

# Restriction Enzyme Analysis of Satellite DNA Components from the Bovine Genome

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A restriction enzyme analysis was performed on satellite DNA components, isolated, as described in the preceding paper, from the bovine genome by a combination of  $\text{Cs}_2\text{SO}_4/\text{BAMD}$  and  $\text{Cs}_2\text{SO}_4/\text{Ag}^+$  density gradient centrifugation. Such an analysis has led to the unambiguous identification of eight satellite DNA components and to new information on their repeat units; this indicates that identical repeat lengths are shared by them, a fact strongly suggesting a common origin.

Investigations from our laboratory [1–6], as well as from a number of other laboratories, have shown that buoyant density gradient centrifugation techniques are an extremely powerful tool for the analysis of mammalian genomes. In particular, the combination of  $\text{Cs}_2\text{SO}_4/\text{BAMD}$  and  $\text{Cs}_2\text{SO}_4/\text{Ag}^+$  density gradients has recently allowed us to prepare eight satellite and 11 minor DNA components from the bovine genome [5,6]. Several of these dG + dC-rich DNA components exhibit the same buoyant density in CsCl and the same behavior in  $\text{Cs}_2\text{SO}_4/\text{BAMD}$  or  $\text{Cs}_2\text{SO}_4/\text{Ag}^+$  density gradients. This encouraged us to use another method, namely the electrophoretic analysis of the fragments produced by restriction enzymes, to assess the identity of the DNA components. We report here the results obtained so far on the satellite components. Although our initial aim in this investigation was simply to identify in an unambiguous way different satellite components, our data have provided interesting new information on the repeat units of some satellites and show that identical repeat lengths are found in several of them. This finding supports the idea of a common origin for the satellite DNA components present in a given species.

*Abbreviations.* BAMD, 3,6-bis(acetato-mercurimethyl)dioxane. Satellite DNA components are indicated by their buoyant density in CsCl density gradient, as determined in the preceding paper [6]. Restriction enzymes are indicated according to Smith and Nathans [7].

*Enzymes.* Restriction endonucleases (EC 3.1.4. –).

## MATERIALS AND METHODS

### *DNA Samples*

The DNA samples investigated here were fractions obtained from the combined  $\text{Cs}_2\text{SO}_4/\text{BAMD}$ ,  $\text{Cs}_2\text{SO}_4/\text{Ag}^+$  density gradient centrifugations previously described [5,6]. The fractions are identified by their buoyant density in CsCl and their code number [6].

### *Restriction Enzyme Digestions*

Restriction endonucleases *EcoRI*, *HaeIII*, *HhaI* were used; *HaeIII* and *HhaI* were prepared according to a method developed in our laboratory [8]; *EcoRI* was prepared following the method of Yoshimori [9]. DNA samples (5–15  $\mu\text{g}$ ) were digested to completeness using conditions already described [10]. Gel electrophoresis, ethidium bromide staining, photography of the gel, and scanning of the negative pictures were done as in previous work [10]. Band stoichiometry was estimated by integration of the areas under the peaks of the scans.

The molecular weight of the fragments obtained by electrophoresis of digested DNA samples was determined using as a secondary standard the fragments of total calf thymus DNA digested with the enzyme used; this standard was calibrated against primary standards formed either by *EcoRI* fragments of  $\lambda$ -phage DNA or by *HindII* + III or *HaeIII* fragments of SV40 DNA [10].  $R_F$  values were normalized according to Prunell *et al.* [10].

