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A Defect in the Cytochrome *b* Large Subunit in Complex II Causes Both Superoxide Anion Overproduction and Abnormal Energy Metabolism in *Caenorhabditis elegans**

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A *mev-1(kn1)* mutant of the nematode *Caenorhabditis elegans* is defective in the cytochrome *b* large subunit (Cyt-1/ceSDHC) in complex II of the mitochondrial electron transport chain. We have previously shown that a mutation in *mev-1* causes shortened life span and rapid accumulation of aging markers such as fluorescent materials and protein carbonyls in an oxygen-dependent fashion. However, it remains unclear as to whether this hypersensitivity is caused by direct toxicity of the exogenous oxygen or by the damage of endogenous reactive oxygen species derived from mitochondria. Here we report important biochemical changes in *mev-1* animals that serve to explain their abnormalities under normoxic conditions: (i) an overproduction of superoxide anion from mitochondria; and (ii) a reciprocal reduction in glutathione content even under atmospheric oxygen. In addition, unlike wild type, the levels of superoxide anion production from *mev-1* mitochondria were significantly elevated under hyperoxia. Under normal circumstances, it is well known that superoxide anion is produced at complexes I and III in the electron transport system. Our data suggest that the *mev-1(kn1)* mutation increases superoxide anion production at complex II itself rather than at complexes I and III. The *mev-1* mutant also had a lactate level 2-fold higher than wild type, indicative of lactic acidosis, a hallmark of human mitochondrial diseases. These data indicate that Cyt-1/ceSDHC plays an important role not only in energy metabolism but also in superoxide anion production that is critically involved in sensitivity to atmospheric oxygen.

Mitochondria are specialized organelles whose primary function is to synthesize ATP via oxidative phosphorylation in aerobic eukaryotes (1). During this process, electrons are transferred ultimately to oxygen after their passage through four membrane-bound complexes. Electrons enter the electron transport system through either complex I (NADH-CoQ[1] oxidoreductase) or complex II (succinate-CoQ oxidoreductase). Via two, single-electron reductions, they are transferred to CoQ (coenzyme Q or ubiquinone), thereby reducing CoQ first to ubisemiquinone and then to ubiquinol (dihydroubiquinone). At complex III ($CoQH_2$-cytochrome *c* oxidoreductase) molecules of ubiquinol undergo two sequential and spatially separate one-electron oxidations, a process called Q cycle. These reducing equivalents are then transferred to the remainder of the electron transport chain: cytochrome *c*, complex IV (cytochrome *c* oxidase), and finally to oxygen. Mitochondrial deficiencies cause a variety of diseases, including human congenital neurodegenerative diseases like MELAS (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes), MERRF (myoclonic epilepsy with ragged red fibers), KSS (Kearns-Sayre syndrome), CPEO (chronic progressive external ophthalmoplegia), NARP (neuropathy, ataxia, and retinitis pigmentosa), MILS (maternally inherited Leigh syndrome), and LHON (Leber hereditary optic neuropathy) (2–4). Many of the ultimate manifestations of these diseases are triggered by a metabolic imbalance known as lactic acidosis, which is characterized by a high lactate/pyruvate ratio and is a characteristic feature of a variety of other metabolic disorders in addition to mitochondrial diseases. In contrast to the well defined pathologies resulting from complex I, III, and IV deficiencies, the effects of complex II defects in humans are not as completely understood (5–13). A complex II deficiency has been described that is associated with a mitochondrial disease called Leigh syndrome and resulted in metabolic changes leading to lactic acidosis (5, 6). Another mutation in the flavoprotein subunit of complex II has recently been shown to cause late onset optic atrophy, ataxia, and myopathy (10, 11). Finally, individuals with an inherited propensity for vascularized head and neck tumors (*i.e.* paragangliomas) have been recently demonstrated to contain one of several mutations in the small and large subunits of cytochrome *b* in succinate-ubiquinone oxidoreductase (14, 15). In addition to its role in electron transport, the succinate dehydrogenase (SDH) moiety of complex II plays an essential role in the trichloroacetic acid cycle, catalyzing the conversion of succinate to fumarate. Complex II is composed of four subunits, named SDHA through SDHD in *Escherichia coli* or human and SDH1 through SDH4 in yeast. SDHA/SDH1 is a flavoprotein subunit, and SDHB/SDH2 is an iron-sulfur protein subunit. Two other hydrophobic membrane-anchored subunits (consisting of large and small subunits of cytochrome *b*) are named SDHC/SDH3/CybL and SDHD/SDH4/CybS. We will

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[1] The abbreviations used are: CoQ, coenzyme Q or ubiquinone; SDH, succinate dehydrogenase; ROS, reactive oxygen species; SMP, submitochondrial particles; MCLA, *cypridina* luciferin analog, 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-α]pyrazin-3-one.

refer to the *Caenorhabditis elegans* orthologs as Cyt-1/ceSDHC through ceSDHD.

