CHAPTER

45

Motor Neuron Diseases

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OUTLINE

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

Motor neuron diseases affect about 5 out of 100,000 worldwide. They may affect both upper and lower motor neurons (ALS), upper motor neurons only (hereditary spastic paraplegias) or lower motor neurons only (spinal motor atrophy, SMA). Approximately 90% of MND are sporadic and they can range in severity from rapidly progressing fatal diseases to slowly progressing chronic diseases. The identification of causative mutations in specific genes in cases of human MND, including familial ALS (fALS) and SMA (Puls et al., 2003; Anderson & Talbot, 2003) has provided new opportunities, using transgenic and gene targeting approaches, to investigate the molecular participants in disease processes. In autosomal dominant fALS, the mutant proteins often acquire toxic properties that directly or indirectly affect the functions and viability of neurons (Bruijn et al., 2004), and introduction of mutant genes into mice reproduces some features of these diseases. In contrast, autosomal recessive diseases, like SMA, which usually lack the functional protein encoded by the mutant gene (*Survival Motor Neuron* or *SMN* in SMA), can often be modeled by gene targeting strategies. Study of some types of MND in animals, including progressive motor neuronopathy, have lead to discovery of mutant genes/proteins,

whose roles in neurons illuminate potential disease mechanisms in humans (Martin et al., 2002b).

In models of MND, manipulation of expression of selected genes in specific cell populations (Lambrechts et al., 2003a), creation of chimeric animals to test whether abnormalities are cell autonomous (Clement et al., 2003), administration of trophic factors to prevent cell death (Koliatsos et al., 1994), and testing of a variety of drug therapies have been used to try to ameliorate phenotypes and thus provide insights into disease mechanisms and potential treatment strategies. 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 reviews (Anderson & Talbot, 2003; Price et al., 2000; Le et al., 2005).

The disease is characterized clinically by weakness, muscle atrophy and spasticity affecting both upper and lower motor neurons

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 23 per 100,000 people (Bruijn et al., 2004;). 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 is 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. 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 die usually within two to five 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, which affect particularly the spinal and corticospinal motor neurons, appear to evolve through a series of stages influencing size, shape, content, metabolism and physiology of these cells. Years ago, it was observed that proximal axonal segments were swollen with maloriented arrays of neurofilaments (NF) (Carpenter, 1968; Delisle et al., 1984). Similar abnormalities were documented in experimental models (Clark et al., 1984). The investigations described below tested the hypothesis that this type of pathology was the result of defects in axonal transport. Moreover, it was hypothesized that impairments in transport could also be associated with a "dying-back" phenomenon (i.e., degeneration proceeding retrograde from distal nerve terminals to proximal cell body), which was hypothesized to be related to impaired delivery of essential constituents to the most distant segments of axons and the axon terminals (Griffin & Price, 1976). 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 dissociation of neurons from their targets, retrogradely transported trophic support to neuronal cell bodies is compromised, which, in turn, affects the viability of these cells (Kolatsios et al., 1994). 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 (Ince, 2000). Later, neurons may become atrophic. There is Wallerian degeneration of motor axons. In the final stages, motor neurons exhibit several features of apoptosis, which are discussed below (Martin et al., 2005) (see also Apoptosis in Ch. 37). Ultimately, the numbers of motor neurons in brainstem nuclei and spinal cord are reduced and there is a loss of large pyramidal neurons in motor cortex associated with secondary degeneration of the corticospinal tracts (Ince, 2000).

Excitotoxicity has been suggested to be a mechanism by which motor neurons are damaged in ALS. 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 (Rothstein et al., 1995). Reductions in levels of activity of this major glutamate transporter lead to increased extracellular concentrations of glutamate at synapses and evidence of excitotoxicity exists in some patients with ALS (see Excitotoxicity in Ch. 17).

The mechanisms of cell death, including apoptosis and necrosis, are the subjects of very active research discussed in Ch. 37. In ALS, current evidence indicates that apoptosis plays a role in the degeneration of motor neurons, albeit perhaps in a non-classical form.

Thus, in ALS, motor neuron degeneration evolves over time, showing the following: chromatolysis, appearance of inclusions and aggregates, somatodendritic atrophy, and accumulations of vacuoles in mitochondria in dendrites and cell bodies. In late stages, DNA fragmentation is evident and cytochrome C and cleaved caspase-3 appear in cytoplasm. The increase in caspase-3 activity and the activation of endonuclease leads to the appearance of internucleosomal fragmentation of genomic DNA in cytoplasm, an increase in levels of Bax and Bak, and a decrease in levels of Bcl-2 in mitochondrial membrane-enriched fractions derived from selectively vulnerable motor regions (Martin et al., 2005). Some investigations support the idea of an inappropriate re-emergence of a programmed cell death 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 is linked to a p53-driven, intrinsic mitochondrial caspase-3-dependent apoptotic pathway, possibly involving a Bax channel model. In studies of mouse models of ALS there is not a clear consensus on the type or mechanism of cell death. (See references in Martin & Liu, 2004, 2005.)

