domain, intracellular domain associations would be required to bring these groups together into an adhesive patch with a sufficient number of intercellular bonds to resist shear forces.

References and Notes

13. See supporting data on Science Online.
41. We thank Y. Ji, M. Auer, and K. MacDonald for help in preparing samples; F. Macaluso and J. Ault for assistance with high-pressure freezing; and K. Taylor, E. Branlund, N. Kisseberth, and D. Mastronarde for providing facilities and support for tomographic data collection and image reconstruction. Coordinates for Fig. 2 G to I, have been deposited in the Protein Data Bank with accession codes 1Q5S, 1Q5A, 1Q5B, and 1Q5C. Tomographic reconstructions have been submitted to the electron microscopy database of the European Bioinformatics Institute with accession codes EMD-1051, EMD-1052, and EMD-1053. Supported by NIH grant R01 GM47429 (P.C.).

Supporting Online Material

www.sciencemag.org/cgi/content/full/302/5642/109/ DC1

Materials and Methods

SOM Text

Figs. S1 to S5

Movies S1 to S3

SOM References

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Wild-Type Nonneuronal Cells Extend Survival of SOD1 Mutant Motor Neurons in ALS Mice

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The most common form of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting adult motor neurons, is caused by dominant mutations in the ubiquitously expressed Cu-Zn superoxide dismutase (SOD1). In chimeric mice that are mixtures of normal and SOD1 mutant–expressing cells, toxicity to motor neurons is shown to require damage from mutant SOD1 acting within nonneuronal cells. Normal motor neurons in SOD1 mutant chimeras develop aspects of ALS pathology. Most important, nonneuronal cells that do not express mutant SOD1 delay degeneration and significantly extend survival of mutant-expressing motor neurons.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder in which motor neurons die beginning in mid–adult life. About 10% of cases are dominantly inherited; about 20% of these arise from mutations in the gene for Cu-Zn superoxide dismutase (SOD1) (1). Transgenic mice (2–4) and rats (5, 6) that express mutant SOD1 develop a progressive motor neuron disease that shares many features with human ALS; the complete absence of SOD1 in mice does not cause such disease (7). Because toxicity is neither accelerated nor ameliorated by reducing wild-type SOD1 activity (8) and is either unaffected (9) or enhanced (9) by increasing wild-type SOD1 activity, mutant SOD1 must cause disease through acquisition of toxic properties. These may include aberrant oxidative chemistry catalyzed by SOD1-bound copper (10–14) or poisoning of a cellular process (or processes) by abundant SOD1 protein aggregates (15–17). This triggers a cell death pathway in motor neurons that includes activation of caspase 3 (18, 19).

Damage to nonneuronal cells may be involved in toxicity. Before onset of disease in SOD1 mutant mice, there is an inflammatory response, including activation of microglia (20–22) and astrocytes (3, 20); the anti-inflammatory compound minocycline extends survival in mouse models of ALS (23–25), although whether this reflects action on microglia, astrocytes, or more directly on motor neurons is not established (24). Although accumulation of mutant SOD1 damages motor neurons in culture (26), SOD1 mutant expression only in neurons (27, 28) or glia (29) has not provoked disease in mice. Thus, fundamental unanswered questions are whether motor neuron express mutant SOD1 develop a progressive motor neuron disease that shares many features with human ALS; the complete absence of SOD1 in mice does not cause such disease (7). Because toxicity is neither accelerated nor ameliorated by reducing wild-type SOD1 activity (8) and is either unaffected (9) or enhanced (9) by increasing wild-type SOD1 activity, mutant SOD1 must cause disease through acquisition of toxic properties. These may include aberrant oxidative chemistry catalyzed by SOD1-bound copper (10–14) or poisoning of a cellular process (or processes) by abundant SOD1 protein aggregates (15–17). This triggers a cell death pathway in motor neurons that includes activation of caspase 3 (18, 19).

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death is caused by toxicity of mutant SOD1 acting solely within motor neurons, whether cells expressing mutant SOD1 damage neighboring wild-type motor neurons, and whether wild-type nonneuronal cells can protect motor neurons expressing ALS-causing SOD1 mutations.

To resolve these issues, we generated chimeric animals composed of mixtures of normal cells and cells that express a human mutant SOD1 polypeptide at levels sufficient to cause fatal motor neuron disease when expressed systemically in mice. Forty-two chimeras were produced by injection of wild-type embryonic stem (ES) cells that constitutively express yellow fluorescent protein (YFP) into blastocysts of SOD1G37R/YFP chimeras. (A) Chimeras generated by injection of E-YFP–expressing ES cells into blastocysts of SOD1G37R or SOD1G37R (line 42) (4) mutant blastocysts (Fig. 1, A and B). Percent chimerism (ranging from ~5 to ~90% wild-type cells) was determined by multiple measures (table S1), including assessment of coat color, immunoblotting of tail extracts for accumulation of mutant SOD1 and YFP (Fig. 1B), and by the proportion of cross-sectional area in spinal cords with detectable YFP (Fig. 1, C and D).

