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# Diverse functions of the prion protein – Does proteolytic processing hold the key?☆

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ABSTRACT

Proteolytic processing of the cellular and disease-associated form of the prion protein leads to generation of bioactive soluble prion protein fragments and modifies the structure and function of its cell-bound form. The nature of proteases responsible for shedding, α-, β-, and γ-cleavage of the prion protein are only partially identified and their regulation is largely unknown. Here, we provide an overview of the increasingly multifaceted picture of prion protein proteolysis and shed light on physiological and pathological roles associated with these cleavages. This article is part of a Special Issue entitled: Proteolysis as a Regulatory Event in Pathophysiology edited by Stefan Rose-John.

## 1. Introduction

Proteolytic cleavages are irreversible posttranslational modifications involved in much more than just cellular protein degradation. They rather represent important regulatory events affecting various physiological and pathological processes. In general, proteolytic cleavage events may not only alter biological functions of a given precursor substrate but, in many instances, they also produce protein fragments harboring specific intrinsic properties thus contributing to higher biological complexity. As such, proteolytic processing events have gained attention since they might offer potential therapeutic options in various pathological conditions. Consequently, as in many other fields of biomedical research, cleavage of certain key proteins is a highly relevant issue with regard to neurodegenerative diseases.

The proteolytic processing of one of those “key proteins”, namely the cellular prion protein ($PrP^C$), as well as its physiological and pathological consequences are in the focus of this review. This evolutionary conserved glycoprotein, which is highly expressed in the nervous system but also present in most other tissues in mammals, is best known for its detrimental role in fatal and transmissible prion diseases. In these neurodegenerative conditions, which include Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (“mad cow disease”) in cattle and scrapie in sheep, the conformational misfolding of $PrP^C$ into a pathogenic isoform ($PrP^{Sc}$) represents the underlying disease mechanism. Important pathomechanistic features include: (i) aggregation and deposition of $PrP^{Sc}$, (ii) neurotoxicity resulting in synaptic impairment and neuronal loss, (iii) formation of proteinaceous infectious particles (termed “prions”) mainly composed of $PrP^{Sc}$ and (iv) progression of pathology [1–6]. Due to the data obtained from the analysis of valuable animal models in prion research and given that several (though not all) of the above mentioned features are likewise relevant in other neurodegenerative diseases, prion diseases may be seen as a “prototype” proteinopathy [7–10].

In addition to prion diseases, $PrP^C$ is also involved in Alzheimer's disease (AD), the most common cause of dementia. Here, $PrP^C$ located at the neuronal surface acts as a high affinity receptor for toxic oligomers of the amyloid-β peptide (Aβ), which itself is produced by subsequent proteolytic cleavages from a much larger neuronal protein, the amyloid precursor protein (APP). While this detrimental interaction between $PrP^C$ and Aβ has been experimentally worked out, challenged and also reviewed in great detail since its discovery in 2009 [11–22], another commonality regarding these two disease-associated proteins appears interesting: Conserved proteolytic cleavage events of both, APP and $PrP^C$, have been noticed decades ago [23–31]. However, while extensive research has been performed on the processing of APP and its biological and pathogenic consequences, comparably few studies have been conducted and relatively little knowledge has been gained on the physiological and pathological relevance of the cleavage events

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occurring on $PrP^C$. Lately, this situation has fortunately changed as evidenced by an increasing amount of studies focusing on proteolytic processing $PrP^C$ and the relevance of the resulting fragments in health and disease.

It is now increasingly anticipated that generation of specific fragments by proteolytic cleavages indeed represents a key for understanding parts of the biological complexity associated with this protein. And, as for APP [32,33], this complexity is even increasing with the identification of so far unrecognized cleavage events [34]. Importantly, apart from its relevance in neurodegenerative diseases, a whole variety of biological functions has been suggested for $PrP^C$ [35–39].

In the subsequent paragraphs we will first provide an overview of the most relevant $PrP^C$ cleavages including their initial description, resulting fragments and – as far as known – responsible proteases and regulatory aspects. Next, we will concentrate on physiological functions to end with the pathological roles associated with these cleavages. We will not only review the literature for what is known so far, but – whenever appropriate – also speculate on functions of physiological fragments that might be extrapolated or deduced from studies using artificial analogues such as recombinant proteins.

## 2. A variety of conserved $PrP^C$ cleavages occurs

Four cleavages appear to be conserved and currently represent the best investigated processing events on $PrP^C$ (Fig. 1). This, however, does not exclude the existence of alternative and additional cleavage events in different species, tissues or experimental settings [40–42]. Moreover, it should be noted that the terminology of these cleavages (especially for the α-, β- and γ-cleavage), which has been chosen by attributing the historical sequence of discovery and by somewhat adopting the nomenclature of the processing of APP, may lead to confusion regarding mechanistic aspects, responsible proteases and functional outcomes. Thus, this review also aims at establishing some order based on what is known to date.

