Chapter 18

Neuronal Autophagy and Prion Proteins

Audrey Ragagnin, Aurélie Guillemain,
Nancy J. Grant and Yannick J. R. Bailly

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55646

1. Introduction

Protein and organelle turnover is essential to maintain cellular homeostasis and survival. Removing and recycling cell constituents is achieved by autophagy in all cells, including neurons. Autophagy contributes to various physiological processes, such as intracellular cleansing, cellular homeostasis, development, differentiation, longevity, tumor suppression, elimination of invading pathogens, antigen transport to the innate and adaptative immune systems, and counteracting endoplasmic reticulum (ER) stress and diseases characterized by the accumulation of protein aggregates [1]. Autophagy plays a role in a number of infectious and inflammatory diseases, in addition to protein unfolding and misfolding diseases that lead to neuron, muscle and liver degeneration or heart failure [2-4]. Whereas autophagy has long been defined as a form of non-apoptotic, programmed cell death [5], recent findings suggest that autophagy functions primarily to sustain cells, and only defects in autophagy lead to cell death [6].

2. Autophagy in neuronal physiology

Autophagy was initially identified and characterized in a few cell types including neurons. The distinct vacuoles which feature this self-eating process were originally described at the ultrastructural level [7, 8]. The formation of autophagosomes was associated with chromatolysis of a restricted neuroplasm area, free of organelles, but filled with various types of vesicles [9]. The function of autophagy in mature neurons , however, is still debated. In comparison with other organs, rodent brains show high expression levels of autophagy-related (Atg) proteins and low levels of autophagy markers such as autophagosome number and LC3-II. Indeed, even under prolonged fasting conditions, the number of autophagosomes does not

increase in neurons, probably because their nutrient supply from peripheral organs is maintained [10]. However, mice with CNS defects in their autophagic machinery exhibit neurological deficits, such as abnormal limb-clasping reflexes, locomotor ataxia, and lack of motor coordination, in addition to a significant loss of large pyramidal neurons in the cerebral cortex and Purkinje cells (PCs) in the cerebellar cortex [11-13].

Macroautophagy (hereafter referred to as autophagy) is initiated when a portion of the cytoplasm is sequestrated within a double-membrane organelle, the so-called autophagosome [14]. The autophagic machinery has been extensively detailed at the molecular level in a number of reviews including several chapters of this book [14-17]. Atg and several non-Atg proteins have been identified as regulators of key steps leading to the degradation of cytosolic components in lysosomes: initiation and nucleation of phagophores, expansion of autophagosomes, maturation of autophagosomes into amphisomes/autolysosomes, and execution of autophagic degradation [18]. The endosomal sorting complex required for transport (ESCRT) pathway functions in the sorting of transmembrane proteins into the inner vesicles of multivesicular bodies (MVB) during endocytosis. Also it is conceivably an essential part of the basal autophagy process in neurons because ubiquitin- or p62/SQSTM1 (p62)-labelled inclusions and autophagosomes accumulate in neurons deficient in ESCRT components [19]. Increasing evidence suggests that autophagy is regulated in a cell type-specific manner and as such autophagy may serve a distinct function in neurons and may show difference in the molecular machinery underlying basal autophagy (Fig. 1).

2.1. Axonal autophagy

In neurons, autophagy occurs in axons, suggesting that it may be uniquely regulated in this compartment and specifically adapted to local axonal physiology [20]. In primary dorsal root ganglion neurons, autophagosomes initiate distally in nerve terminals and mature during their transport toward the cell soma [21, 22]. In non-neuronal cells, the autophagosomal membrane has multiple possible origins, including endocytosed plasma membrane (amphisome), ER, mitochondria, and trans-Golgi membranes [18, 21, 23- 32]. In contrast, the origin of autophagosomes in the axons is likely to be restricted to the sources of membrane available in the terminals such as smooth ER and plasma membrane [33, 34], excluding rough ER and Golgi dictyosomes.

As observed in ultrastructural studies of axotomized neurons [9, 35], analysis of Purkinje cell (PC) degeneration in lurcher mutant GluRδ2^{Lc} [36] demonstrates autophagosomes in their axonal compartment. In this study, an excitotoxic insult mediated by GluRδ2^{Lc} triggered a rapid and robust accumulation of autophagosomes in dystrophic axonal swellings providing evidence that autophagy is induced in dystrophic terminals and that autophagosome biogenesis occurs in axons [37]. The molecular scenario underlying the initiation of this axonal autophagy is unclear. Liang *et al.* [38] suggested that autophagy in lurcher PCs could be directly activated by an interaction between the postsynaptic GluRδ2^{Lc}, nPIST and beclin 1 an important regulator of autophagy. Nevertheless, how activation of this postsynaptic signaling pathway in dendrites initiates autophagosome formation in axon compartments is uncertain. PC death that is correlated with early signs of autophagy appears to be independent of depolarization

http://dx.doi.org/10.5772/55646

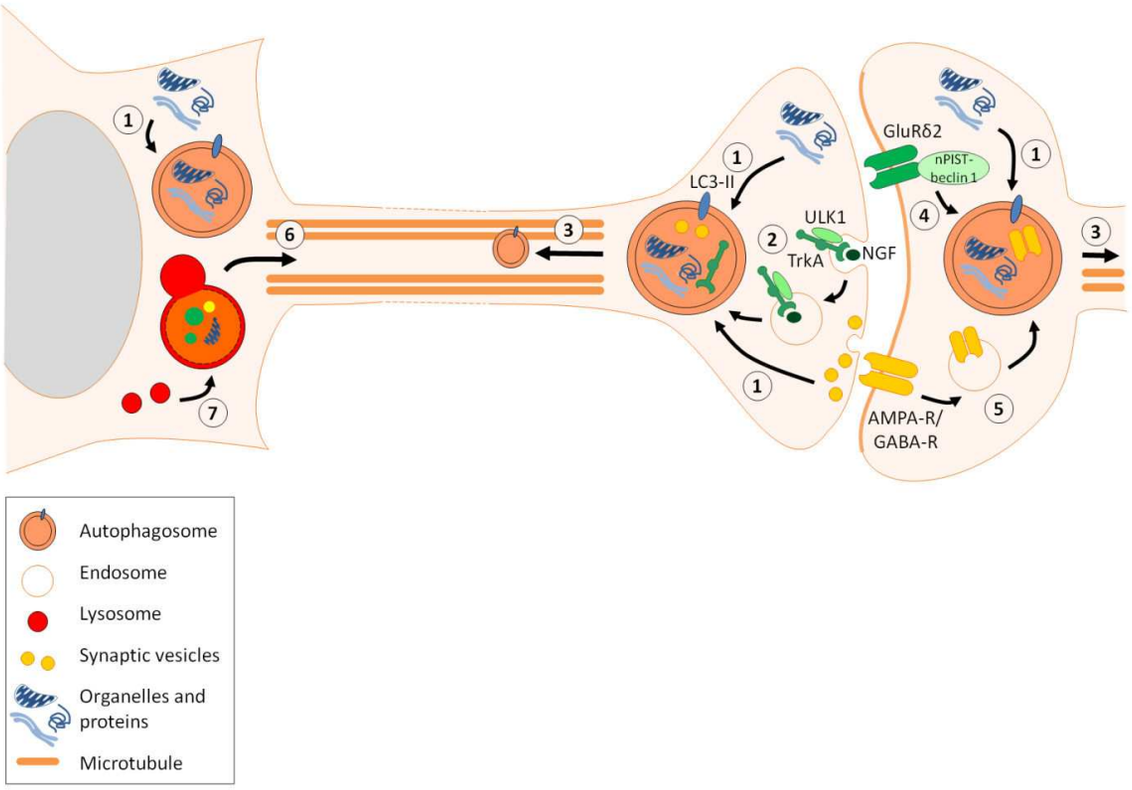

Figure 1. Physiological neuronal autophagy. Autophagy recycles synaptic components to sustain neuronal homeostasis and regulate synaptic plasticity and growth. **1.** Autophagic degradation of organelles, synaptic vesicles and proteins. **2.** ULK1-mediated autophagy of endocytosed NGF-bound TrkA receptors. **3.** Dynein-mediated retrograde transport of autophagosomes. **4.** GluRδ2 activation of beclin 1-dependent autophagy via nPIST. **5.** Targeting of postsynaptic receptors to autophagosomes via endocytosis. **6.** Kinesin-dependent anterograde transport of autophagosomes. **7.** Formation of autophagolysosomes by fusion of autophagosomes with lysosomes.

in the heteroallelic mutant Lurcher/hotfoot bearing only one copy of the lurcher allele and no wild-type GRID2 [39]. However, in the lurcher mutant bearing only one copy of the lurcher allele and one copy of the wild-type GRID2 allele, the leaky channel of GluRδ2^{Lc} depolarizes the neuron and this could transduce an electrical signal to the distal ends causing rapid physiological changes within axons. This effect combined with the local changes in postsynaptic signaling in dendrites may promote autophagosome biogenesis [37, 40].

2.2. Microtubule-dependent dynamics of neuronal autophagy

Previous data indicate that autophagy is a microtubule-dependent process. In cultured sympathetic neurons, autophagosomes formed in the distal ends of axon undergo retrograde transport along microtubules to the cell body where lysosomes that are necessary for the degradation step of autophagy are usually located [21]. Consistent with these observations, prominent retrograde transport of GFP-LC3-labelled autophagosomes has been observed in the axons of primary cerebellar granule cells [36]. In serum-deprived PC12 cells, autolysosomes formed by fusion of autophagosomes with lysosomes move in both anterograde and retro-

grade directions in neurites, and this trafficking requires microtubules [41]. Furthermore, both pharmacological and siRNA-based inhibition of directional microtubule motor proteins kinesin and dynein partially block respectively, anterograde and retrograde neuritic transport of autophagosomes, indicating that they participate in this transport. Recent observations in primary dorsal root ganglionic neurons support a maturation model in which autophagosomes initiate distally, engulfing mitochondria and ubiquitinated cargo, and move bidirectionally along microtubules driven by bound anterograde kinesin and retrograde dynein motors [22]. Fusion with late endosomes or lysosomes may then allow autophagosomes to escape from the early distal pool by robust retrograde dynein-driven motility. The involvement of the dynein-dynactin complex in the movement of autophagosomes along microtubules to lysosomes has also been demonstrated in non-neuronal cells [42]. Consistent with the formation of an autolysosomal compartment, autophagosomes increasingly acidify as they approach the cell soma, thereby fueling the catalysis of the degradation of their engulfed contents. Fully acidified autolysosomes undergo bidirectional motility suggesting reactivation of kinesin motors [22, 41].

The interaction of the autophagic membrane marker Atg8/LC3 with the microtubule-associated protein 1B (MAP1B) [43] implicates microtubule-dependent, axon-specific regulation of autophagosomes. Overexpression of MAP1B in non-neuronal cells reduces the number of LC3-associated autophagosomes without impairing autophagic degradation. The scarcity of LC3-labelled autophagosomes in CNS neurons under normal conditions may be explained by their high expression levels of MAP1B [10, 36]. By modifying microtubule function, the LC3-MAP1B interaction has been proposed to accelerate the delivery of LC3-autophagosomes to lysosomes, thereby promoting efficient autophagic turnover [37]. The exact mechanism underlying the involvement of microtubule in autophagosome formation, as well as targeting and fusion with lysosomes is open to debate [44, 45]. Based on (*i*) the absence of obvious changes in LC3 autophagosomes when they are associated with phosphorylated MAP1B-P, (*ii*) the elevated level of MAP1B-P bound to LC3 in dystrophic terminals containing a large number of autophagosomes [36] and, (*iii*) the conserved role of MAP1B-P in axonal growth and repair during development or injury (which implicates autophagy in remodelling axonal terminals during regeneration) [46], the interactions of LC3 with MAP1B and MAP1B-P have been proposed to represent a regulatory determinant of autophagy in axons under normal and pathological conditions respectively [37].

2.3. Functions of neuronal autophagy

Neurons, as non-dividing cells, are more sensitive to toxic components than dividing cells. Therefore, their survival and the maintenance of their specialized functions under physiological and pathological conditions is crucial requiring a tight quality control of cytoplasmic components and their degradation. Autophagy is believed to be of particular importance in the synaptic compartments of neurons where high energy requirements and protein turnover are necessary to sustain synaptic growth and activity. The CNS displays relatively low levels of autophagosomes under normal conditions, even after starvation, but requires an indispensable turnover of cytosolic contents by autophagy even in the absence of any disease-associated

mutant proteins [10, 47, 48]. The scarcity of immature autophagosomes in neurons is likely to reflect a highly efficient autophagic degradation in the healthy brain. Accordingly, inhibition of autophagy causes neurodegeneration in mature neurons suggesting that autophagy may regulate neuronal homeostasis [11, 12]. For example, abnormal protein accumulation and eventual neurodegeneration are observed in the CNS of mice lacking the *atg5* or the *atg7* genes. This implies that basal autophagy is normally highly active and required for neuronal survival [11, 12]. The cardinal importance of autophagy in central neurons is further supported by recent studies showing a rapid accumulation of autophagosomes in cortical neurons when lysosomal degradation is inhibited. Thus, constitutive autophagy apparently plays an active role in neurons even under nutrient-rich conditions [49, 50].

2.3.1. Axonal homeostasis

Constitutive autophagy is probably essential for axonal homeostasis. Suppression of basal autophagy by either deleting an *atg* gene or inhibiting autophagic clearance in neurons disrupts axonal transport of vesicles destined for lysosomal degradation, and causes axonal swelling and dystrophy [11, 12, 37, 50]. For examples, Atg1/Unc-51 mutants in *C. elegans* show defaults in axonal membranes [51], and Unc-51.1, the murine homologue of Unc-51 is necessary for axonal extension, suggesting a possible role for these proteins in axonal membrane homeostasis [20, 52, 53]. In the cerebellum, neuron-specific deletion of FIP200, a protein implicated in autophagosome biogenesis, causes axon degeneration and neuronal death [13]. Altogether, these data suggest that autophagy is essential to maintain axonal structure and function through retrograde axonal transport [16]. The degree of vulnerability and the formation of intracellular inclusions vary significantly among the different types of CNS neurons in mutant brains deficient in Atg5 or Atg7 suggesting disparate intrinsic requirements for autophagy and relative levels of basal autophagy [20]. For example, while ubiquitinated inclusions are rare in the Atg5- or Atg7-deficient PCs, these cells are among the most susceptible neurons to *Atg 5/7* gene deletion [54, 55]. ULK1, the human homologue of Atg1 is incorporated into the active NGF-TrkA complex after its K-63 polyubiquitination and association with p62 [52, 56]. The subsequent interaction of ULK1 with endocytosis regulators allows trafficking of NGF-bound TrkA receptors into endocytic vesicles [57] providing a possible mechanism of crosstalk between autophagy and endocytosis. By fusing with autophagosomes, some membrane compartments, including endosomes, can be removed from axons and degraded in lysosomes. This process maintains the homeostasis of the axonal membrane networks and as such is essential for axonal physiology [20, 53].

Indeed, dysfunctional autophagy has been implicated in axonal dystrophy. Axonal swellings occur in autophagy-deficient mouse brains [11, 12] and genetic ablation of *Atg7* provokes cell-autonomous axonal dystrophy and degeneration, inferring that autophagy is crucial for membrane trafficking and turnover in axons [53]. In Atg5- or Atg7-deficient PCs, axonal endings exhibit an accumulation of abnormal organelles and membranous profiles much earlier than the somato-dendritic compartment [53, 54]. Axonal degeneration is increasingly believed to precede somatic death by a non-apoptotic auto-destructive mechanism [58, 59]. The “dying-back” progressive retrograde degeneration of the distal axon is a likely model of

the chronic injury observed in neurodegenerative diseases [59]. NGF-deprivation induces autophagosome accumulation in the distal tips of neurites of PC12 cells, and knocking down *Atg7* or *beclin 1* expression delays neurite degeneration of NGF-deprived sympathetic neurons [60]. This suggests that overactive or deficient autophagy contributes to axonal degeneration in a dying-back manner due to the fragility of the axonal tips [20].

2.3.2. Dendritic autophagy

Early autophagosomes have also been observed in dendrites and the cell body of neurons suggesting that axon terminals are not be the only sites where neuronal autophagosomes form, and that autophagy may play a regulatory function in dendrites under physiological and pathological conditions [19]. Along this line, mTOR a key regulator of the autophagic pathway, modulates postsynaptic long-term potentiation and depression, suggesting that autophagy may critically control synaptic plasticity at the postsynaptic, dendritic compartment [61]. Further investigations are required to determine the specific roles of autophagy in dendrites and axons.

Since autophagosomes can fuse with endosomes and form amphisomes, there is a link between autophagy and endocytosis [62]. ESCRT proteins have recently been implicated in normal autophagy [19, 63, 64]. The endocytic pathways, in particular multi-vesicular bodies (MVBs) may serve as critical routes for autophagosomes to reach lysosomes, because defects in ESCRT function prevents fusion or maturation of autophagosomes. The ESCRT-MVB pathway could represent the primary, if not the only, route for delivering autophagosomes to lysosomes in some cell types [20]. In neurons, a large part of the endocytosed cargo merges with the autophagic pathway prior to being degraded by lysosomes [65]. Alterations in ESCRT function have also been linked to autophagy-deficiency in fronto-temporal dementia (FTD) and amyotrophic lateral sclerosis (ALS). In these cases, the particular vulnerability of the neurons appears to be associated with a dysfunction in the autophagosome-MVB pathway in the dendritic compartment [19].

