Invited.01: Transmission of misfolded proteins in neurodegenerative disorders: A common mechanism of disease progression

Virginia M.Y. Lee

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The accumulation of misfolded proteins is a fundamental pathogenic process in neurodegenerative diseases. These hallmark proteinaceous lesions include extracellular senile plaques comprised of the Aβ peptide and intracellular neurofibrillary tangles consisted of tau proteins in Alzheimer disease as well as α-synuclein (α-syn) containing Lewy bodies and Lewy neurites in Parkinson disease. We hypothesized that templated recruitment of endogenous proteins by misfolded conformers follow by cell-to-cell spreading of the pathology are a common disease mechanism that account for the progression of these age-related disorders. In both tauopathies and synucleinopathies, we demonstrate that pre-formed fibrils (pffs) generated from recombinant tau or α-syn enters cultured primary neurons as well as transgenic and wild-type mice, promoted recruitment of soluble endogenous proteins into insoluble protein aggregates resembling the pathology in their human counterparts. Pathologic misfolded aggregates propagated along major central nervous system (CNS) pathways to regions far beyond injection sites and appear to follow neuroanatomical interconnectomes. Thus, synthetic α-Syn or tau pffs are wholly sufficient to initiate neurodegenerative disease pathology and transmit disease in primary neurons in vitro and in mice in vivo. Thus, these data support a prion-like cascade in neurodegenerative disease protein spreading whereby cell-to-cell transmission and propagation of misfolded proteins underlie the CNS proliferation of disease pathology. These findings open up new avenues for understanding the progression of neurodegenerative diseases and for developing novel therapeutics.

Invited.02: Healthy animals, healthy Canada: An expert assessment of approaches to animal health risk assessment

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Keywords: animal health, risk assessment, socioeconomic impacts

In September 2001, the Council of Canadian Academies released an expert panel assessment of approaches to animal health risk assessment in Canada. The expert panel was asked to assess “the state and comprehensiveness of risk assessment techniques in animal health science, specifically pertaining to risks which may impact human health.” This presentation will briefly summarize the key findings of this report. The Panel’s major finding was that an integrated, multidimensional approach that considers the appropriate range of
potential animal, human, socioeconomic and environmental consequences, as well as risk management outcomes, in the risk assessment process would contribute to assessments that provide increased value to risk managers, decision-makers, and stakeholders. Many risks to animal health have economic, ecological, and social implications beyond those directly affecting domestic animal health. A full range of potential consequences should be identified early in the risk assessment process using input from risk managers, risk assessors, and relevant stakeholders. Selection of consequences to consider in the risk assessment should be a formal element of the process. Risk-based decision-making and subsequent risk communication and management could benefit from a greater engagement of stakeholders in establishing risk assessment questions, scope, and consequences, and from improved access to expertise and knowledge among risk assessment practitioners. Because risk assessment is part of a broader risk analysis process that comprises hazard identification, risk assessment, risk communication, and risk management, all four phases need to be effectively performed to maximize the benefits of the risk assessment component.

Through the assessment process, the Panel found that Canada is well-equipped to meet the needs of importation and international trade obligations. However, adopting an integrated, multidimensional approach to risk assessment would help to serve both these areas and the broader goals of risk assessment—that is, to better inform decisions about current risks, emerging threats, and optimal risk management strategies. The majority of risk assessments conducted are qualitative and, while they may consider a range of consequences, the major focus is on the economic and trade consequences of introducing animal disease into Canada. In reviewing risk assessments from other countries, the Panel observed that several countries were taking a broader view of the consequences of animal health events. Canada is also moving toward this broader view.

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Invited.03: Understanding knowledge mobilization in a prion science context

Ralph Matthews

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The processes through which knowledge is created, communicated, evaluated, accepted and implemented are researchable social processes. These social processes involve far more than what is sometimes referred to as “knowledge translation” (KT) with its implication that medical scientists and other professionals have knowledge that others should learn to accept. In contrast, “knowledge mobilization” (KM) is a nonlinear, discursive, reflexive, multi-directional and socially constructed process. To understand KM requires an awareness of how the development and acceptance of scientific knowledge is influenced by: cultural values; assessments of benefits, risks and costs; social organizational structures; institutional cultures; and, political processes. That is, knowledge “in the wild” involves a constant series of translation and reconstructions about its meaning and relevance and it is these that are the subject matter of KM research.

