PERSPECTIVE

Metabolism, ubiquinone synthesis, and longevity

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"You have made your way from worm to man, but much within you is still a worm."

—Nieztsche

Until recently, the mechanisms that control the aging process were thought to be immensely complex and nearly impossible to dissect at the molecular level. However, genetic analysis, primarily in model organisms such as yeast, worms, and flies, is dramatically changing this view. It seems that at least three distinct genetic networks control the aging process. These networks include the insulin/IGF-1 signaling pathway, signaling that arises from the mitochondrial electron transport chain, and finally, mechanisms that govern the response to dietary restriction. The use of invertebrates as model organisms to study aging has been extensive, since the ease of genetic manipulation and short life span give them desirable characteristics. However, the question often remains as to whether we can draw the same conclusions in higher organisms. Some of the life-lengthening pathways discovered in invertebrates (i.e., knocking down insulin/IGF-1 signaling) are conserved in mammals; however, distinguishing between species-specific models of aging and universal processes remains a major challenge in the field. Indeed, the conservation of such processes is key to the validation of the use of model organisms to study the aging process. Hekimi and colleagues (Liu et al. 2005) in this issue of *Genes and Development* substantiates the use of the round worm, *Caenorhabditis elegans*, as an excellent model for the study of the role of mitochondria in the aging process of mammals.

Mitochondria and longevity

Mitochondria have been implicated in the aging process for several decades. Measuring the metabolic rates of several species during the 1920s, Pearl (1928) discovered a correlation between metabolic rate and life span: Animals with lower metabolic rates consumed less O_2 per gram of tissue and lived longer than animals with higher metabolic rates. Exceptions have been discovered since Pearl's initial observations, such as the high metabolic rates of some long-lived birds (Holmes et al. 2001); however, Pearl's initial observation led to the formulation of the "rate of living theory of aging" (Pearl 1928). The theory suggests that reduced metabolic rates in an animal should result in an increased life span.

Several years later, the "rate of living theory of aging" became more refined with the formulation of the "oxygen radical theory of aging" proposed by Denham Harman (Harman 1956). Harman reasoned that reactions using molecular oxygen create, on occasion, toxic $O_2^{\bullet-}$ radicals. Harman hypothesized that lower levels of oxygen free radicals, by reducing metabolic activities that use molecular oxygen (O_2), would result in increased longevity.

The mitochondrial electron transport chain (ETC) is the major consumer of molecular oxygen within a cell. The ETC is situated within the inner membrane of the mitochondria. It is a complex cascade of redox reactions that allow the phosphorylation of ADP, forming ATP (the main energy carrier in the cell) using the energy derived from various substrates through central metabolism (glycolysis and the TCA cycle) contained in reducing equivalents (NADH or $FADH_2$) (Fig. 1). Electrons contained in these molecules enter complex I or complex II of the ETC and are then transferred on to complexes III and IV. The cytochrome c oxidase (complex IV) catalyzes the last redox reaction of the cascade by reducing diatomic oxygen to water. The particularity of this pathway is that the electron flux through the different ETC complexes is coupled to a proton translocation across the inner mitochondrial membrane against the existing gradient, reinforcing it. Thus, this process transduces energy from a chemical (chemical bonds) to a physical form (gradient). Finally, the gradient is used by the ATP synthase, complex V, to phosphorylate ADP to ATP (Mitchell 1961).

The ETC is an extremely efficient engine. However, ~0.4% to 4% of molecular oxygen is only partially reduced, producing the superoxide ion ($O_2^{\bullet-}$) (Boveris and Chance 1973; Turrens and Boveris 1980; Boveris 1984; Imlay and Fridovich 1991; Hansford et al. 1997). Within the mitochondria, the primary molecule responsible for producing superoxide is ubisemiquinone (Turrens et al. 1985). Ubiquinones are redox active, lipophilic essential