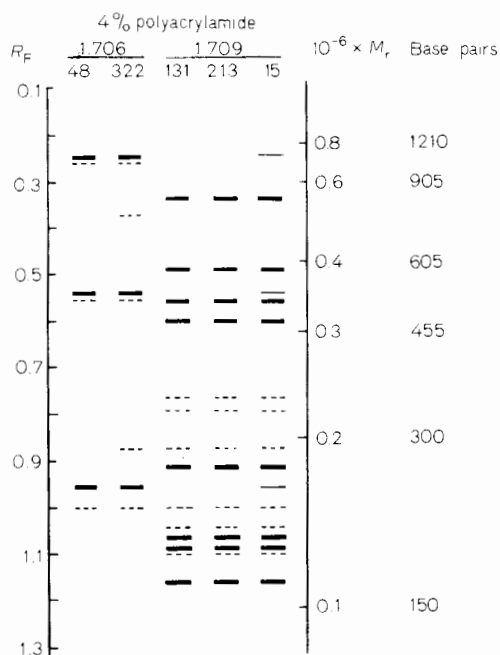


Fig. 1. Restriction endonuclease *HaeIII* digestion pattern of 1.706 and 1.709- $\text{g}/\text{cm}^3$  satellites. The buoyant density and code number of each fraction (according to [6]) is given on top of each pattern. The relative mobilities and molecular weights of the fragments and the polyacrylamide concentration are indicated. Different yields of the fragments are represented by a different band thickness; broken lines indicate faint bands

The presence of single-stranded oligonucleotides in restriction enzyme digests was investigated by chromatography on hydroxyapatite [10].

## RESULTS

### Restriction Patterns of $\text{Cs}_2\text{SO}_4/\text{Ag}^+$ Density Gradient Fractions Obtained from Calf Thymus DNA

A number of fractions obtained by centrifuging calf thymus DNA in  $\text{Cs}_2\text{SO}_4/\text{BAMD}$  and  $\text{Cs}_2\text{SO}_4/\text{Ag}^+$  were investigated in their restriction patterns (Table 1 of [6]). The results concerning the fractions containing satellite DNA components are given below.

The *HaeIII* restriction enzyme digestion pattern of DNA fractions containing the 1.706- $\text{g}/\text{cm}^3$  satellite revealed their identity and their degree of purity. Fig. 1 shows the patterns obtained for the two fractions containing most of this satellite. They were characterized by three major bands and three accompanying faint bands. Fraction 322 showed two additional faint bands compared to fraction 48; these correspond to two major bands of satellite 1.711a, which contaminates fraction 322 (Fig. 5 of [6]). The other fractions tested (Table 1 of [6]) exhibited the same digestion pattern, in some cases with additional bands belonging

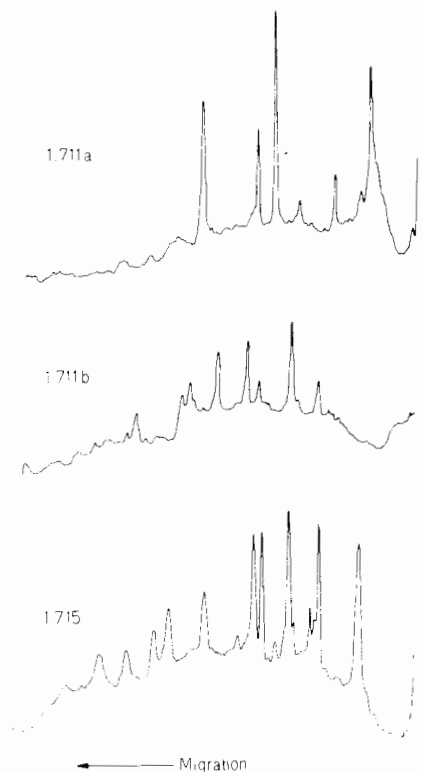


Fig. 2. Gel electrophoresis of *HaeIII* fragments from digested satellites 1.711a, 1.711b and 1.715. Satellites 1.711a and 1.711b were run on a 4% polyacrylamide gel; satellite 1.715 on a 6% polyacrylamide gel. Expanded scans [10] were used for the estimates of fragment lengths

to satellite components present in neighboring fractions. Interestingly, fraction 24, containing the minor component at 1.7064  $\text{g}/\text{cm}^3$ , showed no banding pattern, but just a smear when digested with endo R · *HaeIII*. The 1.706- $\text{g}/\text{cm}^3$  satellite was also degraded by endo R · *HhaI*, as indicated by the fact that it entered a 1.5% agarose gel only after degradation, yet it did not show well-defined bands, as already observed by Roizès [11].

