Volume 5 Number 6 June 1978 Nucleic Acids Research

The properties of gene Dm 225, a representative of dispersed repetitive genes in Drosophila melanogaster

N.A. Tchurikov*, Yu.V. Ilyin, E.V. Ananiev+ and G.P. Georgiev

Institute of Molecular Biology, and Institute of Molecular Genetics, USSR Academy of Sciences, Moscow, USSR

Received 7 February 1978

ABSTRACT

The properties of Dm 225 DNA, a fragment of D.melanogaster genome 2.9 kb in length excised by EcoRI endonuclease and cloned in the λ gt phage or pMB9 plasmid, are described. The DNA hybridizes to a significant portion (0.8%) of total polysomal poly(A)+RNA (mRNA). The size of the hybridizing mRNA is about 2.3 kb (19S); it is present in the fraction of heavy polysomes. Dm 225 DNA fragments obtained with the aid of Hae III endonuclease have been mapped. mRNA hybridizes with all the fragments. In one of the end fragments, the 3'-end of mRNA has been localized and thus the direction of transcription determined. About 250 copies of the gene Dm 225 are present in the haploid genome of D.melanogaster, and all of them have the same size upon restriction with EcoRI endonuclease. On the other hand, the sequences of the genome adjacent to Dm 225 DNA are different and may vary from one cell line to another as evidenced by experiments in which the D.melanogaster DNA was restricted by Hind III endonuclease. In combination with in situ hybridization data /1,2/ the results obtained in this paper demonstrate that the structural gene present in Dm 225 DNA is a representative of a multiple gene family dispersed throughout the whole genome of D.melanogaster.

INTRODUCTION

For a long time it was believed that most of the structural genes were represented by only one copy per genome. A few exceptions like genes for histones /3/ were found to be present in many copies clustered in one particular region of a chromosome. However, a novel type of arrangement of certain structural genes in the D.melanogaster genome has been discovered recently. These are multiple dispersed genes, several representatives of which were isolated with the aid of genetic engineering techniques. Two of such families, Dm 351 and Dm 412, were isolated by Hogness and coworkers /4/ and three

of them, Dm 118, Dm 225 and Dm 234, in our laboratory /1/. These dispersed structural genes are efficiently transcribed in Drosophila culture cells and repeated several dozens or hundreds times per genome. They were detected by in situ hybridization in 20 to 40 sites on different chromosomes. Their location on chromosomes is rather unstable /1,2/. In the present paper, the properties of D.melanogaster DNA present in the clone λgt-Dm 225 are described.

MATERIALS AND METHODS

Transfer of D.melanogaster DNA from λgt - Dm 225 to plasmid pMB9. Originally Dm 225 was cloned in a λgt bacteriophage vector /5/. For extensive biochemical studies, we needed higher amounts of Dm 225 DNA which could be more easily obtained if working with plasmid pMB9 /6/. For this reason, a Dm 225 DNA fragment was transferred to the plasmid. λgt - Dm 225 DNA was prepared as described previously /7/. DNA from pMB9 was obtained according to /8,9/.

pMB9 and λgt - Dm 225 DNAs were treated with endonuclease EcoRI and electrophoresed in 1% agarose gel /10/. The gels were stained with ethidium bromide and analyzed in long-wave length UV light, then DNAs of pMB9 and Dm 225 (2.9 kb fragment) were eluted according to Thuring et al. /11/. Ethidium bromide was removed by treatment with Dowex (AG50W-X8 Bio-Rad) in a solution containing 0.5 M NaCl, 0.01 M Tris·HCl buffer, pH 8.0 and 5 mM EDTA. The supernatant was extracted after such a treatment with water-saturated phenol, pH 8.0, and after extensive dialysis concentrated in a rotor evaporator.

pMB9 DNA treated in the same way by EcoRI endonuclease retained some residual transforming activity equal to 0.005% of the transforming activity of uncleaved DNA. Ligation of restricted and purified DNAs of Dm 225 and pMB9 was performed in 10 µl of a solution containing: 0.1 M NaCl; 8 mM $MgCl_2$; 0.05 M Tris·HCl buffer, pH 7.5; 0.08 mM ATP; 7 mM β-mercaptoethanol; 0.5 µg of serum albumin; 0.5 µg of Dm 225 DNA; 0.01 µg of plasmid DNA and T4 DNA ligase /12/. After 1 hr incubation (dimerization step) at 12°C, the reaction volume was in-

creased ten-fold by adding a solution containing: 0.1 M NaCl; 8 mM $MgCl_2$; 0.05 M Tris·HCl buffer, pH 7.5; 0.08 mM ATP; 7 mM β-mercaptoethanol; 50 μg/ml of serum albumin and T4 DNA ligase. The incubation at 12°C was continued for extra 16 hr for a DNA ring to be formed. To provide optimum conditions for this two-step ligation, DNA concentrations were calculated for each step as described by Dugaiczyk et al. /13/. Following ligation, the mixture was used for transformation of Ca^{2+} treated E.coli K12802 rK^-mK^+ cells /3,14/. Prior to plating, the cells were incubated for 1 hr at 37°C in L-broth with agitation. About 10^5 transformant per 1 μg of plasmid DNA were obtained and ~90% of them contained pMB9 - Dm 225 recombinant DNA.

The work has been performed in P3 conditions.

