



# Prion 2015

*Fort Collins*

## Program Guide





## **Welcome**

The International Organizing Committee and the Prion Research Center at Colorado State University welcome you to Prion 2015. This Congress will build on the tradition of past meetings to include keynote presentations on the history of prions and future directions in prion research by pioneers Byron Caughey and Reed Wickner, and add a yeast prion workshop to the traditional pre-congress animal and human TSE workshops. The main congress also features three plenary talks by Claudio Soto, Karen Ashe and John Collinge, 22 invited and 20 selected talks from leading researchers from around the world.

We are pleased that you are joining us for Prion2015. We encourage you to visit [Prion2015.org](http://Prion2015.org) or scan the QR code on the back of this guide, to access detailed, up-to-date information about the meeting. Prion2015 is supported by the Alberta Prion Research Institute, NeuroPrion, The Creutzfeld-Jakob Disease Foundation, The Centers for Disease Control, The Prion Research Center at Colorado State University, CSU Ventures and registered attendees like you. Thank you for your support.



### **The Local Organizing Committee:**

Glenn Telling, PRC Director  
Ed Hoover, Co- Founding  
Richard Bessen  
Eric Ross  
Candace Mathiason  
Mark Zabel



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K E Y N O T E S P E A K E R S

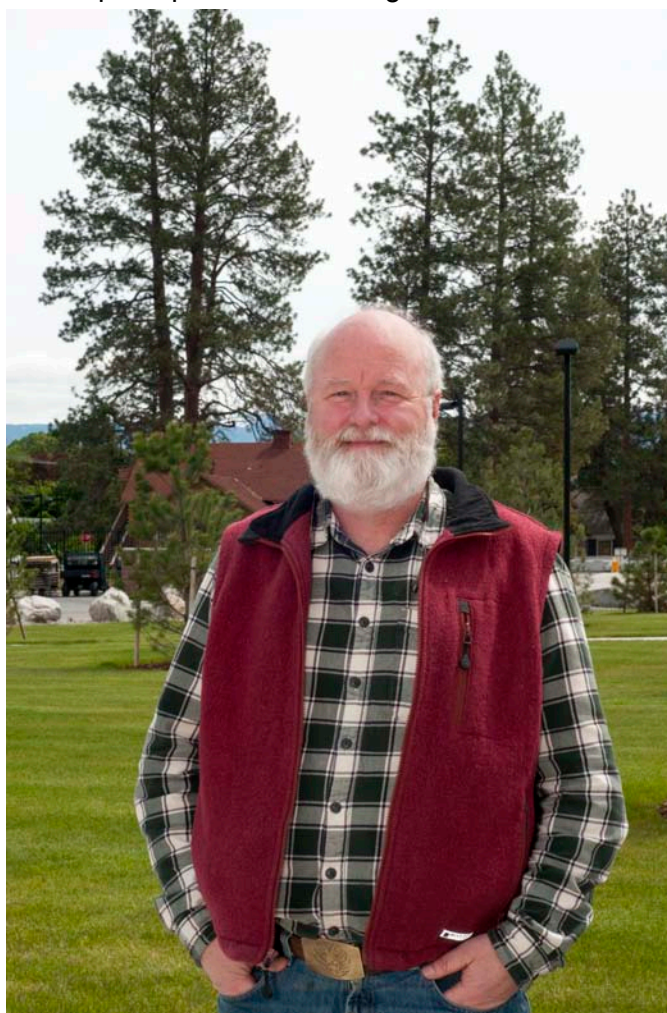
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## Byron Caughey

Dr. Caughey is a Senior Investigator and Chief of the TSE/Prion Biochemistry Section of the Laboratory of Persistent Viral Diseases, Rocky Mountain Laboratories (RML) in Hamilton, Montana. RML is part of the National Institute of Allergy & Infectious Diseases (NIAID), National Institutes of Health. Dr Caughey has been on the editorial boards of several journals and is currently an editor for the *Journal of Virology*. Moreover, he is a Fellow of the American Academy of Microbiology and has served, or is serving, on the scientific advisory boards of agencies that fund or perform TSE/prion disease research.

Dr. Caughey earned his Ph.D. in biochemistry from the University of Wisconsin-Madison in 1985. After post-doctoral research in neurochemistry at Duke University, he joined the Rocky Mountain Labs as a post-doc for Bruce Chesebro in 1986 to begin studies of the transmissible spongiform encephalopathies (TSEs) or prion diseases.

Dr. Caughey now leads one of the several TSE research labs at Rocky Mountain Labs. Key questions that his lab has addressed include the following: What is the infectious agent of prion/TSE diseases of humans and animals? How is prion protein refolded and spread to cause prion diseases? How can we prevent prion protein misfolding? How can we better diagnose and treat prion diseases?



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K E Y N O T E S P E A K E R S

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## Reed Wickner

After receiving his B.A. at Cornell in mathematics and M.D. from Georgetown, Reed Wickner was a postdoc with Herb Tabor at NIH studying enzymology, and then with Jerry Hurwitz at Albert Einstein studying DNA replication. He began his independent work at NIH studying the genetics of dsRNA viruses in *S. cerevisiae*.

In 1994, he discovered that yeast have prions, infectious proteins analogous (but not homologous) to the mammalian PrP-based disease agents. He showed that proteins can act as genes, and that the prion amyloids (filamentous protein polymers) have an in-register parallel beta sheet structure that can explain how proteins can template their conformation (just as DNA templates its sequence), explaining their ability to serve as genetic material. Recently his group has investigated the biological roles of yeast prions, finding that the prions [PSI<sup>+</sup>] and [URE3] can be lethal, and even their mildest forms are detrimental to yeast. Currently Wickner's lab is focused on the roles of chromosomal genes in eliminating prions, and protecting cells from their untoward effects.



## INVITED SPEAKER ABSTRACTS

### K1

#### PrP conformational conversions and the detection of prions

Byron Caughey

Rocky Mountain Labs, NIAID, NIH, Hamilton, Montana, USA

The multimeric structures of TSE prions that allow for strain-specific propagation remain unclear. All of the current models of infectious prion architecture have features that appear to be incongruent with certain empirical observations. Nonetheless, we have built detailed models of prion amyloid fibrils based on the parallel in-register intermolecular  $\beta$ -sheet architecture that has been shown to occur in various synthetic PrP amyloids. Our modeling suggested structural features that might strongly influence PrP<sup>Sc</sup>-like folding toward the N-terminus of the protease-resistant amyloid core; namely prolines 102 and 105, mutations of which are linked to genetic human prion diseases, and a nearby cluster of lysines within residues 101–110. We have now shown empirically that substitution of these residues with alanines or asparagines results in recombinant PrP amyloid fibrils with extended proteinase-K resistant  $\beta$ -sheet cores and infrared spectra that are more reminiscent of *bona fide* PrP<sup>Sc</sup>.

Based on the ability of prions to seed polymerization of recombinant PrP, we and others have developed ultrasensitive RT-QuIC assays for a wide variety of prions in many types of samples. Notably, RT-QuIC assays of CSF and nasal brushings from CJD patients can provide diagnoses with >95% sensitivity and nearly 100% specificity. Recently improved assays can now provide results in a matter of hours rather than days. Appropriate selection of recombinant PrP substrates and conditions have allowed us to sensitively detect all types of human and animal prions at our disposal ( $n=26$ ). Moreover, by comparing seeding activities with different substrates, prion strains can be discriminated.

### K2

#### Yeast prions: How proteins template their conformation, anti-prion systems, prion clouds.

Reed B. Wickner

Laboratory of Biochemistry and Genetics, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892

In 1994, we formulated genetic criteria for infectious proteins (prions) of yeast, and showed that two long-known non-chromosomal genetic elements of *S. cerevisiae*, [URE3] and [PSI], were actually prions of Ure2p and Sup35p, respectively. We defined the Ure2p prion domain, as Ter-Avanesyan did for Sup35p. Work by many labs showed that the [PSI] and [URE3] prions were amyloid forms of Sup35p and Ure2p prion domains. We described [BETA], a prion form of Prb1p, as simply the active form of this vacuolar protease, so non-amyloid prions are also possible. Using solid-state NMR, we have shown that infectious amyloids of Sup35p, Ure2p and Rnq1p prion domains are in-register parallel beta sheet structures, folded along the long axis of the filaments. We proposed that different locations of the folds are at least part of the differences between prion variant amyloids, and can now explain how proteins can template their (amyloid) conformation, just as DNA templates its sequence.

We found that overproduction of the yeast paralogs Btn2p and Cur1p cure [URE3-1], the strong variant of Lacroute usually studied. In Btn2p curing [URE3-1], Ure2p aggregates are collected to one site in the cell co-localizing with the single dot of Btn2p, suggesting a sequestration mechanism of curing. [URE3] spontaneously arises at higher frequency in a *btn2 cur1* mutant than in wild type, and nearly all of the [URE3] variants arising are cured by simply restoring the normal level of these proteins.



Thus, these proteins are part of an anti-prion system that normally eliminates arising prions. The [URE3] variants cured by normal levels of Btn2p and Cur1p have much lower seed/propagon number than those needing overproduced Btn2/Cur1 to be cured, supporting the sequestration mechanism of curing.

Wild *S. cerevisiae* show three groups of Sup35p prion domain sequences. Each polymorph can form [PSI], but transmission between polymorphs is limited. This natural protection from 'catching a prion' is analogous to the protection afforded M/V129 PrP heterozygous humans, and may have been similarly selected for that reason. Transmission across this 'intraspecies barrier' defines different [PSI] variants. We showed that segregation of variants, and spontaneous variant mutation occur without any selection being imposed. This constitutes the first proof of Collinge's Prion Cloud concept.

#### PL1

**Using in vitro prion replication for high sensitive detection of prions and prion-like proteins and for understanding mechanisms of transmission.**

Claudio Soto

*Mitchell Center for Alzheimer's diseases and related Brain disorders, Department of Neurology, University of Texas Medical School at Houston.*

Prion and prion-like proteins are misfolded protein aggregates with the ability to self-propagate to spread disease between cells, organs and in some cases across individuals. In Transmissible spongiform encephalopathies (TSEs), prions are mostly composed by a misfolded form of the prion protein (PrP<sup>Sc</sup>), which propagates by transmitting its misfolding to the normal prion protein (PrP<sup>C</sup>). The availability of a procedure to replicate prions in the laboratory may be

important to study the mechanism of prion and prion-like spreading and to develop high sensitive detection of small quantities of misfolded proteins in biological fluids, tissues and environmental samples. Protein Misfolding Cyclic Amplification (PMCA) is a simple, fast and efficient methodology to mimic prion replication in the test tube. PMCA is a platform technology that may enable amplification of any prion-like misfolded protein aggregating through a seeding/nucleation process. In TSEs, PMCA is able to detect the equivalent of one single molecule of infectious PrP<sup>Sc</sup> and propagate prions that maintain high infectivity, strain properties and species specificity. Using PMCA we have been able to detect PrP<sup>Sc</sup> in blood and urine of experimentally infected animals and humans affected by vCJD with high sensitivity and specificity. Recently, we have expanded the principles of PMCA to amplify amyloid-beta (A $\beta$ ) and alpha-synuclein ( $\alpha$ -syn) aggregates implicated in Alzheimer's and Parkinson's diseases, respectively. Experiments are ongoing to study the utility of this technology to detect A $\beta$  and  $\alpha$ -syn aggregates in samples of CSF and blood from patients affected by these diseases.

Recently, we have been using PMCA to study the role of environmental prion contamination on the horizontal spreading of TSEs. These experiments have focused on the study of the interaction of prions with plants and environmentally relevant surfaces. Our results show that plants (both leaves and roots) bind tightly to prions present in brain extracts and excreta (urine and feces) and retain even small quantities of PrP<sup>Sc</sup> for long periods of time. Strikingly, ingestion of prion-contaminated leaves and roots produced disease with a 100% attack rate and an incubation period not substantially longer than feeding animals directly with scrapie brain homogenate. Furthermore, plants can uptake prions from contaminated soil and transport them to different parts of the plant tissue (stem and leaves). Similarly, prions bind tightly to a variety of environmentally-relevant surfaces, including stones, wood, metals, plastic, glass, cement, etc. Prion contaminated surfaces efficiently transmit prion disease when these materials were directly injected into the brain of animals and

strikingly when the contaminated surfaces were just placed in the animal cage. These findings demonstrate that environmental materials can efficiently bind infectious prions and act as carriers of infectivity, suggesting that they may play an important role in the horizontal transmission of the disease.

Since its invention 13 years ago, PMCA has helped to answer fundamental questions of prion propagation and has broad applications in research areas including the food industry, blood bank safety and human and veterinary disease diagnosis.

## PL2

### **Temporal, spatial and structural relationships of Type 1 and Type 2 A $\beta$ oligomers in the brain**

Peng Liu<sup>1</sup>, Miranda Reed<sup>1</sup>, Linda Kotilinek<sup>1</sup>, Marianne Grant<sup>1</sup>, Colleen Forster<sup>1</sup>, Wei Qiang<sup>3</sup>, Samantha Shapiro<sup>1</sup>, John Reich<sup>1</sup>, Angie Chiang<sup>4</sup>, Joanna Jankowsky<sup>4</sup>, Carrie Wilmot<sup>1</sup>, James Cleary<sup>1,2</sup>, Kathleen Zahs<sup>1</sup>, Karen Ashe<sup>1,2</sup>

<sup>1</sup>University of Minnesota, Minneapolis, MN, USA, <sup>2</sup>Minneapolis VA Medical Center, Minneapolis, MN, USA, <sup>3</sup>Binghamton University, Vestal, NY, USA, <sup>4</sup>Baylor College of Medicine, Houston, TX, USA

Amyloid fibrils containing in-register  $\beta$ -sheets accumulate as A $\beta$ -containing amyloid plaques in Alzheimer's disease (AD). A wide variety of A $\beta$  oligomers (A $\beta$ o) coexist with amyloid fibrils in AD. I will discuss the results of recent studies comparing the spatial, temporal and structural relationships of A $\beta$ o to amyloid fibrils and their effects on cognition in transgenic mouse models of A $\beta$  amyloidosis. Brain-derived A $\beta$ o can be classified as Type 1 and Type 2. Type 1 A $\beta$ o, reactive to A11 antibodies, have no spatio-temporal relationship with A $\beta$  plaques. Type 2 A $\beta$ o, reactive to OC antibodies, emerge after, and accumulate around A $\beta$  plaques. Type 2 A $\beta$ o contain in-register parallel  $\beta$ -sheets. Type 2 A $\beta$ o, despite being highly abundant, do not impair cognition in mice when present at levels comparable to those in AD. Type 2 A $\beta$ o may negatively regulate Type 1 A $\beta$ . These results advance our understanding of

A $\beta$ o in the brain, and may be important in developing effective therapies for AD.<sup>11</sup>

## PL3

### **TBA**

John Collinge

*MRC Prion Unit, London*

## I1

### **TBA**

Marc Diamond

University of Texas, Southwestern Medical Center

## I2

### **Intercellular dissemination routes of self-templating protein aggregates**

Ina Vorberg<sup>a, b</sup>

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<sup>b</sup>Rheinische Friedrich-Wilhelms-Universität Bonn, Sigmund-Freud-Str. 25, 53127 Bonn, Germany

The prion protein PrP is the first identified protein with infectious properties. A growing body of evidence now argues that the conformational transition of normally soluble proteins into self-templating aggregates constitutes a biological principle that underlies diverse processes in both health and disease. Cell-to-cell transmission has been observed for disease-related protein particles, but the cellular mechanisms that mediate their transfer to bystander cells remain ill-defined. Several proteins of yeast can self-associate and propagate in a prion-like fashion. While yeast prions share little sequence homology with mammalian prions, the unusual Q/N rich amino acid composition of fungal prion domains has also been found in many mammalian proteins. We have shown previously that the yeast prion domain NM derived from Sup35 also propagates in mammalian cells and constitutes an ideal



model protein to study cytosolic prion-like phenomena. Here we explore the different cellular pathways utilized by these Q/N rich proteins to infect neighboring cells. We demonstrate that NM prions preferentially infect neighboring cells by direct cell-to-cell contact. However, a subset of yeast prions is also associated with exosomal fractions that transmit the prion phenotype to uninfected cells. The relative ease with which artificial protein aggregates are shared between cells suggests that proteinacious particle transfer may play a more general role in cell-to-cell communication.

