Hydrogen sulfide increases thermotolerance and lifespan in *Caenorhabditis elegans*

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Hydrogen sulfide (H_2S) is naturally produced in animal cells. Exogenous H_2S has been shown to effect physiological changes that improve the capacity of mammals to survive in otherwise lethal conditions. However, the mechanisms required for such alterations are unknown. We investigated the physiological response of *Caenorhabditis elegans* to H_2S to elucidate the molecular mechanisms of H_2S action. Here we show that nematodes exposed to H_2S are apparently healthy and do not exhibit phenotypes consistent with metabolic inhibition. Instead, animals exposed to H_2S are thermotolerant and long-lived. These phenotypes require SIR-2.1 activity but are genetically independent of the insulin signaling pathway, mitochondrial dysfunction, and caloric restriction. These studies suggest that SIR-2.1 activity may translate environmental change into physiological alterations that improve survival. It is interesting to consider the possibility that the mechanisms by which H_2S increases thermotolerance and lifespan in nematodes are conserved and that studies using *C. elegans* may help explain the beneficial effects observed in mammals exposed to H_2S.

Early life arose in highly reducing conditions where hydrogen sulfide (H_2S) was abundant and molecular oxygen (O_2) was scarce (1, 2). Early organisms were likely to have extracted energy from the environment initially by anoxygenic photosynthesis using chemicals, including sulfide, as electron donors, followed by anaerobic respiration using sulfate as an electron acceptor (resulting in the production of sulfide), and, finally, by aerobic respiration (1). The success of primordial eukaryotes may have resulted from their ability to take advantage of chemically unstable mixtures of H_2S and O_2. In modern marine sediments, eukaryotic microbes accumulate at locations where O_2 and H_2S coexist, allowing for maximal energy production by redox chemistry (1, 3).

The importance of O_2 in biology is widely recognized; however, the ancient nature of H_2S suggests that it also might impact fundamental aspects of biological processes. H_2S is naturally produced by animal cells, and endogenous H_2S affects various aspects of physiology (4, 5). In addition, recent evidence suggests that H_2S can have dramatic effects on mammalian physiology (6–8). Mice exposed to H_2S enter into a physiological state that allows them to endure periods of low metabolic rate and decreased core body temperature without apparent ill effects (6). The H_2S-induced state also allows for mice to survive exposure to otherwise lethal hypoxic conditions (7). In rats, H_2S improves outcome after severe hemorrhage (50). These studies raise the possibility that exogenous H_2S could be of clinical benefit, especially for pathologies resulting from decreased oxygen perfusion. It is unlikely that there is a single cellular target of H_2S that mediates all observed physiological alterations. H_2S is a strong reducing agent that could reduce disulfide bonds of proteins (or maintain thiol groups in the reduced state), interact with active-site cysteine residues, affect cellular redox balance, or bind to metal atoms in enzymes (4, 9). In addition, it was recently demonstrated that H_2S can act as an electron donor and increase production of ATP in metazoans (10, 11), as was shown previously for the gutless clam *Solemya reidi* (12, 13). These examples illustrate that H_2S will probably have many cellular targets, just as O_2 interacts with myriad cellular factors to influence diverse cellular processes.

To investigate the nature of H_2S-induced physiological alterations in animals, we exposed the genetically tractable nematode *Caenorhabditis elegans* to atmospheres containing H_2S. As expected, we found that high concentrations of H_2S are toxic. However, exposure to low concentrations of H_2S results in physiological alterations that allow for increased thermotolerance and lifespan. We show that these phenotypes require the NAD^+-dependent deacetylase *sir-2.1*, suggesting that one cellular activity of H_2S is to increase the activity of SIR-2.1.

