Shedding light on prion disease
Markus Glatzel, Luise Linsenmeier, Frank Dohler, Susanne Krasemann, Berta Puig, and Hermann C Altmeppen*
Institute of Neuropathology; University Medical Center Hamburg-Eppendorf; Hamburg, Germany

ABSTRACT. Proteolytic processing regulates key processes in health and disease. The cellular prion protein (PrPC) is subject to at least 3 cleavage events, α-cleavage, β-cleavage and shedding. In contrast to α- and β-cleavage where there is an ongoing controversy on the identity of relevant proteases, the metalloprotease ADAM10 represents the only relevant PrP sheddase. Here we focus on the roles that ADAM10-mediated shedding of PrPc and its pathogenic isoform (PrPSc) might play in regulating their physiological and pathogenic functions, respectively. As revealed by our recent study using conditional ADAM10 knockout mice (Altmeppen et al., 2015), shedding of PrP seems to be involved in key processes of prion diseases. These aspects and several open questions arising from them are discussed. Increased knowledge on this topic can shed new light on prion diseases and other neurodegenerative conditions as well.

KEYWORDS. ADAM10, neurodegeneration, prion disease, proteolytic processing, shedding

INTRODUCTION

The cellular prion protein (PrPC) is a membrane-anchored glycoprotein that is highly expressed in neurons of the central and peripheral nervous system. The N-terminal part of PrPC is highly flexible and contains an octameric repeat region, a neurotoxic domain and a hydrophobic core. The C-terminal part is structured and comprises α-helices, β-strands, loop domains, 2 N-linked glycosylation sites and a glycosylphosphatidylinositol (GPI)-anchor for attachment to the outer leaflet of the plasma membrane.1,2 Conformational conversion of PrPC into a misfolded isoform (PrPSc) is critically involved in initiation and progression of fatal neurodegenerative prion diseases such as Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle and chronic wasting disease (CWD) in deer.3,4 PrPSc is regarded as an essential component of the transmissible entity of prion diseases, namely the prion, which appears to be devoid of nucleic acids.5–7

Long before it has been realized that proteolytic cleavage of PrPC occurs constitutively under physiological and pathological conditions, proteolytic processing regulates key processes in health and disease. The cellular prion protein (PrPC) is subject to at least 3 cleavage events, α-cleavage, β-cleavage and shedding. In contrast to α- and β-cleavage where there is an ongoing controversy on the identity of relevant proteases, the metalloprotease ADAM10 represents the only relevant PrP sheddase. Here we focus on the roles that ADAM10-mediated shedding of PrPc and its pathogenic isoform (PrPSc) might play in regulating their physiological and pathogenic functions, respectively. As revealed by our recent study using conditional ADAM10 knockout mice (Altmeppen et al., 2015), shedding of PrP seems to be involved in key processes of prion diseases. These aspects and several open questions arising from them are discussed. Increased knowledge on this topic can shed new light on prion diseases and other neurodegenerative conditions as well.

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conditions, PrP has been subjected to proteolytic processing for diagnostic and research purposes. In laboratories, artificial proteolysis of PrP is achieved by proteinase K to assess presence of PrP\textsuperscript{Sc} and to gain insight into its structural features\textsuperscript{8}. In addition, release of full-length PrP\textsuperscript{C} is accomplished by GPI-anchor cleavage upon treatment with phosphatidylinositol-specific phospholipase C (PI-PLC)\textsuperscript{9,10}.

The relevance of naturally occurring cleavage events has only recently been realized and it seems that PrP cleavage regulates PrP\textsuperscript{C} levels and functions. Moreover, generated PrP fragments are bioactive\textsuperscript{11–17}. Here, we will focus on the release of nearly full-length PrP\textsuperscript{C} by the metalloprotease ADAM10 while leaving other cleavage events, such as \(\alpha\)-cleavage and \(\beta\)-cleavage, aside as these have recently been reviewed in detail\textsuperscript{18–20}.