The results for the three fractions (Table 1 of [6]) containing the 1.709- $\text{g}/\text{cm}^3$  satellite are also shown in Fig. 1. All of them show the same endo R · *HaeIII* digestion pattern, except for fraction 15 which appears to be contaminated by the 1.706- $\text{g}/\text{cm}^3$  satellite. In contrast with the 1.709- $\text{g}/\text{cm}^3$  satellite, the digestion of the major component at 1.709- $\text{g}/\text{cm}^3$  (fraction 122) revealed a continuous fragment distribution with no bands.

The restriction enzyme analysis confirmed that the two 1.711- $\text{g}/\text{cm}^3$  satellites are different, as indicated by their different behavior in  $\text{Cs}_2\text{SO}_4/\text{BAMD}$  and  $\text{Cs}_2\text{SO}_4/\text{Ag}^+$  density gradients. The 1.711a satellite showed an endo R · *HaeIII* pattern characterized by higher-molecular-weight bands compared to 1.711b satellite and there were no common bands between the two satellites (Fig. 2 and 4). Fractions 543 and 314

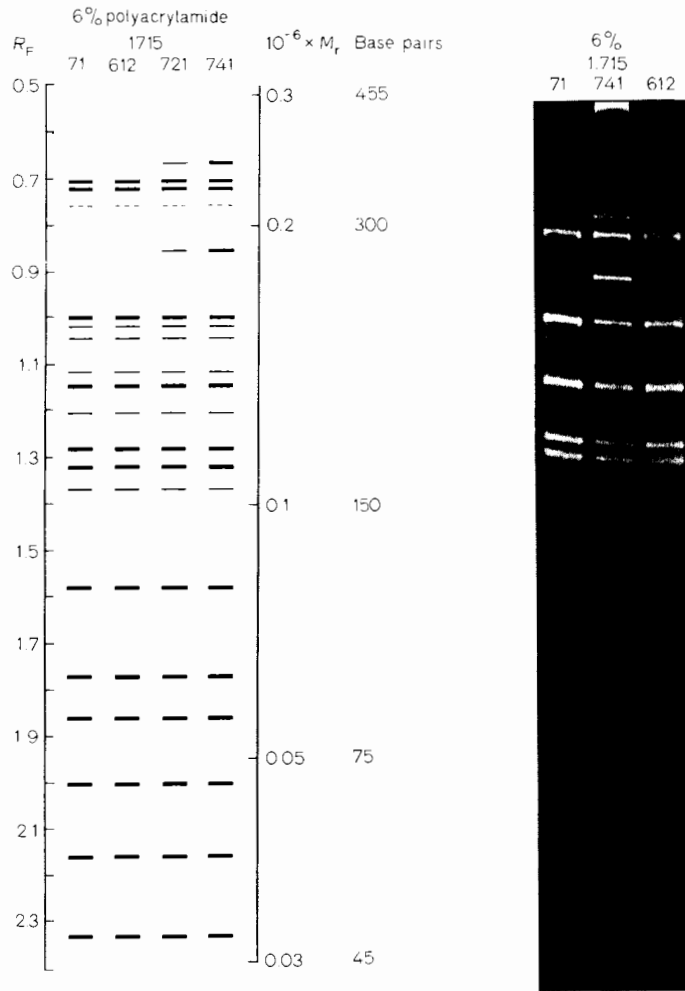


Fig. 3. Restriction endonuclease *HaeIII* digestion pattern of 1.715-g/cm<sup>3</sup> satellite. The picture of a 6% polyacrylamide gel containing fractions 71, 741 and 612 is also shown. The last band is barely visible in the picture, but it is evident in the scan of Fig. 2. Details as in Fig. 1

(Table 1 of [6]) containing the 1.711a satellite showed an identical endo R · *HaeIII* digestion pattern. The same observation holds for the fractions 30, 412 and 521 which contain the 1.711b satellite.