### I3 **A $\beta$ Strains and Alzheimer's Disease**

Lary Walker

*Emory University, Atlanta, GA, USA*

An essential early event in the development of Alzheimer's disease is the misfolding and aggregation of A $\beta$ . Enigmatically, despite the extensive deposition of human-sequence A $\beta$  in the aging brain, nonhuman primates do not develop the full pathologic or cognitive phenotype of Alzheimer's disease, which appears to be unique to humans. In addition, some humans with marked A $\beta$  accumulation in the brain retain their cognitive abilities, raising the question of whether the pathogenicity of A $\beta$  is linked to the molecular features of the misfolded protein. I will present evidence for strain-like molecular differences in aggregated A $\beta$  between humans and nonhuman primates, and among end-stage Alzheimer patients. I will also discuss a case of Alzheimer's disease with atypical A $\beta$  deposition to illustrate heterogeneity in the molecular architecture of A $\beta$  assemblies, and how this variability might influence the nature of the disease. As in the case of prion diseases, strain-like variations in the molecular architecture of A $\beta$  could help to explain the phenotypic variability in Alzheimer's disease, as well as the distinctively human susceptibility to the disorder.

This research was conducted in collaboration with Harry LeVine, Rebecca Rosen, Amarallys Cintron, David Lynn, Yury

Chernoff, Anil Mehta and Mathias Jucker and colleagues. Supported by AG040589, RR165/OD11132, AG005119, NS077049, the CART Foundation and MetLife.

### I4 **Prion protein sialylation and prion diseases**

Ilija Baskakov

*University of Maryland School of Medicine, Baltimore, MD, USA*

Cellular prion protein or PrP<sup>C</sup> is a sialoglycoprotein in which terminal sialic acids are attached to galactose residues of two N-linked glycans via  $\alpha$ 2-3 and  $\alpha$ 2-6 linkages. Due to diverse structure and composition of oligosaccharides, PrP<sup>C</sup> primary structure consists of more than 400 different glycoforms. Upon conversion to PrP<sup>Sc</sup>, the posttranslational modifications of PrP<sup>C</sup>, including its N-linked glycans, are carried over giving rise to sialylated PrP<sup>Sc</sup>. Since the discovery that the PrP<sup>Sc</sup> and PrP<sup>C</sup> glycans are sialylated more than 25 years ago, the role of sialylation in prion replication or its pathogenesis remains uncertain.

In mammals, sialic acids are the most abundant terminal residues of cell membrane glycans. Sialic acids play an essential role in a broad range of cellular functions, but are especially important for neuronal plasticity and immunity. Sialic acids on a surface of mammalian cells act as a part of "self-associated molecular pattern" helping immune system to recognize "self" from "altered self" or "non-self".

In the first part of this presentation, a novel hypothesis that sialylation of PrP<sup>Sc</sup> controls the fate of prions in an organism will be discussed. The second part will present the new data on changes of PrP<sup>Sc</sup> sialylation in secondary lymphoid organs and implications of these findings for prion transmission and replication. The third part will demonstrate that the PrP<sup>Sc</sup> glycoform ratio, which is an important feature used for strain typing, is not only controlled by prion strain or host but also the sialylation status of PrP<sup>C</sup>.

## **I5 Pathogenic properties of synthetically generated prions**

Jiyan Ma

*Van Andel Research Institute, Grand Rapids, Michigan, USA*

Synthetically generating prions with bacterially expressed recombinant prion protein (recPrP) strongly supports the prion hypothesis. Yet, it remains unclear whether the pathogenic properties of synthetically generated prions (rec-Prion) fully recapitulate those of naturally occurring prions. A series of analyses including intracerebral and intraperitoneal transmissions of rec-Prion in wild-type mice were performed to determine the characteristics of rec-Prion induced diseases. Results from these analyses demonstrated that the rec-Prion exhibits the same pathogenic properties with naturally occurring prions, including a titratable infectivity that can be determined by end-point titration assays, capability of transmitting prion disease via routes other than the direct intra-cerebral inoculation, causing ultra-structural lesions that are specific to prion disease, and sharing a similar manner of visceral dissemination and neuroinvasion with naturally occurring scrapie and chronic wasting disease. These findings confirmed that the disease caused by rec-Prion in wild-type mice is bona fide prion disease or transmissible spongiform encephalopathies, and the rec-Prion contains similar pathogenic properties as naturally occurring prions.

the respiratory musculature drives the rate of disease progression. In cognitive disorders, such as Alzheimer's disease, one can find similarly find evidence of spreading dysfunction and pathology. One mechanism to account for this spread of disease from one neural structure to another is by evoking prion-like propagation of a toxic misfolded protein from cell to cell. Recent studies in animals that model aspects of Alzheimer's Disease, Parkinson's Disease, and Tauopathy, have bolstered the arguments in favor of prion-like, although in most of these models the mice do not develop overt "clinical" symptoms. Recently, Jacob Ayers demonstrated that the symptoms of ALS can be transmitted from a strain of mice that expresses mutant SOD1-G93A at high levels to a second transgenic strain that expresses mutant SOD1 at low, nontoxic, levels. This model showed many prion-like features including evidence of host-adaptation (earlier and more penetrant disease upon second passage). Interestingly, homogenates from paralyzed mice expressing the G37R variant of SOD1 transmitted poorly, a finding suggestive that different SOD1 variants may exhibit strain-like properties. These "inducible" models of human neurodegenerative disease enable the generation of models that do not require extraordinary levels of transgene expression and provide a more precise means of initiating the disease process, advances that may translate into more predictive pre-clinical models.

## **I6 Transmissible protein toxins in neurodegenerative disease**

Jacob Ayers, David Borchelt  
*University of Florida, Gainesville, FL, USA*

Amyotrophic lateral sclerosis (ALS) is an obvious example of neurodegenerative disease that seems to spread along anatomical pathways. The spread of symptoms from the site of onset (e.g. limb) to

## **I7 Early Trafficking and Dissemination of CWD Prions in Deer**

Edward A. Hoover<sup>1\*</sup>, Clare E. Hoover<sup>1</sup>, Davin M. Henderson<sup>1</sup>, Nathaniel D. Denkers<sup>1</sup>, Kristin A. Davenport<sup>1</sup>, Shannon Bartelt-Hunt<sup>2</sup>, Alan M. Elder<sup>1</sup>, Anthony E. Kincaid<sup>3, 4</sup>, Jason C. Bartz<sup>3</sup>, Mark D. Zabel<sup>1</sup>, Candace K. Mathiason<sup>1</sup>

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Omaha, Nebraska, USA, <sup>3</sup>Department of Medical Microbiology and immunology Creighton University, Omaha, Nebraska, USA <sup>4</sup>Department of Pharmacy Science, Creighton University, Omaha, Nebraska, USA

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Efficient horizontal infection is a hallmark of chronic wasting disease (CWD) in free-ranging cervids. The mechanisms and pathways that enable this remarkable process, however, remain incompletely understood--in particular the facile trans-mucosal entry, exit, and environmental persistence of CWD prions. We have focused on trans-mucosal CWD infection in white-tailed deer, specifically on early prion tissue tropism and later stage prion shedding and association with environmental constituents using modifications of real-time quaking-induced conversion combined with amplified immunohistochemistry. We have documented very early trans-mucosal prion passage (within hours), followed by uptake and amplification in upper digestive tract lymphoid tissues (4 weeks), and dissemination to more distant lymphoid and non-lymphoid tissue sites (8-12 weeks). We have used quantitative approaches to real-time conversion to estimate the relatively low (i.e. vs. tissues) prion concentrations in body fluids and excreta; i.e. >100 (cervidized mouse) LD<sub>50</sub> are shed daily in the urine of one CWD infected deer. Using similar methods, we have also demonstrated and quantified the impressive affinity of CWD prions for both silty clay loam (a major environmental soil constituent) and metal surfaces. Mucosal uptake, excretion, and environmental interactions are central to this most transmissible of prion diseases.

## **I8** **A $\beta$ Oligomer Induced Signal Transduction at the Post-Synaptic Density**

Stephen Strittmatter

*Yale, New Haven, USA*

Alzheimer's disease is characterized by A $\beta$  accumulation, with soluble oligomers being most synaptotoxic. We characterized A $\beta$  forms throughout the lifespan of various Alzheimer model mice, and in post-mortem human brain. A $\beta$  exists in several populations, where PrPC-interacting oligomers are a HMW A $\beta$  assemblies. Levels of PrPC-interacting A $\beta$  match closely with memory impairment. Once formed, A $\beta$  acts to impair synapses through PrPC and mGluR5 activation of Fyn. We sought to repurpose a kinase inhibitor oncology compound for AD. Fyn kinase inhibitor treatment blocks A $\beta$ -induced signaling and phosphorylation of the AD risk gene product, Pyk2, and of NR2B. Fyn inhibitor dosing of APP/PS1 mice fully rescues memory deficits and synaptic depletion, without altering APP or A $\beta$  metabolism.

## **I9** **Mechanisms of PrP<sup>C</sup>-Mediated Neurotoxicity**

David Harris, Alex McDonald, Bei Wu

*Boston University School of Medicine, Boston, MA, USA*

Considerable evidence indicates that the cellular prion protein (PrP<sup>C</sup>) serves as a cell-surface transducer of neurotoxic signaling initiated by both PrP<sup>Sc</sup> and A $\beta$  oligomers. We have been interested in the mechanism underlying this process. We have shown previously that certain mutant forms of PrP<sup>C</sup> create spontaneous ionic currents when expressed in neurons and other cell types, a phenomenon that results from abnormal interactions of the flexible, N-terminal domain of PrP<sup>C</sup> with the plasma membrane. We now report that the isolated N-terminal domain, when fused to an unrelated protein (EGFP), induces ionic currents with similar properties. In addition, antibodies directed against specific epitopes in the structured, C-terminal domain induce spontaneous currents in cells expressing wild-type PrP<sup>C</sup>, and also cause severe dendritic degeneration of cultured neurons in a PrP<sup>C</sup>-dependent fashion. Ligands such as pentosan sulfate, which bind



to the N-terminal domain, inhibit both current activity and dendritic changes induced by C-terminal antibodies. These results have implications for the mechanisms of PrP<sup>C</sup>-mediated neurotoxicity, and they suggest that deleterious side effects may accompany the therapeutic application of anti-PrP antibodies.

## I10

### One Prion, Two Prion, Red Prion, Blue Prion..

Heather True

Washington University School of Medicine,  
St. Louis, MO

Protein aggregates are a hallmark of several protein conformational disorders. Misfolded proteins aggregate in a variety of degenerative diseases, including neurodegenerative diseases such as Alzheimer's Disease, Parkinson's Disease, and prion diseases. More recently, protein aggregates have also been associated with certain degenerative muscle diseases such as Limb Girdle Muscular Dystrophy. Genetic mutations, sporadic changes in protein structure, or a compromise in the protein quality control system can all lead to the accumulation of aggregated protein and cause disease. Cellular toxicity often attributed to misfolded or aggregated protein species can make the causal effect difficult to discern. For this reason, model systems that utilize non-toxic protein aggregates have been a valuable tool for elucidating cause and consequence with both protein misfolding disorders and chaperonopathies. Prion disease models, and the study of non-toxic prion proteins in fungal systems have paved the way to understanding basic concepts of protein conformational disorders. We utilize prion proteins endogenous to *Saccharomyces cerevisiae* to elucidate the contribution of intragenic and extragenic effectors of protein aggregation. Moreover,

amyloidogenic proteins tend to adopt several distinct aggregate structures, which contributes to the complexity, and is thought to play a key role in disease pathology. We have begun to determine how a variety of self-propagating amyloid structures can be formed from a single polypeptide sequence. We have found surprising effects of chaperones and extracellular environment on the generation and selection of specific protein aggregate structures. In addition, we have utilized yeast prion proteins as substrates to investigate how mutations in chaperones associated with human disease alter proteostasis. Strikingly, we recently found that some disease-associate mutations alter client processing in a conformer-specific manner.

## I11

### Structural Determinants for Cross-Species Transmission of Elk CWD Prions

Christina Sigurdson<sup>1</sup>, Tim Kurt<sup>1</sup>, Lin Jiang<sup>2</sup>, Natalia Fernandez-Borges<sup>3</sup>, Cyrus Bett<sup>1</sup>, Terry Spraker<sup>4</sup>, Joaquin Castilla<sup>3</sup>, David Eisenberg<sup>2</sup>, Qingzhong Kong<sup>5</sup>

<sup>1</sup>UC San Diego, La Jolla, CA, USA, <sup>2</sup>UCLA, Los Angeles, CA, USA, <sup>3</sup>CIC bioGUNE, Derio, Spain, <sup>4</sup>Colorado State University, Fort Collins, CO, USA, <sup>5</sup>Case Western University, Cleveland, OH, USA

Chronic wasting disease (CWD) is a highly prevalent, fatal prion disease of deer and elk in the U.S. and Canada. Humans are exposed to CWD prions by consuming infected meat, yet the risk of prion infection is unknown. Public concern over potential CWD transmission to humans has existed for decades and has resulted in major surveillance and research efforts to understand the potential threat for transmission to humans. Here we report a strong species barrier between humans and cervids due to specific amino acid residues in human prion protein (PrP). Transgenic mice expressing human PrP resist CWD, yet mice expressing human PrP with elk residue

substitutions are susceptible, supporting specific residues as causal for an apparent block in elk prion transmission to humans. Additionally, transgenic mice expressing the human-elk chimeric prion protein show a delay in developing human Creutzfeldt-Jakob disease prion infection as compared to mice expressing human PrP. In vitro conversion experiments additionally revealed a surprising contributor to the determinants of species barriers, the amyloid-forming propensity of a segment, which helps to explain why some species, such as the bank vole, are highly susceptible to prions from other species despite having sequence differences. Collectively this work reveals key amino acids that constitute a substantial structural barrier for CWD transmission to humans, and helps illuminate the molecular requirements for cross-species prion transmission.

**I12**

**Mono-nucleated blood cell lines display different efficacy to transmit TSE by the transfusion route**

Jean-Yves Douet<sup>1</sup>, Caroline Lacroux<sup>1</sup>, Hugh Simmons<sup>2</sup>, Hervé Cassard<sup>1</sup>, Olivier Andreatti<sup>1</sup>

<sup>1</sup>INRA, UMR 1225, Interactions Hôtes Agents Pathogènes, Ecole Nationale Vétérinaire de Toulouse, 23 chemin des Capelles, Toulouse, France, <sup>2</sup>AHVLA Weybridge, New Haw, Addlestone, UK

Recent studies demonstrated that despite an apparent low infectivity level whole blood and White blood Cells have a high efficacy to transmit Prion disease by the transfusion route. These results supported the view that leuco-depletion is a beneficial measure for preventing the risk of preventing prion disease by transfusion.

In this study using a sheep Scrapie models and blood transfusion we showed that the transfusion of 100µL but not 10µL of whole blood from an infected incubating donor can transmit the disease. Animals that received 105 fresh white blood cells transmitted the disease through intravenous route developed the disease while while lower number of cells

remained healthy.

