Serotonin Mediates Behavioral Gregarization Underlying Swarm Formation in Desert Locusts

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Serotonin Mediates Behavioral Gregarization Underlying Swarm Formation in Desert Locusts

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Desert locusts, *Schistocerca gregaria*, show extreme phenotypic plasticity, transforming between a little-seen solitarious phase and the notorious swarming gregarious phase depending on population density. An essential tipping point in the process of swarm formation is the initial switch from strong mutual aversion in solitarious locusts to coherent group formation and greater activity in gregarious locusts. We show here that serotonin, an evolutionarily conserved mediator of neuronal plasticity, is responsible for this behavioral transformation, being both necessary if behavioral gregarization is to occur and sufficient to induce it. Our data demonstrate a neurochemical mechanism linking interactions between individuals to large-scale changes in population structure and the onset of mass migration.

Phenotypic plasticity, the differential expression of alternative phenotypes from a single genotype depending upon environmental conditions, is of considerable evolutionary importance, but controlling mechanisms remain elusive (*1*). Changes in population density can be a substantial source of environmental variability and can trigger phenotypic changes that equip animals for increased competition for resources as well as for dispersal or migration (*1*–*3*). Desert locusts undergo an extreme and economically devastating form of this kind of phenotypic plasticity, changing reversibly between two extreme forms or phases (*4*–*6*). These differ extensively in morphology and physiology, but behavior is the key to both establishing and maintaining each phase (*7*). Swarming begins with a rapidly induced switch from mutual repulsion in solitarious locusts to attraction and aggregation after just a few hours of forced crowding (*7*, *8*). Although the sensory stimuli triggering behavioral gregarization have recently been identified (*9*–*11*), it was unknown how they mediated their effect.

Solitarious locusts acquire full gregarious behavioral characteristics within the first 2 hours of forced crowding [Fig. 1, A to C, fig. S1, and supporting online material (SOM) text]. This period coincides with a substantial but transient (<24 hours) increase in the amount of serotonin [5-hydroxytryptamine (5-HT)] specifically in one region of the central nervous system (CNS), the thoracic ganglia, but not the brain (*12*) (Fig. 1D). To determine whether this increase caused gregarization, we first analyzed the relationship between the degree of behavioral gregarization and the amount of serotonin in individual locusts crowded for different times (*13*). Behavior was characterized by using a binary logistic regression model (*8*), which produced a single probabilistic metric of gregariousness P_{greg} that encompassed four different variables (table S1). A P_{greg} of 0 meant an animal behaved solitariously, whereas a P_{greg} of 1 indicated fully gregarious behavior. Amounts of serotonin in the thoracic ganglia were measured by using high-performance liquid chromatography (HPLC).

We crowded solitarious locusts for 0, 1, or 2 hours to generate the entire gamut of behavior, from solitarious to gregarious (Fig. 2A). The amount of serotonin was significantly positively correlated with the extent of gregarious behavior across this entire range (analysis of covariance, 5-HT $\log_e$ transformed; $F_{1,35} = 21.817$, $r^2 = 0.429$, $P < 0.001$). Locusts that behaved the most gregariously ($P_{greg} > 0.8$) had approximately three times more serotonin (12.78 ± 1.85 pmol; mean ± SD, $n = 10$ locusts) than more solitariously behaving ($P_{greg} < 0.2$) locusts (4.18 ± 0.27 pmol; $n = 7$ locusts). Furthermore, the amount of serotonin only corresponded with the degree of gregarization but not the duration of crowding, per se ($F_{3,35} = 1.218$, $P = 0.318$).

