Human, Animal and Yeast Prion Workshops

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ANIMAL TSEs ABSTRACTS

Infectious recombinant prions: In vitro generation and propagation of different strains

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Prion diseases are a group of fatal neurodegenerative diseases that affect humans and animals and whose main characteristic is its infectious nature. PrPSc, a misfolded variant of the endogenous PrPC, is the solely pathogenic agent. The infectivity of the misfolded protein was amplified/propagated in vitro since a decade ago using Protein Misfolding Cyclic Amplification (PMCA), a technology that has had an enormous impact in the prion field1. Recently, a version of PMCA using recombinant PrP (rec-PrP) as substrate (rec-PMCA) has been developed to generate highly PK resistant PrP (rec-PrPres). The infectivity showed by a diversity of rec-PrPres generated in vitro by different groups using a variety of co-factors and modified procedures was also diverse. These results confirm: i) the GPI and glycosylation components are not necessary in enciphering an infectious conformation and ii) rec-PrPres can be also structured in the form of different recombinant prion strains with robust in vitro self-replicating abilities but dissimilar infectious features in vivo.

Our study has been focused on understanding the infectivity and the effect of different cofactors of recombinant prions generated using the polymorphic variant of the bank vole PrP (109I). This model was used as the shortest incubation time model for prion diseases2 and because of its outstanding susceptibility to propagate most of the existing prion strains from different species3.

This study shows the in vitro generation of infectious recombinant bank vole prions and how cofactors influence over the propagation of certain prion strains with specific infectious features.


Propagation of diverse prion strains in bank vole PrP transgenic mice

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The interspecies transmission of prions is typically an inefficient process, a phenomenon referred to as the species
barrier. Despite expressing a prion protein (PrP) sequence that differs from mouse PrP at only eight positions, pioneering work from the Agrimi and Nonno labs has demonstrated that bank voles are uniquely susceptible to a wide range of prion strains isolated from many different species. Using transgenic (Tg) mice expressing bank vole PrP (BVPPrP), we have recently determined that expression of BVPPrP is sufficient to engender enhanced susceptibility to a diverse range of prion isolates. Efficient transmission of 16 prion isolates from 8 different species was observed in Tg(BVPrP) mice, suggesting that BVPPrP may be a “universal acceptor” for prions. Many strain-specified properties of the prion isolates—including the size of proteinase K-resistant PrP Sc, the pattern of cerebral PrP Sc deposition, and the conformational stability—were conserved upon serial passage in Tg(BVPrP) mice. Passage of hamster, elk, and human prion isolates in Tg(BVPrP) mice generated prions that transmitted efficiently to Tg mice expressing mouse PrP, implying that Tg(BVPrP) mice may be useful for facilitating the adaptation of prion strains to the mouse PrP sequence. However, many Tg(BVPrP)-passaged prion isolates were not transmissible to Tg mice expressing hamster, elk, or human PrP, arguing that BVPPrP-adapted prions are not “universally infectious”.

Early generation of PrPres after brain microinjection of scrapie in C57BL mice and PrP-KO mice

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In prion diseases protease-resistant prion protein (PrPres) accumulates in the CNS and is often associated with neuropathology. PrPres is also infectious and responsible for transmission and spread of the infection in vivo. Here we studied early spread of PrPres in C57BL mice after microinjection of scrapie into the striatum. At 30 min injected PrPres was visible in Prnp-null (PrP-KO) mice and C57BL mice near the needle track and on nearby blood vessels suggesting rapid transport of inoculum by brain interstitial fluid flow. At 3 and 7dpi, in PrP-KO mice PrPres in the inoculum was mostly gone; however, in C57BL mice at these same times, generation of new PrPres was detectable by immunohistochemistry, immunoblot and RT-QuIC assay. Such early detection of new PrPres has not been observed previously, and showed that PrPres propagation in vivo was rapid. In addition, these experiments provide evidence for early catabolism of inoculated PrPres in PrP-KO mice.

Host determinants of prion strain diversity independent of prion protein genotype

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In prion diseases phenotypic diversity can be specified by prion strains in which biological traits are propagated through an epigenetic mechanism mediated by distinct PrPSc conformations. Here we investigated the role of host factors on phenotypic diversity of chronic wasting disease (CWD) in different host species that express the same Prnp. Two CWD strains that have distinct biological, biochemical, and pathological features were identified in transgenic mice that express the Syrian golden hamster (SGH) Prnp. The CKY strain of CWD had a ~30 day shorter incubation period than the WST strain of CWD in transgenic mice, but following transmission to SGH, the incubation period of CKY CWD was ~150 days longer than WST CWD. Limited proteinase K
digestion revealed strainspecific PrPSc polypeptide patterns that were maintained in both transgenic mice and SGH, but the solubility and conformational stability of PrPSc differed for the CWD strains in a host-dependent manner. WST CWD produced large PrPSc amyloid plaques in the brain of SGH that were partially insoluble and stable at a high concentration of protein denaturant. However, in transgenic mice, PrPSc from WST CWD did not assemble into plaques, was highly soluble, and had low conformational stability. Similar studies using the HY and DY strains of transmissible mink encephalopathy only resulted in minor differences in prion biological and PrPSc properties between transgenic mice and SGH, suggesting that the altered phenotypes of the CWD strains upon interspecies transmission were not the result of differences in Prnp expression between the rodents. These findings indicate that host-specific pathways that are independent of Prnp can alter the PrPSc conformation of certain prion strains, leading to changes in the biophysical properties of PrPSc, neuropathology, and clinical prion disease.

