Mitochondrial Expression and Function of GAS-1 in Caenorhabditis elegans

A mutation in the gene gas-1 alters sensitivity to volatile anesthetics, fecundity, and life span in the nematode Caenorhabditis elegans. gas-1 encodes a close homologue of the 49-kDa iron protein subunit of Complex I of the mitochondrial electron transport chain from bovine heart. gas-1 is widely expressed in the nematode neuromuscular system and in a subcellular pattern consistent with that of a mitochondrial protein. Pharmacological studies indicate that gas-1 functions partially via presynaptic effects. In addition, a mutation in the gas-1 gene profoundly decreases Complex I-dependent metabolism in mitochondria as measured by rates of both oxidative phosphorylation and electron transport. An increase in Complex II-dependent metabolism also is seen in mitochondria from gas-1 animals. There is no apparent alteration in physical structure in mitochondria from gas-1 nematodes compared with those from wild type. These data indicate that gas-1 is the major 49-kDa protein of complex I and that the GAS-1 protein is critical to mitochondrial function in C. elegans. They also reveal the importance of mitochondrial function in determining not only aging and life span, but also anesthetic sensitivity, in this model organism.

Volatile anesthetics are compounds that are used extensively to produce reversible unconsciousness and relief of pain. It is quite remarkable that their mechanism of action is not understood (1, 2). Our laboratory exploits a very simple animal model, the nematode Caenorhabditis elegans, to investigate the molecular mechanism of volatile anesthetic action. We have established that the interactions of multiple genes are crucial in controlling the behavior of C. elegans in volatile anesthetics (3, 4).

At least seven genes interact to control the response of C. elegans to volatile anesthetics (3, 4). Mutations in one gene, gas-1 (for general anesthetic-sensitive) cause hypersensitivity to all inhalation anesthetics tested as well as to ethanol. gas-1 overrides the effects of the other genes on sensitivity to volatile anesthetics. Nematodes with this mutation are also temperature-sensitive embryonic lethals, have a reduced life span, slow growth rates, and an increased sensitivity to the deleterious effects of free radicals and hyperoxia (5). However, they move quite normally in air, indicating a functional neuromuscular system.

Previously, we cloned the gas-1 gene and identified a point mutation in the allele fc21 (6). Sequence comparison strongly suggested that gas-1 encoded a homologue of the bovine 49-kDa(IP) subunit of NADH-ubiquinone oxidoreductase (Complex I), the first protein complex of the mitochondrial electron transport chain. Previous studies from other investigators indicated that Complex I was the most sensitive complex to inhibition by volatile anesthetics (7, 8).

The 49-kDa(IP) proteins are common to both the very complicated eukaryotic Complex I (41 different subunits) and to the much simpler Paracoccus enzyme (only 15 subunits). Both enzyme complexes catalyze the same reaction, i.e. proton-pumping across the mitochondrial membrane, driven by the transfer of electrons from NADH to a quinone (9–10). A knockout mutant of the “49-kDa(IP) gene” in Neurospora completely lacked NADH-dehydrogenase activity, because the “matrix arm” of the enzyme complex failed to assemble (11). All of the Complex I mutants in Neurospora were reported to have reduced growth rates, and their conidia were less viable. This is reminiscent of the reduced growth rate, life span, and brood size of fc21. The matrix arm of Complex I contains the binding site for NADH as well as all but one of the redox centers. Lastly, the 49-kDa(IP) subunit from Rhodobacter has been implicated in binding the head group of quinones (such as the electron acceptor, ubiquinone) and quinone-like inhibitors of Complex I. Thus, 49-kDa(IP) proteins seem to be essential for the core function of Complex I (10, 12). gas-1(fc21) is the first known mutation of this subunit in animals.

The fact that, in gas-1(fc21) animals, a strictly conserved amino acid residue is affected in a subunit essential for Complex I function suggests that the activity of the mutant Complex I is decreased or abolished. However, it has not been proven that the GAS-1 protein has a mitochondrial function. In addition, during the sequencing of the genome of C. elegans a second homologue of the 49 kDa(IP) subunit, T26A5.3, was identified (13). Because the relative importance of these two genes in mitochondrial function is not known, we studied their expression and the effects of the gas-1(fc21) mutation on mitochondrial function.

