Two distinct intervening sequences in different ribosomal DNA repeat units of *Sciara coprophila*

Renate Renkawitz-Pohl*, Lloyd Matsumoto** and Susan A. Gerbi**†

*Institut für Molekulare Genetik, Im Neuenheimer Feld 230, Universität Heidelberg, 6900 Heidelberg, GFR, and **Division of Biology and Medicine, Brown University – Box G, Providence, RI 02912, USA

Received 31 March 1981

ABSTRACT

We have prepared a partial gene library of sheared DNA from the fungus fly, *Sciara coprophila*, by dA-T tailing and insertion into pBR322. Two ribosomal DNA clones which differ from the usual ribosomal DNA organization in this organism were studied in detail. Clone pBc 1L-1 has an intervening sequence of 1.4 kb, and clone pBc 6D-6 has an intervening sequence of 0.9 kb. These intervening sequences occur in about the same position in 28S rDNA, but do not appear to share sequence homology with one another. Previously we found that 90% of *Sciara* ribosomal DNA is homogenous and lacks an intervening sequence, and our present data explains the size heterogeneity found in most of the remaining 10%. We have found no evidence of size heterogeneity in the nontranscribed spacer.

INTRODUCTION

Intervening sequences (IVS) are found within the 28S portion of ribosomal RNA genes (rDNA) of several eukaryotic species. For example, all of the repeated copies of rDNA in some strains of *Tetrahymena* (1, 2) and probably all of the rDNA in *Physarum* (3, 4) contain intervening sequences. IVS⁺ rDNA has also been described in the organellar genomes of chloroplasts (5) and mitochondria (6-9). Since in all of the aforementioned cases all rDNA copies are IVS⁺, these genes must necessarily be utilized for transcription, and the transcribed portion of the precursor rRNA corresponding to the IVS must be subsequently spliced out. Indeed, rRNA precursors containing the IVS transcript have been described in many of the above cases (10-12), and the splicing reaction has even been performed in vitro (13).

IVS⁺ rDNA has also been found in the nuclear genomes of insects, namely, *Drosophila melanogaster* (14-17), *Drosophila virilis* (18), *Drosophila hydei* (19) and *Calliphora erythrocephala* (20). However, in these examples only some of the repeated rDNA copies are IVS⁺ and the remainder are IVS⁻. In these cases where the cell has a choice between these two rDNA types found within the same genome, it is of interest to inquire if one class is prefer-

entially transcribed. In fact, the IVS^+ rRNA genes in D. melanogaster are transcribed only at very low levels if at all (21-24). It could be argued that carrying such silent genes may be a burden to the organism, and the observation that the D. melanogaster IVS^+ rDNA is underreplicated during polyploidization could support such a view (25). In D. melanogaster there are about 200 reiterated rRNA genes in the nucleolus organizer region on the X or Y chromosome. On the X chromosome 49% of the rDNA copies have type 1 IVS^+ and on the X or Y chromosome 16% have type 2 IVS^+ (26). Thus there are still 140 uninterrupted rRNA genes (IVS^-) in adult females, and 240 uninterrupted rRNA genes (IVS^-) in adult males, available for transcription.

We wanted to investigate whether intervening sequences occur in the rDNA of the fungus fly, Sciara coprophila, as this organism has only 45 rRNA genes per X chromosome (27). Since the genetic constitution of male flies is XO, they only have 45 rRNA genes total per cell, which is among the lowest rDNA reiteration value known for a multicellular eukaryote. Therefore, if it is disadvantageous for a dipteran to have intervening sequences in some of its rRNA genes, one might expect Sciara to have primarily IVS^- genes. In this paper we report our studies on this question, using clones from a partial gene library of Sciara male DNA constructed by dA-T tailing of sheared DNA and insertion into pBR322.

Previously we had prepared and characterized an 8.4 kb Eco RI complete rDNA unit inserted into the Eco RI site of pBR322; this clone was named pBc 2 (28). Using the rDNA insertion of pBc 2 as a probe for Southern blots of genomic DNA, we discovered that about 90% of the Sciara rDNA repeated units had the same size and organization as this probe. By S1 nuclease digestions using the method of Berk and Sharp (29) we found that this major class of rDNA lacked an intervening sequence. However, in addition to this major class of rDNA, there were minor classes representing about 10% of the total, which were somewhat larger in size than the usual 8.4 kb unit. We will show in this paper that these minor classes are due in part to 2 other types of rDNA units, each with a different kind of intervening sequence present in the 28S rDNA. We have found no evidence for spacer heterogeneity which would have been an alternative explanation for these minor classes. Thus, although Sciara males contain only 45 rRNA genes/cell, they have not only one but in fact two types of rDNA intervening sequences. We present data here that these two types of intervening sequences do not share appreciable sequence homology with one another.

