A Complex II Defect Affects Mitochondrial Structure, Leading to ced-3- and ced-4-dependent Apoptosis and Aging*

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The mev-1(ken1) mutation of Caenorhabditis elegans is in Cyt-1, which encodes a subunit of succinate-coenzyme Q oxidoreductase in the mitochondrial electron transport chain. Mutants are hypersensitive to oxidative stress and age precociously in part because of increased superoxide anion production. Here, we show that mev-1 mutants are defective in succinate-coenzyme Q oxidoreductase, possess ultrastructural mitochondrial abnormalities (especially in muscle cells), show a loss of membrane potential, have altered CED-9 and Cyt-1 protein levels under hyperoxia, and contain ced-3- and ced-4-dependent supernumerary apoptotic cells. These defects likely explain the failure of mev-1 to complete embryonic development under hyperoxia as well as its reduced life span.

The oxidative phosphorylation system or electron transport chain is located within the mitochondrial inner membrane and is intimately responsible for three important processes: (i) ATP production, (ii) generation of reactive oxygen species (ROS), and (iii) regulation of programmed cell death or apoptosis. The proximal ROS is superoxide anion (O2•−), which derives from the Q cycle at rates estimated between 0.1 and 2% of the total electron flow (reviewed in Refs. 1 and 2). A variety of enzymatic and nonenzymatic defense mechanisms have evolved to either quench free radicals or ameliorate their resultant oxidative damage. Apoptosis (or programmed cell death) occurs by a mechanism that is conserved from nematodes to humans (reviewed in Refs. 3 and 4). In C. elegans, a largely invariant cell lineage (including 131 specific somatic cells destined to undergo apoptosis during hermaphroditic development) has provided fertile ground for the study of apoptosis. In other organisms, mitochondria are thought to provide a major initiation signal for apoptosis via the opening of the mitochondrial permeability transition pore, which leads to the release of a number of cell death-promoting factors, including cytochrome c. In C. elegans, normal, developmentally regulated apoptoses are not mediated by cytochrome c leakage from mitochondria.

Mitochondrial oxidative phosphorylation system defects can cause a perplexing variety of diseases that exhibit a significant degree of clinical heterogeneity (reviewed in Refs. 5 and 6). For the most part, the pathophysioligies present themselves in tissues with high energy demands, such as heart and muscle. The interrelationship of the three activities described above (e.g. ATP generation, ROS production, and apoptosis initiation) may explain some of the perplexing pathophysiological features of mitochondrial diseases.

We have previously described the mev-1(ken1) mutant of the nematode C. elegans as containing a point mutation in Cyt-1 (a large subunit of cytochrome b in complex II) (7). Isolated by screening for hypersensitivity to oxidative damage (8), mev-1 ages precociously at high oxygen concentrations and accumulates markers of aging (e.g. fluorescent materials and protein carbonyls) more rapidly compared with the wild type (9–11). We have recently shown that there are two distinct pathophysiological in mev-1 animals, viz. increased superoxide production from mitochondria and abnormal energy metabolism (12). Here, we report the results from histochemical and ultrastructural examinations of mev-1 mutants that revealed severe defects in succinate-CoQ oxidoreductase and in the membrane potential (ΔΨm) of mitochondria. In addition, mev-1 was found to possess morphological abnormalities in muscle mitochondria, particularly under hyperoxia. CED-9 and Cyt-1 protein levels were also abnormal in the mev-1 mitochondrial membrane. This may explain our electron microscopic and Nomarski-DIC microscopic observations of supernumerary apoptosis in mev-1 embryos. The abnormal apoptosis was suppressed by mutations in either ced-3 or ced-4, indicating that the inappropriate signal in mev-1 embryos stimulates induction of the normal ced-9/ced-4/ced-3 apoptotic pathway. Furthermore, the mev-1,ced-3 double mutant lived longer than mev-1, suggesting that the supernumerary apoptoses contribute to the phenotype of life shortening in mev-1.

