Reactive oxygen species production in the mitochondrial matrix: implications for the mechanism of mitochondrial mutation accumulation

Aubrey de Grey
Department of Genetics, University of Cambridge
Downing Street, Cambridge CB2 3EH, UK
Email: ag24@gen.cam.ac.uk

Abstract

The vicious cycle theory postulates that typical mitochondrial DNA (mtDNA) mutations cause their host mitochondria to generate more superoxide and other reactive oxygen species (ROS) than do normal mitochondria, thereby promoting the occurrence of additional mtDNA mutations at an ever-accelerating rate. However, nearly all the loss-of-function mtDNA mutations seen *in vivo* are large deletions, which (as the original statement of the theory indeed noted, though this has been widely overlooked) should not trigger a vicious cycle because they will prevent the assembly of the potentially superoxide-generating enzyme complexes. Consistent with this is the observation that each cell exhibiting loss of mtDNA-encoded function *in vivo* contains copies of a single, evidently clonally expanded, mutant mtDNA species, whereas the vicious cycle theory predicts a spectrum of mutant forms in each cell. Two recent papers, however, unveil a way in which mtDNA mutations could indeed promote ROS production of their host mitochondria. MtDNA mutations probably shift the intramitochondrial NAD^+/NADH redox couple towards NADH, and this is now shown *in vitro* to cause ROS production by alpha-ketoglutarate dehydrogenase, an essential enzyme of the TCA cycle. This does not revive the vicious cycle theory, but it has complex implications for the two most plausible more recent theories, known as "survival of the slowest" and "crippled mitochondria". It may also prove to explain other recent observations in mitochondrially mutant cells *in vivo*.

In 1989, seventeen years after Harman had suggested the same idea in an evidently over-obscure forum,[1] Linnane and colleagues proposed that mitochondrial DNA (mtDNA) mutations might accumulate during life as a side-effect of respiration and contribute substantially to age-related degeneration.[2] This theory was thereafter widely cited and discussed throughout biogerontology and became one of the dominant schools of thought in the field. A year later, Bandy and Davison put forward a mechanistic elaboration of this hypothesis, which soon became known as the "vicious cycle theory" of mtDNA mutation accumulation.[3] Their idea, summarised in Figure 1, was that typical mtDNA mutations might have the same effect on the respiratory chain (subunits of which are all that the mtDNA encodes) as various small-molecule inhibitors of respiration: namely, to stimulate the one-electron reduction of molecular oxygen to superoxide by electrons caught in the "traffic jam" caused by the inhibitor. This, they reasoned, would stimulate the accelerated occurrence of further mutations in neighbouring mitochondria, creating a positive feedback loop that would soon mutate all the cell's mtDNA into oblivion.

Not all respiratory chain inhibitors stimulate superoxide production, however, and Bandy and Davison carefully noted that the same would be true of mutations. Specifically, they pointed out that a mutation preventing the synthesis of cytochrome b would actually abolish any superoxide production at Complex III that a normal mitochondrion might exhibit, because without cytochrome b in place, the complex cannot be assembled. This soon turned out to be highly relevant to the plausibility of the vicious cycle theory in normal aging, because it was reported that respiration-deficient cells of several tissues predominantly possessed mutations that would indisputably preclude assembly of both the

enzyme complexes known to be prone to generate superoxide, namely Complexes I and III.[4-7] These mutations were large deletions, which eliminated the genes for at least a couple of respiratory chain subunits, but more importantly also removed at least one transfer RNA (tRNA) gene. There is no redundancy of tRNA genes in the mtDNA, so the loss of any such gene abolishes the synthesis of all 13 mtDNA-encoded proteins, of which one (cytochrome b, just mentioned) is part of Complex III and fully seven are parts of Complex I.[8]