MATERIALS AND METHODS

Cloning *Sciara* DNA

DNA was isolated from adult *Sciara* males as they have a genotype of XO; by contrast, two genotypes are found in females: X'X and XX, and it would be undesirable to have a mixture of two genotypes in a genome library. Separation of these two genotypes using the phenotypic markers swollen and Wavy wings would have been feasible but tedious. In order to remove mitochondria and gut bacteria (30), nuclei were prepared and DNA then isolated, basically as previously described (19). DNA was further purified by sedimentation through a layer of CsCl ($\rho = 1.400$ gm/cc) containing 10mM Tris-HCl (pH 8.0), 10mM EDTA in a SW-41 rotor at 34,000 rpm for 10 hours at 5°C. Under these conditions of short centrifugation time, RNA and protein remain in the upper half of the gradient since they are low molecular weight, and high molecular weight DNA is in the bottom 0.5 ml. This DNA was pelleted in 10mM Tris-HCl (pH 8.0), 10mM EDTA in a SW-41 rotor 15 hours at 35,000 rpm and 5°C and resuspended in the same Tris-EDTA buffer. DNA was sheared by passage through a 26G needle (ca. 50 times) until gel electrophoresis showed the size to be somewhat greater than 15 kb, and was further size selected on a 5-30% (w/v) sucrose gradient made up in 10mM Tris-HCl (pH 8.0), 10mM NaCl, 1mM EDTA and spun at 33,600 rpm for 12 hours at 5°C in a SW-41 rotor. Fractions were sized by electrophoresis in neutral and alkaline gels (31); the latter is important to check that there are very few nicks, as homopolymer tails could also add onto nicked ends.

pBR322 was used as a vector since all restriction sites are known from its DNA sequence (32). Supercoiled pBR322 was purified (28), opened at the single Bam HI site, and tailed with dATP; *Sciara* DNA was tailed with dTTP (33, 34). Calf thymus terminal transferase was a generous gift from Dr. Robert Ratliff (Los Alamos), and we found it to be free of nicking activity at 15°C as assayed by incubation with supercoiled pBR322 which was not converted into relaxed circles. We stopped the reaction when tails were about 100 nucleotides long, as judged by tritiated precursor uptake.

0.1 μ g/ml of tailed pBR322 was annealed with an equimolar amount of tailed *Sciara* DNA by incubation in 0.2M NaCl, 0.5mM EDTA, 10mM Tris-HCl (pH 8.0) at 60°C for 2 minutes followed by 45°C for 90 minutes. Dialysis in the same buffer but at 10mM NaCl followed, since high salt is inhibitory to transformation. Ligation was judged to be unnecessary, because it presumably occurs *in vivo* after transformation has taken place. Annealed DNA was used to transform *E. coli* K12 strain HB101 (35, 36).

Transformants of pBR322 containing Sciara inserts were ampicillin resistant and tetracycline sensitive; more than 95% of these clones contained Sciara DNA. A partial gene library of about 4000 clones was transferred to 144-well microtiter dishes, and Whatman #541 filter replicas were screened (37, 38). As a probe we used a complete rDNA unit from Sciara isolated as an 8.4 kb Eco RI fragment from the pBR322 vector of clone pBc 2 (28); this probe was ³²P-labelled by nick-translation (39, 40) as previously (28).

All DNA cloning was performed under P2+EK1 conditions as previously specified in the NIH guidelines.

Restriction Endonuclease Digestion

Enzyme incubations and subsequent gel electrophoresis analysis were done as described before (28).

Two-Dimensional Hybridization

Two-dimensional hybridization mapping of restriction fragments was performed by a method of Clyde Hutchison III (personal communication and ref. 41). Briefly, this involved electrophoresis of an unlabelled restriction digest in a wide lane, photography after ethidium bromide staining, and transfer to a square sheet of Millipore HA nitrocellulose paper by the method of Southern (42), followed by baking at 80°C in a vacuum oven for two hours. The second dimension consisted of a restriction digest of the 8.4 kb Eco RI fragment from pBc 2 which was ³²P-labelled by nick-translation (28, 39, 40) and electrophoresed in a wide lane. After brief autoradiography, the second dimension was denatured, neutralized, and transferred to the same filter paper containing the first dimension. The paper was laid down such that the bands of the first dimension crossed those of the second dimension at right angles, and transfer was done under hybridization conditions (4X SSC, 0.1% SDS) at 65°C for 20 hours.

