Genomic organization of low copy number sequences that are associated with deca-satellite DNA in the monkey genome

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ABSTRACT
A previously described segment of African green monkey DNA (cloned in phage λMK2) contains deca-satellite linked to DNA sequences that are estimated to occur once per genome. Sequences homologous to the low copy number sequences in λMK2 are also associated with species-specific satellite DNAs in the human and mouse genomes. A second clone, λMK8, contains a monkey DNA region that is colinear and homologous to a portion of the low copy number sequences in λMK2, but no satellite sequences. The two cloned segments are markedly different starting at a point proximal to the satellite DNA region in λMK2. DNA-blotting experiments indicate that λMK8 but not λMK2 represents the typical genomic organization and that the low copy number segments occur only once per haploid genome. The data suggest that rearrangements such as deletions or inversions occurring in monkey cells account in part for the structure of λMK2. Additional rearrangements may have occurred during cloning in E. coli. This unique chromosomal region may be particularly susceptible to recombination.

INTRODUCTION
The long tandem repeats of DNA sequences that are known as satellite DNAs occur in most eukaryotes where they are concentrated in the heterochromatic regions around centromeres and telomeres. The repetitive nature of these sequences and the rapid rate at which they change in evolution (for a current review, see reference 1) and among individuals of a given species (2-5) complicate experimental approaches to questions regarding the origin, maintenance and function of satellite DNA. The isolation and characterization of low copy number DNA sequences that are adjacent to satellite DNA in the genome might help to address some of these questions. For example, genomic segments that flank satellite DNA may play a role in the generation of tandem arrays and in the events that lead to the rapid rearrangements of satellite DNA that are manifested as intraspecies polymorphisms.

Recently, we described a cloned segment of African green monkey (Cercopithecus aethiops) DNA containing low copy number sequences flanking deca-satellite, a previously characterized monkey centromeric satellite (6).
Deca-satellite is known to be highly polymorphic among individual monkeys (2,3). The structure of the cloned recombinant, \( \Lambda \text{MkA} \), is shown in Figure 1. DNA-blotting experiments demonstrated that at least portions of the low copy number segments (LC-A) in \( \Lambda \text{MkA} \) are conserved in the human and mouse genomes (6). Moreover, the data suggested that not only portions of LC-A but also their association with satellite DNA are conserved in primates and rodents.

A second, independent monkey library was also screened with sub-cloned probes from within LC-A. Several of the newly isolated monkey clones annealed with probes containing the species-specific deca- and \( \alpha \)-satellite sequences. These observations confirmed the association of at least a portion of LC-A with satellite DNA in the monkey genome. Other of the phage selected from the second library with LC-A probes contained no satellite (e.g., \( \Lambda \text{MkB} \)).

We report here two kinds of experiments designed to test whether the segment cloned in \( \Lambda \text{MkA} \) represents the arrangement of homologous sequences in the monkey genome. First, we analyzed two types of phage selected from the second, independent monkey genomic library with a subcloned probe from within LC-A: recombinants with \( (\Lambda \text{Mk20 and } \Lambda \text{Mk31}) \) and without \( (\Lambda \text{Mk8}) \) satellite DNA. Second, DNA-blot analyses were made of restriction endonuclease digests of monkey DNA using several probes subcloned from \( \Lambda \text{MkA} \) and \( \Lambda \text{Mk8} \).

The experiments indicate that the arrangement of sequences at a junction between LC-A and deca-satellite in \( \Lambda \text{MkA} \) is not colinear with the homologous segment in the genome. Two explanations for this are proposed: 1) that the rearrangement observed in \( \Lambda \text{MkA} \) reflects recombinational events during the propagation of the phage in \( E. \text{ coli} \) and 2) that the sequence also tends to rearrange in the monkey genome itself.

