Extensive telomere repeat arrays in mouse are hypervariable

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ABSTRACT

In this study we have analysed mouse telomeres by Pulsed Field Gel Electrophoresis (PFGE). A number of specific restriction fragments hybridising to a $(TTAGGG)_4$ probe in the size range 50–150kb can be detected. These fragments are devoid of sites for most restriction enzymes suggesting that they comprise simple repeats; we argue that most of these are likely to be $(TTAGGG)_n$. Each discrete fragment corresponds to the telomere of an individual chromosome and segregates as a Mendelian character. However, new size variants are being generated in the germ line at very high rates such that inbred mice are heterozygous at all telomeres analysable. In addition we show that specific small (~4–12kb) fragments can be cleaved within some terminal arrays by the restriction enzyme MnlI which recognises 5'(N_7)GAGG3'. Like the complete telomere-repeat arrays (TRA's) these fragments form new variants at high rates and possibly by the same process. We speculate on the mechanisms that may be involved.

INTRODUCTION

The ends of mammalian chromosomes, like those of simple eukaryotes, terminate with stretches of G-rich repeats (1, 2, 3,). The special structures adopted by these repeats may render the termini (telomeres) resistant to degradation and fusion (4, 5, 6, 7). In ciliates and humans, at least, these repeat arrays are added to the ends of chromosomes de novo by a ribonucleoprotein enzyme, telomerase (8, 9, 10, 11, 12, 13, 14). In *S.cerevisiae* TRA's at the ends of linear plasmids participate in intermolecular exchanges, converting each other at high frequency, but it is not known whether this occurs between natural chromosome ends (15, 16, 17).

The termini of human chromosomes are composed of 10–15kb arrays which are mainly of the form TTAGGG (18, 19, 20, 21, 22); at the proximal end of the arrays are clusters of degenerate repeats such as $(TTGGGG)_n$ or $(TGAGGG)_n$ (3, 23). On conventional agarose gels the human TRA's can only be revealed as heterogeneous smears in Southern analysis using a labelled $(TTAGGG)_4$ probe. This is, presumably, because 46 different chromosome ends are being detected simultaneously and any particular end is likely to vary from cell to cell. Thus it has not been possible to follow the genetic behaviour of individual telomeres.

In a previous study, we showed that mouse TRA's which also hybridised to $(TTAGGG)_4$ were very much larger than those of human, failing to enter conventional agarose gels (23). Here we show that it is possible using PFGE to characterise the terminal fragments of specific mouse chromosomes and to follow their inheritance.

MATERIALS AND METHODS

Total genomic mouse DNA

(a)Strains used

Total spleen and kidney DNA's extracted from *Mus musculus, Mus domesticus, Apodemus sylvaticus* and the inbred Mus musculus strain AKR/J mice were a gift from R Hill. *Mus spretus* DNA was a gift from N Copeland and N Jenkins. The *Mus caroli* mouse was a gift from J Ansell. The *Mus musculus, Mus domesticus* and *Apodemus sylvaticus* mice were trapped wild in Greece, Denmark and locally respectively. The *Mus spretus* and *Mus caroli* mice were trapped and then held in the laboratory for a period of time. The inbred strains used were DBA/2, C57BL/6 and AKR/J. DBA/2 mice were obtained from colonies in Hull and Oxford. C57BL/6 mice were obtained from both local and Hull colonies. Cross strain matings were set up between Hull DBA/2 and local C57BL/6 mice. The F1 progeny were then brother/sister mated to obtain the F2 generation.

(b)DNA isolation

Two different methods were used:

(1)Nuclei were isolated and DNA recovered as described by Hill *et al.* (24) from mouse spleen, liver and kidney. This method was used for DBA/2 and C57BL/6 DNA used in Figure 4.

(2)The spleen, liver or kidney from a freshly killed mouse was dropped into buffer (100 mM NaCl, 50 mM Tris, 10 mM EDTA, pH8.0) and processed for 10 seconds with a Silversun homogenizer. Next, proteinase K to 100 μg/ml and SDS to 0.5% were added and the whole incubated for 16 hours at 37°C. Next day, the preparations were extracted with phenol (1 volume (vol)), phenol (0.5 vol)/chloroform (0.5 vol), and chloroform (1 vol). The DNA was precipitated with 0.3 vol of 7.5M NH_4OAc and 2.5 vols of ethanol and recovered by spooling into TE (1 mM EDTA, 10 mM Tris, pH8.0). This method was used for the *Mus*

caroli mouse and those mice involved in the cross strain mating experiment.

Southern blotting

Conventional gel electrophoresis

Restriction enzyme digests were performed according to the manufacturer's recommended conditions. Restricted DNA was separated in a 20 cm by 20 cm, 0.8% agarose gel at 50 volts in TAE buffer (0.8 M Tris, 0.4 M NaOAc, 0.02 M EDTA pH8.2) for 16 hours. The separated DNA fragments were then transferred to Hybond-N (Amersham) using a Vacugene vacuum transfer apparatus (LKB).

Pulse field gel electrophoresis (PFGE)

Restriction enzyme digests were performed as above, with extra care taken to ensure minimal shearing of the liquid DNA. Restricted DNA was separated using a Chef DR II Biorad pulse field system in a 1% agarose gel, run in 0.5 ×TAE at 6 volts/cm, using a 5 second pulse time at 10°C for 23 hours. Transfer to Hybond-N was as described above.

