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Human satellite I sequences include a male specific 2.47 kb tandemly repeated unit containing one Alu family member per repeat

M.Frommer, J.Prosser and P.C.Vincent

Kanematsu Memorial Institute, Sydney Hospital, Macquarie Street, Sydney, NSW 2000, Australia

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ABSTRACT

A portion of human satellite I DNA is digested by HinfI into three fragments of 775, 875 and 820bp in length which form a tandemly repeated unit 2.47kb in length, specific to male DNA. One Alu family member per repeat is found within the relatively G+C rich 775bp fragment. The 875 and 820bp fragments are highly A+T rich and consist of long stretches of poly dAdT and related sequences.

INTRODUCTION

Satellite I is the most A+T rich of the three major cryptic satellites of human DNA (1-3). It constitutes 1-2% of the human genome and behaves as a simple sequence by DNA fingerprinting (4). We previously reported that satellite I contains sequences with recognition sites for a number of restriction endonucleases, including HinfI, which cleaves a portion of it into three fragments of approximate lengths 770bp, 850bp and 950bp (3). We report here that the three HinfI fragments (with corrected sizes of 775bp, 820bp and 875bp) form a tandemly repeated sequence of unit length 2.47kb which is specific to male DNA. The sequence consists of a "noisy" region (with many restriction sites) within the G+C rich 775bp fragment and a "silent" region (with very few restriction sites) within the other two fragments of 820bp and 875bp. The silent region is highly A+T rich, containing long stretches of poly dAdT and related sequences. A nearly complete and well-conserved primate Alu family sequence is contained within the noisy region, and therefore forms part of the 2.47kb tandemly repeated unit.

METHODS

DNA preparation

Satellite DNA was prepared as previously described from placental DNA of male births (1, 2). Male and female total DNA was also of placental origin.

Restriction enzyme digests

Restriction enzymes were purchased from commercial suppliers and used according to their directions. In addition to the enzymes listed in (3) the following enzymes were tested on purified satellite I DNA: AhaIII, AluI, HhaI, HincII, HphI, KpnI, MboII, MspI, PstI, PvaI, RsaI and SmaI. Those which cut are described in the text or shown in Figure 1. Restriction fragments with single stranded 5' ends were labelled with α-^{32}P nucleoside triphosphates using reverse transcriptase. Fragments were separated by electrophoresis in vertical gels of 4% or 12% polyacrylamide (0.5 mm thick) or 1.5% agarose (0.8 mm or 3.0 mm thick). α-^{32}P labelled fragment were visualised by autoradiography. Other digestion products were stained with ethidium bromide (EtBr) and visualised under ultraviolet light. Various restriction endonuclease digests of the plasmid pBR 322 and λC1857 S7 were used as standards.

DNA sequencing

The consensus sequence of uncloned DNA fragments eluted from polyacrylamide gels was determined by the method of Maxam and Gilbert (5). The sequence of fragments cloned in M13 mp 8 and 9 (6) was determined by the method of Sanger et al. (7). Sequence libraries were searched by computer for regions homologous to satellite DNA (8).

Southern transfers and hybridisation

DNA fragments were transferred from 1.5% agarose gels to nitrocellulose filters by the method of Southern (9). Prehybridisation (2 hours) and hybridisation (20 hours) were carried out in 3 x SSC, 1 x Denhardts, 0.1% SDS at 65° C, followed by washing in 0.1 x SSC, 0.1% SDS at 50° C. Probes were labelled with α-^{32}P nucleoside triphosphates by nick translation (10) or by primed synthesis on single stranded templates (6).