The fractions containing the 1.715-g/cm<sup>3</sup> satellite were identical, as judged by the restriction enzyme analysis shown in Fig. 3. The two additional bands in fractions 721 and 741 derive from contaminating 1.720a and 1.723 satellites (see Fig. 4).

The two 1.720-g/cm<sup>3</sup> satellites showed completely different endo R · *HaeIII* and endo R · *HhaI* digestion patterns. The results for the 1.720a satellite are shown in Fig. 4 and 5. In contrast with these results, the 1.720b satellite gave a smear in the high-molecular-weight region with endo R · *HaeIII*, and very-low-molecular-weight fragments with endo R · *HhaI* (not shown).

Fig. 4 and 5 show the endo R · *HaeIII* and endo R · *HhaI* restriction patterns obtained with total calf thymus DNA and the purified satellite DNA com-

ponents. Most of the bands shown by the satellite components are also visible in the unfractionated DNA. Differences in the multiplicity of bands and in the relative amount of satellite components account for the fact that some satellite bands do not appear in the total DNA digests. Another factor is the intensity of the background smear of total DNA, particularly in the high-molecular-weight region. Our results on total calf thymus DNA are in good agreement with those published by Mowbray *et al.* [12] and Philippsen *et al.* [13].

*Repetitive Units of Calf Thymus Satellite DNAs*

**1.706-g/cm<sup>3</sup> Satellite.** Digestion of this satellite with endo R · *HaeIII* (Fig. 1 and 4) yields three major bands in equimolar amounts; the corresponding fragments contain 250, 535 and 1100 base pairs, respectively. Three very minor bands corresponding to 225, 500 and 1060 base pairs accompanied the major bands.

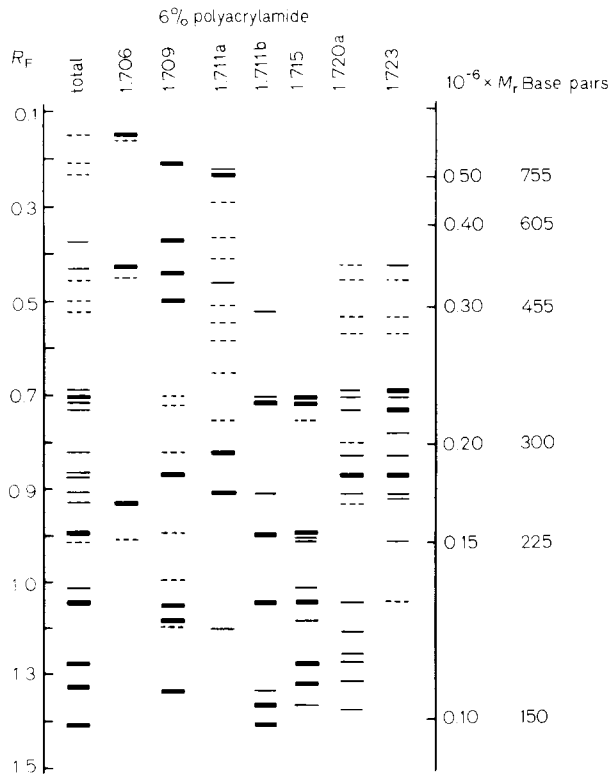


Fig. 4. Restriction endonuclease *HaeIII* digestion pattern of total and satellite DNAs from calf thymus. Details as in Fig. 1

These results are in agreement with those of Streeck and Zachau [14] obtained with endo R · *BsuI*, an enzyme having the same specificity as endo R · *HaeIII*. Also in agreement with Streeck and Zachau [14], the three major bands as well as the three minor accompanying bands do not correspond to integral multiples of the same repeat unit. This multiplicity could exist, however, if short segments 15-nucleotides long had been cut off at the ends of each repeat units. Such short segments have not been detected, however, as single-stranded fragments when running the digests of hydroxyapatite columns. For this reason, the repeat unit should be considered to be equal to the sum of the three major bands namely 1900 base pairs. It should be pointed out that our values are systematically lower than those of Streeck and Zachau [14]. This discrepancy is only in part due to the different molecular weight used for SV40 DNA ( $3.4 \times 10^6$  in our case, versus  $3.3 \times 10^6$  in the case of Streeck and Zachau [14], since there is no simple linear relationship between the two series of data.

