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News & Views

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More is not always better: hyperglutamylation leads to neurodegeneration

Anna Akhmanova[1] & Casper C Hoogenraad[1,2]

Post-translational modifications of tubulin can regulate the dynamics and mechanical properties of microtubules and their interactions with different proteins, such as molecular motors. Two studies now demonstrate that excessive accumulation of a specific modification, polyglutamylation, leads to neurodegeneration in mice and humans, likely due to defects in axonal microtubule-based transport.

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See also: **MM Magiera *et al*** (December 2018) and **V Shashi *et al*** (December 2018)

Microtubules are cytoskeletal filaments controlling many aspects of cell architecture, such as cell shape and polarity, as well as the distribution and transport of various organelles. To support very diverse cellular functions, microtubule structure, organization, and dynamics can be adapted between cell types, developmental stages, and even different microtubule subsets within the same cell. Such microtubule specialization relies on the differential use of tubulin isotypes, microtubule-associated proteins (MAPs), and post-translational modifications of tubulin.

An important tubulin modification is (poly)glutamylation, the addition of polyglutamate tails to the C-termini of α-tubulin or β-tubulin (Magiera *et al*, 2018b). Polyglutamylation is initiated by adding a glutamic acid residue to the γ-carboxyl group of a gene-encoded glutamate. The new branch of the polypeptide chain can then be elongated by further glutamate addition. Branch formation and extension are catalyzed by different members of the tyrosine tubulin ligase-like (TTLL) family of enzymes, which have different specificities (Magiera *et al*, 2018b). Glutamylation is reversible—glutamate residues can be removed by the members of cytosolic carboxypeptidase (CCP) family (Rogowski *et al*, 2010). Tubulin is the major target of this post-translational modification, but multiple other substrates are known (van Dijk *et al*, 2008).

Polyglutamylation affects the structure and increases the negative charge of the intrinsically disordered tubulin tails, which are important for microtubule interactions with a broad variety of MAPs. Tubulin polyglutamylation is detected on spindle and midbody microtubules during cell division and is strongly enriched on centrioles, cilia, and neuronal microtubules (Magiera *et al*, 2018b). The function of this modification can vary depending on the cell type and cellular compartment; for example, it is required for the motility and function of cilia (Magiera *et al*, 2018b).

The first indication that the levels of polyglutamylation are critical for neuronal function and survival came from the studies of the *Purkinje cell degeneration* (*pcd*) mouse model (Rogowski *et al*, 2010). This mouse lacks functional CCP1, and this defect leads to degeneration of cerebellar Purkinje cells. Since CCP1 is a negative regulator of polyglutamylation, this work showed that levels of this modification appear to be critical for neuronal survival. However, many important questions remained unanswered. CCP1 removes not only polyglutamate chains, but also gene-encoded C-terminal acidic residues from different proteins, including tubulin (Tanco *et al*, 2015; Magiera *et al*, 2018b), and the phenotype of the *pcd* mouse might be due to the lack of the latter activity. It also remained unclear whether the sensitivity to CCP levels is a general property of neuronal cells, and if so, what the underlying mechanism would be (Fig 1).

To address these questions, Magiera *et al* now combined conditional Purkinje cell-specific knockouts in genes encoding CCP1 and TTLL1, a major brain polyglutamylase (Magiera *et al*, 2018a). Whereas the loss of CCP1 causes Purkinje cell death soon after birth, the concomitant deletion of TTLL1 fully reversed this defect. TTLL1-deficient mice had strongly reduced levels of polyglutamylation of tubulin. This result leads to several important conclusions. First, the loss of CCP1 activity is toxic due to the increased polyglutamylation and not because of the increased removal of gene-encoded glutamates of tubulin or other proteins. Second, increased but not decreased levels of polyglutamylation compromise neuronal survival. This finding fits with the observation that blocking of polyglutamylation of the predominant α-tubulin isoform in flies does not compromise their viability (Jenkins *et al*, 2017). Third, hyperglutamylation causes neuronal death in a cell-autonomous manner.

If excessive polyglutamylation causes neuronal death, why does not this defect manifest itself in the cerebral cortex and

