A cadmium-hypersensitive mutant of the fission yeast *Schizosaccharomyces pombe* was found to accumulate abnormally high levels of sulfide. The gene required for normal regulation of sulfide levels, *hmt2*,” was cloned by complementation of the cadmium-hypersensitive phenotype of the mutant. Cell fractionation and immunocytochemistry indicated that HMT2 protein is localized to mitochondria. Sequence analysis revealed homology between HMT2 and sulfide dehydrogenases from photosynthetic bacteria. HMT2 protein, produced in and purified from *Escherichia coli*, was soluble, bound FAD, and catalyzed the reduction of quinone (coenzyme Q1) by sulfide. HMT2 activity was also detected in isolated fission yeast mitochondria. We propose that HMT2 functions as a sulfide:quinone oxidoreductase. Homologous enzymes may be widespread in higher organisms, as sulfide-oxidizing activities have been described previously in animal mitochondria, and genes of unknown function, but with similarity to *hmt2*,” are present in the genomes of flies, worms, rats, mice, and humans.

The oxidation of sulfide can provide energy for chemolithotrophic or photosynthetic growth of bacteria. This capacity allows some bacteria to thrive in such unlikely environments as hot sulfur springs and deep-sea thermal vents. Sulfide-based anoxygenic photosynthesis appeared quite early in evolution. Today, it is widespread in the green and purple phototrophic anoxygenic photosynthesis appeared quite early in evolution. This led to the cloning and characterization of a new gene encoding a mitochondrial enzyme that can oxidize sulfide. The protein has sequence homology to sulfide-oxidizing enzymes of bacterial photosynthesis, suggesting a common evolutionary origin of sulfide metabolism between prokaryotes and eukaryotes. Interestingly, potential homologues of this enzyme appear in the genomes of nematodes, fruit flies, mice, rats, and humans. This raises the possibility that sulfide oxidation is more widespread among organisms, and might occur in a wider diversity of habitats, than has previously been imagined.

**EXPERIMENTAL PROCEDURES**

**Geneic Materials—***S. pombe* strains Sp223 (h+, ade6.216, ure4.294, leu1.32) and B1048 (h+, ade7.50, ure4.294) have been described (14). JS21 (h+, ure4.294, leu1.32) and JS23 (h+, ure4.294, leu1.32) were derived from the mating of Sp223 with B1048. A cadmium-hypersensitive mutant of JS21 was crossed to JS23 to yield JS563 (h+, ure4.294, leu1.32, hmt2+ and JV7 (h+, ure4.294, leu1.32, hmt2+). JV5 (h+, ure4.294, leu1.32, hmt2+; URA3+) harbors a homologous insertion, whereas JV11 (h+, ure4.294, leu1.32, URA3+) harbors a random insertion of an hmt2+ disruption construct bearing the Saccharomyces cerevisiae *URA3* gene. DS31 (h+, ure4.294, leu1.32, sir1::LEU2) was obtained by disruption of the sulfide reductase gene with a S. cerevisiae LEU2+ fragment (D. Speiser, USDA-ARS, Albany, CA). JV3 (ure4.294, leu1.32, sir1::LEU2, hmt2+) was obtained from the mating of DS31 with JV7.

**Growth Conditions—**Cells were grown at 30 °C on complete medium YG (2% glucose, 0.67% yeast nitrogen base without amino acids; supplemented with 20 μg/ml uracil and 100 μg/ml leucine as needed). JS21 cells were mutagenized by a 45-min exposure to 1.5 μg/ml MNNG. Media for comparison of growth on glucose or glycerol consisted of either 1% yeast extract, 2% glucose or 1% yeast extract, 4% glycerol, 0.1% glucose, 0.67% yeast nitrogen base without amino acids; supplemented with 20 μg/ml uracil and/or 100 μg/ml leucine as needed).
**Sulfate Oxidation in Mitochondria**

**Sulfide Oxidation—** S²⁻ was collected and assayed as described (14). S²⁻ content was first normalized to the dry weight of the cell culture and, to minimize day-to-day assay variability, was subsequently normalized to each day’s value obtained from wild-type cells grown without cadmium. Mitochondria were isolated using published procedures (19, 20). As this locus affects heavy metal tolerance, it was given the genetic designation hmt2. When compared with the wild-type parent strain JS21, which can grow at up to 800 μM cadmium, the hmt²⁻ mutant ceases to grow at 100 μM cadmium and shows reduced yield with as low as 5 μM cadmium. A difference in growth rate was not found in the presence of high NaCl or sucrose concentrations, but a reduction relative to the wild type was observed after heat shock at 47°C or in the presence of hydrogen peroxide, the thiold-oxidizing agent diamide, or the nonfermentable substrate glycerol. Because the catabolism of glycerol requires a functional respiratory pathway, the defect was suspected to be associated with mitochondrial function.

