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Retrograde axonal transport and motor neuron disease

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Abstract

Transport of material between extensive neuronal processes and the cell body is crucial for neuronal function and survival. Growing evidence shows that deficits in axonal transport contribute to the pathogenesis of multiple neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS). Here we review recent data indicating that defects in dynein-mediated retrograde axonal transport are involved in ALS etiology. We discuss how mutant copper-zinc superoxide dismutase (SOD1) and an aberrant interaction between mutant SOD1 and dynein could perturb retrograde transport of neurotrophic factors and mitochondria. A possible contribution of axonal transport to the aggregation and degradation processes of mutant SOD1 is also reviewed. We further consider how the interference with axonal transport and protein turnover by mutant SOD1 could influence the function and viability of motor neurons in ALS.

Keywords

amyotrophic lateral sclerosis; axonal transport; dynein; motor neuron; superoxide dismutase

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease that leads to paralysis and death, typically within 5 years after onset because of loss of motor neurons in the spinal cord, brainstem, and motor cortex. Approximately 10% of ALS cases are inherited and of these approximately 20% are caused by dominantly inherited mutations in the Cu, Zn-superoxide dismutase 1 (*SOD1*) gene (Deng *et al*. 1993; Rosen *et al*. 1993). To date, more than 100 mutations scattered throughout the SOD1 protein have been identified and it has been established that SOD1 mutants acquire toxic properties (Gaudette

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et al. 2000). However, the nature of the toxicity and how the toxicity causes preferential motor neuron death is still debated. Several hypotheses of how mutant SOD1 could cause neurodegeneration including aberrant redox chemistry, mitochondrial damage, excitotoxicity, microglial activation, and inflammation as well as SOD1 aggregation have been proposed (Bruijn *et al*. 2004; Pasinelli and Brown 2006; Shaw and Valentine 2007).

Recent studies have shown that motor neurons are highly sensitive to defects in axonal transport, and reduced transport has been suggested to cause or contribute to the degeneration in ALS. Mutations in the retrograde motor complex dynein and in the dynein interacting complex dynactin cause motor neuron degeneration in humans and mice (Hafezparast *et al*. 2003; Puls *et al*. 2005). Decreased kinesin-mediated (anterograde) and dynein-mediated (retrograde) axonal transport have been observed both in ALS patients and in transgenic animal models (Breuer *et al.* 1987; Breuer and Atkinson 1988; Collard *et al.* 1995; Sasaki and Iwata 1996a; Williamson and Cleveland 1999; Ligon *et al.* 2005). In G93A SOD1 transgenic mice, a considerable inhibition of retrograde axonal transport was observed at a very early stage of disease before animals became symptomatic (Ligon *et al.* 2005). However, the mechanisms by which mutant SOD1 affects axonal transport have not been established. We recently showed that compared with wild-type (WT) SOD1, several SOD1 mutants interact with the dynein-dynactin complex much more stably (Zhang *et al.* 2007). Furthermore, we and others have shown that dynein co-localizes with protein inclusions formed by mutant SOD1 in ALS (Ligon *et al.* 2005; Zhang *et al.* 2007).

In this review, we will focus on recent data linking axonal transport defects to motor neuron degeneration and ALS etiology. We will discuss how mutant SOD1 and an aberrant interaction between mutant SOD1 and dynein-dynactin could influence neuronal survival by impairing retrograde transport of neurotrophic factors and mitochondria, as well as the potential role of this interaction in the aggregation process of mutant SOD1.

Intracellular transport in neurons

Motor neurons are highly specialized cells with extensive dendritic arbors and axonal processes that can extend up to 1 m from the cell body. The ability of the neuron to maintain this specialized morphology depends on cytoskeletal elements and continous transport of proteins and organelles to and from the cell body. The neuronal cytoskeleton comprises networks of microtubules, actin filaments, and neurofilaments (Fig. 1). Microtubules provide stability and polarity to the axonal compartment of the neuron as they are polarized with a slow-growing minus end directed toward the cell body and a fast growing plus end directed peripherally in the axon. Actin which is enriched at the cell membrane contributes mainly to the integrity of the cell periphery and plays a role in resistance to stress in mature neurons. Neurofilaments, which assemble from three subunits NF-L, NF-M, and NF-H primarily provide structural stabilization and regulate the axonal caliber, thereby controlling the speed of impulse conduction along the axon. Neurofilaments are particularly abundant in motor neurons with large caliber axons that depend on fast impulse conduction.

In addition to their structural roles, both microtubules and actin filaments provide conduits for intracellular transport. The microtubules provide long-range pathways for fast

anterograde movement (away from cell body) of kinesin motor proteins and the retrograde movement (toward cell body) of the dynein motor complex (Fig. 1). Actin filaments are used by myosin motor proteins for short-range, dispersive distribution of vesicles, and/or organelles to the cell periphery. Transport on microtubules and actin is coordinated to distribute organelles such as mitochondria throughout the cell body and neurites.