**MATERIALS AND METHODS**

All materials and methods were as described previously unless indicated otherwise (3,6). The various subclones of \( \Lambda \text{MkA} \) and \( \Lambda \text{MkB} \) that are used as probes are described in Figure 1. The DNA-bLOTS shown in Figure 2 utilized nitrocellulose filters as described (6) while those in Figure 3 utilized zeta-probe nylon membranes (BioRad) and modified hybridization conditions. Briefly, the zeta-probe membranes were prehybridized in 3 x SSC, 4 x Denhardt solution, 0.1% (w/v) sodium lauryl sulphate, 100 \( \mu \)g of sheared and denatured salmon sperm DNA and 1 mg/ml of yeast soluble RNA. After a period of 36h at 65°C the membranes were hybridized in a fresh solution of the same composition but containing 10 \( \mu \)g/ml of salmon sperm DNA, 200 \( \mu \)g/ml of yeast.
soluble RNA and $10^6$/cpm per ml of denatured $^{32}\text{P}$-nick translated DNA probe (1 to $5 \times 10^8$ cpm/µg). The membranes were then washed for five 45 min periods at $52^\circ$C in 0.1 SSC and 0.1% sodium lauryl sulphate. These conditions are more stringent than those used for the selection of phage AMk8 from the library (6): annealing in 6 x SSC, 5 x Denhardt's, 0.1% SDS, 25 µg/ml of total yeast RNA, 25 µg of sheared and denatured Salmon sperm DNA and $2 \times 10^5$ cpm/ml of probe at $68^\circ$C for 18 hours; washing (twice) for 30 min at $68^\circ$C in 4 x SET (1 x SET: 0.15 M NaCl, 0.03 M Tris pH 8.0, 0.002 M EDTA), twice for 30 min at $55^\circ$C in 2 x SET and finally twice for 30 min at $55^\circ$C in 1 x SET.

The map of restriction endonuclease EcoRI sites on AMk8 was determined by partial digestion and end labeling of the products at the right cohesive terminus of the lambda phage vector (7). The dodecanucleotide homologous to the right hand cohesive end and other required reagents were purchased from Collaborative Research, Inc., Lexington, MA. Other sites were placed by standard methods using either the phage or subcloned regions. The arrangement of sequences homologous to subcloned probes from within LC-A was determined by DNA-blotting.

RESULTS
Comparison of Independently Cloned Monkey Fragments Containing Sequences Homologous to LC-A

A subcloned probe from within LC-A, p5 (Figure 1), was used to select recombinant phage from a different genomic library than that used for the isolation of AMkA itself (see Table III in reference 6). Seven phage were obtained and all seven annealed to probes p2, p4 and p5 derived from LC-A; four also annealed to monkey-specific satellite DNA probes representing both alpha- and deca-satellites while three did not anneal to either satellite probe. As previously observed (6) every phage that contains sequences homologous to LC-A (including AMkA itself) gives a poor yield of DNA. We prepared and analyzed DNA from those phage that gave the better yields: one without (AMk8) and two with (AMk20 and AMk31) homology to satellite DNA.

Phage DNAs were digested with several restriction endonucleases and DNA blots were tested for hybridization with subcloned regions of LC-A and with satellite DNA probes. On the basis of these data, a map of AMk8 was constructed (Figure 1). As for AMkA (6), the experiments leading to the map were unambiguous and we observed no indication of any instability in the isolated monkey DNA insert.

