Double-stranded sequences in RNA of *Drosophila melanogaster*: relation to mobile dispersed genes

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ABSTRACT

Double-stranded RNA (dsRNA) sequences were isolated from either total RNA or cytoplasmic poly(A)⁺RNA of *D. melanogaster* culture cells using a method described previously /1,2/. Virtually all dsRNA was found to be of high molecular weight (>200 base pairs) and unable to snap back after RNA melting. Thus, it corresponds to one of dsRNA classes found in mouse cells, namely, to dsRNA-A /3/. Three different cloned DNA fragments of *D. melanogaster* which hybridized to melted dsRNA were selected among 100 randomly taken. All of them efficiently bound poly(A)⁺RNA and high percentage of total cellular DNA. According to these and other properties, they were assigned to a group of mobile dispersed genes of *D. melanogaster*. DsRNA hybridizes to all subfragments of the two mobile dispersed genes tested (mdg 1 and mdg 3). Thus, complete transcripts of mobile dispersed genes are present in dsRNA. In total RNA, transcripts from one strand are more abundant than those from another one. DsRNA is heavily enriched in the transcripts from mobile dispersed genes as compared to total or poly(A)⁺RNA of the cytoplasm. It has been suggested that dsRNA in *D. melanogaster* is formed as the result of symmetric transcription of mobile dispersed genes. At least in the cytoplasmic fraction, two complementary strands are separated in vivo and may combine during the isolation of RNA.

INTRODUCTION

Long double-stranded RNA sequences were found in nuclear pre-mRNA (hnRNA) from mammalian cells /1,2,4/. They were fractionated further into several classes. Two of them, viz. dsRNA-A (very long ds sequences about 300-800 base pairs or more unable to fold back after melting of hnRNA) and dsRNA-B (ds sequences 100-200 base pairs long able to snap back) /2,3/ were characterized in more detail. Although a number of data on the structural organization of dsRNA sequences have been obtained since that time /5-11/, the function of these struc-

tural elements remains obscure. Therefore we have decided to continue this work on D. melanogaster cells where the genome is much smaller and a lot of genetic information is available.

In the present work, we found that the RNA of D. melanogaster culture cells contained dsRNA sequences. In contrast to mouse cells, the great majority of dsRNA sequences belongs to class A. They are unable to fold back after RNA melting and have a large size comparable with that of mRNA. The cloned D. melanogaster DNA fragments able to hybridize to dsRNA at the same time efficiently bind abundant poly(A)⁺RNA and total DNA, the latter fact indicating their transcription from repetitive DNA sequences. The sequences to which dsRNA hybridized were identified as mobile dispersed genes /12-20/. The detailed mapping performed in two cases has shown that dsRNA is transcribed from the whole region of the genes. One strand is transcribed from ten to twenty times more efficiently than another.

MATERIALS AND METHODS

Cells and labeling. The cell line 67J25D growing in monolayer /21/ was used. The cells were labeled either with carrier-free [³²P]orthophosphate (The Radiochemical Centre, Amersham; England; 10 mCi per plate containing about 2x10⁸ cells in 20 ml of a phosphate-free medium, 2 hrs incubation) or with [³H]uridine (Isotope, USSR; 5 mCi per plate, 40 min incubation). In some experiments, [³H]uridine was added 20 and 4 hrs before the end of the incubation, and [³²P]orthophosphate 10-15 hrs before it.

RNA isolation. Total cellular RNA was prepared using the phenol procedure at 65°C according to Scherrer *et al.* /22/, deproteinized additionally by chloroform, and treated with DNAase I.

For isolation of cytoplasmic RNA, the cells were subjected to lysis according to the procedure of McKenzie et al. /23/ and, after removal of the nuclei and mitochondria, to deproteinization with phenol in the cold. Poly(A)⁺RNA was separated with the aid of poly(U) Sepharose chromatography /24/.

Isolation of dsRNA sequences. The method of Ryskov *et al.* /1,2/ was applied either to total RNA or to poly(A)⁺RNA. In the latter case, the RNA was self-annealed before RNAase treatment to reconstitute ds sequences melted during poly(U) Sepharose chromatography. Electrophoresis of dsRNA was performed in 5% polyacrylamide gel according to Loening /25/.

