Volume 8 Number 16 1980 Nucleic Acids Research

Variability in the molecular organization of the 5S RNA genes among strains of Drosophila melanogaster

Nikolaj Junakovic*

MRC Mammalian Genome Unit, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK

Received 23 May 1980

ABSTRACT

The organization of the 5S RNA cluster has been analyzed in four strains of Drosophila melanogaster by the Southern technique. In some of the strains the 5S RNA cluster appears to be interrupted by an unrelated sequence. In other strains a continuous cluster is present.

INTRODUCTION

In the fruit fly Drosophila melanogaster there are about 160 5S RNA genes per haploid genome (1, 2) located at locus 56 F on chromosome two (3, 4). The single repeat unit consists of a 5S gene plus spacer DNA and is about 375 base pairs long (4, 5). The molecular organization of these genes can be studied by using appropriate restriction enzymes that fail to cut within the 5S repeat unit and therefore excise the whole cluster. The fragments so generated can be separated by gel electrophoresis and identified by the Southern technique (6). This approach has been adopted in several laboratories but the results obtained have been so far conflicting. An early attempt led to the conclusion that the 5S genes are grouped in two clusters roughly equal in size separated by an unrelated sequence (7). Although there was some genetic evidence to support this result (2), technical reasons, namely the electrophoretic conditions used, did not allow a firm conclusion. More recently the same problem has been reinvestigated by two groups who have presented evidence

that on the contrary the 5S RNA genes are all contiguously arranged to form a single uninterrupted cluster 160 repeats long (4, 8).

In this report the analysis has been extended to four inbred strains of Drosophila using a variety of restriction enzymes. The results show that there are differences among strains. In two of the strains all the enzymes tested devided the 5S cluster into two parts whereas in the other two, only one enzyme was found to split the cluster. In addition, variability within a strain was detected by comparing restriction patterns of the 5S cluster in individual flies.

MATERIALS AND METHODS

Drosophila strains

The flies were maintained at Molecular Biology (Edinburgh) and provided by D. Finnegan. Cambridge (Oregon-R) is a line which originates from Vancouver, has been maintained for at least 6 years in Cambridge and 2 years in Edinburgh. Yale (Oregon R) originates from Yale University and has been maintained for one year at Imperial College (London) and for 2 years in Edinburgh. The Canton S originates from Oak Ridge Laboratory and has been maintained for at least 20 years in Cambridge. No further information is available to date for the gtw^a strain.

DNA preparation

Single flies were roughly dissociated with dissecting needles under a microscope and transferred to 100 µl of 50 mM Tris, 100 mM EDTA pH 7.8 containing 100 µg/ml pronase on ice. After 10-15 min, the samples were made 1% SDS and heated to 65°C for 30 min. An additional aliquot of pronase was added (200 µg/ml final) and the incubation continued at 37°C for 3 h. The samples were diluted to 0.5 ml with T.E. (10 mM Tris, 1 mM EDTA pH 8) and extracted gently with phenol and then with phenol-chloroform 1:1.

Extensive dialysis against T.E. and ethanol precipitation followed. The pelleting of the ethanol precipitate was carried out in a swinging rotor. This prevented some of the DNA from sticking to the tube walls. The tubes were dried and the DNA allowed to resuspend for 1 h in T.E. before adding the salts and the enzymes. The amount of DNA extracted from single flies was estimated by running the samples in a 1% gel at 10 V/cm. This compacted the DNA in a very narrow area of the gel and allowed to compare the fluorescence intensity with known amounts of lambda DNA run in the same gel. The recovery was from 0.1 to 0.5 µg per fly. The mobility of the DNA was slightly lower than T4 DNA in a 0.3% gel.

DNA from pools of flies was prepared following essentially the same procedure except that the flies were homogenized in a Dounce homogenizer. The plasmid 12D1 was prepared and donated by D. Finnegan (Molecular Biology, Edinburgh).

DNA restriction and gel electrophoresis

Digestion with EcoRI, Bam I, Hind III, Bgl II, Pst I, Hpa I, Sal I were carried out in 25 µl according to the conditions described by Robert (11). The enzymes were provided by B. Smith (this laboratory). Reaction mixtures were incubated for 3-12 h and the digestion terminated by the addition of 5 µl of 100 mM EDTA, 20% Ficoll and Orange G in electrophoresis buffer (12).

Vertical, 0.3% agarose gels (20x20x0.3 cm) were used. To prevent such a dilute gel from shrinking, the gel box was immersed in electrophoresis buffer. At 30 V, in 20 h the separation between the full size lambda and T4 DNA was 1 cm.

