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Isolation and organization of calf ribosomal DNA

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ABSTRACT

Ribosomal DNA (rDNA) from calf was isolated by three density gradient centrifugations. The first centrifugation in Cs_2SO_4/BAMD was used to obtain partially resolved dG+dC-rich fractions from total DNA. The second and third centrifugations, in Cs_2SO_4/Ag^+, led to the isolation of an rDNA fraction characterized by a symmetrical band in CsCl, ρ= 1.724 g/cm^3. This new procedure appears to be generally suitable for the isolation of rDNA and other dG+dC-rich repeated genes.

The organization of isolated calf rDNA has been studied by restriction enzyme digestion and by hybridization with cloned rDNA from Xenopus laevis. The repeat unit of calf rDNA has a molecular weight of $21x10^6$ and is split by EcoR1 into two fragments, $16x10^6$ and $5.0x10^6$, and by BamHI into seven fragments. EcoRI and BamHI sites have been mapped. Most of the 18S and 28S RNA genes and the transcribed spacer are contained in the small EcoRI fragment, while the non-transcribed spacer is localized on the large EcoRI fragment. This spacershowed length heterogeneity within a single individual; such heterogeneity is limited to two regions of the spacer.

INTRODUCTION

Most of the available information on the organization of the nuclear ribosomal genes comes from extensive studies carried out on the ribosomal DNA (rDNA) of Xenopus (for references see 1,2). In this species,the genes for the two large ribosomal RNAs (rDNA) are tandemly repeated in several hundred units, ranging in molecular weight from 7 to $10x10^6$.
Each repeat unit contains a transcribed spacer, an 18S gene, an internal transcribed spacer, a 28S gene, and a non-transcribed spacer, in that order. While the nucleotide sequences of ribosomal genes appear to have been highly conserved during evolution (3), the non-transcribed spacers appear to differ in length and in sequence not only in closely related species, but also within a single individual (4-6).

Our knowledge of mammalian rDNA is much more limited. Isolation of rDNA has only been done in the case of calf, by selective heat denaturation and S1 nuclease digestion followed by centrifugation in CsCl/actinomycin density

gradients(7). In addition, a restriction fragment of mouse rDNA has been isolated by cloning rDNA fractions enriched in ribosomal genes by reverse phase chromatography (8). Some information on the organization of rDNA has been obtained from these isolated DNAs. Additional data have been obtained using restriction fragments of total DNA and hybridization with cloned heterologous rDNA or with rRNA (9,10). An obvious handicap of the latter approach is that only restriction fragments containing coding regions are detected and the construction of a map of the repeat unit is indirect and subject to some ambiguities.

In the present work, we have used a new technique to isolate rDNA and we have studied,by restriction mapping and hybridization with heterologous rRNA or cloned rDNA,the organization of ribosomal genes in rDNA isolated from calf.

MATERIALS AND METHODS

DNA preparations

Calf thymus DNA was preparation CTRII (11), which was obtained from a single thymus, according to the method of Kay et al. (12), and further purified by hydroxyapatite chromatography (11). The sedimentation coefficient ($s_{20,w}$) was determined by band centrifugation and found to be 27S±1.8S.

Density gradient centrifugations

These experiments were carried out as described in previous papers (13,14).

Digestion with restriction endonucleases and gel electrophoresis

Restriction enzymes were purchased from Biolabs (Beverly, Ma., USA), with the exception of EcoRI which was prepared following Thomas and Davis (15). EcoRI digestions were carried out according to Polisky et al. (16). BamHI digestions were done in 50mM NaCl, 10mM Tris-HCl (pH 7.4), 7mM $MgCl_2$, 7mM 2-mercaptoethanol ; HindIII and HaeIII digestions in 10mM NaCl, 10mM Tris-HCl (pH 7.4), 7mM $MgCl_2$, 7mM 2-mercaptoethanol. Vertical agarose (Sigma, St. Louis, Mo., USA) slab gels (0.4x20x20cm) were used. Gel electrophoresis, ethidium bromide staining, and photography of the gel were done according to Prunell et al.(17). The EcoRI and HindIII fragments of phage λ DNA and HaeIII fragments of SV40 DNA were used as molecular weight markers (15,18, P. Philippsen and R. Davies, personal communication).

RNA-DNA hybridization

This was done essentially according to Gillespie and Spiegelman (19).