Comparing the maps of AMkA and AMk8, the two cloned segments are essen-
Figure 1. Maps of two monkey recombinant phage, λMkA and λMk8, and of the monkey genomic region that contains homology to the LC-A sequences (LC-A is composed of two portions, LC-Aright and LC-Aleft). A map of λMkA was described previously (6), but the present map is considerably more detailed. The approximate size in kbp of the inserts in the two recombinant phage are: λMkA, 9.2 and λMk8, 15. Subcloned regions of λMkA and λMk8 are indicated by number below the maps and referred to in the text as p1, p2, etc. Some of the subclones were described previously (6). Plasmids p8 and p9 were cloned in pBR322 and pUC8c, respectively, using the restriction endonuclease sites indicated in the figure. Regions of the 3 maps that anneal to specific subclones are indicated by common markings, as follows: ☐, p9; cross-hatch, deca-satellite; triangles, p8; x, p1; +, p2; o, p3; horizontal lines, p4 or p5. The genomic map was constructed using the data in Figures 2 and 3 and reference 6 as well as similar, unpublished experiments. Restriction endonuclease sites are as follows: E, EcoRI; B, BamHI; P, PstI; K, KpnI; X, XmnI; H, HindIII; B, BgiII; BII, BgiIII; SII, SacII; Xb, XbaI. The BgiII sites in λMkA that are indicated by dotted lines with a superimposed bar have not been precisely mapped. Several BgiII and SacII sites occur in p8 but have not been mapped; there is a single SacII site in p0. It is not known whether the XbaI site present in the p2 region of λMkA and genomic DNA occurs in λMk8. Restriction endonuclease sites discussed in the text are encircled. The LC-A region (low copy number sequences in λMkA) includes the two segments, LC-Aleft and LC-Aright, that flank the sequences that anneal with deca-satellite).
tially the same in the region to the right of the encircled BamHl site in each insert except that the monkey sequence in AMk8 extends about 10 kbp further. Several minor differences were observed to the right of the BamHl site. 1) The EcoRI fragment (subcloned in p3 and labeled 3 on the map of AMkA) is 420 bp long in AMkA as determined by sequencing (not shown) while it is about 30 bp shorter in AMk8 as determined by relative mobility on a single electrophoresis gel (not shown). 2) The 1.3 kbp EcoRI fragment in AMkA hybridized to a poly[d(GT)·d(CA)] probe and 16 tandem CA dinucleotides were found by sequence analysis but the corresponding fragment of AMk8 failed to hybridize to poly[d(GT)·d(CA)] (not shown). 3) The nucleotide sequences of the regions corresponding to p1 differ in AMkA and AMk8 in a few clustered and three dispersed positions. 4) AMk8 does not contain a PstI site corresponding to the encircled PstI site in the 1.3 kbp EcoRI fragment in AMkA (Figure 1). These differences can probably be explained on the basis of individual polymorphisms since the two phage were isolated from libraries constructed with DNA from two different individuals. It is also possible that they are related to the rearrangements postulated to explain the structure of AMkA (see below).

To the left of the BamHl sites, AMkA and AMk8 are distinctly different. First, as already noted, AMk8 does not anneal to the deca-satellite probe. Second, the 2.2 kbp EcoRI/BamHl fragment contained in AMk8 (p8) does not occur in AMkA nor does the 1.4 kbp PstI fragment of AMkA occur in AMk8. The subclones p0 and p8, from AMkA and AMk8, respectively, cross-hybridize only poorly (data not shown) and p9 (containing the 1.4 kbp PstI fragment) does not cross-hybridize to AMk8. Thus, within a short distance to the left of the common BamHl site, the two cloned segments diverge markedly. The implications of this observation will be discussed below.

The restriction endonuclease digests of AMk20 and AMk31, two phage that annealed to p2, p4, p5 and monkey satellite DNA, gave unusual results. First, several restriction endonuclease fragments expected from the arms of the ACharon4A vector were not produced. Second, the sequences that annealed with p5, deca- and alpha-satellite probes were all contained in a single BamHl fragment (see Figure 2 of reference 6). This result is inconsistent with the structure of both AMkA and AMk8 and with the genomic organization of the sequences that is described below. The unexpected properties of both the vector and insert segments suggested that gross rearrangements had occurred in these recombinants during replication in E. coli, and they were not studied further.
DNA-blot Experiments with Restriction Endonuclease Digests of Monkey DNA and Subcloned Probes from AMkA and AMk8

Previously, we showed that several of the subcloned probes from within AMkA anneal to specific endonuclease HindIII and EcoRI bands in digests of total liver DNA from the monkey designated M2 (6). More extensive experiments designed to determine the organization of the LC-A and p8 sequences in two different monkey DNAs, M1 and M2, were carried out and representative experiments are displayed on Figures 2 and 3. Neither one of these animals was the source for the DNA used to construct the monkey genomic libraries from which AMkA or AMk8 were selected. Our analysis of these data is chiefly concerned with whether or not the segments cloned in AMkA and AMk8 each represent a genomic sequence. If this were true, then more than one copy per haploid genome of the DNA segments common to these phage (segment to the right of the circled BamHI site on Figure 1) is expected and different copies should have different flanking sequences on the left of the BamHI site. We note that the low copy number sequences (LC-A) in AMkA flank the region containing deca-satellite. For convenience we designate the portion of LC-A that lies to the right of the encircled BamHI site as LC-Aright and the portion that lies to the left of the deca-satellite as LC-Aleft (see Figure 1).

