UC Irvine
UC Irvine Previously Published Works

Title
Sequence arrangement of the rRNA genes of the dipteran Sarcophaga bullata

Permalink
https://escholarship.org/uc/item/5s4979mn

Journal
Nucleic Acids Research, 9(11)

ISSN
0305-1048

Authors
French, Cynthia K
Fouts, DL
Manning, JE

Publication Date
1981

DOI
10.1093/nar/9.11.2563

Peer reviewed

Nucleic Acids Research

Sequence arrangement of the rRNA genes of the dipteran Sarcophaga bullata

Cynthia K.French[1], D.L.Fouts and J.E.Manning

Department of Molecular Biology and Biochemistry, University of California at Irvine, Irvine, CA 92717, USA

ABSTRACT

Velocity sedimentation studies of RNA of Sarcophaga bullata show that the major rRNA species have sedimentation values of 26S and 18S. Analysis of the rRNA under denaturing conditions indicates that there is a hidden break centrally located in the 26S rRNA species. Saturation hybridization studies using total genomic DNA and rRNA show that 0.08% of the nuclear DNA is occupied by rRNA coding sequences and that the average repetition frequency of these coding sequences is approximately 144. The arrangement of the rRNA genes and their spacer sequences on long strands of purified rDNA was determined by the examination of the structure of rRNA:DNA hybrids in the electron microscope. Long DNA strands contain several gene sets (18S + 26S) with one repeat unit containing the following sequences in the order given: (a) An 18S gene of length 2.12 kb, (b) an internal transcribed spacer of length 2.01 kb, which contains a short sequence that may code for a 5.8S rRNA, (c) A 26S gene of length 4.06 kb which, in 20% of the cases, contains an intron with an average length of 5.62 kb, and (d) an external spacer of average length 9.23 kb.

INTRODUCTION

The DNA sequences coding for the major ribosomal RNA species (18S and 28S) in several eucaryotes (3,4,5,6,7,8,9,10,11) have been found to be repeated 50-500 fold per haploid equivalent of DNA and are clustered in the nucleolar organizer region(s) of the chromosomes. These clustered arrays of rRNA coding sequences are composed of numerous individual repeated units, each of which contains an 18S gene region, an adjacent small transcribed spacer sequence, which may contain a 5.8S coding sequence, and a 28S gene region, followed by an external non-transcribed spacer sequence. The sequential organization of these repeated units have been found to exist in two different structural arrangements. In the protists, Tetrahymena (12), Physarum (13), and Dictyostelium (14), the repeat units are arranged in a head-to-head palindromic array, while in Xenopus (15), Drosophila (7), Sciara (11), and Leishmania (16), the repeat units are present in a head-to-tail tandem array.

A second major difference between the organization of the rRNA genes in different organisms is the structure of the 28S gene. This difference involves the presence or absence of a noncoding segment of DNA (intron) which interrupts the 28S gene region at a unique site. With respect to the presence or absence of introns, three patterns of 28S gene structures have been observed: those without any detectable introns in the 28S gene, such as in Xenopus (15), Bombyx (17), and mouse (5); those which contain introns in all of the 28S genes, such as in Tetrahymena (18,19); and, as in Sciara (11) and Drosophila, those organisms in which only some of the 28S rRNA genes contain an intron (6,7,8,9).

The structural organization of the introns in the 28S gene has been most extensively analyzed in D. melanogaster. Here, the introns are composed of two distinguishable classes. Type I introns include 0.5, 1.0 and 5.0 kb length classes. Type II introns range in length from 1.5 to 6.0 kb and have no sequence homology to the Type I class (20). Transcriptional studies done in D. melanogaster to determine whether the intron-containing classes of cistrons are transcribed have shown that only a very limited number of rDNA transcripts (1-2 copies/cell) contains the transcribed intron segment (21,22). With respect to the position of the intron within the 28S gene, it is seen that in the dipterans D. melanogaster (6,7,8,9), D. virilis (23), and Calliphora erythrocephala (24), the position of the intron relative to the junction of the 28S gene and internal spacer is highly conserved. The origin and function of these introns are, however, unknown.

We report here our studies on the number, size and sequence arrangement of the rRNA genes in a close relative of Drosophila and Calliphora, Sarcophaga bullata. The arrangement of ribosomal cistrons was studied by first enriching for double stranded DNA containing the rRNA genes from S. bullata DNA by application of the avidin-biotin method of gene enrichment (25). The arrangement of rRNA coding sequences in the enriched nuclear DNA was determined by use of the T4 gene 32-ethidium bromide technique (26) and electron microscopic analysis. We have found that the organization and lengths of the rDNA gene sequences are similar to that in D. melanogaster, and that S. bullata contains an intron in at least 20% of the 26S rRNA coding sequences.

