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A comparative study of G+C-rich satellite components of sheep and goat DNA

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ABSTRACT

Restriction fragment patterns of G+C-rich satellites of sheep and goat DNA were compared. The 1.712 g/cm^3 satellites of both species appear homologous, consisting of repeats 760 base pairs long and showing coincidence of position of primary$^+$ EcoRI, BamHI and most BspRI restriction target sites. The EcoRI and BamHI endonucleases produce mostly monomers of the repeating unit, while oligomers prevail in the AluI and BglII digests. Species-specific differences in the frequency, position and mode of distribution of secondary$^+$ restriction target sites for EcoRI, BglII and AluI were observed. Unlike the 1.712 g/cm^3 satellites, the 1.723 g/cm^3 component of sheep DNA and the 1.719 g/cm^3 material from goat DNA appear species--specific, since no homologous material could ever be detected in the DNA of the other species.

INTRODUCTION

DNA of many eukaryotes contains satellite components usually characterized by distinct base composition and repetitive nucleotide sequences[1,2]. Introduction of base- or sequence-specific ligands[3,4] has improved the resolving power of density gradient centrifugation and permitted purification and characterization of many new components of DNA. In spite of their widespread occurrence the phylogenetic origin and biological function of the satellites is a matter of conjecture. Studies employing in situ hybridization technique have shown that many satellites are localized in centrometric heterochromatin[5,6], although others may occur in euchromatin[7]. In any case it appears that the satellites do not carry any coded message determining the structure of proteins, so that they are not subjected to selection pressure due to conservation of functional state of proteins. Comparative studies of satellites

may therefore throw some light on the trends and mechanism of reorganization of eukaryotic genome and provide independent criteria for tracing phylogenetic relation among taxonomically close species or genera. This approach has been successfully applied for elucidating phylogenetic relationship among hominoid primates[8,9]. Sheep and goat, both representatives of the tribe Caprini, provide an interesting model for studying possible correlation between the occurrence of satellites and chromosomal architecture, since the main difference between the two species lies in their karyotypes. Curtain et al.[10] demonstrated sequential homology of the most abundant G+C-rich satellites of sheep and goat using cross-hybridization technique. In the present communication restriction fragment patterns[11-14] of sheep and goat DNA-satellites are compared.

MATERIALS AND METHODS

Netropsin hydrochloride was kindly provided by Dr. C.Zimmer, Institute of Microbiology and Experimental Therapy, Jena. Pronase (B grade) and ethidium bromide (B grade) were from Calbiochem.

Restriction endonucleases. EcoRI restriction endonuclease was isolated from E. coli SSR 11 as described by Greene et al.[15], following the purification procedure up to the phosphocellulose chromatography step; at this stage of purity the enzyme was free of non-specific endonucleases. The BamHI endonuclease was prepared from B. amyloliquefaciens using a modified procedure of Wilson and Young[16]. The BspRI endonuclease, isoschizomeric with BsuRI or HaeIII, was purified from B. sphaericus R as described by Kiss et al.[17]. AluI was isolated according to Roberts et al.[18] and BglII was purified as described by Pirrotta[19].

Preparation and fractionation of DNA. Liver was excised from single male adult animals immediately after slaughter, frozen and kept at -20°C until use. Liver nuclei were prepared as described[20]. DNA was extracted from the nuclei with 4M guanidine hydrochloride[21] and deproteinated by repeated shaking with chloroform-isoamylalcohol followed by treatment with self-digested pronase.

DNA was fractionated on kieselguhr-methylated serumalbumin[22] (MAK), using 70 x 130 mm column for 25 mg DNA. The first, G+C-rich fraction, eluted with 0.70 - 0.72M NaCl and comprising about 20% of the DNA, was fractionated on CsCl-netropsin gradient[4], using Ti 60 rotor of a Beckman L5-65 centrifuge. Fractions forming separate peaks or shoulders on the densitograms were pooled and their buoyant density profiles were determined using analytical CsCl gradient centrifugation on Spinco E. Fractions containing satellite DNA were dialyzed and subjected to cleavage by restriction endonucleases. The buffer for incubation with EcoRI and AluI contained 50 mM NaCl, 10 mM Tris pH 7.5; for BamHI, BspRI and BglII 10 mM NaCl, 10 mM Tris pH 8.0 was used; all buffers contained 10 mM $MgCl_2$.