The electron transport system is also the major endogenous source of reactive oxygen species (ROS) such as superoxide anion ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($^{\cdot}OH$). The ROS produced during aerobic respiration cause cumulative oxidative damage that results in aging and age-related degenerative diseases (16–18). It is known that oxygen is initially converted to superoxide anion by complexes I and III (16, 18). Conversely, there is no evidence that complex II is a site of superoxide anion production.

We have previously described a *mev-1(kn1)* mutant of the nematode *C. elegans* that has a missense mutation in Cyt-1/ceSDHC in mitochondrial complex II (19). The mutant, isolated by screening for hypersensitivity to oxygen, ages precociously (19, 20). We have also reported that the *mev-1* mutant accumulates markers of aging (*e.g.* fluorescent materials and protein carbonyls) more rapidly than wild type (21–23) at high oxygen concentrations. In addition, the *mev-1* mutant has much lower succinate-cytochrome *c* oxidoreductase activity than wild type (19). However, the reason the *mev-1* mutant is hypersensitive to hyperoxia as well as the role of Cyt-1/ceSDHC was completely unclear. We now demonstrate that *mev-1* animals manifest a series of biochemical defects that serve to explain their oxygen-hypersensitive cellular and organismal mutant phenotypes. In addition, the data also suggest that, contrary to the current sentiment in the scientific literature, significant amounts of superoxide anion may be produced at complex II.

EXPERIMENTAL PROCEDURES

Mitochondrial Isolation—To prepare stage-synchronized animals of either wild type (N2: Bristol strain) or *mev-1(kn1)*, isolated embryos were cultured on nematode growth medium (NGM) at 20 °C under atmospheric (21%) or hyperoxic (40%) conditions. Young adult animals were harvested 72 h later by centrifugation and were homogenized (10% w/v) in isolation buffer (210 mM mannitol, 70 mM sucrose, 0.1 mM EDTA, and 5 mM Tris-HCl, pH 7.4) with a Teflon homogenizer. Mitochondria were isolated by differential centrifugation (24) and suspended in Tris-EDTA buffer (0.1 mM EDTA, 50 mM Tris-HCl, pH 7.4). Submitochondrial particles (SMP) were obtained by sonicating freeze-thawed mitochondria twice for 20 s with 1-min intervals in a model U200S sonicator (IKA Labortechnik). SMP were washed twice and suspended in isolation buffer.

Biochemical Analyses—The activities of complex I plus III (NADH-cytochrome *c* oxidoreductase) and complex II plus III (succinate-cytochrome *c* oxidoreductase) in mitochondria isolated from wild-type and *mev-1* young adult animals were measured as described (24).

Mitochondrial Superoxide Anion Generation Assay—Superoxide anion generation was measured using the chemiluminescent probe MCLA (*cypridina* luciferin analog, 2-methyl-6-(*p*-methoxyphenyl)-3,7-dihydroimidazo[1,2-α]pyrazin-3-one). Forty micrograms of either intact mitochondria or SMP were added to 1 ml of assay buffer (50 mM HEPES, pH 7.4, 2 mM EDTA) containing 0.5 mM MCLA. For the measurement of superoxide anion generation from mitochondria with substrate and inhibitor, 1.5 mM succinate as a complex II substrate was added in the mitochondrial solution, and 100 μM antimycin A as a complex III inhibitor was added after addition of the mitochondria and succinate. The solution was placed into the photon counter with a H-R550 photomultiplier (Hamamatsu Photogenic Co. Ltd.) and measured at 37 °C. The rates of superoxide anion production were expressed as counts per second. The amount of superoxide anion production was calculated by subtracting the optical density of samples in the presence of 10 μg/ml bovine Cu,Zn-superoxide dismutase from that in the absence of Cu,Zn-superoxide dismutase.

GSH Assay—The levels of total thiol groups (total SH) and reduced glutathione (GSH) in whole lysates from wild-type and *mev-1* young adult animals were measured by the rate of formation of 5,5′-dithio-(2-nitrobenzoic acid) as described (25).

