A new *copia*-like transposable element found in a *Drosophila* rDNA gene unit

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Received 9 April 1985; Accepted 6 May 1985

ABSTRACT

We have discovered a member of a new family of *copia*-like transposable elements inserted into the non-transcribed spacer between two ribosomal genes (rDNA). This family, which we call 3S18, consists of at least 15 elements which are scattered throughout the *Drosophila melanogaster* genome. The elements of this family are approximately 6.5 kb long and have 0.5 kb terminal direct repeats. All of the elements appear to have the same restriction sites. The element is mobile as the size pattern of homologous fragments varies among different strains. *In situ* hybridization results confirm the scattered location and transposable qualities of 3S18. The element is not transcribed into abundant RNA.

INTRODUCTION

Most of the moderately repetitive DNA of *D. melanogaster* is composed of families of chromosomally dispersed sequences. Several of these families have been studied in detail and many of them are known to be transposable elements (1). The prototype family *copia* and its related families (such as 412, 297, mdg 1, mdg 3, gypsy and others) share several properties. Each family consists of numerous elements found in widely scattered locations in *Drosophila* chromosomes. The sequences of the elements are highly conserved within the family and often contain direct repeats at their termini. There are differences in their number and location in different strains of *Drosophila* suggesting that they undergo transposition. They often code for relatively abundant cellular RNA's (2).

Here we present evidence for a new family of *copia*-like transposable elements, one copy of which is inserted into the non-transcribed spacer region of a *Drosophila* ribosomal DNA (rDNA) gene. Over half of the 28S genes in *D. melanogaster* are interrupted by non-coding DNA sequences. Thus far two general types of 28S rDNA insertions have been studied (Type I and Type II). Both types of insertion have homologous sequences in several different locations in the chromocenter but are rarely found in euchromatin. They show

only a low level of rearrangement among different strains. The Type I insertion sequence is found in very low abundance in cytoplasmic RNA's (3,4). These properties distinguish the 28S rDNA insertions from the highly mobile copia-like transposable elements such as the one we have found in an rDNA gene.

MATERIALS AND METHODS

Subclone Construction

C2 DNA (5) and pBR322 DNA were digested with Bam HI to completion and ligated at a 1:1 molar ratio with T4 DNA ligase (BRL) at 0.01 Weiss units/ μ g. E. coli HB101 was then transformed with the ligation solution (6). Transformants that were Ap^rTc^s were selected for rapid electrophoretic screening (7) and plasmids of the correct sizes were tested for the presence of the expected fragments in Bam HI digestions.

Preparation of Plasmid DNA

Fifty milliliter M9CA + 0.2 mg/ml thiamine (8) cultures were amplified with 170 μ g/ml chloramphenicol at 2×10^8 cells/ml and further incubated 12-18 hrs. Cultures were harvested and lysed as described by Sharp et al. (9). Lysates were sheared 15 times through a 5 ml pipet and transferred to Type 50Ti polycarbonate bottles. TES (0.05 M Tris-HCl, 0.05 M NaCl, 0.005 M EDTA, pH 8.5) was added to a total of 4.0 g, followed by 0.26 g of 10 mg/ml ethidium bromide and 4.0 g CsCl. Gradients were formed at 40,000 rpm for 40-65 hr at 15°C in a Beckman Type 50Ti rotor. After removal of the upper chromosomal bands, the lower plasmid bands were further purified by an additional equilibrium centrifugation in CsCl at 37,000 rpm for 20-24 hr at 15°C. Ethidium bromide was removed from the covalently closed circular DNA by extraction with isoamyl alcohol followed by dialysis against a cation exchange resin (Dowex 50-W).

Restriction and Cross-hybridization Analysis

DNA from clone 2 (C2) was isolated as previously described (1). The 17 kb insertion of pDm103 (Dm103'), containing a ribosomal RNA repeat unit with a Type I insertion in the 28S gene (10) was generously provided by Drs. D. Fouts and J. Manning and the ribosomal protein gene clone HR0.6 (11) was generously provided by Dr. M. Rosbash.

