNA binding (Table 1). Thus, the mRNAs which bind to different DNA fragments are really diverse mRNA species.

A conclusion may be drawn that at least four other genes encoding cytoplasmic poly(A)$^+$RNA occur in different combinations in close neighbourhood to Dm225 DNA copies.

3. The neighbours of Dm225 DNA are mobile dispersed genes

Some properties of these novel genes were studied. They encode abundant classes of mRNA, accounting to from 0.1 to 1% of the total (Table 1).

The DNA from clones H27, H52, H34 and H47 was hybridized to total D. melanogaster DNA labeled by nick translation. 1% or more of total DNA was hybridized to immobilized DNA of any clone (Table 1). Only 0.75% of total DNA was bound to the 2.9 + 1.4 kb fragments. The rest hybridized to other DNA sequences present in the clones. This result suggests that other sequences present in the clones are also repetitive. If total label-

Table 1. Hybridization properties of the DNA fragments containing D. melanogaster genome

Clones	Fragments	% of [^{32}P]mRNA hybridizes	% of [^{32}P]pre-mRNA hybridized	% of [^{32}P]cDNA hybridized	% of [^{32}P]cDNA depleted in repeats[x]	% of total [^{32}P]labeled Drosophila DNA
H34	7.5 kb	1.5 1.2[xx] 0.1[xxx]	0.5	0.8	0.02	1.2
H47	7.0; 6.3 kb	1.2 1.1[xx] 0.15[xxx]	0.4	1.2	0.025	0.8
H27	1.8 kb	0.3 0.3[xx] 0.01[xxx]	0.3	0.2	0.005	0.15
H52	1.8 kb	0.1 0.1[xx] 0.01[xxx]	0.1	0.1	0.004	0.25
H130	2.2; 2.9; 1.4 kb	1.1 1.1[xx] 0.12[xxx]	0.3	0.5	0.008	0.9

DNA fragments used in the hybridization experiments were eluted from 0.9% agarose gels and immobilized on nitrocellulose filters. Sectors of the filters containing 0.1-0.5 μg DNA were separately incubated with different labeled RNA and DNA preparations in 50-100 μl of a solution containing 2xSSC - 0.2% SDS and 10 μg of poly(U). In most cases, the annealing was performed at 65°C for 15 hrs. Each figure represents the average of three separate results with samples of the same RNA or DNA. The background (determined as hybridization to filters containing 0.5 μg of pBR322 or EcoRI DNAs) in all cases was very low (~0.001% of input counts). [^{32}P]RNAs have specific activity ~0.8×10^6 cpm/μg. [^{32}P]labeled cDNA and total Drosophila DNA (isolated from cell culture) were about 8×10^7 cpm/μg and 2×10^7 cpm/μg, respectively.

[x]About 300 μg of the total Drosophila DNA immobilized on the filters was hybridized to 10^7 cpm of [^{32}P]cDNA in 400 μl of a solution containing 6xSSC - 0.2% SDS at 65°C for 40 hrs. The cDNA remaining in the solution was used in the experiments.

[xx]The hybridization was performed in the presence of all other indicated in the table fragments used as the competitors (about 1-5 μg of each).

[xxx]The self-competition experiment (about 5 μg of competitor immobilized on the filter) was used.

ed DNA is hybridized to the restricted DNA of clones immobilized on the Southern filtersm all fragments of the latter bind the label (Fig. 6).

In the other experiments, we hybridized [^{32}P]cDNA prepared on total cytoplasmic mRNA from the Drosophila cultured cells to total isolated Drosophila DNA immobilized on the filters in order to remove cDNA sequences which were present in many copies in the genome. The cDNA preparation depleted in repeats was used for hybridization to DNA fragments of clones H27, H52, H34 and H47 containing new genes (Table 1). Only a small amount of [^{32}P]cDNA was hybridized in this case. Probably this is due to incomplete exhaustion of the repeats. On the other hand, this depleted cDNA preparation efficiently hybridized to the DNA of the unique Drosophila gene which had