**EXPERIMENTAL PROCEDURES**

**General Methods and Strains—** Culturing techniques have been described previously (13). PH126 (ced-3[n1286] mev-1[kn1] unc-50[e306]) was constructed by crossing MT3002 (ced-3[n1286]) hermaphrodites with mev-1 unc-50 heterozygous males and scoring for second generation uncoordinated animals, who gave rise exclusively to third generation embryos with no apoptotic cells. PH137 (ced-4[n1162] dpy-17[e164] mev-1[kn1] unc-50[e306]) was constructed by crossing MT2551 (ced-4[n1162] dpy-17[e164]) hermaphrodites with mev-1[kn1] unc-50[e306] heterozygous males and picking Dpy Unc second generation progeny. Age synchronous populations were prepared as described previously (9–11). Hatching was determined as described previously (9–11). The life spans of individual adult hermaphrodites were determined on agar plates. To prevent progeny production, 5-fluoro-2′-deoxyuridine

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**The abbreviations used are: ROS, reactive oxygen species; CoQ, coenzyme Q; DIC, differential interference contrast.
Fig. 1. Succinate-CoQ oxidoreductase activity in wild-type and mev-1
C. elegans. Immunocytochemistry was performed to detect succinate-CoQ oxidoreductase activity in situ. Enzyme activity was readily apparent in the wild type (A), but virtually undetectable in the mev-1 mutant (B). The left and right arrows point to body wall muscle and an embryo, respectively. Longitudinal sections are presented in both panels, with a cross-section also included in A.

Preparation of Monoclonal Antibodies against Cyt-1 and CED-9—To raise antibodies, peptides were first synthesized using an automated peptide synthesizer. The peptides consisted of either amino acids 40–220 of C. elegans Cyt-1 or amino acids 206–223 of C. elegans CED-9 with an additional cysteine residue in the COOH terminus. The synthesized peptides were then conjugated to the adjuvant keyhole limpet hemocyanin. The synthesized peptide synthesizer. The peptides consisted of either amino acids 40

Preparation of Mitochondrial Fractions for Western Blot Analysis—Young adults were harvested by centrifugation and washed five times with S basal buffer and once with isolation buffer (210 mM mannitol, 70 mM sucrose, 0.1 mM EDTA, and 5 mM Tris-HCl (pH 7.4)). The worms were homogenized (10%, w/v) in isolation buffer with a Teflon-glass homogenizer. Mitochondria were isolated by differential centrifugation at 600 × g to remove debris and then at 7200 × g to pellet mitochondria. Mitochondrial protein was measured using the BCA protein assay kit (Pierce).

Western Blot Analysis—Mitochondrial and cytosolic fractions were boiled in sample buffer (50 mM Tris-HCl (pH 6.8), 2% SDS, 0.14 M 2-mercaptoethanol, 10% glycerol, and 0.01% bromphenol blue) and separated by electrophoresis on 10 or 12.5% SDS-polyacrylamide gels. Proteins were electroblotted onto Immobilon polyvinylidene difluoride membranes in buffer consisting of 48 mM Tris, 39 mM glycine, 0.036% SDS, and 20% methanol and probed with 1 μg/ml KM2408 or 3 μg/ml KM2422. Immunoreactive proteins were visualized using the enhanced chemiluminescence system (ECL, Amersham Biosciences).

RESULTS

Succinate-CoQ Oxidoreductase Activity—Histochemical analysis of succinate-CoQ oxidoreductase activity was performed on frozen sections of wild-type and mev-1 young adults (Fig. 1). Enzyme activity was widespread throughout wild-type animals, including embryos, with a concentration in body wall muscles. Conversely, there was a complete absence of staining in mev-1 animals. This is consistent with the previous observation of reduced levels in mev-1 cell extracts (7).

