A Metabolic Model for Life Span Determination in *Caenorhabditis elegans*

Commentary

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Summary

Several studies with the nematode *Caenorhabditis elegans* have made the unexpected discovery that certain hypomorphic mutations in genes encoding mitochondrial proteins result in life span extension. These mutations appear to act independently of the other known pathway that regulates life span extension, the dauer-specifying insulin/IGF-1-like pathway. Here we present a hypothesis that unifies the effects of these two classes of genes on longevity. The central concept is that energy generation in *C. elegans* occurs by differential flux through two coexisting mitochondrial metabolic pathways—aerobic respiration and fermentative malate dismutation. In the latter process, fumarate is terminally reduced at complex II to succinate. We suggest that most, if not all, long-lived mutants in *C. elegans* utilize malate dismutation, a byproduct of which is the generation of fewer radical species.

Introduction

The nematode *Caenorhabditis elegans* has been used extensively as a model organism for aging research (Guarente and Kenyon, 2000). On the premise that the fundamental processes of aging are conserved across species, the identification of single-gene mutations that extend the life span in *C. elegans* (gerontogenes) has been seen by many as heralding the potential that pharmacological intervention might one day also be used to extend human longevity. A perplexing result to have arisen from the nematode studies concerns the actual number of genes that, when mutated, lead to life span extension (the Age phenotype). Indeed this number is now well in excess of 100 and represents almost 1% of the *C. elegans* genome. How can such a large number of genes normally act to "limit" life span (Wood and Johnson, 1994)? In this manuscript we have attempted to address this issue. Beyond simply invoking evolutionary arguments, such as antagonistic pleiotropy and the like, we propose that most such mutations invoke the same compensatory biochemical response—the ultimate outcome of which is longer life. We suggest that many, if not all, *C. elegans* life span-enhancing mutations induce the utilization of an alternative, anaerobic, fermentative metabolism (normally present in the dauer state), which leads to a reduction in the generation of life-shortening radical oxygen species (ROS) (Finkel and Holbrook, 2000; Ishii et al., 1998; Melov et al., 1998). This

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model provides a simple and common endpoint to the complex transcriptional regulatory networks previously suggested to control longevity in *C. elegans* (Guarente and Kenyon, 2000; Hsin and Kenyon, 1999). Hence, a discrete metabolic alteration could be responsible for the increased longevity seen in many Age mutants.

Long Life and the Dauer Connection

C. elegans normally transits four larval stages (L1–L4) before becoming a self-fertilizing, egg-laying adult. Under adverse environmental conditions juveniles can progress into an alternative L3 larval stage known as the dauer larvae (Riddle, 1988). Surviving for 3–6 months (4–8 times its normal life span), dauer larvae appear limited only by the resources stored before entry. In *C. elegans*, dauer formation is controlled by three parallel signaling pathways, all of which channel sensory information into the DAF-12 protein (Riddle et al., 1981). The insulin/IGF-1-like signaling pathway (IIL), comprising *daf-2* (an insulin/IGF receptor homolog), *age-1* (a PI-3 kinase), *daf-18* (a PTEN phosphatase), *daf-16* (a FOXO family transcription factor), and other genes, is of particular interest because attenuation of this pathway allows reproductive development yet also significantly extends adult life span (Gems et al., 1998; Kenyon et al., 1993). Complete inactivation of this pathway leads to a dauer-constitutive (Daf-c) phenotype. *daf-16* mutants are epistatic to *daf-2* mutants and abolish the ability to form dauer larvae as well as all life span enhancement mediated by the IIL pathway. Importantly, the decision to become a dauer is made midway through the L1 larval stage, and this pathway is regulated, indisputably, at the transcriptional level.