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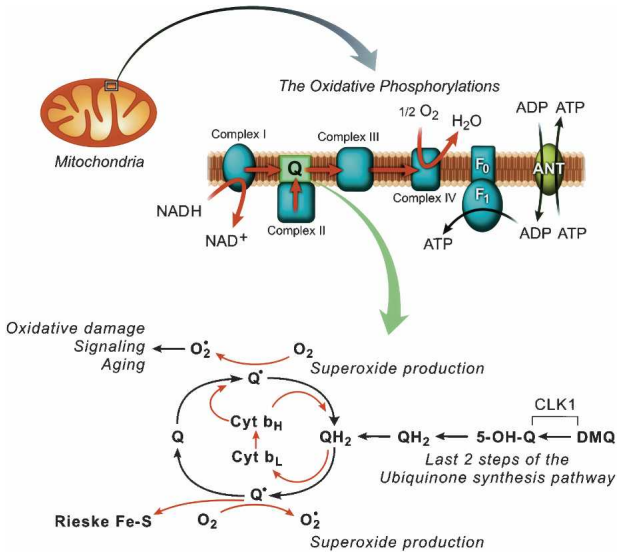

Figure 1. Ubiquinone synthesis is controlled by the gene *CLK1*. Depicted is a mitochondrion with magnification showing the mitochondrial electron transport chain, which is localized within the inner mitochondrial membrane. Electrons are donated to either complex I or complex II by NADH or $FADH_2$. From complexes I and II, electrons are transferred to ubiquinone (Q), complex III, and finally complex IV. Within the ETC, the Q-cycle can be found and is expanded as shown. When Q becomes QH_2 or vice versa, the ubiquinone goes through an intermediate form that is a radical: the ubisemiquinones ($Q^{\bullet}$). The ubisemiquinone radical can donate its free electron to the Rieske Iron Sulfur center, but can also reduce oxygen to superoxide ($O_2^{\bullet-}$), the main source of ROS. The *CLK1* gene encodes 3-methoxy-6-methyl-5-polyprenyl-benzoquinone-hydroxylase and is required for the synthesis of 5-hydroxy-quinone (5-OH-Q) from DMQ. Mutations of *clk1* lead to increased levels of DMQ.

electron carriers of the ETC involved in the Q-cycle (Fig. 1; Trumpower 1990). Briefly, the reduced form of the ubiquinone ($CoQH_2$) undergoes a nonenzymatic two-cycle reoxidation involving the ubisemiquinones as a "stable" radical intermediate. Ubisemiquinone is a radical intermediate that is formed when the oxidized form of the ubiquinone is reduced (and vice versa). Although ubisemiquinones are highly reactive toward O_2, they are very short lived, thereby partially alleviating their toxic effects within the cell. Furthermore, Reactive Oxygen Species (ROS) generated by misplacement of electrons from the ETC by ubisemiquinones are quickly and effectively detoxified by both superoxide dismutases and catalases. ROS that are able to escape detoxification can react with larger macromolecules, leading to the appearance of aberrant molecules, such as protein carbonyls or peroxidated lipids, which are dysfunctional and potentially impair processes essential for cell maintenance and survival. Such modifications have been shown to increase with age in all species tested so far and provide tools to study the aging process (Oliver et al. 1987; Sohal et al. 1993, 1994, 1995; Agarwal and Sohal 1996; Eisenstark et al. 1996; Dukan and Nystrom 1998; Adachi and Ishii 2000; Aguilaniu et al. 2003).

In addition to proteins and lipids, ROS can damage DNA and in particular, mitochondrial DNA (mtDNA). mtDNA encodes for several subunits of the electron transport chain and many mitochondrial specific tRNAs required for protein translation within the mitochondria. Therefore, damage to mtDNA can lead to the production of mutated forms of mitochondrial components. Consequently, the ETC itself can be altered and function in an abnormal manner. One of the possible consequences of such aberrant behavior is excessive ROS production. This would, in turn, lead to more oxidative modifications to other macromolecules, leading to a "catastrophe" scenario in which ROS production leads to more ROS produced.

Several pieces of data support a model in which mtDNA mutations contribute to the aging process. First, cytochrome-c-oxidase-deficient clones appeared in aging post-mitotic tissues (Muller-Hocker 1989). Second, pathogenic mtDNA point mutation 8993T → G led to an inhibition of the oxidative phosphorylation machinery and increased ROS production (Geromel et al. 2001; Mattiazzi et al. 2004). Third, Trifunovic et al. (2004) created mice that expressed a proofreading-deficient version of PolgA (the mitochondrial DNA polymerase). Consequently, these mice accumulated mitochondrial mutations at an accelerated rate, and aged faster (Trifunovic et al. 2004).