Reassociation of DNA. Samples of DNA were dialyzed against 0.01x SSC and denatured by adding 0.1 volume of 1M NaOH. After 10 min at room temperature the solution was neutralized with 0.1 volume of 2M NaH_2PO_4. The DNA was annealed at 63°C to c_0t 0.39 as described by Britten and Kohne[23].

Agarose gel electrophoresis was performed according to Thomas and Davis[24] using a horizontal slab gel apparatus (20 x 20 x 0.3 cm) constructed in this laboratory. Composite acrylamide-agarose gels were prepared according to Peacock and Dingman[25]. After staining with ethidium bromide (1 µg/ml) the gels were photographed using a Hanovia lamp and an orange filter. Molecular weights of fragments were estimated from electrophoretic mobility, using EcoRI digests of λ phage DNA[24] and EcoRI or BspRI digests of λdv plasmid[26], or of the 1.714 g/cm^3 calf satellite DNA[11] as standards.

RESULTS

The two-step separation procedure (Figs 1, 2) used here to purify the G+C-rich DNA permitted the isolation of the 1.712 and 1.723 g/cm^3 satellites from sheep DNA and the 1.712 and 1.719 g/cm^3 components from goat DNA, while the 1.705-1.706 g/cm^3 components from both species were incompletely separated. No peak at 1.723 g/cm^3 was ever seen in goat DNA.

The 1.712 g/cm^3 satellites of both species and the 1.723 g/cm^3 component of sheep DNA as well as the 1.719 g/cm^3 mate-

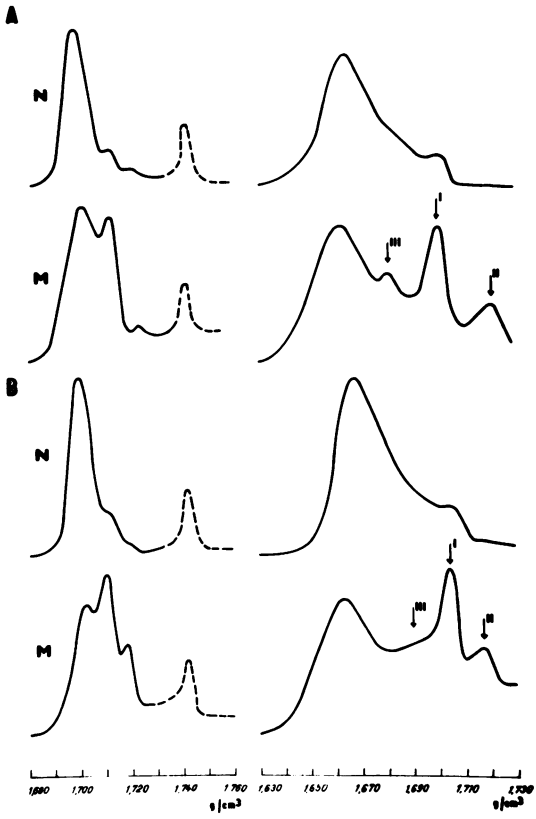

Fig.1. Analytical density gradient centrifugation of sheep (A) and goat (B) DNA in CsCl (left) and CsCl--netropsin (right). N, whole DNA; M, first fraction from MAK-column, eluted with 0.70-0.72M NaCl. The peaks or shoulders of satellite components are marked with arrows: I, 1.712 g/cm^3 satellites; II, 1.723 g/cm^3 satellite of sheep or 1.719 g/cm^3 satellite of goat; III, 1.705-1.706 g/cm^3 satellites; the indicated densities do not correspond to the scale on the abcissa, since density values in the presence of netropsin are lower than those of pure DNA.

rial from goat DNA renatured nearly completely at low c_0t values (Tab. 1) indicating a highly repetitive character. The 1.705 - 1.706 g/cm^3 components of both species renatured incompletely, which may be due to contamination with main-peak nonrepetitive DNA. No distinct bands could ever be obtained from this material with restriction endonucleases EcoRI and BspRI, so that in its present form it is not amenable to restriction analysis.