Restriction enzymes were obtained from Bethesda Research Labs, Inc. Restriction digests were separated on agarose gels in 40 mM Tris base, pH 8.0, 5 mM sodium acetate, 1 mM EDTA. DNA was transferred to nitrocellulose filters using the procedure of Southern (12). The molecular sizes of the restriction

fragments were determined from marker fragments (Hind III digest of λ phage DNA). DNA fragments were isolated by electroelution (13). DNA was labelled with [^{32}P]-dCTP using the nick translation kit of New England Nuclear.

Hybridizations were carried out according to the procedure of Rigby (14). The filters were prehybridized in 10 mls 10X Denhardt's (0.2% Ficoll, 0.2% polyvinylpyrrolidone, 0.2% BSA), 3X SSC, 0.1% SDS, and 100 $\mu\text{g}/\text{ml}$ denatured salmon sperm DNA for 6-10 hrs at 68°. The nick-translated probe was then added and allowed to hybridize for 40-48 hrs in the prehybridization solution. The filters were washed in 2X SSC, 0.1% SDS, 0.1% NaPPi (several changes) at 68° followed by a final wash in 2X SSC.

Analysis of Genomic DNAs

Drosophila embryos were dechorionated (15) and then dounced in 30 mM Tris (7.4), 100 mM NaCl, 5 mM MgCl₂, 250 mM Sucrose. NP 40 was added to 0.5% and the solution was swirled for two min after which it was centrifuged for 10 min at 12,000g. High molecular weight DNA was extracted from the pellet (16), digested to completion with various restriction enzymes and fractionated in 0.7% agarose gels. DNA fragments were transferred to nitrocellulose (12) and probed with (^{32}P)-labelled plasmid DNA as described above.

RNA Isolation

Cellular RNAs were isolated (17) from dechorionated Drosophila embryos and either digested with RNase-free DNase (Bethesda Research Laboratories) or selected on an oligo-dT cellulose column (18).

Analysis of Cellular mRNAs

mRNAs were glyoxylated (19), fractionated in 1.4% agarose gels, transferred to nitrocellulose (20), and probed with ^{32}P -labelled plasmid DNA as described above.

In situ Chromosomal Hybridization

The salivary glands from larvae bearing the mutation giant (gt, 1-0.9) were dissected and the chromosomes prepared for hybridization according to the method of Spradling *et al.* (21). ^{35}S -labelled plasmid DNA (specific activity 0.5×10^8 cpm/ μg DNA) was prepared by the nick translation method of Maniatis *et al.* (16) and used for *in situ* hybridization as follows: The ^{35}S -labelled DNA was denatured by boiling for 10 minutes shortly before hybridization. Between 1.5×10^5 and 2.5×10^5 cpm were used per slide in 25 μl of hybridization mix which contained the following: 10 mM PIPES, pH 6.8 - 0.45 M NaCl - 0.045 M sodium citrate (3X SSC) - Denhardt's solution - 10 mM DTT - 1 mM GTP - 50% (v/v) deionized formamide and 2 μg (per slide) E. coli carrier DNA. Hybridization was performed at 38°C for 18 hours. The slides were then

washed 3X 20 minutes in 3X SSC - 50% formamide (v/v) at 36°C, 3X 10 minutes at room temperature in 2X SSC, 5 minutes in 20% ethanol, 5 minutes in 100% ethanol and finally air-dried. Kodak NTB2 emulsion was used for autoradiography. The slides were exposed for 30 days and the chromosomes stained with Giemsa (Flinka, suihleud) diluted 1:20 with 10 mM phosphate pH 6.8.

RESULTS

C2 Has Regions of Homology to rDNA

Preliminary experiments with clone 2 (C2) DNA had shown that it contained within its 14 kb insert, regions with homology to rDNA, to ribosomal protein mRNAs and to regions of middle repetitive DNA which were not homologous to rDNA (5,22). To better examine these regions of C2 DNA we subcloned three contiguous Bam HI fragments containing most of the *Drosophila* DNA into pBR322 (see Figure 1). pAB19 contains 6 kb of the *Drosophila* DNA and 13 kb of λ DNA while pAB4 and pAB2 contain 4 kb and 3 kb of *Drosophila* DNA, respectively.