DNA transfer hybridization

DNA was transferred to nitrocellulose filters (Shleicher and Schuell BA85) in 20 x SSC overnight. Filters were rinsed briefly in 2 x SSC, blotted dry and baked in a vacuum oven at 80°C for 1 h. Preincubation was at 65°C in Denhart's solution (13) for

4-6 h. The plasmid 12D1 was labeled by nick translation (14) and denatured by boiling for 5 min.

Hybridization was carried out overnight at 65°C in 20-30 ml of Denhart's solution containing 0.5% SDS and about 10^7 cpm ^{32}P labeled p12 D1. After hybridization filters were washed at 65°C in 2 l of 2 x SSC containing 0.5% SDS for 6 h with two changes. The filters were dried and exposed to flash sensitized X-ray film (Fuji RX) with intensifying screens (Ilford fast tungstate) at -70°C (15). Exposure time was overnight.

RESULTS

Variability among strains

DNA was extracted from the strains $gtw^a/T(y:2)Cyo$ (gtw^a), Yale, Cambridge and Canton S and digested with the enzymes indicated. The fragments were separated by gel elctrophoresis and transferred to nitrocellulose filters. The probe used to identify the fragments containing the 5S RNA genes was a ^{32}P labeled cloned segment of homologous 5S DNA originating from an internal region of the 5S cluster (4). The results are shown in Fig. 1. It is apparent that each strain exhibits a unique restriction pattern. In particular three features vary: i) the number of fragments bearing 5S sequences, ii) the mobility of the fragments, iii) the relative intensity of the bands.

i) In two of the strains analyzed (Fig. 1A,B) all the enzymes, alone or in combination, produced two bands. In the other two strains (Fig. 1C,D) only Hind III and Eco RI produced two bands whereas the other enzymes gave rise to one band. A third faint band can be seen in the EcoRI digest of the Yale strain. It is probably due to the presence of variants in the populations (see below).

ii) The size of the fragments containing 5S DNA varies among the strains from 27 to 52 Kb in gtw^a, from 23 to 72 in Yale, from