Results and Discussion

C. elegans is not adversely affected when grown in atmospheres containing 50-ppm H_2S (0.005%) in room air (hereafter referred to simply as H_2S) [supporting information (SI) Fig. 5]. We chose 50-ppm H_2S because this concentration has been shown to affect mammalian physiology (6) but is not apparently toxic to the worms. Animals raised in H_2S are visually indistinguishable from untreated controls and produce statistically identical numbers of progeny (221 ± 35 in H_2S, compared with 234 ± 15 in control conditions; $P > 0.05$; $n = 5$–10 in each group). Neither embryonic nor postembryonic development is delayed by H_2S (Table 1). In addition, the rate of egg-laying is not significantly different in H_2S (Table 2). The rate of egg-laying is tightly correlated with oocyte production (14), an energetically expensive activity that is a sensitive readout of metabolic capacity. Consistent with this, we observe a 2-fold decrease in the rate of egg-laying when ambient O_2 tension is reduced to 2% (from 21% O_2 in room air), a perturbation that was previously shown to decrease the metabolic rate of worms by ≈30% (15). H_2S does not further alter the rate of egg-laying in environments with reduced ambient O_2 (Table 2). These data contrast with hypometabolic phenotypes commonly observed in nematodes with defective, including *clk-1*(*qm30*) mitochondrial function, including *clk-1*(*qm30*) animals (16–19). Our experiments demonstrate that apparent metabolic output is not appreciably changed when animals are raised in H_2S. However, we cannot definitively conclude that mitochondrial energy production has not been affected in these conditions. In addition, we observe that H_2S exposure does not induce expression of several transgenes driven by heat-shock promoters, including *hsp-16.2*::*GFP* and *hsp-4*::*GFP* (SI Fig. 6) (20–23). Together these data indicate that animals grown in H_2S are as healthy as untreated controls, and that in our conditions this concentration of H_2S does not affect apparent metabolic rate.

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Conflict of interest statement: The authors acknowledge a potential conflict of interest in that both authors are named as inventors on at least one patent that was licensed to a private company, founded by Mark Roth, to commercialize this technology.

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Table 1. Developmental rate in H_2S

	Developmental time, h ± SEM (*n*)	
	Embryogenesis	Postembryonic
Untreated	12.6 ± 0.4 (43)	49.6 ± 0.2 (15)
H_2S	13.3 ± 0.4 (34)	49.3 ± 0.2 (15)
clk-1(*qm30*)	16.9 ± 2.0 (17)*	69.0 ± 0.3 (15)*

*Developmental time is significantly different ($P < 0.05$) from untreated controls by log-rank analysis.

We observed that animals grown in H_2S are more thermotolerant than untreated controls (Fig. 1). At high temperature, animals grown in H_2S have a mean survival time up to 8-fold longer than untreated controls. Although the maximum extension of survival time observed varied between experiments, the effect was quite robust, with an average of 77% of H_2S-treated animals alive when all untreated animals had died (15 independent experiments) (SI Fig. 7). In this experiment, animals were raised in H_2S and challenged with high temperature in the presence of H_2S. In fact, we observe that animals grown in room air were more sensitive to thermal stress in H_2S (Fig. 1*B*). Thus, H_2S does not act directly to prevent damage associated with thermal stress. Moreover, unlike thermotolerance induced by prior stress such as heat shock (24) or azide (25), continuous exposure to H_2S is required for increased thermotolerance (Fig. 1*C*). These data suggest that H_2S exposure initiates physiological alterations, one manifestation of which is increased survival at high temperature.

In *C. elegans*, resistance to high temperature is often correlated with increased lifespan (24). Indeed, we observe that animals grown in H_2S are long-lived compared with controls (Fig. 2). The mean lifespan of animals grown in H_2S is 9.6 days greater than the untreated population, an increase of 70%. Maximum lifespan was similarly increased as H_2S-treated animals reached 75% mortality 10 days after the control population. Increased lifespan is not observed when animals are moved into H_2S at the beginning of the lifespan experiment as L4 larvae. In fact, the lifespan of these animals is slightly shorter than untreated controls (Fig. 2*B*). These data suggest that H_2S cannot act postdevelopmentally to slow the rate of aging (see also SI Fig. 7). However, these animals produce normal numbers of progeny, suggesting that overall physiological function is not impaired (227 ± 18 progeny when moved to H_2S, compared with 208 ± 16 in room air; $P > 0.05, n = 5$ in each group). Lifespan extension requires the continuous presence of H_2S in the atmosphere insofar as animals grown in H_2S but moved to room air have a normal lifespan (Fig. 2*C*). This finding indicates that H_2S exposure solely during development is insufficient for increased lifespan. We conclude that the increase in lifespan is another manifestation of the physiological alterations resulting from the exposure to H_2S.

