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Mini review

Metabolism in the *Caenorhabditis elegans* Mit mutants

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Abstract

In many eukaryotes oxidative phosphorylation via the mitochondrial electron transport chain provides the major means of ATP production. Complete removal of this capacity often results in premature death. Recent studies using the nematode *Caenorhabditis elegans* are surprising because they have revealed that disruption of many of the key components of the normal mitochondrial energy-generating machinery do not result in death, rather they result in adult life span extension. Such mutants have been collectively termed Mit mutants. In this short review, the potential use of alternate metabolic pathways for energy generation by Mit mutants will be considered. The effects of using such pathways on residual mitochondrial functionality, reactive radical species production, and longevity will also be explored.

Keywords: Aging; Caenorhabditis elegans; Mitochondria; Mit mutants; Oxidative stress; Membrane potential; *clk-1*; *isp-1*; *atp-3*; *lrs-2*

1. Introduction

Mitochondria are the metabolic heart of most eukaryotic cells. In addition to their key role in oxidative phosphorylation and ATP production, they also perform important roles in pyrimidine biosynthesis, fatty acid metabolism, steroid biogenesis, [Fe–S] cluster assembly, and calcium homeostasis. Mitochondria are highly specialized, the innermost of their double membranes embedded with the protein machinery necessary for ATP production. Food molecules are predominately oxidized in the central mitochondrial matrix by the tricarboxylic acid (TCA) cycle. Electrons from these molecules are then tunneled down a chain of inner membrane-bound redox centers, and are ultimately used to reduce molecular oxygen to water. This electron transport chain (ETC) is comprised of several components, including (in order of electron transport) complexes I and II, which oxidize NADH and succinate, respectively, ubiquinone (Q), complex III, cytochrome *c*, and finally complex IV, which reduces oxygen to water. During electron transport, redox energy is captured in the form of a proton gradient which is subsequently harvested by a fifth protein complex, termed complex V or F_oF_1 ATPase, to generate ATP.

In humans, alterations in mitochondrial functionality underlie an extraordinary ensemble of clinical problems (Wallace, 1999). These diseases commonly involve tissues that have high energy requirements such as heart, muscle, or the renal and endocrine systems. Many of these diseases have been linked to specific DNA mutations (both nuclear or mitochondrial) that inhibit mitochondrial bioenergetics or biogenesis, or disrupt mitochondrial DNA synthesis or stability (Brandon et al., 2005). Increasing evidence also implicates mitochondrial dysfunction in aging. Several studies have recorded an increase in the number of somatic mtDNA mutations that accumulate with age (Melov et al., 1995; Welle et al., 2003). Furthermore, a recent study, using healthy human volunteers, revealed a distinct decrease in mitochondrial bioenergetic capacity with advancing age (Short et al., 2005). The free radical theory of aging (Harman, 1956), places mitochondria at the seat of aging since this organelle is the largest generator of harmful reactive radical species in the cell.

Unexpectedly, many mutants in the nematode *Caenorhabditis elegans* that are defective in their ability to generate components of their ETC are, paradoxically, long-lived—the so-called Mit mutants. These mutants, and possible reasons for their unexpected life extension, form the topic of the present discussion.

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2. The Mit mutants

The Mit class of long-lived mutants generally contain loss of function, or reduced-in-activity, alterations in components of the canonical ETC (Fig. 1). Most exhibit a 20–40% increase in mean adult life span. Mutations affecting all five ETC complexes, as well as the intermediary electron carrier, ubiquinone, have been identified (reviewed in Rea and Johnson, 2003). In addition, mutations affecting transporters of TCA-cycle substrates, pyruvate dehydrogenase, and other components modulating ETC substrate availability have similarly been shown to lengthen life (Dillin et al., 2002; Lee et al., 2003; de Jong et al., 2004; Kayser et al., 2004a). Genetic epistasis experiments indicate that almost all of the Mit mutants act independently of the other major pathway known to extend adult lifespan in worms—the insulin-like *daf*-2/*daf*-16 signaling pathway (Lee et al., 2003).

