

Minireview

Cytoplasmic dynein could be key to understanding neurodegeneration

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Abstract

A new mouse mutation, *Sprawling*, highlights an essential role for the dynein heavy chain in sensory neuron function, but it lacks the ability of other known heavy-chain mutations to ameliorate neurodegeneration due to defective superoxide dismutase.

Eukaryotic cells transport molecules, complexes and organelles around the cell by means of energy-dependent motor proteins. The main motor responsible for movement of cargos to the minus end of microtubules is cytoplasmic dynein. This is a huge multisubunit protein complex that interacts with many intracellular pathways and whose multifarious roles in the cell are far from being completely understood. In neurons, dynein is the major retrograde motor, moving cargoes from the synapse along the axon and back to the cell body. Previous mutations in the core of this motor - the dynein heavy chain - are known to ameliorate neurodegeneration in mouse models of amyotrophic lateral sclerosis (ALS). A recent paper by Chen, Popko and colleagues [1] reporting a new mouse mutant for the dynein heavy chain extends our knowledge of the effects of dynein mutations on the nervous system, but the mystery of dynein's relation to neurodegenerative disease thickens.

Cytoplasmic dynein is a large complex of proteins whose constituent members are the heavy chain (encoded by a single gene), the intermediate chains (two genes), the light-intermediate chains (two genes), and the light chains (three genes) [2]. The precise stoichiometry of the intact complex is not known, but at its core lies a homodimer of heavy chains. This dimer binds to microtubules and enables dynein to move in an ATP-dependent manner [3]. The other dynein subunits are thought to maintain the stability of the complex, to modulate its activity and to interact with accessory and cargo proteins (Figure 1a) [4-10]. Cytoplasmic dynein may also perform tasks other than transporting

cargos; for example, endosomes depend on dynein not just for their motility, but also for their maturation, morphology and receptor sorting [11].

The cytoplasmic dynein heavy-chain protein has a mass of 532 kDa and is encoded by a 78-exon gene, *DYNC1H1*; no splice isoforms are known (Figure 1b). A *Dync1h1* mouse knockout results in no detectable phenotype in heterozygotes and early embryonic lethality in null animals [12]. Two mouse mutants - Legs at odd angles (*Loa*) and Cramping 1 (*Crat*) - have been described previously, both of which are due to point mutations in *Dync1h1* (Figure 1b) [13]. These single amino-acid substitutions result in similar phenotypes: heterozygous animals show clenching of the hindlimbs when held by the tail (Figure 1c) and an obvious gait disorder, and homozygotes die at or before birth. Histological studies of the spinal cord of heterozygotes reveal a progressive loss of motor neurons. Retrograde axonal transport as measured by the movements of a fluorescent tetanus toxin fragment is normal in heterozygous *Loa* embryonic motor neurons but is slowed down in homozygotes [13,14].

Sprawling, a new mouse dynein heavy-chain mutation

The new mutation described by Chen *et al.* [1] is a radiation-induced dominant mutation that arises from a 9-bp deletion in *Dync1h1* that changes the four residues from position 1,040-1,043 into a single alanine, and it lies close to the *Crat* mutation (see Figure 1b). Called *Sprawling* (*Swl*), the

