A Family of Sequences with Regional Homology to Bovine 1.715 Satellite DNA

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The organization of sequences homologous to the bovine 1.715 satellite DNA was studied in the bovine genome. A novel family of sequences, containing different numbers of copies of the 1.4 kb satellite period alternating with 1.2 kb sequence not related to this satellite, was found. Possible mechanism of the generation of these sequences is proposed.

Introduction

Eight satellite DNAs, representing 23% of genomic DNA, have been detected in the bovine genome [1]. Their percent contribution in the genome is as follows: 1.706 satellite (satellite III) – 4.2%, 1.709 satellite – 4.6%, 1.711a satellite – 1.7%, 1.711b satellite – 7.1%, 1.715 satellite (satellite I) – 5.1%, 1.720 satellite – 0.14%, 1.720b satellite – 0.11% and 1.723 satellite (satellite II) – 0.51% [1]. Most of them were subjects of extensive restriction analyses [2–6] and investigated by other techniques [7, 8]. The sequences of the repeated units of four of them, namely: 1.706 [9], 1.711a [10], 1.715 [11] and of one of the 1.720 satellites [4] are already known. Three of them, 1.706, 1.711a and 1.720, in spite of revealing quite different buoyant densities and long range periodicities (2350 bp, 1413 bp and 46 bp respectively), appear to evolve from a common ancestor sequence. The repeated unit of the 1.711a satellite, in addition to the sequences shared in common with the 1.706 satellite, contains an unrelated 611 bp long sequence [10]. The scheme for the development of this family of sequences has been proposed [4, 9, 10].

In this paper we present our results on the organization of sequences homologous to the 1.715 satellite DNA. Data presented in this communication allow us to propose a scheme of molecular events that have led to the generation of the present day organization of sequences homologous to the 1.715 satellite in the bovine genome.

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Materials and Methods

Calf thymus DNA was isolated as described previously [12]. The high molecular weight DNA preparation enriched in 1.711b satellite sequences was prepared as follows: total calf thymus DNA preparation was precipitated by complexing it with homologous histone H1 [13]. The initial H1/DNA ratio (w:w) was adjusted to 0.4. The complex was sedimented, DNA was isolated from the supernatant [14] and was subject to the second precipitation step at H1/DNA ratio = 0.4. DNA was isolated from the precipitate and, after dialysis to 5 mM borate, pH = 9.2, was centrifuged in the Ag^+/Cs_2SO_4 gradient to separate the 1.706 and 1.711 a satellites [1]. Fractions from the main peak were pooled together. This DNA preparation (MW > 15 kb) had a buoyant density value of 1.710 g/cm³ and was devoid ot the 1.706, 1.723 and of ca. 80% of the 1.715 satellite sequences as judged on the basis of analytical CsCl density gradient centrifugation. It was also free of the 1.711 a satellite on the basis of the Ag^+/Cs_2SO_4 gradient profile. The above preparation was digested with the appropriate restriction endonucleases and used in several hybridization experiments.

Restriction endonucleases

*Hin*d III, *Hin*d II, *Msp* I, *Pst* I, *Sau* 96, *Taq* I, *Hha* I, *Sst* I and *Hae* III were purified according to the published procedures or their slight modifications [14–16]. *Alu* I, *Sau* 3 AI, and *Eco* RI were purchased from POCh (Poland). Digestion conditions for *Taq* I were as in [14], for *Sau* 96 as in [17] and for *Eco* RI as in [7]. For all other enzymes the incu-

bations were done in the medium of 6 mM Tis-HCl, pH = 7.6, 6 mM $MgCl_2$ and 6 mM mercaptoethanol.

