Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 326 (2005) 18–22
BBRC
www.elsevier.com/locate/ybbrc

# Prion disease incubation time is not affected in mice heterozygous for a dynein mutation

Majid Hafezparast[a,*], Sebastian Brandner[b], Jackie Linehan[b], Joanne E. Martin[c], John Collinge[a,b], Elizabeth M.C. Fisher[a]

[a] *Department of Neurodegenerative Disease, Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK*

[b] *Medical Research Council Prion Unit, Institute of Neurology, National Hospital for Neurology and Neurosurgery, Queen Square, London WC1N 3BG, UK*

[c] *Department of Histopathology, Neuroscience Centre, Queen Mary University of London, The Royal London Hospital, Whitechapel, London E1 1BB, UK*

Received 25 October 2004

**Abstract**

A mechanism for transmission of the infectious prions from the peripheral nerve ends to the central nervous system is thought to involve neuronal anterograde and retrograde transport systems. Cytoplasmic dynein is the major retrograde transport molecular motor whose function is impaired in the Legs at odd angles (*Loa*) mouse due to a point mutation in the cytoplasmic dynein heavy chain subunit. *Loa* is a dominant trait which causes neurodegeneration and progressive motor function deficit in the heterozygotes. To investigate the role of cytoplasmic dynein in the transmission of prions within neurons, we inoculated heterozygous *Loa* and wild type littermates with mouse-adapted scrapie prions intracerebrally and intraperitonially, and determined the incubation period to onset of clinical prion disease. Our data indicate that the dynein mutation in the heterozygous state does not affect prion disease incubation time or its neuropathology in *Loa* mice.


*Keywords:* Prion disease; PrP; *Loa*; Cytoplasmic dynein; Retrograde transport

Prion diseases are a group of transmissible and fatal neurodegenerative diseases characterised by accumulation of an abnormal isoform of the prion protein ($PrP^{Sc}$) in the central nervous system, widespread neuronal loss, and spongiform change. Prion diseases may be transmitted by a variety of routes, but in variant Creutzfeldt–Jakob disease (vCJD) it is thought that the main route of infection is through the ingestion of prions, followed by prion accumulation in lymphoreticular tissues [1]. Follicular dendritic cells (FDCs) within the spleen have been shown to play a major role in subsequent neuroinvasion by direct or indirect presentation of the infectious agent to the sympathetic nerve endings that innervate the spleen [2,3].

The transmission of $PrP^{Sc}$ from the peripheral nervous system (PNS) to the central nervous system (CNS) is not well understood. However, the direct route of $PrP^{Sc}$ neuroinvasion through PNS resembles some viral infections such as rabies, herpes, and poliomyelitis [4–6]. Upon infection and gaining entry into peripheral nerves, the viral particles utilise neuronal retrograde transport to invade the CNS [7–9]. Indeed, several reports have highlighted the involvement of intracellular retrograde transport of the normal cellular form of prion protein ($PrP^{C}$), raising the possibility that axonal

* Corresponding author. Present address: Department of Biochemistry, School of Life Sciences, University of Sussex, Falmer, Brighton BN1 9QG, UK. Fax: +44 1273 678121.
*E-mail address:* m.hafezparast@sussex.ac.uk (M. Hafezparast).

0006-291X/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2004.10.206

retrograde transport in peripheral nerves may provide the means of prion entry into the CNS [10–13].

Cytoplasmic dynein is the main motor protein involved in axonal retrograde transport. We have previously shown that dominantly inherited motor function deficit in the Legs at odd angles (*Loa*) mouse is caused by a point mutation in the heavy chain subunit of cytoplasmic dynein. Homozygous *Loa*/*Loa* exhibit excessive neurodegeneration and die within a day after birth. Heterozygous *Loa*, however, have a normal life span, albeit succumbing to progressive motor function deficit and an abnormal gait [14,15]. Although the *Loa* mutation does not compromise the housekeeping functions of cytoplasmic dynein, it impairs the cytoplasmic dynein mediated fast retrograde transport in spinal cord motor neurons, as shown by studies of tetanus toxin binding and uptake [14].

We hypothesised that if cytoplasmic dynein is involved in the neuronal retrograde transport of prions from peripheral nerves to the CNS, the incubation times in *Loa* mice should be increased. Here we show that heterozygous *Loa* incubation period from peripheral infection until the onset of clinical symptoms of prion disease is not significantly different from that of their wild type littermates.