2.3.3. Protein homeostasis

Neurons deficient in Atg5 or Atg7 exhibit an accumulation of polyubiquitinated proteins in inclusion bodies even though the proteasome function is normal, suggesting that basal autophagy prevents spontaneous protein aggregation and plays an essential role in protein clearance and homeostasis in neurons. Such a function is even more critical in neurons expressing disease-related proteins like the aggregate-prone mutant α-synuclein and poly-glutamine-containing proteins [66-68], although how autophagy selectively degrades these disease-related proteins is unclear. The ubiquitin-associated protein p62 is a likely candidate, providing a link between autophagy and selective protein degradation. Indeed, p62 binds numerous proteins through multiple protein-protein interacting motifs, including one for LC3 [55, 56] and the ubiquitin-associated C-terminal domain which binds ubiquitinated proteins. The relationship between p62 and autophagy is further supported by the observation that a marked accumulation of p62 and LC3 occurs only when lysosomes, but not proteasomes, are blocked. Furthermore, p62 protein levels are elevated in autophagy-deficient neurons [36, 55].

This argues that p62 is a specific substrate of autophagic degradation rather than a molecule involved in autophagosome formation since p62-knockout mice display intact autophagosomes and slower protein degradation. Autophagy-deficient cells and neurons accumulate ubiquitin- and p62-positive inclusions, and this accumulation is greatly reduced by ablating p62 [55, 69]. p62 with mutations in the LC3 recognition sequence escape autophagic degradation, leading to the formation of inclusions, whereas those with mutations in the self-oligomerizing domain PB1 are poorly degraded, but no protein inclusions form. Thus increased levels and oligomerization of p62 are required for the formation of inclusion bodies, and their degradation is facilitated by oligomerization. Ubiquitinated aggregates induced by proteasome inhibition are also reduced in p62-deficient cells suggesting that p62 is a general mediator of inclusion formation and normally functions as an adaptator targeting proteins for autophagic degradation [20].

2.3.4. Neuronal autophagy in synapse development, function and remodeling

Neuronal autophagy has been recently shown to play an important role in synapse development. The ubiquitin-proteasome system negatively regulates growth of the neuromuscular junction (NMJ) in *Drosophila* [70] whereas NMJ is positively regulated by neuronal autophagy; a decrease or an increase in autophagy correspondingly affects synapse size [71]. Indeed, an overexpression or a mutation of *Atg1*, a gene involved in autophagy induction, respectively enhanced or decreased NMJ growth. Furthermore, this positive effect of autophagy on NMJ development is mediated by downregulating Hiw, an E3 ubiquitin ligase which negatively regulates synaptic growth by downregulating Wallenda (Wnd), a MAP kinase kinase [70- 72]. Although autophagy is considered as a nonselective bulk degradation process, it can regulate specific developmental events in a substrate-selective mode [73, 74]. In *C. elegans*, when presynaptic afferences are removed from postsynaptic cells, GABAA receptors are selectively targeted to autophagosomes [73]. Accordingly, Hiw could traffic to autophagosomes via a still unknown mechanism, although Hiw could be unselectively degraded by autophagy along with other presynaptic proteins. Interestingly, the synaptic density in mice carrying an atg1 mutation is decreased due to excessive activity of the MAP kinase ERK, suggesting that activated ERK negatively regulates synapse formation and that Atg1 regulates synaptic structure by downregulating ERK activity [75]. As pointed out by Shen and Ganetzky [71], autophagy is a perfect candidate to modulate synaptic growth and plasticity in function of environmental conditions, resulting in plausible consequences in learning and memory.

Autophagy has recently been shown to regulate neurotransmission at the presynaptic level [76]. Besides enhancing protein synthesis via the mTORC1 complex, mTOR activity inhibits autophagy by an Atg13 phosphorylation-induced blockade of Atg1 [77]. In the nervous system, mTORC1 promotes learning and synaptic plasticity dependent on protein synthesis [78- 80]. Conversely, the mTOR inhibitor rapamycin impedes protein synthesis and blocks cell injury-induced axonal hyperexcitability and synaptic plasticity, as well as learning and memory [81, 82]. In prejunctional dopaminergic axons, inhibition of mTOR induces autophagy as shown by an increase in autophagosome formation, and decreases axonal volume, synaptic vesicle number and evoked dopamine release. Similarly, non-dopaminergic striatal terminals also

display more autophagosomes and fewer synaptic vesicles. Conversely, chronic autophagy deficiency in dopamine neurons increases dopaminergic axon size and evoked dopamine release, and promotes rapid presynaptic recovery. Thus mTOR-dependent axonal autophagy locally regulates presynaptic structure and function. In cultured brain slices, the occurrence of autophagosomes in presynaptic terminals isolated from their cell bodies confirms that autophagosomes are locally synthesized [83], and supports the view that this autophagy may serve to modulate presynaptic terminal function by sequestrating presynaptic components [76]. The global stimulating effect of chronic autophagy deficiency on dopaminergic neurons is consistent with the implication of autophagy in neurite retraction of sympathetic neurons *in vitro* [84] and neuritic growth in developing neurons [21].

There are only a few other reports indicating that autophagy may participate in synapse remodeling. In the cerebellar cortex of the (*Bax*$^{-/-}$;*GluRδ2*^{Lc}) double mutant mouse (Fig. 2A), prominent autophagic profiles are evident in parallel fiber terminals subjected to intense remodeling in the absence of the PCs, their homologous target neurons [85]. As mentioned above, endocytosed GABAA receptors are selectively targeted to autophagosomes *in C. elegan*s neurons [73], whereas autophagy promotes synapse outgrowth in *Drosophila* [71]. Autophagy may also modulate synaptic plasticity as recently demonstrated in mammalian hippocampal neurons [61]. Here, neuronal stimulation by chemical LTD induces NMDAR-dependent autophagy by inhibiting the PI3K-Akt-mTOR pathway. Enhanced autophagosome formation in the dendrites and spines of these neurons targets internalized AMPA receptors to lysosomes suggesting that autophagy contributes to the NMDAR-dependent synaptic plasticity required to maintain LTD and assure certain brain functions [61]. A possible mechanism for this formation of autophagosomes and autophagic degradation of AMPARs in dendritic shafts and spines may involve a change in endosome cycling. The formation of more amphisomes due to the fusion of endosomes with autophagosome [86, 87] would reduce the recycling endosome population, and direct more AMPAR-containing endosomes to autophagosomes for lysosomal degradation. Another alternative, but not exclusive actor is p62. This autophagosomal protein is important for LTP and spatial memory [88], interacts with AMPAR and is required for the trafficking of AMPAR [89]. AMPAR via its interaction with p62 would be trapped in autophagosomes as their number increased [61]. mTOR regulates protein turnover in neurons by functioning at the intersection between protein synthesis and degradation. During learning and reactivation in the amygdale and hippocampus, rapamycin inhibition of mTOR has recently been shown to impair object recognition memory [90], implicating signaling mechanisms involved in protein synthesis, synaptic plasticity and cell metabolism in this cognitive function.

2.4. Few autophagosomes, a feature of basal neuronal autophagy

Neurons are highly resistant to large-scale induction of autophagy in response to starvation, probably due to the multiple energy sources available to assure their function [48]. Interestingly, the activity of mTOR, a negative regulator of autophagy is significantly reduced in hypothalamic neurons from mice after a 48h starvation [91], although there are reports that autophagy in neurons can be regulated independently of mTOR [92, 93]. For example, insulin

impairs the induction of neuronal autophagy *in vitro*, but in its absence induction of autophagy is mTOR-dependent. Furthermore, a potent Akt inhibitor provokes robust autophagy [92]. Thus insulin signaling maintains a low level of autophagosome biogenesis in healthy neurons constituting a critical mechanism for controlling basal autophagy in neurons. In addition to insulin signaling, multiple parallel signaling pathways including the mTOR pathway can regulate autophagy in neurons. From these data, Yue and collaborators [20] have proposed that basal autophagy in CNS neurons is regulated by at least two mechanisms: (1) a non-cell-autonomous mechanism whereby regulators (nutrients, hormones and growth factors) are supplied by extrinsic sources (glia, peripheral organs), (2) a cell-autonomous mechanism controlled by intrinsic nutrient-mediated signaling or specific factors expressed in neurons.

Neurons may depend less on autophagy to provide free amino acids and energy under physiological conditions given their quasi exclusive use of blood-born glucose as a source of carbon and energy for protein synthesis. Accordingly, the primary function of neuronal autophagy may be different than a primary response to starvation, and autophagy regulatory mechanisms are likely to be specific in neurons. Furthermore, gender differences in autophagic capacity have been suggested by the faster autophagic response to starvation of cultured neurons from male rats compared to those from females [94]. While *in vivo* evidence of neuronal autophagy mediated by nutrient signaling is still missing, a number of stress-related signals, neuron injuries and neuropathogenic conditions trigger prominent formation and accumulation of autophagosomes in neurons. During this process, neurons may undergo a significant change in autophagy regulation, involving a deregulation that allows neurons to switch from basal level (neuron-specific process featured by a low number of autophagosomes) to an activated state (well-conserved induced autophagy with large-scale biosynthesis of autophagosomes) [20]. Hypoxic-ischemia [95, 96], excitotoxicity [97-99], the dopaminergic toxins, methamphetamine and MPP+ [65, 100, 101], proteasome inhibition [102-104], lysosomal enzyme/lipid storage deficiencies [105-108] are examples of these pathological inducers of neuronal autophagy (see below).

3. Autophagy in neuronal physiopathology

Autophagy normally protects effect against neurodegeneration, but defects in the autophagy machinery are sufficient to induce neurodegeneration. Indeed, neuron-specific disruption of autophagy results in neurodegeneration [11, 12]; for example PC-specific Atg7 deficiency impedes axonal autophagy via an important p62-independent axonopathic mechanism associated with neurodegeneration [55]. Furthermore, specific defects in selective autophagic components or in the cargo selection process can induce neurodegeneration. This hypothesis is supported by the studies of cargo recognition and degradation components, such as p62, NBR1, or ALFY [109, 110]. Defects at any one of the autophagic steps can cause an abnormal accumulation of cytosolic components and lead to disease states. Therefore, each step of the autophagic process needs to be tightly regulated for efficient autophagic degradation.

The housekeeping role of neuronal autophagy is more evident when neurons are loaded with pathogenic proteins [67]. In many neurodegenerative disorders, cytoplasmic, nuclear and

extracellular inclusions composed of aggregated and ubiquitinated proteins are believed to contribute to organelle damage, synaptic dysfunction and neuronal degeneration. The autophagic process in diseased neurons participates in the clearance of abnormal aggregate-prone proteins such as the expanded glutamine (polyQ)-containing proteins (e.g. mutant huntingtin in Huntington's disease (HD)), mutant forms of α-synuclein in familial Parkinson disease (PD), different forms of tau in Alzheimer's disease (AD), tauopathies and FTD, mutant forms of SOD1 in motor diseases such as ALS, and mutant forms of PMP22 in peripheral neuropathies are cleared from diseased neurons by autophagy [19, 20, 55, 56, 66, 67, 111-115]. However, accumulation of these intracellular aggregates is believed to play a significant role in the etiology of neurodegenerative diseases including prion diseases (PrD) [3, 67]. One common feature is the dramatic cyto-pathological accumulation of autophagosomes in injured and degenerating neurons [116-121]. Such signs of defects in autophagy have been interpreted as a result of an "autophagic stress", or in other words an imbalance between protein synthesis and degradation [116]. This has traditionally been viewed as a highly destructive cellular mechanism, driving the cell to death [117]. In these diseases, it is now accepted that autophagy eliminates aggregate-prone proteins and damaged organelles more efficiently than the proteasome machinery. Since the proteasome is unable to degrade them [122], the clearance of misfolded, aggregated proteins originating from neuropathologic deficits is highly dependent on autophagy. However, a blockade of the autophagic flux is likely to impede the clearance of these proteins. The accumulation of aggregated proteins and organelles within the diseased neurons then contributes to cell dysfunction and in the end results in cell death [16], (Fig. 3). Indeed, pharmacological upregulation of autophagy reduces neuronal aggregates and slows down the progression of neurological symptoms in animal models of tauopathy and HD [123], AD [41, 124, 125] and PrD [126, 127].

The mechanisms that determine the activation of autophagy for the removal of aggregated proteins are not clearly understood, but failure of the other proteolytic systems to handle the altered proteins seems to at least partly underlie autophagy activation. Thus oligomers and fibers of particular proteins can block the proteolytic activity of the ubiquitin-proteasome system and chaperone-mediated autophagy (CMA) that results in autophagy upregulation [128, 129]. In addition, sequestration of negative regulators of autophagy in the protein aggregates could also provoke activation of this pathway. Thus it has been shown that blockage of autophagy in neurons leads to the accumulation of aggregated proteins and neurodegeneration even in the absence of aggregate-prone proteins [11, 12]. Although the specific reasons for the failure of the proteolytic systems are unknown, factors such as enhanced oxidative stress and aging seem to precipitate entry into a late failure stage when the activity of all degradation systems are blocked or decreased, leading to accumulation of autophagic vacuoles and aggregates and finally cell death [130].

Autophagy protects against cell death in the case of growth factor withdrawal, starvation and neurodegeneration, but it is required for some types of autophagic cell death [131-134]. However, the role of autophagy as a positive mediator of cell death is not well understood in mammalian systems, although many studies suggest that impaired autophagy sensitizes cells and organisms to toxic insults. Atg1-dependent autophagy restricts cell growth [135]. Cells

http://dx.doi.org/10.5772/55646

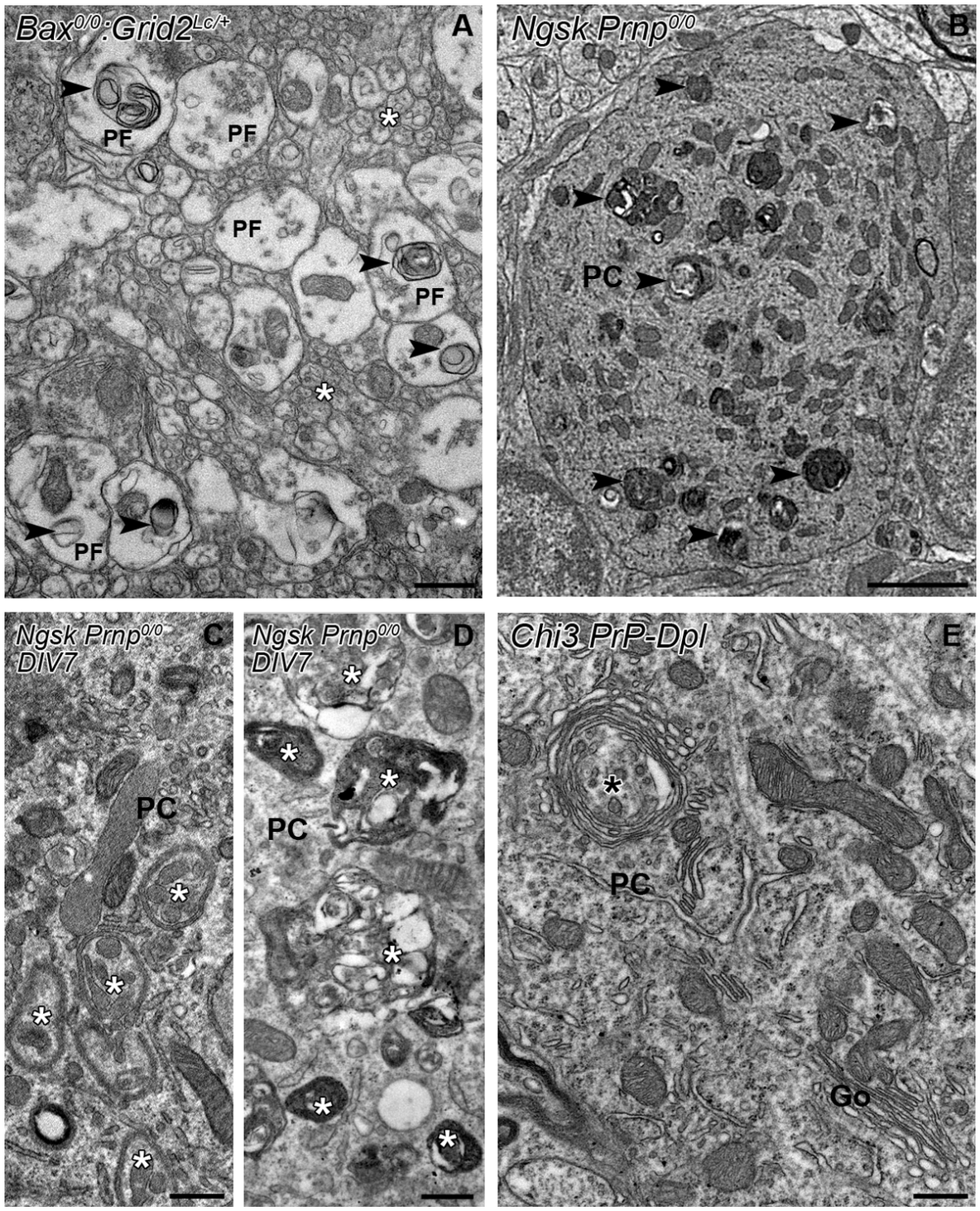

Figure 2. Autophagy in cerebellar neurons.A. Neuronal autophagy in the cerebellum of a Purkinje cell-deficient *Bax*$^{0/0}$*;Grid2*$^{Lc/+}$ double mutant mouse. Autophagic-like profiles (arrowheads) in presynaptic parallel fiber boutons (PF) in the cerebellar molecular layer. * intervaricose parallel fibers. **B.** Autophagolysosomes (arrowheads) characteristic of neuronal autophagy in the soma of a cerebellar Purkinje cell (PC) of a prion protein-deficient Ngsk *Prnp*$^{0/0}$ mouse. **C-D.** Phagophores, autophagosomes (* in C) and autophagolysosomes (* in D) in the soma of cerebellar Purkinje cells (PC) from prion protein-deficient Ngsk *Prnp*$^{0/0}$ mouse maintained 7 days *in vitro* (DIV7) in organotypic culture. **E.** Autophagosome (*) forming from a Golgi dictyosome in the Purkinje cell soma (PC) of a transgenic mouse expressing a neurotoxic Chi3 PrP-Dpl chimera. Go, normal Golgi dictyosome. Scale bars = 500 nm in A, C-E, 2 µm in B.

deficient in Pdk1, a positive regulator of mTOR pathway [136], display autophagy and reduced growth. The increased growth capacity that results from disrupting autophagy may contribute to the tumorigenicity of cells mutant for tumor suppressors [38, 137, 138]. Overexpression of Atg1 leads to apoptotic cell death [135]. Cells undergoing autophagic cell death display signs of apoptosis [139], as do Atg1-null cells [135]. Thus, elevated levels of autophagy promote cell death and the role of autophagy in cell death is likely to be context-dependent.

