ANTIOXIDANTS & REDOX SIGNALING
Volume 10, Number 3, 2008

DOI: 10.1089/ars.2007.1760

Comprehensive Invited Review

Amyotrophic Lateral Sclerosis: From Current Developments in the Laboratory to Clinical Implications

MAURO COZZOLINO,[1] ALBERTO FERRI,[1,2] and MARIA TERESA CARRÌ[1,3]

Reviewing Editors: Michael Benatar, Jeffrey Keller, and Angelo Poletti

ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a late-onset progressive degeneration of motor neurons occurring both as a sporadic and a familial disease. The etiology of ALS remains unknown, but one fifth of instances are due

[1] Fondazione Santa Lucia IRCCS; [2]Department of Psychobiology and Psychopharmacology, CNR Institute for Neuroscience; and [3]Department of Biology, University of Rome "Tor Vergata," Rome, Italy.

to specific gene defects, the best characterized of which is point mutations in the gene coding for Cu/Zn superoxide dismutase (SOD1). Because sporadic and familial ALS affect the same neurons with similar pathology, it is hoped that understanding these gene defects will help in devising therapies effective in both forms. A wealth of evidence has been collected in rodents made transgenic for mutant SOD1, which represent the best available models for familial ALS. Mutant SOD1 likely induces selective vulnerability of motor neurons through a combination of several mechanisms, including protein misfolding, mitochondrial dysfunction, oxidative damage, cytoskeletal abnormalities and defective axonal transport, excitotoxicity, inadequate growth factor signaling, and inflammation. Damage within motor neurons is enhanced by noxious signals originating from nonneuronal neighboring cells, where mutant SOD1 induces an inflammatory response that accelerates disease progression. The clinical implication of these findings is that promising therapeutic approaches can be derived from multidrug treatments aimed at the simultaneous interception of damage in both motor neurons and nonmotor neuronal cells. *Antioxid. Redox Signal.* 10, 405–443.

I. INTRODUCTION

AMYOTROPHIC LATERAL SCLEROSIS (ALS, also known as Lou Gehrig disease) was described for the first time in 1869 by the distinguished French neurologist Jean-Martin Charcot as a progressive, late-onset motor neuron disease (65). Charcot observed degeneration and death of upper and lower motor neurons, indicated by "myelin pallor," representing loss of the axons of upper motor neurons as they descend from the brain to connect onto the lower motor neurons within the spinal cord.

The most typical feature of this progressive lethal disease is the degeneration of cortical, bulbar, and spinal motor neurons, except for those that control the bladder and eye movement. This leads to generalized muscle weakness and atrophy, speech and swallowing disabilities, and progressive paralysis until death is caused by respiratory failure.

ALS is one of the most common neurodegenerative disorders, with an incidence of 1 to 2 per 100,000 and a prevalence of 4 to 6 per 100,000; as many as 30,000 Americans have the disease at any given time. The average age at onset is 50 years, but juvenile cases also are observed.

With the current standard support therapy, including nighttime breathing assistance early in the course of the disease and application of alternate feeding options once swallowing becomes difficult, the typical survival time for patients after diagnosis is 3–4 years, although large deviations have been observed.

Approximately 10% of ALS cases are inherited (familial ALS, fALS), but ALS is sporadic in the vast majority of patients (sALS). The clinical expressions of sporadic and familial ALS are very similar. Although ALS patients show some degree of heterogeneity as far as symptoms, age at onset, and disease duration are concerned, fALS cases are indistinguishable from sALS on the basis of clinical and pathologic criteria (364).

This review focuses on the clinical implications of the evidence generated thus far that ALS arises through a combination of several mechanisms. These mechanisms act through concurring damage inside motor neurons and their neighboring nonmotor neuronal cells. Therefore, ALS is considered both a multifactorial and multisystemic disease that affects several cell types, similar to other disorders such as spinal cord injury, Alzheimer's disease, Huntington's disease, and Parkinson's disease, in which many factors (a large number of which overlap with those that have a role in ALS) contribute to neurodegeneration and in which single-drug approaches have proven insufficient for effective treatment.

A. ALS is a multifactorial disease

The pathogenesis of ALS was considered quite obscure for more than a century after its first description. Anatomic and morphologic examination of postmortem samples conveyed the notion of the selective vulnerability of motor neurons mainly in the anterior horns of the spinal cord, associated with the activation of astrocytes and microglia and muscle atrophy. Pioneering biochemical studies examined alterations in the cerebrospinal fluid (CSF) of patients and in samples from spinal cord and brain. However, many studies were hampered by the difficulty of working with stored material and by the low sensitivity of available methods.

A major step forward in the understanding of the molecular mechanisms underlying ALS was provided in 1993 by the observation that mutations in the gene coding for the antioxidant enzyme Cu,Zn superoxide dismutase (SOD1; EC 1.15.1.1) are carried by one fifth of fALS patients (*i.e.*, 2% of all ALS cases) (94, 327). This enabled the development of novel experimental models, such as transgenic organisms carrying the mutant protein (most notably, transgenic mice expressing the G93A mutant SOD1), and allowed the collection of a wealth of data on the molecular and cellular alterations induced by the presence of the mutant protein. Although many studies merely confirmed what was already known from observations in postmortem samples, others offered a new rationale for this obscure pathology.

Increasing evidence indicates that cellular functions impaired as a consequence of the expression of mutant SOD1 converge on pathways (Fig. 1) that could be activated in sporadic ALS by other toxic factors, and it is hoped that therapies effective in mutant SOD1 models will translate to sporadic and non-SOD1–linked ALS.

1. Oxidative stress. In aerobic organisms, minimal levels of potentially toxic reactive oxygen species (ROS), such as superoxide, hydrogen peroxide, and hydroxyl radical, are produced during the reduction of molecular oxygen by mitochondria. Additionally, production of H_2O_2 by oxidases (*e.g.*, monoamine oxidase) can result in greater oxidative stress in tis-

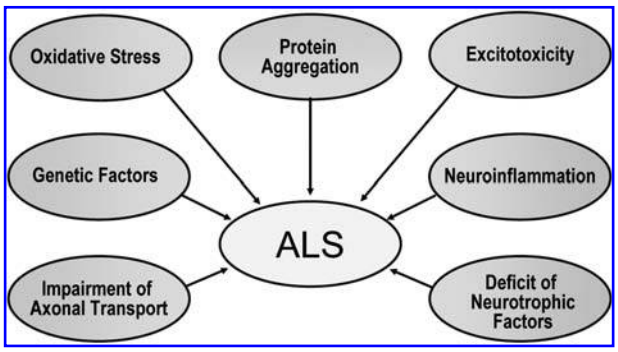

FIG. 1. ALS is a multifactorial disease. Damage within motor neurons occurs through several concurring pathways, including protein misfolding and aggregation, oxidative damage and mitochondrial dysfunction, cytoskeletal abnormalities and defective axonal transport, and genetic factors. Such damage is enhanced by noxious signals originating from non-neuronal neighboring cells, where mutant SOD1 induces excitotoxicity, inadequate growth factor signaling, and an inflammatory response that accelerates disease progression.

sues enriched in these enzymes, such as the central nervous system (CNS). In the CNS, stress conditions may also be promoted by the generation of nitric oxide, used as a second messenger in neurotransmission and signalling. NO is released from activated microglia along with superoxide, and accumulating levels of diffusible NO and superoxide give rise to peroxynitrite. In turn, peroxynitrite and related reactive nitrogen species (RNS) may perform both oxidation chemistry and nitration of the aromatic side chains of amino acids, promoting a condition of nitrosative stress (340).

As in other tissues, the intracellular antioxidant defense in the CNS includes enzymatic activities and low-molecular-weight antioxidant species, plus more complex forms of protection such as induction of genes through the control of redox-regulated transcription factors, control of the expression of NO synthases, and systems for metal transport and buffering. However, the CNS is more susceptible to oxidative and nitrosative stress than are other tissues for several reasons (157). Therefore, it is not surprising that several common neurodegenerative conditions (*e.g.*, Alzheimer's disease and Parkinson's disease) are associated with this kind of stress, although in many cases, it is not clearly established whether oxidative stress is a cause, consequence, or correlate of neurodegeneration.

A role for ROS-mediated oxidative stress both in sporadic and familial ALS patients was proposed in many studies reporting the occurrence of typical oxidation products. Protein carbonyl levels, lipid peroxidation, and protein glycoxidation were investigated as markers of oxidative damage in the spinal cord from patients with sporadic ALS and found to be significantly increased (348, 349).

In 1997, the group of Flint Beal conducted a systematic survey, examining markers of oxidative damage to protein, lipids, and DNA in several areas of the CNS (motor cortex, parietal cortex, and cerebellum) from control subjects, fALS patients with and without known SOD1 mutations, and sALS patients. Protein carbonyl and nuclear DNA 8-hydroxy-2′-deoxyguanosine (8-OHdG) levels were increased in sALS motor cortex but not in fALS patients, and malondialdehyde levels showed no significant changes. However, immunohistochemical studies showed increased neuronal staining for hemoxygenase-1, malondialdehyde-modified protein, and 8-OHdG in both sALS and fALS spinal cord [*i.e.*, in the area that is most affected in the disease (45, 116)], and biochemical measurements showed increased concentrations of 3-nitrotyrosine and 3-nitro-4-hydroxyphenylacetic acid in the lumbar and thoracic spinal cord of ALS patients (31).

Similar measurements were repeated in the transgenic mice overexpressing mutant SOD1, the best model available for ALS (40), and confirmed that the occurrence of oxidative and nitrosative stress is indicated by measures of the level of production of "hydroxyl radical–like" species (46) and other ROS (236), oxidative damage to proteins (16, 234), membrane lipids (155, 234), and DNA (234), and by the occurrence of nitrotyrosine (64).

More recently, several investigators have suggested that oxidative stress in muscle may also contribute to ALS pathogenesis. In a study by Mahoney and colleagues (239), markers of oxidative stress were detected in the skeletal muscle of mice expressing mutant SOD1. Elevations in malondialdehyde and protein carbonyls and upregulation of antioxidant enzymes SOD1, SOD2, and catalase were found in skeletal muscle from symptomatic mice.

The observation that mutations in the antioxidant enzyme SOD1 are reported in patients with fALS was also considered an indication that oxidative stress is involved in the pathogenesis of ALS (327). As discussed, mutant SOD1 does not necessarily lose its antioxidant, superoxide-scavenging function. Nonetheless, administration of several antioxidant molecules has been proven beneficial in the mouse model for SOD1-linked fALS, indicating that oxidative stress is indeed a component of this pathology. For instance, *Gingko biloba* extract, which exerts protective effects against mitochondrial damage and oxidative stress, is effective in delaying the onset or prolonging survival (or both) of ALS mice (117). Preventing free radical propagation with the spin-trapping molecule 5′,5′-dimethylpyrroline-*N*-oxide (DMPO) or with an antioxidant porphyrin (iron 5,10,15,20-tetrakis-4-carboxyphenyl porphyrin; manganese [III] tetrakis [*NN*′-diethylimidazolium-2-yl]porphyrin) that catalytically scavenges a wide range of ROS, including peroxynitrite, also significantly delays paralysis and increases survival of transgenic mice overexpressing mutant G93A-SOD1 (88, 237, 416). Finally, exacerbation of superoxide-induced oxidative stress through deficiency of the mitochondrial enzyme Mn superoxide dismutase (SOD2) is detrimental and aggravates the pathologic phenotype in mice overexpressing human mutant SOD1 (15).

That the simple administration of antioxidants may be beneficial for patients is denied by common clinical practice, because many of them receive high doses of CoQ10, vitamin E, vitamin C, or acetylcysteine with no relevant effect (115, 130, 285). Nonetheless, conventional antioxidants may play a role in ALS prevention, because long-term users of vitamin E have less than half the risk of dying of ALS than do nonusers (21).

It must be pointed out that "unconventional" antioxidants may possess better therapeutic properties than the ones commonly used and prove more useful. For instance, treatment with melatonin, an ampiphilic molecule with a unique spectrum of

antioxidative effects, had positive effects both in models (cultured motor neuronal cells and in the genetic mouse model of ALS) and also in a clinical safety study on 31 patients, in whom high-dose melatonin treatment normalized circulating serum protein carbonyls to control values (404). The efficacy and safety of edaravone, a free radical scavenger previously approved for treatment of acute cerebral infarction, was assessed in 20 ALS patients during a 6-month treatment period. The decline in the values on a functional rating scale was significantly less than that in the 6 months before edaravone administration, and CSF 3-nitrotyrosine was markedly reduced to almost undetectable levels in almost all patients at the end of the 6-month treatment period (430). However, a word of caution is necessary in recommending trials with new antioxidants if they possess more than one effect *in vivo*. In a recent study, Ahtoniemi *et al*. (6) experimented with pyrrolidine dithiocarbamate (PDTC) in rats expressing mutant SOD1. PDTC is both a metal chelator and an antioxidant that has several actions *in vivo*, including inhibition of nuclear transcription factor κB and induction of NRF2 (a transcription factor that binds to the antioxidant response element of numerous genes), and it has been proven to have beneficial effects in animal models of various diseases through its antioxidant and antiinflammatory activity. Surprisingly, PDTC treatment significantly decreased the survival of ALS rats because it completely blocked the induction of immunoproteasome and increased the level of ubiquitinated proteins and copper concentration in the spinal cord, enhancing the toxicity of mutant SOD1 and oxidative stress.

Antioxidant administration may have a better effect when in combination with drugs targeting other pathogenic pathways activated in ALS (see later). For instance, markers of oxidative damage were synergistically reduced in the lumbar spinal cord of the mutant SOD1 mice when an agent inhibiting apoptosis, the histone deacetylase inhibitor phenylbutyrate, was administered in combination with the catalytic antioxidant AEOL 10150 (295). Similarly, oral administration of Neu2000, a potent antioxidant that also prevents NMDA neurotoxicity (151), together with lithium carbonate, a mood stabilizer that prevents apoptosis, significantly enhanced survival time and motor function of the mutant SOD1 mice, and together, they had an additive effect (351).

Targeting antioxidants to the actual site of damage may also constitute a promising approach to treating ALS. Reasoning that a major proportion of cellular ROS is generated at the inner mitochondrial membrane (IMM) by the respiratory chain, a novel peptide antioxidant (SS-31) was targeted to the IMM, and its therapeutic effects were assessed both *in vitro* and *in vivo* in the mouse model of SOD1-linked fALS. SS-31 protected neuronal cells expressing mutant SOD1 against cell death induced by hydrogen peroxide and improved survival and motor performance of SS-31–treated ALS mice, which showed a decreased cell loss and a decrease in immunostaining for markers of oxidative stress in the lumbar spinal cord (295).

It must be noted that many antioxidants were found beneficial, at least in experimental systems, in a wide range of pathologic conditions, including several common neurodegenerative diseases. This may be interpreted as indicating that a generalized oxidative stress may not be considered specific for ALS, but rather a phenomenon preceding or accompanying neurodegeneration. For instance, membrane lipid peroxidation and oxidative modification of various membrane-associated proteins occur in a range of neurodegenerative disorders, and this form of oxidative stress is promoted by transition metals like iron and copper (251). In aerobic life, correct handling of copper and iron is crucial, because those metals are able to undergo "redox cycling" (*i.e*., alternate oxidization and reduction, during which toxic ROS are generated). Both "free" copper and "free" iron have the ability to promote, *via* the Fenton reaction, the formation of hydroxyl radicals, and therefore, living organisms have developed a stringent control of the metabolism of these metals. *In vivo*, they are normally sequestered within complexes or bound protectively to enzymes, proteins, or carriers, because a complex mechanism of iron and copper buffering has evolved to avoid metal-mediated oxidative stress (27, 167, 193, 303). Under normal conditions, intracellular "free" copper ions virtually do not exist (308), whereas a "labile" iron pool is detectable and may increase in some conditions, mostly derived from iron mishandling and subtle changes in the pool of redox-active metal ions and in metal compartmentalization (27, 306, 361).

Several studies have addressed the question of environmental metal toxicity in ALS, because epidemiologic evidence indicates that occurrence of sporadic ALS is growing in many countries. Several studies that have considered lead exposure as a risk factor for sporadic ALS have found some effect (189, 190, 277), whereas studies on the relation between exposure to other metals (including copper and iron) and development of ALS could not demonstrate conclusively an association. However, a tendency of copper levels to increase with the progression of the disease was observed in patients, indicating that some dismetabolism of this metal may be occurring in ALS (43).

With the rationale that copper overload might be a culprit in the generation of oxidative stress, copper chelators such as diethyldithiocarbamate, D-penicillamine, and bathocuproine have been used in several experimental paradigms for ALS *in vitro* and *in vivo* and found beneficial in ameliorating the pathologic phenotype (74, 127, 172, 408). Furthermore, genetically decreased spinal cord copper concentration prolongs life in the mutant SOD1-G93A transgenic mouse model of ALS (201), and copper chelators can rescue elevation of ROS levels observed in lymphoblasts from fALS patients (332).

Copper mishandling may be deleterious also through disregulation of Cu-containing enzymes. Besides SOD1, several copper-containing enzymes are found in the brain, including dopamine β-hydroxylase, tryptophan-2,3-dioxygenase, lysine oxidase, cytochrome oxidase, monoamine oxidases, tyrosinase, D-amino levulinate dehydratase, and an astrocyte-specific form of ceruloplasmin, which are prominent for their roles in the nervous system. Their possible involvement in ALS has not been assessed, except for cytochrome *c* oxidase, which has been reported to be decreased in the spinal cords of patients with ALS (124).

Iron ions are also an essential but potentially toxic constituent of most organisms, and their metabolism is meticulously regulated at both the cellular and systemic levels. In higher eukaryotes, these include transport protein transferrin (Tf), storage protein ferritin (Ft), and ceruloplasmin (Cp), a copper ferroxidase that is expressed as a glycosylphosphatidylinositol-anchored form in the mammalian central nervous system (293).

In many eukaryotic systems, gene expression of Tf and Ft is tightly regulated through the coordinate action of the IRE/IRP (iron-responsive element/iron regulatory protein) machinery but also by non–IRP-mediated mechanisms. Oxidative stress may directly interfere with the IRE/IRP machinery and induce alteration of iron homeostasis. We recently studied the effect of the modulation of SOD1 levels on iron metabolism in a cultured human glial cell line and in a mouse motor neuronal cell line and found evidence of a link between the level of SOD1 activity and the expression of Tf receptor and Ft. However, no clear link was found between iron metabolism and the expression of mutant SOD1s typical of ALS (92). Nonetheless, it must be mentioned that early evidence suggests that iron may be implicated in the pathogenesis of sporadic ALS (194, 224, 425). In particular, iron levels are elevated in some regions of brain from sALS patients (194), in which Ft-H (ferritin heavy chain) and Ft-L (ferritin light chain) are increased. Furthermore, Ft is upregulated in transgenic mice expressing mutant SOD1 just before end-stage disease, indicative of excessive Fe deposition (282).

Another mechanism by which cells fail to regulate their iron status properly is through a mutation in the HLA-linked hemochromatosis (high Fe, Hfe) gene. Mutations in the Hfe gene are associated with the iron-overload disease, hemochromatosis (135), and two studies reported an increased incidence of the Hfe mutation in ALS patients and association of the H63D polymorphism in Hfe with sporadic ALS (137, 401).

In this context, iron chelators may have beneficial effects in ALS, including induction of the hypoxia-inducible factor-1 (HIF-1), which would cause the transcription of *VEGF* (vascular endothelial growth factor), a gene that has been related to ALS (see later).

2. Protein aggregation and impairment of axonal transport. Protein aggregates are a hallmark of all types of ALS and are frequently found in motor neurons of the spinal cord; they include ubiquitinated skein-like inclusions, Bunina bodies, and hyaline inclusions rich in proteins of neurofilaments. Many different proteins have been found associated with these abnormal aggregates, including intermediate filaments proteins like neurofilaments and peripherin (222), SOD1, kinases involved in signal transduction like p38MAPK (39) and Cdk5 (271), cystatin C (a cysteine protease inhibitor that plays an important role in regulating extracellular protein homeostasis in the CNS) (280), TDP43 (a nuclear factor that functions in regulating transcription and alternative splicing) (19), and many others [(415) and references therein)]. To which extent all these proteins are directly involved in aggregate formation or are simply entrapped within these proteinaceous aggregates remains to be established.

Several hypotheses have been put forward to explain the formation and function of such intracellular aggregates in ALS. Misfolded proteins may be generated as a consequence of conditions increasing the burden of oxidative stress and producing carbonylated, glycated, nitrated, or otherwise modified forms of abundant proteins such as SOD1 or those participating in the cytoskeleton network (62, 73, 87, 116, 175, 350, 367). Conversely, intraneuronal aggregates may arise through impaired degradation of misfolded protein, either because of impairment of the ubiquitin-proteasome pathway or of altered chaperone activity or both. Ubiquitin-containing inclusions are the most frequently reported inclusions in ALS (both sporadic and familial). Aggregates of ubiquitin-immunoreactive material are also reactive to a series of intermediate filaments, such as neurofilaments, peripherin, and α-internexin, suggesting that an alteration in the handling of these proteins by the ubiquitin-proteasome system (UPS) is associated with the disease (413). Dorfin, a RING-finger–type E3 ubiquitin ligase, shows a distribution pattern parallel to that of ubiquitin in both familial ALS with SOD1 mutation and sporadic ALS, suggesting that Dorfin participates in the formation of ubiquitylated inclusions (170, 273). p62, a protein that has been proposed to act as a shuttling factor of polyubiquitinated proteins to the proteasome, selectively interacts with mutant SOD1s, but also localizes in ubiquitinated skein-like inclusions in sporadic, non–SOD1-linked ALS cases (26, 345). Interestingly, Bunina bodies, known to be ubiquitin-negative, do not contain p62. These observations point to a role of p62 in forming the inclusions both in fALS and in sALS, likely by linking misfolded mutant SOD1 molecules or other cellular proteins to the UPS (129, 262).

Mutant SOD1 is indeed a major component of inclusions associated with fALS (415). Inclusions that are strongly immunoreactive with SOD1 antibodies are a common feature in transgenic mice expressing various mutant SOD1s (G93A, G85R, and G37R) and resemble those seen in human patients with familial ALS (56). SOD1 itself is degraded by the UPS (188, 384), and oxidative damage increases the level of ubiquitination of mutant SOD1. The proteasome catalytic activity is decreased in the spinal cord of mutant SOD1 mice, in motor neurons and astrocytes of patients with ALS, and in cultured cells expressing mutant SOD1 (69, 187, 253, 384). Accordingly, proteasome inhibition induces selective death of motor neurons in primary mixed cultures derived from embryonic spinal cord, suggesting that these neurons are particularly sensitive to UPS-function blockage (384), although a more recent study has shown that long-term proteasome inhibition causes degeneration of both motor neurons and interneurons, with no significant difference in their survival (395). On this basis, it has been proposed that oxidative damage to key proteins, and to mutant SOD1 in familial ALS, leads to their misfolding, polyubiquitination, accumulation, and aggregation, eventually inhibiting the UPS and generating a sort of self-sustaining vicious cycle (384). Although attractive, the link among misfolding of SOD1, impairment of the UPS, and cell death, has been questioned by recent data, which provided additional insight into the interplay between SOD1 aggregation, ubiquitination, and pathogenesis of the disease. Basso *et al*. (30) showed that SOD1 is mono- and oligoubiquitinated during motor neuron disease progression in the G93A mouse model of fALS. However, ubiquitination occurs only after SOD1 aggregation: insoluble SOD1, which accumulates at presymptomatic stages of the disease, is essentially unmodified, whereas at symptomatic stages, insoluble SOD1 is ubiquitinated. On the basis of these results, the authors suggested that oligoubiquitination may occur as a secondary event, not necessary for aggregate formation, but affecting proteasomal function. Similar to other mono- and oligoubiquitinated proteins that are inefficiently targeted to the proteasome (372), aberrantly accumulated, insoluble SOD1 would be poorly ubiquitinated and thus inefficiently recognized

by proteasomes, thus leading to overload and dysfunction of the ubiquitin-proteasomal system (30).

Obviously, understanding the role of protein aggregates in ALS requires further research, and whether protein aggregates are themselves pathogenic or may even be beneficial to neurons by sequestering toxic factors is a question that has been posed for several other neurodegenerative conditions and remains unsolved.

Protein aggregates may be toxic for motor neurons because they entrap proteins critical for viability, or because they cause a mechanical hindrance interfering with intracellular transport. The former possibility is well illustrated by the work of Pasinelli and co-workers (292), who showed that the antiapoptotic protein Bcl-2 binds to aggregates containing mutant SOD1 that are present in human fALS patients and in spinal cord mitochondria from mutant SOD1 transgenic mice, and suggested that entrapment of Bcl-2 by large SOD1 aggregates may deplete motor neurons of this protein, priming motor neurons for cell death. The latter hypothesis is exemplified by the issue of "axonal strangling." Axonal strangling by protein aggregates would obviously result in impairment of axonal transport, and defects in slow orthograde transport, including the slowing of neurofilament (NF) transport, have been observed in three different mouse models of ALS before disease onset, and NF inclusions increase in parallel with a reduction in axon caliber and with the onset of motor weakness (412, 433). Consistent with the hypothesis that NF aggregates impair axonal transport, overexpression of neurofilament subunits in transgenic mice is sufficient to inhibit transport, resulting in degeneration of motor neurons (81, 421). In line with these reports, transgenic mice overexpressing peripherin, an intermediate filament, showed massive age-related motor neuron degeneration preceded by the presence of neuronal cytoplasmic peripherin aggregates, and similar peripherin inclusions were observed in a mouse model of ALS expressing mutant SOD1 (33).

Defects in axonal retrograde transport have also been observed as one of the earliest phenotypes in mice expressing the SOD1-G93A mutation, in which cytoplasmic dynein co-localized with aggregates of mutant SOD1 in motor neurons (229). A proposed role for dynein is the removal of misfolded or degraded proteins from the cell periphery, and the transport of these proteins back to the cell body for degradation through the dynein–dynactin machinery. In this context, it is noteworthy that mutations in dynactin have been identified in patients with a slowly progressive form of familial ALS (265, 304) (see later) and that the expression levels of dynein-associated polypeptides and dynamin-1 are modulated in models of oxidative stress like chronic ethanol (333) and nicotine treatment (174) and in aging (299).

To test the hypothesis that perturbation of axonal transport may be involved the pathogenic mechanism of ALS, Holzbaur and co-workers (221) engineered a targeted disruption of dynein–dynactin complex in motor neurons of transgenic mice by the overexpression of dynamitin (p50), a dynactin subunit able to disassemble the dynactin complex. These mice showed signs of slow degeneration of motor neurons and denervation of muscle paralleling neurofilament accumulations and a significant inhibition of retrograde transport. In line with this work, mice with a missense point mutation in cytoplasmic dynein heavy chain, called Loa (legs at odd angles) mice, display a progressive motor neuron degeneration resembling some histologic traits of human ALS (153). However, mutations have not been found in the most conserved exons of dynein in ALS cases (5), and defects in cytoplasmic dynein may ameliorate rather than exacerbate the motor neuron degeneration induced by expression of mutant SOD1. Mice obtained by crossing SOD1G93A with Loa mice survived up to 35 days longer than mice expressing the SOD1G93A transgene in a wild-type dynein background, and no transport defects were observed in cultured motor neurons from the SOD1G93A/Loa mice (202). The mechanisms involved in the recovery of transport and the improvement in life span of these double transgenic mice have not been clarified.

3. Excitotoxicity. Among the first hypotheses on the mechanisms of ALS, excitotoxicity due to excessive glutamate levels was proposed on the basis of a seminal article reporting increased levels of glutamate in the CSF of many sALS patients (328). Excess glutamate may be the consequence of decreased reuptake of this neurotransmitter, which, in turn, may result from inactivation or loss of its transporters.

In patients with ALS, Rothstein *et al*. (329) found a marked decrease in the maximal velocity of transport for high-affinity glutamate uptake in synaptosomes from spinal cord, motor cortex, and somatosensory cortex. In a later study by immunohistochemical analysis, the same authors reported that glutamate transporter EAAT2 was severely decreased in ALS brain tissue, both in the motor cortex and in the spinal cord. As no loss of astroglial was found, the authors hypothesized a primary defect in the EAAT2 protein (330). This finding was supported by other studies reporting that >50% of sALS patients have a dramatic loss of EAAT2 compared with controls (230, 244).

Although no quantitative change in mRNA occurs for glutamate transporters EAAT1, EAAT2, or EAAT3 in ALS motor cortex, even in patients with a large loss of EAAT2 protein and decreased tissue glutamate transport (52), decreased activity of EAAT2 may arise by genetic mutation. The N206S mutation of the human glutamate transporter EAAT2 was reported in a heterozygous sALS patient (17). This mutation caused reduced glycosylation of the transporter, decreased uptake activity, and transport rate, together with a reduction of EAAT2 in the plasma membrane (380). This finding, however, has never been generalized to a significant number of patients and therefore is hardly related to the pathogenesis of ALS.

The loss of functional EAAT2 may also derive from aberrant or alternative splicing of the mRNA coding for this protein. Lin *et al*. (230) identified multiple aberrant mRNAs in neuropathologically affected areas of the nervous system and in the CSF of ALS patients. *In vitro* expression studies suggested that proteins translated from these aberrant mRNAs may undergo rapid degradation or produce a dominant-negative effect on normal EAAT2, or both, resulting in loss of protein and activity. However, alternatively spliced mRNA variants of EAAT2 were found also in postmortem brain specimens of patients with AD, with Lewy body disease (171) and also in control subjects (123), indicating that these EAAT2 variants are not causally linked to ALS.

Interestingly, glutamate transporters are oxidatively modulated. In 1997, Trotti *et al*. (380) found that three Na^+-dependent rat glutamate-transporter subtypes, EAAT1 (GLAST),

EAAT2 (GLT-1) and EAAT3 (EAAC1), undergo opposite functional changes in response to oxidation or reduction of their reactive sulfhydryls. When expressed in *Xenopus* oocytes, GLT1 was inactivated by hydrogen peroxide and by two different ALS mutant SOD1s. This inactivation was prevented by chelation of the copper ion or by the use of an antioxidant [Mn(III)TBAP], suggesting that Cu-dependent pro-oxidative chemistry catalyzed by mutant SOD1 may exert its toxic function through inhibition of this transporter (379).

In an astrocyte model system, the expression and the amount of protein of the three EAAT transporters remained unchanged under conditions of oxidative stress. However, L-glutamate uptake was significantly lower, and partial recovery was obtained in the presence of DTT and GSH ethyl ester, suggesting that oxidative stress alters transporter activity without changing transporter expression (259). This hypothesis was supported by a study in another cell model, human neuroblastoma SH-SY5Y cells expressing a mutant SOD1, in which we observed no difference in EAAT1, EAAT2, and EAAT3 glutamate transporter mRNAs and immunoreactive proteins compared with parental cells. However, the V_{max} of glutamate uptake was reduced in mutant cells, with no change in K_m, suggesting that one or more transporters were functionally inactivated, possibly because of increased oxidative stress induced by the mutant SOD1 (335).

A different hypothesis was proposed from data obtained in polarized epithelial canine kidney cell lines, in which stable expression of mutant SOD1 causes the downregulation of GLT-1 by increasing the internalization and degradation of the surface transporter (390).

On this basis, it is not clear whether the loss of EAAT2 is a primary cause of ALS or a consequence of oxidative damage or some other fortuitous stress. One possibility is that the impairment of its function contributes only to the rapid progression of the disease. Overexpression of EAAT2 in the SOD1 mutant mice was reported to delay the grip-strength decline and motoneuron loss (148), but the onset and the outcome of ALS were not modified, a fact that clearly indicates that loss of EAAT2 may contribute to, but does not cause, motor neuron degeneration in ALS. This was supported by a time-course study in which the transgenic mutant SOD1 mouse had a significant decrease in the level of the glial glutamate transporter, but not its mRNA, only at the advanced stage of the disease (38).

Conversely, an early decrease of GLT1 was observed in transgenic rats expressing high levels of mutant SOD1 well before neuronal damage (173). A β-lactam antibiotic (ceftriaxone), that acts as potent stimulator of the transcription of the gene coding for EAAT2 in the brain, has been reported to delay decline in motoneurons and muscle strength and increase survival in mutant SOD1 transgenic mice (331). This finding has encouraged new hopes for a valid therapeutic agent. However, it must be recalled that the same antibiotic already had been tested in ALS patients in an open trial without substantial evidence of efficacy in terms of improvement of muscle strength and disability (35). Finally, it is worth mentioning that EAAT2 loss occurs in other neurodegenerative diseases like Alzheimer's disease (227) and other neurologic conditions (91), and therefore its misregulation may not be considered specific for ALS.

Changes in glutamate receptors, in particular the GluR2 subunit of the AMPA receptor, have also been involved in ALS because treatments with AMPA-receptor antagonists can slow the disease and prolong the survival of mutant SOD1 transgenic mice [(318) for review]. The evidence indicating that reduction in RNA editing of GluR2 occurs specifically in the motor neurons of patients with sALS (197) was not confirmed in transgenic rats carrying SOD1 mutants at the symptomatic stage (198), and GluR2 mRNA in motoneurons of ALS patients did not differ from those in the control group (196). In other studies in SOD1 mutant mice, the overexpression or deficit of GluR2 AMPA receptors ameliorates or accelerates disease progression, respectively (370, 388).

The relevance of excitotoxic processes as initiators of ALS pathogenesis has been questioned, not only because they may become relevant relatively late in the course of the disease, but also because several antiglutamate drugs have been proposed for clinical treatment of ALS, with no relevant benefit. The only noteworthy exception is represented by riluzole, which is also the only drug currently approved for ALS treatment. However, although prolonged treatment with riluzole is relatively well tolerated, >10 years of use has clearly demonstrated that it prolongs survival very modestly. This information, together with the knowledge that riluzole has several other effects, including a mild antioxidant activity (209, 362), cast doubt on the relevance of glutamate toxicity in ALS.

4. Deficit of neurotrophic factors and neuroinflammation. A wealth of data exist indicating that neurotrophic factors (NTFs), which act as survival and differentiation factors in the nervous system, can rescue damaged neurons, including motor neurons in models *in vivo* and *in vitro* [for a review, see (106)]. As in other neurodegenerative conditions, a deficit of NTFs has been proposed as a component of the complex mechanisms underlying the pathogenesis of ALS. However, it is not clear whether imbalance of neurotrophic levels is indeed involved in the pathology of ALS. For instance, when the immunoreactivity for neurotrophin-4/5 (NT-4/5), neurotrophin-3 (NT-3), and brain-derived neurotrophic factor (BDNF) was studied in postmortem motor cerebral cortex, no difference in number or intensity of immunostained neurons was found between ALS and controls (101). In a later study, the expression of nerve growth factor (NGF), BDNF, NT-3, and NT-4/5 was investigated in postmortem muscle tissue of the biceps from patients with ALS, and both mRNA and protein levels of all four neurotrophins were found to be increased (216), whereas other studies have indicated that both NGF and its high-affinity receptor TrkA are not expressed in spinal cords from ALS patients (4). Motor neurons in ALS patients might be sufficiently supplied with endogenous BDNF from other neuronal subpopulations in the spinal cord (199), and GDNF but not BDNF was found to be increased in CSF in ALS patients (143). The expression of the high-affinity functional receptor for BDNF, TrkB, was increased in ALS spinal cords but much less phosphorylated on tyrosine residues than on those of controls (267). GDNF expression was found to be increased in muscle biopsies from ALS patients, whereas GDNF receptor-α mRNA levels were not changed significantly in the diseased muscles (142, 422).