* This work was supported in part by National Institutes of Health Grants GM58881 and GM45402 (to M.M.S. and P.G.M.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ Supported by the Veterans Administration Hospital Medical Services and by National Institutes of Health Grant PO1 AG15885.

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1 The abbreviations used are: gas-1, general anesthetic-sensitive gene; Complex I, the first protein complex of the electron transport chain in mitochondria; PCR, polymerase chain reaction; bp, base pairs; GFP, green fluorescence protein; EGF, enhanced GFP; MOPS, 4-morpholinepropanesulfonic acid; BSA, bovine serum albumin; ADP/O, number of ADP molecules converted to ATP per oxygen atom respirated; ETC, electron transport chain; kb, kilobase(s); SDH, succinate dehydrogenase; NMJ, neuromuscular junction; ACh, acetylcholine; NFR, NADH-ferricyanide reductase; FP, flavoprotein; IP, iron protein; TTFA, thienyl trifluoroacetone, an inhibitor of electron transport through Complex II.
In these studies we show that gas-1 is abundantly expressed in multiple tissues, including those most likely to confer anesthetic-induced immobility: neurons and body wall muscle. Expression of the second homologue of the 49-kDa(IP) subunit, T28A5.3, could not be detected via a promoter reporter. Pharmacological studies with aldicarb (14) indicate that the effect of gas-1 on anesthetic sensitivity is partially presynaptic and most consistent with neuronal effects. We also show that isolated mitochondria from gas-1 animals have reduced Complex I enzymatic activities, as measured by rates of both oxidative phosphorylation and electron transport. An increase in Complex II-dependent metabolism also is seen in gas-1(fc21) animals. These results confirm that GAS-1 is the major isofrom of the 49-kDa(IP) subunit in Complex I, and that it is a crucial component of electron transport and oxidative phosphorylation in *C. elegans*.

**EXPERIMENTAL PROCEDURES**

**Nomenclature**—The conventions for *C. elegans* nomenclature have been followed throughout (15). Gene names are italicized 3-letter abbreviations followed by a hyphen and a number, e.g. gas-1, the gene. This designation can also specify worms homozygous for a mutation in this gene, e.g. gas-1(e5), the mutant worm. The wild type allele is indicated by a superscript plus following the gene name, e.g. gas-1+. Individual allele names are represented by a combination of one or two letters and a number, either alone or added in parentheses, e.g. fc21 or in gas-1(fc21). Non-italized, all capital letters indicate the protein, e.g. the protein GAS-1. Bracketed indicate transgenic constructs. For example, (gas-1+) are animals carrying the wild type gas-1 gene as a result of microinjection into a mutant gas-1 background.

**Nematode Strains**—The wild type *C. elegans*, N2, as well as the mutants *mnDp1; unc-3(e151) and unc-7(e5)* were obtained from the Caenorhabditis Genetics Center in Minneapolis, MN. *gas-1(fc21)* was isolated in a screen for immobile worms in 3.5% enflurane (4) after maturation of N2 with ethylmethylsulfonate (N2 is immobilized by 6.5% enflurane). Standard techniques were used for growing and maintaining cultures of *C. elegans* and for constructing double mutants, e.g. *unc-7 gas-1* as a result of microinjection into a mutant gas-1 background.

**Microinjection**—Transgenic nematodes such as *unc-7 gas-1*; rol-6(su1006) had been created by microinjection as previously described (6). In these studies we show that gas-1 is abundantly expressed in multiple tissues, including those most likely to confer anesthetic-induced immobility: neurons and body wall muscle. Expression of the second homologue of the 49-kDa(IP) subunit, T28A5.3, could not be detected via a promoter reporter. Pharmacological studies with aldicarb (14) indicate that the effect of gas-1 on anesthetic sensitivity is partially presynaptic and most consistent with neuronal effects. We also show that isolated mitochondria from gas-1 animals have reduced Complex I enzymatic activities, as measured by rates of both oxidative phosphorylation and electron transport. An increase in Complex II-dependent metabolism also is seen in gas-1(fc21) animals. These results confirm that GAS-1 is the major isofrom of the 49-kDa(IP) subunit in Complex I, and that it is a crucial component of electron transport and oxidative phosphorylation in *C. elegans*.