**Sulfide Hyperaccumulation and Hypersensitivity—** A noticeable trait of the mutant is that the colonies turn bright yellow in the presence of cadmium, suggesting the formation of CdS. Over an 18-h period, JS563 accumulated ~6-fold as much acid-labile sulfide as the wild-type JS23 (Table 1). S. pombe is known to produce sulfide production during cadmium stress and a higher level of sulfide production was observed in both strains grown in 200 μM cadmium, but the hmt²⁻ JS563 continued to exceed wild-type levels. Exogenous sulfide is toxic to S. pombe at concentrations in the 100 μM range. Because the

**RESULTS**

**Mutant Phenotype—** S. pombe mutant strain JS563 bears a cadmium-hypersensitivity trait that segregates as a single locus. As this locus affects heavy metal tolerance, it was given the genetic designation hmt2. When compared with the wild-type parent strain JS21, which can grow at up to 800 μM cadmium, the hmt²⁻ mutant ceases to grow at 100 μM cadmium and shows reduced yield with as low as 5 μM cadmium. A difference in growth rate was not found in the presence of high NaCl or sucrose concentrations, but a reduction relative to the wild type was observed after heat shock at 47°C or in the presence of hydrogen peroxide, the thiold-oxidizing agent diamide, or the nonfermentable substrate glycerol. Because the catabolism of glycerol requires a functional respiratory pathway, the defect was suspected to be associated with mitochondrial function.

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**Sulfide Oxidation in Mitochondria**

**TABLE I**

| Strain | Plasmid | Media | 0 μM Cd | 200 μM Cd |
|--------|---------|-------|---------|-----------|
| JS23   | pART1   | SG    | 1       | 3.2 (±0.8) |
| JS563  | pART1   | SG    | 6.8 (±3.1) | 9.8 (±3.8) |
| JS563  | pJV26   | SG    | 1.6 (±0.09) | 3.6 (±1.6) |
| JV5    |         | SG    | 8.4 (±1.3) | 15.2 (±4.0) |
| JS23   | YG      | 1     |         |           |
| JS563  | YG      | 6.4 (±0.5) |         |
| DS31   | YG      | 0.4 (±0.04) |         |
| JV3    | YG      | 1.1 (±0.05) |         |

**hmt2** mutant already exhibits an elevated level of endogenous sulfide, it could be more sensitive to an exogenous supply of this substance. JS563 showed impaired growth relative to the wild-type strain in the presence of Na₂S, with the greatest differential observed at 200 μM Na₂S.

**Defect Not in Sulfate Assimilation**—One described route of sulfide production in *S. pombe* is the sulfur assimilatory pathway, where inorganic sulfur is routed from sulfate to sulfite to sulfide and then to cysteine. A defect in this pathway might increase the sulfide pool through either attenuating sulfide incorporation to cysteine or by overproducing sulfide directly. Both possibilities were examined by the following experiments.

First, when cultured in minimal medium, JS563 grew as well as the wild type, suggesting that cysteine production is sufficient for cell growth. Further supplementation with cysteine (100 μM) did not increase its growth rate. Therefore, a defect in the incorporation of S²⁻ into cysteine seems unlikely.

Second, if sulfide overaccumulation were because of hyperactivity in sulfate assimilation, a genetic block in this pathway should abolish sulfide hyperaccumulation. The double mutant JV3 contains mutations in both hmt2 and sulfite reductase. Like the parent sulfite-reductase mutant strain DS31, JV3 is unable to convert sulfate to cysteine and therefore requires cysteine supplementation. For this reason, sulfide assays were carried out on cells grown in the complete YG medium. DS31 accumulated far less sulfide than either wild-type or JS563 (Table I), as expected from its lack of sulfite reductase activity. However, DS31 also accumulated ~3-fold less sulfide than JV3, suggesting that the hmt2 locus in JV3 enhances sulfide accumulation through a pathway separate from inorganic sulfur assimilation.