**SHEDDING OF PrP\textsuperscript{C}**

Soluble forms of nearly full-length PrP\textsuperscript{C} have been described in human CSF\textsuperscript{21} and blood\textsuperscript{22,23}, yet insight into the molecular mechanisms on the generation of these PrP\textsuperscript{C} species were only gained recently. The release of extracellular domains of membrane-bound proteins by proteolytic cleavage is termed “shedding”. Shedding of PrP\textsuperscript{C} was initially described by Borchelt et al. in primary cultures of neonatal Syrian hamster brain\textsuperscript{24}. Additional experiments in primary lymphoid and neuronal cells and in cultured cell lines showed that this mechanism is conserved across cell types and species\textsuperscript{25,26}. By using inhibitors of proteases, the first hints that the sheddase belongs to the family of metalloproteases have been followed\textsuperscript{24,27} and finally ADAM10 was identified as the PrP\textsuperscript{C} sheddase\textsuperscript{28}. Digestion experiments in a cell-free system using recombinant proteins recently confirmed these findings\textsuperscript{29}. Additionally, it has been shown that ADAM9 regulates the activity of ADAM10 and thus indirectly influences PrP\textsuperscript{C} shedding\textsuperscript{30–32}. The cleavage site could be mapped between Gly(228) and Arg(229) (in murine PrP\textsuperscript{C}) thus locating merely 3 residues distant to the GPI-anchor attachment\textsuperscript{28,33}. In contrast to the conclusive data concerning shedding of PrP\textsuperscript{C} gained from cell culture studies, in vivo data regarding this important processing step are not as detailed. Transgenic mice moderately overexpressing ADAM10 have reduced PrP\textsuperscript{C} levels and present with decreased PrP\textsuperscript{Sc} amounts and prolonged incubation times upon challenge with prions\textsuperscript{34}.

The availability of conditional ADAM10 knockout mouse models circumvented the problem of early embryonic lethality occurring in complete ADAM10 knockout mice\textsuperscript{35} and enabled us to study the contribution of this protease to the shedding of PrP\textsuperscript{C} in vivo\textsuperscript{35,36}. Using 2 different conditional ADAM10 knockout mouse lines with deletion of ADAM10 in either neuronal precursors\textsuperscript{35} or forebrain neurons\textsuperscript{37}, we were able to confirm ADAM10 as the relevant PrP\textsuperscript{C} sheddase in vivo\textsuperscript{38,39}.

These mice gave further valuable insights into the effects that lack of PrP\textsuperscript{C} shedding has in membrane homeostasis of PrP\textsuperscript{C} and in the context of prion disease. Firstly, lack of ADAM10-mediated shedding leads to disturbed posttranslational processing and membrane homeostasis of PrP\textsuperscript{C} at the neuronal plasma membrane, where—as a consequence—levels of PrP\textsuperscript{C} increase. Secondly, due to impaired shedding, PrP\textsuperscript{C} also accumulates in the early secretory pathway while its mRNA levels remain unaffected.

**SHEDDING OF PrP\textsuperscript{C} IN PRION DISEASE**

Resistance of cells that express only anchorless PrP toward chronic prion infection has been demonstrated\textsuperscript{40}. In line with this, cell culture based experiments gave evidence that forced artificial release of full-length PrP\textsuperscript{C} by exposure to PtdIns-PLC not only prevents prion infection of susceptible cell lines but also cures chronically prion-infected cell lines from producing PrP\textsuperscript{Sc}\textsuperscript{41,42}. In vivo data on the role of shedding of PrP\textsuperscript{C} or PrP\textsuperscript{Sc} lagged behind when compared to data obtained from cell culture experiments. The first hints that soluble versions of PrP\textsuperscript{C} have profound effects on the pathophysiology of prion disease came from transgenic mice expressing soluble PrP\textsuperscript{C}.
dimers. In these mice, soluble PrP<sub>C</sub> dimers were able to antagonize PrP<sup>Sc</sup> propagation and did not form PrP<sup>Sc</sup> themselves.43 Since in this model PrP<sup>C</sup> was fused to the relatively large Fc<sub>y</sub> tail of human IgG<sub>1</sub> (PrP<sup>PC-Fc</sup>) and was dimerized, the data cannot be directly transferred to bona fide shed PrP<sup>C</sup>. Other transgenic mice expressing PrP<sup>C</sup> with a stop codon inserted C-terminal to the putative ADAM10 cleavage site more closely mimicked transgenic expression of shed PrP<sup>PC</sup>.44,45 In contrast to PrP<sup>PC-Fc</sup>, anchorless PrP<sup>C</sup> can be converted to PrP<sup>Sc</sup> and this results in an altered type of prion disease upon challenge with prions.44,45 Later it turned out that higher expression levels of anchorless PrP<sup>C</sup> can even give rise to spontaneous generation of prions.46 However, none of these studies investigated the role of physiological shedding of PrP<sup>C</sup> in prion disease.