Behavioral gregarization can be acquired via two distinct sensory pathways: a thoracic pathway driven by mechanosensory stimulation of the hind legs as locusts jostle each other and a cephalic pathway in which the combined sight and smell of other locusts is the necessary stimulus (*9*–*11*). Locusts stimulated via either sensory pathway displayed similar levels of gregarious behavior after 2 hours (Fig. 1C, fig. S1, and SOM text). We tested whether gregarization induced by these separate pathways showed the same relationship between serotonin and behavior that we saw in crowded solitarious locusts. The thoracic pathway was activated by either stroking a hind femur (*10*) or electrically stimulating metathoracic nerve 5, which simulated mechanosensory stimulation (*11*). In both instances, the amount of serotonin in the thoracic ganglia significantly increased and was correlated with the extent of

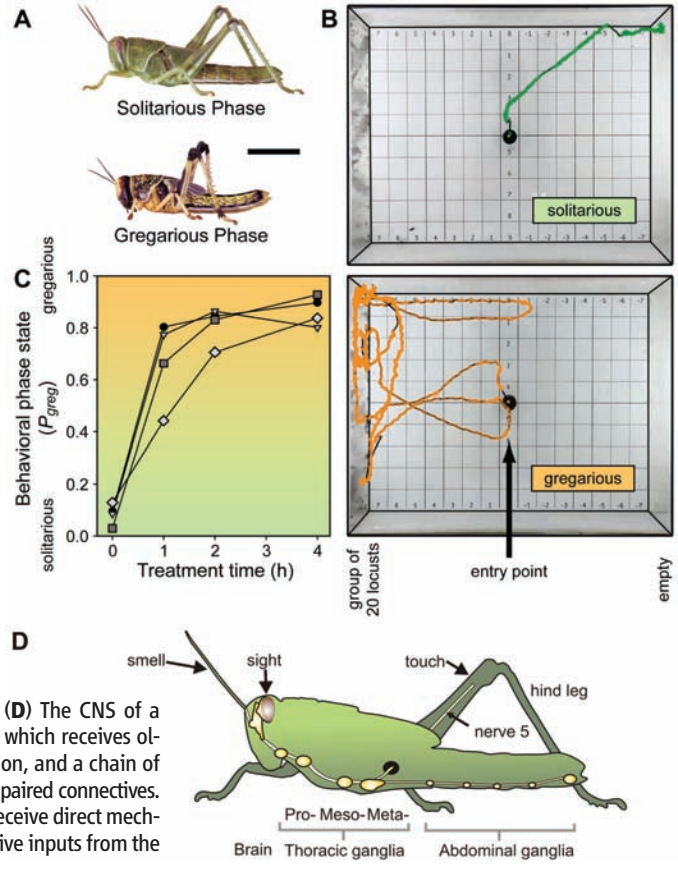

Fig. 1. (**A**) Final larval instar solitarious and gregarious locusts. Scale bar, 1 cm. (**B**) Trajectories (over 500 s) of a solitarious (upper) and gregarious (lower) locust in the behavioral arena. A group of 20 long-term gregarious-phase locusts was placed behind a clear partition on the left. (**C**) Solitarious locusts undergo rapid behavioral gregarization with appropriate stimulation; median P_{greg} of locusts treated for 0 to 4 hours by either forced crowding with gregarious locusts (circles), stroking a hind femur (squares), electrically stimulating the principal hind-leg nerve (diamonds), or exposure to the sight and smell of other locusts (triangles). See SOM text for analysis. (**D**) The CNS of a locust consists of the brain, which receives olfactory and visual information, and a chain of segmental ganglia linked by paired connectives. The three thoracic ganglia receive direct mechanosensory and proprioceptive inputs from the legs.

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gregarization [linear regressions; in stroked locusts, $F_{1,24} = 15.027$, $P = 0.001$, $r^2 = 0.39$ (Fig. 2B); in electrically stimulated locusts, $F_{1,13} = 7.457$, $P = 0.017$; $r^2 = 0.37$ (Fig. 2C)]. Likewise, gregarization through exposure to just the sight and smell of other locusts led to an increase in the amount of serotonin in the thoracic ganglia that correlated with the degree of behavioral gregarization [$F_{1,11} = 23.065$, $P = 0.001$, $r^2 = 0.7$ (Fig. 2D)]. These results indicate that gregarizing stimuli from both sensory pathways converge in the thoracic ganglia, but it is unknown whether they have a cumulative effect on serotonin production.