Temporal parameters of prionemia in hamsters and deer following oral, nasal, or intravenous inoculations—minutes post inoculation through terminal clinical disease

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Infectious prions traverse epithelial barriers to gain access to the circulatory system, yet the temporal parameters of transepithelial transport and persistence in the blood over time remains unknown. We used wbRT-QuIC to analyze whole blood collected from TSE-inoculated deer and hamsters throughout the entire incubation period for the presence of PrPC-conversion competent amyloid (prionemia). We observed prionemia in the blood of TSE-inoculated hosts throughout disease course from minutes post exposure to terminal disease.

Scrapie transmits to white-tailed deer by the oral route and has a molecular proﬁlesimilar to chronic wasting disease and distinct from the scrapie inoculum

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The purpose of this work was to determine susceptibility of white-tailed deer (WTD) to the agent of sheep scrapie and to compare the resultant PrPSc to that of the original inoculum and chronic wasting disease (CWD). We inoculated WTD by a natural route of exposure (concurrent oral and intranasal (IN); n=5) with a US scrapie isolate. All scrapie-inoculated deer had evidence of PrPSc accumulation. PrPSc was detected in lymphoid tissues at preclinical time points, and deer necropsied after 28 months post-inoculation had clinical signs, spongiform encephalopathy, and widespread distribution of PrPSc in neural and lymphoid tissues. Western blotting (WB) revealed PrPSc with 2 distinct molecular profiles. WB on cerebral cortex had a profile similar to the original scrapie inoculum, whereas WB of brainstem, cerebellum, or lymph nodes revealed PrPSc with a higher profile resembling CWD. Homogenates with the 2 distinct profiles from WTD with clinical scrapie were further passaged to mice expressing cervid prion protein and intranasally to sheep and WTD. In cervidized mice, the two inocula have distinct incubation times. Sheep inoculated intranasally with WTD derived scrapie developed disease, but only after inoculation with the inoculum that had a scrapie-like profile. The WTD study is
ongoing, but deer in both inoculation groups are positive for PrP\textsuperscript{Sc} by rectal mucosal biopsy. In summary, this work demonstrates that WTD are susceptible to the agent of scrapie, two distinct molecular profiles of PrP\textsuperscript{Sc} are present in the tissues of affected deer, and inoculum of either profile readily passes to deer.

**Studying the Biochemical Basis for Prion Propagation**

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Prion is the unorthodox infectious agent in transmissible spongiform encephalopathies (TSEs, also known as prion diseases). The seeding process, by which the pathogenic prion protein conformer (PrP\textsuperscript{Sc}) conveys its structural properties to its normal counterpart (PrP\textsuperscript{C}), is central to the prion concept. However, the mechanistic basis for the seeding process of prion propagation is poorly understood. Using the serial protein misfolding cyclic amplification (sPMCA) technique, we have synthetically generated infectious prions with purified bacterially expressed recombinant PrP (recPrP) plus two cofactors, synthetic phospholipid POPG and total RNA from mouse liver. The synthetically generated prions (rec-Prion) can be propagated indefinitely in vitro via sPMCA. Using this relatively simple system, we have dissected the prion propagation process with a variety of tools. Our results indicate distinct but critical roles of cofactors and sPMCA procedure in prion propagation. The implication of our findings will be discussed.

**Deformed templating and its role in evolution of synthetic prions**

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What are the origins of strain mutation and new structural variants? How do prions mutate? A new model referred to as deformed templating model postulates that changes in replication environment play an active role in generating new PrP\textsuperscript{Sc} variants (Makarava & Baskakov, PLoS Pathogen V. 9(12) 2013). Changes in replication environment involve changes in cellular/biochemical environment (new co-factors, presence of anti-prion drugs) or transmission to a new host (changes in PrP\textsuperscript{C} sequence). Deformed templating is responsible for generating conformationally diverse prion populations, from which conformers that are fit to replicate in a particular cellular environment are selected.

The role of deformed templating in evolution of the strains of synthetic origin will be discussed. A direct illustration of the deformed templating mechanism by which a self-replicating state can seed altered self-replicating states with different folding patterns will be presented.

**Key steric zipper segments govern conversion by mouse and elk prions**

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The molecular mechanism by which PrP\textsuperscript{C} is converted to PrP\textsuperscript{Sc} remains poorly understood, yet is clearly influenced by (1) the conformation of the PrP\textsuperscript{Sc} and (2) PrP\textsuperscript{C} and PrP\textsuperscript{Sc} sequence similarity. Sequence complementarity is also an important determinant of seeding in other amyloid proteins, such as amyloid-\(\beta\), \(\alpha\)-synuclein and tau. How do specific amino acid side chains influence prion conversion? Eisenberg and
colleagues have shown that the atomic-level structure of amyloid fibrils formed by peptides from PrP, amyloid-β, tau and other amyloidogenic proteins consists of a repetitive motif: β-sheets arranged with self-complementary interdigitating amino acid side chains at the interface.

We hypothesize that sequence complementarity within key short segments of PrP impacts prion conversion across many strains and species. In support of this hypothesis, we have found that amino acid substitutions associated with conformational changes within the β2-α2 loop, or with long-distance interactions between the loop and the third helix, do not impact conversion. Instead, seeded prion conversion appears to be controlled by amino acid sequence within key segments of the host PrP. Interestingly, PrPc variants with substitutions in certain segments are efficiently converted by some mouse prion strains but not others, suggesting that the steric zippers involved in prion conversion may vary by PrPSc conformation.

This work provides important insights into species barriers to prion transmission as well the molecular basis for self-templating amyloid formation.