MATERIALS AND METHODS
    Preparation of DNA. High molecular weight DNA from Sarcophaga

bullata (purchased from Carolina Biological Supply) was extracted from a crude nuclear pellet prepared from organisms 24-36 hours after puparium formation (25,27). The DNA was further purified by centrifugation to equilibrium in a neutral cesium chloride buoyant density gradient.

Preparation of rRNA. Monosomes were isolated from late third instar larvae by a modification (28) of the procedure of Jackson et al. (29). The rRNA was then extracted by the standard SDS-phenol/chloroform methods, and fractionated by velocity sedimentation through a 5-20% sucrose gradient. Following this procedure, the individual RNAs were pelleted through a 6M CsCl cushion (30). The purified RNAs were analyzed on polyacrylamide gels and stored at -70°C.

Coupling of rRNA and cytochrome c-biotin. Modification of Sarcophaga bullata rRNA with cytochrome c-biotin was done exactly as described by Manning et al. (31) for Drosophila rRNA. Coupling conditions resulted in a final ratio of 130 nucleotides per bound cytochrome c-biotin.

R-loop formation and hybrid isolation. Double stranded nuclear DNA (final concentration 400 µg/ml) was mixed with cytochrome c-biotin modified rRNA (final concentration 35.5 µg/ml) and dialyzed for 5 hrs at 4°C against 90% formamide, 0.01 M sodium borate (pH 8.5), 0.5 M NaCl and 0.002 M EDTA. This rRNA concentration represents a 15-fold RNA excess over the rDNA coding sequences present. The reaction mixture was then incubated at 40°C for 16 hours (Rot reached = 0.48 moles sec l^{-1}). Following R-loop formation, the reaction mixture was dialyzed for 2 hrs at 4°C against 100 volumes of 1.0 M NaCl, 0.02 M sodium borate (pH 8.0) and 0.005 M EDTA. 200 µl of avidin spheres (25,27) at 20 mg/ml in 1.0 M NaCl, 0.001 M EDTA, were added and the dialysis continued for 2 hr at 4°C. This solution was then added slowly, with gentle mixing, to a solution of cesium chloride in 0.005 M EDTA (pH 8.0) to give a final volume of 5.0 ml and a density of 1.45 g/cc. The mixture was centrifuged at 35,000 rpm for 48 hrs at 15°C in a Spinco SW50.1 rotor and the gradient was fractionated into 0.5 ml aliquots. The DNA pellet was resuspended in SSC. The DNA present in each of the aliquots, as well as the pellet, was assayed for rDNA sequences as described below.

rRNA:rDNA hybridization and preparation for the electron microscope. rRNA:rDNA hybrids were prepared for electron microscope mapping studies as follows: 20 µl of enriched double strand rDNA (6 µg/ml) was denatured by addition of 5 µl of 1.0 N NaOH, after 30' at 25°C the mixture was neutralized on ice by addition of 2.0 M Hepes acid. To the denatured rDNA, 10 µl

of rRNA (2.48 mg/ml) was added and the mixture was incubated for 20' at 65°C. The mixture was then dialyzed for 2 hr at 4°C against 0.01 $\underline{M}$ sodium phosphate (pH 7.4), 0.001$\underline{M}$ EDTA, and the rRNA:rDNA hybrids were prepared for examination in the electron microscope by use of the T4 gene-32 protein spreading technique (26). Both double stranded and single stranded $\emptyset$X174 DNA were used as molecular length internal standards.

Preparation and hybridization of ^{125}I-labeled rRNA. ^{125}I-labeled rRNA was used to assay the amount of rDNA coding sequences in the CsCl enrichment gradient fractions. ^{125}I-labeled rRNA was prepared exactly as described by Commerford (32). Following the iodination procedure, labeled RNA was purified by Sephadex G-100 column chromatography followed by sec-butanol extraction of the unincorporated [125]I. Specific activities obtained ranged from 3.0 - 6.0 x 10^7 cpm/µg. Hybridization was performed by reacting a 60-fold mass excess of purified ^{125}I-labeled rRNA with total nuclear DNA or DNA from the CsCl gradient fractions. High salt reactions were conducted in 0.75 $\underline{M}$ NaCl, 0.0025 $\underline{M}$ PIPES (pH 6.8), low salt reactions were conducted in 0.18 $\underline{M}$ NaCl, 0.0025 $\underline{M}$ PIPES (pH 6.8), and both reactions were incubated at 65°C. Hybridized samples were resuspended in 1.0 ml of 1 X SSC and treated with 50 µg/ml RNase at 25°C for 30 minutes. 10 µl aliquots were removed before digestion to measure input radioactivity. RNase-resistant hybrids were TCA precipitated and collected on GFC filters (Whatman), washed, dried and counted in a toluene-based scintillating cocktail (4 gram omniscint/liter).

