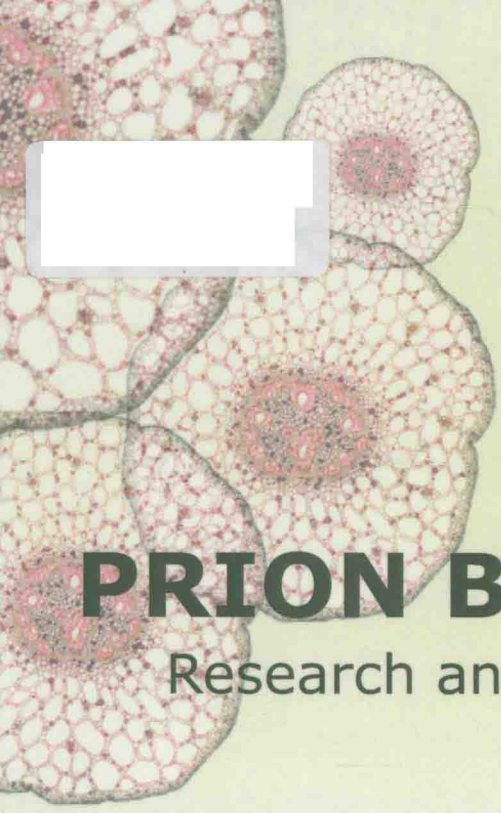

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Research and Advances

Vincent Béringue, PhD
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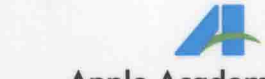

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PRION BIOLOGY

Research and Advances

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Dr. Vincent Béringue has been working in the prion field for almost twenty years. He obtained his PhD in 1998 from the AgroParisTech Institute in Paris, in the laboratory of late Dr. Dominique Dormont. After an MRC-funded postdoctoral position in the laboratory of Professor John Collinge and Dr. Simon Hawke at Imperial College School of Medicine in London, he joined Dr. Hubert Laude's laboratory at INRA (National Institute for Agricultural Research) in Jouy-en-Josas in 2001, as permanent staff scientist. He is now head of the laboratory in the Molecular Virology Immunology Department at INRA. His primary research interests include the biochemistry, diversity and evolution of animal and human prions.

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List of Abbreviations

GPI	Glycosylphosphatidylinositol
NBH	Normal hamster brain homogenate
PI-PLC	Phosphatidylinositol-specific phospholipase C
PK	Proteinase K
PMCA	Protein misfolding cyclic amplification
PNGase F	Peptide:N-glycosidase F
PrP	Prion protein
PrP^{C}	Cellular prion protein
PrPres	Proteinase K-resistant form of prion protein
PrP^{Sc}	Disease-associated scrapie isoform of prion protein
rPrP	Recombinant prion protein
rShaPrP	Recombinant full-length Syrian hamster prion protein
SBH	263K scrapie-infected hamster brain homogenate
TSEs	Transmissible spongiform encephalopathies

Introduction

Mammalian prions are infectious pathogens responsible for transmissible spongiform encephalopathies (TSEs), a group of fatal neurodegenerative disorders affecting farmed and wild animals, as well as humans. Scrapie in sheep and goats, bovine spongiform encephalopathy (BSE), chronic wasting diseases in cervids, and human Creutzfeldt-Jakob disease (CJD) are among the most prevalent TSEs worldwide. Long considered in the middle of the last century as "slow viruses" of the central nervous system, prions now stand firmly as a novel class of proteinaceous infectious pathogens. Prions are essentially composed of PrP^{Sc}, a misfolded, aggregation-prone form of the ubiquitously expressed, host-encoded cellular prion protein (PrP^{C}). Due to these unique properties, prions have attracted a strong research interest. Since the "mad cow" crisis, they also have had a profound impact on human and animal health policy and far-reaching implications on the precautionary principle. This book's chapters are aimed at better understanding prion structure and biology.

PrP^{C} is a glycolipid-anchored cell membrane syaloglycoprotein expressed abundantly at the cell surface of neural cells. While evolutionary conserved among mammals, suggesting thus an important role, its physiological function(s) remains poorly understood. PrP^{C} has been potentially involved in many cellular functions, including neuroprotection, response to oxidative stress, cell proliferation, and differentiation, synaptic function, and signal transduction pathways such as those involving response to pain, as shown by Gadotti and Zamponi (chapter 1). One of the complicating factors in elucidating PrP^{C} function is that PrP-knockout mice, cattle, and goat behave normally and show only subtle if any physiological alterations. Chadi et al. (chapter 2) discuss the potential role PrP may have during embryonic mouse development following the marginal differences in the cellular pathways between wild-type and PrP^{C}-ablated mice at zygotic or adult stage, as observed by transcriptomic analyses of 25,000 mouse genes.