Duplicate DNA aliquots from density gradient centrifugation fractions were denatured with NaOH, diluted 20 times with 2M NaCl (20), 0.1M KCN, and loaded onto nitrocellulose filters BA85 (Schleicher and Schüll, Dassel, West Germany). DNA fixation was done by heating the filters at 80° for 2-3 hr. Hybridization was carried out at 64° for 12-15 hr in 2xSSC (SSC is 0.15M NaCl, 0.015M Na citrate) containing 0.2 % sodium dodecyl sulfate (SDS) with ^{3}H-rRNA from Xenopus laevis (final concentration 4 µg/ml ; specific activity 1-5x10^{5} cpm/µg). Three washings were done at room temperature with 2xSSC for 30 min. Filters were then incubated for 30 min at 37° in 2xSSC containing 50 µg/ml of RNase A (preheated 10 min at 100°) and 0.5 U/ml of RNase TI. Filters were washed 4 times in 2xSSC, dried, and counted in a scintillation counter. Background values obtained with DNA-free filters were substracted.

DNA-DNA hybridization

a) DNA probes

Two recombinant pBR313 plasmids, HM123 and HM456, containing the 3x10^{6} and the 4x10^{6} fragments of Xenopus laevis rDNA, respectively, were used. These recombinants are equivalent to the CD18 and CD42 plasmids of Morrow et al.(21); the restriction maps of the rDNA inserts are shown in fig. 5B.

The 1.5x10^{6} EcoRI-BamHI fragment and the 3.7x10^{6} EcoRI-HindIII fragment (6) were prepared from the HM123 and HM456 plasmids, respectively, by digestion with the corresponding restriction enzymes and preparative electrophoresis on 0.5 % agarose gel. Slices containing the DNA fragments were cut under ultraviolet light after ethidium bromide staining. Agarose was melted in 7.5M $NaClO_4$ at 65° and DNA was recovered by chromatography on hydroxyapatite at the same temperature (22). These fragments will be referred to as the 28S probe and the 18S probe, respectively. They are shown in fig. 5B as black segments. The plasmids and the derived fragments were labelled with ^{32}P by nick-translation (23) at a specific activity of 2-20x10^{6} cpm per µg.

b) Hybridization

Filter hybridization. Aliquots of the different pooled fractions enriched in rDNA were loaded onto nitrocellulose filters as described above. Filters were soaked at 65° for 30 min in 3xSSC, for 3 hr in 3xSSC containing 0.2 % each of Ficoll 400, bovine serum albumin and polyvinyl pyrrolidone (24), and for 1 hr in the same solution made 0.1 % in SDS. Hybridization was carried out at 65° for 22 hr in 0.6M NaCl, 0.2M Tris-HCl pH 8, 0.01M EDTA, 0.1 % SDS and 0.2 % each of Ficoll 400, bovine serum albumin and polyvinyl pyrrolidone containing 4x10^{5} cpm per ml of denatured ^{32}P DNA probe. Filters were first washed extensively in the last soaking

solution, then twice in SSC, 0.1 % SDS at 64°. They were allowed to dry and were counted with scintillation mixture.

Gel transfer and hybridization. Digested DNA from agarose gels was transferred (after denaturation) onto nitrocellulose sheets 0,45µm (BA 85, Schleicher & Schüll) by blotting (25), using 3M NaCl, pH11 as the transfer solution. The filters were heated at 80° for 2-3 hr and then soaked as described above. Hybridization was performed in the last soaking solution containing the denatured DNA probes. Washing of the filters was done as indicated above. After drying, autoradiography was done using ND 54 films (Eastman-Kodak, Rochester, N.Y.) with an Ilford (Basildon, Essex, UK) fast tungstate screen at -20° for 24 hr. Nitrocellulose sheets already used for hybridization were re-used with different probes. The radioactive annealed probe was removed by boiling the filters in SSC for 10 min ; filters were then rinsed in 3xSSC and allowed to dry. Control autoradiography was done in order to test the absence of residual radioactivity.