**Al dicarb /Levamisole Assay**—Aldicarb and levamisole were added to agar plates on which an *Escherichia coli* lawn had been grown as in previous studies (14, 18). 24 h after addition of the drugs, nematodes were passed on the agar plates. Nematoses were scored through a dissecting microscope for contracted immobility 2 h after exposure to the drugs, during which a steady-state response had been reached. They were scored as immobile if no locomotion was seen for 10 s. EC50 values were determined as described previously (3, 4). At least 50 animals were scored at each concentration of drug and the EC50 values were calculated from the responses of 50 nematodes at two to five different concentrations of drug, done in duplicate. N, the number of assays, is stated in parentheses. Values are given ± S.D. Values were compared using student’s t test.

**Preparation of Mitochondria**—All preparations were done at 4 °C. Clean worms were suspended in MSM-E (220 mM mannitol, 70 mM sucrose, 5 mM MOPS, 1 mM EDTA, pH to 7.4 with KOH). A polytron (Brinkman Instruments) was used for initial rupture: 20 s at 14,000 rpm. 5 mg/g of worms) protein type XVII (Sigma, St. Louis, MO) was added to the homogenate and stirred for 10 min. Immediately afterward the slurry was homogenized in a glass Potter/Elvehjem tissue grinder with a Teflon pestle. After adding 1 volume of MSM-E containing 0.4% BSA, the homogenate was centrifuged (300 × g, 10 min, 4 °C). The supernatant was filtered through three layers of gauze and recentrifuged (7000 × g, 10 min, 4 °C). The mitochondrial pellet was resuspended in 100 μl of MSM-E. Total protein was determined by the Lowry assay with BSA as a standard (21).

**Oxidative Phosphorylation**—Polarigraphic measurement of oxidative phosphorylation was performed as previously described (22). Briefly, oxygen uptake was followed with a Clark type electrode (Yellow Springs Instruments, YSI) connected to a chart recorder via a YSI Oxygen monitor. 500 μg of mitochondria was injected into 500 μl of air saturated incubation media (100 mM KC1, 50 mM MOPS, 1 mM EGTA, 5 mM potassium phosphate, 1 mg/ml defatted BSA) kept at 30 °C. The supernatant was filtered through three layers of gauze and recentrifuged (7000 × g, 10 min, 4 °C). The mitochondrial pellet was resuspended in 100 μl of MSM-E. Total protein was determined by the Lowry assay with BSA as a standard (21).

**Gas-1 Promoter Reporter Assay**—The genomic DNA sequence immediately upstream of the gas-1 coding sequence was amplified by PCR using the cosmid K09A9 as a template. The primers introduced an artificial ApaI site on one end of the resulting 818-bp PCR fragment and a BamH1 site on the other. This sequence was cloned directionally into the pBluescript II K+ (Stratagene) and named pKEK. Correct assembly of pKEK was confirmed by sequencing the construct. pKEK was microinjected into *mnDp1; unc-7 gas-1* yielding unc-7 gas-1[pKEK, rol-6] offspring, which were tested for sensitivity to halothane. For a detailed description of the rationale behind using the nematode strain, *mnDp1; unc-7 gas-1* to demonstrate neutral rescue of gas-1 (see Ref. 6). The localization of the GAS-1:EGFP internal fusion protein in animals that were mobile in 2% halothane was determined by epifluorescence microscopy.

**Electron Transport Chain (ETC) Assays**—Frozen (−60 °C) samples of mitochondria preparations were solubilized with cholate, and zero order rates were determined spectrophotometrically for the following enzyme activities: Citrate synthase, rotenone-sensitive NADH-cytochrome c reductase, succinate-cytochrome c reductase, antimycin A-sensitive deucylubiquinol-cytochrome c reductase, NADH-ferricyanide reductase, rotenone-sensitive NADH-decyubiquinone reductase, succinate-DCPIP reductase, and duroquinone-stimulated TFFA-sensitive succinate DCPIP reductase. The first order rate constant was determined for cytochrome c oxidase. Assays and calculations were performed as described by Hoppel et al. (25).
Electron Microscopy—Freshly prepared mitochondria were prepared for transmission electron microscopy as described by Hoppel et al. (25).