Mitochondrial metabolic rate versus quality of ubiquinone pool

The free radical theory of aging has been tightly linked with the assumption that increased electron flow (i.e., oxygen consumption rates) results in higher production of ROS. However, this assumption is slowly being overturned. In fact, it is often at very low oxygen consumption rates (i.e., low metabolic rates) that ROS production is the highest. For example, in isolated mitochondria that are functioning in a nonphosphorylating mode (because ADP was not provided), ROS production is higher (Korshunov et al. 1997). In yeast, when oxygen consumption rates are low due to limited activity of ATP synthase, protein carbonyl levels are higher (Aguilaniu et al. 2001). Indeed, when the conversion of ADP to ATP slows, oxygen consumption rate decreases, and membrane potential and reduced forms of cytochrome b are increased. The resulting higher levels of reduced cytochrome b create a larger pool of ubisemiquinones, resulting in a higher potential for interaction among ubisemiquinone and O_2, leading to higher levels of ROS (Fig. 1).

It appears that it is not the pace at which the ETC works that determines ROS production rate, but rather the redox state of the ubiquinone pool. Therefore, the quality of the ubiquinone pool may be more important than the rate of metabolism in determining longevity. In this issue of *Genes and Development*, Hekimi and colleagues (Liu et al. 2005) report that modification of the ubiquinone pool in mice, by directly perturbing the ubiquinone synthesis pathway, causes increased longevity and a higher resistance to oxidative stress.

Mutations and perturbations that alter mitochondrial function and life span

Mitochondria are the primary sites of oxygen free-radical production. Therefore, according to the "free radical theory of aging," reduced ROS production and/or increased protection against ROS should correlate with increased longevity. Consistent with this prediction, yeast, worms, flies, or mice that overexpress antioxidant defenses live longer (Melov et al. 2000; Fabrizio et al. 2003; Sun et al. 2004; Schriner et al. 2005). Although these studies showed that protection against ROS results in increased life span, the use of genetic approaches to alter mitochondria to study the aging process is not straightforward since, a priori, this organelle is indispensable for life. However, research in nematodes identified the mitochondrial oxidative phosphorylations as regulators of the aging process. Three studies demonstrated that reduction of function of several mitochondrial genes extends the life span of fully developed, adult worms. Siegfried Hekimi's lab showed that a single mutation in an iron sulfur component of complex III, *isp-1*, increased longevity (Feng et al. 2001). This mutation decreases oxygen consumption, suggesting that it lowers the activity of the electron transport chain (Feng et al. 2001). *isp-1* mutant worms have delayed development and reduced rates of other physiological processes, including eating, movement, and defecation (Feng et al. 2001). Two independent RNA interference (RNAi)-based screens, in the Ruvkun and Kenyon laboratories, also showed that components of the mitochondrial electron transport chain increased longevity when they were inactivated using RNAi (Dillin et al. 2002; Lee et al. 2003). This knockdown also resulted in reduced ATP levels, O_2 consumption, and slowed the rate of development and other physiological processes, including eating, movement, and defecation (Dillin et al. 2002; Lee et al. 2003). Mutations in another worm gene, *clk-1*, is discussed in greater detail below.

Not all mitochondrial ETC lesions increase longevity. The *mev-1(kn1)* mutation is probably the best example of a mutation that decreases mitochondrial activity, but does not increase longevity. *mev-1* encodes the cytochrome b large subunit of complex II. The *mev-1(kn1)* mutation was identified in a genetic screen to identify mutations that resulted in worms that were more sensitive to the drug methyl viologen (paraquat). Cells treated with paraquat produce excess oxygen free radicals. *mev-1(kn1)* mutant animals are short lived and have reduced mitochondrial respiratory rates (Ishii et al. 1998). Additionally, *mev-1(kn1)* mutant animals have higher levels of oxygen free radicals compared to wild-type animals (Senoo-Matsuda et al. 2001). Similar to *mev-1(kn1)* mutant animals, *gas-1(fc21)* mutant animals are also hypersensitive to paraquat, short lived, have reduced ETC activity, and have higher levels of oxygen free radicals compared to wild-type animals (Senoo-Matsuda et al. 2001). *gas-1* encodes the 49-kDa iron-containing subunit of mitochondrial electron transport chain complex I (Kayser et al. 1999). Besides *mev-1* and *gas-1* mutations, *nuo-1(ua1)* and *atp-2(ua2)* mutant worms also have deficient mitochondrial respiratory chains (Tsang et al. 2001). *nuo-1(ua1)* and *atp-2(ua2)* mutant animals arrest during development and do not grow to reproductive adulthood (Tsang et al. 2001). *nuo-1* encodes the NADH- and FMN-binding subunit of complex I, and *atp-2* encodes an active-site subunit of complex V, the ATP synthase (Tsang et al. 2001).