Ultrastructural Abnormalities in mev-1—Electron micrographs of wild-type body wall muscles revealed normal mitochondrial morphologies, including extensive cristae, at 21% oxygen (Fig. 2A) as well as at 50 and 10% oxygen (Fig. 2, B and C). Conversely, the mitochondria from mev-1 body wall muscles had progressively disorganized cristae as oxygen was increased (Fig. 2, D–F). Many such mitochondria also contained electrondense and amorphous inclusions at 21% oxygen (Fig. 2D), with additional paracrystalline inclusions apparent in animals reared under 50% oxygen (Fig. 2E). Similar ultrastructural abnormalities were present in hypodermal cells, including cuticles of mev-1 (but not wild-type) animals (data not shown). These abnormalities did not appear to 10% oxygen (Fig. 2F).
Similar ultrastructural changes also characterized *mev-1* intestinal cells. Although reasonably normal at 21% oxygen (Fig. 3C), *mev-1* intestinal mitochondria were noticeably swollen and rounded after animals were incubated under 50% oxygen (Fig. 3D). Unlike with *mev-1* mitochondria in muscle, no inclusion bodies were present. Again, hyperoxia had no detectable effect on the wild type (Fig. 2B). Is it possible that the more pronounced ultrastructural abnormalities in *mev-1* muscle versus intestinal tissue reflect the higher levels of oxidative phosphorylation in the former.

$\Delta \Psi_m$—The ability of mitochondria to generate a membrane potential was monitored by fluorescence microscopy after adding the dye JC-1. This dye is taken up by mitochondria as a monomer and forms J-aggregates when concentrated in highly polarized mitochondrial membranes. This transition is reflected by green fluorescence for monomers and by red fluorescence for dimers. In Fig. 4 (A and B), images of the wild type and *mev-1* loaded with JC-1 are shown, respectively. The image of *mev-1* showed a similar profile of monomers, but there were markedly fewer J-aggregates, indicating that $\Delta \Psi_m$ was significantly reduced in the *mev-1* mutant.

CED-9 and Cyt-1 Protein Levels Are Abnormal in *mev-1* Mitochondria—Using antibodies raised against peptides that correspond to portions encoded by the *ced-9* and *cyt-1* genes, Western blotting was performed using mitochondrial preparations from wild-type and *mev-1* animals grown at different oxygen concentrations (Fig. 5A). Densitometric analyses revealed that the CED-9 and Cyt-1 protein levels were different in the two strains (Fig. 5B). The level of Cyt-1 in *mev-1* mitochondria was remarkably reduced under hyperoxia. In contrast, hyperoxia induced CED-9 protein levels in wild-type mitochondria, but caused a statistically insignificant reduction in *mev-1* mitochondria. The levels of *ced-9* and *cyt-1* mRNAs in the wild type and *mev-1* remained unchanged when oxygen was varied between 21 and 50% (data not shown); thus, the reduction of CED-9 and Cyt-1 protein levels in *mev-1* mitochondrial membranes may result from a structural abnormality of mitochondria.

Apoptosis, Embryogenesis, and Life Span—Given the importance of CED-9 in triggering apoptosis, its abnormal expression in *mev-1* suggested that this mutant might have an altered pattern of apoptosis. Indeed, the following data indicate that the *mev-1* mutation causes supernumerary embryonic apoptotic cells, especially under hyperoxia. Wild-type and *mev-1* embryos were scored using Nomarski-DIC microscopy at the "comma" stage (~400 min after the first cell division) for the presence of cells with the distinctive and easily recognizable raised and rounded morphology that characterizes apoptotic cells. The
and therefore, that fluorescence was ends of the C indicates less membrane potential (F) than in the wild type (C). The eggs at the right ends of the mev-1 panels were inviable, and therefore, that fluorescence was artifactual.