RESULTS

Purification of rDNA

Preliminary experiments on calf thymus DNA, as centrifuged in Cs_2SO_4/ BAMD density gradient, r_f = 0.18 (r_f is the ligand/nucleotide molar ratio), showed that rDNA is localized in the light fractions (15-19, fig.5, ref. 13). Resolution in this density region is, however, highly increased when using higher r_f values (see fig. 6, ref. 13). On the basis of these results, an r_f value of 0.25 was chosen for the first step in the purification of rDNA (fig. 1a) ; this led to the pelleting of 90 % of loaded DNA. The hybridization of the DNA contained in each fraction with Xenopus laevis ^{3}H-rRNA showed that rDNA was present in fractions 2 through 10. The enriched fractions 3 through 9 were pooled for further purification ; they represented 4.4 % of the starting material. Contamination by satellite DNAs was checked by CsCl centrifugation and comparison of the EcoRI restriction patterns of the pooled rDNA fractions with those of purified satellite DNAs. A high level of contamination by satellite DNAs 1.715, 1.711a and 1.711b (14) was revealed by the CsCl profile (fig. 1a) and the EcoRI restriction pattern (fig. 2). A contamination by the 1.711a satellite (see next paragraph) was not detected at this stage because this satellite shows no band pattern when degraded with EcoRI (fig.2) and has the same buoyant density as the 1.711b satellite.

Two additional centrifugations(figs.1b,1c) were done using Cs_2SO_4/Ag^+

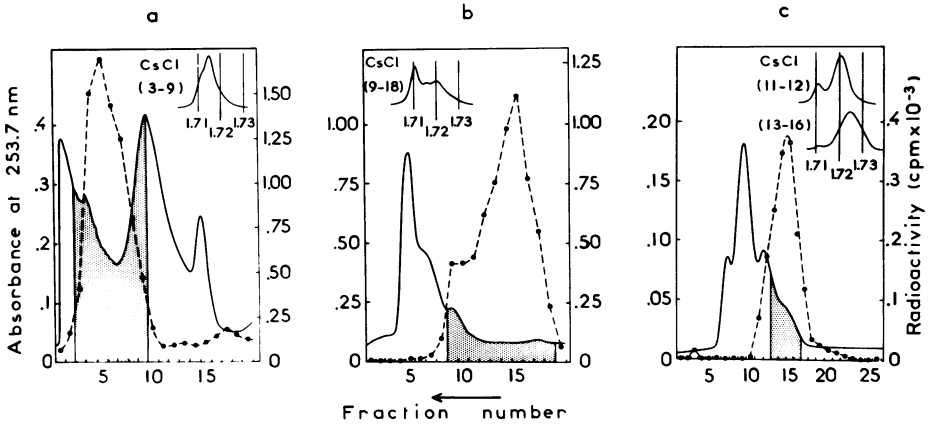

Fig. 1 Purification of calf rDNA a) Total calf thymus DNA (210 A_{260}units) in 0.1M Na_2SO_4, 5mM $Na_2B_4O_7$ containing BAMD (r_f = 0.25) and Cs_2SO_4 (ρ = 1.47 g/cm^3) was centrifuged in a Beckman type 30 rotor at 25° for 110 hr at 25,000 rpm.
b) Enriched ribosomal DNA (18 A_{260}units) from a (grey area) was centrifuged in 5mM $Na_2B_4O_7$ pH 9.2 containing Ag^+ (r_f = 0.36) and Cs_2SO_4 (ρ = 1.50 g/cm^3) under the conditions mentioned above.
c) Purified ribosomal DNA (1 A_{260}unit) from b (grey area) was centrifuged in 5mM $Na_2B_4O_7$ pH 9.2 containing Ag^+ (r_f = 0.40) and Cs_2SO_4 (ρ = 1.51 g/cm^3) in a Beckman type 65 rotor at 25° for 90 hr at 35,000 rpm.
The solid line represents the absorbance profile as derived from the transmission recording at 253.7 nm with an LKB Uvicord (Stockholm, Sweden) equipped with a cell of 0.3 cm diameter.
The analytical CsCl band profile of pooled fractions are shown in inserts. The arrow indicates the direction of the centrifugal field in the preparative experiments. The radioactivity of hybridized ^{3}H-rRNA is indicated by solid points and the dashed line. The aliquots of centrifugation fractions loaded on nitrocellulose filters contained the same amount of DNA : 5, 1, 0.1 µg in a, b and c, respectively. Fractions indicated by the grey area were used for the restriction enzyme analysis of rDNA ; these will be indicated as fractions a, b and c, respectively.