Taken together, a clear correlation between mitochondrial metabolic activity and longevity cannot be derived from these studies. For example, *clk-1* mutant animals are long-lived, but have normal respiratory rates, and RNAi of several ETC components results in increased longevity and decreased metabolic rates. Therefore, defining the role that mitochondria play in the aging process will be essential to elucidating the link between metabolic rates and longevity.

clk-1

In this issue of *Genes and Development*, it is reported that a loss-of-function mutation in the gene *mclk1* leads to a life-span extension in mice (Liu et al. 2005). *clk-1* is directly linked to mitochondria and their metabolism since it encodes for an enzyme that is crucial to ubiquinone synthesis (Jonassen et al. 1998). As explained above, these molecules are central to both mitochondrial respiration and superoxide production. Over the past decade, the mechanism by which the ubiquinone synthesis pathway influences longevity has been the focus of much investigation. Although a great deal of headway has been made, the mechanism by which *clk-1* imparts its beneficial effects is unknown.

clk-1 mutations were identified in an EMS screen for maternal-effect mutations that affected development and behavior in *Caenorhabditis elegans* (Wong et al. 1995). *clk-1* animals are slow growing, exhibiting twofold lengthened larval stages compared to wild type (Wong et al. 1995). The protracted embryonic cell cycle and larval development eventually result in fertile adults that continue to display slowed biological functions. These animals show a reduction in egg laying rate (as well as a decreased number of eggs produced). Defecation rates were slowed by 1.8-fold, and pharyngeal pumping was decreased 1.4-fold in the *clk-1* null mutants (*qm30*). Most notably, the mean and maximum life span were increased in the three *clk-1* mutants.

Interestingly, *clk-1* mutations can be fully rescued by maternal-derived *clk-1*$^{+}$. *clk-1*$^{-/-}$ mutants that descend from self-fertilizing *clk-1*$^{-/+}$ hermaphrodites are rescued of nearly all mutant phenotypes including life span (Wong et al. 1995). From these results, it appears that *clk-1* may act early during the animal's life cycle to help set the rate of living of the entire organism for the remainder of its life. The various phenotypes, especially the life-span extension, inspired further investigation of *clk-1* to determine the function and the degree of conservation between species.

The worm *clk-1* gene was mapped to chromosome III, and the 187-residue protein was determined to be highly

similar in structure and function to the *Saccharomyces cerevisiae* Coq7p protein and conserved among eukaryotes, including humans (Ewbank et al. 1997; Vajo et al. 1999). Yeast coq7p is an enzyme (3-methoxy-6-methyl-5-polyprenyl-benzoquinone-hydroxylase) required for the penultimate step in ubiquinone (Q) biosynthesis. Cells lacking coq7p cannot produce ubiquinone and accumulate dimethoxy-ubiquinone (DMQ) (Stenmark et al. 2001). *coq7*Δ mutants are respiration deficient, leading to the inability to grow on nonfermentable carbon sources (Jonassen et al. 1996). The *coq7*Δ mutant yeast strain is rescued by the *C. elegans clk-1* gene, or the homologous *COQ7* genes from rat or human (Jonassen et al. 1996; Ewbank et al. 1997; Vajo et al. 1999). Because of the respiration defects of yeast lacking coq7p, it was difficult to reconcile that *clk-1* mutant worms are able to perform mitochondrial respiration at near normal levels.

Jonassen et al. (2001) understood that the *C. elegans clk-1* mutants must obtain ubiquinone from their diet. When they grew worms on *Escherichia coli* strains unable to produce ubiquinone, the *clk-1* mutant worms arrested at the L2 larval stage of development and failed to develop into reproductive adults (Jonassen et al. 2001). Further studies revealed that it was the length of the isoprenyl tail of ubiquinone that was critical to the severity of the phenotypes observed (developmental arrest and/or sterility). The shorter the isoprenyl chain, the more severe the phenotype (Hihi et al. 2003; Jonassen et al. 2003).