Metabolite Analyses—Young adult animals were cultured under atmospheric (21%) or hyperoxic (50%) conditions for 16 h, and whole lysates were prepared as described below. Animal lysates were collected by centrifugation at 10,000 × *g* for 10 min. The pellets were suspended in an equal volume of 10% trichloroacetic acid and homogenized with ultra-Turrax T8 (IKA Labortechnik) on ice and then sonicated with a Sonifier 450 (Branson). The homogenates were clarified by centrifugation at 10,000 × *g* for 10 min. The supernatants then were neutralized with 4 N KOH and centrifuged at 10,000 × *g* for 10 min. Lactate and pyruvate were measured using Sigma diagnostic kits. Citrate, succinate, and glutamate were measured with an enzymatic bioanalysis/food analysis kit (Roche Diagnostics). ATP was measured using an ATP bioluminescence assay kit CLS II (Roche Diagnostics).

TABLE I
Activities of NADH-cytochrome c oxidoreductase and succinate-cytochrome c oxidoreductase in wild-type and mev-1 animals under atmospheric oxygen

Each value reported is the mean ± S.D. of three different experiments.

Strain	NADH-cytochrome *c* oxidoreductase	Succinate-cytochrome *c* oxidoreductase
	nmol min^{-1} *mg*$^{-1}$	
Wild type	312.8 ± 84.1	40.2 ± 24.6
mev-1	306.6 ± 86.6	5.0 ± 1.8[a]

[a] $p < 0.05$ for wild type as compared with *mev-1* mutants by paired Student's *t* test.

RESULTS

Confirmation of a Complex II Defect in mev-1 Mutants—We first measured the activities of complex I and complex II in wild-type and *mev-1* genetic backgrounds to directly confirm the genetic evidence that the *mev-1* mutant is normal for NADH-cytochrome *c* oxidoreductase (complex I plus III) activity but has a severely reduced succinate-cytochrome *c* oxidoreductase (complex II plus III) activity (Table I). The activity of succinate-CoQ oxidoreductase (complex II) in *mev-1* mutant mitochondria was also markedly lower than that in wild type (data not shown). By extension, these data suggest that the various biochemical manifestations of the *mev-1* mutation derive from a defect in the electron transfer between complex II and complex III. Because Northern and Western blots revealed roughly equal mRNA and protein levels of Cyt-1/ceSDHC in wild-type and mutant animals (data not shown), the *mev-1* mutation most likely compromises enzyme activity *per se* as opposed to affecting complex assembly.

Production of Superoxide Anion from Mitochondria Is Elevated in mev-1 Mutants—The specific mechanism by which a complex II deficiency causes hypersensitivity to exogenous oxygen in *mev-1* mutants has not been directly addressed. There is no evidence in the literature to suggest that complex II contributes directly to ROS production. Indeed, it is currently thought that the majority of ROS are produced in mitochondria at complex III (16–18). To determine the effects of the *mev-1* mutation on mitochondrial ROS production, we examined mitochondria from *mev-1* and wild-type animals cultured under atmospheric and hyperoxic conditions. The levels of superoxide anion production were found to be approximately 2-fold higher in intact mitochondria and SMP from *mev-1* animals as compared with wild type under atmospheric oxygen (Fig. 1). SMP were employed because they are known to be depleted in the mitochondrial superoxide dismutase (MnSOD) that transforms superoxide anion to hydrogen peroxide.

As a next step, we examined the effect of hyperoxia on superoxide anion production from intact mitochondria (Fig. 2). The level of superoxide anion production from intact *mev-1* mitochondria (without stimulation by exogenous succinate and antimycin A) was higher than in wild type and was significantly increased under hyperoxia (40% O_2). Specifically, it was ~3.4-fold higher than the wild type under 40% O_2 and ~1.6-fold higher than *mev-1* under 21% O_2. On the other hand, superoxide anion production from the wild-type mitochondria was relatively independent of oxygen concentration. These re-

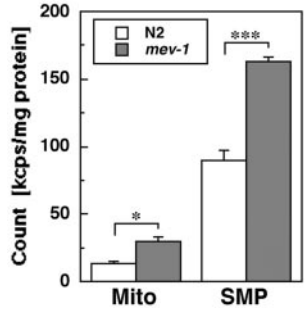

FIG. 1. **Superoxide anion production in mitochondria or submitochondrial particles isolated from wild-type or *mev-1* young adult animals.** Each value reported is the mean ± S.D. of four different experiments. *, $p < 0.05$; ***, $p < 0.0005$ by paired Student's *t* test. *Mito*, mitochondria.