Table 2. Rate of egg-laying in H_2S

	Rate of egg-laying, no. of embryos per h ± SD			
	Room air	H_2S	2% O_2	2% O_2 plus H_2S
Untreated	7.8 ± 1.5	8.3 ± 3.0	4.2 ± 1.3	4.5 ± 1.6
H_2S	nd	7.8 ± 2.0	4.9 ± 1.4	4.7 ± 1.2
clk-1(*qm30*)	4.2 ± 0.6*	3.2 ± 1.1*	2.4 ± 0.8*	2.5 ± 1.0*

nd, not done.

*Rate of egg-laying is significantly different ($P < 0.05$) than untreated controls in same conditions by Student's *t* test.

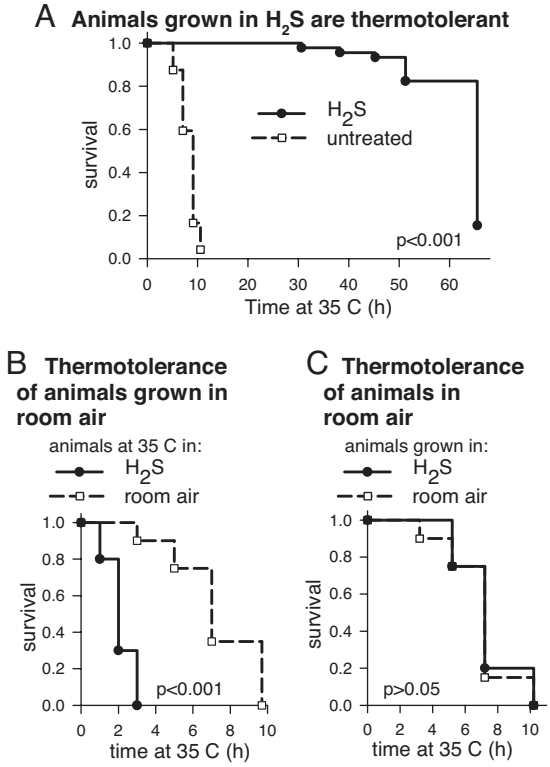

Fig. 1. H_2S increases thermotolerance in wild-type *C. elegans*. (*A*) Animals exposed to H_2S survive longer than untreated controls at high temperature. Nematodes were moved to 35°C in the same gaseous atmosphere in which they had been cultured (SI Fig. 5). The mean survival time of animals grown in H_2S was 65.5 h (solid line; $n = 136$), compared with 9.1 h ($n = 96$) for untreated controls (dashed line). (*B*) Prior exposure to H_2S is required to survive high temperature in H_2S. All animals were grown in room air without H_2S and then moved to 35°C in the presence or absence of 50-ppm H_2S. Animals first exposed to H_2S at high temperature had a mean survival time of 2.1 h (solid line; $n = 20$), whereas the control group exposed in room air survived for 7.3 h (dashed line; $n = 20$). (*C*) The continuous presence of H_2S in the atmosphere is required for increased survival at high temperature. Animals were exposed to 35°C in room air. Animals grown in H_2S before heat shock survived 7.3 h (solid line; $n = 20$), which is not significantly longer than untreated controls (dashed line; 7.0 h; $n = 20$). Indicated *P* values were determined by log-rank analysis.

Most genes that influence lifespan can be categorized into one of three genetically independent pathways in *C. elegans* (26–28). We considered the possibility that exposure to H_2S and associated physiological alterations may modulate one or more of these pathways. To evaluate this possibility, we tested whether exposure to H_2S caused increased thermotolerance in mutant animals with defects in these pathways.