This strong correlation between phase state and serotonin levels in the thoracic ganglia led us to ask whether blocking the action of serotonin could prevent behavioral gregarization. A mixture of two 5-HT receptor antagonists, ketanserin (1 mM) (*14*, *15*) and methiothepin (1 mM) (*16*, *17*), or a saline control were injected directly into the meso- and metathoracic ganglia of solitarious locusts. The locusts then received either mechanosensory or olfactory and visual gregarizing stimuli for 1 hour. The locusts injected with the antagonists failed to gregarize in response to either stimulus regime (Fig. 3A), in contrast to the saline-injected controls [analysis of variance (ANOVA) of normal rank transformed data, $F_{1,49} = 17.169$, $P < 0.0005$; there was no interaction between stimulus regime and degree of gregarization, $F_{1,49} = 0.001$, $P = 0.987$]. Median P_{greg} in the antagonist-injected locusts was 0.27 for the thoracic and 0.07 for the cephalic pathways (Fig. 3A). By contrast, the median P_{greg} values of the saline-injected controls were 0.91 and 0.74, respectively. Next, we inhibited serotonin synthesis by using α-methyltryptophan (AMTP), a competitive antagonist of tryptophan hydroxylase (*18*, *19*). Locusts were given repeated systemic injections of either 40 μl of 0.1 mM AMTP in locust saline or just saline (controls) over 5 days (*13*). The AMTP-injected locusts (Fig. 3B) showed little behavioral gregarization after having their hind-femora stroked for 2 hours (median $P_{greg} = 0.13$), in strong contrast to the controls (median $P_{greg} = 0.91$; Mann-Whitney $U = 30.500$, $n = 23$ locusts, $P = 0.032$).

We next determined whether serotonin or serotonin receptor agonists were sufficient to induce behavioral gregarization in the absence of stimuli associated with other locusts. Serotonin (1 mM) in saline or a saline control were topically applied to the exposed thoracic ganglia over 2 hours. Serotonin-treated locusts (Fig. 4A) were significantly more gregarious in behavior (median $P_{greg} = 0.6$) than control animals, which remained highly solitarious (median $P_{greg} = 0.07$; Mann-Whitney $U = 41$, $n = 30$ locusts, $P = 0.004$). In a second experiment, animals injected in the thoracic ganglia with a mixture of two serotonin receptor agonists, 1 mM α-methylserotonin (*20*, *21*) and 1 mM 5-carboxamidotryptamine (*16*), showed a significant shift toward gregarious behavior (median $P_{greg} = 0.4$) as compared with saline-injected controls after 1 hour [median $P_{greg} = 0.13$; Mann-Whitney $U = 63$, $n = 30$ locusts, $P = 0.042$ (Fig. 4B)].

Gregarizing stimuli cause serotonin to increase in the thoracic CNS, and exogenous serotonin increases the likelihood of locusts behaving gregariously. We next asked whether enhanced endogenous serotonin synthesis could amplify the effect of stimuli presented for a brief period. Locusts were given single 40-μl injections in the thoracic haemocoel of either the serotonin precursor 5-hydroxytryptophan (5-HTP) (10 mM) (*22*, *23*) or saline controls, and their behavior was assayed after either 30 min of further solitude or 30 min of crowding. Treatment regime had a significant effect on behavior [ANOVA of normal rank transformed P_{greg} data; $F_{3,59} = 5.6$, $P = 0.002$ (Fig. 4C)]. Control locusts that just received saline and were kept in isolation remained highly solitarious in behavior (median $P_{greg} = 0.17$). The median P_{greg} of crowded saline-injected locusts was 0.46, suggesting some change in