Each of the subcloned probes anneals to only a single major genomic band in the digests carried out with single restriction endonucleases (Figures 2 and 3) except in the EcoRI digest annealed with p4 (Figure 2) and the BgII digest annealed with p9 (Figure 3). In the EcoRI digest two genomic bands are seen with p4 as expected from the structures of both AMkA and AMk8. In the BgII digest, p9 anneals to two bands (5.6 and 7.6 kbp) in

Figure 2. A. DNA-blot analysis of 2 individual monkey DNAs with sub-cloned probes from AMkA and AMk8. DNAs from monkey livers M1 and M2 were digested with enzymes HindIII, HindIII plus XmnI, EcoRI or BamHI plus XmnI. For each digest, 25 µg portions were loaded in multiple identical lanes on a single gel and fractionated by gel electrophoresis: HindIII, HindIII plus XmnI and BamHI plus XmnI on 1% agarose and EcoRI on 1.4% agarose. After transfer of the fragments to nitrocellulose, the filters were cut into strips corresponding to the lanes and the strips were annealed with nick translated probes p1, p2, p3, p4, p6 and p8 as indicated by the numbers below each lane. The sizes indicated in kbp to the left of each autoradiogram are the determined sizes of the major bands. The size of the bands was estimated using as molecular weight markers wild type lambda phage DNA digested with HindIII and pX174 DNA digested with HaeIII. The autoradiograms were exposed for 2 weeks with an intensifying screen. The smear in the lanes annealed with p4 result from the poly[d(GT)-d(CA)] stretch in p4 that was previously described (6).
monkey M1 (Figure 3). With monkey M2, p9 annealed to a single 7.6 kbp BglII fragment (not shown). The two fragments in M1 DNA each anneal at about half the intensity of the other major bands on the blot, suggesting the presence of two polymorphic alleles of a single copy sequence in the region containing p9. This was the only difference in major bands observed between monkey M1 and M2 in all the experiments. In addition to the discrete genomic fragments, p4 anneals to a smear of fragments in all four digests (Figure 2). The smear results from the presence of the (CA)$_{16}$ stretch contained in the probe (see above and reference 2). Poly[G-T]-[d(C-A)] segments are repeated and interspersed in all eukaryote genomes (8).

Given the amounts of genomic DNA used in these blots (25 µg in Figure 2 and 20 µg in Figure 3), the specific radioactivity of the probes (greater than 1 x 10$^8$ cpm per µg) and the exposure times, the data indicate a copy number of close to one for all the major homologous fragments in the haploid
genome (except the satellite sequences). Quantitative dot blots with p2 and 
p8 confirm this conclusion (R.E. Thayer and M.F. Singer, unpublished data). 
If there is more than one genomic copy of the subcloned segments, then all 
copies must be highly homologous and have essentially identical restriction 
endonuclease sites.

As described more fully below, all of the DNA-blot data fit a single, 
coherent genomic map that is consistent with the structure of LC-Aright and 
all of the AMk8 insert. However, beside the major bands, several of the 
probes annealed weakly to additional genomic fragments. The intensity of 
the minor bands is well below what is expected for a single-copy sequence 
and the pattern of minor bands was not identical in the two monkeys (not 
shown). The minor bands are not consistent with the maps of either AMkA or 
AMk8. For example, p6, p1, p2 and p3 anneal weakly with 2 EcoRI bands about 
4 kbp in length. However, p1 and p6 are separated by 6 kbp in AMkA.

Organization Within the Monkey Genome of Sequences Homologous to LC-Aright

The DNA-blot experiments using a single or two restriction endo-
nuclease (Figure 2) establish that the DNA sequences in LC-Aright are 
linked in the genome and the segment is colinear with AMkA and AMk8. The 
extreme EcoRI sites defining the 9.2 kbp long insert in AMkA were generated 
during construction of the Alul-HaeIII library (3,6) and thus the 1.1 kbp 
EcoRI fragment at the far right of AMkA is contained in a 1.9 kbp EcoRI 
fragment in the genome (6) and in AMk8. The colinearity of the genome and 
LC-Aright was confirmed by several addition restriction endonuclease digests 
(unpublished). These experiments demonstrated that the PstI site (circled 
in Figure 1) found in AMkA but not AMk8 does not occur in M2 DNA (M1 DNA was 
not tested). The map of the genomic region homologous to LC-Aright is shown 
in Figure 1.