Several reports have recently found that hypomorphic mutations in, or RNAi knockdown of, numerous mitochondrial proteins can also lead to life span extension, but in a *daf-16*-independent manner (Dillin et al., 2002; Lee et al., 2003). Almost all of these mutations affect components of the energy-producing electron transport chain (ETC) and normally would be expected to have deleterious effects. Few have an obvious paralogue in the nematode genome, ruling out redundancy as the reason for an absence of a lethal phenotype. We collectively term all such mitochondrial mutants "Mit." A more detailed description of several of these mutations is provided in Supplemental Table S1 at http://www.developmentalcell.com/cgi/content/full/5/2/197/DC1. Of particular relevance is *clk-1* (Wong et al., 1995), a Mit mutant defective in synthesis of the important, mitochondrial electron-transporting lipid ubiquinone. Mutations in the CLK-1 protein variably affect its ability to synthesize demethoxy-ubiquinone, the next to penultimate precursor of ubiquinone (Rea, 2001). Biochemical studies hint, however, that the ultimate defect in *clk-1* worms is potentially a major reduction in the ability of the citric acid cycle to operate efficiently (Miyadera et al., 2001; Jonassen et al., 2001, 2002). Within our model for life span extension in *C. elegans*, the paradoxical life-lengthening effects of mutations such as *clk-1* become
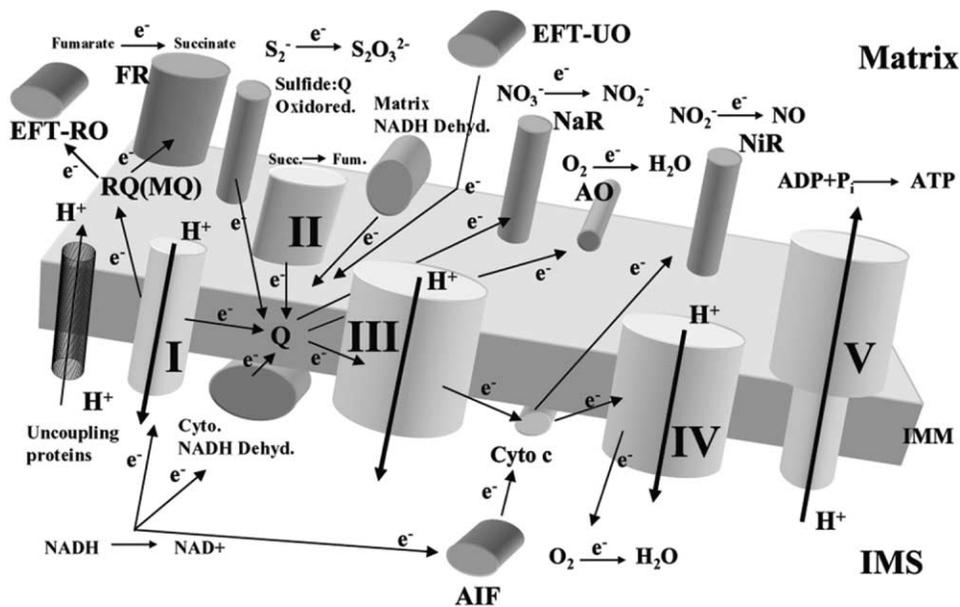
Figure 1. Alternative Mitochondrial Electron Sinks and Sources

The inner mitochondrial membrane (IMM) of a generic eukaryotic mitochondrion is depicted. Complexes I–V are labeled. Proteins providing new functionality to the electron transport chain are shown at rear. Heavy dark arrows represent proton (H^+) flow through their respective complexes. Additional arrows indicate directionality of electron (e^-) flow. AIF, apoptosis-inducing factor; AO, alternative oxidase, Cyto c, cytochrome c; Dehyd, dehydrogenase; EFT-RO, electron transfer flavoprotein:rhodoquinone oxidoreductase; EFT-UO, electron transfer flavoprotein:ubiquinone oxidoreductase; Fum, fumarate; FR, fumarate reductase; IMS, intermembrane space; Matrix, mitochondrial matrix; MQ, menaquinone (in bacteria); NaR, nitrate reductase; NiR, nitrite reductase; Oxidored, oxidoreductase; Q, ubiquinone; RQ, rhodoquinone; Succ, succinate.

readily explainable. Furthermore, a previously unrecognized relationship between the Mit and Daf classes of mutants is suggested.

Mitochondrial Function in *C. elegans*

The energy-generating machinery of mammalian mitochondria is comprised of five multisubunit proteins, termed complexes I–V (Wallace, 1999). Typically, reducing equivalents derived from the tricarboxylic acid (TCA) cycle enter either complex I (NADH) or complex II (FADH$_2$). Electrons are then shunted to complex III via ubiquinone (UQ), then from complex III onto complex IV via cytochrome c, and, finally, onto dioxygen. An electrochemical gradient across the inner mitochondrial membrane is maintained by proton pumping at complexes I, III, and IV. Controlled dissipation of this gradient through complex V results in ATP production. Many nonmammalian mitochondria also contain components that extend the utility of the ETC for ATP production (Van Hellemond et al., 2003). All such modifications permit either new electron sources to be tapped (e.g., sulfide:ubiquinone oxidoreductase of the mussel *Geukensia demissa* [Doeller et al., 2001]) or provide access to a new sink for electron disposal, such as through mitochondrial fumarate reductase (FR; Figure 1).

Many parasitic worms, such as *Fasciola hepatica* and *Ascaris suum*, encounter both aerobic and anaerobic environments during various stages of their life cycles (Komuniecki and Harris, 1995; Tielens et al., 2002). In the absence of oxygen many of these species employ anaerobic mitochondrial fermentation for energy production (Figure 2). This process involves carboxylation of the glycolytic intermediate phosphoenolpyruvate into oxaloacetate and subsequent conversion into malate. Transfer of malate into the mitochondrial matrix, with subsequent dismutation into acetate and succinate, allows for both redox balance and generation of reducing equivalents that can be passed into the ETC for ATP production. A specialized complex II that operates in reverse as a fumarate reductase and requires the use of rhodoquinone (RQ) provides for succinate formation. Rhodoquinone is a benzoquinone with a redox potential sufficiently negative so that the fumarate/succinate couple can be reduced and is therefore an indispensable component of the FR assembly. Both acetate and succinate are excreted (Tielens et al., 2002).