Restriction fragment patterns of 1.712 g/cm^3 satellites of sheep and goat DNA. Restriction endonucleases AluI, BglII, EcoRI and BamHI all produced monomers and oligomers of a fundamental unit, which in both species had the same length of 760 base pairs (Fig. 3 and 4). Oligomers were much more frequent in the AluI and BglII digests in comparison with the

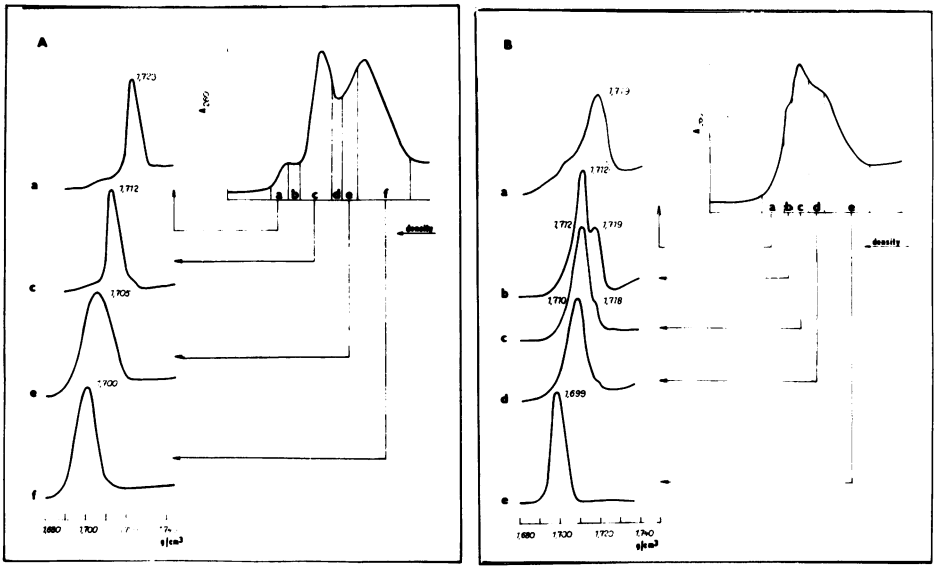

Fig.2. Preparative CsCl-netropsin density gradient centrifugation and pycnographic analysis of the fractions.
A, sheep DNA; B, goat DNA. The densitographs a-f represent density profiles (without netropsin) of single fractions taken from preparative CsCl-netropsin gradients (upper right).

Table I. Renaturation of satellites at low c_0t value
Buoyant densities (ρ) of native, denaturated and renaturated (at c_0t = 0.39) DNA were measured by centrifugation in CsCl gradient.

Species	ρ of native satellite DNA g/cm^3	ρ after renaturation g/cm^3	ρ of denatured satellite DNA g/cm^3
sheep	1.712	1.713	1.727
	1.723	1.726	1.733
	1.705	1.715	1.726
goat	1.712	1.716	1.726
	1.719	1.724	1.730
	1.706	1.717	1.722