Although no conclusive evidence exists that growth factors are limiting in ALS, NTFs administration in rodent models of motor neuron disease yielded a remarkable effect on survival

of degenerating motor neurons and rescue of axotomized motor neurons. For instance, treatment with BDNF has been shown to be neuroprotective for motor neurons undergoing degeneration in wobbler mice (176, 381), and ciliary neurotrophic factor (CNTF) prevents degeneration of motor neurons in mice with a progressive motor neuronopathy (346). On this basis, treatment with NTFs has been tried in the transgenic ALS mice. Adenoviral (AVR) and adeno-associated viral (AAV) vectors have been used to introduce GDNF into muscles of the SOD1-G93A mouse model of ALS. In this model, GDNF modestly, but significantly, prevents motor neurons from degeneration, delays the disease onset, and prolongs the life span in the treated mice (3, 399), whereas intramuscular injection of GDNF protein does not produce any improvement in clinical data (241). Spinal motor neurons in the SOD1-G93A mouse model of ALS were rescued by systemic administration of leukemia inhibitory factor (LIF) (22) or *VEGF* (402, 438), a growth factor originally identified for its ability to affect angiogenesis [for a review, see (44)].

These data prompted a number of preclinical trials, and some of these promising NTFs have been administered to groups of ALS patients. None of the tested factors has succeeded in altering the outcome of the disease. In a phase III trial using treatment with BDNF in a large cohort of ALS patients, the primary end-point analysis failed to demonstrate a statistically significant survival effect of BDNF in ALS, although *post hoc* analyses showed that a subset of patients showed statistically significant benefit (2). In another study, the intrathecal delivery of BDNF in doses of up to 150 μg/day was well tolerated, but the small number of patients did not allow any conclusions about the efficacy of the treatment (275).

Ciliary neurotrophic factor (CNTF) is another NTF that has been suggested to play a role in ALS, because abolishing CNTF gene expression in mice causes progressive atrophy and loss of motor neurons in adult mice, accompanied by a small but significant reduction in muscle strength (248). A patient with SOD1-linked familial ALS who had a homozygous CNTF gene defect showed significantly earlier disease onset (134). This trait could be reproduced in the SOD1 transgenic mice (134), suggesting that CNTF acts as a modifier gene that leads to early onset of disease; however, this may be true only in SOD1-linked ALS, because among 400 patients with ALS, Al-Chalabi *et al*. (9) found no difference in age at onset, clinical presentation, rate of progression, or disease duration for those with one or two copies of the null allele, suggesting that CNTF is not a major disease modifier in ALS. CTNF had been previously tried in a double-blind, placebo-controlled trial in 570 patients with ALS, by using increasing doses of recombinant human CNTF. No beneficial effect on any measure of ALS progression was observed, whereas adverse events and increased deaths were observed with the highest doses of rhCNTF (256).

A possible explanation for the lack of clinical efficacy of NTFs is that it is not clear which NTFs receptors are expressed in (human) motor neuron in adult life. As reported in a study on mice (435), the expression of neurotrophic factor receptors in postnatal spinal motor neurons is not static, but undergoes dynamic changes. Expression of some receptors remains relatively stable in embryonic and postnatal spinal motor neurons, whereas others are progressively downregulated in postnatal life until they are lost in the adult, and this process is faster in transgenic mice expressing mutant SOD1.

The choice of NTFs may also be very relevant. For instance, in a cell model consisting of organotypic spinal cord cultures, glutamate-induced motor neuron degeneration is prevented by IGF-I, GDNF, and NT-4/5, but not by BDNF, CNTF, and NT-3 (79). This concept is in line with recent experiments in purified cultured corticospinal motor neurons, whose damage contributes to the loss of motor function in ALS, showing that IGF-1 specifically enhances the extent and rate of axon outgrowth, whereas BDNF enhances branching and arborization, but not axon outgrowth (288).

As discussed in two consecutive articles by the group of Luis Barbeito (63, 294), fibroblast growth factor-1 (FGF-1) can be released by motor neurons in response to oxidative stress to induce astrocyte activation. Although FGF-1 is known to be neuroprotective after spinal cord injury or axotomy, they found that it could activate spinal cord astrocytes in a manner that decreased motor neuron survival in co-cultures and in the degenerating spinal cord of transgenic mutant SOD1 mice. This may be explained by the complex interaction between astrocytes and motor neurons in ALS.

Several studies from the last two decades reported indications of inflammatory reactions in ALS patients. Although these kinds of studies are difficult to conduct, and contrasting results have sometimes been obtained, CSF, plasma, and epidermis from ALS patients were found to have large amounts of cytokines and chemokines, such as interleukin-6 (IL-6), tumor necrosis factor α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), monocyte colony-stimulating factor (MCSF), and transforming growth factor β1 (TGF-β1) (163, 177, 283, 296).

Compelling evidence of an association between inflammation and the progression in the pathogenesis arises also from studies in murine models of fALS. In both the mutant SOD1 transgenic mice and rats, several authors reported an increase in the expression of proinflammatory factors before the onset of the disease, with a sustained microglial activation throughout the active phase of the disease progression. For instance, both TGF-β1 and MCSF expression are increased in mice during the presymptomatic phase, whereas TNF-α expression appeared to increase well before beginning of motor deficits (10, 109, 156, 164, 165, 420, 429). The specific role of TNF-α in neurodegeneration of murine ALS models was recently questioned by Julien and colleagues (139). They showed that the genetic ablation of TNF-α failed to influence both the onset and the progression of ALS-like disease caused by SOD1 mutations in two different murine ALS models.

Moreover, the absence of TNF-α did not prevent the appearance of gliosis in those mice, implying that the neuroinflammation process in ALS pathogenesis is not due to a single cytokine pathway, but originates from a complex cellular network in which several active molecules participate to build a crosstalk between neuronal and non-neuronal cells.

Other paracrine factors may be relevant in ALS, such as lipid-derived eicosanoids, including prostaglandin E2 (PGE2), a product of cyclooxygenase-2 (COX-2), that are significantly increased in murine ALS models as well as in ALS patients [see for review Hensley (166)].

Significant microglial activation has been reported in presymptomatic mice (109). This phenomenon is associated with an increased level of COX2 mRNA and protein and an in-

crease in PGE2 content limited to lesioned CNS regions (11). The same authors observed a marked increase in COX2 expression in postmortem spinal cord samples from sALS patients (11). These results were confirmed by Maihofner and colleagues (240), who observed an increase in COX2 expression in spinal cord specimens of human sALS cases that parallels an increase of PGE2. COX1 expression did not differ between sALS and control tissues.

In this context, it is noteworthy that enzymatic inhibition of COX2 activity by pharmacologic approaches protects motor neurons and increases survival in mutant SOD1 transgenic mice (100, 297), and this treatment also decreases the levels of PGE2. However, the role of PGE2 was recently questioned by Almer *et al*. (12), who reported that the genetic ablation of COX1 with a subsequent marked decrease of PGE2 levels in an ALS mouse model fails to attenuate neurodegeneration. It should be noted, moreover, that none of the COX inhibitors tested so far has shown efficacy in human ALS patients (89).

The robust inflammatory response found in CNS tissue of several murine ALS models provides an alternative explanation for oxidative stress in these animals. Indeed, it is now well established that some cytokines can trigger microglia to produce copious reactive oxygen and nitrogen species through assembly of NADPH oxidase (NOX), induction of nitric oxide synthase (iNOS), and transcriptional upregulation of lipid-oxidizing enzymes such as COX2 (76). Additionally, other cell types, including astrocytes, express nonphagocytic analogues of NOX that contribute to increase oxidative stress during the inflammatory process (219).

B. Genetics of ALS

Familial ALS can be inherited either as an autosomal dominant or autosomal recessive trait. Linkage studies indicated that both types of inheritance are represented by more than one distinct genetic entity. Dominant fALS is clinically and pathologically indistinguishable from sALS, and like sALS, it is a disease of adulthood, although juvenile cases are occasionally seen.

Several loci have been identified by genetic analysis, but only a few of them have been assigned to specific genes (Fig. 2). These include genes coding for copper/zinc superoxide dismutase [SOD1, (94, 327)], alsin (152, 424), senataxin (68), dynactin 1 (265, 304), VAMP (vesicle-associated membrane protein)–associated protein B (VAPB) (272), and angiogenin (140, 141). These genes encode proteins involved in a wide range of cellular processes, from oxidation to axonal transport, RNA processing, DNA repair, vesicular transport, and angiogenesis.

1. Mutation in SOD1. ALS1 is located on chromosome 21 and was shown to be the locus coding for SOD1.

Wild-type SOD1 (wtSOD1) is a very well characterized, abundant homodimeric enzyme present in virtually every cell type. Each monomer binds one zinc and one copper ion, with the Cu atom playing the active role in the removal of superoxide through a well-described mechanism (Fig. 3), and the Zn atom conferring structural stability to the enzyme. Most of wtSOD1 remains in the cytoplasm after synthesis, whereas small fractions localize to mitochondria (278), in nuclei (339), or are secreted (385).

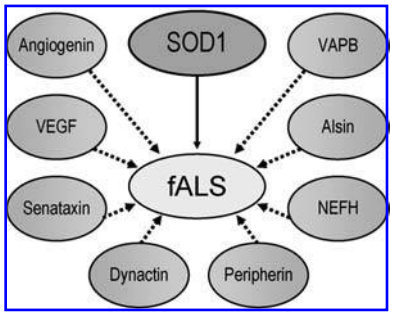

FIG. 2. Genetics of human ALS. The only gene conclusively associated with familial ALS by genetic analysis is the one coding for SOD1. Several other loci have been identified, but only a few of them have been assigned to specific genes. These genes encode proteins involved in a wide range of cellular processes, from oxidation to axonal transport, RNA processing, vesicular transport, and angiogenesis. Despite some disagreement about the relevance of such candidate genes as a primary cause for ALS, their identification has contributed to suggesting pathways that may be altered and may play a role in the pathogenesis of the disease.

More than 130 different SOD1 mutations were reported in fALS families with various frequencies in the population (14). Most of these mutations act dominantly, except one that is linked to recessive inheritance; recessive forms of ALS usually have juvenile onset and may have a very long course. The vast majority are point mutations, except a few that cause deletion, insertion, or premature termination and truncation of a short C-term portion of the protein.

Mutations are distributed in all five exons of the gene and result in alteration of amino acids scattered throughout the protein structure: whereas some mutations affect the active site, others affect proper folding or stability of the dimer. Biophysical, biochemical, and bioinorganic investigations have shown that ALS-associated mutant SOD1 proteins are extremely heterogeneous (347).

The absence of neurodegeneration in SOD1-knockout mice, together with the normal enzymatic activity of many SOD1 variants *in vitro* and *in vivo* (see later), demonstrate that mutation in SOD1 results in toxicity by imparting an additional function on the variant SOD1 protein rather than by losing or diminishing superoxide scavenging activity. Therefore, SOD1-linked fALS is considered a gain-of-function rather than a loss-of function disease.

2. Other genes. Besides SOD1, a number of genes potentially involved in fALS have been described. In most cases, because of the low prevalence of ALS, genetic analyses have been performed on relatively small populations, and although many gene association studies have been performed in ALS, only a few have led to the identification of candidates with repeatable result (355). In most cases, either linkage studies have not been confirmed in multiple kindreds and in wider populations or mutated genes have been found associated also with other neurodegenerative disease and therefore should be viewed as possible risk factors, more than truly causative of ALS. Nonetheless, the discovery of mutations in those candidate genes in

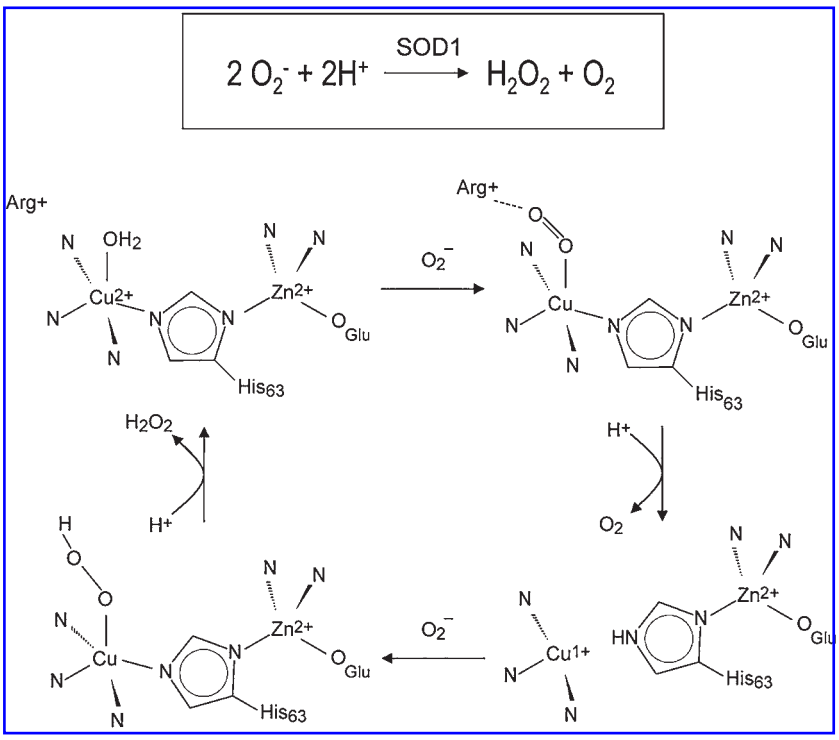

FIG. 3. SOD1 reaction and mechanism of action. SOD1 catalyzes the disproportionation of superoxide (O_2^-) to give molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) in a two-step process: one molecule of O_2^- first reduces the Cu^{2+} ion to form dioxygen, and then a second molecule of O_2^- reoxidizes the Cu^{1+} ion to form hydrogen peroxide. In the oxidized (Cu^{2+}) form of the enzyme, the imidazolate group of His63 acts as a bidentate ligand, bridging the copper and zinc ions. In the reduced form (Cu^{1+}), copper moves away from the His63 nitrogen, which continues binding the zinc ion. Arg143 plays an important role in the catalytic process in positioning superoxide.

some patients or in specific populations may greatly contribute in suggesting pathways that may be altered and play a role in the pathogenesis of the disease.

A few cases exemplifying this concept are worth mentioning. The chromosomal region 14q11.2 was identified as a candidate region for fALS by Hayward *et al*. (160), and Greenway *et al*. (140) reported an association of the SNP rs11701 in the single gene coding for angiogenin (ANG). This was extended in a later study (141), in which the rs11701 SNP was genotyped in 1,629 individuals with ALS and in 1,264 controls from five independent populations. This study confirmed the association in the Irish and Scottish populations with ALS, although no association was observed in the populations from the United States, England, or Sweden. No association was found in Italy (93) and Turkey (N. Basak, personal communication). ANG is a hypoxia-regulated gene coding for a 123-residue, 14.1-kDa protein and a member of the pancreatic ribonuclease A superfamily. Its RNase activity is important for the angiogenic activity in many tissues, but ANG is expressed also in the neuroaxis, including in the motor neurons, and therefore may represent an inducer of neuroneovascularization *in vivo*. Some ANG mutations found in ALS patients are located within the catalytic core, and one of them is in the nuclear localization signal; therefore, one could expect that ALS is associated with a loss of function of the mutated angiogenin. However, patients with ALS exhibited higher, rather than diminished serum angiogenin than did control subjects already at diagnosis (85). Although these data suggest that elevation in serum angiogenin may be used as a biomarker for ALS, it is not clear what may be the exact role of ANG in ALS. Interestingly, in endothelium, angiogenin can modulate activity of *VEGF*, because downregulation of ANG decreases the level of mRNA transcription induced by *VEGF* and decreased nuclear translocation of ANG abolishes the angiogenic activity of *VEGF*. *VEGF* is a cytokine that controls new blood vessel growth. In the nervous system, however, it also supports the growth and survival of neurons *via* angiogenic, neurotrophic, gliotrophic, and antiapoptotic activity and therefore exerts an important protective function. *VEGF* is considered a risk factor in the pathogenesis of fALS because studies of 600 individuals with ALS and 1,000 case controls showed that ALS at-risk haplotypes in the *VEGF* promoter exist in the Swedish, Belgian, and English populations with ALS (220). Subjects homozygous for some haplotypes in the *VEGF* promoter/leader sequence had a 1.8 times greater risk of ALS. These "at-risk" haplotypes resulted in reduced circulating *VEGF* levels *in vivo* and reduced *VEGF* gene transcription, *VEGF* expression, and translation of a different *VEGF* isoform. However, association of *VEGF* promoter polymorphisms with sporadic ALS was not found in a Dutch population (389), in North American subjects (67, 371), and in Chinese patients (437). Although *VEGF* is a putative modifier of ALS, mutations in that gene have not been found in Italian individuals with ALS (93), in Turkish patients (N. Basak, personal communication), and in German patients, unless a gender-dependent association is considered (114). Nonetheless, the expression of *VEGF* and of its major receptor (*VEGFR2*) is reduced in anterior horn cells from patients with ALS (53), and *VEGF* is reduced also in the CSF (186). Furthermore, dysregulation of *VEGF* through deletion of its hypoxia-regulatory element causes motor neuron degeneration and an ALS-like phenotype in mice (284).

Among candidate genes for ALS, some are involved in RNA metabolism. For instance, dominant mutations within SETX, the gene coding for senataxin, are the cause of ALS4 (68), a rare juvenile form with a slow rate of progression. Recessive mutations in this gene had been previously identified as a cause

for ataxia-oculomotor apraxia type 2, a condition characterized by cerebellar ataxia, oculomotor apraxia, peripheral neuropathy, and immunodeficiency (264). The existence of two different phenotypes associated with a different pattern of inheritance of the same gene strongly suggests that motor neuron degeneration associated with SETX mutations arises from a gain-of-function mechanism of the mutated protein. Wild-type senataxin may have both RNA and DNA helicase activities and may have a role in RNA processing. What may be the role of alteration of RNA processing in neurodegeneration associated in ALS is not clear. However, it is interesting that the survival motor neuron (SMN) protein, another protein that functions as an assembly factor for snRNPs and has therefore been implicated indirectly in general cellular RNA processing, is associated with degeneration of motor neurons in spinal muscular atrophy (SMA) (324). Several studies on the association of SMN with ALS had controversial results (84); evidence suggests that SMN genotypes producing less SMN protein increased susceptibility to and severity of sporadic ALS, and alterations in the number of copies of SMN1 or SMN2 was found significantly frequently among ALS patients compared with controls (393). However, in a more recent and larger study of 600 patients with sporadic ALS and 621 controls, SMN1, but not SMN2, gene copy number was found to be a risk factor for sporadic ALS (78). In this context, it is worth mentioning that mitochondrial damage modulates alternative splicing in neuronal cells (243), and the unbalance in a vast number of mRNA isoform may contribute to neurodegeneration in ALS, where mitochondrial damage is an early sign preceding motor neuron loss.

Involvement of impaired intracellular transport in the pathogenesis of ALS is suggested by the work of Parkinson and colleagues (291), which identified a heterozygous mutation in exon 6 of the gene coding for CHMP2B, a member of the chromatin-modifying protein/charged multivesicular body protein (CHMP) family in a 75-year-old man with a rapidly progressive form of ALS and no evidence of dementia or extramotor neurologic involvement. The CHMP proteins are components of ESCRT-III (endosomal sorting complex required for transport III), a complex involved in degradation of surface receptor proteins and formation of endocytic multivesicular bodies. CHMP2B is expressed in all neuronal populations, especially in the hippocampus, frontal and temporal lobes, and cerebellum, but not in astrocytes or oligodendrocytes (356). However, mutations in this gene have been associated with frontotemporal dementia (356), a condition only occasionally seen in ALS patients.

The involvement of impaired axonal transport in ALS is suggested also by genetic studies. ALS8, an early-onset, slowly progressive, lower motor–predominant form of ALS accompanied by an unusual tremor, was found linked to a dominant missense mutation in the VAMP (vesicle-associated membrane protein)–associated protein B (VAPB) (272). The P56S mutation of VAPB was also found in other kindreds with phenotypes ranging from late-onset spinal muscular atrophy to late-onset atypical ALS with slow progression (245). VAPB is involved in vesicle trafficking in the endoplasmic reticulum to Golgi transport but may also be involved in axonal transport of membrane components because it associates with microtubules. The P56S mutation induces a shift in the localization of VAPB from the endoplasmic reticulum to other compartments. Kanekura and collaborators (191) demonstrated that overexpression of wild-type VAPB promotes unfolded protein response (UPR), an ER reaction to suppress accumulation of misfolded proteins, whereas siRNA for VAPB attenuates UPR, and expression of P56S-VAPB inhibits UPR by inducing aggregate formation and mislocalization of wt-VAPB (191). However, families with the P56S VAPB mutations were all on the same haplotype, suggesting a founder effect that occurred before the Portuguese colonization of Brazil, and in a more recent study, the association between VAPB mutations and sporadic ALS was ruled out (204).

As mentioned earlier, mutations in the gene coding for dynactin are another proposed susceptibility factor for ALS. Dynactin binds directly to microtubules and to cytoplasmic dynein, and its primary role in motor neurons is retrograde axonal transport of vesicles and organelles along microtubules. A point mutation in the largest polypeptide of the dynactin complex was identified in a family with an autosomal dominant form of distal motor neuropathy (304), and three other dynactin mutations were subsequently found in German ALS patients (266). Although the families analyzed were too small to conclusively prove genetic segregation, the latter study suggested that dynactin mutations may be a susceptibility factor for ALS.

A contribution of impaired axonal transport is also suggested by the fact that different isoforms of peripherin, a neuron-specific type III intermediate filament protein, may be involved in ALS. Overexpression of wild-type peripherin in mice resulted in massive degeneration of motor axons and formation of inclusions in motor neurons and neurites (33). In mouse, three isoforms are found: Per58, Per56, and Per61. Per61 contains a 32-amino acid insertion, and it is unable to assemble properly, forming intracellular aggregates *in vitro* that are neurotoxic to motor neurons. *In vivo*, Per61 expression was detected in motor neurons of transgenic ALS mice expressing the SOD1 G37R mutation and in pathologic spinal cord lesions in familial ALS patients (323) and was identified in Lewy body–like ubiquitinated inclusions in motor neurons from patients with ALS (161). A homozygous mutation in the peripherin gene was found in a patient with ALS. The mutation resulted in large aggregates within the cell bodies of residual spinal motor neurons and was also immunoreactive with antibodies to the neurofilament proteins (223).

Depositions of neurofilaments occur in the perikarya and proximal axons of motor neurons in ALS patients and may cause axonal degeneration by impeding the transport of components required for axonal maintenance. Deletions and insertions within the neurofilament heavy-subunit (NEFH) gene have been found by several independent studies in different populations (7, 121, 376), both in a few patients with sporadic ALS and in a family with autosomal dominant ALS. This observation was not confirmed in other studies that also extended the analysis to other neurofilament proteins (326, 392), reporting that polypeptide sequence variants were found at comparable frequency in DNAs from normal individuals and that no variant cosegregated with familial disease. However, absence of NF-H in mice does not significantly affect the number of neurofilaments or axonal elongation or targeting in peripheral motor and sensory axons, but it does affect the efficiency of survival of motor and sensory axons (316). On the whole, those

studies suggested that alteration of NEFH content may represent a mechanism involved in loss of motor neurons and that NEFH mutations can be a primary, albeit uncommon, cause of ALS.

Mutations in the locus ALS2 may represent another factor of susceptibility to ALS. Seven different mutations in ALS2 have been associated with different forms of juvenile ALS (110, 111, 152, 212, 424), although this has been discussed by studies that found no significant association between variants in the ALS2 gene and sporadic ALS (8, 158), and mutations in ALS2 are associated with other conditions such as primary lateral sclerosis (289). The ALS2 gene is expressed in various tissues and cells, including neurons throughout the brain and spinal cord, and codes for a protein called alsin, a guanine-nucleotide exchange factor that specifically binds to small GTPase Rab5 (287). *In vitro* and *in vivo* studies performed with full-length and truncated forms of alsin protein support its role in endosomal dynamics (96, 377) and trafficking of mitochondria, but also a function in AMPA-receptor trafficking (217). All ALS2 mutations reported so far generate protein truncation. The truncating nature of the mutations and the recessive pattern of inheritance suggest that motor neuron degeneration is the result of a loss of function. However, loss of ALS2 function is insufficient to trigger motor neuron degeneration; mice genetically deprived of alsin failed to recapitulate clinical or neuropathologic phenotypes consistent with motor neuron disease by 20 months of age and showed signs typical of hereditary spastic paralysis rather than ALS (423), but neurons are more susceptible to oxidative stress (57). Finally, deficiency in the ALS2 gene does not affect the motor neuron degeneration in mutant SOD1 transgenic mice (231), although alsin knockdown by RNA interference causes death of embryonic rat spinal motor neurons (180).

The relation between genetic susceptibility and the mechanisms operating in sporadic ALS is not always obvious. A very interesting exception may be represented by the recent report that the Ser326Cys polymorphism of the human 8-oxoguanine DNA glycosylase 1 (hOGG1) gene is associated with an increased risk of sporadic ALS in males and with a reduced DNA-repair activity (77). OGG1 releases free 8-hydroxyguanine from oxidized DNA and introduces a chain break in a double-stranded oligonucleotide specifically at an 8-hydroxyguanine residue base-paired with cytosine and therefore is involved in repair of the major mutagenic base lesion in DNA caused by exposure to ROS (325). This observation supports the idea that, besides increased oxidative burden, decreased repair activity also caused by mutations in DNA repair enzymes may be linked to the pathogenesis of sporadic ALS.

From that reported earlier, it is clear that our understanding of the genetics of familial ALS is still very incomplete, and further studies are needed.

Genetic studies also contributed to a better understanding of sporadic ALS, in which a combination of a genetic component or an environmental factor on the background of an aging nervous system (or both) are involved. This view has prompted a recent genome-wide association study using samples from unrelated, white, non-Hispanic U.S. patients with sporadic ALS and neurologically normal controls (344). Although 34 potentially associated candidate single-nucleotide polymorphisms (SNPs) were identified, in this study, no single locus was definitively associated with increased risk of developing disease; nonetheless, extension of this kind of study to other populations will most probably allow a better understanding of sALS.

II. MUTANT SOD1 AND ALS

A. Toxicity of mutant SOD1

The first evidence of the involvement of SOD1 in familial ALS was provided by Siddique *et al*. (353), who demonstrated the linkage of a gene causing fALS to a locus on chromosome 21. Rosen *et al*. (327) identified 11 different SOD1 missense mutations in 13 different ALS families and proposed two possible mechanisms by which mutations in SOD1 could cause fALS.

Mutations could reduce enzyme superoxide dismutase activity, leading to an accumulation of the toxic superoxide radical, or cause the appearance of a new toxic function leading the mutant protein to catalyze aberrant reactions or to promote toxic protein aggregation.

The loss-of-function hypothesis was quickly questioned by several works. High-level expression of a mutant SOD1 that retains a wild-type–like enzyme activity (G93A), led to an ALS-like pathology in transgenic mice (150). These mice, although characterized by a systemic increase of total SOD activity, clearly showed traits of motor neuronal degeneration. Conversely, SOD1-deficient mice developed normally and showed no overt motor deficits by 6 months of age, although these mice exhibited marked vulnerability to motor neuron loss after axonal injury, indicating that SOD1 is required under stressful conditions (320). Furthermore, elevation of wild-type SOD1 was found to have no effect on mutant-mediated disease in transgenic mice (56), and most loss-of-function mutations cause a recessive rather than a dominant effect.

Two hypotheses have been proposed to explain the gain of toxic function of mutant SOD1 proteins. The "oligomerization/aggregation" hypothesis maintains that mutant SOD1 proteins are, or become, misfolded and consequently oligomerize into increasingly high-molecular-mass species that ultimately lead to the death of motor neurons [see for review (387) and later]. The "oxidative damage" hypothesis proposes that ALS mutant SOD1s catalyze oxidative reactions that damage substrates critical for viability of the altered cells or, alternatively, they promote their own oxidative damage. In an early article, Wiedau-Pazos *et al*. (408) reported that two different mutant SOD1s catalyzed the oxidation of a model substrate by hydrogen peroxide at a higher rate than that seen with the wild-type enzyme. Catalysis of this reaction by these two mutant SOD1s was more sensitive to inhibition by two copper chelators (diethyldithiocarbamate and penicillamine) that also reversed the proapoptotic effect of mutant enzymes expressed in a neural cell line.

A mechanism explaining the prooxidant properties of mutant SOD1 has been proposed by Yim and co-workers (426, 427). The authors, analyzing some biochemical properties of two mutant SOD1s, which retain wild-type–like SOD activity, proposed that the enhanced free radical–generating function of mutant SOD1s relative to that of the wild-type enzyme, particularly at lower H_2O_2 concentrations, is due to a small decrease in the

value of K_m for H_2O_2 for the mutant SOD1s. The SOD1 prooxidant activity has been further characterized by Sankarapandi and Zweier (337), who established that SOD1 acquires peroxidase activity at physiologic pH only in the presence of HCO_3^- or structurally similar anions. In a later study, Elam and coworkers (107) discovered that the bicarbonate ion enhances the rate of inactivation of SOD1 by hydrogen peroxide, damaging amino acid residues at the active site.

The relevance of SOD1 prooxidant activity in ALS pathology is still widely debated. However, Zhang and colleagues (434) showed that bicarbonate-mediated SOD1 peroxidase activity induces covalent aggregation of human SOD1 *in vitro* through a self-oxidation reaction. These data suggest a link between the oligomerization/aggregation hypothesis and the oxidative-damage hypothesis and provide a possible explanation for SOD1-linked phenotype, because intracellular SOD1 aggregation is a typical hallmark of pathology in several ALS models, as well as in patients (415).

A tight correlation between exogenous oxidative stress with *in vivo* formation of mutant SOD1 aggregates has been proposed by Oeda and colleagues (276). These authors generated transgenic *Caenorhabditis elegans* strains expressing three different mutant human SOD1 proteins, which retain wild-type–like SOD1 activity. These transgenic strains showed greater vulnerability to oxidative stress induced by a superoxide generator than did a control strain that expressed wild-type human SOD1. In the absence of oxidative stress, mutant SOD1s were degraded more rapidly than the wild-type human SOD1 in *C. elegans*. In the presence of oxidative stress, however, this rapid degradation was inhibited, and the transgenic *C. elegans* expressing mutant human SOD1 in muscle tissues demonstrated discrete aggregates in the adult stage (276).

The oxidative-damage hypothesis would require that copper (or some other redox active metal ion) be bound to the mutant SOD1 protein to promote the oxidation reaction. However, several ALS mutations affect the metal-binding capacity of the enzyme, and therefore the role of copper-mediated oxidative damage in neuronal SOD1-mediated toxicity has been questioned. Subramaniam and colleagues (365) removed the gene encoding the copper chaperone for SOD1 (CCS) in transgenic mice expressing fALS-linked mutant SOD1; although the absence of CCS led to a significant reduction in the amount of copper-loaded mutant SOD1, it did not modify the onset and progression of motor neuron disease in SOD1-mutant mice. Further to test the Cu-dependent SOD1 toxicity hypothesis, Wang and colleagues (398) made transgenic mice expressing a mutant SOD1 characterized by the disruption of active site through artificial mutation of four histidines (SOD1-Quad). Although completely inactive, SOD1-Quad induced a motor neuron disease similar in clinical and pathologic appearance to other mouse models of SOD1-linked fALS (398), and histologic analysis of SOD1-Quad mouse tissues revealed the presence of SOD1 aggregates similar to detergent-insoluble forms of mutant SOD1 detected in cultured cells and mice expressing fALS-SOD1 variants (104, 181, 352, 397). This strengthens the hypothesis that motor-neuron–specific toxicity does not require copper bound to a normally configured active site and that the propensity of mutant SOD1 to oligomerize or to form aggregates is the only common (toxic?) property of several investigated mutant SOD1s.

B. Misfolding of mutant SOD1

SOD1 accumulates in many neuronal populations and is particularly abundant in motor neurons, as described by Pardo and collaborators (290). This evidence offered support for the view that high levels of a toxic species of SOD1 (represented by either mutant SOD1 or wild-type SOD1 under stress conditions) may place motor neurons selectively at risk in this disorder. Human wild-type SOD1 is an exceptionally stable protein, characterized by a melting temperature of 95°C, but physiologic metal-catalyzed oxidation causes destabilization and aggregation of wild-type SOD1 *in vitro* [(see for review (312)]. The propensity of oxidized wild-type SOD1 to form oligomers/aggregates has been studied by the group of Chakrabartty (310), which described the propensity of zinc-deficient wild-type SOD1 and mutant SOD1s to form visible aggregates on oxidation, as compared with the wild-type holoprotein (310) and that oxidation of either mutant or wild-type SOD1 leads the enzyme to dissociate into monomers before aggregation (311). Mutant SOD1s displayed greater aggregation propensity than wild-type holo-SOD1 when treated with oxidants (Fig. 4). On the whole, these data imply that wild-type SOD1 may also be involved in oxidation-mediated formation of aggregates, and thus may provide a link between sporadic and familial ALS. The relevance of these processes *in vivo* comes both from the discovery of oxidized SOD1 in a mouse model of ALS (16, 298) and from the presence of monomer/misfolded SOD1 (detected through an antibody that recognizes a buried epitope in the SOD1 native homodimer interface) in three different ALS mouse models and in a human individual with an A4V SOD1 mutation (313).

In line with the notion that a mild oxidative stress increases the propensity of mutant SOD1 to form aggregates, Tiwari and Hayward (375) showed that different classes of mutant SOD1, treated as *holo*proteins with reducing agents, exhibited greater accessibility of cysteine residues to alkylation by iodoacetamide with respect to wild-type SOD1, and that SOD1 cysteines in tissue lysates from ALS transgenic mice exhibited increased accessibility to modifying agents. Furthermore, Furukawa and O'Halloran (125) showed that on incubation *in vitro* with a low ratio between reduced glutathione and oxidized glutathione (GSH/GSSG), an immature state of wild-type SOD1 (characterized by both metal depletion and reduction of the disulfide bond between cysteine residues 57 and 146) formed oligomers, which disappeared when samples were treated with thiolic reductants. The same immature mutant SOD1 formed similar oligomers when its concentration increased toward physiologic conditions. These data suggest that SOD1 multimers are stabilized through the formation of nonspecific intermolecular disulfide bonds, in a process involving the oxidation state of cysteine residues 57 and 146.

