Review article

The plasma membrane redox system: a candidate source of aging-related oxidative stress

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Abstract

The plasma membrane redox system (PMRS) is an electron transport chain in the plasma membrane that transfers electrons from either intra- or extracellular donors to extracellular acceptors. Unlike the superoxide-generating NADPH oxidase of phagocytes and the homologous (but much less active) enzymes found in some other cells, the PMRS is still incompletely characterised at the molecular level. Much is known, however, concerning its function and affinity for both physiological and non-physiological substrates. A role for it in aging, the ‘reductive hotspot hypothesis’ (RHH), was proposed in 1998 as part of an explanation for the apparently indefinite survival *in vivo* of cells that have entirely lost mitochondrial respiratory capacity as a result of the accumulation of mitochondrial mutations. Stimulation of the PMRS might allow the cell to maintain redox homeostasis even while continuing to operate the Krebs cycle, which may be advantageous in many ways. However, the PMRS may, like the mitochondrial respiratory chain, be prone to generate superoxide when thus dysregulated – and in this case superoxide would be generated outside the cell, where antioxidant defences are more limited than inside the cell and where much highly oxidisable material is present. Cascades of peroxidation chain reactions initiated by this process may greatly amplify the oxidative stress on the organism that is caused by rare mitochondrially mutant cells. Since such cells increase in abundance with aging (though remaining rare), this is an economical hypothesis to explain the rise in oxidative stress seen in (and generally believed to contribute substantially to) mammalian aging. In an extension of previously published accounts of RHH, I propose here that the lysosomal toxicity of oxidised cholesterol derivatives (oxysterols) may contribute to the toxicity of mitochondrial mutations by affecting lysosomal function in many cell types in the same way as they have been proposed to do in arterial macrophages.

Background: The ‘simple glycolysis’ model and its inadequacy

The bulk of our cells’ ATP synthesis capacity derives from the mitochondrial electron transport chain, a highly intricate system of enzyme complexes in the inner mitochondrial membrane that consumes electrons from various sources and delivers them to molecular oxygen to make water. Energy is released in this process and is directed into the synthesis of

ATP by the chemiosmotic coupling of the electron transport chain to the ATP synthase: protons are pumped out of the mitochondrial matrix by three components of the electron transport chain and return via the ATP synthase (Nicholls and Ferguson 2002). The electron transport chain and the ATP synthase are collectively termed the respiratory chain. There is a well-known alternative source of ATP within all cells, however: glycolysis, the bifurcation of glucose to make two molecules of pyruvate. This occurs in

the cytosol, and two molecules of ATP are generated in this process. Though this is much less than the estimated 36 ATP molecules which are subsequently made by the respiratory chain as a result of the further metabolism of those two pyruvate molecules, cells can get by quite well – temporarily – by using glycolysis alone, and do so *in vivo* in certain circumstances such as during cell division (Brand 1997).

The respiratory chain does something in addition to generating ATP, however, as already noted: it consumes electrons. These electrons are generated by a wide variety of cellular processes, including glycolysis and the Krebs cycle, and any diminution of respiratory chain activity must therefore be compensated, pretty quickly, in one of two ways: a corresponding down-regulation of these processes, or a stimulation of some other system for consuming the electrons they release.

The widespread assumption of biologists over the years has been that cells adopt the former course. Restricting ourselves for the moment to the process of nutrient metabolism for ATP synthesis, it is supposed (though usually only tacitly) that cells lacking respiratory chain activity make ATP only via glycolysis and shut down both pyruvate dehydrogenase and the Krebs cycle. Hereafter this will be termed the 'simple glycolysis' model of metabolism in respiration-deficient cells; it is represented in Figure 1. The result of such an action in terms of the electron budget is that only four electrons are released for each glucose molecule metabolised, and the presumption is that these are re-consumed by reduction of the two pyruvate molecules to lactate, which is then exported from the cell. The accumulation of lactate in the extracellular fluid of over-exercised muscle is consistent with this model: one aspect of over-exercise is the depletion of oxygen for use by the respiratory chain, which necessarily inhibits it. Further support is found in the ability (King and Attardi 1989) to maintain mammalian cells in culture even after permanently removing their respiratory chain by eliminating their mitochondrial DNA. Culture of mtDNA-deficient cells can be done merely by providing extra pyruvate in the medium (Note: uridine must also be provided, but for reasons not related to the present paper), and this is interpreted as working because cells use this extra pyruvate as a sink for electrons generated in relatively low amounts by processes other than glycol-

ysis (Figure 1B). Respiration-deficient yeast cells grow (albeit poorly) without even any extra pyruvate (Whittaker 1979), indicating that they can balance the electron books by other means.

