Comparative structure and evolution of goat and sheep satellite I DNAs

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ABSTRACT

The satellite I DNAs of domestic goat (Capra hircus) and domestic sheep (Ovis aries) have been studied using molecular hybridisation and restriction enzyme analysis. Both satellite DNAs are composed of repeat units of 820 base pairs in length, but their restriction maps, although similar, differ in certain respects. Thus the majority of sheep satellite I repeat units have two EcoRI sites and one AluI site, whereas the majority of goat satellite I repeat units have one EcoRI site and two AluI sites. The sheep satellite I repeat units with the two EcoRI sites are much more homogeneous than the repeats forming the remainder of the satellite, as judged by the difference in the melting temperatures of native and reassociated molecules. DNAs from species of wild sheep and goats were screened for the presence of these repeat units, and they appear to have been amplified during the radiation of the Ovis genus. Goat satellite I is composed of a single sequence type which has changed through base substitution until the sequence now shows considerable heterogeneity. It is proposed that the major sequence types of these two satellite DNAs were amplified by different saltatory replication events at different times in the evolution of the group.

INTRODUCTION

Satellite DNAs have been identified in the sheep and goat which have similar buoyant densities (1), and which will cross-hybridise with chromosomes of the other species (2,3). It was therefore considered of interest to see what structural similarities there were between the cross-hybridising satellite sequences of these species, and to see if this would shed some light on the evolution of these sequences.

Preliminary restriction analysis of sheep and goat satellite I DNAs shows that they are composed of long range repeat units of the same length and with similar restriction maps (4). The present paper examines in greater detail the structure of, and relationship between, the satellite I DNAs of these species using molecular hybridisation and restriction enzyme analysis. It confirms the fact that they are closely related, but also shows that there are differences which occur between the major fractions

of the two satellites. It is argued that it is most unlikely that these differences are the result of unequal crossing-over, and so it is proposed that these satellite DNAs were amplified for the most part by the separate replication of repeat units from the same sequence family.

MATERIALS AND METHODS

a) Animals

Tissues (liver and blood) were obtained from the following species: Saanan goats (Capra hircus), Scottish blackface sheep (Ovis aries), aoudad (Barbary sheep) (Ammotragus lervia), European mouflon sheep (Ovis musimon), Afghan urial sheep (O.vignei cycloceros), and Siberian ibex (Capra ibex sibirica). [The taxonomic classification for wild sheep is that used by (5)]. Frozen livers of wild species were kindly supplied by Dr. O. Ryder of San Diego Zoo, and blood samples of O. musimon, and of the Surrey breed of domestic sheep (which dates back almost 3,000 years), were kindly supplied by Dr. G. Lincoln of the MRC Centre for Reproductive Biology, Edinburgh.

b) Satellite DNA preparation

DNA was prepared from the above tissue samples by phenol extraction and treatment with ribonuclease A and proteinase K (6). Satellite DNAs were prepared by caesium chloride centrifugation at a DNA concentration of 70µg/ml in the Sorvall TV850 vertical rotor. Gradients were collected from the bottom by paraffin displacement. Buoyant densities were measured on a MSE Centriscan analytical ultracentrifuge (7) using Micrococcus lysodeikticus as reference ($\rho = 1.731 \text{ gm/cm}^3$). Pure satellite DNAs were dialysed against 10mM tris-HCl, pH 8, 1mM EDTA prior to pelleting overnight. DNA pellets were resuspended in 10mM tris-HCl pH 7.5.

c) Restriction of DNA, gel electrophoresis and DNA:DNA hybridisation

Restriction enzymes were purchased from New England Biolabs and Bethesda Research Laboratories and used under the conditions recommended by the manufacturers. Digests were run in slab gels prepared in 40mM tris-acetate, pH 8.2, 20mM sodium acetate, 1mM EDTA. Molecular weights were estimated by tracing photographic negatives with a Joyce-Loebl scanning densitometer, and with reference to restriction fragments of ϕ X174 RF DNA (8) and bacteriophage λ DNA (9) molecular weight standards. In order to determine the relative number of copies of different DNA fragments in a particular restriction digest, a HaeIII digest of ϕ X174RF DNA was used to construct a standard curve of the relative fluorescence of

different molecular weight DNA fragments present in equimolar amounts (10)