The presence *in vivo* of these immature states of mutant SOD1 characterized by alterations in native disulfide bond and prone to oligomerization has been confirmed by several recent works. Marklund and co-workers (184) showed that most of three different mutant SOD1s in the CNS of ALS transgenic mice were inactive because of insufficient Cu charging, and all contained subfractions with a reduced C57-C146 intrasubunit disulfide bond (184). Furukawa *et al*. (126) demonstrated the presence of insoluble disulfide-linked SOD1 multimers, which can incorporate also wild-type SOD1, in the spinal cord of

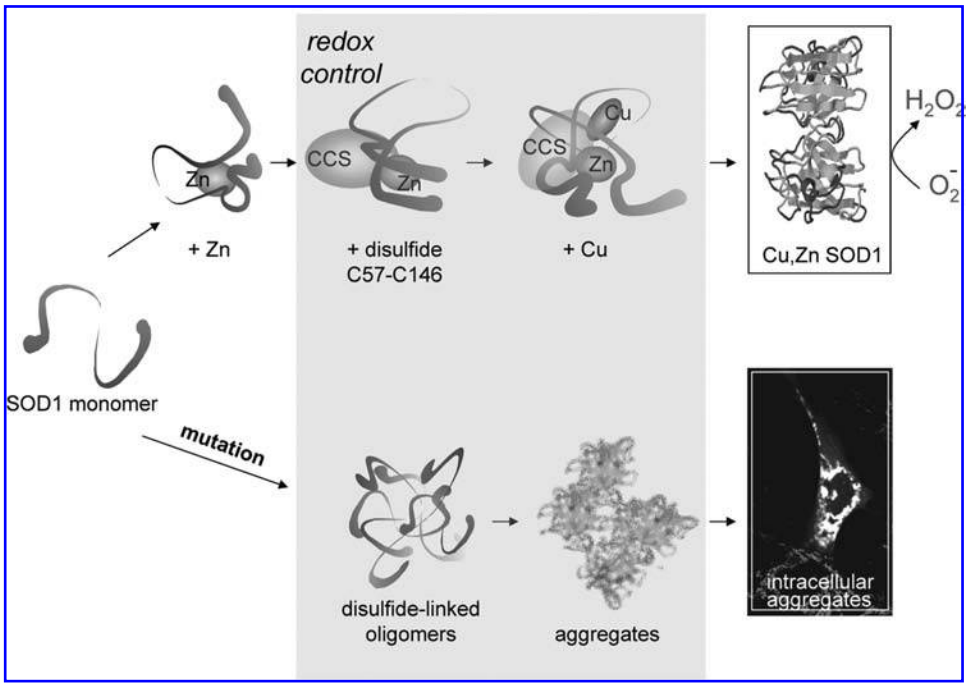

FIG. 4. SOD1 protein folding and aggregation pathways. SOD1 is synthesized as a disulfide-reduced, metal-free monomer. The sequential acquisition of a zinc ion, the formation of a disulfide bridge between cysteines in position 57 and 146 (human SOD1 numbering), and the acquisition of a copper ion are the key events that make SOD1 a completely folded, dimeric, and active enzyme. The two latter steps of the folding process are catalyzed by the copper chaperon for SOD1 (CCS) and require an appropriate redox environment. Any alteration of these processes, due to mutation in the SOD1 polipeptide and/or in variation of the redox environment, could result in the inability of the native protein to fold correctly, and hence in the accumulation of immature forms of SOD1. Immature SOD1 molecules could undergo an oligomerization process mediated by the formation of non-native disulfide bridges. Oligomeric SOD1 could be noxious *per se*, or could work as nucleation center for the formation of larger multimers and ultimately of insoluble aggregates, which could represent the form of SOD1 that is actually toxic for cells. Insoluble aggregates may be visualized by fluorescent antibodies against SOD1 in neuronal cells overexpressing mutant SOD1.

symptomatic ALS transgenic mice, but not in unaffected tissue such as brain cortex and liver. The same disulfide-linked SOD1 multimers were observed in the mitochondrial fraction of both spinal cord of ALS transgenic mice and cultured motor neuron–derived cells (95, 119). As discussed in the next section, these observations may be explained by the findings of Culotta and colleagues (120) that only the most immature form of SOD1 (characterized by metal depletion and reduction of C57-C146 intrasubunit disulfide bond) is taken up into intermembrane space of mitochondria.

The hypothesis that oxidative damage induces wild-type SOD1 to misfold and form aggregates finds further support in an article by Choi and collaborators (72). The authors showed that SOD1 is a target of oxidative damage in Alzheimer's disease (AD) and Parkinson's disease (PD) brains, where it is selectively accumulated as a carbonylated form. Moreover, Cys-146, a cysteine residue of SOD1 that is mutated in familial ALS, is oxidized to cysteic acid in AD and PD brains (72), suggesting an involvement of misfolded wild-type SOD1 in other neurodegenerative pathologies in which oxidative damage has been widely described [see for review (315)].

Zinc binding to the protein appears to be an important event that increases stability of both mutant and wild-type SOD1. Binding of zinc to the enzyme compensates for the loss of stability caused by reduction of the disulfide bond and promotes retention of the dimeric state (20), and zinc binding to metal-depleted and cysteines-reduced proteins decreases the extent of disulfide-linked multimerization of SOD1s (125). Thus, different abilities of mutant SOD1 to coordinate zinc could favor the steady-state levels of the reduced protein, leading to aberrant protein–protein interaction *via* nonnative disulfide bond formation. This condition, as shown by Furukawa and O'Halloran (125), could favor abnormal interactions of mutant SOD1 with itself or with other cellular constituents.

This hypothesis has been questioned by Shaw and Valentine (347), who observed that five mutant SOD1s (E100K, D101N, N139K, D90A, and N86S) among the many ALS-associated SOD1 variants that have been purified and studied so far, although closely similar to wild-type protein in terms of stability and metal coordination properties, are prone to form aggregates more readily than the wild type in different ALS experimental models (42, 119, 383).

To account for this apparent incongruence, Oliveberg and collaborators (232) proposed an alternative mechanism by which mutant SOD1 forms aggregates. Several ALS-associated SOD1 mutations decrease the net negative charge of the SOD1 polypeptide, and this reduction in net charge could promote aggregation regardless of mutant SOD1 affinity for metals. Indeed, alterations of net charge are known to be a factor contributing to protein aggregation (58, 71, 211), and the decrease

in net charge of some pathogenic proteins seems to be important in other familiar diseases associated with protein aggregation [see for review (347) and references therein].

C. Properties of SOD1 aggregates

The biochemical properties of mutant SOD1 aggregates have been largely studied in several recent works, mostly *in vitro*. For instance, DiDonato *et al*. (98) examined the propensity of the structurally destabilized H43R and A4V mutant SOD1s to form aggregates. Dynamic light-scattering and electron-microscopy studies indicated that both mutant proteins aggregated at low pH values, with the greatest amount of aggregation occurring at pH 3.5. Moreover, filamentous aggregates of mutant SOD1s bind Congo red, suggesting the presence of amyloid-like structures.

The aggregation process may occur through a multistep reaction, as proposed by Kare and Dokholyan (200). The authors described, through a dynamic simulation of wild-type SOD1 and three structurally diverse fALS mutants (A4V, G37R, and H46R), that a common effect of mutations on SOD1 dimer was the mutation-induced disruption of dynamic coupling between monomers. Although these observations are in line with the previously mentioned model of aggregation induced by oxidation (310, 311), the relevance *in vivo* of these aggregation processes is debated, because both studies were carried out at nonphysiologic pH, an experimental condition that may affect dimer stability (159).

Further evidence strengthening the relevance of SOD1 quaternary structure in the aggregation process comes from the data of Ray *et al*. (319). The authors demonstrated that a mutant SOD1 (A4V) aggregates *in vitro*, forming amyloid pores structures, whereas wild-type SOD1 is stable. Furthermore, to test the hypothesis that dimer dissociation may be necessary to the first step of aggregation, an intersubunit disulfide bond between symmetry-related residues was introduced at the A4V dimer interface; this disulfide bond stabilized the A4V dimer and completely abolished aggregation.

An accurate description of crystal forms of mutant SOD1s was given by Hart and collaborators (108). Mutant SOD1 crystallized in three different forms that resembled amyloid-like filaments and water-filled nanotubes. All of the described structures depended on aligned beta-sheet interactions that may be promoted by nonnative conformational rearrangement in the metal-depleted mutant enzyme.

Despite all of these studies, whether any of the oligomeric structures described are similar to the SOD1 aggregates that may be causative of ALS remains uncertain. Wang *et al*. (397) reported the presence of ubiquitin immunoreactive inclusions and intracellular fibrillar thioflavin-S-positive, suggesting the presence of amyloid-like inclusions, in several ALS mouse models. By using dynamic imaging analysis of living cells to analyze the aggregation and growth properties of different pathogenic proteins, Matsumoto *et al*. (249) reported that mutant SOD1 (G85R/G93A) forms a porous aggregate structure, through which other nascent proteins may diffuse. Moreover, such mutant SOD1 aggregates colocalized with HSP70, a chaperon protein that participates in the proteasome-mediated degradation of proteins under stress conditions. The latter evidence suggests a link between aggregates formation and the protein quality control performed by protein-degradation machinery. In this context, Bonetto and collaborators (30) recently characterized SOD1 isoforms in a detergent-insoluble fraction from spinal cord of G93A SOD1 mice at different stages of the disease. The authors established that mutant SOD1 accumulates in spinal cord of G93A SOD1 presymptomatic mice as detergent-insoluble aggregates. Part of the insoluble SOD1 was recovered as mono- and oligoubiquitinated forms at symptomatic stages of the disease only. These data imply a central role for the decrease of proteasome activity during the course of the disease, and the authors suggested that the short polyubiquitin chain may have a protective role in limiting further aggregation or proteasome overload or both.

III. MITOCHONDRIAL DYSFUNCTION: A PRIMARY TARGET OF MUTANT SOD1

Mitochondria are "the powerhouse of the cell" because of their ability to convert nutrients into ATP, and they also play an essential role in intermediate metabolism and in maintaining cellular Ca^{2+} homeostasis. However, mitochondria are also the main source of reactive oxygen species (ROS) and function as gatekeepers in the intrinsic apoptotic processes. Thus, mitochondrial dysfunction can result in cell death, either by bioenergetics failure or by apoptosis.

A common trait of many neurodegenerative diseases, including ALS, is damage to mitochondria that contributes to the degenerative phenotype (32). Indications that mitochondrial dysfunctions may participate in the pathogenesis of ALS came from studies conducted essentially in postmortem tissues from sALS patients (242). Morphologic defects along with metabolic deficits in the activities of the respiratory chain complexes have been described in mitochondria from ALS patients, both sporadic and familial (51, 338, 354, 409, 410). Mitochondrial degeneration was not immediately recognized as an important component of motor neuron pathology in familial or sporadic ALS. Only the evidence obtained from seminal studies on animal models of the disease was able to catch the attention of the scientific community on mitochondria as the core of the processes that actually lead motor neurons to death [for a review, see (242)].

In 1995, Wong and colleagues (414) showed that in four lines of mice overexpressing the G37R SOD1 mutant, the most prominent cellular abnormality associated with the clinical phenotype of motor neuron disease was the presence of membrane-bound, intracytoplasmic vacuoles in neuronal processes (both axons and dendrites) and soma of motor neurons, which appeared to derive from degenerating mitochondria. Similar vacuolar abnormalities were not present in motor neurons of mice expressing comparable levels of wild-type SOD1, nor in the processes of the majority of nonmotor neurons and in adjacent glial cells, further suggesting that mitochondria are implicated in the tissue specificity of the cell-death process induced by mutant SOD1. Mitochondrial abnormalities were concurrently described in the G93A mouse model of the disease (90). A more detailed analysis of the disease progression in this mouse model indicated that mutant SOD1 toxicity and the resulting functional decline in motor neurons is likely mediated by early damage to

mitochondria. Jiming Kong and Zuoshang Xu (210) showed that mitochondrial changes, including dilated and disorganized cristae, leakage of the outer membrane, broken outer membrane, and vacuoles clearly originating from mitochondria are evident even at a presymptomatic stage, during which muscle strength is normal. As long as the disease progresses, motor neurons undergo a massive vacuolation, which is higher at the symptoms onset and then declines as the disease reaches the end stage. More-recent evidence has further sustained early alterations in the morphology and deficits in the activity of mitochondria of fALS-SOD1 mice (37, 185, 205, 247, 250), thus arguing for a direct role of mitochondrial degeneration in the pathogenesis of ALS. The possible mechanisms whereby SOD1 mutants cause mitochondrial damage and the functional outcomes of this damage are discussed in the following sections.

A. SOD1 localization into mitochondria

A major advance in our understanding of the mechanism of mitochondrial damage induced by mutant SOD1 derived from the observation that a small fraction of the wtSOD1, as well as of the mutant SOD1s, associates with various mitochondria compartments.

The subcellular distribution of SOD1 was first explored in the early 1970s by Irwin Fridovich. Fractionation studies showed that the cytosol from chicken liver contains Cu,Zn-SOD activity, whereas the mitochondrial matrix has Mn-SOD activity. Cu,Zn-SOD activity was also reported in the intermembrane space of mitochondria and in nuclei (405, 406), but lysosomal contamination of subcellular fractions was advocated to question these localizations (133). Almost 30 years later, the localization of SOD1 was reinvestigated by the same group in rat liver, and results were consistent with the previous reports (278). Most recently, by electron-microscopy analysis and examination of fractions enriched for mitochondria derived from models and a patient with ALS, both wild-type and fALS-related human SOD1s were found in the intermembrane space and the matrix, as well as in both the inner and outer membranes of spinal cord and brain mitochondria (42, 95, 169, 179, 235, 292, 394).

Whether fALS SOD1s accumulate to the same extent as wtSOD1 is an issue that is still debated. Liu *et al*. (234) studied the localization of SOD1 in spinal cord mitochondria isolated from symptomatic mice expressing various ALS-linked SOD1 mutants, as well as human wtSOD1. All mutant SOD1s analyzed, but not wtSOD1 or endogenous mouse SOD1, were present in the spinal cord mitochondrial fractions. Moreover, even though highly divergent as to their biochemical characteristics and cytoplasmic levels of expression, all the mutant SOD1s analyzed associated with similar amounts with mitochondrial fractions, including an unstable SOD1 mutant found in human ALS that accumulates only to trace cytoplasmic levels. These observations have recently been extended by us, analyzing 12 different fALS-mutant SOD1s that have all been found associated with mitochondria of motor neuronal cells to a much greater extent than wtSOD1 (119), suggesting that the specific association of mutant SOD1s, but not wtSOD1, with mitochondria of motor neuronal cells represents the common toxic property of these mutants. Conversely, many works have shown that both wt and mutant hSOD1s localize in mitochondria (169, 179, 250, 278, 394). This apparent contradiction may be explained by the observation that mitochondria from mice and cultured cells expressing mutant and wild-type human SOD1 have different shapes and sizes (169, 309) and may thus behave differently, depending on the method used for mitochondrial purification, as discussed in detail by Vijayvergiya *et al*. (394). Nonetheless, all the cited reports agree that mutant SOD1 proteins localize to mitochondria, consistent with an important contribution of this phenomenon to pathogenesis. A word of caution must be given about such a conclusion, because in a recent article, Bergemalm *et al*. (42) proposed that the high expression rates of hSOD1s in transgenic ALS models, like the G93A-expressing mice, may cause artificial loading of the hSOD1s into mitochondria, and only small amounts of mutant SOD1s could be found associated with mitochondria in mice expressing low-level unstable mutants G85R and G127X. Interestingly, these mice do not show the mitochondrial swelling and widespread vacuolar pathology that characterize other transgenic ALS mice, suggesting other primary locations of injury (42). In a well-characterized neuronal cell model (NSC-34), however, mutant SOD1s localize into mitochondria and cause mitochondrial impairment even when a relatively low level of mutant protein is expressed (119, 254).

The significance of the presence of wild-type SOD1 inside mitochondria is unclear, considering that the dismutation of superoxide generated by the mitochondrial respiratory chain is provided by the matrix-associated Mn-SOD (SOD2). O'Brien *et al*. (274) suggested that SOD2 and mitochondrial SOD1 may have both unique and overlapping functions in protecting yeast mitochondrial proteins from oxidative damage, and SOD1 downregulation by small interference RNA has been shown to lead to severe damage of mitochondria of human neuroblastoma cultured cells (18, 274). These observations suggest an important biologic role of SOD1 in the preservation of mitochondrial homeostasis.

Conversely, data indicate that mutant SOD1 toxicity descends from its localization and deposits inside mitochondria. For instance, the obligatory accumulation of two mutant SOD1s (G93A and G85R), but not wild-type SOD1, in mitochondria of mouse neuroblastoma cells was reported as significantly more toxic than that obtained with the normal cytosolic versions of the same mutants. No effects were obtained when mutant SOD1s were specifically overexpressed in nuclei or in the endoplasmic reticulum. The localization of mutant SOD1 in the mitochondria triggers the release of cytochrome *c* and a cell-death pathway that is dependent on cytochrome *c* release and caspase activation, but independent of Bcl-2 family proapoptotic proteins. Moreover, overexpression of Dorfin, a RING-finger–type ubiquitin ligase that enhanced the degradation of SOD1, significantly reduced the amount of mutant SOD1 in the mitochondria, the release of cytochrome *c*, and the activation of the caspase cascade, thus preventing neuronal cell death (368, 369). Cytosolic CCS overexpression in G93A-SOD1 mice highly enriched the amount of mutant SOD1 within mitochondria. Importantly, this effect strongly correlated with a dramatic acceleration of neurologic deficits and enhancements of mitochondrial pathology (360). Altogether, these observations imply that the localization of mutant SOD1 in the mitochondria is critical in the pathogenesis of fALS.

How SOD1 enters mitochondria of mammalian cells is essentially unknown, because of the lack of any obvious presequence signal. By using *in vitro* mitochondrial import assays conducted in yeast, the group of Culotta (120) proposed a model whereby only a SOD1 polypeptide that is *apo-* for both copper and zinc and reduced in the conserved disulfide can efficiently enter the mitochondria. The retention of SOD1 within mitochondria is provided, according to this model, by the conversion of the immature polypeptide into an active *holo-* enzyme through the intervention of CCS, which is highly expressed in the IMS of yeast mitochondria (Fig. 5). Similar outcomes were obtained in experiments in which mouse mitochondria were incubated with human SOD1 (279) and are consistent with the cited observations from CCS-overexpressing fALS mice (360). Interestingly, the IMS of mitochondria has been recently shown to contain machinery consisting of small Tim (translocase of the inner mitochondrial membrane) proteins and the recently described Erv-1 and Mia40 proteins. This system efficiently traps precursors incoming from the Tom (translocase of the outer mitochondrial membrane) complex by introducing disulfide bonds into newly imported precursor proteins, thereby locking them in a folded conformation [reviewed in (168)]. However, experimental evidence for a role of this machinery in the oxidative folding and retention of SOD1 inside mitochondria is still unavailable.

B. Oxidative stress in mitochondria

Because oxidative phosphorylation is thought to generate high levels of intracellular free radicals (286), the vulnerability of mitochondria in ALS might arise from locally and aberrantly generated free radicals because of dysfunctions in the activity of respiratory complexes. Indeed, alterations in the mitochondrial respiration have been described in cellular and animal models of ALS, and also in patients. In neuronal cultured cells, we reported that the expression of mutant SOD1s induces a significant loss of mitochondrial membrane potential, suggestive of respiratory chain dysfunction (60, 82). Similarly, decreased ATP levels, impaired function of respiratory chain enzymes, and increased production of ROS have been reported in neuroblastoma and motor neuron–like NSC-34 cells expressing mutant SOD1s (41, 119, 254). These effects have been shown to be related to a shift in the mitochondrial redox balance (GSH/GSSG ratio) toward a more oxidizing state, a condition that could strongly enhance mitochondrial SOD1 accumulation and toxicity (119). Mitochondrial respiratory chain dysfunction, due to a decrease in the enzymatic activity of complex I+III, II+III, and IV, was also evident in the G93A mouse model of fALS (185, 250). These alterations seem to affect mitochondria in the spinal cord more severely than in other tissues and to develop at an advanced stage of the disease, although Kirkinezos *et al*. (205) reported an early decrease in complex IV activity, even before the disease onset.

Mitochondria are not only a major source of ROS generation, but they are also a sensitive target for the damaging effects of oxygen radicals (Fig. 6). Among the major targets, ROS may affect sulfhydryl groups of mitochondrial proteins, causing intramolecular cross-linking and formation of protein aggregates. Moreover, hydroxyl radicals can generate peroxi-

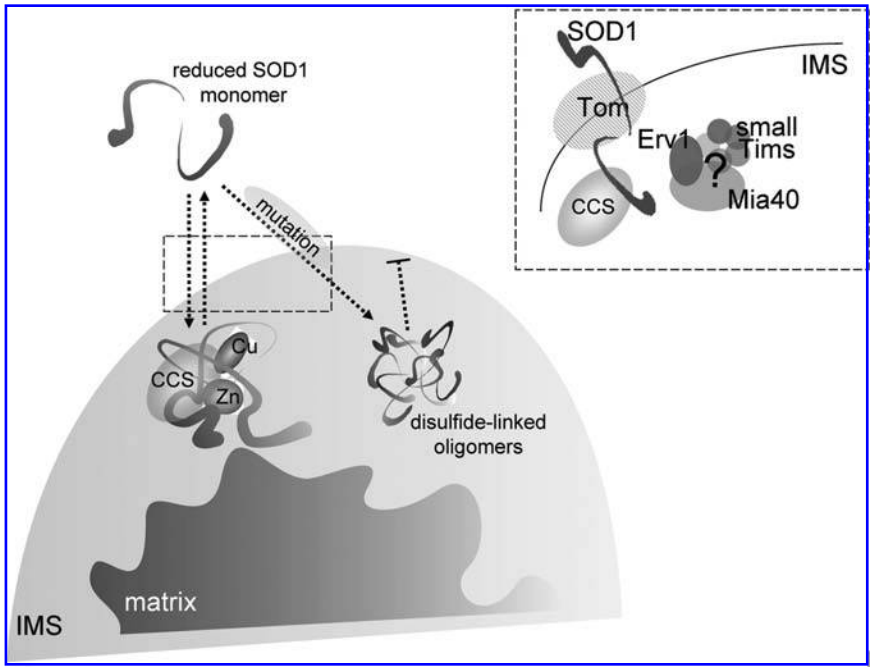

FIG. 5. Models for SOD1 import and accumulation into mitochondria. SOD1 enters mitochondria as a disulfide-reduced, metal-free monomer. The retention of SOD1 within the IMS is likely provided by the conversion of the immature polypeptide into an active holoenzyme through the intervention of CCS, accordingly to a "folding trap" mechanism that has recently been characterized for other IMS proteins. In this mechanism (inset), an intermembrane space import and assembly machinery consisting of Tom (translocase of the outer mitochondrial membrane) and small Tim (translocase of the inner mitochondrial membrane) proteins, and the recently described Erv-1 and Mia40 proteins, catalyzes the import of proteins into the IMS through an oxidative folding mechanism. This system efficiently traps incoming precursors through the introduction of disulfide bonds into newly imported precursor proteins, thereby locking them in a folded conformation. The presence of fALS mutation in SOD1 polipeptide and/or the accumulation of oxidative stress in fALS mitochondria could direct the newly imported polypeptide to an oligomerization/aggregation pathway, similar to that hypothesized for cytosolic SOD1, leading to the entrapment and accumulation of mutated, misfolded SOD1 inside mitochondria. Recent *in vivo* and *in vitro* observations report evidence for oxidatively crosslinked aggregates in mitochondria of ALS models.

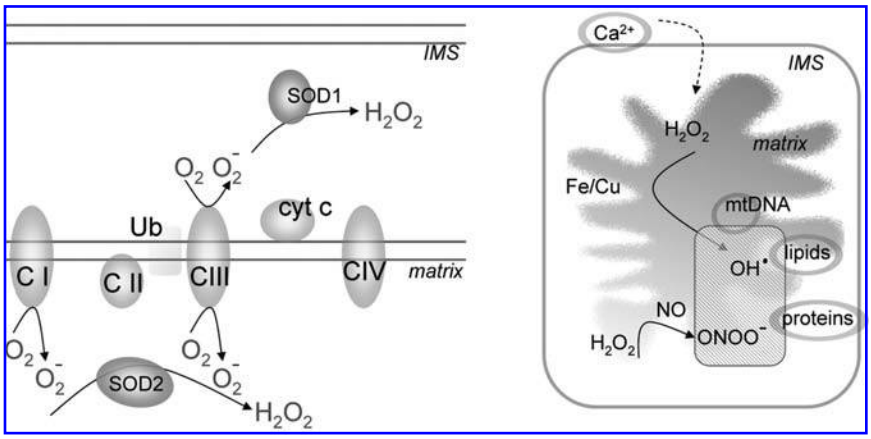

FIG. 6. Generation of oxidative stress in mitochondria. Respiratory complexes leak electrons to oxygen generating mainly superoxide anions (O_2^-). These anions are converted to H_2O_2 and oxygen by superoxide dismutases, which are present in the intermembrane space (IMS) of mitochondria (SOD1) and in the matrix (SOD2). Superoxide anions may reduce transition metals, which in turn react with H_2O_2, producing hydroxyl radicals ($OH^\cdot$), or may directly react with nitric oxide (NO) to produce peroxynitrite ($ONOO^-$). $OH^\cdot$ and $ONOO^-$ are strongly reactive against many substrates: they may oxidize mitochondrial proteins, causing intramolecular cross-linkings and formation of protein aggregates, generate peroxidation of membrane lipids, increase the release of calcium from mitochondria, and damage mitochondrial DNA (mtDNA). CI-CIV, respiratory complexes I-IV; Ub, ubiquinone; cyt c, cytochrome *c*.

dation of membrane lipids and generate peroxyl-radical intermediates. Oxidants are also known to increase the release of calcium from mitochondria and to damage mitochondrial DNA (mtDNA) easily.

Indeed, robust oxidative damage to mitochondrial proteins and lipids has been described in fALS mice (205, 247, 250), and formation of disulfide-linked SOD1 multimers in mitochondria has recently emerged as a property of cellular and mouse models of fALS (95, 119). Significantly higher levels of mutant mtDNA were found in the spinal cord of ALS patients compared with controls (410), and oxidative damage to mitochondrial DNA is indicated by the presence of 8-hydroxy-2-deoxyguanosine (8-OHdG), one of the best markers of the oxidative DNA damage, in the spinal cord of transgenic mutant SOD1 mice (403). Transfer of mtDNA from ALS patients to mtDNA-depleted human neuroblastoma cells recapitulated the defects previously observed in ALS subjects, such as altered electron-transport chain function, increases in free radical–scavenging enzyme activities, perturbed calcium homeostasis, and altered mitochondrial ultrastructure (366). Conversely, mtDNA from platelets of ALS patient was able to restore normal respiratory function in mitochondria-depleted non-neuronal cells, indicating that the link between mtDNA mutations and impaired mitochondrial respiration is limited to a neuronal background (128).

An increase in cytoplasmic calcium levels was reported in neuroblastoma cells expressing the mutant SOD1 (60), and sustained elevations of intracellular calcium levels have also been reported in cultured primary motor neurons from G93A transgenic mice (214). Perturbation of calcium metabolism, besides linking mitochondrial dysfunction to glutamate excitotoxicity, could be relevant to the issue of oxidative stress, because it has been shown that exposure to elevated Ca^{2+} concentrations induces production of free radicals in isolated mitochondria, thus making this process self-sustaining (105).

Consistent with the view that mitochondria-dependent oxidative stress contributes to the pathologic mechanisms of ALS, molecular approaches aimed at increasing mitochondrial antioxidative activity have been significantly successful in protecting NSC-34 motor neuron–like cells against mutant SOD1-induced cell death (237), and oral administration of creatine, which protects from the increase of oxidative damage, produced an improvement in motor performance and extended survival in G93A transgenic mice (207).

C. Mitochondria-dependent cell death

Mitochondria-dependent cell death represents another potential connection between ALS pathology and the physiology of mitochondria. Mitochondria are central in the mechanisms underlying cell survival, with mitochondrial ROS being crucial in the regulation of the apoptotic process. ROS are very well known inducers of cell death: they regulate early and late steps of apoptosis, and inhibition of ROS production also protects against apoptosis [(122) and refs. therein]. Conversely, ROS are also byproducts of the apoptotic process, because caspases exert their cleavage activity also on complex I and II in the electron-transport chain, thus damaging mitochondrial function and generating ROS (322) (Fig. 7).

However, whether apoptosis is the leading mechanism of death of spinal cord motor neurons in ALS is still an open question. The uncertainty originates mostly from morphologic data, which could never report reliable evidence about the numeric relevance of this phenomenon (37, 246, 247, 255), probably because of the paucity of apoptotic dying motor neurons at a given time point. Indeed, it has been estimated that in end-stage transgenic G93A mice, with ~50% of their anterior horn motor neurons lost, about two apoptotic cells are present per tissue section of 1/25 mm of the lumbar spinal cord, with the majority of these apoptotic cells no longer exhibiting definite phenotypic characteristics allowing their identification as neurons or glia (302). Nevertheless, a great body of biochemical evidence has been provided about the recruitment of mitochondria-dependent programmed cell death in ALS (146). In particular, the process has been carefully detailed at the molecular level in the G93A model of the disease. A sequential cascade of Bax translocation from the cytosol to the mitochondria, cytochrome *c* release from the mitochondria to the cytosol, where it binds the scaf-

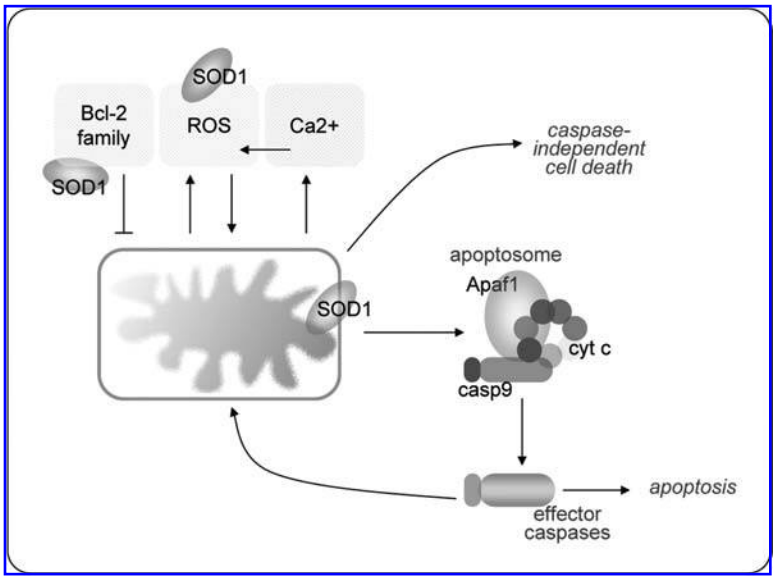

FIG. 7. Mitochondria-dependent cell death. According to a general model, death signals converging on mitochondria induce the release of cytochrome *c*, which participates, together with the scaffold protein Apaf-1 and the caspase9 enzyme, in the formation of the apoptosome in the cytosol. Apoptosome drives the activation of effector caspases, such as caspase3, leading to apoptosis. Cleavage activity of caspase3 may also feed back on mitochondria, contributing to damage mitochondrial function and to generate reactive oxygen species (ROS). The intermembrane space (IMS) of mitochondria also contains proapoptotic factors that participate to caspase-independent mechanisms of cell death. Mutant SOD1s might hinder mitochondrial function and activate the apoptotic program directly, because of their localization in the IMS, or indirectly, by interfering with the antiapoptotic activity of Bcl-2 or through the generation of ROS.

fold protein Apaf-1, activation of the initiator caspase9 and the executor caspase3 and caspase7, and cleavage and inactivation of the inhibitor of apoptosis XIAP have been described in the spinal cord of transgenic G93A SOD1 mice during the progression of the disease by the group of Przedborski (145). Similar mechanisms participate in the apoptotic cell death that occurs when mutant SOD1s are expressed in cultured cells (83, 307). Consistent with the relevance of these events, prevention of cytochrome *c* release from mitochondria extends the life span of transgenic G93A mice (440), and the broad-range caspase inhibitor benzyloxycarbonyl-Val-Ala-Asp(*O*-methyl)-fluoromethylketone (zVAD-fmk) has a modest but significant effect on the survival of the same animals (226). The Bcl-2 family seems to be widely involved in the cell-death process, with members of the pro- and antiapoptotic subtypes coordinately deregulated, in both transgenic mice and human cases (302). Bcl-2a1, another member of the Bcl-2 family, is upregulated selectively in spinal motor neurons of mice transgenic for G93A-SOD1, already at the asymptomatic stage. Interestingly, Bcl-2a1 has protective effects against mutant SOD1-induced caspase3 activation when overexpressed in neuronal cells, suggesting an antiapoptotic significance of the upregulation observed in transgenic mice, but its overexpression increases the level of cell death after exposure to TNF-α in the same cells, consistent with a reinforcing role of Bcl-2a1 in the progressive loss of motor neurons occurring in ALS (86, 305). Pasinelli *et al*. (292) provided evidence of a direct link between SOD1 and an apoptotic pathway by demonstrating that both wtSOD1 and mutant SOD1 bind the antiapoptotic protein Bcl-2 in ALS models and patients, possibly impairing Bcl-2–dependent regulation of the mitochondrial membrane potential and the binding and inhibition of proapoptotic protein. Yet, interfering with the apoptotic cascade through Bcl-2 overexpression or caspase inhibition is unable to rescue motor neurons completely in fALS mutant mice (23, 192, 226, 396). A plausible interpretation is provided by recent data suggesting that clinical symptoms in the mice model of ALS might result specifically from damage to the distal motor axon and not from activation of the death pathway. By crossing Bax-deficient mice with mice expressing mutant SOD1, Gould *et al*. (138) showed that Bax deletion failed to prevent neuromuscular denervation and mitochondrial vacuolization, although motor neurons were completely rescued from mutant SOD1-mediated death. Thus, therapeutic strategies aimed at disrupting common mechanisms of denervation should be used along with those aimed at blocking cell death to be more effective.

