Review Article
Proteolytic processing of the prion protein in health and disease

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Abstract: A variety of physiological functions, not only restricted to the nervous system, are discussed for the cellular prion protein (PrP^C). A prominent, non-physiological property of PrP^C is the conversion into its pathogenic isoform (PrP^{Sc}) during fatal, transmissible, and neurodegenerative prion diseases. The prion protein is subject to posttranslational proteolytic processing and these cleavage events have been shown i) to regulate its physiological functions, ii) to produce biologically active fragments, and iii) to potentially influence the course of prion disease. Here, we give an overview on the proteolytic processing under physiological and pathological conditions and critically review what is currently known about the three main cleavage events of the prion protein, namely α-cleavage, β-cleavage, and ectodomain shedding. The biological relevance of resulting fragments as well as controversies regarding candidate proteases, with special emphasis on members of the A-disintegrin-and-metalloproteinase (ADAM) family, will be discussed. In addition, we make suggestions aimed at facilitating clarity and progress in this important research field. The better understanding of this issue will not only answer basic questions in prion biology but will likely impact research on other neurodegenerative diseases as well.

Keywords: Prion protein, proteolytic processing, α-cleavage, β-cleavage, ectodomain shedding, ADAM10, ADAM17

Introduction

The mature cellular prion protein (PrP^C), coded by the *PRNP* gene, is a membrane-anchored protein of 208 amino acids and a molecular weight of approximately 35 kDa (in humans and mice) with two variably occupied N-glycosylation sites. The flexible N-terminal part harbors an octameric repeat region (OR), a neurotoxic domain (ND, discussed later), and a hydrophobic core (HC) (**Figure 1**). The C-terminal part of the protein, on the other hand, is more structured and comprises three alpha-helices, two beta-strands, loop domains, up to two N-glycans, a disulfide bond, and the glycosylphosphatidylinositol (GPI)-anchor for attachment to the outer leaflet of membranes [1, 2]. Due to its GPI-anchor the protein is mainly located within cholesterol and sphingolipid-rich microdomains, termed lipid rafts [3, 4].

PrP^C is discussed to fulfil several physiological functions [5-7]. These range from involvement in neuro-, synapto-, and neuritogenesis as well as differentiation [8-10], cell adhesion [11, 12], neuroprotection [13, 14], and copper-homeostasis [15], to receptor properties and participation in cellular signalling pathways. In signalling, PrP^C can either have a central role [16-19] or act as a regulatory cofactor [20]. In both situations, accessory molecules are required since PrP^C does not span the plasma membrane and is thus unable to transduce signals into the cytosol.

A non-physiological property of PrP^C is its conversion into the pathogenic isoform (PrP^{Sc}; Sc for scrapie, a prion disease of sheep) giving rise to prion diseases or transmissible spongiform encephalopathies (TSE). Prion diseases are fatal neurodegenerative conditions of sporadic or genetic aetiology or may be acquired by exposure to infectious prions [21]. They affect – in different subtypes and peculiarities – humans [22] and other mammalian species [23]. PrP^{Sc}, produced by a template-driven conformational
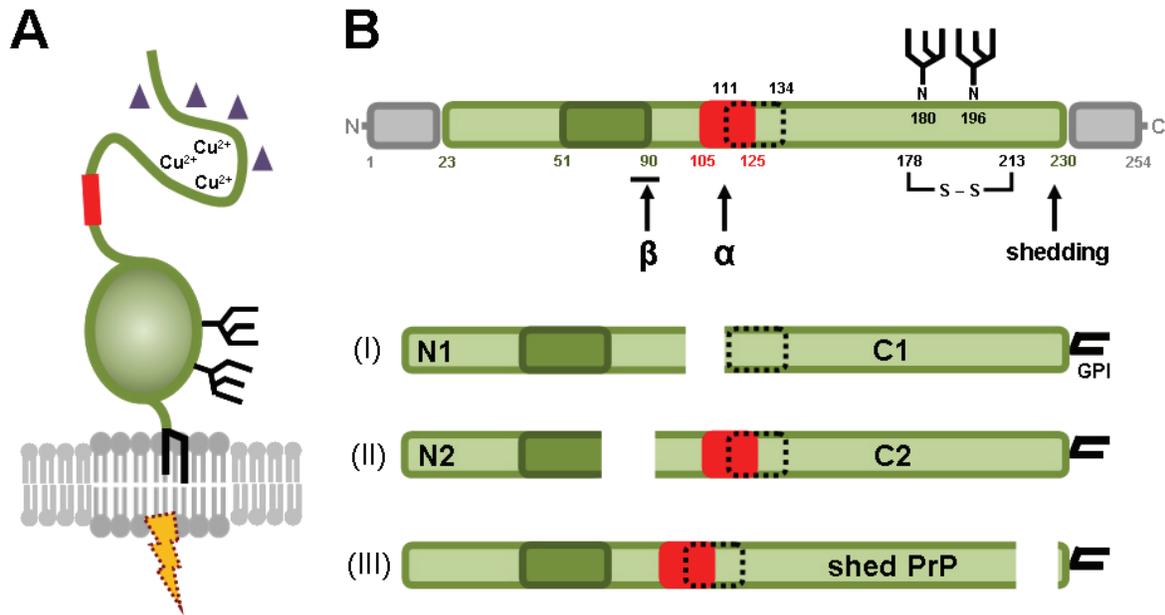
Figure 1. Schematic representation of the prion protein. (A) The prion protein is located in lipid rafts and attached to the outer leaflet of the cellular membrane via a GPI-anchor. The flexible N-terminal part of the protein – among other features – harbors a neurotoxic domain (red box) and is able to bind copper ions and oligomeric amyloid β (purple triangles). The C-terminal part of PrPc has a globular structure and comprises up to two N-glycan side chains. Involvement of PrPc in protective or toxic signalling (dotted thunderbolt) requires accessory molecules (not shown) to bypass the lipid bilayer. (B) Linear representation of the primary sequence of murine PrPc showing important protein domains. After removal of the N-terminal signal sequence (aa 1-22; grey box) by signal peptidases in the ER and the C-terminal signal sequence for the attachment of the GPI-anchor (aa 231-254; grey box), the mature prion protein comprises an octameric repeat region (aa 51-90; dark green), a neurotoxic domain (aa 105-125; red box), a hydrophobic core (aa 111-134; dotted box), a disulfide bridge (between aa 178 and 213), and two variably occupied N-glycosylation sites (aa 180 and 196). The three most important cleavage events are indicated by arrows. (I) α-cleavage gives rise to a soluble N1 fragment of 11 kDa and a membrane-bound C1 fragment of 18 kDa. Of note, this cleavage destroys the neurotoxic domain. (II) β-cleavage at the end of the octameric repeat region produces N2 (9 kDa) and C2 (20 kDa) fragments. (III) Ectodomain shedding close to the GPI-anchor results in the release of nearly full-length PrP from the membrane. References are given in the text.