density gradients, the optimal r_f values at pH 9.2 being determined by analytical centrifugation in each case. In the first centrifugation,the 1.711b satellite DNA was removed, as revealed by the EcoRI restriction pattern (fig.2,lane b),but another contaminating satellite DNA having the same density and identifiable with the 1.711a satellite was still present,as shown by the CsCl profile. A small amount of 1.715 satellite DNA was also present. The contaminant satellites were removed in the second Cs_2SO_4/Ag^+ centrifugation, as shown by the analytical CsCl centrifugation(fig.1c) and the res-

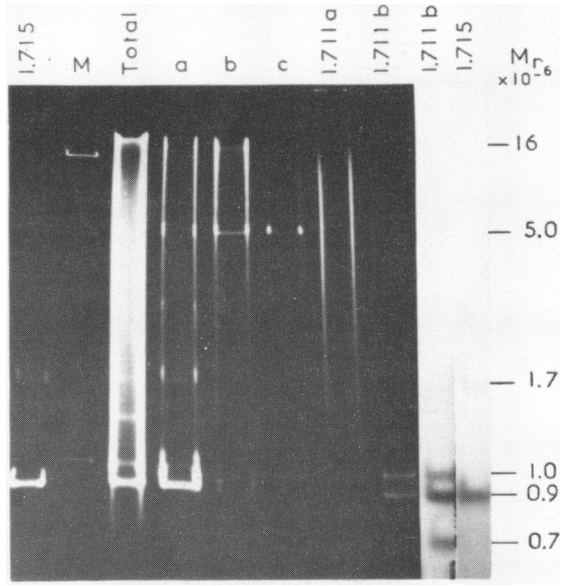

Fig. 2 Left: Gel electrophoresis of EcoRI digests of rDNA fractions as obtained at different purification steps. rDNA fractions a, b and c (see fig. 1), total DNA, and satellites 1.711a, 1.711b and 1.715 were digested to completion by EcoRI and electrophoresed on a 0.8 % agarose gel. DNA loads were 2µg for a, 0.2µg for b and c, 5µg for total DNA and 1µg for satellite DNAs. A mixture of a λ DNA EcoRI digest and a SV40DNA HaeIII digest was used as molecular weight marker (M). The molecular weights (M_r) of rDNA and satellite EcoRI fragments are indicated.

Right: Hybridization of plasmid HM123 to EcoRI digests of satellites 1.715 and 1.711b. ^{32}P-HM123 was hybridized to EcoRI fragments transferred onto nitrocellulose sheets as described in the Materials and Methods section.

triction enzyme analysis (fig.2,lane c). Following the procedure just described, 100 µg of highly purified calf rDNA were obtained from 125mg of total calf thymus DNA. On the basis of hybridization experiments with rDNA from Xenopus, it could be estimated that this DNA was enriched at least 400 times in ribosomal cistrons and showed a buoyant density of 1.724 g/cm^3 when centrifuged in CsCl. The first step in the purification procedure just described for calf DNA was also successfully used for mouse and human DNA.

Characterization of rDNA

Total calf DNA was digested with different restriction enzymes(fig.3A,C)

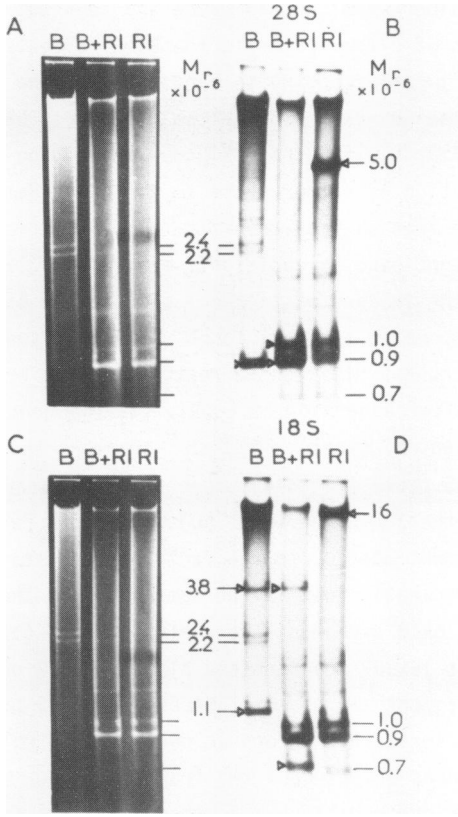

Fig. 3 Detection of rDNA fragments in restriction endonucleases digests of total calf DNA. Total calf DNA (5µg) was digested to completion by EcoRI or by BamHI, or by both enzymes, and electrophoresed on a 0.8% agarose gel (A and C). Hybridization was carried out as described in the Materials and Methods section with the isolated 28S and 18S radioactive probes (B and D,respectively). The fragments showing hybridization with satellite DNAs are indicated by bars. The fragments which were found to correspond to rDNA are indicated by arrows (see fig.4).