Molecular weight determinations of rRNA. Sarcophaga rRNA was analyzed by electrophoresis on non-denaturing 2.4% polyacrylamide gels as described by Loening (33). After electrophoresis, the gels were scanned using a Gilford gel scanning accessory on a Beckman DU spectrophotometer. rRNA was also analyzed by electrophoresis on 1.2% agarose gels containing 10 m$\underline{M}$ methylmercury hydroxide as described by Bailey and Davidson (34). After electrophoresis, the gels were soaked in 50 m$\underline{M}$ β-mercaptoethanol for 20 min, stained in EtBr (1 µg/ml) for 30 min and visualized using a UV transilluminator. The molecular weights of the rRNA species were determined from plots of RNA electrophoretic mobility (in arbitrary units) versus the logarithm of molecular weight (33). RNAs utilized as molecular weight standards were $\underline{E}$. $\underline{coli}$ 16S rRNA (0.56 X 10^6 daltons), HeLa 18S rRNA (0.70 X 10^6 daltons) and HeLa 28S rRNA (1.75 X 10^6 daltons) (33). The molecular weights of the Sarcophaga rRNAs were then converted to $S^o_{20,w}$ values using the equation of Spirin (35).

RESULTS

    Analysis of S. bullata rRNA by velocity sedimentation and gel electro-
phoresis. The size of the major RNAs in Sarcophaga was determined by both
cosedimentation of Sarcophaga rRNA in a 5-20% linear sucrose gradient with
^{3}H-uridine labeled 26S and 18S D. melanogaster rRNA as internal standards
(Fig. 1A); and polyacrylamide gel electrophoresis using HeLa 28S and 18S
rRNAs as external standards (data not shown). The molecular weight values
obtained by gel electrophoresis for the 26S and 18S rRNAs are 1.44 and 0.75
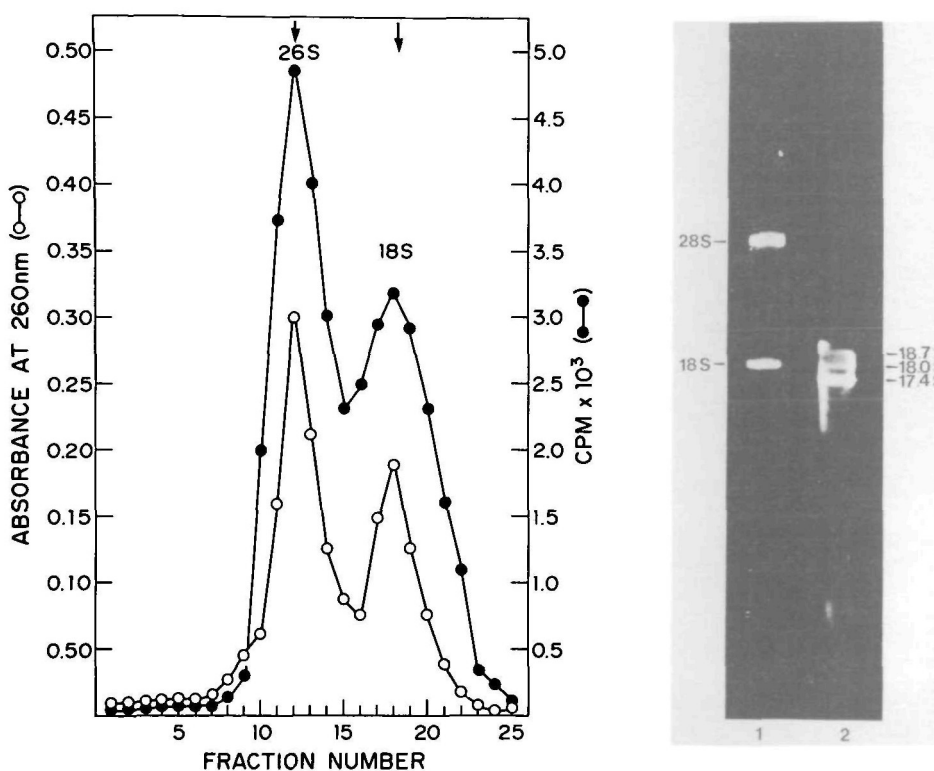

Figure 1. (A) Sucrose density gradient analysis of rRNA. OD quantities
of S. bullata were cosedimented with ^{3}H-labeled D. melanogaster 26S and
18S rRNA in linear 5-20% sucrose gradients, at 22,000 rpm for 16 hrs
at 4°C in an SW41 rotor. (-O-) denotes S. bullata 26S and 18S rRNA,
(-●-) denotes ^{3}H D. melanogaster rRNA. (B) Methylmercury gel analysis.
26S and 18S S. bullata rRNA was fractionated on 0.6 x 15 cm 1.2% agarose
gels containing 10 mM Methylmercury hydroxide. Lane 1, ethidium bromide
stained pattern of HeLa 28S and 18S ribosomal size markers. Lane 2, position
of migration of 26S and 18S S. bullata rRNA. Three prominent bands are
resolved at 18.7S, 17.4S, and the intact 18S.

x 10^6 daltons, respectively. These values are approximately the same as those observed for D. melanogaster rRNA by Loening (33), and are in agreement with the results of the velocity sedimentation analysis which indicates that the two major rRNA species in S. bullata have sedimentation coefficients similar to those observed for D. melanogaster. Also, from the measurements of RNA:DNA-hybrid regions (see below), we estimate values of 2.12 + 0.14 and 4.06 + 0.32 kb for the lengths of the Sarcophaga 18S and 26S genes, respectively. Since these values are in reasonable agreement with those obtained by the electrophoretic and sedimentation analysis, we take the molecular lengths of the 18S and 26S rRNA of S. bullata to be 2.12 and 4.06 kb, respectively.