An additional 23 chimeras (Fig. 1H) were produced by using aggregation (31) of morulae from wild-type embryos with morulae carrying transgenes for another mutant SOD1 (SOD1G93A) (2) and for ubiquitously expressed β-galactosidase (lacZ) (32). Comparable estimates were obtained for the amount of mutant SOD1 by using either coat color or immunohistochemistry of spinal cord sections to identify cells expressing lacZ. For example, animals determined to be ~50% chimeric by coat color had a corresponding ~50% of spinal cord areas expressing lacZ, including large spinal motor neurons (Fig. 1I). For comparison, spinal cord sections from wild-type and germline SOD1G93A mice are unstained or completely stained for lacZ (Fig. 1, J and K).

The presence of wild-type cells in the SOD1G37R/YFP and SOD1G85R/YFP chimeras delayed disease onset with average extensions of 1.6 months (P = 0.0033; n = 17; one-tailed Mann-Whitney test) for SOD1G85R and 1.2 months (P = 0.0007, n = 17) for SOD1G37R chimeras (Fig. 1E). Compared with germline SOD1 mutant mice, there was an average extension of life-span of 1.8 months (P = 0.04, n = 13) for SOD1G85R and 1.1 months (P = 0.0001, n = 17) for SOD1G37R chimeras with a maximum delay of 7.8 and 3.3 months, respectively (Fig. 1F). Both measures correlated well with age at onset (Fig. 1G) and disease duration (Fig. 1H).
with the proportion of wild-type (YFP-expressing) cells within each spinal cord (Fig. 1G).

A robust extension in life-span was also seen in the SOD1<sup>G37R</sup>/hNF-L<sup>A22</sup> mice. Eleven of the 23, including 5 with ~30% contribution from wild-type cells, survived disease-free until they were killed at ~10 months of age (Fig. 1, L and M), an age at least twice that of the longest-lived SOD1<sup>G93A</sup> littersmates (Fig. 1I). Immunoblotting of spinal cord and motor roots of two of these revealed that 67% (chimera 45; Fig. 1N) and 77% (chimera 67) of motor neurons contained mutant SOD1, but there was no degeneration or axonal loss in thoracic roots of either chimera and only the earliest signs of degeneration in some lumbar roots of chimera 67. This contrasts with germline SOD1<sup>G93A</sup> animals in which 100% of the motor neurons express mutant SOD1 (Fig. 1O), and half of these are lost by 5 months (2).

Extended survival of mutant-expressing motor neurons was also seen in chimeras generated by aggregation of morulae from a SOD1<sup>G37R</sup> transgenic line (line 29, which has a later disease onset) (4) with morulae whose wild-type neurons were marked by expression of very low levels of the smallest human neurofilament subunit (hNF-L) (3) (Fig. 2A). Immunobots for the SOD1<sup>G37R</sup> mutant and hNF-L in spinal cord extracts revealed ~30 and ~90% mutant cells in two SOD1<sup>G37R</sup>/hNF-L chimeras (Fig. 2B). Chimera 7, with the higher wild-type content, did not develop disease even 5 months beyond the age of the longest-lived germline SOD1<sup>G37R</sup> mice. As seen with antibodies specific for human SOD1 (Fig. S2), hNF-L, or all neurofilaments (to identify mutant and wild-type axons), 30% of ventral root axons (Fig. 2, C to E) were mutant. However, there was no sign of axonal degeneration or loss in the L5 ventral root (Fig. 2F), in which 978 axons remained, a number consistent with the 927 ± 99 (n = 26) seen in age-matched wild-type mice. Furthermore, in contrast to parental SOD1<sup>G37R</sup> mice (Fig. 2, H, K, and N), even the earliest pathologic signs of disease, including astrogliosis and microgliosis, were absent in this chimera (Fig. 2, I, L, and O), just as they were in normal mice (Fig. 2, G, J, and M).

