Structural organization of the two main rDNA size classes of *Ascaris lumbricoides*

Eduard Back, Fritz Müller* and Heinz Tobler+

Zoologisches Institut der Universität Freiburg, Pérolles, CH-1700 Freiburg, Switzerland

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ABSTRACT

The two main rDNA size classes in the genome of *Ascaris lumbricoides* consist of 8.8 kb and 8.4 kb long repeating units present in a quantitative ratio of roughly 10:1. They both contain the genes coding for 18S, 5.8S and 26S ribosomal RNAs. The length heterogeneity is due to a 450 bp long spacer region localized in the longer repeating unit which begins 870 bp upstream of the 5'-end 18S gene. A few additional microheterogeneities in base sequence occur at the 5'-end of the 26S gene. The 18S, 5.8S and 26S coding regions have been mapped on both the 8.8 kb and 8.4 kb repeating units and the localization of the 5'- and 3'-ends of the 18S and 26S genes has been performed by S1 protection. No intervening sequences are present in either coding region of the two main rDNA size classes.

INTRODUCTION

The genes coding for ribosomal RNA have been studied in a great number of organisms (see 1-4, for review). Among nematodes, rRNA genes have only been characterized in some detail in *Caenorhabditis elegans* and in the closely related *Caenorhabditis briggsae* (5). In these two species, the rDNA is organized in a rather homogeneous tandem array of very short 7000 bp repeating units. In contrast, the present report shows that the 300 ribosomal genes (6) of *Ascaris lumbricoides*, a DNA eliminating nematode, occur in two main rDNA size classes of 8800 bp and 8400 bp length. Moreover, we recently found a third rDNA class containing intervening sequences in its 26S rRNA coding region (Back et al., in preparation). The organization and structural analysis of the ribosomal genes of *Ascaris lumbricoides* presented in this and the joined paper will serve as a base for further investigations on the expression of these genes.
MATERIALS AND METHODS

Restriction Nucleases, Enzymes, Radioactive Compounds and Autoradiography

Most restriction enzymes, T4-polynucleotide kinase, and DNA polymerase I large fragment were obtained from New England Biolabs. S1 nuclease was purchased from Sigma. Some restriction enzymes, T4 DNA ligase, α-amylase, ribonuclease T1, pancreatic ribonuclease and calf alkaline phosphatase were supplied by Boehringer Mannheim, the latter was further purified by Sephadex G-75 chromatography as described (7). γ-32P-ATP ( > 5000 Ci/m mole), α-32P dCTP and α-32P dGTP (each 3000 Ci/m mole) were purchased from Radiochemical Centre, Amersham, U.K. Autoradiography was done in contact with Kodak X-Omat S films at room temperature or with intensifying screens (Ilford) and preflashed X-Omat S films at -70° C (8).

Preparation of DNA from Ascaris Larvae

Adult Ascaris lumbricoides worms were collected from infected pigs in the local slaughterhouse (Micarna Ltd., Courtepin, Switzerland) and shipped to the laboratory. The rearing of moving larvae and the DNA preparation from larvae was carried out as previously described (9).

Purification and Labelling of 18S and 26S rRNA

18S and 26S rRNA were extracted from oocytes according to the method of Kirby (10) and separated by centrifugation in a 15-30% (w/v) sucrose gradient made up in 0.1 M NaCl, 0.02 M NaAc, 1 mM EDTA, 0.2% SDS, pH 5.0. Gradients were centrifuged in an AH 627 rotor (Sorvall) at 25000 rpm and 22° C for 19h. 18S and 26S rRNA was labelled by γ-32P-ATP and T4-polynucleotide kinase (11) after partial alkali degradation (12). Specific activity was 1 to 3 x 10^7 cpm/ug RNA.

Construction of Recombinant Plasmids

Gel-purified DNA fragments (13) containing single rDNA repeating units from Bam HI-digested DNA of Ascaris lumbricoides larvae were cloned using the plasmid vector pBR 322. The plasmid was opened at the single Bam HI site, treated with alkaline phosphatase and its linear form was isolated from a preparative low melting agarose gel. 300 ng of Ascaris DNA were mixed with 1 ug of vector DNA and ligated in 60 ul of 50 mM Tris HCl, 10 mM
MgCl₂, 50 µg/ml BSA, 10 mM DTT, 0.1 mM ATP, pH 7.6 at 4°C for 5h with 0.5 Weiss units of T4 DNA ligase. After transfection of CaCl₂-treated E. coli K12 HB101 cells, ampicillin-resistant clones were analyzed by the method of Grunstein and Hogness (14) with the use of ³²P-labelled 18S and 26S rRNA as probes. Recombinants containing the Ascaris rDNA inserts were ultimately identified by digestion with the restriction enzymes Bam HI, Eco RI and Xba I of the plasmid DNA in cleared lysates (15). All DNA cloning was performed according to the NIH guidelines using the P2-E.K.1 level of containment.