Although most cases ALS are sporadic, mutations in several genes may cause familial ALS

Approximately 10% of patients with ALS are familial and, in the majority of these cases, the disease is inherited as an autosomal dominant (see Bruijn et al., 2004 for references). 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 ALS1, mutations in the Cu/Zn superoxide dismutase (SOD1) gene occur in ~5–10% of autosomal dominant cases of fALS. Mutations in Dynactin p150glued have been linked to autosomal dominant MND (Puls et al., 2003) and may, as an allelic variant, serve as a risk factor in ALS (Munch et al., 2004). In ALS2, autosomal recessive deletion mutations have been identified in the ALS2 gene that encodes Alsin, a protein that regulates GTPases (Hadano et al., 2001; Yang et al., 2001). In ALS4, a rare autosomal dominant form of juvenile ALS, mutations have been identified in the gene (SETX) that encodes senataxin (Chen et al., 2004), which contains a DNA/RNA helicase domain with homology to other proteins known to have roles in the processing of RNA (Moreira et al., 2004). 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 (Oosthuyse et al., 2001a), it has been reported that individuals homozygous for certain haplotypes in the VEGF promoter have an increased risk for ALS (Lambrechts et al., 2003a). Thus, VEGF, a cytokine involved in angiogenesis but with many other functions, may play a role as a susceptibility gene for ALS (Cleveland, 2003).

ALS1 is caused by 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 superoxide dismutase type 1 (SOD1), a 153–amino acid enzyme that, as a homodimer, catalyzes the conversion of $\cdot O^-_2$ to O_2 and H_2O_2. To date, investigators have identified approximately 100 mutations (http://alsod.org), all of which lead to autosomal dominant fALS (Bruijn et al., 2004); the exception is homozygous *D90A SOD1*, which is inherited recessively.

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 (Borchelt et al., 1994; Bowling et al., 1995). It is thought that the mutant enzyme causes selective neuronal degeneration through gain of a toxic property (Wong et al., 1995; Bowling et al., 1995), consistent with the dominant pattern of inheritance. Supporting this concept are the following observations: SOD1-/- mice do not develop motor neuron disease (Reaume et al., 1996); the levels of enzyme activity do not correlate with disease (Borchelt et al., 1994); and, in transgenic mice, increasing wild-type SOD1 activity does not ameliorate the disorder (Bruijn et al., 2004). The presence of aggregates containing mutant SOD1 in affected neurons has generated several hypotheses regarding disease pathogenesis including that essential molecules are sequestrated by mutant proteins in aggregates; that damaging mutant peptides are mislocated (i.e., to mitochondria) and cause problems at these sites; malfolded proteins in aggregates are toxic and affect molecular motors, axonal transport, proteosomal degradation and glutamate transport; and mutant SOD1 participates in aberrant copper chemistry. Some of the potential mechanisms are discussed later.

ALS2 is linked to mutant Alsin

In several families with autosomal recessive juvenile ALS, mutations have been identified in *ALS2* (chromosome 2), encoding Alsin. This illness, which was originally described in a Tunisian kindred, is characterized by spasticity (involvement of upper motor neurons) and weakness/amyotrophy (involvement of lower motor neurons) (Hadano et al., 2001). 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 (see Ch. 21). Alsin appears to be a guanine–nucleotide exchange factor (GEF) for at least the RAB5 family of GTPases. The *ALS2* mutations are believed to be unstable (Yamanaka et al., 2003), and it has been hypothesized that loss of functional activities of the GEF domains of Alsin could impact on signal transduction pathways, regulation of the cytoskeleton and/or intracellular trafficking (Hadano et al., 2001; Yang et al., 2001). However, at present the mechanisms whereby mutations in this gene cause MND are unknown.

ALS4 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 (Chen et al., 2004), the mammalian ortholog of a yeast RNA helicase (Moreira et al., 2004). This disease is manifested by distal weakness beginning at approximately 25 years of age, slow progression and a normal lifespan. There is some evidence of partial denervation of muscle and some affected individuals have signs of involvement of upper motor neurons. Sensation is normal. At autopsy, the number of spinal motor neurons is found reduced and swelling of axons is noted among a variety of populations of neurons (Rabin et al., 1999). 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 (Chen et al., 2004). 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 (AOA2). Interestingly, infantile spinal muscular atrophy with respiratory distress type 1, manifested as weakness and difficulty with respiration at age 1–6 months, results from mutations in another helicase gene, which encodes the immunoglobulin M-binding protein (IGH MBP2) on chromosome 11 (Grohmann et al., 2003).

Angiogenic factors may be linked to ALS

Mutations in the *angiogenin* (*ANG*) gene have been linked to both fALS and sALS (Greenway et al., 2006). ANG, a member of the ribonuclease A (RNase A) superfamily, is a potent

inducer of angiogenesis (Fett et al., 1985). Expressed in motor neurons, ANG can be upregulated by hypoxia and is thought to stimulate rRNA transcription of endothelial cells (Moroianu et al., 1994), is critical for cellular proliferation induced by other angiogenic proteins, including vascular endothelial growth factor (VEGF) (Kishimoto, et al, 2005). The discovery of an angiogenesis gene linked to ALS is important because previous work surprisingly showed that mice harboring a deletion of the hypoxic response element within the VEGF promoter exhibit a motor neuron disease-like behavior (Oosthuyse et al., 2001b). Subsequently, haplotypes in the VEGF promoter that influence VEGF transcription has been identified at higher rates in some cohorts with ALS (Lambrechts et al., 2003). Moreover, lentiviral delivery of VEGF to motor neurons (Azzouz et al., 2004) or intracerebroventricular delivery of VEGF (Storkebaum et al., 2005) provides protection in rodent models of ALS. Taken together, these findings further strengthen the view that alterations in hypoxia-inducible genes play an important role in ALS and have encouraged investigators to develop VEGF-based therapies for ALS and to assess the potential role of angiogenic factors as risk factors for this illness.