RESULTS

Satellite I HinfI fragments form a 2.47kb repeat consisting of a primate Alu sequence, another G+C rich sequence and a region of highly A+T rich DNA

Fragments resulting from complete digestion of satellite I with HinfI are illustrated in Figure 5a. The gel shows three prominent bands of size 775bp, 820bp and 875bp. These correspond respectively to the fragments of 770bp, 850bp and 950bp previously reported by us based on estimates from acrylamide gels (3). The lengths of the 820/850bp and 875/950bp fragments have not been determined by sequencing, but the estimates from agarose

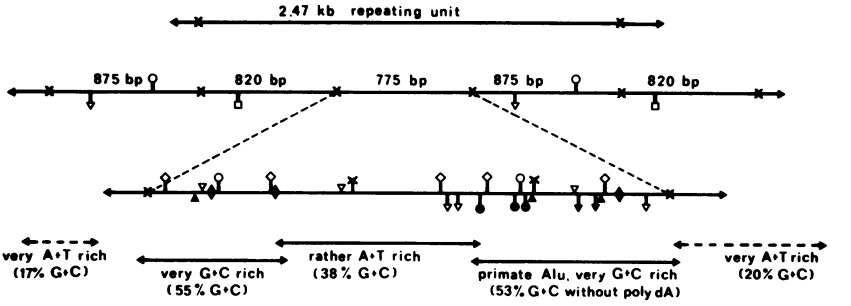

Figure 1. The 247kb repeating unit and a restriction map of the region. The 820bp, 775bp and 875bp fragments are defined by HinfI sites. Within the 775bp fragment (enlarged) and the 820bp and 875bp fragments, sites for the following restriction enzymes are shown: AluI ↓, AvaII ♦, DdeI ◇, EcoRII ▲, Fnu4HI ▽, HaeIII ⚹, HinfI x, HphI ♀, MboI ●, MspI ⏃, RsaI ⏚.

gels, 775bp, 820bp and 875bp, are probably more accurate and will be used in this paper. We showed previously that, of a range of restriction endonucleases, no enzyme cleaved the 820bp or 875bp fragments, but that a number of them cleaved the 775bp fragment. We have subsequently located a single site for each of the enzymes RsaI and HphI in the 875bp fragment which has allowed us to establish that the three fragments form a tandemly repeated unit of 2.47kb, which maps as shown in Figure 1. The 820bp fragment of the 2.47kb repeat is only partially digested by MspI.

To investigate the sequence relationship between the "noisy" and the adjacent "silent" region of the 2.47kb repeated unit, we have sequenced the 775bp HinfI fragment and 130bp of the following 875bp fragment. We have also sequenced a 450bp portion which includes the last 130bp of the 875bp fragment and 320bp of the adjoining 820bp fragment. The sequencing strategy for the 775bp fragment is outlined in Figure 2; the sequences themselves are presented in Figure 3.

The 2.47kb repeat unit contains an Alu family member located at the 3' end of the 775bp fragment from position 504 to position 803 in Figure 3. The satellite Alu sequence is a typical dimer, as found for interspersed Alu sequences in all primate DNAs (11)(Figure 4). The poly dA tail of the second half of the dimer extends into the 875bp fragment. The entire Alu sequence is flanked by A+T rich imperfect direct repeats (ATATG[G]AT$^{C}_{A}$T, shown overlined in Figure 3) and appears to have been inserted into A+T rich DNA. The entire sequence was obtained by consensus sequencing of satellite

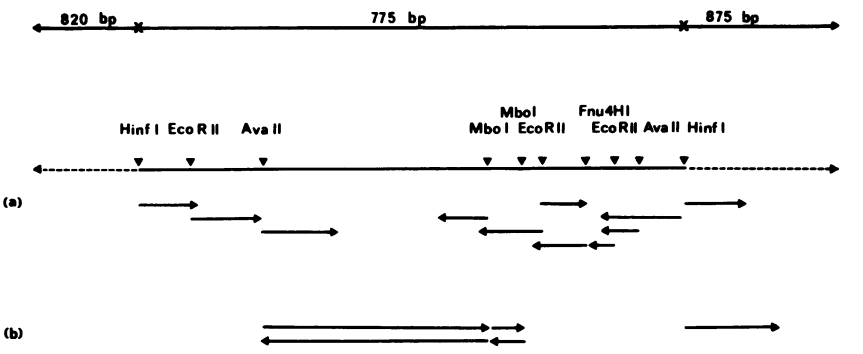

Figure 2. Sequencing strategy for the 775bp and part of the 875bp fragments: (a) consensus sequenced regions, and (b) M13 cloned and sequenced regions.

I restriction fragments and is, therefore, part of the 2.47kb satellite repeat.

