Multiple Deletions Are Detectable in Mitochondrial DNA of Aging Mice*

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Mammalian mitochondrial DNA (mtDNA) is a closed circular DNA molecule of approximately 16 kb; several thousand mtDNA molecules are present in the average somatic cell. Deletions in human mtDNA have been associated with several mitochondrial disorders, including Kearns-Sayre syndrome (KSS), progressive external ophthalmoplegia, and Pearson’s marrow-pancreas syndrome (1–3; for review see Ref. 4). These mtDNA deletions vary in size but usually delete genes encoding proteins essential for oxidative phosphorylation as well as tRNAs and rRNAs needed for their synthesis. Specific deletion events have been characterized in a number of patients. The most common KSS deletion (4977 kb) is flanked by a 13-base pair direct repeat (5, 6). Other deletions are also flanked by exact or inexact direct repeats. Deletion junctions may occur precisely at repeat boundaries or some distance away (7–9). The severity of symptoms in KSS patients seems to depend in part on the ratio of normal to deleted mtDNA molecules, with affected individuals often containing more than 50% deleted mtDNA molecules (10).

Recent studies found the 4977-bp KSS mtDNA deletion at very low levels in normal human tissues; the levels seemed to increase with increasing age of the source (11–15). Other mtDNA deletions have also been detected in elderly human patients (16, 17). Estimates of the common KSS deletion in the elderly range from 0.007 to 0.1% (heart) (11, 13), 0.001 to 12% (brain) (18, 19), to 0.02 to 0.1% (muscle) (14, 20). Differences between individuals and in measurement methods may account for some of the variability; in addition, significant regional variation was seen in brain. The low levels seen in aged individuals, compared to the much higher amounts in affected patients, make the role of mtDNA deletions in the aging process unclear. Using a PCR approach, we detected deletions in the mtDNA of aged mice; their location and abundance were predicted from the presence of direct DNA repeats in the mtDNA sequence. The observation of mtDNA deletions in mice makes possible a systematic examination of these deletions and their physiological significance.

MATERIALS AND METHODS

DNA Isolation, Sequencing, and Cloning of PCR Products—Tissue samples from 13 aged (18.5–27 months old) and 12 young (6 week old) C57 or NMRI mice were homogenized, digested with proteinase K in 0.5% SDS, and total DNA purified by phenol-chloroform extraction and ethanol precipitation (21). PCR products were purified using QiAquick PCR purification columns (Qiagen) and sequenced using a “fmo” DNA Sequencing System (Promega). Individual deletion products were cloned into a pGEM-T vector (Promega) and the plasmid DNA sequenced.

Identification and Evaluation of DNA Direct Repeats—Direct repeats in the mouse and rat mtDNA sequences were found using the Genetics Computer Group, Inc. program Repeat (22–24). Initial selection was by GC content and the distance between repeats. The relative stability ($\Delta G_{29}^\circ$) of a helix formed between two direct repeats was estimated by summing the individual nearest-neighbor interactions (25, 26); $\Delta G_{29}^\circ$ for helix initiation was omitted as it is constant for each helix. The destabilizing contribution of a single-base mismatch (internal loop) in DNA and RNA model oligonucleotides ranges from $-$0.6 to +2.1 kcal/mol; it depends on both the mismatched base and surrounding base pairs (26–28). Because values have not been determined for any of the mismatch/surrounding base pair examples studied here, existing data were averaged to give the $\Delta G_{29}^\circ$ of +1.2 kcal/mol used in our estimates.

PCR Conditions—PCR primers (Table I) were selected with the computer program Oligo 4.0 (National Biosciences, Inc.). Those mtDNAs containing a deletion between repeats spaced 3–5 kb apart were specifically amplified using a short PCR cycle (Taq DNA polymerase (Promega); 94°, 40 s; 50°, 20 s; 72°, 40 s; 30 cycles) to prevent synthesis of a full-length product from undeleted mtDNA. A “hot start” (29) procedure with paraffin wax (Aldrich) as a barrier was used to increase accuracy and sensitivity.