Preparation of DNA and its restriction fragments. Growing of p225 was performed as described by Tanaka and Weisblum /8/. Lysates were prepared by the method of Clewell and Helinski /9/ and used for CsCl-ethidium bromide centrifugation for 48 hrs at 40,000 rpm, 18°C, in a Spinco Ti50 rotor. The band of supercoiled DNA as vizualized with UV light was collected.

The plasmid DNA free of ethidium bromide, RNA and CsCl was prepared by passing 3 ml of the collected solution through a two-step column packed with Dowex (AG50W-X8, Bio-Rad) bed volume 1.5x5 cm, and Bio-Gel A15m agarose. The eluent was dialyzed against 10 mM Tris·HCl, 1 mM EDTA, pH 7.4.

DNA from the culture cells was isolated as described earlier /7/. DNA from salivary glands was isolated by the method of Locker and Marrakechi /15/.

The incubation with restriction endonucleases was performed in the following media: for EcoRI and Hae III, in 0.05 M NaCl, 0.04 M Tris·HCl, pH 7.4, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol (20 min at 37°C); for Hind III, in 50 mM Tris·HCl, pH 7.5, 50 mM $MgCl_2$, 50 mM β-mercaptoethanol (3 hrs at 37°C); for Hpa I, in 0.05 M Tris·HCl, pH 7.5, 0.05 M $MgCl_2$, 10 mM KCl, 10 mM β-mercaptoethanol. The reactions were stopped by heating for 3 min at 70°C.

Gel electrophoresis. Dm 225 DNA and its fragments were

fractionated by electrophoresis in 5% polyacrylamide gels /17/. SV-40 DNA treated with Hae III endonuclease was used as a marker. 1.4-4% agarose slab gels, 5-12 cm wide were used for blotting. The DNA was transferred to nitrocellulose filters (HA "Millipore", 0.22 μ) according to Southern /18/, strips 5-7 mm in width, were treated according to Denhardt /19/ and used for hybridization.

Preparation of mRNA. Cytoplasmic and polysomal mRNAs from the culture cells of D.melanogaster were prepared by the method of McKenzie et al. /16/. For ^{32}P labeling of RNA, the cells growing on a C-45 insect medium (USSR) were collected by low speed centrifugation at room temperature, extensively washed with the phosphate free Earle medium containing 30 mM Hepes and calf serum (dialyzed against 0.14 M NaCl), and incubated in this medium overnight. The cells were collected again and suspended in a new portion of the phosphate--free medium. Then ^{32}P orthophosphate (Amersham) was added (~200 μCi/ml) and the cells were incubated during 8 hrs. The [^{32}P] mRNA was prepared as described above. The specific activity of [^{32}P] mRNA obtained was 0.5-1.0x10^6 cpm/μg.

The labeling of DNA with ^{32}P. Nick translation was used for DNA labeling /20/. The reaction mixture contained: 50 mM Tris·HCl, pH 7.8; 5 mM $MgCl_2$; 10 mM β-mercaptoethanol; 50 mg/ml of serum albumin; 14 μM dGTP; 14 μM dCTP, 14 μM ^{32}P dATP and 14 μM ^{32}P dTTP (200 Ci/mmole; Amersham); 10-100 μg/ml of DNA and DNA polymerase I. After incubation for 1 hr at 15°C the DNA was precipitated with ethanol in the presence of a tRNA carrier and purified by chromatography on a hydroxyapatite column. The material eluted with 0.5 M sodium phosphate at 60°C was dialyzed against 0.1 M NaCl, 0.01 M Tris·HCl (pH 7.5), 0.005 M EDTA, precipitated with ethanol in the presence of a tRNA carrier dissolved in a solution containing 0.1 M NaCl; 10 mM Tris·HCl (pH 7.5); 1 mM EDTA, and 0.1% SDS, denatured (0.3 M NaOH, 15 min, 100°C), neutralized and used for hybridization. The specific activity of DNA was 20x10^6 cpm/μg.

Hybridization. For hybridization, filters containing DNA were incubated in 50-300 μl of a solution containing 2-4x

xSSC, 0.2% SDS and RNA or DNA for 16 hrs at 65°C. In some experiments, competitors were added (poly(A); unlabeled mRNA). The filters were washed after hybridization as described in /7/, dried and exposed to X-ray film (RT-1, USSR) for 1-10 days and/or dissected and counted in a liquid scintillation spectrometer SL-30 (Intertechnique, France).

RESULTS

1. Dm 225 DNA produces high amounts of mRNA in vivo. In previous experiments, Dm 225 DNA was shown to hybridize with a significant proportion of the cytoplasmic poly(A)$^+$RNA. Now the purified polysomal poly(A)$^+$RNA was also used and hybridization was effective again. Hybridization was performed in saturating conditions, i.e. in the presence of excess Dm 225 DNA, and repeated several times to exhaust the sample of mRNA. As a result, from 0.7 to 0.9% of labeled RNA was bound to DNA. As the background of the hybridization reaction was very low (~0.001%) and since no further hybridization of RNA to new portion of Dm 225 DNA took place, we may conclude that this figure reflects the real content of Dm 225 sequences in the polysomal mRNA.

The specificity of hybridization has been also proved in rehybridization experiments. For this purpose, the total mRNA was hybridized to DNA of Dm 225; the hybridized RNA (without the RNase treatment of filters) was eluted by boiling for 5 min in a solution containing 50 μg/ml of yeast tRNA, cooled, treated with DNAase I, deproteinized and hybridized with a new portion of DNA. One may see (Table I) that purified mRNA efficiently hybridizes to its own DNA.