We will focus our attention on the four best-studied Mit mutants-*clk*-1 (Wong et al., 1995), *isp*-1 (Feng et al., 2001), *lrs*-2 (Lee et al., 2003) and *atp*-3 (Dillin et al., 2002). The first three mutants are defined by known point mutations while the fourth, like many Mit mutants, has been identified by RNAi-mediated disruption of its transcript. Disruption of the canonical ETC occurs at different locations in each of these four mutants. In *clk*−1, a defective demethoxyubiquinone (DMQ) mono-oxygenase prevents synthesis of 5-hydroxyubiquinone, the penultimate intermediate of ubiquinone formation, leading to the accumulation of DMQ_9 instead of Q_9 (the subscript refers the number of isoprenoid groups present on the quinone ring) (Rea, 2001; Jonassen et al., 2001). *isp*-1 contains a mutation in the Rieske iron–sulfur protein subunit of complex III (Feng et al., 2001). The long-lived *isp*-1*(qm150)* mutant allele corresponds to a missense point mutation which may affect the redox potential of the [2Fe–2S] cluster in the head region of the ISP-1 protein. This region normally acts to transfer single reducing equivalents within complex III from ubiquinol to cytochrome c_1. Feng et al. (2001) suggest that fewer electrons move down this high affinity arm of the Q-cycle (Mitchell, 1975) and onto cytochrome *c*. *lrs*-2 contains a mutation in mitochondrial leucine-tRNA synthetase and was identified in a screen for genetic alterations that increased nematode life span independently of the *daf*-2/*daf*-16 pathway (Lee et al., 2003). The *lrs*-2*(mg312)* allele encodes a truncated protein that is predicted to be inactive and animals with this mutation exhibit a 200% increase in mean adult life span relative to wild-type animals. The mitochondrial genome of *C. elegans* encodes 12 polypeptides, all components of the ETC: cytochrome *b*, subunits I–III of complex IV, the a-chain of the F_oF_1 ATPase and finally subunits 1–6 and 4 L of complex I (Okimoto et al., 1992). Loss of all these proteins is predicted to occur in *lrs*-2. Lastly, the *atp*-3 mutant is deficient in the delta subunit of the F_oF_1 ATPase (Dillin et al., 2002). This subunit forms part of the stator that anchors the $\alpha_3\beta_3$ subunit of F_1 relative to the inner mitochondrial membrane and permits other components of the complex to rotate during the ATP generation cycle.

Not all *C. elegans* mutants with defects in their mitochondrial ETCs are long-lived. The *mev*-1*(kn1)* mutant, for example, contains a missense mutation in the large cytochrome *b* subunit of complex II, resulting in an inability to transfer electrons to Q, increased superoxide radical

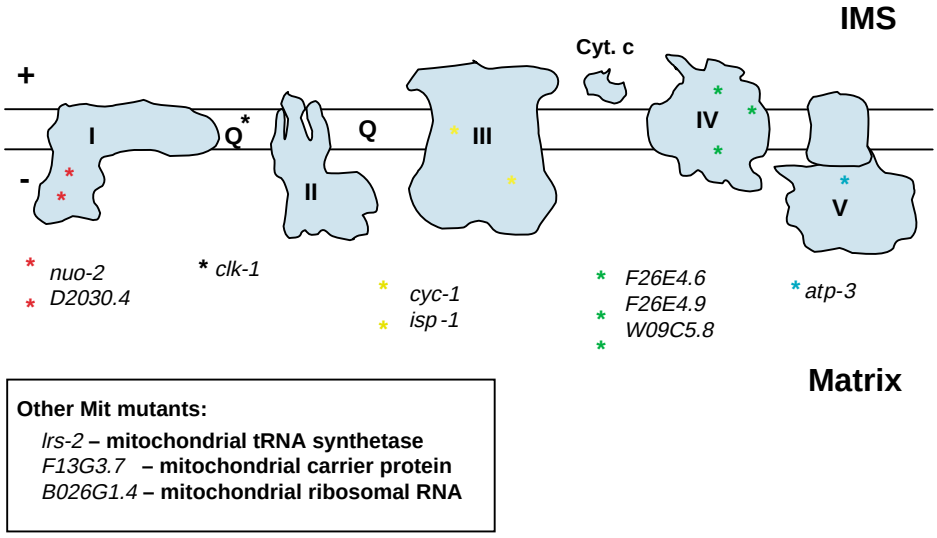

Fig. 1. Location of altered mitochondrial proteins in Mit mutants of *C. elegans*. Complexes I–V of the ETC are depicted schematically (shapes correspond to predicted quaternary structures). Each complex is a multi-protein assembly, and by analogy to other eukaryotes, thought to be comprised of 43, 4, 11, 13 and 16 subunits, respectively. Subunits affected by Mit mutations are marked with an asterisk and corresponding gene names are listed. Only mutations affecting the ETC are shown. Many more Mit mutations are not depicted. (cyt. c, cytochrome *c*; IMS, inner membrane space; Q, ubiquinone; +/-, orientation of $\Delta\varphi$).