Neuronal autophagy is currently believed to constitute a protective mechanism that slows the advance of neurodegenerative disorders, and that its inhibition is associated with neurodegeneration [130]. Substantial attention is currently being focused on the molecular mechanisms underlying the autophagic fight against neurodegeneration, the role of autophagy in early stages of pathogenesis and therapeutical approaches to upregulate protective neuronal autophagy. It is unclear whether accumulation of autophagic vacuoles in degenerating neurons results from increased autophagic flux or impaired flux. A chronic imbalance between autophagosome formation and degradation causes "autophagic stress" [140]. Due to obvious therapeutic consequences, it is imperative to understand how autophagic stress occurs in each autophagy-associated neurodegenerative condition: either a cellular incapacity to support an excessive autophagic demand or a defective degradation (lysosomal) step [141].

4. Autophagy in prion diseases

4.1. Prion diseases

4.1.1. Infectious and familial prion diseases

Prion diseases (PrD) are transmissible spongiform encephalopathies (TSEs) which are fatal neurodegenerative diseases in humans (Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker syndrome (GSS), variant CJD (vCJD), fatal familial insomnia (FFI) and kuru) and in animals (bovine spongiform encephalopathy (BSE), transmissible mink encephalopathy (TME), chronic wasting disease (CWD) and scrapie). In humans, PrD manifest after a long incubation period free of symptoms as a rapid progressive dementia that leads inevitably to death. Severe loss of neurons with extensive astrogliosis and moderate microglial activation, characteristic of all TSEs, results in a progressive spongiform degeneration of the brain tissue which is reflected by ataxia, behavioral changes and, in humans, a progressive cognitive decline [142-145]. According to the protein-only hypothesis [146], TSEs are caused by prions that are believed to be proteinaceous infectious particles mainly consisting of PrP^{SC}, an abnormal isoform of the normal, host-encoded prion protein (PrP^{C}), [142]. Prions are able to catalyze a switch from PrP^{C} conformation into an aggregated misfolded conformer PrP^{SC} which collects throughout the brain according to a prion strain-specific anatomo-pathologic signature. These PrDs share a protein misfolding feature with other neurodegenerative diseases (e.g. AD, PD and HD), [147].

The central role played by PrP^{C} in the development of PrD was first illustrated by the observation that disruption of the PrP gene (PRNP) in mice confers resistance to PrD and impairs

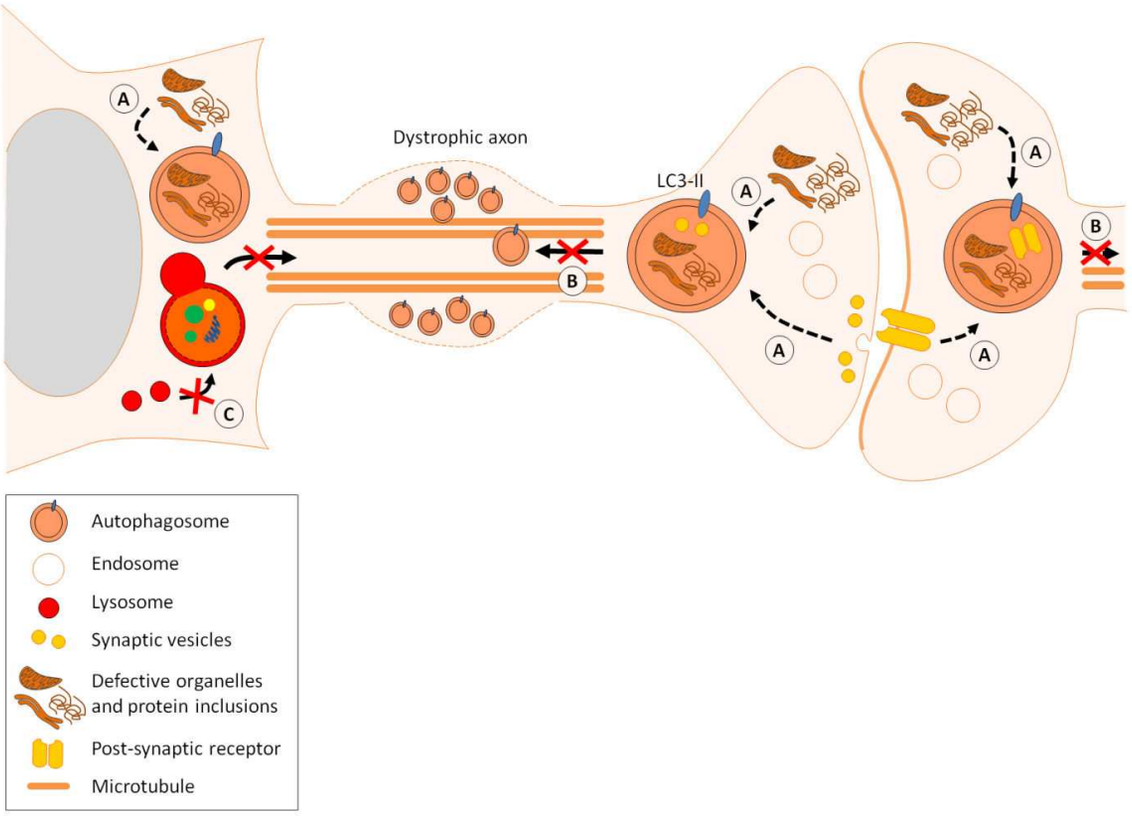

Figure 3. Impaired steps of neuronal autophagy in neurodegenerative disorders.A. Defective autophagosome biogenesis. **B.** Blockage of retrograde transport and accumulation of autophagosomes in dystrophic neurites. **C.** Failure of autophagosomes to fuse with lysosomes.

the propagation of infectious prions [148], while PrP-overexpressing (*tga20*) mice exhibit reduced incubation periods when compared with wild-type mice [149]. Overall, the current data argue for a primary role of the neuronal, GPI-anchored PrP^{C} in prion neuropathogenesis [150].The subversion of PrP^{C} function(s) as a result of its conversion into PrP^{SC} is assumed to account for prion-associated toxicity in neurons [151]. Whether PrP^{SC} triggers a loss of PrP^{C} physiological function (loss-of-function hypothesis) or promotes a gain of toxic activity (gain-of-function hypothesis), or both, is an ongoing debate in the TSE field [152]. Elucidating the roles of PrP^{C} in neurons should help to answer this question. Knockout experiments, however, have failed to reveal any obvious physiological role for PrP^{C}. Mice devoid of PrP^{C} are viable and display only minor phenotypic or behavioural alterations that vary according to the null strain, and hence, these results do not permit one to assign a specific function to PrP^{C}. *Ex vivo* studies support the involvement of PrP^{C} in copper homeostasis [153]. In addition, the localisation of PrP^{C} on the cell membrane and its affinity for the neuronal cell adhesion molecule (N-CAM), laminin, and the laminin receptor [154, 155] have implicated PrP^{C} in cell adhesion. Such properties may reflect the involvement of PrP^{C} in the outgrowth and maintenance of neurites, and even cell survival. Indeed, recent experimental evidence showing that PrP^{C} interacting with β1 integrin controls focal adhesion and turnover of actin microfilaments in neurons substantiates a role for PrP^{C} in neuritogenesis. Of note, integrins are well known

inducers of autophagy (see review in chapter by Nollet and Miranti). Remarkably, during neuronal differentiation, the downregulation of Rho kinase (ROCK) activity by PrP^C is necessary for neurite sprouting [156]. A stress-protective activity has also been assigned to PrP^C based on results obtained with primary neuronal cultures. Neuronal cells derived from PrP-knockout mice are more sensitive to oxidative stress and serum deprivation than wild type cells [157-159]. Moreover, after ischemic brain injury, PrP^C-depleted mice revealed enlarged infarct volumes [160-162]. This neuroprotective role of PrP^C has been linked to cell signaling events. The interaction of PrP^C with the stress inducible protein (STI-1) generates neuroprotective signals that rescue cells from apoptosis [163]. Previous studies of both neuronal and non-neuronal cells substantiate the coupling of PrP^C to signaling effectors involved in cell survival, redox equilibrium and homeostasis (e.g. ERK1/2, NADPH oxidase [164], cyclic AMP-responsive element binding protein (CREB) transcription factor and metalloproteinases [165, 166]. According to these data, PrP^C has been proposed to function as a « dynamic cell surface platform for the assembly of signaling modules » [167]. Despite these overall advances, the sequence of cellular and molecular events that leads to neuronal cell demise in TSEs remains obscure [168, 169]. At present, one envisions that neuronal cell death results from several parallel, interacting or sequential pathways involving protein processing and proteasome dysfunction [170], oxidative stress [159, 171], apoptosis and autophagy [172].

4.1.2. Autophagy in prion-infected neurons

Prion propagation involves the endocytic pathway, specifically the endosomal and lysosomal compartments that are implicated in trafficking and recycling, as well as the final degradation of prions. Shifting the equilibrium between propagation and lysosomal clearance impairs the cellular prion load. This and the presence of autophagic vacuoles in prion diseased neurons [173, 174] suggest a role for autophagy in prion infection (reviewed in [172]). Indeed, the high numbers of autophagic vacuoles observed in neurons from experimentally prion-infected mice (Fig. 4A, B, Fig. 5) and hamsters is indicative of a robust activation of autophagy [175, 176]. Furthermore, autophagic vacuoles and multivesicular bodies have been detected in prion-infected neuronal cells in vitro [177].The formation of autophagic vacuoles has recently been observed in neuronal pericarya, neurites and synapses of neurons experimentally infected with scrapie, CJD and GSS [174], as well as in neuronal synaptic compartments in humans with certain PrD [173]. PrDs are further correlated with autophagy given that the Scrg1 protein (encoded by the scrapie responsive gene1, *Scrg1*) is upregulated in scrapie and BSE-infected brains, as well as in brains of patients with sporadic CJD [178-180] and is associated with neuronal autophagosomes [181, 182]. This Scrg1 protein is thus, a new marker for autophagic vacuoles in prion-infected neurons (Fig. 4A, B). In the brains of CJD and FFI patients and experimentally scrapie-infected hamsters, increased cytoplasmic levels of LC3-II-immunostained autophagosomes have been demonstrated in neurons, again indicating autophagy activation. In addition, the decreased p62 and polyubiquitinated proteins levels in hamster and human brains infected with prion suggest an upregulation of autophagy with enhanced autophagic flux and protein degradation. Downregulation of the mTOR pathway and upregulation of the beclin 1 pathway in these infected tissues provide further evidence of autophagy activation [183]. On the basis of these observations, Xu *et al.* [183] propose that

neuronal autophagy is an intricate element of prion infections. They suggest that once PrP^{SC} enters host cells and is delivered to endosomes, it accumulates in amphisomes via fusion with autophagosomes and then with lysosomes. At this initial stage of infection, PrP^{SC} does not co-localize with autophagosomes, probably because PrP^{SC} levels are too low to be detected due to their rapid degradation in autophagolysosomes. In agreement with this explanation, blocking the fusion of autophagosomes with lysosomes using bafilomycin A1 permits the visualization of PrP-PG14 and PrP^{SC} in autophagosomes [183], as is the case for Aβ1-42 [184].

The role of lysosomes in PrDs is still controversial. Although autophagic lysosomal degradation of PrP^{SC} in infected neurons is supposed to clear prion aggregates and inhibit PrP^{SC} replication, there are indications that PrP^{SC} may subvert the autophagic-lysosomal system to promote the conversion of PrP^{C} into PrP^{SC}. Lysosomal inhibitors prevent the build-up of PrP^{SC} [126] and agonists of the autophagy-lysosome pathway enhance the clearance of PrP^{SC} [185, 186, 126]. However, as PrP^{SC} production increases, the accumulating PrP^{SC} may saturate the clearance capacity of the system causing lysosomal disruption and release of PrP^{SC} aggregates into the neuroplasm. In turn this would cause cell stress and over-activate autophagy, as has been reported in prion-diseased brain tissue [183].

The octapeptide repeats region of PrP^{C} has been shown to negatively influence autophagy. As measured by LC3-II expression, autophagy induced by serum deprivation occurs earlier and to a greater extent in hippocampal neurons from ZH-I *PrnP*$^{-/-}$ compared with those from wild type mice. Reintroduction of PrP^{C}, but not *PrP*^{C} lacking its N-t octapeptide region, into ZH-I $PrnP^{-/-}$neurons delays this upregulation of autophagy [187]. The transconformation of PrP^{C} into PrP^{SC} could interfere with the function of this domain and as a consequence, upregulate autophagy. It is conceivable that the activation of autophagy observed in PrD models reflects a defense mechanism designed to degrade prions and resist oxidative stress. A reduction in autophagy combined with endosomal/lysosomal dysfunction has indeed been proposed to contribute to the development of PrD [188]. Furthermore, the anti-cancer drug imatinib has been shown to activate lysosomal degradation of PrP^{SC} [186] and is a potent autophagy inducer [189]. When administered early during peripheral infection, imatinib delays both PrP^{SC} neuroinvasion and the onset of clinical disease in prion-infected mice [190]. Upregulation of autophagy has beneficial effects on the clearance of aggregate-prone proteins in PrD and other neurodegenerative diseases [66, 111-115, 191, 192]. Both lithium and trehalose enhance PrP^{SC} clearance from prion-infected cells by inducing autophagy, as demonstrated by increases in LC3-II protein and the number of GFP-LC3 puncta [193, 126]. Furthermore, PrP^{SC} can be cleared not only by mTOR-independent autophagy (lithium and trehalose), but also by the mTOR-dependent route because the mTOR inhibitor rapamycin also causes a decrease in cellular PrP^{SC}. Lithium-induced autophagy also reduces PrP^{C} levels. This treatment causes internalization of PrP^{C} [194], and the consequent reduction of available membrane-bound PrP^{C} is known to decrease its conversion into pathologic PrP^{SC} [195-198]. This would provide an additional, indirect way to reduce PrP^{SC} by reducing of PrP^{C} with lithium treatment.

Whether autophagy-inducing compounds are candidates for therapeutic approaches against prion infection has recently been investigated in prion-infected mice. Starting in the last third of the incubation periods, treatment with rapamycin and to a lesser extent with lithium

significantly prolonged incubation times compared to mock-treated control mice [126, 172]. Along this line, activation of the class III histone deacetylase Sirtuin 1 (Sirt1) has been shown to mediate the neuroprotective effect of resveratrol against prion toxicity [199] and prevent prion protein-derived peptide 106-126 (PrP106-126) neurotoxicity via autophagy processing [200]. Moreover, Sirt1-induced autophagy protects against mitochondrial dysfunction induced by PrP106-126, whereas siRNA knockdown of Sirt1 sensitizes cells to PrP106-126-induced cell death and mitochondrial dysfunction. Finally, knockdown of Atg5 decreases LC3-II protein levels and blocks the effect of a Sirt1 activator against PrP106-126-induced mitochondrial dysfunction and neurotoxicity. Thus inducing Sirt1-mediated autophagy may be a principal neuroprotective mechanism against prion-induced mitochondrial apoptosis. Nevertheless, understanding the mechanisms underlying Sirt1-mediated autophagy against prion neurotoxicity and mitochondrial damage merits further investigation, in particular determining the Sirt1-mediated dowstream signaling network, including FOXOs, p53 and PGC-1α. More recently, the mTOR inhibitor and autophagy inducer rapamycin has been shown to delay disease onset and prevent PrP plaque deposition in a mouse model of the Gerstmann-Sträussler-Scheinker PrD [127]. Here, the reduction in symtom severity and prolonged survival correlate with increases in LC3-II levels in the brains of treated mice, suggesting that autophagy induction enhances elimination of misfolded PrP before plaques form. This is in agreement with the well known neuroprotective effects of rapamycin in various models of neurodegenerative diseases with misfolded aggregate-prone proteins (e.g. PD [111], ALS [201], HD [115], spinocerebellar ataxia [66, 202], FTD [203] and AD [41, 124, 125].

4.2. Doppel-expressing prion protein-deficient mice

Research efforts to determine the function of PrP^C using knockout mutant mice have revealed that large deletions in the PrP^C genome result in the ectopic neuronal expression of the prion-like protein Doppel (Dpl) causing late onset degeneration of PCs and ataxia in *PrnP*$^{-/-}$mouse lines, such as Ngsk [204], Rcm0 [205], ZH-II [206] and Rikn [207].