This presentation will provide an overview of knowledge mobilization particularly as it relates to various aspects of prion science investigation, both in veterinary and medical science. In doing so, it will set out a framework for the study of KM, distinguishing between the “knowledge issues” and the “social impact issues” involved in prion science investigation. The result is a framework for assessing what is sometimes described as a “knowledge ecosystem”.

Using the perspective so developed, the presentation will conclude with an overview of a complex of three studies (funded through PrioNet and other sources) that seek to examine the processes of KM in the prion science area. It will also point to the relevance of these studies to understanding innovation and the commercial application of scientific knowledge.

Invited.04: Toward rational, risk-based policies for BSE: Maintaining perspective

Noel Murray

Canadian Food Inspection Agency; Ottawa, ON Canada

While there may be some potentially important gaps in our knowledge, particularly for atypical strains of BSE, we have learnt a great deal since it first leapt onto the world stage in the 1980s. As our understanding of its pathogenesis continues to evolve in tandem with the development of increasingly sensitive assays, perhaps one of the greatest challenges is to ensure that new found knowledge does not lead to calls for measures that are disproportionate to the attendant risks. Excessive measures ultimately divert scarce and finite resources from other competing public and animal health priorities with potentially significant adverse socio-economic impacts.

Ultra-sensitive assays, whether they target PrPSc itself or infectivity are capable of detecting vanishingly small amounts that may be millions of times less than a single bovine oral infectious dose. Observations that PrPSc or infectivity is clearly present do not, in and of themselves, justify concerns that the scope of existing measures, such as the exclusion of specified risk materials (SRM) from the human food supply or the animal feed chain, may be inadequate. Perspective, as always, is essential. The fundamental risk-based question is how informative these results are to real life exposure scenarios, particularly those involving oral ingestion.

As assays have become increasingly more sensitive, there has been a growing list of tissues that for the first time have been identified as harboring PrPSc and/or infectivity. They include peripheral nerves, skeletal muscle, saliva, tongue as well as tissues all along the digestive tract from the esophagus to the rectum. Conclusions are invariably drawn that such findings may
represent previously unrecognized transmission risks. But do they?

It is perhaps worth re-visiting earlier work, which formed the basis for establishing the SRM list. Wild type mice were inoculated intra-cerebrally with an extensive range of tissues, many of which were the same as those now being reported for the first time to contain PrP\textsuperscript{Sc} and/or infectivity. Even though a species barrier was confirmed when cattle were also inoculated intra-cerebrally, infectious dose estimates indicate that these mice were still about 600 times more sensitive than orally challenged cattle. Considering this, it would be reasonable to conclude that those tissues in which infectivity was not detected in these mouse assays would likely pose a negligible risk under most, if not all realistic exposure scenarios.

**Invited.05: Alzheimer disease: Future outlook**

Joseph B. Martin

Harvard Medical School; Harvard University; Boston, MA USA

Despite the extraordinary advances in our appreciation of the biologic processes that result in Alzheimer disease, the most common of the neurodegenerative disorders, there remain huge unsettled issues about the next appropriate steps in finding effective treatments to not cure, but slow down the pace of the disease. The reality of this failure to discern a point of treatment deviation in the disease course in humans leads to the grave concerns about the extent of the costs anticipated for the care of an increasingly aging population, whose treatments for other conditions lead to a situation of chronic care and eventual requirement for long-term care for an increasing number of the population. This talk will review the economic factors we face, touch upon the current efforts in clinical trials seeking effective treatments, and conclude with a few points about whether AD is a prion-like disorder.