Mutant dynactin p150^{Glued} causes fALS

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 p*150^{glued}* subunit of dynactin (Munch et al., 2004; Puls et al, 2003), a motor protein which, along with dynein, plays a role in retrograde axonal transport (Ch. 8). 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 (Puls et al., 2003). Subsequently, weakness and atrophy appear in the distal lower extremities. The dynactin complex, which includes p150 and dynamitin, provides a linker between cargos, microtubules and dynein. The mutation in the index family occurs in a motif in the p150^{glued} subunit that binds to microtubules; modeling studies suggest that this mutation affects protein structure to create steric hindrance and distortion of the folding of the microtubule binding domain. Consistent with this concept is the observation that the mutant protein binds less well to microtubules (Puls et al., 2003). 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 subunit can confer risk in ALS (Munch et al., 2004).

Taking advantage of the discovery of this fALS-linked mutation in a motor protein, several groups have created mice expressing either wild-type or mutant (G59S) human dynactin p*150^{glued}* (Lai et al., 2007; Laird et al., 2008) as well as mice with targeted deletion of *Dctn1* encoding dynactin p150 (Lai et al., 2007) in efforts to determine the mechanism whereby mutant p150 causes MND. Interestingly, neuronal expression of mutant, but not of wild-type, dynactin p150^{Glued} is sufficient to elicit clinical and pathological evidence of motor neuron disease in mice. In addition to loss and degeneration of motor neurons, these mutant mice exhibited alterations in vesicular transport in cell bodies of motor neurons, axonal swelling and axo-terminal degeneration (Laird et al., 2008). There was evidence of autophagic cell death associated with this mouse model. The fact that genetic deletions of autophagy genes in neurons are sufficient to cause neurodegeneration would support the idea that defects in basal autophagy may contribute to the pathogenesis of motor neuron disease (Hara et al., 2006; Komatsu et al., 2006).

Studies in cell culture suggested that mutant dynactin-linked MND can be explained by a loss- or gain-of-function mechanism (Levy & Holzbaur, 2006; Levy et al., 2006). Although haplo-insufficiency could explain disease arising from G59S mutants, the observation that mice with heterozygous deletion of *Dctn1* were normal (Lai et al., 2007) would not be consistent with this view. Studies of the G59S knock-in mice revealed that this mutation destabilized the dynactin subunit and altered the normal physiology of the dynein/dynactin complex necessary for early embryonic development (Lai et al., 2007). While the heterozygous knockout mice were normal, the heterozygous G59S knock-in mice exhibited a mild motor phenotype (Lai et al., 2007), results that are consistent with the notion that mutant dynactin-associated MND arises through a dominant-negative mechanism. Altering the dose of wild-type p150^{Glued} alleles in *G59S dynactin* mice should clarify this issue in future studies.

VAPB associated with ALS is a ligand for eph receptors

A missense mutation (P56S) in the ALS8 gene encodes a highly conserved ~30kDa protein with an amino-terminal major sperm protein (MSP) domain termed vesicle-associated membrane protein-binding protein B (VAPB) has been associated with ALS and late-onset spinal muscular atrophy in several large Brazilian families (Nishimura et al., 2004). VAP proteins (VAPA and VAPB) are type II integral membrane proteins targeted to the cytosolic surface of endoplasmic reticulum (ER). Cell culture studies showed that ALS-associated VAPB mutant caused protein aggregation and recruits wild-type VAPs into these aggregates in the ER (Teuling et al., 2007). Studies in drosophila revealed that the MSP domains of VAP proteins are released from membranes and serve as secreted ligands for Eph receptors. However, VAP mutant failed to secrete the MSP domain and accumulated as inclusions in the ER, leading to an unfolded protein response, findings that are interpreted to indicate that mutant VAPB may cause disease through a cell-autonomous pathway in the ER as well as a non–cell-autonomous Eph receptor signaling mechanism (Tsuda et al., 2008).

ALS is linked to two genes involved in RNA metabolism: TDP-43 and FUS

The recent discovery of two new ALS-associated genes, *TAR DNA-binding* protein *(TDP-43)* (Kabashi et al., 2008; Sreedharan et al., 2008; and *fused in sarcoma (FUS)* (Kwiatkowski, Jr. et al., 2009; Vance et al., 2009), that encode RNA-binding proteins raised the intriguing possibility that alterations in RNA metabolism could play a critical role in the pathogenesis of ALS. Missense mutations in *TDP-43* and *FUS* have been identified, respectively, in ~3% and ~4% of fALS. Significantly, *TARDBP* mutations have also been identified in ~1.5% of sALS cases. Most identified mutations in *TARDBP* and *FUS* to date are clustered within the highly conserved C-terminal regions encoded by these two genes. The important role of TDP-43 in

the central nervous system was initially shown in pathological analysis of neurodegenerative diseases as a component of ubiquitinated protein aggregates in cases of amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration with ubiquitinated inclusions (FTLD-U) (Neumann et al., 2006). For both diseases, TDP-43 is depleted from the nuclei, but accumulated in the ubiquitinated inclusions of affected neurons, suggesting that loss of normal function of TDP-43 as a nuclear protein or, alternatively, gain of a toxic function by TDP-43 aggregates play significant roles in the pathogenesis of ALS and FTLD-U. However, the exact mechanisms by which mutant TDP-43 or FUS contributes to ALS remain elusive. See (Lagier-Tourenne & Cleveland, 2009) for references and review.