production at complex II, and a profound shortening of life span that can be reversed by lowering ambient oxygen concentrations (Senoo-Matsuda et al., 2001). Similarly, *gas*-1*(fc21)* contains a missense mutation in the 49 kDa subunit of complex I, which leads to a decrease in the maximal rate of oxidative phosphorylation at complex I, a reciprocal rate increase at complex II, an increase in total reactive oxygen species (ROS)-mediated damage to mitochondrial proteins, and finally a shortening of lifespan (Kayser et al., 2004b). Such findings imply that there must be something peculiar about the long-lived Mit mutants and the specific mutations they harbor. How is it that one mitochondrial mutation can disrupt the ETC and shorten lifespan while another, seemingly related one, can increase lifespan?

3. Mitochondrial bioenergetics

Two major factors define the 'health' of a mitochondrion—ROS production and the protonmotive force (Δp). Both affect long-term mitochondrial viability, while Δp is also intimately linked to ATP metabolism.

3.1. Mitochondrial ROS production

Undesired production of reactive radical species (superoxide, hydroxy radical, nitric oxide, lipid peroxides, xenobiotic intermediates etc.), are thought by many to be the cause of senescence (reviewed in Martin et al., 1996; Sohal, 2002). Studies in multiple species indicate that the largest source of ROS derives from normal operation of the mitochondrial electron transport chain. Transient formation of flavinsemiquinone and ubisemiquinone within components of the ETC (Skulachev, 1996), provide sites from which electrons can be prematurely lost to molecular oxygen to form the highly unstable superoxide radical (O_2^-). Estimates from several species suggest that from 0.02% to as much as 2% of electrons may be lost in this manner (Cadenas et al., 1977; Herrero and Barja, 1998). The higher values are probably overestimates and likely reflect the non-physiological, in vitro oxygen concentrations used during measurement (Imlay and Fridovich, 1991). Superoxide is an extremely unstable molecule and rapidly dismutates into H_2O_2 (spontaneous, 10^5 $[mol/L]^-1$ and SOD-catalyzed, 10^9 $[mol/L]^{-1}$ (Tarpey and Fridovich, 2001). H_2O_2, on the other hand, is relatively stable and can traverse mitochondrial membranes; however, if not degraded catalytically, or in the presence of transition metals (such as iron and copper), H_2O_2 can form highly destructive hydroxyl radicals that can lead to lipid peroxidation and various other detrimental radical chain reactions that result in irreversible damage to host molecules and structures (Knight, 2000).

In *C. elegans*, as in most eukaryotes, mitochondria appear to provide the bulk of energy production under normoxic conditions. Tsang and Lemire (2002) have shown that in the hermaphrodite, mitochondrial DNA (and presumably therefore mitochondria) undergoes a massive expansion during larval development, increasing some 30 fold. The bulk of this expansion corresponds to germline development in the fourth-larval and young adult stages. In the worm, complexes I and III, and possibly complex II under some conditions, are likely to be the major sites of ROS production based on biochemical, sequence conservation and cross-species studies (Senoo-Matsuda et al., 2001; Kristal and Krasnikov, 2003; Ishiguro et al., 2001).

3.2. Mitochondrial Δp

The inner mitochondrial membrane acts as a capacitator that stores potential energy in the form of an electrical (Δφ), and to a lesser extent, chemical (ΔpH) gradient (Nicholls and Ferguson, 2002). Mitchell (1961) expressed the Gibbs free energy available in this electrochemical gradient in terms of voltage to define the term protonmotive force (Δp). Under normal circumstances mitochondria maintain a Δp of about 180–200 mV by pumping protons and/or separating charge as electrons are passed along the ETC—specifically, through complexes I, III and IV. Full reduction of the ETC results in a peak Δp of about 220 mV, beyond which non-specific proton leakage limits Δp. Many key mitochondrial activities, such as ATP production, Ca^{2+} regulation and apoptosis are modulated by Δp or its components. ROS production at complexes I and III is also strongly dependent upon these parameters (Nicholls and Ferguson, 2002).

