Motor Neuron Diseases

Donald L. Price
Steven Ackerly
Lee J. Martin
Vassilis Koliatsos
Philip C. Wong

AMYOTROPHIC LATERAL SCLEROSIS IS THE MOST COMMON ADULT ONSET MOTOR NEURON DISEASE 731

Motor neuron disease is characterized clinically by weakness, muscle atrophy and spasticity. 732
Some cases of amyotrophic lateral sclerosis are familial. 733
Several genes have been shown to cause amyotrophic lateral sclerosis. 733

NON-TRANSGENIC, INDUCED MODELS OF MOTOR NEURON DISEASE 734

Interruption of the communication between the motor neuron cell body and axon by transection, crush or avulsion induces motor neuron injury. 734

SELECTED GENETIC MODELS OF RELEVANCE TO AMYOTROPHIC LATERAL SCLEROSIS 735

Transgenic mice expressing wild type or mutant neurofilament genes develop motor neuron disease and neurofibillary pathology. 736
Familial-amytrophic lateral sclerosis-linked mutant SOD1 mice reproduce many of the clinical and pathological features of amyotrophic lateral sclerosis. 736
Lines of mice with mutant genes encoding motor proteins develop an amyotrophic lateral sclerosis-like phenotype. 736
A variety of experimental therapeutic strategies have been tested in mutant SOD1 mice. 737
Mouse models offer opportunities for discovering disease mechanisms and for testing novel treatment strategies. 739

AMYOTROPHIC LATERAL SCLEROSIS IS THE MOST COMMON ADULT ONSET MOTOR NEURON DISEASE

The motor neuron diseases (MND), including amyotrophic lateral sclerosis (ALS), are chronic, progressive illnesses characterized by severely disabling clinical features involving motor systems (weakness, muscle atrophy and, in ALS, stiffness); relatively selective involvement of lower motor neurons and, in classical ALS, upper motor neurons; disease-related abnormalities associated with, in some instances, the presence of intracellular protein aggregates (inclusions); alterations in axonal transport; and death of motor neurons. Some forms of the disease are inherited as autosomal dominants, others as recessives. In some instances, the presence of specific gene products confer risk. The majority of cases appear to be sporadic. Although symptomatic treatments are available, there are, at present, no effective mechanism-based therapies [1-4]. The MND represent challenges for science and medicine, not because of their prevalence but because of the tragedy of these illnesses for affected individuals, their families and caregivers. Recent research, particularly studies utilizing animal models, has provided new insights into the mechanisms of these disorders, identified potential new targets for therapy and allowed design and testing of treatments.

Before discovery of mutant genes linked to MND, scientists created and studied surrogate models of dysfunction/death of motor neurons [4, 5], including: axotomy models (axonal transection and avulsion); models of neurofilamentous abnormalities of axons induced by exposure to toxins; and spontaneously occurring animal models, such as hereditary canine spinal muscular atrophy (HCSMA), a genetic disease occurring in Brittany spaniels. Although useful for testing hypotheses about pathogenic mechanisms, these models have variable direct relevance to human MND. However, because investigations of these models set the stage and established experimental strategies for later work, we briefly describe studies...
of several of these disorders as examples of the types of investigations that lead to investigations of the new models, many of which were developed following the exciting discoveries of the roles of mutant genes in MND.

The identification of causative mutations in specific genes in cases of human MND, including familial ALS (fALS) and spinal muscular atrophy (SMA) [6–8], has provided new opportunities, using transgenic and gene targeting approaches, to investigate the molecular participants in disease processes [1, 3]. In autosomal dominant fALS, the mutant proteins often acquire toxic properties that directly or indirectly impact on the functions and viability of neurons [5, 4, 7, 9, 10], and introduction of mutant genes into mice reproduces some features of these diseases [4, 11–13]. In contrast, autosomal recessive diseases, such as SMA, which usually lack the functional protein encoded by the mutant gene (SMN – ‘survival motor neuron’ – in SMA), can often be modeled by gene targeting strategies [1, 4]. Studies of some MND in animals, including progressive motor neuronopathy (PMN) [14], have led to discovery of mutant genes/proteins whose roles in neurons illuminate potential disease mechanisms in humans [14].

In models of MND, therapeutic manipulations, manipulation of expression of selected genes in specific cell populations [15, 16], creation of chimeric animals to test whether abnormalities are cell autonomous [17], administration of trophic factors to prevent trophic cell death [18–20] and testing of a variety of drug therapies [21–24] have been used to try to ameliorate phenotypes and thus provide insights into disease mechanisms and potential treatment strategies [1, 3, 4, 15, 25, 26]. Results of these studies are being used to design novel therapies to be tested in clinical trials in humans.

In this review, we focus on ALS, particularly its genetic variants, and relevant model systems with the belief that understanding these inherited illnesses will help to clarify the mechanisms of the more common sporadic forms of MND. SMA, a major cause of MND in infancy and childhood, is beyond the scope of this chapter and is the subject of several recent reviews [4, 8, 27].