Three different mono-nucleated cell blood populations, namely CD14, CD45R and CD4/8cell population displayed comparable infectivity as measured by Intracerebral inoculation in a rodent model, intravenous administration of fresh CD14 was unable to transmit the disease while sheep that received lymphocytes (CD4/8 or CD45R) from the same donor sheep developed the disease.

This work confirms the high efficacy of WBC in transmitting TSE by the transfusion route. It also provides indication with regards to leuco-depletion performance requirements when mitigating blood borne Prion disease transmission risk. Our results also indicate that the ability of WBC to transmit the disease following intravenous is lineage dependent which suggest that it is specifically associated to their biological functions.

**I13**

**The contribution of microglia to the pathogenesis of prion disease**

V.Hugh Perry

University of Southampton, Southampton, UK

During progression of a number of neurodegenerative disease such as Alzheimer's disease, Parkinson's disease, and prion diseases there is an innate immune response in the brain. This innate immune response is characterised by an increase in the density of the microglia, and their 'activation' as judged by alterations in their morphology and the upregulation or de novo synthesis of macrophage antigens. We have studied the innate immune response in prion disease, an animal model of chronic neurodegeneration. As the disease progresses there is evidence of microglia activation and their phenotype is dominated by an hybrid pro- and anti-inflammatory profile, including the expression of TGF-beta PGE<sub>2</sub>, and CCL2. Proliferation and priming of the microglia is driven by colony-stimulating-factor-1 (CSF1) and interleukin-34 (IL-34) binding to the CSFR1. Blockade of CSFR1 leads to a reduction in the numbers of microglia, that is accompanied by a reduction



in neuronal degeneration, a delay in the onset of behavioural deficits and a modest extension of survival. Microglia contribute to prion disease pathogenesis which is further exacerbated by systemic inflammation acting on the primed microglia.

#### I14

**Neuronal death induced by misfolded prion protein is due to NAD<sup>+</sup> depletion and can be relieved *in vitro* and *in vivo* by NAD<sup>+</sup> replenishment.**

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The mechanisms of neuronal death in protein misfolding neurodegenerative diseases (PMNDs) such as Alzheimer, Parkinson and prion diseases are poorly understood. We used a highly toxic misfolded prion protein (TPrP) model to understand neurotoxicity induced by prion protein misfolding. We show that abnormal autophagy activation and neuronal demise is due to severe, neuron-specific, NAD<sup>+</sup> depletion. TPrP-exposed neuronal cells exhibit dramatic reductions of intracellular NAD<sup>+</sup> followed by decreased ATP production, and are completely rescued by treatment with NAD<sup>+</sup> or its precursor nicotinamide because of restoration of physiological NAD<sup>+</sup> levels. TPrP-induced NAD<sup>+</sup> depletion results from PARP1-independent excessive protein ADP-ribosylations. *In vivo*, TPrP-induced degeneration of hippocampal neurons is prevented dose-dependently by intracerebral injection of NAD<sup>+</sup>. Intranasal NAD<sup>+</sup> treatment of prion-infected sick mice significantly improves activity and delays motor impairment. Our study reveals NAD<sup>+</sup> starvation as a novel mechanism of autophagy activation and neurodegeneration induced by a misfolded amyloidogenic

protein, and suggests NAD<sup>+</sup> replenishment as a novel strategy for neuroprotection.

#### I15

**Prion protein and amyloid- $\beta$  precursor protein: Functions & interactions revealed using the zebrafish model system**

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Envisioning treatments for Prion and Alzheimer diseases would benefit from more fully appreciating the function and normal biology of the key proteins central to their etiology. We seek to understand the normal functions of prion protein and amyloid-beta precursor protein, including impactful interactions between the two, using integrative analysis of their loss of function in zebrafish. Targeted mutagenesis has enabled a concerted disruption of each gene. Behavioural, electrophysiological, and molecular phenotyping combined with advanced imaging have been applied to reveal that prion protein has ancient roles in cell adhesion and regulation of neuronal activity towards neuroprotection. Further, our genetic and biochemical studies reveal a specific interaction between PrP<sup>C</sup> and APP (amyloid- $\beta$  precursor protein) holoprotein that expands the potential role for PrP<sup>C</sup> in Alzheimer Disease.

#### I16

**Immunotherapeutic approaches for prion and related disorders**

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Many active and passive immunization approaches have been developed with limited success, for Alzheimer's disease (AD); however, immunomodulation has been less studied for prion diseases. Previously, we have demonstrated we could prevent transmission of prions in a proportion of susceptible mice with a mucosal vaccine. Chronic wasting disease (CWD) infects large numbers of deer and elk, with the potential to infect humans. CWD spreads via an oral-fecal route making a mucosal vaccine an ideal method to try to prevent infection. We have tested a mucosal vaccine in white tail deer, using vaccine strain *Salmonella* expressing cervid PrP. Once a mucosal response was established, the vaccinated animals were boosted orally and locally by application of polymerized recombinant PrP onto the tonsil and rectal mucosa. Blood, saliva and feces were collected regularly to assess IgA, IgM and IgG titers to PrP. The vaccinated and control animals were then challenged orally with CWD-infected brain homogenate. Tonsil and rectal biopsies plus blood, saliva, feces and urine were collected every three months post-infection to assay for PrP<sup>CWD</sup>, as an indicator of infection. Three years post CWD oral challenge all control deer developed clinical CWD while among the vaccinated there was a prolongation of the incubation period and one deer remained CWD free clinically and by RAMALT and tonsillar biopsies. Western blots showed that immunoglobulins from this vaccine react to PrP<sup>CWD</sup>. We have also developed a novel immunomodulatory approach using a pBri peptide. We tested this approach in APP/PS1, 3xTg and TgSwDI AD models and have documented pBri as an immunogen reduces: amyloid plaques, vascular amyloid deposits and neurofibrillary tangles. pBri vaccination in human PrP expressing mice also induces an immune response that recognizes PrP<sup>Sc</sup>. We have also used pBri immunization to develop monoclonal antibodies (mAbs) that recognize multiple pathological proteins, including PrP<sup>Sc</sup>. These mAbs are also able to reduce PrP<sup>Sc</sup> infectivity in tissue culture models. We hypothesize that active and passive immunization that specifically targets pathological conformers has potential as a

therapeutic approach in a number of neurodegenerative disorders including AD and prion diseases.

#### I17

##### **Functional prion-like signaling in mammals**

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Prion-like switches have been proposed to functionally encode molecular memories and to transduce cellular signals. We recently demonstrated the first functional, prion-based signaling activity in mammals. Specifically, we found that two members of the death domain superfamily of mammalian signaling proteins, MAVS and ASC, form prion-like polymerized states upon activation that functionally commit cells to antiviral and inflammatory responses, respectively. The domains responsible for polymerization, CARD and PYRIN, could form bona fide prions when expressed in yeast. Their prion forms could be specifically triggered by transient co-expression of respective upstream signaling proteins. Mutations in MAVS and ASC that disrupted their prion activities in yeast also abrogated their ability to signal in mammalian cells. Moreover, prion formation was sufficient for activation, as a yeast prion domain functionally replaced CARD and PYRIN in mammalian cell signaling. Finally, we found that a conserved fungal NOD-like receptor and prion pair can functionally substitute for the inflammatory signaling activities of PYRIN domains in mammalian cells. These results indicate that prion-like polymerization is a conserved signal transduction mechanism in innate immunity and inflammation.

#### I18

##### **Prion Initiation: A Multistep, Chaperone-Regulated Pathway In vivo Prion Initiation: A Multistep, Chaperone-Regulated Pathway In vivo**

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The prion hypothesis provides an explanation for a collection of previously inexplicable biological phenomena ranging from the infectivity of neurodegenerative diseases, such as mad cow disease, Creutzfeldt-Jakob disease, and scrapie, in mammals to the non-Mendelian inheritance of unique traits in fungi. According to this idea, prion-associated phenotypes are determined epigenetically by alternative conformations of normal, host-encoded proteins and persist because the alternative conformers are stabilized by their assembly into amyloid, which can template the continued refolding of other conformers of the prion into a like state. *In vivo*, the efficiency of conformational self-replication is modulated by cellular processes that impact aggregate dynamics.

In contrast to the well-studied process of prion persistence, our mechanistic understanding of prion appearance lags far behind. To gain this insight, our studies have focused on the Sup35/[PSI<sup>+</sup>] prion of *S. cerevisiae*. The spontaneous appearance of [PSI<sup>+</sup>] is enhanced by Sup35 overexpression in yeast strains propagating the [RNQ<sup>+</sup>] prion, suggesting that [PSI<sup>+</sup>] appearance is limited by forces that can be overcome by Rnq1 aggregates. The predominant model suggests that nucleation is rate-limiting for [PSI<sup>+</sup>] appearance, and Rnq1 aggregates promote this event by providing a heterologous template. An alternative, but not mutually exclusive, possibility is that [RNQ<sup>+</sup>] mediates its effects through a system-based pathway. Indeed, our studies *in vivo* have uncovered a complex interplay between prion conformation and chaperone availability in which Rnq1 aggregates titrate chaperone activity into a range that is compatible with [PSI<sup>+</sup>] appearance, protecting nascent Sup35 aggregates from clearance and promoting conformation-specific amplification of aggregates.

**I19**

**The determinants of protein oligomer toxicity**

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The conversion of proteins into fibrils propagating in a prion-like manner is associated with a number of neurodegenerative diseases. Oligomers forming early or released from the fibrils are toxic to cells, but the structural and biological factors responsible for such toxicity are poorly understood.

Using different conditions, mutagenesis and chaperones we have converted a protein into oligomers featuring different levels of toxicity. Exposure/flexibility of hydrophobic residues on the oligomer surface and oligomer size are key determinants of their toxicity, with the most toxic aggregates displaying the highest hydrophobic exposure and smallest size. Using size-directed mutagenesis we have been able to form oligomers with different size and hydrophobic exposure, and found that oligomer toxicity correlates with a combination of both small size and high hydrophobicity in a 3D-plot.

Oligomer toxicity also arises from the ability of the extracellularly added oligomers to interact with the cell membrane and cause a dramatic calcium influx with consequent apoptosis. Nontoxic oligomers can become toxic, and viceversa, by modulating the content of membrane GM1 ganglioside, thus altering the affinity of the oligomers to the membrane. When equal amounts of toxic and nontoxic oligomers are bound to the membrane under different conditions of GM1 content, similar toxicities were observed.

Overall, the data indicate that the cytotoxicity of extracellular protein oligomers depends on both structural determinants present in the oligomers, such as their small size and high hydrophobic exposure, and the composition of the membrane, such as the presence/quantity of the GM1 ganglioside, which ultimately determine oligomer-membrane binding.

**I20**

**Using rationally-designed mutations to modulate prion activity**



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Numerous prions have been identified in yeast that result from the structural conversions of proteins into an amyloid form. Most of the yeast prion proteins contain glutamine/asparagine (Q/N) rich prion domains that drive prion conversion. However, many other Q/N-rich domains show no detectable prion-like activity. To better understand the sequence basis for prion activity, we previously used a quantitative mutagenesis method to determine the prion propensity of each amino acid in the context of Q/N-rich domains, and used these values to develop the prion prediction algorithm PAPA. Using these data, we can design rational mutations to dramatically increase or decrease the prion activity of known prion domains. Additionally, we tested whether we could generate new prion domains through rational mutation of Q/N-rich domains that had previously been shown to have no detectable aggregation or prion activity. We were consistently able to design mutations to increase the aggregation propensity of these domains, and in some cases these mutants actually acquired the ability to support bona fide prion activity, suggesting that many Q/N-rich domains may be just a few mutations away from being able to form and propagate prions. Collectively, these results further define the sequence basis for prion activity, and may aid in the identification of new prion proteins.

## I21

**Experimental evidence that the infectious prion protein contains a four-rung beta-solenoid structure.**

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The structures of PrP<sup>Sc</sup> and of truncated PrP 27-30 have eluded experimental determination due to their insolubility and propensity to aggregate. Nevertheless, the repeating nature of some aggregates (e.g. of 2D crystals and amyloid fibrils) has allowed the collection of structural data from both PrP<sup>Sc</sup> and PrP 27-30. Here, we summarize the experimental evidence that the infectious prion protein contains a four-rung beta-solenoid structure at its core.

X-ray fiber diffraction patterns from all prion strains (rodent-adapted natural isolates, synthetic and mutant prions) exhibited the same repeating unit of 19.2 Å per molecule, represented by a series of meridional diffraction signals at 4.8, 6.4, and 9.6 Å. The underlying structure for all prion strains was identified as a four-rung beta-solenoid core in a cross-beta arrangement. PrP peptides of varying length are often used as facile surrogates, but their structures do not reach the structural complexity of PrP<sup>Sc</sup>. Even recombinant PrP amyloids did not adopt the prion structure with detailed fidelity, and in some cases their structures were radically different from that of pathogenic PrP<sup>Sc</sup>.

Cryo electron microscopy, in concert with 3D reconstructions, revealed the protofilament organization of PrP 27-30 amyloid. Unprocessed electron micrographs routinely displayed 4.8 Å spacings, confirming the presence of beta-strands in a cross-beta configuration. Averaged fibrils showed periodicities of ~2 and ~4 nm. The former measurement is consistent with the molecular height of PrP 27-30 along the fibril axis, while the latter may represent higher order structures that reveal the underlying quaternary structure.

## I22

**Prion tropism is influenced by both prion formation and prion clearance in LRS tissues**

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between PrP<sup>Sc</sup> formation and clearance may contribute to strain tissue tropism.

A prion strain is operationally defined as a phenotype of disease under a fixed set of agent and host parameters. The strain-specific phenotype of disease is defined by differences in neuropathology but can differ in incubation period, clinical signs of disease, host-range and tissue tropism. The hyper (HY) and drowsy (DY) strains of hamster-adapted transmissible mink encephalopathy (TME) differ in susceptibility to infection by extraneural routes of infection and prion tropism. While it is known that DY TME is not detected in secondary lymphoreticular system tissues of infected hosts, the underlying mechanism is not understood. To explore the strain-specific differences in tissue tropism, we systematically compared the pathogenesis of these two strains at early time points post infection by various extraneural routes of infection. We found that similar to the lymphotropic strain HY TME, DY TME is able to rapidly cross epithelia, enter draining lymphatic vessels, circulate in the blood and is transported to LRS tissues. Since DY TME can cause disease once it enters the peripheral nervous system, the restriction in DY pathogenesis is not due to a failure of transport to the LRS but rather is due to the inability of DY TME to establish infection in the LRS. We hypothesize the rate of DY PrP<sup>Sc</sup> clearance exceeds PrP<sup>Sc</sup> formation in LRS tissues. To test this hypothesis we used protein misfolding cyclic amplification (PMCA) of HY and DY PrP<sup>Sc</sup> and determined that DY PrP<sup>Sc</sup> formation is 100 fold less efficient compared to HY PrP<sup>Sc</sup> formation. Additionally, using PMCA, we found that the LRS environment does not contain inhibitors to DY PrP<sup>Sc</sup> formation. Next we determined that DY PrP<sup>Sc</sup> is more susceptible to proteolytic degradation compared to other prion strains that can establish infection in the LRS. Overall, we conclude that i) PrP<sup>Sc</sup> transport is not involved in tropism and ii) the balance



## LATE-BREAKING ABSTRACTS

**O18**

### **Zoonotic Potential of CWD Prions**

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Chronic wasting disease (CWD) is a widespread and expanding prion disease in free-ranging and captive cervid species in North America. The zoonotic potential of CWD prions is a serious public health concern. Current literature generated with in vitro methods and in vivo animal models (transgenic mice, macaques and squirrel monkeys) reports conflicting results. The susceptibility of human CNS and peripheral organs to CWD prions remains largely unresolved. In our earlier bioassay experiments using several humanized transgenic mouse lines, we detected protease-resistant PrP<sup>Sc</sup> in the spleen of two out of 140 mice that were intracerebrally inoculated with natural CWD isolates, but PrP<sup>Sc</sup> was not detected in the brain of the same mice. Secondary passages with such PrP<sup>Sc</sup>-positive CWD-inoculated humanized mouse spleen tissues led to efficient prion transmission with clear clinical and pathological signs in both humanized and cervidized transgenic mice. Furthermore, a recent bioassay with natural CWD isolates in a new humanized transgenic mouse line led to clinical prion infection in 2 out of 20 mice. These results indicate that the CWD prion has the potential to infect human CNS and peripheral lymphoid tissues and that there might be asymptomatic human carriers of CWD infection.

