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Hypothesis

Why do mammalian mitochondria possess a mismatch repair activity?

P.A. Mason, R.N. Lightowlers*

School of Neurology, Neurobiology and Psychiatry, University of Newcastle upon Tyne, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH, UK

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Abstract All nucleated mammalian cells contain mitochondrial DNA, a small (approximately 15–17 kb) circular genome found in the matrix. This molecule is present in multiple copies, with numbers routinely exceeding 1000 per cell. Many pathogenic mutations of this genome have been reported, with the vast majority being highly recessive. A mismatch repair activity has been recently described in mitochondria that shows no strand bias for correcting point mutations. What could be the physiological function of such an activity? Mammalian mtDNA is remarkable in being a patchwork of many short repeat sequences. With reference to several recent publications, we hypothesise that the function of this activity is to preserve the mitochondrial genome by repairing short loop out sequences that would otherwise be lost as mitochondrial DNA polymerase gamma replicates the mitochondrial genome.

Key words: Mitochondrial DNA; MtDNA repair; Mitochondrial mismatch repair

1. Introduction – repair of mitochondrial DNA

Most eukaryotic cells contain mitochondria, the organelles housing the multi-subunit respiratory complexes that produce ATP via oxidative phosphorylation. A subset of these component polypeptides are encoded in the matrix of the organelle by the mitochondria's own genome (mtDNA), a circular molecule found in many copies per cell. The mammalian mitochondrial genome is small (15–17 kb), intronless and compact, with very little non-coding sequence and is believed to be more prone to mutation than the nuclear genome.

Historically, as there are multiple copies of mtDNA, it was thought unlikely that mitochondria possessed DNA repair capabilities, as damaged molecules could merely be degraded or lost during cell division. Furthermore, UV- and cisplatin-induced intrastrand crosslinks were shown not to be repaired in mitochondria, consistent with the absence of a nucleotide excision repair (NER) mechanism, which functions to remove such lesions in the nucleus [1,2]. In fact, although the lack of NER has not been challenged, mitochondria are now known to repair many other lesions using repair activities similar to those found in the nucleus (for a review, see [3], [4]). Several mitochondrial DNA glycosylases involved in these pathways are expressed as nuclear and mitochondrial isoforms encoded by the same gene, first described for the human base excision repair (BER) protein uracil DNA-glycosylase encoded by the UNG gene [5]. Reactive oxygen intermediates formed as a consequence of respiration in the mitochondrion are believed to promote oxidative damage to mtDNA. BER proteins involved in the repair of these lesions, such as the MutY homologue and OGG1 are also present as multiple isoforms, which have been shown to localise to mitochondria in rat neurones [6] and human cells [7,8].

2. Mismatch repair (MMR) – mitochondrial MMR (mt-MMR) is not strand biased

There are clearly numerous mitochondrial proteins that are known to repair faulty bases, but are there mitochondrial enzymes that can promote MMR? The mammalian nucleus uses a MMR system with components similar to the bacterial MutHLS paradigm (for a review see [9]), albeit without any MutH homologue [10]. Base/base MMR is carried out by MutSα, a heterodimer consisting of the MutS homologues MSH2 and MSH6 in tandem with MutLα, comprising MutL homologue MLH1 and (h)PMS2. Small loops are repaired by MutSβ, again involving MSH2 heterodimerically bound to MSH3, and MutLβ consisting of MLH1 and (h)PMS1 [11,12]. If mt-MMR does exist in mammals, one might expect to find MSH and MLH as central players. Indeed, yeast mitochondria (both *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*) have their own MSH, called MSH1 [13]. Yeast MSH1 shares sequence similarity to the other MSHs, possessing both DNA-binding and ATPase domains. Strains lacking MSH1 exhibit gross mtDNA damage [14], and the protein has been shown to be a DNA-stimulated ATPase in vitro [15]. It must be noted, however, that no MSH1 homologue has been identified in any mammalian genome.

Strand bias is also essential for a true MMR system, as, unlike repair of damaged bases, the two bases in a mismatch are unflawed and a mechanism must exist for recognising the inadvertently incorporated base. Recognition of hemi-methylated DNA is used by many prokaryotes, where the nascent strand after replication is unmethylated, allowing the

*Corresponding author. Fax: (44)-191-222 8553.
E-mail address: r.n.lightowlers@ncl.ac.uk (R.N. Lightowlers).

Abbreviations: NER, nucleotide excision repair; BER, base excision repair; MMR, mismatch repair; MSH, MutS homologue; MLH, MutL homologue; LLR, long loop repair; mt-MMR, mitochondrial mismatch repair

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MutHLS machinery to recognise and facilitate the removal of the incorrect base in a mismatch [16]. The mammalian system of strand discrimination in the nucleus is not fully understood, but seems to be based upon the recognition of strand ends [17] and may be co-ordinated by proliferating cell nuclear antigen (PCNA), a polymerase processivity factor [18,19].