Following heat denaturation at 60°C for 15 minutes, or denaturation by methylmercury hydroxide (34), the 26S rRNA is converted into two molecular weight species. In methylmercury hydroxide gels these two species migrate at 18.7S and 17.4S, respectively (Fig. 1B). The size of these two 26S fragments are similar to those previously reported for denatured 26S rRNA in D. melanogaster (36,37), suggesting the presence of a hidden break in the S. bullata 26S rRNA.

Number of rRNA coding sequences. The fraction of nuclear DNA homologous to rRNA was determined by rRNA/DNA saturation hybridization experiments. As shown in Fig. 2, the rRNA hybridizes at saturation with 0.08% of the nuclear DNA. Using a nuclear genome size of 5.48 X 10^8 nucleotide pairs (38), and taking the sum of the molecular lengths of 26S
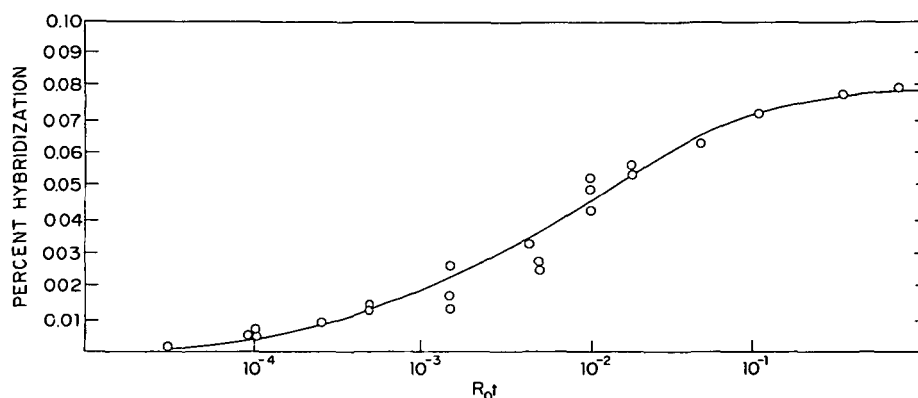

Figure 2. Kinetic analysis of ribosomal RNA. Results of a solution hybridization of excess denatured S. bullata total cellular DNA hybridized to ^{125}I-labeled 26S + 18S rRNA.

and 18S rRNA to be 6.2 kb, the rRNA gene set (26S and 18S) is repeated 144
times in the Sarcophaga genome.

Enrichment of rDNA. The rRNA coding sequences in S. bullata were
enriched by forming rDNA:rRNA R-loop hybrids with cytochrome c-biotin
modified rRNA. The hybrid molecules were then partially purified from the
remaining nuclear DNA by reaction with avidin spheres and buoyant banding
in a neutral CsCl density gradient.

The amount of enrichment attained by this procedure was determined
by solution hybridization of DNA from the various CsCl gradient fractions
with ^{125}I-labeled rRNA and the results are summarized in Figure 3. The
DNA from the pellet of the enrichment gradient (depleted fraction) saturates
at 0.018%, approximately a four-fold reduction in sequences as compared to
the saturation value obtained for unfractionated nuclear DNA (0.06% and
Figure 2). The DNA from the region of the gradient containing the avidin
spheres (the enriched fraction) saturates at 1.56%, indicative of a 26-fold
enrichment for coding sequences versus unfractionated DNA.

Sequence arrangement of Sarcophaga rDNA. Following hybridization of
rRNA with the enriched rDNA from the CsCl gradient, the distribution of
DNA:rRNA hybrid regions on the long DNA strands was observed by electron
microscopy using the T4 gene 32-ethidium bromide staining technique. A
schematic of the spatial arrangement of the rRNA coding sequence on the
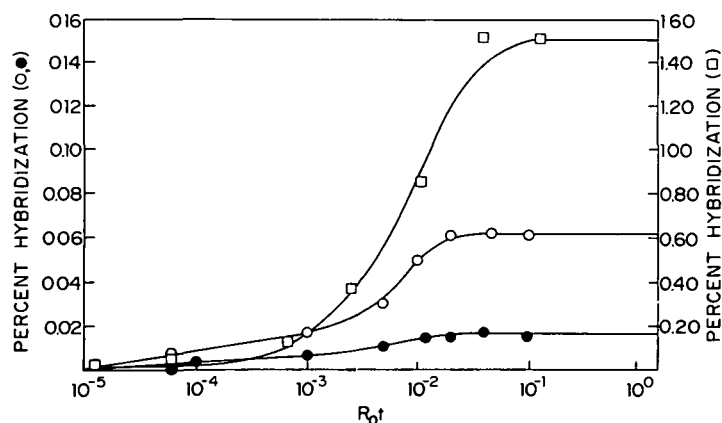
Figure 3. Kinetic analysis of DNA's from various fractions of the
enrichment gradient. Results of hybridization of ^{125}I-labeled rRNA to
nuclear DNA before fractionation (-O-), to DNA from the enriched fraction
(-□-) and to the depleted fraction (-●-) of DNA from the CsCl enrich-
ment gradient.