IV. NON–CELL-AUTONOMOUS DEATH OF MOTOR NEURONS

One of the most striking features of ALS is the cell specificity: motor neurons are selectively affected in patients, whereas mutant SOD1 is expressed ubiquitously. However, the neurotoxic effect of mutant SOD1 may be not just a direct consequence of its expression inside the neuron, but may require functional alteration of non-neural cells (47). This hypothesis has been demonstrated by several independent groups with ALS mouse models. To study the contribute of astrocytes to motor neuronal degeneration in ALS, Elliott and co-workers (136) generated multiple lines of transgenic mice expressing G86R mutant SOD1 restricted to astrocytes. Mice expressing mutant SOD1 under the control of an astrocyte-specific promoter (GFAP-mutant SOD1 mice) exhibited significant astrocytic hypertrophy and GFAP reactivity, increasing with age. However, GFAP-mutant SOD1 transgenic mice developed normally and experienced neither spontaneous motor deficits with increasing age nor alterations in neuronal and microglial morphology. These data indicate that mutant SOD1 expression in astrocytes alone is insufficient to evoke motor neuron degeneration in mouse, but is sufficient to cause at least some degree of astrocytosis that is not reactive but primary. The evidence that the expression of mutant SOD1 restricted to neurons of transgenic mice is not sufficient to produce the typical neurodegeneration of ALS comes from the work of Pramatarova *et al*. and Lino

et al. (233, 301). The authors showed that transgenic mice expressing mutant SOD1s in neuronal compartment developed regularly and did not experience motor neuron deficits. Moreover, the accumulation of mutant SOD1s in postnatal motor neurons failed to induce fALS-associated pathology in motor neurons or neighboring cells in the spinal cord (233). More definitive evidence for an active role of neighboring cells in the ALS neurodegenerative process comes from an elegant work of Clement and co-workers (75). The authors constructed and analyzed chimeric mice that were mixtures of normal cells and cells expressing mutant human SOD1 and observed that nonneuronal cells that do not express mutant SOD1 delay degeneration and significantly extend the survival of mutant-expressing motor neurons. The hypothesis of an interaction occurring between motor neurons and glia cells in ALS was supported also by the observation, in glia/neurons cocultures, that ROS produced by motor neurons inactivate glial glutamate transport (428). After these articles, several studies explored the role of glial cells in the ALS pathogenesis and thus the involvement of the neuroinflammatory processes in the pathology [see later and (47) for review].

Finally, signals from the muscle may interfere with the cascade of events that leads to motor neuron degenerations (99, 228). On the whole, these data establish the relevance of a complex interplay among different cell types in the pathogenesis of ALS, where the integration of intercellular cross-talk may create a paracrine milieu inconsistent with healthy neural function.

A. Microglial cells: dictating disease progression

Microglia in the CNS are immunologically active and capable of responding to events associated with the formation of the neuronal–glial environment. In response to a variety of insults, microglia transform from a resting state into active, phagocytic cells that release several factors governing the inflammatory response.

Evidence that microglial cells are involved in the course of ALS pathogenesis emerged from several works. Activated microglia are typically found in ALS patients and in the mouse models of ALS as well [see for review (47)]. The group of Friedlander (440) reported that treatment of ALS mice with the antibiotic minocycline delays both disease onset and mortality of animals. Minocycline is a second-generation tetracycline, capable of inhibiting microglial activation, with distinct antibiotic and antiinflammatory properties (13, 407). Minocycline appears to act both on the microglia, where the upregulation of iNOS is inhibited, and on motor neurons, where the release of mitochondrial cytochrome *c* (and thus the initiation of a proapoptotic pathway) is prevented (213, 431, 432).

The role of microglia expressing mutant SOD1 in the noxious cellular cross-talk that leads to neurodegeneration of ALS mice has been recently clarified by elegant work from the group of Cleveland (48). By using mice carrying a deletable ("floxed") mutant SOD1 gene that can be excised by the action of the Cre recombinase, the authors identified differential contribution of mutant SOD1 within motor neurons and microglial cells in triggering disease onset and progression. The expression within motor neurons is involved in the determination of disease onset and of an early phase of progression, whereas diminishing the levels of mutant SOD1 in microglia had almost no effect on the early phase but significantly slowed disease progression. These results prompt the hypothesis that the action of mutant SOD1 within different cell types regulates distinct ALS phases, onset, and progression, to generate non–cell autonomous killing of motor neurons.

Similar results were obtained by Beers and co-workers (34), by using a different approach. They crossed PU.$1^{-/-}$ mice, which are unable to develop myeloid and lymphoid cells because of deletion of the transcription factor PU.1, with mutant SOD1 mice (mutSOD1^{G93A}), and the entire myeloid lineage of mutSOD1^{G93A}/PU.$1^{-/-}$ mice was replaced by transplantation of bone marrow from wild-type mice or from mutSOD1^{G93A} mice. Transplantation at birth with mutSOD1^{G93A} mutant–expressing myeloid cells produced onset and survival typical of the SOD1-G93A mutant strain. Replacement of the microglial, monocyte, and macrophage lineages with normal cells from wild-type mice had no effect on disease onset but slowed disease progression after onset, and transplantation of microglial cells expressing mutant SOD1 into $PU^{-/-}$ mice did not induce an ALS-like disease, demonstrating that macrophages/microglial cells expressing mutant SOD1 are not sufficient to cause motor neuron death by themselves (34).

On the whole, these results demonstrated that mutant SOD1 within macrophages/microglial cells modifies disease progression, whereas expression of mutant SOD1 within the motor neurons is a primary determinant of onset and early disease (Fig. 8). However, pharmacologic or genetic approaches to inhibit microglial activation may be still relevant for therapy in ALS patients in whom onset has occurred (long) before the time of diagnosis, and slowing the progression would undoubtedly be a major success.

In this scenario, several studies focused on the cross-talk between neurons and glial cells to unmask the molecular signals that evoke the activation of microglia [see for review (166)]. These cells produce increased quantities of pro-inflammatory cytokines, reactive nitrogen species (RNS), reactive oxygen species (ROS), and glutamate, in response to stimuli from neurons and astrocytes such as pro-inflammatory cytokines and neurotrophic factors [see for review (263)].

Surprisingly, mutant SOD1 is able to induce microgliosis *in vitro*. As shown by Julien and colleagues (385), cultured BV2 microglial cells became active, producing TNF, COX2, and inducible nitric oxide synthase (iNOS), when mutant SOD1 is present in the cellular medium (385). Mutant SOD1 localized into the endoplasmic reticulum and was secreted into the extracellular space both in spinal cord from ALS mice model and in cultured cells, and vaccination with mutant SOD1 delayed disease onset and mortality in G37R mice, a strain characterized by a low transgene expression (386). Altogether, these data indicate that the secreted fraction of mutant SOD1 may contribute to the intercellular signaling involved in the pathogenesis of ALS.

B. Astrocytes: inducing excitotoxicity

Astrocytes represent a different class of glial cells that becomes reactive in areas of motor neuron degeneration in ALS. These cells play a central role in supporting and sustaining proper neuronal function through the regulation of extracellular glutamate levels.

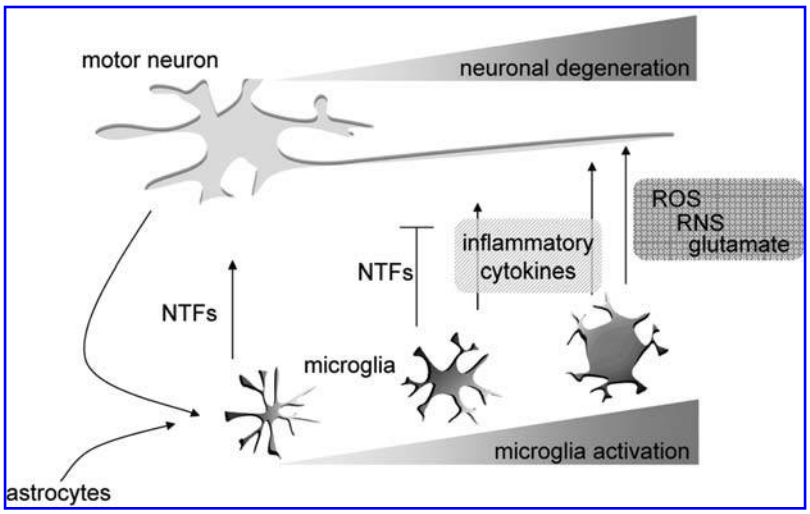

FIG. 8. Contribution of microglial cells. In response to signals originating from damaged neurons and the surrounding astrocytes, microglia downregulate the production of neurotrophic factors (NTFs), which sustain neurons in resting condition, and start to produce proinflammatory cytokines. As long as microglia activation proceeds, inflammatory cytokines accumulate, together with reactive oxygen (ROS) and nitrogen (RNS) species, and glutamate. The resulting inflammatory environment contributes to the degeneration of motor neurons and to the progression of the disease.

Astrocytosis is a typical hallmark of the neurodegenerative process in several ALS experimental models, as well as in patients (156, 225, 341). Midfrontal, inferior parietal, temporal, cingulate, and occipital cortices, as well as the motor cortex from ALS patients, are affected by a widespread astrogliosis (215). Evidence for a functional role of astrocytes in the neurodegenerative process of ALS came from a study on the G85R mouse model (55) (Fig. 9). In these animals, the first signs of damage were observed in the astrocytes, where the authors reported a 50% decrease in the astrocyte-specific glutamate transporter GLT-1 (homologous to human EAAT2), which is the major glutamate transporter in the spinal cord, in parallel with neuronal loss and astrogliosis. A focal loss of GLT-1 was also observed in the ventral horn of the spinal cord from a rat model of ALS, where gliosis paralleled with a 90% decrease in GLT-1 at end stage of disease (173).

Boston-Howes and co-workers (50) reported that a constitutive, nonlethal activation of caspase-3 in astrocytes from the previously mentioned rat model is responsible for cleavage and inactivation of GLT-1.

Although the evidence is controversial (see earlier), levels of glutamate transporter EAAT2 are also reduced in the motor cortex and spinal cord of ALS patients, possibly decreasing glutamate transport and subsequently increasing extracellular glutamate concentration. Besides having a role in glutamate metabolism, astrocytes play a role in neuroinflammation. Like microglia, reactive astrocytes express inflammatory markers and can produce pro-inflammatory mediators, participating directly in inflammatory reactions (263). The evidence that concentrations of MCP-1 (monocyte chemoattractant protein-1), a factor critical for the migration of monocytes in areas of injury, are increased in both serum and CSF from ALS patients, suggests that astrocytes play an important role in mediating the inflammatory response to injury in ALS (29).

The first evidence that an active exchange of inflammatory cytokines between glia and neurons occurs in ALS, leading to a non–cell autonomous death of neurons induced by fALS-SOD1, came from the work of our group conducted in an *in vitro* cellular model (118). By using the U373 astrocytoma cell line as a model system for astrocytes, we devised a model of

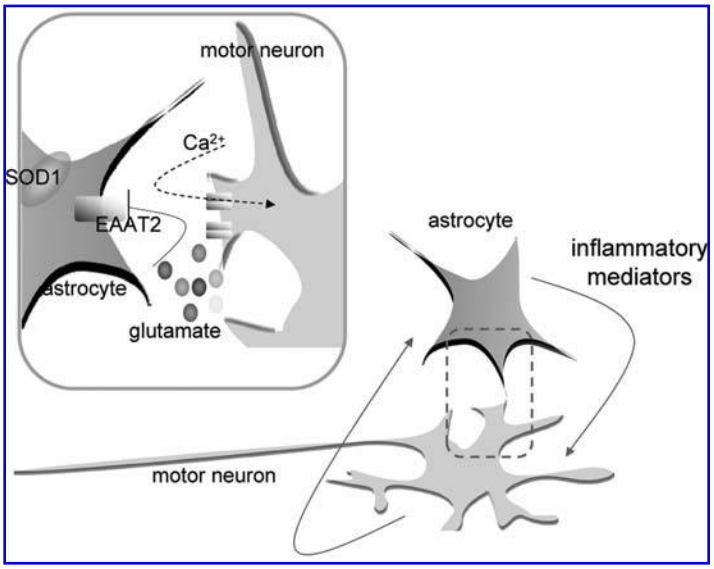

FIG. 9. Contribution of astrocytes. Alterations in the expression levels and/or inactivation of astrocyte glutamate transporters EAAT2 decrease glutamate reuptake and subsequently increase extracellular glutamate concentration, thus leading to excitotoxicity. Astrocytes also play a role in neuroinflammatory processes. In response to signals originating from neighboring motor neurons, reactive astrocytes express inflammatory markers and can produce proinflammatory mediators, participating directly in inflammatory reactions.

human origin, in which interactions occurring in co-cultures of neuroblastoma cells and glioblastoma cells, both expressing mutant SOD1 at a low level, have been studied thoroughly. The co-culture condition, mimicking the situation in SOD1-linked fALS patients, triggered a cascade of events in both cell types. Expression of mutant SOD1 induced activation of fALS-glioblastoma cells, as indicated by an increase in expression of GFAP, which responded by increasing the level of intracellular inflammatory markers (*e.g.*, COX2), inducing nNOS activity and releasing NO and IFN-γ into the culture medium. Both released factors acted on neuroblastoma cells expressing mutant SOD1, inducing activation of caspase1 and release of IL-1β. This cytokine diffused to glioblastoma cells inducing NF-κB–mediated upregulation of COX2 and nNOS, thus contributing to the inflammatory response elicited by the expression of mutant SOD1. Sequentially, NO-dependent caspase3 activation and apoptotic death of neuroblastoma cells were triggered. In line with these results, two articles were recently published strengthening the indication that an active cross-talk between astrocytes and motor neurons is generated in ALS, in which diffusible factors from both cell types induce neurons to enter the apoptotic pathway (97, 268). These articles reported that astrocytes expressing ALS-linked mutant SOD1 kill murine motor neurons derived from embryonic stem cells or primary mouse spinal motor neurons. This effect was increased by the simultaneous expression of mutant SOD1 in motor neurons; no indication of which diffusible factors were involved in the cellular cross-talk was provided.

C. Muscle involvement in ALS

Functional evidence for an active role of muscle in the non–cell autonomous motor neuron degeneration comes from studies by Dobrowolny *et al*. (99). The authors generated transgenic mice expressing in skeletal muscle a specific isoform of insulin-like growth factor-1 (IGF-1), which is locally active and does not enter the bloodstream. Crossing these mice with mutant SOD1 mice yielded offspring characterized by a remarkable increase of life span. These results were recently questioned in a study demonstrating through muscle-specific silencing that mutant SOD1-mediated damage within muscles was not a significant contributor to non–cell autonomous pathogenesis of ALS (258). In addition, enhancement of muscle mass and strength provided no benefit in slowing disease onset or progression (258). The authors concluded that most likely the synthesis of IGF-1 by muscle primarily benefits SOD1 mutant–mediated disease by affecting the innervating neuron at the neuromuscular junction, where IGF-1 can have profound effects (258).

Thus, the relations between muscles and motor neurons and the contribution of muscle to ALS pathogenesis are still debated. Studies from Loeffler and colleagues (113) on the protein Nogo-A have brought new insights into this subject. Nogo-A is a well-known repellent for axonal regeneration and is synthesized by oligodendrocytes in the CNS (113, 342). Knocking out Nogo-A increased mutant SOD1 mouse life span and protected motor neurons, whereas overexpressing Nogo-A in adult mouse muscle fibers led to neuromuscular junction instability and nerve terminal retraction (183). In patients, Nogo-A is selectively expressed in muscle, and the expression level is correlated with the functional status of the patients (103, 182) (Fig. 10). More recently, muscle expression of Nogo-A has been proposed as a marker of ALS. In patients with pure lower motor neuron syndrome (LMNS) in whom typical ALS later developed, the detection of Nogo-A before the onset may predict progression to ALS (300). These data suggest that Nogo-A might be a molecular player underlying neuromuscular junction destabilization in ALS, revaluing the role of muscle in non–cell autonomous pathogenesis of ALS.

V. THERAPEUTIC APPROACHES

A. Pharmacologic therapies in ALS

Studies in transgenic animals expressing mutant SOD1 have provided a wealth of information about the pathologic mechanisms of SOD1-linked fALS and possible explanations for the selective vulnerability of motor neurons in this disease (Fig. 11). Because sporadic ALS clinically overlaps with familial ALS (both SOD1-linked and non-SOD1 linked), and because evidence suggests that pathways altered by mutant SOD1 are activated in sALS by other toxic factors, over the past 10-year period, many clinical trial using drugs belonging to different categories (*e.g.*, antioxidants, antiapoptotics, antiinflammatories, antiexcitotoxic, and neurotrophic factors) have been based on successful experimentation in mutant SOD1 transgenic mice. This choice was dictated by the obvious consideration that those mice (and, more recently, mutant SOD1 transgenic rats) were the only animal model available and that clinical and basic research had pointed out to several possible mechanisms for the pathogenesis of ALS.

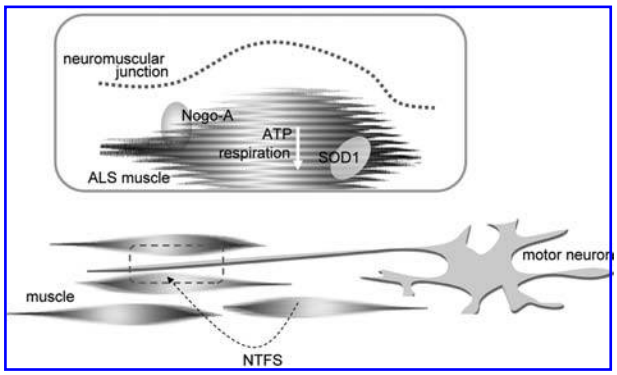

FIG. 10. Muscle involvement in ALS. Retraction of motor axons from synaptic connections to muscle is among the earliest presymptomatic events of ALS, suggesting that muscle is a likely primary source for toxicity. The relations between muscles and motor neurons and the contribution of muscle to ALS pathogenesis are, however, still controversial. In ALS, affected muscles may be impaired in their ability to support motor neurons through the production of neurotrophic factors (NTFs). Moreover, the decrease in respiration and in production of ATP may be responsible for significant muscle energy defects. Nogo-A, a well-known repellent for axonal regeneration that is upregulated in ALS muscles, might be another molecular player in the destabilization of neuromuscular junctions.

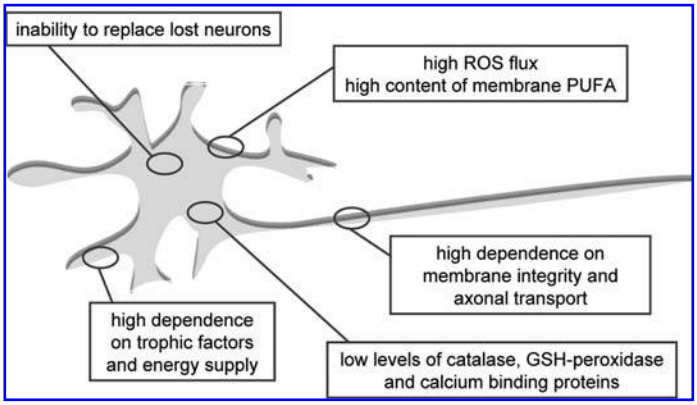

FIG. 11. Vulnerability of motor neurons. As other neuronal populations, motor neurons are very susceptible to oxidative and nitrosative stress because of a low level of some antioxidant enzymes, a high content of easily oxidized substrates (*e.g.*, membrane polyunsaturated fatty acid, PUFA), and an inherently high flux of ROS generated during neurochemical reactions, such as dopamine oxidation and inability to replace lost neurons (although synaptic plasticity may partially help to overcome damage). The functionality of motor neurons is highly dependent on membrane integrity, axonal transport, support from exogenous trophic factors, and energy supply. Low levels of calcium-binding proteins may contribute to decreased ability to counteract calcium loading due to excitotoxic mechanisms.

Unfortunately, all past attempts to translate evidence in mice to benefit in patients has failed. One reason may be that tests in animal models have limited predictive value because of anatomic, genetic, and physiologic differences between humans and mice and because most, but not all, features of human ALS are reproduced in the mutant SOD1 rodents (61). Another reason may be that those drugs may work in SOD1-linked fALS cases, but not in all other non–SOD1-linked ALS cases that constitute the vast majority of patients (36). In this view, the recent reports that, under certain conditions (*e.g.*, oxidative stress, metal deprivation), wild-type SOD1 behaves "aberrantly," like a mutant SOD1 (28, 112) and that it is possible to show a common molecular signature in SOD1 for both sporadic and familial ALS (144) strengthen our hopes that what is found in mutant SOD1 models will ultimately prove true in all ALS patients.

Most probably, however, the main reason for the failure to move from bench to bedside is that many clinical trials underrated the fact that ALS is a multifactorial disease, and several alterations work together in contributing to its pathogenesis. Furthermore, as described earlier, ALS is also a multisystemic disease that affects several cell types. In this light, ALS therapies should be aimed at the simultaneous interception of multiple aspects of the pathogenesis, rather than at a single-drug treatment.

This view is supported by the observation that >700 compounds have been proposed for single pharmacologic treatment of ALS, and almost 100 reached therapeutic trials (http://www.als.net/). All drugs have failed so far, regardless of the category, except one (riluzole, the only compound approved for use in ALS), which prolongs survival by 3 months only, and it does not evidently improve the quality of life of patients (257).

In a recent study (36), the question of the utility of preclinical data in mice to identify therapeutic agents for further study in humans has been raised, and data from the literature have been subject to a meta-analysis of 167 published studies to identify the most promising agents. This study remarked the existence of a "publication bias" (*i.e.*, that small negative studies fail to be published, whereas small positive studies are far more likely to be published). Despite the observation that "most treatment trials in the mouse model of ALS are of limited methodologic quality" (36), among those studies reporting outcome in terms of both survival and the survival interval, beneficial effects were consistently greater for agents with an antioxidant or neurotrophic mechanism of action, especially when used in combination with some other compound.

Indeed, studies in animals have shown that combination therapies often have synergistic effects (61) and that compounds that have more than one activity and therefore target multiple mechanisms operating in ALS may be worth consideration. This is well exemplified by the beneficial effect of minocycline, a drug that effectively crosses the blood–brain barrier and is neuroprotective in a number of experimental paradigms for neuronal injury (66, 417, 431, 432). Minocycline inhibits microglia activation and the apoptotic cascade through mechanisms that include inhibition of caspases, mitochondrial cytochrome *c* release, iNOS activity, and protein tyrosine nitration (373, 374, 407, 440), and its effect in ALS mice is greater when it is administered together with creatine, which improves energy metabolism (436).

Multifunctional drugs may allow overcoming a well-known problem with the design of multidrug therapies (*i.e.*, the possibility of negative interactions between drugs). In this context, it may be noticed that simultaneous treatment with different molecules could be safer and even more effective if they are delivered selectively to their target-cell type instead of systemically.

B. Growth factor therapies in ALS

As mentioned earlier, it is believed that lack of neurotrophic support might contribute to the pathogenesis of ALS, because several attempts have been successful in the mouse model. For instance, it is known that *VEGF* overexpression prolongs survival by 30% in mutant SOD1 transgenic mice and rats (24, 363, 402, 438). These findings suggest that *VEGF* may eventually be used as a therapy for ALS. Normally, growth factors do not cross the blood–brain barrier, but spinal cord motor neuron axons and nerve terminals lie outside the barrier and thus may be targeted by systemic administration of protein growth factors. Ciliary neurotrophic factor (1, 256), growth hormone (357), thyrotropin-releasing hormone (54, 59, 178, 261), insulin-like growth factor (218), and brain-derived neurotrophic factor (2) have been tested, but evidence in humans is that none of the growth factors tested has succeeded in altering the outcome of the disease.

One practical problem when using trophic factors as a therapy in patients is that cells produce several types of these fac-

tors, and it is not always clear which type, or which combination of these types, is required for a beneficial action.

This is well exemplified by the treatment with insulin-like growth factor (IGF-1), which was attempted because IGF-1 was found to reduce the apoptotic death of motor neurons *in vivo* after axotomy or spinal transection and to increase motor neuronal sprouting and muscle endplate size in rodents. Furthermore, IGF-1 levels were found to be significantly reduced in serum of ALS patients (378), and free IGF-1 was also found to be much lower in spinal motor neurons (411), possibly because of specific increases in some, but not all, IGF-binding proteins that are represented in the spinal cord and play an important part in regulating the bioavailability of IGF-1. Interestingly, it has been proposed that oxidative stress–induced neuronal death due to hydrogen peroxide overload may act through inhibition of IGF-1 signaling and may constitute an additional pathway contributing to ROS neurotoxicity (439).

In mutant SOD1 transgenic mice, retrograde delivery of recombinant adeno-associated virus IGF-1 injected into muscle increased the life span, helped to maintain the integrity of motoneurons, and decreased gliosis (195). However, randomized clinical trials with the recombinant circulating form of human IGF-1 in ALS have yielded conflicting results (49, 218, 260). A possibility, as yet to be explored, is that better results could be obtained by treatment with the muscle-specific form of this factor, as suggested by the previously mentioned study in mutant SOD1 mice in which expression of local IGF-1 reduced inflammation in the spinal cord and enhanced motor neuronal survival, thus delaying the onset and progression of the disease (99).

Another problem with the clinical use of NTFs in ALS is that method of delivery may be limiting the efficacy of treatment with this class of molecules. For instance, muscle-derived GDNF had profound effects on muscle innervation and axonal degeneration in the transgenic mutant SOD1 mice, whereas overexpression of GDNF in astrocytes in the CNS failed to demonstrate any neuroprotective effects. These data suggest that retrograde transport and signaling of GDNF is more physiologic and effective for ALS treatment than anterogradely transported GDNF (228) and indicate that the effectiveness of this NTF may depend significantly on its route of delivery to motor neurons.

Little or no benefit has been reported for IGF-1 when administered subcutaneously (49, 218). However, high-dose intrathecal infusion of IGF-1 extended survival in mice (269), and a phase I clinical trial using the same strategy showed some benefit to patients with no adverse effects (270).

C. Gene therapies in ALS

1. Silencing toxic genes. A nonpharmaceutical approach to the interception of damage is to silence the synthesis of specific genes coding for proposed mediators in the pathogenesis of ALS. Small interfering RNAs (siRNAs) provide a powerful tool to decrease or abolish the expression of a single gene (Fig. 12). This approach has given very promising results in experimental models. For instance, intraspinal injection or muscle injection of a lentiviral vector for the expression of interfering RNA molecules specifically targeting the human SOD1 gene resulted in an efficient and specific reduction of SOD1 expression in transgenic ALS mice and improved survival of vulnerable motor neurons in the brainstem and spinal cord, thus resulting in improved motor performance, considerable delay in the onset of ALS symptoms, and extension in survival (314, 317, 418). Furthermore, antisense oligonucleotides to SOD1 significantly slowed disease progression in the mouse model of ALS by reducing both SOD1 protein and mRNA levels throughout the brain and spinal cord when continuously infused intraventricularly (358).

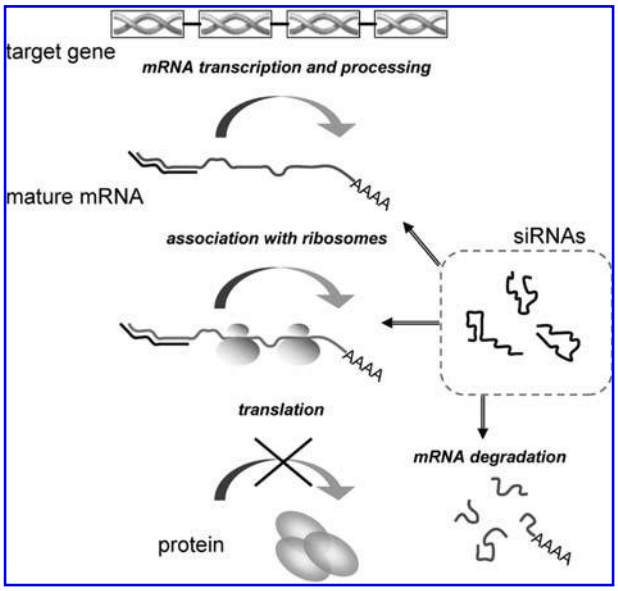

FIG. 12. Mechanisms of siRNA. RNA silencing has been established as a strong research tool, with potentially powerful therapeutic implications. This technique is based on the use of small interfering RNAs (siRNAs), which promote the degradation of selected target mRNAs, thus affecting the steady-state expression levels of a protein. Viral vectors expressing short hairpin RNAs, which are processed into functional siRNAs, have been proved useful in downregulating the expression of G93A mutant SOD1 in mouse models of the disease.

In the case of dominant, gain-of-function human diseases such as ALS, siRNAs should be designed specifically to silence the mutant disease allele (*e.g.*, mutant SOD1), while leaving expression of the wild-type allele unperturbed. This approach is feasible *in vitro* (343).

One major drawback of this system is the existence of the "shutdown phenomenon" of transgenic siRNA. In the case of mutant SOD1, this phenomenon has been prevented in transgenic mice, and a stable knockdown effect of siRNA on SOD1 expression has been obtained for more than four generations (334).

RNA interference has been used effectively in the transgenic mutant SOD1 mice to silence the gene coding for the proapoptotic protein prostate apoptosis response-4 (Par-4), a protein that was found to be enriched in synaptosomes and postsynaptic density from the ventral horn of the spinal cord and increased significantly during declining stages of muscle strength in those mice (419). Silencing of Par-4 inhibited mitochondrial dysfunction and caspase-3 activation and protected spinal motor neurons from apoptosis.

Similar results were obtained by administration of antisense peptide nucleic acid targeting either the death-signalling p75 neurotrophin receptor (382) or GluR3, a subunit of glutamate receptors (321), which are upregulated in the transgenic mouse model.

In other cases, the silencing of genes suspected to play a role in familial ALS has given unexpected results, because of our incomplete knowledge of the real function of their protein products. For instance, silencing of dynactin resulted in apparently normal amounts of dynein associated with membrane compartments, but anterograde and retrograde organelle movement in axons was completely disrupted (154).

2. Delivering trophic factors. Another problem with trophic factors is that their delivery to the CNS is often hindered by the toxic side effects that result from oral administration of recombinant proteins and from a scarce bioavailability (25). In an attempt to overcome this problem, Guillot *et al*. (147) used lentiviral vectors to deliver GDNF by facial nucleus or intraspinal injection in the mutant SOD1 transgenic mice. These vectors allowed the sustained expression of GDNF in motor neurons. GDNF expression induced a significant rescue of degenerating cells in the facial nucleus; intriguingly, it failed to prevent the loss of spinal motor neurons and muscle denervation of transgenic mutant SOD1 mice. This may be interpreted as an indication that facial nucleus *versus* spinal motor neurons have different vulnerability in ALS or that GDNF has differential effects on the two populations, but also that the method of delivery may be important if the correct population is to be reached. In this context, it must be noted that treatment with IGF-1 or *VEGF*, retrogradely transported in motoneurons through viral vectors that were injected into muscles, produced two of the best results obtained in the transgenic mutant SOD1 mouse model, even when these NTFs where delivered after onset of symptoms. In the previously mentioned study, Kaspar *et al*. (195) used the retrograde transport ability of AAV in the G93A mouse model of ALS to test the efficacy of IGF-1. By injecting an AAV engineered to express IGF-1 into respiratory and motor limb muscles, they were able to target the affected motor neurons directly. In the same mouse model, a single injection of a *VEGF*-expressing lentiviral vector into muscles delayed onset and slowed progression of the disease. The therapeutic efficacy of late IGF-1 or *VEGF* delivery is particularly significant for human disease, opening potential applications for clinical trials. Unfortunately, therapy with viral vectors (Fig. 13) in humans is still made complicated by the difficulty in choosing the right vector to target the relevant tissue or cell type and to obtain a prolonged expression of the gene, making the gene-therapy approach with viral vectors still remote from the clinic (25).

D. Stem cell therapies in ALS

Cell therapy has been widely suggested as a treatment for multiple diseases including ALS. In some experimental systems, such as in models of diabetes (208), Parkinson's disease (149, 359), Huntington's disease (102, 391), Alzheimer's disease (281, 400), and muscular dystrophy (336), cell therapy has been successful, although translation to patients has sometimes yielded unexpected effects. A variety of donor cells have been tested for treatment of motor neuron diseases, including isolated preparations from bone marrow and embryonic spinal cord, and the issue of the number of cells to be transplanted has been addressed.

The rationale is to replace lost cells or to support the function of damaged cells and prevent further loss. Stem cells are thus used as a source of neuroprotective proteins and function as "nurse cells" (Fig. 14).

In ALS, replacement of lost motor neurons is quite unfeasible, not only because of the difficulty of finding out which population of stem cells should be used, but also because of the time required for axon growth and muscle reinnervation and because of the rapid progression of the disease. Therefore, efforts have been focused on discovering appropriate nurse cells to implant in patients. This treatment may reinforce a natural response to degeneration of motor neurons that is known to promote proliferation and migration in the spinal cords of neural progenitor cells (NPCs) in ALS mice (70), although the populations of NPCs and neural stem cells (NSCs) that are normally present in mammalian forebrain may be altered in ALS transgenic mice (238).

We have demonstrated that NPCs can be efficiently engineered to inhibit apoptosome activation, and that in this condition, they retain their potential to differentiate and are able to

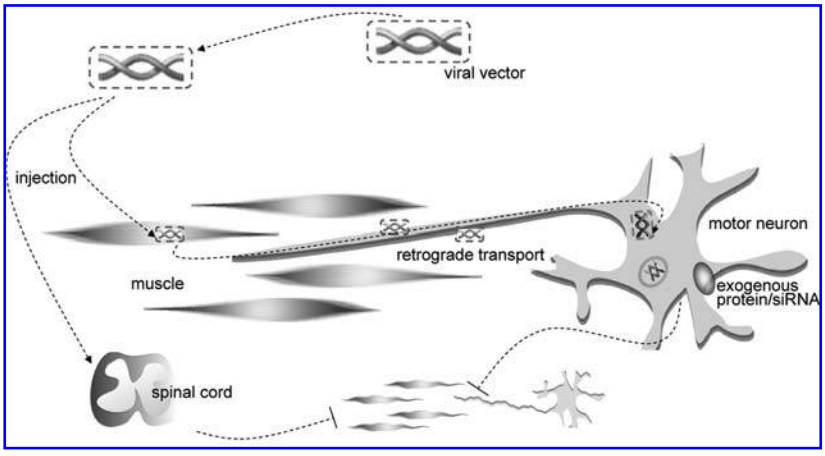

FIG. 13. Gene therapies in ALS. Viral particles are injected into muscle, taken up by motor neurons synapses, and retrogradely transported to the cell body. The viral genome, coding for either trophic factors supporting motor neurons or siRNA targeting specific mRNAs, such as mutant SOD1 mRNA, is then transcribed in the nucleus. Viral particles may be also injected into the spinal cord, possibly extending the efficacy of gene therapy also to nonneuronal cells surrounding motor neurons.