3.3. Mitochondrial ATP production

F_oF_1 ATPase is a multi-subunit enzyme complex (Nicholls and Ferguson, 2002) consisting of a membrane embedded domain (F_o), and a hydrophilic catalytic domain (F_1) that provides for reversible phosphorylation of ADP. F_o operates as a molecular motor, coupling proton translocation across the inner mitochondrial membrane to the interconversion of ADP and ATP (Reid et al., 1966). Under normal circumstances, mitochondria maintain their Δp and matrix ADP/ATP ratio at levels such that they favor the forward reaction—that is, ATP synthesis. Pathological conditions, that alter this balance, can turn the F_oF_1 complex into an ATPase.

With these bioenergetic principles in mind we can begin to understand that the mitochondrial mutations present in the long-lived Mit mutants presumably function to modulate ROS production, Δp and/or ATP production 'better' than their wild type gene products. This may occur at a regulatory level, or by invoking compensatory metabolic responses that change how one, or all, of these parameters are generated.

4. Energy production in the Mit mutants

While mitochondrial oxidative phosphorylation regenerates much of the ATP and NAD^+ in many eukaryotes, numerous other pathways have also co-evolved to provide for both energy production and reducing equivalent recycling. Such pathways range from simple cytosolic fermentations (like lactate production in exercising human