To find out how large is the RNA hybridizing to Dm 225, the cytoplasmic poly(A)$^+$RNA was prepared and fractionated into size classes using sucrose gradient ultracentrifugation. Dm 225 DNA was hybridized to the fractions obtained. One may see from Fig. I that hybridization takes place with an RNA fraction having the sedimentation coefficient of ~19S. Thus, Dm 225 DNA hybridizes with a certain fraction of RNA about 2300 nucleotides (2.3 kb) long. This figure is somewhat lower than the size of Dm 225 DNA itself (2.9 kb).

To prove further the messenger nature of poly(A)$^+$RNA

Table 1

The hybridization properties of Dm 225 DNA

Exp. N	Material hybridized to Dm 225 DNA	Total cpm	Hybridization cpm	Hybridization % of input
1	Polysomal [^{3}H] mRNA	190000	1672	0.88
2	Polysomal [^{3}H] mRNA	160000	1312	0.82
3	[^{3}H] labeled Dm 225 mRNA rehybridization x)	2500	2125	85
4	Total Drosophila [^{32}P] DNA from cell culture	210000	945	0.45
5	Total Drosophila [^{32}P] DNA from cell culture	160000	704	0.44
	Total Drosophila [^{32}P] DNA from cell culture +100 µg of total unlabeled mRNA	160000	192	0.12
6	Total Drosophila [^{32}P] DNA from salivary glands	180000	666	0.37
7	Total [^{32}P] Drosophila DNA from salivary glands	150000	525	0.35
8	3' Ends of [^{3}H] Dm 225 mRNA	820	305	37
	3' Ends of [^{3}H] Dm 225 mRNA + A and C Hae III fragments of Dm 225	820	285	35
	3' Ends of [^{3}H] Dm 225 mRNA + B, D and E Hae III fragments of Dm 225	820	53	6.5

pMB9 - Dm 225 DNA was immobilized on HA nitrocellulose filters and the sectors of filters containing 0.1-0.5 µg DNA were incubated with labeled [^{3}H] mRNA (specific activity $0.8 \cdot 10^6$ cpm/µg) or denatured [^{32}P] DNA (specific activity $2 \cdot 10^7$ cpm/µg) in 50 µl of 2xSSC - 0.2% SDS. Poly(A) (10 µg per sample) was added. In most cases the annealing was performed at 65°C for about 15 hrs. Each figure represents the average of three separate results with samples of the same mRNA (or DNA).

The hybridization was repeated one or two times with new filters containing p225 DNA. Usually the counts bound were added to the first figure. The background (determined as a hybridization to filters containing 0.5 µg pMB9 DNA) in all cases was very low (~0.001% of input counts).

x) Bound mRNA was eluted from filter, treated with DNAase I, deproteinized and used for the second hybridization.

hybridizing to Dm 225 DNA, we tried to elucidate whether this RNA was present in a particular fraction of polysomes. The isolated polysomal material was fractionated by ultracentrifugation in a sucrose gradient. Although the 80S peak was most prominent, a significant part of the label was still found in polysomes. Hybridization of Dm 225 DNA with frac-

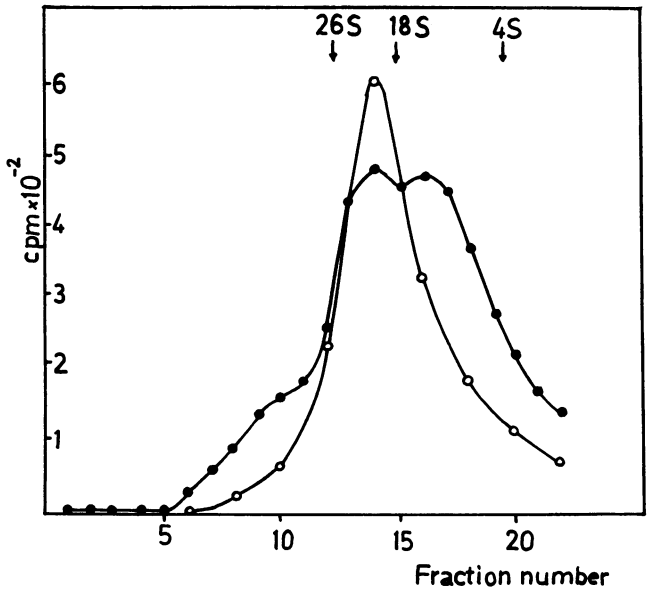

Fig. 1. The size of Dm 225 mRNA.

Cytoplasmic ^{3}H poly(A)$^{+}$mRNA was isolated from the cultivated cells of Drosophila melanogaster by the method of McKenzie et al. /16/ and fractionated by 5-20% sucrose gradient ultracentrifugation (SW-50.1 rotor, 90 min, 44000 rpm). Gradient fractions (0.2 ml) were hybridized to excess of Dm 225 DNA immobilized on nitrocellulose filters.

●–● total radioactivity (2 µl aliquots)
○–○ radioactivity of RNA hybridized to Dm 225 DNA.

tions along the gradient revealed two peaks: one in the 80S monosome region, and the second in heavy polysomes containing ~ 20 ribosomes per particle (Fig.2). The presence of hybridizing RNA in a certain polysome fraction also supports the idea that this is the real mRNA which is not only efficiently transcribed but also translated in the cytoplasm of D.melanogaster culture cells.