The first microtubule-based anterograde transport motor protein identified was kinesin 1, also called conventional kinesin. By hydrolyzing ATP, kinesin 1 moves cargo toward the plus end of microtubules (anterograde transport). To date, more than 45 members of the kinesin family have been identified in humans. Although a majority of the kinesins transport cargo in the anterograde direction, a few have also been shown to function in the retrograde direction, whereas others play a role in regulating microtubule dynamics rather than transport (for review see Miki *et al.* (2005, 2001).

Cytoplasmic dynein is the major motor driving retrograde transport in cells. Dynein is a large complex (approximately two million daltons) and consists of two dynein heavy chains (DHC), two dynein intermediate chains (DIC), four dynein light intermediate chains (DLIC), and various dynein light chains (DLC). The dimeric DHC forms the core of the dynein complex. Each DHC subunit folds to form a globular head containing the motor domain and a flexible stalk that is involved in dimerization of the two heavy chains as well as interaction with other dynein subunits [reviewed in Pfister *et al.* (2006)]. *In vivo*, dynein requires the co-complex dynactin for most functions. Dynactin functions both to increase the motor efficiency of dynein and to serve as an adaptor between dynein and various cargos [reviewed in Schroer (2004)]. The dynactin assembly compises multiple subunits that form a distinct structure: a filament base and a projecting sidearm linked to the base by a shoulder domain. The base of dynactin is formed by a small filament of the actin-related protein, Arp1, capped by other subunits such as CapZ, Arp11, p25, and p27. The projecting sidearm of dynactin is formed by two dimeric $p150^{Glued}$ subunits and is involved in the interaction with dynein via the DIC, but it also interacts with microtubules. A tetramer of the dynamitin-p50 subunit forms the shoulder of dynactin that links the sidearm to the filament base. Over-expression of p50 competitively dissociates the $p150^{Glued}$ sidearm from the filament base (Melkonian *et al.* 2007).

Recent studies suggest that dynein and kinesin can function in an interdependent manner, such that disruption of movement in one direction will affect movement in the opposite direction (Brady *et al.* 1990; Waterman-Storer *et al.* 1997; Martin *et al.* 1999). Moreover, many cargos can move bi-directionally along microtubules [reviewed in Welte (2004)]. In some cases the overall direction of motility is regulated, but in other cases the movement appears to be stochastic with multiple apparently random changes in direction. Several models have been proposed to explain the bi-directionality and interdependency of cargo transport [reviewed in Gross *et al.* (2002) and Holzbaur (2004)]. In the ‘tug-of-war’ model, kinesins and dynein are suggested to bind to the cargo simultaneously and the direction of movement is determined by the dominant motor at any given time. In another model, either kinesin or dynein is active at a certain time. The slow transport of neurofilaments has been suggested to occur in the ‘tug-of-war’ manner whereas cargos such as peroxisomes and vesicles have been suggested to be transported in a coordinated manner (Gross *et al.* 2002;

Holzbaur 2004). The coordination of the motor activities could be regulated by direct interaction between motor proteins, by adaptor proteins linking different motors, or by other regulatory pathways. Recent studies have supplied evidence for all of the proposed mechanisms. A direct interaction between kinesin-1 and dynein has been observed (Ligon *et al.* 2004). In addition, dynactin has been reported to interact with both dynein and several kinesins (Blangy *et al.* 1997; Deacon *et al.* 2003). Furthermore, microtubule associated proteins such as tau might also differentially influence the motility of kinesin and dynein motors (Dixit *et al.* 2008).

Dynein defects and motor neuron disease

Dynein and dynactin have multiple cellular house-keeping roles, including participation in mitosis and endoplasmic reticulum to Golgi vesicular trafficking. However, the dynein-dynactin machinery is also required for multiple neuron-specific processes, such as neuronal migration (Sasaki *et al.* 2000), neurite outgrowth, and synapse formation (Barakat-Walter and Riederer 1996; Cheng *et al.* 2006) as well as retrograde axonal transport of proteins and organelles (Schnapp and Reese 1989; He *et al.* 2005). Disruption of dynein or dynactin is therefore expected to compromise severely the function and health of neurons with long axons, including motor neurons.

Two independent *N*-ethyl-*N*-nitrosourea-induced missense mutations in the stalk domain of the DHC1 gene, *Loa* and *Cra1*, decreased retrograde transport in motor neurons and produced late-onset motor neuron degeneration in heterozygous mice (Hafezparast *et al.* 2003). Furthermore, a mutation (G59S) in the $p150^{Glued}$ dynactin subunit was recently linked to a slowly progressive form of motor neuron disease in a North American family and heterozygous knock-in mice as well as transgenic mice carrying this mutation also developed motor neuron degeneration (Puls *et al.* 2003, 2005; Lai *et al.* 2007; Laird *et al.* 2008). Moreover, impaired dynein-dynactin complexes upon over-expression of the dynactin p50 subunit in mice also cause motor neuron disease (LaMonte *et al.* 2002).