### 2.1. α-Cleavage separates the N-terminal from the C-terminal half

A highly relevant and the most studied cleavage event under physiological conditions occurs in the middle part of the mature $PrP^C$ (between amino acids 109 and 112 in murine and human sequences; Fig. 1A) and has been termed α-cleavage [31,43]. This cleavage releases the N-terminal flexible part of $PrP^C$ (N1 fragment of ~11 kDa) while leaving the globular C-terminal domain (C1 fragment of ~16 kDa) attached to the membrane via its glycosylphosphatidylinositol (GPI) anchor [30,31,44] (Fig. 1B). As for the full-length $PrP^C$ (fl-$PrP^C$), two N-glycosylation sites may be variably occupied by respective glycan moieties resulting in the typical three-banding pattern of the C1 fragment in Western blot analyses. Deglycosylation is therefore often performed prior to such analyses in order to discriminate between fl-$PrP^C$ and its C1 fragment while the soluble N1 fragment – despite a relatively low stability – can be readily immunoprecipitated from bodily fluids, tissue homogenates or cell culture supernatants [30,45,46]. Initially, this cleavage has been located to acidic endosomal compartments [47,48]. Later, it has been demonstrated that this cleavage takes place during the vesicular trafficking of $PrP^C$ along the secretory pathway [49–51] (Fig. 1B).

Artificial dimerization of $PrP^C$ increases its membrane transport and α-cleavage [52]. After reaching the plasma membrane, C1 forms complexes [53] and has a significantly higher stability and persistence at the cell surface than fl-$PrP^C$ [47]. The latter, in contrast, is known for its

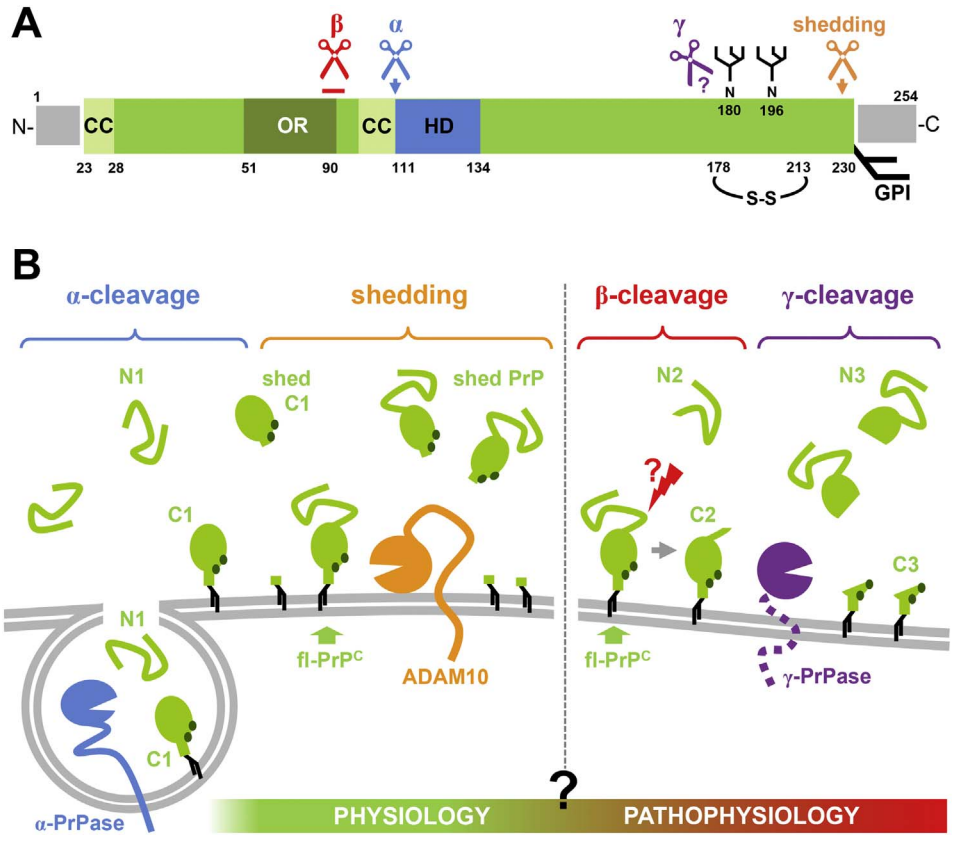


**Fig. 1.** (A) Linear representation of the $PrP^C$ sequence and domain structure (murine sequence). N- and C-terminal signal sequences (grey boxes) are removed and a GPI anchor is added for C-terminal membrane attachment during biosynthesis. Mature $PrP^C$ (ranging from aa 23 to 230) then comprises: (i) an octameric repeat domain (OR; dark green), (ii) two charged cluster regions (CC; bright green), (iii) a hydrophobic domain (HD; blue), (iv) a disulfide bridge (linking aa 178 with aa 213), and (v) up to two N-glycans (at position 180 and 196). Scissors indicate the sites (or suggested regions) of conserved α- (blue), β- (red), γ-cleavage (purple) and shedding (orange).
(B) Schematic overview of the four conserved cleavage events of $PrP^C$ and the resulting fragments discussed in this review. The mature full-length prion protein (fl-$PrP^C$; green arrows) is attached to the outer leaflet of the plasma membrane via its GPI anchor (black). A considerable fraction of $PrP^C$ undergoes α-cleavage during transport to the cell surface. This cleavage in the middle part of the protein yields the soluble and flexible N1 and a globular membrane-bound C1 fragment and is performed by a proteolytic entity termed α-PrPase (blue). $PrP^C$ as well as its C1 fragment can be released from the plasma membrane by ADAM10-mediated shedding (orange) in close proximity to the GPI anchor. Under pathological conditions and in response to oxidative stress, β-cleavage (red) is increased. Proteolytic activity as well as reactive oxygen species (ROS; red thunderbolt) can be responsible for this cleavage and the generation of the resulting N- (N2) and C-terminal fragments (C2). Lastly, unglycosylated $PrP^C$ can be subject to γ-cleavage by a γ-PrPase (purple) within the far C-terminal part resulting in a soluble N3 and a short C3 fragment. The dashed line indicates that the exact cellular localization of β- and γ-cleavage are not exactly defined but intracellular compartments are to be considered. Moreover, the line aims to separate the prominent cleavages under physiological conditions from the ones that are rather present (or at least increased) in a pathological context. However, it is questionable whether such a clear separation can be drawn and this assignment should not be misconceived as exclusive. Dark green dots within the C-terminal part of $PrP^C$ indicate the position of two N-glycosylation sites (that may or may not be occupied by sugar moieties) and illustrate that only shedding releases glycosylated fragments.