DNA preparation and analysis. The DNA of *D. melanogaster* was prepared from the culture cells by the phenol detergent method /24/. The DNA of pBR322 plasmid or of hybrid plasmids was isolated according to Bolivar *et al.* /26/. DNA preparations were labeled with [³²P]nucleoside triphosphates (The Radiochemical Centre, Amersham) using the reaction of nick translation /27/.

DNA was digested with endonucleases Bsu, HindIII, EcoRI or their combinations. The fragments were separated by 1% or 2% agarose (Calbiochem) gel electrophoresis /28/. After staining with ethidium bromide and photographing, the DNA was transferred to nitrocellulose filters according to Southern /29/ and was hybridized to RNA as described /30/.

Cloning experiments. For cloning, pBR322 plasmid was used /26/. Its DNA was cut with HindIII and ligated with the HindIII restricted total DNA of *D. melanogaster* (ratio 1:100) /31/. The latter was underrestricted and long fragments (>5 kb) were taken for ligation. The Ca²⁺-treated E.coli K12 802 rK⁻mk⁺ cells were transformed /32/ and colonies containing recombinant DNA were selected using the ampicillin - tetracyclin test /26/. Hundred clones containing recombinant DNA were used for selection of DNA fragments hybridizing to dsRNA.

In the first series of experiments, combinations of DNA from five clones were used and then the clones from combinations giving positive results were tested individually /24/. The isolated dsRNA was melted by heating before annealing.

In separate experiments, DNA samples were tested for hybridization with poly(A)⁺RNA and with total cellular DNA labeled with [³²P] by nick translation and sheared to fragments 100-200 nucleotides long by alkaline treatment.

RESULTS

1. Isolation of double-stranded sequences from Drosophila melanogaster and their properties

The technique elaborated previously for the isolation of ds sequences from the nuclear RNA of mammalian cells which included RNAase treatment at relatively high ionic strength and gel chromatography through Sephadex G-75 was applied to RNA of D. melanogaster /1,2/.

In the first series of experiments, the total pulse-labeled RNA from the culture cells of D. melanogaster was analyzed. The RNA was treated with RNAase at a high salt concentration. About 1% of the labeled material was resistant to RNAase and was recovered in the void volume upon gel filtration through a Sephadex G-75 column (Fig. 1a). This material was collected and again treated with a mixture of RNAase A and RNAase T1 either at high (2xSSC) or at low (0.01xSSC) salt concentration. At high salt, all the label remained acid insoluble. At low salt, it readily degraded to acid soluble material. The pretreatment with DNAase I did not influence the sensitivity of RNAase in different salt solutions. One may conclude that the material recovered in the first chromatographic peak indeed represents double-stranded RNA (dsRNA).

It was shown previously with mouse cells that dsRNA recovered in the void volume consisted of two types of sequences: dsRNA-B able to fold back and dsRNA-A unable to fold back after melting of original RNA. The same experiment was performed with dsRNA of D. melanogaster (Fig. 1b). If RNA was melted before RNAase treatment, ds sequences disappeared almost completely from it. Less than 0.05% of the RNAase resistant material could be detected. Thus, in contrast to dsRNA from mouse cells, virtually all the dsRNA sequences in D. melanogaster are unable to snap back upon RNA melting. One may conclude that strands forming ds structures are not the parts of the same RNA chain but are present in different molecules (like dsRNA-A in mouse).

Most of the rapidly labeled RNA is located in the cell nucleus. To check whether dsRNA sequences are also located in

Fig. 1. Sephadex G-75 chromatography of *D. melanogaster* RNA preparations treated with a mixture of RNAase A and RNAase T1 in 2xSSC.

(a) Total pulse-labeled RNA was treated with the RNAase mixture.

(b) The same, but RNA was denatured before RNAase digestion at 100°C for 3 min in 0.1xSSC.

(c) Poly(A)⁺RNA prepared from long-labeled cytoplasmic RNA; before RNAase digestion, it was annealed for 15 hrs at 65°C (2 µg RNA in 50 µl of 2xSSC - 0.2% SDS).