2. Restriction map of Dm 225 DNA. To analyze further the relationship between Dm 225 DNA and the corresponding mRNA, the DNA was fragmented into smaller pieces with restriction enzymes. Dm 225 DNA which was originally cut from the D.melanogaster genome with EcoRI endonuclease was not found to contain the Hind III and Hpa I sites. However, Hae III endonuclease cut Dm 225 DNA into four sites generating five

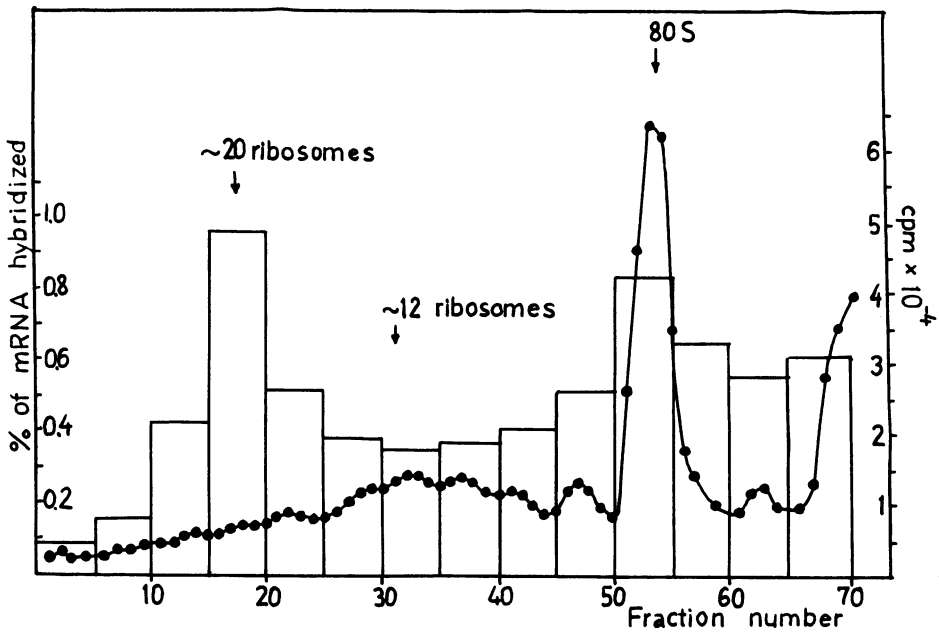

Fig. 2. The size of polysomes containing Dm 225 mRNA.

The polysomes were isolated from [^{3}H]uridine labeled D.melanogaster cells and ultracentrifuged in a sucrose gradient as described by McKenzie et al. /16/. The fractions were pooled, ^{3}H poly(A)$^+$mRNA was isolated from 14 pools and used for hybridization to excess of Dm 225 DNA immobilized on nitrocellulose filters.

●—● radioactivity of 1 µl aliquots of fractions collected.

Open bars represent the percentage of poly(A)$^+$mRNA hybridized to Dm 225 DNA.

fragments of 1200 , 760, 505, 320 and 100 bp (Fig. 3). The end fragments were detected by comparing DNA fragments obtained after digestion of pMB9 - Dm 225 DNA with Hae III alone or by a combination of Hae III and EcoRI. The results showed fragments B and C to be localized at the ends of Dm 225 DNA. The location of the other fragments was deduced from the analysis of Dm 225 DNA partially digested with Hae III endonuclease.

As a result, the following restriction map of Dm 225 DNA was obtained (Fig. 3).

3. Transcriptional mapping of Dm 225 DNA. The purpose of the following experiments was to detect from which frag-

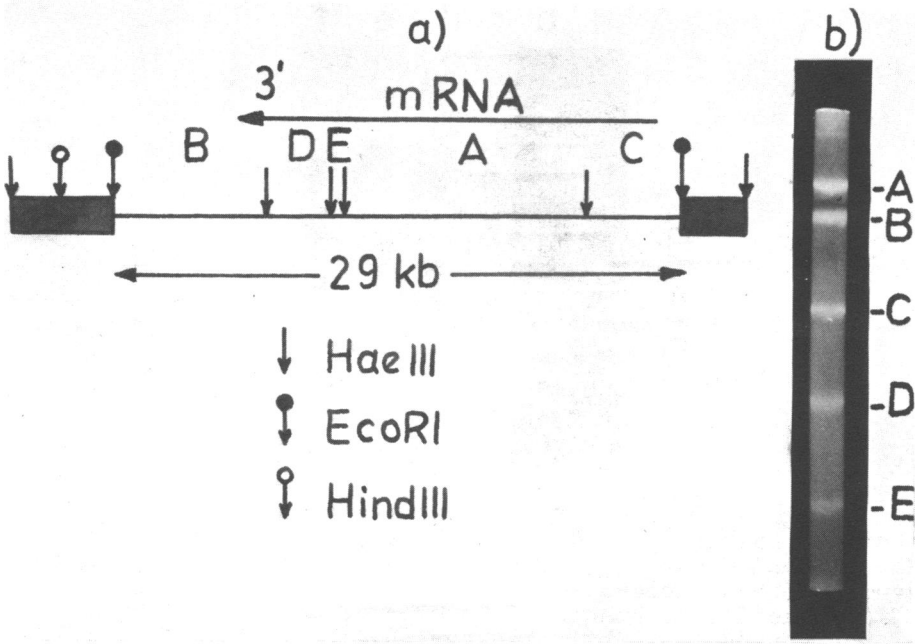

Fig. 3. Restriction enzyme mapping of Dm 225 DNA.

a) The schematic presentation of the results on mapping of Dm 225.

b) Electrophoretic separation of the fragment obtained by Hae III endonuclease treatment of Dm 225 DNA excised from p225 with the aid of EcoRI endonuclease (5% polyacrylamide gel was used).