**Kinetics of Sulfide Consumption**—To address if the hmt2⁻ mutation affects the consumption of S²⁻, cells were pulse-labeled with ³⁵SO₄²⁻. During the brief 15-min labeling period, both wild-type and mutant cells converted ³⁵SO₄²⁻ into ³⁵S²⁻ (Fig. 1, inset). A shorter transcript in JV3 can be attributed to the truncation of the hmt2 gene on the disruption construct itself because this band is also present in JV11, a strain bearing a random integration of a *S. cerevisiae* URA3" marker (Fig. 2). Polymerase chain reaction and Southern analysis indicated that, in the disruption strain JV5, the coding region of the gene is intact, but two copies of the disruption construct have integrated in tandem upstream of the *hmt2* coding region, disrupting the promoter and possibly part of the untranslated leader sequence. As a result, the 1.9-kb mRNA is deficient in JV5 (Fig. 3B). A shorter transcript in JV5 can be attributed to the truncated hmt2 gene on the disruption construct itself because this band is also present in JV11, a strain bearing a random integration of the disruption construct. An additional transcript in JV5 of ~4 kb hybridizes faintly to both URA3" (not shown) and hmt2" probes. This band might represent transcriptional read-through from the upstream URA3" gene through hmt2". Neither transcript detected in JV5 is expected to yield a functional hmt2" product.

The disruption phenotype in JV5 is very similar, though not identical, to that of the original mutant. As with JS563, JV5 is hypersensitive to cadmium, hyperaccumulates sulfide (Table I), and exhibits poorer growth after heat shock, or in the presence of hydrogen peroxide or Na₂S. Surprisingly, no significant difference is observed in the presence of diamide or with glyceral as the major carbon source. This anomaly could be attributed to a leaky allele. For example, the transcription of a functional mRNA in JV5, though severely reduced, might not...
be entirely abolished. When JV5 was crossed to wild-type, the disruption phenotype segregated as a single locus and was linked with the URA3 marker (53 cadmium-hypersensitive, yellow, URA3 progeny: 46 cadmium-resistant, white, URA3 progeny, \(x^2 = 0.64\)). When crossed to JS563, wild-type recombinants were not recovered (49 cadmium hypersensitive, yellow, URA3 progeny: 51 cadmium hypersensitive, yellow, URA3 progeny, \(x^2 = 0.04\)). This genetic linkage between the original mutant locus and the disruption locus is consistent with the cloned gene corresponding to the genetic lesion in JS563.

**Sequence Change from hmt2\(^+\) to hmt2\(^--\)**—The hmt2\(^+\) genomic and cDNA clones were sequenced across the entire coding region. Comparison with the wild-type sequence revealed a G to A transition that changed amino acid 396 from glutamate to lysine. When the mutant allele, in the vector pART1, is reintroduced into JS563, it is unable to complement (Fig. 2, pJV30). When a 300-base pair region surrounding amino acid 396 is replaced by wild-type sequence, the hybrid allele is fully functional (Fig. 2, pJV34). This result demonstrates that this single point mutation is sufficient to account for the mutant phenotype.

**Similar Genes**—BLAST searches (26) of protein and nucleotide sequence data bases found sequence similarity between the encoded protein, HMT2, and a variety of oxidoreductases, some of which are listed in Table II. The two best matches, *C. vinosum* flavocytochrome c (6, 27) and *R. capsulatus* sulfide quinone reductase (28), are bacterial enzymes that oxidize sulfide. Overall sequence identity between HMT2 and these two enzymes is low (–20%). However, potentially functional features are conserved among these sequences (Fig. 4; and see the Discussion).

The sequences with greater similarity to HMT2 have unknown function. Apparent full-length genes from human, mouse, *Caenorhabditis elegans*, and the cyanobacterium *Synechocystis* sp. PCC6803 all appear to have >30% overall sequence identity with HMT2. A fragmentary expressed sequence tag (EST) from rat appears similarly well conserved. ESTs from *Drosophila, Chloroflexus*, and *Schistosoma* all have >20% identity with HMT2. These proteins are predicted to have unusually high pl: human, pl = 9.5; mouse, pl = 9.1; *C. elegans*, pl = 9.8; *Synechocystis*, pl = 8.9. This feature is shared with HMT2 (pl = 9.9), sulfide quinone reductase (pl = 9.5), and NADH dehydrogenase (pl = 9.4).