The satellite Alu sequence is approximately 80% homologous to the consensus human Alu sequence, obtained as the average sequence of ten human Alu clones (12, 13). This is a level of homology not strikingly different from that of individual clones of interspersed Alu sequences of man, which are, on average, 87% homologous to the consensus sequence (12). The satellite Alu sequence shows no more homology than the human consensus Alu sequence to Alu sequences from African green monkey (14). The only major difference between the satellite Alu sequence and the consensus human interspersed Alu sequence is that near the 5' end of the second unit of the dimer, the satellite Alu contains an extra 15bp of sequence, which appears to have been formed by the loss of a 10bp region of hairpin symmetry and the duplication of 14 or 15bp of the resulting sequence. In addition, the satellite sequence is missing 12 base pairs at the 5' end of the first unit of the dimer, and there are four deletions or insertions/duplications of 1-3bp (Figure 4). All primate Alu family members, including the satellite Alu sequence, contain in the second unit of the dimer a 31bp insert relative to the first unit of the dimer (13). This insert is lacking in the mouse and CHO Alu monomers (14). The satellite Alu sequence gives an indication of the origin of the primate 31bp insert: 8bp direct repeats are present at the boundaries of the sequence which are missing from the mouse Alu monomer (Figure 4).

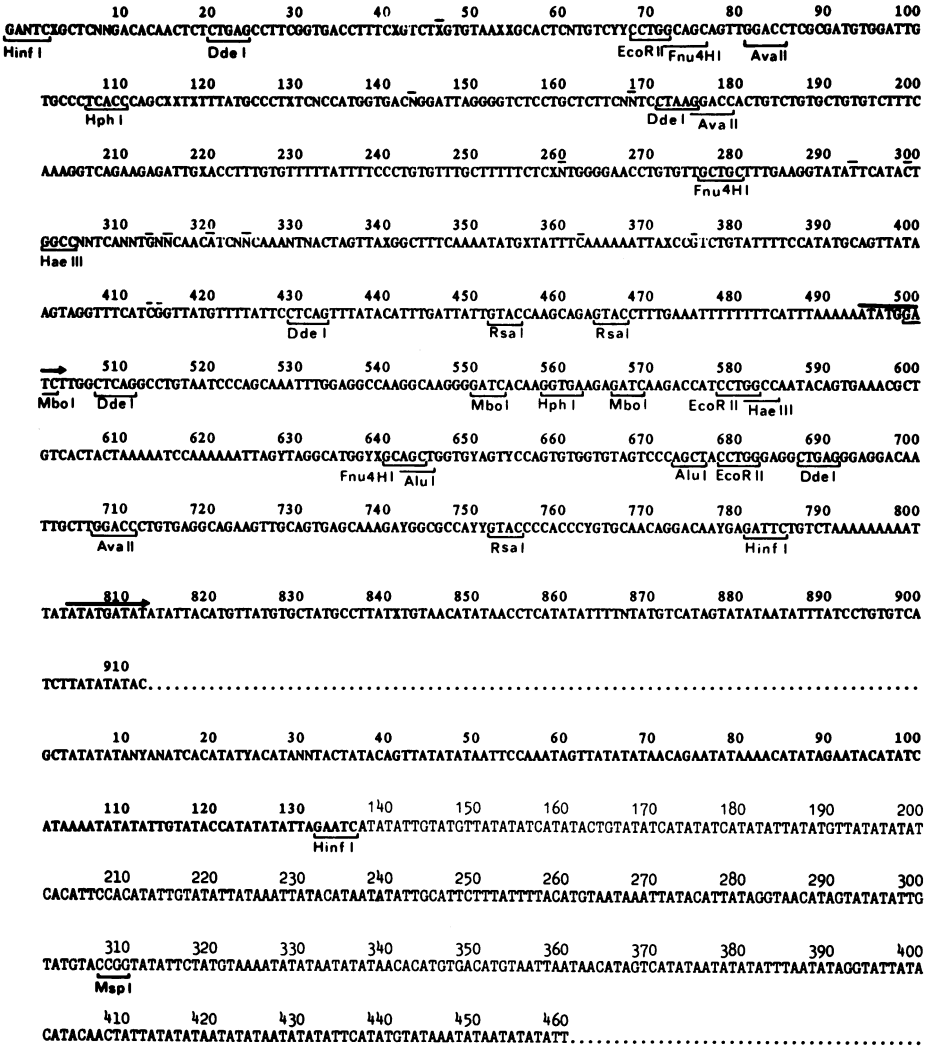

Figure 3. Sequence of the entire 775bp fragment beginning from the HinfI site and leading into the first 130bp of the 875bp fragment. The last 130bp of the 875bp fragment lead into the first 350bp of the 820bp fragment. The direct repeats at either end of the inserted Alu sequence are overlined with arrows. Where the sequencing gel was unclear X = purine, Y = pyrimidine, N = not known, N = nucleotide may or may not be present. The locations of mapped restriction sites are noted.

The remainder of the 775bp fragment does not appear to be related to the Alu family. Upstream from the satellite Alu sequence there is a region about 300bp in length which is A+T rich (38% G+C) relative to the very G+C

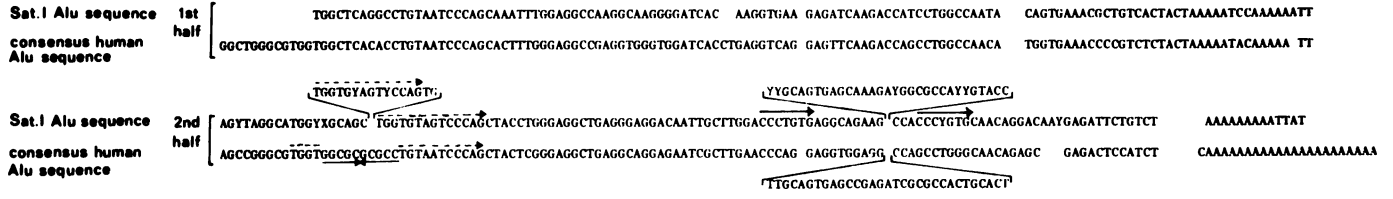

Figure 4. Comparison of satellite Alu sequence and consensus human Alu sequence (12). The two monomers of the Alu sequence are aligned to maximise homology between them. ⟶ ⟶ mark the direct repeats flanking the insert of 31bp which is found in primate Alu sequence but absent from the mouse Alu monomer (14). - - - - - - ▸ marks the duplication absent from human consensus Alu sequence. ⟶ ⟵ marks a sequence with hairpin symmetry absent from satellite I Alu sequence.

