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Deletions of muscle mitochondrial DNA in mitochondrial myopathies: sequence analysis and possible mechanisms

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ABSTRACT

Forty per cent of patients with mitochondrial myopathies, a diverse group of multisystem diseases predominantly affecting skeletal muscle and the brain, have large deletions of a proportion of muscle mitochondrial DNA (mt DNA). These appeared to be identical in 13 of 28 cases, contained within the region 8286−13595 bp. Analysis of the deletion junction in two cases showed a 13 nucleotide sequence which occurred in the normal genome as a direct repeat flanking the region deleted in the mutant mt DNAs. Mt DNA deletions may arise from recombination or slippage between short sequence repeats during replication.

INTRODUCTION

Defects of the mitochondrial genome have recently been described in two human diseases, mitochondrial myopathies[1] and Leber's optic atrophy[2]. Human mt DNA is a circular double stranded molecule 16569 bp in length[3] which is exclusively maternally transmitted. It codes for two ribosomal RNAs, 22 tRNAs, and 13 of the 67 or so subunits of the mitochondrial respiratory chain and oxidative phosphorylation system: seven subunits of NADH CoQ reductase (complex I); cytochrome b (complex III); subunits I, II, and III of cytochrome oxidase (complex IV); and two subunits, referred to as 6 and 8, of H^+ATPase[4,5].

The mitochondrial myopathies are clinically and biochemically heterogeneous. Clinical presentations include muscle weakness, particularly involving the extraocular muscles, and multisystem neurological syndromes mainly affecting the central nervous system. In vitro studies of mitochondrial metabolism show respiratory chain defects, predominantly involving complex I, III, or IV, in most patients[6]. Large deletions of a proportion of muscle, but not leukocyte, mt DNAs have been observed in about 40 per cent of cases of mitochondrial myopathy[1,7−9]. Two patients with tandem duplications of one population of leukocyte mt DNAs have also been reported[10].

We have investigated 72 cases of mitochondrial myopathy to date, and 30 have deletions involving 20−90 per cent of muscle mt DNAs[9]. Restriction mapping in 28 cases showed that all but one of the deletions were in the region 7 000−15 000 bp[1,9]. Within the limits of this technique (+/− 100 bp), 13 appeared to be identical and were 5 kb in length, involving a minimum region of 8587−13367 bp (Rsa I and Cfo I sites lost), and upper limits of 8286−13595 bp (Xba I and Cfo I sites retained). These and other authors' data[8] suggest that there is a deletion hotspot in mt DNA. We therefore analysed the deletion junction in two cases to determine whether such deletions are identical, and if the sequence data indicate a possible mechanism for mt DNA deletions.

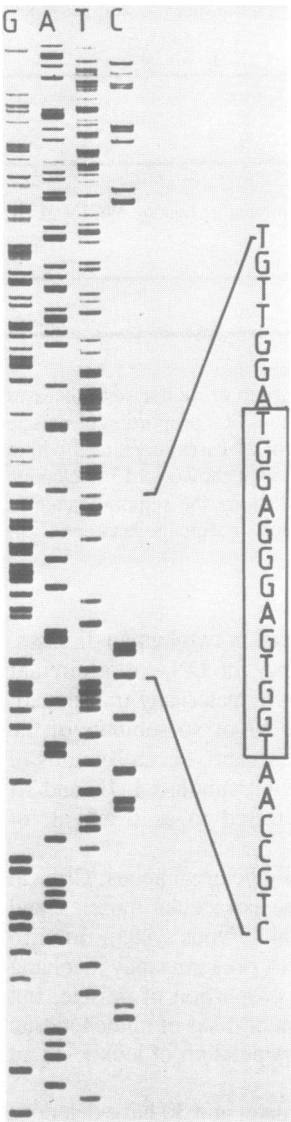

Figure 1. H strand sequence of amplified mt DNA (8172–13525 bp) from a patient with mitochondrial myopathy, showing the deletion junction. Boxed region shows residual 13 nucleotide repeat.

METHODS

Mt DNA was extracted from the muscle of two patients with apparently identical deletions as described previously[1]. The region 8171–13525 bp was amplified by means of the polymerase chain reaction (PCR)[11], using thermostable Taq polymerase (Perkin Elmer Cetus), primers TGCTCTGAAATCTGTGGAGC (8171–8190) and CGATGATGTGGTCTTTGGAG (13525–13506), and a Hybaid Intelligent Heating Block. DNA was amplified in 36 three step cycles; denaturation (91.6°C, 80 seconds), annealing

(56°C, 100 seconds) and extension (71.5°C, 120 seconds). The amplified DNA was purified and concentrated prior to filling in ragged ends with dNTPs using 1 unit Klenow at 37°C for 30 minutes. Blunt ended ligation into Sma I cut M13mp18 was mediated by T4 DNA ligase (Amersham) at 17°C overnight. The ligation reaction was transformed into $CaCl_2$ treated E. coli JM 109 cells; clear colonies were cultured and single stranded M13 purified by polyethylene glycol precipitation. Clones were sequenced by the dideoxy method[12] using a Sequenase kit (USB) and alpha ^{35}S-dATP (Amersham). Labelled oligonucleotides were resolved on 8M urea polyacrylamide gels (8%) by electrophoresis at 2500V (2−4 hours).

RESULTS

Sequence data obtained from one clone from one patient and two from the second were identical and are shown in Figures 1 and 2. Sequences matching the published mt DNA sequence were observed up to 8482 and down from 13460 bp; these nucleotides were bridged by a 13 nucleotide (nt) direct repeat occurring at 8470−8482 and 13447−13459 bp of the published sequence. As this 13 nt sequence may be derived from either side of the flanking region, or be contributed in part by both sides, the exact breakpoint of the deletion could not be determined. The deletion joins parts of two reading frames, ATPase 8 and NADH dehydrogenase 5. The abnormal junction leads to a frameshift (Fig. 2). If these fused genes are transcribed and translated, the product of ATPase 8, a 68 amino acid polypeptide, would be normal as far as amino acid 39, but the frameshift would lead to the addition of three altered amino acids prior to termination at position 43 (Fig.2).