Axonal transport defects have been suggested to produce motor neuron degeneration in ALS. Studies both in ALS patients and in transgenic animals have revealed decreased axonal transport in both anterograde and retrograde directions (Breuer *et al.* 1987; Breuer and Atkinson 1988; Collard *et al.* 1995; Sasaki and Iwata 1996a; Williamson and Cleveland 1999; Ligon *et al.* 2005). In motor nerve specimens from ALS patients, decreased axonal transport of organelles such as mitochondria was observed (Breuer *et al.* 1987; Breuer and Atkinson 1988). In ALS transgenic mice, slowed anterograde transport of cargos such as neurofilaments appeared prior to disease onset (Warita *et al.* 1999; Williamson and Cleveland 1999). Interestingly, inhibition of retrograde transport has been suggested to be one of the earliest events in the G93A SOD1 mouse model of ALS. In these mice, decreased retrograde transport was evident long before mice showed visible symptoms. However, this reduction in retrograde transport coincided with the onset of neuromuscular junction destabilization and onset of muscle weakness (Ligon *et al.* 2005). Furthermore, defects in retrograde transport have been demonstrated even in embryonic motor neurons isolated from G93A embryos (Kieran *et al.* 2005).

Surprisingly, crossing heterozygous *Loa* and *Cra1* mutant mice with G93A SOD1 mice ameliorated the transport defect, delayed disease onset, and slowed disease progression in G93A mice (Kieran *et al.* 2005; Teuchert *et al.* 2006). Though the precise mechanism(s) leading to this effect remain unclear, several explanations have been suggested. One hypothesis states that the dynein mutations alter intracellular transport and thereby change the subcellular localization of SOD1 or the interaction of SOD1 with other proteins or organelles (Kieran *et al.* 2005; El-Kadi *et al.* 2007). For example, it is possible that decreased interaction of mutant SOD1 with mitochondria could improve cell survival by reducing apoptosis or other downstream consequences (Wong *et al.* 1995; Menzies *et al.* 2002; Liu *et al.* 2004; Pasinelli *et al.* 2004). Interestingly, SOD1-positive aggregates have been observed in homozogous *Loa* mice suggesting that this dynein mutation can affect WT SOD1 distribution in the cell (Hafezparast *et al.* 2003). A second hypothesis is that the decreased retrograde transport rates caused by the DHC *Loa* or *Cra1* mutations might counterbalance an inhibition of anterograde transport caused by G93A mutant SOD1, thereby restoring the balance between anterograde and retrograde transport (Kieran *et al.* 2005; El-Kadi *et al.* 2007).

Other studies, however, have reported that disrupting dynein-dynactin by means other than *Loa* and *Cra1* mutations did not influence survival or disease progression in G93A mice. Lai *et al.* (2007) reported that mice with the G59S mutation in the dynactin subunit p150^{Glued} showed signs of reduced motor neuron axonal transport and developed motor neuron disease which were similar to *Loa* and *Cra1* mice. However, when heterozygous G59S knock-in mice were crossed with G93A mice, no improvement of the G93A phenotype could be observed. Furthermore, a recent study showed that a novel DHC mutation, *Swl*, caused proprioceptive sensory neuropathy without causing motor neuron deficits (Chen *et al.* 2007). Crossing the *Swl* mice with G93A SOD1 mice did not affect ALS disease onset or progression either. The same study also reported that *Loa* and *Cra1* mice not only developed late-onset motor neuron disease, but also suffered from sensory neuropathy that occurred prior to the onset of motor symptoms (Chen *et al.* 2007).

The studies crossing different dynein and dynactin mutant mice with G93A mice are summarized in Table 1. Two hypotheses could be envisioned to explain the various effects on motor neuron viability and G93A SOD1 mice phenotype by the different dynein and dynactin mutants. First, the various dynein and dynactin mutations could inhibit dynein transport of all cargos but to different degrees. The *Swl* mutation could affect dynein-mediated transport to a less extent, thus causing harm only to very sensitive sensory proprioceptive neurons. *Loa* and *Cra1* on the other hand could cause a higher level of dynein inhibition, thereby causing both sensory and motor neuron degeneration. It is possible that only reduction of dynein transport within a certain range could have beneficial effect on G93A SOD1 mice. To address this hypothesis, the rate of axonal transport in motor and sensory neurons in the *Swl* and G59S p150^{Glued} mice needs to be determined.