To address mechanisms of motor neuron degeneration in mutant TDP-43- associated ALS, investigators have developed various model systems. For examples, overexpression of human TDP-43 induced formation of TDP-43 inclusions that were toxic to yeasts (Johnson et al., 2008). Pan-neuronal overexpression of human TDP-43 in *c. elegans* resulted in uncoordinated movement and aberrant synapses in motor neurons (Ash et al., 2010). Overexpression of human TDP-43 in motor neurons of *Drosophila* also resulted in abnormal neuronal morphology, function and reduction in number of neurons (Li et al., 2010). Overexpression of human wild-type (Wils et al., 2010) or ALS-linked mutant (A315T or M337V) TDP-43 (Wegorzewska et al., 2009; Zhou et al., 2010) in rodents resulted in abnormal motor function and a shorter life span. However, the ubiquitin-positive cytoplasmic inclusions, characteristic of human ALS pathology, were infrequently found in affected motor neurons. Because overexpression of wild-type TDP-43 is associated with significant toxicity in a variety of model systems, it remains unresolved as to how ALS-associated mutant TDP-43 contributes to pathogenesis of motor neuron disease.

Toward the goal of understanding the physiological and cellular functions of TDP-43, investigators utilized knockout or knockdown strategies. Constitutive TDP-43 knockout mice established that TDP-43 is essential for early embryogenesis (Kraemer et al., 2010; Sephton et al., 2010; Wu et al., 2010). RNAi knockdown studies of TDP-43 revealed histone deacetylase 6 as a target of TDP-43 (Fiesel et al., 2010). Our group has taken both "gain-of-function" and "loss-of-function" approaches in mice to determine the physiological role of TDP-43 in the adult animal. A conditional TDP-43 knockout mouse model in which *TDP-43* can be deleted after development exhibited a strikingly lean phenotype, indicating that this RNA-binding protein is critical for fat storage in the adipocytes (Chiang et al., 2010). Furthermore, a cellular TDP-43 knockout model with high-throughput RNA sequencing identified a set of downstream genes regulated by TDP-43 (Mortazavi, et al., 2008). The TDP-43–dependent transcriptome revealed a gene, Tbc1d1, that is highly expressed in skeletal muscle, significant for regulating leanness and linked to human obesity (Chiang et al., 2010). This result suggests that the reduction of Tbc1d1 in skeletal muscle is responsible for the increased fat metabolism and leanness observed in our conditional TDP-43 knockout mice. The observation of hypermetabolism in cases of ALS further suggests that hypermetabolism may confer increased risk for ALS (Dupuis et al., 2004).

Using methodology with high-resolution digital DNA sequencing, investigators mapped the interactions of TDP-43 binding sites with RNA in the mouse brain. Such analysis of the TDP-43 dependent transcriptome of the striatum revealed that mRNAs most dramatically decreased by the reduction of TDP-43 were those harboring very long introns (Polymenidou et al., 2011; Tollervey, 2011). In mice either lacking endogenous TDP-43 or expressing human TDP-43 in motor neurons, it was found that TDP-43 is important for regulating the proper distributions of mitochondria in the cytoplasm and of FUS and SMN-associated Gemini of coiled bodies (GEMs) in the nuclei of motor neurons (Shan et al., 2010).

The majority of FUS mutations associated with motor neuron disease cluster within the extreme C-terminal region, which is highly enriched in arginine. Mutations in all five arginine residues have been reported in fALS cases (Lagier-Tourenne & Cleveland, 2009). It is suggested that this region represents a non-classical PY-type nuclear localization signal (PY-NLS) and that mutations in this region disrupt proper nuclear import of FUS, resulting in redistribution of FUS into stress granules in the cytoplasm. The degrees of defective import into nuclei of each mutation in the C-terminus are inversely correlated with the age of onset in the familial cases, implying either that the loss of FUS in the nuclei or its accumulation in the cytoplasm contributes to disease pathogenesis (Dormann et al., 2010). FUS mutants co-accumulate with TDP-43 in the stress granules and in the nuclear bodies formed by overexpressing TDP-43 in motor neurons (Shan et al., 2010). These observations suggest that FUS and TDP-43 participate in a common biochemical pathway essential for the survival of motor neurons.

That a large number of cases of sporadic ALS are associated with cytoplasmic inclusions containing aggregates of ubiquitinated TDP-43 suggests that potential genetic modifiers of TDP-43 may affect the risk for ALS. Using the yeast model as a screening platform, investigators identified Pbp1, the yeast ortholog of ataxin 2 (ATXN2), as a genetic modifier of TDP-43 toxicity. ATXN2, a polyglutamine (polyQ) protein mutated in spinocerebellar ataxia type 2 (see in Ch. 48) is mislocalized in spinal motor neurons of cases of ALS whereas TDP-43 is aberrantly localized in spinocerebellar ataxia type 2. These investigators found that the intermediate-polyQ length of 27–33 glutamines in *ATXN2* was associated with ALS, supporting the view that *ATXN2* is a genetic modifier that increases risk for ALS (Elden et al., 2010).