Our conditional forebrain-specific ADAM10 knockout (ADAM10 cKO) mice enabled us to perform prion inoculation experiments in mice lacking the ability to generate neuronally shed PrP<sup>C</sup>.39 Briefly, besides elevated PrP<sup>C</sup> membrane levels, depletion of ADAM10 resulted in increased PrP<sup>Sc</sup> formation and shortened incubation time to prion disease. While lack of ADAM10 did not influence prion infectivity our data suggested a role of ADAM10-mediated shedding in the spread of prion-associated pathology within the brain. Thus, findings obtained with this mouse model touch several aspects that are currently discussed in the prion field (Fig. 1) and provide insight into several open questions discussed in more detail below.

**THE DIFFERENT FACETS OF ADAM10-MEDIATED SHEDDING IN PRION DISEASE**

**PrP<sub>C</sub> Membrane Levels and the Execution of Neurotoxicity in Prion Diseases**

Expression of PrP<sub>C</sub> is an absolute requirement for the establishment of prion diseases.27–49 The importance of GPI-anchored PrP<sub>C</sub> for both PrP<sup>Sc</sup> conversion and neurotoxicity, 2 mechanismally different yet related aspects in prion diseases,50,51 has been revealed by several studies (for a review see refs. 52–54).

However, it is not exactly clear how membrane-bound PrP<sup>C</sup> mediates neurotoxicity. PrP<sub>C</sub> acts as a receptor for various oligomeric β-sheet-rich protein species associated with different neurodegenerative diseases such as Alzheimer’s (AD) and Parkinson’s disease (PD).15,55–57 Binding of these toxic conformers—in the case of prion diseases presumably specific assemblies of PrP<sup>Sc</sup>—to PrP<sub>C</sub> at the neuronal surface may initiate signaling pathways ultimately leading to loss of synapses and neurons.15,55,56–60 Another mechanism of neurotoxicity is thought to arise from membrane pores,61,62 formed either directly by PrP<sup>Sc</sup> aggregates or by the N-terminus of PrP<sup>PC</sup> upon structural changes.63,64 Such pores would then disturb the neuronal ion homeostasis and

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**FIGURE 1.** ADAM10 influences several aspects of prion diseases. As revealed by our recent study using ADAM10 cKO mice (as a model for depleted neuronal shedding of PrP) and Tga20 mice (as a model for efficient PrP shedding) the sheddase ADAM10 controls membrane levels and production of anchorless PrP (center) and, by doing so, seems to influence PrP<sup>Sc</sup> formation (A) as well as neurotoxicity and incubation times (B). Moreover, spread of prion-associated pathology throughout the brain appeared to be affected (C). In contrast, our study did not indicate involvement of the protease in the production of infectious prions and thus on transmissibility (D). Details are discussed in the main text.
calcium influx leading to activation of calpain and possibly uncontrolled downstream proteolysis. While we indeed observed a correlation between increased PrPSc amounts and upregulation of calpain indicative for increased pore formation in our ADAM10 cKO mice, we could not find evidence for activation of toxic signaling cascades supporting the receptor model.39 However, this might have been overlooked in our study. To elucidate this point, a more detailed investigation might be necessary (e.g., analysis of synapotosome preparations). In fact, it is conceivable that more mechanisms of neurotoxicity exist and that they all act in concert in prion diseases.