Organization Within the Monkey Genome of LC-Aleft and Sequences in Subclone 
p8

The DNA blot experiments in Figures 2 and 3 indicate that the region to 
the left of the BamHl site in AMkA is different from the major homologous 
genomic segments while the map of AMk8 is colinear with the genomic organi-
ization (Figure 1). For example, p1 anneals with a 2.6 kbp genomic EcoRI 
fragment, as it does in digests of AMk8, but not with a 6 kbp genomic EcoRI 
fragment as it does in AMkA.

DNA-blot experiments indicate that deca-satellite sequences begin a 
short (but unknown) distance to the left of the SacII site in AMkA. At the 
far left of the insert the deca-satellite sequence is flanked by low copy
TABLE 1
Comparison of Monkey DNA Restriction Endonuclease Fragments
That Anneal with p9 and Those Predicted by AMkA.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Observed Genomic Fragment</th>
<th>Expected from AMkA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kbp</td>
<td>kbp</td>
</tr>
<tr>
<td>EcoRI</td>
<td>5.8</td>
<td>6.1</td>
</tr>
<tr>
<td>HindIII</td>
<td>11</td>
<td>&gt;6.1</td>
</tr>
<tr>
<td>BamHI</td>
<td>17</td>
<td>&gt;5.7</td>
</tr>
<tr>
<td>KpnI</td>
<td>4.8</td>
<td>&gt;9.2</td>
</tr>
<tr>
<td>XmnI</td>
<td>2.0 and 1.2</td>
<td>7.3</td>
</tr>
<tr>
<td>PstI</td>
<td>1.4</td>
<td>1.4</td>
</tr>
<tr>
<td>BglII</td>
<td>7.6 (M2)</td>
<td>&gt;6</td>
</tr>
<tr>
<td></td>
<td>7.6 and 5.6 (M1)</td>
<td></td>
</tr>
<tr>
<td>HindIII/KpnI</td>
<td>2.6</td>
<td>&gt;6.1</td>
</tr>
<tr>
<td>BamHI/HindIII</td>
<td>11</td>
<td>&gt;6.1</td>
</tr>
</tbody>
</table>

number sequence (LC-Aleft). The map of AMkA predicts that a single genomic HindIII fragment should anneal to p1, p6 and p9. Instead, p1 anneals to a 13 kbp fragment (Figure 2) while p6 (Figure 2) and p9 (Figure 3) anneal with 11 kbp HindIII fragments. Because p6 and p9 anneal to the same size genomic HindIII fragment and p9 anneals to a 1.4 kbp PstI fragment (not shown) as predicted from the map of AMkA, we conclude that LC-Aleft is colinear with genomic sequences. No known satellite DNA sequences are seen by sequence analysis of p6 or of the 200 bp at either end of p9 (not shown). The DNA-blot data in Figure 3 are consistent with a single copy of the p9 segment in the monkey genome. None of the major genomic bands detected by p9 fit the map of AMkA (Table I) except for the 1.4 kbp PstI fragment. The data show that LC-Aleft and LC-Aright are each genomic fragments that probably occur only once in the monkey genome. However, in the genome, LC-Aright is always contiguous with the p8 sequence as it is in AMkB while LC-Aleft can not be linked to p8 by any of the data we have been able to establish. In addition, none of the genomic digests provide evidence for the linkage of LC-Aleft (represented in p6 and p9) with the sequence in p1 or p2. The genomic blots shown in Figure 3 indicate that the p9 fragment is at least 17 kbp from the circled BamHI site because a 21 kbp genomic KpnI fragment anneals...
to p8 but not to p9. We assume that this represents a fragment beginning at the Kpnl site in A.Mk8 (Figure 1) and extending to the left.