Oligomer hybridizations

The $(TTAGGG)_4$ synthetic oligonucleotide was manufactured on an Applied Biosystems 381A DNA Synthesizer by D Chambers. For a standard reaction, 25 ng of the oligonucleotide was labelled in the presence of 5O uCi of gamma ^{32}P ATP (Amersham, 5000 Ci/ml) using 10 units of Polynucleotide Kinase (BCL) in the recommended buffer. Hybridizations were performed in 5×SSC, 5 ×Denharts, 0.1% SDS and 0.1% NaPPi at 48°C for 16 hours. The filters were washed four times for 15 minutes in 4×SSC, 0.1% SDS and 0.1% NaPPi at 56°C.

Random prime hybridizations

The 196 mouse major satellite double strand DNA probe was a gift from A Mitchell originally isolated by Pietras *et al.* (25). It was radiolabelled with alpha^{32}p CTP (Amersham; 800 Ci/mMol) to a specific activity greater than 10^8 cpm using a random priming kit (BCL). Hybridizations were performed under the conditions of Church and Gilbert at 68°C (26). Filters were washed four times for 15 minutes in 2×SSC, 0.1% SDS and 0.1% NaPPi at 68°C.

Bal31 time course

The reaction mix contained DBA/2 total genomic DNA at a concentration of 200 μg/ml which was digested with 40 units/mi of Bal31 (BCL) in the manufacturers recommended buffer at 30°C. Initially, the mix, without Bal31, was preincubated at 30°C for 10 minutes, one aliquot removed and incubated in the absence of Bal31 for the duration of the time course (timepoint = 0). Subsequently, the Bal31 was added and aliquots were removed at the required timepoints. The reaction was stopped by the addition of 0.1 vols of 200 mM EGTA pH8.0. Each aliquot was then extracted once each with phenol (1 vol), phenol (0.5 vol) /chloroform (0.5 vol), and chloroform (1 vol). The DNA was recovered by precipitation with 0.3 vol of NH_4OAc and 2.5 vols of ethanol and spooled into TE pH8.0. This was then digested with HaeIII. For the complete timecourse 390 μg of total genomic DBA DNA was used; each aliquot contained 30 μg of DNA.

Determination of origin of MnlI specific DNA fragments

(a) Analysis of total telomeric smear

Total mouse genomic DNA of the required individual was digested with the restriction enzyme HaeIII (BCL). The sample was separated by conventional gel electrophoresis in 1% low melting point (LW), agarose (BRL). DNA in the size range > 23 kb was purified as follows; the gel slice was melted to 68°C in an equal volume of 100 mM NaCl, 10 mM Tris pH8.0, ImM EDTA for 30 minutes. The molten gel was cooled to 37°C and digested for 16 hours with 5 units/ml of agarose (Calbiochem). The DNA was then extracted once each with phenol (1 vol), phenol (0.5 vol)/chloroform (0.5 vol), and chloroform (1 vol) and recovered by precipitation with 0.3 vols of NH_4OAc and 2.5 vols of ethanol. The DNA obtained was then digested with Mn1l (New England Biolabs) and the fragments separated by conventional gel electrophoresis.

(b) Analysis of a specific MnlI DNA fragment

Total genomic DNA from the required individual was digested with HaeIII and the fragments separated by PFGE (as above) into 1% LMP agarose. The position of the required band was determined and separated from the rest of the gel. The plug was redigested with 10−20 units of MnlI in an equal volume of the manufacturers recommended buffer together with 1 mM spermidine and 0.1% Triton×100 for 16 hours at 37°C. The plug was washed in ice cold TE PH8.0, the digest stopped with 10 mM EDTA and then subjected to conventional gel electrophoresis as previously described.

RESULTS

A telomere probe recognises discrete high molecular weight fragments in mouse DNA.

DNA from different mouse species and inbred strains was digested with the 4bp-cutting restriction enzyme HaeIII, which should not cut within the TRA, and electrophoresed on a conventional 0.8% agarose gel. Following Southern transfer, hybridization with a $(TTAGGG)_4$ probe showed that, in all cases, with the exception of *Mus caroli* and *Mus spretus*, the TRA's ran as an unresolved band > 23 kb (Fig 1). The telomeres from *Mus caroli* and *Mus spretus* were very much smaller, ranging in size from 20−30 kb and 5−20 kb respectively.

To determine the size of the TRA's in *Mus musculus*, DNA from the inbred strains C57BL/6 and DBA/2 was digested with a series of 4bp-cutting restriction enzymes and subject to PFGE. Hybridisation to the $(TTAGGG)_4$ probe revealed a series of discrete bands in DBA/2 mice ranging in size from 50−150 kb (Fig 2). The majority of the probe hybridized to a complex mixture of poorly resolved fragments between 30−50 kb. An identical pattern of fragments was observed with several different 4bp-cutting restriction enzymes (data not shown); this is to be expected if the telomeres are composed mainly of TTAGGG stretches which contain no sites for any of these enzymes. Fewer bands were observed in C57BL/6 animals in the size range > 50 kb.

The high molecular weight fragments are terminal and behave as highly variant Mendelian loci

It seemed highly likely that these very long fragments hybridizing to $(TTAGGG)_4$ were terminal fragments; also the discrete nature of each band suggested to us that it derived from an individual chromosome end. To confirm these suspicions, we carried out both biochemical and genetic analysis.