*1.709-g/cm<sup>3</sup> Satellite.* When digested with endo R · *HhaI* (Fig. 5 A), this satellite gives a very simple electrophoretic pattern with a major band of very high multiplicity at 3200 base pairs and two other bands present in equimolar amounts at 1900 base pairs and 1300 base pairs. The sum of the molecular weights of

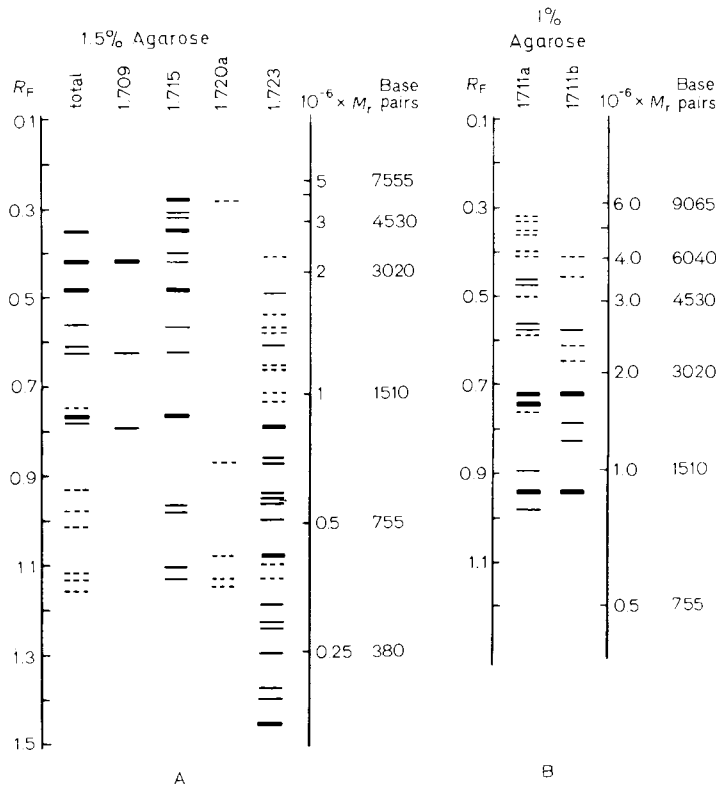


Fig. 5. Restriction endonuclease *HhaI* digestion patterns of total and satellite DNAs from calf thymus. Details as in Fig. 1

Table 1. Restriction endonuclease *Hae*III fragments of satellite 1.711a

Fragment	Size (base pairs)	Multiplicity
a	185	4
b	253	2
c	290	4
d	490	1
e	755	2
f	830	1

these bands is equal to that of the major band. On the other hand, endo R · *Hae*III (Fig. 1 and 4) gives a complex pattern composed of eight fragments present in equimolar amounts (845, 575, 520, 465, 275, 195, 185, 160 base pairs) and a series of fragments obtained in low yield. The sum of the molecular weights of the fragments present in equimolar amounts adds up to 3220 base pairs, the basic repeat length found with endo R · *Hha*I.

**1.711a Satellite.** The endo R · *Hha*I digestion pattern (Fig. 5B) of this satellite reveals a basic unit of 1360 base pairs; multiples of this band are found at 2720, 4000 and 5400 base pairs. Another band is found at 1230 base pairs. Bands apparently derived from the addition of this band to the basic repeat unit or its multiples are also found. The main endo R · *Hae*III fragments (Fig. 2 and 4) obtained from the 1.711a satellite are listed on Table 1. The sum of the products of the molecular weights of the bands by their relative multiplicity is 5240 base pairs which is about 4 times a basic repeat unit of 1310 base pairs. Very interestingly, four combinations of *Hae*III fragments correspond to the basic repeat of 1310 base pairs: 2b + e, 2c + e, d + f, 4a + 2c. This is a very strong indication that this arrangement of segments exists in the 1.711a satellite DNA.