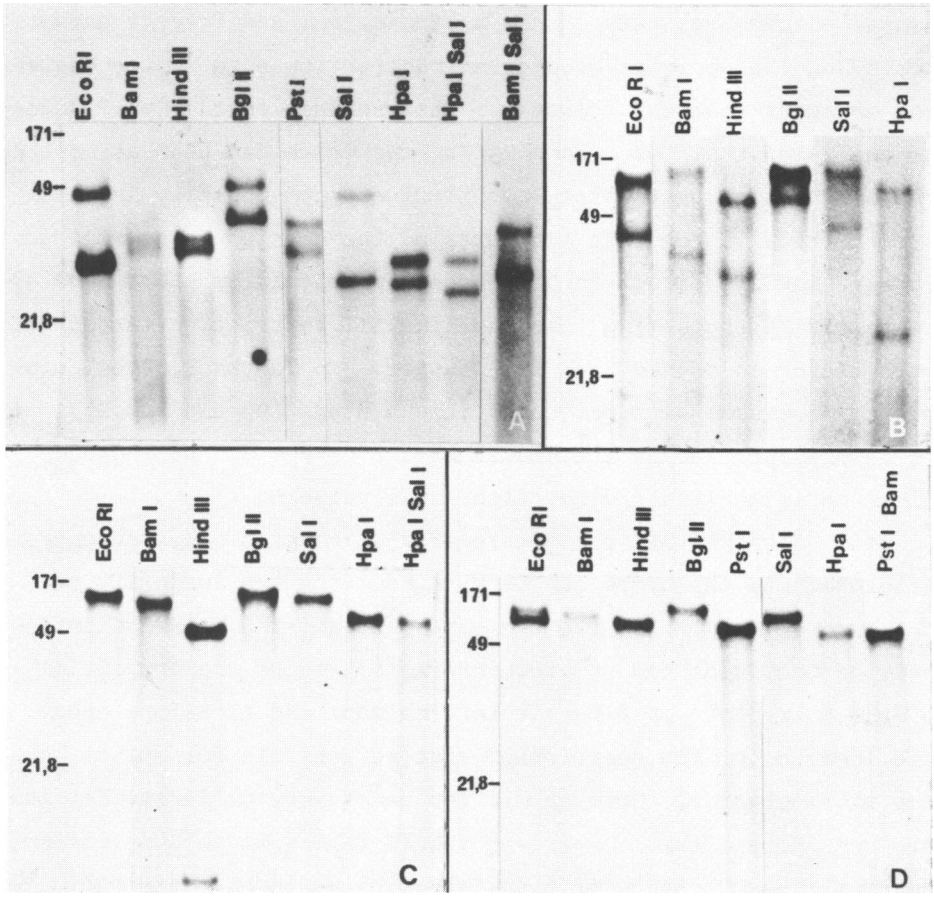

Fig. 1 Variability of the restriction patterns among strains. For each strain the DNA was purified from a pool of 100 flies and the equivalent of two flies (about 0.5 µg) digested with a restriction enzyme. The ^{32}P labeled probe is the plasmid 12 D1 containing a segment of DNA originating from the internal region of the 5S cluster (4). A) gtw^a strain; B) Yale strain; C) Cambridge strain; D) Canton S strain. The size markers are T4 DNA (16), full size lambda DNA and lambda DNA digested with EcoRI and Hind III. The same size markers are used throughout.

17 to 87 Kb in Cambridge and from 67 to 100 Kb in Canton S. These size ranges are roughly consistent with a length of the full cluster of approximately 60 Kb in Cambridge and Canton S and ap-

proximately half a cluster in gtw^a and Yale. The differences in length of the fragments within a strain, using different enzymes, depend on the position of a given restriction site in the sequences adjacent to the 5S cluster. The presence in the Hpa I digest of the Yale strain of a band 23 Kb long which can bear at most 60 repeat units suggests that in this digest and possibly in this strain the division into two parts of the cluster is asymmetric. In the Hind III digest in Cambridge a particularly short band appears (17 Kb) suggesting that the Hind III restriction target is much closer to one edge of the cluster. In Canton S, EcoRI produces two close, high molecular weight bands (90 and 100 Kb). A threefold increase in the amount of the enzyme and prolonged times of incubation did not alter this pattern.

iii) A quantitative estimate of the amount of the sequences homologous to the probe can be obtained from the intensity of the bands. Namely, in the two band patterns the relative intensity of the bands can provide information about the proportion of the 5S repeats in a particular fragment and therefore about the location of the restriction site responsible for splitting the whole cluster. However, if two bands very different in size are considered, this comparison should take into account differences in the efficiency of transfer which could bias the ratio in favour of the faster migrating band (6). The two bands in the Bam I and Hind III digests of the gtw^a strain (Fig. 1A, see also Fig. 3) are very close and apparently equal in intensity suggesting a balanced division of the cluster. The differences in intensity in most of the other digests are probably due to the differential transfer efficiency. On the contrary, in the Yale strain, in four out of six digests the upper band is more intense. This feature, coupled with the presence of a particularly short fragment in the Hpa I digest strongly suggests an unbalanced division in this strain.

Physical linkage of the 5S clusters

Since in gtw^a and Yale strains all the enzymes tested produced a two band pattern, the question arises whether the two bands are alleles or physically linked. The pattern resulting from a partial digestion can distinguish between the two alternatives (Fig. 2). Assuming that the sequences flanking the 5S cluster are random, the spacing between consecutive restriction targets 6 base pairs long is on average 4 Kb. Therefore if the two bands are alleles, a partial digestion is expected to produce a ladder of bands progressively increasing by about 4 Kb. On the contrary, in case of physical linkage, the joining of the two halves of the cluster will produce a saltatory increase of the size of the partially digested fragments. Obviously the use of only one enzyme is not sufficient because the actual distribution of any particular restriction target on either side of the 5S cluster is not known.

The results of a partial digestion, using 6 enzymes, of the gtw^a strain DNA is shown in Fig. 3 and the resulting molecular weights are reported in the legend. In all the partial digests a high molecular weight band appears and its aize is either the

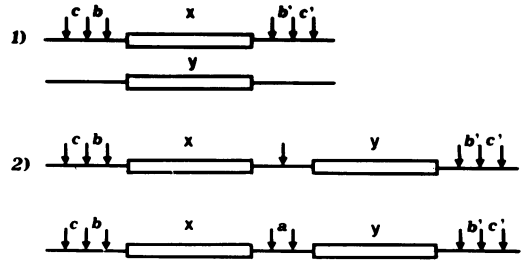

Fig. 2 Prediction about the partial digestion fragments. 1) the two bands being alleles, 2) physical linkage. X and Y are fragments bearing 5S RNA genes; b,b',c,c' and a are stretches of adjacent DNA sequences defined by restriction sites. The presence or absence in the partial digest of fragments bearing X and Y should allow to distinguish between the two alternatives.