number of such cell corpses in mev-1 embryos was 2-fold higher than in wild-type embryos (Fig. 6). The supposition that these were indeed supernumerary apoptoses is supported by the observation that null mutations in either ced-3 or ced-4 completely suppressed this (Fig. 6). These two genes are integral elements of the ced-9ced-4ced-3 apoptotic pathway in C. elegans and are known to completely suppress the normal, developmentally regulated apoptoses for which this nematode is so well known. In addition to supernumerary apoptosis, we observed grossly malformed mev-1 embryos that contained evidence of necrosis, particularly under hyperoxia (data not shown). As described previously (8), the life span of the mev-1 mutant was shorter than that of the wild type (Fig. 7). The survival curve of the mev-1,ced-3 double mutant was similar to that of the wild type during the first one-forth or so of life and then resembled that of mev-1 during the later half (Fig. 7). This is numerically evidenced by the fact that the times needed to reduce survival to 20% were similar in mev-1 (21.5 days) and mev-1,ced-3 (21.4 days), but not in the wild type (25 days). Thus, a portion of the mev-1-specific reduction in life span was abrogated by a mutation that suppresses apoptosis, suggesting that the supernumerary apoptoses in mev-1 contribute to its shortened life span.

As expected, the majority of wild-type animals completed embryonic development within 12 h after fertilization and hatched to become L1 larvae (Fig. 8). Conversely, hyperoxia negatively impacted this process in mev-1 embryos (Fig. 8).

**DISCUSSION**

One of the greatest challenges in the molecular genetics era has been to elucidate the mechanisms by which specific mutations manifest themselves as phenotypic changes. This challenge increases significantly as one moves to progressively higher orders of biological organization. We have previously established that mev-1(kn1) is a missense mutation in a complex II subunit of the electron transport chain (7). Since that discovery, a significant challenge has been to account for the varied pathologies imposed by this mutation. We present them in the deliberate order below, reflecting the notion that each

**FIG. 4.** ΔΨm as evidenced by JC-1-specific fluorescence in the wild type (A–C) and mev-1 (D–F). Three different images of the same preparation are shown for both the wild type (A–C) and mev-1 (D–F). A and D are Nomarski-DIC images. B and E show green fluorescence at 530 nm (indicative of monomers). C and F show fluorescence at 690 nm (indicative of dimers). The arrowheads point to animals that were alive when this experiment was conducted. The differences in fluorescence intensity indicate substantial dysfunction in the mitochondrial membranes of mev-1 animals. Most notably, there was significantly less red fluorescence in mev-1 mitochondria, which indicates less membrane potential (F) than in the wild type (C). The eggs at the right ends of the mev-1 panels were inviable, and therefore, that fluorescence was artifactual.