Plasmid Controls and Measurement of Deleted mtDNA Molecules—Plasmid pDM (Deleted MtDNA Mimic) is a positive control for detection and measurement of a mtDNA lacking the DNA between the “D-1” direct repeats (Table II). pDM contains mouse mtDNA sequences (from 8420 to 9226 joined to 12,932–14,251) inserted into pBS (Stratagene). The PCR product amplified from pDM is 142 bp longer than from mtDNA with a D-1 deletion.
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Detection of Deletions in Mouse mtDNA—The mtDNA deletions observed in patients with certain mitochondrial disorders or at low levels in older humans are often associated with direct sequence repeats. We searched mouse and rat mtDNA (22, 23) for direct repeats; numerous pairs of exact (or inexact) repeats (12 nucleotides or longer) are present. Several repeat pairs (Table I!) were selected that: 1) were relatively G/C-rich, 2) did not span either the light or heavy strand origins of mtDNA replication, and 3) were sufficiently distant that a deleted mtDNA could be distinguished from an intact molecule by a short-cycle PCR protocol (11). The positions of these repeats in relation to important features of the mouse mtDNA genome are shown in Fig. 1.

Mouse mtDNA repeat D-1 is the longest exact match and has a high estimated stability. Relevant features of the D-1 direct repeats, several other repeat pairs, and PCR primers are shown in Tables I and II. PCR primers PL51/PL52 amplify a 4614-bp product from undeleted mtDNA under standard PCR conditions; mtDNA with a D-1 deletion yields a product of 748 bp. PCR amplification using a short PCR cycle to suppress synthesis of product from wild-type mtDNA revealed a possible D-1 deletion product in several tissues of a 19.5-month-old NMRI mouse (Fig. 2A). Southern blotting and DNA sequencing (not shown) confirmed that the 748 bp bands are products of mtDNA with one D-1 repeat and the intervening DNA deleted. Most tissues contained deleted mtDNA. We emphasize that this result is not quantitative; both the actual amount of DNA in 1 O.D. unit of a “standard” DNA preparation (21) can vary between samples, and different tissues have different mtDNA to nuclear DNA ratios. For example, heart (Fig. 2A), appears to have low levels of the D-1 deletion. Measurement by the quantitative assay described below showed this DNA sample to be low in both amplifiable mtDNA and nuclear DNA; the actual percentage of deleted mtDNA was similar to that of lung mtDNA.

Detection of Deletions—Several other aged mice had the same relative levels of the 3867 bp D-1 mtDNA deletion seen in Fig. 2A (data not shown). Based on this result, 12 additional aged mice were examined for the presence of the 748-bp D-1 deletion product in tissues of varied embryonic origin and metabolic function (brain, heart, and liver; Fig. 1, B-D). All 13 aged mice had deleted mtDNA, although not all tissues were positive in every animal.

A similar search was made for D-1 deletions in several tissues of one young (6 week) C57 mouse (Fig. 3A) and in liver, heart, and brain DNA of 11 additional young C57 mice. In several independent PCR assays, no D-1 deletion products were seen in tissues of this one mouse nor in the other 11 heart or liver DNAs (data not shown). Faint 748 bp bands were visible in some young brain DNA samples (Fig. 3B); they hybridized to a mtDNA probe and were amplified by PCR primers internal to the original PL51/PL52 primer pair (data not shown). Occasionally, PCR amplification of other young mouse tissue DNAs showed very faint D-1 deletion bands. In these tissues, the amount of total mtDNA in young and old animals was comparable (data not shown). Estimates of deleted mtDNA amounts are discussed below.

RESULTS

Detection of Deletions in Mouse mtDNA—The mtDNA deletions observed in patients with certain mitochondrial disorders or at low levels in older humans are often associated with direct sequence repeats. We searched mouse and rat mtDNA (22, 23) for direct repeats; numerous pairs of exact (or inexact) repeats (12 nucleotides or longer) are present. Several repeat pairs (Table I!) were selected that: 1) were relatively G/C-rich, 2) did not span either the light or heavy strand origins of mtDNA replication, and 3) were sufficiently distant that a deleted mtDNA could be distinguished from an intact molecule by a short-cycle PCR protocol (11). The positions of these repeats in relation to important features of the mouse mtDNA genome are shown in Fig. 1.