enriched strands of rDNA is shown in Figure 4. A single repeat unit
contains the following sequences:

a) an 18S gene of length 2.12 + 0.14 kb.

b) an internal spacer (Spl' + 5.8S + Spl") which can be identified
   as: Spl', 1.20 + 0.16 kb; 5.8S gene, 0.21 + 0.02 kb; and Spl",
   0.60 + 0.03 kb.

c) a 26S gene. Approximately 80% of the 26S genes are unbroken, or
   continuous, with a length of 4.06 + 0.32 kb as shown in the upper
   arrangement of Fig. 4. However, some of the 26S genes contain an
   insertion of an additional DNA segment that is not complementary
   to the rRNA. This arrangement is referred to as the "intron"
   arrangement.

d) An external spacer, Sp 2, which is heterogeneous in size, with an
   an average length of 9.24 + 5.81 kb.

The presumed 3' to 5' polarity of the coding strand of rDNA indicated
in Fig. 4 is assigned from the determination of the polarity of the 40S rRNA
precursor of Xenopus (39). Fig. 5 is an electron micrograph showing such a
repeat unit on a single strand of rDNA after hybridization with 18S and
26S RNA and preparation for electron microscopic observation by the T4 gene
32-ethidium bromide procedure. Further detailed discussion of the indivi-
dual components of the rDNA gene set is presented below.

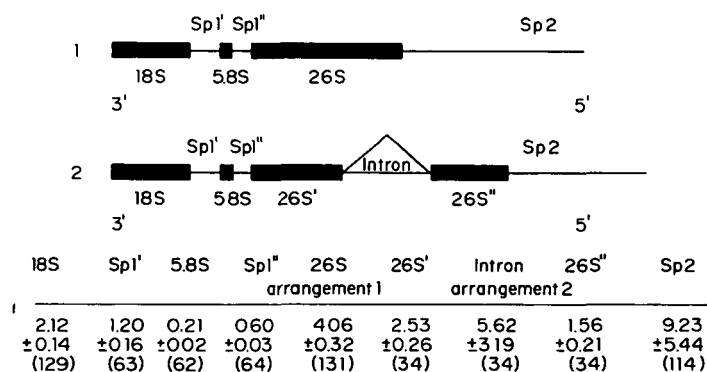| 18S	Spl'	5.8S	Spl"	26S	26S'	Intron	26S"	Sp2
				arrangement 1		arrangement 2		
2.12	1.20	0.21	060	406	2.53	5.62	1.56	9.23
±0.14	±016	±002	±003	±0.32	±0.26	±319	±0.21	±5.44
(129)	(63)	(62)	(64)	(131)	(34)	(34)	(34)	(114)

Figure 4. Schematic diagram of the repeat unit of S. bullata rDNA.
The 18S, 5.8S, and 26S gene regions are depicted by heavy bars, while
the spacer sequences (Sp) and the "intron" sequences are denoted by
thin lines. The lengths of all sequences are given in kilobase units
and are shown below the diagram. The number of molecules measured are
given in parentheses. The 3' and 5' designation in the diagram refers
to the presumed polarity on the coding strand of rDNA.
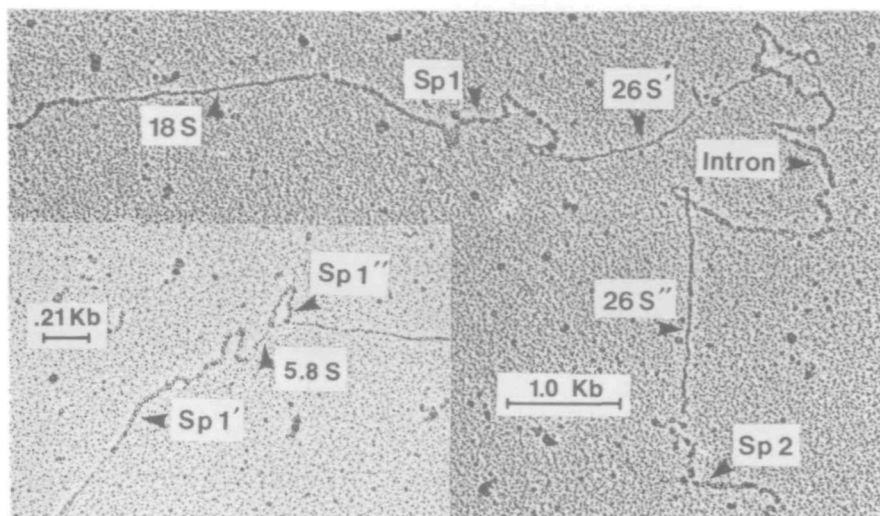
Figure 5. Electron micrographs of rDNA:rRNA hybrid molecules prepared for electron microscopy by the T4 gene 32-protein technique. A micrograph of a molecule showing a gene set with an intron. Inset, high magnification of the transcribed spacer and the presumed 5.8S gene.