**O19**

### **FAMILIAL PRION PROTEIN MUTANTS INHIBIT HRD1-MEDIATED RETROTRANSLOCATION OF PROTEINS BY DEPLETING MISFOLDED PROTEIN SENSOR BiP**

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Similar to many proteins trafficking through the secretory pathway, cellular prion protein (PrP) retrotranslocates from the endoplasmic reticulum to the cytosol through the endoplasmic reticulum-associated degradation (ERAD) pathway in an attempt to alleviate the accumulation of misfolded cellular PrP. Surprisingly, familial PrP mutants fail to retrotranslocate and simultaneously block normal cellular PrP retrotranslocation.

That impairments in retrotranslocation of misfolded proteins could lead to global disruptions in cellular homeostasis prompted further investigations into PrP mutant retrotranslocation defects. A gain- and loss-of-function approach identified E3 ubiquitin ligase, Hrd1, as a critical regulator of PrP retrotranslocation in mammalian cells. Expression of familial PrP mutants, V210I129V and M232R129V, not only abolished PrP retrotranslocation, but also that of Hrd1-dependent ERAD substrates, transthyretin TTRD18G and  $\alpha$ 1-anti-trypsin A1AT-NHKKKK. Mutant PrP expression decreased BiP levels by 50% and attenuated ER stress-induced BiP by increasing BiP turnover 6 fold. Overexpression of BiP with PrP mutants rescued retrotranslocation. These results show that PrP mutants hijack the Hrd1-dependent ERAD pathway, an action that would result in misfolded protein accumulation especially in terminally differentiated neurons. This could explain the age-dependent neuronal degeneration in familial prion diseases.

**O20**

### **Locating folds of the in-register parallel $\beta$ -sheet of the Sup35p prion domain infectious amyloid**

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The [PSI<sup>+</sup>] prion is a self-propagating amyloid of the translation termination factor, Sup35p, of *Saccharomyces cerevisiae*. The N-terminal 253 residues (NM) of this 685-residue protein normally function in regulating mRNA turnover but spontaneously form infectious amyloid *in vitro*. We converted the three Ile residues in Sup35NM to Leu and then replaced 16 single residues with Ile, one by one, and prepared Ile-1-<sup>13</sup>C amyloid of each mutant, seeding with amyloid formed by the reference sequence Sup35NM. Using solid-state NMR, we showed that 10 of the residues examined, including six between residues 30 and 90, showed the ~0.5-nm distance between labels diagnostic of the in-register parallel amyloid architecture. The five scattered N domain residues with wider spacing may be in turns or loops; one is a control at the C terminus of M. All mutants, except Q56I, showed little or no [PSI<sup>+</sup>] transmission barrier from the reference sequence, suggesting that they could assume a similar amyloid architecture *in vitro* when seeded with filaments of reference sequence Sup35NM. Infection of yeast cells expressing the reference SUP35 gene sequence with amyloid of several mutants produced [PSI<sup>+</sup>] transfectants with similar efficiency as did reference sequence Sup35NM amyloid. Our work provides a stringent demonstration that the Sup35 prion domain has the folded in-register parallel  $\beta$ -sheet architecture and suggests common locations of the folds. This architecture naturally suggests a mechanism of inheritance of conformation, the central mystery of prions.

#### P166

**Progressive accumulation of prion protein and spongiform encephalopathy in the obex and peripheral tissues of Rocky Mountain elk (*Cervus elaphus nelsoni*) with chronic wasting disease**

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Twenty-two neurological regions of brain stem at the level of the dorsal motor nucleus of the vagus nerve from 85 chronic wasting disease positive elk were examined in order to delineate a possible specific pattern of spread of prion accumulation and spongiform degeneration through this one histological section. The purpose was to formulate a scoring system of spread of the prion and spongiform change by using this single section. A consistent pattern of spread of prion and spongiform degeneration could be documented within these 22 neuro-anatomical regions which allowed positive elk to be placed in 10 categories (obex scores). Next approximately 125 peripheral tissues from 35 chronic wasting disease positive adult elk were examined for the present of prion. It was found that the obex scores correlated well with the present of prion in a relatively orderly fashion in these peripheral tissues.

#### P167

**Polymorphisms in prion protein amino acid 170 do not alter the high susceptibility of red-backed voles (*Myodes gapperi*) to chronic wasting disease**

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The  $\beta 2$ - $\alpha 2$  loop structure of the cellular prion protein (PrP<sup>C</sup>) has been identified as a potential determinant of a host's susceptibility to chronic wasting disease (CWD). The NMR structures of PrP<sup>C</sup> from species that possess an asparagine at position 170 (170N; deer, elk, bank voles) show a  $\beta 2$ - $\alpha 2$  loop that is more rigid than PrP<sup>C</sup> possessing a serine at that same residue (170S; mice, cattle, humans). The 170N genotype is also associated with CWD susceptibility whereas 170S appears to contribute to CWD resistance. We identified and captured a population of red-backed voles that display a natural asparagine/serine polymorphism at position 170. We bred these voles to produce pups with homozygous (170SS, 170NN) or heterozygous (170SN) genotypes and intracerebrally-challenged them with white-tailed deer (*Odocoileus virginianus*) CWD. Following challenge, all three genotypes of voles displayed high attack rates of disease (100% for 170NN and 170SN cohorts, and 89% for 170SS cohort) and statistically-indistinguishable survival times ( $333 \pm 28$  days post-infection (dpi),  $334 \pm 53$  dpi, and  $349 \pm 30$  dpi, respectively; median survival time  $\pm$  95% confidence interval). Clinical signs of disease were similar across experimental challenge groups and included lethargy, ataxia, and decreased burrowing activity. The glycoform profiles and deposition of the abnormal prion protein were assessed by immunoblot and immunohistochemistry analyses. Our work suggests that susceptibility to white-tailed deer CWD in red-backed voles is not reduced by the presence of 170S in the  $\beta 2$ - $\alpha 2$  loop of PrP<sup>C</sup>.

#### P168

##### **Plant-derived vaccine against Chronic Wasting Disease using multimeric deer PrP<sup>C</sup> and its derivatives as immunogens**

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Chronic wasting disease (CWD) is a prion disease that affects free-ranging and farmed cervids. It is the most contagious prion disease, transmissible with direct animal to animal contact or indirect exposure to prions in the environment. The prevalence of CWD has increased rapidly in North America and high infection rates can be seen in captive and free-ranging cervids. Thus, it is apparent that there is an urgent need to control and prevent this disease from spreading.

One potential means of controlling CWD is through active vaccination using cellular prion protein (PrP<sup>C</sup>) as the immunogen. Moreover, one technology receiving attention is the use of plant-based expression systems to produce recombinant proteins as vaccines. This platform provides an economical and viable alternative to other vaccine production systems such as microbial and cell culture systems. Plants act as natural bioreactors with infrastructure for cultivation and harvesting readily available. Vaccines can stably accumulate in seed plants such as *Arabidopsis thaliana* and *Brassica napus* (Canola) and the seeds can be transported and stored at ambient temperature without refrigeration.

Our strategy is to produce a plant-derived vaccine using cervid multimeric PrP<sup>C</sup>.

##### **Our preliminary results show that plant-derived deer PrP<sup>C</sup> multimers can stably accumulate in seeds of *A. thaliana* plants.**

To our knowledge this is the first report of expression of recombinant PrP<sup>C</sup> in a plant system. Several transgenic lines are currently being scaled-up for biochemical analysis and immunization experiments. Our overall goal is to develop an effective vaccine against CWD and prion-related diseases.

#### P169

##### **TSE strainotyping: the first step is the most important**



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**Introduction.** In the past, the bio-assay in mice has proved to be an important and valuable tool for the characterisation and typing of TSE strains. However, little attention has been paid to the selection of the individual mouse from the primary passage whose brain is used for subpassage. We show here that the correct selection for secondary passage, is crucial to the outcome of the bio-assay and the identification of the TSE strains present in the original sheep brain.

**Methods.** Groups of 20-25 RIII or Vm mice were inoculated intracerebrally with a 10% brain homogenate of scrapie affected sheep. Incubation periods to terminal disease/ death were calculated and the brains examined individually by PrP<sup>Sc</sup> profiling as reported previously.

**Results.** PrP<sup>Sc</sup> profiling of individual mouse brains detected up to 3 different profiles after primary passage from a single inoculum. In the case of multiple PrP<sup>Sc</sup> profiles, the mice that significantly preceded the death of the majority of the mice, consistently showed the PrP<sup>Sc</sup> profile of the fastest strain known in the mouse line: ME7 in the RIII mice and 87V in the VM mice. In addition, in RIII mice a new TSE strain was identified that has not been reported before.

**Conclusion.** Individual examination of all mice from a primary passage by PrP<sup>Sc</sup> profiling is vital to the correct identification of

all strains present in the original inoculum. Blind transmission from the first mouse to come down with scrapie leads to an overestimation of the fastest strains in each mouse line.

## P170

### Clinical Stage of Infection is Critical in the Antemortem Diagnosis of Chronic Wasting Disease in Deer and Elk

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Chronic wasting disease (CWD) is an efficiently transmitted spongiform encephalopathy of cervids (e.g. deer, elk, and moose), and is the only known prion disease affecting both free-ranging wildlife and captive animals. The antemortem detection of CWD and other prion diseases has proven difficult, due in part to difficulties in identifying an appropriate peripheral tissue specimen and complications with conventional test sensitivity. At present, biopsies of the recto-

anal mucosal-associated lymphoid tissues (RAMALT) have shown promising sensitivity and are not impractical to collect in live animals. Nasal brush collections have likewise proven both sensitive and practical for identification of prion infections in humans. In this study, we evaluated both RAMALT and nasal brush collections by real time quaking-induced conversion (RT-QuIC), and compared our findings to RAMALT immunohistochemistry as well as conventional postmortem evaluation of obex and retropharyngeal lymph node tissues from over 700 captive and free-ranging deer and elk in areas with endemic CWD. We correlated our results with various clinical findings, including pathological stage of infection as determined by obex scoring, PrP genotype, age, and sex. While the sensitivity of RAMALT RT-QuIC analyses exceeded that of RAMALT IHC (69-80% vs. >44%) and nasal brush collections (15-30%), the sensitivity of both biopsy and nasal brush analyses were dependent primarily on clinical stage of disease, although PrP genotype was also an important predictor of sample positivity. Our findings further demonstrate the potential and limitations of antemortem sample analyses by RT-QuIC in the identification and management of prion diseases.

**P171**

**Prion peripheralization is a host-driven trait of prion infection, independent of strain**

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Chronic wasting disease (CWD), like scrapie of sheep, is a horizontally transmissible spongiform encephalopathy.

Proposed natural routes of transmission for both agents include saliva, urine, and feces, and are likely related to an accumulation of misfolded prion proteins in peripheral excretory tissues, including salivary glands and both gastrointestinal and urogenital tracts. In recent years, prion research has focused on the prion strain (e.g. bovine spongiform encephalopathy – BSE, scrapie, or CWD) as the source code for pathogenic information – encoded by tertiary conformation for example – with strain commonly used as the predominant criteria for classifying prions. In the present study, we compared the peripheral distribution of CWD prions in two experimental host species: transgenic cervidized mice and cattle. Following inoculation and demonstrable infection with CWD, a variety of central and peripheral tissues were collected at necropsy, including brain and both peripheral lymphoid and excretory tissues, and analyzed for prion seeding activity by real time quaking-induced conversion (RT-QuIC). While transgenic mice displayed broad and elevated levels of CWD prions in the periphery, as has been demonstrated with scrapie in mice and CWD in deer, the peripheralization of CWD prions in cattle was very limited and similar to that reported in BSE. Our findings imply that prion peripheralization is likely a trait imparted by the host, independent of the prion strain, though additional research investigating the peripheralization of BSE prions in cervids, for example, is required. It also remains to be seen if other prion phenotypes, e.g. zoonotic potential, are host-derived in nature.

**P172**

**ACTIVATION OF THE NUCLEAR RECEPTOR NUR77 BY A NOVEL DIINDOLYL METHANE ANALOG SUPPRESSES INFLAMMATORY GENE EXPRESSION IN PRIMARY ASTROCYTES**

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Inflammatory activation of glial cells is involved in the progressive loss of dopaminergic neurons in Parkinson's disease (PD). Astrogliosis is accompanied by activation of NF $\kappa$ B, which coordinately regulates the expression of neuroinflammatory genes associated with PD including inducible nitric oxide synthase (iNos), tumor necrosis factor alpha (Tnf $\alpha$ ), and interleukin 1 $\beta$  (Il1 $\beta$ ). These observations suggest that inhibition of NF $\kappa$ B in glial cells could be a promising therapeutic target for the prevention of neuroinflammatory injury. Nuclear orphan receptors in the NR4A family, including NR4A1 (Nur77) and NR4A2 (Nurr1), are reported to antagonize the effects of NF $\kappa$ B on inflammatory gene expression. However, high affinity pharmacologic ligands of these receptors have been lacking. A novel ligand of Nur77, 1,1bis (3'indolyl)1(pmethoxyphenyl) methane (CDIM5), activates Nur77 in cancer cells and causes nuclear degradation of the transcriptional coactivator C/BP (p300), which is also required for the transcriptional activity of NF $\kappa$ B. We therefore postulate that activation of Nur77 by CDIM5 in astrocytes suppresses NF $\kappa$ B dependent inflammatory gene expression induced by the 1methyl4 phenyl1, 2, 3, 6tetrahydropyridine (MPTP) and the inflammatory cytokines interferon gamma (IFN $\gamma$ ) and tumor necrosis factor alpha (TNF $\alpha$ ). CDIM5 increased expression of Nur77 mRNA and suppressed neuroinflammatory gene expression. CDIM5 also inhibited the expression of multiple NF $\kappa$ B regulated inflammatory and apoptosis genes in qPCR array studies but did not prevent p65 translocation to the nucleus, suggesting a nuclear-specific mechanism of inhibition. These data demonstrate that CDIM5 prevents production of neurotoxic inflammatory mediators in glial cells through NF $\kappa$ B inhibition, suggesting that this series could be a useful modality in preventing neuroinflammation.

**P173**

**NOVEL SMALL MOLECULE ACTIVATOR OF THE NUCLEAR RECEPTOR NURR1 (NR4A2) PROMOTES A DOPAMINERGIC PHENOTYPE AND PROTECTS AGAINST NEUROTOXICITY IN A MOUSE MODEL OF PARKINSON'S DISEASE.**

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Parkinson's disease (PD) is characterized by the degeneration of dopaminergic neurons of the ventral midbrain. In previous studies, the orphan nuclear receptor Nurr1 (NR4A2) has been shown to both suppress inflammatory gene expression in glial cells and to regulate many genes associated with the production/release of dopamine (DA) in neurons. The phytochemical-based compound, 1,1-bis(3'-indolyl)-1-(p-chlorophenyl) methane (C-DIM12) activates Nurr1 in cancer cells, suppresses inflammatory gene expression in primary astrocytes and protects against loss of dopaminergic neurons in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) model of PD in mice. Quantification of mRNA by qPCR demonstrated C-DIM12 induced expression of Nurr1 in two neuronal cell lines and significantly increased expression of the Nurr1-regulated genes tyrosine hydroxylase (TH) and the vesicular monoamine transporter (VMAT2) in a time- and dose-dependent manner. Knockdown of Nurr1 expression by RNAi prevented C-DIM12-mediated induction of regulated genes, including TH and VMAT2, indicating that Nurr1 is required for gene activation by C-DIM12. Co-treatment with C-DIM12 also preserved cell viability following exposure to 6-OHDA. In vivo, mice were treated (+/-) MPTP every four days and (+/-) C-DIM12 every day for 2-weeks. Neurological behavior associated with PD in mice was monitored during the time of treatments by utilizing an innovative automated real-time gait analysis system. Preliminary data from this study confirms a significant preservation of TH positive neurons by design-based (3D) stereological methods, and TH protein levels analyzed by western blot. mRNA isolated from brain tissue also demonstrated suppression of neuroinflammatory gene expression.