Motor neuron disease is characterized clinically by weakness, muscle atrophy and spasticity. This illness, often termed Lou Gehrig’s disease in the United States, is the most common adult-onset form of MND with a prevalence of approximately 2–3 per 100,000 people [1–3, 10, 25, 28]. Each year in the United States, in excess of 5,000 people are diagnosed with ALS. In parts of the United Kingdom, 1 in >500 deaths are attributed to some form of MND. The principal clinical signs of ALS include progressive limb weakness, which may be symmetrical or asymmetrical; atrophy of appendicular, bulbar and respiratory muscles; and spasticity [1, 2, 26, 28]. The paralysis/muscle atrophy and spasticity are the result of degeneration of motor neurons in the spinal cord/brain stem and motor cortex respectively. The onset of this illness is typically in the fifth or sixth decade of life; affected individuals usually die within 2–5 years of appearance of symptoms. Both sporadic (sALS) and familial (fALS) forms of illness exist; familial cases make up approximately 5–10% of the total. While the causes of the majority of cases of ALS are yet to be identified, the shared features of the clinical presentations and pathologies occurring in both sporadic and familial cases suggest the existence of some common disease mechanisms.

The pathological processes impact particularly on the spinal and corticospinal motor neurons.

These processes appear to evolve through a series of stages influencing size, shape, content, metabolism and physiology of these cells [28–30]. Years ago, it was observed that proximal axonal segments were swollen with maloriented arrays of neurofilaments (NF) [31, 32]. Similar abnormalities were documented in experimental models [5, 33–37]. Investigations, described below, tested the hypothesis that this type of pathology was the result of defects in axonal transport [5, 38–43] (see Ch. 28 for discussion of axonal transport). Moreover, it was hypothesized that impairments in transport could also be associated with ‘a dying back’ phenomenon (i.e., degeneration proceeding retrogradely from distal nerve terminals to proximal cell body), which was hypothesized to be related to impaired delivery of essential constituents to the most distant axons and terminal [5]. In MND, it was hypothesized that these abnormalities become more extensive over time with clinical signs becoming increasingly evident. Moreover, as a part of the dying back process and discrimination of neurons from targets, retrogradely transported trophic support to neuronal cell bodies is compromised, which, in turn, impacts on the viability of these cells [18, 20, 44]. In ALS, motor neurons show a variety of abnormalities, including chromatolysis (enlargement of cell bodies with dispersal/margination of Nissl substance and protein aggregates and inclusions, which are often ubiquitinated [28]. Later, neurons may become atrophic. There is Wallerian degeneration of motor axons (see Ch. 30). In the final stages, motor neurons exhibit several features of apoptosis (see Ch. 35), which are discussed below [4, 29, 30, 45–47]. Ultimately, the numbers of motor neurons in brainstem nuclei and spinal cord are reduced and there is also a loss of large pyramidal neurons in motor cortex associated with secondary degeneration of the corticospinal tracts [28].

Excitotoxicity (see Chs 15 and 32) has been suggested to be a mechanism by which motor neurons are damaged in ALS [25, 48, 49]. About 60–70% of sporadic ALS patients have a 30–95% reduction in the levels of the astroglial glutamate transporter EAAT2 (excitatory amino acid transporter 2), also termed GLT-1, in motor cortex and spinal cord [25, 48, 49]. Reduction in level of activity of this major glutamate transporter leads to increased extracellular concentrations of glutamate at synapses and evidence of excitotoxicity exists in some patients with ALS.

The mechanisms of cell death are the subject of various research [29, 30, 45–47, 50–53]. Three variants of cell death have been described: apoptosis or programmed...
cell death (PCD); necrosis associated with cytoplasmic swelling and loss of membrane integrity; and autophagy (an intracellular catabolic process that occurs by lysosomal degradation of damaged or expendable organelles). The morphological distinctions between these different forms of cell death are blurred and it has been suggested that the death process may involve a continuum with varying overlapping contributions, particularly of apoptosis and necrosis. In ALS, current evidence indicates that apoptosis plays a role in the degeneration of motor neurons, albeit, perhaps, in a nonclassical form [4, 29, 30, 45-47, 50, 54-56].

Thus, in ALS, motor neuron degeneration evolves over time showing the following: chromatolysis; appearance of inclusions and aggregates; somatodendritic atrophy; and accumulation of vacuoles in mitochondria in dendrites and cell bodies. In late stages, nuclear fragmentation is evident and cytochrome c and cleaved caspase-3 appear in cytoplasm. The increase in caspase-3 activity and the activation of DNA fragmentation factor endonuclease (DFF-45) leads to the appearance of internucleosomal fragmentation of genomic DNA in cytoplasm, an increase in levels of Bak and Bak and a decrease in levels of Bcl-2 in mitochondrial membrane-enriched fractions derived from selectively vulnerable motor regions [29, 30, 45, 50, 55, 57]. Some investigations support the idea of inappropriate re-emergence of a PCD mechanism involving p53 activation and cytosol-to-mitochondria redistributions of cell death proteins. In this scenario, it has been suggested that death of motor neurons in ALS appears to be linked to a p53-driven, intrinsic mitochondrial caspase-3-dependent apoptotic pathway, possibly involving a Bak channel model [30, 57]. In studies of mouse models of ALS [47, 54, 58-63], there is no clear consensus on the type or mechanism of cell death [30] and some of these issues are discussed below.

Some cases of amyotrophic lateral sclerosis are familial. Approximately 10% of cases of ALS are familial and, in the majority of these cases, the disease is inherited as an autosomal dominant [1, 3, 25]. Several of the genes that cause or confer risk in ALS are reviewed below because the information serves as important background for subsequent discussion of genetically engineered models. In ALS-1, mutations in the superoxide dismutase 1 (SOD1) gene occur in ≈5-10% of autosomal dominant cases of ALS. Mutations in dynactin p150glued have been recently linked to autosomal dominant ALS [7] and may, as an allelic variant, serve as a risk factor [64]. In ALS-2, autosomal recessive deletion mutations have been identified in ALS-2, which encodes alsin, a protein that regulates GTPases [65, 66]. In ALS-4, a rare autosomal dominant form of juvenile ALS, mutations have been identified in the gene (SETX) that encodes senataxin [67], which contains a DNA/RNA helicase domain with homology to other proteins known to have roles in the processing of RNA [68]. Finally, following an observation that deletion of the hypoxia response element in the promoter of the vascular endothelial growth factor (VEGF) gene causes degeneration of motor neurons in mice [69], it has been reported that individuals homozygous for certain haplotypes in the VEGF promoter have an increased risk for ALS [15]. Thus, VEGF, a cytokine involved in angiogenesis but with many other functions, may play a role as a susceptibility gene for ALS [70].