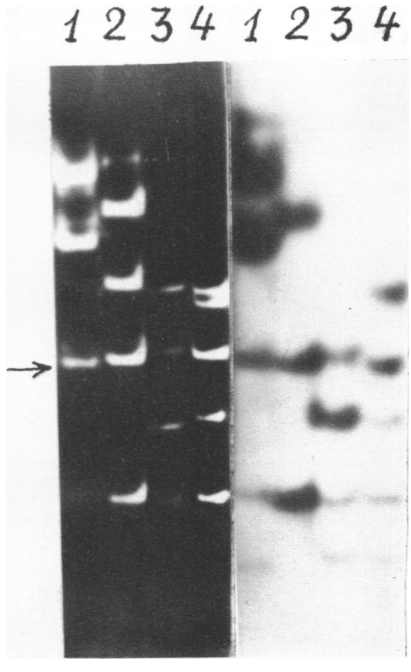

Fig. 6. Hybridization of [^{32}P]labeled total D. melanogaster DNA to DNA fragments of some selected clones.

The DNA of clones H34, H52, and H27 (numbers 2, 3, 4, respectively) was digested by EcoRI and HindIII restriction enzymes and fractionated in 1% agarose slab gel. The DNA of clone H47(I) was digested by EcoRI enzyme alone to have better separation between the 6.3 kb and 7.0 kb fragments.

The electrophoretic patterns and radioautographs are presented. The arrow indicates the position of the 2.9 kb DNA fragment.

been isolated by us (data not shown). In other words, virtually all DNA sequences located around the Dm225 gene, in particular those responsible for mRNA transcription, are represented by many copies in the genome of D. melanogaster.

The exact number of copies cannot be calculated accurately on the basis of labeling DNA binding as some internal heterogeneity may exist within the fragments.

The chromosomal location was determined for the 7.0 kb

and 7.5 kb fragments of clones H47 and H34, respectively, using the *in situ* hybridization of [^{3}H]cRNA transcribed from these DNA fragments to polytene chromosomes of *D. melanogaster* salivary glands.

Fig. 7 demonstrates that cRNA transcribed from the 7.0 kb fragment of H47 DNA hybridizes to many sites in *D. melanogaster* chromosomes. Their total number is about 80. Thus, the gene is scattered. In the unpaired chromosome region, the lo-

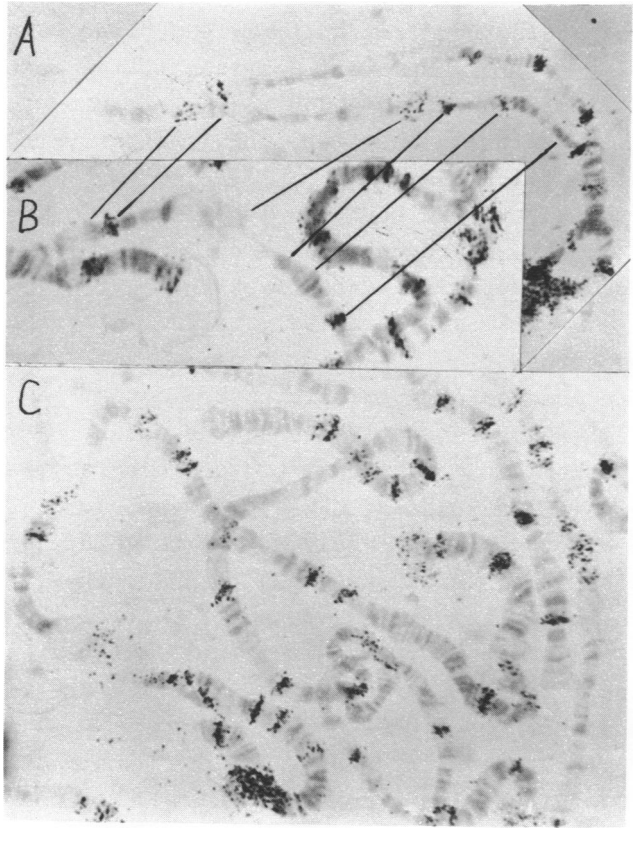

Fig. 7. *In situ* hybridization of [^{3}H]RNA complementary to the 7.0 kb DNA fragment isolated from clone H47 with the polytene chromosomes of *D. melanogaster* salivary glands.