There are some curious shortcomings of the simple glycolysis model, however. First we can note some teleological ones. Shutting down the Krebs cycle is a rather drastic step for a cell to take, in view of its role in many key cellular processes apart from respiration, such as amino acid synthesis. Further, the Krebs cycle is itself a generator of ATP, as the conversion of succinyl CoA to succinate creates GTP from GDP (Sanadi and Littlefield 1953) and the intramitochondrial tri- and diphosphate pools of all nucleotides are kept in equilibrium by the nucleoside diphosphate kinase (Postel 1998). This not only doubles the amount of substrate-level (i.e., non-chemiosmotic) ATP synthesis from carbohydrates but also allows cells to generate ATP from fatty acid oxidation without chemiosmosis.

If we find teleology distasteful, empirical data may be more telling – and the results of most relevance here are also those of most relevance to aging. Since 1983 it has been known that the skeletal muscle of sufferers of mitochondriopathies (diseases caused by mitochondrial mutations) accumulates mutant mtDNA in a non-uniform distribution, with some fibre segments exhibiting essentially normal respiratory capacity as determined histochemically and others exhibiting complete loss of that capacity (Müller-Höcker et al. 1983; Johnson et al. 1983). In 1989 this was likewise shown in normal aging (Müller-Höcker 1989) (though the proportion of respiration-negative fibres was far lower); over the next few years the same was identified in a variety of other tissues (Müller-Höcker et al. 1996, 1997; Itoh et al. 1996). The problem here was that in at least some such tissues we could be absolutely sure that these cells were surviving in their respiration-negative state for a very long time – many years, in the case of humans. Two pieces of evidence showed this. Firstly, these cells increase in number with age (Müller-Höcker 1989; Müller-Höcker et al. 1996, 1997; Itoh et al. 1996). This is not conclusive, as it could be simply that they were appearing at an accelerating rate at older ages and dying equally quickly at any age. But secondly, *in situ* hybridisation and single-cell PCR analysis showed that within a given respiration-deficient muscle fibre segment there was always only one species of mutant DNA, which had clonally expanded

Figure 1. The 'simple glycolysis' model for how cells maintain ATP supply and redox homeostasis when their respiratory chain is inactive. A Glucose metabolism with an active respiratory chain. B Glucose metabolism without an active respiratory chain.

(Müller-Höcker et al. 1993; Charlton et al. 1998). Since such fibre segments were up to a couple of millimetres long (Lee et al. 1998), it seemed clear that the longer ones must have become respiration-deficient in a shorter segment some time ago and spread along the fibre due to some kind of selective advantage. What that advantage actually is remains unclear (Yoneda et al. 1992; de Grey 1997), but whatever it is, it can only occur if the respiration-deficient segment does not die in the meantime. More direct evidence for this interpretation is now available, with the finding that older monkeys have some respiration-deficient fibre segments much longer than any seen in younger monkeys (Lopez et al. 2000). Some respiration-deficient fibre segments do apparently atrophy

and die, but this seems to be a stochastic event that many affected fibre segments can escape indefinitely.

Why is the survival of respiration-deficient cells *in vivo* evidence against the simple glycolysis model? Because *in vitro*, mammalian respiration-deficient cells need exogenous pyruvate to survive – and the extracellular milieu *in vivo* does not contain appreciable amounts of pyruvate, nor of any other substance that might similarly act as an electron sink by being imported, reduced and exported by respiration-deficient cells.

Even this is not the most direct evidence against the simple glycolysis model. As mentioned above, *in situ* determination of respiration deficiency in various tissues has been performed by histochemistry –

usually to cytochrome c oxidase (COX), three of whose subunits are encoded in the mtDNA and are thus absent in mitochondria whose DNA contains large deletions eliminating at least one essential tRNA gene, which are the commonest class of mtDNA mutation seen in normal aging. Such assays clearly require suitable controls, and the principal one is to perform simultaneous histochemistry on some enzyme present in the same subcellular location as COX but lacking mtDNA-encoded subunits. The enzyme generally used is succinate dehydrogenase (SDH), since that is located in the inner mitochondrial membrane. Usefully, SDH is a component of the Krebs cycle. Thus, the simple glycolysis model predicts that it would probably be absent or down-regulated in cells lacking COX activity, or at least that it would be at normal levels. In fact, however, it is reproducibly elevated (Müller-Höcker 1989; Müller-Höcker et al. 1993, 1996, 1997; Itoh et al. 1996; Shoubridge et al. 1990; Moraes et al. 1992). This cannot be explained by a general proliferation of mitochondria, because the rise in SDH activity clearly exceeds any rise in mtDNA concentration (Moraes et al. 1992).