Gel electrophoresis, DNA standards

Polacrylamide slab gels, 3–8% (acrylamide:bisacrylamide ratio of 19:1), were run in the Tris-borate-EDTA [19] or Tris-acetate [20] buffer systems. Agarose (horizontal or vertical) 1.2% or 1.6% slab gels were run in Tris-acetate buffer. After the electrophoresis the gels were stained and photographed as described [21]. The *Hha* I or *Hae* III digests of pBR 322 [22], or the *Alu* I digest of 1.706 bovine satellite DNA [3] served as length standards in the range of 20–500 bp, whereas the partial *Hind* III digest of *C. aethiops* DNA and the partial *Pst* I and *Eco* RI digests of the 1.715 bovine satellite DNA in the 172–12000 bp range [3, 24]. The precise lengths of all the DNA standards used were known from their sequences. The reproducibility of the length determinations was within the limits of ± 2% in various experiments.

Isolation of DNA fragments from preparative gels

10 mg of total bovine DNA was digested with an excess amount of *Eco* RI and electrophoresed on six 3% polyacrylamide gels (0.5 × 15 × 23 cm). 1000 bp, 1400 bp and 1600 bp long fragments were recovered from the gel by diffusion [3] and separated from the acrylamide monomer on DEAE-cellulose columns. 2.6 kb and 4.0 kb *Hind* III fragments were isolated from the DNA preparation enriched in these sequences (see above) after *Hind* III digestion and preparative electrophoresis on the horizontal 1.2% agarose gels (2.0 × 14.0 × 23.0 cm) as described [7].

Blotting, hybridization and DNA probes

DNA was transferred from agarose gels onto nitrocellulose filters (Millipore or Sartorius), according to the procedure of Southern [24]. Hybridization and subsequent washings of the filters were done in condition previously described [25]. The 1.711a unique sequence [10] was isolated from the 1.711a satellite DNA in form of the 584 bp long *Sau* 3 AI fragment. After labeling 5′ ends with – $^{32}P\gamma ATP$ (1000 Ci/mM, prepared according to [26] from $H_3{}^{32}PO_4$, Swierk, Poland) and polynucleotide kinase (Miles), the fragment was digested with *Sst* I, which makes a single cut [10], and the two resulting fragments were separated from minor contaminations by electrophoresis on a 4% polyacrylamide gel. The 506 bp long end fragment was used as the hybridization probe.

As a source of the 125 bp *Hae* III/*Hind* III fragment served, cloned in another experiment [27], 850 bp long *Eco* RI/*Hind* III fragment derived from the 1.6 kb *Eco* RI fragment. It was labeled as described above.

The details of the preparation of 1.715 satellite DNA *Hae* III fragments used as probes in several hybridization experiments will be given elsewhere [11].

Results

The occurrence of sequences homologous to 1.715 satellite repeated unit outside 1.715 satellite DNA

The repeated sequence of the 1.715 bovine satellite DNA is 1399 bp long [11]. The sequences recognized by *Sst* I and *Eco* RI are present once per period. However, when radioactively labeled satellite I DNA was used as a probe in hybridization experiments with Southern transferred *Eco* RI and *Sst* I digests of total bovine DNA, we also found, in addition to the 1.4 kb fragment and its multimers, two *Eco* RI fragments: 1.0 kb and 1.6 kb long, and a 2.6 kb *Sst* I fragment, all hybridizing with satellite I sequences (Fig. 1). *Hind* III is known to possess only irregular sites, if any, in the period of the satellite I DNA [8, 27]. After double digestion with *Eco* RI and *Hind* III, the hybridization to 1.6 kb *Eco* RI fragment was no longer observed, but a 0.85 kb fragment hybridizing to the probe appeared. Digestion of bovine DNA with several other restriction endonucleases resulted in generation of fragments with sequence homology to satellite I DNA, although their lengths cannot be simply related to satellite I periodicity (Fig. 1 and data not shown).

A similar hybridization experiment, but with *Hind* III digested total bovine DNA revealed a ladder of fragments with sequence homology to satellite I DNA. The smallest one was 2.6 kb long, the others differed from it by multiples of 1.4 kb, *i.e.* by the multiples of the period length of the 1.715 satellite DNA (Fig. 2). As it was mentioned above, there are no regular sites recognized by *Hind* III in the satellite I repeated sequence.