RESULTS

Aldicarb/Levamisole Assay—Using contracted immobility as an end point, sensitivity to aldicarb was decreased in gas-1 animals, whereas sensitivity to levamisole was unchanged from that of N2 (Table I). Levamisole is a cholinergic agonist, whereas aldicarb inhibits acetylcholine degradation. These agents are used in C. elegans to distinguish pre- from postsynaptic effects of mutations. Our results indicate that at least part of the effect of gas-1 results from a presynaptic effect (18).

Promoter Reporter Assay—Previously, we were able to rescue the hypersensitivity of gas-1(fc21) to halothane by introducing a 4.6-kb SfuI restriction fragment from the cosmid K09A9 into an unc-7 gas-1(fc21) animal (6). Thus, the fragment contained not only the wild type coding sequence for gas-1 but also a functional promoter. To visualize the tissues in which this gas-1 promoter is active, the 818-bp sequence immediately upstream of the gas-1 start codon (gas-1 promoter) was cloned in front of the promoterless coding sequence of a green fluorescence protein, EGFP. Wild type nematodes made transgenic for this reporter construct show green fluorescence in tissues that normally express the gas-1 gene.

The strongest fluorescence was regularly observed in the pharyngeal muscles and widely distributed in the tail. Body wall muscles, vulva muscles, and parts of the nervous system were also labeled, although with more variability (Fig. 1). Autofluorescence of the gut granules made it impossible to

![Figure 1](image)
determine whether EGFP was expressed in the intestinal cells. Gonads and eggs never expressed the reporter, suggesting silence of the microinjected gas-1 promoter in these tissues. All developmental stages of larvae expressed the construct, which was seen as early as L1.

Rescue with a Fluorescent Fusion Protein—To assess whether GAS-1 is indeed directed into mitochondria, a plasmid, pKEK, was constructed that expressed an EGFP-labeled GAS-1 protein under the control of the gas-1 promoter. We reasoned that to rescue the mutant phenotype, the GAS-1::EGFP fusion protein must reach the mitochondria. All mitochondrial proteins encoded in the nucleus require an N-terminal transit peptide to be routed into the mitochondrion; this peptide is believed to be cleaved off once the protein reaches its destination. In the case of GAS-1, an algorithm described by Gavel and von Heijne (26) predicted such a cleavage site after amino acid residue number 32. Thus, the EGFP tag had to be inserted downstream of this site. On the other hand, inserting the tag into the sequence of the mature part of GAS-1 might disrupt GAS-1’s function. To solve this potential dilemma, the fusion in pKEK was designed to repeat the first 9 amino acids beyond the cleavage site with EGFP sandwiched between these repeats (Fig. 2A). The rationale was to provide the transit peptidase with a familiar peptide sequence around its cleavage site and to have the full sequence of the mature GAS-1 downstream of the tag.

EGFP-containing transformants, unc-7 gas-1 [gas-1Δ·egfp; rol-6], showed subcellular punctate green fluorescence rather than the fluorescence-filled cells seen with the promoter reporter transformants (Fig. 2B). In body wall muscle these fluorescenting dots are neatly arranged in lines parallel to the myofibrils. This suggests mitochondrial localization of the fusion protein. This pattern was seen in all stages of development, as early as L1. A simpler construct with the EGFP appended to the C terminus of GAS-1 yielded a comparable staining pattern but did not rescue the anesthetic phenotype (data not shown).

T26A5.3—The C. elegans sequencing consortium (13) identified a potential isogene to gas-1. Because no mutations of this gene are known, it is designated by its associate cosmid name: T26A5.3. The predicted gene product would be a protein that is 96% identical to GAS-1 and is 65% identical to the bovine homologue (transit peptides excluded). Worms transgenic for T26A5.3 (unc-7 gas-1 [T26A5.3; rol-6], originating from mnDp1; unc-7 gas-1 microinjected with the cosmid T26A5), were immobile in 2% halothane in all (12 out of 12) transformed lines. In contrast, worms transgenic for a chimeric gene consisting of T26A5.3 coding sequence under the control of the gas-1 promoter were rescued to normal anesthetic behavior (data not shown). Thus, when under the control of the gas-1 promoter, the T26A5.3 protein can functionally replace GAS-1.