and hybridized with the 28S or 18S probes(fig.3B,D). Hybridization of either probe to the EcoRI digests showed three bands ($1x10^6$,$0.9x10^6$ and $0.7x10^6$)and several faint bands. The three bands correspond to the main EcoRI fragments exhibited by satellite DNAs 1.715 and 1.711b(fig.2;3A,C) seen on the gel after ethidium bromide staining. The faint bands could not be identified, except for a band which appears to correspond to the dimer of the 1.715 EcoRI repeat unit. In addition,a $5.0x10^6$ band was obtained with the 28S probe and a

$16x10^6$ band was obtained with the 18S probe and does not correspond to UV bands. Hybridization of either probe to the BamHI digests, showed two bands ($2.4x10^6$ and $2.2x10^6$) corresponding to bands seen on the gel. In addition, hybridization with the 18S probe showed two additional bands ($3.8x10^6$ and $1.1x10^6$),and hybridization with the 28S probe showed one additional band ($0.9x10^6$). Hybridization of either probe to the EcoRI+BamHI digests showed the same major bands seen on EcoRI digests, with an increased intensity of the lowest molecular weight band.In addition,a band($3.8x10^6$)appears only upon hybridization with the 18S and was already seen in the BamHI digest.Other weaker bands, corresponding to EcoRI bands, were also seen.

Purified calf rDNA exhibited the restriction and hybridization patterns shown in figure 4. After digestion by EcoRI, two fragments of $16x10^6$ and $5.0x10^6$ were seen (central panel). The $5x10^6$ fragment bound plasmid HM123 (fig. 4A) and the 28S probe (fig. 4C). The $16x10^6$ fragment bound plasmid HM456 (fig. 4B) and the 18S probe (fig. 4D). The broad pattern of hybridization on this fragment was due to the fact that the starting molecular weight of the DNA preparation was in the same range as the fragment size and therefore truncated $16x10^6$ fragments were also present (17). No hybridization was found between the $16x10^6$ fragment and plasmid HM123 or the 28S probe, or between the $5x10^6$ fragment and plasmid HM456 or the 18S probe. The existence of other EcoRI sites in spacer regions in calf rDNA is ruled out since the ultraviolet pattern does only reveal two fragments.

The BamHI restriction pattern is composed of five fragments (a,b,e,f,g), a broad band (c) corresponding to fragments of $3.2\text{-}3.3x10^6$ and a weak, diffuse band (d) ; table I presents the molecular weights of the BamHI fragments. The a fragment has a molecular weight of $5.3x10^6$, does not contain an EcoRI site, as shown by the double digest, and does not bind any of the probes.Thus it must originate from a spacer region contained in the $16x10^6$ EcoRI fragment. Similarly, the c fragment ($3.2\text{-}3.3x10^6$) does not contain an EcoRI site and does not bind any probe ; it cannot originate from the $5x10^6$ EcoRI fragment because its size is larger than that of the internal spacer. Therefore, fragment c should also originate from a spacer region contained in the $16x10^6$ EcoRI fragment. Fragment b has a molecular weight of $3.8x10^6$; it does not hybridize with plasmid HM123 or the 28S probe, but it does hybridize with the plasmid HM456 and with the 18S probe. This fragment is preserved in double BamHI+EcoRI digests and therefore derives from the $16x10^6$ EcoRI fragment. The weak, diffuse band d shows a hybridization in the region $3.0\text{-}3.8x10^6$ where it exhibits two distinct bands at 3.2 and $3.7x10^6$ with both plasmids