Mutations in OPTN were identified in several japanese patients with ALS

Mutations in the *OPTN* (optineurin) gene have been discovered in eight Japanese patients with ALS (Maruyama et al., 2010). Optineurin is a ubiquitously expressed cytoplasmic protein that participates in a number of signaling pathways, including nuclear factor kappa B (NF-kB) signaling and vesicular trafficking (Sahlender et al., 2005). Cell culture studies revealed that the nonsense and missense mutations of *OPTN* identified in ALS patients compromised NF-kB signaling whereas the E478G mutant optineurin altered its cytoplasmic distribution. Optineurin immunoreactive cytoplasmic inclusions can be seen in motor neurons of an individual carrying the E478G mutation as well as in some TDP-43 or SOD1 positive inclusions in ALS (Maruyama et al., 2010). Although optineurin inclusions are found only in a minority of TDP-43

linked ALS and are infrequently observed in other neurodegenerative diseases (Hortobagyi et al., 2011), the findings mentioned above implicate the involvement of optineurin in the pathogenesis of ALS.

Identification of valosin-containing protein (VCP) is linked to fALS by exome sequencing

Using an emerging technology termed *exome sequencing*, an approach that exploits the massive parallel sequencing capability to analyze rapidly at high resolution the small percentage (~1%) of the human genome that encodes proteins for rare variants, researchers have identified missense mutations in the valosin-containing protein (VCP) gene as a cause of fALS (Johnson et al., 2010). VCP mutations may be responsible for 1–2% of fALS. In addition, alterations in VCP have been linked to a genetic syndrome of inclusion body myopathy, Paget disease of bone and/or frontotemporal dementia (Custer et al., 2010). VCP, a member of the highly conserved family of ATPases associated with diverse cellular activities (AAA+), is thought to be critical for maturation of autophagosomes. Mutant VCP can cause mislocalization of TDP-43 protein as cytoplasmic aggregates in spinal motor neurons (Custer et al., 2010). These findings further support the notion that alterations in the ubiquitin, proteasome and autophagy degradation systems may contribute to the pathogenesis of ALS.

MODELS OF MOTOR NEURON DISEASE INDUCED BY EXPERIMENTAL NERVE INJURY HAVE BEEN INSTRUCTIVE

Interrupting the communication between the motor neuron cell body and axon by transection, crush or avulsion induces motor neuron 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. Following axotomy, the proximal and distal stumps become enlarged with accumulations of membranous elements; shortly thereafter the distal stump begins to undergo Wallerian degeneration (Griffin et al., 1977). The cell bodies of axotomized neurons may show chromatolysis and synaptic disconnection (Price & Porter, 1972); alterations in levels of specific mRNAs, changes in the synthesis and transport of specific proteins, and aberrant distributions of cytoskeletal proteins (i.e., phosphorylated neurofilaments appear in perikarya) (Muma et al., 1990). 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 and degeneration (Koliatsos et al., 1994). During outgrowth of regenerating axons, fast transport delivers membranous constituents required for new growth cones and the axolemma. 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 and others (Muma et al., 1990; Hoffman et al., 1987).

Proximal axotomies (including rhizotomies) of facial and spinal motor neurons, particularly in young animals, causes death of neurons. These models have proved to be very useful for examining the influences of trophic factors on the survival of axotomized motor neurons (Koliatsos et al., 1994). For example, brain-derived neurotrophic factor (BDNF), a survival factor for motor neurons (Ch. 29), is expressed in the local environment and in muscle targets of motor neurons; expression in muscle is upregulated by denervation. Significantly, motor neurons express the gene encoding $p145^{trkB}$, a receptor involved in BDNF signal transduction, and it is believed that BDNF and phosphorylated TrkB are carried by retrograde axonal transport to motor neurons from skeletal muscles. 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. The administration of neurotrophins may have beneficial effects in models of MND. If delivery problems and biological toxicities can be overcome, trophic approaches may offer potential for treatment in certain types of MND. See (Yuen et al., 1996) for review and references.

IDPN induces neurofilamentous axonal pathology

Administration of IDPN (β,β'-Iminodipropionitrile} produces pathology in proximal axons similar to that described in ALS. IDPN selectively impairs slow transport, particularly of the three NF proteins, and the transport of tubulin and actin, 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 (Griffin et al., 1983b). 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. Secondary to the axonal abnormalities, changes occur in Schwann cells and myelin sheaths (Griffin et al., 1983b). 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. Moreover, other investigations demonstrated that retrograde axonal transport was also important in the disease (Price et al., 1975). See Chapter 8 for further discussion of axonal transport and neuronal function. (Morfini et al., 2009).

SELECTED GENETIC MODELS OF RELEVANCE TO ALS AND OTHER MOTOR NEURON DISEASES HAVE BEEN IDENTIFIED OR GENERATED

Hereditary canine spinal muscular atrophy (HCSMA) is a naturally occurring mutation that produces motor neuron disease

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 (Sack, Jr., et al., 1984). 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; intermediately 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. Axonal transport of NF proteins, and to a lesser extent, microtubules 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 resemble the abnormalities described in cases of ALS. To date, the genetic basis for the canine disorder has not been defined.

Some transgenic mice expressing wild-type or mutant *NF* genes develop motor neuron disease and neurofibrillary pathology

Neurofilaments (NF) are assembled as obligate heteropolymers of three subunits, including NF-L (68 kD), NF-M (95 kD), and NF-H (115 kD). NFs are an important determinant of axonal caliber (see Ch. 6). 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 two-fold overexpression of wild-type (wt) mouse *NF-L* is not associated with an overt phenotype (Monteiro, et al., 1990), but greater elevations of NF protein leads to accumulations of NF in cell bodies, accumulations of NF in distended axons and evidence for denervation. Animals die within 3–4 weeks of birth. Doubling NF-H content by overexpression of wt 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 (Collard et al., 1995).