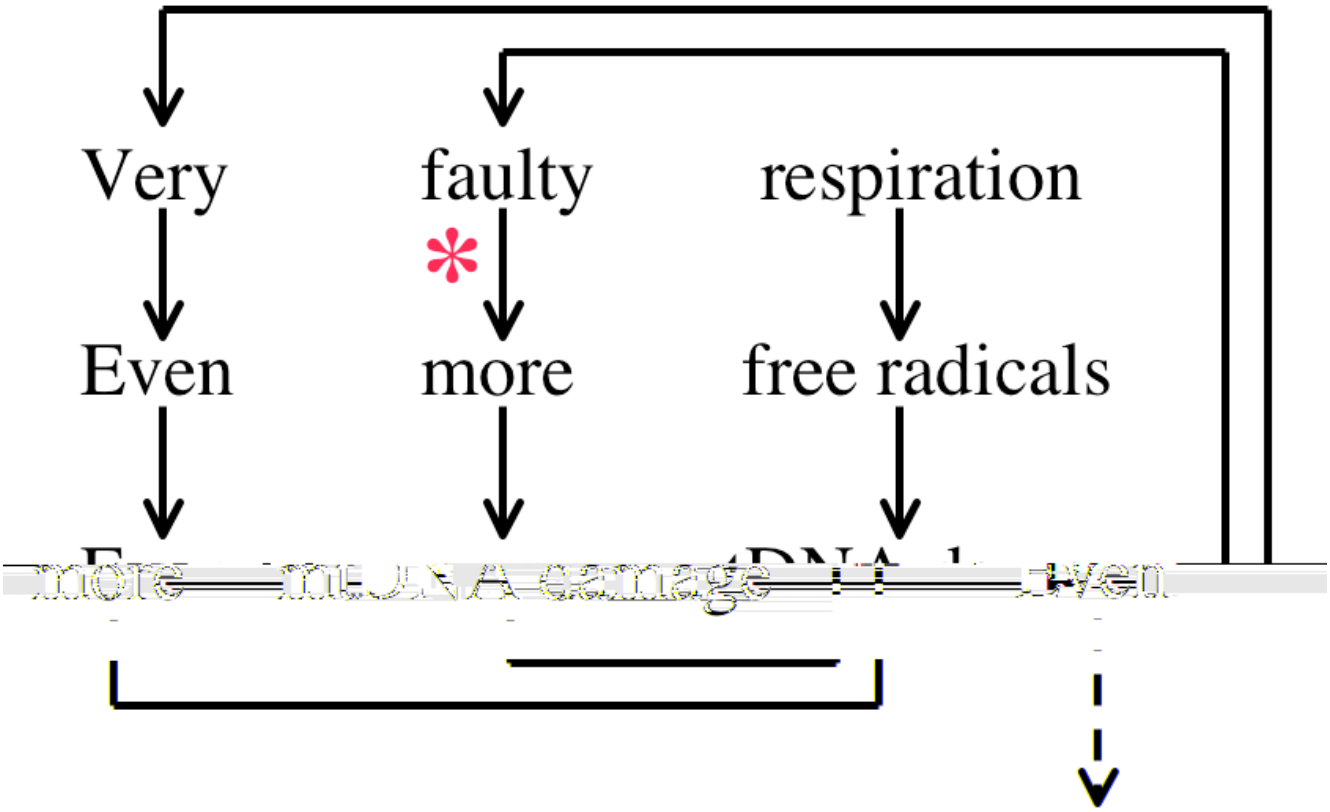

Figure 1. The vicious cycle theory. The asterisk marks the crux of the theory: that typical mutations elevate ROS production.

It was generally overlooked that this finding challenged the vicious cycle theory, even as the predominance of deletions among loss-of-function mtDNA mutations *in vivo* was eventually determined to be virtually complete. A second observation, however, challenged it even more (though this also escaped most commentators). This was that within each respiration-deficient cell there was always only one mutant mtDNA species (generally together with a residue of wild-type mtDNA). This showed that the mutant molecule had clonally expanded – and done so quite rapidly, far faster than genetic drift would allow – at the expense of the wild-type molecules in the cell, but had *not* stimulated (to a detectable degree, anyway) the occurrence of more mutations.[6,7] Different cells had different mutations, so it was not just that one particular mutation was immensely more common than any other.

In due course, other mechanistic models for mtDNA mutation accumulation were devised. Many of them were inconsistent with available evidence, but two were at least not conclusively so. The present author suggested in 1997 that it is precisely the loss of superoxide production that gives mutant mitochondria and their DNA a selective advantage and drives their clonal expansion: in this "survival of the slowest" (SOS) hypothesis,[9] mitochondrial turnover by autophagy is driven by self-inflicted free radical damage to the mitochondrial membranes, so a mutant mitochondrion is "less suicidal" and is more often replicated simply because it is more long-lived (Figure 2). A few years previously, Attardi's group picked up[10] on an idea which Shoubridge had suggested but immediately rejected:[11]

that the internal biochemistry of mutant mitochondria somehow stimulates them to replicate. This became known as the "crippled mitochondrion" (CM) hypothesis.