## Materials and methods

*Mice.* C57BL/6J mouse strain was obtained from Harlan UK. Breeding and genotyping of the *Loa* mice have been described elsewhere [14,16]. The *Loa* strain was maintained in the C57BL/6J background and the progeny were genotyped to identify heterozygous *Loa* (*Loa*/+) and wild-type (+/+) animals used in this study. *Care of animals was in accordance with the United Kingdom Home Office guidelines.*

*Inoculations, phenotyping, and data analysis.* Chandler/RML mouse adapted scrapie prions was passaged in CD1 Swiss mice (Harlan UK). Brains from these animals were used to generate a 1% homogenate in PBS, which was used as the inoculum for all subsequent experiments. For inoculations, mice were anaesthetised with halothane/$O_2$ and inoculated intracerebrally (i.c.) or intraperitonially (i.p.) with 30 or 100 μl of the inoculum, respectively. They were examined daily and were culled as soon as they showed clinical signs of scrapie or signs of distress. Criteria for clinical diagnosis of scrapie in mice were as described [17]. Incubation times were measured by the number of days from inoculation to the onset of clinical scrapie. The incubation times of each group of i.c. or i.p. inoculated mice were converted to percent scrapie-free animals between 140 and 230 days with 5-day intervals. These values were then compared using the Mann–Whitney test.

*Histopathology.* Mice were culled using $CO_2$ asphyxiation. Mouse brains were fixed in 10% buffered formol saline and then immersed in 98% formic acid for 1 h. The fixed tissues were paraffin wax embedded. Serial sections of 4 μm nominal thickness were cut and pre-treated with autoclaving, formic acid, and 4 M-guanidine thiocyanate. Abnormal PrP accumulation was examined using anti-PrP monoclonal antibody ICSM-35 (D-Gen, UK). Gliosis was detected by a rabbit antiserum against glial fibrillary protein (GFAP). Immunodetection was accomplished on a Ventana automated immunostaining system (www.ventanamed.com). Photographs were obtained on a ColorView II digital camera (www.soft-imaging.de) mounted on a ZEISS Axioplan microscope and composed in Adobe Photoshop.

## Results

Upon peripheral prion infection, the infectious agent accumulates in the lymphoid organs, in particular the FDCs in spleen. Neuroinvasion is believed to occur as a result of a direct uptake of prions by the sympathetic nerve fibres which innervate the spleen, or by an as yet unknown cell-mediated delivery system to the nerve termini of the sympathetic nerves. In either case the internalised prions will have to gain entry into the CNS. One proposed mechanism for such entry is the retrograde transport of prions within the neurons.

As *Loa* mice carry a point mutation in cytoplasmic dynein, which is the main molecular motor involved in retrograde axonal transport, we used prion incubation time as a parameter to test whether the impaired cytoplasmic dynein function in *Loa* would affect the onset of prion disease. As homozygous *Loa* mice die within a day after birth, we inoculated *Loa*/+ and their +/+ littermates with Chandler/RML, intraperitoneally (i.p.) and intracerebrally (i.c.) (Table 1). In addition, as the *Loa* mutation is on a largely C57BL/6J background, we inoculated mice from the C57BL/6J strain to be able to account for any variation in the incubation times due to differences in mouse strains. The i.c. inoculation was set up to provide control groups for each genotype, as in these samples the CNS invasion of infectious prions would be independent of the rate of axonal transport within the peripheral nerves, and thus we would expect to see no significant difference in the incubation times between different i.c. inoculated genotypes.

Table 1
Summary of incubation periods in i.c. and i.p. inoculated mice

| Mice | i.c. incubation period (days ± SD) | | | i.p. incubation period (days ± SD) | | |
|---|---|---|---|---|---|---|
| | Shortest | Longest | Mean | Shortest | Longest | Mean |
| *Loa*/+ | 150 | 173 | 156 ± 9 ($n = 14$) | 173 | 229 | 202 ± 13 ($n = 12$) |
| +/+ | 147 | 173 | 153 ± 8 ($n = 13$) | 188 | 205 | 202 ± 7 ($n = 6$) |
| C57BL/6J | 141 | 151 | 149 ± 3 ($n = 9$) | 173 | 205 | 200 ± 14 ($n = 6$) |

i.c., intracerebrally; i.p., intraperitoneally; SD, standard deviation; and *n*, number of mice showing prion disease clinical symptoms in each group (some mice died of intercurrent illness during the course of this study).