**P174**

**Prion formation, but not clearance, is supported by PMCA**

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The prion agent is comprised of a misfolded isoform, PrP<sup>Sc</sup>, of the normal, host encoded isoform, PrP<sup>C</sup>. Formation of PrP<sup>Sc</sup> is thought to occur in a 3-step process, first PrP<sup>C</sup> binds to PrP<sup>Sc</sup>; second, PrP<sup>Sc</sup> directs the conversion of PrP<sup>C</sup> to PrP<sup>Sc</sup>, resulting in the elongation of the PrP<sup>Sc</sup> fiber; and third, fragmentation of PrP<sup>Sc</sup> fibers results in the formation of additional free ends for further PrP<sup>C</sup> binding. The net accumulation and infectivity of prions is dictated by the balance between the rate of PrP<sup>Sc</sup> accumulation and clearance.

Protein misfolding cyclic amplification (PMCA) is an *in vitro* technique that recapitulates the propagation of PrP<sup>Sc</sup> through a series of incubation and sonication cycles. PMCA propagated prion strains retain their biochemical and biological properties. Little is known about the mechanism of PrP<sup>Sc</sup> propagation during PMCA, where localized high temperature and pressure from sonication have the potential to inactivate and/or decrease the infectivity of prions.

In this study we investigate if degradation of PrP<sup>Sc</sup> and loss of prion infectivity occurs during PMCA. To accomplish this we performed PMCA under conditions that do not support PrP<sup>Sc</sup> formation and did not observe either a reduction in PrP<sup>Sc</sup> abundance or an extension of prion incubation period, compared to untreated control samples. These results indicate that prion clearance does not occur during PMCA. These data have significant implications for the interpretation of PMCA based experiments such as prion amplification rate, adaptation to new species and strain interference where production and clearance of prions can affect the outcome.

**P175**

**Nasal associated lymphoid tissue of the Syrian golden hamster expresses high levels of the cellular isoform of the prion**

**protein**

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The presence of the normal isoform of the prion protein, designated PrP<sup>C</sup>, is required for replication and neurodegeneration during the course of prion pathogenesis. Extraneural exposure to prions results in accumulation of misfolded prion protein in peripheral lymphatic tissues prior to neuroinvasion. The primary objective of this study was to compare the total amount of PrP<sup>C</sup> in different hamster lymphoid tissues involved in the early pathogenesis of prion disease. Lymphoid tissues were collected from golden Syrian hamsters and Western blot analysis was performed to quantify total PrP<sup>C</sup> levels. PrP<sup>C</sup> immunohistochemistry (IHC) of paraffin embedded tissue sections was performed to identify spatial distribution of PrP<sup>C</sup> in tissues of the lymphoreticular system. Nasal associated lymphoid tissue contained the highest amount of total PrP<sup>C</sup> followed by Peyer's patches, mesenteric and submandibular lymph nodes, and spleen. The relative levels of PrP<sup>C</sup> expression in IHC processed tissue correlated strongly with the Western blot data, with high levels of PrP<sup>C</sup> corresponding with a higher percentage of PrP<sup>C</sup> positive B cell follicles. Lymphoid tissues were also shown to contain distinct molecular weight PrP<sup>C</sup> populations with different ratios of full length and truncated PrP<sup>C</sup> present. High levels of PrP<sup>C</sup> in lymphoid tissues closely associated with the nasal cavity could contribute to the relative increased efficiency of the nasal route of entry of prions, compared to oral infection.

**P176**

**Mutations at residue 22 in A $\beta$  modify the deposition of wild-type A $\beta$  in the brains of transgenic mice**

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In Alzheimer's disease (AD) pathogenesis, the amyloid- $\beta$  (A $\beta$ ) peptide exhibits many properties that are reminiscent of PrP prions. Increasing experimental evidence argues that distinct strains of A $\beta$  prions exist and are capable of producing different patterns of cerebral A $\beta$  deposition in susceptible mice. Heritable forms of AD caused by a point mutation within the A $\beta$  coding region are thought to produce a more pathogenic form of A $\beta$  that manifests unique clinicopathologic features, but whether the mutant peptide behaves as a distinct A $\beta$  prion in vivo remains unclear. Here, we report the characteristics of two strains of A $\beta$  prions formed from synthetic A $\beta$ 40 peptides containing either the Arctic (E22G) or Dutch (E22Q) mutation. To investigate whether mutant A $\beta$  prions modulate wild-type A $\beta$  aggregation in vivo, we inoculated synthetic A $\beta$ 40(E22G) or A $\beta$ 40(E22Q) fibrils into the brains of transgenic mice overexpressing wild-type A $\beta$ . Histopathological analyses with high-resolution confocal microscopy showed that both mutant A $\beta$  peptides markedly increased the accumulation of A $\beta$ 38 within the induced plaque deposits compared to wild-type A $\beta$ 40. Interestingly, inoculation of Arctic A $\beta$  peptide, but not the Dutch A $\beta$  peptide, resulted in significantly lower A $\beta$ 42 levels within the induced plaques compared to controls. Similar results were found in parallel experiments using brain homogenates derived from AD patients harboring the Arctic or Dutch mutation. We conclude from these results that the mutant A $\beta$  prion alone is sufficient to modulate the cerebral deposition of wild-type A $\beta$ .

#### P177

#### **Real-Time Quaking-Induced Conversion Detection and Discrimination of Classical and Atypical L-type Bovine Spongiform Encephalopathy**

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Cattle are susceptible to both classical bovine spongiform encephalopathy (C-BSE) and atypical forms of BSE. The latter appears to be of sporadic origin and therefore may never be eradicated. Prion surveillance would greatly benefit from a practical and sensitive tests for routine BSE detection and strain discrimination. The Real-Time Quaking-Induced Conversion (RT-QuIC) assay, which is based on prion-seeded fibrillization of recombinant prion protein (rPrP<sup>Sen</sup>), is known to be highly specific and sensitive for detection of multiple human and animal prion diseases, but not BSE.

We have developed RT-QuIC conditions for the detection of C-BSE and atypical L-type bovine spongiform encephalopathy (L-type BSE or L-BSE) using brain samples from C-BSE and L-BSE Italian cattle. We found that both BSE forms can be sensitively detected and distinguished using particular rPrP<sup>Sen</sup> substrates within the same test run. Specifically, L-BSE was detected using multiple rPrP<sup>Sen</sup> substrates while C-BSE was much more selective and could only be detected using Ha-S rPrP<sup>Sen</sup>. This RT-QuIC substrate-based approach suggests a diagnostic strategy for specific, sensitive, and

rapid detection and discrimination of at least some BSE forms.

**P178**  
**Regional distribution of amyloid  $\beta$ -peptide binding to prion protein in patients with Alzheimer's disease**

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In Alzheimer's disease the generation of oligomeric species of amyloid  $\beta$ -peptide is causal to disease initiation and progression. Oligomeric species of amyloid  $\beta$ -peptide bind to the N-terminus of plasma membrane-bound cellular prion protein (PrP<sup>C</sup>). This binding may be associated to synaptic degeneration. Composition of bound amyloid  $\beta$ -peptide oligomers, binding domains within PrP<sup>C</sup> and modifiers of this binding have mostly been studied in cell culture or murine models of Alzheimer's disease. Our understanding on the PrP<sup>C</sup> amyloid  $\beta$ -interaction in patients with Alzheimer's disease is limited regarding to occurrence of binding, binding domains within PrP<sup>C</sup>, and sizes of bound oligomeric species of amyloid  $\beta$ -peptide.

Here we employed the combination of a novel Amyloid- $\beta$ -PrP<sup>C</sup> interaction assay (APIA), co-immunoprecipitation and size exclusion chromatography to study regional distribution of PrP<sup>C</sup> amyloid  $\beta$ -peptide binding on neuropathologically characterized Alzheimer's disease and control brains.

Amyloid  $\beta$ -peptide binding to PrP<sup>C</sup> was always via the N-terminus of PrP<sup>C</sup> and showed regional differences.

**P179**  
**Detection of CWD prion in fecal samples by RT-QuIC**

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Chronic wasting disease (CWD) is a prion disease which mainly affects captive and wild cervids in North America. To date, cases in elk, mule deer, white-tailed deer and moose have been reported. In CWD, infectious *prions* are transported from the CNS into a wide range of peripheral tissues, body fluids, excreta and eventually shed into the environment.

In order to detect the disease, the use of easily accessible specimens such as feces would be a practical way for prion detection. However, those excretions and secretions harbor relatively low concentrations of prions which challenge current diagnosis methods. In an earlier study, we demonstrated that CWD prions are detectable in urine collected from pre-symptomatic deer and in fecal extracts by real-time quaking-induced conversion assay (RT-QuIC). RT-QuIC employs minute amounts of PrP<sup>Sc</sup> as a seed to initiate conformational transition of recombinant PrP (rPrP) by vigorous intermittent shaking.

In this study, we aim to improve the detection of CWD prions in fecal extracts by RT-QuIC, and to determine the shedding pattern in feces of elk which were challenged orally with CWD prions in an experimental study. We used methanol precipitation, ultracentrifugation and sodium phosphotungstic acid (NaPTA) precipitation to purify and to concentrate CWD prions in feces. We found that NaPTA precipitation of fecal extracts does not interfere with seeding activity, but increases the sensitivity of detection. Our data demonstrate that concentration and purification of PrP<sup>Sc</sup> enhances detection of CWD prions in feces,



which will eventually enable the use of RT-QuIC for CWD surveillance.

#### P180

##### **Detection of cellular prion protein in exosome derived from ovine plasma**

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**Background.** Cellular prion protein (PrPC) is present at extremely low levels in the blood of animals and its detection might be masked by proteins or soluble components of plasma. In order to study the potential of plasma-derived exosomes as a tool for prion diseases diagnosis we established a simple method to significantly enrich plasma-derived PrPC after exosome extraction in blood.

**Material and Methods.** Plasma samples were collected from five naturally Scrapie infected sheep and three healthy controls. Exosomes were precipitated by a polymeric mixture from 1ml of all plasma samples. Exosome pellets were lysed using RIPA buffer and assayed for exosomal protein marker flotillin-1 by Western Blotting (WB). In order to concentrate PrPC, samples were precipitated with thyroglobulin (TG) and methanol and then centrifuged. The expression of PrPC was assessed by WB using the anti-PrP antibody P4.

**Results.** First, we have observed that flotillin-1 was enriched in lysed exosome fraction compared with brain homogenate. These data confirmed that by polymeric precipitation it was possible to extract sufficient purified amount of exosomes. Second, the TG/methanol precipitation permitted to eliminate the majority of plasma

protein contaminants and to observe the diglycosylated band at 37kDa in all tested samples and all three PrPC bands in five samples.

**Conclusions.** In this study, it was possible to extract PrPC from plasma derived exosomes by a simple, fast method without the use of ultracentrifuge steps and to visualize it by standard WB assay, reducing the presence of the majority plasma proteins.

#### P181

##### **Effects of *PRNP* gene mutations on experimental and natural transmission of scrapie in a goat herd**

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Although horizontal transmission of scrapie in goats has been repeatedly observed, little it is known with regard to the factors that govern the transmission of scrapie within an affected herd.

We report the data collected in a herd of 37 goats orally infected with a scrapie brain homogenate from a pool of ARQ/ARQ<sup>wildtype</sup> sheep. After infection, recipient goats were mated to obtain 31 F<sub>1</sub> goats; moreover 10 F<sub>2</sub> goats were obtained from the F<sub>1</sub> group. The F<sub>1</sub> and F<sub>2</sub> progenies were kept in the same environment to allow natural exposure to scrapie. Occurrence of scrapie was observed in the experimentally infected goats and only in one subset of the offspring naturally exposed. In both groups, goats carrying the R154H, H154H, R211Q and P168Q-P240P dimorphisms succumbed to scrapie after a



longer incubation period compared to the R154R211Q222 (wildtype), G37V, Q168Q-P240P, and S240P carriers. D145D and Q222K goats were resistant to infection.

Although the age of the F<sub>1</sub> goats at the onset of clinical scrapie suggests a peri-partum infection, no statistically effect of the lag time between the birth of each kid and the clinical onset of scrapie in the dams was found. Moreover, only one out of 25 placentas was detected with PrP<sup>Sc</sup> deposition by western blotting and immunohistochemical means.

Our results confirm that in both experimental and natural conditions K222 and D145 mutations protect goats against scrapie, while other mutations affect the length of the incubation period.

#### P182

##### **<sup>1</sup>H NMR brain metabolite fingerprinting of scrapie exposed sheep**

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We investigated, by multivariate statistical data analysis, variations in the high resolution <sup>1</sup>H NMR brain metabolite profile of sheep exposed to scrapie agent in an affected flock. On the basis of clinical examinations and western blotting analysis for pathological prion protein (PrP<sup>Sc</sup>) in brain tissues, sheep were catalogued as not infected (H), infected with clinical signs (S), and infected without clinical signs (A). By pair wise discriminant analysis of spectral data, comparing S vs H, we found a different metabolite distribution, with inosine, cytosine, creatine, and lactate being higher in S than in H brains, while the branched chain amino acids (leucine, isoleucine, valine), phenylalanine, uracil, tyrosine, gamma-amino butyric acid, total aspartate (aspartate + N-acetyl aspartate)

being lower in S. On the basis of a soft independent modelling of class analogy, 1 out of 3 A samples were assigned to H class. Furthermore, A brains were found to be higher in choline and choline-containing compounds. By means of partial least squares regression, a good correlation ( $R^2 = 0.99$ ) was found between PrP<sup>Sc</sup> amount and <sup>1</sup>H NMR metabolite profile of infected (S and A) sheep, the metabolite mostly correlated with PrP<sup>Sc</sup> was alanine. The overall results, obtained by different chemometric tools, were able to describe a brain metabolite profile of infected sheep with and without clinical signs, compared to healthy ones, and indicated alanine as a biomarker for PrP<sup>Sc</sup> amounts in scrapie brains.

#### P183

##### **Novel Detection of PrP<sup>CWD</sup> on Plants Collected from Rocky Mountain National Park**

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Chronic wasting disease (CWD) affects animals such as elk, deer, and moose and has become endemic over the last decade. The disease is one of many transmissible spongiform encephalopathies which occur due to the accumulation of an abnormally folded, proteinase K resistant, form of the normal cellular prion protein PrP<sup>C</sup>. This abnormally folded form, PrP<sup>CWD</sup>, seeds conversion of PrP<sup>C</sup> into PrP<sup>CWD</sup> and eventually forms amyloid fibrils. The exact mechanisms behind transmission and spread of CWD are unknown but research has shown that it can be spread through horizontal, vertical, and indirect/environmental routes. PrP<sup>CWD</sup> has been found in both soil and water. Additionally, PrP<sup>CWD</sup> is very resistant to degradation which makes it stable in the environment for long periods of time. A study has shown that the

abnormal prion protein can remain viable in the environment for as long as 16 years. We wanted to further explore the latter and determine whether prions could be detected in grasses and other plants in Rocky Mountain National Park (RMNP) by use of the protein misfolding cyclic amplification (PMCA) assay.