We have recently described an activity in rat liver mitochondrial lysates that functions to repair GT and GG mismatches [20]. Similar to the classical bacterial MutHLS system, this mitochondrial activity is mismatch selective, bi-directional, and ATP dependent, but it does not show strand bias. This activity is much less efficient than nuclear repair. Furthermore, in the purified mitochondrial extract we were unable to detect any MSH2, the MSH that is an essential protein in all mammalian nuclear MMR (reviewed in [10]). In the absence of mammalian mitochondrial MSHs such as MSH2, it seems unlikely that classical MMR mechanisms exist in these organelles. There has been one report, however, that mammalian mitochondria harbour MSH2 [21]. This was consistent with their data that mitochondrial lysates could repair mismatches using chimeroplasty, a technique utilising a DNA–RNA hybrid molecule [22,23]. Whilst this may be independent evidence for mt-MMR, in our study no MSH2 could be identified in our MMR-proficient purified rat liver mitochondria by Western analysis. Furthermore, total cell lysates from human LoVo cells, a line deficient in functional MSH2 protein [24], show approximately one-fifth of the MMR activity of our mitochondrial lysates. Given that mitochondrial enrichment is often estimated as five-fold, this suggests that LoVo mt-MMR has a similar activity to rat mt-MMR which may not require MSH2. Interestingly, LoVo cells have also been shown to be deficient in both MSH3 and MSH6 protein, both proteins that dimerise with MSH2 for repair function [25,26], and are likely to be unstable in its absence. Thus, if LoVo and rat liver mt-MMR activities are similar, the activity is likely to be independent of a mechanism similar to the classic MMR found in the nucleus.

3. Mitochondrial loop repair may also repair mismatches

Crucially, as mentioned above, the mt-MMR capability inherent to the rat mitochondrial lysates is not strand biased [20], suggesting that the repair mechanism is different to nuclear DNA repair. MtDNA does not have obvious strand-discrimination features such as methylation that are used in bacterial MMR and although PCNA may be directed to the mitochondrion in some fungi [27], it does not appear to have a mitochondrial targeting sequence and it is currently unknown whether it is imported. Why would mitochondria have retained a MMR capability that is unable to identify and remove the mis-incorporated nucleotide? Although the activity is potentially an evolutionary artefact, it is more likely to have been retained for some other function to which the repair activity measured in [20] is just an adjunct.

The human mitochondrial genome is notable for being composed of short near-perfect direct repeats and large deletions of the mitochondrial genome are common [28], possibly due to polymerase slippage at these repeat sequences during replication [29]. Smaller deletions, however, are far less common than would be predicted, even accepting an element of ascertainment bias. For example, of the deletions listed in [28], three describe deletions of 5–20 bp whilst more than ninety describe larger deletions of approximately 2 kb or more. Only one medium-sized deletion (of 264 base pairs) is described. Of the deletions larger than a few nucleotides, more than 85% are flanked by direct repeats. Therefore, it is highly likely that loop repair pathways, especially ones that preferentially repair tracts of 20–2000 nt, are operating in mammalian mitochondria. If base/base mismatches are also repaired by loop repair mechanisms, a MMR function might persist without any direct selection pressure.

So, if the mt-MMR activity that has been detected in mammalian mitochondria is actually an activity to repair loop outs, are there any other precedents for such a mechanism? There are a number of loop repair pathways in the nucleus of yeast and higher eukaryotes (called long loop repair, or LLR to distinguish them from MMR-driven repair of short loops, see Fig. 1) described in the literature that recapitulate some of the functions that would be required for repairing loops in mitochondria. Classic MMR itself repairs short loops (up to about 12 nt) using the MSH2:MSH3 (MutSβ) dimer [30,31]. Loops of 8–16 nt are repaired by both MMR and LLR, with the contribution of MMR decreasing on larger loops. Long loops (greater than approximately 15 nt) are repaired by different LLR mechanisms according to their size, structure, and sequence context. For example, in yeast, one repair activity employs MSH2 and a NER protein, RAD1, to correct loops of 30 nt during meiotic recombination [32] and an MSH3:RAD1 activity has been shown to repair a 92 nt loop formed during slipped replication [33].

Similar LLR pathways also operate in mammals, with loops of from 12 to 283 nt being repaired both in vitro [31] and in vivo [34,35].

4. Could there be LLR in mammalian mitochondria?

If there was indeed a loop repair mechanism in the mammalian mitochondria, our previous results suggest this activity must be independent of MSH2 and would be expected to retain loops when they are encountered. One repair activity that appears to be both MMR independent and NER independent has been characterised in mammalian CHO cells [35,36]. It has been shown to repair templates with shorter palindromic loops of 16 and 26 nt, preferentially retaining the loop, whilst resolving longer palindromes (40 nt) and non-palindromic loops of any size with a preference for loop loss. It is possible that a modified form of this type of LLR is found in the mitochondrion, however, the available data is not supportive of an exact copy of this mechanism. In human mtDNA, it is clear that loops would be retained. Further, whilst there are many repeat elements in the mammalian mitochondrial genome, these are generally not palindromic but are direct repeats (an exception is the unstable short palindromic repeat in porcine mtDNA [37]).