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Measurement of mtDNA Deletions in Aged and Young Mice—Total mtDNA was measured by amplifying a 354-bp product from mtDNA (using primers PL47 and PL48) from 10 ng of total mouse DNA in the presence of increasing amounts of pTMM (Fig. 4A). The concentration of pTMM where the two product bands are of equal intensity (Fig. 4A, lane 5) indicates that equal numbers of the two targets were present. This amount was adjusted for size differences in the PCR products and the template molecules. Similarly, deleted mtDNA was measured by co-amplifying (using primers PL51 and PL52) 1...
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TABLE I

| Primers | Positions | Primers | Positions |
|---------|-----------|---------|-----------|
| PL48/PL47 | 645(21)/999(21) | PL77/PL78 | 7586(20)/13352(20) |
| PL51/PL52 | 8484(21)/13098(21) | PL79/PL80 | 11491(21)/15188(21) |
| PL66/PL67 | 7916(21)/13410(21) | PL84/PL83 | 11619(21)/15077(20) |
| PL69/PL68 | 8265(20)/13375(20) | PL85/PL86 | 979(20)/5650(20) |
| PL69/PL70 | 8265(20)/13684(20) | PL87/PL88 | 1903(20)/3699(20) |
| PL71/PL76 | 7586(20)/13263(20) | PL88/PL89 | 979(20)/5650(20) |

Compatible PCR primer pairs listed, L strand primer first.
Positions: primer 5’ end is listed; oligonucleotide length is in parenthesis.
PL66/PL67 was used for rat mtDNA; PL66 also hybridizes to mouse mtDNA at 7939, with 1 mismatch.

TABLE II

| DNA repeat | Sequence | Positionsa | Deletion sizeb | −ΔG°c | PCR primers | Product size |
|------------|----------|------------|----------------|-------|-------------|--------------|
| D-1        | AGCCCTACTAATTAC | 9,089–9,103 | 3,867 | 24.7 | PL51/PL52 | 748 |
|            | 12,956–12,970 | 4,236 | 23.6 | PL68/PL69 | 1,244 |
| D-2        | TCTTTGCAGGATT | 8,844–8,896 | 4,236 | 23.6 | PL68/PL69 | (875)d |
|            | 13,120–13,132 | 4,742 | 24.3 | PL68/PL69 | 1,385 |
| D-3        | CTCCTGCTTACAT | 9,553–9,566 | 3,726 | 24.3 | PL68/PL69 | 1,385 |
| D-13       | CTCCTGCTTACAT | 8,677–8,691 | 4,974 | 23.0 | PL69/PL70 | 445 |
| D-14       | CAAATGTTACCTCCAATCG | 7,964–7,984 | 5,252 | 23.1 | PL51/PL52 | 748 |
|            | 12,956–12,970 | 4,236 | 23.6 | PL68/PL69 | 1,244 |
| D-15       | TACCTGCTACATTTTCC | 11,881–11,901 | 2,976 | 23.1 | PL51/PL52 | 748 |
|            | 14,857–14,877 | 4,567 | 24.3 | PL51/PL52 | 748 |
| D-16       | ACTATGCTGCTACCTGCC | 1,094–1,113 | 3,821 | 27.4 | PL51/PL52 | 748 |
|           | 4,915–4,934 | 5,022 | 25.2 | PL51/PL52 | 748 |
| D-17       | AGAACCTCCAGCTTGCTAGG | 2,979–3,008 | 651 | 19.6 | PL51/PL52 | 748 |
|            | 3,630–3,659 | 661 | 19.6 | PL51/PL52 | 748 |
| Rat        | CCTAGCTCCATGGAATTTAC | 8,103–8,118 | 4,834 | 29.3 | PL51/PL52 | 748 |
|            | 12,937–12,952 | 661 | 19.6 | PL51/PL52 | 748 |
| Human      | ACCTCCCTCACAACCA | 8,470–8,482 | 4,977 | 25.2 | PL51/PL52 | 748 |
|           | 13,447–13,459 | 661 | 19.6 | PL51/PL52 | 748 |

a Coordinates in mtDNA (22, 23).

b Base pairs lost if one repeat and intervening sequence are deleted.
c Relative stability, in kcal/mol, calculated from nearest-neighbor parameters.
d Predicted product not observed.

Fig. 2. PCR detection of deletion D-1 in mtDNA of aged mice. A, PCR amplification of mtDNA from tissues of one aged mouse. B–D, PCR amplification of mtDNA from the liver (B), brain (C), and heart (D) of 13 aged mice. Each reaction contained 1.0 μg of total DNA template, primers PL51/PL52. Arrows indicate the 748-bp PCR product amplified from deleted mtDNA. Ta = tail; Sk = skin; Li = liver; Br = brain; He = heart; Sp = spleen; Lu = lung; Ki = kidney; FO = skeletal muscle (forequarter); HO = skeletal muscle (hindquarter); 1–13 = individual mice. Lanes marked 10 and 100 contain PCR reactions with 10 and 100 fg of pDMM template as a positive control. Lane C is a control PCR (no DNA template); lane M is an Alu I digest of pBR322 (910, 659, 656, 521, 403, and 281–226 bp). The high molecular weight band in some reactions in panels B–D comes from one DNA stock used as a diluent (see “Materials and Methods”).