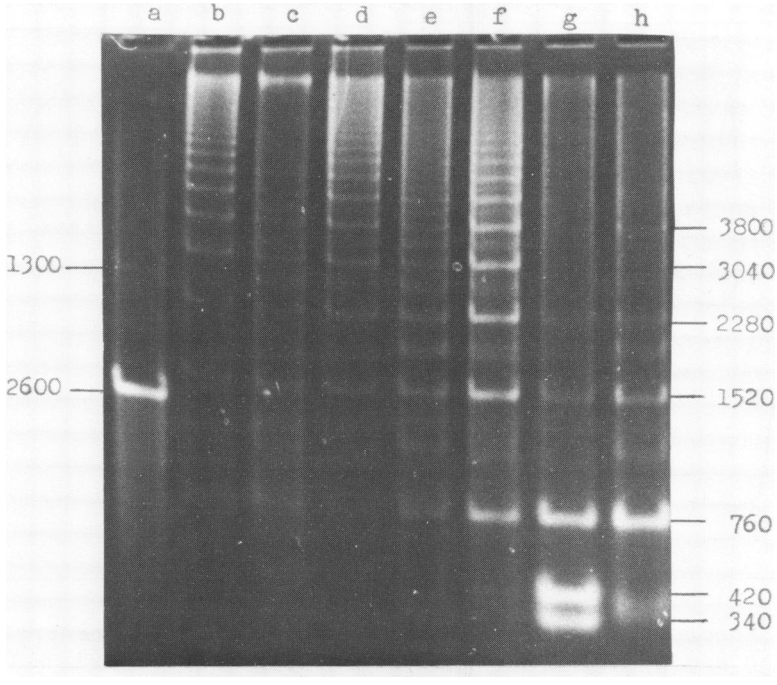

Fig.3. Electrophorograms of 1.712 g/cm^3 sheep and goat DNA--satellites digested with restriction endonucleases.
a, EcoRI digest of 1.714 g/cm^3 calf satellite (reference); b,c, digests with AluI of sheep (b) and goat (c) satellites; d,e,f, Digests with BglII of sheep (d,f) and goat (e) satellites; g,h, EcoRI digests of sheep (g) and goat (h) satellites. 1% agarose gel was used.

EcoRI and BamHI digests; the characteristic multiple-band patterns with AluI and BglII did not change upon addition of portions of fresh enzyme, indicating that the digestion was complete. In exhaustive digests of both satellites with EcoRI and BamHI the frequency of oligomers was very low in general, but significantly more di- and trimers were present in the digests of goat satellite in comparison with the satellite from sheep DNA. The EcoRI digests of the satellites of goat DNA showed a distinct band of pentamers but no band corresponding to tetramers of the fundamental unit; this anomaly was reproducible.

Besides the integral oligomers, some digests contained sets

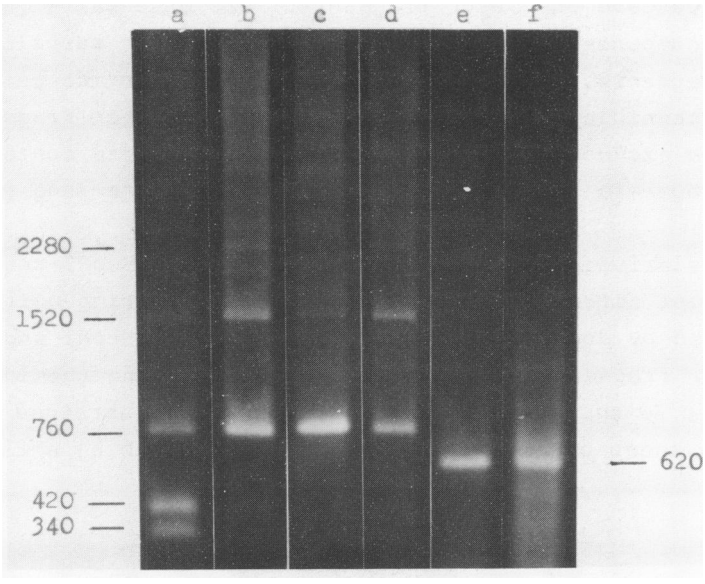

Fig.4. Electrophorograms of 1.712 g/cm^3 sheep and goat DNA-satellites digested with EcoRI, BamHI and BspRI.
a,b, digests with EcoRI of sheep (a) and goat (b) satellites;
c,d, digests with BamHI of sheep (c) and goat (d) satellites;
e,f, digests with BspRI of sheep (e) and goat (f) satellites.
1% agarose gel was used.