(i) ability to leave lipid rafts, (ii) relatively high endocytosis rate, (iii) repeated cycling between cellular surface and intracellular compartments, and (iv) a comparably fast turnover rate [47,54,55]. These differences can be explained by the fact that the flexible N-terminal part of $PrP^C$, which is lacking in C1, is required for important interactions with binding partners (such as LRP1 [56,57]) that regulate re-uptake and trafficking of $PrP^C$ [58–60].

It is intriguing that, although a complete knockout of the *Prnp* gene (which encodes for $PrP^C$) in mice is well tolerated [61–63], expression of various $PrP^C$ versions with deletions including the α-cleavage site on a *Prnp* knockout background is toxic in cells and mice [64–67] (reviewed in [68,69]). While this indicates the biological relevance of this cleavage event and its fragments (which will be discussed later), it could also provide an explanation for the difficulties in identifying the responsible protease(s). Indeed, during the last decade there has been an ongoing controversy regarding the nature of the so called α-PrPase [70–72], which we briefly summarize below:

Whereas initial reports on a relevant contribution of the serine protease plasmin [73,74] could not be supported in plasminogen-deficient mice [75], other *in vitro* studies suggested involvement of two members of the *A disintegrin and metalloproteinase* (ADAM) family, namely ADAM10 and – upon stimulation – ADAM17 [76,77]. Of note, the term α-cleavage is reminiscent of the non-amyloidogenic α-secretase-mediated cleavage of APP, which prevents formation of Aβ, and could indicate involvement of identical proteases. In fact, ADAM10 is currently regarded as the main physiologically relevant α-secretase of APP [78–81]. Regarding $PrP^C$, other studies also found ADAM10 and ADAM17 to correlate with the α-cleavage in human brain [82], mouse models and neuronal cultures [83,84], respectively. Another ADAM family member, ADAM8, has also been shown to play a role in $PrP^C$ α-cleavage in muscle [85]. A role of ADAM8, 10 and 17 in the α-cleavage has been supported more recently in a biophysical study [86]. Using recombinant proteins this study also indicated a higher complexity with different cleavage possibilities dependent on the coordination state of the $PrP^C$ N-terminal part with divalent ions [86]. However, it must be noted that cleavages occurring on a recombinant substrate by a recombinant protease in a test tube may not always have physiological correlates in more complex systems. Moreover, species differences between substrate (murine $PrP^C$) and ADAM proteases (human versions) used in that study [86] may possibly contribute to the complex findings. In fact, while ADAM8 is not likely to play a significant role in this cleavage in the central nervous system [72], several other studies also questioned a physiologically relevant involvement of ADAM10 and 17 [52,87–91].

Thus, in our opinion the identity of the α-PrPase is anything but solved. Complexity is even increased by a surprisingly high tolerance of this cleavage towards modifications (such as point mutations and deletions) around the suggested cleavage site [70]. This latter tolerance together with the evolutionary conservation and biological relevance [92] (discussed later) may support the view that a redundant system involving more than one protease has been established to ensure the proper processing of $PrP^C$ into C1 and N1 fragments. This, in turn, could well explain the seemingly controversial results obtained with different experimental approaches so far. Lastly, the possibility of species-, tissue- and cell type-specific differences has to be considered.

### 2.2. Different processes result in β-cleavage

β-Cleavage of $PrP^C$ has been identified N-terminal to the α-cleavage region [31,43,45] (Fig. 1A). It takes place around amino acid position 90 and, in contrast to α-cleavage, is mainly observed under pathological conditions and much less present or even absent in normal conditions. In consequence of this cleavage, an N-terminal fragment of ~9 kDa (termed N2) is released whereas a corresponding C2 fragment of 18–20 kDa (depending on the glycosylation status) remains bound to the cellular membrane (Fig. 1B). Depending on the pathophysiological situation or experimental model both, cleavage by calpains [93] or lysosomal proteases [42,94] and direct cleavage performed by reactive oxygen species (ROS), have been proposed for this region [43,71,95–97]. With regard to the latter, it is intriguing that a similar copper-dependent cleavage by radicals in a Fenton type reaction has been shown for APP [43,98]. The ROS-mediated cleavage of $PrP^C$ is thought to represent the mechanistic basis of the protective response linked to $PrP^C$ against oxidative stress [95,96,99,100]. Regarding proteases involved in β-cleavage it should be mentioned that the protease-resistant core of $PrP^{Sc}$ (often referred to as PrP27–30) found in prion diseases has a similar length as C2, which indicates involvement of identical cellular proteases in the regular production of C2 and in the cellular attempt to break down $PrP^{Sc}$ [101,102]. Moreover, the diagnostically established digestion of tissue samples with proteinase K also yields this PrP27–20 fragment in the case of individuals affected by prion disease [101,102]. In conclusion, quite different biological processes generate C2-like PrP fragments and are currently summed up under the term "β-cleavage", which consequently should be used with care.