In *C. elegans*, the insulin/IGF signaling (IIS) pathway regulates the decision to enter into an alternative third larval stage, the dauer, upon exposure to unfavorable conditions, such as high population density, low food, or high temperature (29). Mutations in the insulin-like receptor DAF-2 that reduce IIS increase the probability of entry into the dauer state and, in adults, increase thermotolerance and lengthen lifespan even without entry into dauer (30–32). All known phenotypes of *daf-2* mutants can be suppressed by mutations in the DAF-16 FOXO transcription factor (26, 30, 31). Our data suggest that exposure to H_2S does not result in decreased IIS. First, H_2S exposure starting in adulthood does not increase lifespan (Fig. 2*B*), whereas knockdown of *daf-2* by RNAi starting in adulthood is sufficient to increase lifespan (32). Second, *daf-2*-mutant animals are more resistant to high temperature when grown in H_2S (Fig. 3*A*). Third, H_2S does not induce entry into the dauer state in wild-type nematodes because postembryonic development time is not extended (Table 1), nor does it affect entry into or exit

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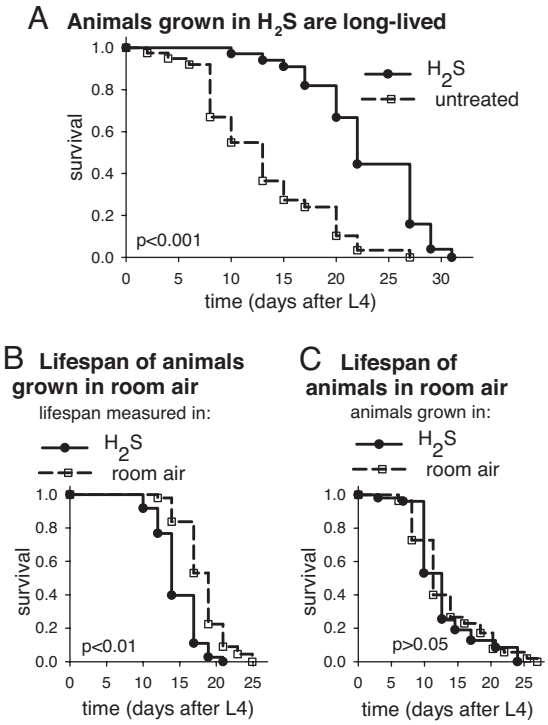

Fig. 2. H_2S increases lifespan in *C. elegans.* (*A*) Animals grown in H_2S live longer than untreated controls. The lifespan of animals was monitored in the same conditions in which they had developed. The mean lifespan of animals in H_2S was 22.6 ± 1.0 days (solid line; $n = 80$), compared with 13.0 ± 1.0 days for untreated controls in room air (dashed line; $n = 40$). Maximum lifespan also was increased. (*B*) Exposure to H_2S beginning as L4 does not increase lifespan. All animals were from populations grown in room air. The lifespan of animals moved into H_2S-containing environments at the beginning of the lifespan experiment (solid line) is 14.8 ± 0.3 days ($n = 73$), which is slightly shorter than controls that remained in house air (dashed line; mean lifespan 18.2 ± 0.4 days; $n = 48$). (*C*) Increased lifespan requires continuous exposure to H_2S. The lifespan of all animals was monitored in room air. The lifespan of animals raised in H_2S until L4 (solid line; 12.8 ± 0.7 days; $n = 52$) was indistinguishable from untreated controls (dashed line; 13.2 ± 0.7 days; $n = 59$). All lifespan experiments were performed at room temperature.