**Invited.06: New approaches to understanding and preventing neurodegenerative diseases**

Christopher M. Dobson

Department of Chemistry; University of Cambridge; Cambridge, UK

Neurodegenerative disorders such as Alzheimer and Parkinson diseases arguably represent the greatest challenge to the social fabric and health care systems of much of the modern world. The predominant reason for their rapidly increasing prevalence is the increase in longevity that has resulted from the tremendous advances in public health and hygiene and in medical and surgical interventions over the last century. But the nature of neurodegenerative disorders is quite different from those of most other types of disease, and indeed there are at present no cures or even highly effective treatments. Very significant advances have, however, been made recently in our understanding of the fundamental molecular origins of these conditions, and are now suggesting new and rational therapeutic strategies by which to combat their onset and progression. This talk will discuss recent approaches to this end that we are currently exploring in the context of molecular and cellular biophysics.

**Invited.07: Cofactor molecules maintain infectious conformation and restrict strain properties in mammalian prions**

Surachai Supattapone\textsuperscript{1,4}, Nathan R. Deleault\textsuperscript{1},

Daniel J. Walsh\textsuperscript{1}, Justin R. Piro\textsuperscript{1}, Fei Wang\textsuperscript{2}, Xinhe Wang\textsuperscript{2}, Jiyan Ma\textsuperscript{2} and Judy R. Rees\textsuperscript{3}

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Non-proteinaceous cofactor molecules are required for the propagation of infectious prions in vitro. Several recent discoveries in this area will be discussed, including: (1) The identification of a novel endogenous prion propagation cofactor from mouse brain (phosphatidylethanolamine = PE). Synthetic PE can serve as a solitary cofactor for the formation of infectious recombinant prions from multiple animal species.\textsuperscript{1} (2) The role of cofactor molecules in maintaining the infectious conformation of PrP\textsuperscript{Sc}. Withdrawal of cofactor molecules during serial propagation of purified recombinant prions caused adaptation of PrP\textsuperscript{Sc} structure accompanied by $> 10^4$-fold reduction in specific infectivity to undetectable levels, despite the ability of adapted “protein only” PrP\textsuperscript{Sc} molecules to self-propagate in vitro.\textsuperscript{2} (3) The role of cofactor molecules in maintaining prion strain properties. Propagation in the presence of only one functional cofactor (PE) induced the conversion of three distinct strains into a single strain with unique infectious properties and PrP\textsuperscript{Sc} structure.\textsuperscript{2} Taken together, these in vitro studies show that cofactor molecules can regulate the defining features of mammalian prions: PrP\textsuperscript{Sc} conformation, infectivity, and strain properties.

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The structures of the infectious prion protein, PrP\textsuperscript{Sc}, and that of its proteolytically truncated homolog, PrP\textsuperscript{27–30}, have eluded experimental determination due to their insolubility and propensity to aggregate. Molecular modeling has been used to fill this void and to predict the structures of both forms. The various modeling approaches have produced vastly different models, which indicate the limitations of this methodology. Over the years, in absence of a three-dimensional (3D) structure, a variety of experimental approaches have been used to gain insights into the molecular fold of this medically important isoform.

Here, we present an overview of recently published experimental results, which provided new insights into the structures of PrP\textsuperscript{Sc} and PrP\textsuperscript{27–30}. Negative stain electron microscopy and X-ray fiber diffraction argued that the β-sheets of the infectious prion conformer form a β-helix or β-solenoid structure with a height of four β-strands (rungs) per molecule of PrP\textsuperscript{Sc}/PrP\textsuperscript{27–30} \((\approx 19.2\,\text{Å per molecule})\). Results obtained by hydrogen/deuterium exchange and limited proteolysis followed by mass spectrometry claimed that none of the α-helices, which are characteristic for the structure of PrP\textsuperscript{C}, remain after conversion to the infectious state. Analyses of misfolded, recombinant forms of PrP provided insights into the structural pleomorphism that characterizes PrP, and which has also been seen with other amyloidogenic proteins. Stop codon mutants of PrP were found to adopt a β-helical conformation, supporting earlier predictions.