While the worm can obtain enough ubiquinone from its diet to metabolically function and develop to adulthood, the *mclk1*$^{-/-}$ mutant mice fail to survive past embryonic day 10.5 (E10.5), although the heterozygous mice are able to synthesize ubiquinone and are viable (Levavasseur et al. 2001; Nakai et al. 2001). Like *coq7*Δ mutant yeast cells, *mclk1*$^{-/-}$ mutant embryonic mouse cells do not synthesize ubiquinone; rather, they accumulate DMQ. Whether mitochondrial respiration is affected is unclear, since Levavasseur et al. (2001) reported that DMQ was able in vitro to sustain 65% of wild-type respiration. More direct experiments are still needed to clarify this issue.

In addition to accumulating DMQ, *mck1*$^{-/-}$ embryonic stem (ES) cells are resistant to oxidative stress, have lower levels of lipid oxidative damage and are slow to multiply. This is similar to the phenotypes observed in the worm, suggesting a conserved function from nematodes to mammals. In addition, the tendency of *mclk1*$^{-/-}$ ES cells to differentiate under low leukemia inhibitory factor treatment is much less than in wild-type animals. Finally, the *mclk1*$^{-/-}$ cells seem to be resistant to apoptosis triggered by a set of pro-apoptotic agents. This is consistent with the fact that the apoptotic pathway is known to be dependent on ROS concentration (Chan 2005).

Since homozygous *mclk1*$^{-/-}$ mice are not viable, the authors study the viable heterozygous *mclk1*$^{+/-}$ mice. Surprisingly, they are long lived even though they possess the enzyme for ubiquinone synthesis. Therefore, the role of *clk-1* in longevity is conserved from worms to mice, and the high degree of similarity of the murine and human *clk-1* gene suggests that this mechanism may be conserved in humans. Interestingly, in the liver of these mice, loss of heterozygosity (LOH) occurs, leading to the appearance of *mclk1*$^{-/-}$ clones that expand and that would likely resemble *mclk*$^{-/-}$ ES cells phenotypically. However, it remains to be clearly shown whether the liver is the only organ where LOH occurs and, if so, if this phenomenon is responsible for the extended longevity that is observed. If this proves to be true, it raises many new and interesting questions as to the tissue specificity of the mitochondrial role of aging and, in this particular case, its role through the synthesis of ubiquinone.

How does reduced mitochondrial activity alter longevity?

One possibility is that mitochondrial activity inhibits the insulin/IGF-1 signaling pathway (Guarente and Kenyon 2000). In worms, reducing the activity of DAF-2, an insulin/IGF-1 receptor homolog, or downstream signaling components, extends life span approximately twofold. This life-span extension requires activity of the forkhead-family transcription factor DAF-16. However, *isp-1* mutations, *clk-1* mutations, or RNAi of respiratory chain components extend the life span of *daf-16* mutants (Wong et al. 1995; Feng et al. 2001; Dillin et al. 2002; Lee et al. 2003). In addition, the already long life span of *daf-2(e1370)* mutants is further extended by *isp-1* mutations, *clk-1* mutations, or RNAi of respiratory chain components (Wong et al. 1995; Feng et al. 2001; Dillin et al. 2002; Lee et al. 2003). Moreover, unlike reduction of respiratory chain activity, reduction of insulin/IGF-1 signaling is known to cause a significant increase in ATP levels (Braeckman et al. 1999; Dillin et al. 2002). Finally, both *daf-2* and *daf-16* act exclusively in adults to regulate lifespan (Dillin et al. 2002). Together these findings indicate that respiratory-chain RNAi does not increase life span by inhibiting the DAF-2 pathway. Similar experiments have not been performed yet in vertebrates. However, the insulin/IGF-1 signaling pathways affects mitochondrial ROS steady state, since all animals with an attenuated activity of this pathway are oxidative stress resistant. This is probably due to the fact that they overproduce protective enzymes such as SODs and catalases (Honda and Honda 1999; Holzenberger et al. 2003).