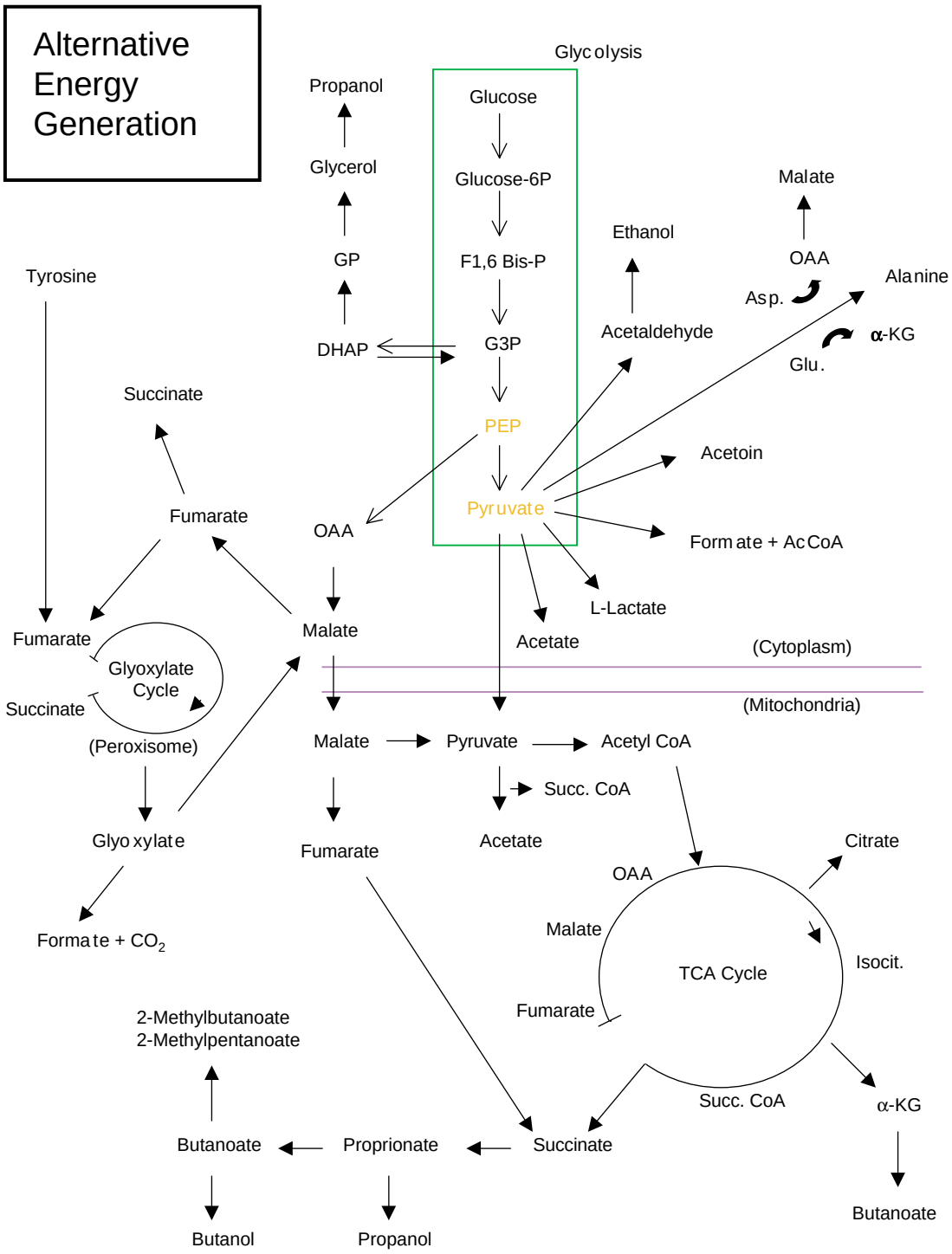

Fig. 2. Alternate energy production pathways in helminthes. ATP production in helminthes can proceed by the many pathways depicted (some remain hypothetical), but not all are common to every species. Redox balance is maintained by regulating the amounts of various reduced and oxidized end-products excreted. Only terminal end-products and key metabolic intermediates are shown. The reader is referred to Tables 1 and 2 I of Barrett (1984) for a detailed discussion of the amounts of ATP and NADH produced in the various pathways. (GP, *s,n*-glycerophosphate; DHAP, dihydroxyacetone phosphate; PEP, phosphoenolpyruvate; OAA, oxaloacetate; F1,6 BisP, fructose 1,6 Bisphosphate; G3P, glycerol-3-phosphate; Asp, aspartic acid; Glu, glutamic acid; α-KG, α-ketoglutarate; AcCoA, acetyl coenzyme A; Succ., succinate; Isocit., isocitrate; TCA, tricarboxylic acid).

muscle cells), through more complex pathways that involve specialized organelles (such as the hydrogenosome and chloroplast), to elegant modifications of ordinary mitochondria that result in branched electron transport chains capable of conferring anaerobic growth.

Many worm species spend all or part of their life cycle in hypoxic environments. Several pathways by which members of this large group of animals produce energy in the presence of low or no oxygen are already known. Most are fermentations falling broadly into two categories—those that produce end-products derived from pyruvate and those that produce end-products derived from phosphoenolpyruvate (Fig. 2). A typical worm species can make use of several such pathways simultaneously (Barrett, 1984). The availability of such choice affords these animals a number of advantages, including: the ability to modulate redox couples (so that synthetic reactions can be sustained in the absence of oxygen), the option of sending metabolic intermediates into overflow pathways when glycolytic flux is enhanced, modulation of ATP production rate and efficiency, end-product storage (for both carbon recycling and evasion of the metabolic costs of excretion), avoidance of end-product toxicity, pH control, nitrogen excretion, osmotic regulation, and the option of operating different pathways in a tissue-, age- and sex-specific manner.

In its natural soil environment, *C. elegans* is regularly exposed to bouts of hypoxia and anoxia. Studies by Foll et al. (1999) have shown that *C. elegans* can employ several alternative energy-generating pathways when cultured anaerobically. The potential ability to invoke these alternate pathways to generate both ATP and NADH provides the first clue to how Mit mutants may be supplementing deficits in their mitochondrial energy budgets. Nonetheless, many of the mutations are predicted to cause profound effects on Δp and mitochondrial ROS production. Clearly, there must be other pieces to the longevity puzzle.

5. Hypotheses for Mit mutant life extension

A common characteristic of Mit mutants is slowed embryonic development and larval growth, as well as decreased pharyngeal pumping and defecation rates. Several investigators (Hekimi et al., 2001; Branicky et al., 2000; Lakowski and Hekimi, 1996) have used this point to argue Mit mutants are long-lived simply because they 'live slower', equating slowed rhythmic behaviors with similar reductions that are observed at lower temperatures, and consequently assuming there must also be a reduction in overall metabolic rate. Such thoughts have led to notions of reduced rates of damage accumulation because 'burning a candle at only one end should double its lifetime'. But is life in the Mit mutants really so simple? Is it reasonable to assume all metabolic reactions have slowed down given that growth temperature remains unaltered? An alternative is that compensatory metabolic pathways are activated in an attempt to remove the build-up of upstream metabolites. Perhaps use of such pathways produces less 'toxicity' and this is what results in slower aging. In the following paragraphs we will explore some of the potential compensatory reactions that might be invoked in the various Mit mutants and we will place them in the context of how they could relate to life extension.