Initial experiments showed that pAB19 and pAB2 both hybridized to an rDNA clone (DM103') but pAB4 did not hybridize to DM103' (data not shown). Figure 2A shows the results of an experiment designed to determine the regions within pAB19 and pAB2 which are homologous to DM103'. Digests of pAB19 and pAB2 were blotted and probed with DM103'. The results show that all of pAB19 except the 1.1 kb Pst I-Bam HI fragment (fragment b) and all of pAB2 except the 0.5 kb Pst I-Bam HI fragment (fragment c) hybridize to DM103'. Thus there are two regions of C2 which are homologous to rDNA and these two regions are separated by more than 6 kb of DNA, stretching from the Pst I site in pAB19 to the Pst I site in pAB2 (see Figure 1), which has no homology to rDNA. The strength of

Figure 1. Restriction endonuclease map of C2. The broad hatched lines represent λ vector DNA. The line underneath the map shows which Bam HI fragments of C2 were isolated and subcloned into pBR322 (pAB19, pAB4 and pAB2). The heavy dark lines labelled a, b, c and d represent the restriction fragments which contain the ends of the transposable element 3S18.

Figure 2. Regions at either end of C2 are homologous to rDNA but the middle of C2 is not homologous to rDNA. (A) Hybridization of DM103', a rDNA clone, to restriction endonuclease digestions of 0.5 μ g of pAB2 and 1.0 μ g of pAB19. The first lane shows the ethidium bromide stained gel and the second lane shows an autoradiogram of the filter. The letters a, b, c and d represent the same restriction fragments as in Figure 1 and show that fragments b and c are the only fragments which do not hybridize to DM103'. (B) Hybridization of pAB2 to an Eco RI-Hind III digestion of 0.5 μ g of DM103'. The restriction fragments labelled e and f are the only non-vector fragments of DM103' which hybridize to pAB2 (Figure 3 shows the positions of e and f in DM103').

the hybridization of DM103' to the 5 kb Hind III-Pst I fragment (fragment a) and to the 1.5 kb Pst I-Hind III fragment (fragment d) suggests that both of these fragments consist primarily of DNA homologous to rDNA. Thus the region not homologous to rDNA probably extends only a short distance past the Pst I sites into each of these fragments.

To determine which region of rDNA was homologous to the C2 DNA DM103' was digested, blotted and then probed with pAB2 (see Figure 2B). The 6 kb Hind III-Hind III fragment (fragment e) and the 0.9 kb Hind III-Hind III fragment (fragment f) hybridized to pAB2. The 0.9 kb fragment of DM103' is part of the 28S β gene while the 6 kb fragment contains the non-transcribed spacer and part of the 18S gene (see Figure 3). It should be noted that the restriction map for C2 presented here differs from what we previously reported (22). The several Hind III sites and two R1 sites we had located in pAB4 were found to be artifactual when subclones were examined and are therefore deleted in this map.

Figure 3. Restriction map comparing C2 and DM103' and showing the direct repeats at the ends of 3S18. The crosshatched bars represent areas with homology to rDNA. The open bar represents the inserted element 3S18, the open boxes with an enclosed arrow at each end of 3S18 represent the direct repeats which border the element. The diagonal lines drawn from the ends of 3S18 down to the map of DM103' show the presumed region in the rDNA sequence where 3S18 was inserted. The heavy solid lines labelled e and f represent the fragments of DM103' which hybridize to pAB2 as shown in Figure 2B.