The arrows indicate cleavage sites for the restriction enzymes EcoRI (⇩), Hind III (⇩), and Hae III (↓). Location of mRNA sequences and direction of its transcription is shown on top of the map.

Only Hae III fragments of pMB9 DNA close to Dm 225 insertion are shown on the scheme.

ments mRNA is transcribed and what is the direction of transcription. The pMB9 - Dm 225 DNA was digested with both Hae III and EcoRI endonucleases, fractionated in 4% agarose slab gel, and transferred to nitrocellulose filters /18/.

In separate samples either ^{32}P-labeled nick-translated Dm 225 DNA, or ^{32}P-labeled total Drosophila DNA, or ^{32}P-labeled mRNA purified by hybridization with total Dm 225 DNA were hybridized to these filters. One may see (Fig. 4) that both [^{32}P] DNAs hybridized to all the Hae III-fragments almost proportionally to their size with an exception of fragment E which was too small for the Southern procedure.

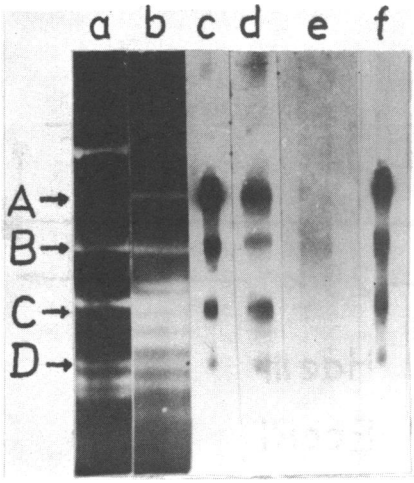

Fig. 4. Transcriptional mapping of Dm 225. (a,b)- electrophoretic patterns (in 4% agarose gel) of SV-40 DNA cleaved by Hae III endonuclease (a) and p225 DNA cleaved by both Hae III and EcoRI endonucleases (b). The arrows indicate the positions of A-D Hae III fragments of Dm 225, (c, d, e,f)- radioautographs of the strips (b) hybridized to [^{32}P]Dm 225 DNA (c), [^{32}P]mRNA (d), [^{32}P]- 3'-ends of mRNA (e) and total [^{32}P]Drosophila DNA (f). Poly(A) was added to the hybridization mixture to avoid possible oligo(dT)-poly(A) hybridization.

Purified Dm 225 mRNA also hybridizes to these four fragments. Thus, almost all of the Dm 225 DNA sequences are represented in the polysomal mRNA. It is not surprising since Dm 225 DNA is only slightly larger than the corresponding mRNA. There is no strict correlation between the binding of radioactive RNA and the size of the DNA fragment, this suggesting that some small parts of DNA are not represented in mRNA. For example, hybridization of mRNA to fragment B is relatively low and probably depends on the ending of mRNA sequences within this fragment.

To determine the direction of transcription, hybridization with the 3'-ends of mRNA was performed. Fragmented Dm 225 mRNA was fractionated by centrifugation in a sucrose gradient followed by poly(U)-Sepharose chromatography. Light ($\leqslant$ 6S) polyadenylated fragments were used for hybridization. In this case, only B fragment bound the radioactive RNA.

The same results were obtained in another experiment. 3' Ends of [^{3}H]mRNA were hybridized to Dm 225 DNA in the pre-

sence of Hae III fragments of Dm 225 as competitors. Only the left part of Dm 225 (fragments B, D, E) is a strong competitor while the right part of Dm 225 (fragments A and C) has practically no influence on hybridization of the 3'-ends of [^{3}H]mRNA (Table 1). One may conclude that the direction of transcription is from fragment C to fragment B. In all likelihood the DNA sequence coding for mRNA terminates within subfragment B of the Dm 225 DNA. It starts either within subfragment C or possibly outside but close to the Dm 225 DNA sequence.

4. Gene 225 is represented by homogeneous DNA sequences repeated ~250 times per genome. To determine the number of Dm 225 copies per genome the ^{32}P-labeled nick-translated DNA of D.melanogaster was hybridized to the excess Dm 225 DNA (Table 1). About 0.5% of the total DNA forms hybrids with Dm 225 DNA. It was shown in the previous section that [^{32}P]DNA hybridized efficiently to all fragments of Dm 225 DNA. Therefore, such a high hybridization reflects the repetitiveness of not only some small part of Dm 225 DNA but of all its sequence. It is possible to calculate from the hybridization percentage and the size of DNA that Dm 225 DNA is repeated about 250 times in the haploid genome of D.melanogaster.

In this experiment we also determined which part of Dm 225 DNA was transcribed into mRNA. For this purpose, the excess mRNA was added to a hybridization mixture of Dm 225 DNA and the ^{32}P-labeled total DNA of D.melanogaster. In these conditions, a four-fold decrease of DNA binding was observed (Table 1). Thus, at least about 75% of Dm 225 DNA is transcribed into mRNA whereas the rest may represent sequences located outside the structural gene. The result also indicates that the structural gene itself is multiple.

It was important to know whether the Dm 225 DNA content was the same in the cell culture and in the tissues of D.melanogaster, in particular in those tissues where most of the DNA belonged to polytene chromosomes. Therefore, DNA was prepared from the salivary glands of larvae and, after nick translation, hybridized to escess Dm 225 DNA. The amount of bound DNA was equal to ~0.4% (Table 1).