### 2.3. Unglycosylated $PrP^C$ can be substrate for γ-cleavage

Most recently, γ-cleavage of $PrP^C$ has been identified and described as a "previously underappreciated and overlooked" processing step [34]. The exact cleavage site remains to be identified, but molecular weights of the resulting fragments, i.e. a released N-terminal fragments (N3) of ~20 kDa and a small GPI-anchored C3 fragment of ~5 kDa, indicate cleavage in a region between amino acids 170 and 200, possibly just N-terminal of the first N-glycosylation site (Asn181 in the human and Asn180 in the murine sequence) [34,103] (Fig. 1). Of note, one previous study had described a fragment similar to C3 [104]. Interestingly, γ-cleavage seems to exclusively occur on unglycosylated $PrP^C$ indicating that glycans lead to sterical hindrance of the responsible protease, possibly a member of the matrix metalloproteases (MMP) [34,105]. Golgi and trans-Golgi network as well as the endocytic recycling compartment have been suggested as likely locations for this cleavage. While prevalence and relevance of this cleavage in different species, tissues and cell culture models require further exploration, the finding of increased C3 amounts in CJD brain samples might point towards a pathophysiological role of this cleavage [34].

### 2.4. Shedding releases soluble PrP from the plasma membrane

Finally, a far C-terminal cleavage close to the membrane, which releases nearly fl-$PrP^C$ from the cell surface while only leaving a short stump consistent of the GPI anchor and few amino acids behind (Fig. 1), has been described decades ago [27–30,50]. This proteolytic shedding must not be confounded with experimental phospholipase C-mediated cleavage within the GPI anchor structure [55,106]. Shed PrP was sometimes termed "N3" fragment, however, due to the recent description of γ-cleavage (with corresponding C3 and N3 fragments) we recommend to use the term "shed PrP" when referring to the most C-terminally released cleavage product. Shed PrP was first identified in preparations of prion-infected hamster brain where 15% of total $PrP^{Sc}$ was found to end with Gly228 [27]. An identical cleavage site (between Gly228 and Arg229) and the responsible protease were later also found [87] and confirmed [86] for murine $PrP^C$ using recombinant human ADAM10. The confirmation of ADAM10 as the relevant sheddase of murine $PrP^C$ *in vivo* has been shown using conditional ADAM10 knockout mice [89]. There is evidence that not only fl-$PrP^C$ but also C1 [90] and C2 [107] fragments (resulting from α- and β-cleavage, respectively) are shed from the plasma membrane, which further diversifies the variety of $PrP^C$-derived proteolytic fragments (Fig. 1B).

The similarity between $PrP^C$ shedding and α-secretase cleavage of APP has already been acknowledged at a time when the responsible protease was still unknown [29]. Since ADAM10 turned out to exert the

main proteolytic activity in both cases [78–81,87,89], this not only indicates an interesting link between these two cleavage events, but also highlights structural and topological preferences of the protease. In fact, membrane-proximal cleavages at the cellular surface (such as the non-amyloidogenic cleavage of APP within the Aβ sequence or the shedding of $PrP^C$ (Fig. 1B)) mirror what is currently known about ADAM10`s substrate specificity [108]. Indeed, this could be seen as another argument speaking against its suggested involvement in the membrane-distant α-cleavage of $PrP^C$ (discussed above). While knowledge on the regulation of ADAM10 at various levels has increased significantly (reviewed in [108–110]), the mechanistic fine-tuning of $PrP^C$ shedding clearly requires a better understanding. In this regard, it would be interesting to study the influence of known binding partners and regulators of both, protease (e.g. tetraspanins (reviewed in [109,111,112])) and substrate (reviewed in [35,60]), on this specific cleavage. For a detailed discussion of ADAM10 biology, including regulatory aspects as well as the variety of substrates and (patho)physiological consequences, we also commend the interested reader to comprehensive reviews published along with this article in the same special issue [113,114].

## 3. $PrP^C$ cleavage products and their roles in physiology

Physiological functions of $PrP^C$ are diverse and $PrP^C$ has recently been described as a multivalent scaffold protein binding several extracellular and transmembrane ligands [35]. This ability may explain its multiple functions ranging from trophic support [36,37] and cell adhesion [115,116] to signalling [38,39].

Proteolytic processing of $PrP^C$ may regulate these dynamic assemblies and thereby influence $PrP^C$-associated functions. Moreover, fragments generated by these cleavages may act as soluble ligands enabling protein interactions in distance which could represent a basis for intercellular communication (Fig. 2). In that regard, the diversity of $PrP^C$ fragments described above could well provide a means to convey different signals and, thus, to confer biological complexity. It should also be considered that the diverse proteolytic processing events are in part initial factors regulating the degradation and therefore the half-life of the full length prion protein.

In the following, the main cleavage events are described in more detail regarding their known or anticipated physiological functions.