These considerations led the present author to wonder whether respiration-deficient cells might in fact adopt the other of the two options listed earlier: not to shut down electron-releasing processes but to up-regulate some alternative route to the respiratory chain for their disposal. (The option of down-regulating other electron-releasing processes but not the Krebs cycle seems inadequate, as the Krebs cycle releases so many electrons.) A candidate was rapidly identified: the plasma membrane redox system (PMRS), or plasma membrane oxidoreductase (PMOR) as it was sometimes known in those days.

Before describing the details of the PMRS, it is necessary to explain how a system located in the plasma membrane could even in principle be relevant to the activity of mitochondria (see Figure 2). As mentioned above, the PMRS can only maintain redox homeostasis in OXPHOS-deficient cells that are still performing the TCA cycle (as the histochemical evidence concerning SDH indicates) if the reducing equivalents generated by the TCA cycle can gain access to the plasma membrane. There are two such reducing equivalents to consider: the NADH generated at three points in the cycle and the FADH₂ generated by SDH. Intramitochondrial NADH can be reoxidised to NAD⁺ by a reversal of the usual mode of action of the malate–aspartate shuttle: normally

this shuttle oxidises cytosolic NADH and reduces matrix NAD⁺, but it operates close to thermodynamic equilibrium and can thus be reversed by only modest shifts in substrate concentrations (Burat et al. 1984). The situation concerning FADH₂ is more speculative, as FADH₂ is trapped within the SDH enzyme and is thus constrained to deliver its electrons to ubiquinone within the mitochondrial inner membrane. If complexes III and IV are absent as a result of mitochondrial mutations, the resulting ubiquinol can be reoxidised only by a reversal of enzymes that normally provide electrons to ubiquinone from cytosolic sources. The most straightforward candidate for this function is the glycerophosphate shuttle, but there is as yet no direct evidence for this process. However, given the absence of alternative explanations for the high level of SDH in OXPHOS-negative cells *in vivo*, this must currently be regarded as the most economical interpretation of available data.

The mammalian PMRS *in vitro*

What respiration-deficient cells appear to need in order to maintain Krebs cycle activity is an electron transport system in the plasma membrane that oxidises cytosolic NADH and/or NADPH and reduces extracellular electron acceptors. (Additionally they

Figure 2. The way in which export of electrons from the cell can allow respiration-deficient cells to maintain redox homeostasis while also maintaining the Krebs cycle. Note the need to reverse the malate/aspartate and (probably) glycerophosphate shuttles. TCA: tricarboxylic acid or Krebs cycle; MAS: malate/aspartate shuttle; GS: glycerophosphate shuttle; II: Complex II (succinate dehydrogenase).

need to reverse the normal import of glycolysis-generated electrons into mitochondria by the malate/aspartate and glycerophosphate shuttles, as just discussed.) An enzyme that does this at a high rate is well known to biomedical researchers: the phagocyte NADPH oxidase (Cross and Segal 2004). This enzyme is at first sight an excellent candidate for the way a cell might relieve itself of surplus electrons, because it has high capacity ($V_{\max}$); moreover, because its extracellular electron acceptor is molecular oxygen and the product is superoxide, its upregulation might cause oxidative stress. In fact, however, it is not found in the cell membrane of muscle fibres, neurons or other cell types in which respiration-deficiency is mainly seen in aging. Homologues of its catalytic subunit have been found in many cell types (Meier et al. 1991; Lambeth et al. 2000), but these have much lower $V_{\max}$, not plausibly sufficient to maintain normal Krebs cycle rates in respiration-deficient cells.

An entirely distinct electron export system was discovered in the 1970s, however, and is altogether more widely expressed – indeed, no cell type is known that lacks it. This is the plasma membrane redox system (PMRS) (Crane et al. 1985). The PMRS exists in plants and has in fact been more extensively characterised there than in animals, but there are major differences so the plant PMRS will not be discussed here. In mammals the PMRS was first identified as a system by which cultured cells could reduce ferricyanide (Crane and Löw 1976). Since ferricyanide is not present in the extracellular milieu, what this meant for the PMRS's physiological role was unclear, as indeed it still is. The same applied to the finding that the PMRS's electron source could be extracellular (exogenous NADH, specifically) rather than intracellular (O'Donnell and Azzi 1996), since there is essentially no NADH in the extracellular medium either. The latter observation was of considerable note, however, because the electron acceptor in this case was molecular oxygen and the product was superoxide, and especially because the rate of superoxide production exhibited by cultured fibroblasts compares with that of phagocytes' NADPH oxidase during the respiratory burst (O'Donnell and Azzi 1996).