Neurotoxicity seems to be the determinant of incubation times in prion disease and is likely dependent on the amount of PrP C at the plasma membrane. The connection between amounts of membrane-bound PrP C and survival times is strengthened by our study since levels of surface PrP C expression (Tga20 > ADAM10 cKO > wild type) inversely correlated with incubation times (wild type > ADAM10 cKO > Tga20).39 Of note, lack of ADAM10-mediated shedding reduced the incubation time by approximately 30%,39 whereas overexpression of the protease leads to prolonged survival.34 Additionally, in mice expressing anchorless PrP C, coexpression of membrane-bound PrP C results in accelerated clinical prion disease.44,46

Taken together, these findings clearly support the view that maintenance of plasma membrane levels of PrP C is a critical factor for susceptibility toward prion infection and suggest ADAM10 as a key modulator.

**Inhibition of PrP Sc Formation by Shed PrP?**

Conversion of PrP C to PrP Sc is likely to occur at the cell surface, yet a considerable amount of newly formed PrP Sc then rapidly traffics to intracellular compartments.65,66 Our ADAM10 cKO mice showed significantly higher levels of PrP Sc when compared to controls39 arguing that, as observed in cell culture based experiments,65,66 also in vivo conversion of PrP C to PrP Sc mainly occurs at the plasma membrane. However, a connection between surface PrP Sc levels and efficiency of PrP Sc formation cannot be generalized since Tga20 mice, despite increased PrP C membrane levels, only show poor PrP Sc conversion.39,67 Until to date, there are no plausible explanations for this curious and puzzling finding in this widely used PrP C-overexpressing mouse model. We suggest that shed PrP blocks PrP Sc formation in the extracellular space and that increased production of shed PrP (as shown earlier for these mice)38,39 may explain the phenomenon of high PrP C levels and poor PrP Sc generation in Tga20 mice.67 This protective effect of soluble PrP would likely be active in cis (i.e., referring to the same cell) and in trans (i.e., providing protection to neighboring or –by potential distribution via the brain interstitial flow (ISF)– even to distant cells). In fact, some studies support the view that anchorless versions of PrP C either directly inhibit the conversion to PrP Sc or at least are less efficiently converted compared to membrane-anchored forms.43,68–71 Fitting to this model, impaired production of shed PrP C in our ADAM10 cKO mice (possibly in connection with increased PrP Sc surface levels) resulted in increased PrP Sc formation,39 whereas overexpression of ADAM10 leads to decreased production of PrP Sc.34 Further support for this model might come from bank voles: this animal model has raised attention in prion research due to its high susceptibility to different sources of prions.72,73 Accordingly, transgenic mice expressing bank vole PrP C are highly susceptible to prion infection. Although a detailed analysis is required, this might in part be attributed to known alterations in the amino acids sequence of bank vole PrP C in direct vicinity of the putative ADAM10 cleavage site, potentially resulting in impaired shedding74 and consequently lack of protective shed PrP along with increased membrane PrP C levels.

**Prion Spreading—A Potential Role of ADAM10-Mediated Shedding?**

Prion diseases and other neurodegenerative proteinopathies, such as AD or PD, have in
common that pathology spreads throughout the brain following a defined pattern.\textsuperscript{75–77} For acquired forms of prion diseases initiated by uptake of infectious prions (e.g., via the digestive tract) spread from the periphery to the CNS, a process termed “neuroinvasion,” is required to establish disease.\textsuperscript{78,79} Although the precise mechanisms are not fully understood, it is thought that certain conformers of pathogenic PrP act as “seeds” or “nucleating particles” critical for the spread from affected to unaffected cells, tissues and brain regions.\textsuperscript{75–77}

Different mechanisms of spread have been suggested in the past. \textit{In vitro} experiments proposed a role of direct cell-to-cell fusion and formation of tunneling nanotubes for the intercellular spread of prion seeds.\textsuperscript{80} There is evidence that released membranous structures such as viral particles\textsuperscript{81,82} and especially exosomes\textsuperscript{83,84} participate in dissemination of prion seeds. In addition, transsynaptic cell-to-cell transfer of pathogenic prion seeds and transport along neurites seems to be relevant.\textsuperscript{79,85}