To rule out that T26A5.3 is a non-transcribed pseudogene, RNA from a mixed stage culture of wild type worms was amplified using RT-PCR with primer pairs designed to either specifically amplify the gas-1 mRNA or the presumed T26A5.3 mRNA. In both cases products of the expected size were obtained (1633 and 1423 bp, respectively), indicating that T26A5.3 is transcribed (data not shown). Re-amplification of the products with nested primers was successful if primers specifically designed for the respective cDNA were employed. However, amplification failed when gas-1 primers were used on the PCR product obtained with T26A5.3 primers (and vice versa). This ruled out cross-reactivity of the primers with the
mRNA from either gene.

To visualize where and when T26A5.3 is expressed, a promoter reporter construct was made analogous to the one for gas-1. We assumed that the promoter of T26A5.3 is situated upstream of the predicted start codon but no farther away than the next predicted gene upstream of T26A5.3; this entire 1.5-kb fragment was placed upstream of the EGFP code. When this construct was introduced into a wild type nematode, no fluorescence above background could be detected in the transformants at any stage of development (data not shown). We conclude that T26A5.3 is naturally expressed at very low levels or in tissues where the assay cannot detect it, such as the germ line or gut.

**Oxidative Phosphorylation**—Oxidative phosphorylation allows assessment of the impact of gas-1 on the proton transport capacity of the whole respiratory chain. In intact mitochondria, electron transport, as measured by oxygen uptake (respiration), and generation of ATP (phosphorylation) are tightly coupled by the proton gradient across the inner mitochondrial membrane.

Mitochondria from wild type nematodes can metabolize malate, glutamate, pyruvate, or succinate (27). Oxidative phosphorylation, measured as oxygen uptake, is maximal when electron donor substrates (malate, glutamate, pyruvate, or succinate) and substrates for the F$_0$F$_1$-ATPase (ADP and inorganic phosphate) are present in saturating concentrations (state 3 respiration). Thus, the state 3 rate represents the maximum respiratory capacity of the mitochondrion. Malate, glutamate, and pyruvate are oxidized by mitochondrial enzymes, which produce NADH, which in turn feeds electrons into the respiratory chain via Complex I. When Complex I is blocked by rotenone, none of these substrates promotes oxygen uptake (data not shown). In gas-1 state 3 respiration with either glutamate, malate, or pyruvate/malate as the substrate is lower than in the wild type by 66%, 64%, and 62%, respectively (Fig. 3A and Table II). Similarly, the ADP/O ratio was decreased in the gas-1 mutant (Fig. 3B and Table II). On the other hand, in mitochondria from gas-1 animals, succinate-dependent state 3 respiration is enhanced relative to that of N2. Succinate is directly oxidized by Complex II rather than Complex I (28).

We also wished to show that restoration of gas-1 function could correct the defects in oxidative phosphorylation seen in gas-1(pKEK rol-6). Therefore, we measured metabolism in transgenic animals carrying the translational fusion, pKEK, which rescues gas-1(pfc21). This fusion is carried as an extrachromosomal array, that is, it is not incorporated into the genome. Thus not all offspring of a rescued parent remain wild type and not all animals that appear as wild type express the wild type gene in all cells. We tried to enrich for the presence of gas-1+ by picking animals that strongly express the EGFP fusion, i.e. that strongly glow green under fluorescence, to seed the culture flasks. These animals may be genetic mosaics but are of the identical genotype (albeit mosaic) or offspring that are purely as controls (Table III). Values for Complex I-dependent metabolism were significantly higher in mitochondria from these cultures of mixed genotype to all cells. We tried to enrich for the presence of gas-1+ by picking animals that strongly express the EGFP fusion, i.e. that strongly glow green under fluorescence, to seed the culture flasks. These animals may be genetic mosaics but are of the identical genotype (albeit mosaic) or offspring that are purely gas-1(pKEK rol-6) animals; they throw offspring of the next predicted gene upstream of T26A5.3; this entire 1.5-kb fragment was placed upstream of the EGFP code. When this construct was introduced into a wild type nematode, no fluorescence above background could be detected in the transformants at any stage of development (data not shown). We conclude that T26A5.3 is naturally expressed at very low levels or in tissues where the assay cannot detect it, such as the germ line or gut.