Electron Microscopy

R-loops were formed at 48.5°C according to the procedure of Thomas et al. (43). For this purpose 28S rRNA was prepared from Sciara as previously detailed (28). DNA and RNA were each at a concentration of 5 µg/ml, and were diluted ten-fold for mounting for electron microscopy by the formamide technique (44), using a 20% formamide hypophase and 50% formamide hyperphase.

Heteroduplexes were formed between clones pBc 1-L1 and pBc 6D-6 and between each of these DNAs with clone pBc 2 as follows: 2.5 µg/ml of each DNA were denatured at 49.5°C in 70% formamide, 10mM Tris-HCl (pH 8.0), 100mM NaCl, 10mM EDTA for 2 hours. An equal volume of formamide was added and renaturation proceeded at 37°C for 3 hours. The heteroduplex mixture

was diluted one fifth for mounting for electron microscopy by the same formamide technique as above.

Relaxed circular pBR322 DNA was used as an internal size marker. Electron micrographs were taken with a Philips EM 201 at 60 kv.

RESULTS

1. Cloning *Sciara coprophila* DNA

In order to study variants of *Sciara* rDNA organization, we constructed a partial gene library of randomly sheared *Sciara* DNA which was inserted via dA-T tails into the Bam HI site of pBR322. 4000 clones with insertions of 5-15 kb *Sciara* DNA were screened (38) using nick-translated ^{32}P -*Sciara* rDNA which was obtained from the clone pBc 2 (28). Of the 4000 clones we screened, 9 were found to be positive, containing 1-2 rDNA units inserted into the plasmid. This represents 0.23% positive clones, which is greater than that expected, since only 0.04% of the *Sciara* male genome contains rDNA (27).

2. Constant size of the nontranscribed spacer in *Sciara*

Clones containing rDNA were characterized by restriction endonuclease digestions (Eco RI, Hae II, Hind II, Hind III). Because these clones contain randomly sheared *Sciara* DNA in which the exact junction between the *Sciara* DNA and the vector is not a restriction site, the number of rDNA units inserted is not an integral multiple. Usually an incomplete rDNA unit in addition to a complete one was found. As shown in Fig. 1, seven clones showed the same arrangement of restriction fragments as reported previously for clone pBc 2 which represents the majority of the rDNA repeat units (28). A Hae II fragment of 1.4 kb contains the majority of the nontranscribed spacer (NTS) and no coding regions. Digestion by Hae II as well as by other enzymes showed that these 7 clones contained together a total of 9 nontranscribed spacers which were all constant in size. We have not observed size heterogeneity in the spacer of *Sciara* rDNA such as has been found in several other organisms (16, 45-51). Moreover by Southern blots, all nontranscribed spacers of these 7 *Sciara* clones hybridize with nick-translated pBc 2 rDNA, which means that not only is their size constant, but also they contain homologous sequences (data not shown). Finally, the transcribed regions of these 7 clones show no evidence for length or gross sequence heterogeneity.

3. Heterogeneity caused by intervening sequences in *Sciara* 28S rDNA

Two of the 9 positive rDNA clones we obtained showed restriction patterns which did not agree with the structure of the bulk rDNA found in clone pBc 2. As we will describe in detail below, this was due to two

Fig. 1: Schematic map of seven *Sciara* rDNA clones. Vertical bar between 18S and 28S rRNA coding regions is the internal transcribed spacer and includes the region coding for 5.8S rRNA. 28S rRNA is split into α and β chains (28).

different intervening sequences: one found in clone pBc 1L-1 and the other in clone pBc 6-D6. The sizes of these two types of IVS account for most of the minor size heterogeneity we previously observed by genomic Southern blots (28). Moreover, like the seven clones in Fig. 1, the three complete nontranscribed spacer regions contained within these two clones also showed no size heterogeneity.