New approaches are being employed to analyze the structures of PrP\textsuperscript{Sc}, delta-GPI PrP\textsuperscript{Sc}, and of other variants. In particular, the helical periodicity that is inherent to most amyloid fibrils can be used to generate a 3D structure from two-dimensional (2D) images. Higher-resolution structures require electron micrographs to be recorded using cryo low-dose imaging techniques. Image processing based on the helical periodicity of fibrillar aggregates then allows the reconstruction of a 3D volume from 2D images. Once the parameters of the helical periodicity are determined, the image data can be averaged and back-projected into a 3D volume. Preliminary, helical reconstructions of PrP\textsuperscript{27–30} fibrils show repeating densities along the fibril axis spaced at \(-20\,\text{Å}\), in good agreement with the earlier X-ray fiber diffraction results.
**Invited.10: Epigenetic dominance of prion conformers**

Eri Saijo, Jifeng Bian, Hae-Eun Kang, Seahun Kim, Jürgen A. Richt, Jason Bartz, Tracy Nichols, Terry Spraker, Nora Hunter and Glenn C. Telling

1Prion Research Center (PRC) and Department of Microbiology; Immunology and Pathology; Colorado State University; Fort Collins, CO USA; 2Department of Microbiology; Immunology and Molecular Genetics; University of Kentucky; Lexington, KY USA; 3Kansas State University College of Veterinary Medicine; Manhattan, KS USA; 4Department of Medical Microbiology and Immunology; Creighton University; Omaha, NE USA; 5US Department of Agriculture; National Wildlife Research Center; Fort Collins, CO USA; 6The Roslin Institute and the University of Edinburgh; Midlothian, UK

To address the poorly understood mechanism by which distinct prion conformations and host prion protein primary structures interact to influence pathogenesis, we produced transgenic (Tg) mice encoding different scrapie susceptibility alleles. Tg mice expressing OvPrP-A136 infected with SSBP/1 scrapie prions propagated a relatively stable (S) prion conformation, which accumulated as punctate aggregates in the brain, and produced prolonged incubation times, while infected Tg mice expressing OvPrP-V136 developed disease rapidly, and the converted prion was comprised of an unstable (U), diffusely deposited conformer. Infected Tg mice expressing both alleles manifested properties consistent with the U conformer, suggesting that this dominant effect resulted from exclusive conversion of OvPrP-V136 but not OvPrP-A136. Surprisingly, however, studies with mAb PRC5, which discriminates OvPrP-A136 from OvPrP-V136, demonstrated substantial conversion of OvPrP-A136, and that the resulting prion acquired the characteristics of the U conformer. These results, substantiated by in vitro analyses as well as studies in the natural host, indicate that co-expression of OvPrP-V136 altered the conversion potential of OvPrP-A136 from the S to the otherwise unfavorable U conformer by physical interaction of allele products during infection. This epigenetic mechanism expands the range of conformations adoptable by a PrP primary structure, and thus the variety of options for strain propagation.

**Invited.11: BSE: What else is new?**

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**Keywords:** C-type & atypical BSE, regulation, science

Almost 30 years after the initial detection of classical (C-type) BSE and its subsequent spread through the western hemispheres, the number of C-type BSE cases are now steadily declining related to the implementation of effective control measures. This seems to be a regulatory success story and there is a strong push to call the case of “Bovine Spongiform Encephalopathy” solved and to be closed. While this might be true from a regulatory point of view, this presentation provides evidence that—in the presence of many open questions—from a scientific point of view BSE is far from being solved and why a precautionary approach remains necessary.

**Invited.12: Polymorphic and polyphenotypic behavior of scrapie**

Jan Langeveld

Central Veterinary Institute of Wageningen UR Centre; Lelystad, The Netherlands

**Keywords:** scrapie, goat, sheep, prion

In sheep and goats, prion disease (or TSE) is known for a long time as scrapie. In classical scrapie infection occurs horizontally in the field through ingestion (especially placenta) or during lactation or sometimes vertically during pregnancy; Nor98/atypical scrapie seems to develop spontaneously. The effectiveness of transmission is influenced by polymorphisms in prion protein (PrP), tissue tropism, PrP expression levels, and possibly additional host factors.

Phenotypic parameters of TSEs in the natural host are e.g., incubation time, brain lesion profile, tissue tropism, PrPSc tissue distribution, PrPSc biochemical properties, and rodent bioassay characteristics. In this way different strains have been identified, which even can change their phenotype. Mixtures of phenotypes are also being considered. One example is CH1641 scrapie in sheep and goat, a form of classical scrapie that has some PrPSc molecular resemblance to BSE but differing from it in its tissue tropism and transmissibility to rodents. These cases though rare do occur probably on different continents.1,4 Because of some similarities in rapid tests between CH1641 scrapie and BSE, and the uncertainty of the source of the BSE epidemic that arose since the 1980s, TSEs in small ruminants remain a subject for alertness. Also, in contrast to scrapie, BSE exhibits a very stable phenotype and is transmissible to many mammalian species including primates (vCJD in humans).