If these long fragments derive from individual chromosome ends they might be expected to segregate as single genetic loci in families. Thus we followed the segregation of these TRA bands

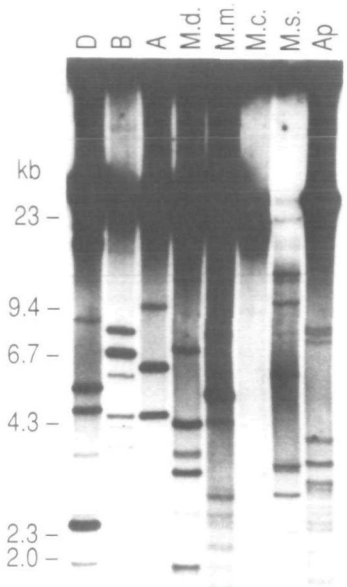


Figure 1. Analysis of TRAs in different mice strains and species by conventional gel electrophoresis. 10 μg samples of DBA/6 (D), C57BL/6 (B), Akr/J (A), *Mus domesticus* (M.d.), *Mus musculus* (M.m.), *Mus caroli* (M.c.), *Mus spretus* (M.s.), and *Apodemus sylvaticus* (Ap) DNA were digested with HaeIII, electrophoresed through a 0.8% agarose gel and transfered to a nylon filter. The filter was then hybridised with $(TTAGGG)_4$ as described in the materials and methods. HindIII digest of lambda DNA is indicated as size marker (kb).

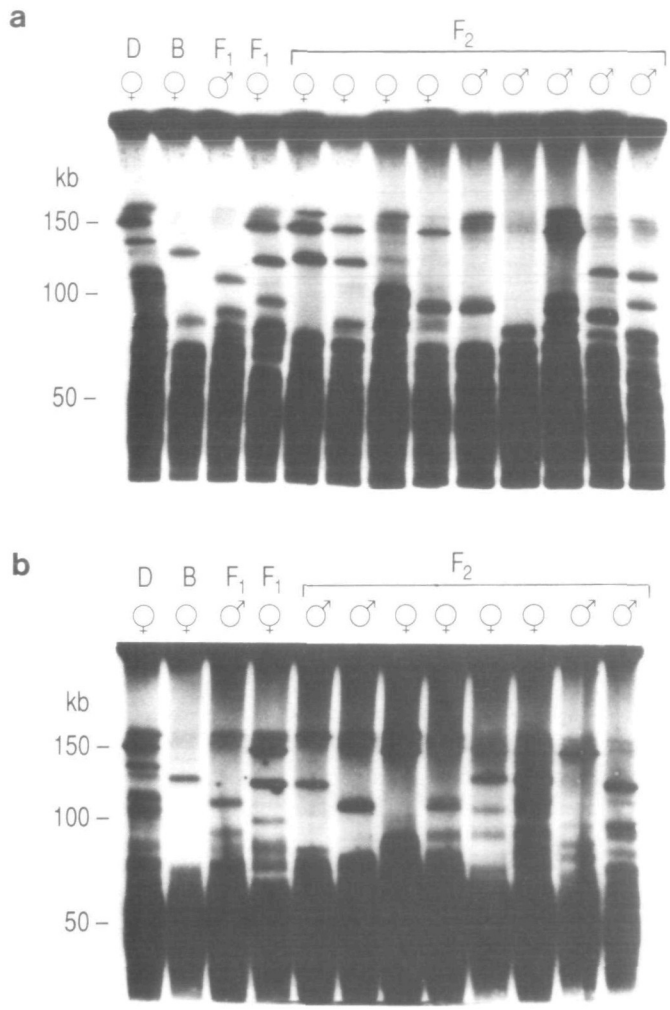


Figure 2. Analysis by PFGE of the inheritance of mouse TRAs. (a) and (b) Cross strain mating between a DBA/2 mouse (D) and a C57BL/6 (B) mouse. From the resultant F1 progeny a brother × sister pair was set up to generate the F2 offspring. (a) and (b) show the same initial DBA/2 and C57BL/6 pair and the same F1 brother×sister pair but 2 sets of F2 offspring respectively. 10 μg of total genomic spleen DNA from each mouse was digested with HaeIII. The restricted fragments separated by PFGE, transferred to a nylon filter and hybridized with $(TTAGGG)_4$ as described in the materials and methods. Lambda concatemer size markers are indicated (kb).

in several generation crosses between C57BL/6 and DBA/2 inbred strains. As shown in Figure 2 (a and b) the sibling F1's were not identical, inheriting a different combination of bands from their parents. Not one of the parental fragments was found in all the F1's (only four shown). Each F1 received about half of the DBA parent's bands. The most plausible explanation for these observations is that the 2 allelic terminal restriction fragments for any particular chromosome differ in size even in inbred mice. For this to be the case new length variants would have to be generated in the germ line at high frequency. It is possible to see new size bands in the F2 generation upon careful examination of the data but these are very close in size to bands inherited from the parents. The analysis is more difficult by PFGE than for small fragments on conventional gels. A more clear-cut case of a new variant observed in the F1 generation is shown below. It was quite easy to follow the stable segregation of most bands through several generations to approximately 50% of offspring as expected for a single genetic locus (Fig 2a and b). All possible combinations of DBA/2 fragments were found in the F1 generation so none of these bands is allelic with another. We must assume that the alleles of these termini are within the 30–50 kb range.

To test the terminal nature of the fragments the DNA was treated with the exonuclease Bal31 for various lengths of time prior to digestion with the restriction enzyme. As shown in Figure 3 these very long fragments become progressively smaller with increasing duration of Bal31 digestion. The discrete high molecular weight fragments had reduced in size by 30–40 kb by the final time point. The same can be said for the bulk of the telomeres represented by the 30–50 kb smear, also there is a considerable reduction in signal strength within the smear by the final time point. The rate of reduction in the length of the TRA's is approximately 30 kb in 90 minutes using 1 unit of Bal31 per 5 μgs of DNA. This compares favourably with our previous experiments showing a rate of loss of 15 kb in 90 minutes for human telomeres using a lower concentration of Bal31 (1 unit per 13.3 μgs of DNA)(18). The progressive decrease in size of these larger fragments with time and with the expected kinetics argues against non-specific degradation during the Bal31 digestion. There is no ideal control to test for degradation over this size range. However, the smear hybridising to a mouse satellite probe and extending up to 70 kb was not reduced in size and intensity during the course of the Bal31 digestion (Fig 3b).