DNA Mapping

The restriction site mapping of the rDNA inserts was performed essentially following the method of Smith and Birnstiel (16). The plasmids pAlr8 and pAlr13 were cut with Bam HI, dephosphorylated with calf alkaline phosphatase, phenol-extracted and precipitated with ethanol. The DNA (9 µg) was end-labelled with γ-³²P-ATP and T4-polynucleotide kinase (11), phenol-extracted, passed over a G-50 Sephadex column and precipitated with ethanol. After resuspension in the appropriate buffer, one half of each sample was cleaved with Bgl II, the other half with Xba I. The fragments were separated on a 1% horizontal low melting agarose gel (Sigma Type VII) in 0.04 M Tris-Ac, 0.02 M NaAc, 1 mM EDTA, pH 8.0. The Bgl II-Bam HI 8.0 kb A-fragment and the Bam HI-Xba I 3.1 kb B-fragment (see Figure 2) were recovered from the gel (13) and precipitated with ethanol. After resuspension in the appropriate buffer, the fragments were partially digested with the restriction endonucleases indicated in Figure 2. The resulting fragments were electrophoresed on a 0.8% or 1% agarose gel in 0.2 M glycine, 0.015 M NaOH buffer (pH 8.3) which was then dried and autoradiographed. ³²P-end-labelled Eco RI + Hind III digested λ DNA and Hinf I digested pBR 322 DNA were used as size markers.

Electrophoresis, Southern blot Hybridization and DBM blotting

Enzyme digestions were performed according to the conditions specified by the manufacturers. Agarose gel electrophoresis was carried out in 0.2 M glycine, 0.015 M NaOH, pH 8.3.

Southern transfer was done by the standard technique (17) and filters were hybridized with RNA probe in 50% (v/v) deionized
formamide, 6 x SSC at 48° C (4) or with DNA probe in 2 x SSC, 10 x Denhardt's solution (18) at 65° C in plastic bags. After specified incubation times, filters were washed 2 times with 250 ml of 0.1 x SSC at room temperature for 10 min and 3 times with 250 ml of 2 x SSC at 68° C for 15 min. Filters were dried for 30 min at 80° C in a vacuum oven and exposed as described above.

The DBM paper was prepared by the method of Levy et al. (19).  

**32P Labelling of DNA**

In order to label 5'-ends of restriction fragments, the DNA was treated with calf alkaline phosphatase and labelled with γ-32P-ATP and T4-polynucleotide kinase (11).

DNA was labelled at the 3'-ends with α-32P-dNTP and E. coli DNA polymerase I large fragment (Klenow) as described (20).

Nick translation of DNA was carried out essentially according to the method of Rigby et al. (21). Specific activities ranged from 10⁷ to 10⁸ cpm/ug DNA.

**Heteroduplex Analysis**

We used the BAC spreading method of Sogo et al. (22) for mounting heteroduplexes for electron microscopy. Clone pAlr13, linearized with Hind III, and gel-purified rDNA repeats from clone pAlr8, about 0.3 ug of each, were mixed in 100 ul of 70% (v/v) formamide, 0.3 M NaCl, 10 mM triethanolamine-HCl, 5 mM EDTA, pH 8.0, denatured at 90° C for 5 min and renatured for 30 min at 40° C. To 10 ul of this sample, 1 ul of 0.2% BAC stock solution in formamide was added and the mixture was spread over a hypophase of redistilled water. The DNA-BAC film was picked up with carbon coated grids, which were pretreated by floating for 15 min on droplets containing 30 ug/ml ethidium bromide. The excess ethidium bromide solution was removed by drying on a filter paper. The grids were washed by floating on redistilled water for 10 min, stained with uranyl acetate by immersing for 15 sec into a fresh solution containing 1 mM uranyl acetate, 1 mM HCl and 98% ethanol. For the removal of excess stain, the grids were dipped into 90% ethanol and dried on filter paper. Specimens were rotary shadowed with platinum-carbon at an angle of 3° with 1500 Hz. Micrographs were taken on a Siemens Elmiskop 101 at 80 kV and a magnification of 8000. The magnification was
Nucleic Acids Research determined with a carbon grating replica of 2160 lines/mm from Balzers Union (Liechtenstein).