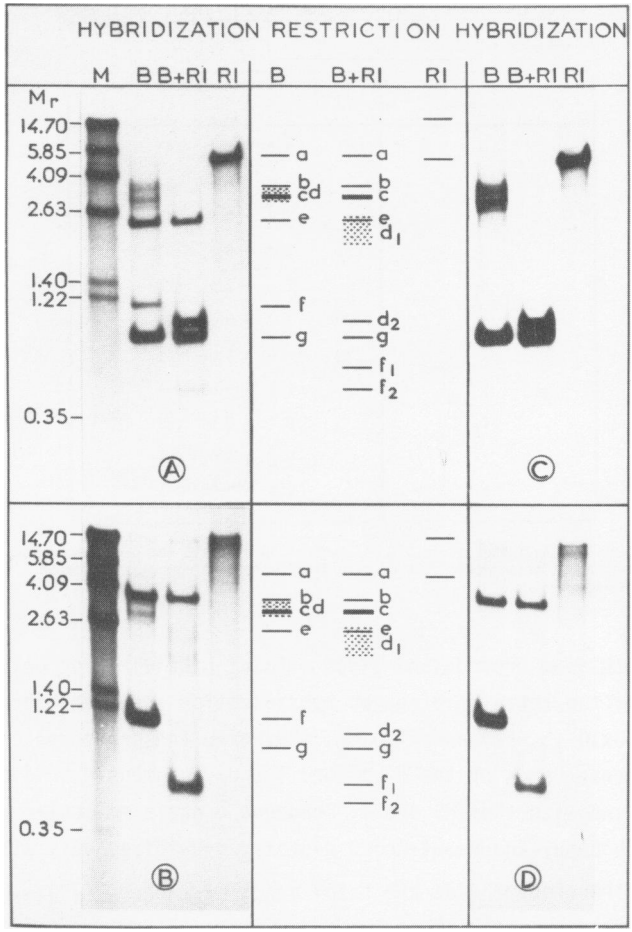

Fig. 4 Restriction endonuclease pattern and hybridization of calf rDNA
Purified calf rDNA (0.25 µg) was digested to completion by EcoRI, BamHI or by BamHI+EcoRI, and electrophoresed on a 1 % agarose gel. ^{32}P-Labelled plasmids HM 123 and HM 456 were hybridized on the restriction fragments (A and B). The same nitrocellulose sheets were re-used (see Materials and Methods) for hybridization with the isolated 28S and 18S probes (C and D). A scheme of the restriction patterns obtained with the different endonucleases is shown in the central panel. Fragments are labelled a to g. HindIII fragments of phage λ^{32}P-labelled DNA were used as molecular weight markers (M).

and with the 18S probe. In double digests BamHI+EcoRI, band d produces a diffuse band d_1 and a sharp band d_2. The former one is barely seen in the gel

Table I. Molecular weights of BamHI and BamHI+EcoRI restriction fragments of calf rDNA.

Fragment	$M_r \times 10^{-6}$
a	5.3 ± 0.05 (4)
b	3.8 ± 0.09 (6)
c	3.2 - 3.3 (4)
d	3.2 - 3.7 (3)
d_1	2.1 - 2.6 (2)
d_2	1.0 ± 0.03 (3)
e	2.4 ± 0.06 (6)
f	1.1 ± 0.03 (3)
f_1	0.7 ± 0.01 (2)
f_2	0.6 ± 0.02 (2)
g	0.9 ± 0.02 (6)

Fragments are indicated as in fig. 4. Numbers in brackets indicate the number of independent measurements. Bands c, d and d_1 are heterogeneous in length (see text).

stained with ethidium bromide and gives a faint hybridization only with plasmid HM456 ; two zones of stronger hybridization are found in d_1 at 2.1×10^6 and 2.6×10^6 ; when added to d_2, they give the exact molecular weights of the two intense bands of the d region. The d_2 fragment hybridizes with plasmid HM123 and with the 28S probe. Fragment e has a molecular weight of 2.4×10^6 in both BamHI and BamHI+EcoRI digests, hybridizes only with plasmid HM123, and is therefore inside the 5×10^6 EcoRI fragment at a distance corresponding to at least 1.5×10^6 (the size of the 28S probe) from the 28S EcoRI site. Fragment f has a molecular weight of 1.1×10^6 and contains one EcoRI site, the splitting of which gives fragments f_1 and f_2 of molecular weights 0.7×10^6 and 0.6×10^6, respectively. The hybridization results reveal that the f fragment contains part of the 18S gene, f_1 belonging to the 16×10^6 and f_2 to the 5×10^6 EcoRI fragments. Fragment g, of molecular weight 0.9×10^6, hybridizes only with plasmid HM123 and the 28S probe ; it must be, therefore, localized between fragments e and d_2. These results suggest a model where the EcoRI site producing d_1 and d_2 is inside the 28S gene, d_2 being the part of the gene which is contained in the 5×10^6 EcoRI fragment, and d_1 being heterogeneous in size and part of the spacer in the 16.1×10^6 EcoRI fragment (fig. 5). Fig. 5 summarizes the mapping results described so far. The relative position of