Given the rate of acquisition of new variants and heterozygosity of these fragments we should expect that different colonies of the same inbred strain and even members of the same colony would show different patterns; this was indeed found to be the

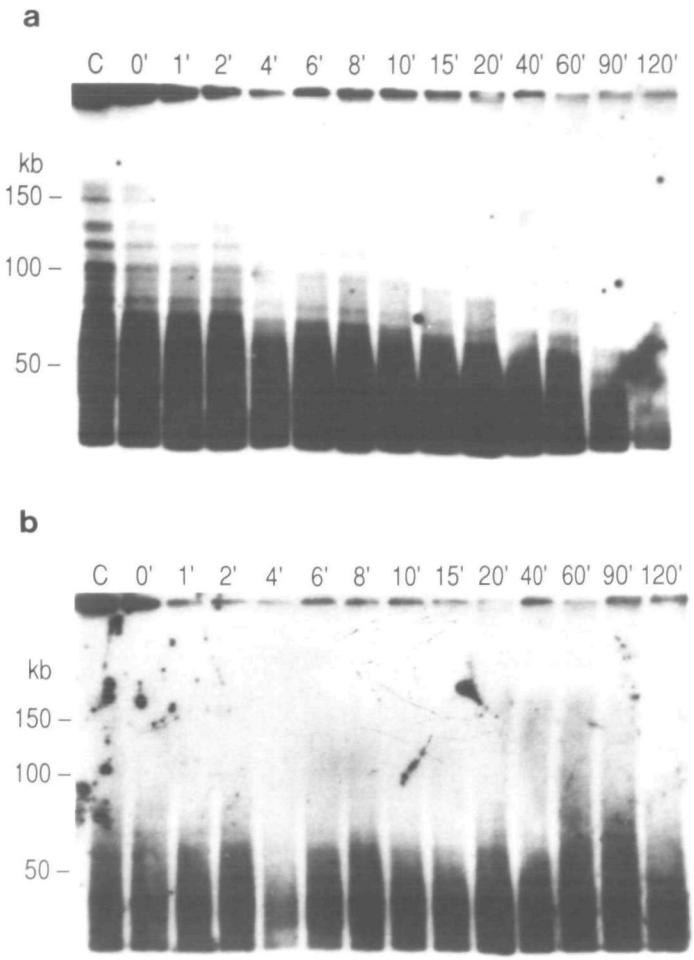


Figure 3. Bal31 sensitivity of mouse TRAs. The Bal31 time course was performed on total genomic spleen DNA from a DBA/2 mouse as described in the materials and methods. 10 μg of DNA from each time point was digested with HaeIII. The restricted fragments were separated by PFGE transferred to a nylon filter and hybridized with (a) $(TTAGGG)_4$ and (b) the mouse 196 major satellite as described in the materials and methods. Between the two hybridizations the filter was boiled for 10 minutes in distilled water. Track C refers to the starting DBA/2 DNA digested with HaeIII. 0', 1' etc refer to the time of exposure to Bal31. Lambda concatemer DNA size markers are indicated (kb).

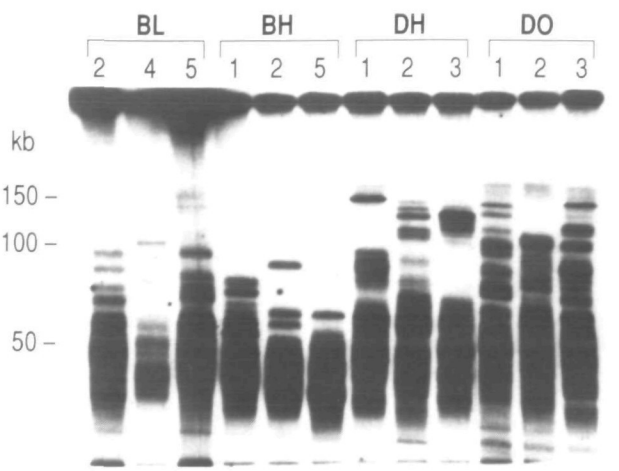


Figure 4. PFGE comparing TRAs of different individuals of C57BL/6 and DBA/2 mouse strains from different inbred colonies. Total genomic DNA from each individual was digested with HaeIII, subjected to PFGE, transferred to a nylon filter and hybridized with $(TTAGGG)_4$ as described in the materials and methods. B refers to the C57BL/6 and D to the DBA/2 mouse strains. L refers to a local inbred colony of mice, H to colonies in Hull and O to a colony in Oxford. The numbers above each lane refer to the number given to each individual mouse received at different times and bear no relation to other mice of the same number from different colonies. Lambda concatemer DNA size markers are indicated (kb).

case as shown in Figure 4 but it was also clear that the mice in the 2 different DBA colonies (one maintained in Hull, the other in Oxford) were more similar to each other in banding pattern than they were to any of the C57BL/6 mice, whose fragments were smaller.