The regions of pAB2 and pAB19 which hybridize to rDNA also show good restriction site homology to regions of DM103' (see Figure 3). Examination of Figure 3, showing detailed restriction maps of C2 and DM103', shows that pAB19 has the same restriction sites as parts of the 18S gene and the non-transcribed spacer while pAB2 has the same sites as the 5' end of a 28S gene with a Type I insert. If the approximately 6 kb of C2 DNA which does not hybridize to DM103' were removed then C2's restriction map would agree with the restriction map of DM103' over the region containing the 18S gene, the nontranscribed spacer, the 28S β gene and part of a Type I IVS. The most likely explanation for these results is that C2 is the result of the insertion of an app. 6 kb piece of DNA into the nontranscribed spacer of a rDNA gene unit. We shall refer to this inserted DNA as 3S18.

3S18 is Bordered by Direct Repeats

Figure 4 shows the results of probing a Bam HI, Pst I and Hind III digest of pAB19 with different fragments isolated from pAB2. This reveals that, in addition to the pBR322 bands, there is some homology between pAB2 and pAB19. When fragment c is isolated from pAB2 and used as a probe it hybridizes only to fragment a of pAB19. When fragment d is used as the probe it hybridizes to both fragments a and b. The hybridization of fragment c to fragment a and of

Figure 4. Hybridization of restriction fragments of 3S18 to endonuclease digestions of 0.5 μ g of pAB2 and 1 μ g of pAB19. pAB2 was digested with Bam HI, Hind III and Pst I while pAB19 was digested with Bam HI, Eco RI and Pst I. (A) The filter was probed with 32 P-labelled fragment c (isolated from a digestion of pAB2). The autoradiogram shows that fragment c hybridizes only to itself and to fragment a. (B) The filter was probed with 32 P-labelled fragment d (isolated from a digestion of pAB2). Fragment d hybridizes to itself and to both fragments a and b. The other fragment which shows hybridization is the result of a small amount of vector contamination in the probe.

fragment d to fragment b suggest that the DNA inserted into the nontranscribed spacer is bordered by direct repeats which have an internal Pst site (the hybridization of fragment d to fragment a of pAB19 is probably due to the presence of a number of tandem Alu repeats in the nontranscribed spacer (23)).

There Are Several Genomic Copies of 3S18 Scattered Throughout the Genome

As preliminary experiments had indicated that the 4 kb Bam HI fragment (pAB4) was moderately repetitive DNA (22), genomic DNA was digested with various restriction enzymes, blotted and probed with pAB4. The Bgl II digest (there are no Bgl II sites in 3S18) reveals at least 15 copies of this portion of 3S18 in the genome (lane 3 of Figure 5A). Digestion of the genomic DNA with Bam gives one strongly hybridizing 4 kb band which is the same size as the band which would result from a Bam digestion of 3S18 (lane 2 of Figure 5A). The Eco-Bam digestion also results in hybridizing bands of the same size and number as would be found after digesting 3S18 DNA (see lane 1 of Figure 5A). These results indicate that the restriction sites within 3S18 are

Figure 5. There are multiple copies of 3S18 scattered throughout the genome. (A) Hybridization of pAB4 to various restriction digests of *D. melanogaster* genomic DNA (3 μ g per lane). As 3S18 has no Bgl II restriction sites the number of Bgl II fragments hybridizing to pAB4 should correspond to the number of copies of 3S18 in the genome. (B) *In situ* hybridization of pAB4 to *D. melanogaster* chromosomes demonstrates the presence of 3S18 at many different chromosomal sites. The insert in the lower left corner shows where two sister chromatids had separated and only one strand hybridized to the probe (at band 6A). The insert in the lower right corner shows band 68BC in which it appears that one of the sister chromatids may have a different copy number (fewer hybridizing sequences) of 3S18.

preserved in most of the genomic copies of 3S18.

In situ hybridization of pAB4 to larval polytene chromosomes of *D. melanogaster* (gt 1 $\times$ gtX11) shows that this sequence is localized in 12 euchromatic sites and in the chromocenter (see Figure 5B). The lack of hybridization to the nucleolus implies that 3S18 is not inserted into a nucleolar rDNA gene in these strains. The lower half of Figure 5B shows one example where 3S18 hybridizes to only one sister chromatid. In this case the sister chromatids are separated with 3S18 hybridizing to band 6A on only one chromatid. In another case, 3S18 hybridizes to band 68BC much less on one side of the chromosome. These results show that the chromosomal locations of some of the 3S18 copies are different in the two strains gt 1 and gtX11.