Substantial evidence suggests that *C. elegans* contains the machinery for anaerobic mitochondrial fermentation in addition to that for the TCA cycle. First, rhodoquinone is present in all stages of the *C. elegans* life cycle (Takamiya et al., 1999). Second, database mining (http://www.ncbi.nlm.nih.gov) reveals that *C. elegans*, like the parasitic nematode *A. suum* (Kita et al., 2002), contains two complex II flavoprotein (Fp) subunits (*C03G5.1* and *C34B2.7*). The Fp subunit contains the fumarate/succinate catalytic site and largely dictates whether complex II will function as a succinate dehydrogenase (SDH) or a fumarate reductase. Furthermore, *C. elegans* also contains a small, soluble FR (*F48E8.3*) that is homologous to OSM1 (*osm*otic sensitive protein 1) and FRDS (*f*umarate *red*uctase, *s*oluble) of the yeast
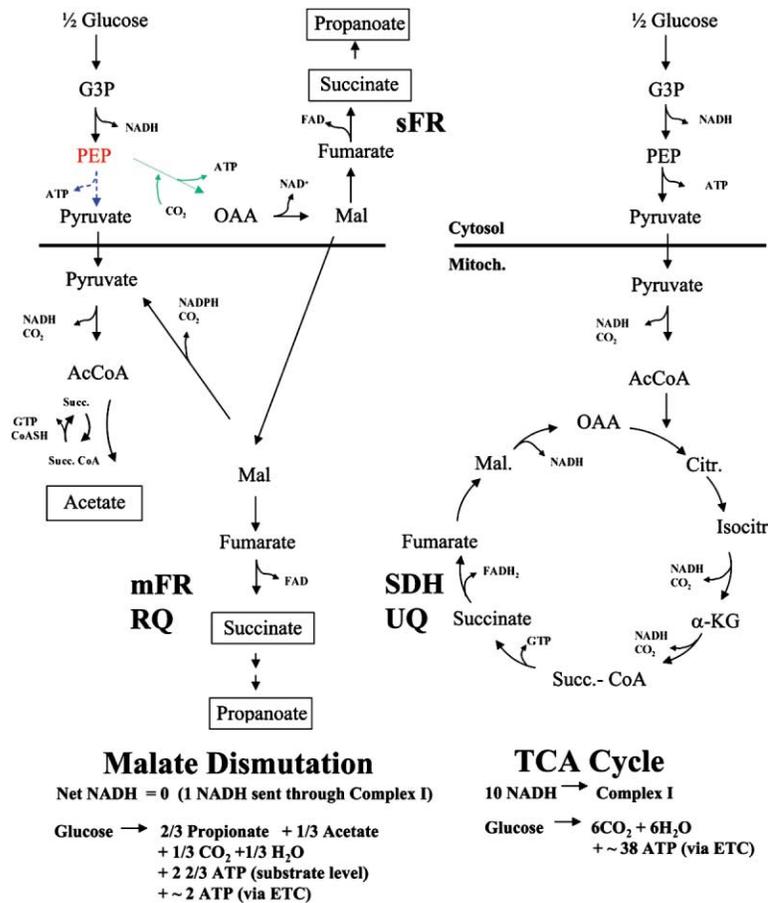
Figure 2. Comparative Analysis of Malate Dismutation and the Tricarboxylic Acid Cycle

Left, malate dismutation (MD) as a proposed mechanism for energy generation in dauer larvae of *C. elegans*. Many facultative anaerobes employ reduction of fumarate as an anaerobic terminal electron sink. MD occurs during proprionate fermentation in the helminth *F. hepatica* (Van Hellemond et al., 2003). Right, the tricarboxylic acid (TCA) cycle as utilized during reproduction and growth. Note that mitochondrial fumarate reductase (mFR) and succinate dehydrogenase (SDH) catalyze reverse reactions. Glycolytic reactions occurring in the cytosol are shown above the heavy black line, and mitochondrial reactions are shown below the line. Note the positions of the small fumarate reductase (sFR) and mFR. NAD(P)H generated inside the mitochondria is sent through complex I before reaching mFR. Waste metabolites are boxed. The key control point of MD: phosphoenolpyruvate (PEP, red) can be either carboxylated to oxaloacetate (OAA) by phosphoenolpyruvate carboxykinase (green arrow) or dephosphorylated to pyruvate by pyruvate kinase (broken blue arrow). Conversion of pyruvate to acetate proceeds by way of a GTP-generating step involving two enzymes, acetate:succinate CoA-transferase and succinyl-CoA synthase. (C05G5.4 encodes an α subunit of the latter enzyme. Interestingly, at the mRNA level, this protein is coordinately regulated with the SDH/mFR subunit C03G5.1 [Kim et al., 2001] and is 2-fold elevated in dauers versus a mixed-stage population [Jones et al., 2001].) Bottom, the stoichiometry of each reaction is illustrated. α-KG, α-ketoglutarate; AcCoA, acetyl coenzyme A; Citr, citrate; G3P, glyceraldehyde-3-phosphate; Isocitr, isocitrate; RQ, rhodoquinone; Succ. CoA, succinyl coenzyme A; UQ, ubiquinone.