Another LLR activity has been described that is not compromised in *msh2-msh3*, or *mlh1-pms1* mutants, and is also independent of NER (*RAD* genes) [38]. This activity repairs long loops at an efficiency of 85–95% in vivo compared to wild-type MMR-proficient cells. Although described in yeast, it has been mirrored in mammalian systems in nuclear cell-free extracts [31]. This human mechanism is also independent of MMR, being seen in extracts that are deficient in MSH2 and MSH6, MLH1, PMS2, or the RAD homologues ERCC1 and ERCC4. A 5′ flanking nick directs efficient repair to the

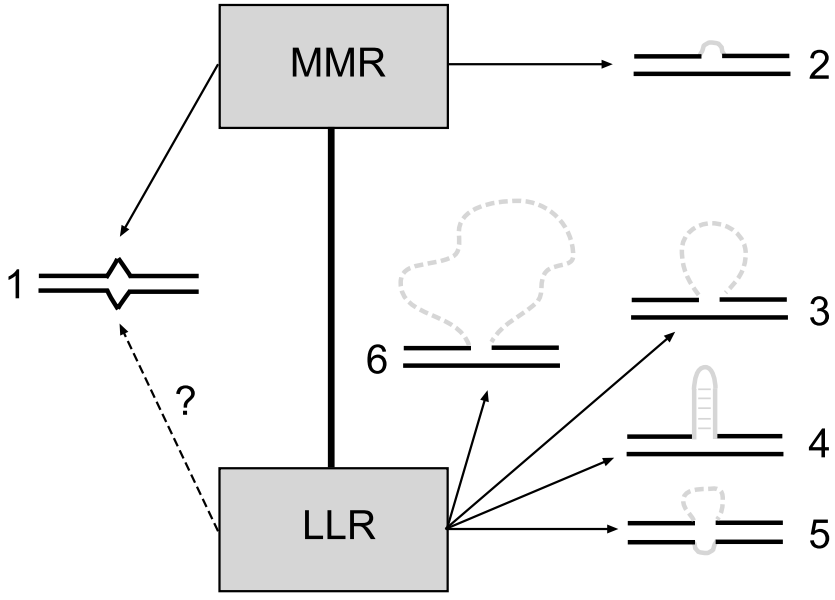

Fig. 1. DNA mismatch and loop repair mechanisms. Classic MutHLS-mediated DNA repair (MMR) recognises and resolves base/base mismatches (1) and small loops (2) up to approximately 12 nt, using a strand-specific mechanism. LLR recognises loops ranging from 8 nt upwards as substrates, preferentially retaining small inverted repeat hairpins (4) and the smaller of the two opposing loops (5), whilst preferentially removing larger loops (3). Very large loops of more than 2 knt may also be repaired, favouring the loss of the loop (6). LLR may repair base/base mismatches via co-repair with a loop or by another mechanism. Mitochondria may possess modified versions of these mechanisms, but are unlikey to process very large loops (6). Full line, retained sequence following repair; dashed line, sequence lost following repair.

nicked strand whereas a 3′ nick (or no nick) reduces repair sharply. In contrast to the LLR activity described in [36], loop secondary structure inhibits this activity, and sequence context also seems to affect recognition by enzymes involved in the pathway. Intriguingly, in this study, repair of single-base mismatches, although sharply reduced in the MMR-deficient background, was reported to be retained at approximately 25% activity of control levels, similar to the repair levels seen in [20]. In addition, single-base mismatches were repaired more efficiently when loops were present, suggesting that co-repair was occurring after initiation at the loops. As the activity is MSH2 independent and is capable of repairing single nucleotide loops, it is tempting to conclude that a similar repair pathway exists in mammalian mitochondria. However, again it cannot be identical, as preferential removal of loops is favoured 2:1 over retention, an activity that would not be consistent with retention of loops, as seen in human mtDNA.

5. Summary

It is possible that evolution has shaped LLR in the mitochondrion, with important players selected from various DNA repair mechanisms. Our work suggests that MSH2 is not targeted to mitochondria, yet other MMR factors may be imported and may contribute to the important LLR activity. For example, MSH6 has a putative mitochondrial targeting sequence, although the majority clearly localises to the nucleus. Further, an alternatively transcribed isoform of MSH6 (GTBP-alt) is missing the carboxy-terminal portion, which encompasses a HLH motif, and an ATPase domain [39], both of which are involved in multimerisation in MutS [40,41]. This isoform might thus be a stable monomer that does not need MSH2 to function. MMR-independent mechanisms may also be involved. Each could possess different characteristics such as the preference for a 5′ nick, or for loop retention on encountering substantial secondary structure. By designing the correct templates containing nicks and loops of varying sizes, it will be possible to identify the presence and form of LLR pathway in mammalian mitochondria.

In this paper, we have referred to the numerous types of LLR mechanisms that have been reported in the nucleus of eukaryotes. We believe that LLR in mammalian mitochondria is a crucial mechanism for retaining intact genomes, but until candidates are identified and repair systems are reconstituted, this belief will remain merely an hypothesis.

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