internalization of PrP^C in N2a cells is relatively rapid as demonstrated by Morris's group, with more than 25% of the surface PrP^C internalized within 10 minutes [79]. In conclusion, evidence indicates that N2a cells exhibit proper intracellular trafficking of PrP^C, which may be required for PrP^C to PrP^{Sc} conversion.

In this scenario, prions (i.e. PrP^{Sc}) may enter cells by binding to PrP^C on the cell surface and then PrP^C transports prions (i.e. PrP^{Sc}) to the PNC, which seems to be either an endosomal compartment or the Golgi. Whether the massive conversion to prion occurs at the cell surface or in the PNC is unknown, but Tabrizi's group reported strong detection of PrP^{Sc} at the plasma membrane and in the PNC in murine N2a cells, suggesting that both are candidate locations for conversion. In addition, they suggested that prion conversion occurs at the cell surface within 1 min, indicating that strong association of exogenous PrP^{Sc} with PrP^C occurs at the plasma membrane [80]. However, artificial GPI-anchorless PrP expressed in transgenic mice on a *Prnp*$^{0/0}$ background was still converted to PrP^{Sc} after prion inoculation into the mice [12], indicating 1) that the conversion occurs in another compartment beyond the cell surface and 2) that there must be another endocytic pathway for PrP^{Sc} to enter cells.

The α-cleavage of PrP^C may occur after expression on the surface, because both physiological and artificial GPI-anchorless PrPs are not cleaved [23]. Thus, if PrP^C is cleaved to generate C1, C1 does not take prions to the intracellular compartment, since it does not seem to be internalized. We have also found that PrP^C is rapidly internalized in N2a cells, but not in HpL3-4 cells, which are competent and incompetent for prion infection, respectively (data not shown), although other group has shown that PrP^C in HpL3-4 cells are rapidly internalized and HpL 3-4 cells are able to replicate prions [81]. HpL 3-4 cells that we obtained might have lost these phenotypes, suggesting that competent cells for prion propagation should have features of rapid PrP^C internalization and PrP^C accumulation in the Golgi. It would be interesting to check if other competent cell lines such as rat pheochromocytoma PC12, hypothalamic neuronal GT-1 cells, and the rabbit epithelial cell line RK13 [82] show similar endocytic features as N2a cells.

The identity of the cellular proteases responsible for α-cleavage seems to be important to address the location of α-cleavage event. In 1992 and 1995, Prusiner's group suggested that a chymotryptic protease is responsible for the degradation of PrP^C to generate PrP^C-II, because tosyl phenylalanyl chloromethyl ketone (TPCK), but not normal protease inhibitor cocktail, reduced the production of PrP^C-II [19,20]. Alternatively, Prelli's group reported in 1998 that the metal-chelating agents EDTA and EGTA show the highest activity to block α-cleavage of PrP^C [83], suggesting the involvement of metalloproteases, although the operative enzymes were not identified. In 2001, however, Checler's group [84] provided the first evidence implicating the responsible metalloproteases as the disintegrins ADAM10 (A disintegrin and metalloprotease) and TACE (tumor necrosis factor a-converting enzyme or ADAM17) by using an *in vitro* cell culture system and a pharmacological approach, although they failed to completely block the generation of C1 and N1. In 2011, however, Glatzel's group used neuron-specific *Adam* 10 knockout mice to show that ADAM10 does not have a direct role in the α-cleavage of PrP^C, but rather it is responsible for the shedding of PrP^C from the surface of cell [85]. ADAM9 and ADAM10 are likely both involved in the shedding of PrP^C as supported by work from Hooper's group [86]. Recently, Kong's group demonstrated that ADAM8 is also a candidate for the α-cleavage of PrP^C [87]. Since mice with ADAM8 deficiency still exhibited residual C1 [87] and male

and female *Adam8* -/- mice were viable and fertile [88], compensatory mechanism may be involved. Collectively, much work remains to clarify the exact enzyme mediating α-cleavage of PrP^C. The best evidence currently available suggests that metalloproteases are involved. Interestingly, involvement of an enzyme responsible for the generation of PrP^{Sc} or C2, a pathogenic form of PrP^C, has also been suggested [43]. This study reported that pharmacological inhibitors of calpains, caspases, and the proteasome, prevented the production of C2, while lysosomal proteases were not involved. Thus, C2 as well as C1 might be generated via physiological machinery. This needs further investigation because calpain-like activity is also suggested in normal processing of PrP^C [89].