### 3.1. Beneficial roles of $PrP^C$ α-cleavage

A function of $PrP^C$ in myelin maintenance of peripheral nerves was reported, since mice lacking $PrP^C$ showed a chronic demyelinating polyneuropathy (CDP) [64,66,117]. This function has initially been linked to the C1 fragment as mice expressing PrP mutants disabling α-cleavage also suffer from CDP. Accordingly, C1 accumulates in sciatic nerves of healthy wild type mice [117], but how exactly C1 contributes to myelin maintenance is currently unknown. It is conceivable that axonal C1 may interact with the membrane of the surrounding Schwann cell by (i) binding Schwann cell C1 in a homophilic or a specific receptor in a heterophilic interaction or by (ii) integrating into the Schwann cell membrane via its hydrophobic domain (HD, Fig. 1A) providing direct mechanical linkage [71]. Interestingly, recent data show that the flexible N-terminal part of $PrP^C$ (and presumably the corresponding released N1 fragment) acts as a specific ligand for the G protein-coupled receptor Adgrg6, thus promoting myelin homeostasis [118]. Thus, the best characterized function of $PrP^C$ to date (i.e. a role in myelin maintenance) seems to be substantially linked to its α-cleavage. Combined with the published role of $PrP^C$ in cell adhesion [115,116], further research on α-cleavage may provide a clue to unravel the mechanism of this clinically relevant $PrP^C$ function.

Additionally, neuroprotective functions originally linked to fl-$PrP^C$ [36,119–121] may, in part, be attributed to N1. In fact, N1 acts neuroprotective by inducing anti-apoptotic effects through the p53

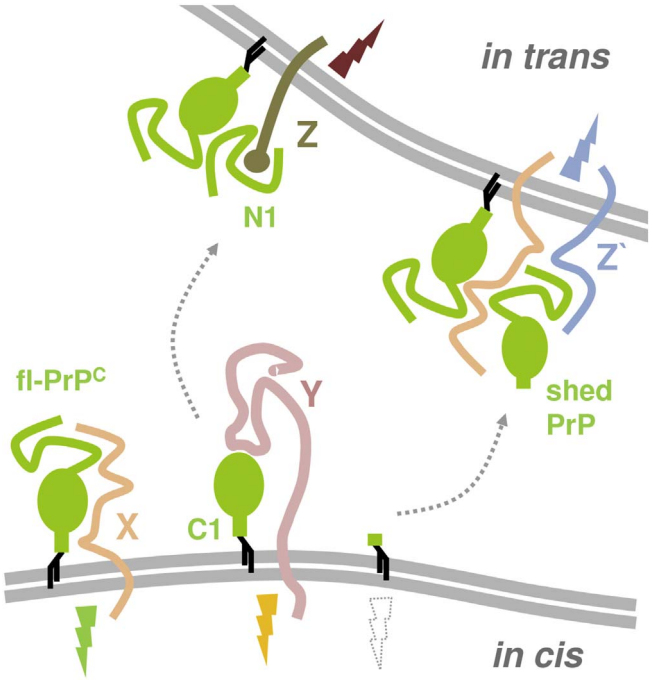


**Fig. 2.** $PrP^C$ cleavages influence protein interactions and functions.
The full-length prion protein (fl-$PrP^C$) is known to interact with a variety of transmembrane proteins (X) *in cis* to elicit physiologically relevant signals (green thunderbolt). Proteolytic fragmentation (as examplified here for the α-cleavage) may inhibit association with some (X) while possibly allowing for new interactions with other binding partners (Y), thereby altering functional outcome (orange thunderbolt). Shedding by ADAM10 may then be one mechanism to downregulate $PrP^C$-mediated signals and functions *in cis* (dotted/vanished thunderbolt). On the other hand, released fragments (such as N1 and shed PrP shown here) comprise specific domains that may allow for binding to receptors (Z) or even formation of functional complexes (Z`) via crosslinking on neighboring or distant cells (*in trans*). This intercellular communication might be relevant in processes such as (i) neuroprotection, (ii) myelination, (iii) neurite outgrowth, axon guidance and synapse formation, as well as in (iv) inflammatory responses. For some of these processes, membrane-bound $PrP^C$ seems to be an important (co-)receptor on the recipient cell. Of note, most experimental data in this regard was obtained using recombinant PrP fragments that might mimic physiologically generated cleavage products. Details and references are given in the main text.

pathway [122]. The mechanism behind this has to be further investigated, but it does not require internalization of N1 [122], which also indicates binding of N1 to a surface receptor and downstream signalling.

Interestingly, the N-terminal half of $PrP^C$ is able to interact with a broad range of binding partners (reviewed in [60]) due to its charged polybasic cluster region (for binding to glycosaminoglycans) and its copper-binding octameric repeat domain [123] (Fig. 1A). Owing to these characteristics N1 is a 'sticky' peptide binding diverse membrane receptors and thereby contributing to cellular communication [59]. By using NMR it was shown that N1 is able to insert deeply into the membrane bilayer with just minimally disrupting it [124–126]. The latter ability especially relies on the octameric repeats and the polybasic region [125] and this peptide-lipid-interaction may be functionally relevant for the overall bioactivity of N1.

In contrast to the protective effects of α-cleavage described above, cell culture data has revealed that the corresponding membrane-bound C1 fragment enhances p53-dependent apoptotic signalling [127]. On the other hand, since transgenic mice expressing C1 in the absence of $PrP^C$ do not show any signs of increased apoptotic cell death, the *in vivo* relevance of this finding remains unclear [128].