Selected aspects of the genetics of MND are reviewed in greater detail below with the belief that understanding of the biology of some of the proteins encoded by these genes and subsequent generation of mouse models are of value in clarifying the molecular mechanisms of selective dysfunction and death of motor neurons in MND, and, eventually, for identifying targets for new treatments.

Several genes have been shown to cause amyotrophic lateral sclerosis.

**Amyotrophic lateral sclerosis 1 is caused by expression of mutant SOD1.** Approximately 15-20% of patients with autosomal dominant FALS (i.e., ≈2% of all ALS cases) have mutations in the gene (chromosome 21) that encodes cytosolic Cu/Zn SOD1, a 153 amino acid enzyme that, as a homodimer, catalyzes the conversion of O₂⁻ to O₂ and H₂O₂ [3, 6]. To date, investigators have identified approximately 100 mutations (see on line at: alsod.org), all of which lead to autosomal dominant FALS [3]; the exception is homozygous D90A SOD1, which is inherited as recessive. These mutations are scattered throughout the structure of SOD1 and are not preferentially localized near the active site or the dimer interface. While some FALS SOD1 mutants show reduced enzymatic activities, many mutant proteins retain activity [9, 71]. It is thought that the mutant enzyme causes selective neuronal degeneration through a gain of toxic property [11, 71], consistent with the dominant pattern of inheritance. Supporting this concept are the following observations: SOD1 T37m mice do not develop disease [72]; the levels of enzyme activity do not correlate with disease [9]; and, in transgenic mice, increasing wild-type SOD1 activity does not ameliorate the disorder [3, 73]. The presence of aggregates containing mutant SOD1 in affected neurons has generated several hypotheses regarding disease pathogenesis including: the sequestration of essential molecules by mutant proteins in aggregates; damaging mutant peptides are mislocated (i.e. to mitochondria) and cause problems at these sites; malformed proteins in aggregates are toxic and impact on molecular motor, axonal transport, proteasomal degradation and glutamate transport; and mutant SOD1 participates in aberrant copper chemistry. Some of the potential mechanisms are discussed below.

**Amyotrophic lateral sclerosis 2 is linked to mutant alsin.** In several families with autosomal recessive juvenile ALS, mutations have been identified in ALS2 (chromosome 2), encoding alsin [65, 66]. This illness, which was originally described in a Tunisian kindred, is characterized by spasticity (involvement of upper motor neurons) and weakness/anatomy (involvement of lower
motor neurons) [65]. The functions of alsin are not well understood, but the protein has several sequence motifs that have homologies to GTPase regulatory proteins important in cell signaling and in protein trafficking. Alsins appear to be a guanine—nucleotide exchange factor (GEF) for at least the RAB5 family of GTPases. The ALS2 mutations are believed to be unstable [74] and it has been hypothesized that loss of functional activities of the GEF domains of alsins could impact on signal transduction pathways, regulation of the cytoskeleton and/or intracellular trafficking [65, 66]. However, at present the mechanisms whereby mutations in this gene cause MND are unknown.

Amyotrophic lateral sclerosis 4 is linked to mutations in a helicase gene. This rare autosomal dominant form of juvenile ALS has been linked to mutations in SETX (chromosome 9), encoding senataxin [67], the mammalian ortholog of a yeast RNA helicase [68]. This disease is manifest by distal weakness (beginning at approximately 25 years of age), slow progression and a normal lifespan [75]. There is some evidence of partial denervation of muscle and some affected individuals have signs of involvement of upper motor neurons [75]. Sensation is normal. At autopsy, the number of spinal motor neurons is reduced and swelling of axons occurs among a variety of populations of neurons [75]. The corticospinal tract shows evidence of degeneration. Sensory neurons exhibit some mild abnormalities. Senataxin has a helicase domain homologous to that of other proteins known to have roles in the processing of RNA [67, 76–78]. Significantly, recessive mutations in SETX, which are believed to result in a truncated protein (loss of function), have also been reported in ataxia–oculomotor apraxia type 2. Interestingly, infantile spinal muscular atrophy with respiratory distress type 1 manifests as weakness and difficulty with respiration at age 1–6 months, results from mutations in another helicase gene, which encoded the immunoglobulin M-binding protein (IGH MBP) on chromosome 11 [79, 80].