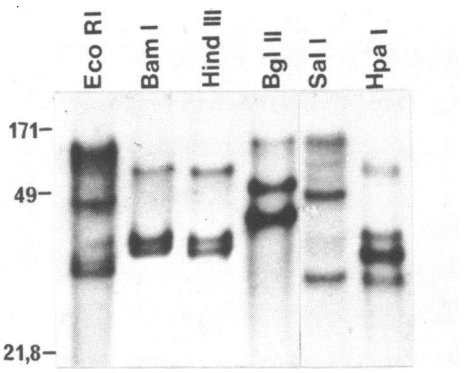

Fig. 3 Partial digestion of the DNA from the gtw^a strain. As expected in case of physical linkage, in all the digests a high molecular weight band appears which is due to the joining of the two halves of the 5S cluster. The molecular weights expressed in Kb are: EcoRI 28, 42.5, 100; Bam I 32, 33, 67; Hind III 31.5, 33, 62; Bgl II 37.5, 52, 127; Hpa I 27, 31, 33, 67.

sum of the two total digest bands (Bam I, Hind III) or higher. This result is consistent with the physical linkage hypothesis and provides some indirect evidence about the number of cuts operated by a particular enzyme between the two clusters. Bam I and Hind III probably cut once because the partial band equals in length the sum of the two bands resulting from the complete digestion. The other enzymes cut more than once because an intermediate ladder is visible and because the size of the high molecular weight band exceeds the sum of the two band pattern due to the addition of segments from the adjacent sequences (a,b,b', c,c' in Fig. 2).

Intrastock analysis

From the analysis of single flies of the gtw^a strain (Fig. 4) it appears that there is also some intrastock polymorphism which however does not seem to be related to the variability among the strains.

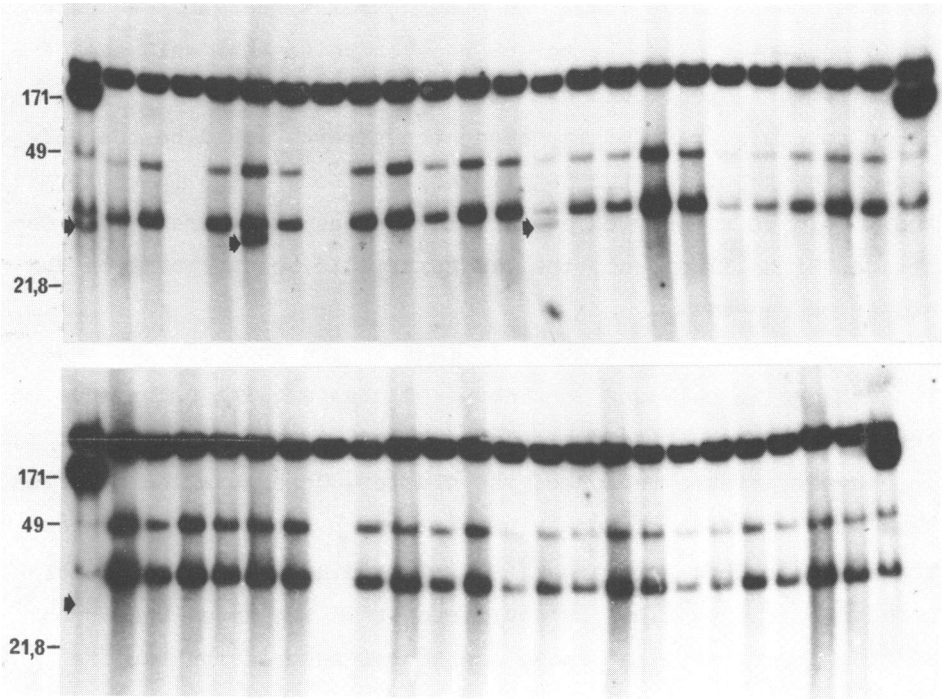

Fig. 4 Intrastock analysis. DNA of individual flies from the gtw^a strain were digested with EcoRI. The handling of a 0.3% gel involves distorsions. To ensure that all the samples would be comparable, about 2 h after the start of the run in all the slots an aliquot of T4 DNA was added and labeled T4 DNA included in the probe. Three variants with an extra band 27 Kb and one variant with an additional faint band 26 Kb long are observed.

DISCUSSION

In this report a variety of restriction enzymes have been used to study the organization of the 5S cluster in Drosophila. It is shown that the number and the size of the fragments containing 5S genes varies among the strains.