The individuals of Oregon RC (A, and C) and gt w^a (B) stocks were used for the experiments.

cation of the gene in two homologs of Oregon RC stock is different indicating the mobility of the corresponding DNA sequence (Fig. 7A). Fig. 7B presents the same zone of the chromosome from gtw^a D. melanogaster stock. One can see that different stocks have an utterly different distribution of the gene too.

DISCUSSION

In this paper, sequences adjacent to the Dm225 sequence were studied. We learned from the previous paper that they were heterogeneous /5/. However, upstream to the coding sequence of the Dm225 gene, we found a 1.6 kb region homogeneous for all of the ten clones studied. This region hybridized to abundant cytoplasmic mRNA.

It is quite possible that the three fragments (2.9 kb, 0.25 kb and 1.4 kb) represent the same transcriptional unit. The heterogeneity appears at the right end of the 1.4 kb fragment (Fig. 2) as well as immediately to the left from the Dm225 sequence itself.

The main conclusion from this paper is that the Dm225 genes are flanked by repetitive sequences occurring in high frequency in the genome, and many of them are responsible for the transcription of abundant mRNAs. However, some of them do not hybridize to cytoplasmic poly(A)$^+$RNA but bind rapidly--labeled presumably nuclear pre-mRNA (hnRNA). Thus, they are also transcribed. Possibly, only a small part of the newly synthesized RNA enters the cytoplasm while the big one represents the non-informative regions of RNA which are located outside the cloned fragment.

Rather long regions of the genome are constructed in such a way. We found that clones containing 12-20 kb insertions completely consisted of repetitive sequences (Dm34 and 47). The existence of such blocks containing repetitive genes separated by spacers (also repetitive) can explain the specific structure of the D. melanogaster genome, the main feature of which is alternation of long repetitive and very long unique sequences without significant interspersion /17/. So far,

nine different multi-gene families have been cloned in our laboratory (including those described in this paper). Taken together with those described by Hogness et al. /2/ they can account for at least 5% of the total DNA of D. melanogaster. This is not far from 13% that is the content of middle repetitive DNA in the D. melanogaster genome. The dispersed genes of D. melanogaster are mobile. This was well documented for the Dm225 gene and some other genes /1,4/. The same is also true of the new genes described in this paper (Tchurikov et al. paper in preparation). Therefore, the information content of the regions containing such genes varies. In different strains or even individuals of the same stock, the same zone of chromosome may contain different sets of mobile dispersed genes. However, for some unknown reason, this occurrence does not influence the morphology of the corresponding chromosome regions. The detailed organization of the regions also remains obscure. We did not find any redundancy of the Dm225 sequences within the cloned DNA fragment. On the other hand, the total number of Dm225 copies seems to be higher than the number of sites found by the in situ hybridization suggesting some kind of repetition of the genes.

Finally, one may suggest that at least long regions of the genome consisting of clustered mobile dispersed genes can correspond to intercalary heterochromatin /1,4/. Due to the existence of many different families of multiple dispersed genes in these regions, the processes of excision and insertion may be facilitated and occur more often during evolution in heterochromatin than in other parts of the genome.

ACKNOWLEDGEMENT

The authors are greatly indebted to Prof. H.Boyer for a gift of pBR322 plasmid and E.coli HB101, to Dr. V.Nosikov for PstI and HindIII restriction endonucleases, and to Dr. G.Dolganov for T4-induced DNA ligase. We also thank Dr. M.Evgen'ev and Dr. E.Ananiev for valuable advices in performing the in situ hybridization experiments, to Prof. A.Zelenin, Dr. A. Kushch, Dr. N.Stepanova and Dr. V.Barsky for their valuable advices.

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