(a) Clone pBc 1-L1

Eco RI digestion of pBc 1-L1 resulted in three fragments of 9.8 kb, 4.8 kb and 4.3 kb (Fig. 2a). Since there is one Eco RI site in the vector pBR322, and one Eco RI site mapped in the *Sciara* 18S rRNA coding region (28), the result of three rather than two Eco RI fragments suggests that perhaps two 18S rRNA coding regions are present in this clone (Fig. 9). If this is the case, then the observed 9.8 kb fragment would represent an rDNA unit which is 1.4 kb larger than the usual 8.4 kb. In order to clarify which part of the rDNA unit is responsible for this length difference, Hae II plus Eco RI double digestions were performed. These enzymes cut clone pBc 2 (one rDNA unit in pBR322) into five fragments (Fig. 2b): the bulk of the molecule

Fig. 2: Restriction enzyme profiles of clone pBc 1L-1 and clone pBc 2. Panel (a) shows Eco RI digestion products after electrophoresis in a 1% gel. Panel (b) shows Eco RI + Hae II products in a 1.5% agarose gel. This and all gels shown were run in the buffer described by Helling *et al.* (52); the negative image of the ethidium bromide profile is shown for better contrast.

in the 2.4 kb band codes for β 28S rRNA, the 2.3 kb band mainly for α 28S rRNA, the 2.0 kb band contains mostly sequences coding for 18S rRNA, the 1.6 kb band represents part of pBR322, and the 1.4 kb band contains exclusively nontranscribed spacer sequences (28). Figure 2b also shows the Hae II plus Eco RI restriction enzyme pattern of pBc 1-L1 in comparison to pBc 2; the staining density indicates that the 18S RNA coding region (2.0 kb) and the nontranscribed spacer (1.4 kb) occur twice in pBc 1-L1, as will be proven later in this paper. Although a 2.3 kb band corresponding to α 28S rDNA occurs once, a 2.4 kb molecule of β 28S rDNA is missing --- instead two additional bands appear at 1.3 kb and 3.8 kb. After Southern transfer, these two additional bands hybridize with rDNA (data not shown). In order to clarify the nature of the sequences within the 1.3 kb and 3.8 kb fragments we took advantage of the two dimensional hybridization mapping of restriction fragments described by Hutchison (41). pBc 1-L1 was digested with Hae II,

and was transferred to a nitrocellulose filter. For the second dimension the rDNA Eco RI insert in pBc 2 was ^{32}P -labelled by nick-translation, digested with Eco RI + Hae II, electrophoresed and transferred to the same nitrocellulose filter but perpendicular to the first dimension containing pBc 1-L1. The transfer was performed under annealing conditions, so that points where homologous regions in pBc 2 and pBc 1-L1 cross one another gave rise to hybridization visualized by autoradiography (Figure 3). The ^{32}P -DNA of the second dimension does not stick to the nitrocellulose paper except at such cross-points of homology. This experiment showed that the 4.3 kb Hae II band of pBc 1-L1 contains 18S and α 28S rRNA coding parts as expected, the 2.6 kb band also contains 18S rDNA which proves our supposition that pBc 1-L1 contains 18S rDNA twice, and the 1.4 kb fragment hybridizes with the spacer as expected. The two additional, unknown bands of 1.3 kb and 3.8 kb are identified by this hybridization as homologous to the β 28S rDNA region in pBc 2. We showed above that clone pBc 2 has an Eco RI unit of 8.4 kb (Fig. 2a); if we subtract from this 2.4 kb which is the Hae II fragment containing β 28S rDNA and substitute for the latter the 3.8 kb fragment found in pBc 1-L1 which hybridized with β 28S (Fig. 3), we arrive at a sum of 9.8 kb which also is the observed Eco RI unit for clone pBc 1-L1 (Fig. 2a). Thus, this 9.8 kb Eco RI unit of pBc 1-L1 must contain a Hae II fragment of 3.8 kb rather than a 2.4 kb β 28S rDNA. These data imply that the 3.8 kb β 28S rDNA fragment has an intervening sequence of 1.4 kb. This will be confirmed below by electron microscopy. Moreover the other β 28S rDNA fragment of 1.3 kb (Fig. 3) cannot be included in the 9.8 kb Eco RI unit, but must be from the next adjacent gene (Fig. 9); this will be visualized later in this paper by R-loop mapping.

Our other restriction digests also concur with the notion of a 1.4 kb intervening sequence within β 28S rDNA of clone pBc 1-L1. Hind III digestions give rise to a fragment which spans β 28S rDNA, nontranscribed spacer and part of the 18S rDNA. This fragment is also 1400 bases longer in pBc 1-L1 than in pBc 2 (data not shown). Additional digestions using Eco RI + Hind II + Hind III and Eco RI + Hae II + Hind II demonstrated that a Hind II site occurs within the 1.4 kb intervening sequence (Fig. 9).