The shortest and longest incubation times for each group of mice are shown in Table 1. As expected, i.c. versus i.p. inoculations overall gave rise to shorter incubation times. There was, however, an overlap between the longest i.c. (173 days) and the shortest i.p. incubation times (also 173 days). Mice inoculated i.c. and i.p. with PBS did not show any signs of prion disease >300 days post-inoculation.

Mean incubation-times and survival plots are shown in Table 1 and Fig. 1, respectively. As expected, there was no significant difference between mean incubation times in i.c. inoculated *Loa*/+ versus +/+ ($P = 0.72$) and C57BL/6J ($P = 0.80$) mice. In addition, there was no significant difference between +/+ and C57BL/6J incubation times ($P = 0.80$).

The mean incubation times in the i.p. inoculated mice were $202 \pm 13$ days, $202 \pm 7$ days, and $200 \pm 14$ days for *Loa*/+, +/+, and C57BL/6J, respectively. Statistical analysis of these values did not show any significant differences in the incubation times between *Loa*/+ versus +/+ ($P = 0.10$) and C57BL/6J ($P = 0.62$) mice. The incubation times in +/+ and C57BL/6J mouse strains were not significantly different either ($P = 0.17$).

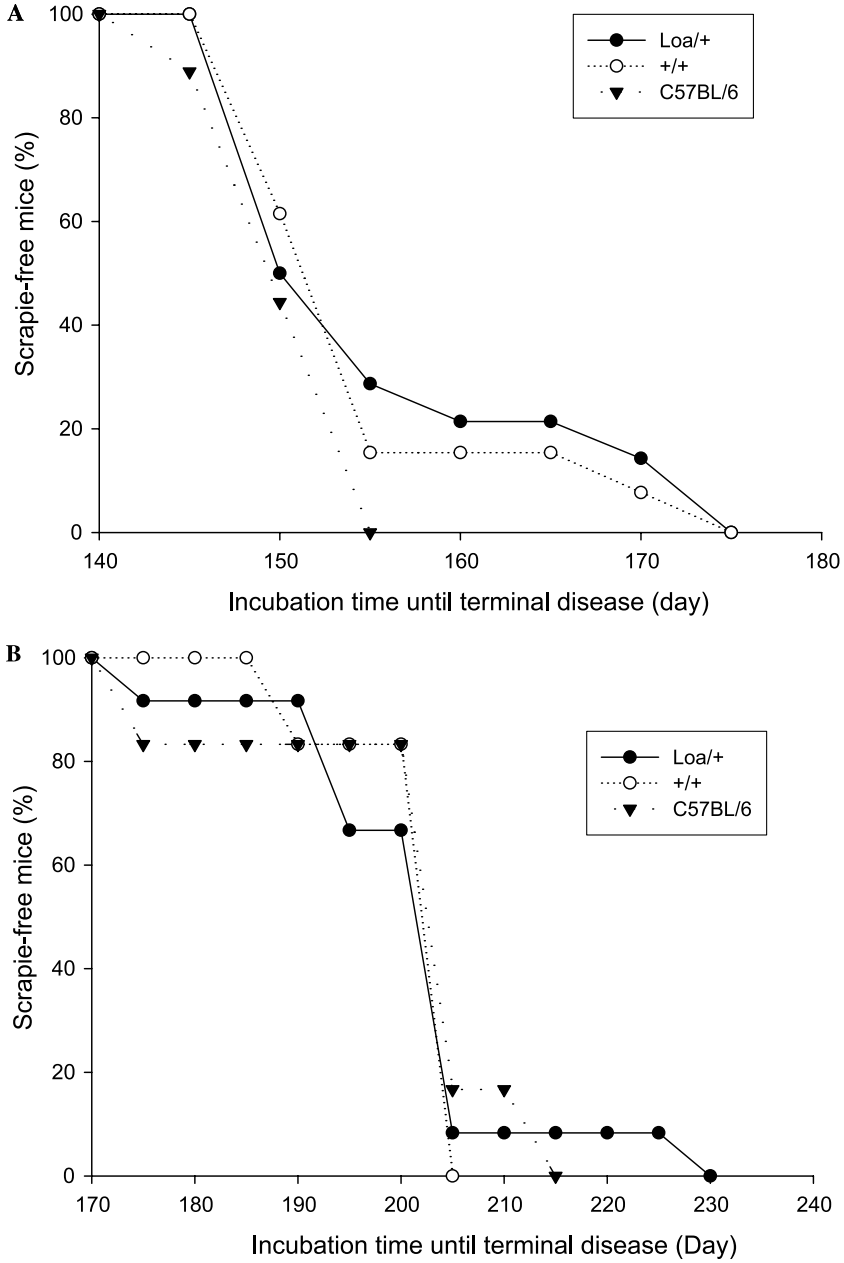


Fig. 1. Survival plots of RML i.c. (A) and i.p. (B) inoculated mice. The incubation times are presented as percentage of sprapie-free mice between 140 and 230 days with 5-day intervals.