Thus, the content of Dm 225 DNA is approximately the

same in the salivary gland cells which have polytene chromosomes and in the cultivated cells.

The next question is how homogeneous, throughout the genome, are sequences hybridizing to Dm 225 DNA. Dm 225 is known to have been originally cut out from the D.melanogaster genome by an EcoRI restriction enzyme /7/.

Therefore, the first experiments to be performed were those in which the total DNAs from two cell lines of D.melanogaster 67J25D and 67J25G /21/ were restricted by EcoRI endonuclease, fractionated by electrophoresis in 1.4% agarose gel, and transferred to nitrocellulose filters according to Southern /18/. The filters were hybridized to [^{32}P]Dm 225 DNA (Fig. 5).

On radiograms (Fig. 5) obtained with DNA of both cell lines virtually all the hybridized labeled DNA is located in one sharp band with the same mobility as that of purified Dm 225 DNA. Direct counting of dissected filters demonstrates that 97% of the label is present in this band.

Similar experiments were performed with the total cellular DNA from cells of the 67J25G line restricted with the mixture of EcoRI and Hae III endonucleases in conditions optimized for complete digestion. Again, hybridization takes place only with the DNA Hae III•EcoRI fragments indistinguishable in size from the Dm 225 Hae III fragments (Fig. 6) thus, confirming the homogeneity of the Dm 225 sequence in D.melanogaster genome. One can conclude that all the structural genes of the Dm 225 gene family with short adjacent sequences are identical at least in respect to their size and location of the EcoRI and Hae III sites. These results give a clear cut evidence for the homogeneity of all the 250 DNA sequences related to Dm 225 DNA present in the D.melanogaster genome. In other words, all the structural genes of the Dm 225 gene family with short adjacent sequences are identical at least in respect to their size and location of the EcoRI and internal Hae III sites.

5. Sequences adjacent to Dm 225 DNA are heterogeneous and vary among different cell lines of D.melanogaster. To analyse sequences adjacent to the Dm 225 fragment in the

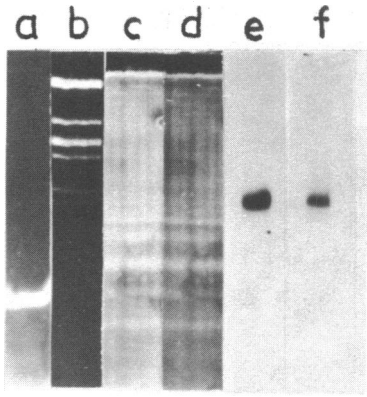

Fig. 5. Homogeneity of Dm 225 sequences throughout the D.melanogaster genome.

a) SV-40 DNA digested by Hae III endonuclease (all fragments except A run off the gel in these conditions).
b) λ CI 857 DNA cleaved by EcoRI endonuclease.
c,d) Total DNAs isolated from D.melanogaster cell lines 67J25G (c) or 67J25D (d) after EcoRI endonuclease digestion.
(a-d) Staining with ethidium bromide.
e,f) Radioautographs of the strips containing EcoRI-restricted DNA from 67J25G (e) or 67J25D (f) cells hybridized to ^{32}P Dm 225 DNA.DNA for blotting procedure was fractionated in 1.4% slab agarose gel.

D.melanogaster genome, we used the restriction enzyme which does not cut Dm 225 DNA, namely Hind III. The DNAs of the two cell lines, 67J25G and 67J25D, were restricted by Hind III, the Southern filters were prepared, hybridized to [^{32}P]Dm 225, and autoradiographed. One can see from Fig. 7 that the radiograms are smeared, i.e. fragments hybridizing to Dm 225 DNA are heterogeneous in size. On the other hand, the distribution of hybridizing DNA fragments from the cell line 67J25G is different from that of the line 67J25D. Particularly, with the DNA from the line 67J25D no discrete bands of hybridization can be seen whereas discrete bands about 12 and 10 kb can be resolved on the background of the smeared material in the preparations of DNA from the line 67J25G.

The 12 kb band accounts for about 25% of the total hybridization. Thus, the sequences adjacent to Dm 225 fragment are heterogeneous and vary among different cell lines although some of them may also be repeated up to 80 times.

Another approach to the study of adjacent sequences, in

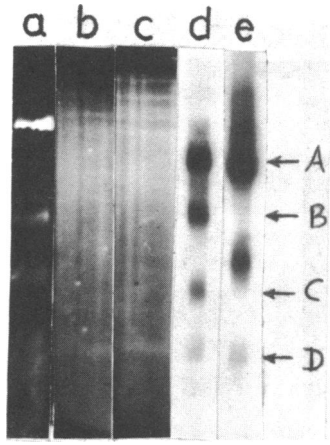

Fig. 6. Properties of Hae III fragments of the total D.melanogaster DNA isolated from 67J25G cell line.

a) SV-40 DNA digested by Hae III endonuclease used as a marker.

b,c) The total D.melanogaster DNA treated by Hae III and EcoRI endonucleases (b) or by Hae III endonuclease alone (c). The samples were stained with ethidium bromide.

d,e) Radioautographs of the strips containing Hae III EcoRI cleaved DNA (d) or Hae III cleaved DNA (e) hybridized with [^{32}P] Dm 225 DNA.