Similar PC degeneration is observed when the N-terminal truncated form of PrP is expressed (ΔPrP) in *Prnp*-ablated mouse lines [208] and when Dpl is overexpressed [209, 210]. Of note, full-length PrP^C antagonizes the neurotoxic effects of both Dpl and ΔPrP [208-212], but not PrP^C lacking the N-terminal residues 23-88 [213]. These results imply that Dpl and ΔPrP induce cell death by the same mechanism, likely by interfering with a cellular signaling pathway essential for cell survival and normally controlled by full-length PrP^C [209, 214]. The mechanism underlying Dpl-induced neurotoxicity is still under debate. PrP-deficient neurons undergo Dpl-induced apoptosis in a dose-dependent, cell autonomous manner [215]. Oxidative stress is a likely candidate to play a role in the death of these neurons because NOS activity is induced by Dpl both in vitro and in vivo [212, 216]. Endogenous, as well as exogenous PrP^C has been shown to inhibit Dpl-induced apoptosis, a neuroprotective function that has been attributed to its BCL-2-like properties [158]. Like BCL-2, PrP^C antagonizes mitochondrial apoptotic pathways, thereby protecting neurons from cell death [217- 219]. In BAX-induced apoptosis [220, 221], PrP^C probably acts by preventing the conformational changes in BAX that are necessary for its activation [222]. In primary cultures, Dpl-induced apoptosis of *Prnp*$^{+/+}$ as

http://dx.doi.org/10.5772/55646

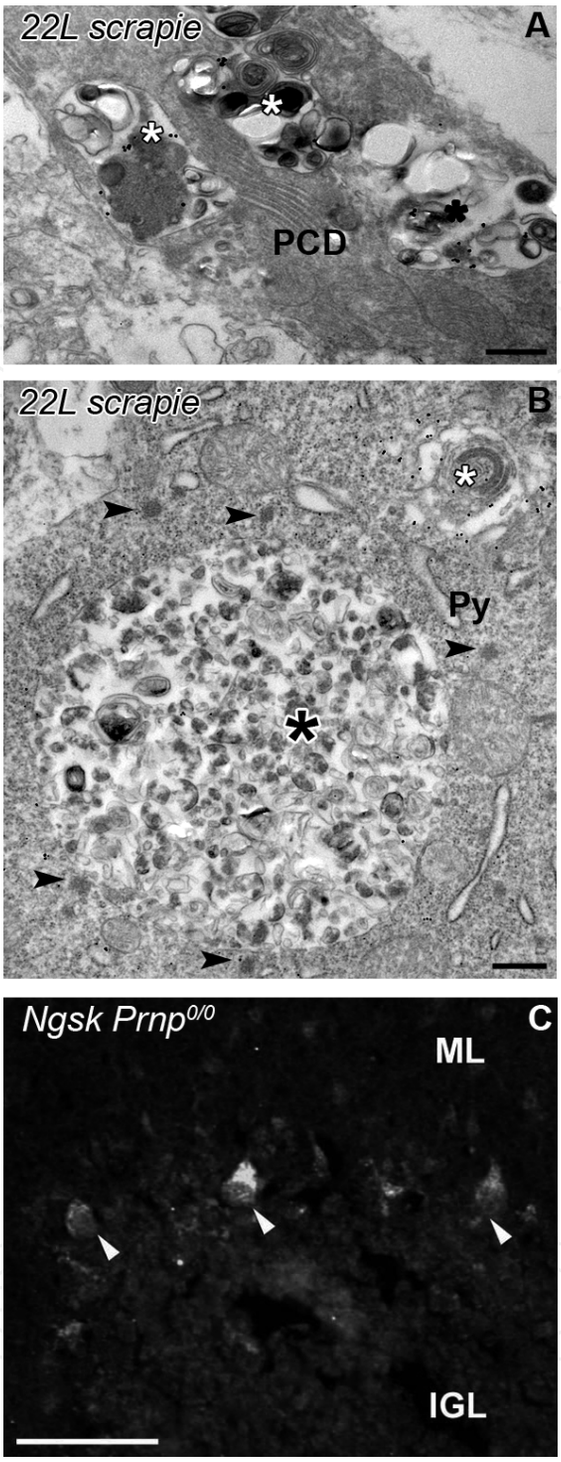

Figure 4. Scrapie responsive gene 1 (Scrg1)-immuno-cytochemistry in prion-infected and prion protein-deficient neurons.A-B. Scrg1 immunogold labeling in central neurons of a clinically ill 22L-scrapie-infected mouse. Scrg1-bound immunogold particles label autophagolysosomes (* in A) in a Purkinje cell dendrite (PCD) and an autophagosome forming from a Golgi dictyosome (white asterisk in B) in the somatic neuroplasm of a pyramidal neuron (Py) of the CA3 field of the hippocampus. In this neuron, lysosomes (arrowheads) and immunogold particles labelling Scrg1 surround a large autolysosome-like vacuole (black asterisk). **C.** Scrg1 immuno-fluorescent labeling of Purkinje cells (arrowheads) in the cerebellar cortex of a prion protein-deficient Ngsk *Prnp*$^{0/0}$ mouse. IGL, internal granular layer; ML, molecular layer. Scale bars = 500 nm in A-B and 50 µm in C.

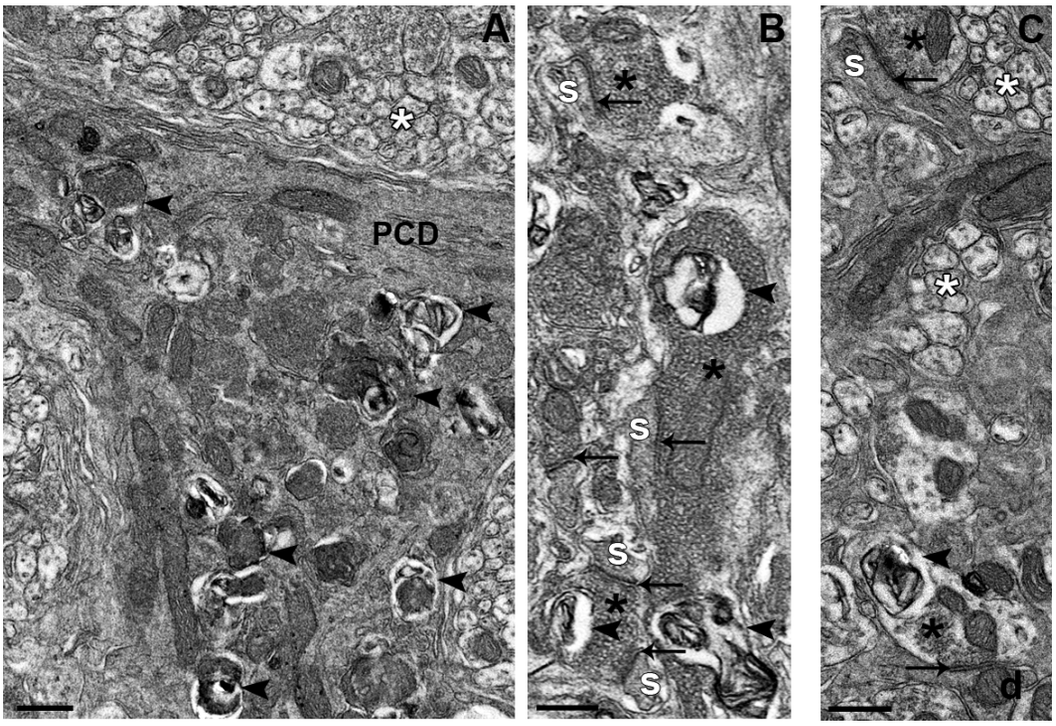

Figure 5. Neuronal autophagy in cerebellar neurons of a clinically ill 22L-scrapie-infected mouse. A. Accumulation of autophagosomes (arrowheads) in a main dendrite of a Purkinje cell (PCD) in the cerebellar molecular layer. *, parallel fibers. **B.** Autophagosomes (arrowheads) in presynaptic axon terminals (black asterisks) establishing synapses (arrows) on postsynaptic Purkinje cell dendritic spines (s). **C.** An intact parallel fiber bouton (black asterisk) makes a synapse (arrow) on a Purkinje cell spine (s) in the upper part of the picture and another parallel fiber bouton (black asterisk) containing an autophagosome (arrowhead) makes a synapse (arrow) on a putative interneuron dendrite (d) in the bottom of the picture. Scale bars = 500 nm.

well as *Prn*P$^{-/-}$ granule cells, has recently been shown to be inhibited by BAX deficiency or pharmacologically blocking caspase-3 suggesting that it is mediated by Bax and caspase-3 [223]. These results further confirm *in vivo* data concerning the effects of Bax expression on PC survival in the cerebellum of the Dpl-overexpressing Ngsk *PrnP*$^{-/-}$mouse that we reported several years ago [224]. In these mice, PC death is already significant as early as 6 months of age. During aging, quantification of PC populations shows that significantly more PCs survived in the Ngsk *PrnP*$^{-/-}$:*Bax*$^{-/-}$ double mutant mice than in the Ngsk *Prn*P$^{-/-}$mice. However, the number of surviving PCs is still lower than wild type levels and less than the number of surviving PCs in *Bax*$^{-/-}$ mutants. This suggests that neuronal expression of Dpl activates both BAX-dependent and BAX-independent pathways of cell death. Interestingly, a partial rescue of Ngsk *PrnP*$^{-/-}$PCs is observed in Ngsk *PrnP*$^{-/-}$*-Hu-bcl-2* double mutant mice, in a proportion similar to that found in Ngsk *PrnP*$^{-/-}$:*Bax*$^{-/-}$ mice, strongly supporting the involvement of BCL-2-dependent apoptosis in Dpl neurotoxicity [225]. The capacity of BCL-2 to apparently compensate for the deficit in PrP^{C} by partially rescuing PCs from Dpl-induced death suggests that the BCL-2-like property of PrP^{C} may counteract Dpl-like neurotoxic pathway in wild-type neurons. Although not exactly identical to BCL-2, PrP^{C} may functionally replace BCL-2 as it decreases in the aging brain [222]. The N-terminal domain of PrP^{C} which is partially homologous to the BH2 domain of BCL-2 family of proteins [226, 227] is probably responsible for the

neuroprotective functions of PrP^C because BAX-induced apoptosis cannot be counteracted by N-terminally truncated PrP. BCL-2 antagonizes the pro-apoptotic effect of BAX by interacting directly with this BH2 domain [228-230], and this domain is missing in both Dpl and the neurotoxic mutated forms of PrP: ΔPrP [208, 214, 231] and Tg(PG14)PrP [232]. Interestingly, expression of Dpl fused to a BH2-containing octapeptide repeat and the N-terminal half of the hydrophobic region of PrP^C makes cells resistant to serum deprivation [233]. Furthermore, N-terminal deleted forms of PrP^C have been reported to activate both BAX-dependant and BAX-independant apoptotic pathways [231].

4.2.1. Autophagy in prion protein-deficient mice

The Dpl-activated, BAX-independent cell death mechanism may involve neuronal autophagy as we have detected the expression of Scrg1, a novel protein with a potential link to autophagy in the Ngsk *PrnP*$^{-/-}$PCs (Fig. 4C), [181]. Both neuronal Scrg1 mRNA and protein levels are increased in prion-diseased brains [179, 180], and Scrg1 is associated with dictyosomes of the Golgi apparatus and autophagic vacuoles in degenerating neurons of scrapie-infected Scrg1-overexpressing transgenic and WT mice (Fig. 4A, B), [181, 182]. Both before and during PC loss, protein levels of Scrg1 and the autophagic markers LC3-II and p62 are increased in Ngsk *PrnP*$^{-/-}$PCs, whereas their mRNA expression is stable, suggesting that the degradation of autophagic products is impaired in these neurons [234, 235]. Autophagic profiles collect in somato-dendritic and axonal compartments of Ngsk *PrnP*$^{-/-}$ (Figs. 2B, 6), but not wild-type PCs. The most robust autophagy occurs in dystrophic profiles of the PC axons in the cerebellar cortex (Fig. 6D) and at their preterminal and terminal levels in the deep cerebellar nuclei (Fig. 6A-C) suggesting that it initiates in these axons. Taken together, these data indicate that Dpl triggers autophagy and apoptosis in Ngsk *PrnP*$^{-/-}$ PCs. As reflected by the abundance of autophagosomes in the diseased Ngsk PCs, Dpl neurotoxicity induces a progressive dysfunction of autophagy, as well as apoptosis. Whether this autophagy dysfunction triggers apoptotic cascades or provokes autophagic cell death independent of apoptosis remains to be resolved. In the Ngsk *PrnP*$^{-/-}$ PCs, the increased expression of LC3-II and p62 at the protein level, without any change in mRNA levels, suggests that the ultimate steps of autophagic degradation are impaired. This is further confirmed by the prominence of autophagolysosomes in these neurons which indicate that the fusion of autophagosomes with lysosomes occurs normally, but downstream, the autophagic flux is blocked.

To further investigate the neurodegenerative mechanisms induced by Dpl in Ngsk cerebellar PCs, we are using an organotypic cerebellar culture system which allows an easier way to approach mechanistic questions than in vivo models [236]. For this purpose, we have assessed the growth and viability of PCs in cerebellar organotypic cultures from Ngsk and ZH-I *PrnP*$^{-/-}$ mice using morphometric methods to measure PC survival and development [237]. The timing and amplitude of PC growth impairment and neuronal death are similar in Ngsk and ZH-I *PrnP*$^{-/-}$ cultures (Fig. 7). In addition, increased amounts of autophagic (LC3-II, Fig. 8) and apoptotic (caspase-3, Fig. 9) markers are detected in protein extracts from both cultures indicating that both apoptosis and autophagy (Fig. 2C, D) contribute to PC death in Ngsk [235] and ZH-I cultures. This suggests that PrP^C -deficiency, rather than Dpl expression, is respon-

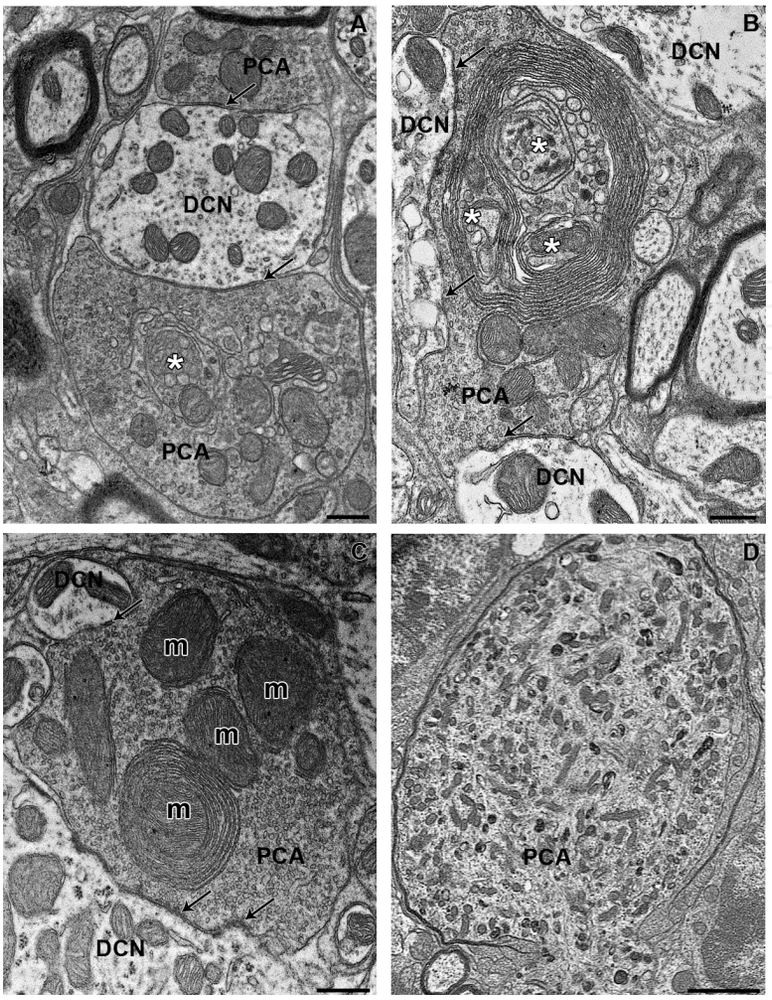

Figure 6. Neuronal autophagy in the cerebellar deep nuclei of a prion protein-deficient Ngsk *Prnp*$^{0/0}$ mouse.A. A presynaptic terminal of a Purkinje cell axon (PCA) establishes symmetric synapses on a postsynaptic dendrite from a neuron of the interpositus deep cerebellar nucleus (DCN) and contains an autophagosome (*). **B.** A double membrane wrap sequesters autophagosomes (*) in a Purkinje cell axon varicosity (PCA) symmetrically synapsing (arrows) on dendrites from neurons of the dentate deep cerebellar nucleus (DCN). **C.** Mitophagy by double membranes wrapping around mitochondria (m) in a Purkinje cell presynaptic axon terminal (PCA) making symmetric synapses (arrows) on postsynaptic dendrites from dentate deep nuclear neurons (DCN). **D.** Dystrophic Purkinje cell axon (PCA) filled with electron-dense autophagic profiles in the cerebellar internal granular layer. Scale bars = 500 nm in A-C, 2 µm in D.

sible for the neuronal growth deficit and loss in these cultures. For presently unknown reasons, the neurotoxic properties of Dpl do not seem to contribute to the degeneration of Ngsk PCs in these organotypic cultures. As the neurotoxicity induced by Dpl takes about 6 months to develop *in vivo*, it is possible that organotypic cultures are not mature enough to model 6-month-old cerebellar tissue. Nevertheless, *ex vivo* cerebellar organotypic cultures do provide a suitable system for analyzing the mechanisms underlying the neurotoxic effects of PrP^C-deficiency and prion infections [238] using pharmacological and siRNA-based approaches.