MnlI releases small highly variable fragments from some telomeres

No restriction enzymes will cleave tandem arrays of TTAGGG. However, the type IIS restriction enzyme MnlI, which recognises the nonpallindromic sequence $5'(N)_7GAGG3'$, will cut within the TRA where there is a single T-G transition, caused either by occasional misincorporation by the telomerase or by mutation, resulting in a TGAGGG repeat. We demonstrated previously that a cluster of MnlI sites resides within the proximal end of human TRA's (23). Figure 5 shows PFGE analysis of DBA/2,C57BL/6 and F1's DNA cut with HaeIII or MnlI and probed with $(TTAGGG)_4$. The very large fragments of 50 kb and greater seen with HaeIII (and other 4bp-cutting restriction enzymes tested) were not observed upon MnlI digestion. Also, there has been a shift in the size of the smear to a lower molecular weight range. The most likely exphination is that MnlI is cutting at least once within most telomeres. If this were the case we might expect that small MnlI fragments released from some of the TRA's could now be resolved on conventional agarose gels. This was found to be the case. As shown in Figure 6 fragments ranging in size from 1–12 kb were observed when DNA was cut with MnlI and probed with $(TTAGGG)_4$. All the bands were variant between DBA/2 and C57BL/6 animals. The cluster of bands between 1 and 3 kb long behaved like most other genetic loci in the F1 generation; that is, all the bands of both parents were represented in all the F1's. However some bands from 4 kb and upwards are behaving quite differently. These bands were found in only a proportion of F1's, indicating that like the entire TRA'S, they are heterozygous even in inbred mice and therefore segregate in the F1 generation. We have observed bands of 1–10 kb hybridizing to the $(TTAGGG)_4$ probe with a variety of 4bp-cutting restriction enzymes, including HaeIII, as shown in Figure 6a. These HaeIII banding patterns differed between inbred strains but not between individuals within a strain (data not shown). Also, unlike the MnlI fragments of 4 kb and upwards (Fig. 6b), all of the bands from both parents were detected in all F1 progeny suggesting that the inbred mice are homozygous at these loci (Fig 6a).

To explain the degree of heterozygosity of the MnlI fragments >4 kb in size new variants must be generated at high frequency. In support of this expectation a new variant of ~12 kb can be seen in one of the F1's (Fig 6b). The signal strength for this new fragment is less than expected in relation to the smaller fragments. This could either mean that the band is not represented in all spleen cells, and hence is a somatic variant; or that it does not comprise TTAGGG alone or that the repeats are degenerate. Provocatively, the mouse with this ~12 kb variant was the F1 in which a new TRA length variant of 140 kb was seen, raising the possibility that they have arisen on the same chromosome and by the same process (Fig 5a). Therefore, we next tested

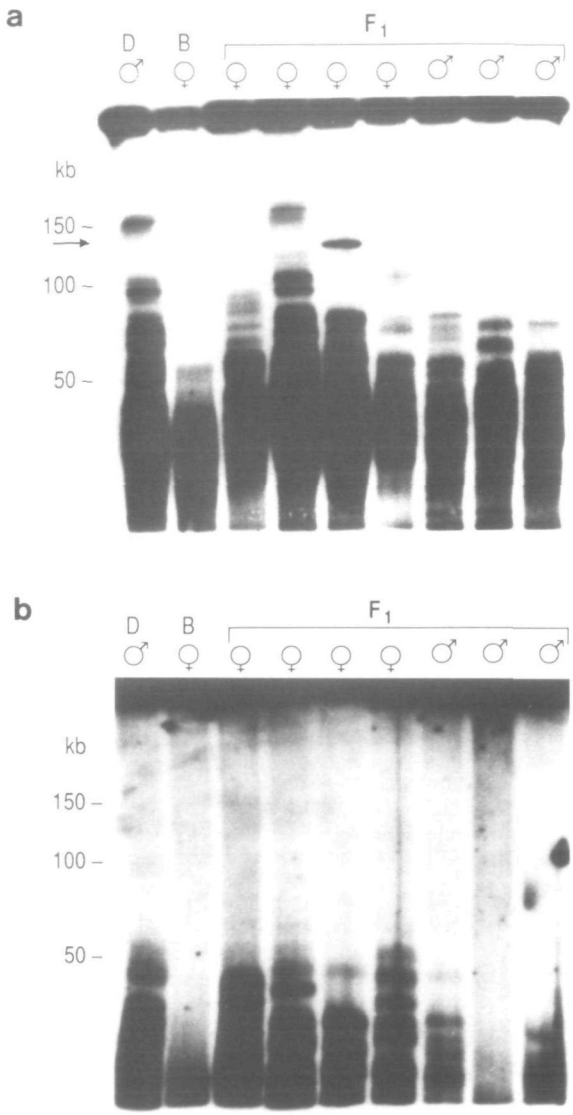


Figure 5. PFGE analysis of TRAs following digestion with (a) HaeIII and (b) MnlI. The C57BL/6 and DBA/2 individuals were different from those used previously. Total genomic spleen DNA of the above and their F1 offspring were digested with (a) HaeIII and (b) MnlI. The restricted fragments were separated by PFGE, transferred to a nylon filter and hybridized as described in the materials and methods. Lambda concatemer DNA size markers are indicated (kb).

whether the 140 kb TRA length variant and the 12 kb MnlI variant could be co-transmitted through the germ line to the next generation. As shown in Figures 7a and b both these variants were indeed co-transmitted to 7 out of 10 F2 mice. It is not clear whether the signal strength with the 12 kb variants is lower than expected relative to other fragments. Sequencing will be necessary to determine whether this fragment diverges from the $(TTAGGG)_n$ pattern.