change of PrPc, is partially resistant to proteinase K (PK) digestion, has amyloidogenic properties, tends to aggregate, and is thought to be the main, if not the sole, component of the transmissible agent termed "prion" [24]. However, recent data indicates that neurotoxicity is not necessarily linked to transmissibility and the nature of the neurotoxic agent in prion diseases remains to be defined [25]. Loss of physiological functions of PrPc is also assumed to contribute to the neurodegeneration seen in prion disease [26].

In addition to its pivotal role in prion disease [27, 28], PrPc might play a role in other neurodegenerative conditions such as Alzheimer`s disease (AD) (reviewed in [2]). For instance, recent studies indicate an influence of PrPc on the

neurotoxicity of oligomeric species of amyloid β (Aβ) [20, 29], a peptide causally involved in disease initiation and progression of AD, and of other β-sheet-rich conformers produced in neurodegenerative diseases [19]. If and how binding of oligomeric Aβ to PrPc really leads to neurotoxic effects in AD is currently under discussion [29-33]. Furthermore, PrPc negatively regulates the activity of beta-site APP-cleaving enzyme 1 (BACE1) thereby reducing the amyloidogenic processing of the amyloid precursor protein (APP) to Aβ [34, 35].

PrPc is subject to proteolytic processing (**Figure 1B**). Accumulating data indicate the importance of these processing steps which seem to regulate the multiple functions of PrPc under physiological conditions and produce biologically ac-

tive fragments. In addition, processing of the prion protein is thought to influence not only the course of prion diseases but also other neurodegenerative diseases. Here, we review the three most important cleavage events, i.e. the α-cleavage, β-cleavage, and ectodomain shedding, with regard to their biological relevance, role in disease, and responsible proteases. Regulating these cleavages might be of therapeutic interest. We will not discuss artificial proteolytic processing of the prion protein such as treatment with PK or thermolysin which is routinely performed for research and diagnostic purposes [36-39].

Alpha-cleavage of PrP^C

Description

The mature prion protein is composed of two structurally divergent parts, each of them making up roughly one half of the full-length protein: the less-structured N-terminal part with its flexible N-terminus and the compact and globular C-terminal domain (**Figure 1**). Since, in general, structure determines protein function, it was reasonable to expect that a cleavage in the middle of PrP^C, separating these two unequal parts, would produce two fragments with diverse functions distinct from the function of full length PrP^C.

Indeed, as initially reported for chicken PrP and later extended for mammalian PrP^C, a released soluble fragment of approximately 11kD (termed N1) and a N-terminally truncated membrane-attached counterpart of approximately 18 kD (termed C1, which after deglycosylation runs at approximately 16 kD [40, 41]) were found in transfected cell lines and, more importantly, under physiological conditions in brain homogenates and cerebrospinal fluid [42-44]. The corresponding cleavage site was termed α (discriminating it from another upstream β-cleavage site [see below]) and was identified by sequencing and epitope mapping at positions K110/H111 or H111/M112 (human sequence) located directly upstream of the hydrophobic core domain [44-46]. In fact, α-cleavage is the main proteolytic processing event yielding C1 fragments that accumulate at the plasma membrane and make up 5-50% of total PrP^C levels depending on the cell type and brain region [43, 44, 47]. Several cellular compartments have been suggested for this cleavage event. Since

Brefeldin A and lysosomal inhibitors [43] as well as cholesterol depletion [48] block production of N1 or C1 fragments, α-cleavage was thought to occur in acidic endosomal compartments or in detergent resistant microdomains of the plasma membrane respectively. In contrast to this, a more recent study using different constructs of mammalian PrP indicates that α-cleavage does not require lipid raft localization and seems to take place while PrP^C is traversing the late secretory pathway [49]. This finding is also supported by a cell-culture based study investigating processing of bovine PrP^C [50].

