Review

# Cytoplasmic dynein heavy chain: the servant of many masters☆

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**Cytoplasmic dynein is the main retrograde motor in all eukaryotic cells. This complex comprises different subunits assembled on a cytoplasmic dynein heavy chain 1 (DYNC1H1) dimer. Cytoplasmic dynein is particularly important for neurons because it carries essential signals and organelles from distal sites to the cell body. In the past decade, several mouse models have helped to dissect the numerous functions of DYNC1H1. Additionally, several *DYNC1H1* mutations have recently been found in human patients that give rise to a broad spectrum of developmental and midlife-onset disorders. Here, we discuss the effects of mutations of mouse and human DYNC1H1 and how these studies are giving us new insight into the many critical roles DYNC1H1 plays in the nervous system.**

## Introduction

Cytoplasmic dynein 1 is a large (~1.5 MDa), multisubunit motor complex (Figure 1A) that moves towards the minus end of microtubules in eukaryotic cells [1]. It belongs together with the axonemal dyneins and cytoplasmic dynein 2 to the dynein superfamily. Axonemal dyneins are responsible for the movement of cilia and flagella, whereas cytoplasmic dynein 2 has a role in intraflagellar transport and is required for cilia and flagella assembly [1].

The core of the cytoplasmic dynein 1 complex is the heavy chain (DYNC1H1) dimer (Figure 1A). Each heavy chain is enormous – a half-megadalton protein – and, perhaps unsurprisingly, serves multiple purposes. Towards the N terminal it has a long tail domain with binding sites for other structural and regulatory components of the dynein complex and docking sites for cargoes including adaptor proteins (Figure 1B and Table 1); at the C terminal, DYNC1H1 folds into a daisy-like structure comprising six ATPase domains associated with diverse cellular activities (AAA+) and a microtubule-interacting stalk region [2,3] (Figure 1A,B). This motor domain drives the entire complex and its cargoes along microtubules [2,4], although it is not completely understood how ATP hydrolysis is coupled to force generation or even the total number of ATP molecules bound to DYNC1H1 at any given time [4–6].

DYNC1H1 is highly conserved and is an essential protein in higher eukaryotes because the dynein complex has housekeeping functions in all cells, including orientation of the mitotic spindle, nuclear positioning, Golgi maintenance, and endosomal dynamics [7–9]. In the nervous system, the complex takes on additional roles specific to neurons; driven by the heavy-chain motor, it transports cargoes within dendrites and is the sole motor supporting retrograde transport in axons – carrying signalling complexes that affect gene expression, development, and regeneration, misfolded proteins, and organelles from the synapse back to the cell body. Interestingly, the analysis of motor function in axons revealed that motor proteins, including cytoplasmic dynein, strictly rely for axonal transport on the local synthesis of ATP by glycolytic enzymes, which are bound to transported organelles [10,11], and not on mitochondrial ATP.

In mammals, new insight into DYNC1H1 function came from characterising a set of dynein heavy chain mouse mutations. Now mutations in humans are also being found. Both mouse and human data show that the dynein heavy chain is essential for normal function of the nervous system and even single conservative amino acid substitutions in this > 500-kDa protein can result in neurological abnormalities.

## An allelic series of DYNC1H1 mutants for understanding the role of cytoplasmic dynein in the nervous system

### *Mouse Dync1h1 mutant strains*

A phenotype arising from a single point mutation gives a snapshot of protein function. By working with multiple mutations of one protein, an 'allelic series', we gain a much

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*Keywords:* amyotrophic lateral sclerosis; axonal transport; Cramping 1; Legs at odd angles; motor neurons; neurodegeneration; Sprawling.