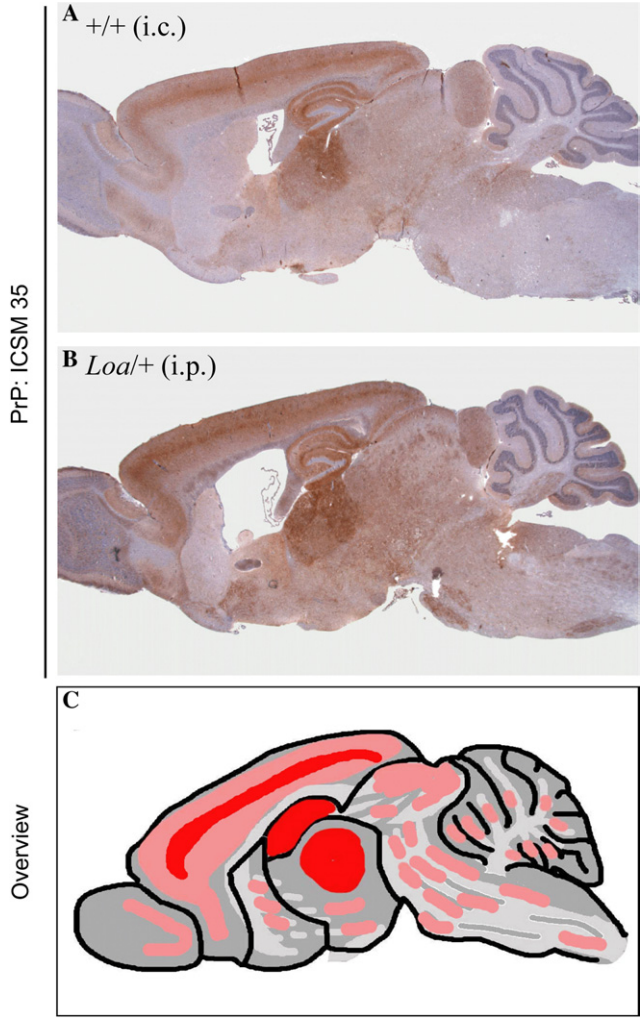


Fig. 2. ICSM-35 immunostained brain sections of RML i.c. and i.p. inoculated +/+ and *Loa*/+ mice, respectively, show prion protein depositions throughout the brain, with a subtle difference between i.c. and i.p. inoculated mice, which may be explained by the route.

We used haematoxylin and eosin staining, and GFAP and ICSM-35 immunostains to analyse the brains of all the i.c. and i.p. inoculated mice. This analysis revealed that all RML-inoculated mice which showed clinical symptoms had spongiosis, gliosis (data not shown), and abnormal prion protein deposition throughout the brain (Fig. 2). However, we did not see any difference between *Loa*/+ and +/+ mice. Although there might be a subtle difference between i.c. and i.p. inoculated mice, there was no difference between the genotypes, indicating that any difference between i.c. and i.p. inoculated groups is explained by the route.

## Discussion

Two mechanisms are likely candidates for the transport of prion within peripheral nerves and into the CNS, where the main pathological damage is caused upon prion infection. One model postulates that prions propagate spatially by a 'domino' mechanism which is initiated when $PrP^{C}$ on the axolemmal surface is converted to $PrP^{Sc}$, upon the entry of the infectious agent into the peripheral nerves [2,18]. The second mechanism may utilise anterograde and retrograde axonal transport involving the molecular motor kinesins and cytoplasmic dynein.