**FIG. 5.** Protein levels of Cyt-1 and CED-9 in mitochondrial preparations from wild-type and mev-1 young adults. A, Western blots after immunological detection of Cyt-1 and CED-9; B and C, densitometric scans showing the relative protein levels, with 1.0 taken as the wild-type density at 21% oxygen. Hypoxia substantially reduced Cyt-1 association with the mitochondrial membrane in mev-1, but not in the wild type. In addition, CED-9 was not induced by hypoxic treatment in mev-1. Each value is the mean ± S.D. of three different experiments. Student’s t tests were performed on all quantitative analyses with two significant differences noted: p < 0.05 (*) and p < 0.005 (**) for mev-1 mutants at 50% oxygen compared with the wild type at 21% oxygen (p < 0.05 † and p < 0.005 ‡‡) for mev-1 mutants at 50% oxygen compared with mev-1 mutants at 21% oxygen). N2, wild type.
successive pathology is the consequence of those preceding it. First, it is clear that the mev-1 mutation initially manifests itself as three distinct biochemical pathologies (12): (i) elevated ROS levels in mev-1 mutants (specifically, superoxide anion levels in both intact mitochondrial and submitochondrial particles were approximately two times greater in mev-1 mutants than in the wild type); (ii) depletion of the ROS scavenger glutathione in mev-1 animals, which presumably renders mev-1 animals more susceptible to the increased ROS levels mentioned above; and (iii) a higher ratio of lactate to pyruvate in mev-1 mutants, suggesting that a metabolic imbalance known as lactate acidosis occurs in these animals. Second, these three biochemical pathologies, especially the first two, result in significantly higher levels of oxidative stress in mev-1 animals, which presumably renders mev-1 animals more susceptible to the increased ROS levels mentioned above; and (iii) a higher ratio of lactate to pyruvate in mev-1 mutants, suggesting that a metabolic imbalance known as lactate acidosis occurs in these animals. Second, these three biochemical pathologies, especially the first two, result in significantly higher levels of oxidative stress in mev-1 animals, which presumably correlates with the relative rates of respiration and hence the levels of oxidative stress. Many electron-dense and amorphous inclusions at 21% oxygen (Fig. 2D) or additional paracrystalline inclusions apparent in animals reared under 50% oxygen (Fig. 2E) may be mitochondrial creatine kinase (16). Perhaps even more importantly, $\Delta W_m$ was severely compromised in mev-1 (Fig. 4). This suggests that the ultrastructural abnormalities lead to a loss of mitochondrial functionality in mev-1 animals. It is also possible that the decreased $\Delta W_m$ was not due to increased oxidative stress per se, but to another abnormal consequence of electron transport by the complex II defect in mev-1. Fourth, we have demonstrated that mitochondrially localized CED-9 protein levels are altered in a mev-1 genetic background (Fig. 5). CED-9, the structural and functional homolog of Bcl-2, is a mitochondrially bound protein that controls apoptosis by regulating CED-4 and CED-3 activity (reviewed in Refs. 3 and 4). CED-9 was induced in wild-type mitochondria by hyperoxia, but this induction was not observed in mev-1 mitochondria. As the mRNA levels of CED-9 and Cyt-1 were unchanged, the differences between the wild type and mev-1 reflect some sort of post-translational
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alteration. Again, it is possible that these differences are a secondary consequence of the increased oxidative stress that characterizes mev-1. Alternatively, they may reflect a more direct consequence of the mev-1 mutation that acts to directly alter the mitochondrial membrane such that CED-9 cannot be properly retained. The lower amount of mitochondrially localized CED-9 in mev-1 may in turn be insufficient to protect against the oxidative damage induced by hyperoxia. It is also possible that this MEV-1 protein may be destabilized in the mutant. Indeed, mRNA levels were unchanged, but this protein appeared only in mitochondria, not in the cytosol (data not shown). The increase in the CED-9 levels in the wild type may be due to up-regulation responding to oxidative stress, and mev-1 may not have this ability. Fifth, the observation of supernumerary apoptosis in mev-1 embryos (Fig. 6) is a logical and perhaps inevitable consequence of the pathologies articulated above. Its suppression by null mutations in either ced-3 or ced-4 indicates that, although the trigger is different from that normally employed by C. elegans during developmental pruning, the basic apoptotic machinery is the same. All of these pathologies ultimately explain two important organismal consequences of the mev-1 mutation. The first was that mev-1 embryos failed to complete development under hyperoxia. Increased oxidative stress, abnormal mitochondrial structure/function, and ced-9-mediated supernumerary apoptosis may all have contributed to this. The second of these organismal consequences was the shortened life span perpetrated by the mev-1 mutation. Again, increased oxidation stress and abnormal mitochondrial structure/function may be important in this organismal phenotype. In addition, the ability of ced-3 to partially restore the life span of mev-1 to that of the wild type provides strong evidence that at least a portion of the reduced life span of mev-1 animals is due to abnormal apoptosis. In summary, it is now possible to sort the various phenotypes associated with the mev-1 mutation into a hierarchical scheme that progresses through increasing levels of biological complexity.