Extended survivals of SOD1 mutant–expressing motor neurons in the chimeras could arise, at least in part, from a protective effect of wild-type motor neurons. To test this, two SOD1<sup>G37R</sup>/YFP chimeras were identified that developed without wild-type motor neurons; all motor neurons of multiple lumbar levels (Fig. 3, B and C, arrows) and motor roots (Fig. 3E) accumulated mutant SOD1. Both of these also displayed a striking left-right asymmetry in the proportion of wild-type (YFP-positive) neurons that are not motor neurons.
nonneuronal cells in the two halves of their spinal cords. Germline SOD1<sup>G93A</sup> mice at end-stage disease uniformly exhibit symmetric loss of two-thirds of their large motor neurons in both halves of the lumbar spinal cord (Fig. 3A). However, although all motor neurons were mutant in these two SOD1<sup>G93A</sup>/YFP chimeras, there was an asymmetric loss of motor neurons (Fig. 3A; P < 0.001; paired Student’s t test with n > 4 sections per animal) and axons, with more than twice as many large-caliber (> 3.5 μm diameter) surviving axons (Fig. 3D; 187 on the left versus 89 on the right) in the less-affected side. In both chimeras, the side with higher neuronal survival had a higher proportion (25 versus 2% in chimera 646; 30 versus 10% in chimera 213) of wild-type (YFP-expressing) nonneuronal cells throughout the lumbar cords. Thus, even when all motor neurons are mutant, an environment having a higher proportion of wild-type, nonneuronal cells reduces motor neuron mortality.

To assess whether SOD1 mutant nonneuronal cells can influence neighboring wild-type neurons, spinal cord sections of chimeric animals were analyzed at end-stage disease for pathologic signs of neurodegeneration. A hallmark for damage to neurons in human patients is the appearance of ubiquitin-positive protein aggregates (34, 35). These are also seen as an early sign for damaged neurons in SOD1<sup>G93A</sup> (3, 8) and SOD1<sup>1G37R</sup> (4) mice, but do not appear in motor neurons of wild-type mice (Fig. 4, A to C). Ubiquitin aggregates appear in neuronal processes and, less prominently, in cell bodies (Fig. 4B; fig. S3). Similar ubiquitinated epispots were never seen in age-matched wild-type littermates (Fig. 4A; fig. S3). In contrast, in both SOD1<sup>G13T</sup> and SOD1<sup>G85R</sup> chimeras (n = 4), some wild-type neurons (YFP-containing; arrows in Fig. 4, D and E, and G and H) in end-stage chimeras accumulated ubiquitinated epitopes in neuronal processes (Fig. 4F, arrows) and cell bodies (Fig. 4I, arrow), which indicates that a deficit in ubiquitin-dependent protein degradation is acquired by these wild-type neurons. The intensity of such ubiquitin staining in wild-type axons (Fig. 4F, arrows) and motor neuron cell bodies (Fig. 4I, arrow) frequently exceeded that of neurons expressing mutant SOD1 (Fig. 4F, boxed areas; Fig. 4I).

We found that expression of mutant SOD1 in motor neurons at levels that cause disease in parental mice is not sufficient to trigger their degeneration or the development of pathologic abnormalities. Rather, wild-type nonneuronal cells, in some cases representing a small minority of total cells, can ameliorate degeneration and death of SOD1 mutant–expressing motor neurons compared with those in parental SOD1 mutant mice. That SOD1 mutant neurons survive longer when surrounded by a wild-type environment supports the view that damage to adjacent nonneuronal cells by mutant SOD1 is a major contributor to disease caused by SOD1 mutations. Damaged glial cells and neurons, therefore, could act in concert to provoke disease, consistent with failure of mutant expression in single cell types to induce motor neuron degeneration (27–29). It is also consistent with the failure of increased levels of mutant SOD1 within neurons to accelerate disease caused by ubiquitous expression of SOD1<sup>G93A</sup> (29). Indeed, we know of no compelling in vivo evidence that the genotype of the motor neurons themselves has any bearing on the probability of their death in ALS; motor neuron death could in principle be provoked solely by damage to multiple types of adjacent cells such as interneurons, astrocytes, and microglia. Further work is critical to evaluate this possibility.

Fig. 4. Acquisition of abnormal ubiquitination in wild-type neurons adjacent to SOD1 mutant–expressing cells. Confocal micrographs of spinal cord cross sections from the lumbar region of (A) normal (C57/B6), (B) SOD1<sup>G13T</sup> (line 148), and (C) SOD1<sup>G85R</sup> (line 42) mice after staining with (red) neurofilament (SMI32) and (blue) ubiquitin antibodies. Ubiquitin-containing aggregates in neuronal processes (arrows) are present in SOD1<sup>G13T</sup> and SOD1<sup>G85R</sup> germine animals, as well as in the cell bodies (block arrow). (D to I) Triple labeling of spinal cord sections from (D to F) SOD1<sup>G13T</sup> and (G to I) SOD1<sup>G85R</sup> chimeras stained with (D and G) SM132 to identify neurons (red), (E and H) GFP to detect wild-type cells (green), and (F and I) ubiquitin (blue). Arrows in (D to F) point to a wild-type axon with elevated levels of ubiquitin compared with a mutant axon highlighted in the boxed region. Arrow in (G to I) points to a wild-type neuronal cell body with high ubiquitin accumulation compared with an adjacent SOD1<sup>G85R</sup> motor neuron. Additional examples are in fig. S5.
Thalamic Control of Visceral Nociception Mediated by T-Type Ca\(^{2+}\) Channels