Restriction fragments separated electrophoretically in agarose gels were transferred to Sartorius 16 x 16cm nitrocellulose filters (11) except that the transfer was performed with 10 x SSC, 10mM KOH (12). For filter disc hybridisations, nitrocellulose filters were each loaded with 1ng of total satellite DNA in the presence of 3µg bacterial DNA (13). Filters were incubated in 2 x SSC, pH 7.2, 0.1% SLS, 2 x Denhardt's solution (14) at 65°C for 3-5 hours prior to incubation (3-16 hrs) with ³²P- labelled nick translated satellite DNAs (15) under the same conditions. After hybridisation, filters were washed twice in 2 x SSC, once at 65°C and once at 20°C, and then rinsed briefly in 3mM tris, not pHed (i.e. pH=9.4), before being exposed to Kodak X-Omat H X-ray film at 20°C. Hybrids on filter discs were melted by transferring them from one screw-top vessel to another, each vessel containing 1-2ml of 0.3xSSC equilibrated to the next temperature point. Elution was monitored by Cerekov radiation.

d) Thermal denaturation studies

Melting temperatures of native satellite DNAs were determined by heating in a quartz glass cuvette (1cm light path) in a Pye Unicam SP1750 spectrophotometer fitted with SP874 and SP876 heating accessories. For ΔT_m determination of total satellite DNA preparations, 10µg of DNA were sheared to single-strand lengths of approximately 1kb in 1ml of 0.3xSSC (16). About 3µg of individual restriction fragments were used, again in 1ml of 0.3xSSC. After denaturation of the native DNA, the sample was sealed in a glass ampoule and incubated to $Cot\ 10^{-1}$ (Cot =moles nucleotide/litre second), when it was cooled, returned to the cuvette, and remelted.

RESULTS

a) Restriction analysis of satellite DNAs

In our hands the buoyant densities of sheep and goat satellite I DNAs (hereafter referred to as SI and GI respectively) were both 1.713gm/cm³. Restriction endonucleases EcoRI, BamH-I, SstI and AluI were all found to restrict about 70% of the SI DNA preparation to a 820 base pairs (bp) monomer and multiples thereof. Restriction with EcoRI also produced fragments of 360 and 460bp in length (fig. 1 and cf.(4)). All but SstI also cleaved GI DNA to fragments based on a 820 bp monomer, and digestion with AluI also produced fragments of 340 and 480bp in length (fig. 2). Both the 360 and the 460bp SI/EcoRI and the 340 and 480bp

Fig. 1. Restriction fragments of sheep satellite I DNA separated in a 1.5% agarose gel. Lanes (1), (10) and (11) : reference fragments produced by λ /Hind III, ϕ X174RF/Hae III and λ /Hind III/EcoRI digests respectively. Lane (2) : SI digested with AluI, Lane (3) SI/SstI, (4) SI/BamH-I, (5) SI/EcoRI, (6) SI/EcoRI/SstI, (7) SI/EcoRI/BamH-I, (8) SI/EcoRI/AluI, (9) SI/MboI. The DNAs in digests involving SstI (Lanes 3 and 6) are only partially restricted.

GI/AluI fragments total the length of a repeat unit, and measurements carried out on the relative amounts of these fragments (see Materials and Methods) show that in both cases the two fragments are present in equimolar amounts. In the case of SI, the ratio of repeats containing two EcoRI sites, to the combined total of repeats containing one EcoRI site and those present as dimers after EcoRI digestion is 21:7, and so 75% of SI repeat units contain two EcoRI sites. In the case of GI, the ratio of repeats containing two AluI sites to those containing only one is

Fig. 2. Restriction fragments of goat satellite I DNA separated in a 1.5% agarose gel. Lane (1) λ DNA digested with Hind III and EcoRI, and (10) ϕ X174RF DNA digested with Hae III (marker DNAs), (2) GI digested with AluI, (3) GI/SstI, (4) GI/BamH-I, (5) GI/EcoRI, (6) GI/BamH-I/EcoRI, (7) GI/EcoRI/AluI, (8) GI/MboI, (9) GI/PstI. The arrowheads indicate the 1300 and 1160 bp fragments in the AluI digest.

approximately 2:1, and so about 66% of GI repeat units contain two AluI sites.