Finally, it is worth noting that three overlapping but distinct types of apoptosis have now been described in C. elegans. The first and most extensively explored is the developmentally regulated process in which specific cells are predestined to undergo programmed cell death in C. elegans. Specifically, 131 specific somatic cells are destined for apoptosis during normal development of hermaphrodites. Second, Gartner et al. (17) have described widespread apoptosis that can occur in the C. elegans germ line in response to DNA damage. Third, we have presented evidence of supernumerary apoptosis in somatic cells of mev-1 embryos (Figs. 5 and 6), a likely consequence of the increased oxidative stress experienced by this mutant. There are a number of obvious similarities between these three types of apoptosis. First, they are morphologically similar (i.e. the apoptotic cells appear identical under Nomarski-DIC microscopy). Second, all three types of apoptosis are executed using the same core machinery, i.e. they are each dependent upon the ced-9/ced-4/ced-3 gene products. There are also some clear differences between the three types of apoptosis. The first type of apoptosis is developmentally regulated and is restricted to specific cells in the lineage. It is not triggered by oxidative stress, having evolved as a “hardwired” characteristic to selectively eliminate specific cells in the C. elegans lineage. Conversely, the latter two are flexible and are responses to cellular damage. Both are likely evolutionary devices more akin to that in mammals, in which apoptosis serves as a mechanism to eliminate incapacitated cells that might threaten the whole organism (reviewed in Ref. 18). It is likely that both the mev-1-stimulated apoptosis and the germ line apoptosis share the same or similar triggering mechanisms as employed by mammals to eliminate grossly damaged cells. However, in addition to the fact that one involves somatic cells and the other a germ line, they exhibit genetic differences. Specifically, the germ line apoptosis requires functional rad-5 and him-7 gene products, i.e. DNA damage-induced germ line apoptoses do not occur in rad-5 and him-7 mutants (17). The rad-5 and him-7 mutants are not hypersensitive to oxidative stress, as is mev-1.2 In addition, neither mutant shows supernumerary apoptoses in embryos.2 Thus, it appears that the initial events that trigger these two types of apoptosis are different from one another. Further research will undoubtedly reveal more similarities and differences between these three types of apoptosis. As summarized in Fig. 9, the data in this and a previous report (12) go a long way toward clarifying the progressive consequences of the mev-1 mutation.

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REFERENCES
1. Beckman, K. B., and Ames, B. N. (1999) Physiol. Rev. 78, 547–581
2. Finkel, T., and Holbrook, N. J. (2000) Nature 408, 239–247
3. Liu, Q. A., and Hengartner, M. C. (1999) Ann. N. Y. Acad. Sci. 887, 92–104
4. Meier, F., Finch, A., and Evan, C. (2000) Nature 407, 796–801
5. Morgan, J. A., and Hann M. G. (1999) Biochim. Biophys. Acta 1410, 125–145
6. Wallace, D. G. (1999) Science 283, 1482–1488
7. Iishi, N., Michikihko, F., Hartman, P. S., Tsuda, M., Yasuda, K., Senoo-Matsuda, N., Yanae, S., Ayusawa, D., and Suzuki, K. (1998) Nature 394, 694–697
8. Iishi, N., Takahashi, R., Tornita, S., Keino, T., Honda, S., Yoshino, K., and Suzuki, K. (1996) Natl. Acad. Sci., USA 93, 165–171
9. Honda, S., Iishi, N., Suzuki, K., and Matsu, M. (1993) J. Gerontol. 48, B57–B61
10. Adachi, H., Fujisawa, Y., and Iishi, N. (1998) J. Gerontol. 53A, B240–B244
11. Higashio, H., Iishi, N., Ishida, H., Ichimori, K., Nakazawa, H., and Suzuki, K. (1994) Mech. Ageing Dev. 74, 161–170
12. Senoe-Matsuda, N., Yasuda, K., Tsuda, M., Okubo T., Yoshimura, S., Nakazawa, H., Hartman, P. S., and Iishi, N. (2001) J. Biol. Chem. 276, 41553–41558
13. Brenner, S. (1974) Genetics 77, 71–94
14. Mitchell, D. H, Stiles, J. W., Satelli J., and Sanadi, D. R. (1979) J. Gerontol. 35, 28–36
15. Sulston, J. E., Schierenberg, E., White, J. G., and Thomson, N. (1983) Dev. Biol. 100, 64–119
16. Stadhouders, A. M., Jap, P. H. K., Winkler, H.-P., Eppenberger, H. M., and Wallimann, T. (1994) Proc. Natl. Acad. Sci. USA 91, 5089–5093
17. Gartner, A., Milestein, S., Ahmed, S., Hodgkin, J., and Hengartner, M. O. (2000) Mol. Cell 5, 435–443
18. Evan, G., and Littlewood, T. (1998) Science 281, 1317–1322

2 P. S. Hartman, unpublished data.
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