Identifying the protease

Controversy remains regarding the identity of the protease(s) responsible for α-cleavage. Initial reports suggested an involvement of lysosomal serine proteases [43]. However, the use of chicken PrP and potential occurrence of PrP fragments resulting from lysosomal degradation impede interpretation of these data. The serine protease plasmin is yet another enzyme that has been suggested for this cleavage *in vitro*, yet *in vivo* data could not confirm this [51]. Inhibitor-based *in vitro* experiments analyzing the α-cleavage of fluorescently labelled PrP^C suggested involvement of calpain-like activity, but again *in vivo* proof is still lacking [52].

Most of the current work on the identification of the responsible protease and probably best experimental support so far focuses on the A-disintegrin-and-metalloproteinase (ADAM) family of proteases. Many of these membrane-bound and zinc-dependent proteases of the adamalysin subfamily perform proteolytic ectodomain shedding of a broad range of substrates in various physiological and pathological processes [53, 54]. Mainly two members of this group, ADAM10 and ADAM17 (TNFα-converting enzyme, TACE), have been suggested to be responsible for α-cleavage of PrP^C. At first, it was shown that production of N1 and C1 fragments could be increased in HEK cells upon phorbol-ester-driven activation of protein kinase C (PKC) [46]. This observation was systematically followed by inhibitor studies that suggested ADAM10 and ADAM17 as PrP^C-cleaving enzymes with ADAM10 being responsible for the constitutive α-cleavage while processing via ADAM17 was stimulus-dependent [55]. ADAM9, another member of the ADAM family, was suggested to contribute indirectly to the production

of N1/C1, namely by shedding of ADAM10 from the membrane [56]. These results could be confirmed by overexpression of ADAM10 in cell culture [57] and by the finding that inhibitors of metalloproteases were able to block α-cleavage [58]. A role of ADAM10 in the constitutive α-cleavage was then further supported by a study investigating the content of PrPᶜ fragments in human cerebral cortex. Despite inter-individual variations, this study found a positive correlation between C1 amounts and levels of active ADAM10 [59].

However, there is also strong controversy. An initial opposing report challenging the role of ADAM10 in this cleavage came from a study where neither ectopic expression nor siRNA depletion of ADAM10 in HEK cells affected C1 levels [60]. Another study was likewise unable to detect enhanced amounts of specific cleavage products of PrPᶜ in transgenic mice that overexpressed ADAM10 in neurons [61]. Recently, ADAM8 and not ADAM10 was identified as the main contributor to α-cleavage in skeletal muscle, although it should be noted, that expression of ADAM10 in muscle is only 2% of its level in brain and thus the situation in the CNS might differ [62]. These authors also described a self-regulatory loop with PrPᶜ being able to upregulate ADAM8 thereby modifying its own cleavage and the production of C1/N1 fragments. Finally, *in vivo* data showed unaltered levels of C1 and N1 fragments in mice deficient in ADAM10 in neuronal precursor cells [41]. Thus, involvement of this protease in α-cleavage remains controversial although it is certain that interspecies and inter-tissue differences exist and a certain degree of redundancy [54] complicates these investigations as it is possible that additional proteases might take over ADAM10-functions in the absence of ADAM10.

As mentioned before, ADAM17 is another candidate protease for the α-cleavage of PrPᶜ. Not only its contribution to cleavage but also the mechanism of regulation has been investigated in detail (reviewed in [63]). In brief, studies on cell lines and primary neurons showed that stimulation of muscarinic receptor subtypes M_1 and M_3 by cholinergic agonists induces a cascade of events involving the activation of certain isoforms of protein kinase C as well as extracellular regulated kinase-1 (ERK-1). The latter leads to phosphorylation of ADAM17 thereby upregulating its activity, which then culminates

in increased α-cleavage of PrPᶜ [64, 65]. Furthermore, ERK-1 not only controls proteolysis of PrPᶜ but also its expression levels via promoter transactivation in a regulatory cascade involving the transcription factor AP-1 [66]. A similar transcriptional control of PrPᶜ has previously been shown for the amyloid intracellular domain (AICD) that is produced by γ-secretase mediated cleavage of APP and acts on PrPᶜ transcription via p53 upregulation [67]. However, neither the regulation of PrPᶜ by AICD nor the involvement of ADAM17 in PrPᶜ endoproteolysis could be confirmed by follow-up studies using cell culture models or transgenic mice [60, 68].

While the primary sequence around the cleavage site was reported by one group to be of significant importance for the generation of N1 and C1 [69], studies of others indicate that the protease is surprisingly tolerant towards different kinds of modifications within the PrPᶜ sequence but might depend on the highly conserved HC domain as well as on PrPᶜ membrane-anchoring [70, 71]. Of note, sequence differences at the cleavage site (H111/M112 in humans compared to H110/V111 in mice) may account for interspecies differences regarding the α-cleavage with ADAM17 showing preference for murine PrPᶜ [69, 72]. However, since there is uncertainty on the true identity of the protease responsible for α-cleavage, the term "α-PrPase" seems justified [70]. This also avoids confusion with the "α-secretase" that performs the non-amyloidogenic processing of APP and has recently been identified to be ADAM10 [73-76].