With the aim to establish the relationship between satellite I DNA and *Hind* III fragments, two

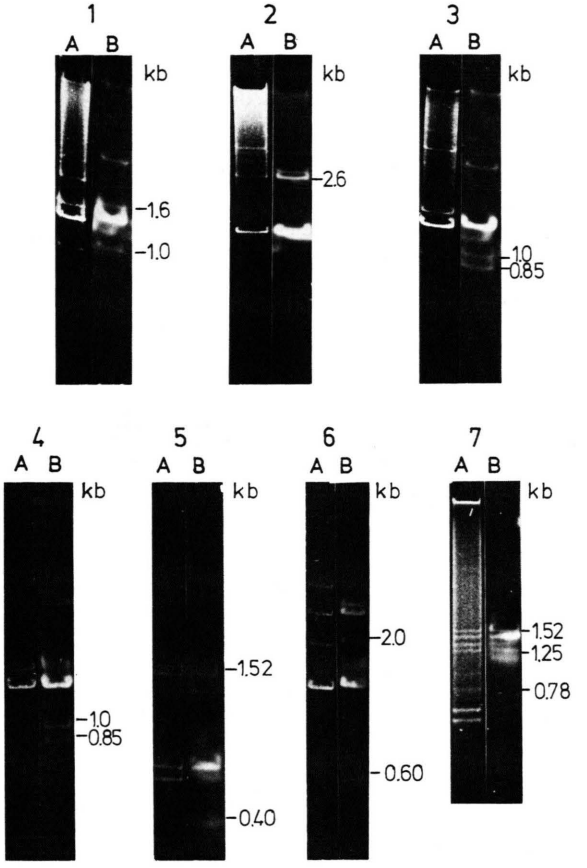

Fig. 1. The 1.5 µg samples of total bovine DNA were digested to completion with *Eco* RI [1], *Sst* I [2], *Eco* RI + *Hin*d III [3, 4], *Eco* RI + *Pst* I [5, 7], *Sst* I + *Hin*d III [6], separated on 1.4% agarose gel, transferred onto the nitrocellulose filter, and hybridized to S2 probe [1–3] or H4 probe [4–6], derived from 1.715 satellite DNA, and to 125 bp *Hae* III/*Hin*d III fragment (HH-probe) derived from 2.6 kb *Hin*d III fragment [7] (see Fig. 3 for the localization of the probes). A, The photograph of the fel stained with ethidium bromide. B, the autoradiogram of the filter.

of them – the 2.6 kb and the 4.0 kb – were isolated from the gel and mapped with *Eco* RI, *Pst* I and *Sst* I. Two *Eco* RI fragments: the 1.6 kb, carrying a single *Hin*d III site, and the 1. Okb, were also isolated and mapped with the same enzymes.

From these experiments (data not shown) it was obvious that the 2.6 kb *Hin*d III fragment consists of the 1.6 kb and the 1.0 kb *Eco* RI fragments. The 4.0 kb *Hin*d III fragment is closely related to the 2.6 kb one. In addition to the 1.6 kb and 1.0 kb *Eco* RI fragments it contains also one 1.4 kb *Eco* RI fragment indistinguishable in these tests from the 1.4 kb *Eco* RI repeated sequence of the 1.715 satellite DNA.

To define the range of sequence homology between the 2.6 kb *Hin*d III fragment and satellite I DNA, the 1.6 kb and 1.0 kb *Eco* RI fragments were mapped with several restriction enzymes whose cleavage postions on satellite I DNA are known from its sequence [11].