of fractional oligomers. These appeared in the AluI digest of goat satellite (Fig. 3c) in the BglII digests of satellites from both species (Fig. 3e,f) and in the EcoRI digest of goat DNA (Fig. 3h). Most fractional oligomers formed double lines of approximately equal intensity (e.g. Fig. 3c and f) suggesting that the internal restriction target site is located asymmetrically with respect to the primary target sites of the same enzyme. In the EcoRI digest of sheep satellite most monomers of the repeating unit were split into two unequal fragments, 340 and 420 base pairs long, indicating that most copies of the EcoRI repeating unit contained an internal EcoRI target site. No fractional oligomers, homologous to these fragments, were discernible (Fig. 3g) although they were clearly visible in the digest of goat satellite (Fig. 3h).

With BspRI restriction endonuclease both goat and sheep

satellites gave one major fragment of 620 base pairs (Fig. 4 e,f), accompanied by several fragments; in goat satellite two short fragments, consisting of 90 and 40 nucleotide pairs, were discernible and the presence of even shorter fragments cannot be excluded. The digest of sheep satellite contained short fragments about 70 and 40 nucleotide pairs long (Fig. 5 a,d).

The relative positions of EcoRI, BamHI and BspRI restriction target sites within the fundamental repeating units were determined by double digestion (Fig. 5). With EcoRI and BspRI two major fragments, 210 and 410 base pairs long (besides the short 90, 70 and 40 base-pair fragments) were obtained from both the sheep and goat DNA satellites (Fig. 5b,e) while the

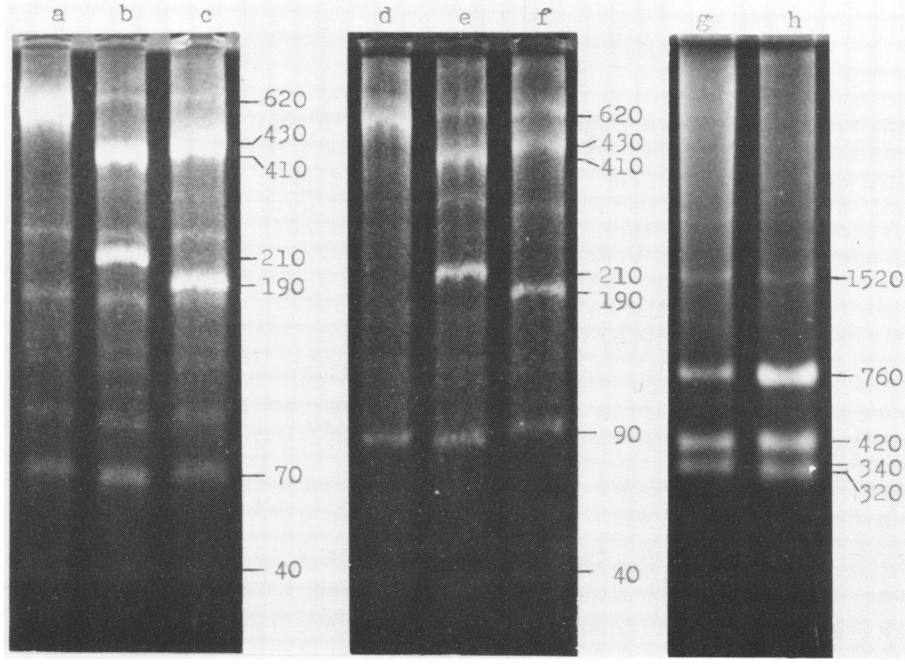

Fig.5. Electrophorograms of double digests of sheep and goat 1.712 g/cm^3 satellites.
a,b,c sheep satellite digested with BspRI, BspRI+EcoRI and BspRI+BamHI, respectively ; d,e,f, goat satellite digested with BspRI, BspRI+EcoRI and BspRI+BamHI, respectively; g,h, sheep satellite digested with EcoRI and EcoRI+BamHI; a-f, 6% acrylamide gel was used; g,h, 1% agarose gel was used.