Arrows indicate the positions of A-D Hae III fragments of Dm 225.

In all cases 4% slab gel was used for electrophoresis and blotting procedure.

particular, of those closest to the Dm 225 fragment was the analysis of the total Drosophila DNA from the 67J25G cell line restricted by Hae III endonuclease alone (Fig. 6). Dm 225 - [^{32}P] DNA hybridized in this case to two bands corresponding to the internal fragments A and D and to a rather homogeneous material localized between C and B. The latter obviously contained the end fragment C and a short adjacent sequence which was identical in most of the copies of the Dm 225 gene. Hybridization with a band corresponding to the end fragment B also disappeared. Instead of this, some smeared hybridizing material moving slower than the fragment A could be observed. Thus, the sequences closely adjacent to the end of the structural part of gene Dm 225 were already heterogeneous.

Additionally, Hind III DNA fragments from D.melanogaster have been cloned in a pBR322 plasmid (which was constructed

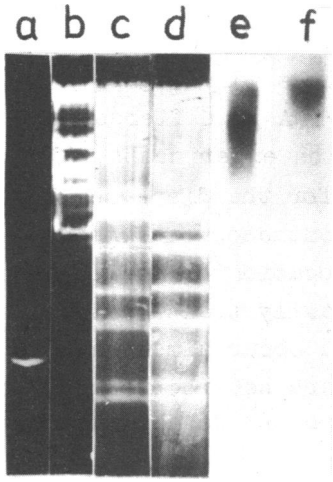

Fig. 7. Heterogeneity of Hind III fragments containing Dm 225 DNA in the total D.melanogaster DNA isolated from the two different cell lines.

a) λ CI857 DNA treated by EcoRI endonuclease.
b,c) DNAs from 67J25G (b) or 67J25D (c) cell lines after digestion with Hind III endonuclease.
d,e) Radioautograph of the strips containing 67J25G (d) or 67J25D (e) DNAs cleaved by Hind III endonuclease obtained after hybridization with [^{32}P] Dm 225 DNA.
1.4% slab gel was used for DNA fractionation and blotting procedure.

by Boyer) and screened for the existence of Dm 225 DNA by means of colony hybridization. Seven clones with Dm 225 DNA sequences were detected. In each case, the Drosophila DNA insertions are characterized by different sizes of 7 kb to 16 kb and different EcoRI restriction patterns. A detailed description of these patterns will be published elsewhere (Tchurikov, Ilyin and Georgiev, manuscript in preparation). These results convincingly demonstrate a great heterogeneity of the flanking sequences located in the direct neighbourhood of Dm 225 DNA fragments.

DISCUSSION

The results obtained show that the DNA sequence present in λgt-Dm 225 (or pMB9 - Dm 225) clones contains practically the whole structural gene responsible for the formation of abundant 19S mRNA. On the other hand, we have found that the

Dm 225 structural gene is repeated many times in the genome. The Dm 225 sequence is homogeneous throughout the whole genome but the adjacent sequences vary significantly. This suggests the presence of Dm 225 DNA in different sites of the genome. The in situ hybridization experiments /1,2/ provided an independent demonstration for the dispersed localization of Dm 225 genes in different chromosomes of D.melanogaster. It has been shown /1,2/ that the location of Dm 225 genes is very unstable and that they can be easily translocated from one site of the genome to another. They occur almost always in the regions of the so-called intercalary heterochromatin, one of the main characteristics of which is the ability for ectopic pairing /22/.

The number of hybridization sites on chromosomes is equal to ~25 in homozygous animals. This is ten times lower than the number of copies. Therefore, one chromosomal site should contain about 10 copies of the Dm 225 gene on the average. Whether the sequences adjacent to different Dm 225 -DNAs are similar or different within the same site is not yet known. The work with longer DNA fragments containing Dm 225 DNA is in progress now to answer the question.

Thus, the distribution of the structural genes present in Dm 225 DNA is characterized by a novel type of arrangement in the genome. Unlike most of the structural genes which are unique, it is repeated many times. In contrast to some known repeated structural genes like histones /3/ or heat-shock genes /4/, it is not concentrated in a certain locus but is dispersed, being located in many different bands on different chromosomes of D.melanogaster. The Dm 225 gene is not the only representative of genes with such a type of distribution in the genome.

In the Hogness laboratory, two families of multiple dispersed genes, Dm 351 and Dm 412, were isolated /4/. Two more gene families, Dm 118 and Dm 234, were described in our laboratory /1,2/. The main properties of these four genes are rather similar. All are localized in many different sites on chromosomes, represented by a number of copies, and all encode the abundant mRNAs in culture cells. For Dm 118 and Dm 234,the

unstable localization in chromosomes was also described /1/. Apparently, structural genes of this type are also present in other species. Levy and McCarthy /23/ found that about half of abundant mRNAs in Drosophila cells are transcribed from the repetitive DNA sequences although the distribution of these sequences on chromosomes is not known. One may suggest that multiple dispersed genes are widely encountered in eukaryotes.

The nature of multiple dispersed genes remains obscure although it is clear that they belong to a class of actively expressed genes. Dm 225 hybridizes to 0.7-0.9% of labeled mRNA in vivo. For Dm 118 DNA the figure is also high (1%) /1/ Even higher binding of mRNA (up to 5-7%) was obtained for one of the genes described by Hogness /4/. Dm 118 DNA is efficiently transcribed not only in the culture cells. It was found in several puffs present in polytene chromosomes of D.melanogaster. One may suggest that multiple dispersed genes are responsible for the synthesis of proteins produced in high amounts by the cell.