(d) The same, but instead of annealing, RNA was melted as described in (b).

Fractions 4-7 were collected and taken for further analysis. The volume of the column was 85 ml. The volume of a fraction was 3.0 ml.

the cytoplasm, we labeled the cells for a long time (20 hrs) and isolated total cytoplasmic RNA.

Unexpectedly, this RNA was also found to contain a high proportion of dsRNA. From 1 to 3% of it was resistant to RNAase (data not shown). However, this result might be explained by the presence of viral RNA in the samples analyzed. It was found that *D. melanogaster* cell cultures are contaminated with viruses containing dsRNA (Kulguskin, personal comm.; Ananiev, personal comm.). Fortunately, the latter does not hybridize to *D. melanogaster* DNA and can be readily distinguished from cel-

lular RNA by the hybridization test. On the other hand, it is labeled rather slowly. Therefore, the contamination with viral RNA is negligible after pulse labeling, but may be significant after long labeling.

Finally, viral dsRNA is not polyadenylated (Kulguskin, personal comm.); therefore we tested poly(A)⁺ cytoplasmic RNA for the presence of ds sequences. As the isolation of this RNA includes the melting step (during the elution from a poly(U)-Sepharose column) we annealed poly(A)⁺ RNA by incubation in 2xSSC at 65°C ($C_0t \sim 100$) before RNAase treatment.

One can see (Fig. 1c) that poly(A)⁺ cytoplasmic RNA does contain dsRNA sequences comprising 0.4–0.5% of the total material. The melting of RNA before RNAase treatment eliminates all of the resistant material (Fig. 1d). The conclusion can be drawn that complementary sequences are present among poly(A)⁺ RNA of the cytoplasm and that they are located in different RNA molecules.

The size of dsRNA sequences isolated from total RNA or from cytoplasmic poly(A)⁺ RNA was estimated using polyacrylamide gel electrophoresis (Fig. 2). All dsRNA from D. melanogaster was found to have a high molecular weight from 200 b.p. and more. Similar size was obtained in denaturing conditions (data

Fig. 2. Determination of the size of dsRNA prepared from total pulse-labeled RNA (see Fig. 1a) by polyacrylamide gel electrophoresis.

not shown). This is the lower estimate since occasional breaks may take place during RNAase digestion because of possible slight mismatching of RNA sequences. As a result, a decrease in the size of dsRNA should take place.

Thus, according to both the inability to fold back and high molecular weight, the dsRNA sequences isolated from D. melanogaster RNA belong to the class of dsRNA-A. DsRNA-B, the major fraction of dsRNA in mouse cells, is virtually completely absent from D. melanogaster RNA.

2. Isolation and characterization of cloned DNA sequences hybridizing to dsRNA sequences

As has been mentioned above, the cellular origin of dsRNA can be proved unequivocally only by its hybridization to DNA of D. melanogaster. Therefore, we used cloning to isolate DNA fragments hybridizing to dsRNA. DNA samples prepared from about 100 clones containing the HindIII fragments (underrestricted) of D. melanogaster DNA inserted in pBR322 plasmid were tested for hybridization with melted dsRNA and with poly(A)⁺RNA. Four clones were found to contain DNA that bound high amounts of dsRNA. Table 1 shows the results on hybridization experiments with DNA prepared from the three of clones. The fourth one contained the same main sequence (mdg1) as the first clone.

One can see that the DNA fragments binding dsRNA also hybridize to a significant fraction of poly(A)⁺RNA. They also bind a high amount of total D. melanogaster DNA. One can estimate that the latter figures correspond to several hundreds copies of the respective DNA sequences per genome of culture cell. The same estimates were obtained in corenaturation experiments with vast excess of total DNA. Thus, dsRNA sequences are often transcribed from multiple genes responsible for the formation of abundant poly(A)⁺RNAs. Furthermore, we isolated two other clones (Dm87 and Dm88) whose DNA did not hybridize to dsRNA but bound 0.05% of total poly(A)⁺RNA. Their DNA did not bind any detectable amount of total DNA (less than 0.01%) which was compatible with the existence of one or a few copies of the DNA sequences per genome. These DNA fragments can be classified as single-copy genes and their transcripts seem to be absent

from dsRNA. We also tested DNA from the clone containing ribosomal genes for hybridization with dsRNA. No binding could be detected (data not shown). Thus, ribosomal RNA comprising the bulk of total cellular RNA does not interfere with the preparation of dsRNA.