5.1. Antioxidants and hormesis

One simple solution to why Mit mutants are long-lived involves the notion that mitochondria in these animals remain partially functional but there is a mild *increase* in their ROS leak rate. Unlike the situation in *mev*-1 and *gas*-1, this electron leak rate would be effectively counteracted by the induction of endogenous protectants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase; responses mediated by the oxidative-stress regulated transcription factor SKN-1 (An and Blackwell, 2003), might also be evoked. Not only could the increased ROS be detoxified, but levels may be lowered beyond normal values to effectively result in *less* damage accumulation and ultimately increased lifespan. In many regards this idea is equivalent to hormesis—the process whereby an organism previously exposed to a sub-lethal dose of a toxicant or stressor makes a positive response to that treatment (Calabrese and Baldwin, 2002). In this case, the positive response would be longer life.

How might ROS production become elevated? Inhibitor studies using purified mitochondria reveal superoxide production becomes elevated when upstream ETC components become loaded with reducing equivalents. Based on the types of mutations present in *clk*-1, *isp*-1 and *atp*-3, one could easily imagine that ETC complexes upstream of many Mit mutant mutations become loaded with reducing equivalents, turning their mitochondria into overt radical generators. Considering the *isp*-1*(qm150)* mutation, if the redox potential of the ISP-1 [2Fe–2S] cluster was in fact elevated above that of cytochrome c_1 this would not only hinder electron flow down the high affinity arm of the Q-cycle, but, it would also keep the cluster loaded and/or leave ubisemiquinone at Q_P (or Q_N, where the b_H heme would normally off load its electron back to Q). *isp*-1*(qm150)* could respond by elevating protective enzymes such as mitochondrial SOD3, catalase or a putative NAD(P)H: paraquat-type oxidoreductase, hence only appearing to have a reduction in ROS output. Increased sod-3 mRNA levels have been observed in this mutant (Feng et al., 2001). For *clk*-1, the situation appears to be more complicated. Two different studies have reported there is no apparent alteration in SOD3 or catalase levels (Honda and Honda, 1999; Braeckman et al., 2002). On the other hand, in one of these studies (Honda and Honda, 1999), the *clk*-1 mutation was found to potentiate the aberrant activation of SOD3 in a *daf*-2 mutant background. Our own studies on the Nrf-2 related transcription factor SKN-1

reveal that the anti-oxidant system controlled by this protein is constitutively activated in *clk*-1 mutants (manuscript in preparation). These findings hint that *clk*-1 animals may well be experiencing endogenous oxidative stress.

A second reason why Mit mutants may be long-lived relates to an additional major function of ubiquinone—to act directly as an antioxidant (Nohl et al., 2003). Build-up of reducing equivalents in the ETC of Mit mutants may be sufficient to invoke elevated Q production (except in *clk*-1). Excess Q could then act to retrieve free radicals from runaway chain reactions. Ultimately, however, these electrons have to find at least one sink and it is not immediately clear what it would be, especially in Mit mutants with ETC blocks at the level of complex III or beyond—though Q does have other cellular sites of minor function (Nohl et al., 2003). For *clk*-1, other studies (Miyadera et al., 2002) have suggested that DMQ, the Q synthesis intermediate that accumulates in these animals, is a better antioxidant than Q (despite having a lower standard redox potential). DMQ is very likely not conferring the *clk*-1 longevity effect, however, since three *clk*-1 alleles (*e2519, qm30* and *qm50*) have been identified (Wong et al., 1995) that differ greatly in the degree of their lifespan increase but which accumulate almost equivalent amounts of DMQ (Jonassen et al., 2001).