HUMAN TSEs ABSTRACTS

Transgenic mice expressing the fatal familial insomnia mutation recapitulate key pathological features of the human disease without developing prion infectivity

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Fatal familial insomnia (FFI) and a genetic form of Creutzfeldt-Jakob disease (CJD178) are clinically different prion disorders linked to the D178N prion protein (PrP) mutation. The disease phenotype is determined by the 129 M/V polymorphism on the mutant allele, which is thought to influence D178N PrP misfolding, leading to the formation of distinctive prion strains with specific neurotoxic properties. However, the mechanism by which misfolded variants of mutant PrP cause different diseases is not known. We generated transgenic (Tg) mice expressing the mouse PrP homolog of the FFI mutation. These mice synthesize a misfolded form of mutant PrP in their brains and develop a neurological illness with severe sleep disruption, highly reminiscent of FFI and different from that of analogously generated Tg(CJD) mice modeling CJD178 (Bouybayoune et al., 2015; Dossena et al., 2008). No prion infectivity was detectable in Tg(FFI) and Tg(CJD) brains by bioassay or protein misfolding cyclic amplification, indicating that mutant PrP has disease-encoding properties that do not depend on its ability to propagate its misfolded conformation. Tg(FFI) and Tg(CJD) neurons have different patterns of intracellular PrP accumulation associated with distinct morphological abnormalities of the endoplasmic reticulum and Golgi, suggesting that mutation-specific alterations of secretory transport may contribute to the disease phenotype. These results establish a new mouse model of FFI and support the concept that the pathogenic properties of mutant PrP are enciphered in misfolded forms of the protein that are not infectious (Chiesa et al., 2003).


Distinct Strains of Aβ Prions Implicated in Malignant Forms of Alzheimer Disease with Rapid Progression

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Keywords: Sporadic Alzheimer disease, structure of brain β-Amyloid, endophenotypes, disease progression rate.

Because over 75% of phenotypic variance of late onset Alzheimer disease (AD) remains unexplained by currently identified risk genes, we aimed to investigate the prion paradigm of AD, specifically the role of conformational structure of the brain amyloid beta (Aβ) in remarkably variable rates of clinical decline. Extensive experimental data accumulated in prion diseases, and analyses of aging brain samples, indicate that the pathological process underlying prion diseases and Alzheimer’s disease (AD) starts in isolated brain structures, and then spreads through neuronal projections. Accordingly, these findings have raised some fundamental questions in AD, including (i) whether different conformational features of beta Amyloid (Aβ) or tau are responsible for remarkably different progression rates of the disease, and (ii) whether subtle differences in the conformation of Aβ or tau may be responsible for diverse disease phenotypes. Using a tandem of novel biophysical methods, we inventoried and analyzed conformational structural characteristics of Aβ in the cortex of 48 cases of sporadic AD with distinctly different disease durations, and correlated the data with clinical profiles and genetics. The distinct isolates of human brain Aβ were monitored for their potency to propagate and accelerate the pathology in the brains of transgenic model of AD. In both hippocampus and posterior cingulate cortex we identified an extensive array of distinct Aβ42 particles that differ in conformational structure, size, and display of N-terminal and C-terminal domains. Rapidly progressive cases feature distinctly structured strains of brain Aβ42 forming unique spectrum of oligomeric particles that replicate and propagate faster in the transgenic brains upon inoculation. Our data indicate that Aβ42 generates in the AD brain a broad spectrum of different conformational structures – strains – that may have a potentially different toxicity and accumulation rate. The link between rapid clinical decline, levels of Aβ42 with distinct structural characteristics, and their propagation (replication) rate in vivo argue for prion-like mechanism encoding variable propagation tempo and phenotypic characteristics of the disease in distinct structures of Aβ42.

Variable relative contribution of methionine and valine at residue 129 to protease resistant prion protein in heterozygous cases of Creutzfeldt-Jakob disease.

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Sporadic Creutzfeldt-Jakob disease (sCJD) is thought to originate from the spontaneous misfolding of the endogenous host prion protein (PrPc) into its infectious prion isofrom, PrPSc. By contrast, iatrogenic CJD (iCJD) is associated with exposure to an exogenous source of PrPSc. sCJD and iCJD occur as three possible PRNP codon 129 genotypes: patients homozygous for methionine (M129) or valine (V129) and those who are heterozygous at this locus. In CJD patients...
heterozygous at residue 129, the relative contribution of each allotype to PrP Sc is unknown and its influence on prion pathogenesis is poorly understood. We have used mass spectrometry to determine the relative abundance of M129 and V129 in PrP Sc from heterozygous cases of sCJD and iCJD, the latter of which are linked to human growth hormone therapy in the United Kingdom. Our results show that, while the amount of M129 or V129 in PrP Sc is variable in heterozygous sCJD patients, PrP Sc with V129 is most abundant in the majority of heterozygous iCJD patients. The relative abundance of M129 or V129 in PrP Sc did not correlate with CJD type, age at clinical onset, or disease duration. However, the data are consistent with sCJD PrP Sc originating from the stochastic refolding of endogenous PrPC and iCJD originating from a non-stochastic, exogenous source of PrP Sc. Thus, the relative abundance of M129 and V129 in PrP Sc may be indicative of the PrP Sc allotype(s) which best converted PrPC to PrP Sc and may provide a means to trace back to the origin of CJD infection.