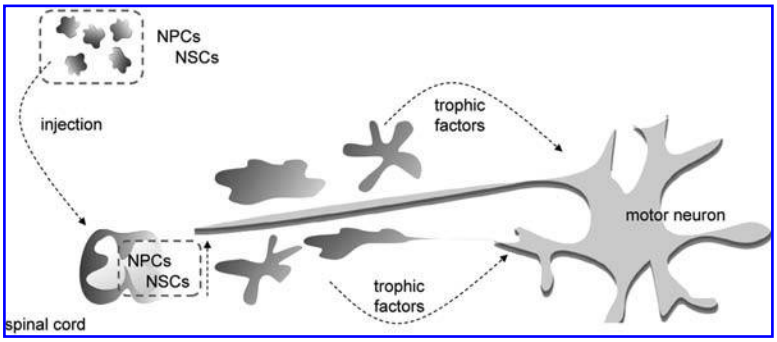

FIG. 14. Stem cell therapies in ALS. Undifferentiated or transdifferentiated neural stem cells (NSCs) and neural precursor cells (NPCs), either genetically manipulated for the release of trophic factors or not, could be a candidate source for local or systemic cell therapies in ALS. The injection of precursor cells may reinforce a natural response to degeneration of motor neurons that is known to promote proliferation and migration in the spinal cords of neural progenitor cells.

resist neurodegenerative inducers such as amyloid-β peptide and mutant SOD1 (82).

In the SOD1 transgenic mouse model, implanted neuron-like cells derived from a human teratocarcinoma cell line (131), testicular Sertoli cells (162), or intravenously administered human umbilical-cord blood cells (132) had a significant neuroprotective benefit to vulnerable motor neurons, most probably as the result of secreted neurotrophic factors. Klein and collaborators (206) demonstrated that human NPCs isolated from the cortex could be expanded in culture and modified by using lentivirus to secrete GDNF. These cells were then transplanted into the lumbar spinal cord of the mutant SOD1 rat model of ALS, and they survived, integrated, and released GDNF within the region of cell survival, but not outside this area. In these studies, neuron survival was increased in proximity to the site of implantation, but this did not modify progression of the disease or the animal life span. Only more recently, Corti *et al*. (80) showed that a subpopulation of neural stem cells that can generate cholinergic motor neuron–like cells on differentiation *in vitro* was able to modify disease progression in transplanted transgenic mice.

A known problem with cell therapy is the number of cells to be transplanted, and their ability to persist in time. In principle, this may be overcome through genetic engineering of NSC clonally derived and immortalized *via* a retroviral vector encoded with v-*myc* oncogene, which would provide a limitless supply of neurons for treatment for patients, a strategy that has been found feasible in mice (203), but not yet tested in humans.

Adverse reaction to stem cell therapy may also constitute a problem. For instance, use of this approach in Parkinson patients was complicated by the development of dyskinesia that persisted after discontinuation of levodopa (359).

Preliminary stem transplantation trials have already been performed in ALS patients. The feasibility and safety of intra–spinal cord implantation of autologous mesenchymal stem cells (MSCs) was evaluated in seven ALS patients, and no patient was reported to manifest major adverse events. Reportedly, a significant slowing of the linear decline of the forced vital capacity was evident in four patients 36 months after MSCs transplantation (252), but no evidence of the fate of the implanted cells has been provided.

VI. CONCLUDING REMARKS

The last decade has witnessed three major advances in our understanding of ALS.

First, a wealth of new evidence on experimental models based on mutant SOD1 has allowed deeper insights into the mechanisms leading to degeneration of motor neurons and of other genes and factors that participate to the pathogenesis of sporadic ALS. Oxidative stress may play diverse roles, acting both directly, through damage of crucial molecules, and indirectly, modulating the level of expression and/or the bioavailability of other important players in the mechanisms of signal transduction that dictate motor neuron survival.

Second, ALS has been recognized as multisystemic disease, and the contribution of signals originating in cells other than motor neurons to the progression of the disease has been clearly assessed. This has changed our perspective on therapeutic strategies worthy of consideration in the near future. In particular, the concept of multidrug approaches has been strongly supported by a number of studies in which interception of neuronal waste has been addressed.

Last, but not the least, a significant step forward has been made in our knowledge of how motor neurons may be targeted through gene therapy, both for the silencing of noxious genes and for delivering trophic factors, whereas stem cell therapy is still an attractive, albeit difficult, option for support to damaged neurons.

ACKNOWLEDGMENTS

This work was supported by grants from MIUR (PRIN 2005057598 “Segnali e dialogo fra cellule neuronali, gliali e muscolari nella sclerosi laterale amiotrofica”), Min. Salute (PF “Meccanismi molecolari e cellulari delle malattie neurodegenerative del sistema motorio,” and PF “Tollerabilità e efficacia di interventi nutrizionali nella SLA: studio in vitro e in vivo”) and by SISAL and Telethon (GGP07018).

ABBREVIATIONS

AAV, Adeno-associated virus; ALS, amyotrophic lateral sclerosis; ANG, angiogenin; BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; CCS, copper chaperone for superoxide dismutase; Cp, ceruloplasmin; COX2, cyclooxygenase2; CSF, cerebrospinal fluid; 8-OhdG, 8-hydroxy-2′-deoxyguanosine; EAAT, excitatory amino acid transporter; fALS, familial amyotrophic lateral sclerosis; Ft, ferritin; GDNF, glia-derived neurotrophic factor; GLT-1, glutamate transporter

1; GSH, glutathione (reduced); GSSG, glutathione (oxidized); Hfe, hemochromatosis gene; iNOS, inducible NO-synthase; IFN-γ, interferon γ; IL, interleukin; IGF-1, insulin-like growth factor 1; IREs, iron regulatory elements; IRP, iron regulatory protein; MAPK, mitogen activated protein kinase; NGF, nerve growth factor; NPCs, neural precursor cells; NSCs, neural stem cells; NT, neurotrophin; NTF, neurotrophic factor; ROS, reactive oxygen species; seRNAs, silencing RNAs; SOD, superoxide dismutase; sALS, sporadic amyotrophic lateral sclerosis; SMN, survival motor neuron; Tf, transferrin; TfR, transferrin receptor; TNF, tumor necrosis factor; *VEGF*, vascular endothelial growth factor.

REFERENCES

1. ALS CNTF Treatment Study Group. A double-blind placebo-controlled clinical trial of subcutaneous recombinant human ciliary neurotrophic factor (rHCNTF) in amyotrophic lateral sclerosis. *Neurology* 46: 1244–1249, 1996.
2. The BDNF Study Group (Phase III). A controlled trial of recombinant methionyl human BDNF in ALS. *Neurology* 52: 1427–1433, 1999.
3. Acsadi G, Anguelov RA, Yang H, Toth G, Thomas R, Jani A, Wang Y, Ianakova E, Mohammad S, Lewis RA, and Shy ME. Increased survival and function of SOD1 mice after glial cell-derived neurotrophic factor gene therapy. *Hum Gene Ther* 13: 1047–1059, 2002.
4. Adem A, Ekblom J, and Gillberg PG. Growth factor receptors in amyotrophic lateral sclerosis. *Mol Neurobiol* 9: 225–231, 1994.
5. Ahmad-Annuar A, Shah P, Hafezparast M, Hummerich H, Witherden AS, Morrison KE, Shaw PJ, Kirby J, Warner TT, Crosby A, Proukakis C, Wilkinson P, Orrell RW, Bradley L, Martin JE, and Fisher EM. No association with common Caucasian genotypes in exons 8, 13 and 14 of the human cytoplasmic dynein heavy chain gene (DNCHC1) and familial motor neuron disorders. *Amyotroph Lateral Scler Other Motor Neuron Disord* 4: 150–157, 2003.
6. Ahtoniemi T, Goldsteins G, Keksa-Goldsteine V, Malm T, Kanninen K, Salminen A, and Koistinaho J. Pyrrolidine dithiocarbamate inhibits induction of immunoproteasome and decreases survival in a rat model of amyotrophic lateral sclerosis. *Mol Pharmacol* 71: 30–37, 2007.
7. Al-Chalabi A, Andersen PM, Nilsson P, Chioza B, Andersson JL, Russ C, Shaw CE, Powell JF, and Leigh PN. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. *Hum Mol Genet* 8: 157–164, 1999.
8. Al-Chalabi A, Hansen VK, Simpson CL, Xi J, Hosler BA, Powell JF, McKenna-Yasek D, Shaw CE, Leigh PN, and Brown RH Jr. Variants in the ALS2 gene are not associated with sporadic amyotrophic lateral sclerosis. *Neurogenetics* 4: 221–222, 2003.
9. Al-Chalabi A, Scheffler MD, Smith BN, Parton MJ, Cudkowicz ME, Andersen PM, Hayden DL, Hansen VK, Turner MR, Shaw CE, Leigh PN, and Brown RH Jr. Ciliary neurotrophic factor genotype does not influence clinical phenotype in amyotrophic lateral sclerosis. *Ann Neurol* 54: 130–134, 2003.
10. Alexianu ME, Kozovska M, and Appel SH. Immune reactivity in a mouse model of familial ALS correlates with disease progression. *Neurology* 57: 1282–1289, 2001.
11. Almer G, Guegan C, Teismann P, Naini A, Rosoklija G, Hays AP, Chen C, and Przedborski S. Increased expression of the pro-inflammatory enzyme cyclooxygenase-2 in amyotrophic lateral sclerosis. *Ann Neurol* 49: 176–185, 2001.
12. Almer G, Kikuchi H, Teismann P, and Przedborski S. Is prostaglandin E(2) a pathogenic factor in amyotrophic lateral sclerosis? *Ann Neurol* 59: 980–983, 2006.
13. Amin AR, Attur MG, Thakker GD, Patel PD, Vyas PR, Patel RN, Patel IR, and Abramson SB. A novel mechanism of action of tetracyclines: effects on nitric oxide synthases. *Proc Natl Acad Sci U S A* 93: 14014–14019, 1996.
14. Andersen PM. Amyotrophic lateral sclerosis associated with mutations in the CuZn superoxide dismutase gene. *Curr Neurol Neurosci Rep* 6: 37–46, 2006.
15. Andreassen OA, Ferrante RJ, Klivenyi P, Klein AM, Shinobu LA, Epstein CJ, and Beal MF. Partial deficiency of manganese superoxide dismutase exacerbates a transgenic mouse model of amyotrophic lateral sclerosis. *Ann Neurol* 47: 447–455, 2000.
16. Andrus PK, Fleck TJ, Gurney ME, and Hall ED. Protein oxidative damage in a transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem* 71: 2041–2048, 1998.
17. Aoki M, Lin CL, Rothstein JD, Geller BA, Hosler BA, Munsat TL, Horvitz HR, and Brown RH Jr. Mutations in the glutamate transporter EAAT2 gene do not cause abnormal EAAT2 transcripts in amyotrophic lateral sclerosis. *Ann Neurol* 43: 645–653, 1998.
18. Aquilano K, Vigilanza P, Rotilio G, and Ciriolo MR. Mitochondrial damage due to SOD1 deficiency in SH-SY5Y neuroblastoma cells: a rationale for the redundancy of SOD1. *FASEB J* 20: 1683–1685, 2006.
19. Arai T, Hasegawa M, Akiyama H, Ikeda K, Nonaka T, Mori H, Mann D, Tsuchiya K, Yoshida M, Hashizume Y, and Oda T. TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Biophys Res Commun* 351: 602–611, 2006.
20. Arnesano F, Banci L, Bertini I, Martinelli M, Furukawa Y, and O'Halloran TV. The unusually stable quaternary structure of human Cu,Zn-superoxide dismutase 1 is controlled by both metal occupancy and disulfide status. *J Biol Chem* 279: 47998–48003, 2004.
21. Ascherio A, Weisskopf MG, O'Reilly E J, Jacobs EJ, McCullough ML, Calle EE, Cudkowicz M, and Thun MJ. Vitamin E intake and risk of amyotrophic lateral sclerosis. *Ann Neurol* 57: 104–110, 2005.
22. Azari MF, Galle A, Lopes EC, Kurek J, and Cheema SS. Leukemia inhibitory factor by systemic administration rescues spinal motor neurons in the SOD1 G93A murine model of familial amyotrophic lateral sclerosis. *Brain Res* 922: 144–147, 2001.
23. Azzouz M, Hottinger A, Paterna JC, Zurn AD, Aebischer P, and Bueler H. Increased motoneuron survival and improved neuromuscular function in transgenic ALS mice after intraspinal injection of an adeno-associated virus encoding Bcl-2. *Hum Mol Genet* 9: 803–811, 2000.
24. Azzouz M, Ralph GS, Storkebaum E, Walmsley LE, Mitrophanous KA, Kingsman SM, Carmeliet P, and Mazarakis ND. VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature* 429: 413–417, 2004.
25. Azzouz M. Gene therapy for ALS: progress and prospects. *Biochim Biophys Acta* 1762: 1122–1127, 2006.
26. Babu JR, Geetha T, and Wooten MW. Sequestosome 1/p62 shuttles polyubiquitinated tau for proteasomal degradation. *J Neurochem* 94: 192–203, 2005.
27. Balamurugan K and Schaffner W. Copper homeostasis in eukaryotes: teetering on a tightrope. *Biochim Biophys Acta* 1763: 737–746, 2006.
28. Banci L, Bertini I, Durazo A, Girotto S, Gralla EB, Martinelli M, Valentine JS, Vieru M, and Whitelegge JP. Metal-free superoxide dismutase forms soluble oligomers under physiological conditions: a possible general mechanism for familial ALS. *Proc Natl Acad Sci U S A* 104: 11263–11267, 2007.
29. Baron P, Bussini S, Cardin V, Corbo M, Conti G, Galimberti D, Scarpini E, Bresolin N, Wharton SB, Shaw PJ, and Silani V. Production of monocyte chemoattractant protein-1 in amyotrophic lateral sclerosis. *Muscle Nerve* 32: 541–544, 2005.
30. Basso M, Massignan T, Samengo G, Cheroni C, De Biasi S, Salmona M, Bendotti C, and Bonetto V. Insoluble mutant SOD1 is partly oligoubiquitinated in amyotrophic lateral sclerosis mice. *J Biol Chem* 281: 33325–33335, 2006.
31. Beal MF, Ferrante RJ, Browne SE, Matthews RT, Kowall NW, and Brown RH Jr. Increased 3-nitrotyrosine in both sporadic and familial amyotrophic lateral sclerosis. *Ann Neurol* 42: 644–654, 1997.
32. Beal MF. Mitochondria take center stage in aging and neurodegeneration. *Ann Neurol* 58: 495–505, 2005.

33. Beaulieu JM, Nguyen MD, and Julien JP. Late onset of motor neurons in mice overexpressing wild-type peripherin. *J Cell Biol* 147: 531–544, 1999.
34. Beers DR, Henkel JS, Xiao Q, Zhao W, Wang J, Yen AA, Siklos L, McKercher SR, and Appel SH. Wild-type microglia extend survival in PU.1 knockout mice with familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 103: 16021–16026, 2006.
35. Beghi E, Bendotti C, and Mennini T. Merits of a new drug trial for ALS? *Science* 308: 632–633; author reply 632–633, 2005.
36. Benatar M. Lost in translation: treatment trials in the SOD1 mouse and in human ALS. *Neurobiol Dis* 26: 1–13, 2007.
37. Bendotti C, Calvaresi N, Chiveri L, Prelle A, Moggio M, Braga M, Silani V, and De Biasi S. Early vacuolization and mitochondrial damage in motor neurons of FALS mice are not associated with apoptosis or with changes in cytochrome oxidase histochemical reactivity. *J Neurol Sci* 191: 25–33, 2001.
38. Bendotti C, Tortarolo M, Suchak SK, Calvaresi N, Carvelli L, Bastone A, Rizzi M, Rattray M, and Mennini T. Transgenic SOD1 G93A mice develop reduced GLT-1 in spinal cord without alterations in cerebrospinal fluid glutamate levels. *J Neurochem* 79: 737–746, 2001.
39. Bendotti C, Atzori C, Piva R, Tortarolo M, Strong MJ, DeBiasi S, and Migheli A. Activated p38MAPK is a novel component of the intracellular inclusions found in human amyotrophic lateral sclerosis and mutant SOD1 transgenic mice. *J Neuropathol Exp Neurol* 63: 113–119, 2004.
40. Bendotti C and Carri MT. Lessons from models of SOD1-linked familial ALS. *Trends Mol Med* 10: 393–400, 2004.
41. Beretta S, Sala G, Mattavelli L, Ceresa C, Casciati A, Ferri A, Carri MT, and Ferrarese C. Mitochondrial dysfunction due to mutant copper/zinc superoxide dismutase associated with amyotrophic lateral sclerosis is reversed by N-acetylcysteine. *Neurobiol Dis* 13: 213–221, 2003.
42. Bergemalm D, Jonsson PA, Graffmo KS, Andersen PM, Brannstrom T, Rehnmark A, and Marklund SL. Overloading of stable and exclusion of unstable human superoxide dismutase-1 variants in mitochondria of murine amyotrophic lateral sclerosis models. *J Neurosci* 26: 4147–4154, 2006.
43. Bergomi M, Vinceti M, Nacci G, Pietrini V, Bratter P, Alber D, Ferrari A, Vescovi L, Guidetti D, Sola P, Malagu S, Aramini C, and Vivoli G. Environmental exposure to trace elements and risk of amyotrophic lateral sclerosis: a population-based case-control study. *Environ Res* 89: 116–123, 2002.
44. Bogaert E, Van Damme P, Van Den Bosch L, and Robberecht W. Vascular endothelial growth factor in amyotrophic lateral sclerosis and other neurodegenerative diseases. *Muscle Nerve* 34: 391–405, 2006.
45. Bogdanov M, Brown RH, Matson W, Smart R, Hayden D, O'Donnell H, Flint Beal M, and Cudkowicz M. Increased oxidative damage to DNA in ALS patients. *Free Radic Biol Med* 29: 652–658, 2000.
46. Bogdanov MB, Ramos LE, Xu Z, and Beal MF. Elevated "hydroxyl radical" generation in vivo in an animal model of amyotrophic lateral sclerosis. *J Neurochem* 71: 1321–1324, 1998.
47. Boillee S, Vande Velde C, and Cleveland DW. ALS: a disease of motor neurons and their nonneuronal neighbors. *Neuron* 52: 39–59, 2006.
48. Boillee S, Yamanaka K, Lobsiger CS, Copeland NG, Jenkins NA, Kassiotis G, Kollias G, and Cleveland DW. Onset and progression in inherited ALS determined by motor neurons and microglia. *Science* 312: 1389–1392, 2006.
49. Borasio GD, Robberecht W, Leigh PN, Emile J, Guiloff RJ, Jerusalem F, Silani V, Vos PE, Wokke JH, and Dobbins T. A placebo-controlled trial of insulin-like growth factor-I in amyotrophic lateral sclerosis: European ALS/IGF-I Study Group. *Neurology* 51: 583–586, 1998.
50. Boston-Howes W, Gibb SL, Williams EO, Pasinelli P, Brown RH Jr, and Trotti D. Caspase-3 cleaves and inactivates the glutamate transporter EAAT2. *J Biol Chem* 281: 14076–14084, 2006.
51. Bowling AC, Schulz JB, Brown RH Jr, and Beal MF. Superoxide dismutase activity, oxidative damage, and mitochondrial energy metabolism in familial and sporadic amyotrophic lateral sclerosis. *J Neurochem* 61: 2322–2325, 1993.
52. Bristol LA and Rothstein JD. Glutamate transporter gene expression in amyotrophic lateral sclerosis motor cortex. *Ann Neurol* 39: 676–679, 1996.
53. Brockington A, Wharton SB, Fernando M, Gelsthorpe CH, Baxter L, Ince PG, Lewis CE, and Shaw PJ. Expression of vascular endothelial growth factor and its receptors in the central nervous system in amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 65: 26–36, 2006.
54. Brooke MH, Florence JM, Heller SL, Kaiser KK, Phillips D, Gruber A, Babcock D, and Miller JP. Controlled trial of thyrotropin releasing hormone in amyotrophic lateral sclerosis. *Neurology* 36: 146–151, 1986.
55. Bruijn LI, Becher MW, Lee MK, Anderson KL, Jenkins NA, Copeland NG, Sisodia SS, Rothstein JD, Borchelt DR, Price DL, and Cleveland DW. ALS-linked SOD1 mutant G85R mediates damage to astrocytes and promotes rapidly progressive disease with SOD1-containing inclusions. *Neuron* 18: 327–338, 1997.
56. Bruijn LI, Houseweart MK, Kato S, Anderson KL, Anderson SD, Ohama E, Reaume AG, Scott RW, and Cleveland DW. Aggregation and motor neuron toxicity of an ALS-linked SOD1 mutant independent from wild-type SOD1. *Science* 281: 1851–1854, 1998.
57. Cai H, Lin X, Xie C, Laird FM, Lai C, Wen H, Chiang HC, Shim H, Farah MH, Hoke A, Price DL, and Wong PC. Loss of ALS2 function is insufficient to trigger motor neuron degeneration in knock-out mice but predisposes neurons to oxidative stress. *J Neurosci* 25: 7567–7574, 2005.
58. Calamai M, Taddei N, Stefani M, Ramponi G, and Chiti F. Relative influence of hydrophobicity and net charge in the aggregation of two homologous proteins. *Biochemistry* 42: 15078–15083, 2003.
59. Caroscio JT, Cohen JA, Zawodniak J, Takai V, Shapiro A, Blaustein S, Mulvihill MN, Loucas SP, Gudesblatt M, Rube D, and et al. A double-blind, placebo-controlled trial of TRH in amyotrophic lateral sclerosis. *Neurology* 36: 141–145, 1986.
60. Carri MT, Ferri A, Battistoni A, Famhy L, Gabbianelli R, Poccia F, and Rotilio G. Expression of a Cu,Zn superoxide dismutase typical of familial amyotrophic lateral sclerosis induces mitochondrial alteration and increase of cytosolic Ca2+ concentration in transfected neuroblastoma SH-SY5Y cells. *FEBS Lett* 414: 365–368, 1997.
61. Carri MT, Grignaschi G, and Bendotti C. Targets in ALS: designing multidrug therapies. *Trends Pharmacol Sci* 27: 267–273, 2006.
62. Casoni F, Basso M, Massignan T, Gianazza E, Cheroni C, Salmona M, Bendotti C, and Bonetto V. Protein nitration in a mouse model of familial amyotrophic lateral sclerosis: possible multifunctional role in the pathogenesis. *J Biol Chem* 280: 16295–16304, 2005.
63. Cassina P, Pehar M, Vargas MR, Castellanos R, Barbeito AG, Estevez AG, Thompson JA, Beckman JS, and Barbeito L. Astrocyte activation by fibroblast growth factor-1 and motor neuron apoptosis: implications for amyotrophic lateral sclerosis. *J Neurochem* 93: 38–46, 2005.
64. Cha CI, Chung YH, Shin CM, Shin DH, Kim YS, Gurney ME, and Lee KW. Immunocytochemical study on the distribution of nitrotyrosine in the brain of the transgenic mice expressing a human Cu/Zn SOD mutation. *Brain Res* 853: 156–161, 2000.
65. Charcot JM and A J. Deux cas d'atrophie musculaire progressive avec lésions de la substance grise et des faiseaux antérolatéraux de la moelle épinière. *Arch Physiol Neurol Path* 2: 744–754, 1869.
66. Chen M, Ona VO, Li M, Ferrante RJ, Fink KB, Zhu S, Bian J, Guo L, Farrell LA, Hersch SM, Hobbs W, Vonsattel JP, Cha JH, and Friedlander RM. Minocycline inhibits caspase-1 and caspase-3 expression and delays mortality in a transgenic mouse model of Huntington disease. *Nat Med* 6: 797–801, 2000.
67. Chen W, Saeed M, Mao H, Siddique N, Dellefave L, Hung WY, Deng HX, Sufit RL, Heller SL, Haines JL, Pericak-Vance M, and Siddique T. Lack of association of VEGF promoter polymorphisms with sporadic ALS. *Neurology* 67: 508–510, 2006.
68. Chen YZ, Bennett CL, Huynh HM, Blair IP, Puls I, Irobi J, Dierick I, Abel A, Kennerson ML, Rabin BA, Nicholson GA, Auer-Grumbach M, Wagner K, De Jonghe P, Griffin JW, Fischbeck

KH, Timmerman V, Cornblath DR, and Chance PF. DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). *Am J Hum Genet* 74: 1128–1135, 2004.
69. Cheroni C, Peviani M, Cascio P, Debiasi S, Monti C, and Bendotti C. Accumulation of human SOD1 and ubiquitinated deposits in the spinal cord of SOD1G93A mice during motor neuron disease progression correlates with a decrease of proteasome. *Neurobiol Dis* 18: 509–522, 2005.
70. Chi L, Ke Y, Luo C, Li B, Gozal D, Kalyanaraman B, and Liu R. Motor neuron degeneration promotes neural progenitor cell proliferation, migration, and neurogenesis in the spinal cords of amyotrophic lateral sclerosis mice. *Stem Cells* 24: 34–43, 2006.
71. Chiti F, Calamai M, Taddei N, Stefani M, Ramponi G, and Dobson CM. Studies of the aggregation of mutant proteins in vitro provide insights into the genetics of amyloid diseases. *Proc Natl Acad Sci U S A* 99(suppl 4): 16419–16426, 2002.
72. Choi J, Rees HD, Weintraub ST, Levey AI, Chin LS, and Li L. Oxidative modifications and aggregation of Cu,Zn-superoxide dismutase associated with Alzheimer and Parkinson diseases. *J Biol Chem* 280: 11648–11655, 2005.
73. Chou SM, Wang HS, Taniguchi A, and Bucala R. Advanced glycation endproducts in neurofilament conglomeration of motoneurons in familial and sporadic amyotrophic lateral sclerosis. *Mol Med* 4: 324–332, 1998.
74. Ciriolo MR, De Martino A, Lafavia E, Rossi L, Carri MT, and Rotilio G. Cu,Zn-superoxide dismutase-dependent apoptosis induced by nitric oxide in neuronal cells. *J Biol Chem* 275: 5065–5072, 2000.
75. Clement AM, Nguyen MD, Roberts EA, Garcia ML, Boillee S, Rule M, McMahon AP, Doucette W, Siwek D, Ferrante RJ, Brown RH Jr, Julien JP, Goldstein LS, and Cleveland DW. Wild-type nonneuronal cells extend survival of SOD1 mutant motor neurons in ALS mice. *Science* 302: 113–117, 2003.
76. Colton CA, Snell J, Chernyshev O, and Gilbert DL. Induction of superoxide anion and nitric oxide production in cultured microglia. *Ann N Y Acad Sci* 738: 54–63, 1994.
77. Coppede F, Mancuso M, Lo Gerfo A, Carlesi C, Piazza S, Rocchi A, Petrozzi L, Nesti C, Micheli D, Bacci A, Migliore L, Murri L, and Siciliano G. Association of the hOGG1 Ser326Cys polymorphism with sporadic amyotrophic lateral sclerosis. *Neurosci Lett* 420: 163–168, 2007.
78. Corcia P, Camu W, Halimi JM, Vourc'h P, Antar C, Vedrine S, Giraudeau B, de Toffol B, and Andres CR. SMN1 gene, but not SMN2, is a risk factor for sporadic ALS. *Neurology* 67: 1147–1150, 2006.
79. Corse AM, Bilak MM, Bilak SR, Lehar M, Rothstein JD, and Kuncl RW. Preclinical testing of neuroprotective neurotrophic factors in a model of chronic motor neuron degeneration. *Neurobiol Dis* 6: 335–346, 1999.
80. Corti S, Locatelli F, Papadimitriou D, Del Bo R, Nizzardo M, Nardini M, Donadoni C, Salani S, Fortunato F, Strazzer S, Bresolin N, and Comi GP. Neural stem cells LewisX+ CXCR4+ modify disease progression in an amyotrophic lateral sclerosis model. *Brain* 130: 1289–1305, 2007.
81. Cote F, Collard JF, and Julien JP. Progressive neuronopathy in transgenic mice expressing the human neurofilament heavy gene: a mouse model of amyotrophic lateral sclerosis. *Cell* 73: 35–46, 1993.
82. Cozzolino M, Ferraro E, Ferri A, Rigamonti D, Quondamatteo F, Ding H, Xu ZS, Ferrari F, Angelini DF, Rotilio G, Cattaneo E, Carri MT, and Cecconi F. Apoptosome inactivation rescues proneural and neural cells from neurodegeneration. *Cell Death Differ* 11: 1179–1191, 2004.
83. Cozzolino M, Ferri A, Ferraro E, Rotilio G, Cecconi F, and Carri MT. Apaf1 mediates apoptosis and mitochondrial damage induced by mutant human SOD1s typical of familial amyotrophic lateral sclerosis. *Neurobiol Dis* 21: 69–79, 2006.
84. Crawford TO and Skolasky RL Jr. The relationship of SMN to amyotrophic lateral sclerosis. *Ann Neurol* 52: 857–858; author reply 858–861, 2002.
85. Cronin S, Greenway MJ, Ennis S, Kieran D, Green A, Prehn JH, and Hardiman O. Elevated serum angiogenin levels in ALS. *Neurology* 67: 1833–1836, 2006.
86. Crosio C, Casciati A, Iaccarino C, Rotilio G, and Carri MT. Bcl2a1 serves as a switch in death of motor neurons in amyotrophic lateral sclerosis. *Cell Death Differ* 13: 2150–2153, 2006.
87. Crow JP, Ye YZ, Strong M, Kirk M, Barnes S, and Beckman JS. Superoxide dismutase catalyzes nitration of tyrosines by peroxynitrite in the rod and head domains of neurofilament-L. *J Neurochem* 69: 1945–1953, 1997.
88. Crow JP, Calingasan NY, Chen J, Hill JL, and Beal MF. Manganese porphyrin given at symptom onset markedly extends survival of ALS mice. *Ann Neurol* 58: 258–265, 2005.
89. Cudkowicz ME, Shefner JM, Schoenfeld DA, Zhang H, Andreasson KI, Rothstein JD, and Drachman DB. Trial of celecoxib in amyotrophic lateral sclerosis. *Ann Neurol* 60: 22–31, 2006.
90. Dal Canto MC and Gurney ME. Neuropathological changes in two lines of mice carrying a transgene for mutant human Cu,Zn SOD, and in mice overexpressing wild type human SOD: a model of familial amyotrophic lateral sclerosis (FALS). *Brain Res* 676: 25–40, 1995.
91. Danbolt NC. Glutamate uptake. *Prog Neurobiol* 65: 1–105, 2001.
92. Danzeisen R, Achsel T, Bederke U, Cozzolino M, Crosio C, Ferri A, Frenzel M, Gralla EB, Huber L, Ludolph A, Nencini M, Rotilio G, Valentine JS, and Carri MT. Superoxide dismutase 1 modulates expression of transferrin receptor. *J Biol Inorg Chem* 11: 489–498, 2006.
93. Del Bo R, Scarlato M, Ghezzi S, Martinelli-Boneschi F, Corti S, Locatelli F, Santoro D, Prelle A, Briani C, Nardini M, Siciliano G, Mancuso M, Murri L, Bresolin N, and Comi GP. Absence of angiogenic genes modification in Italian ALS patients. *Neurobiol Aging* 2006. [Epub ahead of print.]
94. Deng HX, Hentati A, Tainer JA, Iqbal Z, Cayabyab A, Hung WY, Getzoff ED, Hu P, Herzfeldt B, Roos RP, Warner C, Deng G, Soriano E, Smyth C, Parge HE, Ahmed A, Roses AD, Hallewell RA, Pericak-Vance MA, and Siddique T. Amyotrophic lateral sclerosis and structural defects in Cu,Zn superoxide dismutase. *Science* 261: 1047–1051, 1993.
95. Deng HX, Shi Y, Furukawa Y, Zhai H, Fu R, Liu E, Gorrie GH, Khan MS, Hung WY, Bigio EH, Lukas T, Dal Canto MC, O'Halloran TV, and Siddique T. Conversion to the amyotrophic lateral sclerosis phenotype is associated with intermolecular linked insoluble aggregates of SOD1 in mitochondria. *Proc Natl Acad Sci U S A* 103: 7142–7147, 2006.
96. Devon RS, Orban PC, Gerrow K, Barbieri MA, Schwab C, Cao LP, Helm JR, Bissada N, Cruz-Aguado R, Davidson TL, Witmer J, Metzler M, Lam CK, Tetzlaff W, Simpson EM, McCaffery JM, El-Husseini AE, Leavitt BR, and Hayden MR. Als2-deficient mice exhibit disturbances in endosome trafficking associated with motor behavioral abnormalities. *Proc Natl Acad Sci U S A* 103: 9595–9600, 2006.
97. Di Giorgio FP, Carrasco MA, Siao MC, Maniatis T, and Eggan K. Non-cell autonomous effect of glia on motor neurons in an embryonic stem cell-based ALS model. *Nat Neurosci* 10: 608–614, 2007.
98. DiDonato M, Craig L, Huff ME, Thayer MM, Cardoso RM, Kassmann CJ, Lo TP, Bruns CK, Powers ET, Kelly JW, Getzoff ED, and Tainer JA. ALS mutants of human superoxide dismutase form fibrous aggregates via framework destabilization. *J Mol Biol* 332: 601–615, 2003.
99. Dobrowolny G, Giacinti C, Pelosi L, Nicoletti C, Winn N, Barberi L, Molinaro M, Rosenthal N, and Musaro A. Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. *J Cell Biol* 168: 193–199, 2005.
100. Drachman DB, Frank K, Dykes-Hoberg M, Teismann P, Almer G, Przedborski S, and Rothstein JD. Cyclooxygenase 2 inhibition protects motor neurons and prolongs survival in a transgenic mouse model of ALS. *Ann Neurol* 52: 771–778, 2002.
101. Duberley RM, Johnson IP, Anand P, Leigh PN, and Cairns NJ. Immunocytochemical studies of neurotrophins in cerebral motor cortex in amyotrophic lateral sclerosis. *Brain Res* 763: 259–263, 1997.
102. Dunnett SB and Rosser AE. Stem cell transplantation for Huntington's disease. *Exp Neurol* 203: 279–292, 2007.
103. Dupuis L, Gonzalez de Aguilar JL, di Scala F, Rene F, de Tapia M, Pradat PF, Lacomblez L, Seihlan D, Prinjha R, Walsh FS, Meininger V, and Loeffler JP. Nogo provides a molecular marker