N1-fragment

Despite the enigma concerning the nature of the α-PrPase, several recent findings highlight the physiological importance of α-cleavage of PrPᶜ. Firstly, several functions of PrPᶜ have been attributed to the N-terminal part of the protein and binding of a variety of ligands was shown to take place to different motifs of this part (reviewed in [77]). Therefore, α-cleavage can be seen as a way to negatively regulate these functions. Secondly, produced N1 and C1 fragments have intrinsic functions. For soluble N1, a role in intercellular communication and neuroprotective functions were suggested [78] (**Figure 2**). Moreover, N1 production was shown to interfere with the neurotoxicity of Aβ oligomers, the proposed neurotoxic species in AD. Recently, two
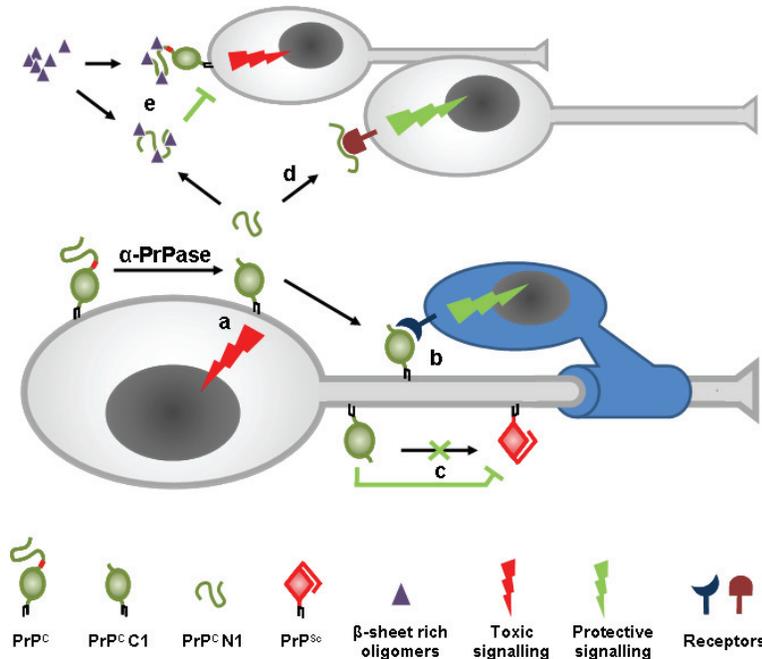
Figure 2. Model of the α-cleavage of PrP^C. The α-PrPase produces membrane-attached C1 and soluble N1 fragments. C1 plays a dual role by initiating apoptotic signalling (a) and its involvement in trophic signalling onto Schwann cells to maintain the myelin sheath (b). Due to the loss of the neurotoxic domain, C1 is unable to misfold into the pathogenic isoform PrP^{Sc} and, in addition, might be a dominant negative inhibitor of the conversion process (c). The released N1 fragment is involved in neuroprotective signalling (d). While β-sheet-rich oligomers are thought to bind to the N-terminus of PrP^C to induce toxic signalling, α-cleavage not only prevents this interaction but produces N1 that might bind these oligomers and block their toxicity (e). References are given in the text.

stretches (residues 23-27 and 95-110) within the N-terminus of PrP^C have been reported to build a high-affinity platform for the binding of Aβ oligomers [29, 79]. Thus, in addition to the neuroprotective signalling, this effect might in part be achieved by binding of soluble N1 to amyloid β-oligomers thereby blocking neurotoxic signalling pathways [80] (**Figure 2**). Interestingly, this blocking and neuroprotective function of N1 might not be limited solely to Aβ oligomers but could be a more general mechanism of competing with toxic β-sheet-rich conformers found in several neurodegenerative diseases [19, 81]. By releasing the N-terminus of PrP^C, α-cleavage might have a dual protective function: producing the neuroprotective catabolite N1 as well as inhibiting neurotoxic signalling which is thought to require full-length PrP^C not only in prion disease [28, 82] but also in other neurodegenerative conditions [19, 29, 30, 83]. In line with this, expression of N-terminally truncated or deleted constructs that are unable to undergo this cleavage lead to toxicity in transgenic mice [84, 85].

C1-fragment

The physiological function of the membrane-attached C1 fragment is controversial (**Figure 2**). On the one hand, cell culture based studies suggest that C1, similar to full-length PrP^C [86,

87], is able to initiate a p53-dependent apoptotic cascade resulting in increased caspase-3 activation [88]. In contrast to full-length PrP^C, this effect of C1 does not depend on clathrin-mediated endocytosis and seems to involve a different pathway leading to p53 activation [89].

On the other hand, a protective function of the C1 fragment has recently been shown [40]. More than a decade ago, it was already found that transgenic mice expressing N-terminal deletion mutants of PrP^C show diverse signs of neurotoxicity and demyelination, which, although milder, could also be observed in *PRNP$^{0/0}$* mice [84, 90, 91]. Interestingly, all of these deletions included the α-cleavage site and could be rescued by coexpression of PrP^C. It was postulated that axonal C1 expression is linked to the maintenance of the myelin sheath in the peripheral nervous system [40]. Since expression of C1 at the axonal surface is able to rescue myelination, C1 acts *in trans* on adjacent Schwann cells to initiate protective signalling that helps to maintain the myelin sheath. It might be hypothesized, that these signals should be rather longstanding than transient. In line with this assumption, C1 was shown to have a much longer half-life than PrP^C and to accumulate on the cellular surface [43]. In fact, the study of Bremer *et al.* found that sciatic nerves contain even more C1 than full-length

PrP^C [40]. Unlike PrP^C, C1 is unable to undergo copper-stimulated [92-94] and clathrin-mediated endocytosis, which has been shown by several groups to depend on the N-terminus [95-97] and its interaction with LRP1 [98, 99].