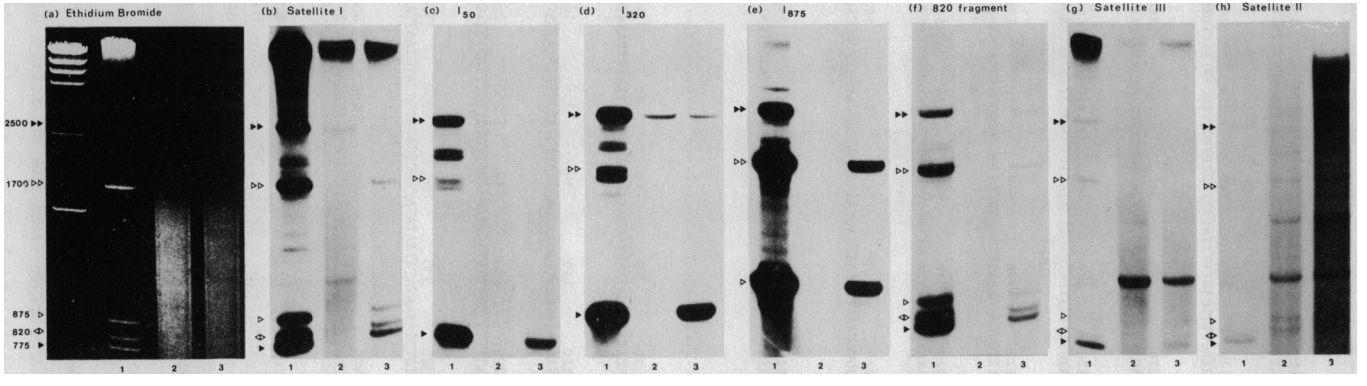

Figure 5. (a) Ethidium bromide stained digest of marker DNA (pBR 322 cut with Sau961 and λ cut with HindIII) and HinfI digests of (1) purified male satellite I, (2) whole female and (3) whole male DNA. (b) to (h) Southern hybridisations of α-^{32}P labelled probes to (1), (2) and (3). The probes used (indicated above each blot) are (b) nick translated whole satellite I, (c) I_{50} probe, (d) I_{320} probe, (e) I_{875} probe, (f) nick translated 820bp fragment, (g) nick translated whole satellite III and (h) nick translated whole satellite II. Photographic prints of the Southern hybridisations are contact prints of individual autoradiographs. Because the same size bands are not in identical positions the relevant bands for each gel are indicated: ▶▶ 2.5kb, ▷▷ 1.7kb, ▷ 875bp, ⌔ 820bp and ▶ 775bp.

rich regions on either side of it and which contains short regions of poly dT and poly dNdT. Upstream of this, the first 200bp of the 775bp fragment consists of a highly G+C rich region (55% G+C) similar to the Alu sequence in restriction site profile (Figure 1) and base composition (the Alu sequence, not including the poly dA tail, is 53% G+C) but without any clear homology to it. The sequences at the 5' and 3' ends of the 875bp fragment and the 5' end of the 820bp fragment are very similar to each other (Figure 3); all are highly A+T rich (15-20% G+C) and contain a large proportion of poly dAdT. Sequences similar to the direct repeats bounding the satellite Alu sequence are frequent. The sequence at the 3' end of the 820bp fragment is more G+C rich than the other sequenced regions of the fragment.

To establish sequence variation between members of the 2.47kb repeat, the sequences of fragments cloned in M13 were compared with the consensus sequence. Three independent clones containing the entire 875bp fragment were compared with the consensus sequence at both ends of this fragment. Five independent clones of a 50bp region within the Alu sequence were compared with each other and with the consensus sequence. Sequences of individual clones were found to differ from each other and from the consensus by only 1-2%. This indicates either a high degree of conservation of the 2.47kb unit, or, more likely, the recent tandem duplication of this sequence.

The fact that MspI only partially digests the 2.47kb repeat seems unlikely to be due to a highly diverged segment and is more probably due to modification at the MspI site. This is in agreement with the results of Cooke et al. who have suggested that the MspI site may be methylated (15). It has recently been shown that MspI is inhibited by methylation of either the internal or external cytosine in the sequence CCGG in certain subsets of this sequence (16, 17).

The 2.47kb satellite I repeat with internal HinfI sites is specific to male DNA

A digest of satellite I with HinfI yields fragments other than the three which constitute the 2.47kb repeat (Figure 5a). There are fairly prominent fragments of approximate length 1.7kb and 2.5kb, plus a large amount of material at high molecular weight (15-25kb). Fragment sizes resulting from digestion of purified satellite I DNA with AvaII, EcoRII, Fnu4HI and MboI and from double digestion with HinfI and each of RsaI, HphI and AluI showed that the 2.5kb and the 1.7kb HinfI bands consist of sequences which are homologous to the 2.47kb repeating unit but are lacking

internal HinfI sites. To investigate the components of the HinfI digest of satellite I, probes were prepared from various satellite sequences and hybridised to Southern blots of HinfI digested male total DNA, female total DNA and satellite I purified from male DNA. Probes used were:

(1) nick-translated total satellite I;
(2) the 50bp MboI fragment contained within the Alu sequence of satellite I, cloned in M13 (I_{50});
(3) the 320bp AvaII/MboI fragment contained within the 775bp fragment and adjoining the Alu sequence, cloned in M13 (I_{320});
(4) the A+T rich 875bp HinfI fragment, cloned in M13 (I_{875});
(5) nick-translated 820bp HinfI fragment eluted from 4% acrylamide gels;
(6) the 100bp HinfI ladder fragment of satellite III, rich in the sequence ATTCC (III_{100});
(7) nick-translated total satellite III;
(8) nick-translated total satellite II.