The internal spacer and 5.8S gene. The short spacer region separating the 18S and 26S genes consistently contains a short duplex region as shown in the inset in the electron micrograph in Fig. 5. It is assumed that this RNA:DNA hybridized region contains a rRNA gene similar to the 5.8S gene which has been characterized by Jordan et al. (40) in Drosophila. Consistent with this observation, Spiers and Birnstiel (39) show that in Xenopus the 5.8S rRNA gene occurs in the transcribed spacer between the 18S and 28S genes. Since both the position and size of the small duplex region observed in Sarcophaga rDNA are like that found in Xenopus and Drosophila rDNA, we presume that the RNA:DNA hybrid region is due to the presence of a gene which codes for an RNA molecule similar to the 5.8S gene found in those organisms.

The Intron. Approximately 20% of all 26S molecules observed in our studies contained an intron. A histogram of the length distribution of the intron is shown in Fig. 6. The introns are heterogeneous in length and vary in size from 1.29 to 12.3 kb. The distribution profile shows three general size classes: 1.0 - 3.0 kb, 5.0 - 7.0 kb, 9.0 - 11.0 kb. The difference between the means of the first two distributions is 3.9 kb,
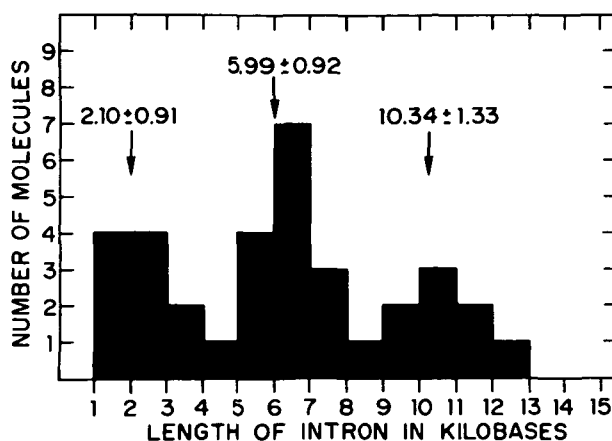
Figure 6. A histogram of the observed distribution of the length of the intron segment from S. bullata. The average lengths are indicated at the top of the histogram.

while that between the middle and largest size class is 4.3 kb. Thus, within experimental error, the size classes differ in length from each other by successive intervals of ~4.1 kb. Although we do not know the sequences present in these size classes, it is possible that the smaller introns have sequences in common with the larger ones and may actually be conserved within the latter. In this regard, an interesting numerical coincidence is the observation that the addition of two of the smaller class introns to that class generates the mean of the middle class. A further addition of two more of the smaller class introns would then generate the mean of the largest class of introns.

The presence of distinct size classes of introns has previously been observed in the 28S genes of D. melanogaster (7). Here, the introns also exhibit three size classes (7), with the mean of each being 1-3 kb smaller than those observed in Sarcophaga. Interestingly, the Type 1 introns present in Drosophila share sequence homologies within this class (20). Although we do not yet know whether introns in Sarcophaga share similar sequences, this possibility is slightly strengthened by the observation that intron sequences in Drosophila rDNA exhibit sequence homology with Sarcophaga rDNA (23).

The position of the intron within the 26S gene may be determined by examining the length distribution of the two parts of the 26S gene. The intron inserts at a position 2.53 $\pm$ 0.26 kb (26S', Fig. 4) from the junction of the 26S gene with the internal spacer, and 1.56 $\pm$ 0.21 kb

(26S", Fig. 4) from the junction of the 26S gene with the external spacer. The length distribution of the two components of the 26S gene, 26S' and 26S", each show a standard deviation of about 10%, which is approximately the expected deviation if each segment is homogeneous in length. Consistent with this interpretation is the observation that the 26S' and 26S" regions add up to the length observed for the intact 26S gene. We, therefore, conclude that the intron occurs at a unique and reproducible site within the total 26S gene.

The external spacer. Fig. 7 shows the observed length distribution for the external spacer (Sp 2). This spacer is heterogeneous is length with a size range of 1 kb to 32.2 kb and an average length of 9.24 ± 5.81 kb.