Restriction maps of these satellites DNAs (fig.3) were constructed from single and double digestion experiments, some of which are shown in figs. 1 and 2. The two maps are very similar (cf.4) but the majority of the repeat units of the two satellites differ.

b) Cross-hybridisation and thermal denaturation studies

Cross-hybridisation reactions were initially performed by hybridising nick translated satellite DNAs to restriction fragments of satellites I and II from both species immobilised on nitrocellulose sheets (11). Fig. 4b shows the results of hybridising SI DNA to one such

Fig. 3. Restriction maps of the major fractions of sheep and goat satellite I DNAs, derived from single and double restriction digests of the kind shown in figs. 1 and 2. Sizes of restriction fragments are in base pairs.

filter, and the major hybridisation is to SI and GI sequences, with minor hybridisation to the GII sequences. Hybridisation of GI DNA to a replica filter produced a similar result (fig. 4c). Note that SI and SII do not cross-hybridise, whereas GI and GII do. The fragments hybridising to GI in the GII track have similar molecular weights to GI sequences cleaved

Fig. 4. Hybridisation of nick translated SI and GI DNAs to satellites I and II of both species. ~1.6 μ g of each satellite DNA was digested with the appropriate restriction enzyme, and half of the products run on a 1.5% agarose gel (a). Lane (1) : SI/BamH-I, (2) SII/MboI, (3) GI/BamH-I, (4) GII/PstI. ~250ng of the remainder of each digest were run on separate gels, transferred to nitrocellulose sheets and hybridised to (b) SI, and (c) GI DNAs, ~2 ng/ml, 4.10⁷ dpm/ μ g. Exposures were (b) 1 hr, and (c) 6 hr at 20°C.

Fig. 5. Melting of ^{32}P -labelled (a) SI and (b) GI hybrids from nitrocellulose discs. (o) immobilised SI DNA, (Δ) immobilised GI DNA. For details of hybridisation and melting see Materials and Methods.

with this enzyme, indicating that the two satellite preparations are slightly cross-contaminated. Note also that the sequences remaining near the origin of the gels in the SI and GI tracks do not hybridise to SI or GI, indicating contamination of the satellite I sequences by other relatively GC-rich sequences.

If the SI hybrids shown in fig. 4(b) are melted in 0.3 x SSC, the heterologous hybrids melt before the homologous ones. This is not the case with the GI hybrids in fig. 4(c), which all appear to melt simultaneously (data not shown). This was investigated quantitatively by carrying out similar reactions with total satellite DNAs immobilised on filter discs, and then melting the hybrids in 0.3 x SSC as described in Materials and Methods. Fig. 5 shows that GI/GI hybrids, and both types of heterologous hybrids have T_{ms} of $\sim 74^\circ\text{C}$, whereas the homologous SI hybrids have a T_m of 80°C . Thus, the divergence between GI sequences is as great as the divergence between GI and SI DNAs.

c) Optical thermal denaturation of native and reassociated satellites and restriction fragments.

It has been mentioned in section (a) that 75% of all SI repeat units contain two EcoRI sites and 66% of all GI repeat units contain two AluI sites. In GI/AluI digests, fragments that result from the loss of two adjacent AluI sites are seen (fig. 2 arrows and fig. 7b), whereas fragments resulting from the loss of two adjacent EcoRI sites are not seen in EcoRI digests of SI (figs. 1 and 7a). This suggests that repeat units with two EcoRI sites are not interspersed among those with just one, but form a distinct part of the satellite. This proposition was tested directly by excising the SI/EcoRI 360 and 460bp fragments and the SI/EcoRI monomer from preparative gels, and determining their ΔT_m s optically. It

Fig. 6. Optical melts of native (●) and reassociated (○) satellite DNAs and restriction fragments. (a) total GI (b) GI/EcoRI/BamH-I monomer (c) total SI (d) SI/EcoRI 360bp fragment (e) SI/EcoRI 460bp fragment (f) SI/EcoRI monomer.

can be seen from fig. 6 (d and e) that not only are the ΔT_m s of the SI/EcoRI 360 and 460bp fragments small, but also that they are the same (1.8°C). The ΔT_m of SI/EcoRI monomer, on the other hand (fig. 6f), is $\sim 7.5^\circ\text{C}$ and shows the presence of both well- and poorly-matched sequences. The former will be enriched by homogeneous sequences moving into the monomer class through the loss of EcoRI sites, but it is not clear to what extent.

As expected, the value for the total satellite falls between those for the separate SI/EcoRI fragments (fig. 6c). Total GI has a large ΔT_m , which confirms the result previously obtained in section (b), which indicated that reassociated GI forms poorly-matched duplexes. It should be noted here, however, that the values obtained for ΔT_m s by the optical method do not agree with those obtained by melting off nitrocellulose. It has been noted before that satellite cRNA/DNA hybrids form very unstable