Nuclease S₁ Protection Mapping

Nuclease S₁ mapping was carried out according to Berg and Sharp (23). Appropriate end-labelled restriction fragments described in the text (2-10 ng, 10000 cpm) were mixed with 2 ug 18S or 26S rRNA in 80% (v/v) deionized formamide, 0.4 M NaCl, 0.4 M PIPES, 10 mM EDTA, pH 6.4 in a final volume of 40 ul. After heating at 70° C for 10 min, hybridization was allowed to proceed for 1h at 50° C. The samples were diluted 10 fold into ice cold S₁ buffer (0.25 M NaCl, 0.03 M NaAc, 1 mM ZnSO₄, pH 4.6 and 20 ug/ml denatured E. coli DNA). 40 or 120 units of nuclease S₁ were added and the mixture was incubated at 30° C for 30 min. Then 10 ug of yeast RNA were added as a carrier and the hybrids were precipitated with ethanol. Fragments were analyzed by alkaline gel electrophoresis as described by McDonell et al. (24) or on 4% sequencing gels following the method of Maxam and Gilbert (25).

RESULTS

Ascaris rDNA Repeating Units in Genomic and Cloned DNA

Genomic DNA extracted from whole Ascaris larvae was digested with Bam HI, Sma I and Bgl II, respectively. The resulting DNA fragments were separated on an agarose gel, transferred to a nitrocellulose filter by the Southern procedure (17) and hybridized with ³²P-labelled 18S and 26S rRNA which has been isolated from Ascaris oocytes. All three restriction enzymes create a prominent band of 8.8 kb and a minor band of 8.4 kb (Figure 1, a-c). Moreover, a third faint band of 13.3 kb is visible in Figure 1,a and b. These results are compatible with Ascaris lumbricoides rDNA being organized in at least three different size classes, each of them occurring in tandem arrays. The three size classes are represented in a proportion of approximately 20:2:1, as has been estimated by comparing the relative intensity of the bands using different concentrations of DNA (data not shown). The third size class of 13.3 kb in length which is an 8.8 kb type rDNA repeat containing a 4.5 kb intervening sequence
Pig. 1a-c: Autoradiograph of a Southern blot from genomic DNA of *Ascaris* larvae. 3 ug aliquots of total larval DNA were digested, separated on a 1% agarose slab gel and transferred to a nitrocellulose filter. Hybridization with $^{32}$P-labelled 18S and 26S rRNA (1 ug each, 1.6 x $10^7$ and 0.8 x $10^7$ cpm) was carried out in 8 ml 50% formamide, 6 x SSC for 20h at 48°C. The lengths of the DNA fragments in this and all other Figures are given in bp, unless stated otherwise.

d and e: The clones pAlr8 and pAlr13 were digested with Bam HI (resulting in 8.8 kb and 8.4 kb rDNA repeating units plus the pBR 322 vector DNA), separated on a 1% agarose slab gel and stained with 1 ug/ml ethidiumbro-mide.

f: Same as in a-c, but digested with Bam HI + Eco RI.

g and h: Same as in d and e, but digested with Bam HI + Eco RI. Bands are designated in alphabetical order according to their electrophoretic mobility.
We have cloned the 8.8 kb and the 8.4 kb rDNA repeating units of a Bam HI digest in the pBR 322 plasmid vector (see Material and Methods). Figure 1d and e show the ethidium bromide staining of Bam HI digested clones, one carrying a 8.8 kb fragment (denoted as pAIr8) and the other one carrying a 8.4 kb fragment (denoted as pAIrl3). A Bam HI and Eco RI digest of pAIr8 and pAIrl3 reveals a banding pattern identical to an autoradiograph of equally digested genomic DNA with the length heterogeneity being located on the Eco RI A-fragment (Figure 1,f-h).

Both the 8.8 kb and the 8.4 kb repeating units were subjected to detailed restriction mapping by the partial restriction mapping procedure of Smith and Birnstiel (16). Figure 2 presents the restriction map of the 8.8 kb repeat, a representative of the main rDNA size class. The 8.4 kb rDNA repeating unit shows an identical restriction pattern with the following exceptions: it lacks a 450 bp long region (dotted area in Figure 2) in the 26S coding region, will be subject of a separate publication (Back et al., in preparation).