The existence of multiple dispersed genes may influence the interpretation of some genetic data. Mutations in these genes should not lead immediately to phenotypic changes and therefore they would be missed in the course of complementation analysis of the genome /24/. Therefore, at least for some regions of a chromosome, the "one band - one gene" concept may not be true. If these genes are present together with other, presumably unique, genes in the same bands, one may expect their coexpression and this can generate novel regulatory mechanisms for their transcription. It is interesting that although the location of the gene 225 is very unstable /1/ the number of copies per genome is invariant for several cell lines and for different stages of development. High homology between different copies of the gene located in different sites of the genome and in the different surrounding is also remarkable. Questions about mechanisms to support such a constancy arise. These and many other problems created by the finding of multiple dispersed genes are under investigation now.

ACKNOWLEDGEMENTS

The authors are greatly indebted to Dr V.Nosikov for a gift of Hae III and Hind III endonucleases and to Dr P.Chumakov for SV-40 DNA. We thank Drs A.Kolchinsky and O.Preobrazhenskaya for guidance on Drosophila cells propagation and Dr D.Kramerov for valuable advices.

*Full address: Dr N.A. Tchurikov, Institute of Molecular Biology, USSR Academy of Sciences, Vavilov street 32, Moscow B-312 U.S.S.R.

ABBREVIATIONS:

Dm 225 fragment of D.melanogaster DNA from the clone λgt-Dm 225 or pMB9 - Dm 225 (p225) containing the structural gene; kb - a unit of length equal to 1000 bases or base pairs (bp) in single- or double-stranded nucleic acids, respectively; poly(A)+mRNA - poly(A)-containing cytoplasmic RNA; SSC - 0.15 M NaCl, 0.015 M sodium citrate, pH 7.2.

REFERENCES

1 Yu.V.Ilyin, N.A.Tchurikov, E.V.Ananiev, A.P.Ryskov, G.N.Yenikolopov, S.A.Limborska, N.E.Maleeva, V.A.Gvozdev and G.P.Georgiev. Cold Spring Harbor Symp. Quant. Biol. (1977) in press.

2 G.P.Georgiev, Yu.V.Ilyin, A.P.Ryskov, N.A.Tchurikov, G.N.Yenikolopov, V.A.Gvozdev and E.V.Ananiev. Science (1977) 195, 394-397.

3 M.L.Birnstiel, E.Weinberg, M.-L.Pardue. In: Symposium on Molecular Cytogenetics, B.Hamkalo, S.Papaconstantinou, eds. Plenum, 1973.

4 D.S.Finnegan, G.M.Rubin, M.Young and D.S.Hogness. Cold Spring Harbor Symp. Quant. Biol. (1977) in press.

5 M.Thomas, R.W.Davis. Proc.Natl.Acad.Sci. USA (1974) 71, 4579.

6 R.L.Rodriguez, F.Bolivar, H.M.Goodman, H.W.Boyer, M.Betlach. In: Proceedings of the ICN-UCLA Symposia on Molecular and Cellular Biology. D.P.Nierlich, W.S.Rutter, C.F.Fox, eds. (Academic Press, San Francisco, 1977), vol.5.

7 Yu.V.Ilyin, N.A.Tchurikov and G.P.Georgiev. Nucleic Acids Res. (1976) 8, 2115-2127.

8 T.Tanaka, and B.Weisblum. J.Bacteriol. (1975) 121, 354-362.

9 D.B.Clewell and D.R.Helinski. Proc. Natl.Acad.Sci.USA (1969) 62, 1159-1166.

10 P.A.Sharp, B.Sugden and J.Sambrook. Biochemistry (1973) 12, 3055-3063.

11 R.W.S.Thuring, S.P.M.Sanders and P.Borst. Anal. Biochem. (1975) 66, 213-220.

12. A.Panet, S.H. van de Sande, P.C.Loewen, H.G.Khorana, A.S.Raac, J.R.Lillehand, K.Kleppe. Biochemistry (1973) 12, 5045-5056.

13 K.Dugaiczyk, H.W.Boyer, H.M.Goodman. J.Mol.Biol. (1975) 96, 171-184.

14 W.B.Wood. J.Mol.Biol. (1966) 16, 118-133.
15 D.Locket, M.Marrakechi. Mol. General Genetics (1977) 154, 249-254.
16 S.L.McKenzie, S.Henikoff and M.Meselson. Proc.Natl.Acad. Sci. USA (1975) 72, 1117.
17 U.Loening. Biochem. J. (1969) 113, 131.
18 E.M.Southern. J.Mol.Biol. (1975) 98, 503-517.
19 D.T.Denhardt. Biochem. Biophys. Res. Commun.(1966) 23, 641.
20 P.W.S.Rigby, M.Dieckmann, C.Rhodes and P.Berg. J.Mol.Biol. (1977) 113, 237-252.
21 V.T.Kakpakov, V.A.Gvozdev, T.P.Platonova, L.G.Polukarova. Genetics (USSR) (1969) 5, N12, 67-76.
22 B.P.Kaufmann, M.K.Iddles. Portugaliae Acta Biol. (1963) 7, 225-248.
23 W.B.Levy, E.J.McCarthy. Biochemistry (1975) 14, 2440-2446.
24 B.H.Judd, M.W.Young. Cold Spring Harbor Symp. Quant.Biol. (1973) 38, 573-579.