Of note, all of the above mechanisms are currently thought to depend on membrane anchoring of PrPC and, indeed, the importance of membrane-bound PrP for neuroinvasion and neural spread has been described.\textsuperscript{86} Nevertheless, mice expressing anchorless PrPC also spread the disease within the CNS albeit with an altered pattern.\textsuperscript{87,88} These studies also discovered a contribution of the brain ISF on the dissemination of anchorless pathogenic prion seeds. Thus, we investigated in our mouse model whether proteolytically shed PrP has a similar role in the spread of prion pathology. In contrast to the disease-accelerating function that lack of PrP\textsubscript{C} shedding has, we were surprised to see that lack of ADAM10-mediated shedding of PrP\textsubscript{C} impaired spreading of prion pathology from the inoculation site to distant brain regions.\textsuperscript{39} In contrast, efficient shedding in Tga20 mice was associated with efficient spread throughout the brain. This is in line with data from the mouse model expressing anchorless PrP\textsubscript{E} where extensive spread of PrP\textsubscript{Sc} also to regions outside the brain has been documented.\textsuperscript{89}

Our finding of clearly reduced prion-associated pathology in cerebellum and brain stem of intracerebrally inoculated ADAM10 cKO mice could indicate that—in addition to the mechanisms of prion spread listed above—the protease ADAM10 contributes to the dissemination of pathology within the CNS.\textsuperscript{39} This could potentially occur by releasing a specific disease-associated cluster or conformer of PrP (as discussed in the next chapter) into the extracellular space, which—via the brain ISF or other routes—is able to nucleate misfolding of PrP\textsubscript{C} molecules in distant brain areas. Since both PrP\textsubscript{C} and its sheddase are ubiquitously expressed throughout the body, this might also bear relevance for the neuroinvasion of exogenously acquired prions.

**Disparity Between PrP\textsubscript{Sc} Levels, Formation of Infectious Prions and Spread of Pathology**

According to the protein-only hypothesis, infectious prion particles are devoid of nucleic acids and mainly—if not solely—composed of misfolded prion protein (PrP\textsubscript{Sc} or PrPres).\textsuperscript{90} In fact, many studies supported the concurrence of PrP\textsubscript{Sc} and prion infectivity.\textsuperscript{91–93} The potential need of non-PrP cofactors for the formation of prion particles\textsuperscript{94–96} has recently been questioned when infectious prions were generated from recombinant prion protein.\textsuperscript{97} We were interested to study whether lack of PrP shedding in our ADAM10 cKO mice would also influence the formation of infectious prions. In view of the significantly increased PrP\textsubscript{Sc} levels found in prion-infected ADAM10 cKO mice, we were surprised to find unchanged infectivity titers.\textsuperscript{39} Thus, our data might indicate that amounts of PrP\textsubscript{Sc} on the one hand and prion infectivity on the other hand are at least not directly congruent. In this respect our study fits to other reports showing some degree of disparity between these 2 sides of the same coin.\textsuperscript{98–104} While the necessity of PrP\textsubscript{Sc} for the formation of transmissible prions is largely undoubted, the exact composition and structure of these particles is still unknown.\textsuperscript{105} Accordingly, while lack of ADAM10-mediated shedding in our study clearly affects PrP\textsubscript{Sc} production, it does not seem to directly impact on generation of prion infectivity.\textsuperscript{39} Generation of prions seems to depend on more than just
formation of PrPSc and likely requires its assembly into unique structures. A recent study described higher infectivity upon prion-challenge in mice expressing anchorless PrPSc compared to wild type mice. However, since in this study the effect was only seen in recipient mice with dramatic overexpression of PrPSc, results cannot be compared to our study where physiological shedding of PrPSc is only abolished in a small subset of total brain cells.