This characteristic of C1 might account for its involvement in longstanding signalling events that might not be limited to the role mentioned above.

In contrast to the findings of Bremer *et al.*, another group reported that N1 rather than C1 might be responsible for the myelinotrophic effects and that C1 is not neuroprotective [100]. However, the α-cleavage seems to be fundamental in this regard.

Since α-cleavage takes place directly N-terminal of the HC, the resulting C1 fragment with its exposed stretch of hydrophobic amino acids is reminiscent of some viral fusion proteins (e.g. those of paramyxoviruses) which after proteolytic activation also harbor a free stretch of approximately 20 hydrophobic amino acids, termed the fusion peptide. This fusion peptide inserts into the host-cell membrane to initiate fusion [101, 102]. Although data in this regard is lacking, we hypothesize that the N-terminus of C1 might likewise be able to insert into membranes, for instance of neighbouring cells. Given the suggested role of PrP^C in cell adhesion [11, 12], this aspect might deserve further investigation.

Alpha-cleavage protects PrP^C from misfolding

The α-cleavage site is located within the neurotoxic domain (ND) of PrP^C (amino acids 106-126 in human and 105-125 in murine sequence) which constitutes a structural prerequisite for the conformational conversion to PrP^{Sc} and is thought to be a main contributor to the amyloidogenic properties of PrP^{Sc} [21, 44, 103]. Interestingly, a synthetic peptide spanning this short region tends to form fibrils, is neurotoxic, and can induce proliferation of astrocytes [104, 105]. In addition to this neurotoxic domain, the N-terminus of PrP^C might play a substantial role in prion pathogenesis as indicated by a recent report [106]. Thus, α-cleavage releases N1 and cleaves PrP^C within the ND, thereby preventing misfolding of residual C1 (**Figure 2**). Supporting the importance of an intact ND for the misfolding, one study found that all PK-resistant PrP^{Sc}

cores studied in brains from sCJD or GSS patients exhibited an intact α-cleavage site [58]. A palindromic sequence (112-AGAAAAGA-119) is located directly downstream of the cleavage site and was shown to be of importance for the physical interaction of PrP^C with PrP^{Sc} during the conformational conversion [107]. Although this primary sequence would remain intact, α-cleavage might alter the secondary structure of this region and thus block this interaction.

On the other hand, after conversion of PrP^C to PrP^{Sc}, α-cleavage was found to be impaired. This fact might be explained by a sterical hindrance preventing the protease from getting access to the cleavage site. Recent reports using different experimental setups and prion strains show that increasing the level of C1 relative to full-length PrP^C has protective effects in prion disease [47, 108]. In the latter study, transgenic mice only expressing C1 in the absence of full-length PrP^C were not susceptible to prion infection, did not accumulate PrP^{Sc}, and did not show any signs of neurodegeneration. Moreover, coexpression of PrP^C and C1 led to reduced PrP^{Sc} production and prolonged incubation times after prion inoculation compared to wildtype mice. In view of the unphysiological overexpression of C1 in this study, it remains questionable whether C1 really is a dominant-negative inhibitor of the conversion process [47] (**Figure 2**) or if the observed effect is just due to the dilution of convertible full-length PrP^C. Nevertheless, these data clearly demonstrate the protective effect of the α-cleavage.

Beta-cleavage of the prion protein

An additional but under physiological conditions less prominent cleavage of PrP^C takes place upstream of the α-cleavage site at the end of the octameric repeat region (Q90 in the murine sequence). Cleavage at this β-site produces soluble N2 and membrane-bound C2 fragments of ~9 kDa and ~20 kDa respectively. The latter fragment was found to be the main cleavage product of PrP in prion infected neuroblastoma cells [109] and in brains of CJD patients [44, 58] indicating a pathophysiological relevance. Since C2 shares several characteristics (i.e. its insolubility in nondenaturing detergents and the electrophoretic mobility [110, 111]) it was regarded as the "in vivo homologue" of the protease-resistant core of PrP^{Sc} (referred to as PrP (27-30)) that is experimentally derived after

treatment with PK [44, 112]. In fact, PK cleavage sites within PrP^{Sc} are located at the end of the octameric repeat region [84]. Initially it was thought that the C2 fragment resulted from an incomplete degradation of PrP^{Sc} by lysosomal proteases [109]. Later, using pharmacological and genetic approaches, it was found that C2 was produced by calpains, and Ca^{2+}-regulated cysteine proteases. Inhibition of lysosomal proteases, caspases, and the proteasome instead had no effect on C2 levels [113].