Another possibility is that reduced mitochondrial activity mimics the effects of dietary restriction (DR). Since mitochondria are central to metabolism, it is reasonable to expect that if the efficiency of mitochondrial metabolism is somehow decreased, it could influence and/or mimic DR. However, it was recently shown that, in *Drosophila*, it is not the calorie content of the food ingested that determines life span, but rather the relative content of yeast and sugar in the food (Mair et al. 2005). Furthermore, in the worm, the temporal requirements of reduced mitochondrial activity are confined to early larval development, but DR can be instituted during adult-

hood. Therefore, the temporal requirements of the ETC and DR appear to be in contrast with one another.

It is also possible that signals derived from mitochondria may set the rate of aging independently of either the insulin/IGF-1 pathway or the DR pathway or even of ROS production. Consistent with this model, reduced mitochondrial activity, induced by RNAi of nuclear encoded mitochondrial components, during larval development, but not adulthood, is sufficient to increase longevity (Dillin et al. 2002). This result suggests that a signal established during development is maintained throughout adulthood to result in longer life span. It is unclear what this signal may be; however, the retrograde system in yeast suggests that a similar mechanism could be conserved in higher eukaryotes to establish and maintain a metabolic state that is conducive to increased longevity (Kirchman et al. 1999). Furthermore, Hekimi and colleagues (Liu et al. 2005) find that *mclk-1*$^{+/-}$ mice lose heterozygosity in liver cells, creating highly proliferative tissue. It is interesting to speculate that these clones may be the site of *mclk1*$^{-/+}$-induced longevity and that a second messenger is needed to ensure coordinated aging of the entire organism (Fig. 2). In the future, it will be imperative to understand how this system is established and maintained throughout phyla.

Future thoughts

The paper published in this issue of *Genes and Development* makes significant observations that point to a mechanism that seems to be conserved from worms to mice in the regulation of life span (Liu et al. 2005). It is of note that many model systems that can use respiration can also use substrate level phosphorylation as an efficient ATP source, and it is expected that these models can withstand massive mitochondrial disturbances that mice will not be able to tolerate. Therefore, the creation of the *mclk1*$^{+/-}$ mice that are long lived provides an extremely valuable tool for further studies of the role of mitochondrial metabolism and ubiquinone synthesis on the aging process. In addition to extending life span, the *mclk1*$^{+/-}$ mice raise an interesting question in organismal aging: Indeed, it is puzzling that mice heterozygote for *mclk1*$^{+/-}$ are long lived because, in theory, they retain the capacity to produce ubiquinone normally, since they possess the enzyme necessary for it. However, in the liver of heterozygote mice, some cells go through a LOH. How can such a local modification generate a phenotype as systemic as a life-span extension?

One simple explanation is that liver mitochondria, in this case, can trigger the production of a hormone that