In sheep, breeding for genetic resistance to disease has been shown possible with a selective strategy for PrP with arginine on position 171 (171R in ARR sheep). This unique strategy has led to efficient reduction in classical scrapie prevalence in sheep within 6–7 y, while it also would have been preventive to BSE spread if it were to enter this species. Unfortunately, for atypical/ Nor98 scrapie a decrease is not visible, and breeding programs will not be effective to reduce it since ARR allele carriers also are susceptible to this TSE type. In goats 171R does not occur, yet recently PrP with lysine on position 222 (222K) appears to be a good alternative candidate, though it has a low frequency and is not observed in some breeds.

The polymorphic variability of PrP in small ruminants allows choices to breed for genetic resistance in both sheep and goats.
Prion protein (PrP) is a host-encoded glycoprotein, which is required for susceptibility to prion diseases in humans, ruminants, rodents and other species. Normally PrP is attached to the cell surface by a glycosylphosphatidylinositol (GPI) linkage; however, in some human families, a PrP mutation consisting of a stop codon at positions 145, 160, 163, 226 or 227 results in synthesis of C-terminally truncated secretable PrP lacking the GPI moiety. Individuals with these mutations develop a fatal GSS-like disease with PrP amyloid plaques in brain. To model these patients and to study the role of PrP membrane anchoring, we previously generated a transgenic mouse model (Tg44+/+) where only the GPI-negative anchorless form of PrP is expressed. In uninfected Tg44+/+ animals, we have not seen clinical or pathological brain disease; however, scrapie-infected Tg44+/+ mice develop a slow fatal neurological disease lasting 320–360 d with high infectivity in CNS, heart, brown fat and colon. The neuropathology consists of extensive perivascular PrP\textsuperscript{res} amyloid deposition with cerebral amyloid angiopathy (CAA) in both gray and white matter accompanied by severe astrogliosis and microgliosis. However, these mice lack typical gray matter vacuolation seen in prion diseases of humans and animals with anchored PrP suggesting that a different pathogenic mechanism is present. In previous studies by other groups in humans and mouse models of Alzheimer disease-associated CAA, A\textsubscript{\textbeta} amyloid deposition and deposition appeared to be influenced by interactions between A\textsubscript{\textbeta} and the brain interstitial fluid (ISF) system. In our experiments using scrapie-infected Tg44+/+ mice, study of interactions between the ISF system and PrP\textsuperscript{res} amyloid found that clearance of ISF tracers was delayed significantly by PrP\textsuperscript{res} amyloid. We now hypothesize that in the absence of membrane-anchored PrP, ISF bulk flow might contribute to concentration of PrP\textsuperscript{res} amyloid precursor fibrils in perivascular regions within the CNS, and subsequent generation of additional amyloid fibrils in these areas might cause damage by partially blocking ISF drainage from the brain.

**Invited.14: Preclinical downregulation of PrP\textsuperscript{c} precursor suggests a fundamental mechanism for the slow progression of prion infections**

David Westaway,1,2,3 Charles E. Mays,1 Chae Kim,4,5 Tracy Haldiman,4 Jacques van der Merwe,1 Qingzhong Kong,4,5 Jan Langeveld,4 Debbie McKenzie1,7 and Jiri G. Safar,1,3

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**Keywords:** conformation dependent immunoassay, scrapie cell assay, substrate depletion

**Background.** Prion diseases are unconventional neurodegenerative disorders characterized by incubation periods lasting for up to decades followed by rapid progression in the CNS within a matter of months because no treatment or cure is available.

**Objectives.** We hypothesized that the downregulation of the PrP-like shadoo protein observed pre-clinically during prion infections\textsuperscript{1,2} might also apply to PrP\textsuperscript{c}. Our objective was to specifically measure PrP\textsuperscript{c} levels in different types of prion disease to determine if it was indeed downregulated.