Similar C2 fragments were also found to be drastically increased in cultured cells that were stressed with reactive oxygen species (ROS) and cleavage at the β-site was dependent on copper ions [45, 58, 114]. Of note, PrP^C was attributed to be involved in protection against oxidative stress [115] and, in turn, oxidative stress was thought to contribute to the pathologic events during prion disease [116-119]. Thus, β-cleavage might be one step in the mechanism by which PrP^C fulfils its cellular protection against oxidative stress [120]. Furthermore, it was shown that this cleavage i) can be directly performed by ROS [114], ii) is dependent on specific Trp residues within the octameric repeat region [120, 121], iii) takes place at the cellular surface, and – in contrast to what was published before [113] – iv) seems not to depend on calpains [120].

Thus, cleavage at the β-site at the end of the octameric repeat region can be achieved by two distinct mechanisms: proteolytic processing in prion diseases and cleavage under conditions of oxidative stress (performed by ROS) [45]. To avoid confusion, it would be reasonable to consider a new nomenclature separating these two events. However, in contrast to the C1/N1 fragments derived from α-cleavage, there is no experimental data in favor of a physiological function of the resulting C2/N2 fragments [78, 88].

Shedding and anchorless PrP

A third physiological cleavage of PrP^C occurs in close proximity to its GPI-anchor and results in the release of the almost full-length protein from the plasma membrane. This protease-mediated cleavage at the very C-terminus is termed shedding [122]. This is distinct from experimentally performed cleavage of the GPI-anchor by phospholipases which was fundamental for the identification of the GPI-

anchorage of PrP^C [42, 123]. However, since phospholipases are cytoplasmic enzymes they are unlikely to encounter PrP^C under physiological conditions. In this regard it was surprising when Harris *et al.* studied the processing of chicken PrP in neuroblastoma cells and found that parts of the released full-length protein and C1 fragments still contained some anchor structure [42]. This finding might be explained by the existence of phospholipases in the cell culture serum as observed by others [124].

In cell culture, a small but significant fraction of total PrP^C is slowly but constitutively shed into the media and shed PrP^C lacks any parts of the GPI-modification indicating cleavage by a protease [122]. A shed and soluble form of PrP^C was also found in human CSF [125] and blood [124, 126, 127] indicating a physiological relevance. Thus, shedding of PrP^C not only occurs in neurons but also in lymphoid cells [124]. Although PrP^C was shown to be less sensitive to phospholipase cleavage compared to other GPI-anchored proteins, it has a much shorter half-life at the cell surface [128], a notion that further supported involvement of a "sheddase". Proteolytic shedding was also confirmed for bovine PrP^C in two cell culture models [50].

Identification of the PrP sheddase

Even though the mechanism releasing PrP^C from the surface was apparent, it took some time until candidate proteases for this event were suggested. *In vitro* studies using inhibitors and stimulators of zinc metalloproteases suggested that shedding of PrP^C requires a zinc metalloproteases and that this event is remarkably reminiscent of the α-secretase-mediated cleavage of APP [122, 129]. Finally, cell culture experiments identified ADAM10 as the sheddase of PrP^C [60]. This study confirmed the previously suggested cleavage site by mass spectrometry at position Gly228/Arg229 [130] and found that ADAM9 has an indirect influence on the shedding by regulating ADAM10 activity, a finding that was also observed by others [56, 131, 132]. In addition, ADAM17 does not seem to be involved in PrP shedding [60].

A somewhat opposing report showed that overexpression of a novel sorting nexin, SNX33, interfered with the constitutive endocytosis of PrP^C thereby leading to elevated amounts of surface PrP^C that was counterbalanced by in-

creased release. This release into the culture medium however appeared to be independent of ADAM10 and was rather performed by phospholipase cleavage of the GPI-anchor [57]. *In vivo* data supporting this SNX33-regulated and ADAM10-independent release of PrP^C is lacking to date. Proteolytic processing of PrP^C and the role of ADAM10 was also investigated using mice that moderately overexpressed the protease [61]. In this study, the authors did not find increased production of C1/N1 fragments or shed PrP. Instead they found reduced PrP^C mRNA levels and suggested that ADAM10 overexpression transcriptionally downregulates PrP^C expression.

In contrast, *in vivo* data from our group confirmed ADAM10 as the main functionally relevant sheddase of PrP^C [41]. First, mice with a knockout of ADAM10 in neural precursor cells had increased amounts of PrP^C (**Figure 3A**) while mRNA levels remained unaffected compared to controls. Second, shedding was absent in primary neurons derived from these mice (**Figure 3B**). Third, genetic reintroduction of ADAM10 into neural stem cells of these knockout mice restored shedding of PrP^C after neuronal differentiation. It remains to be elucidated if i) ADAM10 is able to shed PrP^C *in trans* as shown for Ephrin, another substrate of ADAM10 [133, 134], ii) ADAM10 from other cell types contributes to the release of PrP^C from neuronal membranes, and iii) the protease also plays a role in the sorting of PrP^C to specific regions prior to cleavage, as shown for the processing of its substrate CD23 [135, 136]. Additionally, it has recently been shown that ADAM10 activity towards other substrates, i.e. APP, is tightly regulated on different levels including modulation of its expression, its proteolysis, and its trafficking [131, 132, 137, 138]. It deserves further investigations if shedding of PrP^C is likewise regulated.

Is there a physiological relevance for shedding PrP^C?