**Mitochondrial Localization**—The N-terminal 24 amino acids of HMT2 display features characteristic of mitochondrial targeting sequences (29). The PSORT protein-targeting analysis program predicted with up to a 59% probability that HMT2 might be directed to mitochondria (30). In fission yeast extracts, a single anti-HMT2 immunoreactive band shows an apparent molecular mass of ~48 kDa. Abundance of this protein band correlates to hmt2 mRNA accumulation. It is found in both hmt2\(^+\) and in hmt2\(^--\) (JS563) cells, is more abundant in cells containing hmt2\(^+\) on a multicopy plasmid, and is barely detectable in cells containing a disruption of the hmt2 promoter (JV5). The data are consistent with the 48-kDa band being the translation product of the hmt2\(^+\) gene. The ~3-kDa reduction from the predicted size of HMT2 is consistent with the presumed cleavage of the putative mitochondrial targeting sequence during translocation from cytoplasm to mitochondria.

The bulk of HMT2 immunoreactive protein is pelleted by 100,000 \(\times\) g centrifugation (Fig. 5A), suggesting that HMT2 is membrane-associated or enclosed within an organelle. In purified mitochondrial fractions (6-fold enriched in cytochrome oxidase specific activity), HMT2 is >5-fold enriched (Fig. 5B). A mitochondrial localization for HMT2 was supported by immunofluorescence microscopy experiments (not shown). The mitochondrial fractions were further subfractionated at pH 7.4. The mitochondrial membrane-enriched, fumarase-depleted (0.5–2) pellet showed increased immunoreactivity for HMT2, suggesting that HMT2 may be membrane-associated (Fig. 5C). HMT2 is not predicted to have any hydrophobic regions capable of forming transmembrane helices. However, subfractionation at pH 11 increases the yield of soluble HMT2 (Fig. 5D). This property is characteristic of some peripheral membrane proteins.

**HMT2 Purification**—To facilitate purification of HMT2, a gene fusion construct, pJV40, was made to encode a modified HMT2 protein with additional histidine residues. This construct was able to complement fully the hmt2\(^--\) mutant phenotype, suggesting that the additional amino acid residues do not interfere with the *in vivo* activity of the protein. We therefore assumed that the His\(_6\)-tagged protein is suitable material for

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**Fig. 3. Northern analysis of hmt2\(^+\) expression.** A, wild-type (JS21) and mutant (JS563) strains (harboring pART1) were grown in SG and exposed to 200 \(\mu\)M cadmium for 24 h. 30 \(\mu\)g total RNA from these cultures was hybridized with hmt2\(^+\) cDNA, and rehybridized with a probe specific to the 18S rRNA. B, 30 \(\mu\)g total RNA from a wild-type strain (JS21), a strain bearing a random insertion of the disruption construct (JV11), and the disruption strain (JV5) was hybridized to the hmt2\(^+\) cDNA and to an 18S rRNA probe. Cells were or were not exposed to cadmium (200 \(\mu\)M cadmium for 24 h) as indicated. The *arrow* marks the position of the truncated hmt2 transcript originating from the disruption construct itself.

**Fig. 2. Genetic analysis of hmt2\(^2\).** Deletion derivatives of the complementing genomic clone pJV1 were transformed into JS563 and scored for their ability to complement (+) the mutant phenotype. The alcohol dehydrogenase promoter (Padh) of the pART1 expression vector is indicated, and lies upstream of the hmt2 coding region in pJV26, pJV30, and pJV34. The gene disruption construct shown is the XbaI fragment of pJV17, described under “Experimental Procedures.” Thin line, wild-type genomic DNA; thick line, JS563 genomic DNA; dashed arrow, hmt2\(^+\) cDNA; open box, URA3\(^+\) insert; asterisk, site of mutation in JS563.
Deduced proteins having significant primary sequence similarity to HMT2 (p < 0.05 in BLAST searches of the protein and nucleotide data bases) are listed, together with the degree of pairwise amino acid identity with HMT2.