**Materials and Methods.** To specifically separate PrP\textsuperscript{c} from oligomeric and disease-associated forms, we used sucrose gradient fractionation of infected brains in conjunction with quantification by conformation dependent immunoassays, scrapie cell assays and PMCA titrations.

**Conclusion.** Our data reveal that PrP\textsuperscript{c} is (1) reduced quantitatively in vitro models for infection and at endpoint in rodent models used to study scrapie, Creutzfeldt-Jakob disease and chronic wasting disease and (2) altered qualitatively in terms of glyctype profile. Furthermore, in the case of mouse-adapted scrapie, quantitative reduction occurs pre-clinically, as it does for shadoo, implying activation of a generalized host defense mechanism. Since PrP\textsuperscript{c} is an obligatory precursor for the generation of misfolded forms of the prion protein and is also required for pathogenic signaling from misfolded PrP, downregulation likely impacts disease pathogenesis. Specifically, we assert that preclinical depletion of PrP\textsuperscript{c} may be a major factor contributing to the slow evolution of prion diseases.
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Invited.15: The challenges of policy development to manage prion diseases: A reflection on the past to guide us into the future
Linda A. Detwiler
College of Veterinary Medicine; Mississippi State University; Starkville, MS USA

"If you wait, you are too late." Diseases with long incubation periods (years) and no or limited preclinical tests pose a unique challenge for animal and human health policy makers. The emergence of new strains or disease in different species prompts the need for actions of prevention and control. In the past, regulators and public health officials often had to wait years to obtain information on pathogenesis, transmission, species susceptibility and other aspects of the disease. The ramifications between the choices of delaying until study results were known or taking prompt action might result in allowing the silent spread of infection vs. imposing drastic, costly and perhaps unnecessary prohibitions on certain industries. The presentation will examine the relationship between science and the historical policy decisions for the risk management of scrapie, bovine spongiform encephalopathy, chronic wasting disease and variant Creutzfeldt-Jakob disease. Advances in research have definitely aided in our ability to make more informed decisions, but there are still significant gaps. The presentation will also discuss the challenges of the future, potential solutions and ask if public policy regarding the prion diseases is still relevant.

Invited.16: Studies of chronic wasting disease transmission in cervid and non-cervid species
Edward A. Hoover,1 Candace K. Mathiason,1 Davin M. Henderson,1 Nicholas J. Haley,1 Davis M. Seelig,1 Nathaniel D. Denkers,1 Amy V. Nalls,1 Mark D. Zabe,1 Glenn C. Telling,1 Fernando Goni2 and Thomas Wisniewski,2

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How and why some misfolded proteins become horizontally transmitted agents and occasionally cross species barriers are issues fundamental to understanding prion disease. Chronic wasting disease (CWD) of cervids is perhaps a prototype of horizontal prion transmission, encompassing efficient mucosal uptake, lymphoid amplification, neuroinvasion, peripheralization, and dissemination via mucosal excretion. Efficient mucosal transmission of CWD in deer has been demonstrated by oral, nasal, aerosol, and indirect contact exposure. In addition, other studies (Mathiason CK, et al.) reported at the symposium support a significant role for pre- and/or postnatal transmission of CWD from doe to offspring. Accumulating, yet still incomplete, evidence also suggests that the period of relatively covert CWD infection may be longer than originally thought. Given the above, minimally invasive sensitive assays based on body fluids from live animals would aid substantially in understanding the biology of CWD. We have been applying seeded realtime quaking-induced amplification of recombinant PrP substrates (i.e., RT-QuIC methodology) to: (1) investigate antemortem CWD detection, and (2) model PrP-based species barriers and trans-species adaptation–topics we previously explored using sPMCA and in vivo bioassays. At this symposium, we report sensitive and specific detection CWD prions in saliva, urine, blood (Mathiason lab), and rectal and pharyngeal lymph node samples (Haley NJ, et al.) from pre-symptomatic and symptomatic experimentally and naturally exposed deer. Other ongoing studies are employing RT-QuIC methodology to model amplification barriers among CWD, FSE, BSE, and CJD prions using cervine, feline, bovine, human, and promiscuous sPrP substrates and the above species prion seeds, cellular co-factors, and transgenic mice. Finally, in collaboration with the Wisniewski laboratory, we are conducting of experimental CWD vaccination studies in deer employing oral administration of an attenuated Salmonella vector expressing cervid PrP epitopes.