contiguous fragments a and c is not established. Their position on the map is therefore arbitrary. It should be noted, however, that two regions of heterogeneity roughly corresponding to the positions indicated in fig. 5 for fragments d and c exist in the rDNA of Xenopus (2,6).

DISCUSSION

The isolation procedure described here for rDNA is based on previous work from our laboratory (13,14). The procedure is generally suitable for the isolation of dG+dC-rich DNA components, as demonstrated by our previous work (13,14).

The purification of the rDNA obtained by the present method is at least 400-fold, indicating that it represents at most 0.25 % of total calf thymus DNA. On the other hand, the rDNA was obtained in a final yield of 0.08 %. The discrepancy between these figures is due at least in part to a loss of rDNA during the purification. The enrichment may have been underestimated because of the self-reassociation of the DNA probe. The amount of rDNA in the calf genome is rather close to that reported for Xenopus laevis (1). The ribosomal genes themselves, however, only represent 1/7 of the ribosomal repeat

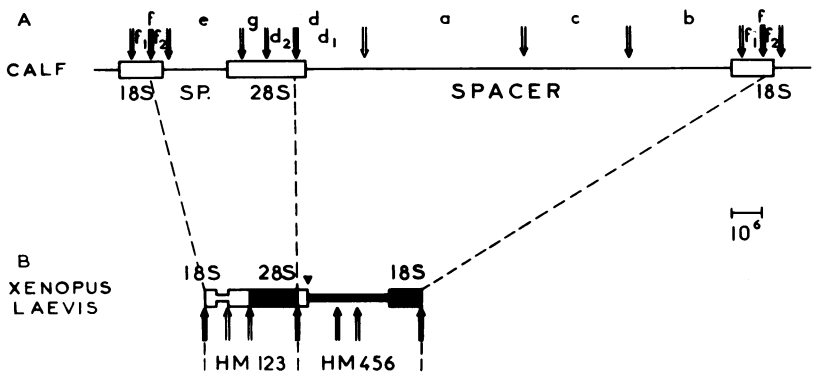

Fig. 5 Restriction maps of the repeating units of calf and Xenopus rDNA
A. Restriction map of the repeating unit of calf rDNA. Black and white arrows indicate EcoRI and BamHI sites, respectively. Fragments are indicated as in fig. 4. Boxes indicate the 18S and 28S genes as localized by hybridization with X. laevis rDNA probes. B. Restriction map of X. laevis rDNA inserts present in plasmids HM123 and HM456. The black regions correspond to the 18S and 28S probes as obtained by EcoRI+HindIII (black triangle) and EcoRI-BamHI digestion, respectively. Other indications as in A. Both repeat units are drawn at the same scale.

unit in calf, whereas they represent 1/2 of it in Xenopus. From these data, we can estimate the number of ribosomal genes per haploid genome to at most 250 in calf (c = 3.6 pg) (26).

The buoyant density of 1.724 g/cm^3 found for purified rDNA corresponds to the value of 1.720 g/cm^3 reported by Blin et al. (7), if the latter is calculated with $\beta_B = 1.19 \times 10^9$ (13) as used in this work.

The finding of a weak, non-specific hybridization of rDNA with at least two dG+dC-rich satellite DNAs is a warning against the danger of such spurious hybridizations. This apparently involves the ribosomal genes rather than the spacer, since it is the same with the 18S and 28S probes, the latter of which only contains coding sequences. The number of spurious bands is such that the information obtained is seriously limited by the possibility that some true hybridization bands are masked by spurious bands. Under these circumstances, mapping of rDNA is totally impossible. It should be stressed however that the spurious hybridization is rather weak and only apparent on the gels, possibly because of the high concentration of satellite DNAs in narrow bands, whereas the hybridization profile of the gradients (in which case dilute solutions were used) never followed the distribution of satellite DNAs.