for diagnosis of amyotrophic lateral sclerosis. *Neurobiol Dis* 10: 358–365, 2002.
104. Durham HD, Roy J, Dong L, and Figlewicz DA. Aggregation of mutant Cu/Zn superoxide dismutase proteins in a culture model of ALS. *J Neuropathol Exp Neurol* 56: 523–530, 1997.
105. Dykens JA. Isolated cerebral and cerebellar mitochondria produce free radicals when exposed to elevated CA2+ and Na+: implications for neurodegeneration. *J Neurochem* 63: 584–591, 1994.
106. Ekestern E. Neurotrophic factors and amyotrophic lateral sclerosis. *Neurodegener Dis* 1: 88–100, 2004.
107. Elam JS, Malek K, Rodriguez JA, Doucette PA, Taylor AB, Hayward LJ, Cabelli DE, Valentine JS, and Hart PJ. An alternative mechanism of bicarbonate-mediated peroxidation by copper-zinc superoxide dismutase: rates enhanced via proposed enzyme-associated peroxycarbonate intermediate. *J Biol Chem* 278: 21032–21039, 2003.
108. Elam JS, Taylor AB, Strange R, Antonyuk S, Doucette PA, Rodriguez JA, Hasnain SS, Hayward LJ, Valentine JS, Yeates TO, and Hart PJ. Amyloid-like filaments and water-filled nanotubes formed by SOD1 mutant proteins linked to familial ALS. *Nat Struct Biol* 10: 461–467, 2003.
109. Elliott JL. Cytokine upregulation in a murine model of familial amyotrophic lateral sclerosis. *Brain Res Mol Brain Res* 95: 172–178, 2001.
110. Eymard-Pierre E, Lesca G, Dollet S, Santorelli FM, di Capua M, Bertini E, and Boespflug-Tanguy O. Infantile-onset ascending hereditary spastic paralysis is associated with mutations in the alsin gene. *Am J Hum Genet* 71: 518–527, 2002.
111. Eymard-Pierre E, Yamanaka K, Haeussler M, Kress W, Gauthier-Barichard F, Combes P, Cleveland DW, and Boespflug-Tanguy O. Novel missense mutation in ALS2 gene results in infantile ascending hereditary spastic paralysis. *Ann Neurol* 59: 976–980, 2006.
112. Ezzi SA, Urushitani M, and Julien JP. Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation. *J Neurochem* 102: 170–178, 2007.
113. Fergani A, Dupuis L, Jokic N, Larmet Y, de Tapia M, Rene F, Loeffler JP, and Gonzalez de Aguilar JL. Reticulons as markers of neurological diseases: focus on amyotrophic lateral sclerosis. *Neurodegener Dis* 2: 185–194, 2005.
114. Fernandez-Santiago R, Sharma M, Mueller JC, Gohlke H, Illig T, Anneser J, Munch C, Ludolph A, Kamm C, and Gasser T. Possible gender-dependent association of vascular endothelial growth factor (VEGF) gene and ALS. *Neurology* 66: 1929–1931, 2006.
115. Ferrante KL, Shefner J, Zhang H, Betensky R, O'Brien M, Yu H, Fantasia M, Taft J, Beal MF, Traynor B, Newhall K, Donofrio P, Caress J, Ashburn C, Freiberg B, O'Neill C, Paladenech C, Walker T, Pestronk A, Abrams B, Florence J, Renna R, Schierbecker J, Malkus B, and Cudkowicz M. Tolerance of high-dose (3,000 mg/day) coenzyme Q10 in ALS. *Neurology* 65: 1834–1836, 2005.
116. Ferrante RJ, Browne SE, Shinobu LA, Bowling AC, Baik MJ, MacGarvey U, Kowall NW, Brown RH Jr, and Beal MF. Evidence of increased oxidative damage in both sporadic and familial amyotrophic lateral sclerosis. *J Neurochem* 69: 2064–2074, 1997.
117. Ferrante RJ, Klein AM, Dedeoglu A, and Beal MF. Therapeutic efficacy of EGb761 (*Gingko biloba* extract) in a transgenic mouse model of amyotrophic lateral sclerosis. *J Mol Neurosci* 17: 89–96, 2001.
118. Ferri A, Nencini M, Casciati A, Cozzolino M, Angelini DF, Longone P, Spalloni A, Rotilio G, and Carri MT. Cell death in amyotrophic lateral sclerosis: interplay between neuronal and glial cells. *FASEB J* 18: 1261–1263, 2004.
119. Ferri A, Cozzolino M, Crosio C, Nencini M, Casciati A, Gralla EB, Rotilio G, Valentine JS, and Carri MT. Familial ALS-superoxide dismutases associate with mitochondria and shift their redox potentials. *Proc Natl Acad Sci U S A* 103: 13860–13865, 2006.
120. Field LS, Furukawa Y, O'Halloran TV, and Culotta VC. Factors controlling the uptake of yeast copper/zinc superoxide dismutase into mitochondria. *J Biol Chem* 278: 28052–28059, 2003.
121. Figlewicz DA, Krizus A, Martinoli MG, Meininger V, Dib M, Rouleau GA, and Julien JP. Variants of the heavy neurofilament subunit are associated with the development of amyotrophic lateral sclerosis. *Hum Mol Genet* 3: 1757–1761, 1994.
122. Fleury C, Mignotte B, and Vayssiere JL. Mitochondrial reactive oxygen species in cell death signaling. *Biochimie* 84: 131–141, 2002.
123. Flowers JM, Powell JF, Leigh PN, Andersen P, and Shaw CE. Intron 7 retention and exon 9 skipping EAAT2 mRNA variants are not associated with amyotrophic lateral sclerosis. *Ann Neurol* 49: 643–649, 2001.
124. Fujita K, Yamauchi M, Shibayama K, Ando M, Honda M, and Nagata Y. Decreased cytochrome c oxidase activity but unchanged superoxide dismutase and glutathione peroxidase activities in the spinal cords of patients with amyotrophic lateral sclerosis. *J Neurosci Res* 45: 276–281, 1996.
125. Furukawa Y and O'Halloran TV. Amyotrophic lateral sclerosis mutations have the greatest destabilizing effect on the apo- and reduced form of SOD1, leading to unfolding and oxidative aggregation. *J Biol Chem* 280: 17266–17274, 2005.
126. Furukawa Y, Fu R, Deng HX, Siddique T, and O'Halloran TV. Disulfide cross-linked protein represents a significant fraction of ALS-associated Cu, Zn-superoxide dismutase aggregates in spinal cords of model mice. *Proc Natl Acad Sci U S A* 103: 7148–7153, 2006.
127. Gabbianelli R, Ferri A, Rotilio G, and Carri MT. Aberrant copper chemistry as a major mediator of oxidative stress in a human cellular model of amyotrophic lateral sclerosis. *J Neurochem* 73: 1175–1180, 1999.
128. Gajewski CD, Lin MT, Cudkowicz ME, Beal MF, and Manfredi G. Mitochondrial DNA from platelets of sporadic ALS patients restores normal respiratory functions in rho(0) cells. *Exp Neurol* 179: 229–235, 2003.
129. Gal J, Strom AL, Kilty R, Zhang F, and Zhu H. p62 accumulates and enhances aggregate formation in model systems of familial amyotrophic lateral sclerosis. *J Biol Chem* 282: 11068–11077, 2007.
130. Galbussera A, Tremolizzo L, Brighina L, Testa D, Lovati R, Ferrarese C, Cavaletti G, and Filippini G. Vitamin E intake and quality of life in amyotrophic lateral sclerosis patients: a follow-up case series study. *Neurol Sci* 27: 190–193, 2006.
131. Garbuzova-Davis S, Willing AE, Milliken M, Saporta S, Zigova T, Cahill DW, and Sanberg PR. Positive effect of transplantation of hNT neurons (NTera 2/D1 cell-line) in a model of familial amyotrophic lateral sclerosis. *Exp Neurol* 174: 169–180, 2002.
132. Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, and Sanberg PR. Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. *J Hematother Stem Cell Res* 12: 255–270, 2003.
133. Geller BL and Winge DR. Rat liver Cu,Zn-superoxide dismutase: subcellular location in lysosomes. *J Biol Chem* 257: 8945–8952, 1982.
134. Giess R, Holtmann B, Braga M, Grimm T, Muller-Myhsok B, Toyka KV, and Sendtner M. Early onset of severe familial amyotrophic lateral sclerosis with a SOD-1 mutation: potential impact of CNTF as a candidate modifier gene. *Am J Hum Genet* 70: 1277–1286, 2002.
135. Golub MS, Germann SL, Araiza RS, Reader JR, Griffey SM, and Lloyd KC. Movement disorders in the Hfe knockout mouse. *Nutr Neurosci* 8: 239–244, 2005.
136. Gong YH, Parsadanian AS, Andreeva A, Snider WD, and Elliott JL. Restricted expression of G86R Cu/Zn superoxide dismutase in astrocytes results in astrocytosis but does not cause motoneuron degeneration. *J Neurosci* 20: 660–665, 2000.
137. Goodall EF, Greenway MJ, van Marion I, Carroll CB, Hardiman O, and Morrison KE. Association of the H63D polymorphism in the hemochromatosis gene with sporadic ALS. *Neurology* 65: 934–937, 2005.
138. Gould TW, Buss RR, Vinsant S, Prevette D, Sun W, Knudson CM, Milligan CE, and Oppenheim RW. Complete dissociation of motor neuron death from motor dysfunction by Bax deletion in a mouse model of ALS. *J Neurosci* 26: 8774–8786, 2006.

139. Gowing G, Dequen F, Soucy G, and Julien JP. Absence of tumor necrosis factor-alpha does not affect motor neuron disease caused by superoxide dismutase 1 mutations. *J Neurosci* 26: 11397–11402, 2006.
140. Greenway MJ, Alexander MD, Ennis S, Traynor BJ, Corr B, Frost E, Green A, and Hardiman O. A novel candidate region for ALS on chromosome 14q11.2. *Neurology* 63: 1936–1938, 2004.
141. Greenway MJ, Andersen PM, Russ C, Ennis S, Cashman S, Donaghy C, Patterson V, Swingler R, Kieran D, Prehn J, Morrison KE, Green A, Acharya KR, Brown RH Jr, and Hardiman O. ANG mutations segregate with familial and "sporadic" amyotrophic lateral sclerosis. *Nat Genet* 38: 411–413, 2006.
142. Grundstrom E, Askmark H, Lindeberg J, Nygren I, Ebendal T, and Aquilonius SM. Increased expression of glial cell line-derived neurotrophic factor mRNA in muscle biopsies from patients with amyotrophic lateral sclerosis. *J Neurol Sci* 162: 169–173, 1999.
143. Grundstrom E, Lindholm D, Johansson A, Blennow K, and Askmark H. GDNF but not BDNF is increased in cerebrospinal fluid in amyotrophic lateral sclerosis. *Neuroreport* 11: 1781–1783, 2000.
144. Gruzman A, Wood WL, Alpert E, Prasad MD, Miller RG, Rothstein JD, Bowser R, Hamilton R, Wood TD, Cleveland DW, Lingappa VR, and Liu J. Common molecular signature in SOD1 for both sporadic and familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 104: 12524–12529, 2007.
145. Guegan C, Vila M, Rosoklija G, Hays AP, and Przedborski S. Recruitment of the mitochondrial-dependent apoptotic pathway in amyotrophic lateral sclerosis. *J Neurosci* 21: 6569–6576, 2001.
146. Guegan C and Przedborski S. Programmed cell death in amyotrophic lateral sclerosis. *J Clin Invest* 111: 153–161, 2003.
147. Guillot S, Azzouz M, Deglon N, Zurn A, and Aebischer P. Local GDNF expression mediated by lentiviral vector protects facial nerve motoneurons but not spinal motoneurons in SOD1(G93A) transgenic mice. *Neurobiol Dis* 16: 139–149, 2004.
148. Guo H, Lai L, Butchbach ME, Stockinger MP, Shan X, Bishop GA, and Lin CL. Increased expression of the glial glutamate transporter EAAT2 modulates excitotoxicity and delays the onset but not the outcome of ALS in mice. *Hum Mol Genet* 12: 2519–2532, 2003.
149. Gupta A and Dawson TM. The role of stem cells in Parkinson's disease. *Neurosurg Clin North Am* 18: 129–142, x-xi, 2007.
150. Gurney ME, Pu H, Chiu AY, Dal Canto MC, Polchow CY, Alexander DD, Caliendo J, Hentati A, Kwon YW, Deng HX, Chen W, Zhai P, Sufit RL, and Siddique T. Motor neuron degeneration in mice that express a human Cu,Zn superoxide dismutase mutation. *Science* 264: 1772–1775, 1994.
151. Gwag BJ, Lee YA, Ko SY, Lee MJ, Im DS, Yun BS, Lim HR, Park SM, Byun HY, Son SJ, Kwon HJ, Lee JY, Cho JY, Won SJ, Kim KW, Ahn YM, Moon HS, Lee HU, Yoon SH, Noh JH, Chung JM, and Cho SI. Marked prevention of ischemic brain injury by Neu2000, an NMDA antagonist and antioxidant derived from aspirin and sulfasalazine. *J Cereb Blood Flow Metab* 27: 1142–1151, 2007.
152. Hadano S, Yanagisawa Y, Skaug J, Fichter K, Nasir J, Martindale D, Koop BF, Scherer SW, Nicholson DW, Rouleau GA, Ikeda J, and Hayden MR. Cloning and characterization of three novel genes, ALS2CR1, ALS2CR2, and ALS2CR3, in the juvenile amyotrophic lateral sclerosis (ALS2) critical region at chromosome 2q33-q34: candidate genes for ALS2. *Genomics* 71: 200–213, 2001.
153. Hafezparast M, Klocke R, Ruhrberg C, Marquardt A, Ahmad-Annuar A, Bowen S, Lalli G, Witherden AS, Hummerich H, Nicholson S, Morgan PJ, Oozageer R, Priestley JV, Averill S, King VR, Ball S, Peters J, Toda T, Yamamoto A, Hiraoka Y, Augustin M, Korthaus D, Wattler S, Wabnitz P, Dickneite C, Lampel S, Boehme F, Peraus G, Popp A, Rudelius M, Schlegel J, Fuchs H, Hrabe de Angelis M, Schiavo G, Shima DT, Russ AP, Stumm G, Martin JE, and Fisher EM. Mutations in dynein link motor neuron degeneration to defects in retrograde transport. *Science* 300: 808–812, 2003.
154. Haghnia M, Cavalli V, Shah SB, Schimmelpfeng K, Brusch R, Yang G, Herrera C, Pilling A, and Goldstein LS. Dynactin is required for coordinated bidirectional motility, but not for dynein membrane attachment. *Mol Biol Cell* 18: 2081–2089, 2007.
155. Hall ED, Andrus PK, Oostveen JA, Fleck TJ, and Gurney ME. Relationship of oxygen radical-induced lipid peroxidative damage to disease onset and progression in a transgenic model of familial ALS. *J Neurosci Res* 53: 66–77, 1998.
156. Hall ED, Oostveen JA, and Gurney ME. Relationship of microglial and astrocytic activation to disease onset and progression in a transgenic model of familial ALS. *Glia* 23: 249–256, 1998.
157. Halliwell B. Oxygen radicals as key mediators in neurological disease: fact or fiction? *Ann Neurol* 32 suppl: S10–S15, 1992.
158. Hand CK, Devon RS, Gros-Louis F, Rochefort D, Khoris J, Meininger V, Bouchard JP, Camu W, Hayden MR, and Rouleau GA. Mutation screening of the ALS2 gene in sporadic and familial amyotrophic lateral sclerosis. *Arch Neurol* 60: 1768–1771, 2003.
159. Hart PJ. Pathogenic superoxide dismutase structure, folding, aggregation and turnover. *Curr Opin Chem Biol* 10: 131–138, 2006.
160. Hayward C, Colville S, Swingler RJ, and Brock DJ. Molecular genetic analysis of the APEX nuclease gene in amyotrophic lateral sclerosis. *Neurology* 52: 1899–1901, 1999.
161. He CZ and Hays AP. Expression of peripherin in ubiquinated inclusions of amyotrophic lateral sclerosis. *J Neurol Sci* 217: 47–54, 2004.
162. Hemendinger R, Wang J, Malik S, Persinski R, Copeland J, Emerich D, Gores P, Halberstadt C, and Rosenfeld J. Sertoli cells improve survival of motor neurons in SOD1 transgenic mice, a model of amyotrophic lateral sclerosis. *Exp Neurol* 196: 235–243, 2005.
163. Henkel JS, Engelhardt JI, Siklos L, Simpson EP, Kim SH, Pan T, Goodman JC, Siddique T, Beers DR, and Appel SH. Presence of dendritic cells, MCP-1, and activated microglia/macrophages in amyotrophic lateral sclerosis spinal cord tissue. *Ann Neurol* 55: 221–235, 2004.
164. Hensley K, Floyd RA, Gordon B, Mou S, Pye QN, Stewart C, West M, and Williamson K. Temporal patterns of cytokine and apoptosis-related gene expression in spinal cords of the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *J Neurochem* 82: 365–374, 2002.
165. Hensley K, Fedynyshyn J, Ferrell S, Floyd RA, Gordon B, Grammas P, Hamdheydari L, Mhatre M, Mou S, Pye QN, Stewart C, West M, West S, and Williamson KS. Message and protein-level elevation of tumor necrosis factor alpha (TNF alpha) and TNF alpha-modulating cytokines in spinal cords of the G93A-SOD1 mouse model for amyotrophic lateral sclerosis. *Neurobiol Dis* 14: 74–80, 2003.
166. Hensley K, Mhatre M, Mou S, Pye QN, Stewart C, West M, and Williamson KS. On the relation of oxidative stress to neuroinflammation: lessons learned from the G93A-SOD1 mouse model of amyotrophic lateral sclerosis. *Antioxid Redox Signal* 8: 2075–2087, 2006.
167. Hentze MW, Muckenthaler MU, and Andrews NC. Balancing acts: molecular control of mammalian iron metabolism. *Cell* 117: 285–297, 2004.
168. Herrmann JM and Kohl R. Catch me if you can! Oxidative protein trapping in the intermembrane space of mitochondria. *J Cell Biol* 176: 559–563, 2007.
169. Higgins CM, Jung C, Ding H, and Xu Z. Mutant Cu, Zn superoxide dismutase that causes motoneuron degeneration is present in mitochondria in the CNS. *J Neurosci* 22: RC215, 2002.
170. Hishikawa N, Niwa J, Doyu M, Ito T, Ishigaki S, Hashizume Y, and Sobue G. Dorfin localizes to the ubiquitylated inclusions in Parkinson's disease, dementia with Lewy bodies, multiple system atrophy, and amyotrophic lateral sclerosis. *Am J Pathol* 163: 609–619, 2003.
171. Honig LS, Chambliss DD, Bigio EH, Carroll SL, and Elliott JL. Glutamate transporter EAAT2 splice variants occur not only in ALS, but also in AD and controls. *Neurology* 55: 1082–1088, 2000.
172. Hottinger AF, Fine EG, Gurney ME, Zurn AD, and Aebischer P. The copper chelator d-penicillamine delays onset of disease and extends survival in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Eur J Neurosci* 9: 1548–1551, 1997.
173. Howland DS, Liu J, She Y, Goad B, Maragakis NJ, Kim B, Erickson J, Kulik J, DeVito L, Psaltis G, DeGennaro LJ, Cleveland

DW, and Rothstein JD. Focal loss of the glutamate transporter EAAT2 in a transgenic rat model of SOD1 mutant-mediated amyotrophic lateral sclerosis (ALS). *Proc Natl Acad Sci U S A* 99: 1604–1609, 2002.
174. Hwang YY and Li MD. Proteins differentially expressed in response to nicotine in five rat brain regions: identification using a 2-DE/MS-based proteomics approach. *Proteomics* 6: 3138–3153, 2006.
175. Hyun DH, Lee M, Halliwell B, and Jenner P. Proteasomal inhibition causes the formation of protein aggregates containing a wide range of proteins, including nitrated proteins. *J Neurochem* 86: 363–373, 2003.
176. Ikeda K, Kinoshita M, Iwasaki Y, Tagaya N, and Shiojima T: Lecithinized superoxide dismutase retards wobbler mouse motoneuron disease. *Neuromuscul Disord* 5: 383–390, 1995.
177. Ilzecka J, Stelmasiak Z, and Dobosz B. Transforming growth factor-Beta 1 (tgf-Beta 1) in patients with amyotrophic lateral sclerosis. *Cytokine* 20: 239–243, 2002.
178. Imoto K, Saida K, Iwamura K, Saida T, and Nishitani H. Amyotrophic lateral sclerosis: a double-blind crossover trial of thyrotropin-releasing hormone. *J Neurol Neurosurg Psychiatry* 47: 1332–1334, 1984.
179. Jaarsma D, Rognoni F, van Duijn W, Verspaget HW, Haasdijk ED, and Holstege JC: CuZn superoxide dismutase (SOD1) accumulates in vacuolated mitochondria in transgenic mice expressing amyotrophic lateral sclerosis-linked SOD1 mutations. *Acta Neuropathol (Berl)* 102: 293–305, 2001.
180. Jacquier A, Buhler E, Schafer MK, Bohl D, Blanchard S, Beclin C, and Haase G. Alsin/Rac1 signaling controls survival and growth of spinal motoneurons. *Ann Neurol* 60: 105–117, 2006.
181. Johnston JA, Dalton MJ, Gurney ME, and Kopito RR. Formation of high molecular weight complexes of mutant Cu, Zn-superoxide dismutase in a mouse model for familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 97: 12571–12576, 2000.
182. Jokic N, Gonzalez de Aguilar JL, Pradat PF, Dupuis L, Echaniz-Laguna A, Muller A, Dubourg O, Seilhean D, Hauw JJ, Loeffler JP, and Meininger V. Nogo expression in muscle correlates with amyotrophic lateral sclerosis severity. *Ann Neurol* 57: 553–556, 2005.
183. Jokic N, Gonzalez de Aguilar JL, Dimou L, Lin S, Fergani A, Ruegg MA, Schwab ME, Dupuis L, and Loeffler JP. The neurite outgrowth inhibitor Nogo-A promotes denervation in an amyotrophic lateral sclerosis model. *EMBO Rep* 7: 1162–1167, 2006.
184. Jonsson PA, Graffmo KS, Andersen PM, Brannstrom T, Lindberg M, Oliveberg M, and Marklund SL. Disulphide-reduced superoxide dismutase-1 in CNS of transgenic amyotrophic lateral sclerosis models. *Brain* 129: 451–464, 2006.
185. Jung C, Higgins CM, and Xu Z. Mitochondrial electron transport chain complex dysfunction in a transgenic mouse model for amyotrophic lateral sclerosis. *J Neurochem* 83: 535–545, 2002.
186. Just N, Moreau C, Lassalle P, Gosset P, Perez T, Brunaud-Danel V, Wallaert B, Destee A, Defebvre L, Tonnel AB, and Devos D. High erythropoietin and low vascular endothelial growth factor levels in cerebrospinal fluid from hypoxemic ALS patients suggest an abnormal response to hypoxia. *Neuromuscul Disord* 17: 169–173, 2007.
187. Kabashi E, Agar JN, Taylor DM, Minotti S, and Durham HD. Focal dysfunction of the proteasome: a pathogenic factor in a mouse model of amyotrophic lateral sclerosis. *J Neurochem* 89: 1325–1335, 2004.
188. Kabuta T, Suzuki Y, and Wada K. Degradation of amyotrophic lateral sclerosis-linked mutant Cu,Zn-superoxide dismutase proteins by macroautophagy and the proteasome. *J Biol Chem* 281: 30524–30533, 2006.
189. Kamel F, Umbach DM, Munsat TL, Shefner JM, Hu H, and Sandler DP. Lead exposure and amyotrophic lateral sclerosis. *Epidemiology* 13: 311–319, 2002.
190. Kamel F, Umbach DM, Hu H, Munsat TL, Shefner JM, Taylor JA, and Sandler DP. Lead exposure as a risk factor for amyotrophic lateral sclerosis. *Neurodegener Dis* 2: 195–201, 2005.
191. Kanekura K, Nishimoto I, Aiso S, and Matsuoka M. Characterization of amyotrophic lateral sclerosis-linked P56S mutation of vesicle-associated membrane protein-associated protein B (VAPB/ALS8). *J Biol Chem* 281: 30223–30233, 2006.
192. Kang SJ, Sanchez I, Jing N, and Yuan J. Dissociation between neurodegeneration and caspase-11-mediated activation of caspase-1 and caspase-3 in a mouse model of amyotrophic lateral sclerosis. *J Neurosci* 23: 5455–5460, 2003.
193. Kaplan J and O'Halloran TV. Iron metabolism in eukaryotes: Mars and Venus at it again. *Science* 271: 1510–1512, 1996.
194. Kasarskis EJ, Tandon L, Lovell MA, and Ehmann WD. Aluminum, calcium, and iron in the spinal cord of patients with sporadic amyotrophic lateral sclerosis using laser microprobe mass spectroscopy: a preliminary study. *J Neurol Sci* 130: 203–208, 1995.
195. Kaspar BK, Llado J, Sherkat N, Rothstein JD, and Gage FH. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 301: 839–842, 2003.
196. Kawahara Y, Kwak S, Sun H, Ito K, Hashida H, Aizawa H, Jeong SY, and Kanazawa I. Human spinal motoneurons express low relative abundance of GluR2 mRNA: an implication for excitotoxicity in ALS. *J Neurochem* 85: 680–689, 2003.
197. Kawahara Y, Ito K, Sun H, Aizawa H, Kanazawa I, and Kwak S. Glutamate receptors: RNA editing and death of motor neurons. *Nature* 427: 801, 2004.
198. Kawahara Y, Sun H, Ito K, Hideyama T, Aoki M, Sobue G, Tsuji S, and Kwak S. Underediting of GluR2 mRNA, a neuronal death inducing molecular change in sporadic ALS, does not occur in motor neurons in ALS1 or SBMA. *Neurosci Res* 54: 11–14, 2006.
199. Kawamoto Y, Nakamura S, Akiguchi I, and Kimura J. Immunohistochemical localization of brain-derived neurotrophic factor in the spinal cords of amyotrophic lateral sclerosis and non-amyotrophic lateral sclerosis patients. *J Neuropathol Exp Neurol* 57: 822–830, 1998.
200. Khare SD and Dokholyan NV. Common dynamical signatures of familial amyotrophic lateral sclerosis-associated structurally diverse Cu, Zn superoxide dismutase mutants. *Proc Natl Acad Sci U S A* 103: 3147–3152, 2006.
201. Kiaei M, Bush AI, Morrison BM, Morrison JH, Cherny RA, Volitakis I, Beal MF, and Gordon JW. Genetically decreased spinal cord copper concentration prolongs life in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci* 24: 7945–7950, 2004.
202. Kieran D, Hafezparast M, Bohnert S, Dick JR, Martin J, Schiavo G, Fisher EM, and Greensmith L. A mutation in dynein rescues axonal transport defects and extends the life span of ALS mice. *J Cell Biol* 169: 561–567, 2005.
203. Kim SU. Genetically engineered human neural stem cells for brain repair in neurological diseases. *Brain Dev* 29: 193–201, 2007.
204. Kirby J, Hewamadduma CA, Hartley JA, Nixon HC, Evans H, Wadhwa RR, Kershaw C, Ince PG, and Shaw PJ. Mutations in VAPB are not associated with sporadic ALS. *Neurology* 68: 1951–1953, 2007.
205. Kirkinezos IG, Bacman SR, Hernandez D, Oca-Cossio J, Arias LJ, Perez-Pinzon MA, Bradley WG, and Moraes CT. Cytochrome c association with the inner mitochondrial membrane is impaired in the CNS of G93A-SOD1 mice. *J Neurosci* 25: 164–172, 2005.
206. Klein SM, Behrstock S, McHugh J, Hoffmann K, Wallace K, Suzuki M, Aebischer P, and Svendsen CN. GDNF delivery using human neural progenitor cells in a rat model of ALS. *Hum Gene Ther* 16: 509–521, 2005.
207. Klivenyi P, Ferrante RJ, Matthews RT, Bogdanov MB, Klein AM, Andreassen OA, Mueller G, Wermer M, Kaddurah-Daouk R, and Beal MF. Neuroprotective effects of creatine in a transgenic animal model of amyotrophic lateral sclerosis. *Nat Med* 5: 347–350, 1999.
208. Kobayashi N. Cell therapy for diabetes mellitus. *Cell Transplant* 15: 849–854, 2006.
209. Koh JY, Kim DK, Hwang JY, Kim YH, and Seo JH. Antioxidative and proapoptotic effects of riluzole on cultured cortical neurons. *J Neurochem* 72: 716–723, 1999.
210. Kong J and Xu Z. Massive mitochondrial degeneration in motor neurons triggers the onset of amyotrophic lateral sclerosis in mice expressing a mutant SOD1. *J Neurosci* 18: 3241–3250, 1998.

211. Konno T. Amyloid-induced aggregation and precipitation of soluble proteins: an electrostatic contribution of the Alzheimer's beta(25-35) amyloid fibril. *Biochemistry* 40: 2148–2154, 2001.
212. Kress JA, Kuhnlein P, Winter P, Ludolph AC, Kassubek J, Muller U, and Sperfeld AD. Novel mutation in the ALS2 gene in juvenile amyotrophic lateral sclerosis. *Ann Neurol* 58: 800–803, 2005.
213. Kriz J, Nguyen MD, and Julien JP. Minocycline slows disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* 10: 268–278, 2002.
214. Kruman, II, Pedersen WA, Springer JE, and Mattson MP. ALS-linked Cu/Zn-SOD mutation increases vulnerability of motor neurons to excitotoxicity by a mechanism involving increased oxidative stress and perturbed calcium homeostasis. *Exp Neurol* 160: 28–39, 1999.
215. Kushner PD, Stephenson DT, and Wright S. Reactive astrogliosis is widespread in the subcortical white matter of amyotrophic lateral sclerosis brain. *J Neuropathol Exp Neurol* 50: 263–277, 1991.
216. Kust BM, Copray JC, Brouwer N, Troost D, and Boddeke HW. Elevated levels of neurotrophins in human biceps brachii tissue of amyotrophic lateral sclerosis. *Exp Neurol* 177: 419–427, 2002.
217. Lai C, Xie C, McCormack SG, Chiang HC, Michalak MK, Lin X, Chandran J, Shim H, Shimoji M, Cookson MR, Huganir RL, Rothstein JD, Price DL, Wong PC, Martin LJ, Zhu JJ, and Cai H. Amyotrophic lateral sclerosis 2-deficiency leads to neuronal degeneration in amyotrophic lateral sclerosis through altered AMPA receptor trafficking. *J Neurosci* 26: 11798–11806, 2006.
218. Lai EC, Felice KJ, Festoff BW, Gawel MJ, Gelinas DF, Kratz R, Murphy MF, Natter HM, Norris FH, and Rudnicki SA. Effect of recombinant human insulin-like growth factor-I on progression of ALS: a placebo-controlled study: the North America ALS/IGF-I Study Group. *Neurology* 49: 1621–1630, 1997.
219. Lambeth JD. NOX enzymes and the biology of reactive oxygen. *Nat Rev Immunol* 4: 181–189, 2004.
220. Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, Wyns S, Thijs V, Andersson J, van Marion I, Al-Chalabi A, Bornes S, Musson R, Hansen V, Beckman L, Adolfsson R, Pall HS, Prats H, Vermeire S, Rutgeerts P, Katayama S, Awata T, Leigh N, Lang-Lazdunski L, Dewerchin M, Shaw C, Moons L, Vlietinck R, Morrison KE, Robberecht W, Van Broeckhoven C, Collen D, Andersen PM, and Carmeliet P. VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nat Genet* 34: 383–394, 2003.
221. LaMonte BH, Wallace KE, Holloway BA, Shelly SS, Ascano J, Tokito M, Van Winkle T, Howland DS, and Holzbaur EL. Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration. *Neuron* 34: 715–727, 2002.
222. Lariviere RC and Julien JP. Functions of intermediate filaments in neuronal development and disease. *J Neurobiol* 58: 131–148, 2004.
223. Leung CL, He CZ, Kaufmann P, Chin SS, Naini A, Liem RK, Mitsumoto H, and Hays AP. A pathogenic peripherin gene mutation in a patient with amyotrophic lateral sclerosis. *Brain Pathol* 14: 290–296, 2004.
224. Leveugle B, Spik G, Perl DP, Bouras C, Fillit HM, and Hof PR. The iron-binding protein lactotransferrin is present in pathologic lesions in a variety of neurodegenerative disorders: a comparative immunohistochemical analysis. *Brain Res* 650: 20–31, 1994.
225. Levine JB, Kong J, Nadler M, and Xu Z. Astrocytes interact intimately with degenerating motor neurons in mouse amyotrophic lateral sclerosis (ALS). *Glia* 28: 215–224, 1999.
226. Li M, Ona VO, Guegan C, Chen M, Jackson-Lewis V, Andrews LJ, Olszewski AJ, Stieg PE, Lee JP, Przedborski S, and Friedlander RM. Functional role of caspase-1 and caspase-3 in an ALS transgenic mouse model. *Science* 288: 335–339, 2000.
227. Li S, Mallory M, Alford M, Tanaka S, and Masliah E. Glutamate transporter alterations in Alzheimer disease are possibly associated with abnormal APP expression. *J Neuropathol Exp Neurol* 56: 901–911, 1997.
228. Li W, Brakefield D, Pan Y, Hunter D, Myckatyn TM, and Parsadanian A. Muscle-derived but not centrally derived transgene GDNF is neuroprotective in G93A-SOD1 mouse model of ALS. *Exp Neurol* 203: 457–471, 2007.
229. Ligon LA, LaMonte BH, Wallace KE, Weber N, Kalb RG, and Holzbaur EL. Mutant superoxide dismutase disrupts cytoplasmic dynein in motor neurons. *Neuroreport* 16: 533–536, 2005.
230. Lin CL, Bristol LA, Jin L, Dykes-Hoberg M, Crawford T, Clawson L, and Rothstein JD. Aberrant RNA processing in a neurodegenerative disease: the cause for absent EAAT2, a glutamate transporter, in amyotrophic lateral sclerosis. *Neuron* 20: 589–602, 1998.
231. Lin X, Shim H, and Cai H. Deficiency in the ALS2 gene does not affect the motor neuron degeneration in SOD1(G93A) transgenic mice. *Neurobiol Aging* 28: 1628–1630, 2006.
232. Lindberg MJ, Bystrom R, Boknas N, Andersen PM, and Oliveberg M. Systematically perturbed folding patterns of amyotrophic lateral sclerosis (ALS)-associated SOD1 mutants. *Proc Natl Acad Sci U S A* 102: 9754–9759, 2005.
233. Lino MM, Schneider C, and Caroni P. Accumulation of SOD1 mutants in postnatal motoneurons does not cause motoneuron pathology or motoneuron disease. *J Neurosci* 22: 4825–4832, 2002.
234. Liu D, Wen J, Liu J, and Li L. The roles of free radicals in amyotrophic lateral sclerosis: reactive oxygen species and elevated oxidation of protein, DNA, and membrane phospholipids. *FASEB J* 13: 2318–2328, 1999.
235. Liu J, Lillo C, Jonsson PA, Vande Velde C, Ward CM, Miller TM, Subramaniam JR, Rothstein JD, Marklund S, Andersen PM, Brannstrom T, Gredal O, Wong PC, Williams DS, and Cleveland DW. Toxicity of familial ALS-linked SOD1 mutants from selective recruitment to spinal mitochondria. *Neuron* 43: 5–17, 2004.
236. Liu R, Althaus JS, Ellerbrock BR, Becker DA, and Gurney ME. Enhanced oxygen radical production in a transgenic mouse model of familial amyotrophic lateral sclerosis. *Ann Neurol* 44: 763–770, 1998.
237. Liu R, Li B, Flanagan SW, Oberley LW, Gozal D, and Qiu M. Increased mitochondrial antioxidative activity or decreased oxygen free radical propagation prevent mutant SOD1-mediated motor neuron cell death and increase amyotrophic lateral sclerosis-like transgenic mouse survival. *J Neurochem* 80: 488–500, 2002.
238. Liu Z and Martin LJ. The adult neural stem and progenitor cell niche is altered in amyotrophic lateral sclerosis mouse brain. *J Comp Neurol* 497: 468–488, 2006.
239. Mahoney DJ, Kaczor JJ, Bourgeois J, Yasuda N, and Tarnopolsky MA. Oxidative stress and antioxidant enzyme upregulation in SOD1-G93A mouse skeletal muscle. *Muscle Nerve* 33: 809–816, 2006.
240. Maihofner C, Probst-Cousin S, Bergmann M, Neuhuber W, Neundorfer B, and Heuss D. Expression and localization of cyclooxygenase-1 and -2 in human sporadic amyotrophic lateral sclerosis. *Eur J Neurosci* 18: 1527–1534, 2003.
241. Manabe Y, Nagano I, Gazi MS, Murakami T, Shiote M, Shoji M, Kitagawa H, and Abe K. Glial cell line-derived neurotrophic factor protein prevents motor neuron loss of transgenic model mice for amyotrophic lateral sclerosis. *Neurol Res* 25: 195–200, 2003.
242. Manfredi G and Xu Z. Mitochondrial dysfunction and its role in motor neuron degeneration in ALS. *Mitochondrion* 5: 77–87, 2005.
243. Maracchioni A, Totaro A, Angelini DF, Di Penta A, Bernardi G, Carri MT, and Achsel T. Mitochondrial damage modulates alternative splicing in neuronal cells: implications for neurodegeneration. *J Neurochem* 100: 142–153, 2007.
244. Maragakis NJ, Dykes-Hoberg M, and Rothstein JD. Altered expression of the glutamate transporter EAAT2b in neurological disease. *Ann Neurol* 55: 469–477, 2004.
245. Marques VD, Barreira AA, Davis MB, Abou-Sleiman PM, Silva WA Jr, Zago MA, Sobreira C, Fazan V, and Marques W Jr. Expanding the phenotypes of the Pro56Ser VAPB mutation: proximal SMA with dysautonomia. *Muscle Nerve* 34: 731–739, 2006.
246. Martin LJ. Neuronal death in amyotrophic lateral sclerosis is apoptosis: possible contribution of a programmed cell death mechanism. *J Neuropathol Exp Neurol* 58: 459–471, 1999.