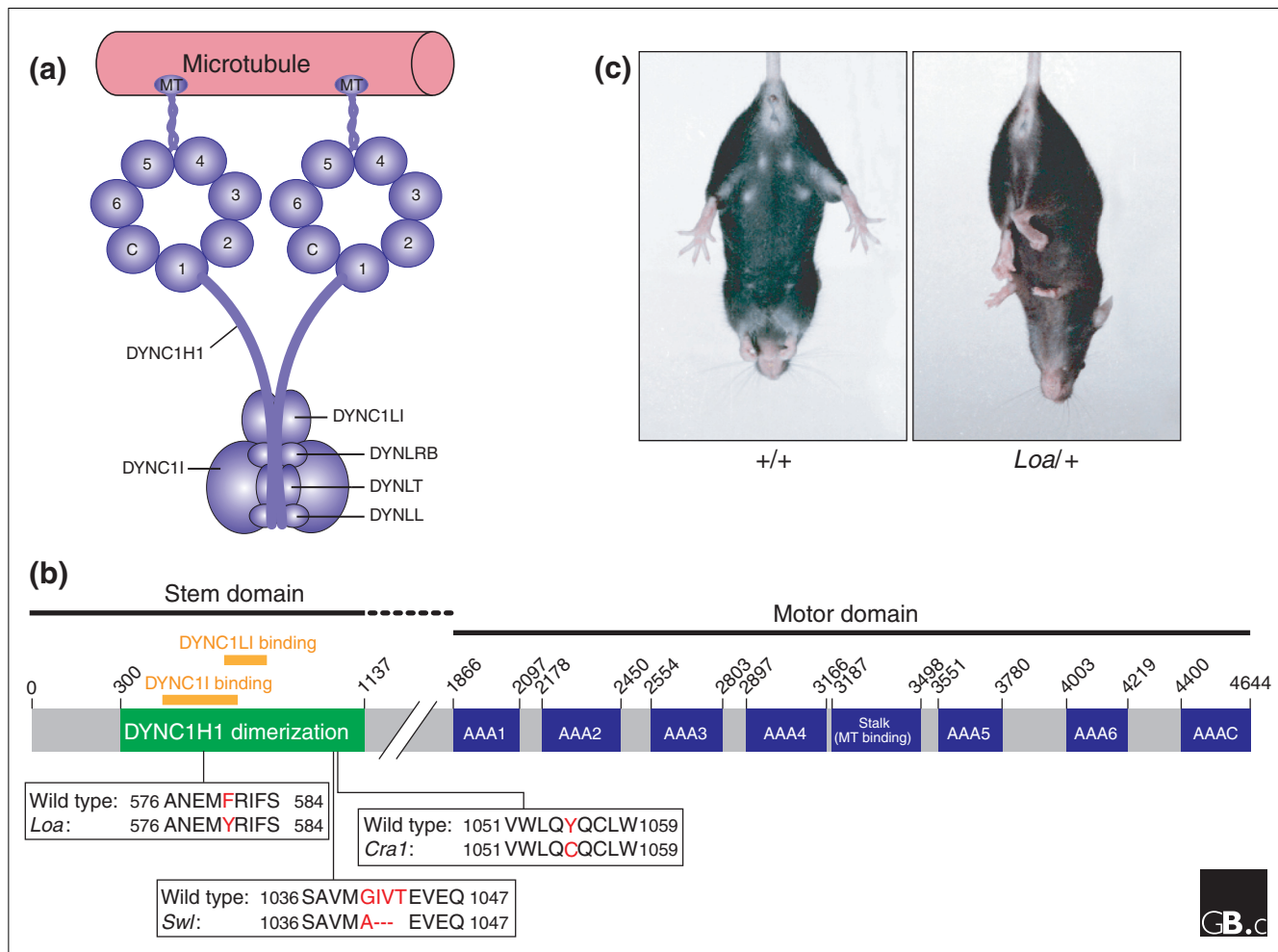


Figure 1
 Heavy-chain dynein mutations. **(a)** A schematic diagram of the cytoplasmic dynein complex. The core of the complex comprises a homodimer of heavy-chain subunits (DYNC1H1), the carboxy-terminal half of which form seven AAA-ATPase domains (labelled 1 to 6 and C). The dynein intermediate (DYNC1I) and light-intermediate (DYNC1LI) chains bind to the amino-terminal domain of the heavy chains. The light chains (DYNLRB, DYNLT and DYNLL) all bind to the intermediate chains. The dynactin complex (not shown) binds to the cytoplasmic dynein intermediate chains. Adapted from [2]. **(b)** Protein domain map of the cytoplasmic dynein heavy chain, showing the location of the mutations *Loa*, *Cra1* and *Swl*. The motor domain consists of the six known AAA-ATPase domains (AAA 1 to 6) and an unrelated seventh domain (AAAC). The microtubule-binding domain lies between AAA4 and AAA5. The amino-terminal half of the protein contains the intermediate (DYNC1I), light-intermediate (DYNC1LI) and heavy (DYNC1H1) chain binding domains [21,22]. The *Loa* mutation falls within both the DYNC1H1 dimerization and DYNC1I binding domains. The *Cra1* and *Swl* mutations fall outside of the DYNC1I binding domain, but still within the DYNC1H1 dimerization domain. **(c)** The hind-limb clasp phenotype of *Loa*+/+ mice. When held by the tail, wild-type (+/+) mice splay their hind legs away from their body. In contrast, *Loa*+/+ mice withdraw their hind limbs, pulling them into their body. *Swl*+/+ mice display a similar phenotype.