6. Conclusions

Proteinse K (PK) treatment of brains infected with prions is the most convincing method to detect PrP^{Sc}. The PK treatment gives rise to an N-terminally truncated form, the size of which is around Mr 27,000-30,000 (PrP 27-30). C1 is a completely different N-terminally truncated form, which is derived from normal cellular PrP^C and may have a physiological function. PrP27-30 is PK resistant and prion-associated, while C1 is PK sensitive and is the product of PrP^C α-cleavage under normal condition. Recently several reports have shown the lack of detectable PK-resistant prion protein (PrPsen *vs.* PrPres) [90], but the infected host suffers from neurodegenerative dysfunction and the infectivity is transmissible [44,91]. These results demonstrate that pathogenic prion proteins are not always PK-resistant or detectable. The byproduct of the "α-cleavage phenomenon" is that PNGase F, which is able to remove almost all types of N-linked glycans, provides a valuable tool to detect both pathogenic PrP^{Sc} and PK-sensitive PrPsen. In some cases, PK treatment might not be necessary to detect abnormal prion proteins, because normal PrP^C shows only two bands, full-length PrP^C and C1, but brains of CJD patients show additional bands (i.e. C2) by PNGase F treatment alone [21].

It seems that there are at least two types of transgenic mouse models: "PrP^{Sc} type" and "ΔF type". The "PrP^{Sc} type" mice generate PrP aggregation and neuropathology that is transmissible to another host, while "ΔF type" mice exhibit direct neurotoxicity, but it is counteracted by PrP^C. Whether a mutation is associated with the "ΔF type" in humans is currently unknown. Alternatively, "ΔF type" mutations mimic neurotoxic signals caused by prions (PrP^{Sc} or PrPsen), since prions require endogenous PrP^C to induce CNS dysfunction.

The "α-cleavage phenomenon" provides an intriguing concept to improve our understanding of prion biology. When α-cleavage is blocked by mutations, it may induce either toxic ΔF type PrP or aggregated PrP such as PrP^{Sc}. Thus, α-cleavage might be a protective mechanism to eliminate detrimental behavior of PrP^C, since anchorless PrP is not cleaved and therefore tends to aggregate when overexpressed [52]. This may explain why endogenous expression of anchorless PrP^C is so minimal [23]. In addition, C1, a naturally occurring fragment of PrP^C, has been shown to protect from prion disease by acting as a dominant negative inhibitor of PrP^{Sc} formation [92]. These reports enhance the importance of the "α-cleavage phenomenon". Despite significant advances in recent years, substantially more research is necessary in the prion field. The interesting questions to be addressed in the future include: 1) What is the mechanism by which Dpl causes male sterility? 2) What is the normal function of Shadoo? 3) What enzymes are responsible to cleave PrP^C or Shadoo? 4) Where

does conversion from PrP^C to PrP^{Sc} occur? 5) Where does α-cleavage occur? 6) What are the normal functions of N1 and C1? 7) What are the normal functions of anchorless PrP?

Acknowledgments

This work was supported in part by MHLW KAKENHI Grant Number H24-Iyaku-Shitei-029 and JSPS KAKENHI Grant Number C24590172. We thank Dr. Kerry S. Campbell, Fox Chase Cancer Center, Philadelphia, USA, for advice and critical review of the manuscript. We also thank Dr. Simon Milling, University of Glasgow, Institute of Infection, Immunity and Inflammation, Glasgow, Scotland, and Dr. Frank Baumann, Hertie Institute for Clinical Brain Research, for critical reading of the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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