To illuminate the possibility that the presence of 3S18 in rDNA was an

artifact of cloning, we offer two lines of evidence. First, we independently isolated a second clone (C3T) from the same library which contains all of C2 DNA and 5 kb more. Heteroduplex analysis of these clones showed complete sequence homology of the two throughout the rDNA and 3S18 regions (data not shown). In addition, randomly sheared (app. 10 kb) fragments of Drosophila DNA, Canton S strain (from which the library was made), hybridized to both clones of rDNA and 3S18 in a sandwich blot (data not shown). These data indicate that Canton S DNA does have the transposable element juxtaposed with rDNA, though this is probably not the case in either strains gt1 or gtX11.

3S18 Is Not Transcribed into an Abundant RNA

When RNAs from Drosophila embryos, larvae, adults and tissue culture cells (Schneider line 2) were size fractionated, blotted and then hybridized to [^{32}P] labelled pAB4 the results showed that there was little, if any, RNA homologous to pAB4 (data not shown). In one experiment with embryo RNA two high molecular weight RNA bands (> 5 kb) were observed but this result could not be reproduced. Even in the one experiment which gave a positive result the hybridization signal was less than one tenth that of the control lanes hybridized to [^{32}P] labelled HRO.6. As HRO.6 is a ribosomal protein gene-containing clone, whose homologous mRNA represents approximately 0.1% of the total mRNA; this places the upper limit for the amount of 3S18 transcripts at less than 0.01% of the total mRNA.

DISCUSSION

Our results clearly show that the 3S18 sequence element located in C2 is large (> 6 kb) and has multiple copies in the Drosophila genome (Figures 5A and 5B). This element has a terminal redundancy of app. 0.5 kb (Figure 4). In situ hybridization of a portion of 3S18 (pAB4) to polytene chromosomes shows the element in widely scattered euchromatic locations and in the chromocenter. Differences in the number and location of 3S18 in different strains of D. melanogaster (Figure 5B) and its presence in one rDNA gene strongly suggest that the element is mobile. These are the same properties as those of several other families of elements generally known as copia -like transposable elements (24). However, unlike the other copia -like transposable elements, 3S18 does not produce an abundant RNA transcript.

The copia -like elements have been shown to have profound effects on Drosophila gene structure and expression (for example 25, 26, 27). Since our identification of this new transposable element it has been discovered at two other locations in the Drosophila genome (28,30). In both cases the presence

of 3S18 is associated with mutations: in the white locus (w^{Zm}) and the Beadex locus (B_x^j).

Our isolate of 3S18 was found to interrupt the non-transcribed spacer between two rDNA units (Figure 2). This is the first example of a copia-like transposable element found in rDNA; however half of the 28S rDNA genes are interrupted by one of two different types of insertion sequences (Type I and Type II). These insertion sequences are characterized by being relatively homogenous, located at a specific site in some of the 28S genes, in permuted arrangements in the chromocenter and a few euchromatic sites, and they lack a high degree of mobility (29).

Clearly, we have identified a new family of copia-like transposable elements, at least one member of which can be found in ribosomal DNA. As more families of nomadic elements are discovered it is becoming clear that a substantial fraction of the Drosophila genome may be mobile.

Finally, it should be noted that C2 was originally isolated because it hybrid-selected mRNA that could be translated in vitro to yield a ribosomal protein (4). We have now identified the fragment of C2 which is responsible for this mRNA selection as containing 28S rRNA sequences (the left portion of pAB2). Further studies to establish the exact regions of homology between ribosomal protein mRNA with these sequences are in progress.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Institutes of Health (GM 24572) and a Dreyfus Teacher-Scholar Grant to M.P. We wish to thank Professor P. S. Chen for his help and Sarah Wright for preparation of this manuscript.

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