A second hypothesis where different dynein and dynactin mutations could affect transport of different dynein-dynactin cargos of varied relevance to different neuronal populations could also be envisioned. Cargos bind to dynein in multiple ways [reviewed in Chevalier-Larsen and Holzbaur (2006); Karcher *et al.* (2002)]. Most known cargos including vesicles and

organelles bind to dynein via the DIC interacting complex dynactin. Other cargos such as neurofilaments can bind directly to the DIC, while a third set of cargos including the Fyn tyrosine kinase and rhodopsin bind via DLCs. A fourth set of proteins including pericentrin bind through DLIC. The G59S $p150^{Glued}$ dynactin mutation could clearly affect the subset of dynein cargos using dynactin as adaptor. Although the *Loa*, *Cra1*, and *Swl* mutations are all predicted to affect homodimerization of DHC, *Loa* is also predicted to effect interaction with DIC, whereas *Swl* is predicted to affect a region important for cargo interaction (Chen *et al.* 2007). It is therefore plausible that the different dynein and dynactin mutations could affect transport of different subsets of cargos. The positive effect of *Loa* and *Cra1* on G93A SOD1 mice could be because of reduction of certain cargos which are not affected by the *Swl* and G59S-$p150^{Glued}$ mutations.

Mutant SOD1, dynein and axonal transport

Although the underlying mechanism is not well understood, it is clear that decreased retrograde axonal transport is a component of familial ALS caused by mutations in SOD1 (Breuer *et al.* 1987; Breuer and Atkinson 1988; Collard *et al.* 1995; Sasaki and Iwata 1996b; Williamson and Cleveland 1999; Ligon *et al.* 2005). Several different mechanisms to explain how mutant SOD1 could cause this can be envisioned and are illustrated in Fig. 2. These mechanisms include: (i) physical blockade of dynein by mutant SOD1 aggregates, (ii) disruption of microtubule formation or stability, (iii) disturbance of dynein motor activity, (iv) disruption of the dynein-dynactin complex integrity, (v) disruption of dynein-dynactin microtubule interaction, or (vi) masking of cargo-binding sites.

Axonal inclusions, including spheroids as large as 20 μm in diameter, have been reported in ALS patients (Kato *et al.* 2003). In ALS transgenic mice, we and others have shown that dynein co-localizes with mutant SOD1 aggregates in motor neuron axons (Ligon *et al.* 2005; Zhang *et al.* 2007). It is possible that mutant SOD1 aggregates could directly hinder dynein transport on microtubules in axons. A similar mechanism was proposed in Huntington's disease after huntingtin aggregates were observed with diameters exceeding those of axons; consistent with this axonal swelling around aggregates and loss of axonal transport was reported in *Drosophila* models (Gunawardena *et al.* 2003; Lee *et al.* 2004).

In a recent study, we showed that mutant SOD1 interacted with dynein more stably than did WT SOD1 in both ALS cell culture and animal models (Zhang *et al.* 2007). Though co-immunoprecipitation of SOD1 and dynein-dynactin showed no evidence suggesting that mutant SOD1 altered the dynein-dynactin subunit interactions, we did observe an increase in the amount of mutant SOD1 that interacted with dynein as the disease progressed (Zhang *et al.* 2007). It is still unclear which subunit(s) of dynein and/or dynactin interacts with mutant SOD1. If mutant SOD1 is a *bona fide* cargo of dynein, the interaction could be mediated by either DLC or dynactin, which are known to bind cargos. However, it is also possible that this aberrant interaction involves novel components distinct from DLC or dynactin. Given the beneficial effects of the *Loa* and *Cra1* DHC mutations on ALS disease (Kieran *et al.* 2005; Teuchert *et al.* 2006), it would be interesting to test whether these dynein mutations affect the interaction of mutant SOD1 with the dynein complex.

On the basis of the data showing increased association of mutant SOD1 with dynein as the ALS progressed in mice (Zhang *et al.* 2007), we hypothesize that the transport capacity of dynein might be overwhelmed by handling mutant SOD1 over time. Interestingly, a recent study in monkeys showed that the dynein-dynactin complex undergoes age-related changes including increased amounts of dynein in nerve endings and a decrease in the dynein-dynactin interaction (Kimura *et al.* 2007). These changes suggest that less functional dynein-dynactin complexes may be available during aging. Both factors, *i.e.* the increase in dynein-mutant SOD1 association and the decrease in functional dynein-dynactin while aging, could contribute to reduced retrograde transport to levels that are no longer able to sustain neuronal survival. This could thereby produce a phenotype of adult onset motor neuron degeneration.