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Sensations from viscera, like fullness, easily become painful if the stimulus persists. Mice lacking \(\alpha1G\)-T-type Ca\(^{2+}\) channels show hyperalgesia to visceral pain. Thalamic infusion of a T-type blocker induced similar hyperalgesia in wild-type mice. In response to visceral pain, the ventroposterolateral thalamic neurons evoked a surge of single spikes, which then slowly decayed as T-type-dependent burst spikes gradually increased. In \(\alpha1G\)-deficient neurons, the single-spike response persisted without burst spikes. These results indicate that T-type Ca\(^{2+}\) channels underlie an antinoceptive mechanism operating in the thalamus and support the idea that burst firing plays a critical role in sensory gating in the thalamus.

Low voltage-activated (LVA) T-type Ca\(^{2+}\) channels play crucial roles in the control of cellular excitability under diverse physiological and pathological processes (1, 2). Recently, studies revealed a novel role of T-type Ca\(^{2+}\) channel in the pain sensory pathway by showing that this channel facilitates pain signals in peripheral nociceptors (3, 4) and in the spinal cord (5). T-type channels are also highly expressed in the thalamus (6), through which noxious signals from the spinal cords must pass before reaching the cortex (7). When the thalamocortical relay neurons receive sensory inputs, they respond in dual firing modes: either in singular action potentials or in a burst of action potentials clustered together as a high-frequency discharge (8–10). T-type Ca\(^{2+}\) channels are known to excite hyperpolarized thalamic neurons to generate bursts of action potentials. There has been much debate about the role of the thalamic burst firing in the sensory processing (11, 12). Therefore, whether thalamic T-type channels would contribute to the nociceptive signal processing as a signal enhancer or a suppressor is an open question.

Mice homozygous for a null mutation of the \(\alpha1G\) (Ca\(V3.1\)) gene showed a functional deletion of T-type currents and lacked low threshold burst firing in the thalamocortical relay neurons (13). We measured the sensitivity of the \(\alpha1G\)-deficient mice (\(\alpha1G^{-/-}\)) by delivering thermal or mechanical stimuli delivered either on the palm or tail (supporting online material). No significant difference was observed between the mutants and their wild-type littermates in these assays (Fig. 1, A to C). Hyperalgesia to cutaneous pain, as measured by the relative enhancement of the pain response by a subcutaneous injection of complete Freund’s adjuvant (CFA) before pain tests (14), also did not significantly differ between the wild type and the mutant (Fig. 1D). Next, we examined the sensitivity of the mice to visceral pain induced by intraperitoneal administration of either acetic acid (Fig. 1E) or MgSO\(_4\) solution (Fig. 1F) as previously described (15). The wild-type mice showed typical pain behaviors characterized by writhing, such as abdominal stretching and constriction in response to these two chemicals, with MgSO\(_4\)-induced pain responses terminated earlier than those by acetic acids (15). However, compared

![Fig. 1. Pain responses of \(\alpha1G^{-/-}\) mice to noxious stimuli. (A) Responses to mechanical stimuli with von Frey filaments. (B) Tail flick responses to thermal stimuli. (C) Paw withdrawal responses to infrared thermal stimuli at two different intensities. (D) One day after injection of CFA (1×) in the left paw, infrared thermal stimuli were delivered either to the injected paw (ipsilateral) or the opposite uninjected paw (contralateral). Visceral pain induced by intraperitoneal injection of either acetic acid (E) or MgSO\(_4\) solution (F). Writhing responses were examined for 20 min after acetic acid injection or for 10 min after MgSO\(_4\) injection. Error bars indicate SEM. Two-tailed t test, *P < 0.01, **P > 0.05.

References and Notes
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CORRECTIONS AND CLARIFICATIONS

ERRATUM
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REPORTS: “Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice” by A. M. Clement et al. (3 Oct. 2003, p. 113). The word “inherited” was deleted from the first sentence of the abstract. It should read as follows: “The most common inherited form of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease affecting adult motor neurons, is caused by dominant mutations in the ubiquitously expressed Cu-Zn superoxide dismutase (SOD1).”