Further analysis of the cloned DNA fragments, including restriction mapping and cross-hybridization with DNA of known clones, has demonstrated that Dm58 DNA as well as Dm6 DNA (which is not shown in Table 1 because its properties are quite similar to those of Dm58 DNA) contain the EcoRI restriction fragment 2.8 kb long described previously as gene Dm225 /14/. This is a gene characterized by efficient transcription, by high degree of reiteration in the genome, and by varying location in different chromosomes /14-18/. It has been also designated as mdg1 (mobile dispersed gene 1). Other clones, Dm86 and Dm11, contain new genes different from those previously described. As was shown above, these sequences also represent the actively transcribed multiple genes (Table 1).

Further analysis of clones Dm86 and Dm11 has shown that they are scattered in the chromosomes of D. melanogaster (Table 1). The localization of the both varies especially if two different strains of D. melanogaster are compared (data not shown). The properties of Dm86 are analyzed in detail in another paper /33/ where we designated the corresponding gene as mdg3. Thus, four randomly selected DNA fragments hybridizing to dsRNA sequences were found to be represented by mobile dispersed genes.

3. Mapping of sequences hybridizing to dsRNA in genes mdg1 and mdg3

The restriction and transcription mapping of DNA present in clones Dm58 and Dm86 will be described elsewhere /33/. The data are summarized in Figs. 3a and 4a to help in analyzing the results shown in Fig. 3 and 4.

To answer the question from which parts of a mobile dispersed gene dsRNA is transcribed, we hybridized melted dsRNA to the Southern filters containing restricted DNA from clones Dm58 and Dm86 (Figs. 3 and 4). Two samples of dsRNA were test-

Table 1. The properties of cloned DNA fragments containing sequences hybridizing to dsRNA of D. melanogaster

Clone, Dm	The length of insertion, kb	Size of poly(A) ⁺ RNA ^x /33/	Hybridization with total pulse-labeled dsRNA, %	Hybridization with cytoplasmic poly(A) ⁺ RNA, %	Hybridization with DNA from culture cells, %	Number of copies per genome (from hybridization)	C t _{1/2} upon co-renaturation with Dm DNA ^{xx}	Number of copies per genome (from C ₀ t analysis)	Number of hybridization sites in polytene chromosomes
58	8.2	29S	5.1	1.2	1.26	262	0.55	220	20-30
86	5.3	26S	2.9	0.7	0.83	235	0.60	200	15
11	4.1	31S	4.0	0.3	0.6	221	0.70	170	5-7

^xThe size of the major transcripts. Also the additional minor transcripts could be detected /14, 33, 39/.

^{xx}Nick-translated DNAs of Dm58, 86 and 11 were denatured and co-renatured with the vast excess of total DNA isolated from D. melanogaster culture cells according to /19/.

ed. They were prepared either from total RNA isolated according to Scherrer or from annealed poly(A)⁺RNA. In both cases, the results were essentially the same. For comparison, we also present the data on hybridization of the same fragments with total heavy (>20S) poly(A)⁺RNA. One can see that dsRNA combined with all the restriction subfragments from which poly(A)⁺RNA is transcribed.

The most clear-cut picture was obtained with Dm86 and Dm38 DNA. The latter is a plasmid containing the Dm86 HindIII restriction fragment and flanking sequences which do not hybridize to poly(A)⁺RNA except small regions at the both sides. Three large HindIII-EcoRI fragments of Dm38 plasmid, A, B, and C, bind melted dsRNA in amounts proportional to their size. D binds a small amount of dsRNA but only part of it belongs to the transcribed region. Upon the restriction of Dm86 or Dm38 plasmids with Bsu in combination with EcoRI or HindIII, one can detect the binding of the label to all short fragments: A, B (B', and

Fig. 3. Hybridization of [³²P] labeled melted dsRNA to the fragments of a mobile dispersed gene present in the Dm86 DNA sequence.