Reduced retrograde transport of cargos such as neurotrophic factors, mitochondria, and membrane vesicles could damage motor neurons by multiple mechanisms. First, reduced retrograde axonal transport of neurotrophic factors could have dire consequences for motor neurons as these factors stimulate neuronal survival. Neurotrophic factors including nerve growth factor, brain-derived neurotrophic factor, and neurotrophin 3 are secreted by target tissues (e.g. muscles) and then bind to Trk receptors on the surface of neurons. The Trk receptor-neurotrophin complex is then internalized and initiates signaling cascades that regulate neuronal cell growth, survival, and repair pathways (Campenot and MacInnis 2004; Bronfman *et al.* 2007). For the neurotrophins to act effectively and promote long-term survival, transport of the neurotrophin-receptor complex to the cell body by the dynein complex may be required (Yano *et al.* 2001; Delcroix *et al.* 2003; Ye *et al.* 2003; Heerssen *et al.* 2004). Some ALS studies have demonstrated a benefit upon administration of exogenous neurotrophic factors, but others have shown little or no effect (Wang *et al.* 2002; Azari *et al.* 2003; Feeney *et al.* 2003; Kaspar *et al.* 2003; Azzouz *et al.* 2004; Zheng *et al.* 2004; Dobrowolny *et al.* 2005; Storkebaum *et al.* 2005; Pun *et al.* 2006; Li *et al.* 2007). Conclusions from such studies may be confounded by issues related to exactly which neurotrophic factors motor neurons requires the timing and the effectiveness of neurotrophic factor delivery.

Second, mitochondrial distribution and dynamics can be affected by reduced axonal transport. Mitochondria are the energy factories of cells and are particularly abundant in areas of neurons with high metabolic demands such as the axon hillock, the nodes of Ranvier, and the synaptic regions. These organelles generate reactive oxygen species (ROS) as a byproduct from energy production. Mitochondria cannot be synthesized *de novo* in cells and relay on constant transport, fission, and fusion to maintain normal morphology and function [see Frazier *et al.* (2006) for review]. Dynein is important not only for axonal transport of mitochondria but also for mitochondrial fission (Frazier *et al.* 2006). Furthermore, damaged mitochondria with reduced membrane potential are degraded by autophagy, an intracellular turnover process in which dynein has been suggested to play a role (Twig *et al.* 2008). Inhibition of dynein function not only could impair energy homeostasis, but also could cause accumulation of damaged mitochondria leading to increased ROS production at nerve terminal regions. Increased levels of ROS could damage synaptic structures and contribute both to the distal neuropathy observed early in ALS and to

impaired re-innervation. Many studies have implicated mitochondria in the pathophysiology of ALS. Although WT SOD1 is traditionally believed to be a cytoplasmic protein, mutant SOD1s also localize on and in mitochondria (Vande Velde *et al.* 2008) and have been suggested to induce mitochondria-mediated apoptosis (Wong *et al.* 1995; Menzies *et al.* 2002; Zhu *et al.* 2002; Liu *et al.* 2004; Pasinelli *et al.* 2004). As both mitochondria and mutant SOD1 interact with dynein, it is possible that locally high concentrations of mutant SOD1 in the proximity of mitochondria may contribute to aggregation of mutant SOD1 in or on mitochondria or to direct impairment of mitochondrial function.

Third, the structural and functional integrity of the endoplasmic reticulum-golgi network is dependent on microtubule-mediated vesicular membrane trafficking. Impairment of dynein-dynactin function by p50 dynamitin over-expression has been shown to interfere with endosome trafficking and cause golgi fragmentation (Burkhardt *et al.* 1997; Valetti *et al.* 1999). Fragmentation of the golgi has been observed in both sporadic and SOD1-mediated familial ALS (Gonatas *et al.* 2006). In ALS mouse models, golgi fragmentation can also be observed before onset of paralysis (Gonatas *et al.* 2006).

Dyneins role in SOD1 protein aggregation and degradation

Dynein-mediated transport has been suggested to play a role in protein degradation and accumulation of misfolded proteins in cells. The observed interaction between mutant SOD1 and dynein could therefore also influence SOD1 aggregation and degradation as discussed below.

The removal of misfolded and/or aggregated proteins may pose a vulnerability for neurons, as suggested by the aberrant accumulation of protein aggregates in many neurodegenerative diseases. Proteins are mainly degraded by two pathways, the ubiquitin-proteasome system (UPS) and macroautophagy (Fig. 3). Proteins destined to be degraded by the UPS are marked for elimination by the covalent attachment of ubiquitin and then shuttled to the proteasome for proteolytic cleavage (Goldberg 2003). As most UPS activity is believed to occur in the cell body, it is possible that dynein-mediated retrograde transport could be involved in shuttling targeted proteins to the proteasome. The proteasome comprises multiple subunits forming a barrel-like chamber within which proteolysis occurs. Because of the restricted opening of the proteasome, larger molecular complexes and organelles cannot enter for degradation by the proteasome (Goldberg 2003).