Saccharomyces cerevisiae (Enomoto et al., 2002) (Supplemental Figure S1). Removal of both OSM1 and FRDS renders yeast unable to grow anaerobically. Third, when starved (to avoid confounding effects of the *Escherischia coli* food source) and incubated under anoxic conditions (oxygen partial pressure <400 Pa), *C. elegans* can survive for periods up to 48 hr by utilizing its carbohydrate stores (Foll et al., 1999). Lactate and acetate were the major excretory products during the first 12 hr, after which acetate, succinate, and proprionate predominated. These data suggest that fermentative pyruvate dismutation and then, later, the more efficient fermentative malate dismutation reactions were likely in operation.

C. elegans is a soil-dwelling nematode, and there are two occasions when it likely requires the use of an anaerobic metabolism. The first is during periods of precipitation, when oxygen levels quickly become limiting (Lee and Atkinson, 1976). At least one other soil-dwelling nematode, the entomopathogen *Steinernema carpocapsae*, has been shown to have a facultative anaerobic metabolism (Shih et al., 1996), and this property will probably hold true for both free-living and parasitic soil-dwelling nematodes in general. Second, we predict that,

beyond its needs for coping with inclement weather, the dauer larvae also has a need for an anaerobic metabolism that is potentially crucial for its longevity. Unlike other larval stages, dauer larvae clearly have a substantial capacity to operate anaerobically when challenged (Scott et al., 2002). Furthermore, relative to mixed-stage populations, dauer larvae have a 10-fold reduction in TCA cycle activity (O'Riordan and Burnell, 1989; Wadsworth and Riddle, 1989), a 3-fold elevation of their small fumarate reductase (*F48E8.3*) RNA, and a 10-fold decrease in RNA encoding the complex II Fp subunit *C34B2.7*. RNA encoding the other Fp subunit, *C03G5.1*, remains unchanged in dauers (http://elegans.bcgsc.bc.ca/SAGE). Such findings strongly suggest that dauers employ FRs for energy balance. Whether dauers indirectly impose a degree of hypoxia within their cells by virtue of both their unique morphology and enzymology (which include a sealed buccal cavity, altered cuticular arrangement, and upregulation of oxygen-catabolizing enzymes, such as catalase, SOD3, and P450 enzymes [Holt and Riddle, 2003; Murphy et al., 2003; Riddle, 1988; Wang and Kim, 2003]), or whether dauers just simply operate an anaerobic metabolic pathway that bypasses the requirement for oxygen as a terminal

acceptor remains to be fully determined, but there is convincing circumstantial evidence to think that at least the latter may be the case.

Mitochondria and Life Span Regulation

In light of the above observations, we propose that mutations residing in the *daf-2* pathway of *C. elegans* lead to aberrant coactivation of a nonoxygen-requiring metabolism—namely, mitochondrial fermentative malate dismutation. Interestingly, a recent study has shown that mutations in *daf-2* confer resistance to hypoxia in a *daf-16*-dependent manner (Scott et al., 2002). Note that, while only kinase- and ligand binding-defective class 2 mutants (Gems et al., 1998) exhibited the Hyp phenotype, we hypothesize that the lack of hypoxia resistance in the class 1 mutants may have arisen by hypoxia inadvertently stabilizing the class 1 mutant DAF-2 proteins—since the latter are all predicted to be trafficking defective, not activity defective.

In mutant *daf-2* animals we speculate that the malate dismutation pathway operates along with a reduced TCA metabolism, as it would in the dauer, and that, in the presence of abundant food (as occurs for *daf-2* mutants and the like), substantial amounts of glycolytic products may be funneled through this pathway. We suggest that the decreased reliance on complex III and IV, coupled with an increased flux of electrons through complex I and the use of RQ, results in a substantial reduction of cellular ROS generation. In the presence of high-oxygen and low-succinate concentrations, mitochondrial FR is known to produce ROS (Imlay, 1995; Turrens, 1987). We suggest that this is the reason dauers express elevated levels of SOD3—specifically to protect against oxygen reperfusion when dauer exit is initiated (if, indeed, the dauer exists in a state of self-imposed hypoxia) or simply to mitigate the effects of FR as a radical generator. In this vein, we also suggest that a series of such additional protective mechanisms (ROS detoxification, heat-shock protein expression, etc.) are normally elicited in conjunction with dauer formation and that it is the combination of reduced ROS generation via malate dismutation and increased repair capacity that synergistically interact to confer increased longevity and stress resistance. Indeed, the dauer-dependent DAF-16 transcription factor may directly sense oxidative stress (Henderson and Johnson, 2001) to activate several stress resistance proteins (McElwee et al., 2003).