**Electron Transport Chain (ETC) Assays**—ETC assays measure electron transport through individual components of the respiratory chain. Table III summarizes the results of the ETC assays. Three of these provide measures of Complex I activity: 1) Rotenone-sensitive NADH-cytochrome-c reductase (I–III in Fig. 4 and Table III) determines the electron transport from the donor NADH through Complex I, ubiquinone (Q), and then Complex III to the acceptor cytochrome c. 2) Rotenone-sensitive NADH-decy ubiquinone reductase (I in Fig. 4 and Table III) measures electron transport through Complex I alone from NADH to decy ubiquinone. Decy ubiquinone is used instead of the natural e–acceptor, ubiquinone, because of its better solubility in water. 3) NADH-ferricyanide reductase (NFR in Fig. 4 and Table III) is an activity of the flavoprotein moiety of Complex I. Electrons from NADH are diverted to the artificial e–acceptor ferricyanide before they could pass through the rotenone-inhibitable section of Complex I.

In the mutant gas-1, all three Complex I-dependent activities are decreased (Fig. 4 and Table III). Thus, the mutant subunit significantly impedes electron transport through each measurable step of Complex I function. The other electron transport steps examined do not require the participation of Complex I. The activities of antimycin A-sensitive decy ubiqui-
ADP/O are indicators of the coupling between respiration and ATP generation. Values were compared using analysis of variance. Mitochondria were uncoupled with 2,4-dinitrophenol. The respiration rate in the presence of ADP (state 3) divided by the one in the absence of ADP (state 4) is the respiratory control ratio (RCR). ADP/O is the number of ADP molecules converted to ATP per oxygen atom respired. Both RCR and ADP/O are indicators of the coupling between respiration and ATP generation. Values were compared using analysis of variance.

Mitochondria looked the same regardless of whether they were isolated from N2 or gas-1 (Fig. 5). Thus, gas-1 does not visibly alter the morphology of the organelle. Furthermore, the amount of contamination appears equal in preparations from both sources, indicating that the lower specific activities seen in the mutant are not caused by a higher fraction of non-mitochondrial or non-nematode proteins.

The presence of E. coli in the mitochondrial preparations raises the question as to the bacterial contribution to the oxygen consumption measured in the oxidative phosphorylation assay. Controls with the food bacteria alone had a constant rate of oxygen uptake, which could not be modulated by addition of any of the substrates used or by ADP or 2,4-dinitrophenol. Mitochondria preps from N2, on the other hand, were responsive to all of the above, indicating that the majority of oxygen uptake is mitochondrial in origin. The bacterial contamination is probably responsible, in part, for respiratory rate measured in the absence of substrates.

**DISCUSSION**

In these studies, we show that 1) gas-1 was expressed and functioned in tissues consistent with its phenotype; and 2) the mutation gas-1(fc21) significantly disrupted mitochondrial function. We evaluated gene expression with EGFP constructs, cellular function with the pharmacological effects of levamisole and aldicarb, and mitochondrial function with measurements of oxidative phosphorylation and electron transport chain activity.

Levamisole is an agonist of the acetylcholine receptor of the neuromuscular junction (NMJ). Any mutation directly impairing the ability of muscle cells to contract is seen as resistance to