**DISCUSSION**

We have been interested in studying the structure and the properties of DNA segments in genomic regions where satellite DNA joins low copy number DNA sequences. The recombinant phage A.MkA appeared to provide such a junction (6). In order to confirm the association of the low copy number region of A.MkA with monkey satellite, sub-cloned portions of LC-A were used to select additional phage from an independent genomic library (AGM library EcoRI-II) and these were then tested for their ability to anneal to satellite (6). Several phage that annealed to both LC-A and to satellite DNA were recovered but because they had clearly suffered rearrangement, their maps could not be constructed. Other monkey phage that were selected with LC-A probes contained no satellite DNA sequences. One of these, A.Mk8, is described in detail here. While it contains a region that is colinear and homologous to 3.5 kbp of the LC-A in A.MkA, the similarity between the two segments ends at a point proximal to the satellite DNA region in A.MkA. Several explanations for the differences between the cloned monkey segments were considered: 1) A.MkA represented the fortuitous joining of satellite DNA and LC-A during the cloning procedures; 2) portions of the LC-A occur more than once and in different contexts in the monkey genome; 3) the monkey used to prepare the Alul/HaeIII library from which A.MkA was isolated had a distinctive arrangement of LC-A; 4) the insert in either A.MkA or A.Mk8 represents a rearrangement of linked genomic sequences. We will discuss the evidence pertinent to each of these alternatives in turn.

The frequency in the monkey EcoRI-II genomic library of phage that anneal to deca-satellite or to the LC-A predicted that fortuitous joining of the two could occur at a frequency of $1.5 \times 10^{-8}$ while the actual frequency was 500-fold higher (6). Moreover, at least portions of the LC-A were shown to be conserved in human and mouse DNA and all of the mouse recombinant phage selected from an appropriate genomic library with monkey LC-Aright annealed to a species-specific satellite DNA probe (6). The frequency with which such phage were detected in the library suggested that LC-A and satellite DNA are associated in mouse DNA as they appear to be in monkey DNA. These results suggested that A.MkA did not represent the fortuitous joining of satellite DNA and LC-Aright during the cloning procedure, but rather indicated the close association of the two in the monkey, as well as the
mouse genome (6). In addition, the absence of any EcoRI sites between the
p6 and p1 regions of AMkA indicates that no fortuitous joining occurred
during the insertion of monkey DNA fragments into the vector.

The second possible explanation for the isolation of two types (plus
and minus satellite) of cloned segments that contain LC-A homology is that
LC-A (or portions thereof) occurs more than once in the monkey genome.
Quantitative dot-blot analysis of genomic DNA with two non-overlapping
subcloned segments, p8 and p2, gave results that are consistent with a
single copy of LC-Aright in monkey DNA (haploid) (R.E. Thayer and M.F.
Singer, unpublished data). In addition, DNA-blotting experiments (such as
those in Figure 2 and 3) using various restriction endonucleases indicate
that only a single major arrangement of the LC-A sequences occur in monkey
DNA. The genomic map is not colinear with the map of AMkA, but is colinear
with AMkB. No evidence for a genomic organization like that in AMkA was
obtained. Thus, if there is more than one copy of the LC-A sequence in
monkey DNA then each copy has the same map, which cannot explain the occur-
rence of two types of cloned insert.

The third possible explanation is that the monkey used to prepare the
AluI/HaeIII library from which AMkA was isolated might have had a distinc-
tive arrangement of LC-Aleft and LC-Aright. We cannot eliminate this possi-
bility. Three monkeys, M1, M2, and the monkey used to construct the library
from which AMkB was isolated appear to have the same arrangements, but other
individuals may have different organizations. Homologous sequences in over
60 samples of human DNA all have virtually identical organizations and yet
here too, cloned representations vary markedly in arrangement (R.E. Thayer,

The data reported here are most consistent with the fourth explanation:
the insert in AMkA represents a rearrangement of genomic segments. Accord-
ing to this model, monkey DNA typically has a genomic arrangement in which
LC-Aleft and a segment including p8 and LC-Aright flank satellite sequences.
The genomic distance between p9 and p8 is unknown and sequences other than
satellite DNA may be included. The structure of AMkA represents a deletion
of genomic sequences that occur between satellite DNA and the p1 segment and
the deleted segments may include additional satellite.

The rearrangement of eukaryotic satellite DNAs upon cloning and replica-
tion in E. coli has been observed frequently (for example, references 2,9,
10). Generally these rearrangements involved the loss or gain of a number
of tandemly repeated units, events that can be explained by unequal cross-
ing-over or intrastrand homologous recombination or slippage during replication. Thus, it is reasonable to suppose that a genomic fragment considerably longer than that in AMkA was inserted in the phage vector and that sequences were lost by recombination during amplification in E. coli, as indeed recombination must have occurred in AMk20 and AMk31, as noted in the section on Results.