This past summer we surveyed three sites within RMNP and collected a total of 32 plants. Plants were collected from both outside and inside enclosures that serve to keep wildlife out and allow for restoration and regrowth of the flora. Plant samples were assayed by PMCA and we are now reporting for the first time the novel detection of PrP<sup>CWD</sup> from the surface on a number of plants assayed.

#### **P184**

#### **Suppression of scrapie prion protein by polyarginine in prion-infected neuroblastoma cells**

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Development of efficient therapeutic intervention for prion disease is difficult and remains as challenge until the effective agent is proven to stop or reverse progression of the disease, inhibiting propagation of prions. Here, we report that poly-L-arginine (PLR) inhibits scrapie prion protein (PrP<sup>Sc</sup>) in ScN2a neuroblastoma cell line permanently infected with prions. Inhibition of PrP<sup>Sc</sup> was dependent on the concentration of PLR and the effective inhibitory concentration was below the cytotoxic levels. When PLRs with different molecular weights were investigated, the polymer with the higher molecular weights exhibited the more potent anti-prion activity. Recently poly-L-lysine (PLK) was discovered to inhibit PrP<sup>Sc</sup>. When anti-prion activity of PLK and PLR polymers with the same polymerization degree was compared, PLR was more effective than PLK. Western blot of proteinase K-resistant PrP<sup>Sc</sup> followed by densitometry analysis showed that the complete elimination of

PrP<sup>Sc</sup> from the cells was accomplished with ~10 nM PLR, while nearly 50% of PrP<sup>Sc</sup> remained in the cells incubated with >30 nM PLK. However, overall cytotoxicity of PLRs and PLKs with the comparable polymerization degrees was the same. Taken together, this study suggests that PLR would be more appropriate than PLK as an option to inhibit PrP<sup>Sc</sup>.

#### **P185**

#### **Axonal changes in hamsters infected with Creutzfeldt-Jakob disease and scrapie recapitulate those following ligation of postganglionic branches of the superior cervical ganglion**

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We compared our electron microscopic data on neuroaxonal dystrophy in hamsters with experimental Creutzfeldt-Jakob disease and scrapie with the detailed ultrastructural neuropathology following ligation of postganglionic branches of the superior cervical ganglion in the rat, published 40 years ago by Margaret Matthews in Philosophical Transactions of the Royal Society of London. The earliest changes observed by Matthews between 6 hours to 2 days in the proximal stump comprised distended proximal axons filled with multivesicular bodies (MVB) and dense cytoplasmic bodies (DCB). Similarly, an increased number of "regular" (round) and "irregular" MVB and some autophagic vacuoles were observed in experimental prion disease. From 12 and 13 hours, clusters of mitochondria were seen in Matthews' experiment followed by increased numbers of DCB, MVB and autophagic vacuoles. By 38 hours following ligation, typical dystrophic neurites filled with "masses of DCB" and large numbers of autophagic vacuoles were observed. Our data in experimental prion diseases closely resembled Matthews' descriptions. MVB were frequent in both Matthews' experiments and in our experimental prion disease models.



MVB that accumulated both in constricted sympathetic nerves and in prion disease tended to be numerous, more complex, irregular in contour and somehow flattened. In this ultrastructural reconstruction, we artificially grouped electron microscopic images according to the framework provided by Matthews in her study of ligated sympathetic nerves. In prion diseases, these changes were observed simultaneously, but we believe that isolated images represented "frozen" processes that affect multiple neurons and their neurites at the same time.

#### **P186**

##### **Atypical and classical BSE differentiated by urine biomarkers**

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Bovine Spongiform Encephalopathy (BSE) is a neurodegenerative disorder in cattle caused by accumulation of the prion protein in the brain. Variant CJD (vCJD) cases were first identified in the UK in the 90s and a correlation was made with eating BSE contaminated meat a decade earlier. The cases of vCJD have reduced tremendously due to removal of specified risk materials and monitoring cattle via BSE rapid testing. The BSE rapid testing procedure involves looking for Proteinase K resistant glycoforms of the prion protein. Widespread testing occurred in response to the vCJD cases and led to the detection of two other forms of BSE with either lower (L-type) or higher (H-type) molecular weight pattern than classical BSE. These atypical cases differ from classical BSE via their pathology, length of disease and transmissibility. The ability to diagnose affected animals pre-mortem via a non-invasive urine based test would be both economically important and cost effective. 2D DIGE was used to compare atypical, classical and aged-matched control urine samples together throughout disease course. This analysis led to the identification of an S100 isoform that was able to distinguish

between atypical BSE and control animals with 100% accuracy. This phenomenon was validated using western blot analysis. Previously, a differentially glycosylated form of clusterin was demonstrated to discriminate between classical BSE and control cattle. Using this novel S100 biomarker concurrently with clusterin, we were able to correctly identify classical BSE, atypical BSE and uninfected test sample in a simple urine test.

#### **P187**

##### **CYCLIN DEPENDENT KINASE 5 PHOSPHORYLATION OF FAMILIAL PRION PROTEIN MUTANTS EXACERBATES CONVERSION INTO AMYLOID STRUCTURE**

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Cyclin dependent kinase 5 (Cdk5) phosphorylation of wild type PrP (pPrP) at serine 43 induces a conversion of PrP into amyloids. Familial prion protein (PrP) mutants undergo conversion from soluble, protease sensitive to insoluble and partially protease resistant proteins. We investigated if Cdk5 phosphorylation of familial PrP mutants would convert into amyloid and if mutants affect the rate of conversion. PrP mutants representing three major familial PrP diseases and different PrP structural domains were studied. We developed a novel In vitro Kinase reaction coupled with Thioflavin T binding to amyloid structures Assay (IKTA) to monitor phosphorylation-dependent amyloid conversion. While non-phosphorylated full-length wild type or PrP mutants did not convert into amyloid, Cdk5 phosphorylation rapidly converted these into Thioflavin T-positive structures following first order kinetics. Dephosphorylation partially reversed conversion. Using circular dichroism spectroscopy, we confirmed phosphorylation-dependent conversion of PrP from alpha helical structures into beta sheet structures and dephosphorylation reversed the conversion.

Non-phosphorylated truncated PrP Y145X and Q160X mutants, converted

spontaneously into Thioflavin T-positive fibrils after a lag phase of over 20 hours, indicating nucleation-dependent polymerization. Phosphorylation reduced the lag phase by over 50% and thus accelerated the formation of the nucleating event. Consistently, pY145X and pQ160X exacerbated conversion in a homologous seeding reaction, whereas pPrP could not seed non-phosphorylated PrP. These results demonstrate an influence of both the N-terminus and the C-terminus of PrP on conversion. We conclude that phosphorylation of the flexible N-terminus of PrP can cause or exacerbate PrP mutant conversion into “amyloid”  $\beta$ -sheet structure.

#### P188

##### **Transmission of amyloid pathology by peripheral administration of misfolded A $\beta$**

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Misfolding and aggregation of Amyloid- $\beta$  (A $\beta$ ) is one of the primary events involved in the pathogenesis of Alzheimer's disease (AD). Recently, it has been proposed that A $\beta$  aggregates can transmit and spread the pathology following a prion-like mechanism. Prions can be exogenously transmitted by many different routes of administration. In the case of A $\beta$ , previous studies showed that intraperitoneal (i.p.) injection of seeds can accelerate cerebral amyloidosis in mouse models. However, other potential routes have not yet been studied. The goal of this work was to assess whether A $\beta$  amyloidosis can be seeded in the brain of a transgenic mouse model of AD by peripheral administration of misfolded particles.

Young tg2576 animals (50 days old) were inoculated with a pool of brain extract coming from old Tg2576 animals (10%w/v) by different routes: i.p. (100 $\mu$ L), eye drops (5 $\mu$ L

each eye, 3 times), intramuscular (i.m., 50 $\mu$ L), and per os (p.o., 1000 $\mu$ L). Animals were sacrificed at 300 days old, and brain samples were analyzed for amyloid pathology by IHC and ELISA.

The i.p., i.m., and eye drops administration of A $\beta$  seeds significantly accelerated pathological features in tg2576. Regardless of the higher volume administered, p.o. treated animals did not show any pathological changes when compared to untreated controls. Differences in the proportion of diffuse, core and vascular deposition was observed within experimental groups. Our data show that peripheral administration of A $\beta$  seeds could accelerate pathological changes in the brain and suggest that an orchestrated cross-talk between the brain and peripheral tissues occurs in AD.

#### P189

##### **TREATMENT WITH iPSC-DERIVED NEURAL PRECURSORS CELLS PRODUCE A CLINICAL AND PATHOLOGICAL IMPROVEMENT IN MICE MODELS OF ALZHEIMER'S AND PRION DISEASE**

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Neurodegenerative diseases (NDs), such as Alzheimer's disease (AD) and Prion diseases (transmissible spongiform encephalopathies), are characterized by the progressive accumulation of misfolded proteins in the brain. The final outcomes of these alterations are a progressive synaptic dysfunction and neurodegeneration that leads to severe brain impairment and disease. NDs impose a tremendous socio-economic burden due to its devastating nature, monetary cost, lack of early diagnostic tools and the lack of effective therapies. Stem cell based therapies offer an



exciting opportunity to treat these diseases and even perhaps recover some of the damage already occurred. The use of induced pluripotent stem cells (iPSCs) as a source of autologous cells for cell grafting therapies offers the possibility to bypass complications due to immune rejection and the use of immunosuppressant. However, to date, this approach has not been tested in animal models of AD and prion disease. To evaluate the therapeutic efficacy of iPSCs to change AD and prion disease progression, we stereotactically injected iPSC-derived neural precursors (iPSC-NP) cells into the mouse brain of a triple transgenic mice model of AD (3xTg AD) and mice intra peritoneal inoculated with the RML prion strain. Interestingly, transplanted iPSC-NP cells at the clinical phase improved AD pathology in aged 3xTg AD and increased animal survival of prion infected mice. No changes were observed in animals injected with PBS (vehicle control). No tumor formation was observed in any mice transplanted with iPSC-NP cells. Our findings suggest that iPSC-NP cells might be a promising cell therapy for NDs.

#### P190

##### **The activated mechanism of Microglia treatment with neurotoxic PrP peptide**

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Microglial activation is a characteristic feature of the pathogenesis in prion diseases. In the present study, we demonstrated that the class B scavenger receptor CD36 is involved in PrP106-126-induced microglial activation through Src tyrosine kinases. CD36 blockade abrogated the PrP106-126-induced iNOS stimulation, reduced caspase-1 cleavage, downregulated IL-1 $\beta$  and IL-6 expression at both mRNA and protein levels, decreased NO production and reduced Fyn phosphorylation. We reported the role of NALP3 inflammasome to microglia activation in prion diseases firstly. We found that PrP106-126-induced caspase-1 cleaved and

IL-1 $\beta$  released depend on NALP3 inflammasome activation, that inhibition of NF- $\kappa$ B activation abrogated PrP106-126-induced NALP3 upregulation, that inflammasome activation is required for the synthesis of pro-inflammatory and chemotactic factors by PrP106-126-activated microglia. Furthermore, our results showed that potassium efflux, production of reactive oxygen species and releasing of Cathepsin B were implicated in PrP106-126-induced NALP3 inflammasome activation in microglia. NAC significantly abrogated PrP106-126-induced NALP3 and ASC upregulation. Higher levels of extracellular K<sup>+</sup> significantly reduced the PrP106-126-induced NALP3 upregulation but had no effect on ASC. Interestingly, Phagocytosis seems to be required for the activation of all major components of the NALP3 inflammasome, whereas lysosomal acidification seems to be indispensable only for the upregulation of ASC, but not NALP3 upregulation. Our findings provide new insights into the mechanisms underlying the activation of microglia by neurotoxic prion peptides and open perspectives for new therapeutic strategies in prion diseases.

#### P191

##### **SPLICED XBP1 AND CLEAVED LUMAN MEDIATE ENDOPLASMIC RETICULUM STRESS-INDUCED PRNP GENE EXPRESSION BY INTERACTING WITH THE ERSE26 PROMOTER REGION.**

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Cellular prion protein (PrP) is essential for transmissible prion diseases but also confers protection against numerous cellular stressors. A thorough understanding of the regulation of human prion protein gene (*PRNP*) expression is necessary to harness these functions of PrP. However, very little is known on the regulation of *PRNP* expression. Recently, endoplasmic reticulum was shown to regulate human *PRNP* expression through the canonical unfolded protein response (UPR). Here, we examined the role of the

non-canonical UPR transcription factor, Luman, in *PRNP* expression.

Overexpression of the transcription factors spliced XBP1 (sXBP1) and cleaved Luman ( $\Delta$ Luman) were found to increased *PRNP* expression and promoter activity, while siRNA-mediated silencing of XBP1 or Luman decreased ER stress-induced PrP mRNA, and protein levels. Site-directed mutagenesis of several putative regulatory elements then helped determine the binding site of sXBP1 and  $\Delta$ Luman to the *PRNP* promoter. Mutation of the newly discovered regulatory element ERSE26 reduced pharmacologically-, sXBP1-, and  $\Delta$ Luman-induced *PRNP* promoter activity and chromatin immunoprecipitation confirmed interaction between XBP1 or  $\Delta$ Luman, and the *PRNP* ERSE26 promoter region. Lastly, the function of ER stress-induced *PRNP* expression on cellular survival was addressed. Comparing condensed chromatin and caspase activation levels in PrP WT or KO neuronal cells following ER stress revealed that PrP protects against ER stress-induced apoptosis.

Therefore, we conclude that the regulation of ER stress-induced *PRNP* expression by sXBP1 and  $\Delta$ Luman constitutes a neuroprotective adaptation against ER stress-induced apoptosis. However, this regulation could collaterally exacerbate prion disease progression by increasing PrP levels available for scrapie conversion.

#### P192

##### Unique features of Genetic prion disease

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Transmissible, sporadic and genetic human prion diseases share important features such as late onset appearance followed by an inevitable fatal outcome, accumulation of disease related PrP forms, and in most cases subsequent transmission of infectivity to corresponding animals. Using our homozygous and heterozygous lines of

TgMHu2ME199K mice, mimicking for genetic CJD linked to the E200K PrP mutation, we investigated the spontaneous generation of fatal disease and de-novo infectivity in genetic prion diseases. We found that in both lines, mutant PrP folds into oligomeric forms since birth, then converts and accumulates as a protease resistant and partially soluble form concomitant with disease progression. De-novo infectivity was generated mostly in heterozygous Tg brains presenting advanced disease, concomitant with the conversion of their wt PrP to a prion like form. This suggests that in cell infection of wt PrP by mutant PrP may be a central feature in disease transmission. Surprisingly, and as opposed to transmissible prion disease, no disease related PrP was found in immune organs of homozygous or heterozygous TgMHu2ME199K mice, regardless of their age or disease status. Our results indicate that mutant PrP may self convert into a disease related form only in the adult CNS environment, indicating a unique mechanism for genetic prion disease. Most important, the fact that no disease related PrP was found in the immune system of out Tg mice suggests that infectivity may be absent from the blood of E200K patients and even more so from healthy carriers, an important conclusion for health authorities.