But what of the large class of Mit mutants? Within the above framework, we suggest that the long-lived Mit mutants normally fulfill their energy requirements as L1, possibly by use of lactate- and/or alcohol-producing fermentative pathways or by supplementation with bacterial UQ, as is the case for *clk-1* (Jonassen et al., 2001). The net result for all Mit mutants is the avoidance of dauer formation and irrevocable commitment to reproductive development. We propose that, in later stages, when the energy requirements of Mit mutants reach a level they cannot meet and in the absence of full *daf-12* and/or hypoxia-mediated activation, these animals are unable to obtain the benefits of a genetic program designed specifically for anaerobic survival. Instead, these animals are forced into using a metabolism that is a biochemical approximation of the streamlined version in the dauer. Such a metabolism could lead to life span extension by way of reduced ROS production due to anaerobic fermentation, albeit less than that observed under optimal *daf-2* inactivation. Consistent with this hypothesis we note that, while the complex III mutant, *isp-1*, shows very little additional life extension when combined with a *daf-2* mutation and grown at 20°C, when cultured at 25°C (and hence placed under a greater mitochondrial load), it becomes Daf-c (Feng et al., 2001). This provides support for our idea that energy level sensing at the L1 stage dictates when an animal will eventually reveal either a Mit phenotype or, instead, transit into a dauer state. Furthermore, our model also provides a straightforward explanation for the synergistic increase in life span that is observed when the *clk-1* and *daf-2* mutations are combined (Lakowski and Hekimi, 1996). We suggest that, in the *clk-1* single mutant, absence of sufficient UQ at a post-L1 stage leads to energy depletion and (inefficient) activation of fermentative metabolism. In contrast, in *daf-2* mutants, this metabolism is constitutively active; hence, *clk-1;daf-2* double mutants respond robustly to *clk-1*-induced energy depletion by powerfully increasing their fermentative metabolism.

Three studies have been published within the last month describing either the transcriptome of dauer larvae (Holt and Riddle, 2003; Wang and Kim, 2003) or that of sterile adults fed *daf-2* RNAi from the first day of adulthood (Murphy et al., 2003). The findings of Holt and Riddle (Holt and Riddle, 2003) concur strongly with our hypothesis that an anaerobic metabolism employing malate dismutation may be functional in dauers. In addition these authors identified what appears to be an additional pyruvate (electron) sink—a predicted alcohol dehydrogenase, *K12G11.3*. The same gene was also identified by Kenyon and colleagues in *daf-2* adults (Murphy et al., 2003). The latter study also made the observation that *daf-2* adults express several antibacterial peptides. The authors argue that a collection of many such stress response genes may be key to dauer longevity. None of the antibacterial genes were identified by Kim and colleagues (Wang and Kim, 2003) in their dauer populations, at least not with their stringent statistical criteria. One possibility is that the antibacterial genes were ectopically activated and then amplified in the *daf-2* adults as a secondary response to their bacterial food source. Dauers would not normally be exposed to such signals in their sealed and food-deficient environment.

Finally, the study of Wang and Kim (2003) and that of Kenyon and colleagues (Murphy et al., 2003) provide evidence that a functional glyoxylate cycle is present in dauer and *daf-2* animals alike. This supports earlier biochemical studies of dauer populations (O'Riordan and Burnell, 1990), where it was also found that these larvae had a marked reduction in their TCA activity relative to adult populations, but their glyoxylate cycle activity remained unchanged. Such observations are important because the glyoxylate cycle normally functions to convert acetyl units from fat reserves into four-carbon compounds, and the process requires an oxidant system to do so. Typically localized to peroxisomes, some of the glyoxylate cycle enzymes utilize oxygen in a catalase-dependent reaction to oxidize reducing equivalents extracted during the retrieval of acetyl units from fat

reserves. Regeneration of oxaloacetate also typically requires an oxidant, usually SDH in mitochondria.

The apparent importance of the glyoxylate cycle to *C. elegans* dauers, coupled with the reported presence of both lipid and glycogen stores in these animals (Gerisch et al., 2001) and the observed temporal usage patterns of lipid and glycogen stores in other soil-dwelling nematode dauers (Wright et al., 1997), leads us to conclude that the precise metabolism dauers in general will employ (aerobic, anaerobic, or combinations thereof) will be species, age, and environment dependent. We suspect that every dauer-forming nematode species will have a unique, time-dependent preference for the use of specific fuel stores while in the dauer larval form. Some, for example, may operate largely aerobically and utilize lipid stores solely. Such species might only tap their glycogen stores anaerobically during periods of precipitation, after which they quickly replenish them when conditions permit via coupled glyoxylate and gluconeogenesis cycles. Given the probable low mitochondrial content of *C. elegans* dauers (Tsang and Lemire, 2003), we predict that energy production in these larvae might proceed by way of both a reduced in activity TCA cycle and by malate dismutation. The soluble fumarate reductase *F48E8.3* and one or both of the two FR/SDH isoforms, *C03G5.1* and *C34B2.7*, may therefore be pivotal in this regard. It is likely that, whatever strategy nematode dauers use, NAD(P)$^+$/NAD(P)H and ADP/ATP ratios will be poised to minimize ROS production and maximize life span.

A Unified Theory for Aging?