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 or 4 weeks and 3 months, depending on levels of a mutant NF-L subunit (Lee, et al., 1994b). The mice develop clinical signs, NF axonal swellings, evidence for motor neuron dysfunction (including altered axonal transport), death, denervation, and muscle atrophy. Thus, perturbations in the biology of NF can cause clinical disease that resembles those features occurring in patients with ALS.

fALS-linked mutant *SOD1* mice reproduce many of the clinical and pathological features of ALS

Transgenic mice harboring mutant human SOD1 develop progressive weakness and muscle atrophy, as well as cellular abnormalities that closely resemble the features of ALS (Bruijn, et al., 1997b and earlier citations therein). For example, the *G37R SOD1* mice, which accumulate up to 3–12 times the normal levels of SOD1 in the spinal cord, develop an MND phenotype (Wong et al., 2002). In some lines of mice, high-molecular-weight complexes of mutant SOD1 accumulate in neural tissues (Wang et al., 2002a & 2002b). Such complexes as these are rarely found outside the nervous system other than in skeletal muscle (Turner, et al., 2003), suggesting that factors in neural tissues either promote the formation or fail to clear these complexes. Motor neurons also exhibit SOD1 inclusions with ubiquitin and phosphorylated NF-H immunoreactivities (Bruijn et al., 1997b; Watanabe et al., 2001). Aggregates are present in neurons, in some mutant lines of mice, and in glial cells (Wang et al., 2002a; Watanabe et al., 2001; Bruijn et al., 1997b; Wong et al., 1995). SOD1, transported with the slow anterograde component (Borchelt et al., 1998) 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 (Williamson & Cleveland, 1999). The presence of Wallerian degeneration correlates with development of weakness (Bruijn et al., 1997b). In mutant mice, cleavage products of caspase-1 and -3 accumulate in spinal cord (early and late, respectively), followed by evidence of cleavage of caspase-9 (Li et al., 2000). However, caspase-1 and caspase-3 activation is not crucial for motor neuron degeneration in mutant SOD1 mice (Kang, et al., 2003). Investigations have defined a motor neuron–specific, cell-autonomous death pathway mediated by Fas signaling and involving p38 (Raoul et al., 2002) (see chapter 37). Interestingly, embryonic murine motor neurons expressing mutant *SOD1* display increased susceptibility to activation of this pathway (but not to deprivation of trophic factors or to excitotoxic manipulations) (Raoul et al., 2002). Eventually, in mutant *SOD1* mice, motor neurons degenerate, and ventral horns show reduced numbers 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 (Borchelt et al., 1994; Bowling et al., 1995), a large number of hypotheses were proposed to explain the ways in which mutations could lead to abnormalities of motor neurons despite normal levels of dismutase activity. However, the mechanism whereby the mutant SOD1 gains an adverse property remains to be proven (Bruijn et al., 2004). See discussion later and in Box 45 for approaches toward elucidating the possible roles of mutant SOD1 in ALS pathogenesis.

Lines of mice harboring other mutant genes may also develop an ALS-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. 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 that are responsible for the anterograde and retrograde transport of cargos along microtubules within axons, as well as dynactin, a large multi-subunit complex involved in dynein-mediated retrograde transport (see Chs. 6–8). Selected investigations relevant to these issues are reviewed below.

Mutant dynactin p150glued transgenic mice have MND-like pathology

The dynein–dynactin complex is a critical component of fast retrograde transport of vesicles and organelles. Dynein is responsible for the minus-end movement along microtubules, and dynactin has been postulated to enhance the processing and efficiency of the motor with $p150^{glued}$ interacting with dynein and tubulin. The overexpression of the dynactin subunit dynamitin disrupts the dynactin complex (presumably by causing dissociation of dynactin at the junction of the $p150^{glued}$ and Arp1 filament). The *in vivo* result is a mouse model displaying a late-onset MND phenotype, including weakness, trembling, abnormal posture and abnormal gait (LaMonte et al., 2002). These mice exhibit denervation and atrophy of muscles and degeneration of motor neurons.

Mutant tubulin-specific chaperone E transgenic mice exhibit progressive motor neuropathy

Progressive motor neuropathy (*PMN*), an autosomal recessive murine disease, manifests as weakness, beginning within several weeks after birth. These mice are homozygous for a Trp 524 gly substitution of *Tbce*, localized to mouse chromosome 13. Tbce 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 Trp 524 residue, which appear to influence protein stability, are believed to affect the biology of tubulin in axons. Transgenic complementation restores the *PMN* mouse line to a normal phenotype. Tbce is an ortholog of human *TBCE*, and mutations in the human gene are associated with two multiorgan system degeneration syndromes, which are distinct from PMN (Martin et al., 2002b).