A thorough discussion of the structure of the PMRS is beyond the scope of this review, but a brief summary is necessary; the reader is referred to de Grey (2003) for more information. Our knowledge of the PMRS resembles our knowledge of the mitochondrial respiratory chain in the 1950s: most of

its components are known, but how they fit together is still largely undetermined. The transfer of electrons from cytosolic NADH or NADPH to the membrane is performed by at least two enzymes, NADH-cytochrome b5 oxidoreductase and DT-diaphorase. Some, but not all, of the routes that these electrons subsequently take involve coenzyme Q. Enzymes involved in the transfer of intra-membranous electrons to extracellular acceptors include the normally mitochondrial protein VDAC and a family of proteins now normally termed CLOX (cycling oxidase) enzymes on account of a remarkable oscillation of their activity between oxidase (or oxidoreductase) and protein disulphide isomerase functions.

Possibly the most significant early observation in regard to the role of the PMRS in aging was published by Lawen's group in 1994 (Larm et al. 1994). They showed that ρ^0 cell cultures – mammalian cells that had been divested of their mitochondrial DNA by culturing for several weeks in ethidium bromide, a potent inhibitor of mtDNA replication – relied on the PMRS for survival even when cultured in medium containing uridine and pyruvate. This was flatly at odds with the simple glycolysis model. The simplest interpretation is probably that pyruvate is (in whole or in part) being reduced exogenously by the PMRS, rather than being imported, reduced by lactate dehydrogenase and exported as the simple glycolysis model suggests.

The mammalian PMRS *in vivo*

Unlike ferricyanide (which is a nonphysiological electron acceptor for the PMRS), pyruvate is actually present in the extracellular medium, but its extracellular concentration *in vivo* still seems clearly insufficient to satisfy the long-term needs of a totally respiration-deficient cell. (The fact that such cells are rare does not help much, as the throughput of the PMRS will still be limited by the rate at which diffusion draws pyruvate towards an affected cell's membrane and lactate away from it.) In fact, only one electron acceptor seems to be present in the required quantity: molecular oxygen (Figure 2).

In order to evaluate the relevance of the PMRS in aging, however, we must first take a step back and consider its role in healthy tissues. It was noted earlier that the PMRS is ubiquitous. Why should it be so? – surely cells that possess a functioning respira-

tory chain will be able to recycle NADH perfectly well? The only plausible answer to this that has so far been proposed is that the cell may need a system to regulate its cytosolic redox state more finely than the respiratory chain can do, and that a plasma membrane escape route for electrons may provide this because it directly alters that state, whereas the respiratory chain mainly communicates with the cytosol via the redox state of the intramitochondrial NAD pool. This is clearly a severely incomplete hypothesis. An alternative is that the PMRS may exist not to help the cell directly but to allow the cell to protect its environment; an example of how this might be arises from the finding that the PMRS is an avid reducer of oxidised ascorbate (i.e., dehydroascorbate) (May 1999). Since dehydroascorbate is rather unstable, breaking down irreversibly with a half-life of only a few minutes if not re-reduced, it makes sense to have a ready source of the necessary extracellular electrons widely distributed throughout all tissues. Moreover, the PMRS can reduce the intermediate oxidation state of ascorbate, ascorbyl radical, thereby helping to prevent the formation of dehydroascorbate by disproportionation of two ascorbyl radicals. But again, whether this really is why the PMRS is so ubiquitous is highly speculative.

The mammalian PMRS in aging

Whatever its *raison d'être*, the PMRS's ubiquity means that it should be considered as carefully as any other ubiquitous cellular component when one is attempting to understand aging and explore ways to postpone it. As noted above, a more specific reason to investigate its potential role emerged a decade ago as it became clear that the simple glycolysis model of the metabolism of respiration-deficient cells *in vivo* was at odds with some important data.