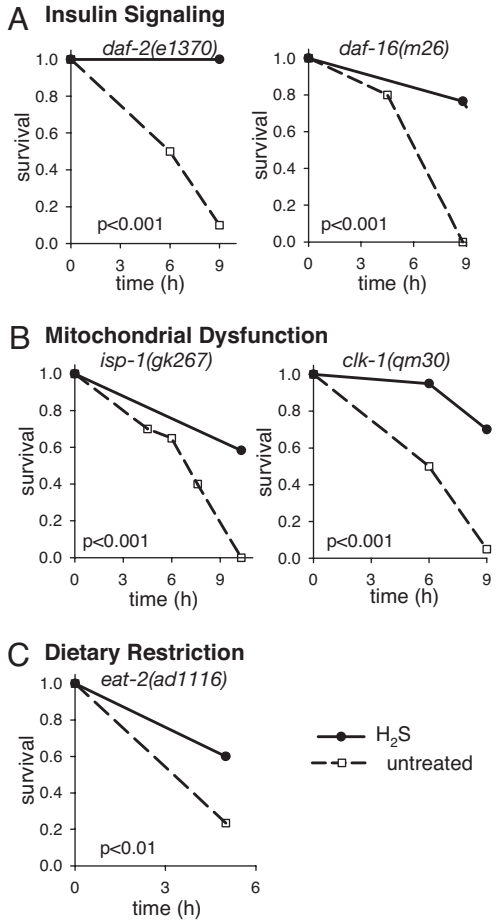

Fig. 3. Thermotolerance of canonical long-lived mutants is increased by H_2S. Just as H_2S increases thermotolerance of wild-type worms (Fig. 1 and SI Fig. 6), long-lived mutants in canonical pathways that influence lifespan (20) also are more thermotolerant when grown in H_2S. All animals grown in H_2S were challenged with high temperature in H_2S (solid line), whereas the thermotolerance of untreated controls was assayed in room air (dashed line). (*A*) H_2S effects are genetically independent of IIS. The thermotolerance of *daf-2*(*e1370*) animals can be enhanced by exposure to H_2S, and *daf-16*(*m26*) mutants, which are defective in IIS, become thermotolerant when grown in H_2S. To facilitate the experiments shown in this figure, strains that show intrinsic thermotolerance, such as *daf-2*(*e1370*) (30), were tested at a slightly higher temperature both in room air and H_2S. However, the thermotolerance at 35°C also is increased when the animals are grown in H_2S (data not shown). (*B*) H_2S-induced thermotolerance is observed in *isp-1*(*gk267*) and *clk-1*(*qm30*) animals that are long-lived as a result of mitochondrial dysfunction. (*C*) H_2S-induced thermotolerance is observed in *eat-2*(*ad1116*) mutant animals, which have defects in pharyngeal pumping that result in dietary restriction.

from dauer in *daf-2*(*e1370*) mutants (data not shown). Finally, *daf-16*-mutant animals become thermotolerant upon exposure to H_2S (Fig. 3*A*). These data suggest that the mechanism by which H_2S increases lifespan and thermotolerance is independent of the IIS pathway.

Reduction of mitochondrial function is a well established mechanism for increasing the lifespan of *C. elegans* (27). *In vitro*, H_2S is an inhibitor of cytochrome *c* oxidase, the terminal enzyme in the electron transport chain (9). However, we do not observe hypometabolic phenotypes in animals grown in H_2S (Tables 1 and 2), suggesting that mitochondrial function is not grossly affected in these conditions. In addition, depletion of mitochondrial components by RNAi only during development increases lifespan (16), whereas animals grown in H_2S, but moved to room air as adults, are not long-lived (Fig. 2*C*). These data suggest that H_2S exposure has characteristics distinct from mitochondrial dysfunction. In support of this premise, we observe that *isp-1*- and *clk-1*-mutant animals, which have defects in mitochondrial function and are long-lived (17, 18), become more resistant to high temperature when grown in H_2S (Fig. 3*B*). We conclude from these data that the effect of H_2S on lifespan is mediated by a genetic mechanism distinct from mitochondrial dysfunction.

Reduced caloric intake or dietary restriction (DR) extends lifespan in a wide range of organisms (33). *C. elegans* subjected to DR appear thin and pale, develop slowly, and have reduced fecundity, which are phenotypes not observed in animals grown in H_2S (Tables 1 and 2) (19, 34). Furthermore, DR can increase lifespan when initiated in adults (35–37), whereas H_2S exposure cannot (Fig. 2*B*). Therefore, we consider it unlikely that H_2S acts through the DR pathway. Consistent with this interpretation, *eat-2*-mutant animals, which are long-lived because of DR (38), become thermotolerant upon exposure to H_2S (Fig. 3*C*). We conclude that H_2S alters the physiology of worms in a manner distinct from DR, suggesting that it acts through a separate mechanism.