Our results have shown that PrP^C has a neuroprotective role in cerebellar PCs. As PCs survive *in vivo* in the cerebellum of the ZH-I mouse, the death of the ZH-I PCs in the organotypic

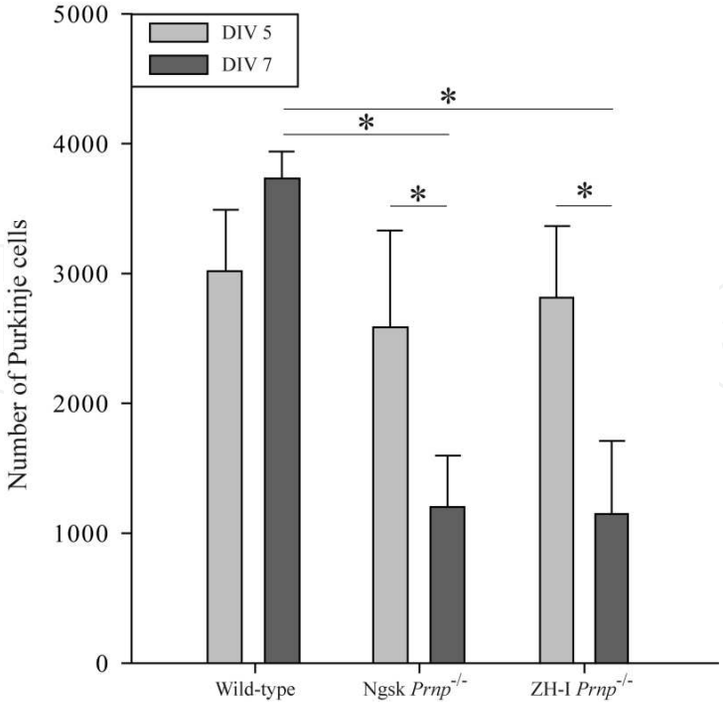

Figure 7. Purkinje cell loss in cerebellar organotypic cultures from wild-type and *Prnp*-deficient mice. PCs stained for calbindin by immuno-fluorescence were counted. This analysis reveals similar, significant reductions in the number of PCs between DIV5 and DIV7 for Ngsk *Prnp*$^{-/-}$ (53.5%) and ZH-I *Prnp*$^{-/-}$ (59%) cultures. During this period, the number of PCs in wild-type cultures is stable ($p > 0.05$). Although the number of PCs is not significantly different between genotypes at DIV5, by DIV7 there are similar decreases in mutant organotypic cultures (Ngsk: 67.8% and ZH-I: 69%) compared to wild-type cultures (two-way ANOVA followed by post-hoc Tukey test; * $p < 0.001$).

cultures is likely to stem from the inherent stress of the *ex vivo* conditions. As mentioned above, PrP^C negatively regulates autophagy as demonstrated by the upregulation of autophagy following serum deprivation in *PrnP*$^{-/-}$ hippocampal neurons when compared to PrP^C-expressing neurons [187]. Recent results suggest that PrP^C can directly modulate autophagic cell death. Using antisens oligonucleotides targeting the *Prnp* transcript, the downregulation of PrP^C expression in glial and non-glial tumor cells induces autophagy-dependent, apoptosis-independent cell death [239]. Previous data have shown that PrP^C acts as a SOD [240] and modulates the activity of Cu/Zn SOD by binding 5 Cu^{++} ions on its N-terminal octapeptide repeat domain [153, 157, 241]. A recent study of the effects of H_2O_2-induced oxidative stress on hippocampal neurons expressing PrP^C or deficient in PrP^C provides further support for the protective role of PrP^C against oxidative stress [242]. Although autophagy and apoptosis occur in both lines, the *Prnp*$^{-/-}$ neurons are less resistant to H_2O_2-induced oxidative stress than the *Prnp*$^{+/+}$ neurons confirming the anti-oxidant activity of PrP^C.

Furthermore, autophagy is more enhanced in *Prnp*$^{-/-}$ neurons than in *Prnp*$^{+/+}$ neurons. In the latter, this is due to H_2O_2-induced enhancement of autophagic flux, and in the former due to H_2O_2-induced impairment of autophagic flux. Similarly, experiments using Atg7 siRNA to inhibit autophagy have revealed that the increased autophagic flux in $Prnp^{+/+}$ neurons protects against H_2O_2 cytotoxicity. Thus a deficiency in Prnp may impair autophagic flux via H_2O_2-

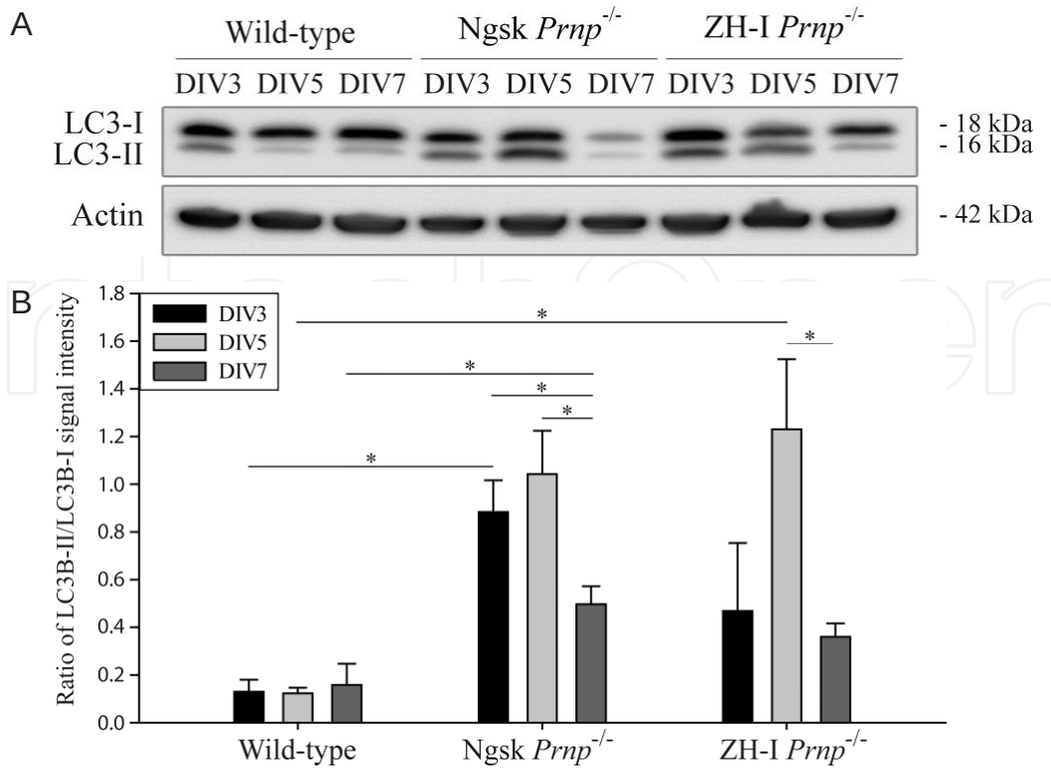

Figure 8. A. Western blot of the autophagic marker LC3B-II, in extracts prepared from organotypic cultures from wild-type, Ngsk and ZH-I *Prnp*$^{-/-}$ mouse cerebellum at DIV3, 5 and 7. Actin was used as a loading control. **B.** Autophagy was measured by quantifying the ratio of the band intensities of LC3B-II and LC3B-I ($n \geq 3$ mice) which reflects the amount of autophagosomes. Compared to wild-type cultures, this ratio increases in mutant cultures at DIV5 suggesting enhanced autophagy and then decreases at DIV7 probably as a result of either autophagic degradation or PC death (Kruskal-Wallis test followed by post-hoc Tukey test; * $p < 0.05$).

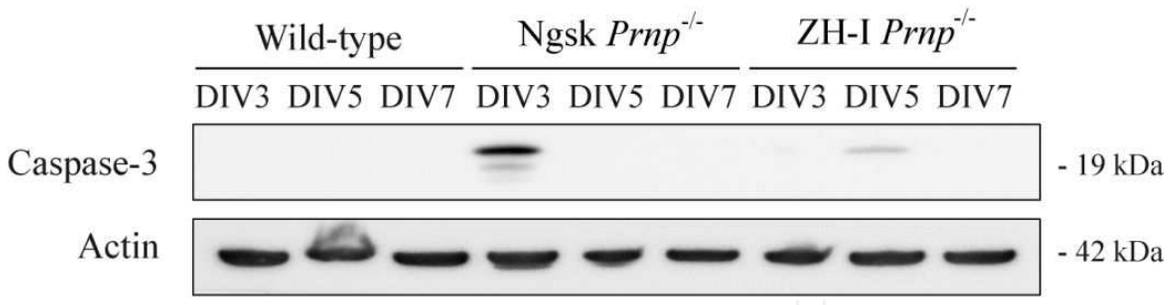

Figure 9. Western blot of the pro-apoptotic activated caspase-3. Activated caspase-3 is detected in extracts of organotypic cultures from Ngsk and ZH-I *Prnp*$^{-/-}$ mouse cerebellum, but not from wild-type mouse cerebellum. Actin serves as a loading control.

induced oxidative stress contributing to autophagic cell death [242]. Since autophagic flux is apparently normal in both *Prnp*$^{+/+}$ and *Prnp*$^{-/-}$ neurons in the absence of stress, the lack of PrP^C only seems to contribute to autophagy impairment under stress-induced conditions, such as H_2O_2 treatment [242], stress-inducing *in vitro* conditions, as well as Dpl-induced toxicity.

4.2.2. Prion protein PrP-doppel Dpl chimeras

When overexpressed ectopically in neurons, mutations within the central region of PrP^C are associated with severe neurotoxic activity, similar to that of Dpl [231, 243]. The absence of these segments, called central domains (CD) is believed to be responsible for neurodegeneration and ataxia. To understand the dual neurotoxicity *vs.* neuroprotective roles of PrP^C, transgenic mice expressing a fusion protein made of the CD of PrP^C inserted within the Dpl sequence have been generated [244]. These mice failed to develop typical Dpl-mediated neurological disorder indicating that this N-terminal portion of PrP^C reduces Dpl toxicity. To further investigate Dpl-like neurotoxicity, Lemaire-Vieille *et al.* recently generated lines of transgenic mice expressing three different chimeric PrP-Dpl proteins [245]. Chi1 (Dpl 1-57 replaced with PrP 1-125) and Chi2 (Dpl 1-66 replaced with PrP1-134) abrogates the pathogenecity of Dpl confirming the neuroprotective role of the PrP 23-134 N-terminal domain against Dpl toxicity. However, when Dpl 1-24 were replaced with PrP 1-124, these Chi3 transgenic mice that express a very low level of the chimeric protein develop ataxia, as early as 5 weeks of age. This phenotype is only rescued by overexpressing PrP^C, and not by a single copy of full-length PrP^C, indicating the strong toxicity of the chimeric protein Chi3. The Chi3 mice exhibit severe cerebellar atrophy with significant granule cell loss and prominent signs of autophagy in PCs (Fig. 2E). We conclude that the first 33 amino acids of Dpl, that are absent in Chi1 and Chi2 constructs, confer toxicity to the protein. This is confirmed *in vitro* by the highly neurotoxic effect of the 25-57 Dpl peptide on mouse embryo cortical neurons. Since this chimeric transgene is not expressed by PCs in the transgenic mice expressing Chi3, the signs of autophagy displayed by these neurons in vitro could result from the neurotoxic effect of the exogenous Chi3 chimeric protein, as well as the deleterious effect of losing their primary afferences (i.e. the granule cells).

5. Perspectives

The beneficial effects that autophagy has on prion infections is currently supported by a growing bulk of evidence from *in vivo* and *ex vivo* data and is strongly promising for future mid-term therapeutic approaches. To further understand the fascinating interplay between autophagy and PrDs, further investigations are necessary to decipher their molecular interactions. Important issues remain. How are the different phases of prion infection physiopathology i.e. propagation, trafficking, recycling and clearance connected with autophagy? Which autophagic pathways are activated by prions - the mTOR-dependent, mTOR-independent or both? The biological function of autophagy per se in prion infection is obscure as the cellular levels of autophagy can apparently modify cell susceptibility to prion infection, although changes in autophagy may be a pre-requisite or a consequence of a prion infection.

Overall, the results point to a need to counteract cell stress and to eliminate toxic aggregate-prone proteins that eventually saturate the usual degradation pathways, including autophagy. These are common features of prion disesase and most of the other neurodegenerative diseases described in this review. Saturation of the autophagic machinery, loss or imbalance of autophagic flux is believed to lead to neurodegeneration. Understanding how autophagy

relates to these diseases is a first step for developing autophagy modulation-based therapies for treating neurological disorders. This implies therapeutic consideration for each type of autophagic default at a precise step of the neurodegenerative disease concerned.

Acknowledgements

The authors are greatly indebted to Dr. Anne-Marie Haeberlé (CNRS UPR3212, Strasbourg) for excellent assistance in transmission electron microscopy and prion-infected tissue handling as well as to Dr. Catherine Vidal (Institut Pasteur, Paris) for intra-cerebellar inoculation of mice with 22L scrapie. A. R. is supported by a doctoral grant from the French Minister of Research and Technology and A. G. is supported by a grant from the French Centre National de la Recherche Scientifique and the AgroParisTech High School.

Author details

Audrey Ragagnin[1], Aurélie Guillemain[1], Nancy J. Grant[2] and Yannick J. R. Bailly[1]

1 Cytologie & Cytopathologie Neuronales, INCI CNRS UPR3212, Université de Strasbourg, Strasbourg, France

2 Trafic Membranaire Dans les Cellules Neurosécrétrices et Neuroimmunitaires, INCI CNRS UPR3212, Université de Strasbourg, Strasbourg, France

References

[1] Mizushima N, Komatsu M. Autophagy: renovation of cells and tissues. Cell (2011) 147:728-41.

[2] Levine B, Deretic V. Unveiling the roles of autophagy in innate and adaptive immunity. Nat Rev Immunol (2007) 7(10):767-77.

[3] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. Cell (2008) 132:27-42.

[4] Mizushima N, Levine B, Cuervo AM, Klionsky DJ. Autophagy fights disease through cellular self-digestion. Nature (2008) 451:1069-75.

[5] Clarke PG. Developmental cell death: morphological diversity and multiple mechanisms. Anat Embryol (1990) 181:195-213.

[6] Kroemer G, Levine B. Autophagic cell death: the story of a misnomer. Nat Rev Mol Cell Biol (2008) 9:1004-10.

[7] Novikoff AB, Beaufay H, De Duve C. Electron microscopy of lysosomerich fractions from rat liver. J Biophys Biochem Cytol (1956) 2:S179-84.

[8] De Duve C. The significance of lysosomes in pathology and medicine. Proc Inst Med Chic (1966) 26:73-6.

[9] Dixon JS. "Phagocytic" lysosomes in chromatolytic neurones. Nature (1967) 215:657-658.

[10] Mizushima N, Yamamoto A, Matsui M, Yoshimori T, Ohsumi Y. In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. Mol Biol Cell (2004) 15:1101-11.

[11] Hara T, Nakamura K, Matsui M, Yamamoto A, Nakahara Y, Suzuki-Migishima R, Yokoyama M, Mishima K, Saito I, Okano H, Mizushima N. Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature (2006) 441:885–9.

[12] Komatsu M, Waguri S, Chiba T, Murata S, Iwata J, Tanida I, Ueno T, Koike M, Uchiyama Y, Kominami E, Tanaka K. Loss of autophagy in the central nervous system causes neurodegeneration in mice. Nature (2006) 441:880-4.

[13] Liang CC, Wang C, Peng X, Gan B, Guan JL. Neural-specific deletion of FIP200 leads to cerebellar degeneration caused by increased neuronal death and axon degeneration. J Biol Chem (2010) 285:3499-509.

[14] Xie Z, Klionsky DJ. Autophagosome formation: core machinery and adaptations. Nat Cell Biol (2007) 9:1102-9.

[15] He C, Klionsky DJ. Regulation mechanisms and signaling pathways of autophagy. Annu Rev Genet (2009) 43:67-93.

[16] Lee J-A. Neuronal autophagy: a housekeeper or a fighter in neuronal cell survival? Exp Neurobiol (2012) 21:1-8.

[17] Klionsky D, et 1268 al. Guidelines for the use and interpretation of assays for monitoring autophagy. Autophagy (2012) 8:445-544.

[18] Mizushima N, Yoshimori T, Ohsumi Y. The role of Atg proteins in autophagosome formation. Annu Rev Cell Dev Biol (2011) 27:107-32.

[19] Lee JA, Beigneux A, Ahmad ST, Young SG, Gao FB. ESCRT-III dysfunction causes autophagosome accumulation and neurodegeneration. Curr Biol (2007) 17:1561-1567.

[20] Yue Z, Friedman L, Komatsu M, Tanaka K. The cellular pathways of neuronal autophagy and their implication in neurodegenerative diseases. Biochim Biophys Acta (2009) 1793:1496-507.

[21] Hollenbeck PJ. Products of endocytosis and autophagy are retrieved from axons by regulated retrograde organelle transport. J Cell Biol (1993) 121:305-15.

[22] Maday S, Wallace KE, Holzbaur EL. Autophagosomes initiate distally and mature during transport toward the cell soma in primary neurons. J Cell Biol (2012) 196:407-17.

[23] Dunn WA Jr. Studies on the mechanisms of autophagy: formation of the autophagic vacuole. J Cell Biol (1990) 110:1923-33.

[24] Axe EL, Walker SA, Manifava M, Chandra P, Roderick HL, Habermann A, Griffiths G, Ktistakis NT. Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum. J Cell Biol (2008) 182:685-701.

[25] Hayashi-Nishino M, Fujita N, Noda T, Yamaguchi A, Yoshimori T, Yamamoto A. A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation. Nat Cell Biol (2009) 11:1433-7.

[26] Ylä-Anttila P, Vihinen H, Jokitalo E, Eskelinen EL. 3D tomography reveals connections between the phagophore and endoplasmic reticulum. Autophagy (2009) 5:1180-5.

[27] Simonsen A & Tooze SA. Coordination of membrane events during autophagy by multiple class III PI3-kinase complexes. J Cell Biol (2009) 186:773-82.