To test the role of NF in mutant SOD1 mice, the latter animals were crossbred to several lines of mice that have altered distributions of NF

The progeny of mutant *SOD1* mice crossed to mice expressing an NF-H-β-galactosidase fusion protein (NF-H-lacZ) (Eyer & Peterson, 1994), which crosslinks NF and prevents its export to axons, show no effect on disease progression. In contrast, mating with *NF-L-/-* mice increases the lifespan of mutant *SOD1* mice (Williamson et al., 1998). However, crosses with mice overexpressing either wild-type *NF-H* or wild-type *NF-L* are associated with slowing of disease progression, increased life span and relative sparing of motor neurons (Kong & Xu, 2000). Given the varied results, it is uncertain as to how 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) influences the growth and permeability of blood vessels

This influence is seen during development and during the response to altered metabolic demands (Carmeliet et al., 2002). VEGF also stimulates survival of motor neurons under certain conditions of stress. Mice homozygous for a targeted disruption of the hypoxia response element (HRE) within the VEGF promoter ($VEGF^{\partial/\partial}$) develop an adult onset progressive MND with clinical and pathological features reminiscent of ALS (Lambrechts et al., 2003a). $VEGF^{\partial/\partial}$ mice show reduced lower basal 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 (Skene & Cleveland, 2001; Rabin et al., 1999). An inability to regulate levels of VEGF has been reported in mutant *SOD1* mice, and the progeny of crosses of $VEGF^{\partial/\partial}$ 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 (Lambrechts et al., 2003a).

The molecular mechanisms whereby mutant *SOD1* causes selective motor neuron death have yet to be defined

Is the toxicity of mutated SOD1 cell-autonomous?

Mutations in *SOD1* do not have obvious impacts on cells in CNS other than motor neurons or on cells in other organs except 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 nor to restricted expression to astrocytes appears to be sufficient to produce disease (Clement et al., 2003). Complex experiments in chimeric mice (with different mixtures of wt 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 prolongs survival in the chimeric mice. These observations suggest that disease may be the result of different populations of cells acting in concert.

Expression of GLT1 is implicated as a possible cofactor

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) (Cleveland & Rothstein, 2001). Using a screen of >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. 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 (Rothstein et al., 2005).

Mutation-induced conformational effects and copper oxidative toxicity have been implicated

As mentioned above, neither elevations nor reductions of SOD1 influence the clinical course or the character of pathology (Bruijn et al., 1998). 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. In this scenario, cell dysfunction and death might be initiated by aberrant oxidative chemistries catalyzed by the Cu bound in the active site of mutant SOD1 (Estévez et al., 1999). Copper within the active site of SOD1 is essential for dismutase activity (Subramaniam et al., 2002). However, Cu is extremely toxic and there is virtually no free intracellular copper. Instead, Cu is delivered to SOD1 by a specific copper chaperone, termed CCS in mammals, which is present within neurons and astrocytes (Rothstein et al., 1999). CCS binds to SOD1 and delivers Cu to the dismutase where it is bound by specific histidine residues. Mice generated with targeted disruption of *CCS* alleles are viable and possess normal levels of SOD1 protein, but levels of SOD1 activity are markedly reduced (Wong, et al. 2000).

The hypothesis that the toxicity of mutant SOD1 involves aberrant chemistry of Cu 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 Cu to SOD1) (Subramaniam et al., 2002); in a second set of experiments, mutant *SOD1* mice also carried mutations in the histidine residues (H46R/H48Q) that disrupt the Cu binding site (Wang et al., 2002b). The *CCS-/-* mutant *SOD1* mice possess virtually no Cu-loaded SOD1 or SOD1 activity. Significantly, these mice exhibit no differences in the onset or progression of disease as compared to mutant *SOD1* mice (Subramaniam et al., 2002). Similarly, 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 (Wang et al., 2002b). Thus, Cu bound within the active site of the enzyme is not essential for mutant SOD1-mediated toxicity. Although proponents of the aberrant Cu chemistry hypothesis suggest that Cu 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 (Wang et al., 2002b).

Accumulating evidence supports the view that fALS-associated mutants facilitate misfolding of wild-type SOD1

The misfolding impacts on proteins important for cellular physiology (Pasinelli et al., 2004; Vande & Cleveland, 2005). In this dominantly inherited disease, only one allele need be mutated to cause disease. Since a wide variety of mutant SOD1 linked to fALS leads to a common pathogenic cascade, it remains plausible that wild-type SOD1 may also participate in the pathogenesis of sporadic ALS. Investigators asked whether misfolded, wild-type SOD1 sharing a common pathogenic mechanism occurring in SOD1-linked fALS could be observed in cases of sALS using a conformation-specific antibody that recognizes both oxidized wild-type and misfolded mutant SOD1 (Bosco et al., 2010). Interestingly, results of these investigations indicated that misfolding of wild-type SOD1 as a consequence of post-translational modifications contributes to pathogenesis of many cases of sALS. Furthermore, the observation that oxidized wild-type SOD1 or wild-type SOD1 purified from cases of sALS inhibited kinesin-based fast axonal transport as found in fALS-linked SOD1 mutants strengthens the view that misfolded wild-type SOD1 represents a step in the pathogenic mechanism in sALS (Bosco et al., 2010). This is discussed further in Box 45-1.

A variety of experimental therapeutic strategies have been tested in mutant *SOD1* transgenic mice

Riluzole is the major drug approved for therapy in human ALS (Bruijn et al., 2004; Cleveland & Rothstein, 2001). However, the benefits are modest. Investigators are examining other ways to ameliorate excitotoxicity, possibly by influencing the levels or activity of GLT-1 (Rothstein et al., 2005). Trials with growth factors were truncated because of evidence of complications (Cleveland & Rothstein, 2001). Trials of anti-aggregation 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 (Martin et al., 2005; Rothstein, 2003).