(a) The restriction and transcription map of Dm86 DNA taken from /33/. It was cloned either in the 5.2 kb fragment limited by HindIII restriction sites (Dm86) or in the larger 15 kb Bam-HI fragment (Dm38). Restriction sites: Bam-HI ▧, HindIII ▣, EcoRI ▢, Bsu |. The transcribed region —

(b-d) Melted dsRNA from cytoplasmic poly(A)⁺ RNA was hybridized to Southern filters containing either EcoRI-HindIII fragments of p38 (b), or Bsu-HindIII fragments of p86 (c), or Bsu-EcoRI

fragments of p86 (d). (e,f) Melted dsRNA from total RNA was hybridized to Southern filters containing either Bsu-EcoRI fragments of p38 (e) or Bsu-HindIII fragments of p38 (f). In the case of p86, the Bsu-EcoRI fragments adjacent to the ends of insertion have the following size D' ~ 0.3, E' ~ 0.33 kb.

b' and e' - the same as (b) and (e), but the total poly(A)⁺ RNA was used for hybridization.

Fig. 4. Hybridization of [32 P]labeled melted dsRNA to the fragments of a mobile dispersed gene present in Dm58 DNA sequences.

(a) The restriction and transcription map of Dm58 DNA taken from /33/. Dm58 DNA consists of two HindIII fragments resulting from underrestriction. Their contiguity in the genome has been proved /33/. Restriction sites: HindIII $\blacksquare$, EcoRI $\blacksquare$, Bsu $\blacksquare$. The transcribed region $\blacksquare$.

(b-d) Melted dsRNA from cytoplasmic poly(A)⁺ RNA was hybridized to Southern filters containing either HindIII (b) or EcoRI+HindIII (c), or EcoRI (d) fragments of p58.

(e,f) Melted dsRNA from total RNA was hybridized to Southern filters containing either Bsu (e) or Bsu+EcoRI (f) fragments of p58, c' and e' - the same as (c) and (e), but the total poly(A)⁺ RNA was used for hybridization.

B''), C, D (D'), E (E') and F (see slots b-f).

The Dm58 fragment is larger and more difficult to be analyzed. The picture is clear with large fragments obtained with the aid of HindIII (A and B), EcoRI (A, B and C) and EcoRI+HindIII (A, B and C'). They bind melted dsRNA proportionally to the size of a fragment. With Bsu and Bsu+EcoRI fragments which are numerous, only the largest fragments were resolved. Although less detailed these data demonstrate the hybridization of all parts of the Dm58 transcriptional unit to dsRNA (Fig. 4 slots b-f). Total poly(A)⁺RNA hybridizes to the same fragments as dsRNA (Fig. 3 slots b', e' and Fig.4 slots c', e').

If dsRNA is not melted before annealing, no binding to DNA fragments takes place. Thus, the hybridization indeed depends on the binding of dsRNA sequences rather than on the interaction of DNA with remnants of single-stranded RNA that withstood the digestion with RNAase.

The data presented in this section suggest the existence of some symmetric transcription of the two mobile dispersed genes explaining the appearance of ds sequences.

4. Some quantitative parameters of hybridization reaction

To determine the fraction of RNA transcribed from different strands of mobile dispersed genes, the DNA of clones Dm58 and Dm86 taken in excess was hybridized to various RNA samples. First, the total pulse-labeled RNA was analyzed (Table 2).