Within our proposed metabolic model for life span determination in *C. elegans*, we can identify two additional advantages. The first is that our model is consistent with the evolutionary theory of aging (Kirkwood, 1977). Specifically, since there are clear disadvantages to reproductive success when utilizing an anaerobic metabolism under normoxia, as revealed by the Mit mutants, such a pathway has obviously not been selected as the primary mode of energy production. It appears, however, to have been selected for in the case of dauer diapause, presumably because it leads to a marked reduction in life-shortening radical species. The second advantage of our model is that it unifies many observations about the Age mutants without requiring a full understanding of the complex transcriptional regulation controlling each. In this light, the suppression of the entire Age phenotype of *clk-1* mutants (back to wild-type N2 levels) by ablation of the gonad (Dillin et al., 2002) can be explained purely metabolically. The gonad rapidly expands in size through the L3 to adult stages and is accompanied by a 30-fold increase in mitochondrial number (Tsang and Lemire, 2002). Most of this cellular expansion derives from the germline precursor cells Z2 and Z3, with only a minor portion coming from the somatic gonad precursor cells Z1 and Z4 (Schedl, 1997). So, for *clk-1* mutants in the absence of complete gonad expansion, the increased metabolic demands are not encountered, and a fermentative pathway is not employed. In the presence of only the Z1- and Z4-mediated expansion, a minor oxidative stress is sensed, but the metabolic response that is initiated not only copes, but

also results in a mild life span increase. Presumably the same applies for the wild-type (N2), since ablation of Z2 and Z3 also leads to life span extension. It must follow then that metabolic benefits induced by Z1/Z4 expansion in N2 must normally be offset by the stress imposed by Z2/Z3 expansion. Previous models (Hsin and Kenyon, 1999) have hypothesized the presence of discrete life span-lengthening and life span-shortening factors continuously emanating from the somatic and germline gonads, respectively. A corollary of our model is that it is simply the level of oxidative stress that is the sole determinant of life span modulation.

We note that mammals do not appear to have a distinct fumarate reductase activity, though several SDH-related sequences do appear to be present in the human genome. Furthermore, under certain conditions, some SDHs can be enticed to operate in reverse (Pershad et al., 1999). Whether mammals can completely alter their mode of energy production to reduce their ROS load remains to be determined. Caloric restriction (CR) leads to life extension in many organisms. Current thoughts on the mechanisms by which CR increases life span ultimately resonate around the efficiency of the mitochondria (Merry, 2002). Hormonal factors affecting lipid desaturases, uncoupling proteins, and the activity of specific ETC complexes are all thought to eventually lead to a reduction in the ROS load emanating from the mitochondrial electron transport chain and as a consequence life span extension. Such an endpoint is very similar to what we are predicting to lead to life span extension in both the *daf-16*-dependent and Mit longevity mutants (the same probably also applies to the Eat mutants). Our hypothesis differs only in that ROS are predicted to be decreased by virtue of the use of a metabolism that operates largely independently of the canonical ETC radical generators. In light of our present hypothesis, it is conceivable that the mode(s) by which CR occurs in different organisms might vary and could involve a related facultative anaerobic metabolic shift and/or procedures that alter the efficiency of the canonical ETC to lower ROS production.

Finally, the success of using *C. elegans* as a fundamental research tool to understand the aging process has been amply illustrated in the enormous number of mutations identified that lead to extended longevity. If, as we propose, longevity determination in this species resides, at least in part, by powering cells with a whole new metabolism, where then does this leave humans and their endeavors to increase their own life span? For one thing, hypotheses remain just that until further tested. Several aspects of our model are readily testable, and studies are already under way to address them. Even if human cells are not capable of anaerobic mitochondrial fermentation, as probably they are not, mitochondrial efficiency appears to be a fundamental control point for life span optimization.

References

Dillin, A., Hsu, A.L., Arantes-Oliveira, N., Lehrer-Graiwer, J., Hsin, H., Fraser, A.G., Kamath, R.S., Ahringer, J., and Kenyon, C. (2002). Rates of behavior and aging specified by mitochondrial function during development. Science *298*, 2398–2401.

Doeller, J.E., Grieshaber, M.K., and Kraus, D.W. (2001). Chemolitho-heterotrophy in a metazoan tissue: thiosulfate production matches ATP demand in ciliated mussel gills. J. Exp. Biol. 204, 3755–3764.

Enomoto, K., Arikawa, Y., and Muratsubaki, H. (2002). Physiological role of soluble fumarate reductase in redox balancing during anaerobiosis in Saccharomyces cerevisiae. FEMS Microbiol. Lett. 215, 103–108.

Feng, J., Bussiere, F., and Hekimi, S. (2001). Mitochondrial electron transport is a key determinant of life span in Caenorhabditis elegans. Dev. Cell 1, 633–644.

Finkel, T., and Holbrook, N.J. (2000). Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Foll, R.L., Pleyers, A., Lewandovski, G.J., Wermter, C., Hegemann, V., and Paul, R.J. (1999). Anaerobiosis in the nematode Caenorhabditis elegans. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 124, 269–280.