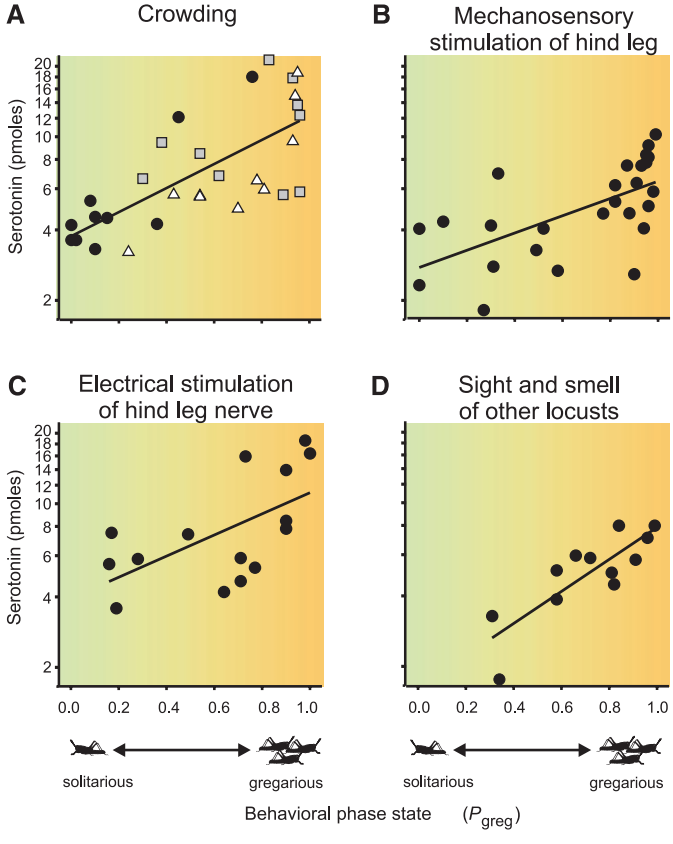

Fig. 2. The amount of serotonin in the thoracic CNS is correlated with the degree of behavioral gregarization. Relationships between the amount of serotonin in the thoracic CNS ($\log_e$ scale) and the degree of behavioral gregarization (P_{greg}) after solitarious locusts have been (**A**) crowded for 1 hour (triangles), 2 hours (squares), or unstimulated controls (circles); (**B**) stroked on the left hind femur for 2 hours; (**C**) given patterned electrical stimulation to metathoracic nerve 5, simulating the effect of mechanosensory stimulation for 2 hours; or (**D**) presented with the sight and smell of ~1000 locusts for 2 hours.

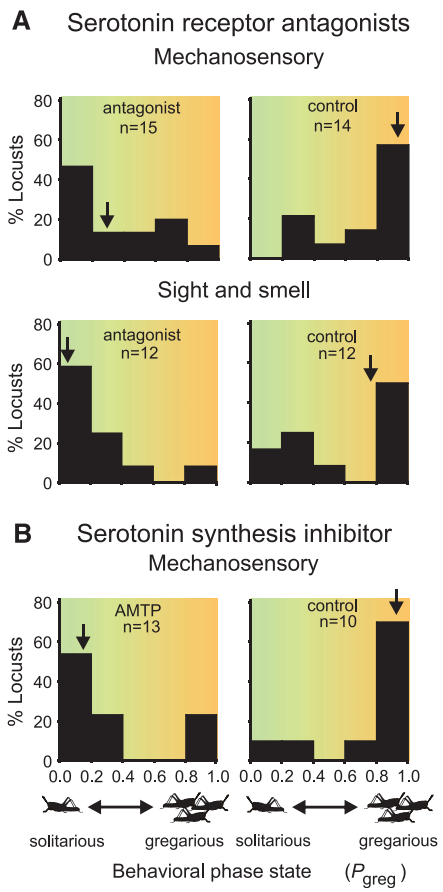

Fig. 3. Serotonin is necessary to induce behavioral gregarization. Behavior of locusts injected with substances that block either the action or the synthesis of serotonin and then exposed to sensory stimuli that normally induce gregarization (left column) is shown. Saline-injected controls are shown in the right column. (**A**) Locusts injected with serotonin-receptor antagonists ketanserin and methiothepin (1 mM) and given either 1 hour of femoral mechanosensory stimulation or 1 hour of olfactory and visual stimulation from other locusts. (**B**) Locusts injected with 0.1 mM AMTP, an inhibitor of serotonin synthesis coupled with 2 hours of mechanosensory stimulation. Arrows indicate median P_{greg} values.