It appeared that there exist two distinct regions in the 2.6 kb fragment: one of about 1.2 kb showing almost perfect homology, and the second, about 1.4 kb long, with no homology to satellite I DNA as revealed by restriction analysis (Fig. 3). On the other hand, two *Hae* III fragments (H4 and H5) derived from 1.715 satellite DNA, localized outside the previously proved region of restriction maps similarity, hybridize to the 2.6 kb *Sst* I fragment, which is equivalent to the 2.6 kb *Hin*d III fragment (the last one carriers a single *Sst* I site). Furthermore, H4 hybridized equally to 1.0 kb *Eco* RI and 0.85 kb *Eco* RI/*Hin*d III fragments, 0.40 kb and 1.52 kb *Eco* RI/*Pst* I fragments, and to 2.0 kb and 0.60 kb *Sst* I/*Hin*d III fragments (Fig. 1). First fragment of the above mentioned pairs corresponds to

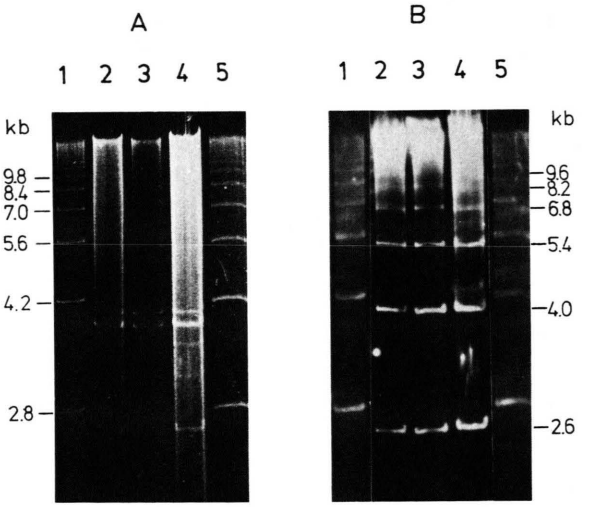

Fig. 2. The 401 bp long *Sau* 3 AI fragment derived from 1.715 satellite DNA (S2 – see Fig. 3) was hybridized to total bovine DNA, and DNA preparations enriched in 1.711b satellite sequences, digested with *Hin*d III. Partial *Eco* RI digest of 1.715 satellite DNA served as DNA standards (1 and 5); 1.5 µg and 3 µg of total bovine DNA (2 and 4 respectively); 0.5 µg of the DNA preparation enriched in 1.711 b sequences [3]. A, the photograph of the gel stained with ethidium bromide. B, the autoradiogram of the filter.