Several in vitro studies have implicated anterograde and retrograde transport mechanisms in trafficking $PrP^{C}$, but the roles of these pathways in the delivery of prions from the PNS to the CNS are unclear [10,12,13]. A mouse model such as *Loa*, which exhibits impaired retrograde transport as a result of a mutation in cytoplasmic dynein, could therefore provide a tool to test the extent of involvement of retrograde transport in the spread of prions from the periphery to the CNS. In order to test our hypothesis that the dynein mutation would affect the speed of prion transport to the CNS and subsequently delay the disease onset, we i.p. inoculated *Loa*/+ and +/+ littermates with prions and showed that there was no difference between incubation times or neuropathology of the mutant versus wild type littermates. This may suggest that the dynein mediated retrograde axonal transport does not play significant role in $PrP^{Sc}$ transport. In this study, however, we were limited to use the *Loa*/+ heterozygotes, as homozygous *Loa*/*Loa* die within a day after birth. The absence of significant difference in incubation times may therefore reflect the limitation of this system, as the impaired retrograde transport in *Loa*/+ mice may not reach the threshold levels to influence the incubation times and to cause delay in disease onset. We note that significant impairment of retrograde transport was seen in *Loa*/*Loa* neurons only, and while there was a trend towards impaired retrograde transport in *Loa*/+ embryonic neurons, this was not statistically significant. In addition, it is possible that i.p. injected prions may gain entry to CNS via systems other than the peripheral nerves.

Cytoplasmic dynein is required for targeting of developing neurons and homozygous mice with a null mutation in dynein die in utero [19]. Homozygous *Loa*/*Loa* mutants of dynein on the other hand are born but die shortly after birth making the use of these mice impractical in analysis of the role of dynein in $PrP^{Sc}$ transmission from periphery to the CNS [14].

LaMonte et al. [20] have generated a transgenic mouse to investigate the role of cytoplasmic dynein in motor neuron degeneration. The dynein in this mouse is specifically disrupted in motor neurons by postnatal overexpression of dynamitin. The lower expressing lines of this mouse do not show pathological symptoms of motor neuron degeneration until 14–18 months of age. It may therefore be possible to i.p. inoculate these mice and investigate the role of cytoplasmic dynein in prion disease before they develop motor neuron degeneration.

The disruption of cytoplasmic dynein in these mice, however, is limited to motor neurons making them less attractive for such an analysis. A transgenic mouse with a targeted postnatal disruption of its neuronal dynein would therefore be a more powerful tool to study the involvement of dynein mediated retrograde axonal transport in $PrP^{Sc}$ pathogenesis.

## Acknowledgments

We thank C. Brown, D. Moore, and M. Desbuslais for technical assistance. This work was supported by the UK Medical Research Council and The Motor Neuron Disease Association.

## References

[1] A.F. Hill, R.J. Butterworth, S. Joiner, G. Jackson, M.N. Rossor, D.J. Thomas, A. Frosh, N. Tolley, J.E. Bell, M. Spencer, A. King, S. Al-Sarraj, J.W. Ironside, P.L. Lantos, J. Collinge, Investigation of variant Creutzfeldt–Jakob disease and other human prion diseases with tonsil biopsy samples, Lancet 353 (1999) 183–189.

[2] M. Glatzel, F.L. Heppner, K.M. Albers, A. Aguzzi, Sympathetic innervation of lymphoreticular organs is rate limiting for prion neuroinvasion, Neuron 31 (2001) 25–34.

[3] M. Prinz, M. Heikenwalder, T. Junt, P. Schwarz, M. Glatzel, F.L. Heppner, Y.X. Fu, M. Lipp, A. Aguzzi, Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion, Nature 425 (2003) 957–962.

[4] R. Etessami, K.K. Conzelmann, B. Fadai-Ghotbi, B. Natelson, H. Tsiang, P.E. Ceccaldi, Spread and pathogenic characteristics of a G-deficient rabies virus recombinant: an in vitro and in vivo study, J. Gen. Virol. 81 (2000) 2147–2153.

[5] M.J. Tomishima, G.A. Smith, L.W. Enquist, Sorting and transport of alpha herpesviruses in axons, Traffic 2 (2001) 429–436.

[6] S. Ohka, A. Nomoto, Recent insights into poliovirus pathogenesis, Trends Microbiol. 9 (2001) 501–506.

[7] S. Mueller, X. Cao, R. Welker, E. Wimmer, Interaction of the poliovirus receptor CD155 with the dynein light chain Tctex-1 and its implication for poliovirus pathogenesis, J. Biol. Chem. 277 (2002) 7897–7904.