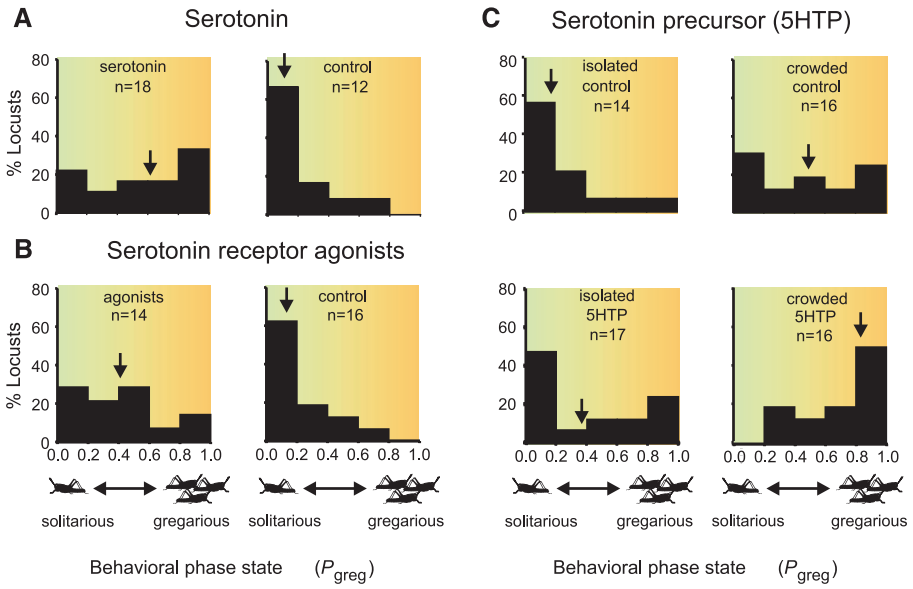

Fig. 4. Serotonin is sufficient to induce gregarious behavior. Behavior of locusts after they have been treated with (**A**) serotonin topically applied to the thoracic ganglia (left column) and paired saline controls (right column); (**B**) a mixture of serotonin agonists (1 mM α-methylserotonin and 1 mM 5-carboxamidotryptamine) and paired saline-injected controls; and (**C**) the serotonin precursor 5-HTP, either with or without 30 min of crowding with other locusts.

behavior; however, the distribution was not significantly different from isolated saline-injected locusts (Dunnet's post-hoc test, $P = 0.383$), which indicates that 30 min was too brief a period of crowding to induce full behavioral gregarization. Locusts that received an injection of 5-HTP but were not crowded also showed a similar but also nonsignificant increase in P_{greg} (median 0.38; $P = 0.448$), which indicates that this treatment was also insufficient to induce full behavioral gregarization. However, locusts that had received both the serotonin precursor and had also been exposed to a brief period of crowding became highly gregarious (median $P_{greg} = 0.81$, $P = 0.001$ as compared with undrugged and uncrowded controls). Thus, 5-HTP can potentiate the effect of gregarizing stimuli applied for a brief period. Unlike the direct application of serotonin, this experiment suggests that endogenous serotonin synthesis driven by sensory stimuli is mechanistically responsible for inducing behavioral gregarization. There are few serotonergic neurons in the locust CNS (*24*), which suggests that the individual neurons driving behavioral gregarization can be identified.