The bands are denoted alphabetically according to their electrophoretic mobility. The top line shows the localization of the 18S, 5.8S and 26S rRNA coding regions.
Fig 3: Hinc II restriction pattern in 8 independently cloned 8.8 kb and 8.4 kb rDNA repeating units. The clones were digested with Hinc II and labelled with $^{32}$P at their 5'-ends. The DNA digests (about 12,000 cpm each) were then separated on a 5% acrylamide gel prepared in 90 min mM Tris-borat pH 8.3, 5 mM MgCl$_2$ and the gel was dried on DE81 paper (Whatman) before exposure.

a-d: 4 clones containing an 8.8 kb rDNA insertion.

e-h: 4 clones containing an 8.4 kb rDNA insertion. The 165 bp band of lanes e-h is cut in two bands of 105 bp and 60 bp length by Hinc II in lanes a-d. In the clone pAlrl3 the rDNA is inserted in the opposite direction.

non-transcribed spacer region. This region is located on the Eco RI A-fragment (cf. Figure 1,g and h) and contains the single Hind III site of the 8.8 kb rDNA. Further proof for the existen-
ce of this 450 bp gap is shown by heteroduplex mapping (Figure 4). Moreover, three restriction sites near the 5'-end of the 26S coding region are only present in the 8.8 kb rDNA repeat (marked by triangles in the Hinc II and Taq I restriction maps, cf. Figure 2). These restriction sites are probably representative for the 8.8 kb rDNA size class and absent in all 8.4 kb rDNA's. That this is the case at least for the Hinc II site has been demonstrated as follows: 8 independently recovered rDNA clones, each containing an 8.8 kb or an 8.4 kb rDNA insertion, were cut with Hinc II, \(^{32}\)P-labelled at their 5'-ends and run on a 5% acrylamide gel. On the autoradiograph of this gel (Figure 3), a 165 bp long DNA fragment is visible in all 8.4 kb rDNA clones (lanes e-h). However, in every 8.8 kb rDNA clone this fragment is cut in two bands of 105 bp and 60 bp length by the enzyme Hinc II (lanes a-d).

In order to check for heterogeneities between the 8.8 kb and 8.4 kb rDNA repeats, a heteroduplex map of the two clones pAlr8 and pAlr13 was constructed. The clone pAlr13 was linearized by digestion with Hind III at its single Hind III recognition site, resulting in the 8.4 kb rDNA flanked asymmetrically by 346 and 4016 nucleotides long pBR 322 vector sequences. The clone pAlr8 was digested with Bam HI and the resulting 8.8 kb rDNA repeat isolated from a preparative agarose gel. Heteroduplexes between these two full-sized rDNA repeating units show a 450 bp long mismatched region near its centre (Figure 4). The length and the location of this loop is in good agreement with the data obtained from restriction enzyme digestions described above, that is, the short rDNA repeat lacks a 450 bp spacer region around the single Hind III site present only in the 8.8 kb rDNA repeating unit (dotted area in Figure 2).

**Mapping of the 5'-and 3'-Ends of 18S and 26S rRNA**

We have localized the 18S and 26S rRNA coding regions on the restriction map (Figure 2), by separately hybridizing \(^{32}\)P-labelled 18S and 26S rRNA to Southern blots of Eco RI + Bam HI digested clones pAlr8 and pAlr13. The respective autoradiograph is shown in Figure 5: 18S rRNA hybridizes strongly to fragments A and A' (lanes a and b) and weakly to fragment B. 26S rRNA gives a strong hybridization signal on the fragments B, D and E.
Fig. 4 Heteroduplex between cloned 8.8 kb and 8.4 kb rDNA repeating units of *Ascaris lumbricoides*. The clone pAlr13 was linearized by Hind III digestion and heteroduplexed with a gel-purified 8.8 kb rDNA repeating unit of clone pAlr8 as described in Materials and Methods. The arrow in the graphical drawing marks the 450 nucleotides long loop of the 8.8 kb rDNA. The 8.4 kb rDNA molecule is asymmetrically flanked by 346 and 4016 nucleotides long pBR 322 vector sequences.
Fig. 5: Localization of the 18S and 26S rRNA coding regions on the clones pAlr8 and pAlr13. 0.5 ug DNA each were digested with Eco RI and Bam HI, separated on a 0.8% agarose slab gel and transferred to a nitrocellulose filter. The filter was divided and one half hybridized with 1 ug (3 x 10^6 cpm) ^32P-labelled 18S rRNA + 50 ug cold 26S rRNA; the other half with 1 ug (1.5 x 10^6 cpm) ^32P-labelled 26S rRNA + 30 ug cold 18S rRNA. Hybridization was carried out in 5 ml 50% formamide, 6 x SSC at 48° C for 15h. (For the arrangement of bands A to E in the clone, refer to Figure 2).