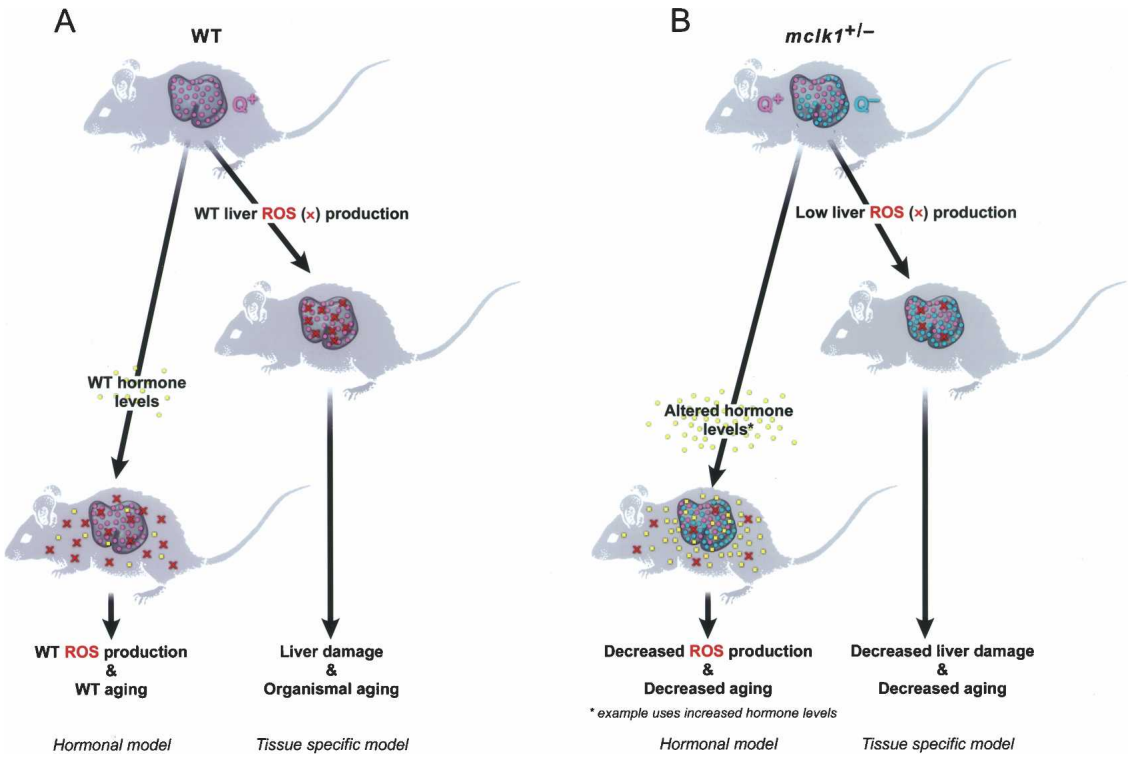

Figure 2. Two possible models of life-span extension in *mclk1*$^{+/-}$ mice. (*A*) The *left* side depicts a hormonal model in which wild-type levels of an unknown hormone (yellow dots) result in wild-type ROS production (red ×s) and aging. The *right* side depicts a tissue-specific model in which the liver is the site of ROS production that is life-span limiting. (*B*) The *left* side shows a hormonal model in which *mclk1*$^{-/-}$ cells cause an alteration in the levels of a hypothetical hormone (yellow dots), synchronizing the mitochondria and decreasing the rate of aging. The *right* side depicts a tissue-specific model in which decreased ROS production (red ×s) by *mclk1*$^{-/-}$ cells in the liver allows for an extended life span. (Ubiquinone-positive cells [Q$^+$] are represented by pink dots; ubiquinone-negative cells [Q$^-$] are represented by blue dots.)

will "synchronize" and set the pace of metabolism for other mitochondria within other organs and tissues (Fig. 2). In the future, it will be interesting to analyze the metabolic differences between mitochondria contained in *mclk1*$^{-/-}$ cells and mitochondria of heterozygous cells from the same animal. If a resetting mechanism does exist, then it would be expected that following the appearance of *mclk1*$^{-/-}$ clones, all mitochondria will quickly acquire the characteristics of *clk1*$^{-/-}$ mutant clones.

It is also possible that mitochondria are not the source of a humoral response, but, rather, liver mitochondria are limiting for life span in the wild-type animals. This is not an entirely new idea, since it was already shown both in worms and flies that insulin/IGF-1-mediated longevity is tissue specific, involving neuronal and intestinal cells (Libina et al. 2003; Giannakou et al. 2004; Hwangbo et al. 2004). The relevant question will then be in which tissues mitochondria and ubiquinone synthesis are limiting for longevity. In the framework of the free radical theory of aging, this scenario is even more intuitive, since the vital organ or tissue that will first reach the point of dysfunction will determine longevity for the whole organism. In the *mclk1* example, a subset of hepatocytes may be the site of altered mitochondrial function that determines life span. One possibility is that *mckl1*$^{-/-}$ patches produce less ROS than their heterozygous counterparts and that this decrease in oxidative damage is enough to increase longevity (Fig. 2B).

Finally, the use of invertebrate model organisms to dissect the molecular details of the aging process and its regulation is invaluable. From insulin/IGF-1 signaling to ubiquinone synthesis, we are quickly learning that these pathways are conserved in mice and perhaps universal in the animal kingdom. In the future, it will be exciting to learn if the synergy observed among these pathways in invertebrates to create exceptionally long-lived animals (e.g., *daf-2*, *clk-1* double-mutant animals) will be recapitulated in mouse models. Within the next few years, could we witness the first 10-yr-old mouse created by combining *igfr1*$^{+/-}$ mutations with *mclk1*$^{+/-}$ mutations?

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