### 3.2. (Potential) functions of $PrP^C$ shedding

Despite the observation of a soluble form of PrP decades ago [29],

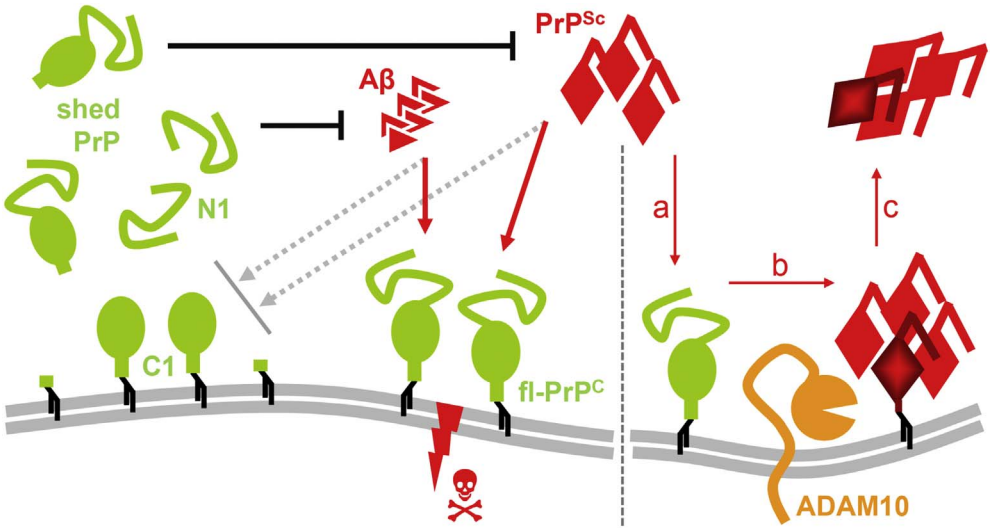


**Fig. 3.** $PrP^{C}$ cleavages impact on neurodegenerative proteinopathies.
Middle part: Toxic protein species, such as oligomers of Aβ in AD and critical seeds of $PrP^{Sc}$ in prion diseases, bind with high affinity (bold red arrows) to the flexible N-terminal part of fl-$PrP^{C}$ at the neuronal surface. This interaction elicits toxic signalling cascades (red thunderbolt and skull). Binding partners of $PrP^{C}$ necessary to transduce signals across the cell membrane are not shown to simplify matters. Left hand side: By cutting the N-terminal half or the nearly full-length prion protein away, α-cleavage and shedding, respectively, reduce this toxic interaction (grey dotted line). In contrast to C2 resulting from β-cleavage (not shown), C1 cannot be misfolded to $PrP^{Sc}$ and even acts as an inhibitor of this conversion. In addition, released N1 and shed PrP may block and detoxify disease-associated oligomers in the extracellular space. Right hand side: Apart from the protective effects described above, shedding may have a dual role in prion diseases: Binding of $PrP^{Sc}$ seeds (a) may lead to templated conversion of membrane-bound $PrP^{C}$ (b). ADAM10-mediated release of the resulting $PrP^{Sc}$ complex (c) may then produce anchorless seeds involved in dissemination of prion pathology. $PrP^{Sc}$ conversion has also been shown for anchorless PrP (not shown). To date it is unclear if already shed PrP can likewise convert and contribute to prion spread.

definite physiological functions of this shed PrP are not known to date. On the other hand, the evolutionary conservation, the relatively high proportion of shed and extracellular liberated PrP [27] and the regulated nature of shedding [129] argue in favour of intrinsic biological functions. As this cleavage occurs at the cell membrane (Fig. 1B), it might function as a mechanism to regulate PrP membrane levels and, thus, functions. This is supported by the observation that shedding is highly increased in models where unphysiologically high levels of $PrP^{C}$ are expressed due to transgenesis [130].

Recombinantly produced fl-$PrP^{C}$ (recPrP) has widely been used as a model for anchorless $PrP^{C}$. While recPrP and shed PrP share identical sequences and have almost the same length, these two entities differ in their biosynthesis and, importantly, in structural aspects and glycosylation state. Nevertheless, we assume that data obtained with recPrP may give an impression of the functional role of physiologically shed PrP. Soluble recPrP impacts on signal transduction, synapse formation and neuroprotection [131]. Moreover, it acts as a guidance cue for axonal growth cones of neurons expressing $PrP^{C}$ [132]. This fits to data showing that $PrP^{C}$ is predominantly expressed in synapses and growth cones [133] and suggests that soluble recPrP and membrane-bound $PrP^{C}$ form a functionally active ligand-receptor complex. Furthermore, in neuron-astrocyte co-cultures, soluble factors produced by $PrP^{C}$-expressing astrocytes are essential for neuronal differentiation and survival [134]. Due to these findings, we hypothesize that shed PrP is the physiological correlate that holds functions in neuroprotection, neurotrophism and chemotaxis. As mentioned earlier, there are obvious analogies between generation of shed PrP and sAPPα. These likely also extend to function as both, sAPPα and shed PrP, may be involved in neurotrophic and neuroprotective processes [131,132,135].

Published functions of the prion protein are predominantly related to the nervous system. However, $PrP^{C}$ is also expressed in immune cells and especially high levels were detected on mast cells [129]. Upon activation of these cells, $PrP^{C}$ shedding is enhanced and, thus, behaves as an inflammatory mediator suggesting an involvement of the prion protein in the inflammatory mast cell response [129,136].