Blood transmission studies of prion infections in the squirrel monkey (Saimiri sciureus)

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Five blood-related vCJD transmissions (3 symptomatic and 2 pre-symptomatic), and an estimated one ‘silent’ infection per 2000 apparently healthy UK residents emphasize the continued need for information about disease risk in humans. We here describe a 4-part study of infectivity in a non-human primate model.

Study 1) Groups of 3-4 monkeys were inoculated ic with 10⁻¹ and 10⁻³ dilutions of pooled brain tissue from UK cases of vCJD (3) or sCJD (2), or a 10⁻¹ dilution of normal human brain: all CJD-inoculated animals became ill after 2-3 year incubation periods, and exosomes extracted from the blood of 5 of 7 of the vCJD-infected animals tested positive by PMCA for PrP TSE (the normal animal tested negative; the test is not yet optimized for sCJD infections).

Study 2) Individual ‘buddy’ monkeys were transfused every 3 months with whole blood taken from each vCJD and sCJD monkey inoculated with 10⁻¹ brain tissue in the above study: none developed disease by clinical, neuropathological, or immunohistological criteria, but blood exosome studies are still in progress.

Study 3) Groups of 2-4 monkeys were inoculated ic or ic/iv with either plasma or buffy coat from UK cases of sCJD and vCJD: none developed symptoms during a 6-8 year period of observation and at autopsy had no signs of disease by either neuropathological or immunohistological criteria.

Study 4) Pairs of monkeys were inoculated ic with various purified blood components from chimp-passaged US cases of GSS (1 case) and sCJD (2 cases), and observed for 5-6 years: only GSS leukocytes transmitted disease (both inoculated animals).

These results, together with other laboratory studies in rodents and non-human primates and epidemiological observations in humans, indicate that GSS (and perhaps other familial forms of TSE) carries a greater transfusion risk than sCJD, and that blood can be infectious during the incubation period of vCJD, but the question of a decades-long asymptomatic carrier state in vCJD remains unresolved.
This work was funded by Baxter Bioscience (Vienna, Austria) and the Fondation Alliance Biosécure (Paris, France)

Hidden prions in the spleen of vCJD-infected humans and macaques

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Immunohistochemistry identified PrPres in 16 among 32,441 appendicectomy samples, suggesting an important prevalence of healthy carriers of variant Creutzfeldt-Jakob Disease (vCJD) in United Kingdom, close to 200-fold more than the number of reported clinical cases. Even this prevalence might be underestimated, as suggested by the absence of PrPres deposition in lymphoid tissues of a vCJD patient. The clinical issue of healthy carriers and the risk of secondary transmission for public health still remain unknown.

Because formalin-induced fixation impairs protein detection, we recently improved our immunohistochemical protocol with novel epitope unmasking methods that detected PrPres in spleens from vCJD patients initially found negative by classical techniques. It also identified new sites of very high PrPres deposition around blood vessels in the spleens of transfusion-infected vCJD primates. This reproducible staining, observed with several anti-PrP antibodies, might correspond to an accumulation of soluble abnormal PrP trapped by newly described mesenchymal cells.

We have recently reported a fatal neurological syndrome in macaques exposed to different BSE/vCJD-infected blood products, corresponding to a myelopathic syndrome in this animal species without classically detectable PrPres. With our new detection technique, these animals exhibited a similar perivascular accumulation of PrPres in their spleens without classical follicular staining.

We hypothesize that vCJD infection might follow different pathophysiological pathways according to the initial source of infection (oral contamination with brain versus transfusion) that may lead to different disease phenotypes. The consequences for public health management will be discussed.


Using patient-specific fibroblasts and iPSC-derived neurons to uncover cellular phenotypes associated with prion diseases

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Cell biology of prion formation and spread remains incompletely understood, largely because of lack of authentic cell models. We report isolation of fibroblasts from skin tissues, derivation of induced pluripotent stem cells (iPSCs), differentiation of iPSCs into mature neurons, and detection of disease-related phenotypes in fibroblasts and
iPSC-derived neurons. Fibroblasts were isolated from skin samples of 23 subjects including nine asymptomatic subjects carrying six different PrP mutations, four patients with sporadic CJD (sCJD), and ten normal controls. Surprisingly, not only protease-resistant PrP was detected with Western blotting but also seeding activity was detected with RT-QuIC in fibroblasts of some PrP mutation carriers or sCJD patients. After iPSCs were derived from fibroblasts of mutation carriers (E200K or D178N) and normal controls, functional mature iPSC-derived neurons were further differentiated, as evidenced by immunostaining with the neuronal marker Map2 and by patch-clamp recording of GABA-induced current. While migration and glycosylation of PrP from fibroblasts were different from those of brain PrP, iPSC-derived neurons exhibited the PrP profile similar to the brain PrP. Notably, shortened neurites and neuritic-beading, characteristics of neurodegeneration, were more readily observed in iPSC-derived E200K neurons or in iPSC-derived neurons challenged with sCJD brain homogenates compared to neurons derived from iPSCs of normal subjects. Our study has generated patient-specific fibroblasts and iPSC-derived neurons that exhibit cellular phenotypes and seem to be authentic cell models for probing human prion diseases. [Supported by the CJD Foundation Award, NIHNS087588, NIHNS062787, and bridge funding from University Hospitals Case Medical Center.]