The very high rate of variation of the MnlI fragments (>4kb) is likely to be due to their location within the TRA'S. To prove that these fragments do arise from the telomere array, DNA from the F1 showing the variant was first cleaved with HaeIII and then run on a conventional agarose gel (Fig 8a). DNA >23 kb was recovered from the gel and then digested with MnlI. This produced several TTAGGG-hybridising bands which corresponded in size to heterozygous bands revealed by MnlI digestion of unfractionated DNA. Also, the new variant band

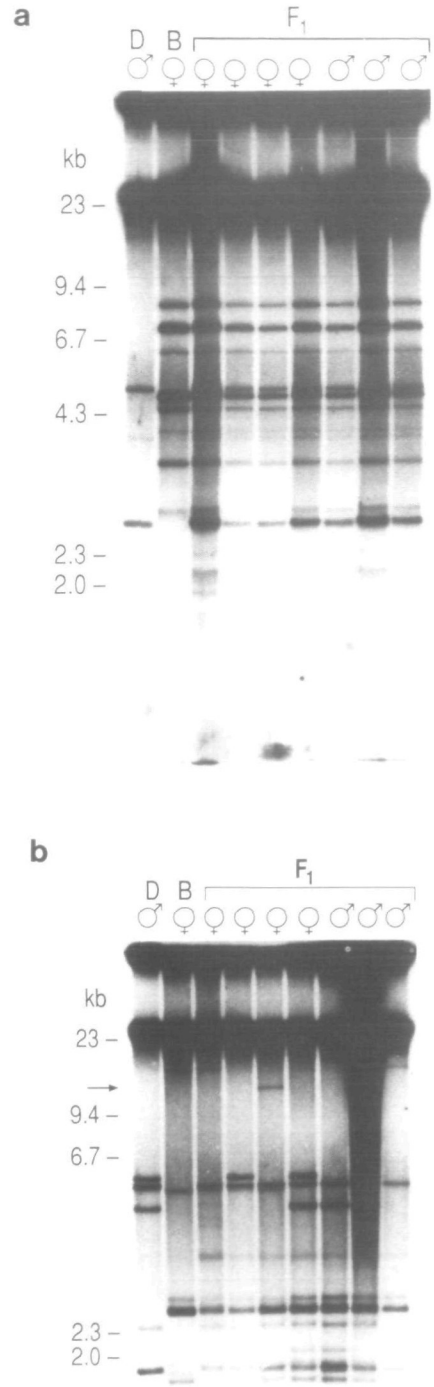
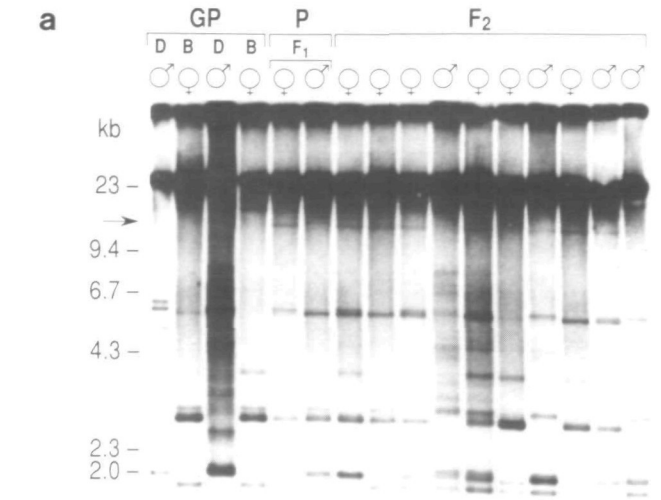


Figure 6. Comparison by conventional gel electrophoresis of TRAs following digestion with (a) HaeIII and (b) MnlI. The same set of genomic DNA's observed in Figure 5 (a) and (b) were used here. The DNA was digested with (a) HaeIII and (b) MnlI. The restricted fragments were separated by conventional gel electrophoresis, transferred to a nylon filter and hybridized with $(T7AGGG)_4$ as described in the materials and methods. HindIII digested Lambda DNA size markers are indicated (kb).

of ~12 kb was released from the TRA by MnlI digestion but it is obscured by the smear. However, the less variant bands which were homozygous in the parental inbred strains were not



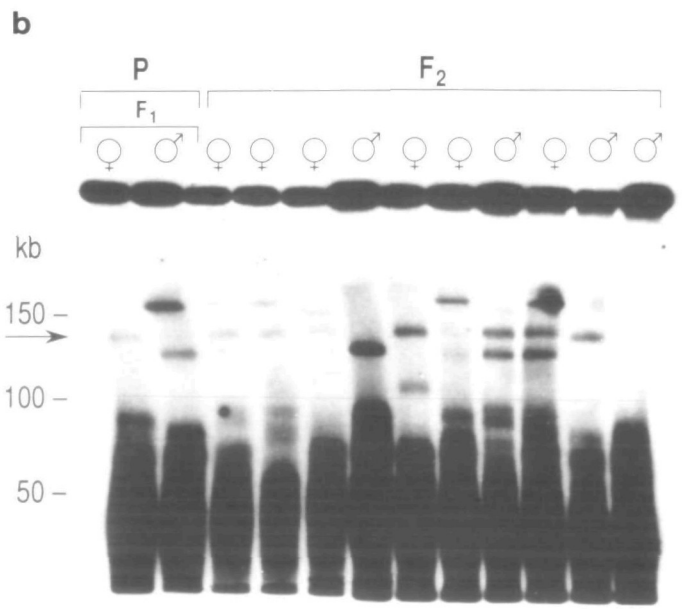


Figure 7. The newly acquired 140 kb TRA length variant and 12 kb MnlI variant are co-transmitted through the germline to the F2 generation. The female F1 mouse showing the variants (Fig 5 and 6) was crossed to another F1 mouse (from a separate C57BL/6×DBA/2 cross), generating 10 offspring. (a) 5 μgs of DNA from the grandparents, the F1's and F2's was digested with MnlI, electrophoresed on conventional agarose gels, blotted and hybridised to a $(TTAGGG)_4$ probe. (b) 10 μg of DNA from the parents an the F2 mice was digested with HaeIII and subjected to PFGE, blotted and hybridised to a $(TTAGGG)_4$ probe. The order of F2 progeny is the same as in (a). GP = grandparents, P = parents.