### 3.3. Knowledge on physiological functions of β-cleavage is limited

Since β-cleavage is predominantly associated with pathophysiological conditions, little is described about potential roles in physiology, where this process – to a minor extent – also occurs. As β-cleavage is highly increased when cells are stressed with ROS, it may be seen as an important step in $PrP^{C}$-mediated protection against oxidative stress [96]. Fitting into this picture, the group of Collins characterized the β-cleavage product N2 as an antioxidant with neuroprotective properties [100]. A study based on PrP deletion mutants identified the N-terminal polybasic region as the responsible part for the neuroprotective activity [137]. As described earlier for N1, N2 also has the ability to interact with membranes due to one charged polybasic cluster region and its octameric repeat domain [103,123]. Thus, as for N1, physiological effects of N2 are likely mediated via the interaction with lipid membranes.

## 4. PrP cleavages impact on diseases

Research on the prion protein is closely linked to prion diseases. In fact, discovery of the gene encoding the prion protein was achieved by searching for genetic elements coding for a protein enriched in brain tissue preparations of prion diseased rodents [101]. Consequently, the vast majority of studies investigating involvement of $PrP^{C}$ in disease contexts have for a long time focused on prion diseases. Yet it is obvious that $PrP^{C}$, due to its expression pattern, subcellular localization and close interactions with other proteins, is likely to play important roles in other disease contexts [35]. In particular, given the roles proteolytically generated prion protein fragments play in physiology (as discussed above), it is likely that they also impact on pathophysiological conditions.

For other proteins involved in neurodegenerative diseases, such as APP in AD, research has predominantly focused on the role of the proteolytic fragment Aβ [138]. For the prion protein, however, the vast majority of pathophysiological aspects has so far been linked to the full-length membrane-bound form and not to its proteolytic descendants. In this part of the review, we therefore focus on (potential) roles of the PrP cleavages in different diseases.

### 4.1. Shed PrP may play a dual role in prion diseases

In a hamster model of prion diseases, around 15% of total $PrP^{Sc}$ presented as an anchorless form ending with Gly228 [27]. Whether this results from ADAM10-mediated shedding of $PrP^{Sc}$ (Fig. 3) [87] or from misfolding of already shed $PrP^{C}$ [139] is unknown, yet both processes are in principal supported by experimental data. In mice expressing GPI-anchorless PrP on a *Prnp* knockout background, generation of $PrP^{Sc}$ was facilitated indicating anchorless PrP as a good substrate for $PrP^{Sc}$ conversion [140]. Upon prion infection, these transgenic mice showed disproportionate deposition of $PrP^{Sc}$ as amyloid not only in the CNS but

also in extracerebral locations [139,140]. In fact, excessive deposition of anchorless $PrP^{Sc}$ in heart muscle causes organ failure possibly due to increased tissue stiffness [141]. On the other hand, membrane anchoring seems to be critically involved in transducing $PrP^{Sc}$-mediated neurotoxicity (Fig. 3) [142,143]. Above mentioned transgenic mice only expressing GPI-anchorless PrP show delayed development of prion disease and atypical clinical symptoms [139,140]. At first sight, anchorless PrP may be regarded as an analogue of shed PrP. However, since there are considerable differences, e.g. with regard to biosynthesis, structure and glycosylation, findings obtained with anchorless PrP mice should not be extrapolated directly to shed PrP which might be a much poorer substrate for conversion [48,144]. Another transgenic mouse model expressing secreted Fc-tagged dimers of $PrP^{C}$ showed significantly less $PrP^{Sc}$ production and prolonged survival upon prion infection and soluble PrP species were consequently suggested as potent anti-prion agents [145]. In fact, recent studies showed that the more $PrP^{C}$ is shed, the less $PrP^{Sc}$ is produced and survival of mice is prolonged [88,130]. In the light of a role of membrane-bound $PrP^{C}$ as a receptor of prion toxicity (Fig. 3) [146], additional protection of shedding is likely conferred by the reduction of $PrP^{C}$ at the neuronal surface [147]. This apparent contradiction of positive and negative effects of anchorless PrP versions, which is likely influenced by structural features such as glycosylation and folding state, cannot be fully explained to date [144,148]. The two transgenic models discussed above have no naturally occurring correlate, whereas shedding of PrP occurs physiologically. This calls for concerted research efforts focusing on this cleavage in prion disease.

Besides a direct impact of shed PrP on $PrP^{Sc}$ production, there is evidence that anchorless PrP contributes to spreading of prion disease pathology but also to prion infectivity. In fact, in mice expressing only anchorless $PrP^{C}$, spreading of prion infectivity and $PrP^{Sc}$ is enhanced [149,150]. Also, humans with prion disease-associated stop mutations, leading to production of anchorless prion protein, show a conspicuous dissemination of prion disease pathology throughout the brain [151]. Finally, in mice lacking the $PrP^{C}$ sheddase ADAM10, spread of prion disease pathology throughout the brain is impaired (Fig. 3) [130].