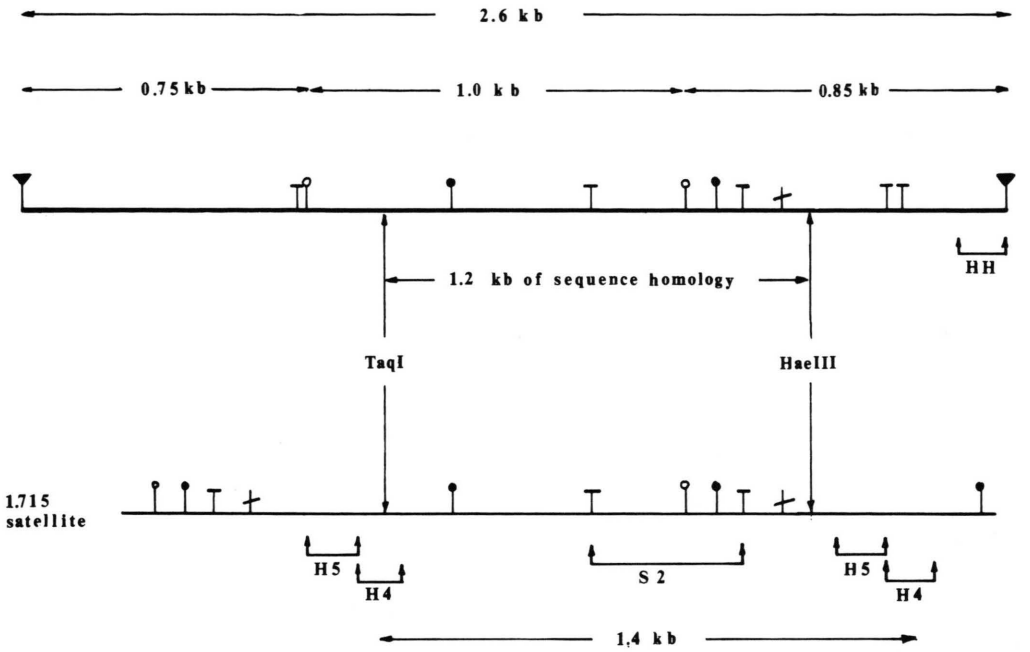

Fig. 3. Comparison of the restriction map of the 2.6 kb *Hind* III fragment with this of the 1.715 satellite 1.4 kb repeated sequence regions of the 2.6 kb *Hind* III fragment suspected to contain the ends of the sequence homologous to the 1.715 satellite repeated unit, *i.e.* 400 bp *Eco* RI/*Pst* I fragment and cloned 850 bp *Eco* RI/*Hind* III fragment carrying, respectively, the left and right end of the homology region were mapped with *Hae* III, *Taq* I, *Sau* 96 I and *Msp* I. Only the *Taq* I site and one *Hae* III site, limiting the region of restriction homology, are given for the clarity of the figure. ♀ , *Eco* RI; ♦ , *Pst* I; ⊤ , *Sau* 3 AI; ⊣, *Sst* I; ▼, *Hind* III. Under the schematic representation of the 2.6 kb *Hind* III fragment and 1.715 satellite DNA the localization of the hybridization probes, used in this study, are given. They are described as follows: **HH**, 125 bp *Hae* III/*Hind* III fragment derived from 2.6 kb *Hind* III fragment, **H4** and **H5**, *Hae* III fragments derived from 1.715 satellite DNA, **S2**, 401 bp *Sau* 3 AI fragment derived from 1.715 satellite DNA.

the right side, and the second to the left side of the 1.2 kb region of restriction homology in the 2.6 kb *Hind* III fragment (Fig. 3). In case of the H 5 fragment we observed hybridization to fragments representing the right side of the region of homology. We conclude that the full lenght of bovine 1.715 satellite repeated sequence is represented in the 2.6 kb *Hind* III fragment.

What is the origin of sequences contiguous to 1.715 sequences in the 2.6 kb unit? From our hybridization date we know that the 0.75 kb *Eco* RI/*Hind* III fragment is not related to any part of 1.4 kb satellite I repeated sequence. Streeck found that the 611 bp "unique sequence" present in the 1.711a bovine satellite DNA hybridizes with this fragment [10]. This result was confirmed in our laboratory. The right end of the 0.85 kb *Eco* RI/*Hind* III fragment, corresponding to the right side of the 2.6 kb *Hind* III fragment hybridizes with neither 1.711a, nor 1.715 satellite DNA probes. To check other possible locations of this sequence in the bovine genome we performed hybridization experiments with 125 bp *Hae* III/*Hind* III fragment as a probe (see Fig. 3 for the localization of this fragment).

It appeared that this sequence is localized predominantly on the DNA fragments originating from the 2.6 kb *Hind* III unit. However, in *Eco* RI/*Pst* I digest there are also two additional fragments, 0.78 kb and 1.25 kb long, hybridizing to 125 bp *Hae* III/*Hind* III probe (Fig. 1). The origin of these fragments is unknown.

The long range organization of the family of Hind III fragments

From the restriction and hybridization analyses of the preparatively isolated 2.6 kb and 4.0 kb *Hind* III fragments it is known that: 1) the 2.6 kb fragment contains a 1.4 kb long sequence homologous to the full period of satellite I DNA; 2) the 4.0 kb fragment