Macroautophagy (or autophagy) is an intracellular process in which proteins, organelles, or protein aggregates are degraded. During autophagy, the substrate is engulfed by membranous structures to form autophagosomes, which then fuse with lysosomes containing hydrolytic enzymes (Mizushima 2007). Autophagy is an important degradation pathway for neurons as mice lacking the autophagy gene *Atg7* develops axonal dystrophy characterized by distal accumulation of membrane structures and swelling of axonal terminals (Komatsu *et al.* 2007b). Dynein has been linked to autophagic clearance of aggregated proteins by several studies (Fig. 3). First, dynein-mediated transport has been shown to collect misfolded proteins from the cell periphery and transport them to the perinuclear area, where they form inclusions called aggresomes; it is suggested that these structures may become

membrane-bound and associate with lysosomes (Johnston *et al.* 2002; Taylor *et al.* 2003). Dynein has also been suggested to play an important role in lysosomal transport and to mediate the fusion of the autophagosome and the lysosome (Burkhardt *et al.* 1997; Ravikumar *et al.* 2005).

Mutant SOD1 is believed to be degraded by both the UPS and autophagy as shown in Fig. 3 (Kabuta *et al.* 2006). We recently demonstrated that while mutant SOD1 strongly interacts with dynein, WT SOD1 does not or to very little extent (Zhang *et al.* 2007). Furthermore, we have shown that mutant, but not WT SOD1 interacts with p62/sequestosome 1 (Gal *et al.* 2007), a protein that has been linked to autophagy (Komatsu *et al.* 2007a; Pankiv *et al.* 2007). Moreover, we showed that over-expression of p62/sequestosome 1 increased the formation of large mutant SOD1 inclusions resembling aggresomes (Gal *et al.* 2007). These data suggest that cells might utilize dynein-mediated transport to collect mutant SOD1 and form aggresomes that are targeted for autophagic degradation. For this purpose, the interaction between mutant SOD1 and dynein might be of beneficial effect to the cell. Supporting the importance of autophagic clearance of aggregates in preventing neuronal toxicity, a recent study showed that blocking of dynein by the *Loa* mutation caused decreased autophagic clearance of mutant Huntingtin protein resulting in increased inclusion/aggre-some burden and toxicity in Huntington's disease model mice (Ravikumar *et al.* 2005). However, this mechanism may not be relevant in SOD1-mediated ALS as this negative effect of *Loa* was not seen in G93A ALS mice (Kieran *et al.* 2005). In fact, as discussed earlier, both *Loa* and *Cra1* had a beneficial effect in the G93A mice and speculations that impairment of aggresome formation/autophagy might even have a positive effect in ALS have been raised (Teuchert *et al.* 2006).

Whether aggregates, aggresomes, and/or inclusions are toxic is still debated. However, multiple mechanisms by which mutant SOD1 aggregates/inclusions could be toxic to cells have been proposed. For instance, inclusions have been suggested to disrupt organelles like mitochondria, cause depletion of essential proteins via co-aggregation or physically block intracellular transport as discussed above.

Conclusions and future investigations

It is clear that dynein-mediated retrograde axonal transport is affected in motor neurons in ALS, however, the underlying mechanism(s) are still unclear. Mutations that perturb the dynein-dynactin machinery cause phenotypes that may influence multiple processes including neurotrophic factor delivery, transport, and homeostasis of mitochondria and protein aggregation or degradation. Disruption of dynein function could therefore link several proposed pathways relevant to ALS pathophysiology, including axonal transport defects, mitochondrial dysfunction, and mutant SOD1 aggregation. Genetic manipulation of *in vivo* models of motor neuron disease continues to provide important mechanistic clues. Crossing *Loa* and *Cra1* mice with G93A SOD1 mice has shown, surprisingly, that disruptions of dynein by some mutations can ameliorate the ALS disease process for reasons that remain to be clarified. A better understanding is needed of how the transport of specific cargos is regulated and how perturbations of both retrograde and anterograde transport are integrated to produce a given phenotype.

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Abbreviations used

ALS	amyotrophic lateral sclerosis
DHC	dynein heavy chains
DIC	dynein intermediate chains
DLC	dynein light chains
DLIC	dynein light intermediate chains
NF	neurofilament
ROS	reactive oxygen species
SOD	superoxide dismutase
Trk	tyrosine kinase
UPS	ubiquitin-proteasome system
WT	wild-type

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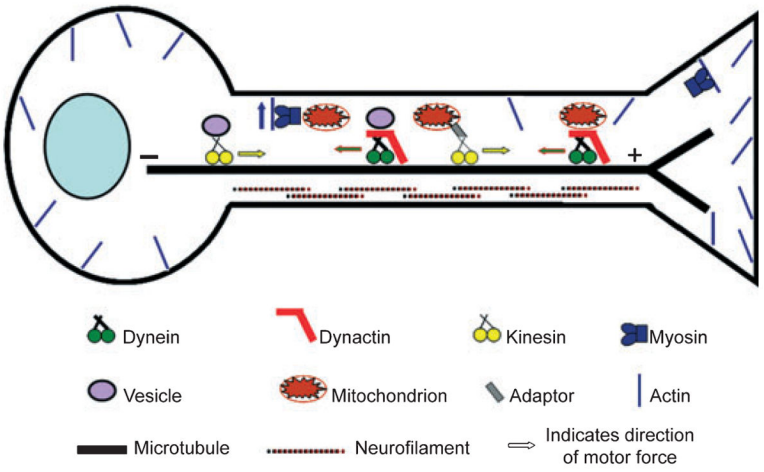

Fig. 1.