#### P193

##### A modern approach to the old kuru brain. The confocal laser microscopy of kuru pathology.

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Kuru was the first transmissible spongiform encephalopathy to be identified in humans, occurring in the Fore linguistic group of Papua New Guinea, and brought to medical attention by D. Carleton Gajdusek. Neuropathologically, kuru-affected brains were characterized by a neuronal loss, astrogliosis and spongiosis. The most striking neuropathologic feature of kuru was the presence of numerous amyloid plaques consisting of pathological form of prion protein. The main objective of the study was description of 3D reconstruction and electron

microscopic structure of amyloid plaques in kuru brain, including co-localization of various proteins. To elucidate the structure of amyloid in the brain we performed immunohistochemistry, confocal laser microscopy, and electron microscopy on archived material from a well-documented 1963 kuru case. The fine structure of plaques was surprisingly well preserved with compact core and radial fibrils at the periphery. Immunohistochemistry and 3D confocal laser microscopy revealed that plaques were built of pathological isoform of prion protein (PrP<sup>Sc</sup>) with abundant astroglial reaction around the core of the plaque. Dystrophic neurites characterized by MAP-tau, APP and p62 immunoreactivity were present but less frequent than in the plaques of other prion diseases. The most interesting finding is that the presence of APP, p62 and MAP-tau immunoreactivity in kuru brain was accentuated at the periphery of the amyloid plaques thus we may speculate that the amyloid fibrils influence the appearance of dystrophic neurites.

#### **P194**

##### **Plasma membrane levels of the prion protein impact on the pathophysiology of prion diseases**

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The cellular prion protein (PrP<sup>C</sup>) is a glycosylphosphatidylinositol (GPI)-anchored protein with two variably occupied N-glycosylation sites, expressed highly in neurons. Its pathological isoform (PrP<sup>Sc</sup>) accumulates in prion diseases and represents an essential component of the transmissible entity, the "prion".

Besides its key role in prion diseases, PrP<sup>C</sup> may play a role in Alzheimer disease. Recent data indicate that plasma membrane levels,

maintained by transport of PrP<sup>C</sup> to the plasma membrane and internalization as well as release of PrP<sup>C</sup> from the plasma membrane by proteolytic shedding are critically involved in the pathophysiology of prion diseases.

We have studied prerequisites for the transport to and correct insertion of PrP<sup>C</sup> at the plasma membrane focussing on GPI-anchor attachment and the role of the PrP<sup>C</sup> C-terminus in cell culture models and mice. Trafficking of PrP with a C-terminal deletion to the plasma membrane is impaired leading to apoptosis in cultured cells and neurodegeneration in mice.

Additionally we assessed effects of disturbed membrane homeostasis of PrP<sup>C</sup>. Impaired shedding of PrP<sup>C</sup> lead to increased levels of PrP<sup>C</sup> at the plasma membrane and greatly enhanced the susceptibility of mice towards prion disease.

#### **P195**

##### **Chronic wasting disease prions detected during early stages of infection by mbPMCA in tissues from white-tailed deer orally inoculated with free and microparticle-bound prions**

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Enhanced oral transmission of rodent-adapted prion disease has been demonstrated with the disease agent bound to several types of mineral microparticles; however, the generalizability of this finding to ruminants has not been established. Contaminated soil is believed to represent a reservoir for environmental prions and may contribute to horizontal transmission of chronic wasting disease (CWD) in captive and wild cervid populations. Here, we examined the impact of CWD agent association with microparticles of montmorillonite, an aluminosilicate clay

mineral that showed the largest disease transmission enhancement in rodent bioassays, on early disease in orally inoculated white-tailed deer. Amplification of prions by PMCA has been achieved from various contaminated organs and excretions at late stages in disease. Using microplate-based PMCA (mbPMCA), we detected different accumulation patterns in white-tailed deer tissues 42 days after oral inoculation with CWD prions bound to montmorillonite. We expected mbPMCA to be more sensitive than immunohistochemistry (IHC) to determine prion accumulations in tissues. Through evaluation of mb-PMCA positive tissues, we found that mbPMCA is more sensitive than IHC by at least a factor of  $10^{6.3}$ , and detected CWD prions in multiple tissue types that were negative by IHC.

These findings suggest that microparticles can enhance the transmission of CWD in white-tailed deer and also demonstrates the consistency and high-throughput utility of the mbPMCA assay. Furthermore, our results indicate that enhanced transmission of microparticle-bound CWD agent warrants consideration in evaluating the relative importance of direct and indirect (environmental) transmission of CWD in natural populations and in disease management.

#### P196

#### **Prion peripheralization is a host-driven trait of prion infection, independent of strain**

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Chronic wasting disease (CWD), like scrapie of sheep, is a horizontally transmissible spongiform encephalopathy. Proposed natural routes of transmission for both agents

include saliva, urine, and feces, and are likely related to an accumulation of misfolded prion proteins in excretory tissues, including salivary glands and both gastrointestinal and urogenital tracts. In recent years, prion research has focused on the prion strain (e.g. bovine spongiform encephalopathy – BSE, scrapie, or CWD) as the source code for pathogenic information – encoded by tertiary conformation for example – with strain commonly used as the predominant criteria for classifying prions. In the present study, we compared the peripheral distribution of CWD prions in two experimental host species: transgenic cervidized mice and cattle. Following inoculation and demonstrable infection with CWD, a variety of central and peripheral tissues were collected at necropsy, including brain and both peripheral lymphoid and excretory tissues, and analyzed for prion seeding activity by real time quaking-induced conversion (RT-QuIC). While transgenic mice displayed broad and elevated levels of CWD prions in the periphery, as has been demonstrated with scrapie in mice and CWD in deer, the peripheralization of CWD prions in cattle was limited and similar to that reported in BSE. Our findings imply that prion peripheralization is likely a trait imparted by the host, independent of the prion strain, though additional research investigating the peripheralization of BSE prions in cervids, for example, is required. It also remains to be seen if other prion phenotypes, e.g. zoonotic potential, are host-derived in nature.

#### P197

#### **Misfolded prion protein (PrP<sup>TSE</sup>) can be detected in the blood of squirrel monkeys infected with variant Creutzfeldt-Jakob disease (vCJD)**

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Variant CJD infections have been recognized in 4 recipients of non-leukocyte-depleted red blood cell (RBC) transfusions (and in a hemophilia patient treated with a plasma-derived coagulation factor). In the UK, PrP<sup>TSE</sup> has been detected in blood of 70% of vCJD patients and in appendices of as many as 1 in 2000 asymptomatic individuals. Reliable tests are needed to detect blood donors infected with vCJD before the appearance of overt illness to protect transfusion recipients. We previously reported the co-localization of PrP<sup>TSE</sup> with extracellular vesicles (EVs) in plasma of vCJD-infected mice. Here we report detecting PrP<sup>TSE</sup> in EVs from archived plasma samples of vCJD-infected squirrel monkeys. Blood was collected from infected and uninfected monkeys into anticoagulant every 3 months and separated into RBCs, buffy coat and plasma. EVs were extracted from plasma using ExoQuick™. PMCA was performed using uninfected TgHu mouse brain homogenate as PrP<sup>C</sup> substrate. We tested samples of blood from 2 groups of monkeys inoculated intracerebrally with vCJD brain diluted 10<sup>-1</sup> (4 monkeys) or 10<sup>-3</sup> (3 monkeys) and from 4 control monkeys inoculated with 10<sup>-1</sup> normal human brain. PrP<sup>TSE</sup> was detected in plasma EV of 5 of 7 vCJD-inoculated monkeys (3/4 10<sup>-1</sup> and 2/3 10<sup>-3</sup>). Multiple tests of control plasma EV samples from the 4 control monkeys were all negative. Whole blood collected from 4 monkeys during incubation and clinical stages of vCJD was transfused into individual recipient monkeys. We continue to test archived plasma samples from donor and transfusion-recipient monkeys.

Baxter and FABS funded the study.

## P198

### MiRNA abundance is altered in synaptoneurosomes during prion infection

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Discrepancies in synaptic plasticity is one of the earliest manifestations of the neurodegenerative state. In prion diseases synapse damage and loss is observed during preclinical disease in both cortical and hippocampal neurons. The underlying mechanisms of synaptic damage have not been identified yet but miRNAs, many of which are known to be enriched at the synapse, may regulate local protein synthesis in rapid response to stressors such as replicating prions. Here we identified miRNAs that are present and/or enriched at neuronal synapses in a prion-induced mouse model of the disease. We used multiplex qPCR to identify the miRNA repertoire and, alternatively, used proteomic strategies to document the protein content. Next, we derived correlations between miRNAs and proteins present at the synapse in order to gain a better understanding of the molecular mechanisms that may contribute to synaptic dysfunction during prion induced neurodegeneration.

## P199

### Copper induced templated misfolding and oligomerization of prion proteins

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Transmissible spongiform encephalopathies are characterized by the templated conversion of normal cellular prion proteins to a misfolded conformation that forms protease resistant neurotoxic aggregates. However,

the biophysical mechanism of prion protein misfolding and aggregation are unclear. Here we resolve, across multiple length scales from single molecules to physiological ensembles, the role of the structured and disordered regions of prion proteins and the effect of different divalent ion co-factors in misfolding and oligomerization. Using a single molecule fluorescence based protease resistance assay, we demonstrate that individual prion protein monomers misfold to a protease resistant conformation before oligomer assembly; the intrinsically disordered N-terminal region and  $\text{Cu}^{2+}$  ions are obligatory for this conformational switching. Using single molecule force measurements with an Atomic Force Microscope (AFM) we show that the misfolded conformation has a 900 fold higher association constant compared to the native conformation. Finally, using real-time quaking-induced conversion (RT-QuIC), we demonstrate that prion proteins misfolded by pre-exposure to  $\text{Cu}^{2+}$  ions, can serve as seeds for templated oligomer formation. Our data demonstrates an exponential relationship between prion aggregation rate and misfolded seed concentration.

## P200

### **Clinical Stage of Infection is Critical in the Antemortem Diagnosis of Chronic Wasting Disease in Deer and Elk.**

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Chronic wasting disease (CWD) is an efficiently transmitted spongiform encephalopathy of cervids (e.g. deer, elk, and moose), and is the only known prion disease affecting both free-ranging wildlife and captive animals. The antemortem detection of CWD and other prion diseases has proven difficult, due in part to difficulties in identifying an appropriate peripheral tissue specimen and complications with conventional test sensitivity. At present, biopsies of the recto-anal mucosal-associated lymphoid tissues (RAMALT) have shown promising sensitivity and are not impractical to collect in live animals. Nasal brush collections have likewise proven both sensitive and practical for identification of prion infections in humans. In this study, we evaluated both RAMALT and nasal brush collections by real time quaking-induced conversion (RT-QuIC), and compared our findings to RAMALT immunohistochemistry as well as conventional postmortem evaluation of obex and retropharyngeal lymph node tissues from over 700 captive and free-ranging deer and elk in areas with endemic CWD. We correlated our results with various clinical findings, including pathological stage of infection as determined by obex scoring, PrP genotype, age, and sex. While the sensitivity of RAMALT RT-QuIC analyses exceeded that of RAMALT IHC (69-80% vs. >44%) and nasal brush collections (15-30%), the sensitivity of both biopsy and nasal brush analyses were dependent primarily on clinical stage of disease, although PrP genotype was also an important predictor of sample positivity. Our findings further demonstrate the potential and limitations of antemortem sample analyses by RT-QuIC in the identification and management of prion diseases.

## P201

### Monitoring the Potential Transmission of Chronic Wasting Disease to Humans

Ermias Belay<sup>1</sup>, Joseph Abrams<sup>1</sup>, Janell Kenfield<sup>2</sup>, Emily Thorp<sup>3</sup>, Ryan Maddox<sup>1</sup>, Lawrence Schonberger<sup>1</sup>

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#### Introduction

Chronic wasting disease (CWD), occurring for decades among wild cervids in Colorado and Wyoming, has now been detected in 17 more U.S. states and 2 Canadian provinces. Monitoring its possible transmission to humans requires conducting epidemiologic and laboratory studies among humans with possible CWD exposure and generating scientific evidence causally linking the two illnesses.

#### Methods

Centers for Disease Control and Prevention collaborated with Wyoming Department of Health, Colorado Department of Public Health and Environment and Wisconsin Department of Health Services to assess CJD incidence among deer and elk hunters by cross-checking their information with mortality data.

#### Results

In Colorado, hunter data include about 6.1 million records of licenses purchased during 1995-2011, representing about 1.2 million hunters; 49.97% of hunters purchased a license to hunt in areas with CWD-positive game units. In Wyoming, data include about 2.2 million records of licenses purchased during 1996-2013, representing about 0.6 million hunters; 28.12% of hunters purchased a license for CWD-positive hunt areas. During the period 11 Colorado hunters (expected: 10.19 cases, 95% CI=4-17) and 4 Wyoming hunters (expected: 4.64 cases, 95% CI=1-9) were identified to have died of CJD. In Wisconsin, over 400 hunters who

consumed venison from CWD-positive cervids are enrolled for follow-up.

#### Conclusions

CJD incidence among hunters in Colorado and Wyoming is not higher than expected. The hunter data, particularly from hunters who consumed CWD-positive venison, are valuable for monitoring potential CWD transmission to humans. Ongoing assessments and long-term follow-up are necessary because of potentially long latency periods.

## P202

### Determine The Mechanism Of Inhibition By Dominant Mutants On Different Prion Conformations

Fen Pei, Tricia Serio

University of Arizona, Tucson, AZ, USA

The prion protein underlies several previously inexplicable phenomena, including transmissible neurodegenerative disease in mammals and non-Mendelian inheritance of unique traits in fungi. Despite efficient and autocatalytic pathway of prion protein misfolding, dominant-negative prion mutants are able to interfere with this pathway for wild-type prion proteins. However, controversies persist about the actual mechanism of dominant-negative inhibition. For example, in the yeast *S. cerevisiae*, the inhibitory mechanism of G58D, a dominant-negative mutant of the prion protein Sup35, was linked to either an enhancement of the fragmentation reaction or to a failure to transmit existing aggregates to daughter cells upon division. These studies used different conformations of the prion protein, raising the possibility that the mutant could impact prion propagation through distinct pathways depending upon Sup35 conformation. To resolve this controversy, we have compared the mechanism of prion curing in strains propagating different conformations ([PSI<sup>+</sup>]<sup>Strong</sup>, [PSI<sup>+</sup>]<sup>Sc4</sup> and [PSI<sup>+</sup>]<sup>Weak</sup>) of the Sup35 protein. Our studies indicate that G58D mutant inhibits propagation of all Sup35 conformations in a dose-dependent manner, leading to a decrease in the steady-state aggregate size and in the number of



transmissible aggregates. However, heterozygous disruption of Hsp104, the cellular factor mediating prion aggregate fragmentation, partially reversed the dominant-negative effects of G58D expression. Together, our studies are consistent with a common mechanism of G58D inhibition of prion propagation: enhanced fragmentation of existing prion aggregates leading to their clearance. Thus, the conformational variation does not modulate the mechanism by which the inhibition occurs, implying a common therapy targeting prion variants in clinical trials.

### P203

#### **Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants.**

Reed Wickner, Evgeny Bezsonov, David Bateman

*NIDDK, Bethesda, MD, USA*

[URE3] is an amyloid prion of the *Saccharomyces cerevisiae* Ure2p, a regulator of nitrogen catabolism. Overproduction of Btn2p, involved in late endosome to Golgi protein transport, or its paralog Cur1p, cures [URE3]. Btn2p, in curing, is colocalized with Ure2p in a single locus, suggesting sequestration of Ure2p amyloid filaments. We find that most [URE3] variants generated in a btn2 cur1 double mutant are cured by restoring normal levels of Btn2p and Cur1p, with both proteins needed for efficient curing. The [URE3] variants cured by normal levels of Btn2p and Cur1p all have low seed number, again suggesting a seed sequestration mechanism. Hsp42 overproduction also cures [URE3], and Hsp42p aids Btn2 overproduction curing. Cur1p is needed for Hsp42 overproduction curing of [URE3], but neither Btn2p nor Cur1p is needed for overproduction curing by the other. Although hsp42Δ strains stably propagate [URE3-1], hsp26Δ destabilizes this prion. Thus, Btn2p and Cur1p are antiprion system components at their normal levels, acting with Hsp42. Btn2p is related in sequence to human Hook proteins, involved

in aggresome formation and other transport activities.