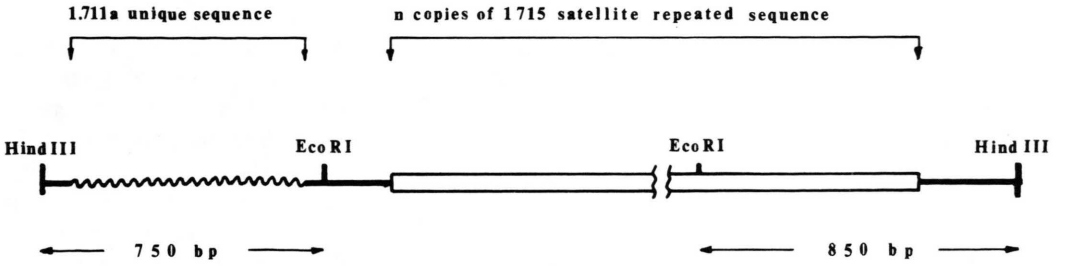

Fig. 4. The schematic presentation of the family of *Hind* III fragments containing "*n*" copies of the 1.715 satellite repeated sequence, flanked with not related sequence regionally homologous to 1.711a satellite unique sequence.

has to be considered as the sum of the 2.6 kb one and the additional copy of the satellite I period. Hybridization of the 1.715 satellite DNA probe to Southern transferred *Hind* III digest of total calf DNA reveal the existance of the family of fragments hybridizing to the probe and differing in their lengths by multiples of 1.4 kb (Fig. 2). We assumed that the observed family can be formally described as it is shown in Fig. 4.

To test this hypothesis we performed hybridization experiment with the Southern transferred *Hind* III digest of total bovine DNA, but using 1.711a satellite unique sequence and the 125 bp *Hae* III/*Hind* III fragment, derived from the right side of 2.6 kb *Hind* III fragment, as probes. In both cases the same ladder of *Hind* III fragments was detected as with the 1.715 satellite DNA probe.

The 2.6 kb and 4.0 kb *Hind* III fragments occur in approximately equimolar amounts. To check, whether these two kinds of repeated units are localized in separate tandems the 1.715 satellite DNA probe was hybridized to partial *Hind* III

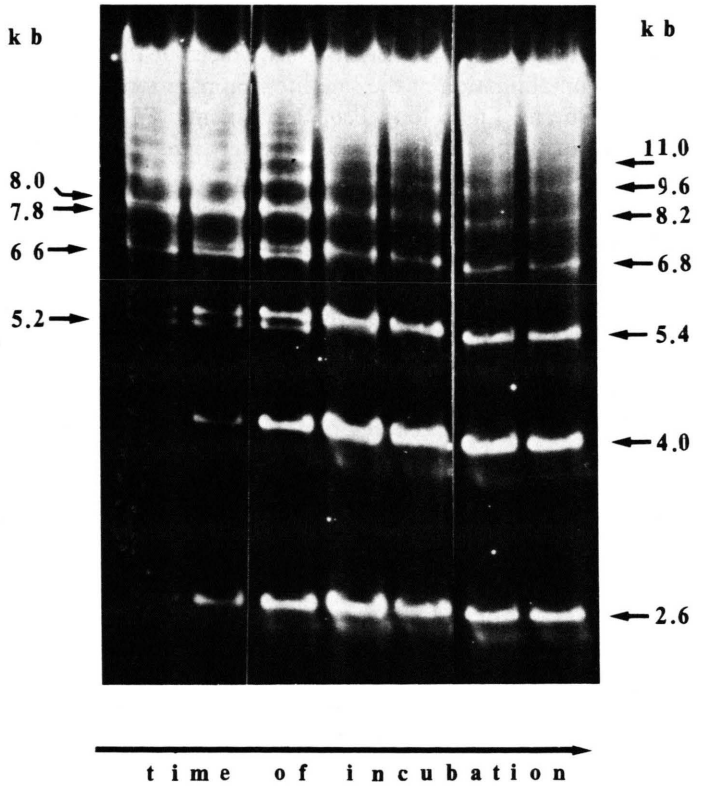

Fig. 5. 10 µg of DNA preparation enriched in 1.711b satellite sequences was incubated with 3 µg of *Hind* III activity for time intervals ranging from 0.5 to 12 h. The digests were separate on 1.2% agarose gel, transferred onto nitrocellulose filter and hybridized to S2 probe derived from 1.715 satellite DNA.