Mutant dynactin p150

\[ \text{causes a form of familial amyotrophic lateral sclerosis.} \]

A family with a slowly progressive autosomal dominant lower motor neuron disease (lacking sensory signs) has recently shown linkage to a G59S mutation in the p150

\[ \text{subunit of dynactin (DCTN1)} \] [7], a motor protein that, along with dynein, plays a role in retrograde axonal transport [81–83]. This inherited disease begins in early adult life with vocal cord paralysis (associated with breathing difficulties), facial weakness and atrophy of muscles in the hands [7]. Subsequently, weakness and atrophy appear in the distal lower extremities. The dynactin complex, which includes p150 and dynamin, provides a linker between cargos, microtubules and dynein. The mutation in the index family occurs in a motif in the p150

\[ \text{subunit that binds to microtubules; modeling studies suggest that this mutation impacts on protein structure to create steric hindrance and distortion of the folding of the microtubule binding domain [7]. Consistent with this concept is the observation that the mutant protein binds less well to microtubules [7]. More recently, heterozygous missense mutations have been described in several familial cases of ALS and one apparently sporadic case; these observations have been interpreted to suggest that variants in the p150

\[ \text{subunit can confer risk in ALS [64].}

Taking advantage of the discovery of this fALS-linked mutation in a motor protein, members of our group (Drs Laird, Ackery, Price, Wong) have recently created a mutant p150

\[ \text{model of fALS with a robust MND phenotype.}

\]

\[ \text{NON-TRANSGENIC, INDUCED MODELS OF MOTOR NEURON DISEASE}

\]

\[ \text{ INTERRUPTION OF THE COMMUNICATION BETWEEN THE MOTOR NEURON CELL BODY AND AXON BY TRANSECTION, CRUSH OR AVULSION INDUCES MOTOR NEURON INJURY.}

Peripheral nerve axonal injury. These highly reproducible models of neuronal injury, which interrupt anterograde and retrograde transport in axons, are associated with distal wallerian degeneration and retrograde responses of neurons [5, 84–86]. Following axotomy, the proximal and distal stumps become enlarged, with accumulations of membranous elements [86]; shortly thereafter the distal stump begins to undergo wallerian degeneration [86]. The cell bodies of axotomized neurons may show: chromatolysis [84]; alterations in levels of specific mRNAs [87–89]; changes in the synthesis and transport of specific proteins; aberrant distributions of cytoskeletal proteins (i.e. phosphorylated neurofilaments appear in perikarya) [88–91]; and synaptic disconnection [85]. Moreover, by choosing certain experimental parameters (i.e. age of animal, motor or sensory neurons, proximal or distal locations of lesion, nature of the lesion (crush, transection or avulsion)), investigators can study responses associated with regeneration (distal lesions) [86, 88, 91–93] and degeneration (proximal avulsions) [18–20, 94]. Over the years, the axotomy model has provided important information concerning the influence of axonal injury on the expression of genes encoding cytoskeletal proteins, including NF proteins, peripherin, tubulin, etc. [88, 89, 91, 92, 95]. For example, following unilateral crush of the sciatic nerve, sensory neurons exhibit reduced levels of NF gene expression [88]; the amounts of NF proteins entering axons are decreased and a wave of reduced axonal caliber moves down axons at the rate of transport of NF proteins [91]. Following regeneration, the synthesis of NF proteins returns to normal and axonal caliber is restored [88, 91]. These experiments provided the first insights into the role of NF as a determinant of axon caliber [91, 95]. Following axonal injury,
the expression of other genes may be increased (i.e. the levels of β-tubulin and peripherin mRNAs increase following axotomy [96] and levels of p75 NGF-R immunoreactivity transiently reappear in motor neurons [97]. Levels of neurotransmitter-related proteins, including choline acetyltransferase, often decrease after axotomy of motor neurons; when reinnervation occurs, these transmitter-related markers usually return to normal. During outgrowth of regenerating axons, fast transport delivers membranous constituents required for new growth cones and the axolemma [93, 98]. When regeneration is complete, levels of these mRNA return to control values. Fast transport delivers many of the proteins essential for resuming normal functions at the neuromuscular junction.

Proximal axotomies (including rhizotomies) of facial and spinal motor neurons, particularly in young animals, cause death of neurons [18–20, 44, 94]. These models have proved to be very useful for examining the influences of trophic factors on the survival of axotomized motor neurons [18–20, 44, 94]. For example, brain-derived neurotrophic factor (BDNF), a survival factor for motor neurons, is expressed in the local environment and in muscle targets of motor neurons; expression in muscle is upregulated by denervation [18]. (Neurotrophic factors are discussed in Ch. 27.) Significantly, motor neurons express the gene encoding p145trkA, a receptor involved in BDNF signal transduction [97] and it is believed that BDNF and phosphorylated TrkB are carried by retrograde axonal transport to motor neurons from skeletal muscles [97, 99]. In the facial nerve axotomy model, human recombinant BDNF, placed in proximity to the proximal stump, reduces cell death as compared to the vehicle-treated group [18, 94, 100]. NT-4/5 has a similar effects on axotomized facial motor neurons [20]. Glial derived neurotrophic factor (GDNF), a member of the TGFβ superfamily, binds to specific receptors and is also a potent trophic factor for motor neurons in vitro and in vivo [19, 101]. The administration of neurotrophins may have beneficial effects in other models of MND. If delivery problems and biological toxicities can be overcome, trophic approaches may offer potential for treatment and certain types of MND [102, 103].