The most fundamental question one must ask concerning the involvement of a system in aging, though by no means necessarily the easiest to answer, is: if it changes in activity with aging, is this part of the problem (a contributor to aging), part of the solution (an attempt by the body to combat aging) or neither (a side-effect with no consequences of its own)? We have seen that it may be sharply up-regulated in respiration-deficient cells, but since these cells are so few and far between *in vivo* even in old age, the third of these options seems indicated.

But is it that simple? A phenomenon that suggests not is well known within free radical biology and medicine: the lipid peroxidation chain reaction. Several major components of the extracellular medium are rich in readily oxidisable lipids. If it happened that the PMRS of respiration-deficient cells generates extracellular free radicals in its haste to dispose of the electrons being delivered to it intracellularly, a consequence would be that circulating lipid-rich material such as high- and low-density lipoproteins (HDL and LDL) would be prone to oxidation of their surface phospholipids as and when they drifted past such cells during their passage through the interstitium. The lipid peroxidation chain reaction is a cycle of reactions that allow a single lipid radical to give rise to arbitrarily many lipid hydroperoxides by incorporation of molecular oxygen and propagation of the radical moiety to neighbouring lipid molecules; this chain is broken only when a lipid radical reacts with a chain-breaking antioxidant such as vitamin E, whose radical form is less reactive with other lipids (Halliwell and Gutteridge 1999). Worse, the globular nature of LDL and HDL greatly diminishes the ability of vitamin E to protect lipids in this way, because in order to do so efficiently its radical form must be recycled by reaction with vitamin C, an aqueous molecule to which it does not have access while it is deep within the lipoprotein particle. Vitamin E radical can react with other lipids, so this actually means that it can act as a chain-propagating pro-oxidant in that context, not a chain-breaking antioxidant at all (Thomas and Stocker 2000). Finally, lipid hydroperoxides are highly prone to cleavage by reduced transition metal atoms, most significantly ferrous iron, which constantly desorbs (as hemin) from erythrocytes and binds to LDL (albeit much more loosely than to albumin and hemopexin, which remove it from the circulation for degradation in the liver) (Miller et al. 1995). Such cleavage creates lipid radicals *de novo*, thereby causing 'branching' of the lipid peroxidation chain reaction and in due course causing even more peroxidation.

This shows us that circulating lipoproteins could become appreciably oxidised by only a scattering of respiration-deficient cells. Further, the effect would be age-dependent, since such cells become more numerous with age. But there is more to do to show that this is a plausible pathway for the contribution of mtDNA mutations to aging. The problem is that ostensibly both HDL and LDL should be prevented by

normal metabolic processes from doing the body any harm as a result of such oxidation. HDL is the particle responsible for reverse cholesterol transport (Tall 1998), so the only cell it seems likely to be able to do any harm to after being oxidised in the interstitium is the hepatocyte that internalises and degrades it, and hepatocytes are not noted for their decline in function with age. LDL, for its part, is taken up by most cells via the Brown–Goldstein receptor, which has a specific affinity for unoxidised LDL and does not bind oxidised LDL; oxidised LDL is internalised via scavenger receptors on macrophages and other cells that again are among the more robust in the body (Horiuchi et al. 2003).

The gap in the above argument is a complication that pervades biology: there is inevitably a threshold of oxidation that determines whether the Brown–Goldstein receptor binds a given particle. Below that threshold, and in particular if no oxidation has occurred to the particle's protein component, it will be internalised by any cell that is short of cholesterol. By this means, as the average level of contamination of circulating LDL rises with age, so does the extent to which cells whose mitochondria are perfectly intact (i.e., the vast majority of our cells) will import toxic material.

This sequence of events therefore constitutes a possibly very large amplification of the toxicity of mitochondrial mutations. Considerable stress can be imposed on cells throughout the body as a side-effect of their internalisation of LDL particles that have been subjected to internal lipid peroxidation initiated by contact with the PMRS of a respiration-deficient cell (de Grey 1998). This proposed chain of events leading from focal mutant mtDNA to systemic oxidative stress (Figure 3) was termed (de Grey 2000, 2002) the 'reductive hotspot hypothesis' (RHH), in recognition that the cells where free radical damage is proposed to begin are in fact foci of reductive stress – excess of electrons – which is converted into oxidative stress only subsequently (if those electrons reduce transition metals which cleave lipids to make lipid radicals).

Might oxysterols poison non-macrophage lysosomes?