μg of mouse DNA in the presence of increasing amounts of pDMM (Fig. 4B). Because the amount of DNA (measured by absorption at 260 nm) in PCR reactions may vary and because the ratio of defective to normal molecules is critical for oxidative phosphorylation potential, the percentage of deleted molecules rather than the amount of deletions in a sample was
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The percentage of the D-1 (3867 bp) deletion in total mtDNA was determined for the tissue DNAs in Fig. 2A, the liver DNAs in Fig. 2B, and for representative brain (Fig. 2C, animals 2, 5, and 12) and heart DNAs (Fig. 2D, animals 1, 2, and 12). For the NMRI mouse shown in Fig. 2A, deleted mtDNA was most abundant in the liver (3.3 \times 10^{-3\%}), kidney (9.25 \times 10^{-4\%}) and lung (5 \times 10^{-4\%}), and less abundant in heart (3.3 \times 10^{-4\%}), skeletal muscle (2 samples, 1.33 \times 10^{-4\%} and 5 \times 10^{-5\%}) and brain (5.8 \times 10^{-5\%}). Although the brain DNA sample had an abundance of deleted molecules, the percentage was low because the brain is rich in mtDNA; this was seen in all brain samples examined. Deleted mtDNAs were too low to measure in tail, skin, and spleen. These same relative levels were also seen in the other animals. Deleted mtDNA was always most abundant in the liver, ranging from 3.3 \times 10^{-3\%} to 6 \times 10^{-2\%}. It was less abundant in the heart (1.8 \times 10^{-4\%} to 3.3 \times 10^{-4\%}) and brain (3.5 \times 10^{-3\%} to 8 \times 10^{-5\%}).

The small amount of deleted mtDNA detected in brain DNA of some young animals was approximately 0.5–2.0 fg, near the detection limit of our PCR assay (the amount in old brain was typically 10-fold higher). The detection limit was determined by measuring the amount of deleted mtDNA in liver DNA of an old mouse and serially diluting this DNA 1:1 with liver DNA from a young mouse (where deletion products were not visible). The minimum detectable level of deleted mtDNA is 0.5–1.0 fg, against a “background” of 1 \mu g of total mouse DNA. We can detect lower levels of plasmid controls in reactions that lack a total DNA background. Primes may possibly bind to either nuclear DNA or unedited mtDNA, effectively reducing the amount available to amplify deleted mtDNA (increasing primer concentration increases non-mtDNA artifact bands).

Presence of Other Deletions in Mouse mtDNA—Other repeated sequences that might produce deletions are present in mouse mtDNA. Relative positions of the repeats are shown in Fig. 1. The potential deletion(s), primes, and PCR product size(s) derived from deleted mtDNA molecules are shown in Table II. We used primer pair PL68/PL69 (Tables I and II) to search for deletions in the liver mtDNA of the same 13 old mice (Fig. 5A). Primers PL68 and PL69 flank at least three repeat pairs (Table II); we predicted products of 875 bp (D-2 repeats), 1244 bp (D-1 repeat), and 1385 bp (D-3 repeat). Multiple bands are visible. Southern blotting and DNA sequencing confirmed the D-1 and D-3 deletion products of 1244 and 1385 bp were present (data not shown). The 875-bp D-2 deletion product was not detectable and could not be detected even after 60 PCR cycles. A band of 675 bp also hybridized with the mtDNA probe; DNA sequencing showed this band resulted from a PCR false priming event with PL69 at position 12711–12720 in mouse mtDNA, a nine out of 10 match occurs at the 3’ end. The remaining bands visible in Fig. 4A were not of mitochondrial origin.