[8] N. Poisson, E. Real, Y. Gaudin, M.C. Vaney, S. King, Y. Jacob, N. Tordo, D. Blondel, Molecular basis for the interaction between rabies virus phosphoprotein P and the dynein light chain LC8: dissociation of dynein-binding properties and transcriptional functionality of P, J. Gen. Virol. 82 (2001) 2691–2696.

[9] H. Raux, A. Flamand, D. Blondel, Interaction of the rabies virus P protein with the LC8 dynein light chain, J. Virol. 74 (2000) 10212–10216.

[10] J. Ma, R. Wollmann, S. Lindquist, Neurotoxicity and neurodegeneration when PrP accumulates in the cytosol, Science 298 (2002) 1781–1785.

[11] F. Beranger, A. Mange, B. Goud, S. Lehmann, Stimulation of PrP(C) retrograde transport toward the endoplasmic reticulum increases accumulation of PrP(Sc) in prion-infected cells, J. Biol. Chem. 277 (2002) 38972–38977.

[12] K.L. Moya, R. Hassig, C. Creminon, I. Laffont, L. Di Giamberardino, Enhanced detection and retrograde axonal transport of PrPc in peripheral nerve, J. Neurochem. 88 (2004) 155–160.

[13] N.S. Hachiya, K. Watanabe, M. Yamada, Y. Sakasegawa, K. Kaneko, Anterograde and retrograde intracellular trafficking of fluorescent cellular prion protein, Biochem. Biophys. Res. Commun. 315 (2004) 802–807.

[14] M. Hafezparast, R. Klocke, C. Ruhrberg, A. Marquardt, A. Ahmad-Annuar, S. Bowen, G. Lalli, A.S. Witherden, H. Hummerich, S. Nicholson, P.J. Morgan, R. Oozageer, J.V. Priestley, S. Averill, V.R. King, S. Ball, J. Peters, T. Toda, A. Yamamoto, Y. Hiraoka, M. Augustin, D. Korthaus, S. Wattler, P. Wabnitz, C. Dickneite, S. Lampel, F. Boehme, G. Peraus, A. Popp, M. Rudelius, J. Schlegel, H. Fuchs, M. Hrabe de Angelis, G. Schiavo, D.T. Shima, A.P. Russ, G. Stumm, J.E. Martin, E.M. Fisher, Mutations in dynein link motor neuron degeneration to defects in retrograde transport, Science 300 (2003) 808–812.

[15] D.C. Rogers, J. Peters, J.E. Martin, S. Ball, S.J. Nicholson, A.S. Witherden, M. Hafezparast, J. Latcham, T.L. Robinson, C.A. Quilter, E.M. Fisher, SHIRPA, a protocol for behavioral assessment: validation for longitudinal study of neurological dysfunction in mice, Neurosci. Lett. 306 (2001) 89–92.

[16] A.S. Witherden, M. Hafezparast, S.J. Nicholson, A. Ahmad-Annuar, N. Bermingham, D. Arac, J. Rankin, M. Iravani, S. Ball, J. Peters, J.E. Martin, D. Huntley, H. Hummerich, M. Sergot, E.M. Fisher, An integrated genetic, radiation hybrid, physical and transcription map of a region of distal mouse chromosome 12, including an imprinted locus and the 'Legs at odd angles' (*Loa*) mutation, Gene 283 (2002) 71–82.

[17] G.A. Carlson, D.T. Kingsbury, P.A. Goodman, S. Coleman, S.T. Marshall, S. DeArmond, D. Westaway, S.B. Prusiner, Linkage of prion protein and scrapie incubation time genes, Cell 46 (1986) 503–511.

[18] R.H. Kimberlin, S.M. Hall, C.A. Walker, Pathogenesis of mouse scrapie. Evidence for direct neural spread of infection to the CNS after injection of sciatic nerve, J. Neurol. Sci. 61 (1983) 315–325.

[19] A. Harada, Y. Takei, Y. Kanai, Y. Tanaka, S. Nonaka, N. Hirokawa, Golgi vesiculation and lysosome dispersion in cells lacking cytoplasmic dynein, J. Cell Biol. 141 (1998) 51–59.

[20] B.H. LaMonte, K.E. Wallace, B.A. Holloway, S.S. Shelly, J. Ascano, M. Tokito, T. Van Winkle, D.S. Howland, E.L. Holzbaur, Disruption of dynein/dynactin inhibits axonal transport in motor neurons causing late-onset progressive degeneration, Neuron 34 (2002) 715–727.