# Animal mitochondrial DNA recombination

Genetic recombination is known to be a source of mitochondrial DNA (mtDNA) variability in plants, fungi and protists[1], but there continues to be a consensus (based on studies of somatic cell hybridization[2] and DNA repair[3]) that such processes do not operate on animal mtDNA[4]. Contrary to this opinion we have now identified and characterized the end-products of recombination in the mitochondrial genome of the phytonematode *Meloidogyne javanica*.

Changes in the copy number of variable number tandem repeat (VNTR) sequences in mitochondrial DNA have occasionally been suggested to occur by recombination[5–8], although other mechanisms could explain such modifications[4]. If a locus consisted of tandem repeats (frequently found in animal mtDNA[9]) interspersed with unique sequences, it would be possible to discriminate recombination from mechanisms that simply change the repeat copy number. Proximity to an origin of replication could also favour the detection of recombination, as displacement loops are associated with mtDNA replication[9] and many models of recombination[10].

The repeat region of *M. javanica* mtDNA (Fig. 1a) meets these criteria and provides an excellent tool with which to detect recombination in animal mtDNA. We analysed the sequences of these nematode VNTRs and frequently found deletions in the centre of the array (Fig. 1b), indicating that genetic recombination might be occurring.

A defining feature of recombination models is the breakage and rejoining of participating DNA strands. The products of intramolecular recombination in mtDNA would be reciprocal, double-stranded subgenomic circles closed by covalent bonds. One such subgenomic circle (a 'minicircle'; Fig. 1c) would contain only the region excised from the mitochondrial genome, whereas its reciprocal partner (a major circle; Fig. 1b) would retain the remainder of the wild-type mtDNA. Mitochondrial genomes containing deletions, similar to major circles, have been observed in several organisms[11], but the presence of subgenomic minicircular end-products (Fig. 1c) has, to our knowledge, not been demonstrated. The existence of these molecules would provide unambiguous support for recombination within animal mtDNA. We have now detected such small circular molecules composed of *M. javanica* mtDNA sequences using the polymerase chain reaction (PCR; Fig. 1c–e) and subsequent sequence analysis.

If recombination occurred, the minicircles would have unique junctions that could not be produced by a deletion process that did not involve circularization of the DNA (Fig. 1d). Sequence analysis of eight cloned minicircle PCR products revealed that, after excision, the 3′ end of the 63-base-pair repeat array merges with the 5′ end of the 102-base-pair repeat array. Moreover, the nucleotide sequences of the two end-products are of the predicted reciprocal arrangement (Fig. 1b,d).

There are many described examples of VNTRs in animal mtDNA[9]. Changes in copy number and the maintenance of heteroplasmy have frequently been attributed to slipped-strand mispairing or illegitimate elongation[12]. The nature of the sequence changes predicted by these models is not consistent with our data.

We have shown that the generation of variability in sequence organization in *M. javanica* mtDNA is entirely consistent with recombination. Sequence polymorphisms and deletions in mtDNA are implicated in many human pathologies[13], so identification of the mechanisms responsible, their frequency and their epidemiology is imperative. Recombination in the mtDNA of higher metazoa remains undocumented, but as it has now been observed in most eukaryotes, its presence seems highly likely.

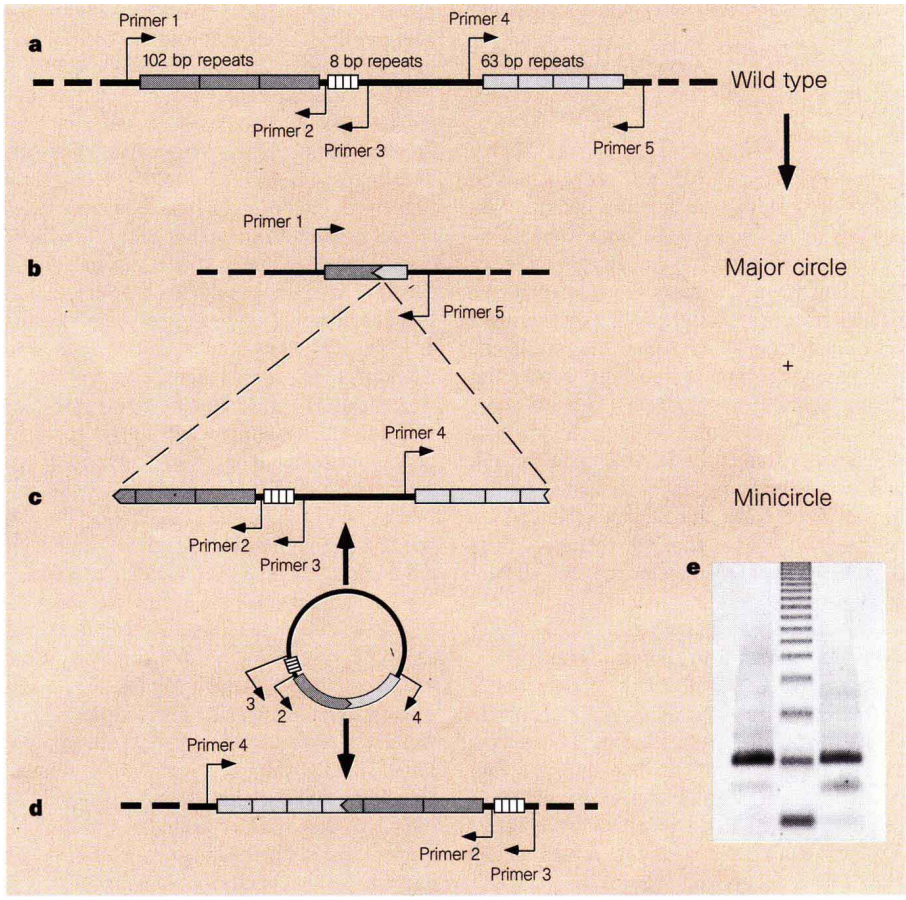


**Figure 1 a**, Organization of the *M. javanica* wild-type mtDNA[14] major non-coding region. PCR primer sites are indicated by arrows, unique flanking sequences by solid black bars and repeat sequences by variously shaded rectangles. The sequence extends beyond the region depicted, and joins to form a circle. **b**, Major-circle and **c**, minicircle recombination end-products. **d**, Primers 2 and 4 are divergent in orientation on the wild-type mtDNA and will only amplify a subgenomic product if the excised fragment forms a minicircle. **e**, Amplified products from this circularity assay (lanes 1 and 3) with a 123-base-pair standard (lane 2). The main products are ~250 base pairs (bp). Sequence analysis of cloned PCR products confirmed that they contained the predicted repeat sequence organizations. Sequences are available on request from the authors.

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