Like α-cleavage and clathrin-mediated endocytosis, shedding can be seen as a mechanism to regulate PrP^C levels at the plasma membrane. This is of outstanding importance regarding the multiple functions discussed for PrP^C, especially its receptor properties and its involvement in toxic signalling cascades. In fact, primary neurons of mice lacking ADAM10 accumulated PrP^C
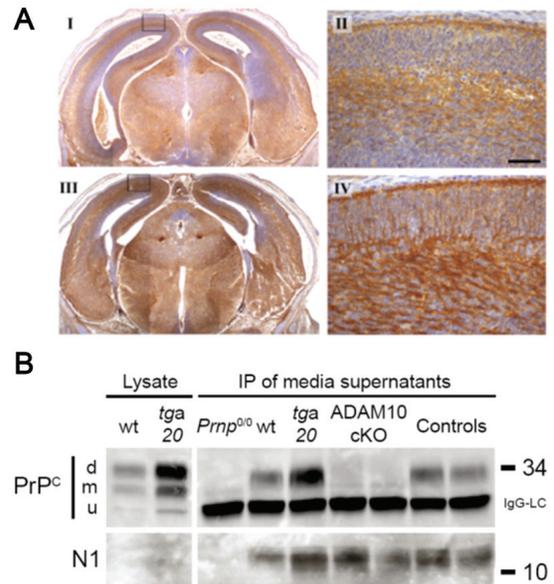
Figure 3. Lack of ADAM10 results in elevated amounts of PrP^C and loss of PrP shedding. (A) Increased immunoreactivity for PrP^C (mouse monoclonal antibody POM1 was used) in brains of embryonic mice with an ADAM10 knockout in neural precursor cells (III and IV) compared to littermate controls (I and II). II and IV represent magnifications of cortex area (see boxes in I and III respectively). Scale bar is 50 μm. (B) Immunoprecipitation (IP) of released PrP fragments in media supernatants of primary neurons derived from embryonic PrP^C knockout (*Prnp*$^{0/0}$), wildtype (wt), and PrP^C-overexpressing (*tga20*) mice as well as from ADAM10 conditional knockout (ADAM10 cKO) and littermate controls reveals a loss of shedding when ADAM10 is lacking while production of the N1 fragment is not affected (d, m, u = di-, mono-, unglycosylated forms of PrP^C; IgG-LC = light chain of capture antibody POM2). (B) is taken from [41] originally published by BioMed Central.

in compartments of the early secretory pathway rather than tolerating increased levels at the cellular surface [41]. Surprisingly, ADAM10-knockout mice also showed reduced activity of BACE1 [74]. This unexpected finding could be explained by the inhibitory effect of PrP^C towards BACE1 in the secretory pathway [34, 35].

In addition, shed PrP itself may have intrinsic functions differing from membrane-attached PrP^C [41, 42, 124]. For instance, it might act as a soluble trophic factor onto neighbouring or distant cells. Furthermore, as discussed for the N1 fragment, shed PrP could potentially bind β-sheet-rich oligomers thereby blocking their dele-
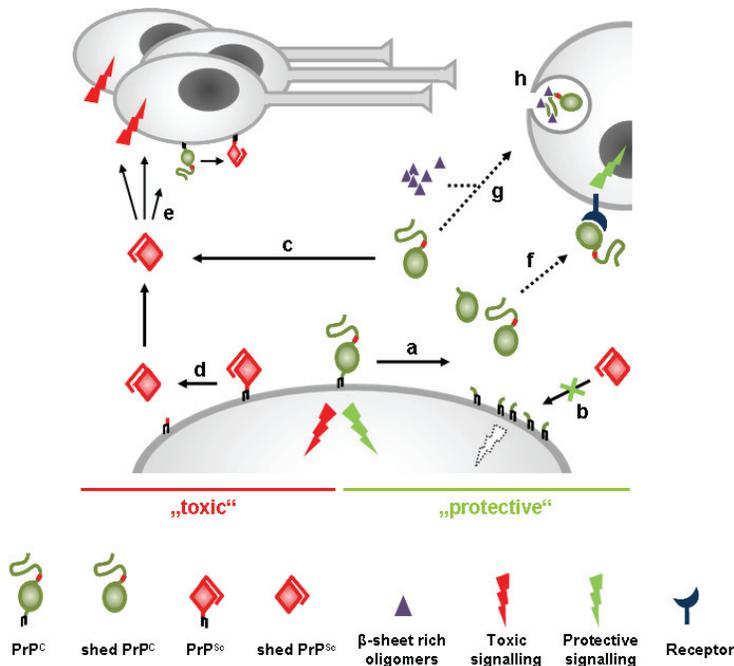
Figure 4. Ectodomain shedding and the role of anchorless PrP. ADAM10-mediated shedding releases nearly full-length PrP (and C1 fragments) from the plasma membrane (a) thereby impairing toxic and protective signaling via PrPc. By reducing the substrate for prion conversion, shedding might have an protective role in prion disease (b). On the other hand, shed PrP was shown to be convertible to PrPSc (c) and shedding might release PrPSc molecules from the membrane (d). Anchorless PrPSc might facilitate the spread of infectivity and induce neurotoxicity and misfolding of PrPc in other cells (e). References are given in the text. It might be speculated that shed PrP acts as a trophic factor and is involved in neuroprotective signalling (f). In addition and as shown for the N1 fragment, shed PrP might likewise be able to bind β-sheet-rich oligomers thereby blocking their toxic effects (g) and potentially guiding them towards phagocytosis and degradation (h).

terious effects and directing them to phagocytosis and degradation (**Figure 4**).