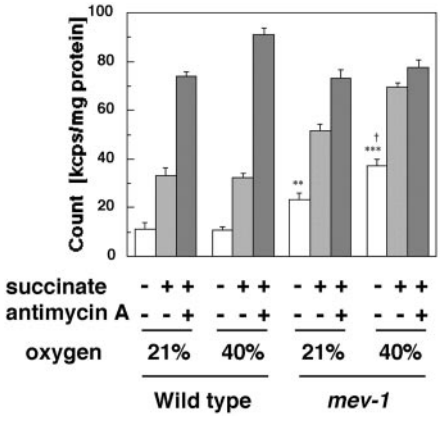

FIG. 2. **The effect of hyperoxia on superoxide anion production.** Wild-type or *mev-1* young adult animals were cultured from embryo stage for 72 h under atmospheric oxygen (21% O_2) or hyperoxia (40% O_2). Superoxide anion production from wild-type and *mev-1* mitochondria was measured in the presence or absence of 1.5 mM succinate and/or 100 μM antimycin A. Each value reported is the mean ± S.D. of three different experiments. For each value as compared with wild type under 21% O_2: **, $p < 0.005$, and ***, $p < 0.0005$. For *mev-1* mutants under 40% O_2 as compared with *mev-1* mutants under 21% O_2: †, $p < 0.05$; ††, $p < 0.005$. Student's *t* tests were done on all quantitative analyses.

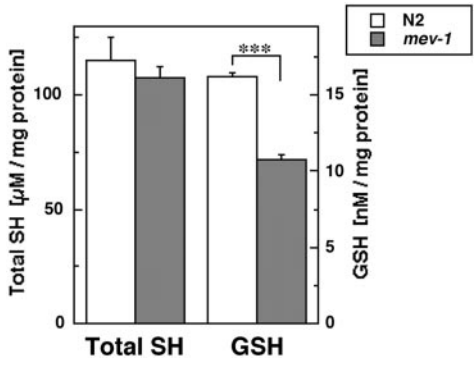

FIG. 3. **The levels of total thiol groups and GSH in whole lysates from wild-type or *mev-1* young adult animals.** The values represent the mean ± SD. of four different experiments. ***, $p < 0.0005$ by paired Student's *t* test. *Total SH*, total thiol groups.

sults suggest that the mutation in this complex II-encoding gene causes cell damage and precocious aging by the endogenous generation of ROS in mitochondria rather than by the direct toxicity of exogenous oxygen.

We further examined superoxide anion production as it is influenced by succinate, a complex II substrate, and antimycin A, a Q cycle (complex III) inhibitor (Fig. 2). Both succinate and antimycin A stimulated superoxide anion production. The proportional increase of the succinate stimulation was approximately the same for wild-type and *mev-1* mutants, although the absolute levels were significantly higher in *mev-1* mutants. In addition, inclusion of antimycin A to succinate-stimulated mitochondria increased superoxide anion production much more in wild-type than in *mev-1* mutants, particularly under hyperoxia.

Glutathione Concentrations Are Reduced in mev-1 Mutants—We also measured total cellular levels of GSH. GSH can either act directly as an antioxidant or as a substrate for the ROS detoxifying enzyme glutathione peroxidase making it an important defense against ROS (26, 27). Only 10–15% of the total cellular GSH is normally found inside the mitochondrial matrix (26). An age-associated increase in superoxide anion and hydrogen peroxide generation by mitochondria and an age-related decline in GSH content are observed in senescent organisms including both mammals and insects (27, 28). Elevated mitochondrial ROS could act as a sink to eventually reduce or deplete total cellular GSH levels, ultimately rendering the entire cell more susceptible to the effects of mitochondrially generated ROS. This in turn could lead to the dual phenotypes of oxygen-hypersensitive precocious aging that are the hallmark phenotypes of *mev-1* mutants. Indeed, total cellular GSH levels were decreased ~30% in *mev-1* mutants when compared with wild type (Fig. 3). Because a thiol group constitutes the reactive moiety of GSH, we also confirmed that total thiol content in *mev-1* mutants was experimentally identical to wild-type levels (Fig. 3). Thus, it is likely that the Cyt-1/ceSDHC deficiency leads to an overproduction of mitochondrial ROS, which in turn reduces GSH levels in both cytosol and mitochondria. In concert these biochemical abnormalities render *mev-1* animals hypersensitive to hyperoxia. Alternatively, the reduced GSH levels in *mev-1* extracts may have resulted from some unknown metabolic changes in *mev-1* mutants *versus* wild type. A stoichiometric comparison of superoxide anion and GSH concentrations would distinguish these alternatives. Such an analysis was not attempted because GSH and the superoxide anion were assayed using different protocols that employed whole lysates in one case and isolated mitochondria in another. However, a variety of data suggest that GSH levels vary in direct response to superoxide anion scavenging rather than to metabolic suppression (26).