released from the TRA. To determine if the variant ~12 kb fragment does indeed arise from the variant 140 kb TRA fragment (Fig. 5a) the latter was purified by PFGE and then cleaved with MnlI. As shown in Figure 8b, the only specific fragment released was one of ~12 kb, corresponding to the variant fragment. The rest of the signal was in the unresolved region of >23 kb.

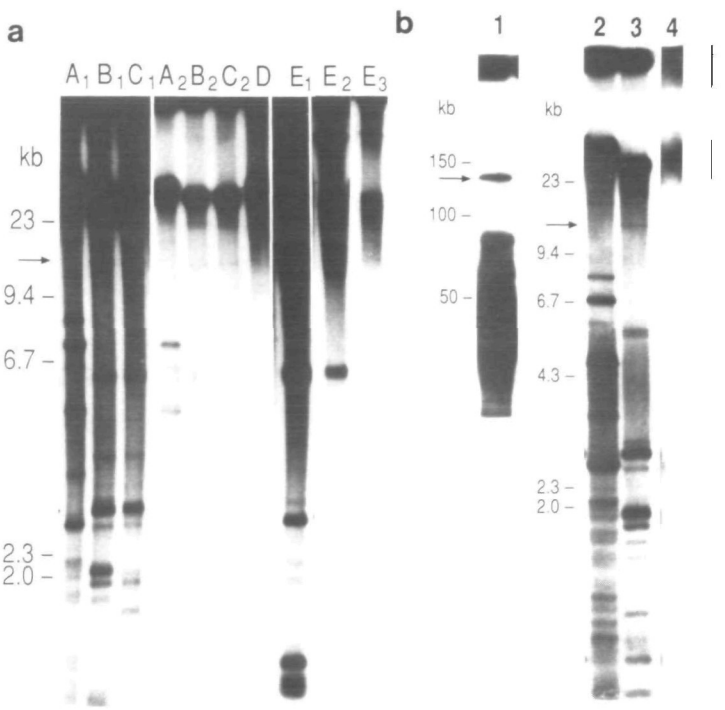


Figure 8. Determination of the origin of MnlI specific variable bands. (a) MnlI specific bands derive from the TRA smear. Total genomic spleen DNA from the F1 female shown in lane 5 of Figures 5a and 6a was initially digested with HaeIII and subjected to conventional gel electrophoresis in 1% LMP agarose gel. The >23 kb telomeric smear at the top of the track was isolated as described in the materials and methods; resultant DNA can be seen in lane D. This was then redigested with MnlI (lanes E1, E2, and E3), and subjected to conventional gel electrophoresis together with genomic DNA of the same individual digested with HaeIII (lanes A1, A2), MnlI (lanes B1 and B2) and HaeIII + MnlI (lanes C1 and C2). The separated fragments were transferred to a nylon filter and hybridized with $(TTAGGG)_4$ as described previously. The repeated lanes show different exposures of the DNA fragments produced by the stated combinations of restriction enzymes. The arrow indicates a new ~12 kb fragment produced by MnlI (see text for further details). HindIII digested lambda DNA size markers are indicated (kb). (b)The ~12 kb MnlI band derives from the 140 kb TRA length variant. Total genomic spleen DNA from the same individual as in (a) was digested with HaeIII and subjected to PFGE in 1% LMP agarose, together with controls to establish the position of the ~140 kb band arrowed (lane 1). This was cut out as a block of gel + DNA and redigested with MnlI as described in the materials and methods. The restriction fragments contained in the plug were separated by conventional gel electrophoresis (lane 4) together with genomic DNA of the same individual digested with HaeIII (lane 2) and MnlI (lane 3). The DNA was then transferred to a nylon filter and hybridized with $(TTAGGG)_4$ as described previously. The ~12 kb band only produced by MnlI and visualized by conventional gel electrophoresis is indicated (see text for further details). Lambda concatemer and HindIII digest size markers are indicated (kb).

DISCUSSION

We have shown that mouse TRA's are extremely long, that individual telomeres can be followed genetically and that new length variants arise at high frequency. They range in size from 30–150 kb, the upper limit being several fold longer than the largest human telomere and 100 fold longer than the TRA's of simple eukaryotes such as *Tetrahymena*. Do the mouse terminal restriction fragments comprise solely TTAGGG repeats or slight variations thereof or could a substantial part of these fragments consist of completely unrelated repeats which also lack sites for most restriction enzymes? A clear understanding will only come from cloning and sequencing of the terminal arrays, which is made difficult by their extreme length. Our data suggests that