5.2. Reduced ETC flux

The antithetical argument to increased ROS production is of course lowered ROS production. Could the Mit mutants be long-lived simply because their mitochondria generate fewer radical species due to reduced flux through their ETCs? This idea seems particularly appealing for the mitochondrial leucine tRNA synthetase mutation *lrs-2(mg312)* which, at first glance, seems to have no functional complexes I, III, IV or V. In a general test of this hypothesis Kayser et al. (2004b) extensively characterized mitochondria from *clk*-1 and N2 worms that had each been cultured in liquid medium and fed wild-type *E. coli*. Their key finding was that mitochondrial complex I from *clk*-1 operated at ~30% of the activity level of N2 mitochondria when examined using endogenous quinone carriers only (that is, DMQ_9, bacterial Q_8 and rhodoquinone). This defect was not due to a reduction in maximal attainable complex I activity, nor to a reduced activity of complex I substrate transporters, but to the specific types of endogenous quinones present. DMQ_9 could not functionally replace Q_9. Oddly, complex II activity remained unaltered. These findings were in direct contrast to earlier studies (Felkai et al., 1999; Miyadera et al., 2001; Jonassen et al., 2003), which reported there were no differences in complex I (NADH oxidoreductase) activity in *clk*-1 animals. As pointed out by Kayser et al. (2004b), these earlier studies presumably either missed the observation due to the absence of appropriate wild-type controls, or they failed to include inhibitors that discounted possible non-specific NADH oxidation. Kayser et al. (2004) also showed that *clk*-1 nematodes display a reduced level of oxidized mitochondrial proteins relative to those from wild-type animals. These findings led to the suggestion that senescence in *clk*-1 animals might, in fact, be delayed because of a lowered electron flux through complex I and a consequent reduction in oxidative damage.

5.3. Fumarate reductase

In humans, complex II is comprised of four subunits and, in conjunction with ubiquinone, normally operates as a succinate:ubiquinone oxidoreductase to form fumarate and ubiquinol (Nicholls and Ferguson, 2002). The enzyme is separable into a hydrophilic catalytic domain and a hydrophobic membrane-associated anchoring domain. Both domains are themselves heterodimers, the hydrophilic one comprised of an FADH-containing (Fp) protein as well as an Iron–Sulfur containing (Ip) protein, and the hydrophobic domain of two small helical bundle-forming proteins. In isolation, and when provided with an appropriate electron acceptor, the hydrophilic catalytic domain can function as a succinate dehydrogenase (SDH). In many species, albeit under a limited set of conditions, this heterodimer can also function in the reverse direction as a fumarate reductase (FR).

In several facultative anaerobic species, duplication and evolution of Fp has allowed one isoform to operate preferentially in the fumarate reductase direction when incorporated into complex II (Iverson et al., 1999). The concomitant evolution of rhodoquinone (RQ), a quinone electron carrier with a standard redox potential below that of the standard succinate/fumarate couple, permits efficient fumarate reduction in such animals under their normal growth conditions. In *C. elegans*, sequence analysis indicates the presence of two Fp isoforms (Rea and Johnson, 2003). Biochemical analysis also reveals the presence of RQ (Takamiya et al., 1999). In principal, then, fumarate reductase coupled to RQ, and/or SDH reversal mediated by a very highly reduced Q pool, might provide a pathway by which an ETC block at Q, III or IV could be by-passed. How might this transfer into life span extension? In bacteria, FR, but not SDH, becomes a radical generator when expressed under normoxic conditions, due to higher electron density on its oxygen-accessible FAD moiety (Imlay, 1995). If the same were true for FR in *C. elegans*, Mit mutants that invoke this pathway may consequently induce an antioxidant response that hormetically increases life span. Alternatively, complex III becomes a ROS generator when $\Delta\varphi$ becomes elevated (Nicholls and Ferguson, 2002); perhaps by-pass of this complex altogether is life span enhancing.

5.4. F_1 ATPase reversal

rho^0 cells, eukaryotic cells that are deficient in mitochondrial DNA, provide an alternative explanation for

why Mit mutants of *C. elegans* could be long-lived. rho^0 cells are able to maintain their inner mitochondrial membrane potential by first synthesizing ATP in their cytoplasm via fermentation (Buchet and Godinot, 1998). Large amounts of ATP are then imported into the mitochondrial matrix by the adenine nucleotide transporter, whereupon it is hydrolyzed via the soluble F_1 fragment of complex V (all subunits of this fragment are nuclearly encoded). Subsequent electrogenic export of the $H_2PO_4^{1-}$ that is released during ATP hydrolysis effectively regenerates the lost proton potential. Mitochondrial inner membrane potential is presumably limiting for many mitochondrial mutants of *C. elegans*; only those that can maintain it above a certain threshold survive. Similar to rho^0 cells, we can hypothesize that several of the Mit mutants (including *atp*-3), might employ cytoplasmic pathways of the type discussed in Section 4, to generate ATP and ultimately regenerate their inner mitochondrial membrane potential. This would have two effects, reduced reliance on their ETCs, and a possible lowering of Δp with a concomitant reduction in ROS production.