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**TABLE II**

Oxidative phosphorylation

| Substrate | Strain | State 3 | DNP | RCR | ADP/O |
|-----------|--------|---------|-----|-----|-------|
| Glutamate | N2     | 72.9 ± 19.0 (14) | 127.9 ± 26.3 (13) | 3.6 ± 0.8 (14) | 2.9 ± 0.6 (14) |
| Malate    |        | 108.1 ± 26.0 (16) | 169.4 ± 38.6 (16) | 3.6 ± 0.5 (16) | 3.3 ± 0.7 (12) |
| Pyruvate + malate | 145.0 ± 16.7 (6) | 127.0 ± 33.3 (5) | 3.1 ± 0.5 (6) | 2.9 ± 1.0 (5) |
| Succinate |        | 167.4 ± 37.7 (13) | 84.8 ± 40.3 (10) | 1.6 ± 0.2 (13) | 2.1 ± 1.7 (10) |
| Succinate/rotenone | 156.5 ± 39.3 (10) | 141.6 ± 40.2 (9) | 1.7 ± 0.2 (10) | 1.9 ± 2.0 (8) |
| Glutamate | gas-1  | 24.6 ± 5.3° (11) | 25.5 ± 10.0° (11) | 1.6 ± 0.3° (11) | 1.9 ± 0.7° (9) |
| Malate    |        | 38.5 ± 5.7° (11) | 31.9 ± 6.6° (11) | 1.9 ± 0.4° (11) | 2.0 ± 0.5° (6) |
| Pyruvate + malate | 54.5 ± 9.5° (5) | 43.4 ± 9.6° (5) | 2.1 ± 0.3° (5) | 1.9 ± 0.6° (3) |
| Succinate |        | 254.8 ± 40.5° (10) | 228.1 ± 44.0° (10) | 1.9 ± 0.3 (10) | 1.3 ± 0.6° (3) |
| Succinate/rotenone | 234.5 ± 21.7° (9) | 221.6 ± 15.8° (9) | 1.9 ± 0.2 (9) | 1.2 ± 0.3 (5) |
| Glutamate | unc-7 gas-1 | 23.4 ± 1.2° (4) | 25.4 ± 9.3° (4) | 1.5 ± 0.1° (4) | 1.9 ± 0.3° (3) |
| Malate    |        | 34.3 ± 7.3° (5) | 29.2 ± 4.1° (5) | 1.9 ± 0.3° (5) | 1.6 ± 0.1° (2) |
| Pyruvate + malate | 50.5 ± 11.8° (4) | 37.9 ± 14.5° (4) | 2.1 ± 0.2° (4) | 1.6 ± 0.0° (2) |
| Succinate |        | 195.2 ± 29.5° (4) | 167.4 ± 23.8° (4) | 1.8 ± 0.3 (4) | 1.0 ± 0.1° (2) |
| Succinate/rotenone | 207.6 ± 22.1° (5) | 194.9 ± 25.1° (5) | 1.6 ± 0.2 (5) | 1.0 ± 0.1° (2) |
| Glutamate | unc-7 gas-1 | 41.0 ± 14.7°(3) | 64.1 ± 4.9°(3) | 2.1 ± 0.2°(3) | 2.5 ± 1.0°(3) |
| Malate    |        | 53.4 ± 13.9°(5) | 51.6 ± 14.9°(5) | 2.3 ± 0.4°(5) | 2.3 (1) |
| Pyruvate + malate | 77.3 ± 6.4°(2) | 65.3 ± 0.2°(2) | 2.1 ± 0.3°(2) | |
| Succinate |        | 110.9 ± 31.8°(5) | 78.7 ± 26.8°(5) | 1.4 ± 0.2 (5) | 0.9 (1) |
| Succinate/rotenone | 137.5 ± 11 (1) | 105.3 ± 1 (1) | 1.6 (1) | |

* Values different from N2, p < 0.05.

† Values different from unc-7 gas-1, p < 0.05.
levamisole in the intact worm. Because gas-1 worms react normally to levamisole, the mutation does not noticeably interfere with muscle contraction/function. Aldicarb inhibits acetylcholine (ACh) esterase. Mutations increasing or decreasing the release of ACh into the NMJ are seen as hypersensitivity or resistance, respectively, to aldicarb in the intact worm. gas-1 worms are slightly resistant to aldicarb, suggesting that the mutation decreases the amount of ACh released by the motor-neurons. Thus, the main effect of the gas-1 mutation is presynaptic.

We also examined cellular expression of gas-1 via microinjection of a promoter reporter construct carrying green fluorescent protein. Microinjected genes normally form extrachromosomal tandem arrays in C. elegans and are lost during mitosis at a high frequency (20). Furthermore, due to the repetitive nature they are subject to gene silencing (29). Thus, fluorescence patterns resulting from transgenic constructs commonly differ between individuals. Furthermore, in C. elegans, micro-injected genes are silenced in the germline, even if their chromosomal counterparts are normally active (30). Thus, although the presence of fluorescence indicates transcription from the gas-1 promoter, the absence of fluorescence does not rule out that the chromosomal gas-1 promoter is active in a tissue. The observed pattern of its reporter indicates that gas-1 is expressed in the muscles and neurons of pharynx, body wall, and vulva. Furthermore, the subcellular distribution of the translational fusion construct, which rescues the mutant phenotype, is consistent with mitochondrial localization of the GAS-1 protein.

Our results indicate that the second 49-kDa(IP)-like gene, T26A5.3, also is expressed in N2. Using a GFP promoter reporter (31), we were unable to find a time or tissue in which this gene was expressed even though we were able to obtain an RT-PCR product of its predicted message. Our interpretation is that gas-1 is the predominant gene for expression of the 49-kDa(IP)-like subunit in C. elegans.