One possible explanation of this variability is that in all the strains studied there are two clusters of 5S genes separated by an unrelated sequence. In some of the strains this sequence would be long and variable enough to accomodate seven restriction

targets 6 base pairs long. In other strains the same sequence would be either much shorter or repetitive so that only one of the enzymes tested would cut it. According to this model, another source of variability among the strains would be the different length of the two 5S clusters. In some cases (gtw^a strain) they would be roughly of equal size whereas in others (Yale, Cambridge, Hind III digest) the clusters would be different in the number of repeats.

An alternative possibility is that in the four strains analyzed there is only one continuous 5S cluster. The occurrence of the two bands would be due to a number of extra restriction sites in a hypervariable region of the cluster. Occasional extra sites have been found in cloned 5S DNA (4) which seem to be preferentially located in some areas (9). According to this second alternative, the variability would result in the type, the number and the distribution of these additional restriction targets within the 5S cluster.

However, in order to fit this alternative with the patterns observed, this hypervariable region should be so heterogeneous in sequence and so well defined in length that in fact this hypothesis overlaps largely with the previous one.

A third possible explanation postulates that the variability in restriction patterns reflects true differences in the organization of the 5S genes in different strains. In some strains the two 5S clusters would be separated by an unknown sequence which would vary from one strain to another in the position it occupies within the cluster. In other strains all the 5S genes would be continguous but some of them would differ from the others in having occasional extra restriction site (Cambridge, Hind III and Canton S, EcoRI digests).

In light of the evidence described in previous reports and here, this seems to be the most likely interpretation.

The genetic evidence suggests that the 5S genes are organized in two functional linked clusters (2). Deficiency mutants containing about half of the 5S gene repeats have been shown to contain a cluster approximately halved in size (8). Cytological studies have shown that in optimal conditions of hybridization and exposure, the *in situ* picture reveals two very close bands at the 56 F locus on chromosome two (10, 4). These data suggest that in some strains the 5S RNA genes are organized in two clusters separated by an unrelated sequence.

On the other hand, from experiments analogous to the ones reported here, it has been concluded that in *Drosophila*, the 5S genes form a continuous cluster (4, 8).

The results described in this report reconcile these two lines of evidence by showing that there are differences among the strains. The molecular basis for this variability would be a segment of DNA which is present within the 5S cluster in some strains and absent in others.

ACKNOWLEDGEMENTS

I thank A. Bird and E. Southern for important discussions, D. Finnegan for providing the flies and the probe, B. Smith for the restriction enzymes and the size markers, P.M.B. Wlaker for hospitality and all the members of the MRC Mammalian Genome Unit for help and the friendly atmosphere.
I was supported by a long term EMBO fellowship.

*Present address: Centro di Studio per gli Acidi Nucleici (C.N.R.), Istituto di Fisiologia Generale, Università di Roma, Roma, Italy.

REFERENCES

1. Tartof, K.D. and Perry, R.P. (1970). J. Mol. Biol. 51, 171-183
2. Procunier, J.D. and Tartof, K.D. (1975). Genetics 81, 515-523
3. Wimber, D.A. and Steffensen, D.M. (1970). Science 170, 639-641
4. Artavanis-Tsakonas, S., Schedl, P., Tschudi, C., Pirrotta, V. Steward, R. and Gehring, W.J. (1977). Cell 12, 1057-1067

5. Hershey, N.D., Conrad, S.E., Sodja, A., Yen, P.H., Cohen, M., Davidson, N., Ilgen, C. and Carbon, J. (1977). Cell 11, 585-598
6. Southern, E.M. (1975). J. Mol. Biol. 98, 503-517
7. Procunier, J.D. and Tartof, K.D. (1976). Nature 263, 255-257
8. Procunier, J.D. and Dunn, R.J. (1978). Cell 15, 1087-1093
9. Pirrotta, V. and Tschudi, C. (1978). In Genetic Engineering (H.W. Boyer and S. Nicosia eds.) pp. 127-134, Elsevier/North Holland Biomedical Press
10. Rudkin, G.T. and Stoller, B.D. (1977). Nature 265, 472-473
11. Roberts, R.J. (1976). CRC Crit. Rev. Biochem. 4, 123-163
12. Loening, U.E. (1969). Biochem. J. 113, 131-138
13. Denhardt, D.T. (1966). Biochem. Biophys. Res. Comm. 33, 641-646
14. Maniatis, R., Jeffrey, A. and Kleid, D.G. (1975). Proc. Nat. Acad. Sci. USA 72, 1184-1188
15. Laskey, R.A. and Mills, R.D. (1977). FEBS Letters 82, 314-316
16. Kim, J.S. and Davidson, N. (1974). Virology 57, 93-111