β,β’-iminodipropionitrile-induced neurofilamentous axonal pathology. Administration of β,β’-iminodipropionitrile (IDPN) produces a pathology in proximal axons [38, 104] similar to that described in ALS [31]. IDPN selectively impairs slow transport, particularly of the three NF proteins [38]; the transport of tubulin and actin are also somewhat slowed, but fast anterograde and retrograde transport appear to be relatively normal. Exposure to IDPN impairs the transport of NF proteins beyond the proximal internodes and the toxin appears to dissociate NF from microtubules [39, 40]. In this model, a relatively selective defect in the transport of NF leads to formation of massive filament-filled proximal axonal swellings and atrophy of distal axons [104]. Secondary to the axonal abnormalities, changes occur in Schwann cells and myelin sheaths [105, 106]. Studies of several toxic neurofilamentous axonopathies, including those induced by IDPN, acrylamide and aluminum, with their highly reproducible pathology, were among the first to be investigated with radiolabeling methods developed in the 1970s. This research demonstrated, for the first time, the roles of impaired axonal transport in generating cytoskeletal axonal abnormalities resembling those identified in ALS [5, 37–43]. Moreover, other investigations were the first to demonstrate that retrograde axonal transport was also important in the disease (i.e. it provided a pathway by which tetanus toxin enters the nervous system [107]). Years after these studies were published, interest in the roles of axonal transport in normal neuronal functions, in the perturbations seen in experimental models (including axotomy, dying back neuropathies and neurodegenerative diseases) and in experimental therapeutics has undergone an extraordinary renaissance [1, 5, 10, 108–112].

Hereditary canine spinal muscular atrophy. This disease of Brittany spaniels, discovered by Dr Linda Cork, manifests as weakness and atrophy of skeletal muscles, with sparing of eye movements and sphincters [34, 113, 114]. Three HCSMA phenotypes have been identified: pups with accelerated disease, produced by mating affected to affected dogs, are tetraplegic by 3–4 months of age; immediately affected dogs become weak at approximately 6 months of age and are paralyzed at 2–3 years of age; and chronically affected dogs become mildly weak later in life and show very slow rates of progression. Neurofilamentous swellings are abundant in the anterior horns involving proximal axons of motor neurons. Moreover, there are reductions in the size of these cells and, possibly, in the content of transmitter markers [34, 36]. Axonal transport of NF proteins and, to a lesser extent, tubulin are impaired. In ventral roots, axonal diameters are smaller than controls and evidence of axonal degeneration is not conspicuous. The clinical phenotypes, selective involvement of motor neurons and cytoskeletal pathology that occur in HCSMA resembles the abnormalities described in cases of ALS. To date, the genetic basis for the canine disorder has not been defined but it would not be surprising if the mutant gene product impacted on the motor axonal transport.

SELECTED GENETIC MODELS OF RELEVANCE TO AMYOTROPHIC LATERAL SCLEROSIS

Information concerning the functions of some of the genes/proteins implicated in causation of and risk for these illnesses and has been of great value for the generation of mouse models, the study of which has enhanced our understanding of the pathogenesis of ALS and its variants.
Transgenic mice expressing wild type or mutant neurofilament genes develop motor neuron disease and neurofibrillary pathology. Neurofilaments are assembled as obligate heteropolymers from three subunits, including NF-L (68 kDa), NF-M (95 kDa) and NF-H (115 kDa) [115–117]. As described above, NFs are an important determinant of axonal caliber [88, 91, 117, 118]. To determine whether increased NF content or the expression of NF transgene (without or with) mutations can cause disease, investigators have generated a variety of lines of NF mice. Approximately twofold overexpression of wild-type mouse NF-L is not associated with an overt phenotype [119] but greater elevations of NF protein leads to accumulations of NF in cell bodies, and accumulations of NF in distended axons and there is evidence of denervation [120]. Animals die within 3–4 weeks of age. Doubling NF-H content by overexpression of wild-type human NF-H results in a similar pathology; however, the onset of signs is later (4–5 months) and the disease progresses more slowly. In this model, axonal transport is impaired [121].

Significantly, mutations in NF genes can cause MND. Mutant NF-L mice with a single amino acid substitution at a conserved residue develop clinical signs between 3–4 weeks and 3 months, depending on levels of a mutant NF-L subunit [122]. The mice develop clinical signs, NF axonal swellings, evidence of motor neuron dysfunction (including altered axonal transport) and death, denervation, and muscle atrophy [122]. Thus, perturbations in the biology of NF can cause clinical disease that resembles those features occurring in cases of ALS.

Familial-amytrophic-lateral-sclerosis-linked mutant SOD1 mice reproduce many of the clinical and pathological features of amyotrophic lateral sclerosis. SOD1 is normally present in neurons (and other cells) [123]. To attempt to model an inherited form of FALS, investigators have introduced mutant SOD1 into mice. These mice develop progressive weakness and muscle atrophy as well as cellular abnormalities which closely resemble the features of ALS [11, 12, 124]. For example, the G37R SOD1 mice, which accumulate three to 12 times the endogenous levels of SOD1 in the spinal cord, develop an MND phenotype [1, 4]. In some lines of mice, high-molecular-weight complexes of mutant SOD1 accumulate in neural tissues [125, 126]; these complexes are rarely found outside the nervous system other than in skeletal muscle [127], suggesting that factors in neural tissues promote the formation or lead to failure of clearance of these complexes. Motor neurons also exhibit SOD1 inclusions, ubiquitin and phosphorylated NF-H immunoreactivities [12, 128, 129]. Aggregates are present in neurons, in some mutant lines of mice, and in glial cells [11, 12, 73, 126, 129]. SOD1, transported with the slow anterograde component [130], accumulates in irregular, swollen, intraparenchymal portions of motor axons and is often associated with vacuolization of mitochondria and disorganized bundles of filaments. Approximately 2–3 months before the appearance of clinical signs, axonal transport appears to be abnormal [131]. The presence of Wallerian degeneration correlates with development of weakness [11, 12]. In mutant mice, cleaved products caspase 1 and 3 accumulate in spinal cord (early and late, respectively), followed by evidence of cleavage of caspase 9 [58], suggesting caspase activation. However, more recent studies demonstrated that caspase-1 and caspase-3 activation is not crucial for motor neuron degeneration in mutant SOD1 mice [132]. Investigations have defined a motor-neuron-specific, cell-autonomous death pathway mediated by Fas signaling and involving p38 [54]. Interestingly, embryonic murine motor neurons expressing mutant SOD1 display increased susceptibility to the activation of this pathway (but not to deprivation of trophic factors or to excitotoxic manipulations) [54]. Eventually, in mutant SOD1 mice, motor neurons degenerate and the anterior horns show reduced number of neurons as well as evidence of local proliferation of glial cells.