0166-2236/$ – see front matter

http://dx.doi.org/10.1016/j.tins.2013.08.001

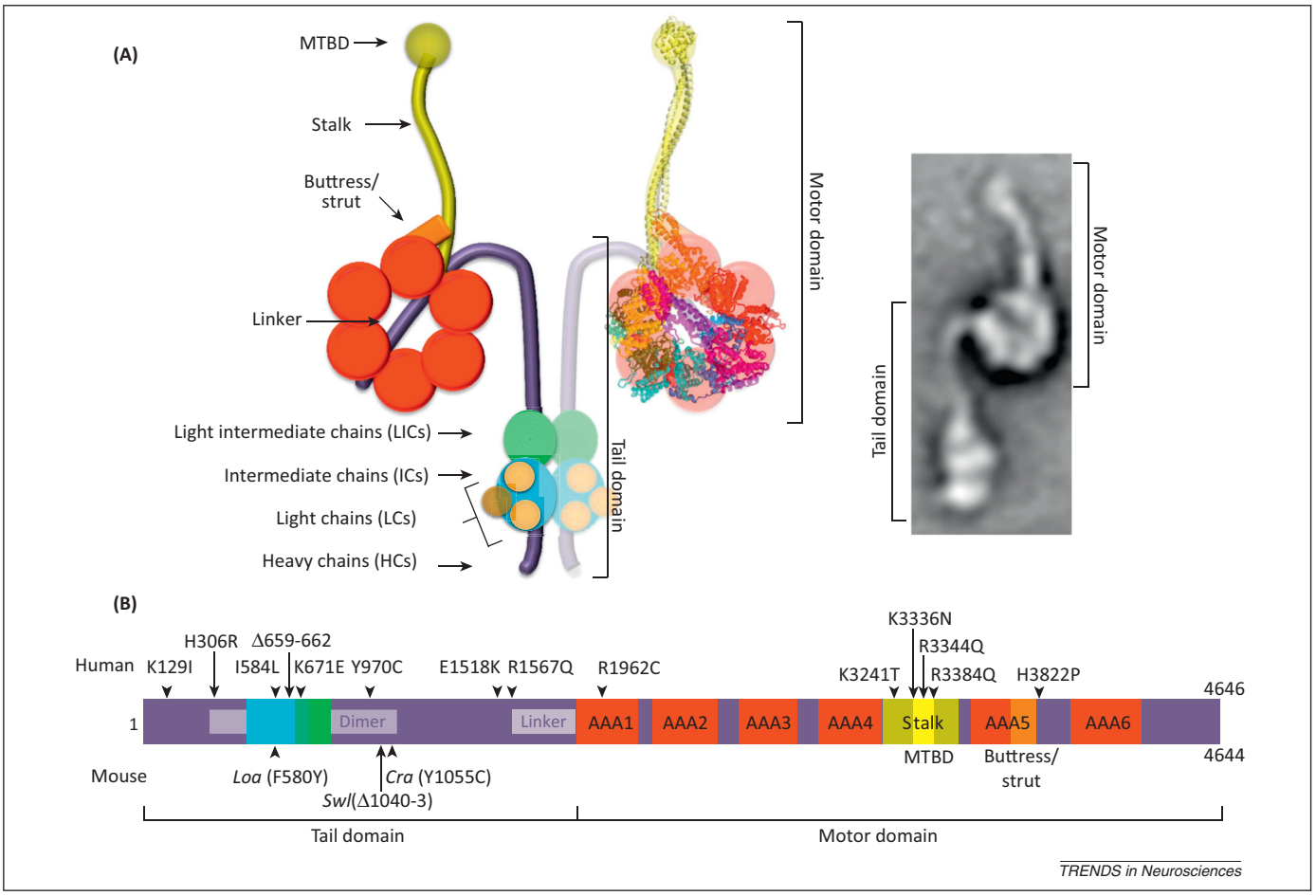


**Figure 1**. The cytoplasmic dynein complex. (**A**) Diagram of the cytoplasmic dynein motor complex including the heavy chain (HC) dimer and its associated subunits. A model of the motor domain [5] built from yeast cytoplasmic dynein (PDB ID 4AKG) and the mouse microtubule-binding domain (MTBD) (PDB ID 3ERR) assembled by Dr A.P. Carter has been overlapped with the schematic of the dynein HC in its apo or post-power stroke form [5,93,94]. Adapted, with permission, from The Company of Biologists (*J. Cell Sci.* 126, 705–713; [4]). The electron micrograph of an isolated molecule of monomeric dynein from *Chlamydomonas reinhardtii* flagella in its pre-power stroke form is shown for comparison on the right. Adapted with permission from Macmillan Publishers (*Nature* 421, 715–718; [93]). Conformational changes driven by ATP hydrolysis in the motor domain, which alter the relative position of the stem and the tail/linker, are hypothesised to lead to the power stroke and progression on microtubules [5,94]. The HCs (in dark violet) contain the six AAA ATPase domains (in red), the stalk region, which includes the MTBD (in dark yellow and yellow, respectively), the buttress (in orange), and the linker region. HCs are associated with light intermediate chains (LICs) (in green), intermediate chains (ICs) (in cyan), and light chains (LCs) (in light yellow). (**B**) Domain composition of the cytoplasmic dynein HC. In addition to the functional domains shown in (A), this scheme displays the homodimerisation region and linker (in white). The positions on the dynein HC of the three mouse mutations (*Loa*, Legs at odd angles; *Cra*, Cramping 1; *Swl*, Sprawling; bottom) and the human mutations discussed in this review (top) are indicated.

richer picture – particularly helpful when trying to dissect the function of a large protein like the cytoplasmic dynein heavy chain.

