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Simple repeated sequences in human satellite DNA

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ABSTRACT

In an extensive analysis, using a range of restriction endonucleases, HinfI and TaqI were found to differentiate satellites I, II and III & IV. Satellite I is resistant to digestion by TaqI, but is cleaved by HinfI to yield three major fragments of approximate size 770, 850 and 950bp, associated in a single length of DNA. The 770bp fragment contains recognition sites for a number of other enzymes, whereas the 850 and 950bp fragments are "silent" by restriction enzyme analysis. Satellite II is digested by HinfI into a large number of very small (10-80bp) fragments, many of which also contain TaqI sites. A proportion of the HinfI sites in satellite II have the sequence 5'GA($^{G}_{C}$)TC. The HinfI digestion products of satellites III and IV form a complete ladder, stretching from 15bp or less to more than 250bp, with adjacent multimers separated by an increment of 5bp. The ladder fragments do not contain TaqI sites and all HinfI sites have the sequence 5'GA($^{A}_{T}$)TC. Three fragments from the HinfI ladder of satellite III have been sequenced, and all consist of a tandemly repeated 5bp sequence, 5'TTCCA, with a non-repeated, G+C rich sequence, 9bp in length, at the 3' end.

INTRODUCTION

Human highly repeated DNA can, in part, be isolated as four cryptic satellites, each of which is separated from main band DNA by isopycnic centrifugation in an appropriate density gradient (1-4). Satellites I, II, III and IV are A+T rich compared to main band DNA (4) and constitute about 5% of the human genome (5).

Satellites II, III and IV have been characterised in terms of the products of digestion with restriction endonucleases EcoRI and HaeIII, whereas satellite I is resistant to digestion by these two enzymes. Several fractions of satellites II, III and IV have been identified by digestion with EcoRI and HaeIII (6). These include: (i) a "ladder" of fragments which are exact multiples in length of a 170 base pair (bp) monomer, (ii) various fragments which do not bear any obvious size relationship to the ladder fragments, (iii) a 3400bp male specific fragment (7, 8), and (iv) sequences

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that are resistant to digestion by either EcoRI or HaeIII or both enzymes. Satellites III and IV have been found to be identical by a range of criteria, including restriction enzyme analyses. Satellite II is similar to satellites III and IV in the size classes of products of digestion with EcoRI and HaeIII, but has been distinguished from satellite III by the observation that different sequence variants of the 170bp ladder material are incorporated into satellite II.

The repeated sequences which make up the 170bp ladder in satellites II and III, particularly the 340bp and 680bp components, are present in large quantities in unfractionated human DNA (9). A 340bp fragment and part of a 680bp fragment, from an EcoRI digest of total DNA, have been directly isolated and sequenced, to provide consensus sequences for a portion of the 170bp ladder material (10). This work has yielded no evidence for any internal repeat within the 170bp monomer.

There is evidence that shorter repeated sequences are also present in the human DNA satellites. The sequence determined for part of a single cloned 1770bp fragment, isolated from an EcoRI digest of satellite III, shows an obvious, though irregular, 4-9bp internal repeat and numerous HinfI and TaqI sites (11). Evidence for relatively simple repeated sequences in satellites I and II has been obtained from DNA fingerprinting studies (12).

We report here that preparations of satellites II, III and IV may vary considerably in their content of the EcoRI or HaeIII 170bp ladder components, but that all preparations of satellites III and IV contain, as a major component, a conserved repeated sequence, 5bp in length. This sequence appears as a ladder, with a 5bp periodicity, in HinfI digests of satellites III and IV. Satellite II, like satellite III, consists mainly of sequences which are identified by the presence of frequent HinfI sites, but the size distribution of fragments obtained by digestion with HinfI is entirely different. In satellite II, unlike satellite III, sequences which contain HinfI sites also contain numerous TaqI sites. Satellite I is characterised by a relative lack of HinfI sites. This satellite contains a sequence which consists of DNA resistant to restriction enzyme digestion, associated with a sequence containing clustered recognition sites for a number of enzymes.