Table 2. Hybridization of different preparations obtained from total pulse-labeled RNA to Dm86 and Dm58 DNAs

RNA preparation	Treatment of RNA	Yield of total RNA (%)	Hybridization to Dm86		Hybridization to Dm58	
			hybridization (% of input)	hybridization calculated per total RNA (%)	hybridization (% of input)	hybridization calculated per total RNA (%)
Total	untreated	100	0.36	0.36	0.6	0.6
	annealed	100	0.33	0.33	0.5	0.5
	melted	100	0.32	0.32	0.56	0.56
dsRNA	nonmelted	1.5	0	0	0	0
	melted	1.5	1.8	0.027	3	0.045

About 0.35% of it is bound to Dm86 DNA and about 0.6% to Dm58 DNA. Its melting practically does not influence the hybridization. Thus, dsRNAs complementary to the corresponding DNAs represent only a small fraction of total RNAs transcribed from them. Moreover, the additional annealing of RNA does not produce any noticeable effect on the following hybridization. It means that the single-stranded RNA fraction contains only one of the two complementary strands present in dsRNA.

Then, dsRNA prepared from pulse-labeled RNA was taken for hybridization (Table 2). If non-denatured it is not bound to DNA, thus indicating the absence of single-stranded regions surviving the RNAase digestion. However, melted dsRNA efficiently hybridizes to cloned DNA fragments. The distribution of hybridizable dsRNA among size classes coincides with that of the total dsRNA (data not shown). The binding percent varies from one experiment to another within the range of 1.5 to 3.0% for Dm86 and from 2.5 to 5.0% for Dm58. This is from five- to tenfold higher than the content of the corresponding sequences in total RNA. Thus, dsRNA is enriched in transcripts from the two particular mobile dispersed genes.

On the other hand, the total amount of transcripts from these two DNAs present in dsRNA is at least ten times lower than in RNA from which ds sequences have been isolated. Therefore, in agreement with the above mentioned data, the transcripts from one strand of Dm86 and Dm58 are much more abundant than the transcripts from another strand.

The second series of hybridization experiments was performed with the fractions of cytoplasmic long-labeled RNA (Table 3). As all RNA fractions are heavily labeled in this case, it is not surprising that the percentage of binding to cloned DNA fragments is very low.

The binding of melted dsRNA from total cytoplasmic RNA to Dm86 and Dm58 DNAs was also very low. Possibly this preparation of dsRNA, in contrast to the pulse-labeled one, contained a lot of viral sequences. An interesting observation is that some hybridization to cloned DNAs takes place only if RNA is annealed before the dsRNA isolation. Otherwise, no labeled RNA could be found in the hybrid. Thus, the complementary strands

Table 3. The hybridization of different preparations obtained from long-labeled cytoplasmic RNA to Dm86 and Dm58 DNAs

RNA preparation	Treatment of RNA	Yield % of total RNA	Hybridization to Dm86 DNA hybridization ion (% of calculated input)	Hybridization to Dm58 DNA hybridization ion (% of calculated input)
Total	untreated melted annealed	100 100 100	0.03 0.04 0.036	0.075 0.082 0.069
dsRNA obtained from total untreated RNA	melted	2.8	0	0
dsRNA obtained from total annealed RNA	melted	2.8	0.06	0.11
Poly(A) ⁺ RNA	untreated melted annealed	4 4 4	0.36 0.39 0.32	0.75 0.78 0.72
dsRNA from annealed poly(A) ⁺ RNA	nonmelted melted	0.02 ^x 0.02	0 5.3	0 10.75

^xThis corresponds to 0.5% of poly(A)⁺ RNA

transcribed from mobile dispersed genes capable of forming dsRNA are separated in the cytoplasm of D. melanogaster.

Poly(A)⁺RNA accounts for about 4% of the total label present in cytoplasmic RNA. One can see that a major part of the sequences hybridizing to Dm86 and Dm58 DNAs present in the cytoplasm belong to this fraction (Table 3). DsRNA prepared from poly(A)⁺RNA is characterized by a very high content of transcripts from Dm86 and Dm58. Again about tenfold enrichment of dsRNA in the transcripts from the two genes as compared to total poly(A)⁺RNA was found. These two mobile dispersed genes in total are responsible for the synthesis of 15% of dsRNA sequences.

The relative representation of the transcripts from two strands of mdg1 and mdg3 was analyzed additionally ⁱⁿ the following experiment. Bsu fragment E of Dm58 and Bsu+EcoRI fragment B' of Dm86 were isolated, denatured and fractionated by polyacrylamide gel electrophoresis. The bands corresponding to two separated strands were eluted, immobilized on nitrocellulose filters and hybridized to melted poly(A)⁺RNA. The ratio of the label bound to one strand to that bound to another strand was equal to 18 for mdg1 and 23 for mdg3 in agreement with the data presented in Table 3.