1 Cell Biology, Department of Biology, Faculty of Science, Utrecht University, Utrecht, The Netherlands. E-mail: a.akhmanova@uu.nl
2 Department of Neuroscience, Genentech, Inc., South San Francisco, CA, USA
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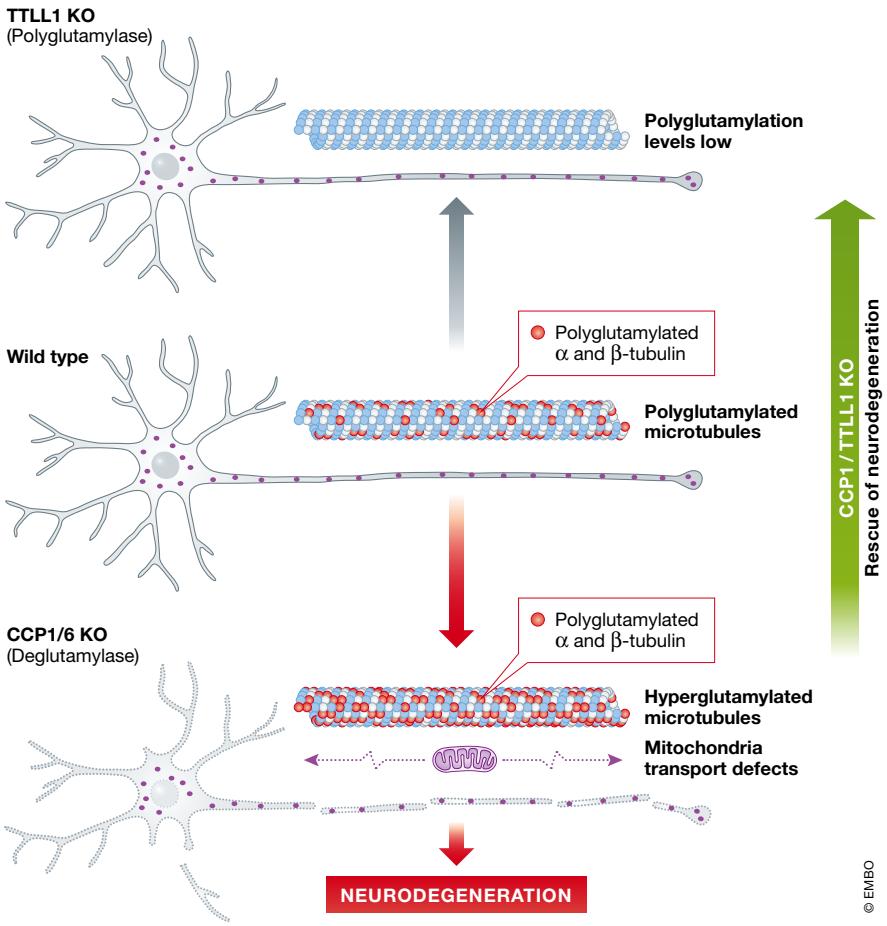

Figure 1. Effects of polyglutamylation on neurodegeneration.
Neurons with reduced deglutamylase activity exhibit defects in mitochondrial motility and degenerate. This effect can be rescued by decreasing polyglutamylase levels, indicating that excessive polyglutamylation, for which microtubules represent a primary target in neurons, is the cause of cell death.

hippocampus? The answer might lie in the fact that the neurons in these brain regions co-express another abundant CCP enzyme, CCP6. Indeed, Magiera *et al* have found that the knockout mouse lacking both CCP1 and CCP6 displayed normal brain development but showed strong signs of progressive neuronal degeneration in cerebral cortex. Sensitivity to increased polyglutamylation seems to be a general neuronal property.

What is the mechanism underlying neuronal cell death in CCP knockout neurons? Histological analysis revealed clear signs of axonal degeneration, whereas the myelin sheets were preserved. A potential candidate to induce this phenotype is spastin —a microtubule-severing enzyme, which is strongly stimulated by microtubule glutamylation (Valenstein & Roll-Mecak, 2016). Magiera *et al* tested spastin involvement but found that spastin knockout did not prevent Purkinje cell degeneration in *pcd* animals, indicating that it is not caused by excessive spastin-mediated microtubule fragmentation. It should be noted, however, that the response of spastin to microtubule glutamylation levels is complex: *in vitro*, tubulin glutamylation can promote the severing activity of the enzyme, but becomes inhibitory after a certain threshold (Valenstein & Roll-Mecak, 2016). Therefore, spastin activity might not be significantly altered or in fact could be decreased in *pcd* mice, a possibility that might be worth exploring since the loss of spastin also causes a neurodegenerative disorder, hereditary spastic paraplegia (Millecamps & Julien, 2013).

Polyglutamylation is also known to affect molecular motors, such as kinesin-1 and flagellar dyneins (Sirajuddin *et al*, 2014; Kubo & Oda, 2017). Therefore, Magiera *et al* next tested whether microtubule-based transport is affected in CCP-deficient cells. Interestingly, they found that a reduction in deglutamylase activity strongly diminished the overall motility of mitochondria in axons, although not the speed or processivity of mitochondria movement. Defects in microtubule-based transport were implicated in many neurodegenerative diseases (Millecamps & Julien, 2013), and it is thus likely that transport perturbation is indeed the cause of neuronal death in CCP knockout cells. In agreement with this view, a very recent study demonstrated that neurons lacking CCP1 have fragmented mitochondria, which exhibit axonal motility defects (Gilmore-Hall *et al*, 2018).

Can these findings be extended to neurodegeneration in humans? Shashi *et al* found biallelic mutations that disrupted CCP1 function and tubulin deglutamylation in 13 patients with infantile-onset neurodegeneration (Shashi *et al*, 2018). A careful comparison of the *pcd* mouse and the pathology observed in human patients showed striking similarities, including abnormalities in the cerebellum, spinal motor neurons, and peripheral nerves. Although one cannot fully discount deglutamylase substrates other than tubulin, these data strongly suggest that an impaired balance in microtubule polyglutamylation has a major effect on neuronal survival in humans. It would be interesting to know whether such imbalance occurs in other neurodegenerative conditions. Since the enzymes controlling this modification are potentially druggable, modulation of their activity might be an interesting avenue for inhibiting neurodegeneration in human patients.

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