Following the discovery that the SOD1 mutations cause disease independent of levels of dismutase activity [9, 71], several hypotheses were proposed to explain the ways in which mutations lead to abnormalities of motor neurons: mutant SOD1 damages cells by carrying out aberrant copper chemistries; the protein becomes mislocalized, perhaps leading to mitochondrial damage; the mutant protein is improperly folded and shows an increased propensity to aggregate; the aggregates of mutant SOD1 cause toxicity, perhaps because high-molecular-weight complexes sequester molecules critical for viability, deplete chaperones essential for proper protein folding and impair proteasomal degradation processes; the mutant protein, by unknown mechanism alters (reduces) glutamate transport leading to excitotoxicity; and the presence of the mutant enzyme is associated with impaired axonal transport, perhaps by interfering with molecular motors. These hypothetical mechanisms are not mutually exclusive. However, the mechanism whereby the mutant SOD1 gains an adverse property remains to be defined [3, 11] (see below).

Lines of mice with mutant genes encoding motor proteins develop an amyotrophic-lateral-sclerosis-like phenotype. For many years, axonal transport has been suggested to play roles in the pathogenesis of human diseases, including ALS, and in various animal models [5, 38, 41, 107]. As described above, these concepts have been validated in experimental investigations of models of traumatic, toxic and neurodegenerative diseases [37, 38, 41, 107, 110]. In recent studies of mutant SOD1 mice, disruption of transport has been shown to antedate the onset of clinical signs [131]; a similar scenario has long been postulated in ALS [5]. Although the mechanisms leading to impaired transport are not entirely clear, the most obvious consequences of impaired transport are the failure of critical proteins synthesized in the cell body and
destined for the synapses to reach their target(s) at terminals, and/or altered retrograde transport of signals, originating in the periphery, to reach cell bodies [18, 81–83, 99, 103, 133]. Extrapolating from investigations of many models, including those related to mutant motor protein, either or both of these abnormalities could lead to clinical signs and to dysfunction and death of motor neurons.

Compelling evidence in support of roles of impaired transport leading to disease comes from recent studies of the impact of mutations on the properties of motor proteins essential for normal axonal transport. These proteins include: the kinesins and the dyneins, members of two superfamilies of molecular motors, which are responsible for the anterograde and retrograde transport of cargos along microtubules within axons; and dynactin, a large multi-subunit complex, involved in dynein-mediated retrograde transport [81–83, 133]. Selected investigations relevant to these issues are reviewed below.

**Mutant dynactin mice.** The dynein–dynactin complex is a critical component of fast retrograde transport of vesicles and organelles [81–83]. Dynactin is responsible for the minus end movement along microtubules and dynactin has been postulated to enhance the processing and efficiency of the motor with p150<sup>glued</sup> interacting with dynein and tubulin [134]. The overexpression of the dynactin subunit dynamin 2 (dynamin 2; GenBank number NM_006400) disrupts the dynactin complex (presumably by causing dissociation of dynactin at the junction of the p150<sup>glued</sup> and Arp1 filament). The *in vivo* result is a mouse model displaying a late-onset MND phenotype, including weakness, tremoring, abnormal posture and abnormal gait [134]. These mice exhibit denervation and atrophy of muscles and degeneration of motor neurons.

**Mutant dynein mice.** Dynein, comprised of heavy, intermediate, light intermediate and light chains [83], is responsible, in conjunction with dynactin complex, for the minus end movement of cargos [81–83]. A late-onset, progressive MND in mice, identified following an N-ethyl-N-nitrosourea-mediated mutagenesis screen, is linked to mutations in the cytoplasmic dynein heavy chain 1 (Dnchc1; GenBank number NM030238) [135]. Heterozygous missense mutations are associated with impaired transport and degeneration of motor neurons.

**Mutant Tbcem1 mice.** Progressive motor neuropathy (PMN), an autosomal recessive murine disease, manifests as weakness beginning within a few weeks of birth [14, 136]. These mice are homozygous for a Trp 524 Gly substitution of Tbcem (tubulin-specific chaperone E), localized to mouse chromosome 13 [14]. Tbcem mRNA is present in neurons in the spinal cord. Degenerative changes are conspicuous in motor axons, and ultrastructural studies of peripheral nerves of PMN mice disclose reduced numbers of microtubules in these axons. Mutations of the highly conserved Trp524 residue, which appears to influence protein stability, are believed to impact on the biology of tubulin in axons. Transgenic complementation restores the PMN mouse line to a normal phenotype [14]. Tbcem is an ortholog of human TBCE and mutations in the human gene are associated with two multigenic system degeneration syndromes, which are distinct from PMN [14].

A variety of experimental therapeutic strategies have been tested in mutant SOD1 mice. Lines of mutant SOD1 mice have been used for pharmacological and 'genetic' therapeutic trials [10, 21, 22, 25, 137–139]. Selected examples of these approaches are briefly described below.