MtDNA deletions may also occur in inexact DNA repeats, i.e., direct repeats with one or more mismatches between them (D-13 to D-18 in Table II). Primer pair PL69/PL70 amplifies a 445-bp product from mtDNA with a D-13 deletion. This product was visible in liver DNA from nine of 13 animals after 30 PCR cycles (Fig. 5B). A second inexact deletion, D-14, was not seen with a single PCR amplification using PL77/PL78 but was easily detectable after a second round of amplification with internal PCR primers (data not shown). Deletions arising from the D-16 repeat have never been detected. All of these deletions occur within base pairs 5,192–15,417 of mtDNA, the region first replicated by the newly initiated heavy strand as it proceeds toward the light strand origin (the “major” region). Direct repeat sequences also exist in the 5191 bp replicated subsequently (the “minor” region, from the light strand origin returning to the heavy strand origin). Two pairs of repeated sequences (D-17, D-18, Table II) within the minor region were examined as potential inducers of deletions. An abundant deletion product was observed for D-17 (Fig. 5C) and confirmed by DNA sequencing. The second pair of direct repeats, D-18, does not seem to induce deletions (see below).

We also searched for D-3, D-13, and D-17 deletions in brain and heart DNA of six old mice and brain and liver DNA of six young mice. These deletions were present in both tissues of old mice, with D-1 and D17 again being the most visible. In contrast, only a few young mice had barely detectable levels of the D-17 deletion in liver and brain DNAs (data not shown).

Finally, to examine the generality of our predictions, we...
Deletions in mtDNA are responsible for a number of human genetic disorders, primarily but not exclusively affecting muscle (1–4). Important questions regarding the involvement of mtDNA deletions in degenerative diseases and aging, i.e. the tissue and cellular distribution of deletions, the kinetics of their appearance, and the underlying mechanisms, are difficult to approach in human subjects. Although speculation is common in the literature that damage to mtDNA is an important factor in aging (32–36), critical proof for a direct relationship is lacking. A decrease in mtDNA integrity could cause an overall decline in cell and tissue function with age. Alternatively, the increased level of mtDNA deletions and point mutations seen in elderly humans may simply reflect an accumulation of repair and replication errors occurring over time, with little relevance to the aging process.

Limited direct evidence exists to support a role for mtDNA damage in aging. While deleted mtDNA molecules are present in aged individuals, they are uncommon, with a single deletion generally constituting much less than 0.1% of total mtDNA. In contrast, human Kearns-Sayre patients have at least 20–80% deleted mtDNA molecules before symptoms of myopathy are seen (10). However, the total damage to mtDNA during aging is difficult to estimate, as many mtDNA deletions and point mutations are possible and could accumulate with age (37). Mouse models suitable for aging studies have been described (38, 39); inbred mouse strains differ in inherent life span and their life span can be further modified by environmental factors such as dietary restriction (40, 41). We show here that it is possible to predict the presence and position of multiple deletions in the mtDNA of aged mice. Knowing the position of such frequently occurring mtDNA deletions will allow accurate measurements of their accumulation with time in these animals and make possible a critical test of their potential role in aging.

Six different deletions were observed in mtDNA of aged mice and rats (Table II). The position and relative abundance of the individual deletions that we detected depend on the thermodynamic stability of direct repeats in the mtDNA sequences. Previous studies examining deletions in mouse mtDNA were not predictive (42–46). Because we searched for deletions at predicted locations, PCR primers could be designed for efficient amplification and optimal product size. This approach tends to reduce or eliminate PCR artifact bands, which is important for accurate quantitation. While this work was in progress, a similar strategy was used to detect the rat mtDNA deletion also observed here (30, 31).

Although mtDNA deletions were readily detected using PCR, the amount of deleted mtDNA present in aged mice was small. Both the absolute level and the percentage of the D-1 deletion in mtDNA of mouse tissues was quite low, ranging from a high of 0.06% (liver) to 2 \times 10^{-5}\% (brain) to undetectable (less than 0.5 fg) in individual animals. Levels of the other deletions were similar or lower, as estimated from relative band intensity and dilution experiments. Deletions were detectable in each of the 13 aged mice examined, although not every mouse was positive in every tissue for every deletion. In agreement with results in human, young mice generally lacked detectable mtDNA deletions, although low levels of some mtDNA deletion products were occasionally seen in brain samples. This is unusual because brain does not accumulate deletions to high levels in aged animals. In contrast, deletions were not detectable in livers of young mice, a tissue that contains abundant deletions in aged mice. The ratio of mitochondrial DNA to nuclear DNA in these two tissues is roughly equivalent.