and a faint one on fragment C (lanes c and d). In order to map the 5'- and 3'-ends of 18S and 26S rRNA, we carried out S1 protection experiments using 18S or 26S rRNA and appropriate ^32P-endlabelled restriction fragments (cf. Figure 2). Eco RI-Eco RI A-fragments, labelled at their 5'-ends, yield the 5'-end of 18S rRNA and Eco RI-Bam HI B-fragments, labelled at their 5'-ends, give the 5'-end of 26S rRNA. On the other hand, Sma I linearized clones, labelled at their 3'-ends, yield the 3'-end of 18S rRNA, whereas Eco RI-Eco RI C-fragments, labelled at their 3'-ends allow to map the 3'-end of the 26S
Fig. 6: Localization of the 5'- (A) and 3'-ends (B) of the 18S and 26S rRNA genes. Nuclease S1 resistant hybrids between endlabelled restriction fragments and 18S or 26S rRNA were separated on either 1.4% alkaline agarose slab gels (a-e, h-j), or on 4% sequencing gels (f and g) and autoradiographed.

a: pAlr13 as 5'-\textsuperscript{32}P-endlabelled marker DNA digested with Bam HI + Eco RI + Xba I.

b and c: S1 resistant hybrids between 18S rRNA and 5'-endlabelled Eco RI A'-and A'-fragments of pAlr13 (b) and pAlr8 (c).

d and e: S1 resistant hybrids between 26S rRNA and 5'-endlabelled Eco RI-Bam HI B'-fragments of pAlr13 (d) and pAlr8 (e).

f and g: S1 resistant hybrids between 18S rRNA and Sma I linearized, 3'-endlabelled clones pAlr8 (f) and pAlr13 (g).

h: pAlr13 as 5'-\textsuperscript{32}P-endlabelled marker DNA, digested with Bam HI + Eco RI.

i and j: S1 resistant hybrids between 26S rRNA and 3'-endlabelled Eco RI C'-fragments of pAlr8 (i) and pAlr13 (j).

Lengths are in nucleotides.
rRNA. The respective hybrids were $S_1$ digested and the size of the protected fragments determined on 1.4% denaturing agarose gels or on 4% sequencing gels. Figure 6 shows two nuclease $S_1$ resistant rDNA fragments for both pAlr13 (lane b) and pAlr8 (lane c). The major band, corresponding to 1480 nucleotides, maps the 5'-end of 18S rRNA relative to the downstream Eco RI cut of fragment A. The minor band, 1850 nucleotides long, indicates a transcription start site, as has recently been shown by $S_1$ mapping and in vitro transcription using a homologous in vitro transcription system developed in our laboratory (Briner et al., in preparation).

The 5'-end of the 26S rRNA maps 930 bp upstream of the Bam HI site in either clone, pAlr13 (Figure 6,d) and pAlr8 (Figure 6,e).

Locating the 3'-end of the 18S rRNA relative to the single Sma I site by an $S_1$ protection experiment, a 150 nucleotides long major band is observed in both clones (Figure 6,f and g). However, a minor band of 510 nucleotides is only present in clone pAlr8 (lane f), whereas a second minor band of 280 nucleotides is more pronounced in pAlr13 (lane g) than in pAlr8 (lane f). Both minor bands are strongly enhanced compared to the major bands in $S_1$ protection assays if nuclear RNA rather than 18S rRNA was used (unpublished data). Moreover, analyses of 8 independently recovered clones confirmed that the different banding pattern is representative for, respectively, the 8.8 kb and the 8.4 kb rDNA size classes (data not shown). The existence of these distinct minor bands reflects a different primary sequence in the internal transcribed spacer of the two rDNA size classes. However, their significance, if there is any, remains at present obscure.