Results obtained during several drug trials have been less than encouraging (vitamin E, selenium, riluzole, gabapentin and p-penicillamine, acopper chelator). Treatment with creatine appear to have modest benefits [138]. Although oral administration of creatine to G93A SOD1 mice results in a dose-dependent improvement in motor tasks and extended survival, a creatine trial in humans showed no beneficial effects compared to placebo [140]. Minocycline, a second generation tetracycline antimicrobial and anti-inflammatory, which acts as a neuroprotectant by upstream inhibition of the activities of caspase-1, caspase-3, inducible NOS matrix metallocproteinases and p38 mitogen-activated protein kinase (MAPK) and, by reducing cytochrome c release, also had a modest effect (i.e. delayed disease onset and prolonged survival) [23, 63]. Because some evidence suggests that several pathogenic pathways converge in ALS, investigators have also turned to combination treatments. Mutant mice received minocycline (which inhibits microglial activation and the pathways described above), riluzole (a glutamate antagonist used to treat patients with ALS) and nimodipine (a voltage-gated calcium channel blocker), beginning in the late preclinical stage. This dietary regimen delays onset, slows progression of weakness and increases survival [22]. Inhibition of inhibitors of intracellular-1β-converting enzyme (ICE) also slows progress of disease in mutant SOD1 mice; in these studies, a mouse line with a dominant negative mutation of ICE was crossed to mutant SOD1 mice. The outcome of the mating results in a modest increase in survival [63].

The reasons that outcome studies in mutant mice may not necessarily translate to benefits for humans with ALS are discussed in a recent editorial [21].

As indicated in the discussion of anatomy models, the beneficial influences of trophic factors on motor neurons provide potential therapeutic opportunities. Although previous human trials have been disappointing (for review see [111, 112]), adenovector delivery of insulin-like growth factor (IGF)-1 to muscle prolongs survival in mutant SOD1 mice [111]. Thus, following intramuscular injection, the retrograde transport of AAV-IGF-1 appears to provide trophic influences to motor neurons.

To test whether increasing or decreasing SOD1 has an impact on disease in the mutant SOD1 mice, these animals were mated with wild-type SOD1 or SOD1<sup>−/−</sup> mice.
Neither elevations nor reductions of SOD1 influence the clinical course or the character of pathology [73]. The molecular mechanisms whereby mutant SOD1 causes selective motor neuron death have not yet been defined. It has been hypothesized that the toxic property of mutant SOD1 may be related to mutation-induced conformational changes in SOD1 that result in aberrant oxidative activities [141]. In this scenario, cell dysfunction and death could be initiated by aberrant oxidative chemistries catalyzed by the copper bound in the active site of mutant SOD1 [141]. Copper within the active site of SOD1 is essential for dismutase activity [16]. However, copper is extremely toxic and there is virtually no free intracellular copper [142, 143]. Instead, copper is delivered to specific proteins by copper chaperones [142, 143]. The copper chaperone for SOD1, termed CCS in mammals and Lys-7 in yeast (where it was discovered), is present within nerve cells, including neurons and astrocytes [144]. CCS binds to SOD1 [142] and delivers copper to the dismutase, where it is bound by specific histidine residues. To determine the role of CCS in copper homeostasis in the nervous system of mammals, mice were generated with targeted disruption of CCS alleles (CCS-/- mice). Although CCS-/- mice are viable and possess normal levels of SOD1, the levels of SOD1 activity are markedly reduced compared to values of control littermates. Metabolic labeling with copper-64 demonstrates that the reduction of SOD1 activity in CCS-/- mice is the result of impaired copper incorporation into SOD1; this effect is specific because no abnormalities are observed in copper uptake, distributions, or incorporation into other copper enzymes. Consistent with this loss of SOD1 activity, CCS-/- mice exhibit increased sensitivity to paraquat and reduced fertility in females, phenotypes that are characteristic of SOD1-/- mice. Moreover, in CCS-/- mice, as in SOD1-/- mice, motor neurons are more vulnerable to axotomy.

The hypothesis that the toxicity of mutant SOD1 involves aberrant copper chemistry bound to SOD1 has been tested by two approaches: in one set of experiments, mutant SOD1 mice were crossed to CCS-/- mice (thus preventing delivery of copper to SOD1) [16]; in a second set of experiments, mutant SOD1 mice also carried mutations in the histidine residues (H46R/H48Q) that disrupt the copper binding site [126]. The CCS-/- mutant SOD1 mice possess virtually no copper-loaded SOD1 or SOD1 activity [16]. Significantly, these mice exhibit no differences in the onset or progression of disease as compared to mutant SOD1 mice [16]. Similarly, the mutant SOD1 mice with the mutated copper binding sites develop pathological features of disease including (fibrillar inclusions) identical to those occurring in mutant SOD1 mice with the normal histidine residues at these sites [126]. Thus, copper bound within the active site of the enzyme is not essential for mutant SOD1-mediated toxicity. Although proponents of aberrant copper chemistry hypothesis suggest that copper bound elsewhere on SOD1 could be involved in such reactions, the results of these studies are more consistent with the idea that the effects of improper folding/aggregation of mutant SOD1 is a critical feature of disease [126].