Serotonin and other monoamines have been implicated in changing behavior after social interactions in a number of contexts, including intraspecific aggression, status, and courtship in many species, including crickets (*25*), crustaceans (*26*, *27*), and rats (*28*). All of these interactions, including behavioral gregarization in locusts, require the interpretation of complex signals from conspecifics leading to long-lasting changes in the way individuals interact during future encounters. Behavioral gregarization therefore resembles memory formation, with specific sensory experiences altering future behavior; in the case of locusts, this entails a suite of changes that creates an integrated behavioral phenotype adapted to a changed biotic environment. Locusts that have been reared gregariously for many generations have lower titers of serotonin than long-term solitarious animals, (*12*) which strongly suggests that gregarious behavior is not maintained by a long-term serotonergic modulation of neuronal circuits. Furthermore, solitarious behavior is acquired more slowly on the isolation of long-term gregarious phase locusts (*8*) than gregarious behavior is acquired by solitarious locusts, implying that gregarious behavior becomes more ingrained during prolonged crowding. This ingraining process may entail serotonin-mediated gene transcription and/or translation-dependent mechanisms similar to those associated with other serotonin-mediated neuronal plasticity (*29*, *30*). Although serotonin clearly mediates the critical change in behavior that drives the early process of gregarization, we do not know whether it directly initiates the full and complex suite of changes associated with the full gregarious phenotype. Serotonin, by providing a rapidly acquired and stable behavioral substrate for group living, may enable slower but fully independent mechanisms of phenotypic change to activate through a process of environmental feedback (*4*, *5*, *7*, *31*).

Could serotonin antagonists be effective locust control agents? Given the ubiquity of serotonin signaling in the animal kingdom, any agent would have to be specific for the serotonin receptor mediating phase change, which is yet to be characterized. To be effective, it would have to be targeted at regions of incipient swarm formation to prevent locusts coalescing further into groups.

The whole multilayered process of phase change depends upon a simple behavioral decision whether to avoid other locusts or band together. Without this initial behavioral choice, no further physiological and morphological change can occur (*12*, *31*), and there is no possibility of further escalation in group size. Our data demonstrate a mechanism by which sensory signals gauging population density alter neuronal circuits underlying this fundamental decision. The consequences ramify upwards into population structure and ultimately provide the essential conditions for mass migration and swarming. Phase change is the defining character of locust biology and is the reason why they make such devastating pests. Serotonin-mediated behavioral plasticity is a pivotal mechanism in this transformation.

References and Notes

1. M. J. West-Eberhard, *Developmental Plasticity and Evolution* (Oxford Univ. Press, Oxford, 2003).
2. J. Buhl *et al.*, *Science* **312**, 1402 (2006).
3. S. Bazazi *et al.*, *Curr. Biol.* **18**, 735 (2008).
4. B. Uvarov, *Grasshopper and Locusts*, vol. 1 (Cambridge Univ. Press, Cambridge, 1966).
5. M. P. Pener, Y. Yerushalmi, *J. Insect Physiol.* **44**, 365 (1998).
6. M. Enserink, *Science* **306**, 1880 (2004).
7. S. J. Simpson, A. R. McCaffery, B. F. Hagele, *Biol. Rev. Camb. Philos. Soc.* **74**, 461 (1999).
8. P. Roessingh, S. J. Simpson, S. James, *Proc. R. Soc. London B Biol. Sci.* **252**, 43 (1993).
9. P. Roessingh, A. Bouaichi, S. J. Simpson, *J. Insect Physiol.* **44**, 883 (1998).
10. S. J. Simpson, E. Despland, B. F. Hagele, T. Dodgson, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3895 (2001).
11. S. M. Rogers *et al.*, *J. Exp. Biol.* **206**, 3991 (2003).
12. S. M. Rogers *et al.*, *J. Exp. Biol.* **207**, 3603 (2004).
13. Materials and methods are available as supporting material on *Science* Online.
14. D. Parker, *J. Neurophysiol.* **73**, 923 (1995).
15. L. Gatellier, T. Nagao, R. Kanzaki, *J. Exp. Biol.* **207**, 2487 (2004).
16. A. J. Tierney, *Comp. Biochem. Physiol.* **128A**, 791 (2001).
17. N. Spitzer, D. H. Edwards, D. J. Baro, *J. Exp. Biol.* **211**, 92 (2008).
18. B. D. Sloley, S. Orikasa, *J. Neurochem.* **51**, 535 (1988).
19. P. A. Stevenson, H. A. Hofmann, K. Schoch, K. Schildberger, *J. Neurobiol.* **43**, 107 (2000).
20. G. Molaei, A. B. Lange, *J. Insect Physiol.* **49**, 1073 (2003).
21. J. F. Colas, J. M. Launay, O. Kellermann, P. Rosay, L. Maroteaux, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 5441 (1995).
22. M. Ureshi, M. Dainobu, M. Sakai, *J. Comp. Physiol. A* **188**, 767 (2002).
23. N. N. Osborne, V. Neuhoff, *Brain Res.* **74**, 366 (1974).
24. N. M. Tyrer, J. D. Turner, J. S. Altman, *J. Comp. Neurol.* **227**, 313 (1984).
25. H. A. Hofmann, P. A. Stevenson, *Nature* **403**, 613 (2000).
26. R. Huber, K. Smith, A. Delago, K. Isaksson, E. A. Kravitz, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 5939 (1997).
27. E. A. Kravitz, R. Huber, *Curr. Opin. Neurobiol.* **13**, 736 (2003).
28. K. A. Miczek *et al.*, *J. Neurosci.* **27**, 11803 (2007).
29. C. Pittenger, E. R. Kandel, *Philos. Trans. R. Soc. London B* **358**, 757 (2003).
30. C. A. Hoeffer, S. Sanyal, M. Ramaswami, *J. Neurosci.* **23**, 6362 (2003).
31. A. I. Tawfik *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 7083 (1999).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/323/5914/627/DC1
Materials and Methods
SOM Text
Fig. S1
Table S1
References