Less uncertain is the key role of PrP^{C} in prion diseases. According to the now widely accepted "protein-only" hypothesis, prion propagation is based on the ability of PrP^{Sc} oligomeric seeds to self-promote and -perpetuate PrP^{C} to PrP^{Sc} conformational conversion, though a nucleated polymerization process, leading to PrP^{Sc} deposition in the brain and sometimes in the lymphoid tissue. The PrP^{Sc} seeds would be contained within the contaminated material in iatrogenic TSE. Etiologically, TSE can also occur sporadically or genetically. Human genetic forms of TSE are associated with autosomal dominant mutations in the PrP gene (>30 mutations described), which are assumed to favor the generation of PrP^{Sc} seeds. Sporadic TSE may reflect a rare stochastic event of spontaneous conversion of PrP^{C} into PrP^{Sc}. Whatever the origin of prion diseases, the mechanisms leading to neurodegeneration are not clear: PrP^{Sc} deposits might be directly noxious; a subversion of PrP^{C} neuroprotective functions due to its enrollment in the conversion process might also be deleterious for neurons. A major breakthrough in the demonstration of the protein-only hypothesis has been the possibility to replicate *in vitro* the PrP^{C} into PrP^{Sc} conversion process by a technique called

protein misfolding cyclic amplification (PMCA). Gonzalez-Montalban et al. (chapter 3) illustrate how PMCA can powerfully amplify minute amounts of abnormal PrP^{Sc} to render the protein detectable by conventional techniques. Mechanistically the process is still poorly understood and, as illustrated by Kim et al. (chapter 4), it appears yet difficult to substitute the substrate containing extractive PrP^{C} by bacterially produced PrP so as to generate "synthetic" prions. However, it is becoming clear that PMCA will be extensively used in the near future as a surrogate for prion infectivity and for much needed diagnostic purposes. Although PrP^{Sc} is known to be enriched in beta-sheet content, the details of structural rearrangement from PrP^{C} to PrP^{Sc} as well as the critical regions important for conversion are not known. The difficulties in purifying PrP^{Sc} in its native form have prevented so far direct structural analyses by X-ray diffraction or Nuclear Magnetic Resonance. Thus, as illustrated by Yam et al. (chapter 5) and Rigter et al. (chapter 6), only indirect evidence can provide clues on the regions that undergo conformational transition or are important in the conversion process.

Another complicating factor in establishing which structural feature(s) confers a biological activity to PrP is that prions, as conventional pathogens, exhibit strain diversity in the same host species. Phenotypically, prion strains display in the same host distinct heritable traits such as the incubation time and a variety of neuropathological and biochemical features, as illustrated by Wemheuer et al. (chapter 7). In the absence of specific nucleic acids, the strain diversity conundrum is assumed to reflect the existence of stable, structurally distinct PrP^{Sc} conformers, at the level of the tertiary and/or quaternary structure. Due to refined detection techniques and to the (large-scale) testing of human and animals for the presence TSE, the spectrum of prion diseases is in constant evolution. For example, it was thought that the prions responsible for the BSE or "mad cow" epidemic were unique in cattle, until the recent discovery of new uncommon BSE forms, showing distinguishable phenotypes in cattle (chapter 8). It came also as a surprise to discover new sheep scrapie prions although the disease was known since more than 250 years. The newly discovered strain is referred to as "atypical" scrapie because it is very difficult to identify by conventional diagnostic tests based on the detection, in the brain, of the protease-resistant core of PrP^{Sc} (chapter 8). This strain is present worldwide and appears equally or even more prevalent than classical scrapie. Intriguingly, putative forms of prion diseases accumulating protease-sensitive PrP^{Sc} have been recently found in human, as highlighted by Rodriguez-Martinez et al. (chapter 9). The transmissible nature of such forms remains to be demonstrated yet.

Prions can transmit from one species to another. Thus animal prions can therefore represent a risk to humans, as shown by the emergence of the variant form of human Creutzfeldt-Jakob disease due to ingestion of BSE prions-contaminated food. Prions cross-species transmission capacities are limited by a barrier commonly referred to as the "species barrier." Its strength is assumed to depend essentially on structural interactions between host PrP^{C} and the infecting prion strain type(s) or conformation(s). Importantly, interspecies transmission is currently unpredictable. Panza et al. (chapter 10) highlight how to circumvent experimental obstacles to study prion transmission barrier between species.

Although PrP is essential for disease to develop, other proteins or factors are likely to contribute to the conversion process. Cell models propagating prions have proved