double digestion with BamHI and BspRI gave major fragments of approximate lengths 190 and 430 base pairs (Fig. 5c,f). Double digests of sheep satellite with EcoRI and BamHI contained fragments of 740, 420, 340 and 320 base pairs; the latter two could not be separated on agarose (Fig. 5h), but were clearly separable on 3% acrylamide (Fig. 7d). On the basis of these results the restriction sites in both satellites could be mapped as indicated in Fig. 6. It appears that the BamHI and the primary EcoRI target sites are very close, approximately 20 base pairs apart; the corresponding short fragment could not be detected because of insufficient quantity of material. For the same reason the location of the small BspRI fragments could not be determined.

Restriction fragment patterns of 1.723 g/cm^3 of sheep DNA and 1.719 g/cm^3 of goat DNA. Our 1.723 g/cm^3 material from sheep DNA was identical with sheep satellite II of Maio et al.[27] as evident from the coincidence of buoyant density and identity of restriction fragment patterns obtained with iso-

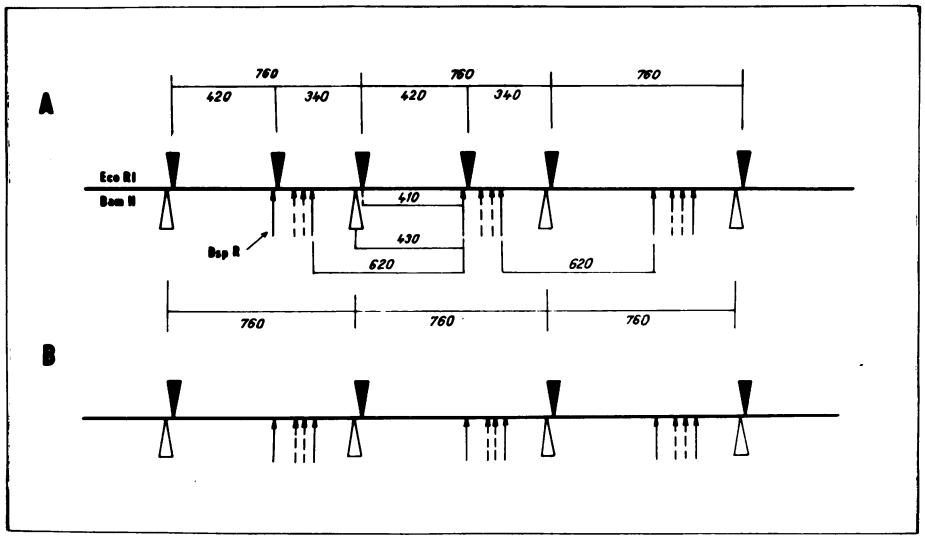

Fig.6. Physical maps of repeated units of 1.712 g/cm^3 satellites.

A, sheep; B, goat satellites.

schizomeric endonucleases HaeIII and BspRI (cf. Fig. 7 b and Fig. 1 f in ref.[27]). Although goat DNA did not contain any distinct 1.723 g/cm^3 component, the presence of a small amount of this DNA in the 1.719 g/cm^3 peak could not be a priori excluded. Therefore we examined the BspRI fragment patterns of this DNA (Fig. 7 c). The pattern obtained consisted of a large number of fragments whose lengths varied between 50 and 2500 base pairs and suggested a periodicity of 20-25 base pairs. Three fragments, 680, 540 and 165 base pairs long, were present in larger amounts than others, but no bands homologous to the three characteristic fragments of the 1.723 g/cm^3 sheep