digest of high molecular weight DNA preparation enriched in 1.711b satellite sequences (Fig. 5). Transient 5.2 kb and 7.8 kb fragments correspond to the dimer and trimer of the 2.6 kb fragment. The 8.0 kb long one, absent in the limit digest, is a dimer of the 4.0 kb fragment. The 6.6 kb fragment is the sum of the 2.6 kb and 4.0 kb fragments. From this experiment it is apparent, that all three different relative arrangements of the 2.6 kb and 4.0 kb fragments do exist.

Discussion

In our studies on the organization of sequences homologous to 1.715 satellite DNA in the bovine genome we have found a novel family of sequences, containing n copies of satellite I period alternating with a sequence not related to this satellite. (This family of sequences will be further referred to as "family".) The molar representation of these sequences is the highest for $n = 1$ and $n = 2$, and decreases for higher values of n, however, we have no data for $n > 9$ because of the limited resolution power of agarose gel electrophoresis. Members of the family containing several copies of the 1.715 satellite period should behave in density gradient centrifugations like 1.715 satellite sequences, leading to a contamination of the satellite I preparations with 1.0 kb and 1.6 kb long *Eco* RI fragments. This probably explains the results of Gautier *et al.* [8] who came to the conclusion that these fragments are integral constituents of the 1.715 satellite. Similarly, Streeck isolated the 1.711 b satellite by repeated BAMD/Cs_2SO_4 gradient centrifugations and found, on the basis of *Hin*d III digestion, that it consists of the 2.6 kb fragment containing a single copy of 1.715 period [10], and the 4.0 kb fragment (personal communication). Possibly, sequences of the same kind but with higher content of the 1.715 period copies were lost in subsequent purification steps, thus we do not known whether his term 1.711 b satellite is equivalent to our definition of family.

Concerning possible mechanisms, which gave rise to the presently existing members of the family (with $n > 2$) we think, that their generation was due to multiple crossing overs among *Hin*d III 4.0 kb sequences containing two copies of satellite I 1.4 kb repeated sequence. The rationale is as follows. If the crossing over events, starting from the 4.0 kb sequence, have to increase or decrease the number of copies of the satellite I 1.4 kb repeated unit between flanking 1.4 kb nonhomologous sequences, then the crossing over points, *i.e.* the regions of homology [28], have to be localizes within two 1.4 kb repeats 1.4 kb apart. In such case the crossing over occurs in register with reference to 1.4 kb internal periodicity, and out of register, *i.e.* as unequal crossing over with regard to higher order (4.0 kb) periodicity. This leads to one 5.4 kb sequence with $n = 3$ and simultaneously to one 2.6 kb sequence with $n = 1$. The internal organization of the 4.0 kb repeated unit should promote the crossing over in register of 1.4 kb 1.715 satellite period length.

The general pattern of the mutual interspersion of the members of the family supports the involvement of crossing overs in the course of evolution of the family.

The sequences studied in this work contain about 1.2 kb long region not related to the surrounding sequences homologous to the 1.715 satellite repeated unit. Some part of this region hybridizes with the 611 bp long so-called unique sequence representing a part of the 1.711a satellite 1413 bp repeated sequence [10]. Streeck suggested that 2.6 kb repeated unit of the 1.711b satellite has been generated by the transposition of the 1.2 kb sequence composed of the 1.711a unique segment linked with a sequence of unknown origin, into the period of the 1.715 satellite DNA [10]. In our opinion it is possible that the fenomenon leading to the insertion of the 1.2 kb nonhomologous sequence into 1.715 satellite sequences was based not on transposition, but on other recombination mechanisms such as, for example, two overlapping inversions. Analysis of the nucleotide sequences at the homology-nonhomology junctions in the 2.6 kb *Hin*d III fragment should allow to verify hypotheses concerning mechanisms responsible for the generation of the complex repeated unit of the 1.711b bovine satellite.

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