Invited.17: A unifying role for prions in neurodegenerative diseases
Stanley B. Prusiner
Institute for Neurodegenerative Diseases; University of California, San Francisco; San Francisco, CA USA

Alzheimer disease, Parkinson disease, fronto-temporal dementias, Creutzfeldt-Jakob disease and amyotrophic lateral sclerosis are all neurodegenerative diseases that share two remarkable characteristics. First, more than 80% of cases are sporadic. Second, the inherited forms of these disorders have a late onset, despite the disease-specific mutant proteins being expressed from early in embryogenesis. This suggests that some event occurs with aging that renders the disease-specific proteins pathogenic; I argue that this event involves a stochastic refolding of the etiologic protein into an alternatively folded, self-propagating state known as a prion. Over the past two decades, studies from many different laboratories have accumulated data arguing that a half dozen proteins producing neurodegeneration are prions: synthetic Aβ peptides have been refolded into prions and bioassayed in transgenic mouse models of Alzheimer disease. Similarly, α-synuclein prions responsible for Parkinson disease and tau prions causing fronto-temporal dementias have been produced from recombinant proteins and bioassayed in transgenic mice. The convergence of data...
implicating prions in the pathogenesis of common neurodegenerative maladies has been remarkable. Many mysteries can now be explained within the paradigm of the prion concept including the steady progression of the disease process as well as the spread from one area of the CNS to another. From our growing knowledge of prions, strategies are emerging for developing informative molecular diagnostics and effective therapeutics for these elusive disorders. Early diagnosis will require reporters, such as positron emission tomography (PET) ligands, to identify prions long before symptoms appear. Meaningful treatments are likely to require drugs that diminish the precursor protein, interfere with the conversion of precursors into prions, and/or clear existing prions.

**Invited.18: Propagated misfolding of SOD1 in ALS: A new prion-like disorder**

_Neil Cashman_

Brain Research Centre; Department of Medicine; University of British Columbia; Vancouver, BC Canada

Approximately 10% of ALS cases are familial, with ~20% of these due to mutations in the gene encoding Cu/Zn superoxide dismutase (SOD1), a ubiquitous free-radical defense enzyme. We sought to molecularly dissect the effects of intracellular obligately misfolded SOD1 mutant proteins on natively structured wild-type SOD1. Expression of the enzymatically inactive, natural familial ALS SOD1 mutations G127X and G85R in human mesenchymal and neural cell lines induced misfolding of wild-type natively-structured SOD1, as indicated by: (1) acquisition of immunoreactivity with SOD1 misfolding–specific monoclonal antibodies; (2) markedly enhanced protease sensitivity suggestive of structural loosening; and (3) non-native disulfide-linked oligomer and multimer formation. Cytosolic mislocalizing mutations of FUS and TDP43, two proteins implicated in familial and sporadic ALS, also triggered SOD1 misfolding. Expression of G127X and G85R in mouse cell lines did not induce misfolding of murine wtSOD1, and a species restriction element for human wtSOD1 was found to constitute ~5% of total SOD1 in spinal cord samples from SOD1 familial as well as sporadic ALS. SOD1 misfolding and toxicity can propagate within and between cells, prompting novel targeted therapies for all forms of ALS. ALS now joins company with Alzheimer, Parkinson, and other neurodegenerative and systemic diseases as a “prion-like” disorder that transmits from cell to cell in the CNS.