In addition to, but perhaps overlapping with, these genetically defined pathways, Sir2 homologues influence lifespan in many organisms, including *C. elegans* (39–42). Overexpression of the *C. elegans* Sir2 homologue, *sir-2.1*, increases lifespan by 18–50%

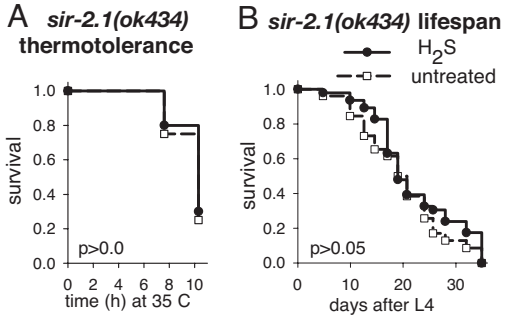

Fig. 4. *sir-2.1* is required for increased thermotolerance and lifespan in H_2S. (*A*) H_2S does not increase thermotolerance of animals that have a deletion in *sir-2.1*. The mean survival time of *sir-2.1*(*ok434*) animals grown in H_2S and exposed to high temperature in H_2S (solid line) is 9.8 ± 0.3 h (n = 20), which is not significantly longer than untreated controls in room air (dashed line; mean survival 9.6 ± 0.3 h; n = 20). (*B*) H_2S does not increase the lifespan of *sir-2.1*(*ok434*) animals. The lifespan of *sir-2.1*(*ok434*) animals raised in H_2S is 20.0 ± 1.6 days (solid line; n = 47), which is statistically indistinguishable from control animals in room air (dashed line; 22.2 ± 1.2 days; n = 26). Indicated P values were determined by log-rank analysis.

(43). Our data indicate that *sir-2.1* is required for increased thermotolerance and lifespan upon exposure to H_2S. In contrast to wild type (Figs. 1*A* and 2*A*), the thermotolerance and lifespan of nematodes harboring a deletion in the *sir-2.1* gene are unchanged when the animals are grown in H_2S (Fig. 4). However, we consider it unlikely that H_2S results in increased lifespan as a result of increased SIR-2.1 expression. H_2S effects on lifespan are independent of *daf-16* (Fig. 3*A*), whereas lifespan extension by overexpression of *sir-2.1* requires DAF-16 activity (43). Indeed, *sir-2.1* transcript levels in animals grown in H_2S are indistinguishable from untreated controls as measured by quantitative RT-PCR, and animals overexpressing *sir-2.1* become more thermotolerant when grown in H_2S (SI Fig. 8). We conclude that H_2S modulates SIR-2.1 activity to impart increased thermotolerance and lifespan in a manner distinct from *sir-2.1* overexpression. The fact that these phenotypes require *sir-2.1* supports the interpretation that the effects of H_2S are distinct from DR because increased lifespan resulting from DR does not require *sir-2.1* (44). Moreover, the finding that SIR-2.1 activity is required for increased thermotolerance and lifespan in H_2S further suggests that these phenotypes do not result from nonspecific metabolic suppression.

Sir2 homologues are NAD^+-dependent deacetylase enzymes that may have a variety of substrates (45). This finding raises the possibility that H_2S shifts redox homeostasis, thereby increasing the available NAD^+ (or the NAD^+/NADH ratio) and resulting in increased SIR-2.1 activity (40, 46). Alternatively, H_2S may directly modify SIR-2.1 to alter its activity (47). It also is possible that SIR-2.1 is indirectly activated by some other aspect of H_2S-induced physiological alterations. Whatever the mechanism by which H_2S-induced physiological alterations are translated into the phenotype of increased lifespan, our studies raise the possibility that endogenous H_2S naturally regulates SIR-2.1 activity. It may be that Sir2 homologues are involved in mediating the physiological alterations observed in mammals exposed to exogenous H_2S. Further investigations of genetic mechanisms that mediate H_2S-induced phenotypes in nematodes may yield insights into similar mechanisms in higher organisms, including humans, with potentially wide-ranging implications in both basic research and clinical practice.