Cyt-1/ceSDHC Affects the Levels of Trichloroacetic Acid Cycle Intermediates—It is possible that the complex II deficiency by the Cyt-1/ceSDHC mutation in *mev-1* mutants might impede flow through the trichloroacetic acid cycle and alter the lactate/pyruvate ratio, resulting in lactic acidosis. Indeed, although pyruvate concentrations were not significantly different in wild type and *mev-1* mutants, there was over twice as much lactate in *mev-1* animals, *i.e.* the lactate/pyruvate ratio in *mev-1* mutants was ~1.6-fold higher than that in wild type (Fig. 4*A* and Table II). Both citrate and succinate levels were normal in *mev-1* animals; however, glutamate was present in reduced amounts in *mev-1* animals at statistically significant levels (Fig. 4*B*). The decrease in glutamate suggests that the trichloroacetic acid cycle intermediate α-ketoglutarate, a precursor of glutamate, is reduced in its content. On the other hand, in the patient with complex II and SDH defects, urine levels of both succinate and α-ketoglutarate were significantly increased (5). An SDH defect may result in a block in the oxidation of succinate to fumarate with accumulation of succinate. Given the elevated lactate levels in *mev-1* mutants, this finding suggests that the flow of pyruvate into the trichloroacetic acid cycle may be reduced. It is possible that these metabolic defects contribute to the *mev-1* phenotypes.

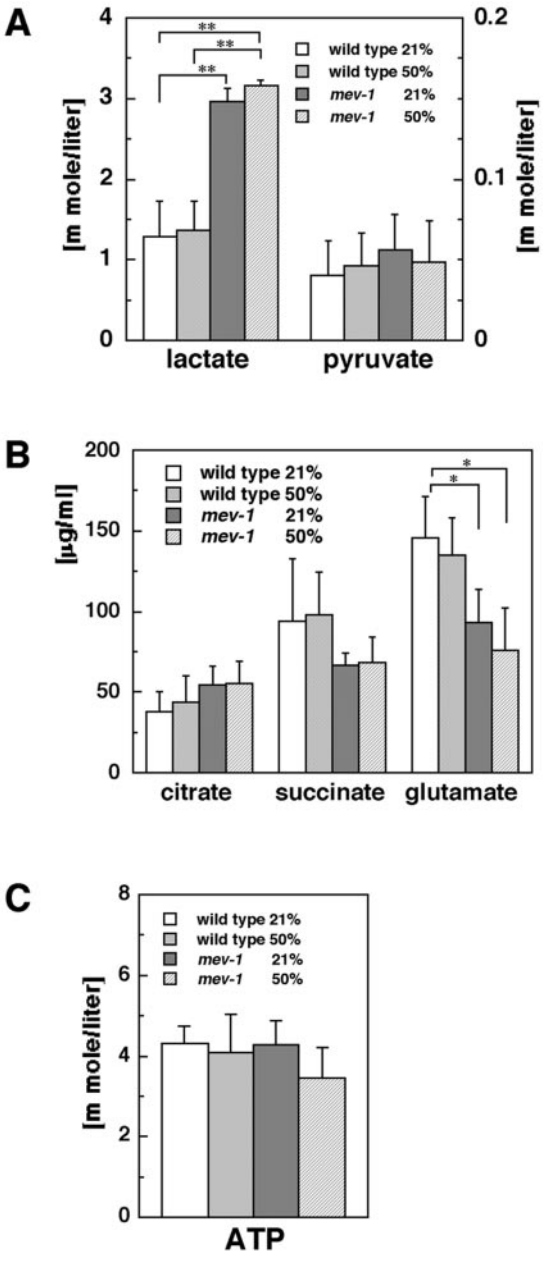

FIG. 4. **Changes in energy metabolism in *mev-1* mutants.** Wild-type or *mev-1* young adult animals were cultured under atmospheric oxygen (21% O_2) or hyperoxia (50% O_2) for 16 h. Concentrations of lactate and pyruvate (*A*), contents of trichloroacetic acid cycle intermediates, citrate, succinate, and glutamate (*B*), and ATP content (*C*) in whole lysates of these animals were measured as described under "Experimental Procedures." Each value reported is the mean ± S.D. of three different experiments. *, $p < 0.05$ and **, $p < 0.005$ by paired Student's *t* test.