The oxidative phosphorylation assay determines the respiration capacity of intact mitochondria by following oxygen uptake under saturating concentrations of substrates for both electron transport and phosphorylation (generation of ATP from ADP and inorganic phosphate). Because both processes are tightly coupled by the proton gradient established by complexes I, III, and IV, oxygen uptake is a measure of ATP generation. In this case also, we see a profound decrease in Complex I activity in the gas-1 mitochondria compared with those of N2. Thus, oxidative phosphorylation assays and ETC assays both indicate that GAS-1 is integral to the function of Complex I. The return toward normal values seen in the rescued line pKEK confirms that the differences seen between gas-1 and N2 are the result of alterations in the GAS-1 protein. Although impairment of Complex I function was expected, two other findings were not: The decrease of NFR activity, and the increase of Complex II activity.

NADH-ferricyanide reductase (NFR) is an activity of the flavoprotein (FP) subcomplex of Complex I and is used as a measure of NADH dehydrogenase activity. NFR is known to function even after perchlorate treatment, which dissociates FP from the other subcomplexes, the iron protein (IP) and the
Mitochondrial Protein in *C. elegans*

hydrophobic protein of bovine Complex I (32). Therefore, wild type GAS-1 protein, which is a subunit of IP, cannot be directly necessary for the flavoprotein (FP)-dependent NFR activity per se. The fact that *gas-1(fc21)* interferes with activity of the FP suggests the mutant subunit prevents proper assembly of the entire Complex I or has an allosteric effect on the FP. The former has been described for a knockout mutation of the 49-kDa(IP) homologue in *Neurospora crassa* (33). Previously, we interpreted a profile of ETC changes similar to those in this study as a deficit in NADH dehydrogenase (34). However, our present results show that a mutation in another functional unit (IP) in Complex I may affect the activity of NADH dehydrogenase. The fact that a subunit of IP affects activity of FP should caution against the interpretation of NFR activity as directly reflecting the status of FP subunits.

The apparent increase of Complex II activity, seen as higher state 3 rates for oxidative phosphorylation, suggests a compensatory up-regulation of Complex II. However, a specific enzymatic change could not be identified in the ETC studies that correlated with the change in Complex II-dependent oxidative phosphorylation. An increase in Complex II activity associated with defects in Complex I has been described in patients with Leber’s Hereditary Optic Neuropathy (35). It would be interesting to know whether the mutant worms actually increase the use of succinate for the regeneration of ATP.

Ishii *et al.* (36) studied *mev-1*, a mutation in a subunit of Complex II, and presented indirect evidence that succinate dehydrogenase activity was decreased in *mev-1* animals. Hartman *et al.* (5) showed that *mev-1* and *gas-1* animals are hypersensitive to hyperoxia and free radical damage, and that life span is shortened in both strains. In contrast, Felkai *et al.* (37) showed that mutations in the *clk-1* gene, which alter a mitochondrial protein and also have decreased Complex II-dependent metabolism, give rise to long-lived animals. Additionally, *mev-1* is not hypersensitive to volatile anesthetics whereas *gas-1* and *clk-1* are hypersensitive to these drugs (4, 5). Therefore, rates of metabolism, aging, and anesthetic sensitivity are not related in a simple manner.

There are a number of plausible hypotheses explaining how mitochondrial function may affect anesthetic sensitivity. It is possible that the anesthetics acutely decrease Complex I-dependent metabolism below a threshold necessary for mobility, which is reached more easily in *gas-1* animals. In support of this possibility, previous reports have indicated that Complex I is the sensitive component of mitochondrial function to the effects of volatile anesthetics (7, 8). We are in the process of testing this possibility by measuring the effects of anesthetics on oxidative phosphorylation in N2 and *gas-1*.

Acknowledgments—We thank Art Zinn, Helen Salz, and Phil Hartman for their helpful discussions. Electron microscopy was done by Medhat Hassan. We also thank Judy Preston, Shawna Boyd, Kalpana Patel, and Hiral Patel for providing invaluable technical assistance. Finally, we thank the members of the Department of Anesthesiology at University Hospitals of Cleveland for their ongoing support in these studies.

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