### 4.2. The role of PrP C-terminal fragments in prion diseases

The C1 fragment cannot be converted to $PrP^{Sc}$ [152]. In fact, transgenic mice overexpressing C1 show delayed development of prion disease since C1 is a dominant negative inhibitor for $PrP^{Sc}$ formation (Fig. 3) [48,128,152,153]. For C2, the inverse scenario is true: C2 can easily be converted to $PrP^{Sc}$ *in vivo* and *in vitro* and is thus regarded to favor progression of prion disease [48,128]. This is in line with data from prion disease models [91,153] and CJD patients [31,45] where increased generation of C2 is associated with prion disease. In fact, in rodent models, C2 is increasingly produced in late disease stages, whereas biosynthesis of $PrP^{C}$ diminishes with disease severity [154]. Differential production of C1 and C2 fragments in prion disease may be caused by structural differences within the octameric repeat domain, potentially resulting from differential $Cu^{2+}$ occupancy that affects endoproteolysis of the prion protein [97,153,155].

Since the γ-cleavage of the prion protein has only recently been described, knowledge on the role of C3 in prion diseases is limited. It is present in a protease resistant form and more abundant in brains of patients succumbing to prion disease [34].

With regard to corresponding N-terminal fragments, there is currently only one study describing a protective effect of a Fc-tagged N1-like fragment in a cell culture model of prion diseases and other proteinopathies [146].

### 4.3. $PrP^{C}$ processing and Alzheimer's disease

The role of $PrP^{C}$ as a specific binding partner for Aβ has been reviewed before [156]. Here, we will only focus on the roles proteolytically generated $PrP^{C}$ fragments may play in AD pathophysiology. Yet before we start to discuss the contributions of individual protein fragments, key aspects of this binding need to be repeated. Binding of Aβ to $PrP^{C}$ (Fig. 3) occurs at two regions within the N-terminus of $PrP^{C}$, with one of them being located just N-terminal of the α-cleavage site [11,15]. Since $PrP^{C}$ does not span the membrane, any signalling event elicited by binding of Aβ requires at least a transient association of $PrP^{C}$ with transmembrane cofactors [14].

A number of studies used recombinantly produced soluble PrP fragments. As mentioned earlier, we are aware that recPrP is not identical with shed PrP. Nevertheless, we assume that data obtained with full-length recPrP give an impression of the role of physiologically shed PrP in AD. Both, recPrP and N1 bind Aβ with comparable specificities and binding strengths [157,158]. Interestingly, both PrP fragments also protect neurons from Aβ-mediated synaptic damage [157,159,160]. Mechanistic insight on this protective action is not fully worked out, yet at least two modes of how proteolytic processing of $PrP^{C}$ confers protection in AD are put forward (Fig. 3): (i) by production of neuroprotective fragments (N1 and shed PrP) that bind and neutralize toxic Aβ extracellularly [16,122,146,157–161] and (ii) by decreasing levels of $PrP^{C}$ as a receptor for Aβ at the neuronal surface [22,130]. Interestingly, $PrP^{C}$ shedding and α-cleavage are increased during the course of AD which suggests that above mentioned mechanisms harbor disease modifying potential [161,162].

An alternative view on the role of proteolysis of $PrP^{C}$ in AD but also in prion disease has been presented by researchers focusing on the role of ADAM17 as the α-secretase of APP and the α-PrPase. In their model, AD and prion disease lead to decreased ADAM17 activity and reduced levels of neuroprotective sAPPα and $PrP^{C}$ N1 fragments which then aggravates stress conditions and initiates a vicious circle leading to neuronal death [83].

### 4.4. $PrP^{C}$ processing may confer protection in hypoxic conditions

A role for $PrP^{C}$ in sensing and responding to oxygen deprivation is firmly established [163]. Moreover, a protective influence of $PrP^{C}$ in cerebral hypoxia has been studied extensively and it is known that expression of $PrP^{C}$ is increased in ischemia with mice lacking $PrP^{C}$ being more sensitive to hypoxic damage [164,165]. In addition, adenovirus-mediated overexpression of $PrP^{C}$ alleviated ischemia-induced injury in a rat model of stroke [166]. Apart from one study showing N1-mediated protection in hypoxic conditions [122], a role of proteolytic processing in this $PrP^{C}$-ischemia-context has not been investigated further. However, we hypothesize that at least parts of the protective functions in hypoxic conditions are executed by $PrP^{C}$ fragments. In fact, protective actions can be observed in regions distant from sites of $PrP^{C}$ upregulation, a mode of action only conceivable for a soluble protein [165]. Furthermore, $PrP^{C}$ upregulation attracts neural progenitor cells in ways that are typically described for soluble homing factors [165]. Finally, for renal ischemia it has been shown that $PrP^{C}$-mediated protection positively correlates with generation of the C1 fragment (and assumingly also with the N1 fragment, although this was not assessed in that study) [167]. Thus, $PrP^{C}$ cleavages may also confer protection in hypoxic conditions outside of the brain (e.g. in renal ischemia or heart failure).

## 5. Conclusion/outlook

The identification of proteolytic processing of the prion protein adds to the complexity of prion protein biology and raises important questions. What is the contribution of soluble prion protein fragments to overall functions associated with $PrP^{C}$? How do the proteolytic modifications of the cell-bound form of the protein modify its function(s) and is proteolytic processing of the prion protein a way to regulate its degradation and half-life? Careful and thorough research analyzing roles of soluble prion protein fragments in health and disease not only

will help to shed light on the function(s) of $PrP^C$, but also may open up new avenues to find alternative therapeutic approaches to interfere with development or progression of AD and prion diseases and, possibly, other pathological conditions.

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