The results are presented in Figure 5. Hybridisation of satellite I to HinfI digested male and female DNA (Figure 5b) shows that the three HinfI fragments which form the 2.47kb repeat are not present in female DNA. The 1.7kb band is also male specific, but the 2.5 band is present in both male and female DNA. There is a large amount of DNA with very few HinfI sites (15-25kb spacing) homologous to satellite I in both male and female DNA.

The Alu fragment (I_{50}) and the non-Alu fragment (I_{320}) from the 775bp region show essentially the same pattern of hybridisation (Figure 5c and d). In male satellite I both probes hybridise strongly to the 775bp fragment and much less strongly to two bands at 1.6 and 1.65kb and to two bands at 1.9 and 1.95kb. The presence of bands at about 1.6 and 1.65kb is consistent with small fractions of size 775 + 820bp and 775 + 875bp, lacking an internal HinfI site. The pattern of hybridisation of each of the probes, I_{50} and I_{320}, to these four bands in total male DNA is identical to that in purified male satellite I, although considerably less intense. It is interesting to note that with the Alu probe (I_{50}) hybridisation to bands at 1.9 and 1.95kb is stronger than hybridisation to bands at 1.6 and 1.65kb, whereas with the non-Alu probe (I_{320}) from this region, the pattern is reversed. It is possible that there has been a duplication of part of the Alu sequence in the 1.9 and 1.95kb fragments. Both probes bind to the 2.5kb band of male and female DNA, indicating that a 2.47kb repeat, like that on the Y

chromosome but lacking internal HinfI sites, is present on the autosomes and possibly the X chromosome. Overall, both probes hybridise less strongly in the 1.7kb region than in the 2.5kb area, indicating that the 1.6, 1.65, 1.9 and 1.95kb sequences are less prevalent in both purified satellite I and total male DNA than sequences in the 2.5kb band.

In male DNA the I_{875} probe hybridises strongly to an 875bp band (and weakly to the 775 and 820bp bands, which contain A+T rich regions). There is strong hybridisation in male DNA to a 1.7kb band (which corresponds to the 1.7kb band visualised in EtBr stained gels of HinfI cut satellite I, Figure 5a) and very faint hybridisation to a band at about 1.6kb. Hybridisation at 2.5kb is weaker than that found at 1.7kb and is found in both male and female DNA.

In male DNA, the nick-translated 820bp fragment hybridises strongly to bands at 775bp, 820bp and 875bp. The 3' end of the Alu sequence, which almost concurs with the HinfI site located between the 775bp and 875bp fragments may constitute a boundary between two different types of DNA, between, for instance, the G+C rich DNA of the 775bp fragment and the A+T rich DNA of the 875bp fragment. The 820bp fragment which lies between the 775bp and 875bp fragments may contain sequences at the 3' end similar to the 775bp fragment and sequences at the 5' end similar to the 875bp fragment, such that it would hybridise to all three bands. From eluting and re-running the 875bp band, we have shown that the 820bp fragment is a minor contaminant of this band (unpublished results) and could account for hybridisation here. Since the 820bp fragment is not a cloned probe, it is possible that contamination of each of the 875bp, 820bp and 775bp bands with their neighbouring band(s) is responsible for the strong hybridisation of the 820bp fragment to all three bands. Because the 820bp fragment appears to hybridise even more strongly to the 775bp band than to itself (similar to the pattern of hybridisation seen with probes of whole satellites I, II and III) it appears that the 775bp fragment is more prevalent in the genome than the other two fragments. Like the I_{875} probe, the 820bp fragment hybridises strongly to a male specific band at 1.7kb and less strongly to a band at 2.5kb which is present in both male and female DNA.

Overall, it appears that all four probes hybridise to the 2.5kb band with approximately equivalent intensity. The 2.5kb band therefore appears to be the entire 820bp + 775bp + 875 bp repeat, lacking internal HinfI sites and found in both male and female DNA. The 1.7kb band, male specific, and more prevalent than the 2.5kb band, appears to be constituted by an 820 + 875bp

fragment, lacking an internal HinfI site, which is amplified (with or without the 775bp fragment) independently of the 2.47kb repeat with three HinfI sites. Fragments of 875 + 775bp and 820 + 775bp, lacking internal HinfI sites are much less prevalent in satellite I DNA, but are also male specific.