Finally to verify the existence of an rDNA intervening sequence, we hybridized linear pBc 1-L1 with 28S rRNA under R-loop conditions (43). As is shown in Fig. 4, 28S rRNA forms two main R-loops, one with the α 28S rDNA and the second with β 28S rDNA. These two R-loops are separated by a "gap" 100 bases in length (28). In addition, one third of the way into

Fig. 3: Two-dimensional Southern blot mapping of clone pBc 1-L1. A ^{32}P -labelled Hae II digest of the 8.4 kb Eco RI fragment from pBc 2 (second dimension, wavy lines) was transferred under hybridizing conditions for 20 hours to a filter containing an unlabelled Hae II digest of pBc 1-L1 (first dimension, straight lines) as described by Clyde Hutchison III (personal communication and ref. 41). The cross-points where hybridization occurred are shown by circles. Eco RI was omitted in the first dimension to prevent a double intensity 18S band at 2.0 kb (cf. Fig. 2b); after just Hae II digestion, 18S rDNA is found in 2.6 kb and 4.3 kb bands (Fig. 9), but all other bands of the 1st dimension are the same as in Fig. 2b. The 1.6 kb fragment in the first dimension contained only vector sequences, and hence did not hybridize with the second dimension.

the β 28S R-loop, there is a stretch of DNA which does not pair with 28S rRNA, representing an intervening sequence of $1370 \text{ bp} \pm 60$ ($n = 9$). This confirms our restriction analysis data that the β 28S rDNA of pBc 1-L1 has an intervening sequence of about 1400 bp. In addition an R-loop of 1.3 kb is visible near one end of the molecule, representing an incomplete β 28S rDNA (as

Fig. 4: R-loop of 28S rRNA hybridized to pBc 1-L1, which was linearized by digestion with Sal I; there is no Sal I restriction site within Sciara rDNA, and only a single Sal I site within pBR322.

proposed in Fig. 3), due to insertion of the randomly sheared molecule into the vector.

(b) Clone pBc 6-D6

The second clone of the nine positive rDNA clones which differed in its organization from that usually found in rDNA was clone pBc 6-D6. Hae II plus Eco RI digestion (Fig. 5) showed that pBc 6-D6 lacked the β28S band (2.4 kb), had a double band at the position of 18S rDNA (2.0 kb) and unknown fragments of 1.3 kb and 0.8 kb besides the expected α28S rDNA (2.3 kb), pBR322 (1.6 kb) and nontranscribed spacer (1.4 kb) fragments. Except for the pBR322 band, all fragments contained rDNA as determined by Southern hybridization with nick-translated rDNA from pBc 2 (data not shown).

Fig. 5: Ethidium bromide stained restriction enzyme profile of clone pBc 6-D6 compared to clone pBc 2. Diagram shows Eco RI + Hae II digestion products after electrophoresis in a 1.4% agarose gel. Other details as in Fig. 2.

With two-dimensional hybridization mapping (Fig. 6) we found that the 2.0 kb band of double intensity from the Hae II and Eco RI digest of pBc 6-D6 had 18S RNA and β 28S RNA coding capacities. The 1.3 kb fragment also contained sequences homologous to β 28S rDNA, while the 0.8 kb band contained nontranscribed spacer material. These data are consistent with clone pBc 6-D6 containing one rDNA unit plus an additional part of a nontranscribed spacer. Normally β 28S rDNA is found in a 2.4 kb Eco RI + Hae II fragment, but in this clone such a fragment is missing. Instead, β 28S rDNA occurs in 2.0 kb and 1.3 kb pieces, the sum of which is 3.3 kb. This is 0.9 kb larger than the usual 2.4 kb fragment, suggesting that an intervening sequence of this size occurs in β 28S rDNA. This was visualized directly by R-loop formation with β 28S rRNA (Fig. 7) which showed an IVS in the β 28S of $850 \text{ bp} \pm 90$ ($n = 11$).

Fig. 6: Two-dimensional Southern blot mapping of clone pBc 6-D6. Details as in Fig. 3.