247. Martin LJ, Liu Z, Chen K, Price AC, Pan Y, Swaby JA, and Golden WC. Motor neuron degeneration in amyotrophic lateral sclerosis mutant superoxide dismutase-1 transgenic mice: mechanisms of mitochondriopathy and cell death. *J Comp Neurol* 500: 20–46, 2007.
248. Masu Y, Wolf E, Holtmann B, Sendtner M, Brem G, and Thoenen H. Disruption of the CNTF gene results in motor neuron degeneration. *Nature* 365: 27–32, 1993.
249. Matsumoto G, Stojanovic A, Holmberg CI, Kim S, and Morimoto RI. Structural properties and neuronal toxicity of amyotrophic lateral sclerosis-associated Cu/Zn superoxide dismutase 1 aggregates. *J Cell Biol* 171: 75–85, 2005.
250. Mattiazzi M, D'Aurelio M, Gajewski CD, Martushova K, Kiaei M, Beal MF, and Manfredi G. Mutated human SOD1 causes dysfunction of oxidative phosphorylation in mitochondria of transgenic mice. *J Biol Chem* 277: 29626–29633, 2002.
251. Mattson MP. Metal-catalyzed disruption of membrane protein and lipid signaling in the pathogenesis of neurodegenerative disorders. *Ann N Y Acad Sci* 1012: 37–50, 2004.
252. Mazzini L, Mareschi K, Ferrero I, Vassallo E, Oliveri G, Boccaletti R, Testa L, Livigni S, and Fagioli F. Autologous mesenchymal stem cells: clinical applications in amyotrophic lateral sclerosis. *Neurol Res* 28: 523–526, 2006.
253. Mendonca DM, Chimelli L, and Martinez AM. Expression of ubiquitin and proteasome in motor neurons and astrocytes of spinal cords from patients with amyotrophic lateral sclerosis. *Neurosci Lett* 404: 315–319, 2006.
254. Menzies FM, Cookson MR, Taylor RW, Turnbull DM, Chrzanowska-Lightowlers ZM, Dong L, Figlewicz DA, and Shaw PJ. Mitochondrial dysfunction in a cell culture model of familial amyotrophic lateral sclerosis. *Brain* 125: 1522–1533, 2002.
255. Migheli A, Atzori C, Piva R, Tortarolo M, Girelli M, Schiffer D, and Bendotti C. Lack of apoptosis in mice with ALS. *Nat Med* 5: 966–967, 1999.
256. Miller RG, Bryan WW, Dietz MA, Munsat TL, Petajan JH, Smith SA, and Goodpasture JC. Toxicity and tolerability of recombinant human ciliary neurotrophic factor in patients with amyotrophic lateral sclerosis. *Neurology* 47: 1329–1331, 1996.
257. Miller RG, Mitchell JD, Lyon M, and Moore DH. Riluzole for amyotrophic lateral sclerosis (ALS)/motor neuron disease (MND). *Amyotroph Lateral Scler Other Motor Neuron Disord* 4: 191–206, 2003.
258. Miller TM, Kim SH, Yamanaka K, Hester M, Umapathi P, Arnson H, Rizo L, Mendell JR, Gage FH, Cleveland DW, and Kaspar BK. Gene transfer demonstrates that muscle is not a primary target for non-cell-autonomous toxicity in familial amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 103: 19546–19551, 2006.
259. Miralles VJ, Martinez-Lopez I, Zaragoza R, Borras E, Garcia C, Pallardo FV, and Vina JR. Na+ dependent glutamate transporters (EAAT1, EAAT2, and EAAT3) in primary astrocyte cultures: effect of oxidative stress. *Brain Res* 922: 21–29, 2001.
260. Mitchell JD, Wokke JH, and Borasio GD. Recombinant human insulin-like growth factor I (rhIGF-I) for amyotrophic lateral sclerosis/motor neuron disease. *Cochrane Database Syst Rev* CD002064, 2002.
261. Mitsumoto H, Salgado ED, Negroski D, Hanson MR, Salanga VD, Wilber JF, Wilbourn AJ, Breuer AC, and Leatherman J. Amyotrophic lateral sclerosis: effects of acute intravenous and chronic subcutaneous administration of thyrotropin-releasing hormone in controlled trials. *Neurology* 36: 152–159, 1986.
262. Mizuno Y, Amari M, Takatama M, Aizawa H, Mihara B, and Okamoto K. Immunoreactivities of p62, an ubiqutin-binding protein, in the spinal anterior horn cells of patients with amyotrophic lateral sclerosis. *J Neurol Sci* 249: 13–18, 2006.
263. Moisse K and Strong MJ. Innate immunity in amyotrophic lateral sclerosis. *Biochim Biophys Acta* 1762: 1083–1093, 2006.
264. Moreira MC, Klur S, Watanabe M, Nemeth AH, Le Ber I, Moniz JC, Tranchant C, Aubourg P, Tazir M, Schols L, Pandolfo M, Schulz JB, Pouget J, Calvas P, Shizuka-Ikeda M, Shoji M, Tanaka M, Izatt L, Shaw CE, M'Zahem A, Dunne E, Bomont P, Benhassine T, Bouslam N, Stevanin G, Brice A, Guimaraes J, Mendonca P, Barbot C, Coutinho P, Sequeiros J, Durr A, Warter JM, and Koenig M. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat Genet* 36: 225–227, 2004.
265. Munch C, Sedlmeier R, Meyer T, Homberg V, Sperfeld AD, Kurt A, Prudlo J, Peraus G, Hanemann CO, Stumm G, and Ludolph AC. Point mutations of the p150 subunit of dynactin (DCTN1) gene in ALS. *Neurology* 63: 724–726, 2004.
266. Munch C, Rosenbohm A, Sperfeld AD, Uttner I, Reske S, Krause BJ, Sedlmeier R, Meyer T, Hanemann CO, Stumm G, and Ludolph AC. Heterozygous R1101K mutation of the DCTN1 gene in a family with ALS and FTD. *Ann Neurol* 58: 777–780, 2005.
267. Mutoh T, Sobue G, Hamano T, Kuriyama M, Hirayama M, Yamamoto M, and Mitsuma T. Decreased phosphorylation levels of TrkB neurotrophin receptor in the spinal cords from patients with amyotrophic lateral sclerosis. *Neurochem Res* 25: 239–245, 2000.
268. Nagai M, Re DB, Nagata T, Chalazonitis A, Jessell TM, Wichterle H, and Przedborski S. Astrocytes expressing ALS-linked mutated SOD1 release factors selectively toxic to motor neurons. *Nat Neurosci* 10: 615–622, 2007.
269. Nagano I, Ilieva H, Shiote M, Murakami T, Yokoyama M, Shoji M, and Abe K. Therapeutic benefit of intrathecal injection of insulin-like growth factor-1 in a mouse model of amyotrophic lateral sclerosis. *J Neurol Sci* 235: 61–68, 2005.
270. Nagano I, Shiote M, Murakami T, Kamada H, Hamakawa Y, Matsubara E, Yokoyama M, Moritaz K, Shoji M, and Abe K. Beneficial effects of intrathecal IGF-1 administration in patients with amyotrophic lateral sclerosis. *Neurol Res* 27: 768–772, 2005.
271. Nakamura S, Kawamoto Y, Nakano S, Ikemoto A, Akiguchi I, and Kimura J. Cyclin-dependent kinase 5 in Lewy body-like inclusions in anterior horn cells of a patient with sporadic amyotrophic lateral sclerosis. *Neurology* 48: 267–270, 1997.
272. Nishimura AL, Mitne-Neto M, Silva HC, Richieri-Costa A, Middleton S, Cascio D, Kok F, Oliveira JR, Gillingwater T, Webb J, Skehel P, and Zatz M. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. *Am J Hum Genet* 75: 822–831, 2004.
273. Niwa J, Ishigaki S, Hishikawa N, Yamamoto M, Doyu M, Murata S, Tanaka K, Taniguchi N, and Sobue G. Dorfin ubiquitylates mutant SOD1 and prevents mutant SOD1-mediated neurotoxicity. *J Biol Chem* 277: 36793–36798, 2002.
274. O'Brien KM, Dirmeier R, Engle M, and Poyton RO. Mitochondrial protein oxidation in yeast mutants lacking manganese-(Mn-SOD) or copper- and zinc-containing superoxide dismutase (CuZnSOD): evidence that MnSOD and CuZnSOD have both unique and overlapping functions in protecting mitochondrial proteins from oxidative damage. *J Biol Chem* 279: 51817–51827, 2004.
275. Ochs G, Penn RD, York M, Giess R, Beck M, Tonn J, Haigh J, Malta E, Traub M, Sendtner M, and Toyka KV. A phase I/II trial of recombinant methionyl human brain derived neurotrophic factor administered by intrathecal infusion to patients with amyotrophic lateral sclerosis. *Amyotroph Lateral Scler Other Motor Neuron Disord* 1: 201–206, 2000.
276. Oeda T, Shimohama S, Kitagawa N, Kohno R, Imura T, Shibasaki H, and Ishii N. Oxidative stress causes abnormal accumulation of familial amyotrophic lateral sclerosis-related mutant SOD1 in transgenic *Caenorhabditis elegans*. *Hum Mol Genet* 10: 2013–2023, 2001.
277. Oh SS, Kim EA, Lee SW, Kim MK, and Kang SK. A case of amyotrophic lateral sclerosis in electronic parts manufacturing worker exposed to lead. *Neurotoxicology* 28: 324–327, 2007.
278. Okado-Matsumoto A and Fridovich I. Subcellular distribution of superoxide dismutases (SOD) in rat liver: Cu,Zn-SOD in mitochondria. *J Biol Chem* 276: 38388–38393, 2001.
279. Okado-Matsumoto A and Fridovich I. Amyotrophic lateral sclerosis: a proposed mechanism. *Proc Natl Acad Sci U S A* 99: 9010–9014, 2002.
280. Okamoto K, Hirai S, Amari M, Watanabe M, and Sakurai A. Bunina bodies in amyotrophic lateral sclerosis immunostained with rabbit anti-cystatin C serum. *Neurosci Lett* 162: 125–128, 1993.
281. Oliveira AA Jr and Hodges HM. Alzheimer's disease and neural transplantation as prospective cell therapy. *Curr Alzheimer Res* 2: 79–95, 2005.

282. Olsen MK, Roberds SL, Ellerbrock BR, Fleck TJ, McKinley DK, and Gurney ME. Disease mechanisms revealed by transcription profiling in SOD1-G93A transgenic mouse spinal cord. *Ann Neurol* 50: 730–740, 2001.
283. Ono S, Hu J, Shimizu N, Imai T, and Nakagawa H. Increased interleukin-6 of skin and serum in amyotrophic lateral sclerosis. *J Neurol Sci* 187: 27–34, 2001.
284. Oosthuyse B, Moons L, Storkebaum E, Beck H, Nuyens D, Brusselmans K, Van Dorpe J, Hellings P, Gorselink M, Heymans S, Theilmeier G, Dewerchin M, Laudenbach V, Vermylen P, Raat H, Acker T, Vleminckx V, Van Den Bosch L, Cashman N, Fujisawa H, Drost MR, Sciot R, Bruyninckx F, Hicklin DJ, Ince C, Gressens P, Lupu F, Plate KH, Robberecht W, Herbert JM, Collen D, and Carmeliet P. Deletion of the hypoxia-response element in the vascular endothelial growth factor promoter causes motor neuron degeneration. *Nat Genet* 28: 131–138, 2001.
285. Orrell RW, Lane RJ, and Ross M. Antioxidant treatment for amyotrophic lateral sclerosis/motor neuron disease. *Cochrane Database Syst Rev* CD002829, 2007.
286. Orrenius S, Gogvadze V, and Zhivotovsky B. Mitochondrial oxidative stress: implications for cell death. *Annu Rev Pharmacol Toxicol* 47: 143–183, 2007.
287. Otomo A, Hadano S, Okada T, Mizumura H, Kunita R, Nishijima H, Showguchi-Miyata J, Yanagisawa Y, Kohiki E, Suga E, Yasuda M, Osuga H, Nishimoto T, Narumiya S, and Ikeda JE. ALS2, a novel guanine nucleotide exchange factor for the small GTPase Rab5, is implicated in endosomal dynamics. *Hum Mol Genet* 12: 1671–1687, 2003.
288. Ozdinler PH and Macklis JD. IGF-I specifically enhances axon outgrowth of corticospinal motor neurons. *Nat Neurosci* 9: 1371–1381, 2006.
289. Panzeri C, De Palma C, Martinuzzi A, Daga A, De Polo G, Bresolin N, Miller CC, Tudor EL, Clementi E, and Bassi MT. The first ALS2 missense mutation associated with JPLS reveals new aspects of alsin biological function. *Brain* 129: 1710–1719, 2006.
290. Pardo CA, Xu Z, Borchelt DR, Price DL, Sisodia SS, and Cleveland DW. Superoxide dismutase is an abundant component in cell bodies, dendrites, and axons of motor neurons and in a subset of other neurons. *Proc Natl Acad Sci U S A* 92: 954–958, 1995.
291. Parkinson N, Ince PG, Smith MO, Highley R, Skibinski G, Andersen PM, Morrison KE, Pall HS, Hardiman O, Collinge J, Shaw PJ, and Fisher EM. ALS phenotypes with mutations in CHMP2B (charged multivesicular body protein 2B). *Neurology* 67: 1074–1077, 2006.
292. Pasinelli P, Belford ME, Lennon N, Bacskai BJ, Hyman BT, Trotti D, and Brown RH, Jr. Amyotrophic lateral sclerosis-associated SOD1 mutant proteins bind and aggregate with Bcl-2 in spinal cord mitochondria. *Neuron* 43: 19–30, 2004.
293. Patel BN and David S. A novel glycosylphosphatidylinositol-anchored form of ceruloplasmin is expressed by mammalian astrocytes. *J Biol Chem* 272: 20185–20190, 1997.
294. Pehar M, Vargas MR, Cassina P, Barbeito AG, Beckman JS, and Barbeito L. Complexity of astrocyte-motor neuron interactions in amyotrophic lateral sclerosis. *Neurodegener Dis* 2: 139–146, 2005.
295. Petri S, Kiaei M, Damiano M, Hiller A, Wille E, Manfredi G, Calingasan NY, Szeto HH, and Beal MF. Cell-permeable peptide antioxidants as a novel therapeutic approach in a mouse model of amyotrophic lateral sclerosis. *J Neurochem* 98: 1141–1148, 2006.
296. Poloni M, Facchetti D, Mai R, Micheli A, Agnoletti L, Francolini G, Mora G, Camana C, Mazzini L, and Bachetti T. Circulating levels of tumour necrosis factor-alpha and its soluble receptors are increased in the blood of patients with amyotrophic lateral sclerosis. *Neurosci Lett* 287: 211–214, 2000.
297. Pompl PN, Ho L, Bianchi M, McManus T, Qin W, and Pasinetti GM. A therapeutic role for cyclooxygenase-2 inhibitors in a transgenic mouse model of amyotrophic lateral sclerosis. *FASEB J* 17: 725–727, 2003.
298. Poon HF, Hensley K, Thongboonkerd V, Merchant ML, Lynn BC, Pierce WM, Klein JB, Calabrese V, and Butterfield DA. Redox proteomics analysis of oxidatively modified proteins in G93A-SOD1 transgenic mice: a model of familial amyotrophic lateral sclerosis. *Free Radic Biol Med* 39: 453–462, 2005.
299. Poon HF, Vaishnav RA, Getchell TV, Getchell ML, and Butterfield DA. Quantitative proteomics analysis of differential protein expression and oxidative modification of specific proteins in the brains of old mice. *Neurobiol Aging* 27: 1010–1019, 2006.
300. Pradat PF, Bruneteau G, Gonzalez De Aguilar JL, Dupuis L, Jokic N, Salachas F, Le Forestier N, Echaniz-Laguna A, Dubourg O, Hauw JJ, Tranchant C, Loeffler JP, and Meininger V. Muscle nogo-a expression is a prognostic marker in lower motor neuron syndromes. *Ann Neurol* 52: 18–20, 2007.
301. Pramatarova A, Laganiere J, Roussel J, Brisebois K, and Rouleau GA. Neuron-specific expression of mutant superoxide dismutase 1 in transgenic mice does not lead to motor impairment. *J Neurosci* 21: 3369–3374, 2001.
302. Przedborski S. Programmed cell death in amyotrophic lateral sclerosis: a mechanism of pathogenic and therapeutic importance. *Neurologist* 10: 1–7, 2004.
303. Puig S and Thiele DJ. Molecular mechanisms of copper uptake and distribution. *Curr Opin Chem Biol* 6: 171–180, 2002.
304. Puls I, Jonnakuty C, LaMonte BH, Holzbaur EL, Tokito M, Mann E, Floeter MK, Bidus K, Drayna D, Oh SJ, Brown RH Jr, Ludlow CL, and Fischbeck KH. Mutant dynactin in motor neuron disease. *Nat Genet* 33: 455–456, 2003.
305. Pun S, Santos AF, Saxena S, Xu L, and Caroni P. Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF. *Nat Neurosci* 9: 408–419, 2006.
306. Puntarulo S. Iron, oxidative stress and human health. *Mol Aspects Med* 26: 299–312, 2005.
307. Rabizadeh S, Gralla EB, Borchelt DR, Gwinn R, Valentine JS, Sisodia S, Wong P, Lee M, Hahn H, and Bredesen DE. Mutations associated with amyotrophic lateral sclerosis convert superoxide dismutase from an antiapoptotic gene to a proapoptotic gene: studies in yeast and neural cells. *Proc Natl Acad Sci U S A* 92: 3024–3028, 1995.
308. Rae TD, Schmidt PJ, Pufahl RA, Culotta VC, and O'Halloran TV. Undetectable intracellular free copper: the requirement of a copper chaperone for superoxide dismutase. *Science* 284: 805–808, 1999.
309. Raimondi A, Mangolini A, Rizzardini M, Tartari S, Massari S, Bendotti C, Francolini M, Borgese N, Cantoni L, and Pietrini G. Cell culture models to investigate the selective vulnerability of motoneuronal mitochondria to familial ALS-linked G93ASOD1. *Eur J Neurosci* 24: 387–399, 2006.
310. Rakhit R, Cunningham P, Furtos-Matei A, Dahan S, Qi XF, Crow JP, Cashman NR, Kondejewski LH, and Chakrabartty A. Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis. *J Biol Chem* 277: 47551–47556, 2002.
311. Rakhit R, Crow JP, Lepock JR, Kondejewski LH, Cashman NR, and Chakrabartty A. Monomeric Cu,Zn-superoxide dismutase is a common misfolding intermediate in the oxidation models of sporadic and familial amyotrophic lateral sclerosis. *J Biol Chem* 279: 15499–15504, 2004.
312. Rakhit R and Chakrabartty A. Structure, folding, and misfolding of Cu,Zn superoxide dismutase in amyotrophic lateral sclerosis. *Biochim Biophys Acta* 1762: 1025–1037, 2006.
313. Rakhit R, Robertson J, Velde CV, Horne P, Ruth DM, Griffin J, Cleveland DW, Cashman NR, and Chakrabartty A. An immunological epitope selective for pathological monomer-misfolded SOD1 in ALS. *Nat Med* 13: 754–759, 2007.
314. Ralph GS, Radcliffe PA, Day DM, Carthy JM, Leroux MA, Lee DC, Wong LF, Bilsland LG, Greensmith L, Kingsman SM, Mitrophanous KA, Mazarakis ND, and Azzouz M. Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. *Nat Med* 11: 429–433, 2005.
315. Rao AV and Balachandran B. Role of oxidative stress and antioxidants in neurodegenerative diseases. *Nutr Neurosci* 5: 291–309, 2002.
316. Rao MV, Houseweart MK, Williamson TL, Crawford TO, Folmer J, and Cleveland DW. Neurofilament-dependent radial growth of motor axons and axonal organization of neurofilaments does not

require the neurofilament heavy subunit (NF-H) or its phosphorylation. *J Cell Biol* 143: 171–181, 1998.
317. Raoul C, Abbas-Terki T, Bensadoun JC, Guillot S, Haase G, Szulc J, Henderson CE, and Aebischer P. Lentiviral-mediated silencing of SOD1 through RNA interference retards disease onset and progression in a mouse model of ALS. *Nat Med* 11: 423–428, 2005.
318. Rattray M and Bendotti C. Does excitotoxic cell death of motor neurons in ALS arise from glutamate transporter and glutamate receptor abnormalities? *Exp Neurol* 201: 15–23, 2006.
319. Ray SS, Nowak RJ, Strokovich K, Brown RH Jr, Walz T, and Lansbury PT Jr. An intersubunit disulfide bond prevents in vitro aggregation of a superoxide dismutase-1 mutant linked to familial amytrophic lateral sclerosis. *Biochemistry* 43: 4899–4905, 2004.
320. Reaume AG, Elliott JL, Hoffman EK, Kowall NW, Ferrante RJ, Siwek DF, Wilcox HM, Flood DG, Beal MF, Brown RH Jr, Scott RW, and Snider WD. Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. *Nat Genet* 13: 43–47, 1996.
321. Rembach A, Turner BJ, Bruce S, Cheah IK, Scott RL, Lopes EC, Zagami CJ, Beart PM, Cheung NS, Langford SJ, and Cheema SS. Antisense peptide nucleic acid targeting GluR3 delays disease onset and progression in the SOD1 G93A mouse model of familial ALS. *J Neurosci Res* 77: 573–582, 2004.
322. Ricci JE, Munoz-Pinedo C, Fitzgerald P, Bailly-Maitre B, Perkins GA, Yadava N, Scheffler IE, Ellisman MH, and Green DR. Disruption of mitochondrial function during apoptosis is mediated by caspase cleavage of the p75 subunit of complex I of the electron transport chain. *Cell* 117: 773–786, 2004.
323. Robertson J, Doroudchi MM, Nguyen MD, Durham HD, Strong MJ, Shaw G, Julien JP, and Mushynski WE. A neurotoxic peripherin splice variant in a mouse model of ALS. *J Cell Biol* 160: 939–949, 2003.
324. Rodrigues NR, Owen N, Talbot K, Ignatius J, Dubowitz V, and Davies KE. Deletions in the survival motor neuron gene on 5q13 in autosomal recessive spinal muscular atrophy. *Hum Mol Genet* 4: 631–634, 1995.
325. Roldan-Arjona T, Wei YF, Carter KC, Klungland A, Anselmino C, Wang RP, Augustus M, and Lindahl T. Molecular cloning and functional expression of a human cDNA encoding the antimutator enzyme 8-hydroxyguanine-DNA glycosylase. *Proc Natl Acad Sci U S A* 94: 8016–8020, 1997.
326. Rooke K, Figlewicz DA, Han FY, and Rouleau GA. Analysis of the KSP repeat of the neurofilament heavy subunit in familiar amyotrophic lateral sclerosis. *Neurology* 46: 789–790, 1996.
327. Rosen DR, Siddique T, Patterson D, Figlewicz DA, Sapp P, Hentati A, Donaldson D, Goto J, O'Regan JP, Deng H-X, Rahmani Z, Krizus A, McKenna-Yasek D, Cayabyab A, Gaston SM, Berger R, Tanzi RE, Halperin JJ, Herzfeldt B, Van den Bergh R, Hung W-Y, Bird T, Deng G, Mulder DW, Smyth C, Laing NG, Soriano E, Pericak-Vance MA, Haines J, Rouleau GA, Gusella JS, Horvitz HR, and Brown Jr RH. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. *Nature* 362: 59–62, 1993.
328. Rothstein JD, Tsai G, Kuncl RW, Clawson L, Cornblath DR, Drachman DB, Pestronk A, Stauch BL, and Coyle JT. Abnormal excitatory amino acid metabolism in amyotrophic lateral sclerosis. *Ann Neurol* 28: 18–25, 1990.
329. Rothstein JD, Martin LJ, and Kuncl RW. Decreased glutamate transport by the brain and spinal cord in amyotrophic lateral sclerosis. *N Engl J Med* 326: 1464–1468, 1992.
330. Rothstein JD, Van Kammen M, Levey AI, Martin LJ, and Kuncl RW. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. *Ann Neurol* 38: 7384, 1995.
331. Rothstein JD, Patel S, Regan MR, Haenggeli C, Huang YH, Bergles DE, Jin L, Dykes Hoberg M, Vidensky S, Chung DS, Toan SV, Bruijn LI, Su ZZ, Gupta P, and Fisher PB. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. *Nature* 433: 73–77, 2005.
332. Said Ahmed M, Hung WY, Zu JS, Hockberger P, and Siddique T. Increased reactive oxygen species in familial amyotrophic lateral sclerosis with mutations in SOD1. *J Neurol Sci* 176: 88–94, 2000.
333. Saito M, Smiley J, Toth R, and Vadasz C. Microarray analysis of gene expression in rat hippocampus after chronic ethanol treatment. *Neurochem Res* 27: 1221–1229, 2002.
334. Saito Y, Yokota T, Mitani T, Ito K, Anzai M, Miyagishi M, Taira K, and Mizusawa H. Transgenic small interfering RNA halts amyotrophic lateral sclerosis in a mouse model. *J Biol Chem* 280: 42826–42830, 2005.
335. Sala G, Beretta S, Ceresa C, Mattavelli L, Zoia C, Tremolizzo L, Ferri A, Carri MT, and Ferrarese C. Impairment of glutamate transport and increased vulnerability to oxidative stress in neuroblastoma SH-SY5Y cells expressing a Cu,Zn superoxide dismutase typical of familial amyotrophic lateral sclerosis. *Neurochem Int* 46: 227–234, 2005.
336. Sampaolesi M, Blot S, D'Antona G, Granger N, Tonlorenzi R, Innocenzi A, Mognol P, Thibaud JL, Galvez BG, Barthelemy I, Perani L, Mantero S, Guttinger M, Pansarasa O, Rinaldi C, Cusella De Angelis MG, Torrente Y, Bordignon C, Bottinelli R, and Cossu G. Mesoangioblast stem cells ameliorate muscle function in dystrophic dogs. *Nature* 444: 574–579, 2006.
337. Sankarapandi S and Zweier JL. Evidence against the generation of free hydroxyl radicals from the interaction of copper,zinc-superoxide dismutase and hydrogen peroxide. *J Biol Chem* 274: 34576–34583, 1999.
338. Sasaki S and Iwata M. Impairment of fast axonal transport in the proximal axons of anterior horn neurons in amyotrophic lateral sclerosis. *Neurology* 47: 535–540, 1996.
339. Sau D, De Biasi S, Vitellaro-Zuccarello L, Riso P, Guarnieri S, Porrini M, Simeoni S, Crippa V, Onesto E, Palazzolo I, Rusmini P, Bolzoni E, Bendotti C, and Poletti A. Mutation of SOD1 in ALS: a gain of a loss of function. *Hum Mol Genet* 16: 1604–1618, 2007.
340. Sayre LM, Smith MA, and Perry G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr Med Chem* 8: 721–738, 2001.
341. Schiffer D, Cordera S, Cavalla P, and Migheli A. Reactive astrogliosis of the spinal cord in amyotrophic lateral sclerosis. *J Neurol Sci* 139 suppl: 27–33, 1996.
342. Schwab ME. Nogo and axon regeneration. *Curr Opin Neurobiol* 14: 118–124, 2004.
343. Schwarz DS, Ding H, Kennington L, Moore JT, Schelter J, Burchard J, Linsley PS, Aronin N, Xu Z, and Zamore PD. Designing siRNA that distinguish between genes that differ by a single nucleotide. *PLoS Genet* 2: e140, 2006.
344. Schymick JC, Scholz SW, Fung HC, Britton A, Arepalli S, Gibbs JR, Lombardo F, Matarin M, Kasperaviciute D, Hernandez DG, Crews C, Bruijn L, Rothstein J, Mora G, Restagno G, Chio A, Singleton A, Hardy J, and Traynor BJ. Genome-wide genotyping in amyotrophic lateral sclerosis and neurologically normal controls: first stage analysis and public release of data. *Lancet Neurol* 6: 322–328, 2007.
345. Seibenhener ML, Babu JR, Geetha T, Wong HC, Krishna NR, and Wooten MW. Sequestosome 1/p62 is a polyubiquitin chain binding protein involved in ubiquitin proteasome degradation. *Mol Cell Biol* 24: 8055–8068, 2004.
346. Sendtner M, Schmalbruch H, Stockli KA, Carroll P, Kreutzberg GW, and Thoenen H. Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuronopathy. *Nature* 358: 502–504, 1992.
347. Shaw BF and Valentine JS. How do ALS-associated mutations in superoxide dismutase 1 promote aggregation of the protein? *Trends Biochem Sci* 32: 78–85, 2007.
348. Shaw IC, Fitzmaurice PS, Mitchell JD, and Lynch PG. Studies on cellular free radical protection mechanisms in the anterior horn from patients with amyotrophic lateral sclerosis. *Neurodegeneration* 4: 391–396, 1995.
349. Shibata N, Nagai R, Uchida K, Horiuchi S, Yamada S, Hirano A, Kawaguchi M, Yamamoto T, Sasaki S, and Kobayashi M. Morphological evidence for lipid peroxidation and protein glycoxidation in spinal cords from sporadic amyotrophic lateral sclerosis patients. *Brain Res* 917: 97–104, 2001.
350. Shibata N, Hirano A, Hedley-Whyte ET, Dal Canto MC, Nagai R, Uchida K, Horiuchi S, Kawaguchi M, Yamamoto T, and Kobayashi M. Selective formation of certain advanced glycation end products in spinal cord astrocytes of humans and mice with superoxide dismutase-1 mutation. *Acta Neuropathol (Berl)* 104: 171–178, 2002.