5.5. GPDH

The *s*,*n*-glycerophosphate dehydrogenase (GPDH) electron shuttle, normally operates to bring cytoplasmic reducing equivalents into the mitochondria at the level of Q (Nicholls and Ferguson, 2002). This system operates by coupling reduction of glycolytically-produced dihydroxyacetone phosphate with cytoplasmically produced NADH to generate *s*,*n*-glycerophosphate. The latter is then re-oxidized by mitochondrial *s*,*n*-glycerophosphate dehydrogenase using Q as the final electron acceptor. In effect, this system by-passes complexes I and II of the ETC and it is conceivable that this by-pass could be important to those Mit mutants with defects upstream of complex III. If complexes III and IV were to remain in tact in these animals then use of this pathway could, in principal, lead to a slight *increase* in Δp across the inner mitochondrial membrane, triggering protective responses that hormetically increase life span as a result. A $\sim$10 mV elevation in Δp is possible because removal of complex I means the higher 'static head Δp' of the complexes III and IV segment of the ETC becomes attainable.

5.6. Food source effects

C. elegans is capable of extracting Q_8 from their bacterial food source for use in their own mitochondrial ETCs. Typically, Q_8 is only accumulated to amounts less than 5% of wild-type Q_9 levels (Jonassen et al., 2003). In the case of *clk*-1, exogenous Q_8 appears to be an essential dietary requirement and is critical for both development and fertility. It has been postulated that endogenously synthesized DMQ_9 may operate in conjunction with bacterial Q_8 in a ping-pong type mechanism at complex III to simultaneously lower non-specific electron loss (via the purported higher antioxidant activity of DMQ_9) and to pass electrons into the ETC (via bacterial Q_8) (Padilla et al., 2004). Importantly, while growth of *clk*-1 in the complete absence of bacterial Q_8 results in developmental arrest, these animals still live longer than wild-type adults implying Q_8, or indeed a Q_8-DMQ_8 interaction, is not essential for their extended longevity (Jonassen et al., 2001).

5.7. Maternal effects

The rapid generation time of *C. elegans* makes it possible that parental factors, stable enough to be transmitted to offspring, can affect viability of adult offspring, a so-called 'maternal effect'. In *clk*-1, maternally supplied mRNA can, for the most part, reverse the long-life of homozygous F1 animals derived from a heterozygous parent (Wong et al., 1995). We predict that this phenomenon might also play a similar, but opposite, role in determining the long-life of homozygous *lrs*-2 animals, which is only derivable from a balanced parental strain (Lee et al., 2003). Residual mRNA and/or mitochondrial ETC activity derived from the parental strain, presumably acts to supplement both ATP production and NADH recycling. Depending on the degree of maternal contribution and the metabolic requirements of *lrs*-2, Δp may attain lower than normal levels, again indirectly leading to a reduction in ROS generation at complexes I and III and increased life span.

6. Conclusion

Several metabolic strategies have been presented that could potentially account for Mit mutant survival and their extended longevity. All approaches have in common the ability to maintain mitochondrial Δp, minimize mitochondrial ROS production (either directly or indirectly), and generate sufficient ATP and NADH for biosynthetic purposes. Many of the strategies could in principal operate cooperatively and indeed additional metabolic 'improvisations' are imaginable. Such ideas illustrate that the Mit mutants are likely a heterogeneous group of longevity mutants, glued together by the commonality of adopting different metabolic pathways that optimize the status quo between key mitochondrial parameters and long-life.

It is often questioned whether the *C. elegans* Mit mutants are a useful model for the study of aging in humans in light of the hundreds of known mitochondrial-dependent diseases. Is the long-life of Mit mutants simply a reflection of that fact that *C. elegans* has at its disposal metabolic pathways that we humans do not? In some cases, the answer will be yes. Despite this, the Mit mutants remain much more informative to aging research for a different reason: As in humans, not all mitochondrial mutations in *C. elegans* result in life extension. When we consider this point, immediately we understand that the Mit mutants must represent a select

set of animals with mitochondrial parameters set optimally for long-life. Defining what these parameters are opens the door to a touchstone that might one day allow scientists to artificially engineer our own mitochondria in order to reap the same longevity benefits.

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