Materials and Methods

Growing Nematodes in H_2S-Containing Atmospheres. Bristol strain N2 (wild-type) and mutant nematode strains were grown at room temperature on nematode growth medium (NGM) plates seeded with live *Escherichia coli* OP50 food (48). Mutant strains obtained from the *C. elegans* genetic stock center were as follows: CB130, *daf-2*(*e1370*); DR26, *daf-16*(*m26*); VC520, *isp-1*(*gk267*); MQ130, *clk-1*(*qm30*); DA1116, *eat-2*(*ad1116*); and VC199, *sir-2.1*(*ok434*).

Plates were maintained in atmospheric chambers sealed with Dow Corning Vacuum Grease (Sigma–Aldrich). Care was taken to ensure that cultures did not starve. Chambers were continuously perfused with room air or 50-ppm H_2S that was freshly mixed into room air (SI Fig. 5). Gasses were hydrated by using gas wash bottles (Fisher) and moved through one-eighth-inch outer diameter FEP tubing (Cole Parmer) with connections by snap connectors (Cole Parmer), stainless-steel quick-connect fittings, or compression fittings (Seattle Fluid Systems). The H_2S-containing atmospheres were constructed by mixing 5,000-ppm H_2S (balanced with N_2) with room air by using mass flow controllers (model no. 810 and Smart-Trak Series 100; Sierra Instruments). All compressed gas mixtures used in this study were obtained from Byrne Gas and were certified standard to within 2% of indicated concentration. Flow tubes (Aalborg) were used to distribute the gas mixture to different chambers. Gas flow rate was 100 cm^3/m to small boxes (100–300 ml) and 800 cm^3/m to the large boxes (1–3 liters) used to culture nematode strains at room temperature. At these flow rates, the gaseous environment of the atmospheric chambers is exchanged every 20–30 min. The concentration of H_2S was monitored with a custom-built H_2S detector (J. Rivera, Fred Hutchinson Cancer Research Center) containing a three-electrode electrochemical Surecell H_2S detector (Sixth Sense). The detector was zeroed with room air and spanned with 100-ppm H_2S before each use. Data were collected by using Chart software with a Powerlab data acquisition unit (ADInstruments) and analyzed with EXCEL. The concentration of H_2S measured was consistently within 10 ppm of the reported value and was stable from day to day. The H_2S-containing atmospheres did not alter the pH of the NGM plates.

Brood Size Measurement. To determine the number of viable progeny produced by nematodes, individual fourth-stage larvae (L4) were transferred to NGM plates with OP50 food at room temperature. Animals were moved daily until they quit laying fertilized eggs. Progeny were counted as L4/young adult.

Measuring Developmental Rates. The time required for embryonic development was determined by measuring the time required for two-cell embryos to hatch. Two-cell embryos were isolated from log-phase adults as previously described (49). Briefly, adults were chopped with a razor blade and ≈20 two-cell embryos were moved to NGM plates without food by mouth pipette. The number of embryos that hatched was monitored every 45–60 min beginning 6–8 h after embryos were picked. Embryos that did not hatch after 36 h were considered dead and were not included in the analysis. Median time of development was determined by log-rank analysis in SigmaStat (Systat). Data from one representative experiment for both embryonic and postembryonic development are shown in Table 1, although each experiment was repeated several times with similar results.

Postembryonic development was measured as the time required for starved first-stage larvae (L1) to become gravid, egg-laying adults. Starved L1 were isolated by picking 30–50 adults from each population into 10 μl of hypochlorite solution (2.5 N KOH, 5% NaOCl) on a small (unseeded) NGM plate. After 5 min, 1 ml of M9 buffer (48) was added to the plate, and the embryos were returned to the atmospheric chambers. After 24–36 h, starved L1 were moved onto NGM plates with OP50, returned to the chamber, and allowed to develop at room temperature. After 30–48 h, individual larvae were moved to NGM plates with a 10-μl spot of OP50. Each worm was monitored every 6–12 h until it began laying eggs (intervals became closer as time progressed and other animals became gravid). If more than one embryo had been laid, the time that the first egg was laid was determined assuming that one egg was laid every 15 min for wild type and every 30 min for the *clk-1*(*qm30*) mutants. This value was determined empirically by counting the number of embryos laid by each worm for the 6-h period after it began egg-laying. Data were analyzed by using log-rank analysis in SigmaStat (Systat).