The high molecular weight material in HinfI digests of satellite I

Material with very few HinfI sites (15-25kb band) forms a large proportion of satellite I, and is plentiful in both male and female DNA (Figure 5a and b). This band is digested by RsaI into a number of regularly spaced fragments, most of which are less than 200bp in length. Preliminary sequence data indicate that many of the fragments are very A+T rich and not unlike the regions of the 820bp and 875bp HinfI fragments which have been sequenced (unpublished data). I_{320} probe, I_{875} probe and nick-translated 820bp fragment hybridise only faintly to the 15-25kb band in purified satellite I (Figure 5d, e and f) indicating that little of the high molecular weight material is homologous to these regions of the 2.47kb male specific repeat. There appears to be no hybdridisation of I_{50} to this high molecular weight band in purified satellite I (Figure 5c) indicating either that all the Alu sequences purified with satellite I are found in the 2.47kb repeating unit, or, that because I_{50} probe gives a weaker hybridisation signal than the other probes used, faint hybridisation to this band is not visible. Of the four probes, I_{50} hybridises most strongly to the 15-25kb band in whole male and female DNA, indicating the probable existence of Alu family members in other non satellite I repeated sequences in the human genome.

Since there is considerable cross-hybridisation between satellite I and satellite III, it seemed possible that the high molecular weight sequences from the HinfI digest of satellite I might contain sequences related to the simple repeat ATTCC, previously identified in satellite III (3). A probe of a 100bp HinfI ladder fragment from satellite III, rich in ATTCC sequence (III_{100}, not shown) gave no hybridisation to any band in the satellite I digest. Nick-translated whole satellite III was also used to probe the satellite I high molecular weight material (Figure 5g). It was found to contain a small amount of the major components of the HinfI digest of satellite I, but did not hybridise particularly strongly to the 15-25kb band. For completeness, satellite II was also used to probe satellite I and was found to contain trace amounts of the major components of the HinfI digest of satellite I (Figure 5h). In summary, the large amount of high molecular weight material in HinfI digests of satellite I (15-25kb) contains very little of the 2.47kb repeat and related sequences. It is present in only

trace amounts in satellite II, but is present in satellite III, along with all the other major components of satellite I, and would account for cross-hybridisation between these two satellites. The 15-25kb band of satellite I is not constituted by the simple repeat ATTCC, but is, like the "silent" region of the 2.47kb repeat, very A+T rich.

DISCUSSION

Sequences homologous to the 2.47kb male specific repeat of satellite I

The 2.47kb male specific sequence which we have extensively characterised here is tandemly repeated and contains three HinfI sites and one Alu family member per 2.47kb unit. There is very little sequence divergence between individual members of the tandem repeat. The 1.7kb repeating unit, also specific to male DNA, is consistent with a fragment composed of the 820 + 875bp fragments missing the internal HinfI site and amplified independently of the 2.47kb repeat which contains two internal HinfI sites. Fragments of the size 820 + 775bp and 875 + 775bp are much less prevalent in the satellite preparation and (together with a proportion of the 1.7kb band) may simply be due to random loss of the HinfI site internal to each of them.

A 2.47kb repeat which contains only one HinfI site per repeating unit is present in approximately equal amounts in male and female DNA. This repeat, on one or more autosomes and perhaps the X chromosome, is present in much lower quantities than the male specific repeat, based on comparison of relative intensities of hybridisation of the 2.47kb male specific sequence probes to Southern blots of HinfI digested satellite I, and of the equivalent EtBr stained gels. The autosomal repeat is probably not greatly diverged from the male specific repeat, since hybridisation of probes from the male specific fragments to the 2.47kb band of male and female DNA and purified satellite I does not decrease with increasingly stringent washes relative to hybridisation to the male specific bands themselves.