| Organism       | Function                        | Length (amino acids) | Identity (%) | Accession        |
|----------------|---------------------------------|----------------------|--------------|------------------|
| R. capsulatus  | Sulfide quinone reductase       | Full (425)           | 20.2         | EMB X97478       |
| C. vinsonum    | Flavocytochrome c               | Full (431)           | 20.2         | SP Q06530        |
| E. coli        | NADH dehydrogenase              | Full (434)           | 19.8         | EMV 00306        |
| S. cerevisiae  | Glutathione reductase           | Full (467)           | 15.6         | GB L35942        |
| H. sapiens     | Unknown                         | Full (450)           | 38.4         | Various          |
| M. musculus    | Unknown                         | Full (452)           | 37.2         | Various          |
| Synecocystis sp. | Unknown                    | Full (409)           | 36.2         | DBJ 90907        |
| C. elegans     | Unknown                         | Full (423)           | 32.6         | Various          |
| R. norvegicus  | Unknown                         | Partial (141)        | 37.6         | DBJ C06990       |
| C. aurantius   | Unknown                         | Partial (115)        | 27.0         | EMV Z49000       |
| S. mansoni     | Unknown                         | Partial (61)         | 21.3         | GB T24144        |
| D. melanogaster| Unknown                         | Partial (143)        | 20.9         | GA A435862       |

\* GB T74801, T64084, T85836, T86387, T95375, R50203, R40496, R13065, N50841, T64005, T74688, T95295, T98295, AA280758, AA281439, W96028, AA100517, A080329, AA082940, AA057016, AA139397, W94661, AA253283, W15319, N54245, AA058685, N58703, AA468172, AA468109, AA536991, AA522752, AA756059; GB T73419; DBJ D82703, D82662; EMB Z19305; EMB D20113; GB T36144, AA353040, AA359423, AA358681, AA359712, AA354477, AA359683, AA383720, AA381822, AA382189, and EST A12244, 125978, 126925, 127444. The full sequence has been deposited in GenBank, with accession number AF042284.

\* GB AA229168, AA184667, AA124108, AA266579, AA089176, AA106635, AA146443, AA245215, AA245216, AA208338, AA509391, AA434617, AA544472, AA499804, AA492716, AA473822.

Substrate Specificity—We tested the ability of other electron acceptors to replace coenzyme Q₂ in the HMT2-catalyzed reaction. Sulfide reacts rapidly and spontaneously with cytochrome c, 2,6-dichloroindophenol, and ferricytochrome, and the addition of HMT2 did not increase reaction rates. Sulfide fails to reduce menadione, NAD⁺, or NADP⁺ spontaneously, and HMT2 was unable to stimulate these reactions. Likewise, sulfate, thiosulfate, cysteine, glutathione, β-mercaptoethanol, succinate, and pyridine nucleotides were all unable to replace sulfide in the HMT2-catalyzed reduction of Q₂. Therefore, HMT2 appears to possess a specific sulfide:quinone oxidoreductase activity.

Reaction Stoichiometry—Sulfide and oxidized quinone concentrations were determined independently at the end of a 10-min reaction of 50 nmol each of sulfide and coenzyme Q₂ with 5 μg of purified HMT2. Sulfide and oxidized quinone were consumed in an approximately 1:1 molar ratio (7.85 ± 1.45 nmol S²⁻/7.6 ± 0.99 nmol quinone, n = 2). Because flavoproteins and quinones both commonly carry two electrons, the stoichiometry of the reaction suggests that sulfide is oxidized to elemental sulfur and donates two electrons to coenzyme Q₂.

Kinetic Parameters—The sulfide:quinone reaction catalyzed by HMT2 is saturable with increasing concentrations of substrate; the apparent Kₘ for coenzyme Q₂ is 2 mM. It is important to note that coenzyme Q₂ is a water-soluble analogue of the more likely physiological electron acceptor, ubiquinone, and therefore the Kₘ obtained may not reflect the affinity of HMT2 for its true substrate. Additionally, because of the insolubility of high quinone concentrations in the reaction buffer, it was not possible to assay Kₘ for sulfide under saturating concentrations of quinone. At lower quinone concentrations (40 μM), Kₘ for sulfide was calculated to be 2 mM. Using saturating S²⁻ (3 mM) and the highest concentration of quinone (1.5 mM) that can be assayed, we obtained an apparent Vₘₐₓ of 1.36 μmol coenzyme Q₂ reduced per μg of enzyme, and Kₘ was 52/sec (assuming an active enzyme concentration of 22.3 nM, based on incomplete FAD binding).