Unique features of genetic prion diseases

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Human prion diseases, which can present in transmissible, sporadic and genetic etiologies, share several important features, such as late onset/long incubation time, an inevitable fatal neurodegenerative outcome, the aberrant accumulation of disease related forms of PrP in the CNS, and in most cases also subsequent transmission of infectivity to corresponding animals. While it was assumed that there is a common mechanism of pathogenesis between all forms of human prion disease, some features may differ, since in the genetic form both fatal disease and de-novo infectivity are generated spontaneously in the absence of an outside infectious trigger. Using our TgMHu2ME199K mice, mimicking for the genetic prion disease linked to the E200K PrP mutation, we have investigated the biochemistry of mutant PrP at different time points and disease status. These include brains as well as organs from the immune system. Our results show that mutant PrP molecules fold into oligomeric forms since birth, long before disease manifestation while accumulation of its protease resistant form appears at several months of age, concomitant with disease progression. This indicates that the mutation itself, at least in the CNS environment induce the self-conversion of the mutant PrP to a disease related form. Interestingly, no disease related PrP could be found in the TgMHu2ME199K mice immune system, an hallmark of the transmissible disease. Our results indicate a unique mechanism for genetic prion disease. Most important no disease related PrP was found in the immune system, suggesting blood or other organs from E200K patients and carriers may not be infectious.

Characteristics of Prion Entry and Spread Following Inhalation Into Nasal Cavity

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To determine if inhalation of prions into the nasal cavity (NC) could cause disease and to describe the characteristics of this route of infection we carried out several sets of experiments. The first experiments involved inoculating hamsters extranasally (en), orally and intracerebrally with HY TME-infected brain homogenate (bh) or mock-infected bh.
to determine incubation periods and titer. The second set of experiments involved collecting NCs and lymphoreticular system (LRS) tissues at 2 week survival intervals till the onset of disease from animals inoculated with either HY or mock-infected bh. The results of these experiments demonstrated that the NC is 10-100 times more efficient as a route of infection compared to per os, with the same incubation period. Surprisingly there was no evidence that prions were associated with olfactory neurons, the olfactory nerve or the olfactory bulb, so that neuroinvasion via the olfactory nerve is not likely. Instead disease-associated prions (PrP\text{d}) were identified in the nasal associated lymphoid tissue (NALT) and submandibular lymph nodes (LN)s at 4 weeks post infection (pi), the cervical LN\text{s} at 8 weeks pi and spleen, Peyer's patches and mesenteric LN\text{s} at 14 wks pi. The next set of experiments were designed to determine how, when and where prions crossed the nasal cavity mucosa following inhalation. Animals were en inoculated with HY TME or mock-infected bh and allowed to survive for a range of time periods (1-180 min), the NCs were collected, decalcified and processed to identify PrP\text{d} or bh. Infected and mock-infected bh was seen crossing via M cells, and between cells of the respiratory, olfactory and follicle associated epithelia between 5-180 min pi. Relatively large amounts of inoculum were identified within the lumen of lymphatic vessels in the lamina propria of the nasal cavity at each time point pi. These results indicate that bh can readily cross the nasal cavity mucosa following inhalation, where it immediately enters lymphatic vessels. Similar experiments completed using mice, DY TME, soil absorbed inoculum and ketamine anesthesia demonstrate that the paracellular transport of prions is not species-specific, strain-specific, affected by soil or is an artifact of gas anesthesia. The lining of the nasal cavity presents no anatomical barrier to inhaled prions, an observation which has been supported by a number of similar findings in deer and sheep.

**Quantitative Real-time Analysis of Disease Specific Tau Amyloid Seeding Activity**

Davin Henderson, Edward Hoover

**Increased Infectivity of Anchorless Mouse Scrapie Prions in Transgenic Mice Overexpressing Human Prion Protein**

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Prion protein (PrP) is found in all mammals mostly as a glycoprotein anchored to the plasma membrane by a C-terminal glycoposphatidylinositol (GPI) linkage. Following prion infection, host protease-sensitive prion protein (PrP\text{sen}) is converted into an abnormal, disease-associated, protease-resistant form (PrP\text{res}).
Biochemical characteristics such as the PrP amino acid sequence and post-translational modifications such as glycosylation and GPI anchoring, can affect the transmissibility of prions as well as the biochemical properties of the PrPres generated. Previous in vivo studies have tested the roles of amino acid sequence and glycosylation on cross-species transmission, but the role of GPI anchoring has not been tested. In the current studies we examined the effect of PrPres GPI anchoring using a mouse-human species barrier model. In this model, anchorless 22L mouse scrapie prions were more infectious than anchored 22L mouse scrapie prions when inoculated into tg66 transgenic mice, which expressed wild-type anchored human PrP at 8-16 fold above normal. Thus the lack of the GPI anchor on PrPres appeared to reduce the effect of the mouse-human PrP species barrier. In contrast, neither form of 22L prions induced disease when tested in a second transgenic mouse which expressed human PrP at 2-4 fold above normal suggesting that PrP expression level also had an impact on our model.

**Glycosylation of PrP<sup>C</sup> is a key factor in determining TSE transmission between species**

Frances Wiseman<sup>1</sup>, Enrico Cancellotti<sup>1</sup>, Pedro Piccardo<sup>1</sup>, Kayleigh Iremonger<sup>1</sup>, Aileen Boyle<sup>1</sup>, Deborah Brown<sup>1</sup>, James Ironside<sup>2</sup>, Jean Manson<sup>1</sup>, Abigail Diack<sup>1</sup>

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The risk of transmission of transmissible spongiform encephalopathies (TSE) between different species has been notoriously unpredictable because the mechanisms of transmission are not fully understood. A transmission barrier between species often prevents infection of a new host with a TSE agent. Nonetheless, some TSE agents are able to cross this barrier and infect new species with devastating consequences. The host PrP<sup>C</sup> misfolds during disease pathogenesis and has a major role in controlling the transmission of agents between species, but sequence compatibility between host and agent PrP<sup>C</sup> does not fully explain host susceptibility. PrP<sup>C</sup> is post-translationally modified by the addition of glycan moieties which have an important role in the infectious process. Here we show in vivo that glycosylation of the host PrP<sup>C</sup> has a significant impact on the transmission of TSE between different host species.