The 3'-end of 26S rRNA maps 470 bp downstream of the Eco RI site in the Eco RI C-fragment in both pAlr8 (Figure 6,i) and pAlr13(Figure 6,j).

No Intervening Sequences are Present in Either of the two Main rDNA Size Classes of Ascaris lumbricoides

In an $S_1$ assay with the 6.9 kb long Bam HI-Sma I A-fragment (cf. Figure 2) of the clone pAlr8, which was labelled at the Bam HI 3'-end and hybridized with 26S rRNA, a 2660 nucleotides long...
Fig. 7: Nuclease S₁ protection in the 26S rRNA gene of *Ascaris lumbricoides*. S₁ resistant hybrids between 26S rRNA and further specified, endlabelled restriction fragments were separated on 1.4% alkaline agarose slab gels and autoradiographed.

a: Hybrids containing Bam HI-Sma I A-fragment of pAlr8, labelled at the Bam HI 3'-end.
b and c: Hybrids containing 5'-endlabelled Eco RI D-fragments of pAlr8 (b) and pAlr13 (c).
d and e: Hybrids containing 5'-endlabelled Eco RI E-fragments of pAlr8 (d) and pAlr13 (e).
f: 3'-32P-endlabelled pAlr8 as marker DNA digested with Bam HI + Eco RI.

region is protected (Figure 7a). This region extends exactly from the labelled Bam HI end to the 26S 3'-end that has been mapped before (Figure 6,i). Hence this rDNA region, covering
about 74% of the total 26S rRNA gene, is not interrupted by any detectable insertion sequence. In a further series of S1 protection experiments with 26S rRNA and the Eco RI D- or E-fragments of pAlr8 and pAlr13, the integral fragment lengths were resistance to nuclease S1 digestions (Figure 7,b-e). These two regions of the Eco RI D- and E-fragments together with the 470 nucleotides that are protected by 26S rRNA on the Eco RI C-fragment (Figure 6,i and j), add up to the 2660 nucleotides long fragment described above. Therefore, the results obtained for pAlr8 and pAlr13 exclude intervening sequences in the 2660 bp downstream region of the 26S gene, as did the results for pAlr8 mentioned above. Thus, taking into account that 930 nucleotides at the 5'-end of the 26S coding region (Figure 6,d and e) are also protected in the S1 experiments, one calculates that the 26S rRNA gene is 3590 nucleotides long. This value agrees well with the length of 26S rRNA measured directly by agarose gel electrophoresis of glyoxal-denatured rRNA (26).

The 18S rRNA coding region is also free of any traceable intervening sequences. Proof for that is furnished by the agreement between the length of the 18S rRNA (about 1700 nucleotides) that has directly been determined and the sum of the S1-protected fragments in the 18S coding region (26).

Localization of the 5.8S rRNA Gene

We have localized the 5.8S rRNA coding region within the internal transcribed spacer by hybridizing a cloned 5.8S rDNA fragment from *Xenopus laevis* to genomic and cloned rDNA of *Ascaris*. The hybridization probe was a 3'-32P-labelled 274 bp Hha I restriction fragment of the Bam HI L-fragment from plasmid pXL212 (27). This DNA fragment covers the 159 bp of the 5.8S rDNA as well as 83 bp and 32 bp flanking sequences, respectively. On a Southern blot of Bam HI + Eco RI digested clones pAlr8 and pAlr13, this DNA probe hybridizes only to the 1980 bp long Eco RI B-fragment (Figure 8,a and b), which covers the internal transcribed spacer region (Figure 2). On a DBM blot of larval DNA digested with Bam HI, Hinf I or Hae III, the same 5.8S rDNA probe hybridizes only to a single band of, respectively, 8.8 kb, 370 bp and 850 bp (Figure 8,c-e). The 8.8 kb band of a Bam HI digest corresponds to a full rDNA repeating unit (the 8.8 kb and
Fig. 8: Localization of the 5.8S rRNA gene in the rDNA repeating unit of *Ascaris lumbricoides*.

a and b: Southern blot hybridization of the Bam HI + Eco RI digested clones pAlr8 (a) and pAlr13 (b) with a $^{32}$P-labelled 5.8S rDNA fragment from *Xenopus laevis* (see text). Hybridization was carried out with $1.8 \times 10^6$ cpm (about 30 ng) DNA in 10 ml 2 x SSC, 10 x Denhardt's solution at 60°C for 21h. The letters correspond to the rDNA fragments of Bam HI + Eco RI digested clone pAlr8 (cf. Figure 2). Only the fragment B hybridizes with 5.8S rDNA.