ATP Levels Are Normal in mev-1 Mutants—Given the complex II deficiency in *mev-1* animals and its various biochemical consequences, it was surprising that ATP levels were experimentally identical in wild type and *mev-1* mutants under atmospheric oxygen (Fig. 4*C*). Even under 50% oxygen the slight reduction in ATP was not statistically significant (Fig. 4*C*). It seems likely that *mev-1* animals rely more heavily on glycolysis for energy acquisition, thus explaining the elevated lactate levels (Fig. 4*A*). However, it is also possible that ATP consumption is decreased in *mev-1* because of some sort of global decrease in the metabolic rate that acts to counterbalance the compromised ATP generation in *mev-1* animals.

TABLE II

The lactate to pyruvate ratio (L/P ratio) in wild-type and mev-1 mutants under either atmospheric oxygen (21%) or hyperoxia (50%)

Data are derived from Fig. 3. Each value reported is the mean ± S.D. of three different experiments.

Strain	Oxygen concentrations	Lactate	Pyruvate	L/P ratio
	%	*mmol liter*$^{-1}$		
Wild type	21	1.28 ± 0.44	0.040 ± 0.022	35.7 ± 16.8
	50	1.37 ± 0.37	0.046 ± 0.021	31.7 ± 5.9
mev-1	21	2.97 ± 0.16[a]	0.056 ± 0.022	58.0 ± 20.9
	50	3.17 ± 0.06[b,c]	0.049 ± 0.025	75.7 ± 32.6

[a] $p < 0.005$ for *mev-1* mutants under 21% O_2 as compared with wild type under 21% O_2.

[b] $p < 0.005$ for *mev-1* mutants under 50% O_2 as compared with wild type under 21% O_2.

[c] $p < 0.005$ for *mev-1* mutants under 50% O_2 as compared with wild type under 50% O_2.

DISCUSSION

Mitochondria are widely accepted as the major intracellular site of ROS production because of the inappropriate single-electron reduction of diatomic oxygen to superoxide anion (16, 18). Because once generated ROS react indiscriminately with a wide variety of cellular constituents, they figure prominently in theories seeking to explain the etiology of aging and neurodegenerative diseases (16–18, 29). As such, it is important to understand the precise mitochondrial location and mechanism of superoxide anion generation. Work with the *mev-1(kn1)* mutation of the nematode *C. elegans* may prove useful in this regard. As articulated in the Introduction, *mev-1* mutants are hypersensitive to oxidative stress and age precociously (19–23). This has been attributed to a missense mutation in the gene encoding Cyt-1/ceSDH3, a large subunit of cytochrome *b*, that compromises complex II activity (19).

We now demonstrate that superoxide anion production is significantly higher in *mev-1* animals, particularly under hyperoxia (Figs. 1 and 2). Along with the reduced GSH levels we have observed in *mev-1* animals (Fig. 3), these data provide the biochemical connection that links the molecular defect in complex II to the organismal phenotypes of ROS hypersensitivity and precocious aging. Perhaps more importantly, the data bear on the question of precisely where in the mitochondria and how superoxide anion is generated.

It is generally believed that most superoxide anions are produced at complex III (16–18). Specifically, the free radical ubisemiquinone is generated during the Q cycle, a series of events that includes a two-step oxidation from ubiquinol to ubisemiquinone to ubiquinone. Ubisemiquinone is alternatively capable of transferring electrons to diatomic oxygen, forming superoxide anion. Given this, how can the increased superoxide production in *mev-1* animals be explained? First, the increase may be an indirect effect of the *mev-1* mutation that uncouples some aspect of electron transport and results in increased superoxide anion at complex III itself. Second, the increase may be a direct effect of the *mev-1* mutation such that superoxide anion is produced at complex II. Two variations on this second scenario could be envisioned. The first is that the complex II defect could affect electron flow such that there is a probable increase of electrons leaking directly from complex II to molecular oxygen. The second is that the mutation results in increased superoxide anion generation because of semiquinone involvement; specifically, the mutation could result in a premature release of the free radical semiquinone before its reduction to ubiquinol.