There are clear differences between the results reported here for aged mice and previous reports from human subjects; such differences suggest that a generalized aging process involving mtDNA deletions may not exist. In mice, the deletions were most abundant in liver, moderately abundant in kidney and lung, and either low or absent in brain, heart, muscle, tail,
skin, and blood. This distribution differs from aged humans where deletions are reportedly most abundant in brain, muscle, and heart, and less abundant in liver (14, 34). Different mtDNA measurement methods and individual diversity among the small number of elderly humans examined may account for some, but not all, of the discrepancy. This distribution difference, as well as the differential accumulation with age noted above for brain and liver, while not completely inconsistent with an involvement of mtDNA deletions in aging, does indicate that differences in induction and accumulation do exist between tissues and between species. It has been hypothesized, based on the human data, that mtDNA deletions are most abundant in tissues with high metabolic and low mitotic activity (19, 47), a suggestion that now appears inaccurate. Most tissues in the adult have low mitotic activity; for example, neither neurons nor hepatocytes divide. More importantly, brain and liver have large and equal oxygen consumption rates, the highest among vertebrate tissues (48). The two diseases do differ markedly in their relative sensitivity to anoxia, but the difference results from the presence of stored substrates for ATP production in liver, not a lower metabolic rate in liver than in brain mitochondria. Thus, on the basis of overall mitochondrial function as measured by oxygen consumption, brain and liver are both metabolically active and mitotically quiet, and would be expected to have high deletion levels, yet they differ dramatically. The observed differences in mtDNA deletion levels seen between tissues in mice and humans suggests that environmental and metabolic differences between these species may affect deletion levels more than the aging process.

The detailed mechanisms producing the deletions and the features of the direct repeats which determine the frequency of a particular mtDNA deletion are not well understood, but the thermodynamic stability of the repeat pairs examined here seems critical to their ability to cause deletions. The most stable repeats that we examined (Table II) were associated with the most abundant deletions. Deletions were detected in both the major and minor regions of the mtDNA genome. D-17, an abundant deletion, is located in the minor region. Apparently, as long as replication origins are intact, repeat stability is more important than genome position. The D-13 and D-14 repeat pairs are less stable and deletions between them are much less abundant. Three pairs of direct repeats (D-2, D-16, and D-18) never produced a detectable level of deletions. D-16 and D-18 are inexact repeats whose estimated relative stability is the lowest of those calculated. D-2 is the shortest repeat we examined (13 nucleotides) and is estimated to be only slightly more stable than the D-13 and D-14 repeats (Table II), which produce very low levels of deletions. Given the uncertainties in nearest-neighbor calculations, the agreement appears excellent. It is clearly possible that other factors, such as distance separating repeat pairs or potential secondary structure in mtDNA, might affect the efficiency of the deletion mechanism(s) and modulate the effects of repeat stability.

We emphasize that deletions may arise by more than one mechanism, some not involving direct repeats. Some studies of deletions associated with mitochondrial disorders in humans have reported associated repeats as short as four nucleotides. The repeats were often not located precisely at deletion junctions and the deletion encompassed neither, one, or both repeat copies (7–9, 16, 49). This type of deletion and associated repeat does not fit the pattern seen above. Indeed, most of these repeats are so short and inexacty spaced that their association with the deletion is likely due to chance.

Several deletion mechanisms have been proposed (3, 6–8), based on the presence of direct repeats at deletion junctions and their sequence characteristics. Sequencing of deletions involving mismatched repeats (D-13, D-14, and D-17) has revealed that deletion events often occur within the repeat rather than precisely at an end, producing a remaining “hybrid” repeat. This result, and the implications of it and secondary structure on the mechanisms responsible for mtDNA deletions, will be discussed separately.²

Finally, the deleted mtDNA molecules present in patients with mitochondrial disorders are believed to arise from a single deleted molecule present very early in development. This deleted molecule is amplified and partitioned during oogenesis and development to comprise the majority population in the affected tissue. DNA sequences of deletion junctions support this hypothesis; patients usually harbor a single deletion. In contrast, our results suggest that the deletions that accumulate in aging mice resulted from independent deletion events because multiple deletions were present in the same tissue. The ability to predict the location of mtDNA deletions in inbred mice now makes it possible to ask whether individual cells within a tissue harbor more than one deletion, how deleted mtDNAs are distributed within the cells of a single tissue, and how the kinetics and abundance of mtDNA deletions are related to aging. Answers to these questions will help us understand the physiological consequences of these deletions and determine if the pattern and rate of accumulation is consistent with a role in aging.

Acknowledgments—We thank Drs. M. S. Kilberg, A. S. Lewin, and D. N. Silverman for comments on the manuscript.

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