Gems, D., Sutton, A.J., Sundermeyer, M.L., Albert, P.S., King, K.V., Edgley, M.L., Larsen, P.L., and Riddle, D.L. (1998). Two pleiotropic classes of daf-2 mutation affect larval arrest, adult behavior, reproduction and longevity in Caenorhabditis elegans. Genetics 150, 129–155.

Gerisch, B., Weitzel, C., Kober-Eisermann, C., Rottiers, V., and Antebi, A. (2001). A hormonal signaling pathway influencing C. elegans metabolism, reproductive development, and life span. Dev. Cell 1, 841–851.

Guarente, L., and Kenyon, C. (2000). Genetic pathways that regulate ageing in model organisms. Nature 408, 255–262.

Henderson, S.T., and Johnson, T.E. (2001). daf-16 integrates developmental and environmental inputs to mediate aging in the nematode Caenorhabditis elegans. Curr. Biol. 11, 1975–1980.

Holt, S.J., and Riddle, D.L. (2003). SAGE surveys C. elegans carbohydrate metabolism: evidence for an anaerobic shift in the long-lived dauer larva. Mech. Ageing Dev. 124, 779–800.

Hsin, H., and Kenyon, C. (1999). Signals from the reproductive system regulate the lifespan of C. elegans. Nature 399, 362–366.

Imlay, J.A. (1995). A metabolic enzyme that rapidly produces superoxide, fumarate reductase of Escherichia coli. J. Biol. Chem. 270, 19767–19777.

Ishii, N., Fujii, M., Hartman, P.S., Tsuda, M., Yasuda, K., Senoo-Matsuda, N., Yanase, S., Ayusawa, D., and Suzuki, K. (1998). A mutation in succinate dehydrogenase cytochrome b causes oxidative stress and ageing in nematodes. Nature 394, 694–697.

Jonassen, T., Larsen, P.L., and Clarke, C.F. (2001). A dietary source of coenzyme Q is essential for growth of long-lived Caenorhabditis elegans clk-1 mutants. Proc. Natl. Acad. Sci. USA 98, 421–426.

Jonassen, T., Marbois, B.N., Faull, K.F., Clarke, C.F., and Larsen, P.L. (2002). Development and fertility in Caenorhabditis elegans clk-1 mutants depend upon transport of dietary coenzyme Q8 to mitochondria. J. Biol. Chem. 277, 45020–45027.

Jones, S.J., Riddle, D.L., Pouzyrev, A.T., Velculescu, V.E., Hillier, L., Eddy, S.R., Stricklin, S.L., Baillie, D.L., Waterston, R., and Marra, M.A. (2001). Changes in gene expression associated with developmental arrest and longevity in Caenorhabditis elegans. Genome Res. 11, 1346–1352.

Kenyon, C., Chang, J., Gensch, E., Rudner, A., and Tabtiang, R. (1993). A C. elegans mutant that lives twice as long as wild type. Nature 366, 461–464.

Kim, S.K., Lund, J., Kiraly, M., Duke, K., Jiang, M., Stuart, J.M., Eizinger, A., Wylie, B.N., and Davidson, G.S. (2001). A gene expression map for Caenorhabditis elegans. Science 293, 2087–2092.

Kirkwood, T.B. (1977). Evolution of ageing. Nature 270, 301–304.

Kita, K., Hirawake, H., Miyadera, H., Amino, H., and Takeo, S. (2002). Role of complex II in anaerobic respiration of the parasite mitochondria from Ascaris suum and Plasmodium falciparum. Biochim. Biophys. Acta 1553, 123–139.

Komuniecki, R., and Harris, B.G. (1995). Carbohydrate and energy metabolism in helminths. In Biochemistry and Molecular Biology of Parasites, J.J. Marr and M. Muller, eds. (London: Academic Press), pp. 49–66.

Lakowski, B., and Hekimi, S. (1996). Determination of life-span in Caenorhabditis elegans by four clock genes. Science 272, 1010–1013.

Lee, D.L., and Atkinson, H.L. (1976). Physiology of Nematodes, Second Edition (London: Macmillan).

Lee, S.S., Lee, R.Y., Fraser, A.G., Kamath, R.S., Ahringer, J., and Ruvkun, G. (2003). A systematic RNAi screen identifies a critical role for mitochondria in C. elegans longevity. Nat. Genet. 33, 40–48.

McElwee, J., Bubb, K., and Thomas, J.H. (2003). Transcriptional outputs of the Caenorhabditis elegans forkhead protein DAF-16. Aging Cell 2, 111–121.

Melov, S., Schneider, J.A., Day, B.J., Hinerfeld, D., Coskun, P., Mirra, S.S., Crapo, J.D., and Wallace, D.C. (1998). A novel neurological phenotype in mice lacking mitochondrial manganese superoxide dismutase. Nat. Genet. 18, 159–163.

Merry, B.J. (2002). Molecular mechanisms linking calorie restriction and longevity. Int. J. Biochem. Cell Biol. 34, 1340–1354.