Cytoskeleton, motor proteins, and intracellular transport in neurons. Neurons depend on the cytoskeleton and molecular motor proteins to maintain cell homeostasis and communication between the cell body and the periphery. The neuronal cytoskeleton comprises microtubules, actin, and neurofilaments. Kinesin motor proteins move cargos along microtubules away from the cell body (anterograde transport), whereas dynein together with the dynactin complex move cargos in the retrograde direction toward the cell body. Myosin motors use actin filaments and are responsible for short-ranged dispersive distribution of cargos. Myosin motors have for instance been implicated in pulling mitochondria away from microtubules and facilitate their anchorage to F-actin. The interaction between a motor and its cargo may be direct or may be mediated by adaptor proteins.

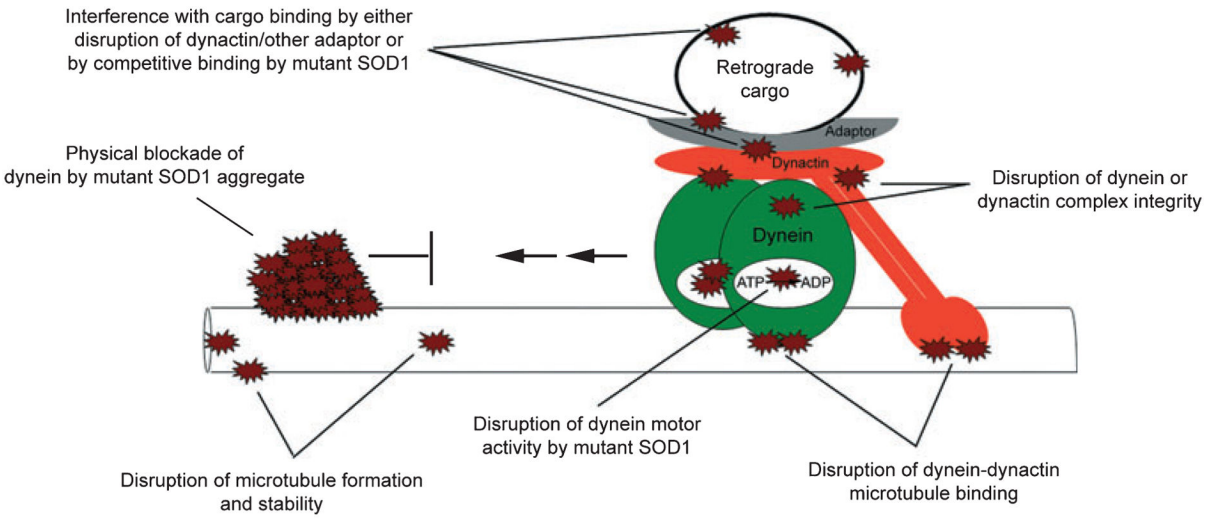

Fig. 2.

Possible mechanisms by which mutant superoxide dismutase 1 could interfere with dynein-dynactin motor activity.