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Survival from Hypoxia in *C. elegans* by Inactivation of Aminoacyl-tRNA Synthetases

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Hypoxia is important in a wide range of biological processes, such as animal hibernation and cell survival, and is particularly relevant in many diseases. The sensitivity of cells and organisms to hypoxic injury varies widely, but the molecular basis for this variation is incompletely understood. Using forward genetic screens in *Caenorhabditis elegans*, we isolated a hypoxia-resistant reduction-of-function mutant of *rrt-1* that encodes an arginyl–transfer RNA (tRNA) synthetase, an enzyme essential for protein translation. Knockdown of *rrt-1*, and of most other genes encoding aminoacyl-tRNA synthetases, rescued animals from hypoxia-induced death, and the level of hypoxia resistance was inversely correlated with translation rate. The unfolded protein response was induced by hypoxia and was required for the hypoxia resistance of the reduction-of-function mutant of *rrt-1*. Thus, translational suppression produces hypoxia resistance, in part by reducing unfolded protein toxicity.

Oxygen requirements of cells and organisms have wide-ranging implications in behavior and disease. Forward genetic screens offer the possibility of discovering genes not previously known to control hypoxic sensitivity. Such genes are likely to play an important role in emergent organismal traits such as habitat range and ability to hibernate. Additionally, these genes may lead to the development of novel therapies for conditions where cellular hypoxic sensitivity is a pathological determinant, such as stroke, myocardial infarction, and cancer. Wild-type *C. elegans* when placed in a severe hypoxic environment (oxygen concentration <0.3 volume percent) become immobile but fully recover when returned to normoxia within 4 hours (*1*). After 4 hours, permanent behavioral deficits and cellular death ensue, and after a 22-hour hypoxic incubation, >99% of wild-type animals are dead. To identify genes that control hypoxic sensitivity, we screened for ethylmethane sulfonate (EMS)–derived mutants that survived a 22-hour hypoxic incubation. In a screen of 3884 F_1 mutant worm genomes, we recovered 14 mutants that had a hypoxia-resistant phenotype (table S1). These mutants fell into 13 complementation groups. We selected *gc47*, one of the strongest hypoxia-resistant mutants, for further characterization and mapping.