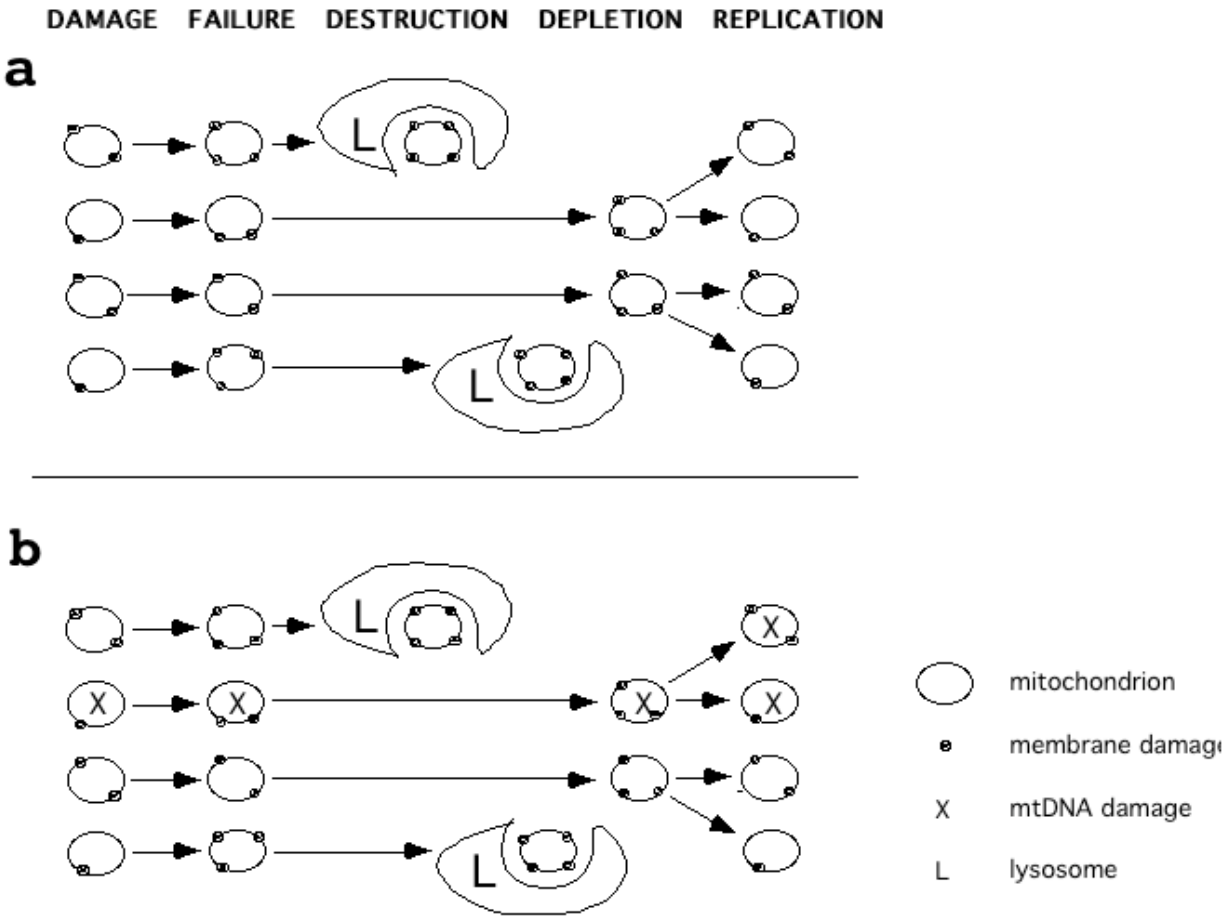

Figure 2. The "survival of the slowest" theory. Mitochondrial turnover in non-dividing cells is driven by self-inflicted membrane damage (a) which is slower in mutant mitochondria, leading to their clonal expansion (b).