The maximum length of any initial insert into the ACharonAA vector carrying AMkA was 15.3 kbp. This is because the vector can accept a maximum of 22 kbp (11) and 6.7 of these are taken up by the internal vector fragment that was included in AMkA (3,6). However, the DNA-blotting data summarized in the map in Figure 1 suggest that the p9 genomic fragment is at least 17 kbp from the (circled) BamHl site because a 21 kbp genomic Kpnl fragment anneals to p8 but not p9 (Figure 3). According to the map, the original monkey insert in the λ-vector would have to have been significantly longer than 15.3 kbp to include sequences in both p9 and p4. Thus, it is difficult to explain the structure of AMkA solely on the basis of rearrangements in E. coli. One possible explanation for the discrepancy is that the genomic segment shown in Figure 1 is prone to rearrangements in the monkey genome as it is in E. coli. Deletions (or deletions plus inversions) might then have occurred in at least two steps, one or more in some of the monkey liver cells from which the library was made and one or more in E. coli. If such deletions had random end points in different monkey cells, then, even if they were frequent, the rearranged single copy segments would not be detectable by genomic blotting.

As already noted, we did observe minor hybridizing bands in genomic DNA blots and the patterns of these differed in the two monkey genomes analyzed (Figures 2 and 3). We are unable to correlate the structure of AMkA with any of these minor bands. It is possible that they represent genomic rearrangements that recur at relatively high frequency in some proportions of the cells, but it is also possible that they represent unrelated, partly homologous genomic sequences.

Altogether, the experiments reported here suggest that the chromosomal region under study may be particularly susceptible to recombination. Because of the instability, it is not now possible to define the precise genomic junction between the LC-A sequences and satellite DNA. Attempts to walk down the genome from regions homologous to p8 and p9 have been hampered by the instability of detected recombinants. Thus, the genomic distances between p8 and deca satellite and between p8 and p9 are unknown.
and sequences other than satellite DNA may be included. We do not know which sequences within the genomic segments schematized in Figure 1 are responsible for the observed instability in *E. coli* or the proposed instability in the monkey genome. A clone such as A\(\lambda\)MkA, that is stable during growth and isolation, may have eliminated some of the sequences that foster instability early during amplification of the phage in *E. coli*. It is possible that the deca-satellite sequences themselves are responsible.

Rearrangements extending into plasmid vector segments were observed in recombinants containing *Drosophila melanogaster* simple sequence satellite DNAs (9).

In general, satellite DNAs in eukaryotic genomes appear to be rather fluid and deca-satellite was previously shown to be unstable in the genome (2,3). In addition, several reports indicate the presence of highly unstable domains in association with satellite DNAs or pericentromeric heterochromatin. Wahl et al. (12) observed that DNA inserts located near the centromeres of hamster chromosomes undergo rearrangements. Recently, Butner and Lo (13) demonstrated a high frequency of rearrangements around a transfected gene that was inserted within mouse centromeric satellite DNA. A middle-repetitive sequence that occurs adjacent to the 1.672 g/cc satellite DNA of *D. melanogaster* has been isolated (14). The sequence is localized to the chromocenter of polytene chromosomes. It is interesting to note that the cloned junction sequence was different from the homologous genomic elements and may thus have been altered in a manner similar to that proposed for A\(\lambda\)MkA. Finally, we note that cloned human DNA segments that contain regions homologous to LC-Aright are, like the monkey segment in A\(\lambda\)MkA, rearranged compared to the human genomic sequences (R.E. Thayer, M.F. Singer and O.W. McBride, unpublished experiments). The human genomic segment that is homologous to LC-Aright has been mapped to region p16 on human chromosome 4 (15). It will be of interest to determine the chromosomal location of the LC-A sequences in the monkey. The bulk of deca-satellite sequences occur in centromeric regions, as shown by in situ hybridization (3). However, short non-centromeric regions of deca-satellite would probably not have been detected. Further investigation of the conserved association of LC-A sequences and species-specific satellite DNAs may provide insights into the role of satellite DNA in promoting genomic fluidity in centromeric as well as non-centromeric locations.
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