4. Comparison of intervening sequences in the β 28S RNA gene of pBc 1-L1 and pBc 6-D6

We demonstrated above that pBc 1-L1 contains an intervening sequence in β 28S of 1400 bp and pBc 6-D6 has a β 28S rRNA gene interrupted by 900 bp. This was also confirmed by heteroduplexes between pBc 2 and pBc 1-L1 (Fig. 8b) as well as between pBc 2 and pBc 6-D6 (Fig. 8c); clearly, the DNA of each respective IVS is missing in our canonical clone pBc 2. We wanted to know if the IVS found in pBc 1-L1 shared sequence homology with the IVS found in pBc 6-D6, and therefore made heteroduplexes between these two clones to answer this question. As can be seen in Fig. 8a, the two intervening sequences do

Fig. 7: R-loop of 28S rRNA hybridized to pBc 6-D6, which was linearized by digestion with Sal I, which cuts at the single site present in the pBR322 vector.

not hybridize with each other. Moreover, the length of each single stranded intervening sequence (Fig. 8a) is approximately the same size as that determined by heteroduplex formation with pBc 2 (Figs. 8b and 8c). Thus, there is no apparent major sequence homology between these two intervening sequences, although we cannot exclude a small bit of homology at either end of the IVS. In addition, as we see only one and not two unpaired regions in Fig. 8a, it seems probable that the positions of intervening sequences within rDNA are similar or even identical in these two clones. This supposition is also supported by our restriction mapping (Fig. 9).

DISCUSSION

By restriction mapping analysis, and R-loop and heteroduplex mapping we found a 1.4 kb IVS in the β28S rDNA of pBc 1-L1 and a 0.9 kb IVS in the β28S rDNA of pBc 6-D6. A summary is presented in Fig. 9. We have reported here that even though *Sciara* males have only 45 rRNA genes/cell, two types of IVS⁺

Fig. 8: Heteroduplex mapping. Plasmids were linearized by digestion with Sal I prior to heteroduplex formation.

(a) Heteroduplex between pBc 1-L1 and pBc 6-D6, where the longer single stranded arm of the unpaired loop is $1540 \text{ bp} \pm 170$ and the shorter arm is $980 \text{ bp} \pm 90$ ($n = 10$). These values compare favorably to our estimates of 1400 bp and 900 bp intervening sequences in pBc 1-L1 and pBc 6-D6, respectively.

(b) Heteroduplex between pBc 1-L1 and pBc 2, showing a single-stranded loop of $1680 \text{ bp} \pm 150$ ($n = 5$), as generally anticipated for a 1400 bp IVS in pBc 1-L1.

(c) Heteroduplex between pBc 6-D6 and pBc 2 showing a single-stranded loop of $940 \text{ bp} \pm 150$ ($n = 4$), in agreement with an IVS in pBc 6-D6 of 900 bp.

genes are found. Such IVS^+ genes represent only about 10% of the total; disregarding possible population polymorphism, this suggests that there are 1-2 copies of each type of IVS^+ rDNA per cell. This would support the notion that IVS^+ rRNA genes might be deleterious to the organism since otherwise one might expect a higher percentage of IVS^+ rRNA genes comparable to *Drosophila*. Allowing for population polymorphism, some animals may have several copies of the pBc 1-L1 type rDNA or else several copies of the pBc 6-D6 type rDNA. Whether both types of rDNA are present in a single organism will be the subject of future work. The only other organism reported so far to have two classes of IVS^+ genes in its rDNA is *Drosophila melanogaster* (26), while all others with interrupted rDNA only have one class of IVS^+ gene.

pBc 2

pBc 1-L1

pBc 6-D6

Fig. 9: Restriction maps of pBc 1-L1 and pBc 6-D6. pBR322 is joined at its Bam HI site to these *Sciara* rDNA inserts. *Sciara* rDNA is not cut by Bam HI nor Sal I. Note that the *Sciara* rDNA orientation of the insert relative to pBR322 is reversed between these two clones. Sizes of larger restriction fragments after Eco RI plus Hae II digestion are indicated (see Figs. 2b and 5). pBc 2 is shown for comparison; in this case the rDNA insert is joined to pBR322 at the Eco RI site.

With so few rRNA genes in Sciara, we would of course like to know if the IVS⁺ rRNA genes are functional. We have found by Miller spreads of Sciara spermatogonia that virtually all rDNA units are transcribed (28), but unfortunately within the error limitations of this method we cannot distinguish whether the few copies of IVS⁺ rRNA genes are also transcribed or not.

Although our heteroduplex data suggests no appreciable sequence homology between the two types of IVS in Sciara rDNA, they appear to be located at about the same point in the β 28S rRNA coding region. This general region of 28S rDNA has been highly conserved during evolution (53) and contains different types of IVS in other organisms (54). However, the exact site differs for the IVS found in Tetrahymena (55), Drosophila virilis (56), Chlamydomonas chloroplast (57) and yeast mitochondria (58). The exact site within β 28S rDNA where the two types of Sciara IVS are found will be decided by future DNA sequencing, and it will be of interest to see if the Sciara IVS sites are identical to that of another dipteran, D. virilis. In the case of D. virilis rDNA, a direct repeat is introduced flanking the IVS (56), and if this is also found for the two types of Sciara IVS, it will suggest a mechanism similar to Drosophila's for evolutionary introduction of the IVS into rDNA.