Interestingly, our study also suggested an influence of ADAM10-mediated shedding on the spread of prion-associated pathology within the brain. In the light of unaltered infectivity titers this could indicate existence of 2 (or more) different entities with bona fide prions accounting for transmission, whereas differently composed or shaped particles might be responsible for the spread of disease throughout the CNS. How could this be explained? It has been shown that not only PrPSc but likewise PrPSc can be proteolytically shed. In contrast, experimental release by PI-PLC can only be achieved for PrPSc indicating that conformational changes in PrPSc still allow for access of the protease ADAM10 but hinder phospholipase cleavage of the GPI moiety. ADAM10-mediated release of a critical seed size of oligomeric PrPSc might –together with other mechanisms mentioned above– contribute to prion spread whereas generation of infectious prions may be independent of the protease’s action. In other words, given that the exact composition and structure of the responsible entities is still unsolved, it is possible that ADAM10 influences intracerebral spread of pathology whereas it is rather not decisive or rate-limiting for the production of prions. Importantly, varying contributions of different cell types and potential cofactors in fine-tuning the production of these different forms in the context of prion diseases cannot be ruled out and deserve further investigations.

**PrP Shedding and its Potential Impact on Other Neurodegenerative Proteinopathies**

As described above, GPI-anchored PrPSc acts as a high affinity receptor for Aβ oligomers at the neuronal membrane and likely does so for different neurotoxic conformers associated with other neurodegenerative diseases. Since ADAM10-mediated shedding reduces PrPSc at the plasma membrane, its stimulation could offer a therapeutic option to reduce neurotoxicity. Moreover, similarly to the inhibitory effect of soluble PrPSc on PrPSc formation in vivo, recombinant or transgenic anchorless PrP binds Aβ oligomers in the extracellular space and blocks their toxic access to neurons. In contrast to these protective effects in AD, one report showed that binding of recombinant PrP to Aβ fibrils leads to their disassembly to more toxic oligomeric forms of Aβ. Thus, effects of anchorless or physiologically shed PrP on these and other toxic protein oligomers, such as α-synuclein, remain to be investigated in more detail.

**OPEN QUESTIONS AND PERSPECTIVES**

Apart from the pathogenic aspects discussed above, it appears likely that PrPSc shedding serves physiological functions. This assumption is especially supported by the high degree of conservation of this cleavage event and the rather ubiquitous expression of protease (ADAM10) and substrate (PrPSc). Given that, surprisingly little is known about potential biological activities of shed PrPSc. In one study, primary neurons were incubated with recombinant PrP and this led to polarization and synapse formation. Although recombinant PrP mimics shed PrP with regard to the lack of a GPI-anchor, other structural features (such as a lack of N-glycans) do not exactly recapitulate shed PrP and, thus, findings obtained with recombinant protein cannot directly be extrapolated to its physiological correlate (shed PrPSc).

So far, PrPSc is one of very few GPI-anchored ADAM10 substrates identified, whereas the majority of substrates contain a transmembrane domain and are thus subject to ectodomain shedding. However, it has been shown that minor fractions of PrP are produced as cytosolic or transmembrane forms. It remains to be investigated whether the latter are subject to membrane-proximate cleavage by ADAM10.
and if this has physiological or pathological consequences. Interestingly, many other ADAM10 substrates in the brain have key roles in one or more of the following aspects: (i) developmental processes (e.g., Notch114), (ii) cell adhesion (e.g., ephrins115 and N-cadherin116), (iii) synapse integrity and function (117; e.g., neurexin-1118) as well as (iv) neuroprotection (e.g., sAPPα derived from APP37,119). ADAM10-mediated shedding plays a major role in regulating these aspects. Interestingly, all of these aspects have also been attributed to PrPSc (for a review see refs. 120, 121). Despite the fact that the physiological roles played by PrPSc are still not fully understood, an interesting speculation may arise from this connection: Could the fact that PrPSc is a conserved substrate of the highly brain-relevant sheddase ADAM10 tell us something about its physiological functions? Moreover, could shed PrPSc (as discussed here) and other truncated forms produced by physiological cleavage events (discussed elsewhere; for a review see refs. 18–20)—at least in part—even represent a key to understanding the multitude of suggested PrPSc functions? And finally, could there be even more yet overlooked cleavage events on PrP that bear physiological or pathogenic relevance (Fig. 2)? Future research should gain deeper insight into these aspects and hopefully provide new therapeutic strategies.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

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