c-e: 2 ug aliquots of larval DNA have been digested, separated on a 2% agarose slab gel and transferred to a DBM paper. Hybridization was performed as in a + b, except that 5 x SSC, 1 x Denhardt's solution, 1 mM EDTA, 0.1% SDS and 2 mg/ml herring sperm DNA was used as medium.

f: DNA length standard. Hae III digested satellite DNA from spermatids of *Ascaris lumbricoides* was hybridized with a $^{32}$P-labelled satellite probe (28). This DNA is sticking on the same DBM paper used for the above hybridization. The DNA monomer is 119 bp long (13).
8.4 kb rDNAs are not separated on this 2% agarose gel). The bands of 370 bp from the Hinf I digest and of 850 pb from the Hae III digest allow to localize the 5.8S rRNA gene in a 340 bp region between the 18S and 26S rRNA genes, if one takes into account the overlap of both restriction fragments (cf. Figure 2).

DISCUSSION

We have cloned several rDNA repeating units of 8.8 kb and 8.4 kb length corresponding to the two main rDNA size classes of *Ascaris lumbricoides*. The length of these repeating units is rather short compared to those published for other organisms. This is due on the one hand to the relatively short coding regions of 1700 and 3600 nucleotides for the 18S and 26S rRNA, and on the other hand to a short spacer sequence between the 26S and 18S genes. Such short but functional rRNA's are readily understood by current models of rRNA secondary structures (29-31). The rDNA repeating unit of *Caenorhabditis elegans*, the only nematode which has been studied except *Ascaris*, shows an even shorter value of 7 kb (5).

Both a cloned 8.8 kb and an 8.4 kb repeating unit were subjected to detailed restriction mapping and shown to be nearly identical with the two following exceptions: The 8.4 kb rDNA lacks a 450 bp region beginning 870 bp upstream of the 18S gene 5'-end. This region was localized by differences in the restriction pattern between the clones pAlr8 and pAlr13. Confirmation of this comes from heteroduplex-mapping using a gel purified 8.8 kb repeating unit and the linearized clone pAlr13. The 450 bp region is probably a deletion in the 8.4 kb rDNA (or, conversely, an insertion in the 8.8 kb rDNA) rather than a duplication. If this were a duplication, one would expect the restriction sites of the 450 bp region to be present in the 8.4 kb and repeated in the 8.8 kb rDNA, which was not observed. The argument, that after a duplication some sequence divergence had occurred by point mutations during evolution is contradicted by the completely identical restriction patterns of pAlr8 and pAlr13 flanking the 450 bp region. Heterogeneities in the rDNA repeating units have been found in several protozoans (32-37)
and in most higher eucaryotes studied so far with a few exceptions (38-40, 5). In all cases the heterogeneity - apart from intervening sequences - is due to length differences in the non-transcribed spacer. This is also true for *Ascaris lumbricoides*. The transcription initiation site in the rDNA repeating unit of *Ascaris lumbricoides* has recently been determined both in vivo by S1 mapping and in vitro using a homologous in vitro transcription system for polymerase I (Briner et al., in preparation). Transcription starts about 500 bp downstream of the observed heterogeneity, thus locating it into the non-transcribed spacer region.

Apart from the missing 450 bp region in the 8.4 kb rDNA, both repeating units are rather homogeneous and show only a few differences in the restriction pattern near the 26S gene 5'-end. These differences seem to be point mutations for the following two reasons: neither are rRNA/DNA hybrids cut by nuclease S1, nor are any loops observed in heteroduplex molecules. However, the observation that these restriction sites are present in all 8.8 kb but in none of the 8.4 kb rDNA's out of 8 independently cloned rDNA repeating units, has implications on the correction mechanism of the two rDNA size classes (this point is discussed in the joined paper).

In *Ascaris lumbricoides*, the two main rDNA size classes of 8.8 kb and 8.4 kb are not interrupted by intervening sequences. This has been demonstrated by nuclease S1 protection with matured 18S and 26S rRNA. However, at this point it is very interesting to note that a third size class of 13.3 kb in length (see Figure 1,a and b) carries an intervening sequence in the 26S rRNA gene (Back et al., in preparation). Thus, the nematode *Ascaris lumbricoides* shows intervening sequences in only a few of the 26S genes, a situation that has so far only been described for some dipteran flies (41-49).

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