A band at 15-25kb hybridises faintly to probes I_{320}, I_{875} and nick-translated 820bp fragment, indicating that there are a few sequences homologous to the 2.47kb male specific repeat which essentially do not contain HinfI sites and are present in very low concentration in males and females. I_{50} probe hybridises more strongly to the 15-25kb band in males and females than either I_{320} or I_{875} probes and presumably means that Alu sequences are found in other, different repeated sequences in the human genome.

The male specific 2.47kb repeated sequence has not previously been

shown to contain an Alu family component. In a genomic library derived from pieces of human DNA 15-20 kb long, Alu sequences characteristic of our repeat would be represented, at most, every 2.47kb or 6 to 8 times. It has been calculated that where Alu sequences are found adjacent to transcribed genes, they average approximately one Alu sequence per 8 or 9kbp (11, 13). Clones have been isolated with a high proportion of Alu sequences; 6 in 18kbp (18), (not unrepresentative of the number expected from our repeat length, but the restriction site profile and spacing of Alu family members in this clone is totally different from that of our DNA), and 10 in 15kbp (19), an even higher frequency of Alu members than we have found.

We have not made any estimates of the copy number of the 2.47kb repeated sequence, either for the Y chromosome or for total male DNA. The sequence must provide a number of Alu family members in addition to the 300,000-500,000 estimate for interspersed Alu sequences, since the satellite Alu sequences would not be included in 300bp renatured material, obtained after S1 nuclease digestion (20), and would be under-represented in most genomic libraries (13). However, it appears that the satellite Alu sequence is low in copy number compared to the copy number for interspersed Alu sequences. Cooke et al. have recently identified a 2.47kb repeat on the Y chromosome, using a cloned 2.1kb male specific fragment from a HaeIII digest of total human male DNA (15). No sequence data are available for this repeat, but the restriction site pattern for the enzymes BstN1 (EcoRII), HaeIII and MspI and the distribution of related sequences in male and female DNA are very similar to the 2.47kb male specific repeat identified here, so that they are almost certainly the same sequence. It was estimated that there are 2,000 copies of the tandemly repeated sequence on the Y chromosome and a lower number of copies on the telomeres of a number of autosomes and the X chromosome.

Evolution of the 2.47kb male specific repeat

The lack of divergence between individual clones of the same segment, and between cloned segments and the consensus sequence of the 2.47kb male specific repeat argues for the recent tandem amplification of this sequence on the Y chromosome. The relatively high level of homology between the satellite Alu sequence and the consensus human interspersed Alu sequence provides further evidence in support of this conclusion. However, sequences homologous to the 2.47kb Y chromosomal repeat described by Cooke et al. have been detected in the DNA of gorilla and chimpanzee. The

sequence in the gorilla is tandemly repeated and has clearly related sites for the enzymes HaeIII and BstNI, but is not present on the Y chromosome, whereas the sequence in the chimpanzee mainly has the characteristics of an interspersed repeat (15). Thus it seems that, although the 2.47kb tandemly repeated sequence on the Y chromosome has a very recent origin, the sequence from which this repeat was formed originated earlier in the evolution of the higher primates. Our hybridisation studies do not establish whether the 2.47kb repeat arose from the tandem amplification of a region of unique or interspersed repeated DNA containing an Alu sequence or from amplification of a pre-existing A+T rich satellite sequence into which a section of G+C rich DNA had been inserted. A single copy of an Alu sequence has previously been found in a tandemly repeated sequence, not related to the 2.47kb repeat, in African green monkey (21). One of the bovine satellite DNAs consists of a tandemly repeated sequence made up of a retrovirus-like element inserted into another satellite sequence (22). It is possible that an A+T rich repeated sequence without an inserted G+C rich region may exist in the satellite I material which remains basically undigested by HinfI (15-25kb band) in male and female DNA. It is not known whether the relatively G+C rich 775bp fragment arose from a single insertion of a piece of DNA containing an Alu sequence or from multiple insertions of G+C rich DNA. Evidence that an Alu family member was inserted into A+T rich DNA is provided by the direct repeats flanking the Alu sequence, ATATGGATCT/ATATGATAT, which are related in sequence to the A+T rich region of the satellite repeat. The origin and diversification of the 2.47kb (Alu-containing) tandem repeat may be clarified by comparing related sequences in man and other primates.

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