Amplification of muscle mt DNA from all 13 cases with apparently identical deletions yielded PCR products of the same size (approximately 350 bp). Analysis of the sequence[3] flanking five other mt DNA deletions in our series, two pairs of which seem identical, showed direct repeats of 11 nt in each case.

DISCUSSION

The use of PCR is ideal for selectively amplifying mt DNA across deletion junctions, as it will not allow amplification of the large (approximately 5 kb) fragment of normal mt DNA between two primers flanking deleted regions. The finding of identical breakpoint sequence in three clones from two patients effectively excludes amplification or cloning artefact, and confirms the earlier indication that a proportion of cases of mitochondrial myopathy have identical mt DNA deletions[9].

The observation of a direct 13 nt repeat in normal mt DNA, retained in a single copy bridging the deletion in these two patients, suggests possible mechanisms for deletion formation, although these may not be the same in all cases. They could arise from recombination events mediated by enzymes that recognize short homologies. However, orthodox recombination is not thought to occur in mt DNA, partly because it does not seem to have DNA repair systems[13]. Short direct repeats flanking sites susceptible to deletion have been observed in bacteria[14] and the human beta globin genes[15]. The deletions often contained only one copy of the repeat and lacked the normal intervening sequence, as observed here. It was proposed that such deletions arose as a result of slippage during replication[14,15]. Once DNA becomes single stranded, repeat 1 is theoretically free to pair with the complementary sequence of repeat 2 downstream. This would produce a single stranded loop containing repeat 1 as well as the sequence between the repeats. After excision

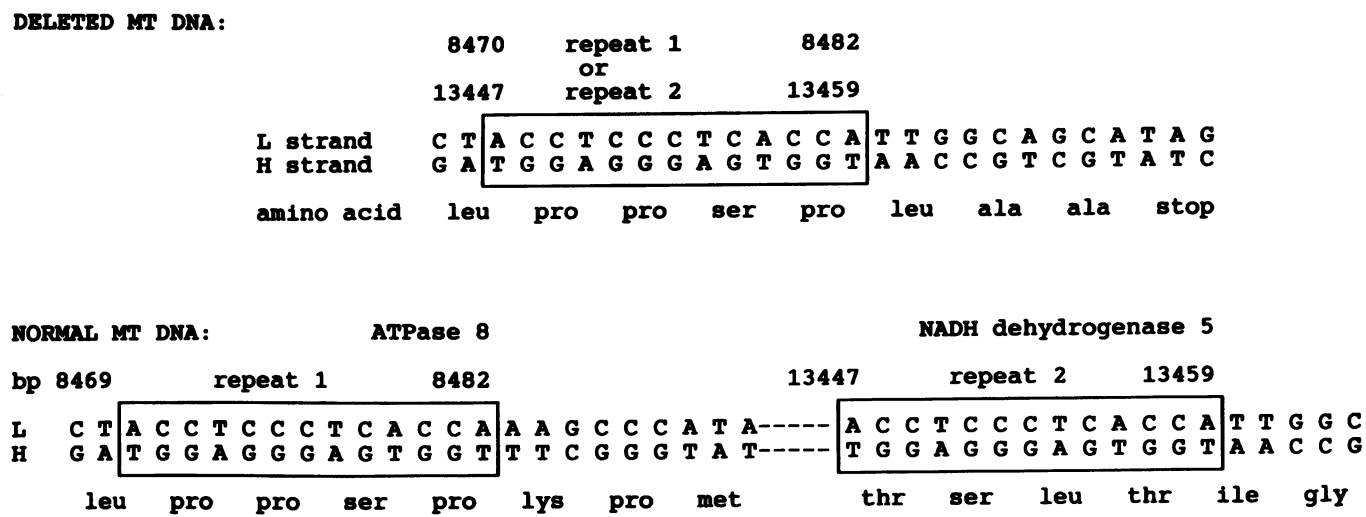

Figure 2. Comparison of the deletion junction in mutant mt DNA (above) with flanking regions in normal mt DNA (below). The deletion causes a frameshift at 13460, and termination of translation occurs three codons downstream. The boxed regions indicate the direct repeat, and the dashed lines normal intervening sequence.

of this loop, subsequent replication would result in sequences such as those shown in Figure 2.

However, this model may not apply to mt DNA as its method of replication is different from that of nuclear DNA. Mt DNA has two origins of replication, one for the heavy strand (O_H) at nt 191, and one for the light strand (O_L) about two-thirds of the way round the circular genome. Replication starts at O_H and proceeds in a clockwise direction; anticlockwise synthesis of the L strand does not begin until the replicating H strand reaches O_L[13]. As this replication system does not lead to large stretches of complementary single stranded DNA, it is difficult to envisage replication slippage as suggested above. Nevertheless, there is some evidence that discontinuous synthesis also occurs in mammalian mt DNAs, for example in sea urchin oocytes[16,17]. Displacement synthesis is extremely slow compared to discontinuous synthesis[13]; it is possible that the latter is preferred during the rapid mt DNA amplification which occurs during oogenesis[18]. As most cases of mitochondrial myopathy and deletions of muscle mt DNA are sporadic[7,8], it is likely that the deletions arise as fresh mutations during oogenesis.

After this paper was submitted, similar findings were described in deleted mt DNAs from lymphocytes of an infant with pancytopenia and lactic acidosis[19], and those in muscle from five patients with mitochondrial myopathies[20].

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