Miyadera, H., Amino, H., Hiraishi, A., Taka, H., Murayama, K., Miyoshi, H., Sakamoto, K., Ishii, N., Hekimi, S., and Kita, K. (2001). Altered quinone biosynthesis in the long-lived clk-1 mutants of Caenorhabditis elegans. J. Biol. Chem. 276, 7713–7716.

Murphy, C.T., McCarroll, S.A., Bargmann, C.I., Fraser, A., Kamath, R.S., Ahringer, J., Li, H., and Kenyon, C. (2003). Genes that act downstream of DAF-16 to influence the lifespan of Caenorhabditis elegans. Nature 424, 277–283.

O'Riordan, V.B., and Burnell, A.M. (1989). Intermediary metabolism in the dauer larvae of the nematode Caenorhabditis elegans. I. Glycolysis, gluconeogenesis, oxidative phosphorylation and the tricarboxylic acid cycle. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 92, 233–238.

O'Riordan, V.B., and Burnell, A.M. (1990). Intermediary metabolism in the dauer larvae of the nematode Caenorhabditis elegans. II. The glyoxylate cycle and fatty-acid oxidation. Comp. Biochem. Physiol. B Biochem. Mol. Biol. 95, 125–130.

Pershad, H.R., Hirst, J., Cochran, B., Ackrell, B.A., and Armstrong, F.A. (1999). Voltammetric studies of bidirectional catalytic electron transport in Escherichia coli succinate dehydrogenase: comparison with the enzyme from beef heart mitochondria. Biochim. Biophys. Acta 1412, 262–272.

Rea, S. (2001). CLK-1/Coq7p is a DMQ mono-oxygenase and a new member of the di-iron carboxylate protein family. FEBS Lett. 509, 389–394.

Riddle, D.L. (1988). The dauer larva. In The Nematode Caenorhabditis elegans, W.B. Woo, ed. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press), pp. 393–412.

Riddle, D.L., Swanson, M.M., and Albert, P.S. (1981). Interacting genes in nematode dauer larva formation. Nature 290, 668–671.

Schedl, T. (1997). Developmental genetics of the germ line. In C. elegans II, D. Riddle, T. Blumenthal, B. Meyers, and J. Priess, eds. (Cold Spring Harbor, New York: Cold Spring Harbor Laboratory Press), pp. 241–269.

Scott, B.A., Avidan, M.S., and Crowder, C.M. (2002). Regulation of hypoxic death in C. elegans by the insulin/IGF receptor homolog DAF-2. Science 296, 2388–2391.

Shih, J.J.M., Platzer, E.G., Thompson, S.N., and Carroll, E.J. (1996). Characterization of key glycolytic and oxidative enzymes in Steinernema carpocapsae. J. Nematol. 28, 431–441.

Takamiya, S., Matsui, T., Taka, H., Murayama, K., Matsuda, M., and Aoki, T. (1999). Free-living nematodes Caenorhabditis elegans possess in their mitochondria an additional rhodoquinone, an essential component of the eukaryotic fumarate reductase system. Arch. Biochem. Biophys. 371, 284–289.

Tielens, A.G., Rotte, C., van Hellemond, J.J., and Martin, W. (2002). Mitochondria as we don't know them. Trends Biochem. Sci. 27, 564–572.

Tsang, W.Y., and Lemire, B.D. (2002). Mitochondrial genome content is regulated during nematode development. Biochem. Biophys. Res. Commun. 291, 8–16.

Tsang, W.Y., and Lemire, B.D. (2003). The role of mitochondria in

the life of the nematode, Caenorhabditis elegans. Biochim. Biophys. Acta *1638*, 91–105.

Turrens, J.F. (1987). Possible role of the NADH-fumarate reductase in superoxide anion and hydrogen peroxide production in Trypanosoma brucei. Mol. Biochem. Parasitol. *25*, 55–60.

Van Hellemond, J.J., Van Der Klei, A., Van Weelden, S.W., and Tielens, A.G. (2003). Biochemical and evolutionary aspects of anaerobically functioning mitochondria. Philos. Trans. R. Soc. Lond. B Biol. Sci. *358*, 205–215.

Wadsworth, W.G., and Riddle, D.L. (1989). Developmental regulation of energy metabolism in Caenorhabditis elegans. Dev. Biol. *132*, 167–173.

Wallace, D.C. (1999). Mitochondrial diseases in man and mouse. Science *283*, 1482–1488.

Wang, J., and Kim, S.K. (2003). Global analysis of dauer gene expression in Caenorhabditis elegans. Development *130*, 1621–1634.

Wong, A., Boutis, P., and Hekimi, S. (1995). Mutations in the clk-1 gene of Caenorhabditis elegans affect developmental and behavioral timing. Genetics *139*, 1247–1259.

Wood, W.B., and Johnson, T.E. (1994). Aging. Stopping the clock. Curr. Biol. *4*, 151–153.

Wright, D.J., Grewal, P.S., and Stolinski, M. (1997). Relative importance of neutral lipids and glycogen as energy stores in dauer larvae of two entomopathogenic nematodes, Steinernema carpocapsae and Steinernema feltiae. Comp. Biochem. Physiol. B Biochem. Mol. Biol. *118*, 269–273.