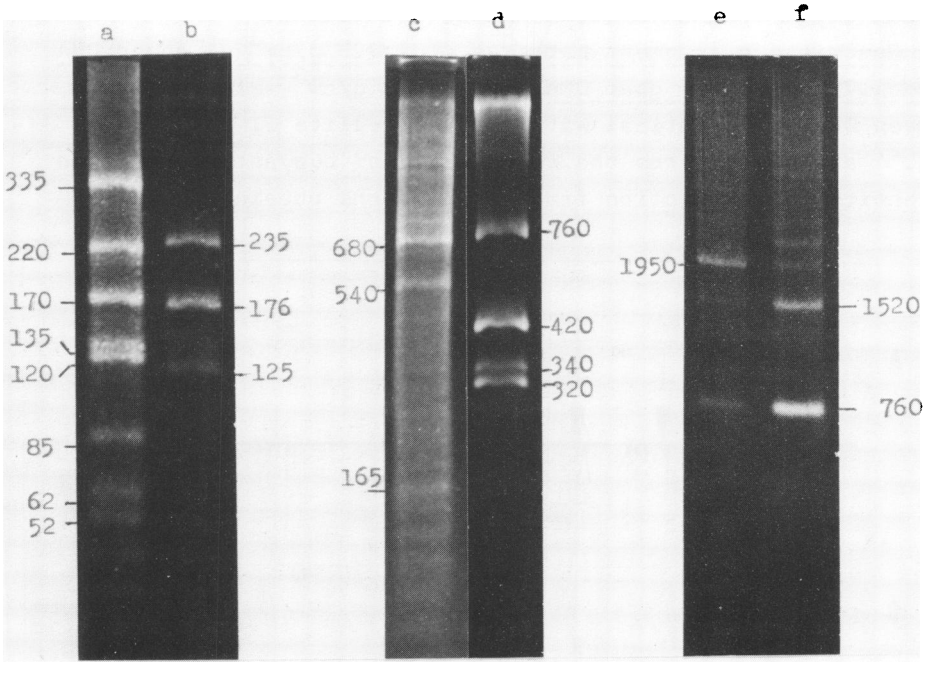

Fig.7 . Electrophorograms of restriction fragments of heavy DNA-satellites of sheep and goat.
a, BspRI digest of calf thymus 1.714 g/cm^3 satellite (reference); b, BspRI digest of sheep 1.723 g/cm^3 satellite; c, BspRI digest of goat 1.719 g/cm^3 component of DNA; d, EcoRI+BamHI digest of 1.712 g/cm^3 sheep satellite (reference); e, EcoRI digest of goat 1.719 g/cm^3 component of DNA; f, EcoRI digest of goat 1.712 g/cm^3 satellite shown for comparison; a,b, 6% acrylamide, c,d, 3% acrylamide, and e,f, 1% agarose gels were used.

satellite II were present.

The 1.719 g/cm^3 component of goat DNA was cleaved with EcoRI restriction endonuclease, producing a major fragment 1950 base pairs long (Fig. 7 e). The faint band of 760 base pairs originated from slight contamination with the 1.712 g/cm^3 satellite. In contrast the 1.723 g/cm^3 satellite from sheep DNA was entirely resistant to EcoRI (not shown).

We may conclude that no satellite homologous to sheep satellite II (1.723 g/cm^3) exists in goat DNA; if homologous sequences are present, they are not sufficiently amplified to permit detection as a separate component of DNA. With respect to complexity of the BspRI fragment pattern of the 1.719 g/cm^3 component of goat DNA this material appears to represent a composite segmented satellite of B-type[12] or might consist of several independent components which happen to have a similar density.

DISCUSSION

Comparison of restriction fragment patterns and mapping of the EcoRI, BamHI and BspRI restriction target sites indicates structural homology between the 1.712 g/cm^3 satellite of sheep and goat; taken in connection with cross-hybridization experiments of Curtain et al.[10], the results leave little doubt of common phylogenetic origin of these DNA-components, which probably originate in the DNA of a common ancestor from which both species began to diverge 1-5 x 10^6 years ago[28,29]. Since it is improbable that amplification of the same component of DNA would proceed independently in an identical manner, it appears that the ancestral precursor already had the character of a repetitive satellite.