DISCUSSION

In S. bullata, the enriched single strands of rDNA contain several repeat units (18S + 26S genes), all arranged in a head-to-tail formation. The repeated units are separated by external spacer sequences which range in length from 1 kb to 32.0 kb, with an average size of 9.24 kb. The size of the average repeat unit is approximately 17.4 kb based on electron microscopy measurements. Thus, the rDNA repeat units in S. bullata are heterogeneous in size like those described for D. melanogaster (6,7,8,9),
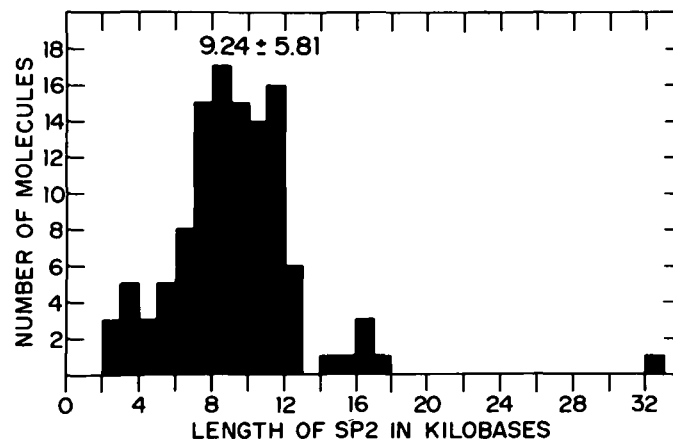

Figure 7. A histogram of the observed distribution of the lengths of the external spacer (Sp2) from S. bullata. The average length of the spacer is indicated at the top of the histogram.

Xenopus laevis (15), and mouse (5), but unlike those repeats described
for Sciara (11), B. mori (17), Tetrahymena (12), and Dictyostellium (14).

The most intriguing feature of the S. bullata rDNA repeat unit is the
presence of an intron in at least 20% of the 26S ribosomal RNA coding
segments. The introns measured range from 1.26 kb to 12.3 kb in length
with an average size of 5.62 kb. Although the length of the intron is
heterogeneous, the position at which it occurs within the total 26S gene
is at a reproducible point. Furthermore, the location at which the
intron is found in S. bullata rDNA appears to be the same as that observed
for the position of the intron in the 26S genes of D. melanogaster (7,8,9),
D. virilis (23), and C. erythrocephala (24). Thus, at least among these
diptera, the location of the intron within the 26S rDNA coding region
has been evolutionarily conserved.

The only other organisms examined which were found to have an intron
in the large ribosomal RNA gene are T. pigmentosa (18), T. thermophila (19),
yeast mitochondrial rDNA (41,42), and N. crassa mitochondrial rDNA (43).
Transcriptional studies in T. thermophila have revealed the presence of
high molecular transcripts containing the intron segment which appear
to be processed to yield functional 26S molecules (19). Transcriptional
studies done in D. melanogaster to determine whether the intron containing
cistrons are transcribed have shown that only a very limited (1-2 copies/
cell) number of transcripts contain an unprocessed intron segment (21,22).
These studies also indicate that those 26S genes containing an intron do
not contribute significantly to the synthesis of the 26S rRNA. With the
widespread occurrence of intron classes of ribosomal cistrons in the order
Diptera, as well as the extremely low level of transcription of this intron-
containing class, questions then arise as to the functional significance of
the intron in the 26S ribosomal gene.

A recent comparison by Tartof (44) of six sibling species within the
melanogaster subgroup of Drosophila indicates that there is a reduction in
the number of ribosomal cistrons among those sibling species which can be
related to the directionality of the phylogeny of the D. melanogaster
subgroup. Concommitant with this reduction in rRNA genes is a selective
decrease in the proportion of 26S rRNA coding sequences which contain an
intron. This observation suggests that those 26S genes which contain
introns may not be functional.

In D. melanogaster, approximately 45-60% of the 200 26S genes contain
introns (44). We have observed that approximately 20% of the 72 26S genes

in Sarcophaga contain an intron. The total number of ribosomal genes is
thus less in S. bullata than D. melanogaster, and with this reduction, a
disproportionate decrease in the number of 26S genes which contain introns
is observed. Consistent with these observations is that by Renkowitz et al.
on Sciara (11). They have found that this dipteran has only 45 rRNA genes
of which less than 10% contain introns (Renkawitz-Pohl, Matsumoto, and Gerbi.
Personal communication). These numerical coincidences invite the specula-
tion that not only are the 26S genes which contain introns non-functional,
but also that there is a relationship between decreasing rDNA gene number
and the percentage of 26S genes which contain introns. This raises the
possibility that, at least in the Diptera, a finite number of rDNA gene
sets is essential, and as this number is approached, those gene sets having
introns are selectively lost.

ACKNOWLEDGEMENTS

This work was submitted by C. French in partial fulfillment of the
requirements for the degree of Doctor of Philosophy, School of Biological
Sciences, University of California at Irvine.