To test the role of NF in mutant SOD1 mice, the latter animals were crossed to several lines of mice that have altered distributions of NF. The progeny of mutant SOD1 mice crossed to mice expressing an NF-H-ß-galactosidase fusion protein (NF-H-lacZ) [118], which crosslinks NF and prevents their export to axons, show no effect on disease progression. In contrast, mating with NF-L-ßgal increases the lifespan of mutant SOD1 mice [145]. However, crosses with mice overexpressing either wild type NF-H or wild type NF-L are associated with slowing of disease progression, increased lifespan and relative sparing of motor neurons [139, 146]. It has been suggested that the common properties shared by these various lines of transgenic mice are the reduced content of assembled NF in the axonal compartment and an increase in NF proteins in cell bodies. Given the varied results, it is uncertain as to the ways by which the distributions of NF influence the mutant SOD1 phenotype. Some of the observed effects may be related to strains of mice used in the experiments.

Vascular endothelial growth factor (VEGF) (VEGF, GenBank number NM 003376) influences the growth and permeability of blood vessels during development and during the response to altered metabolic demands [147]. Importantly, VEGF stimulates survival of motor neurons under certain conditions of stress. Mice homozygous for targeted disruption of the hypoxia response element (HRE) within the VEGF promoter (VEGF-/-) develop an adult onset progressive MND with clinical and pathological features reminiscent of ALS [15, 69]. VEGF-/- mice show reduced lower spinal levels of VEGF and are unable to regulate VEGF levels in response to tissue hypoxia. It has been hypothesized that reduced perfusion of neurons results in chronic ischemia and/or the loss of VEGF-mediated neuroprotection, and that the impaired response may lead to the degeneration of nerve cells [75, 148]. An inability to regulate levels of VEGF has been reported in mutant SOD1 mice, and the progeny of crosses of VEGF-/- mice and mutant SOD1 mice do not survive as well as mice with mutant SOD1 alone. Finally, it has been reported that VEGF may act as a modifier gene for cases of human ALS [15].

It is not known why mutations in SOD1, an ubiquitously expressed protein, cause selective dysfunction and death of motor neurons. Mutations in SOD1 do not have obvious impacts on many other cells in CNS or cells in other organs other than possibly skeletal muscle. The question of whether the toxicity of mutant SOD1 is cell autonomous has been tested by selectively expressing mutant SOD1 in different cell types. Neither restricted expression of mutant SOD1 to neurons to astrocytes appears to be sufficient to produce disease [17]. Complex experiments in chimeric mice (with different mixtures of wild-type or mutant SOD1-expressing cells) disclose that motor neuron toxicity can be influenced by expression of wild-type or mutant SOD1 in glial cells. For example,
in the chimeric mice, normal motor neurons develop abnormalities interpreted to be related to the presence of mutant SOD1 in non-neuronal cells. Moreover, when motor neurons accumulate mutant SOD1, the presence of a higher proportion of wild-type SOD1 in non-neuronal cells reduces the incidence of motor neuron death and prolong survival in the chimeric mice. These observations suggest that disease may be the result of different populations of cells acting in concert.

It has been hypothesized that individuals with ALS have problems with excessive glutamate at synapses and excitotoxicity related to reduced levels of the glutamate transporter GLT1 (EAAT2) [25, 48, 49]. Using a screen of more than 1000 FDA-approved compounds, investigators discovered that β-lactam antibiotics can stimulate expression of GLT1, via increased transcription of the GLT1 gene. When animals were treated with the β-lactam ceftriaxone, the levels of GLT1 in the brain increased, as did the biochemical and functional activity parameters of this transporter [24]. Significantly, this antibiotic, which is neuroprotective in vitro for models of ischemia and neuronal degeneration, delayed the pathology and increased survival in mutant SOD1 mice [24].

Mouse models offer opportunities for discovering disease mechanisms and for testing novel treatment strategies. Riluzole is the major drug approved for therapy in human ALS [3, 25]. However, the benefits are modest. Investigators are examining other ways to ameliorate excitotoxicity, possibly by influencing the levels or activity of GLT-1 [24]. Trials with growth factors were truncated because of evidence of complications [25]. Trials of antiaggregation strategies, novel ways to deliver trophic factors, inhibitors of apoptosis, treatment with gene therapy and the use of engineered cells or stem cells are some of the therapeutic approaches for the future [1, 3, 17, 18, 21, 25, 30, 57, 63, 70, 111, 126, 147, 149, 150].

The identification of genes mutated or deleted in the inherited forms of neurodegenerative diseases, including MND, has allowed investigators to create genetically engineered models of these illnesses [1, 3, 17, 18, 21, 30, 57, 63, 70, 111, 126, 147, 149, 150]. Investigations of these mouse models have greatly increased our understanding of the molecular mechanisms of neurodegenerative diseases critical for identifying potential therapeutic targets. These models are of great value for testing a variety of novel therapeutic approaches. Investigations of the pathogenesis of the neurodegenerative diseases have made spectacular progress over the past few years. We anticipate that knowledge of disease mechanisms will lead to novel treatments for these devastating illnesses.

ACKNOWLEDGMENTS

The authors wish to thank the many colleagues who have worked at JHMI, Drs. David Borchelt, Jack Griffin, Paul Hoffman, Linda Cork, Fiona Laird, Don Cleveland, Michael Lee and Jeffery Rothstein, as well as those at other institutions for their contributions to some of the original work cited in this review and for their helpful discussions. Aspects of this work were supported by grants from the U.S. Public Health Service (5R01NS 1058017, RO1 NS40014, RO1 NS4100) as well as the ALS Association and the Packard Center for ALS.

REFERENCES


27. Le, T. T., Pham, L. T., Butchbach, M. E. et al. SMN2, the major product of the centromeric survival motor neuron (SMN2) gene, extends survival in mice with spinal muscular atrophy and associates with full-length SMN. Hum. Mol. Genet. 14: 845–857, 2005.