The rate of egg-laying was determined for first-day gravid adults from populations cultured in each condition (room air with or without 50-ppm H_2S). Animals were picked as L4 from mixed-stage populations and allowed to develop for 20–30 h in the same conditions at room temperature. Individual worms were then placed onto NGM plates with a 10-μl spot of OP50 food. The number of embryos laid in 3–5 h was counted to determine the rate of egg-laying. To create an atmosphere with 2% O_2, N_2 was mixed with 5% O_2 balanced with N_2. Smart-Trak series 100 mass flow controllers (Sierra Instruments) were used to mix the gas and split it into two atmospheric chambers. Using a model 810 mass flow controller (Sierra Instruments), we then added H_2S to the 2% O_2 that flowed into one of the chambers. Student's t test was

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used to determine whether the rate of egg-laying varied significantly between conditions, assuming two-tailed distributions with unequal variance (EXCEL). In each experiment, 10–15 individuals were included in each group. The data shown in Table 2 are from one experiment that is representative of at least three independent assays.

Thermotolerance Assay. Cultures of nematodes were established in 50-ppm H_2S or room air control conditions and maintained for at least 1 week before thermotolerance measurement. Care was taken to prevent the population from starving. Nematodes were picked from these mixed-stage populations as L4 larvae and allowed to develop for 24–48 h at room temperature. However, treated and control animals were always the same age. For temperature-sensitive *daf-2*(*e1370*) mutants (30), cultures were maintained in H_2S-containing environments at 17°C and moved to room temperature as L4. Groups of 20–30 animals were transferred to NGM plates without food and then placed into an atmospheric chamber perfused with the indicated gas at high temperature in an Echotherm IN35 incubator [National Institute of Standards and Technology (NIST)-traceable; Torrey Pines Scientific] or a VWR incubator model 2005 (VWR International). A ring of palmitic acid around the edge of the plate helped prevent the worms escaping the surface of the agar. Temperature was maintained at 34.5 $\pm$ 1°C, although the temperature was raised slightly to facilitate experiments with thermotolerant strains. In these experiments, the high temperature was chosen so that controls died in $<$10 h [in Fig. 3*A*, *daf-2*(*e1370*) animals were tested at 36.5°C]. HOBO U10 data loggers that were calibrated to a NIST-traceable thermometer (Onset Corporation) were used to monitor the temperature in each chamber. In every experiment, the temperature of the room air- and H_2S-containing chambers was the same. Plates were removed to count the number of animals that had died every few hours. Nematodes were considered dead and removed from the plate when they no longer responded to prodding with a platinum wire. Kaplan–Meier log-rank tests with the program SigmaStat (Systat) were used to evaluate statistical significance. Animals that could not be accounted for were censored from the analysis. Each assay was repeated at least twice with similar results.

Lifespan Measurements. On day 0, groups of 20–30 L4 animals from populations in each condition were picked from mixed-stage cultures onto NGM plates seeded with live OP50 and spotted with 25 μl of 50 μg/ml 5-fluoro-2′-deoxyuridine (Sigma–Aldrich) to prevent growth of progeny. Control experiments indicated that the lifespan of nematodes in H_2S was not affected by the 5-fluoro-2′-deoxyuridine. Plates were placed into atmospheric chambers at room temperature. Every few days, the plates were removed from the chambers to monitor the number of animals remaining alive. Nematodes were considered dead when they no longer responded to repeated prodding with a platinum wire. Data were analyzed with SigmaStat (Systat) by using Kaplan–Meier survival analysis. Each assay was repeated at least twice with similar results.

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