One final component of this process is worth discussing further. It may still be doubted that much

harm would come to cells that take up contaminated LDL, because an LDL particle's first port of call in its destination cells is the lysosomal apparatus, in which the particle is broken down and its cholesterol cargo released for cellular use (Jerome and Yancey 2003). Intuitively it would seem likely that any oxidised molecules within the particle will simply be retained within the lysosome and either broken down or permanently sequestered. The latter option would eventually be detrimental to cellular function, particularly via its effect on general lysosomal function, but one might suppose that the rate of accumulation of such material would be a small fraction of that arriving from intracellular sources such as mitochondrial turnover. However, the nature of this particular toxin suggests that this may be oversimplistic. The core of an LDL particle, and thus the part that can be most extensively oxidised without preventing the particle from being taken up by the Brown–Goldstein receptor, consists largely of cholesterol (mostly in the form of cholesteryl esters). Cholesterol's major oxidation product *in vivo* is 7-ketocholesterol (7-KC). There is considerable evidence that 7-KC is toxic to lysosomes even in small quantities, possibly due to inhibition of the vacuolar ATPase that maintains the low lysosomal pH necessary for efficient activity of numerous lysosomal hydrolases (Jessup et al. 2004). Acidity of the lysosomal compartment is also necessary for chaperone-mediated uptake of many lysosomal substrates (Massey et al. 2004). Accordingly, cells may be profoundly disabled by the adventitious uptake, within LDL particles, of quite modest quantities of oxidised material. The most direct evidence for this is in arterial macrophages, which become unable to process unmodified (native) cholesterol and whose lysosomes therefore become engorged with cholesterol to the extent that the macrophage can no longer function; such cells are termed 'foam cells' and are the first visible stage of the formation of atherosclerotic plaques (Lusis 2000). The macrophage is particularly susceptible to this problem because it has the task of internalising oxidised or otherwise modified lipoproteins inappropriately retained in the artery wall, and it does so however oxidised those particles are (since it expresses a scavenger receptor), but the phenomenon may very well be occurring, albeit less rapidly and less overtly, in many other cell types. Bearing in mind that foam cells begin to be evident in the aorta before puberty (Strong et al. 1958), the possibility that

Figure 3. The reductive hotspot hypothesis: the possible *in vivo* consequences of the accumulation of scattered respiration-deficient cells and muscle fibre segments, if such cells are prone to generate superoxide on their external surface. *PDH*: pyruvate dehydrogenase; *EC-SOD*: extracellular superoxide dismutase; *GSH-Px*: glutathione peroxidase; *P_i*: inorganic phosphate.

multiple cell types are more subtly affected by middle age or later cannot be dismissed.

Conclusion

Despite over 30 years of trying since Harman first suggested the idea (Harman 1972), biogerontologists have not yet been able to determine whether the accumulation of mitochondrial mutations contributes

appreciably to mammalian age-related tissue dysfunction and mortality. The best direct evidence we have is that accelerated mtDNA mutation in mice causes premature tissue dysfunction and death (Zhang et al. 2000; Trifunovic et al. 2004), but there are severe limits on what we can conclude from those results (de Grey 2004a). A major reason why this hypothesis has been so hard to test is that one can imagine so many mechanisms via which the dysfunction of so critical a cellular component could be

bad for us. The 'reductive hotspot hypothesis' described in this article is one candidate mechanism: unattractively elaborate at first sight, but diminishingly so as one appreciates that so many of its components are already well supported by available data, albeit not data with which biologists of aging have historically been familiar.

For many years there was a widespread belief that mitochondrial DNA maintenance and repair systems are extremely primitive or even non-existent. It is now known, however, that their sophistication approaches (though probably does not equal) that for nuclear DNA (Kang and Hamasaki 2002). In other words, we are carrying through evolution quite a lot of genetic machinery to ensure that mitochondrial mutations do not accumulate any faster than they do. This is not a state of affairs that we would expect to obtain for long if a somewhat faster rate of accumulation of those mutations would be harmless; thus, we can be rather confident that mitochondrial mutations contribute to aging in one way or another. RHH is an economical and abundantly testable hypothesis for the mechanism of that contribution – and arguably one of only two such hypotheses that are consistent with all available data, the other being based on mtDNA mutations' possible role in sarcopenia (McKenzie et al. 2002). The details of how mtDNA mutations contribute to aging may bear heavily on what interventions would or would not be expected to mitigate their progeric effects, and even on the prospects for possible side-effects of other biomedical innovations such as somatic cell nuclear transfer, which may involve extensive and possibly mutagenic manipulation of mtDNA in gametes or ES cells (de Grey 2004b). The expeditious testing of these hypotheses is thus a priority.

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