Although fly and worm laboratories have worked at the molecular level with multiple mutant alleles of the dynein heavy chain for many years, the first mammalian DYNC1H1 mutation came in 1998 with a knockout mouse (Table 2) [12]. In mouse and human, DYNC1H1 appears to be a single isoform encoded by 78 exons [13]. Heterozygous knockout mice had no reported phenotype, but homozygous nulls died early in gestation (<8.5 days) with Golgi apparatus, endosome, and lysosome abnormalities, underlining that DYNC1H1 is an essential protein with housekeeping roles.

Insight into the nervous system function of *Dync1h1* came with three further mutants: Legs at odd angles (*Dync1h1*$^{Loa}$), Cramping 1 (*Dync1h1*$^{Cra1}$), and Sprawling (*Dync1h1*$^{Swl}$) mice [14,15] (Table 2 and Figure 1B). *Dync1h1*$^{Loa}$ mice present a single point mutation from a phenylalanine to a tyrosine (F580Y) in the dynein tail domain. Surprisingly, the addition of a single hydroxyl group onto the 532-kDa protein is enough to give rise to a dominant phenotype [15]. This residue lies within the binding site for dynein intermediate chains (ICs) [16] (Figure 1B) and the homodimerisation region [16,17]. *Dync1h1*$^{Cra1}$ mice have instead a single point mutation at the opposite end of the homodimerisation domain

**Table 1. Cytoplasmic dynein-interacting proteins**[a]

| Protein | Type of binding | Site/subunit | Refs |
|---|---|---|---|
| LIS1 | Direct | DYNC1H1 (AAA3/AAA4 junction)<br>DYNC1H1 (AAA4 arginine finger) | [83] |
| NudE | Indirect | Intermediate chain<br>Light chain – LC8 | [84] |
| Dynactin | Indirect | Intermediate chain | [85] |
| Snapin | Indirect | Intermediate chain | [86,87] |
| Htt | Indirect | Intermediate chain | [88] |
| HAP1 | Indirect | Dynactin | [88] |
| BICD1<br>BICD2 | Indirect<br>Indirect | Dynactin<br>Intermediate chain | [89,90]<br>[91] |

[a]The table shows some of the interactors with the cytoplasmic dynein motor complex involved in CNS development and homeostasis.