One might speculate that there are two distinct categories of IVS⁺ rDNA genes. One category is found in the non-insect IVS⁺ rDNAs studied so far; in all these cases the transcribed rDNA is not part of the nuclear chromosomes, but instead is extrachromosomal. Moreover, in all these cases the IVS is transcribed, and a direct repeat flanking the IVS has not been observed by sequence analysis (55, 57, 58). The second category contains IVS⁺ rRNA genes found in insects. In this category, the rDNA is chromosomal; the IVS⁺ rRNA genes represent only a portion of the total rDNA and are apparently not transcribed (at least in D. melanogaster). Finally, in this category the direct repeat flanking the IVS (of at least D. virilis) suggests that the evolutionary origin of this IVS may be via a transposable element.

Finally, in the future we can ask if each of the two IVS types found in Sciara rDNA have a specific location within the 45 tandemly reiterated rRNA genes, which are found within three heterochromomeres which are separable by existing translocations (59).

ACKNOWLEDGEMENTS

We are grateful to Drs. John Morrow and Pieter Wensink for advice on cloning, and to Drs. Richard L. Gourse, and Rainer Renkawitz for helpful comments and discussion. We thank Mrs. Carol King for typing this manuscript. This work was supported by PHS-GM 22882 and PHS-GM 26070 to S.A.G.; S.A.G. was a recipient of Research Center Development Award PHS-GM 00036.

†To whom all correspondence and reprint requests should be sent.

REFERENCES

1. Wild, M.A. and Gall, J.G. (1979) *Cell* 16, 565-573.
2. Din, N. and Engberg, J. (1979) *J. Mol. Biol.* 134, 555-574.
3. Campbell, G.R., Littau, V.C., Melera, P.W., Allfrey, V.G. and Johnson, E.M. (1979) *Nucleic Acids Res.* 6, 1433-1447.
4. Gubler, U., Wyler, T. and Braun, R. (1979) *FEBS Lett.* 100, 347-350.
5. Rochaix, J.D. and Malnoe, P. (1978) *Cell* 15, 661-670.
6. Bos, J.L., Heyting, C., Borst, P., Arnberg, A.C., Van Bruggen, E.F.J. (1978) *Nature* 275, 336-338.
7. Faye, G., Dennebouy, N., Kujawa, C. and Jacq, C. (1979) *Molec. gen. Genet.* 168, 101-109.
8. Heckman, J.E. and RajBhandary, U.L. (1979) *Cell* 17, 583-595.
9. Hahn, U., Lazarus, C.M., Lünsdorf, H., and Küntzel, H. (1979) *Cell* 17, 191-200.
10. Din, N., Engberg, J., Kaffenberger, W. and Eckert, W.A. (1979) *Cell* 18, 525-532.
11. Mannella, C.A., Collins, R.A., Green, M.R., Lambowitz, A.M. (1979) *Proc. Nat. Acad. Sci.* 76, 2635-2639.
12. Gubler, U., Wyler, T., Seebeck, T., and Braun, R. (1980) *Nucleic Acids Res.* 8, 2647-2664.
13. Zaug, A.J. and Cech, T.R. (1980) *Cell* 19, 331-338.
14. Glover, D.M. and Hogness, D.S. (1977) *Cell* 10, 167-176.
15. White, R.L. and Hogness, D.S. (1977) *Cell* 10, 177-192.
16. Wellauer, P.K. and Dawid, I.B. (1977) *Cell* 10, 193-212.
17. Pellegrini, M., Manning, J. and Davidson, N. (1977) *Cell* 10, 213-224.
18. Barnett, T. and Rae, P.M.M. (1979) *Cell* 16, 763-775.
19. Renkawitz-Pohl, R., Glätzer, K.H. and Kunz, W. (1980) *Nucleic Acids Res.* 8, 4593-4611.
20. Beckingham, K. and White, R.L. (1979) *J. Mol. Biol.* 137, 349-373.
21. Long, E.O. and Dawid, I.B. (1979) *Cell* 18, 1185-1196.
22. Glätzer, K.H. (1979) *Chromosoma* 75, 161-175.
23. Jolly, D.J. and Thomas, C.A., jr. (1980) *Nucleic Acids Res.* 8, 67-84.
24. Chooi, W.Y. (1979) *Chromosoma* 74, 57-74.
25. Endow, S.A. and Glover, D.M. (1979) *Cell* 17, 597-605.
26. Wellauer, P.K., Dawid, I.B. and Tartof, K.D. (1978) *Cell* 14, 269-278.
27. Gerbi, S.A. and Crouse, H.V. (1976) *Genetics* 83, 81-90.
28. Renkawitz, R., Gerbi, S.A. and Glätzer, K.H. (1979) *Molec. gen. Genet.* 173, 1-13.
29. Berk, A.J. and Sharp, P.A. (1977) *Cell* 12, 721-732.
30. Gerbi, S.A. (1971) *J. Mol. Biol.* 58, 499-511.
31. McDonnell, M.W., Simon, M.N. and Studier, F.W. (1977) *J. Mol. Biol.* 110, 119-146.
32. Sutcliffe, J.G. (1978) *Nucleic Acids Res.* 5, 2721-2728.
33. Roychoudhury, R., Jay, E. and Wu, R. (1976) *Nucleic Acids Res.* 3, 863-877.
34. Brutlag, D., Fry, K., Nelson, T. and Hunt, P. (1977) *Cell* 10, 509-519.
35. Mandel, M. and Higa, A. (1970) *J. Mol. Biol.* 53, 159-162.
36. Wensink, P.C., Finnegan, D.J., Donelson, J.E. and Hogness, D.S. (1974) *Cell* 3, 315-325.
37. Grunstein, M. and Hogness, D.S. (1975) *Proc. Nat. Acad. Sci.* 72, 3961-3965.
38. Gergen, J.P., Stern, R.H. and Wensink, P.C. (1979) *Nucleic Acids Res.* 7, 2115-2136.