**Invited.19: Prion-like propagation of α-synuclein as a novel therapeutic target in Parkinson disease**

_Edward A. Fon_

Department of Neurology and Neurosurgery; McGill University; Montreal, QC Canada

Recent findings point toward the tantalizing possibility that aggregated α-synuclein can spread from one neuron to another in a prion-like fashion. This process is believed to occur via the release of α-synuclein from one cell followed by its uptake into another. Regardless of the ultimate mechanism of α-synuclein toxicity, we believe that limiting its spread would have major therapeutic implications for patients suffering from PD because it would have the potential to slow disease progression. We have recently developed a cell-based α-synuclein uptake assay that we are using to screen for genes and small-molecules that modulate α-synuclein uptake. Several promising hits obtained so far as well as those that will be obtained from our ongoing screening will be used to help understand how α-synuclein is taken up and processed in cells and to test whether they can influence the spread of α-synuclein in neurons and in vivo. Together, the work has the potential to uncover key regulators and therapeutics targeting the progression of α-synuclein pathology in PD.

**Invited.20: Prion-like aspects of Alzheimer pathology**

_Mathias Jucker_

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Many neurodegenerative disorders are characterized by a predictable temporal progression of specific aggregated proteins in the brain. The hallmark proteopathy is Alzheimer disease in which Abeta is deposited in brain. Abeta-amyloidosis can be exogenously induced by the application of Abeta-containing brain extracts (Meyer-Lüthmann et al., Science 2006; Eisele et al., Science 2010). The amyloid-inducing agent is likely Abeta itself, although in a conformation generated most effectively in the living brain. Once induced, Abeta lesions spread within and among brain regions. The induced amyloid is dependent on the nature of the Abeta seed and of the host, an observation reminiscent
of prion strains. Recently, the concept of prion-like induction of pathogenic proteins has been expanded to include intracellular lesions (Jucker and Walker, Ann Neurol 2011). Nevertheless, the clinical implications of these observations are not yet clear. Our finding that the Abeta-inducing agent is partly soluble (Langer et al., J Neurosci 2011) intensifies the search for protein seeds in bodily fluids that may have diagnostic value and be a novel target for early therapeutic intervention.

Invited.21: Interneuronal spreading of tau pathology in chronic traumatic encephalopathy

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Chronic traumatic encephalopathy (CTE) is a progressive tauopathy that occurs as a consequence of repetitive mild traumatic brain injury.1 In the earliest stages of CTE, focal perivascular clusters of hyperphosphorylated tau (p-tau) neurofibrillary tangles are found at the depths of the sulci in the cerebral cortex. Even in the absence of additional trauma, CTE appears to progress over decades to become a severe tauopathy affecting widespread regions of the cerebral cortex, basal ganglia, diencephalon and brainstem and medial temporal lobes. How focal tau pathology spreads slowly to involve other brain regions in CTE might involve multiple mechanisms, including a prion-like templated misfolding of tau.

Under normal conditions in the mature human central nervous system, tau is primarily associated with microtubules in axons, where it is neither toxic nor associated with neurofibrillary pathology. Brain trauma causes some tau to become dissociated from microtubules in axons via mechanisms that probably include intracellular calcium influx, glutamate receptor-mediated excitotoxicity, and kinase activation mediating hyperphosphorylation of intracellular tau. Tau dissociated from microtubules becomes abnormally phosphorylated, misfolded, aggregated and proteolytically cleaved by calpains and caspases, all of which are associated with neurotoxicity. Direct and indirect evidence for interneuronal tau transfer in animal models has recently suggested that interneuronal spreading of tau pathology may be due to transfer of toxic tau species between neurons.2-5 This might be mediated by either a prionlike templated misfolding of tau, or by calcium dysregulatory effects of oligomeric or toxic N-terminal tau in the receiving neuron.6 While spreading of tau pathology is generally thought to occur in association with neuronal synapses, glial to glial spread, periventricular and diffuse extracellular tau migration patterns involving the cerebrospinal fluid represent additional pathways by which lesion spreading could occur in CTE.7 Cerebrospinal fluid enters the brain parenchyma along the Virchow-Robin spaces surrounding penetrating arteries and brain interstitial fluid is cleared along paravenous drainage pathways. Recent studies have demonstrated that Ab peptide is cleared through this route.8 Clearance through paravenous flow and the cerebrospinal fluid might also regulate extracellular levels of p-tau and explain the frequent perivascular, subpial and periventricular localization of tau protein in CTE. Interneuronal spreading of tau pathology in CTE is complex and likely involves a variety of non-synaptic mechanisms as well as synaptically mediated mechanisms.

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