phenotype of *Swl* heterozygotes (*Swl*/+) is strikingly similar to the limb clenching of *Loa* and *Cra1* heterozygotes. *Swl*/+ mice also develop gait abnormalities and have reduced hindlimb grip strength. But although the outward phenotype of *Swl* heterozygotes is so similar to those of *Loa* and *Cra1* heterozygotes, Chen and colleagues [1] found no reduction in the number of motor neurons in the spinal cord of *Swl*/+ mice (Table 1). Instead they uncovered clear signs of moderate sensory neuropathy. Thus, this paper highlights for the first time the essential

role of the dynein heavy chain in the functioning of mammalian sensory neurons.

On further examination, the authors also found a similar sensory deficit in *Loa*/+ mice, and went on to show that while nociception (the sensing of pain) was unaffected, proprioception (the reception of stimuli produced within the body) was markedly affected in both *Swl*/+ and *Loa*/+ strains, with a striking decrease in the number of proprioceptive sensory receptors. They also found that neuron loss

Table 1**Comparison of *Loa/+*, *Cral/+* and *Swl/+* mice**

	<i>Loa/+</i> [1,13,20]	<i>Cral/+</i> [13]	<i>Swl/+</i> [1]
Mutation	F580Y	Y1055C	[GIVT]I040[A]
Lifespan	Normal	Normal	Normal
Progressive phenotype	Mildly	Mildly	No
Limb clenching	Yes	Yes	Yes
Gait abnormalities	Yes	Yes	Yes
Forelimb grip strength	Reduced	Unknown	Normal
Hindlimb grip strength	Reduced	Reduced	Reduced
Muscle pathology	Normal	Abnormal	Normal
Loss of muscle spindles	Yes	Unknown	Yes
Nociception	*	Unknown	No
Proprioception	Abnormal	Unknown	Abnormal
H reflex	Absent	Unknown	Absent
Loss of lumbar DRG neurons	Yes	Unknown	Yes
Loss of cervical DRG neurons	Mild†	Unknown	No
Size of ventral root	Normal	Unknown	Normal
Size of dorsal root	Thin	Unknown	Thin
Diameter of sciatic nerve	Thin‡	Unknown	Thin
Loss of alpha motor neurons in spinal cord	Mild	Mild	No
Attenuates <i>SOD1^{G93A}</i>	Yes	Yes	No

*Tendency to longer time in tail-flick test, but never shown to be statistically significant (Rogers D, EMCF, Martin JE, unpublished data). †Not statistically significant. ‡(Bros V, EMCF and Greensmith L, unpublished data).

in the dorsal root ganglia was greater in lumbar spinal cord than in the cervical region and that this loss was considerably greater for proprioceptive than for nociceptive sensory neurons. Furthermore, there was degeneration of muscle spindles during late embryonic development that was concomitant with the loss of lumbar proprioceptive neurons in *Loa/+* and *Swl/+* mice, and the dorsal roots of the lumbar segments were also thinner than the ventral roots. Chen *et al.* [1] conclude that the early-onset proprioceptive sensory defect is common to *Swl/+* and *Loa/+*, and that this defect, rather than the motor neuron loss, is likely to account for the movement disorder observed in both mice.