Fig. 7. Hybridisation of ^{32}P -labelled SI to DNAs of wild and domestic breeds of sheep and goats restricted with (a) EcoRI and (b) AluI, run in 2% agarose and transferred to nitrocellulose sheets. (a) (1) domestic sheep (Scottish blackface) (2) domestic sheep (Surrey) (3) and (4) mouflon sheep (5) urial sheep (6) aoudad (7) ibex (8) domestic goat (Saanen). (b) (1) domestic sheep (Scottish blackface) (2) urial sheep (3) aoudad (4) ibex and (5) domestic goat (Saanen). All lanes contain 0.5 μg DNA, except (6) 5 μg , (7) 1 μg , in (a) and (3) 2.5 μg and (5) 1 μg in (b). Molecular weights in base pairs.

duplexes on nitrocellulose (17) and so relative hybrid instability might also be a characteristic of DNA hybrids formed between repeated sequences on nitrocellulose.

d) The presence of the 360 and 460bp SI/EcoRI fragments and the 340 and 480bp GI/AluI fragments in species of wild sheep and goats.

As restriction fragments had been identified which were specific to each satellite, the DNAs of various species of wild sheep and goats were assayed for their presence in an effort to determine when each satellite was generated. Consequently, these various DNAs were digested with EcoRI and hybridised to ^{32}P -labelled SI DNA after electrophoresis and transfer to nitrocellulose (11). It can be seen from fig. 7(a) that cross-hybridising sequences all share the 820bp repeat unit, and that those

Fig. 8. Possible methods of amplification of SI repeat units with two EcoRI sites by a replication mechanism. In (a) an individual repeat unit is amplified, and in (b) limited unequal crossing-over produces a domain of repeat units with identical restriction properties, and a section of this domain, possibly including several repeat units, is amplified.

species most closely related to the domestic sheep also contain the repeat units with two EcoRI restriction sites.

In a similar experiment, AluI-restricted DNAs from these species were hybridised to nick translated GI DNA. Fig. 7(b) shows that the aoudad and ibex contain repeat units with AluI sites separated by 340 and 480 nucleotides.

DISCUSSION

Although our restriction results basically confirm previous findings (4), that SI and GI are closely related sequences, the two sets of results vary in detail. For example, in the previous study the molecular weight determinations were different from ours, a result probably due to the use of different molecular weight markers and electrophoresis buffers in the two studies. Also, GI (and SI) were only cleaved to the higher oligomers of the 820bp repeat series with AluI. The reason for this discrepancy is not clear, but figs. 2 and 7(b) clearly demonstrate that GI repeat units contain two AluI sites.

The present results also show that even though the two satellite DNAs originated from the same sequence family, their physical properties differ considerably. GI repeat units containing two AluI sites are interspersed with those that contain just one, or none at all. Assuming that GI was once composed entirely of repeat units containing two AluI

sites, a minimum of 4.2% divergence would be required to produce the fragments in their present-day ratios. Taking the ΔT_m conversion factor of 1% mismatch per °C (18), the ΔT_m of GI in solution gives 5% divergence, which is in good agreement with the above figure. SI, however, contains two sequence types having different degrees of heterogeneity, which can be distinguished on the basis of EcoRI digestion. These results compare with other studies that have shown that related species contain satellite DNAs with similar long range repeat structures, but which have different physical properties (19) or different restriction maps (20).

In the present study, GI-type sequences were only found in goats and the aoudad, and so it would appear that these sequences were amplified since these species diverged from sheep. The heterogenous sequences of SI do not contain two AluI sites (fig. 7b). The homogeneous sequences of SI were found only in the genus Ovis, and so these sequences have been amplified since the goat/aoudad and sheep lineages diverged. There have therefore been at least two amplifications of this sequence family during goat/sheep evolution.

Very little is known of the phylogeny of this group, but Capra, Ammotragus and Ovis are close serologically (21), and Ammotragus has exactly the same chromosome constitution as O. vignei (22,23). These facts, taken with the above findings, suggest that all three arose almost simultaneously, with the divergence of the sheep lineage slightly preceding the goat/aoudad split, and with the amplification of GI occurring some time between these two events. Amplification of SI sequences could therefore only have occurred at or after the amplification of GI. This argues against the homogeneity of SI being produced entirely by unequal crossing-over (24), as this model predicts that the earlier the sequence comes into being, the longer the chance it has to achieve homogeneity. The formation of SI is best explained by a saltatory replication process (25), either by the amplification of a single repeat unit, or by the spread of the unit with two EcoRI sites by limited unequal crossing-over and the amplification of several consecutive, almost identical, repeat units (fig. 8). Unequal crossing-over and gene conversion (26) could, however, act at low levels to increase copy number further and redistribute sequences within the genome. More knowledge is required concerning their ability to take place within constitutively heterochromatic regions of the genome before their effect on satellite DNA structure and genomic distribution can be fully assessed.

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