Sulfide:Quinone Oxidoreductase Activity in Mitochondria
We tested the native protein in its physiological context for the ability to carry out the same reaction. Mitochondria were isolated from wild-type (JS23) and mutant (JS563) cells. The specific activity of fumarase, an enzyme of the mitochondrial matrix, was equivalent in the two preparations. Mitochondria from both strains were able to reduce exogenous coenzyme Q2 at low rates in the absence of added electron donor (JS23: 75 ± 51 nmol/min·mg; JS563: 68 ± 23 nmol/min·mg). The addition of sulfide significantly increased the rate of quinone reduction in JS23 mitochondria, and the spectrum of the reaction mixture showed a rapid decrease in the 285-nm absorption peak (Fig. 7C, b). Sulfide:quinone reductase activity, corrected for the background rate of quinone reduction, is significantly higher in JS23 (144 ± 45 nmol/min·mg) than in JS563 mitochondria (21 ± 12 nmol/min·mg, p < 0.05). Therefore, fission yeast mitochondria possess a sulfide:quinone oxidoreductase activity that correlates to the presence of functional HMT2 protein.

DISCUSSION

Purified recombinant HMT2 protein is ~25-fold enriched over isolated mitochondria in both protein abundance and in sulfide:quinone oxidoreductase-specific activity. This suggests that the activity of HMT2 itself could account for all of the sulfide:quinone oxidoreductase activity measured in fission yeast mitochondria. The apparent \( K_m \) for sulfide is relatively high (2 mM), when determined with a water-soluble analogue of the more likely electron acceptor, ubiquinone. Assuming this value is physiologically meaningful, HMT2 could nonetheless be adequately supplied with substrate under such conditions as heavy metal exposure, when whole-cell sulfide levels can exceed 1 mM. Quinone should also be available, as ubiquinone is abundant in the mitochondrial inner membrane.

Key features are shared among HMT2 and two sulfide dehydrogenases, flavocytochrome c from \( C. \) \textit{vinosum} and sulfide quinone reductase from \( R. \) \textit{capsulatus}. Flavocytochrome c binds...
a FAD cofactor via a two-part sequence motif (amino acids 34–64 and 314–324; Fig. 4, black bar) (6, 27) that is also present in sulfide quinone reductase and in HMT2. A pair of cysteines (amino acids 191 and 367; Fig. 4, *) forming a disulfide bridge adjacent to the flavin in flavocytochrome c has been implicated to be catalytically involved in the redox reaction (6, 32). Aligning cysteines are also present in sulfide quinone reductase and in HMT2. Finally, the predicted secondary structure of HMT2 bears striking similarities with the known structure of flavocytochrome c. The similarity is greater than to any other protein in the Protein Data Bank as judged by the program PHDthreader (33). Previously, it has been suggested that all flavoenzymes known to be involved in sulfur chemistry may be related (27). HMT2 extends this family of related proteins to the eukaryotes.

It is possible that HMT2 functions in the detoxification of endogenous sulfide. Not all sulfide produced during the assimilation of inorganic sulfur is immediately incorporated into amino acids, and we can detect a low level of acid-labile sulfide even in wild-type cells. Sulfide is a potent inhibitor of cytochrome c oxidase (34), and accumulation of sulfide in mitochondria would be expected to poison respiration. Therefore, a mitochondrial sulfide dehydrogenase might play a role in ensuring that local sulfide concentration near cytochrome c oxidase is kept low. Consistent with this hypothesis, hmt2− cells grow poorly on a nonfermentable carbon source, suggesting that unchecked accumulation of sulfide may interfere with respiration. The exact role of HMT2 in cadmium tolerance is not yet clear, but a likely possibility is to detoxify excess sulfide generated during cadmium stress.

Mitochondrial sulfide oxidation is not unprecedented. Mitochondria of several species of marine animals have been shown to couple sulfide oxidation to the production of ATP (7, 9–12), but the proteins or genes responsible for the initial oxidation step have not been purified or cloned. In the lugworm Arenicola marina, electrons from sulfide appear to enter the electron transport chain at the level of ubiquinone (12), and the authors postulated that the enzyme involved might be similar to the sulfide quinone reductase of R. capsulatus. HMT2, a mitochondrial protein with homology to the R. capsulatus sulfide quinone reductase, strengthens this hypothesis by providing a genetic link between mitochondrial and bacterial sulfide oxidation. Intriguingly, genes similar to hmt2− appear in worms.
flies, mice, rats, and humans. A heat-labile sulfide oxidizing activity has been reported in rat liver mitochondria (35, 36). It is possible that the machinery for capturing electrons from sulfide has been conserved in evolution, although it has been adapted to new physiological roles.

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