mouse telomeres are composed mostly of $(TTAGGG)_n$ repeats from the following observations. Firstly, several lines of evidence support the idea that human telomere repeat arrays include 5–10 kb of monotonous TTAGGG repeats at their distal ends (18, 19, 20, 22). The average size of mouse telomeres is of the order of 40–50 kb, so if the mouse arrays comprise entirely TTAGGG motifs we should expect to see a 5–10 fold increased hybridisation signal for mouse DNA compared to the same amount of human DNA. Our unpublished experiments (JS) suggest that there is an approximately 10 fold increased hybridisation signal using mouse DNA relative to human DNA. Secondly, in the Bal31 experiments shown in Figure 3, the high molecular weight specific bands continue to hybridise to $(TTAGGG)_4$ when 30–40 kb of sequence has been removed; and there is very little reduction in the signal intensity. For the bulk of DNA in the 30–50 kb range the reduction in intensity of hybridisation signal falls off more or less in proportion to the reduction in average size of the fragments. Finally, preliminary Bal31 experiments have shown that the specific variable MnlI fragments which hybridise to $TTAGGG_4$ are not located in the distal 20–30 kb of the TRAs (data not shown). Taken together this data suggests that TTAGGG is found throughout the length of the TRA.

We and others have obtained evidence suggesting that the proximal end of the human TRAs comprise 1–2 kb of degenerate repeats such as $(TTGGGG)_n$ and $(TGAGGG)_n$ (23, 27). Given the length of the mouse TRAs we might expect that they would also have such degenerate arrays and possibly in greater amounts. However, we have not been able to detect hybridisation of radiolabelled $(TTGGGG)_4$ or $(TGAGGG)_4$ probes to mouse TRAs under conditions where they hybridise specifically to human TRAs, (data not shown). Sites for the restriction enzyme MnlI which only requires a single base change within TTAGGG appear to occur every 5–30 kb in the mouse TRA's suggesting little divergence from the consensus repeat. It appears that mouse telomeres are retaining sequence fidelity over much longer regions than those of human. We have no idea why this is the case but it may relate to the frequency of generation of new variants which could maintain these repeats.

It is not clear why mice should have such large telomeres; it does not seem to be essential as two species, *Mus caroli* and *Mus spretus* have TRA's similar in size to those found in humans. It is interesting that all the telomeres of *Mus spretus* appear to be much smaller than those of *Mus musculus*. Is this due to a difference in some aspect of the telomerase apparatus between the two species? It may be possible to address this question through the analyses of crosses between the two species.

Recently it has been shown that there is reduction in TRA length with passage number of human fibroblasts *in vitro* and that cells in a senescent population may lack telomeres at some ends altogether (28). Thus *in vitro*, telomere loss may play a role in senescence, a scenario for which there is evidence in *S. cerevisae* and *Tetrahymena* (29, 30). Some of the mice we have been studying are old in mouse terms, one and a half years, yet they still have TRA's greater than 30 kb in all tissues studied (data not shown). In humans, telomeres shorten with age at a rate of 100 bp per year (31), hence, it is conceivable that the same is happening in the mouse, but the removal of a few 100 bps of terminal DNA during its lifetime would not be detectable.

The most striking aspect of mouse telomeres is their variability. We observed new variants at a minimum rate of 2 in 10 mice in our breeding experiments. If we score this as 2 out of 4 bands clearly analysable in our experiments and assume the rate is the same for all 40 ends, we come to a rate of 1 new variant being generated per end per 20 mice. A similar rate was observed for the specific MnlI fragments contained within the TRA. This is similar to the frequency for the most hypervariable minisatellite described (32). What is the mechanism behind this variation? We must take into account the fact that although the band patterns are different in mice within the same colony and between different DBA colonies, the patterns of the different DBA mice are much more similar to each other than they are to C57BL/6 animals which consistently have fewer bands in the higher molecular weight range (refer to Figure 4). Also new variants are close in size to prexisting bands. This suggests to us that new variants are close in size to the fragments from which they arise; although the new variant of 140 kb shown in Figure 5 must have arisen from a fragment of 100 kb or less suggesting that a 40 kb addition can take place.

Several alternative mechanisms for generating this variation are conceivable. The first could be a recombination/conversion process between sister chromatids or between chromosomes as has been shown for TRA's on plasmids in *S. cerevisae* (15, 16, 17). The very long stretches of TTAGGG may be prone to recombination as proposed by Sen and Gilbert (5). *Tetrahymena* telomeres have nicks and gaps (Reviewed in 1). If such features were found in mouse telomeres it is conceivable that they could promote strand invasion to initiate recombination. A second mechanism could be slippage during conventional replication of the TRA's. A third could be telomere reduction followed by de novo addition by telomerase or vice versa; such expansion and contraction events have been observed for telomeres in *Trypanosoma* and *Tetrahymena* (33, 34). We cannot distinguish between these possibilities. It is interesting though that variants of the MnlI fragments of 4 kb and above within the TRA are arising at about the same frequency as the whole TRA length variants. Elsewhere we will describe how TTAGGG stretches of similar length, but outside and proximal to the TRA generate variants at a frequency of less than 1 in 200 mice. These are typified by the HaeIII bands shown in Figure 6a. It is tempting to speculate that the process which generates variants in the whole TRA simultaneously generates the variants of the MnlI fragments. In fact, we have shown that one MnlI variant of ~12 kb arises within and simultaneously with a 140 kb TRA length variant, supporting this notion. There is only a 1 in 20 probability that both these should arise together independently. In fact the chance of any one of the MnlI fragments of 5–10 kb generating a new variant is close to that for the TRA's, supporting the idea that they arise by the same process. Thus, a process which generates a change in the length of a whole TRA may simultaneously alter the size of an MnlI fragment which makes up only 10% of the TRA length. Again, this could arise through recombination, de novo addition of repeats or a combination of both. Additional experiments will be required to distinguish between these possibilities.

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