Several lines of evidence suggest that the *mev-1* mutation results in superoxide anion production at complex II itself rather than exerting an indirect effect on complex III. Inter-

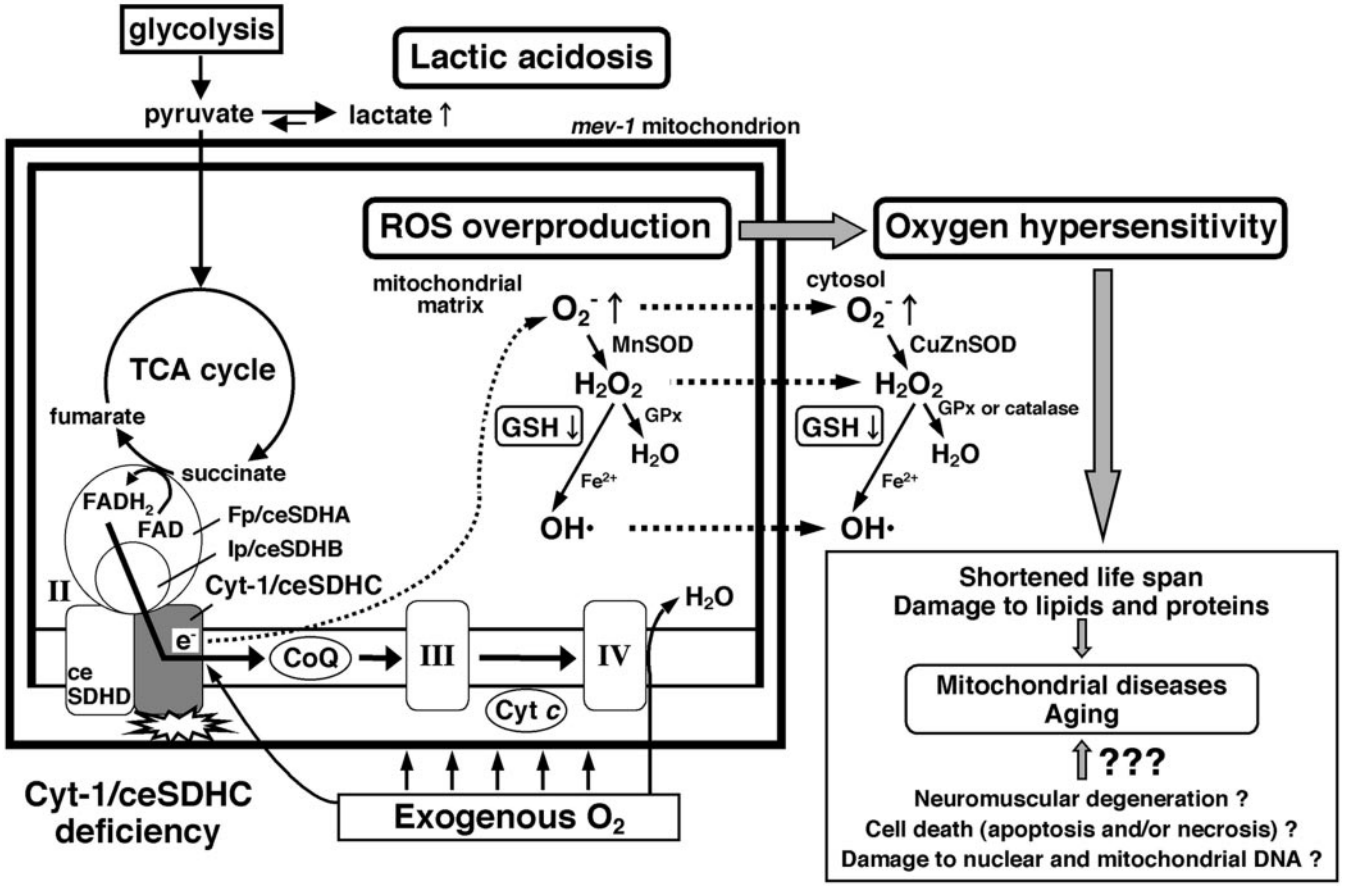

FIG. 5. **Model of Cyt-1/ceSDHC subunit of complex II deficiency in mitochondrial diseases and aging.** The Cyt-1/ceSDHC subunit deficiency causes mitochondrial superoxide anion (O_2^-) overproduction at complex II itself and metabolic changes with lactic acidosis. The superoxide anion overproduction ultimately results in the exogenous oxygen-hypersensitive *mev-1* phenomena: shortened life span and damage to lipids and proteins. Moreover, many kinds of impaired physiological functions associated with mitochondrial diseases and aging (such as neurodegeneration, cell death, and damage to nuclear and mitochondrial DNA) might be caused by the "oxygen hypersensitivity and mitochondrial ROS overproduction mechanism." *ceSDHA* through *ceSDHD* are the four subunits of complex II; *Fp*, flavoprotein subunit; *Ip*, iron-sulfur protein subunit; *III*, complex III; *IV*, complex IV; *Cyt c*, cytochrome *c*; *GPx*, glutathione peroxidase; *TCA*, trichloroacetic acid; *SOD*, superoxide dismutase.