REFERENCES

1. Present address: Department of Biology, Massachusetts Institute of
    Technology, Cambridge, Massachusetts.
2. Abbreviations used: EtBr - ethidium bromide. SSC - sodium citrate
    buffer.
3. Birnstiel, M.L., Wallace, H., Sirlin, J. and Fischberg, M. (1966).
    Nat. Cancer Inst. Monos. 23, 431-444.
4. Brown, D.D., and Weber, C.S. (1968). J. Mol. Biol. 34, 661-680.
5. Arnheim, N., and Southern, E.M. (1977). Cell 11, 363-370.
6. Glover, D.M., Hogness, D.S. (1977). Cell 10, 167-176.
7. Pellegrini, M., Manning, J. and Davidson, N. (1977). Cell 10, 213-224.
8. Wellauer, P.K., and Dawid, I.B. (1977). Cell 10, 193-212.
9. White, R.L., and Hogness, D.S. (1977). Cell 10, 177-192.
10. Gerbi, S.A. (1971). J. Mol. Biol. 58, 499-511.
11. Renkawitz, R., Gerbi, S.A., and Glatzer, K.H. (1979). Molec. Gen.
    Genet. 173, 1-13.
12. Karrer, K.M., and Gall, J.G. (1976). J. Mol. Biol. 104, 421-453.
13. Molgarrd, H.V., Mathews, H.R., and Bradbury, E.M. (1976). Eur. J.
    Biochem. 68, 541-549.
14. Maizels, N. (1976). Cell 9, 431-438.
15. Wellauer, P.K., Dawid, I.B., Brown, D.D., and Reeder, R. (1976).
    J. Mol. Biol. 105, 461-497.

16. Leon, W., Fouts, D.L., and Manning, J.E. (1977). Nuc. Acids. Res. 5, 491-504.
17. Manning, R.F., Samols, D.R., and Gage, L.P. (1978). Gene 4, 153-166.
18. Wild, M.A., and Gall, J.G. (1979). Cell 16, 565-574.
19. Cech, T.R., and Rio, D.C. (1979). Proc. Natl. Acad. Sci. U.S.A. 76, 5051-5055.
20. Wellauer, P.K., Dawid, I.B., and Tartof, K.D. (1978). Cell 14, 269-278.
21. Long, E. O., and Dawid, I.B. (1979). Cell 18, 1185-1196.
22. Jolley, D.J., and Thomas, C.A., Jr. (1980). Nuc. Acids Res. 8, 67-84.
23. Barnett, T., and Rae, P.M.M. (1979). Cell 16, 763-775.
24. Beckingham, K., and White, R. (1980). J. Mol. Biol. 137, 349-374.
25. Pellegrini, M., Holmes, D.S., and Manning, J. (1977). Nuc. Acids Res. 4, 2961-2974.
26. Wu, M., and Davidson, N. (1975). Proc. Natl. Acad. Sci. U.S.A. 72, 4506-4510.
27. Manning, J., Pellegrini, M., and Davidson, N. (1977) Biochem. 16, 1364-1370.
28. Zimmerman, J.L., Fouts, D.L., and Manning, J.E. (1980) Genetics, 95, 673-691.
29. Jackson, A.O., and Larkin, B.C. (1976). Plant Physio. 57, 5-10.
30. Glisin, V., Radomir, C., and Byus, C. (1974). Biochem. 13, 2633-2639.
31. Manning, J., Hershey, D., Broker, T., Pellegrini, M., Mitchell, H., and Davidson, N. (1975). Chromosoma (Berl.) 53, 107-117.
32. Commerford, S.L. (1971). Biochem. 10, 1993-1999.
33. Loening, U.E. (1968). J. Mol. Biol. 38, 355-365.
34. Bailey, J.M., and Davidson, N. (1976). Analy. Biochem. 70, 75-85.
35. Spirin, A.S. (1963). In "Progress in Nucleic Acid Research"., p. 301 J.N. Davidson and W.E. Cohn, eds. Academic Press, New York.
36. Greenberg, J.R. (1969). J. Mol. Biol. 46, 85-98.
37. Sanchez-Lava, P.A. and Puppo, S. (1975). J. Mol. Biol. 95, 9-20.
38. Samols, D., and Swift, H. (1979). Chromosoma (Berl.) 75, 129-143.
39. Speirs, J., and Birnstiel, M. (1974). J. Mol. Biol. 87, 237-256.
40. Jordan, B.R., Jourdan, R., and Jacq, B. (1976). J. Mol. Biol. 101, 85-105.
41. Borst, P. (1978). Cell 15, 705-723.
42. Faye, G., Dennebony, N., Kujawa, C., and Jacq, C. (1979). Molec. Gen. Genet. 168, 101-109.
43. Heckman, J.E. and Rajbhandary, U.L. (1979). Cell 17, 583-595.
44. Tartof, K. (1979). Cell 17, 607-614.