The dynein heavy chain and human amyotrophic lateral sclerosis

The new *Swl* mutation may also help us to a better understanding of the possible involvement of dynein in neurodegenerative disease. The devastating human neurodegenerative disorder amyotrophic lateral sclerosis (ALS) involves progressive loss of motor neurons, resulting in complete paralysis and death, usually 3-5 years after diagnosis. The disease strikes people in mid-life and is

inexorable and incurable. Mental faculties are usually spared while the body becomes progressively immobilized. ALS clearly has a genetic component, but as yet only one major-effect gene is known, superoxide dismutase 1 (*SOD1*), which encodes an enzyme that removes free radicals (reviewed in [15]). ALS-associated mutations in *SOD1* are almost all autosomal dominant with high penetrance; the enzymatic activity of the protein generally remains intact and the mutant protein takes on a dominant gain-of-function, which for unknown reasons kills motor neurons.

In working with the mouse as a model system, we have the ability to set up crosses and see what happens. Chen *et al.* [1] made crosses between their *Swl* heterozygotes and a *SOD1^{G93A}* transgenic strain that models human ALS [16], and between *Loa* heterozygotes and the *SOD1^{G93A}* strain. They report that the survival time of the *Loa*, *SOD1^{G93A}* double heterozygotes is increased, as found in our previous work on this cross [14], but that the *Swl*, *SOD1^{G93A}* double heterozygotes had no difference in survival time compared to their *SOD1^{G93A}* littermates [1]. The difference between the effects of the *Loa* and the *Swl* mutations when combined with the *SOD1^{G93A}* transgene is intriguing, and, as *Loa* also

causes loss of motor neurons as well as a sensory neuron defect, one interpretation of these findings is that the different dynein heavy-chain mutations are differentially affecting pathways in different types of neurons.

The ability of the *Loa* and *Cra1* mutations to attenuate the *SOD1^{G93A}* phenotype and extend lifespan [14,16] is still much of a mystery. In the case of *Loa*, the double heterozygotes lived for around 28% longer than their *SOD1^{G93A}* parents and siblings, and, bizarrely, the rate and flux of retrograde axonal transport were actually increased compared with their siblings. Research investigating interactions between cytoplasmic dynein and mutant *SOD1* includes reports of co-localization of dynein components and mutant *SOD1* in ALS mouse models [17], the interaction of mutant *SOD1* proteins with cytoplasmic dynein [18] and perturbation of transport of mitochondria in motor neurons from *SOD1^{G93A}* mice [19].

Given that *Swl* has no detected motor neuron involvement and does not attenuate the effects of the mutant *SOD1* protein, one exciting possibility arising from the new work [1] is that further insight into the different effects of the various dynein heavy-chain mutations may well help our understanding of *SOD1*-related ALS in humans (Table 1). There is at present no obvious explanation from the sites of the *Loa*, *Cra1* and *Swl* mutations in the dynein gene to why two out of three of them affect the *SOD1^{G93A}* phenotype, and the differences between these mice and the molecular mechanisms of each mutation clearly warrant closer examination.

One intriguing question is whether effects on axonal transport in motor neurons is responsible for this differential effect on the *SOD1* mutant phenotype, and a dissection of axonal transport in live *Loa/+* mice would be of great interest in this context. Chen and colleagues [1] suggest that altered Trk signaling may lead to cell death in *Loa/+* and *Swl/+* mice, raising the question of how cell signaling pathways are altered in these mice in sensory and motor neurons. A further question is whether the *Swl*, *Loa* and *Cra1* phenotypes arise from dysfunction of the complete cytoplasmic dynein complex, or from an as yet unknown function of only the heavy chain. It is likely that this huge protein has more functions that we yet know of. Finally, Chen *et al.* [1] have clearly shown that the ubiquitously expressed cytoplasmic dynein heavy chain is essential for the development and function of a subset of neurons in the sensory nervous system. Why this should be remains a mystery. For all those interested in dyneins, axonal retrograde transport, the nervous system and neurodegeneration, there is an exciting road ahead.

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