METHODS

DNA extraction and preparation of satellite DNAs

DNA was extracted by the method of Marmur (13), from male placentae and

from preparations of leukaemic cells obtained from male patients (with acute myeloblastic or chronic granulocytic leukaemia) who were undergoing leucapheresis for therapeutic purposes. Isolation of human satellites I, II, III and IV, using Ag^{+} or $Hg^{++}/Cs_2\ SO_4$ density gradients, was carried out as previously described (5).

Restriction endonuclease digestion

EcoRII endonuclease was a gift of Dr. Keith Brown. All other restriction endonucleases were purchased from New England Biolabs. Digestions were carried out under the following conditions:-
AvaI, AvaII, BglII, DdeI, HgaI, HindIII, HinfI, MboI, Sau3AI, Sau96I: 6mM Tris-HCl, pH 7.5, 50mM NaCl, 6mM $MgCl_2$, 6mM β-mercaptoethanol, 0.1mg/ml bovine serum albumin (BSA).
BamHI, SalI, XbaI, XhoI: 6mM Tris-HCl, pH 7.9, 150mM NaCl, 6mM $MgCl_2$, 0.1mg/ml BSA.
Fnu4HI, HaeIII, TaqI: 6mM Tris-HCl, pH 7.4, 6mM NaCl, 6mM $MgCl_2$, 6mM β-mercaptoethanol, 0.1mg/ml BSA.
EcoRII, MspI: 10mM Tris-HCl, pH 7.5, 6mM KCl, 10mM $MgCl_2$, 6mM β-mercaptoethanol, 0.1mg/ml BSA.
EcoRI: 100mM Tris-HCl, pH 7.5, 50mM NaCl, 5mM $MgCl_2$, 0.1mg/ml BSA.
Digestions were carried out for 2 hours at 37°, except for TaqI digestions, which were carried out at 65°. Reaction mixtures, of total volume 10µl, contained 0.05-3µg DNA and sufficient enzyme to ensure complete digestion within two hours. Reactions were terminated by heating to 65° for 5 min, followed by rapid cooling to 0°.

Labelling of restriction fragments and gel electrophoresis

With the exception of HaeIII, all the restriction enzymes used in this study cleaved double stranded DNA, to produce single stranded 5' ends which were then filled in by the action of reverse transcriptase, using one or two appropriate ^{32}P-nucleoside triphosphates (14). When digests contained a large number of small fragments, the incubation with labelled nucleoside triphosphate was followed by incubation with an excess (0.1-0.2mM) of all four unlabelled nucleoside triphosphates. The reaction was terminated by heating to 65°, followed by rapid cooling to 0°.

Restriction fragments were separated by electrophoresis in vertical gels of 4% or 12% polyacrylamide (0.5mm thick) or 1.5% agarose (0.8mm or 3mm thick). ^{32}P-labelled fragments were visualised by autoradiography. HaeIII digestion products were stained with ethidium bromide and visualised under ultraviolet light.

Various restriction endonuclease digests of the plasmid PBR322 were used as standards (15). When calculating sizes of small, end-labelled fragments, it was necessary to take into account differences between restriction enzymes in the length of the 5'→ 3' staggered cut and thus in the number of nucleoside triphosphate units added by reverse transcriptase.

DNA sequencing

For sequencing, HinfI fragments of satellite III, containing [α-^{32}P]ATP at the 3' end of each strand, were eluted from 12% polyacrylamide gels. The fragments were denatured, the strands were separated by electrophoresis in 5% polyacrylamide gels at low ionic strength, and the single stranded DNA was sequenced according to Maxam and Gilbert (16). In each case, the more slowly moving strand was eluted from the denaturing gel and sequenced.

Southern transfers and filter hybridisation

DNA fragments were transferred from 1.5% agarose gels to nitrocellulose filters by the method of Southern (17). Hybridisation was carried out using a DNA probe labelled with [α-^{32}P]ATP by nick translation (18).