In contrast to the results obtained with total DNA, the hybridization data from rDNA enabled us to map the repeat unit as shown in fig. 5. The relative position of the restriction fragments and that of the 18S and 28S genes were established as described in the previous section. This was possible because the fragments which could not be detected by hybridization (spacer regions) were seen in restriction patterns. The use of EcoRI probes containing different regions of the 18S and 28S genes allowed the direct localization of most restriction sites. In addition, results obtained with known restriction fragments of the probes allowed the relative positions of some fragments (e.g. fragments e and g) to be established.

A certain degree of length heterogeneity was found, using BamHI and BamHI+EcoRI digests, in fragments c, and d_1 which are localized in the non-transcribed spacer (fig. 5). This was not detected using EcoRI digests because of the lower resolution of high molecular weight fragments. The heterogeneity is localized in fragment d_1, contiguous to the 28S gene and in fragment c, which may or may not be contiguous to fragment d_1 (see Results). The size of fragment d_1 may vary by as much as 0.8×10^6 with two prominent discrete fragment classes ; they differ in molecular weight by 0.5×10^6 and clearly indicate the presence of two major classes of repeats in the rDNA of

the individual calf under consideration. Fragment c exhibits a size variation of 0.1×10^6. This indicates that the degree of heterogeneity of these two fragments is different. The variation in length of fragments c and d seems to be a real variation in length of these spacer stretches. An alternative explanation, not exclusive of the first one, is that the existence of additional BamHI sites could give small fragments which could get lost. The comparison of the molecular weight of the seven BamHI fragments, 20.4×10^6 (taking average values for the heterogeneous fragments), with that of the two EcoRI fragments, 21×10^6 does not permit discrimination between the two explanations even if the second one seems less likely. The length heterogeneity of the non-transcribed spacer in calf rDNA is comparable with that found in mouse (9,10), but it is lower than that found in Xenopus laevis (2).

A comparison (Table II) of the rDNA repeat units of the three mammalian species studied so far, calf, mouse and man, reveals some interesting features. The molecular weight of the repeat unit of mouse rDNA is 3×10^6 to 6×10^6 larger than those of calf and man. This difference in length is definitely due to length variations in the non-transcribed spacer. The EcoRI fragment containing most of the two genes is approximately equal in size in calf and man and only slightly smaller in mouse ; since the lengths of BamHI fragments f_1 (in the 18S gene), g and d_2 (in the 28S gene) are the same, within

Table II. Comparison of restriction fragments of mammalian rDNAs[a]

	CALF		MOUSE				HUMAN
Calf rDNA[b] fragments	This work	Blin et al. (7)	Arnheim and Southern (9)	Cory and Adams (10) (c)	Cory and Adams (10) (d)	Tiemeier et al.(8)	Arnheim and Southern (9)
Total unit	21	21.3	24-25	26.1-27.1	-	-	20
EcoRI small fragment	5.0	4.9	4.7	4.4	-	4.1	4.9
f_1	0.7	-	-	0.9	0.8	-	-
d_2	1.0	-	-	1.2	0.8	0.8	-
g	0.9	-	-	1.1	1.1	0.8	-
$g + d_2$	1.9	-	-	2.3	1.9	1.6	-

(a) The molecular weights of the fragments are given in millions.
(b) See fig. 5 for fragment nomenclature. Mouse rDNA fragments are also listed according to our nomenclature.
(c) Observed values.
(d) Values predicted by the authors on the basis of the length of the repeat unit.

experimental error, in calf and mouse, the slightly shorter length of the small EcoRI fragment in mouse should be due to a slightly shorter transcribed internal spacer in mouse.It should be noted that while the nucleotide sequences of the 18S and 28S gene appear to be highly conserved as judged from DNA-DNA hybridization and, more precisely, by a similar location of BamHI sites on the genes of mouse and calf, the same cannot be said of the spacers. Calf rDNA has one extra BamHI site in the internal transcribed spacer, and mouse and man rDNA have at least one extra EcoRI site in the non-transcribed spacer. The main difference in the latter concerns its length, however, as already mentioned.

Finally, a very interesting feature of the non-transcribed spacers of calf rDNA is the clustering of length variation in two spacer regions. This situation is similar to that previously found in Xenopus rDNA (2,6), possibly also as far as the localization of length variation is concerned.

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Abbreviations : BAMD, 3,6-bis (acetato-mercurimethyl), dioxane ;

c, DNA content per haploid cell.

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