After outcrossing to the wild-type strain N2, the hypoxia resistance of *gc47* was quantified. Immediately after removal from a 20-hour hypoxic incubation, both N2 and *gc47* were paralyzed, but the *gc47* worms recovered the ability to move completely over the next 1 to 2 hours. After a 24-hour recovery, essentially all of the *gc47* animals were alive, whereas almost all wild-type worms failed to survive (Fig. 1, A and B); *gc47* prolonged the hypoxic incubation time required for complete killing by a factor of >3 (Fig. 1C). The hypoxia-resistant phenotype was fully recessive and segregated as a single locus in a Mendelian fashion (Fig. 1D) (*2*). *gc47* was mapped to a 106-kb interval on the left arm of chromosome III (Fig. 2A) (*2*). Double-stranded RNA interference (RNAi) of 29 of the 32 genes in the interval identified only one gene, *rrt-1*, whose knockdown produced high-level hypoxia resistance (Fig. 2B). Simultaneously, five fosmids that together spanned the entire interval were individually injected to attempt transformation rescue of *gc47*. Only one fosmid restored normal hypoxia sensitivity to *gc47* (Fig. 2C); the rescuing fosmid contained the *rrt-1* gene implicated by RNAi. Sequencing *rrt-1* in *gc47* found a single G → A transition, resulting in a change of amino acid residue 271 from an aspartate to an asparagine in *gc47* (D271N, Fig. 2A). Thus, *gc47* is an allele of *rrt-1* and behaves like a reduction-of-function allele.

rrt-1 encodes an arginyl-tRNA synthetase, one of the aminoacyl-tRNA synthetases (AARSs). AARSs catalyze the adenosine triphosphate (ATP)–dependent acylation of their cognate tRNA(s) with a specific amino acid (*3*). AARSs

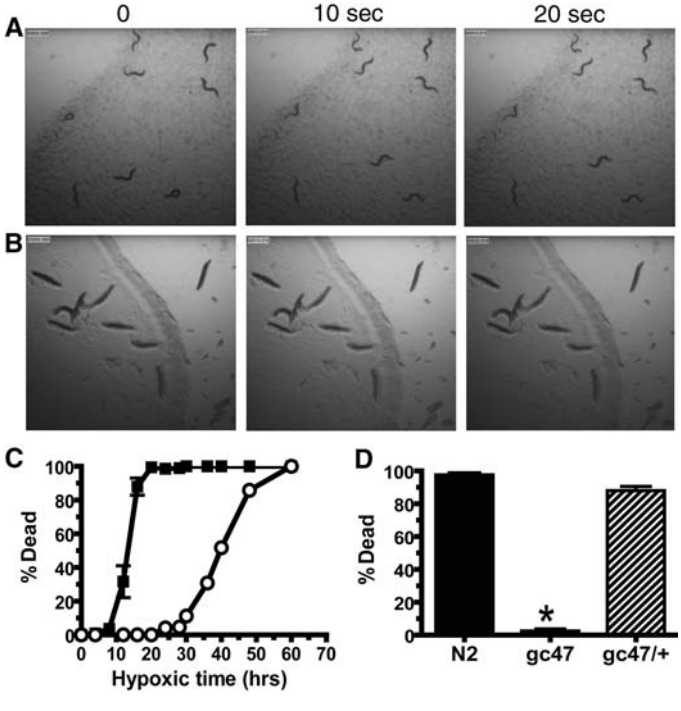

Fig. 1. *gc47* is a potent regulator of hypoxic cell death in *C. elegans*. (**A** and **B**) Time-lapse images (magnification 4×) of *gc47* (A) and N2 (B) adult worms after a 24-hour recovery from a 20-hour hypoxic insult. (**C**) Percent dead animals for N2 (squares) and *gc47* (circles) after a 24-hour recovery as a function of length of hypoxic insult. (**D**) Percent death among homozygous *gc47* (open bar, 13 trials), heterozygous *gc47*/+ (hatched bar, 9 trials), and N2 (solid bar, 16 trials) animals; the results show that *gc47* is recessive. Data are means ± SEM with >30 animals per trial; *$P < 0.01$ (two-tailed *t* test).

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