[28] Tooze SA, Yoshimori T. The origin of the autophagosomal membrane. Nat Cell Biol (2010) 12:831-5.

[29] Hailey DW, Rambold AS, Satpute-Krishnan P, Mitra K, Sougrat R, Kim PK, Lippincott-Schwartz J. Mitochondria supply membranes for autophagosome biogenesis during starvation. Cell (2010) 141:656-67.

[30] Van der Vaart A, Reggiori F. The Golgi complex as a source for yeast autophagosomal membranes. Autophagy (2010) 6:800-1.

[31] Yen WL, Shintani T, Nair U, Cao Y, Richardson BC, Li Z, Hughson FM, Baba M, Klionsky DJ. The conserved oligomeric Golgi complex is involved in double-membrane vesicle formation during autophagy. J Cell Biol (2010) 188:101-14.

[32] Ravikumar B, Moreau K, Jahreiss L, Puri C, Rubinsztein DC. Plasma membrane contributes to the formation of pre-autophagosomal structures. Nat Cell Biol 12:747-57. Erratum in: Nat Cell Biol (2010) 12:1021.

[33] Novikoff PM, Novikoff AB, Quintana N, Hauw JJ. Golgi apparatus, GERL, and lysosomes of neurons in rat dorsal root ganglia, studied by thick section and thin section cytochemistry. J Cell Biol (1971) 50:859-86.

[34] Broadwell RD, Cataldo AM. The neuronal endoplasmic reticulum: its cytochemistry and contribution to the endomembrane system. II. Axons and terminals. J Comp Neurol (1984) 230:231-48.

[35] Matthews MR, Raisman G. A light and electron microscopic study of the cellular response to axonal injury in the superior cervical ganglion of the rat. Proc R Soc Lond B Biol Sci (1972) 181:43-79.

[36] Wang QJ, Ding Y, Kohtz DS, Mizushima N, Cristea IM, Rout MP, Chait BT, Zhong Y, Heintz N, Yue Z. Induction of autophagy in axonal dystrophy and degeneration. J Neurosci (2006) 26:8057-68.

[37] Yue Z. Regulation of neuronal autophagy in axon. Implication of autophagy in axonal function and dysfunction/degeneration. Autophagy (2007) 3:139-141.

[38] Liang XH, Jackson S, Seaman M, Brown K, Kempkes B, Hibshoosh H, Levine B. Induction of autophagy and inhibition of tumorigenesis by beclin-1. Nature (1999) 402:672-6.

[39] Selimi F, Lohof AM, Heitz S, Lalouette A, Jarvis CI, Bailly Y, Mariani J. Lurcher GRID2-induced death and depolarization can be dissociated in cerebellar Purkinje cells. Neuron (2003) 37:813-9.

[40] Yue Z, Horton A, Bravin M, DeJager PL, Selimi F, Heintz N. A novel protein complex linking the delta 2 glutamate receptor and autophagy: implications for neurodegeneration in lurcher mice. Neuron (2002) 35:921-33.

[41] Yang DS, Stavrides P, Mohan PS, Kaushik S, Kumar A, Ohno M, Schmidt SD, Wesson DW, Bandyopadhyay U, Jiang Y, Pawlik M, Peterhoff CM, Yang AJ, Wilson DA, St George-Hyslop P, Westaway D, Mathews PM, Levy E, Cuervo AM, Nixon RA. Therapeutic effects of remediating autophagy failure in a mouse model of Alzheimer disease by enhancing lysosomal proteolysis. Autophagy (2011) 7:788-9.

[42] Jarheiss L, Menzies FM, Rubinsztein DC. The itinerary of autophagosomes: from peripheral formation to kiss-and-run fusion with lysosomes. Traffic (2008) 9:574-587.

[43] Halpain J, Dehmelt L. The MAP1 family of microtubule-associated proteins Genome biology (2006) 7:224-230.

[44] Köchl R, Hu XW, Chan EY, Tooze SA. Microtubules facilitate autophagosome formation and fusion of autophagosomes with endosomes. Traffic (2006) 7:129-45.

[45] Fass E, Shvets E, Degani I, Hirschberg K, Elazar Z. Microtubules support production of starvation-induced autophagosomes but not their targeting and fusion with lysosomes. J Biol Chem (2006) 281:36303-16.

[46] Gonzalez-Billault C, Jimenez-Mateos EM, Caceres A, Diaz-Nido J, Wandosell F, Avila J. Microtubule-associated protein 1B function during normal development, regeneration, and pathological conditions in the nervous system. J Neurobiol (2004) 58:48-59.

[47] Nixon RA, Wegiel J, Kumar A, Yu WH, Peterhoff C, Cataldo A, Cuervo AM. Extensive involvement of autophagy in Alzheimer disease: an immuno-electron microscopy study. J Neuropathol Exp Neurol (2005) 64:113-22.

[48] Boland B & Nixon RA. Neuronal macroautophagy: from development to degeneration. Mol Aspects Med (2006) 27:503-19.

[49] Boland B, Kumar A, Lee S, Platt FM, Wegiel J, Yu WH, Nixon RA. Autophagy induction and autophagosome clearance in neurons: relationship to autophagic pathology in Alzheimer's disease. J Neurosci (2008) 28:6926-37.

[50] Lee S, Sato Y, Nixon RA. Primary lysosomal dysfunction causes cargo-specific deficits of axonal transport leading to Alzheimer-like neuritic dystrophy. Autophagy (2011a) 7:1562-1563.

[51] Sigmond T, Fehér J, Baksa A, Pásti G, Pálfia Z, Takács-Vellai K, Kovács J, Vellai T, Kovács AL. Qualitative and quantitative characterization of autophagy in Caenorhabditis elegans by electron microscopy. Methods Enzymol (2008) 451:467-91.

[52] Okazaki N, Yan J, Yuasa S, Ueno T, Kominami E, Masuho Y, Koga H, Muramatsu M. Interaction of the Unc-51-like kinase and microtubule-associated protein light chain 3 related proteins in the brain: possible role of vesicular transport in axonal elongation. Brain Res Mol Brain Res (2000) 85:1-12.

[53] Komatsu M, Wang QJ, Holstein GR, Friedrich VL Jr, Iwata J, Kominami E, Chait BT, Tanaka K, Yue Z. Essential role for autophagy protein Atg7 in the maintenance of axonal homeostasis and the prevention of axonal degeneration. Proc Natl Acad Sci USA (2007a) 104:14489-94.

[54] Nishiyama J, Miura E, Mizushima N, Watanabe M, Yuzaki M. Aberrant membranes and double-membrane structures accumulate in the axons of Atg5-null Purkinje cells before neuronal death. Autophagy (2007) 3:591-6.

[55] Komatsu M, Waguri S, Koike M, Sou YS, Ueno T, Hara T, Mizushima N, Iwata J, Ezaki J, Murata S, Hamazaki J, Nishito Y, Iemura S, Natsume T, Yanagawa T, Uwayama J, Warabi E, Yoshida H, Ishii T, Kobayashi A, Yamamoto M, Yue Z, Uchiyama Y, Kominami E, Tanaka K. Homeostatic levels of p62 control cytoplasmic inclusion body formation in autophagy-deficient mice. Cell (2007b) 131:1149-63.

[56] Pankiv S, Clausen TH, Lamark T, Brech A, Bruun JA, Outzen H, Øvervatn A, Bjørkøy G, Johansen T. p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem (2007) 282:24131-45.

[57] Zhou X, Babu JR, da Silva S, Shu Q, Graef IA, Oliver T, Tomoda T, Tani T, Wooten M, Wang F. Unc-51-like kinase 1/2-mediated endocytic processes regulate filopodia extension and branching of sensory axons. Proc Natl Acad Sci USA (2007) 104:5842-7.

[58] Coleman M. Axon degeneration mechanisms: commonality amid diversity. Nat Rev Neurosci (2005) 6:889-98.

[59] Raff MC, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration. Science (2002) 296:868-71.

[60] Yang Y, Fukui K, Koike T, Zheng X. Induction of autophagy in neurite degeneration of mouse superior cervical ganglion neurons. Eur J Neurosci (2007) 26:2979-2988.

[61] Shehata M, Matsumura H, Okubo-Suzuki R, Ohkawa N, Inokuchi K. Neuronal stimulation induces autophagy in hippocampal neurons that is involved in AMPA receptor degradation after chemical long-term depression. J Neurosci (2012) 32:10413-22.

[62] Gordon PB, Seglen PO. Prelysosomal convergence of autophagic and endocytic pathways. Biochem Biophys Res Commun (1988) 151:40-7.

[63] Rusten TE, Vaccari T, Lindmo K, Rodahl LM, Nezis IP, Sem-Jacobsen C, Wendler F, Vincent JP, Brech A, Bilder D, Stenmark H. ESCRTs and Fab1 regulate distinct steps of autophagy. Curr Biol (2007) 17:1817-25.

[64] Filimonenko M, Stuffers S, Raiborg C, Yamamoto A, Malerød L, Fisher EM, Isaacs A, Brech A, Stenmark H, Simonsen A. Functional multivesicular bodies are required for autophagic clearance of protein aggregates associated with neurodegenerative disease. J Cell Biol (2007) 179:485-500.

[65] Larsen KE, Fon EA, Hastings TG, Edwards RH, Sulzer D. Methamphetamine-induced degeneration of dopaminergic neurons involves autophagy and upregulation of dopamine synthesis. J Neurosci (2002) 22:8951-60.

[66] Ravikumar B, Duden R, Rubinsztein DC. Aggregate-prone proteins with polyglutamine and polyalanine expansions are degraded by autophagy. Hum Mol Genet (2002) 11:1107-17.

[67] Rubinsztein DC. The roles of intracellular protein-degradation pathways in neurodegeneration. Nature (2006) 443:780-6.

[68] Rubinsztein DC, Gestwicki JE, Murphy LO, Klionsky DJ. Potential therapeutic applications of autophagy. Nat Rev Drug Discov (2007) 6:304-12.

[69] Nezis IP, Simonsen A, Sagona AP, Finley K, Gaumer S, Contamine D, Rusten TE, Stenmark H, Brech A. Ref(2)P, the Drosophila melanogaster homologue of mammalian p62, is required for the formation of protein aggregates in adult brain. J Cell Biol (2008) 180:1065-71.

[70] Collins CA, Wairkar YP, Johnson SL, DiAntonio A. Highwire restrains synaptic growth by attenuating a MAP kinase signal. Neuron (2006) 51:57-69.

[71] Shen W, Ganetzky B. Autophagy promotes synapse development in Drosophila. J Cell Biol (2009) 187:71-9.

[72] Wan HI, DiAntonio A, Fetter RD, Bergstrom K, Strauss R, Goodman CS. Highwire regulates synaptic growth in Drosophila. Neuron (2000) 26:313-29.

[73] Rowland AM, Richmond JE, Olsen JG, Hall DH, Bamber BA. Presynaptic terminals independently regulate synaptic clustering and autophagy of GABAA receptors in Caenorhabditis elegans. J Neurosci (2006) 26:1711-20.

[74] Zhang XD, Wang Y, Wang Y, Zhang X, Han R, Wu JC, Liang ZQ, Gu ZL, Han F, Fukunaga K, Qin ZH. p53 mediates mitochondria dysfunction-triggered autophagy activation and cell death in rat striatum. Autophagy (2009) 5:339-50.

[75] Wairkar YP, Toda H, Mochizuki H, Furukubo-Tokunaga K, Tomoda T, Diantonio A. Unc-51 controls active zone density and protein composition by downregulating ERK signaling. J Neurosci (2009) 29:517-28.

[76] Hernandez D, Torres CA, Setlik W, Cebrián C, Mosharov EV, Tang G, Cheng HC, Kholodilov N, Yarygina O, Burke RE, Gershon M, Sulzer D. Regulation of presynaptic neurotransmission by macroautophagy. Neuron (2012) 74:277–284.

[77] Kamada Y, Yoshino K, Kondo C, Kawamata T, Oshiro N, Yonezawa K, Ohsumi Y. Tor directly controls the Atg1 kinase complex to regulate autophagy. Mol Cell Biol (2010) 30:1049-58.

[78] Huang J & Manning BD. A complex interplay between Akt, TSC2 and the two mTOR complexes. Biochem Soc Trans (2009) 37:217-22.

[79] Long X, Müller F, Avruch J. TOR action in mammalian cells and in Caenorhabditis elegans. Curr Top Microbiol Immunol (2004) 279:115-38.

[80] Richter JD, Klann E. Making synaptic plasticity and memory last: mechanisms of translational regulation. Genes Dev (2009) 23:1-11.

[81] Hu JY, Chen Y, Schacher S. Protein kinase C regulates local synthesis and secretion of a neuropeptide required for activity-dependent long-term synaptic plasticity. J Neurosci (2007) 27:8927-8939.

[82] Weragoda RM, Walters ET. Serotonin induces memory-like, rapamycin-sensitive hyperexcitability in sensory axons of aplysia that contributes to injury responses. J Neurophysiol (2007) 98:1231-9.

[83] Lee S, Sato Y, Nixon RA. Lysosomal proteolysis inhibition selectively disrupts axonal transport of degradative organelles and causes an Alzheimer's-like axonal dystrophy. J Neurosci (2011) 31(21):7817-30.

[84] Bunge MB. Fine structure of nerve fibers and growth cones of isolated sympathetic neurons in culture. J Cell Biol (1973) 56:713-35.

[85] Zanjani SH, Selimi F, Vogel MW, Haeberlé AM, Boeuf J, Mariani J, Bailly YJ. Survival of interneurons and parallel fiber synapses in a cerebellar cortex deprived of Purkinje cells: studies in the double mutant mouse Grid2Lc/+;Bax(-/-). J Comp Neurol (2006) 497:622-35.

[86] Eskelinen EL. Maturation of autophagic vacuoles in mammalian cells. Autophagy (2005) 1:1-10.

[87] Mizushima N, Klionsky DJ.Protein turnover via autophagy: implications for metabolism. Annu Rev Nutr (2007) 27:19-40.

[88] Ramesh Babu J, Lamar Seibenhener M, Peng J, Strom AL, Kemppainen R, Cox N, Zhu H, Wooten MC, Diaz-Meco MT, Moscat J, Wooten MW. Genetic inactivation of p62 leads to accumulation of hyperphosphorylated tau and neurodegeneration. J Neurochem (2008) 106:107-20.

[89] Jiang J, Parameshwaran K, Seibenhener ML, Kang MG, Suppiramaniam V, Huganir RL, Diaz-Meco MT, Wooten MW. AMPA receptor trafficking and synaptic plasticity require SQSTM1/p62. Hippocampus (2009) 19:392-406.

[90] Jobim PF, Pedroso TR, Werenicz A, Christoff RR, Maurmann N, Reolon GK, Schröder N, Roesler R. Impairment of object recognition memory by rapamycin inhibition of mTOR in the amygdala or hippocampus around the time of learning or reactivation. Behav Brain Res (2012) 228:151-8.

[91] Cota D, Proulx K, Smith KA, Kozma SC, Thomas G, Woods SC, Seeley RJ. Hypothalamic mTOR signaling regulates food intake. Science (2006) 312:927-30.

[92] Young JE, La Spada AR. Development of selective nutrient deprivation as a system to study autophagy induction and regulation in neurons. Autophagy (2009) 5:555-7.

[93] Sarkar S, Ravikumar B, Floto RA, Rubinsztein DC. Rapamycin and mTOR-independent autophagy inducers ameliorate toxicity of polyglutamine-expanded huntingtin and related proteinopathies. Cell Death Differ (2009) 16(1):46-56.

[94] Du L, Hickey RW, Bayir H, Watkins SC, Tyurin VA, Guo F, Kochanek PM, Jenkins LW, Ren J, Gibson G, Chu CT, Kagan VE, Clark RS. Starving neurons show sex difference in autophagy. J Biol Chem (2009) 284:2383-96.

[95] Koike M, Shibata M, Tadakoshi M, Gotoh K, Komatsu M, Waguri S, Kawahara N, Kuida K, Nagata S, Kominami E, Tanaka K, Uchiyama Y. Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury. Am J Pathol (2008) 172:454-69.

[96] Adhami F, Liao G, Morozov YM, Schloemer A, Schmithorst VJ, Lorenz JN, Dunn RS, Vorhees CV, Wills-Karp M, Degen JL, Davis RJ, Mizushima N, Rakic P, Dardzinski BJ, Holland SK, Sharp FR, Kuan CY. Cerebral ischemia-hypoxia induces intravascular coagulation and autophagy. Am J Pathol (2006) 169:566-83.

[97] Borsello T, Croquelois K, Hornung JP, Clarke PG. N-methyl-d-aspartate-triggered neuronal death in organotypic hippocampal cultures is endocytic, autophagic and mediated by the c-Jun N-terminal kinase pathway. Eur J Neurosci (2003) 18:473-485.

[98] Wang Y, han R, Liang ZQ, Wu JC,ZhangXD, Gu ZI, Qin ZH. An autophagic mechanism is involved in apoptotic death of rat striatal neurons induced by the non N-methyl-D-aspartate receptor agonist kainic acid. Autophagy (2008) 4:214-226.

[99] Høyer-Hansen M, Jäättelä M. Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium. Cell Death Differ (2007) 14:1576-82.

[100] Castino R, Lazzeri G, Lenzi P, Bellio N, Follo C, Ferrucci M, Fornai F, Isidoro C. Suppression of autophagy precipitates neuronal cell death following low doses of methamphetamine. J Neurochem (2008) 106:1426-39.

[101] Zhu JH, Horbinsky C, Guo F, Watkins S, Uchiyama Y, Chu CT. Regulation of autophagy by extracellular signal-regulated protein kinases during 1-methyl-4-phenylpyridinium-induced cell death. Am J Pathol (2007) 170:75-86.