**1.711b Satellite.** The digestion of this satellite with endo R · *Hha*I (Fig. 5B), gives two major bands of 1360 and 2720 base pairs, the same basic repeat unit of the 1.711a satellite. The other bands present reveal a different arrangement of the sequences in these two satellites. The endo R · *Hae*III digestion pattern (Fig. 2 and 4) is too complex to be analyzed. Nevertheless, the sum of the product of the molecular weight of the bands by their multiplicity is 5442 base pairs which is 4 times a basic repeat unit of 1320 base pairs (Table 2).

**1.715-g/cm<sup>3</sup> Satellite.** According to the endo R · *Hha*I digestion pattern (Fig. 5A), the basic repeat unit is 1360 base pairs in length. The monomer, and its oligomers (2650, 4010, 5500 base pairs) are the most prominent bands in the pattern. The four lowest-molecular-weight fragments (530, 575, 750 and 800 base pairs) can be arranged in pairs (530 + 800 and 575 + 750) giving the basic repeat length. The 530

Table 2. Restriction endonuclease *Hae*III fragments of satellite 1.711b

Size (base pairs)	Multiplicity
80	2
87	3
103	3
148	4
156	4
162	1
199	4
234	2
257	1
340	3
347	1
446	1

and 800-base-pair fragments as well as the 575 and 750-base-pair fragments are in equimolar amounts, but the two pairs of fragments are stoichiometric with each other, the first pair being more abundant. The intermediate pairs of bands found between oligomers can be obtained by addition of the 530 and 800-base-pair fragments to the immediately preceding oligomer. The two fragments in each pair are present in equimolar amounts, but the yield of different pairs is different. These results suggest the existence of two types of basic repeat unit in the 1.715-g/cm<sup>3</sup> satellite, differing in the position of the extra endo R · *Hha*I site. The fact that there are no bands representing other arrangements of the four low-molecular-weight fragments indicates that there is no short interspersion of the two types of repeats. An independent assessment of the basic repeat unit length has been obtained by digestion with endo R · *Eco*RI (not shown). In this case, a very abundant fragment was found at 1360 base pairs in addition to fragments obtained in low yield representing oligomers of this basic repeat unit. The endo R · *Hha*I and endo R · *Eco*RI results are in agreement with those reported by Roizès [11], but the fragment lengths are slightly different, since the reported basic repeat unit was 1460 base pairs and the four lowest-molecular-weight fragments obtained by digestion with endo R · *Hha*I were 560, 600, 800 and 900 base pairs long. These discrepancies are probably due to the different standards used as molecular weight markers. The fragment pattern obtained after endo R · *Hae*III digestion (Fig. 3 and 4) does not show any evidence for the 1360-base-pair repeat unit. Instead, the low-molecular-weight fragments strongly suggest the existence of a short basic repeat unit of 12 base pairs, since bands are found at 48, 60, 72, 84, 96 base pairs. Other bands representing higher multiples of 12 are found, but some are missing. When run on hydroxyapatite, and endo R · *Hae*III digest of this satellite showed the presence of about 6% single-stranded oligonucleotides. Digests obtained at 4 °C

and run on the gel at this temperature revealed an additional band at 36 base pairs; this, however, does not account for all the single-stranded oligonucleotides which appear to be derived also from lower oligomers.

*1.720a Satellite.* After endo R · *Hae*III digestion, this satellite (Fig. 4) shows a major fragment of 275 base pairs and a very large number of fragments which are obtained in lower yield; this makes the analysis difficult. Endo R · *Hha*I gives a smear and five faint bands.