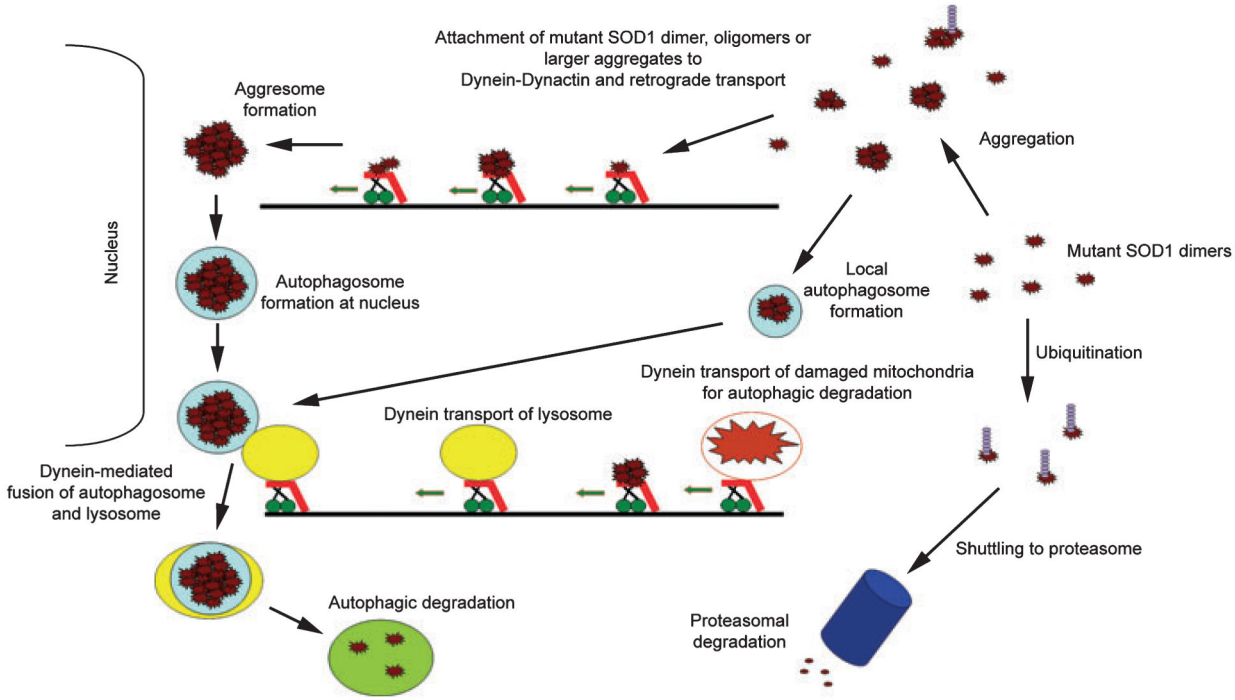

Fig. 3.

Potential role(s) of dynein-dynactin in aggregation and degradation of mutant superoxide dismutase 1 (SOD1). Proteins are mainly degraded by two pathways, the ubiquitin-proteasome system (UPS) and autophagy. Proteins degraded by UPS are ubiquitinated and then shuttled to the proteasome. Dynein could have a potential role in transporting targeted proteins from the cell periphery to the proteasome, as most UPS degradation is believed to occur in the cell body. Autophagy could not only degrade monomeric SOD1, but could also clear SOD1 aggregates. During autophagy, proteins/aggregates are enveloped by membrane and form autophagosomes which are then fused to lysosomes containing hydrolytic enzymes. Dynein is believed to be involved in the fusion of the autophagosome and the lysosome. Dynein transport has also been implicated in the formation of large perinuclear protein aggregates called aggresomes that may be degraded by autophagy. Mutant SOD1 has also been suggested to aggregate on or in mitochondria and cause mitochondrial damage. Dynein transport and dynein-dependent autophagy is used to degrade damaged mitochondria.

Table 1

The effect of different dynein and dynactin mutations on axonal transport and protein aggregation in the G93A ALS mouse model

Mice	Retrograde transport in motor neurons	SOD1 aggregates in motor neurons	Development of motor neuron disease	Phenotypic outcome of crossing	References
G93A	Reduced	Yes	Yes	NA	(Gurney *et al.* 1994; Kieran *et al.* 2005)
Loa +/+	Reduced	Yes, WT SOD1 positive inclusions seen	Yes, severe phenotype, mice die at P0–P1	NA	(Hafezparast *et al*. 2003; Kieran *et al.* 2005)
Loa −/+	No change	ND	Yes, but normal lifespan	NA	(Hafezparast *et al*. 2003; Kieran *et al.* 2005)
Loa −/+ x G93A	No change	ND	Yes	Delayed onset and extended survival of G93A	(Kieran *et al*. 2005)
Cra1+/+	Reduced	No	Yes, severe phenotype, mice die at P0–P1	NA	(Hafezparast *et al*. 2003)
Cra1−/+	ND	ND	Yes, but normal lifespan	NA	(Hafezparast *et al*. 2003)
Cra1 −/+ x G93A	ND	ND	Yes	Delayed onset and extended survival of G93A	(Teuchert *et al*. 2006)
Swl +/+	ND	ND	Embryonic lethal	NA	(Chen *et al*. 2007)
Swl −/+	ND	ND	No	NA	(Chen *et al*. 2007)
Swl −/+ x G93A	ND	ND	Yes	No effect on G93A phenotype	(Chen *et al*. 2007)
G59S $p150^{Glued}$ +/+	ND	ND	Embryonic lethal	NA	(Lai *et al.* 2007)
G59S $p150^{Glued}$ −/+	Reduced (accumulation of cargos, but transport rate not measured)	ND	Yes	NA	(Lai *et al.* 2007)
G59S $p150^{Glued}$ x G93A	ND	ND	Yes	No effect on G93A phenotype	(Lai *et al.* 2007)

ND, not determined; NA, not applicable; ALS, amyotrophic lateral sclerosis; WT SOD, wild-type superoxide dismutase.