We infected mice in which the first (N180T), second (N196T) or both (N180T and N196T) N-glycan attachment sites are disrupted with two human agents (sCJDMM2 and vCJD) and one hamster strain (263K). The absence of glycosylation at both or the first PrP<sup>C</sup> glycosylation site in the host results in almost complete resistance to disease. Absence of the second glycosylation site has a dramatic effect on the barrier to transmission between host species, facilitating the transmission of sCJDMM2 to a host normally resistant to this agent. These results demonstrate that glycosylation of host PrP<sup>C</sup> can dramatically alter cross species transmission and is a key factor in determining the transmission efficiency of TSEs between different species.

**YEAST PRIONS ABSTRACTS**

**Identifying J-protein and yeast prion interactions: diverse chaperone requirements among prions and prion variants/strains.**

Justin K. Hines

Department of Chemistry, Lafayette College, Easton, PA, USA

Most yeast prions are formed of amyloid aggregates that rely on the action of a core set of chaperone proteins (Sis1, Ssa, and Hsp104) for transmission to progeny, and yet some prions exhibit requirements for additional chaperone activities, indicating that prion-chaperone requirements are heterogeneous. Further, prions can form distinct amyloid structures (amyloid structural polymorphisms), called 'strains' in mammalian systems and 'variants' in yeast,
that dictate the intensity of yeast prion-associated phenotypes and stability in mitosis. Recently we and others have uncovered significant complexity in the chaperone requirements of various yeast prions and prion variants. Two specific examples will be discussed: our recent report of distinct prion- and variant-specific requirements for the Hsp70 co-chaperone Sis1, and the unusual requirement of the atypical J-protein Swa2 by at least one variant of the prion [URE3].

W8, a New Sup35 Prion Strain, Transmits Distinctive Information with a Conserved Assembly Scheme

Yu-Wen Huang1,2, Yuan-Chih Chang3, Ruben Diaz-Avalos4, and Chih-Yen King2

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Prion strains are different self-propagating conformers of the same infectious protein. Three strains of the [PSI] prion, infectious forms of the yeast Sup35 protein, have been previously characterized in our laboratory. Here we report the discovery of a new [PSI] strain, named W8. We demonstrate its robust cellular propagation as well as the protein-only transmission. To reveal strain-specific sequence requirement, mutations that interfered with the propagation of W8 were identified by consecutive substitution of residues 5-55 of Sup35 by proline and insertion of glycine at alternate sites in this segment. Interestingly, propagating W8 with single mutations at residues 5-7 and around residue 43 caused the strain to transmute. In contrast to the assertion that [PSI] existed as a dynamic cloud of sub-structures, no random drift in transmission characteristics was detected in mitotically propagated W8 populations. Electron diffraction and mass-per-length measurements indicate that, similar to the three previously characterized strains, W8 fibers are composed of about 1 prion molecule per 4.7-Å cross-β repeat period. Thus differently folded single Sup35 molecules, not dimeric and trimeric assemblies, form the basic repeating units to build the four [PSI] strains.

Mechanism of [PSI+] Prion Curing by Hsp104 Overexpression

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NHLBI, NIDDK, NIH, Bethesda, Md

Yeast prions are propagated by the severing activity of Hsp104. When this activity is inhibited, the prions are cured by the lack of severing of the prion seeds in combination with dilution of the seeds by cell division. Surprisingly, unlike other yeast prions, the [PSI+] prion is also cured by overexpression of Hsp104. The [PSI+] is the amyloid form of Sup35, a component of the translation termination complex. To understand the mechanism by which Hsp104 overexpression cures [PSI+], the curing process was examined using live cell imaging of yeast expressing GFP-labeled Sup35 in combination with standard plating assays. Overexpression of Hsp104 was found to cure [PSI+] by dissolution of the prion seeds, a process that we found is caused by trimming of the seeds by Hsp104. Trimming reduces the size of the prion seeds, perhaps by removing Sup35 monomers from the ends of the prion fibers, but unlike severing, trimming does not increase the number of seeds. Another difference between the trimming and severing activities of Hsp104 is that the trimming activity is inhibited by Ssa1. This
antagonist interaction between overexpression of Ssa1 and the curing by Hsp104 overexpression, which was initially observed by the Chernoff laboratory, is caused by the Ssa1 inhibiting the trimming activity of Hsp104. In contrast, overexpression of a dominant negative Ssa1 mutant has the converse effect; it causes increases trimming of the prion seeds, resulting in faster curing of $[\text{PSI}^+]$. Therefore, Ssa1 concentration plays a critical role in determining whether Hsp104 is able to trim the prion seeds and in turn cure $[\text{PSI}^+]$ yeast when it is overexpressed.