It has been shown that Aβ oligomers damage neurons by the production of ROS in a process that involves activation of the N-methyl-D-aspartate receptor (NMDAR) [139]. This neurotoxic interaction is influenced by copper ions and PrPc with PrPc limiting excessive NMDAR activity by toxic oligomers [20]. An inhibitory effect of PrPc towards NMDAR has previously been shown by others [140]. Interestingly, neurons react to Aβ oligomer treatment by increasing the amount of PrPc at the plasma membrane [141]. Of note, a recent report indicates that activation of NMDAR leads to upregulation of the PrP sheddase ADAM10 in a Wnt/MAPK-dependent cascade [142]. We hypothesize that these initially unrelated findings might combine to a regulatory feedback loop in the control of Aβ oligomer-mediated neurotoxicity. In this scenario, increased surface expression and shedding of PrPc might be a mechanism to block the effects of toxic oligomers.

PrP-shedding and prion disease

The role of shedding in the course of prion disease is not fully understood to date. Two opposing scenarios are conceivable (**Figure 4**): On the

one hand, shedding of PrPc may be protective against prion disease since shedding releases PrPc from the surface and reduces the substrate for conversion [57, 143]. In fact, early experiments with prion-infected cell culture models showed that artificially induced release of surface PrPc by treatment with phospholipase C or with the drug filipin, which both mimic ectodomain shedding, interfered with the formation of PrPSc [143-146]. However, it has to be taken into account that this forced release is much more effective than the physiological shedding. In line with this notion, Taylor *et al.* did not find any alterations in the generation of PrPSc by inhibition or activation of ADAM10-mediated shedding [60]. On the other hand, shedding of PrPc may favor prion disease. In this scenario, shedding of misfolded prions - as released factors - could facilitate prion-spread throughout the nervous system. Accordingly, artificial removal of the GPI-anchor from PrPSc in brain-homogenates from prion-infected mice by cathepsin D did not inhibit further PrPSc formation and infectivity [147]. In contrast to phospholipase C, which was shown to be unable to release PrPSc without prior denaturation of the substrate [147, 148], ADAM10 can shed PrPSc in infected neuroblastoma cells [60]. In addition, anchorless mutant versions of PrPc can be converted to PrPSc in cell-culture and cell-free

systems [149, 150].

Besides all these hints from *in vitro* studies, the role of shedding and anchorless PrP are only poorly understood *in vivo*. Endres *et al*. found prolonged incubation times in prion-infected mice that overexpressed ADAM10 [61]. Although these authors attributed this to transcriptional downregulation of PrP^C by ADAM10, which could not be confirmed in another study [41], this finding underlines the first scenario, with ADAM10-mediated shedding of PrP^C having a protective effect during prion disease. In contrast, the second rather deleterious scenario is supported by results showing that transgenically expressed anchorless, secreted PrP leads to widespread formation of PrP^{Sc} when mice are prion infected [151]. Although some defects in learning and memory were later found in these mice [152], lack of clinical signs of prion disease and a normal life-span were surprising but could later be explained by a low expression of the anchorless PrP (~50% of PrP^C expression in wildtype mice). Indeed, a follow-up study by the same group using mice with a two-fold expression of anchorless PrP found a new type of prion disease upon infection, characterized by a high degree of infectivity and the appearance of dense PK-resistant plaques, whereas grey matter spongiosis was reduced compared to non-transgenic control mice [153]. Another group increased the expression of anchorless PrP in mice 1.7-fold compared to wildtype PrP^C expression and found that these animals spontaneously developed neurological signs reminiscent of Gerstmann-Sträussler-Scheinker (GSS) disease that was accompanied by the generation of anchorless, transmissible *bona fide* prions [106]. In fact, some mutations in the *PRNP* gene resulting in anchorless forms of the proteins were found in patients to cause GSS [154, 155].

Although none of these studies directly investigated ADAM10-mediated shedding, the anchorless version that was used is highly reminiscent of physiologically shed PrP^C. Thus, these findings support the second scenario with shed PrP being able to misfold and contribute to PrP^{Sc} propagation and infectivity. However, since incubation time to terminal prion disease in "anchorless PrP" mice was prolonged, neurodegeneration seems to depend on membrane-anchoring of PrP^C [153], a finding that again indicates the involvement of different entities in neurotoxicity and prion propagation [25].

Outlook

Here we summarized the current knowledge on the proteolytic processing of the prion protein. Cleavage of this protein is of outmost importance for the regulation of the functions of PrP^C and the generation of fragments with autonomous functions such as neuroprotective signalling or myelin maintenance. Furthermore, it has become obvious that at least two cleavage events, i.e. α-cleavage and ectodomain shedding, very likely have the capacity to influence neurodegenerative diseases. Since candidate proteases would provide an attractive goal for therapeutic treatment of these devastating conditions, more research aiming at a deeper understanding of the mechanisms, relevance, and key players of prion protein processing is required. For instance, the therapeutic potential of ADAM inhibition and activation has long been in the focus of researchers in the context of various diseases (as reviewed by [156]). This knowledge should be adopted to and intensified for the field of prion diseases and other neurodegenerative disorders as well.

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