[102] Ding Q, Dimayuga E, Martin S, Bruce-Keller AJ, Nukala V, Cuervo AM, Keller JN. Characterization of chronic low-level proteasome inhibition on neural homeostasis. J Neurochem (2003) 86:489-97.

[103] Pandey UB, Nie Z, Batlevi Y, McCray BA, Ritson GP, Nedelsky NB, Schwartz SL, DiProspero NA, Knight MA, Schuldiner O, Padmanabhan R, Hild M, Berry DL, Garza D, Hubbert CC, Yao TP, Baehrecke EH, Taylor JP. HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS. Nature (2007) 447:859-63.

[104] Bedford L, Hay D, Devoy A, Paine S, Powe DG, Seth R, Gray T, Topham I, Fone K, Rezvani N, Mee M, Soane T, Layfield R, Sheppard PW, Ebendal T, Usoskin D, Lowe J, Mayer RJ. Depletion of 26S proteasomes in mouse brain neurons causes neurodegeneration and Lewy-like inclusions resembling human pale bodies. J Neurosci (2008) 28:8189-98.

[105] Koike M, Shibata M, Waguri S, Yoshimura K, Tanida I, Kominami E, Gotow T, Peters C, von Figura K, Mizushima N, Saftig P, Uchiyama Y. Participation of autophagy in storage of lysosomes in neurons from mouse models of neuronal ceroid-lipofuscinoses (Batten disease). Am J Pathol (2005) 167:1713-28.

[106] Liao G, Yao Y, Liu J, Yu Z, Cheung S, Xie A, Liang X, Bi X. Cholesterol accumulation is associated with lysosomal dysfunction and autophagic stress in Npc1 -/- mouse brain. Am J Pathol (2007) 171:962-75.

[107] Pacheco CD, Lieberman AP. Lipid trafficking defects increase Beclin-1 and activate autophagy in Niemann-Pick type C disease. Autophagy (2007) 3:487-9.

[108] Vergarajauregui S, Connelly PS, Daniels MP, Puertollano R. Autophagic dysfunction in mucolipidosis type IV patients. Hum Mol Genet (2008) 17:2723-2737.

[109] Clausen TH, Lamark T, Isakson P, Finley K, Larsen KB, Brech A, Øvervatn A, Stenmark H, Bjørkøy G, Simonsen A, Johansen T. p62/SQSTM1 and ALFY interact to facilitate the formation of p62 bodies/ALIS and their degradation by autophagy. Autophagy (2010) 6:330-44.

[110] Knaevelsrud H, Simonsen A. Fighting disease by selective autophagy of aggregate-prone proteins. FEBS Lett (2010) 584:2635-45.

[111] Webb JL, Ravikumar B, Atkins J, Skepper JN, Rubinsztein DC. Alpha-synuclein is degraded by both autophagy and the proteasome. J Biol Chem (2003) 278:25009-25013.

[112] Fortun J, Dunn WA Jr, Joy S, Li J, Notterpek L. Emerging role for autophagy in the removal of aggresomes in Schwann cells. J Neurosci (2003) 23:10672-80.

[113] Bjørkøy G, Lamark T, Brech A, Outzen H, Perander M, Overvatn A, Stenmark H, Johansen T. p62/SQSTM1 forms protein aggregates degraded by autophagy and has a protective effect on huntingtin-induced cell death. J Cell Biol (2005) 171:603-14.

[114] Kabuta T, Suzuki Y, Wada K. Degradation of amyotrophic lateral sclerosis-linked mutant Cu/Zn-superoxide dismutase proteins by macroautophagy and the proteasome. J Biol Chem (2006) 281:30524-33.

[115] Berger Z, Ravikumar B, Menzies FM, Oroz LG, Underwood BR, Pangalos MN, Schmitt I, Wullner U, Evert BO, O'Kane CJ, Rubinsztein DC. Rapamycin alleviates toxicity of different aggregate-prone proteins. Hum Mol Genet (2006) 15:433-42.

[116] Petersén A, Larsen KE, Behr GG, Romero N, Przedborski S, Brundin P, Sulzer D. Expanded CAG repeats in exon 1 of the Huntington's disease gene stimulate dopamine-mediated striatal neuron autophagy and degeneration. Hum Mol Genet (2001) 10:1243-54.

[117] Rubinsztein DC, DiFiglia M, Heintz N, Nixon RA, Qin ZH, Ravikumar B, Stefanis L, Tolkovsky A. Autophagy and its possible roles in nervous system diseases, damage and repair. Autophagy (2005) 1:11-22.

[118] Ventruti A, Cuervo AM. Autophagy and neurodegeneration. Curr Neurol Neurosci Rep (2007) 7:443-51.

[119] Nixon RA, Yang DS, Lee JH. Neurodegenerative lysosomal disorders: a continuum from development to late age. Autophagy (2008) 4:590-9.

[120] Lee JH, Yu WH, Kumar A, Lee S, Mohan PS, Peterhoff CM, Wolfe DM, Martinez-Vicente M, Massey AC, Sovak G, Uchiyama Y, Westaway D, Cuervo AM, Nixon RA. Lysosomal proteolysis and autophagy require presenilin 1 and are disrupted by Alzheimer-related PS1 mutations. Cell (2010) 141:1146-1158.

[121] Mariño G, Madeo F, Kroemer G. Autophagy for tissue homeostasis and neuroprotection. Curr Opin Cell Biol (2011) 23:198-206.

[122] Bence NF, Sampat RM, Kopito RR. Impairment of the ubiquitin-proteasome system by protein aggregation. Science (2001) 292:1552-5.

[123] Fleming A, Noda T, Yoshimori T, Rubinsztein DC. Chemical modulators of autophagy as biological probes and potential therapeutics. Nat Chem Biol (2011) 7:9-17.

[124] Nixon RA. Autophagy, amyloidogenesis and Alzheimer disease. J Cell Sci (2007) 120:4081-91.

[125] Spilman P, Podlutskaya N, Hart MJ, Debnath J, Gorostiza O, Bredesen D, Richardson A, Strong R, Galvan V. Inhibition of mTOR by rapamycin abolishes cognitive deficits

and reduces amyloid-beta levels in a mouse model of Alzheimer's disease. PLoS One (2010) 5:e9979.

[126] Heiseke A, Aguib Y, Riemer C, Baier M, Schatzl HM. Lithium induces clearance of protease resistant prion protein in prion-infected cells by induction of autophagy. J Neurochem (2009) 109:25-34.

[127] Cortes CJ, Qin K, Cook J, Solanki A, Mastrianni JA. Rapamycin delays disease onset and prevents PrP plaque deposition in a mouse model of Gerstmann-Sträussler-Scheinker disease. J Neurosci (2012) 32:12396-12405.

[128] Massey AC, Kaushik S, Cuervo AM. Lysosomal chat maintains the balance. Autophagy (2006) 2:325-7.

[129] Iwata A, Riley BE, Johnston JA, Kopito RR. HDAC6 and microtubules are recquired for autophagic degradation of aggregated huntingtin. J Biol Chem (2005) 280:40282-40292.

[130] Martinez-Vicente M, Cuervo AM. Autophagy and neurodegeneration: when the cleaning crew goes on strike. Lancet Neurol (2007) 6:352-61.

[131] Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, Tsujimoto Y. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol (2004) 6:1221-8.

[132] Veneault-Fourrey C, Talbot NJ. Autophagic cell death and its importance for fungal developmental biology and pathogenesis. Autophagy(2007) 3:126-7.

[133] Yu WH, Kumar A, Peterhoff C, Shapiro Kulnane L, Uchiyama Y, Lamb BT, Cuervo AM, Nixon RA. Autophagic vacuoles are enriched in amyloid precursor protein-secretase activities: implications for beta-amyloid peptide over-production and localization in Alzheimer's disease. Int J Biochem Cell Biol (2004) 36:2531-40.

[134] Yu L, Wan F, Dutta S, Welsh S, Liu Z, Freundt E, Baehrecke EH, Lenardo M. Autophagic programmed cell death by selective catalase degradation. Proc Natl Acad Sci USA (2006) 103:4952-7.

[135] Scott RC, Juhász G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr Biol (2007) 17:1-11.

[136] Wullschleger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. Cell (2006) 124:471-84.

[137] Arico S, Petiot A, Bauvy C, Dubbelhuis PF, Meijer AJ, Codogno P, Ogier-Denis E. The tumor suppressor PTEN positively regulates macroautophagy by inhibiting the phosphatidylinositol 3-kinase/protein kinase B pathway. J Biol Chem (2001) 276:35243-6.

[138] Jin S. Autophagy, mitochondrial quality control, and oncogenesis. Autophagy (2006) 2:80-4.

[139] Martin DN, Baehrecke EH. Caspases function in autophagic programmed cell death in Drosophila. Development (2004) 131:275-84.

[140] Chu CT. Autophagic stress in neuronal injury and disease. J Neuropathol Exp Neurol (2006) 65:423-32.

[141] Alirezaei M, Jelodar G, Niknam P, Ghayemi Z, Nazifi S. Betaine prevents ethanol-induced oxidative stress and reduces total homocysteine in the rat cerebellum. J Physiol Biochem (2011) 67:605-12.

[142] Prusiner SB. Prions. Proc Natl Acad Sci USA(1998) 95:13363-13383.

[143] Weissmann C. The state of prion. Nat Rev Microbiol (2004) 2:861-871.

[144] Aguzzi A, Polymenidou M. Mammalian prion biology: one century of evolving concepts. Cell (2004) 116:313-327.

[145] Collinge J. Molecular neurology of prion disease. J Neurol Neurosurg Psychiatry (2005) 76:906-919.

[146] Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science (1982) 216:136-144.

[147] Aguzzi A, Haass C.Games played by rogue proteins in prion disorders and Alzheimer's disease. Science (2003) 302:814-818.

[148] Brandner S, Raeber A, Sailer A, Blättler T, Fischer M, Weissmann C, Aguzzi A. Normal host prion protein (PrPC) is required for scrapie spread within the central nervous system. Proc Natl Acad Sci USA (1996) 93:13148-51.

[149] Büeler H, Aguzzi A, Sailer A, Greiner RA, Autenried P, Aguet M, Weissmann C. Mice devoid of PrP are resistant to scrapie. Cell (1993) 73:1339-47.

[150] Radford HE, Mallucci GR. The role of GPI-anchored PrP(C) in mediating the neurotoxic effect of scrapie prions in neurons. Curr Issues Mol Biol (2010) 12:119-128.

[151] Harris DA, True HL. New insights into prion structure and toxicity. Neuron (2006) 50:353-7.

[152] Winklhofer KF, Tatzelt J, Haass C. The two faces of protein misfolding: gain- and loss-of-function in neurodegenerative diseases. EMBO J (2008) 27:336-49.

[153] Brown DR, Qin K, Herms JW, Madlung A, Manson J, Strome R, Fraser PE, Kruck T, von Bohlen A, Schulz-Schaeffer W, Giese A, Westaway D, Kretzschmar H. The cellular prion protein binds copper in vivo. Nature (1997) 390:684-7.

[154] Gauczynski S, Peyrin JM, Haïk S, Leucht C, Hundt C, Rieger R, Krasemann S, Deslys JP, Dormont D, Lasmézas CI, Weiss S. The 37-kDa/67-kDa laminin receptor acts as the cell-surface receptor for the cellular prion protein. Embo J (2001) 20:5863-75.

[155] Schmitt-Ulms G, Legname G, Baldwin MA, Ball HL, Bradon N, Bosque PJ, Crossin KL, Edelman GM, DeArmond SJ, Cohen FE, Prusiner SB. Binding of neural cell adhesion molecules (N-CAMs) to the cellular prion protein. J Mol Biol (2001) 314:1209-25.

[156] Loubet D, Dakowski C, Pietri M, Pradines E, Bernard S, Callebert J, Ardila-Osorio H, Mouillet-Richard S, Launay JM, Kellermann O, Schneider B. Neuritogenesis: the prion protein controls b1 integrin signaling activity. FASEB J (2012) 26:678-90.

[157] Brown DR, Schulz-Schaeffer WJ, Schmidt B, Kretzschmar HA. Prion protein-deficient cells show altered response to oxidative stress due to decreased SOD-1 activity. Exp Neurol (1997) 146:104-12.

[158] Kuwahara C, Takeuchi AM, Nishimura T, Haraguchi K, Kubosaki A, Matsumoto Y, Saeki K, Matsumoto Y, Yokoyama T, Itohara S, Onodera T.Prions prevent neuronal cell-line death. Nature (1999) 400:225-6.

[159] Milhavet O, Lehmann S. Oxidative stress and the prion protein in transmissible spongiform encephalopathies. Brain Res Brain Res Rev (2002) 38:328-39.

[160] Spudich A, Frigg R, Kilic E, Kilic U, Oesch B, Raeber A, Bassetti CL, Hermann DM. Aggravation of ischemic brain injury by prion protein deficiency: role of ERK-1/-2 and STAT-1. Neurobiol Dis (2005) 20:442-449.

[161] Weise J, Sandau R, Schwarting S, Crome O, Wrede A, Schulz-Schaeffer W, Zerr I, Bähr M. Deletion of cellular prion protein results in reduced Akt activation, enhanced postischemic caspase-3 activation, and exacerbation of ischemic brain injury. Stroke (2006) 37:1296-300.

[162] Mitteregger G, Vosko M, Krebs B, Xiang W, Kohlmannsperger V, Nölting S, Hamann GF, Kretzschmar HA. The role of the octarepeat region in neuroprotective function of the cellular prion protein. Brain Pathol (2007) 17:174-83.

[163] Zanata SM, Lopes MH, Mercadante AF, Hajj GN, Chiarini LB, Nomizo R, Freitas AR, Cabral AL, Lee KS, Juliano MA, de Oliveira E, Jachieri SG, Burlingame A, Huang L, Linden R, Brentani RR, Martins VR. Stress-inducible protein 1 is a cell surface ligand for cellular prion that triggers neuroprotection. Embo J (2002) 21:3307-16.

[164] Schneider B, Mutel V, Pietri M, Ermonval M, Mouillet-Richard S, Kellermann O. NADPH oxidase and extracellular regulated kinases 1/2 are targets of prion protein signaling in neuronal and nonneuronal cells. Proc Natl Acad Sci USA (2003) 100:13326-31.

[165] Pradines E, Loubet D, Schneider B, Launay JM, Kellermann O, Mouillet-Richard S. CREB-dependent gene regulation by prion protein: impact on MMP-9 and beta-dystroglycan. Cell Signal (2008) 20:2050-2058.

[166] Pradines E, Loubet D, Mouillet-Richard S, Manivet P, Launay JM, Kellermann O, Schneider B. Cellular prion protein coupling to TACE-dependent TNF-alpha shed-

ding controls neurotransmitter catabolism in neuronal cells. J Neurochem (2009) 110:912-23.

[167] Linden R, Martins VR, Prado MA, Cammarota M, Izquierdo I, Brentani RR. Physiology of the prion protein. Physiol Rev (2008) 88:673-728.

[168] Moreno JA, Radford H, Peretti D, Steinert JR, Verity N, Martin MG, Halliday M, Morgan J, Dinsdale D, Ortori CA, Barrett DA, Tsaytler P, Bertolotti A, Willis AE, Bushell M, Mallucci GR. Sustained translational repression by eIF2α-P mediates prion neurodegeneration. Nature (2012) 485:507-511.

[169] Ashe KH, Aguzzi A. Prions, prionoids and pathogenic proteins in Alzheimer disease. Prion (2013) 7: in press.

[170] Kristiansen M, Deriziotis P, Dimcheff DE, Jackson GS, Ovaa H, Naumann H, Clarke AR, van Leeuwen FW, Menéndez-Benito V, Dantuma NP, Portis JL, Collinge J, Tabrizi SJ. Disease-associated prion protein oligomers inhibit the 26S proteasome. Mol Cell (2007) 26:175-88.

[171] Pietri M, Caprini A, Mouillet-Richard S, Pradines E, Ermonval M, Grassi J, Kellermann O, Schneider Schneider B. Overstimulation of PrPC signaling pathways by prion peptide 106-126 causes oxidative injury of bioaminergic neuronal cells. J Biol Chem (2006) 281:28470-9.

[172] Heiseke A, Aguib Y, Schatzl HM. Autophagy, prion infection and their mutual interactions. Curr Issues Mol Biol (2010) 12:87-98.

[173] Sikorska B, Liberski PP, Giraud P, Kopp N, Brown P. Autophagy is a part of ultrastructural synaptic pathology in Creutzfeldt-Jakob disease: a brain biopsy study. Int J Biochem Cell Biol (2004) 36:2563-73.

[174] Liberski PP, Brown DR, Sikorska B, Caughey B, Brown P. Cell death and autophagy in prion diseases (transmissible spongiform encephalopathies). Folia Neuropathol (2008) 46:1-25.

[175] Boellaard JW, Schlote W, Tateishi J. Neuronal autophagy in experimental Creutzfeldt-Jakob's disease. Acta Neuropathol (1989) 78:410-418.

[176] Boellaard JW, Kao M, Schlote W, Diringer H. Neuronal autophagy in experimental scrapie. Acta Neuropathol (1991) 82:225-228.

[177] Schätzl HM, Laszlo L, Holtzman DM, Tadzelt J, DeArmond SJ, Weiner RI, Mobley WC, Prusiner SB. A hypothalamic neuronal cell line persistenly infected with scrapie prions exhibits apoptosis. J Virol (1997) 71:8821-8831.

[178] Dron M, Dandoy-Dron F, Guillo F, Benboudjema L, Haw J-J, Lebon P, Dormont D, Tovey MG. Characterization of the human analogue of a scrapie-responsive gene. J Biol Chem (1998) 273:18015-18018.