Formation of a metastable prion by the yeast actin associated protein Lsb2

Tatiana A. Chernova¹, John Shanks¹, Denis Kiktev², Andrew Romanuk², Moiez Ali¹, Yury O. Chernoff²,³ and Keith D. Wilkinson¹

¹Emory University School of Medicine, Atlanta, Georgia, USA, ²Georgia Institute of Technology, Atlanta, Georgia, USA and ³St. Petersburg State University, St. Petersburg, Russia

Amyloid formation in vivo is thought to result from alterations in protein homeostasis and cellular quality control system, however specific mechanisms remain elusive. We have shown that paralogous actin associated proteins Lsb1 and Lsb2 modulate maintenance of the $[\text{PSI}^+]$ prion during thermal stress and that Lsb2 levels and Lsb1 processing are induced by heat-shock. Here we demonstrate that Lsb2 forms prion state $[\text{LSB}^+]$. $[\text{LSB}^+]$ is transmitted through mating and meiosis, and is cured by guanidine-HCl. Detergent resistant Lsb2 aggregates, are detected in the cultures overproducing Lsb2 and are maintained by the $[\text{LSB}^+]$ cells. $[\text{LSB}^+]$ is mitotic instability, and is lost by a significant fraction of cells during growth. In agreement that Lsb2 is a short-leaved protein degraded via ubiquitin-proteasome system, mitotic stability of $[\text{LSB}^+]$ is increased in the cells defective in Lsb2 ubiquitination. Lsb2 derivative, deficient in association with the actin cytoskeleton, is unable to form detergent-resistant aggregates, convert into the $[\text{LSB}^+]$ prion and promote conversion of Sup35 into $[\text{PSI}^+]$ prion. Substitution of the Lsb2 8Q stretch to 8N decreases the average size of Lsb2 polymers and increases efficiency of $[\text{PSI}^+]$ prion induction by overexpression of Lsb2 and Sup35. Lsb2 paralog, Lsb1 cannot induce $[\text{PSI}^+]$, and this difference in prion-inducing abilities between two proteins can be traced to a single amino acid substitution. Our findings directly implicate the role of ubiquitin-proteasome system and actin cytoskeleton in formation of metastable transient prions influencing amyloid formation by other proteins and shed new light on protein-based inheritance mechanisms of protein assembly diseases.

Insights into prion formation from bioinformatic analysis

Paul Harrison

Department of Biology, McGill University, Montreal, Quebec, Canada

We present our latest results on bioinformatic analysis of available genome-scale data, such as how protein interaction data has indicated that prion or ‘prionogenic’ proteins tend to interact with other proteins with ‘prion-like’ character. We discuss how stress-granule assembly arises in bioinformatic analysis as a key functional process for prion/prionogenic proteins. Also, we present a dissection of the molecular evolution of known prion/prionogenic proteins across the fungal kingdom, and discuss the implications of our analyses for the search for further prions in yeast.
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.

A distinct group of human neurodegenerative amyloidoses, including Huntington disease, is caused by expansion of polyglutamine (polyQ) stretches in several otherwise unrelated proteins. Studies in yeast, using the first exon of the human huntingtin (Htt)-encoding gene, demonstrate that the deleterious effect of Htt also correlates with the length of its polyQ. While an N-terminal fragment of mutant Htt with a stretch of 103 glutamine residues (Htt103Q) aggregates and causes toxicity, its wild type variant with a sequence of 25 glutamines (Htt25Q) is not toxic and does not aggregate. Here, we observed that non-toxic polymers of proteins with long polyQ or polyQ interspersed with other residues (polyQX) can seed polymerization of Htt25Q, which causes toxicity. We further showed that toxicity of Htt25Q is related to the ability of its polymers to seed polymerization of the essential glutamine/asparagine (Q/N)-rich Sup35 protein which results not only in depletion of its soluble form but also in sequestration of its essential partner protein, Sup45, through its binding to Sup35 polymers. Prion amyloids of the Q/N-rich Rnq1 protein can also seed Htt25Q polymerization which is accompanied with appearance of Sup35 polymers and growth inhibition. Importantly, just polymers of Htt25Q, but not of Rnq1 or polyQ/QX, seed Sup35 polymerization, suggesting that Htt25Q polymers act as intermediators in seeding Sup35 polymerization. The obtained data provide a novel insight into interactions between amyloidogenic proteins and pathophysiological interrelations between various polyQ disorders.

A protein polymerization cascade mediates the toxicity of seeded amyloids of non-pathogenic huntingtin in yeast

Genrikh Serpionov, Alexander Alexandrov, Michael Ter-Avanesyan
Bach Institute of Biochemistry, Moscow, Russia

Using yeast as a model to understand the mechanisms that underlie protein aggregation, amyloid formation, and prionization
Current knowledge of prion biology has been greatly enhanced by studies in *Saccharomyces cerevisiae*, which contains several epigenetic elements known as yeast prions. The yeast prion [SWI+], whose protein determinant is Swi1, a subunit of an evolutionarily conserved ATP-dependent chromatin-remodeling complex SWI/SNF, was discovered in our laboratory. We showed that the first 38 amino acids of Swi1 were able to aggregate, and maintain and propagate [SWI+]. However, further deletion to the first 32 amino acids resulted in a dramatic reduction in aggregation, indicating that the minimal prion domain (PrD) of Swi1 lies between residues 32 to 38. Further analysis showed that the first 33 amino acids of Swi1 are able to aggregate, and maintain a prion conformation in the absence of full-length Swi1, suggesting that this region is likely the minimal PrD of Swi1. Using a newly designed reporter system that can faithfully report the prion status of Swi1, we conducted high-throughput screens to identify compounds that can eliminate or inhibit [SWI+] and have obtained a number of promising anti-[SWI+] compounds. We are currently elucidating the hit compounds’ mechanism of action and investigating their ability to antagonize PrPSc and inhibit Aβ-induced toxicity in a mammalian cell culture system. These studies will shed light on the mechanisms of protein misfolding, aggregation, and amyloid fiber formation - all of which are relevant to prion diseases and other amyloid-based neurological disorders.