DISCUSSION

First of all, the results of this paper demonstrate the existence of dsRNA sequences in the total rapidly labeled RNA and the cytoplasmic poly(A)⁺RNA of D. melanogaster. The rapidly labeled RNA probably corresponded to heterogeneous nuclear RNA, i.e. pre-mRNA. The hybridization experiments with the cloned fragments of D. melanogaster genome suggest that the major fraction of dsRNA prepared from these sources is of cellular origin. Otherwise, it is difficult to explain the high binding of dsRNA (5% in the case of the total rapidly labeled RNA and up to 15% in the case of poly(A)⁺RNA) to only rather small fragments of D. melanogaster DNA.

Second, significant differences were found in the properties of dsRNA sequences observed in D. melanogaster and those isolated previously from mouse.

We found only one class of long dsRNA sequences in D.melanogaster RNA which corresponded to mouse dsRNA-A. Almost no dsRNA of type B could be observed although of course the existence of very small amounts of dsRNA-B cannot be excluded. Anyhow it is not a major fraction of dsRNA as in mice. This difference may reflect variations in the general plan of genome organization in mouse and Drosophila /34, 35/. The short interspersed repetitive sequences are virtually absent from D. melanogaster genome. On the other hand, most of dsRNA-B belong to sequences just of this type.

It was shown that dsRNA-B sequences did exist in the cells in vivo /36, 37/. Contrary, dsRNA-A may be formed during the isolation of RNA. Actually, the yield of dsRNA-A in mouse depends on the technique used for RNA isolation. In the case of dsRNA from the cytoplasm, we could directly demonstrate that two complementary strands were separated originally.

The third conclusion from that paper is that at least many of dsRNAs are the transcripts from mobile dispersed genes. It is not clear whether dsRNA can or cannot be transcribed from single-copy genes. In two cases (Dm87 and Dm88), the answer is negative. Also the proportion of sequences complementary to mdg1 and mdg3 in dsRNA is much higher than in total poly(A)⁺ RNA. Together they may account for as much as 15% of total dsRNA. One may suggest that the symmetric transcription is a specific feature of mobile dispersed genes.

The mapping of the sequences present in dsRNA along the whole transcribed region and the high molecular weight of dsRNA suggest the symmetric transcription of the whole length of mdg. It may depend either on the existence of two promoters in a mdg itself or in the occasional use of cellular promoters located closely to some of mdg insertions. Another explanation which seems to be less probable, but still should be considered, is the existence of RNA-dependent RNA synthesis in Drosophila culture cells. The size of isolated dsRNA sequences is lower than the size of mdg1 or mdg3. It may depend either on its degradation during RNA isolation or on differential processing of the two transcripts.

The unequal representation of transcripts from the two

strands of genes is probably the result of their unequal transcription. The fast processing of one of the strands is less probable as the ratio between the two strands does not depend on the length of pulse labeling.

In conclusion, the similarity between dsRNA of D. melanogaster and dsRNA-A of mouse should be emphasized. It is not limited by the above mentioned physical properties. It was found previously that dsRNA-A sequences in mouse hybridized to a significant fraction (up to 20%) of cytoplasmic poly(A)⁺RNA. On the other hand, dsRNA-A sequences were represented by the transcripts from repetitive DNA. The analogy with the data obtained in the present paper is obvious. It is interesting that the content of sequences hybridizing to dsRNA-A is much lower in mouse polysomal RNA than in free cytoplasmic poly(A)⁺RNP (the fraction of informosomes) /38/. The distribution of poly(A)⁺RNA transcribed from mobile dispersed genes is similar: the major part of it is present in the fraction sedimenting more slowly than polysomes (Ilyin et al., paper in preparation). An interesting possibility arises, viz. that dsRNA-A sequences in mouse are transcribed from mobile dispersed genes and these genetic elements are also present in higher eukaryotes. The work in this direction is in progress now.

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