39. Maniatis, T., Jeffrey, A. and Kleid, D.G. (1975) *Proc. Nat. Acad. Sci.* 72, 1184-1188.
40. Rigby, P.W.J., Dieckmann, M., Rhodes, C. and Berg, P. (1977) *J. Mol. Biol.* 113, 237-251.
41. Sato, S., Hutchison III, C.A. and Harris, J.I. (1977) *Proc. Nat. Acad. Sci.* 74, 542-546.
42. Southern, E.M. (1975) *J. Mol. Biol.* 98, 503-517.
43. Thomas, M., White, R.L. and Davis, R.W. (1976) *Proc. Nat. Acad. Sci.* 73, 2294-2298.
44. Davis, R.W., Simon, M. and Davidson, N. (1971) in *Methods in Enzymology*, L. Grossman and K. Moldave, eds., vol. 21D pp. 413-428, Academic Press, New York.
45. Wellauer, P.K., Dawid, I.B., Brown, D.D. and Reeder, R.H. (1976) *J. Mol. Biol.* 105, 461-486.
46. Leon, W., Fouts, D.L. and Manning, J. (1978) *Nucleic Acids Res.* 5, 491-504.
47. Blin, N., Sperraza, J.M., Wilson, F.E., Bieber, D.G., Mickel, F.S. and Stafford, D.W. (1979) *J. Biol. Chem.* 254, 2716-2721.
48. Arnheim, N. and Southern, E.M. (1977) *Cell* 11, 363-370.
49. Wellauer, P.K. and Dawid, I.B. (1979) *J. Mol. Biol.* 128, 289-303.
50. Schäfer, M., Wyman, A.R. and White, R., *J. Mol. Biol.* (in press).
51. Kunz, W., Petersen, G., Renkawitz-Pohl, R., Glätzer, K.H. and Schäfer, M. *Chromosoma* (in press).
52. Helling, R.B., Goodman, H.M. and Boyer, H.W. (1974) *J. Virol.* 14, 1235-1244.
53. Gourse, R.L. and Gerbi, S.A. (1980) *J. Mol. Biol.* 140, 321-339.
54. Gourse, R.L. and Gerbi, S.A. (1980) *Nucleic Acids Res.* 8, 3623-3637.
55. Wild, M.A. and Sommer, R. (1980) *Nature* 283, 693-694.
56. Rae, P.M.M., Kohorn, B.D. and Wade, R.P. (1980) *Nucleic Acids Res.* 8, 3491-3504.
57. Allet, B. and Rochaix, J.D. (1979) *Cell* 18, 55-60.
58. Dujon, B. (1980) *Cell* 20, 185-197.
59. Crouse, H.V., Gerbi, S.A., Liang, C.M., Magnus, L. and Mercer, I.M. (1977) *Chromosoma* 64, 305-318.