RESULTS

Satellites I, II and III are extensively cleaved by HinfI

Satellites I, II and III can be effectively differentiated by comparison of the products of digestion with HinfI (Figure 1). Satellite I is broken down into a number of high molecular weight fragments (Figure 1a). The gel shows three prominent bands of approximate size 770bp, 850bp and 950bp, a small amount of undigested material at the origin and a large band at about 3500bp, which, on further analysis in 1.5% agarose gels, proved to be a smear of high molecular weight material. Satellites II and III are almost entirely digested by HinfI (Figure 1, b and c). There is very little undigested material, either at the origin or at the 3500bp position. The low molecular weight fragments in HinfI digests of satellites II and III have been further resolved by electrophoresis in 12% polyacrylamide gels (Figure 2). Satellite II is cleaved by HinfI into a large number of very small fragments (Figure 2b); the smallest fragments are less than 10bp in length, and there are very few fragments of size greater than 80bp. The HinfI digestion products of satellite III form a complete ladder stretching from 15bp or less to more than 250bp (Figure 2j). Adjacent components of the ladder are separated from each other by an increment of 5bp.

Although the HinfI digests shown in Figure 2 were obtained under

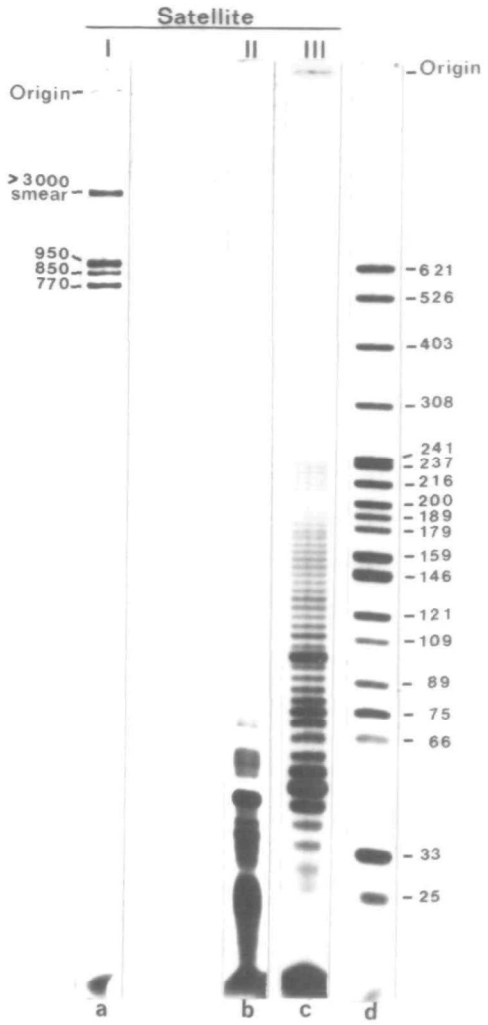

Figure 1. 4% polyacrylamide gels of [α-^{32}P]ATP-labelled fragments produced by digestion of satellites I, II and III with HinfI.
a: HinfI digested satellite I. Fragment sizes, in base pairs, are marked on the left side.
b: HinfI digested satellite II.
c: HinfI digested satellite III.
d: [α-^{32}P]CTP-labelled fragments from digestion of PBR322 with MspI. Fragment sizes, in base pairs, are marked on the right side; these have been adjusted to make them comparable in size with end-labelled HinfI fragments, and give an indication of sizes of fragments in lanes b and c.

conditions which normally result in a complete reaction, the satellite III HinfI ladder has the appearance of a partial digest. However, when samples of both satellites II and III were incubated with increased concentrations of HinfI or for longer times, there was no change in the amount of any of the satellite III ladder components or the satellite II fragments. In addition, individual HinfI ladder components, eluted from a gel, were not further digested by reincubation with an excess of HinfI. We conclude, therefore, that the digestions shown in Figures 1 and 2 were complete, and that the

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