### P204

#### **Influence of habitat and demographic components on exposure to chronic wasting disease in white-tailed deer in the eastern United States**

W. David Walter<sup>1</sup>, Tyler Evans<sup>2</sup>

<sup>1</sup>*U.S. Geological Survey, University Park, Pennsylvania, USA*, <sup>2</sup>*Pennsylvania Cooperative Fish and Wildlife Research Unit, University Park, Pennsylvania, USA*

Chronic wasting disease (CWD) is a transmissible spongiform encephalopathy that was first detected in the northeastern United States in 2005 in New York and West Virginia, and has since been found in Maryland, Virginia, and Pennsylvania. We examined demographic and environmental factors in the central Appalachian region to assess the spatial distribution of CWD in white-tailed deer (*Odocoileus virginianus*). The objectives of our study were to (1) apply Bayesian hierarchical modeling to harvest location data of white-tailed deer tested for CWD in the region since 2005, (2) identify model(s) that best described the spatial distribution of CWD, and (3) map probability of CWD infection across the Northeast. For each deer, environmental covariates were extracted within 6 km<sup>2</sup> grid cells as this size reflected our estimate of the 99% size of home range for white-tailed deer in the region. The model with the most support did not include sex and age but contained random spatial effects and percent habitat and accounted for 94.4% of the overall weight for the candidate set of models. Percent forest cover appeared to have the strongest correlation with the distribution of CWD in the region, with increased risk of CWD occurring in areas that had lower amounts of forest cover. Our results also suggest that the use of age and sex data collected from harvested animals, commonly used in CWD modeling, should be further evaluated considering most are adult males, however, <2 year old deer are commonly diagnosed with CWD in the region.



bioavailability and biodistribution of PrPSc.

## P205

### Absolute Bioavailability of Prions

Akihiko Urayama<sup>1</sup>, Vineetkumar Kharat<sup>2</sup>, Uffaf Khan<sup>1</sup>, Claudio Soto<sup>1</sup>

<sup>1</sup>University of Texas Medical School at Houston, Houston, TX, USA, <sup>2</sup>University of Texas School of Public Health, Houston, TX, USA

Natural prion disease is suspected to arise by per oral (p.o.) infection, and the disease has experimentally been induced by this pathway. However, quantitative information on the absorption of prions, i.e. the bioavailability, have long been a missing piece, although its considerable importance both for basic research and regulatory entities in nations.

To address this, we employed pharmacokinetic approach. Experimentally naïve mice received a p.o. administration of ultra-purified radiolabeled PrPSc, intact levels of PrPSc measured in the gastro-intestinal (GI) tract and colorectum, whole blood, plasma, liver, spleen, and kidney, and data normalized with the injected dose (ID). While PrPSc levels in the stomach, duodenum, and jejunum decreased with time, gradual sustained increases (~10%ID/g) in the ileum and colon peaked at 3-6 hr. Whole blood and plasma PrPSc concurrently reached maximal levels (0.6-1.1%ID/ml) at 3 hr, and blood levels were constantly higher than plasma levels during the course of experiment (0-24hr). Eventually, PrPSc crossed GI-tract immediately became blood-cell-associated (~60% at 30 min) and the ratio increased to ~87% by 24 hr after p.o. administration, while intravenously (i.v.) administrated PrPSc slowly became cell-associated in systemic circulation. This suggests body response to PrPSc differs with the routes of infection. The levels in the liver, spleen, and kidney showed same time profile with the levels in systemic circulation. The intactness of radiolabeled PrPSc was confirmed by acid precipitation and radio-HPLC methods.

Based on the pharmacokinetic analysis for PrPSc following p.o. and i.v. administrations, we will comprehensively discuss the absolute

## P206

### Development of a mouse model of virus-induced parkinsonism

Aaron Phillips<sup>1</sup>, Sean Hammond<sup>1</sup>, Ken Olson<sup>2</sup>, Tawfik Aboellail<sup>2</sup>, Richard Smeyne<sup>3</sup>, Ronald Tjalkens<sup>1</sup>

<sup>1</sup>Department of Environmental and Radiological Health Sciences,, Colorado State University, USA, <sup>2</sup>Department of Microbiology, Immunology, and Pathology, Colorado State University, USA, <sup>3</sup>Department of Developmental Neurobiology, St. Jude Children's Research Hospital, USA

The etiology of Parkinson's disease (PD) has long been thought to involve both genetic and environmental factors. The majority of PD cases are referred to as idiopathic and cannot be explained by genetic variations. There are many environmental factors implicated in the etiology of PD. Such factors include pesticides, heavy metals, and viral infections. Pathogenic contributions from many of these factors are not well-understood, especially when multiple factors are experienced by the same individual. We are developing a mouse model of virally-induced parkinsonism and will use this model to investigate the disease phenotype in animals that had been previously exposed to high levels of the heavy metal manganese. Before we can assess any difference manganese pre-exposure may produce, we must thoroughly characterize the disease phenotype in animals that only receive virus challenge. Through a combination of in vivo imaging and confocal microscopy, we are able to visualize the brain sub-regions of interest such as the substantia nigra pars compacta (SNpc). We show that viral infection results in a progression through the CNS similarly to the typical progression of PD lesions in humans. We show that infection results in neuroinflammation and induces oxidative stress by perturbing calcium-ion homeostasis. We also show that infection does in fact involve the SNpc and TH+ neurons contained within. Lastly, we show that we can control the CNS infection by

performing passive-transfer of immune serum - a point that is important as it potentially allows researchers to 'dial-in' their desired level of viral insult.

## P207

### New determinants of phenotypic diversity in sporadic human prion diseases

Laura Cracco<sup>1</sup>, Ignazio Cali<sup>1,2</sup>, Wen-Quan Zou<sup>1</sup>, Bernardino Ghetti<sup>3</sup>, Man-Sun Sy<sup>1</sup>, Jiri Safar<sup>1</sup>, Byron Caughey<sup>4</sup>, Pierluigi Gambetti<sup>1</sup>

<sup>1</sup>National Prion Disease Pathology Surveillance Center (NPDPSC), Department of Pathology, Case Western Reserve University, School of Medicine, Cleveland, Ohio, USA, <sup>2</sup>Department of Clinical and Experimental Medicine, Second University of Naples, Naples, Italy, <sup>3</sup>Department of Pathology and Laboratory Medicine, Indiana University School of Medicine, Indianapolis, Indiana, USA, <sup>4</sup>Rocky Mountain Laboratories, Institute for Allergy and Infectious Diseases, National Institutes of Health, Hamilton, Montana, USA

The pairing of genotype at codon 129 of the prion protein (PrP) gene (site of a methionine (M)/ valine (V) polymorphism) with type 1 or 2 of scrapie PrP (PrP<sup>Sc</sup>) is the major phenotypic determinant in sporadic human prion diseases. Nevertheless, the sCJDMM2 subtype of sporadic Creutzfeldt-Jakob disease (sCJD) and sporadic fatal insomnia (sFI) are both 129MM with PrP<sup>Sc</sup> type 2 yet they are associated with distinct phenotypes. Conversely, sCJDMM1 and sCJDMMV1 have similar phenotypes despite different genotypes. Physicochemical properties of total (tot) PrP<sup>Sc</sup> (proteinase K (PK)-sensitive (sen) and PK-resistant (res) PrP<sup>Sc</sup>) and resPrP<sup>Sc</sup> were comparatively examined in the four diseases to identify phenotypic determinants.

TotPrP<sup>Sc</sup> from sCJDMM2 and sFI were analysed with i) one- and two-dimensional immunoblots, ii) sedimentation-equilibrium, iii) immunoprecipitation and iv) conformational stability assays. We have uncovered significant differences between totPrP<sup>Sc</sup> conformers associated with sFI and sCJDMM2; they include the presence of (i) a

prominent PK-sensitive population with distinct aggregate density, glycosylation and lipidic component in sFI and of (ii) PK-sensitive, covalently-linked and full-length ~53kDa and ~90 kDa PrP<sup>Sc</sup> components in sCJDMM2. Furthermore, conformational stability also differs. These variations appear to affect primarily senPrP<sup>Sc</sup> as they drastically diminish after PK digestion. In contrast, no differences in either aggregate-density or conformational stability have been observed in preliminary studies of sCJDMMV1 and sCJDMM1.

Our data suggest that new strain features of sensitive PrP<sup>Sc</sup> are the determinant of the phenotype in sCJDMM2 and sFI. A characterization of nature and transmissibility of lipid-carrying aggregates and high molecular weight components is ongoing.

## P208

### Expression of cellular prion protein found in canine cancer cell lines with unique glycosylation patterns

Sophia A Moore<sup>\*1</sup>, Lisa Pang<sup>2</sup>, Wilfred Goldmann<sup>2</sup>, David J Argyle<sup>2</sup>

<sup>1</sup>Prion Research Center at Colorado State University<sup>2</sup> Royal (Dick) School of Veterinary Studies and Roslin Institute, The University of Edinburgh

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Although the abnormal prion protein is known to cause mad cow disease, the role of normal cellular prion protein (PrP<sup>C</sup>) remains unknown. Recent studies have shown an up-regulation of PrP<sup>C</sup> in human cancer types such as colorectal and melanoma. Some studies have proposed multiple roles for PrP<sup>C</sup> in cancer including anti-apoptosis, cell migration and differentiation. While PrP<sup>C</sup> has been investigated in human cancers, it has not been studied in canine cancer. In this study, Western Blot analysis of seven canine cancer cell lines was performed using PrP<sup>C</sup> specific monoclonal antibodies to visualize PrP<sup>C</sup> glycosylation and cleavage patterns. A

time course experiment was then conducted on the J3T canine glioma cell line treated with doxorubicin. Preliminary data show that all canine cancer cell lines express PrP<sup>C</sup>, but some with unique patterns. For the first time it was shown that cancer cell lines, in this case all seven canine cell lines, express the PrP<sup>C</sup>-C1 fragment, which has been described for healthy cells. Interestingly, the Lilly cell line appears to only have the diglycosylated C1 fragment. Future work using the Lilly cell line as a model may reveal the importance of this unique expression of PrP<sup>C</sup> in tumorigenesis of inflammatory mammary carcinoma. Significantly, J3T cancer cells that were exposed to stress induced by doxorubicin had a different glycosylation pattern compared to the DMSO control. Further experiments are required, such as PNGase digestion and epitope mapping, to identify the PrP<sup>C</sup> fragments affected by stress

and their biological relevance. This is the first time PrP<sup>C</sup> has been studied in canine cancer and it has been found to be up-regulated in all seven canine cancer cell lines. Having a canine model for PrP<sup>C</sup> will allow us to explore the potential for PrP<sup>C</sup> as a prognostic tool as well as a synergistic target for patients undergoing chemotherapy.

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*Watermarks found throughout the Prion2015 Guide were designed by CSU PRC members Carla Calvi, Lindsay Parrie, Jifeng Bian, Mark Zabel, Vanessa Selwyn, Connor Hendrich and Aubrey Waechter.*





**Tuesday May 26***Prion2015 Program Guide*07:30 **Check-in and on-site registration**08:30 **Animal, Human and Yeast Prion Workshop**16:00 **Check-in and on-site registration**17:00 **Opening ceremony**

CJD Foundation

CJD International Support Alliance

PRC Organizers

Debbie Yobs

Suzanne Solvyns

17:30 **Keynote Introductions**  
**Keynote Talks**

Alan Rudolph

Byron Caughey

Reed Wickner

CSU Vice President for Research

National Institutes of Health, Hamilton

National Institutes of Health, Bethesda

K1

K2

19:00 **Welcome Cocktail Reception****Wednesday May 27**07:00 **Check-in and on-site registration**08:00 **Propagation**

Check the digital version of this guide for session chairs

Claudio Soto

Marc Diamond

Ina Vorberg

University of Texas Health Sciences Center, Houston

University of Texas, Southwestern Medical Center

German Center for Neurodegenerative Diseases

PL1

I1

I2

10:00 *Coffee break (30 minutes)*

10:30

Larry Walker

Emory University School of Medicine

I3

11:00

Gültekin Tamgüney

German Center for Neurodegenerative Diseases

O1

11:15

Jacob Ayres

University of Florida

O2

11:30

Iliia Baskakov

University of Maryland School of Medicine

I4

12:00

Bruce Chesebro

National Institutes of Health, Hamilton

O3

12:15 *Lunch (One hour, 45 minutes)*

14:00

Jiyan Ma

Van Andel Research Institute

I5

14:30

Fiona Houston

University of Edinburgh

O4

14:45

Jean-Philippe Deslys

Atomic Energy Commission, France

O5

15:00

David Borchelt

University of Florida

I6

15:30

Yury Chernoff

Georgia Institute of Technology

O6

15:45 *Coffee break (30 minutes)*16:15 **Topics in animal prions**

Ed Hoover

Prion Research Center, CSU

I7

16:45

Qingzhong Kong

Case Western Reserve University

O18

17:00

David Berry

University of California, San Francisco

O7

17:15

S. Jo Moore

United States Department of Agriculture, Ames

O8

17:45 **Poster Session I and Cocktail Reception****Thursday May 28**08:00 **Mechanisms of Disease**

Karen Ashe

University of Minnesota

PL2

09:00

Stephen Strittmatter

Yale School of Medicine

I8

09:30

David Harris

Boston University School of Medicine

I9

10:00 *Coffee break (30 minutes)*

10:30

Heather True-Krob

Washington University School of Medicine

I10

11:00

Christina Sigurdson

University of California, San Diego

I11

11:30

Olivier Andreoletti

Ecole Nationale Vétérinaire de Toulouse

I12

12:00

Roberto Chiesa

Mario Negri Institute, Milano

O9

12:15 *Lunch (One hour, 45 minutes)*

14:00

Hugh Perry

University of Southampton

I13

14:30

Corinne Lasmezas

Scripps Research Institute, Florida

I14

15:00

Ted Allison

University of Alberta

I15

15:30

Hermann Altmeppen

University Medical Center Hamburg-Eppendorf

O10

15:45 *Coffee break (30 minutes)*

16:15

Andrea LeBlanc

McGill University

O19

16:30

Jean Manson

University of Edinburgh

O17

16:45 **Structure/Function**

John Collinge

MRC Prion Unit, London

PL3

17:45 **Poster Session II and Cocktail Reception****Friday May 29**

08:00

Thomas Wisniewski

New York University School of Medicine

I16

08:30

Randal Halfmann

University of Texas, Southwestern Medical Center

I17

09:00

Tricia Serio

University of Arizona

I18

09:30

Jesús R. Requena

University of Santiago de Compostela-IDIS

O14

09:45

Anton Gorkovskiy

National Institutes of Health, Bethesda

O20

10:00 *Coffee break (30 minutes)*

10:30

Fabrizio Chiti

University of Florence

I19

11:00

Gerold Schmitt-Ulms

University of Toronto

O15

11:15

Eric Ross

Prion Research Center, CSU

I20

11:45

Lindsay Parrie

Prion Research Center, CSU

O16

12:00

Eri Saijo

National Institutes of Health, Hamilton

O12

12:15 *Lunch (One hour, 45 minutes)*

14:00

Holger Wille

University of Alberta

I21

14:30

Jiri Safar

Case Western Reserve University

O11

14:45

Jason Bartz

Creighton University Medical School

I22

15:15

Edward Pokrishevsky

University of British Columbia, Vancouver

O13

15:30 **Closing Remarks**

PRC Organizers

15:40 **Poster prizes**

PRC Organizers

15:50 **PRION2016 - Japan**

PRION2016 Organizers

16:10 **End of Symposium**17:00 **Brewery Tour**19:00 **Gala Dinner**