Evidence against either SOS or CM is limited and circumstantial. SOS predicts that autophagy is selective for mitochondria with oxidatively damaged membranes, which is contrary to a long-standing belief that it is not selective at all,[12] but that belief has now been discarded – and in fact a recent paper,[13] discussed in another Perspective in this issue,[14] reports the first breakthrough in identifying the genetic basis for this selectivity. CM seems to predict that all loss-of-function mutations would clonally expand, whereas SOS predicts that ones which affect only the ATP synthase would not; there is one report[15] that indeed they do not, but it would be premature to regard that matter as closed. CM also seems to predict that hyperproliferation of mitochondria, manifest as "ragged-red fibres" with the Gomori trichrome stain, will precede or at least be simultaneous with loss of respiratory function in any given cell that is taken over by mutant mtDNA, and this is at odds with the finding that cells affected by mtDNA deletions are often respiration-null and not ragged-red but are almost never the converse[16] – but the mechanistic details of CM are not sufficiently specified to consider this decisive either.

A strong prediction of SOS is that ROS production by the respiratory chain of mutant mitochondria should be eliminated (because the chain is mostly not present). Indeed, ROS production in cultured cells lacking any mtDNA, which should be functionally identical to those with large mtDNA deletions, make less ROS than normal cells.[17] These cells are dividing, however, so may not be representative of the postmitotic cells that most predominantly suffer from mutant mtDNA *in vivo* – and indeed,

respiration-deficient muscle fibre segments stain strongly for oxidatively damaged DNA and/or RNA.[18] This could be due to intracellular ROS production at the cell membrane, which is tantalising in view of the hypothesis that such cells make abundant extracellular ROS to the detriment of circulating oxidisable material and thence the whole body.[19] The key question of the intracellular location of this damage remains to be answered.

Two recent papers[20,21] make this last question even more of a priority than before. Their common finding, by different methods, is that an essential enzyme of the mitochondrial matrix, alpha-ketoglutarate dehydrogenase (AKDH), makes hydrogen peroxide and maybe superoxide when exposed to elevated concentrations of NADH. The mitochondrial NAD pool is normally almost all in the oxidised state, i.e. NAD^+, but elimination of the respiratory chain would be predicted to cause it to become much more reduced – enough, in one hypothesis,[19] to cause a reversal of the malate/aspartate shuttle.

It is very far from simple to interpret the relevance of this finding to the various hypotheses for the accumulation of mutant mtDNA during aging. We can still say confidently that the vicious cycle theory is incorrect, because of the presence of only one mutant species in any affected cell (or muscle fibre segment). We cannot say that this new finding strongly challenges SOS, because matrix-generated ROS may be an epiphenomenon, whose contribution to mitochondrial membrane damage may be minimal. In fact it could be regarded as a pre-emptive reconciliation of SOS with future data – it would mean that a finding of oxidative damage to mutant mtDNA in respiration-deficient muscle fibre segments would not challenge SOS because it would not imply elevated ROS production in the mitochondrial inner membrane, only in the matrix. However, this result also lends new plausibility to CM. As noted above, CM is mechanistically less detailed than SOS and affords a number of possible elaborations. We can describe two classes of such elaboration: one is that mutant mitochondria proliferate because of a misreading of the energy needs of the cell, and the other is that they proliferate to meet an intramitochondrial need. In the former model, for example, mitochondrial biogenesis control factors might sense cellular ATP levels by reference to intramitochondrial ATP, which will be depressed by loss of respiratory function. In the latter, for example, modest levels of damage caused by the dysregulated intramitochondrial biochemistry in mutant mitochondria might stimulate proliferation as "damage dilution" mechanism. Proliferation is seen at the cellular level in the presence of low concentrations of hydrogen peroxide,[22] so this is not out of the question. The identification of a specific candidate for the source of such damage makes this latter option altogether less speculative than before. Finally, it must not be forgotten that the SOS and CM mechanisms could both be operating simultaneously, or some in some cell types and some in others. With total allotopic expression in either the soma[23] or the germ line[24] still some way off (at least for humans), progress in identifying the mechanism of clonal expansion of mutant mtDNA should be a high priority, as it may suggest effective ways to combat the process that can be implemented sooner.

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