pretation of inhibitor data is fraught with danger; however, the antimycin A results (Fig. 2) lend support to the notion that superoxide anion can be produced at complex II, particularly in *mev-1* animals. Specifically, disruption of the complex III-associated Q cycle by antimycin A had comparatively little effect on superoxide anion production in mitochondria derived from *mev-1* animals, suggesting that production occurred at some location other than at complex III. Conversely, the large antimycin A-mediated increase in wild type is in keeping with other evidence implicating complex III as the source of most superoxide anions under normal conditions. That succinate stimulated superoxide anion production more in *mev-1* mutants than wild type is also consistent with our contention that the *mev-1* mutation results in high levels of superoxide anion production at complex II. Similarly, the relative inability of antimycin A to exacerbate the increases mediated by succinate in *mev-1* (but not wild type) also suggests that complex II is the source of most superoxide anion in *mev-1* mutants.

In addition to the inhibitor data, the specific nature of the *mev-1* mutation is consistent with a direct, complex II-mediated production of superoxide anion. Specifically, site-directed mutagenesis has been employed in *E. coli* to demonstrate that amino acid residues 17–33 are likely the ubiquinone-binding sites in the *E. coli* SDHC to which Cyt-1/ceSDHC is the probable ortholog (30). This corresponds to amino acids 59–76 in Cyt-1/ceSDHC. We have shown previously that the *mev-1* mutant has a missense mutation that substitutes glutamic acid for glycine at residue 71 (19). Therefore, it seems reasonable to speculate that this mutation may result in premature release in semiquinone, thereby enabling superoxide anion formation via its subsequent interaction with molecular oxygen. Whatever the case, it seems likely that the *mev-1* mutation exerts an allele-specific effect, that is the missense mutation at position 71 may affect the quinone-binding site whereas other, more severe mutations would not elevate superoxide anion production.

It also seems reasonable to posit that some superoxide anion production might occur at complex II even in wild-type mitochondria. In particular, the observation that succinate, the substrate for complex II, increased superoxide anion production in wild-type mitochondria suggests that complex II might even be a secondary source of superoxide anion production in wild type.

Many reports have implicated that mitochondrial function is reduced in the aging process (1, 16–18, 29). Several studies have demonstrated age-linked declines in the activities of mitochondrial electron transport enzymes NADH-cytochrome *c* oxidoreductase (complex I plus III), succinate-cytochrome *c* oxidoreductase (complex II plus III), ubiquinol-cytochrome *c* oxidoreductase (complex III), cytochrome *c* oxidase (complex IV), and ATP synthase (complex V) in human skeletal muscle (31). Mitochondria are the major source of ROS and are also the first compartment in the cell that is damaged by these ROS. Indeed, aging is accompanied by a decrease in mitochondrial function. That has led to the hypothesis that a deficit in energy metabolism and an increase in ROS production

could be a cause of aging. This hypothesis is strongly supported by the model of short-lived *mev-1* mutants.

In summary, we have shown that *mev-1* animals manifest two distinct biochemical pathophysiologies, namely superoxide anion overproduction from mitochondrial complex II itself (with an attendant decrease in glutathione levels) and metabolic changes with lactic acidosis by the mutation in Cyt-1/ceSDHC subunit (Fig. 5). The metabolic changes in *mev-1* mutants are biochemical and metabolic features associated with human mitochondrial diseases. The superoxide anion overproduction ultimately results in (or strongly contributes to) the exogenous oxygen-dependent *mev-1* phenomena: shortened life span and damage to lipids and proteins (rapid accumulation of fluorescent materials and protein carbonyls) that might be correlated with the pathogenesis of mitochondrial diseases and precocious aging. Elevated ROS by mitochondrial dysfunction is also thought to lead to neurodegeneration, cell death, and damage to nuclear and mitochondrial DNA. The mechanisms are currently poorly understood. Therefore, studies of those cellular and organismal sequelae in *mev-1* mutants might elucidate the pathogenic mechanisms of mitochondrial disease and aging.

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