The post-speciation divergence between the 1.712 g/cm^3 satellites of sheep and goat concerns formation of secondary restriction target site and inactivation of some primary restriction target sites by mutations. The latter effect is responsible for the presence of oligomers, which are rare in the EcoRI and BamHI digests but very frequent in the BglII and AluI digests; therefore no consistent estimate of the overall mutation rate of the DNA can be made from the frequency of

oligomers.

It is characteristic for the 1.712 g/cm^3 satellites of both species that most secondary restriction target sites are located asymmetrically with respect to the primary sites of the same restriction endonuclease, producing fragments of unequal size which on the electrophorograms appear in the form of doublet bands (Fig. 3 c, e, f, g). Unlike the mouse satellite 12, or calf satellite III[30], the sheep and goat 1.712 g/cm^3 DNA component apparently does not contain regions of short-range periodicity within the fundamental repeats of 760 base pairs; other enzymes, however, might detect additional features of repetitivity or symmetry.

Amplification[31] and random interspersion of mutated fundamental units, i.e. of those bearing inactivated primary restriction target sites and of others with secondary target sites is responsible for the appearance of series of fractional oligomers (Fig. 3 c,e,f,h). One notable exception indicating a non-random distribution of mutated fundamental units is the mode of distribution of EcoRI target sites in the satellite of sheep. Although the 420 and 340 base-pair fragments predominate over the intact monomer, no fractional oligomers are seen (Fig. 3 g). In an analogous digest of the goat satellite intact monomers prevail over the fragments, indicating that the overall number of internal restriction target sites is much less than in sheep satellite, yet fractional oligomers are clearly visible (Fig. 3 h). It seems that in sheep satellite the secondary EcoRI target sites are accumulated in a distinct segment where mutations of primary EcoRI target sites are very rare. The predominance of pentamers over tetramers in the EcoRI digest of goat satellite may also indicate the presence of a segment, possibly consisting of a contiguous stretch of fundamental units where every fifth primary EcoRI target site would remain active. Other enzymes used here, however, failed to show any heterogeneity.

If we presume that random spreading of mutated repeating units over the whole satellite occured by inequal crossing over[32], formation of a distinct subclass or segment of satellite DNA should be due to some limitation, preventing free ex-

change of DNA. With respect to high overall contents of the 1.712 g/cm^3 satellites, which account for as much as 12% of total nuclear DNA, it is almost certain that this component must occur in several independent sites located in different chromosomes. Considering this, the satellites of both species appear surprisingly homogeneous, showing few signs of independent development of separate arrays. However, divergence of physically separated arrays of satellite DNA could be counteracted by occasional exchange of repetitive DNA even between non-homologous chromosomes as suggested by Smith[32]. It is possible that some particular feature of sheep karyotype may prohibit such exchange, thus favoring divergent evolution of a distinct segment.

The two heavier satellites, i.e. the 1.723 g/cm^3 satellite of sheep (sheep satellite II[27]) and the 1.719 g/cm^3 component of goat DNA have no amplified homologous counterpart in the other species and therefore probably evolved after both species diverged or were lost in the course of evolution of the other species. Since sheep has six large metacentric chromosomes while goat has none[10], it is tempting to speculate that sheep satellite II (1.723 g/cm^3) could participate in the build-up of the metacentric chromosomes. To verify this hypothesis it would be necessary to determine chromosomal localization of this satellitic DNA. With respect to considerable complexity of restriction fragment patterns of the 1.719 g/cm^3 component of goat DNA any hypothesis concerning its phylogenetic origin and significance would be premature.

DEFINITION

+The fundamental repeating unit is the shortest restriction fragment of DNA whose length is common for several restriction endonucleases and is equal to the interval of periodicity of the series of oligomeric fragments. Primary restriction target sites are those responsible for the formation of these fragments. The secondary or internal restriction target sites are those located in the interior of repeating units as defined by primary target sites of the same endonuclease.

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