Prion-based regulation of the dynamic changes in ribonucleoprotein complexes

Irina Derkatch1, Catherine Potenski2, Xiang Li1, Eric Kandel1,3, Eric Kandel4,5

Prions are over-represented among RNA-binding proteins and components of RNP complexes regulating biogenesis, translation, turnover and cellular distribution of mRNAs. We provide two new lines of evidence that prion-based complexes are engaged in dynamic rearrangements in a network of RNA-processing complexes allowing for rapid switching between RNA storage and degradation, and between inhibition and activation of protein synthesis. Analysis of prion-like aggregation directed by the Q/N-rich prion domain of yeast Lsm4, a P-body-associated activator of mRNA decapping, revealed that Lsm4 forms heritable aggregates. The aggregation, that is controlled by chaperones and induced by environmental changes, such as temperature drop to 4°C, leads to the increase in the number of P-bodies and their clustering around Lsm4 aggregates, indicative of the directed modulation of mRNA turnover. Analysis of prion properties of yeast Pub1 and its mammalian homolog Tia1 revealed that this protein participates in two distinct self-perpetuating structures, both formed through its Q/N-rich prion domain. One is localized to P-bodies and stress granules, consistent with known role of Pub1/Tia1 in stress granule assembly. The other structure is formed co-operatively by Pub1/Tia1 and Sup35/Gspt2, the eRF3 release factor. This heteroprotein prion is normally present in yeast cells, can be visualized as lines forming...
along tubulin cytoskeleton and drives the assembly of an RNP complex implicated in maintaining the integrity of microtubule cytoskeleton. We hypothesize that the complex directs tubulin synthesis to the sites of microtubule assembly, and that Pub1/Tia1 functionally shuffles between two prion-like structures. Support: NIH grant 7R01GM070934-06 (ILD), HHMI (ERK).

**Determining the Mechanism(s) of Dominant-Negative Inhibition of Different Prion Strains**

Fen Pei and Tricia Serio

*Molecular and Cellular Biology, University of Arizona*

The prion mechanism underlies several previously inexplicable phenomena, including transmissible neurodegenerative disease in mammals and the non-Mendelian inheritance of unique traits in fungi. Despite the 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 strains 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 the \([PSI^+]_{\text{Strong}}, [PSI^+]_{\text{Scd}}\) and \([PSI^+]_{\text{Weak}}\) Sup35 strains. Our studies indicate that the G58D mutant inhibits propagation of all Sup35 conformations in a dose-dependent manner, leading to a decrease in aggregate thermodynamic stability. We show that incorporation of G58D into wildtype prion aggregates composed of any of these conformations promotes aggregate disassembly in vivo, leading to a change in the number of transmissible aggregates. Heterozygous disruption of Hsp104, the cellular factor mediating prion aggregate fragmentation, partially reversed the dominant-negative effects of G58D expression. Despite these mechanistic commonalities, the ratio of mutant to wildtype protein required for dominant-negative inhibition correlates with differences in their basal thermodynamic stabilities. Thus, the conformational variation does not modulate the mechanism by which the inhibition occurs but rather the sensitivity of these strains to prion loss.

**Proteins are aberrantly recruited into polyglutamine aggregates via their intrinsically-disordered domains**

Maggie Wear¹, Robert O’Meally², Dmitry Kryndushkin¹, Frank Shewmaker¹

¹Uniformed Services University, Bethesda, MD, USA, ²Johns Hopkins University, Baltimore, MD, USA

Intracellular protein aggregation is the hallmark of several neurodegenerative diseases. Aggregates formed by polyglutamine (polyQ)-expanded proteins, such as Huntingtin, assume amyloid-like structures that are resistant to denaturation. We combined mass spectrometry and a stringent purification procedure to identify the protein species that are trapped within aggregates formed by Huntingtin N-terminal fragments with pathogenic polyQ tracts (>40 glutamines) in both yeast and mammalian (PC12) cells. We found that protein quality-control and RNA-binding proteins were greatly enriched in polyQ aggregates, and despite their evolutionary divergence, there was significant conservation between trapped
proteins identified from yeast and PC12 cells. Notably, in the mammalian cells, a number of neurodegenerative disease-linked proteins were consistently found trapped in the polyQ aggregates. Many of these proteins are found in neuronal inclusions in their respective diseases, suggesting that polyQ aggregates can recruit proteins that are prone to aggregation in different pathological contexts. We also analyzed the primary and secondary structure of our aggregate-associated proteins and discovered a significant enrichment of proteins with very long intrinsically-disordered (ID) domains. When we truncated the ID domains of selected proteins, the proteins no longer co-aggregated with polyQ. The high frequency of ID domains in RNA-binding proteins may explain why these proteins are disproportionately found in pathological inclusions in many neurodegenerative diseases.

Workshop Organizers

Animal Prions
Christina Sigurdson, University of California, Davis
Candace Mathiason, Prion Research Center at Colorado State University

Human Prions
Jason Bartz, Creighton University
Jiri Safar, National Prion Disease Pathology Surveillance Center at Case Western Reserve University
Gianluigi Zanusso, Università di Verona

Yeast Prions
Eric Ross, Prion Research Center at Colorado State University
Heather True-Krob, Saint Louis University

The workshop book cover features Prion2015 logos designed by CSU PRC members Carla Calvi, Lindsay Parrie, Jifeng Bian, Mark Zabel, Vanessa Selwyn, Connor Hendrich and Aubrey Waechter.

Scan the QR code on the back of this book to access Prion2015.org.
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