351. Shin JH, Cho SI, Lim HR, Lee JK, Lee YA, Noh JS, Joo IS, Kim KW, and Gwag BJ. Concurrent administration of Neu2000 and lithium produces marked improvement of motor neuron survival, motor function, and mortality in a mouse model of amyotrophic lateral sclerosis. *Mol Pharmacol* 71: 965–975, 2007.
352. Shinder GA, Lacourse MC, Minotti S, and Durham HD: Mutant Cu/Zn-superoxide dismutase proteins have altered solubility and interact with heat shock/stress proteins in models of amyotrophic lateral sclerosis. *J Biol Chem* 276: 12791–12796, 2001.
353. Siddique T, Figlewicz DA, Pericak-Vance MA, Haines JL, Rouleau G, Jeffers AJ, Sapp P, Hung W-Y, Bebout J, McKenna-Yasek D, Deng G, Horvitz HR, Gusella JF, Brown Jr RH, Roses AD, and Collaborators. Linkage of a gene causing familial amyotrophic lateral sclerosis to chromosome 21 and evidence of genetic-locus heterogeneity. *N Engl J Med* 324: 1381–1384, 1991.
354. Siklos L, Engelhardt J, Harati Y, Smith RG, Joo F, and Appel SH. Ultrastructural evidence for altered calcium in motor nerve terminals in amyotropic lateral sclerosis. *Ann Neurol* 39: 203–216, 1996.
355. Simpson CL and Al-Chalabi A. Amyotrophic lateral sclerosis as a complex genetic disease. *Biochim Biophys Acta* 1762: 973–985, 2006.
356. Skibinski G, Parkinson NJ, Brown JM, Chakrabarti L, Lloyd SL, Hummerich H, Nielsen JE, Hodges JR, Spillantini MG, Thusgaard T, Brandner S, Brun A, Rossor MN, Gade A, Johannsen P, Sorensen SA, Gydesen S, Fisher EM, and Collinge J. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. *Nat Genet* 37: 806–808, 2005.
357. Smith RA, Melmed S, Sherman B, Frane J, Munsat TL, and Festoff BW. Recombinant growth hormone treatment of amyotrophic lateral sclerosis. *Muscle Nerve* 16: 624–633, 1993.
358. Smith RA, Miller TM, Yamanaka K, Monia BP, Condon TP, Hung G, Lobsiger CS, Ward CM, McAlonis-Downes M, Wei H, Wancewicz EV, Bennett CF, and Cleveland DW. Antisense oligonucleotide therapy for neurodegenerative disease. *J Clin Invest* 116: 2290–2296, 2006.
359. Snyder BJ and Olanow CW. Stem cell treatment for Parkinson's disease: an update for 2005. *Curr Opin Neurol* 18: 376–385, 2005.
360. Son M, Puttaparthi K, Kawamata H, Rajendran B, Boyer PJ, Manfredi G, and Elliott JL. Overexpression of CCS in G93A-SOD1 mice leads to accelerated neurological deficits with severe mitochondrial pathology. *Proc Natl Acad Sci U S A* 104: 6072–6077, 2007.
361. Srinivasan C, Liba A, Imlay JA, Valentine JS, and Gralla EB. Yeast lacking superoxide dismutase(s) show elevated levels of "free iron" as measured by whole cell electron paramagnetic resonance. *J Biol Chem* 275: 29187–29192, 2000.
362. Storch A, Burkhardt K, Ludolph AC, and Schwarz J. Protective effects of riluzole on dopamine neurons: involvement of oxidative stress and cellular energy metabolism. *J Neurochem* 75: 2259–2269, 2000.
363. Storkebaum E, Lambrechts D, Dewerchin M, Moreno-Murciano MP, Appelmans S, Oh H, Van Damme P, Rutten B, Man WY, De Mol M, Wyns S, Manka D, Vermeulen K, Van Den Bosch L, Mertens N, Schmitz C, Robberecht W, Conway EM, Collen D, Moons L, and Carmeliet P. Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. *Nat Neurosci* 8: 85–92, 2005.
364. Strong MJ, Kesavapany S, and Pant HC. The pathobiology of amyotrophic lateral sclerosis: a proteinopathy? *J Neuropathol Exp Neurol* 64: 649–664, 2005.
365. Subramaniam JR, Lyons WE, Liu J, Bartnikas TB, Rothstein J, Price DL, Cleveland DW, Gitlin JD, and Wong PC. Mutant SOD1 causes motor neuron disease independent of copper chaperone-mediated copper loading. *Nat Neurosci* 5: 301–307, 2002.
366. Swerdlow RH, Parks JK, Cassarino DS, Trimmer PA, Miller SW, Maguire DJ, Sheehan JP, Maguire RS, Pattee G, Juel VC, Phillips LH, Tuttle JB, Bennett JP Jr, Davis RE, and Parker WD Jr. Mitochondria in sporadic amyotrophic lateral sclerosis. *Exp Neurol* 153: 135–142, 1998.
367. Takamiya R, Takahashi M, Myint T, Park YS, Miyazawa N, Endo T, Fujiwara N, Sakiyama H, Misonou Y, Miyamoto Y, Fujii J, and Taniguchi N. Glycation proceeds faster in mutated Cu, Zn-superoxide dismutases related to familial amyotrophic lateral sclerosis. *FASEB J* 17: 938–940, 2003.
368. Takeuchi H, Kobayashi Y, Ishigaki S, Doyu M, and Sobue G. Mitochondrial localization of mutant superoxide dismutase 1 triggers caspase-dependent cell death in a cellular model of familial amyotrophic lateral sclerosis. *J Biol Chem* 277: 50966–50972, 2002.
369. Takeuchi H, Niwa J, Hishikawa N, Ishigaki S, Tanaka F, Doyu M, and Sobue G. Dorfin prevents cell death by reducing mitochondrial localizing mutant superoxide dismutase 1 in a neuronal cell model of familial amyotrophic lateral sclerosis. *J Neurochem* 89: 64–72, 2004.
370. Tateno M, Sadakata H, Tanaka M, Itohara S, Shin RM, Miura M, Masuda M, Aosaki T, Urushitani M, Misawa H, and Takahashi R. Calcium-permeable AMPA receptors promote misfolding of mutant SOD1 protein and development of amyotrophic lateral sclerosis in a transgenic mouse model. *Hum Mol Genet* 13: 2183–2196, 2004.
371. Terry PD, Kamel F, Umbach DM, Lehman TA, Hu H, Sandler DP, and Taylor JA. VEGF promoter haplotype and amyotrophic lateral sclerosis (ALS). *J Neurogenet* 18: 429–434, 2004.
372. Thrower JS, Hoffman L, Rechsteiner M, and Pickart CM. Recognition of the polyubiquitin proteolytic signal. *EMBO J* 19: 94–102, 2000.
373. Tikka T, Fiebich BL, Goldsteins G, Keinanen R, and Koistinaho J. Minocycline, a tetracycline derivative, is neuroprotective against excitotoxicity by inhibiting activation and proliferation of microglia. *J Neurosci* 21: 2580–2588, 2001.
374. Tikka TM and Koistinaho JE. Minocycline provides neuroprotection against N-methyl-D-aspartate neurotoxicity by inhibiting microglia. *J Immunol* 166: 7527–7533, 2001.
375. Tiwari A and Hayward LJ. Familial amyotrophic lateral sclerosis mutants of copper/zinc superoxide dismutase are susceptible to disulfide reduction. *J Biol Chem* 278: 5984–5992, 2003.
376. Tomkins J, Usher P, Slade JY, Ince PG, Curtis A, Bushby K, and Shaw PJ. Novel insertion in the KSP region of the neurofilament heavy gene in amyotrophic lateral sclerosis (ALS). *Neuroreport* 9: 3967–3970, 1998.
377. Topp JD, Gray NW, Gerard RD, and Horazdovsky BF. Alsin is a Rab5 and Rac1 guanine nucleotide exchange factor. *J Biol Chem* 279: 24612–24623, 2004.
378. Torres-Aleman I, Villalba M, and Nieto-Bona MP. Insulin-like growth factor-I modulation of cerebellar cell populations is developmentally stage-dependent and mediated by specific intracellular pathways. *Neuroscience* 83: 321–334, 1998.
379. Trotti D, Rolfs A, Danbolt NC, Brown RH Jr, and Hediger MA. SOD1 mutants linked to amyotrophic lateral sclerosis selectively inactivate a glial glutamate transporter. *Nat Neurosci* 2: 848, 1999.
380. Trotti D, Aoki M, Pasinelli P, Berger UV, Danbolt NC, Brown RH Jr, and Hediger MA. Amyotrophic lateral sclerosis-linked glutamate transporter mutant has impaired glutamate clearance capacity. *J Biol Chem* 276: 576–582, 2001.
381. Tsuzaka K, Ishiyama T, Pioro EP, and Mitsumoto H. Role of brain-derived neurotrophic factor in wobbler mouse motor neuron disease. *Muscle Nerve* 24: 474–480, 2001.
382. Turner BJ, Cheah IK, Macfarlane KJ, Lopes EC, Petratos S, Langford SJ, and Cheema SS. Antisense peptide nucleic acid-mediated knockdown of the p75 neurotrophin receptor delays motor neuron disease in mutant SOD1 transgenic mice. *J Neurochem* 87: 752–763, 2003.
383. Turner BJ, Atkin JD, Farg MA, Zang DW, Rembach A, Lopes EC, Patch JD, Hill AF, and Cheema SS. Impaired extracellular secretion of mutant superoxide dismutase 1 associates with neurotoxicity in familial amyotrophic lateral sclerosis. *J Neurosci* 25: 108–117, 2005.
384. Urushitani M, Kurisu J, Tsukita K, and Takahashi R. Proteasomal inhibition by misfolded mutant superoxide dismutase 1 induces selective motor neuron death in familial amyotrophic lateral sclerosis. *J Neurochem* 83: 1030–1042, 2002.
385. Urushitani M, Sik A, Sakurai T, Nukina N, Takahashi R, and Julien JP. Chromogranin-mediated secretion of mutant superoxide dismutase proteins linked to amyotrophic lateral sclerosis. *Nat Neurosci* 9: 108–118, 2006.
386. Urushitani M, Ezzi SA, and Julien JP. Therapeutic effects of immunization with mutant superoxide dismutase in mice models of

amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 104: 2495–2500, 2007.
387. Valentine JS and Hart PJ. Misfolded CuZnSOD and amyotrophic lateral sclerosis. *Proc Natl Acad Sci U S A* 100: 3617–3622, 2003.
388. Van Damme P, Braeken D, Callewaert G, Robberecht W, and Van Den Bosch L. GluR2 deficiency accelerates motor neuron degeneration in a mouse model of amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* 64: 605–612, 2005.
389. Van Vught PW, Sutedja NA, Veldink JH, Koeleman BP, Groeneveld GJ, Wijmenga C, Uitdehaag BM, de Jong JM, Baas F, Wokke JH, and Van den Berg LH. Lack of association between VEGF polymorphisms and ALS in a Dutch population. *Neurology* 65: 1643–1645, 2005.
390. Vanoni C, Massari S, Losa M, Carrega P, Perego C, Conforti L, and Pietrini G. Increased internalisation and degradation of GLT-1 glial glutamate transporter in a cell model for familial amyotrophic lateral sclerosis (ALS). *J Cell Sci* 117: 5417–5426, 2004.
391. Vazey EM, Chen K, Hughes SM, and Connor B. Transplanted adult neural progenitor cells survive, differentiate and reduce motor function impairment in a rodent model of Huntington's disease. *Exp Neurol* 199: 384–396, 2006.
392. Vechio JD, Bruijn LI, Xu Z, Brown RH Jr, and Cleveland DW. Sequence variants in human neurofilament proteins: absence of linkage to familial amyotrophic lateral sclerosis. *Ann Neurol* 40: 603–610, 1996.
393. Veldink JH, Kalmijn S, Van der Hout AH, Lemmink HH, Groeneveld GJ, Lummen C, Scheffer H, Wokke JH, and Van den Berg LH. SMN genotypes producing less SMN protein increase susceptibility to and severity of sporadic ALS. *Neurology* 65: 820–825, 2005.
394. Vijayvergiya C, Beal MF, Buck J, and Manfredi G. Mutant superoxide dismutase 1 forms aggregates in the brain mitochondrial matrix of amyotrophic lateral sclerosis mice. *J Neurosci* 25: 2463–2470, 2005.
395. Vlug AS and Jaarsma D. Long term proteasome inhibition does not preferentially afflict motor neurons in organotypical spinal cord cultures. *Amyotroph Lateral Scler Other Motor Neuron Disord* 5: 16–21, 2004.
396. Vukosavic S, Stefanis L, Jackson-Lewis V, Guegan C, Romero N, Chen C, Dubois-Dauphin M, and Przedborski S. Delaying caspase activation by Bcl-2: a clue to disease retardation in a transgenic mouse model of amyotrophic lateral sclerosis. *J Neurosci* 20: 9119–9125, 2000.
397. Wang J, Xu G, and Borchelt DR. High molecular weight complexes of mutant superoxide dismutase, 1: age-dependent and tissue-specific accumulation. *Neurobiol Dis* 9: 139–148, 2002.
398. Wang J, Slunt H, Gonzales V, Fromholt D, Coonfield M, Copeland NG, Jenkins NA, and Borchelt DR. Copper-binding-site-null SOD1 causes ALS in transgenic mice: aggregates of non-native SOD1 delineate a common feature. *Hum Mol Genet* 12: 2753–2764, 2003.
399. Wang LJ, Lu YY, Muramatsu S, Ikeguchi K, Fujimoto K, Okada T, Mizukami H, Matsushita T, Hanazono Y, Kume A, Nagatsu T, Ozawa K, and Nakano I. Neuroprotective effects of glial cell line-derived neurotrophic factor mediated by an adeno-associated virus vector in a transgenic animal model of amyotrophic lateral sclerosis. *J Neurosci* 22: 6920–6928, 2002.
400. Wang Q, Matsumoto Y, Shindo T, Miyake K, Shindo A, Kawanishi M, Kawai N, Tamiya T, and Nagao S. Neural stem cells transplantation in cortex in a mouse model of Alzheimer's disease. *J Med Invest* 53: 61–69, 2006.
401. Wang XS, Lee S, Simmons Z, Boyer P, Scott K, Liu W, and Connor J. Increased incidence of the Hfe mutation in amyotrophic lateral sclerosis and related cellular consequences. *J Neurol Sci* 227: 27–33, 2004.
402. Wang Y, Mao XO, Xie L, Banwait S, Marti HH, Greenberg DA, and Jin K. Vascular endothelial growth factor overexpression delays neurodegeneration and prolongs survival in amyotrophic lateral sclerosis mice. *J Neurosci* 27: 304–307, 2007.
403. Warita H, Hayashi T, Murakami T, Manabe Y, and Abe K. Oxidative damage to mitochondrial DNA in spinal motoneurons of transgenic ALS mice. *Brain Res Mol Brain Res* 89: 147–152, 2001.
404. Weishaupt JH, Bartels C, Polking E, Dietrich J, Rohde G, Poeggeler B, Mertens N, Sperling S, Bohn M, Huther G, Schneider A, Bach A, Siren AL, Hardeland R, Bahr M, Nave KA, and Ehrenreich H. Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. *J Pineal Res* 41: 313–323, 2006.
405. Weisiger RA and Fridovich I. Mitochondrial superoxide simutase: site of synthesis and intramitochondrial localization. *J Biol Chem* 248: 4793–4796, 1973.
406. Weisiger RA and Fridovich I. Superoxide dismutase: organelle specificity. *J Biol Chem* 248: 3582–3592, 1973.
407. Whiteman M and Halliwell B. Prevention of peroxynitrite-dependent tyrosine nitration and inactivation of alpha1-antiproteinase by antibiotics. *Free Radic Res* 26: 49–56, 1997.
408. Wiedau-Pazos M, Goto JJ, Rabizadeh S, Gralla EB, Roe JA, Lee MK, Valentine JS, and Bredesen DE. Altered reactivity of superoxide dismutase in familial amyotrophic lateral sclerosis. *Science* 271: 515–518, 1996.
409. Wiedemann FR, Winkler K, Kuznetsov AV, Bartels C, Vielhaber S, Feistner H, and Kunz WS. Impairment of mitochondrial function in skeletal muscle of patients with amyotrophic lateral sclerosis. *J Neurol Sci* 156: 65–72, 1998.
410. Wiedemann FR, Manfredi G, Mawrin C, Beal MF, and Schon EA. Mitochondrial DNA and respiratory chain function in spinal cords of ALS patients. *J Neurochem* 80: 616–625, 2002.
411. Wilczak N, de Vos RA, and De Keyser J. Free insulin-like growth factor (IGF)-I and IGF binding proteins 2, 5, and 6 in spinal motor neurons in amyotrophic lateral sclerosis. *Lancet* 361: 1007–1011, 2003.
412. Williamson TL and Cleveland DW. Slowing of axonal transport is a very early event in the toxicity of ALS-linked SOD1 mutants to motor neurons. *Nat Neurosci* 2: 50–56, 1999.
413. Wong NK, He BP, and Strong MJ. Characterization of neuronal intermediate filament protein expression in cervical spinal motor neurons in sporadic amyotrophic lateral sclerosis (ALS). *J Neuropathol Exp Neurol* 59: 972–982, 2000.
414. Wong PC, Pardo CA, Borchelt DR, Lee MK, Copeland NG, Jenkins NA, Sisodia SS, Cleveland DW, and Price DL. An adverse property of a familial ALS-linked SOD1 mutation causes motor neuron disease characterized by vacuolar degeneration of mitochondria. *Neuron* 14: 1105–1116, 1995.
415. Wood JD, Beaujeux TP, and Shaw PJ. Protein aggregation in motor neurone disorders. *Neuropathol Appl Neurobiol* 29: 529–545, 2003.
416. Wu AS, Kiaei M, Aguirre N, Crow JP, Calingasan NY, Browne SE, and Beal MF. Iron porphyrin treatment extends survival in a transgenic animal model of amyotrophic lateral sclerosis. *J Neurochem* 85: 142–150, 2003.
417. Wu DC, Jackson-Lewis V, Vila M, Tieu K, Teismann P, Vadseth C, Choi DK, Ischiropoulos H, and Przedborski S. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J Neurosci* 22: 1763–1771, 2002.
418. Xia X, Zhou H, Huang Y, and Xu Z. Allele-specific RNAi selectively silences mutant SOD1 and achieves significant therapeutic benefit in vivo. *Neurobiol Dis* 23: 578–586, 2006.
419. Xie J, Awad KS, and Guo Q. RNAi knockdown of Par-4 inhibits neurosynaptic degeneration in ALS-linked mice. *J Neurochem* 92: 59–71, 2005.
420. Xie Y, Weydt P, Howland DS, Kliot M, and Moller T. Inflammatory mediators and growth factors in the spinal cord of G93A SOD1 rats. *Neuroreport* 15: 2513–2516, 2004.
421. Xu Z, Cork LC, Griffin JW, and Cleveland DW. Increased expression of neurofilament subunit NF-L produces morphological alterations that resemble the pathology of human motor neuron disease. *Cell* 73: 23–33, 1993.
422. Yamamoto M, Mitsuma N, Inukai A, Ito Y, Li M, Mitsuma T, and Sobue G. Expression of GDNF and GDNFR-alpha mRNAs in muscles of patients with motor neuron diseases. *Neurochem Res* 24: 785–790, 1999.
423. Yamanaka K, Miller TM, McAlonis-Downes M, Chun SJ, and Cleveland DW. Progressive spinal axonal degeneration and slowness in ALS2-deficient mice. *Ann Neurol* 60: 95–104, 2006.

424. Yang Y, Hentati A, Deng HX, Dabbagh O, Sasaki T, Hirano M, Hung WY, Ouahchi K, Yan J, Azim AC, Cole N, Gascon G, Yagmour A, Ben-Hamida M, Pericak-Vance M, Hentati F, and Siddique T. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. *Nat Genet* 29: 160–165, 2001.
425. Yasui M, Ota K, and Garruto RM. Concentrations of zinc and iron in the brains of Guamanian patients with amyotrophic lateral sclerosis and parkinsonism-dementia. *Neurotoxicology* 14: 445–450, 1993.
426. Yim HS, Kang JH, Chock PB, Stadtman ER, and Yim MB. A familial amyotrophic lateral sclerosis-associated A4V Cu, Zn-superoxide dismutase mutant has a lower Km for hydrogen peroxide: correlation between clinical severity and the Km value. *J Biol Chem* 272: 8861–8863, 1997.
427. Yim MB, Kang JH, Yim HS, Kwak HS, Chock PB, and Stadtman ER. A gain-of-function of an amyotrophic lateral sclerosis-associated Cu,Zn-superoxide dismutase mutant: an enhancement of free radical formation due to a decrease in Km for hydrogen peroxide. *Proc Natl Acad Sci U S A* 93: 5709–5714, 1996.
428. Yokota T, Miyagishi M, Hino T, Matsumura R, Tasinato A, Urushitani M, Rao RV, Takahashi R, Bredesen DE, Taira K, and Mizusawa H. siRNA-based inhibition specific for mutant SOD1 with single nucleotide alternation in familial ALS, compared with ribozyme and DNA enzyme. *Biochem Biophys Res Commun* 314: 283–291, 2004.
429. Yoshihara T, Ishigaki S, Yamamoto M, Liang Y, Niwa J, Takeuchi H, Doyu M, and Sobue G. Differential expression of inflammation- and apoptosis-related genes in spinal cords of a mutant SOD1 transgenic mouse model of familial amyotrophic lateral sclerosis. *J Neurochem* 80: 158–167, 2002.
430. Yoshino H and Kimura A. Investigation of the therapeutic effects of edaravone: a free radical scavenger, on amyotrophic lateral sclerosis (phase II study). *Amyotroph Lateral Scler* 7: 241–245, 2006.
431. Yrjanheikki J, Keinanen R, Pellikka M, Hokfelt T, and Koistinaho J. Tetracyclines inhibit microglial activation and are neuroprotective in global brain ischemia. *Proc Natl Acad Sci U S A* 95: 15769–15774, 1998.
432. Yrjanheikki J, Tikka T, Keinanen R, Goldsteins G, Chan PH, and Koistinaho J. A tetracycline derivative, minocycline, reduces inflammation and protects against focal cerebral ischemia with a wide therapeutic window. *Proc Natl Acad Sci U S A* 96: 13496–13500, 1999.
433. Zhang B, Tu P, Abtahian F, Trojanowski JQ, and Lee VM. Neurofilaments and orthograde transport are reduced in ventral root axons of transgenic mice that express human SOD1 with a G93A mutation. *J Cell Biol* 139: 1307–1315, 1997.
434. Zhang H, Andrekopoulos C, Joseph J, Chandran K, Karoui H, Crow JP, and Kalyanaraman B: Bicarbonate-dependent peroxidase activity of human Cu,Zn-superoxide dismutase induces covalent aggregation of protein: intermediacy of tryptophan-derived oxidation products. *J Biol Chem* 278: 24078–24089, 2003.
435. Zhang J and Huang EJ. Dynamic expression of neurotrophic factor receptors in postnatal spinal motoneurons and in mouse model of ALS. *J Neurobiol* 66: 882–895, 2006.
436. Zhang W, Narayanan M, and Friedlander RM. Additive neuroprotective effects of minocycline with creatine in a mouse model of ALS. *Ann Neurol* 53: 267–270, 2003.
437. Zhang Y, Zhang H, Fu Y, Song H, Wang L, Zhang J, and Fan D. VEGF C2578A polymorphism does not contribute to amyotrophic lateral sclerosis susceptibility in sporadic Chinese patients. *Amyotroph Lateral Scler* 7: 119–122, 2006.
438. Zheng C, Nennesmo I, Fadeel B, and Henter JI. Vascular endothelial growth factor prolongs survival in a transgenic mouse model of ALS. *Ann Neurol* 56: 564–567, 2004.
439. Zhong J and Lee WH. Hydrogen peroxide attenuates insulin-like growth factor-1 neuroprotective effect, prevented by minocycline. *Neurochem Int* 51: 398–404, 2007.
440. Zhu S, Stavrovskaya IG, Drozda M, Kim BY, Ona V, Li M, Sarang S, Liu AS, Hartley DM, Wu DC, Gullans S, Ferrante RJ, Przedborski S, Kristal BS, and Friedlander RM. Minocycline inhibits cytochrome c release and delays progression of amyotrophic lateral sclerosis in mice. *Nature* 417: 74–78, 2002.

Address reprint requests to:
Maria Teresa Carrì
Dipartimento di Biologia
Università di Roma "Tor Vergata"
Via della Ricerca Scientifica
00133 Rome, Italy

E-mail: carri@Bio.uniroma2.it

Date of first submission to ARS Central, May 29, 2007; date of final revised submission, September 17, 2007; date of acceptance, September 29, 2007.

This article has been cited by:

1. Mauro Cozzolino , Maria Grazia Pesaresi , Valeria Gerbino , Julian Grosskreutz , Maria Teresa Carrì . 2012. Amyotrophic Lateral Sclerosis: New Insights into Underlying Molecular Mechanisms and Opportunities for Therapeutic Intervention. *Antioxidants & Redox Signaling* **17**:9, 1277-1330. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
2. Stacy A. Rudnicki, James D. Berry, Evan Ingersoll, Don Archibald, Merit E. Cudkowicz, Douglas A. Kerr, Yingwen Dong. 2012. Dexpramipexole effects on functional decline and survival in subjects with amyotrophic lateral sclerosis in a Phase II study: Subgroup analysis of demographic and clinical characteristics. *Amyotrophic Lateral Sclerosis* 1-8. [CrossRef]
3. Caterina Bendotti, Marianna Marino, Cristina Cheroni, Elena Fontana, Valeria Crippa, Angelo Poletti, Silvia De Biasi. 2012. Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: Implication for protein aggregation and immune response. *Progress in Neurobiology* **97**:2, 101-126. [CrossRef]
4. Mauro Cozzolino, Maria Teresa Carrì. 2012. Mitochondrial dysfunction in ALS. *Progress in Neurobiology* **97**:2, 54-66. [CrossRef]
5. Serena Carra, Valeria Crippa, Paola Rusmini, Alessandra Boncoraglio, Melania Minoia, Elisa Giorgetti, Harm H. Kampinga, Angelo Poletti. 2012. Alteration of protein folding and degradation in motor neuron diseases: Implications and protective functions of small heat shock proteins. *Progress in Neurobiology* **97**:2, 83-100. [CrossRef]
6. Mariarita Galbiati, Elisa Onesto, Arianna Zito, Valeria Crippa, Paola Rusmini, Raffaella Mariotti, Marina Bentivoglio, Caterina Bendotti, Angelo Poletti. 2011. The anabolic/androgenic steroid nandrolone exacerbates gene expression modifications induced by mutant SOD1 in muscles of mice models of amyotrophic lateral sclerosis. *Pharmacological Research* . [CrossRef]
7. Maria Teresa Carrì, Mauro Cozzolino. 2011. SOD1 and mitochondria in ALS: a dangerous liaison. *Journal of Bioenergetics and Biomembranes* . [CrossRef]
8. M. G. Pesaresi, I. Amori, C. Giorgi, A. Ferri, P. Fiorenzo, F. Gabanella, A. M. Salvatore, M. Giorgio, P. G. Pelicci, P. Pinton, M. T. Carri, M. Cozzolino. 2011. Mitochondrial redox signalling by p66Shc mediates ALS-like disease through Rac1 inactivation. *Human Molecular Genetics* . [CrossRef]
9. P. Piscopo, A. Crestini, A. Adduci, A. Ferrante, M. Massari, P. Popoli, N. Vanacore, A. Confaloni. 2011. Altered oxidative stress profile in the cortex of mice fed an enriched branched-chain amino acids diet: Possible link with amyotrophic lateral sclerosis?. *Journal of Neuroscience Research* **89**:8, 1276-1283. [CrossRef]
10. Elisa Onesto, Paola Rusmini, Valeria Crippa, Nicola Ferri, Arianna Zito, Mariarita Galbiati, Angelo Poletti. 2011. Muscle cells and motoneurons differentially remove mutant SOD1 causing familial amyotrophic lateral sclerosis. *Journal of Neurochemistry* **118**:2, 266-280. [CrossRef]
11. Cinzia Volonté, Savina Apolloni, Maria Teresa Carrì, Nadia D'Ambrosi. 2011. ALS: Focus on purinergic signalling. *Pharmacology & Therapeutics* . [CrossRef]
12. B. Carletti, C. Passarelli, M. Sparaco, G. Tozzi, A. Pastore, E. Bertini, F. Piemonte. 2011. Effect of protein glutathionylation on neuronal cytoskeleton: a potential link to neurodegeneration. *Neuroscience* . [CrossRef]
13. Mario Arciello, Concetta Rosa Capo, Sara D'Annibale, Mauro Cozzolino, Alberto Ferri, Maria Teresa Carrì, Luisa Rossi. 2011. Copper depletion increases the mitochondrial-associated SOD1 in neuronal cells. *BioMetals* **24**:2, 269-278. [CrossRef]
14. Soledad Matus, Laurie H Glimcher, Claudio Hetz. 2011. Protein folding stress in neurodegenerative diseases: a glimpse into the ER. *Current Opinion in Cell Biology* **23**:2, 239-252. [CrossRef]
15. Susanna Amadio, Savina Apolloni, Nadia D'Ambrosi, Cinzia Volonté. 2011. Purinergic signalling at the plasma membrane: a multipurpose and multidirectional mode to deal with amyotrophic lateral sclerosis and multiple sclerosis. *Journal of Neurochemistry* **116**:5, 796-805. [CrossRef]
16. Silvia C. Lenzken, Valentina Romeo, Francesca Zolezzi, Francesca Cordero, Giuseppe Lamorte, Davide Bonanno, Donatella Biancolini, Mauro Cozzolino, Maria Grazia Pesaresi, Alessia Maracchioni, Remo Sanges, Tilmann Achsel, Maria Teresa Carrì, Raffaele A. Calogero, Silvia M.L. Barabino. 2011. Mutant SOD1 and mitochondrial damage alter expression and splicing of genes controlling neuritogenesis in models of neurodegeneration. *Human Mutation* **32**:2, 168-182. [CrossRef]
17. Qian Wang, Xiaojie Zhang, Sheng Chen, Xin Zhang, Sufang Zhang, Moussa Youdium, Weidong Le. 2011. Prevention of Motor Neuron Degeneration by Novel Iron Chelators in SOD1^{G93A} Transgenic Mice of Amyotrophic Lateral Sclerosis. *Neurodegenerative Diseases* **8**:5, 310-321. [CrossRef]
18. Nanette Joyce, Gregory T. CarterMotor Neuron Diseases 1041-1063. [CrossRef]

19. Melissa Nassif , Soledad Matus , Karen Castillo , Claudio Hetz . 2010. Amyotrophic Lateral Sclerosis Pathogenesis: A Journey Through the Secretory Pathway. *Antioxidants & Redox Signaling* **13**:12, 1955-1989. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
20. A. Ferri, P. Fiorenzo, M. Nencini, M. Cozzolino, M. G. Pesaresi, C. Valle, S. Sepe, S. Moreno, M. T. Carri. 2010. Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. *Human Molecular Genetics* **19**:22, 4529-4542. [CrossRef]
21. Johannes M. Herrmann , Jan Riemer . 2010. The Intermembrane Space of Mitochondria. *Antioxidants & Redox Signaling* **13**:9, 1341-1358. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
22. Douglas B. Kell. 2010. Towards a unifying, systems biology understanding of large-scale cellular death and destruction caused by poorly liganded iron: Parkinson's, Huntington's, Alzheimer's, prions, bactericides, chemical toxicology and others as examples. *Archives of Toxicology* **84**:11, 825-889. [CrossRef]
23. Emanuela Cova, Andrea Ghiroldi, Stefania Guareschi, Giuliano Mazzini, Stella Gagliardi, Annalisa Davin, Marika Bianchi, Mauro Ceroni, Cristina Cereda. 2010. G93A SOD1 alters cell cycle in a cellular model of Amyotrophic Lateral Sclerosis. *Cellular Signalling* **22**:10, 1477-1484. [CrossRef]
24. Orly Weinreb , Tamar Amit , Silvia Mandel , Lana Kupershmidt , Moussa B.H. Youdim . 2010. Neuroprotective Multifunctional Iron Chelators: From Redox-Sensitive Process to Novel Therapeutic Opportunities. *Antioxidants & Redox Signaling* **13**:6, 919-949. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
25. S. Pedrini, D. Sau, S. Guareschi, M. Bogush, R. H. Brown, N. Naniche, A. Kia, D. Trotti, P. Pasinelli. 2010. ALS-linked mutant SOD1 damages mitochondria by promoting conformational changes in Bcl-2. *Human Molecular Genetics* **19**:15, 2974-2986. [CrossRef]
26. Melinda Paizs, József I. Engelhardt, Zója Katarova, László Siklós. 2010. Hypoglossal motor neurons display a reduced calcium increase after axotomy in mice with upregulated parvalbumin. *The Journal of Comparative Neurology* **518**:11, 1946-1961. [CrossRef]
27. Chen Benkler, Daniel Offen, Eldad Melamed, Lana Kupershmidt, Tamar Amit, Silvia Mandel, Moussa B. H. Youdim, Orly Weinreb. 2010. Recent advances in amyotrophic lateral sclerosis research: perspectives for personalized clinical application. *The EPMA Journal* **1**:2, 343-361. [CrossRef]
28. Kevin Chen, Frances J. Northington, Lee J. Martin. 2010. Inducible nitric oxide synthase is present in motor neuron mitochondria and Schwann cells and contributes to disease mechanisms in ALS mice. *Brain Structure and Function* **214**:2-3, 219-234. [CrossRef]
29. James Suchy, Sangmook Lee, Ambar Ahmed, Thomas B. Shea. 2010. Dietary Supplementation with S-Adenosyl Methionine Delays the Onset of Motor Neuron Pathology in a Murine Model of Amyotrophic Lateral Sclerosis. *NeuroMolecular Medicine* **12**:1, 86-97. [CrossRef]
30. Mario Arciello, Concetta Rosa Capo, Mauro Cozzolino, Alberto Ferri, Monica Nencini, Maria Teresa Carrì, Luisa Rossi. 2010. Inactivation of cytochrome c oxidase by mutant SOD1s in mouse motoneuronal NSC-34 cells is independent from copper availability but is because of nitric oxide. *Journal of Neurochemistry* **112**:1, 183-192. [CrossRef]
31. Lee J. Martin. 2010. The mitochondrial permeability transition pore: A molecular target for amyotrophic lateral sclerosis therapy. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1802**:1, 186-197. [CrossRef]
32. Sanjoy K. Bhattacharya, James Gomes, Colleen M. Cebulla. 2010. Toward failure analyses in systems biology. *Wiley Interdisciplinary Reviews: Systems Biology and Medicine* n/a-n/a. [CrossRef]
33. Lee J. Martin, Barry Gertz, Yan Pan, Ann C. Price, Jeffery D. Molkentin, Qing Chang. 2009. The mitochondrial permeability transition pore in motor neurons: Involvement in the pathobiology of ALS mice. *Experimental Neurology* **218**:2, 333-346. [CrossRef]
34. Shulamit Naor, Zohar Keren, Tomer Bronshtein, Efrat Goren, Marcelle Machluf, Doron Melamed. 2009. Development of ALS-like disease in SOD-1 mice deficient of B lymphocytes. *Journal of Neurology* **256**:8, 1228-1235. [CrossRef]
35. Livia Pasquali, Patrizia Longone, Ciro Isidoro, Stefano Ruggieri, Antonio Paparelli, Francesco Fornai. 2009. Autophagy, lithium, and amyotrophic lateral sclerosis. *Muscle & Nerve* **40**:2, 173-194. [CrossRef]
36. Caterina Bendotti , Maria Teresa Carrì . 2009. Amyotrophic Lateral Sclerosis: Mechanisms and Countermeasures. *Antioxidants & Redox Signaling* **11**:7, 1519-1522. [Citation] [Full Text PDF] [Full Text PDF with Links]
37. Mauro Cozzolino , Maria Grazia Pesaresi , Ilaria Amori , Claudia Crosio , Alberto Ferri , Monica Nencini , Maria Teresa Carrì . 2009. Oligomerization of Mutant SOD1 in Mitochondria of Motoneuronal Cells Drives Mitochondrial Damage and Cell Toxicity. *Antioxidants & Redox Signaling* **11**:7, 1547-1558. [Abstract] [Full Text PDF] [Full Text PDF with Links]

38. Savina Apolloni, Cinzia Montilli, Pamela Finocchi, Susanna Amadio. 2009. Membrane compartments and purinergic signalling: P2X receptors in neurodegenerative and neuroinflammatory events. *FEBS Journal* **276**:2, 354-364. [CrossRef]
39. A FERRI, M NENCINI, M COZZOLINO, P CARRARA, S MORENO, M CARRI. 2008. Inflammatory cytokines increase mitochondrial damage in motoneuronal cells expressing mutant SOD1. *Neurobiology of Disease* **32**:3, 454-460. [CrossRef]
40. Nizar Souayah, Anna Sharovetskaya, Michael P. Kurnellas, Matthew Myerson, Jeffrey S. Deitch, Stella Elkabes. 2008. Reductions in motor unit number estimates (MUNE) precede motor neuron loss in the plasma membrane calcium ATPase 2 (PMCA2)-heterozygous mice. *Experimental Neurology* **214**:2, 341-346. [CrossRef]
41. S SATHASIVAM. 2008. VEGF and ALS. *Neuroscience Research* **62**:2, 71-77. [CrossRef]