*1.723-g/cm<sup>3</sup> Satellite.* A major fragment of 275 base pairs is also obtained when this satellite is degraded with endo R · *Hae*III (Fig. 4). The two other major bands of 330 and 355 base pairs in size are also common to the 1.720a satellite, but are obtained in different yields. With endo R · *Hha*I (Fig. 5A) a low-molecular-weight band of 260 base pairs is obtained in high yield as well as an oligomeric series of fragments of 645, 1290, 1950 and 2600 base pairs. This result, even if difficult to correlate with the endo R · *Hae*III digestion, strongly suggests the existence of a 645-base-pair repeat unit.

## DISCUSSION

The results obtained by the restriction enzyme analysis confirmed the assignment of components made in the preceding paper [6] on the basis of their behavior in Cs<sub>2</sub>SO<sub>4</sub>/Ag<sup>+</sup> density gradients and their buoyant density in CsCl.

A rather striking new finding obtained in this work concerns the existence of short repeats (multiples of a dodecanucleotide) in the 1.715-g/cm<sup>3</sup> satellite. This situation, apparently missed by previous investigations on this classical satellite, is interesting in that it stresses the need to look for the production of small fragments by restriction enzymes; these may be easily overlooked when using gel electrophoresis.

Another finding concerns the existence of common repeat lengths in several satellite components. As summarized in Table 3, satellites 1.711a, 1.711b and 1.715 g/cm<sup>3</sup> share a 1360-base-pair repeat, as shown in each case by two different restriction enzyme patterns. On the other hand, the 3200-base-pair repeat of the 1.709-g/cm<sup>3</sup> satellite is formed by the sum of two repeats of 1900 and 1300 base pairs, respectively; the first of these repeats is shared by the 1.706-g/cm<sup>3</sup> satellite, and the second is twice the size of the 645-base-pair repeat of the 1.723-g/cm<sup>3</sup> satellite. Finally, the 1.723-g/cm<sup>3</sup> satellite shows a 275-base-pair repeat which is also present in the 1.720a satellite. It should be noted that the identity of repeat lengths in calf satellite components is not perfect. This appears to be due to the following experimental limitations: (a) estimates of repeat lengths of different satellites were not usually obtained from the same gel; (b) differences

Table 3. *Basic repeat length of calf thymus satellites*

Satellite	Basic repeat length (base pairs) after digestion with restriction endonuclease		
	<i>Eco</i> R1	<i>Hae</i> III	<i>Hha</i> I
g/cm <sup>3</sup>			
1.706		1900	
1.709		3220	3200
1.711a		1320	1360
1.711b		1320	1360
1.715	1360	12	1360
1.720a		275	
1.723		275	645

in electrophoretic mobility of fragments of equal length from different satellites could also be due to differences in nucleotide sequences [9]. Real but slight differences in repeat lengths can, however, also contribute to the phenomenon under consideration.

Interestingly, the restriction patterns of satellites showing identical repeats are different. This seems to arise (a) from the presence in such satellites of other non-shared repeats and (b) from the partial or total lack of cleavage of multiples of the basic repeat or of 'addition segments' involving the basic repeat. Such situations may be due either to the addition of different DNA segments into a given satellite DNA or to base changes and/or modifications in the restriction sites. In fact, investigations to be published elsewhere indicate that all satellite DNA components are strongly methylated in the cytosines of dC – dG dinucleotides.

The presence of identical repeat lengths strongly suggests a common origin of satellite components. It is in fact very difficult to explain otherwise how restriction sites separated by identical distances can exist in independently formed satellites. This conclusion is in agreement with the similar one proposed by Gall and Atherton [15] on the basis of the finding that the repeating heptanucleotides of three *Drosophila virilis* satellites differ from each other by one base change. In the present case, however, a lot of divergence has occurred among different satellites. The preservation of identical repeat lengths in spite of such divergence and the consequent differences in base composition (the dG + dC level ranging from 47% to 66%) stresses very strongly the structural role of these DNA components.

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