[179] Dandoy-Dron F, Guillo F, Benboudjema L, Deslys J-P, Lasmézas C, Dormont D, Tovey MG, Dron M. Gene expression in scrapie. Cloning of a new scrapie-responsive

gene and the identification of seven other mRNA transcripts. J Biol Chem (1998) 273:7691-7697.

[180] Dandoy-Dron F, Benboudjema L, Guillo F, Jaegly A, Jasmin C, Dormont D, Tovey MG, Dron M. Enhanced levels of scrapie responsive gene mRNA in BSE-infected mouse brain. Brain Res Mol Brain Res (2000) 76:173-179.

[181] Dron M, Bailly Y, Beringue V, Haeberlé A-M, Griffond B, Risold P-Y, Tovet MG, Laude H, Dandoy-Dron F. Scrg1 is induced in TSE and brain injuries, and associated with autophagy. Eur J Neurosci (2005) 22:133-146.

[182] Dron M, Bailly Y, Beringue V, Haeberlé A-M, Griffond B, Risold P-Y, Tovey MG, Laude H, Dandoy-Dron F. SCRG1, a potential marker of autophagy in transmisible spongiform encephalopathies. Autophagy (2006) 2:58-60.

[183] Xu Y, Tian C, Wang SB, Xie WL, Guo Y, Zhang J, Shi Q, Chen C, Dong XP. Activation of the macroautophagic system in scrapie-infected experimental animals and human genetic prion diseases. Autophagy (2012) 8 (in press).

[184] Hung SY, Huang WP, Liou HC, Fu WM. Autophagy protects neurons from Abeta-induced cytotoxicity. Autophagy (2009) 5:502-510.

[185] Doh-Ura K, Iwaki T, Caughey B. Lysosomotropic agents and cysteine protease inhibitors inhibit scrapie-associated prion protein accumulation. J Virol (2000) 74:4894-4897.

[186] Ertmer A, Gilch S, Yun SW, Flechsig E, Klebl B, Stein-Gerlach M, Klein MA, Schätzl HM. The tyrosine kinase inhibitor STI571 induces cellular clearance of PrPsc in prion-infected cells. J Biol Chem (2004) 279:41918-41927.

[187] Oh JM, Shin HY, Park SJ, Kim BH, Choi JK, Choi EK, Carp RI, Kim YS. The involvement of cellular prion protein in the autophagy pathway in neuronal cells. Mol Cell Neurosci (2008) 39:238-47.

[188] Mok SW, Riemer C, Madela K, Hsu DK, Liu FT, Gültner S, Heise I, Baier M. Role of galectin-3 in prion infections of the CNS. Biochem Biophys Res Commun (2007) 359:672-8.

[189] Ertmer A, Huber V, Gilch S, Yoshimori T, Erfle V, Duyster J, Elsässer HP, Schätzl HM. The anticancer drug imatinib induces cellular autophagy. Leukemia (2007) 21:936-942.

[190] Yun SW, Ertmer A, Flechsig E, Gilch S, Riederer P, Gerlach M, Schatzl HM, Klein MA. The tyrosine kinase inhibitor imatinib mesylate delays prion neuroinvasion by inhibiting prion propagation in the periphery. J Neurovirol (2007) 13:328-337.

[191] Sarkar S, Floto RA, Berger Z, Imarisio S, Cordenier A, Pasco M, Cook LJ, Rubinsztein DC. Lithium induces autophagy by inhibiting inositol monophosphatase. J Cell Biol (2005) 170:1101-11.

[192] Sarkar S, Davies JE, Huang Z, Tunnacliffe A, Rubinztein DC. Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and alpha-synuclein. J Biol Chem (2007) 282:5641-5652.

[193] Aguib Y, Heiseke A, Gilch S, Riemer C, Baier M, Schätzl HM, Ertmer A. Autophagy induction by trehalose counteracts cellular prion infection. Autophagy (2009) 5:361-9.

[194] Sunyach C, Jen A, Deng J, Fitzgerald KT, Frobert Y, Grassi J, McCaffrey MW, Morris R. The mechanism of internalization of glycosylphosphatidylinositol-anchored prion protein. EMBO J (2003) 22:3591-3601.

[195] Marella M, Lehmann S, Grassi J, Chabry J. Filipin prevents pathological prion protein accumulation by reducing endocytosis and inducing cellular PrP release. J Biol Chem (2002) 277:25457-25464.

[196] Parkin ET, Watt NT, Turner AJ, Hooper NM. Dual mechanisms for shedding of the cellular prion protein. J Biol Chem (2004) 279:11170-11178.

[197] Aguib Y, Gilch S, Krammer C, Ertmer A, Groschup MH, Schätzl HM. Neuroendocrine cultured cells counteract persistent prion infection by downregulation of PrPc. Mol Cell Neurosci (2008) 38:98-109.

[198] Heiseke A, Schöbel S, Lichtenthaler SF, Vorberg I, Groschup MH, Kretzschmar H, Schätzl HM, Nunziante M. The novel sorting nexin SNX33 interferes with cellular PrP formation by modulation of PrP shedding. Traffic (2008) 9:1116-1129.

[199] Seo JS, Moon MH, Jeong JK, Seol JW, Lee YJ, Park BH, Park SY. SIRT1, a histone deacetylase, regulates prion protein-induced neuronal cell death. Neurobiol Aging (2012) 33:1110-1120.

[200] Jeong JK, Moon MH, Lee YJ, Seol JW, Park SY. Autophagy induced by the class III histone deacetylase Sirt1 prevents prion peptide neurotoxicity. Neurobiol Aging (2013) 34:146-156.

[201] Fornai F, Longone P, Cafaro L, Kastsiuchenka O, Ferrucci M, Manca ML, Lazzeri G, Spalloni A, Bellio N, Lenzi P, Modugno N, Siciliano G, Isidoro C, Murri L, Ruggieri S, Paparelli A. Lithium delays progression of amyotrophic lateral sclerosis. Proc Natl Acad Sci USA 105:2052-7. Erratum in: Proc Natl Acad Sci USA (2008) 105:16404-7.

[202] Ravikumar B, Vacher C, Berger Z, Davies JE, Luo S, Oroz LG, Scaravilli F, Easton DF, Duden R, O'Kane CJ, Rubinsztein DC. Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet (2004) 36:585-95.

[203] Williams A, Jahreiss L, Sarkar S, Saiki S, Menzies FM, Ravikumar B, Rubinsztein DC. Aggregate-prone proteins are cleared from the cytosol by autophagy: therapeutic implications. Curr Top Dev Biol (2006) 76:89-101.

[204] Sakaguchi S, Katamine S, Nishida N, Moriuchi R, Shigematsu K, Sugimoto T, Nakatani A, Kataoka Y, Houtani T, Shirabe S, Okada H, Hasegawa S, Miyamoto T, Noda

T. Loss of cerebellar Purkinje cells in aged mice homozygous for a disrupted PrP gene. Nature (1996) 380:528-531.

[205] Moore RC, Redhead NJ, Selfridge J, Hope J, Manson JC, Melton DW. Double replacement gene targeting for the production of a series of mouse strains with different prion protein gene alterations. Biotechnology (1995) 13:999-1004.

[206] Rossi D, Cozzio A, Flechsig E, Klein MA, Rulicke T, Aguzzi A, Weissmann C. Onset of ataxia and Purkinje cell loss in PrP null mice inversely correlated with Dpl level in brain. EMBO J (2001) 20:694-702.

[207] Yokoyama T, Kimura KM, Ushiki Y, Yamada S, Morooka A, Nakashiba T, Sassa T, Itohara S. In vivo conversion of cellular prion protein to pathogenic isoforms, as monitored by conformation-specific antibodies. J Biol Chem (2001) 276:11265-71.

[208] Flechsig E, Hegyi I, Leimeroth R, Zuniga A, Rossi D, Cozzio A, Schwarz P, Rülicke T, Götz J, Aguzzi A, Weissmann C. Expression of truncated PrP targeted to Purkinje cells of PrP knockout mice causes Purkinje cell death and ataxia. EMBO J (2003) 22:3095-3101.

[209] Anderson L, Rossi D, Linehan J, Brandner S, Weissmann C. Transgene-driven expression of the Doppel protein in Purkinje cells causes Purkinje cell degeneration and motor impairment. Proc Natl Acad Sci USA (2004) 101:3644-3649.

[210] Yamaguchi N, Sakaguchi S, Shigematsu K, Okimura N, Katamine S. Doppel-induced Purkinje cell death is stoichiometrically abrogated by prion protein. Biochem Biophys Res Commun (2004) 319:1247-1252.

[211] Nishida N, Tremblay P, Sugimoto T, Shigematsu K, Shirabe S, Petromilli C, Erpel SP, Nakaoke R, Atarashi R, Houtani T, Torchia M, Sakaguchi S, DeArmond SJ, Prusiner SB, Katamine S. A mouse prion protein transgene rescues mice deficient for the prion protein gene from Purkinje cell degeneration and demyelination. Lab Invest (1999) 79:689-697.

[212] Cui T, Holme A, Sassoon J, Brown DR. Analysis of doppel protein toxicity. Mol Cell Neurosci (2003) 23:144-155.

[213] Atarashi R, Nishida N, Shigematsu K, Goto S, Kondo T, Sakaguchi S, Katamine S. Deletion of N-terminal residues 23-88 from prion protein (PrP) abrogates the potential to rescue PrP-deficient mice from PrP-like protein/doppel-induced neurodegeneration. J Biol Chem (2003) 278:28944-28949.

[214] Shmerling D, Hegyi I, Fischer M, Blattler T, Brandner S, Gotz J, Rulicke T, Flechsig E, Cozzio A, von Mering C, Hangartner C, Aguzzi A, Weissmann C. Expression of amino-terminally truncated PrP in the mouse leading to ataxia and specific cerebellar lesions. Cell (1998) 93:203-214.

[215] Sakudo A, Lee DC, Nakamura I, Taniuchi Y, Saeki K, Matsumoto Y, Itohara S, Ikuta K, Onodera T. Cell-autonomous PrP-Doppel interaction regulates apoptosis in PrP gene-deficient neuronal cells. Biochem Biophys Res Commun (2005) 333:448-454.

[216] Wong BS, Liu T, Paisley D, Li R, Pan T, Chen SG, Perry G, Petersen RB, Smith MA, Melton DW, Gambetti P, Brown DR, Sy MS. Induction of HO-1 and NOS in doppel-expressing mice devoid of PrP: implication for doppel function. Mol Cell Neurosci (2001) 17:768-775.

[217] Diarra-Mehrpour M, Arrabal S, Jalil A, Pinson X, Gaudin C, Pietu G, Pitaval A, Ripoche H, Eloit M, Dormont D, Chouaib S. Prion protein prevents human breast carcinoma cell line from tumor necrosis factor alpha-induced cell death. Cancer Res (2004) 64:719-727.

[218] Paitel E, Sunyach C, Alves da Costa C, Bourdon JC, Vincent B, Checler F. Primary cultured neurons devoid of cellular prion display lower responsiveness to staurosporine through the control of p53 at both transcriptional and post-transcriptional levels. J Biol Chem (2004) 279:612-618.

[219] Solforosi L, Criado JR, McGavern DB, Wirz S, Sanchez-Alavez M, Sugama S, DeGiorgio LA, Volpe BT, Wiseman E, Abalos G, Masliah E, Gilden D, Oldstone MB, Conti B, Williamson RA. Cross-linking cellular prion protein triggers neuronal apoptosis in vivo. Science (2004) 303:1514-1516.

[220] Bounhar Y, Zhang Y, Goodyer CG, LeBlanc A. Prion protein protects human neurons against Bax-mediated apoptosis. J Biol Chem (2001) 276:39145-39149.

[221] Roucou X, Guo Q, Zhang Y, Goodyer CG, LeBlanc A. Cytosolic prion protein is not toxic and protects against Bax-mediated cell death in human primary neurons. J Biol Chem (2003) 278:40877-40881.

[222] Roucou X, Giannopoulos PN, Zhang Y, Jodoin J, Goodyer CG, LeBlanc A. Cellular prion protein inhibits proapoptic Bax conformational change in human neurons and in breast carcinoma MCF-7 cells. Cell Death Differ (2005) 12:783-795.

[223] Didonna A, Sussman J, Benetti F Legname G. The role of Bax and caspase-3 in doppel-induced apoptosis of cerebellar granule cells. Prion (2012) 6:309-316.

[224] Heitz S, Lutz Y, Rodeau J-L, Zanjani H, Gautheron V, Bombarde G, Richard F, Fuchs J-P, Vogel MW, Mariani J, Bailly Y. BAX contributes to Doppel-induced apoptosis of prion-deficient Purkinje cells. Dev Neurobiol (2007) 67:670-686.

[225] Heitz S, Gautheron V, Lutz Y, Rodeau J-L, Zanjani H, Sugihara I, Bombarde G, Richard F, Fuchs J-P, Vogel MW, Mariani J, Bailly Y. BCL-2 counteracts Doppel-induced apoptosis of prion protein-deficient Purkinje cells in the Ngsk $Prnp^{0/0}$ mouse. Dev Neurobiol (2008) 68:332-348.

[226] Yin XM, Oltvai ZN, Korsmeyer SJ. BH1 and BH2 domains of Bcl-2 are required for inhibition of apoptosis and heterodimerisation with Bax. Nature (1994) 369:321-323.

[227] Roucou X, Gains M, LeBlanc A. Neuroprotective functions of prion protein. J Neurosci Res (2004) 75:153-161.

[228] Oltvai ZN, Milliman CL, Korsmeyer SJ. Bcl-2 heterodimerizes in vivo with a conserved homolg, Bax, that accelerates programmed cell death. Cell (1993) 74:609-619.

[229] Gross A, McDonnell JM, Korsmeyer SJ. BCL-2 family members and the mitochondria in apoptosis. Genes Dev (1999) 13:1899-1911.

[230] Cheng EH, Wei MC, Weiler S, Flavell RA, Mak TW, Lindsten T, Korsmeyer SJ. BCL-2, BCL-X(L) sequester BH3 domain-only molecules preventing BAX- and BAK-mediated mitochondrial apoptosis. Mol Cell (2001) 8:705-711.

[231] Li A, Barmada S, Roth K, Harris D. N-terminally deleted forms of the prion protein activate both Bax-dependent and Bax-independent neurotoxic pathways. J Neurosci (2007) 27:852-859.

[232] Chiesa R, Piccardo P, Ghetti B, Harris DA. Neurological illness in transgenic mice expressing a prion protein with an insertional mutation. Neuron (1998) 21:1339-1351.

[233] Lee DC, Sakudo A, Kim CK, Nishimura T Saeki K, Matsumoto Y, Yokoyama T, Chen SG, Itohara S, Onodera T. Fusion of doppel to octapeptide repeat and N-terminal half of hydrophobic region of prion protein confers resistance to serum deprivation. Microbiol Immunol (2006) 50:203-209.

[234] Heitz S, Grant NJ, Bailly Y. Doppel induces autophagic stress in prion protein-deficient Purkinje cells. Autophagy (2009) 5:422-424.

[235] Heitz S, Grant NJ, Leschiera R, Haeberlé A-M, Demais V, Bombarde G, Bailly Y. Autophagy and cell death of Purkinje cells overexpressing Doppel in Ngsk Prnp-deficient mice. Brain Pathol (2010) 20:119-132.

[236] Dole S, Heitz S, Bombarde G, Haeberlé A-M, Demais V, Grant NJ, Bailly Y. New insights into Doppel neurotoxicity using cerebellar organotypic cultures from prion-protein-deficient mice. Prion 2010. Medimond International Proceedings Eds. (2010) pp7-14.

[237] Metzger F, Kapfhammer JP. Protein kinase C: its role in activity-dependent Purkinje cell dendritic development and plasticity. Cerebellum (2003) 2:206-214.

[238] Falsig J, Sonati T, Herrmann US, Saban D, Li B, Arroyo K, Ballmer B, Liberski PP, Aguzzi A. Prion pathogenesis is faithfully reproduced in cerebellar organotypic slice cultures. PLoS Pathog (2012) 8:e1002985.

[239] Barbieri G, Palumbo S, Gabrusiewicz K, Azzalin A, Marchesi N, Spedito A, Biggiogera M, Sbalchiero E, Mazzini G, Miracco C, Pirtoli L, Kaminska B, Comincini S. Silencing of cellular prion protein (PrPc) expression by DNA-antisens oligonucleotides induces autophagy-dependent cell death in glioma cells. Autophagy (2011) 7:840-853.

[240] Brown DR, Wong BS, Hafiz F, Clive C, Haswell SJ, Jones IM. Normal prion protein has an activity like that of superoxide dismutase. Biochem J (1999) 345:1-5.

[241] Brown DR, Besinger A. Prion protein expression and superoxide dismutase activity. Biochem J (1998) 334:423-429.

[242] Oh JM, Choi EK, Carp RI, Kim YS. Oxidative stress impairs autophagic flux in prion protein-deficient hippocampal cells. Autophagy (2012) 8:1448-1461.

[243] Baumann F, Tolnay M, Brabeck C, Pahnke J, Kloz U, Niemann HH, Heikenwalder M, Rulicke T, Burkle A, Aguzzi A. Lethal recessive myelin toxicity of prion protein lacking its central domain. Embo J (2007) 26:538-547.

[244] Baumann F, Pahnke J, Radovanovic I, Rulicke T, Bremer J, Tolnay M, Aguzzi A. Functionally relevant domains of the prion protein identified in vivo. PLoS One (2009) 4:e6707.

[245] Lemaire-Vieille C, Bailly Y, Erlich P, Loeuillet C, Brocard J, Haeberlé A-M, Bombarde G, Rak C, Demais V, Dumestre-Pérard C, Gagnon J, Cesbron J-Y. Ataxia with cerebellar lesions in mice expressing chimeric PrP-Dpl protein. J Neurosci (2013) (in press).

IntechOpen