It has also been shown in the $SOD1^{G93A}$ transgenic rodents that the knockdown of SOD1 could significant delay the age of onset and slow down the progression of disease either by delivery of lentiviral vector–producing anti-sense RNA against SOD1 (Ralph et al., 2005; Raoul et al., 2005) or by continuous infusion of phosphorothioate-modified anti-sense oligonucleotides against SOD1 (Smith et al., 2006). For clinical applications, however, the integration issue of viral vector, the transient nature of oligonucleotide infusion, and the specificity of the anti-sense RNA remain unsolved issues.

The ability to generate motor neurons from induced pluripotent stem (iPS) cells derived from fALS patients (Dimos et al., 2008) provides the opportunity to generate and analyze motor neurons harboring ALS-linked genes. Moreover, this iPS approach may allow investigators to clarify the early changes associated with these disease-causing mutations. Finally, unlike the non-dividing motor neurons themselves, the proliferating nature of skin cells, fibroblasts, or iPS cells make it possible to modify genetically these cells, providing an infinite source of "corrected" healthy motor neuron precursors for therapeutics in the future (Yan et al., 2007).

AVAILABLE GENETIC MOUSE MODELS WILL AID IN DISCOVERING DISEASE MECHANISMS AND NOVEL MEANS OF THERAPY

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. Investigations of these mouse models have greatly increased our understanding of the molecular

SUPEROXIDE DISMUTASE TYPE 1 AND REDOX SIGNALING

Scott T. Brady

The traditional view of Cu/Zn superoxide dismutase type 1 (SOD1) focuses on its role in managing reactive oxygen species (ROS) by converting superoxide radicals to peroxide as means of preventing oxidative damage to neurons and other cells (Deng et al., 1993). This naturally raised the specter of oxidative damage as a pathogenic mechanism in ALS, a view that continues to receive support (Barber et al., 2010). However, many mutant forms of SOD1 that produce ALS have significant normal enzymatic activity, which raises questions about the role of SOD1 activity in pathogenesis.

Although the abundance of SOD1 in many tissues argued for an important role in controlling reactive oxygen species, SOD1 knockout mice are viable and the phenotype is remarkably mild, with no evidence of motor neuron disease (Turner & Talbot, 2008). The SOD1 knockout did shorten lifespan of mice modestly, but knockouts of five other antioxidant enzymes had no effect on lifespan and overexpression of SOD1 did not extend lifespan (Perez et al., 2009). Moreover, expression of human mutant SOD1 in a mouse with normal SOD1 background still produces motor neuron disease, primarily affecting lower motor neurons in mouse with pathology very similar to human cases of ALS (Kato, 2008). Curiously, although mutant SOD1 is expressed widely in neuronal and nonneuronal tissues for both human cases of familial ALS and many mouse transgenic lines, the primary pathological consequence is motor neuron disease. Regardless, antioxidant therapeutic strategies have failed to significantly alter the course of the disease in humans.

Recent advances in our understanding of the complex biology of SOD1, oxygen and redox signaling pathways argue for a reconsideration of the cellular function of SOD1. Studies in the last decade have revealed additional roles for SOD1, which may be underappreciated. Specifically, redox-dependent signal transduction may be responsible for signaling through some cytokine and growth factor receptors associated with lipid rafts or endoplasmic reticulum/endosomes (see Ch. 7), like interleukin-1β (IL1) and tumor necrosis factor α (TNF), as well as platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and angiotensin II (angII) (Oakley et al., 2009). These redox-active endosomes appear to involve SOD1, NADPH oxidase, peroxiredoxins and chloride channels, as well as more familiar signaling elements like Rac1, NF-κB and tyrosine kinase/phosphatases pathways (Fukai et al., 2011; Oakley et al., 2009).

Signaling through redox-dependent pathways requires endocytosis for activation, where the production of ROS can be compartmentalized effectively. SOD1 appears to be recruited to endosomal surfaces with redox-mediated receptors (Fukai et al., 2011), where it produces H_2O_2 that can activate downstream effectors like Rac1 (Ch. 21) and NF-κB, or may inactivate protein tyrosine phosphatases (Ch. 26). Interestingly, SOD1 has been shown to bind Rac1 directly and to inhibit its activity (Harraz et al., 2008). Many of these pathways are shared with or involved in inflammatory signaling (Ch. 34).

Under normal circumstances, these redox-dependent pathways are limited and highly regulated. Less is known about changes in this aspect of SOD1 functionality with familial ALS-linked mutations, although some mutant SOD1s were found to have reduced binding to Rac1 (Harraz et al., 2008). Questions remain about how the 140 + different mutations can all produce the same disease, but recent studies begin to provide insights. Several groups have generated antibodies that recognize a conformation shared by most and perhaps all familial ALS-linked mutant SOD1 (Urushitani et al., 2007). Remarkably, one of these antibodies also recognizes oxidized forms of wild type SOD1 as well as a conformation of wild-type SOD1 detectable in many cases of sporadic ALS, suggesting that it might recognize a conformation common to all pathogenic forms of SOD1 (Bosco et al., 2010). Such studies support the idea that SOD1 might have a role to play in both familial and sporadic ALS (Kabashi et al., 2007). Significantly, all pathogenic forms of SOD1 examined activated a MAP kinase pathway (see Ch. 25) and inhibited fast axonal transport (Bosco et al., 2010). A better understanding of the normal functions of SOD1 may thus explain the pathogenic gain of function associated with SOD1 in ALS.

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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 pathogeneses of the neurodegenerative diseases have made spectacular progress over the past few years. It is anticipated that knowledge of disease mechanisms will lead to novel treatments for these devastating illnesses.

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