Prion 2015 Oral Abstracts

Propagation

O.01: Transgenic mice expressing human wild-type α-synuclein develop neuropathology after inoculation with brain homogenates from patients with multiple system atrophy or aged subjects without neurological disorder

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Multiple system atrophy (MSA) and Parkinson’s disease are synucleinopathies that are defined by the presence of aggregated and hyperphosphorylated α-synuclein (α-syn) within cells of the central nervous system (CNS).

Recent findings suggest that pathological α-syn may spread prion-like within the nervous system. We investigated prion-like propagation of pathological α-syn in Tg(SNCA)1Nbm/J mice that do not express mouse but low levels of human wt α-syn and do not naturally develop any pathology or neurodegenerative disease.

We inoculated brain homogenate from 2 patients with MSA, from 2 aged control subjects without neurological disorder, or saline intrastriatally into Tg(SNCA)1Nbm/J mice. Challenged mice were sacrificed at 90, 180, and 270 d post inoculation and were analyzed biochemically and immunohistochemically for pathological α-syn.

Brain homogenates from MSA or aged control subjects but not saline triggered progressive accumulation of aggregated α-syn in neurons of inoculated mice. Aggregates of α-syn were hyperphosphorylated and co-stained for p62 that targets proteins for degradation. Aggregates of pathological α-syn were first observable in the ipsilateral brain hemisphere and over time in the contralateral hemisphere and in more rostral and caudal areas.

Our findings show that brain homogenate from MSA patients but not saline induces pathological changes in the CNS of Tg(SNCA)1Nbm/J mice. Our data support that pathological α-syn may propagate prion-like along neuronal networks. Furthermore, human wt α-syn supports propagation of pathological α-syn. Intriguingly, brain homogenate from aged control subjects without neurological disorder equally induced synucleinopathy in brains of Tg(SNCA)1Nbm/J mice suggesting that aged human brains can contain pathological α-syn.

O.02: Experimental transmissibility of mutant SOD1 motor neuron disease

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By unknown mechanisms, the symptoms of amyotrophic lateral sclerosis (ALS) seem to spread along anatomical pathways to engulf the motor nervous system. The rate at which symptoms spread is one of the primary drivers of disease progression. One mechanism by which ALS symptoms could spread is by a prion-like propagation of a toxic misfolded protein from cell to cell along anatomic pathways. Proteins that can transmit toxic conformations between
cells often can also experimentally transmit disease between individual organisms. To survey the ease with which motor neuron disease (MND) can be transmitted, we injected spinal cord homogenates prepared from paralyzed mice expressing mutant superoxide dismutase 1 (SOD1-G93A and G37R) into the spinal cords of genetically vulnerable SOD1 transgenic mice. From the various models we tested, one emerged as showing high vulnerability. Tissue homogenates from paralyzed G93A expressing mice induced MND in 6 of 10 mice expressing low levels of G85R-SOD1 fused to yellow fluorescent protein (G85R-YFP mice) by 3–11 months, and produced widespread spinal inclusion pathology. Importantly, second passage of homogenates from G93A→G85R-YFP mice back into newborn G85R-YFP mice, induced disease in 4 of 4 mice by 3 months of age. Homogenates from paralyzed mice expressing the G37R variant were among those that transmitted poorly regardless of the strain of recipient transgenic animal injected, a finding suggestive of strain-like properties that manifest as differing abilities to transmit MND. Together, our data provide a working model for MND transmission to study the pathogenesis of ALS.

O.03: Role of brain interstitial fluid flow in very early generation and spread of PrPres after microinjection of prion infectivity in C57BL mouse brain

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Prion infectivity typically spreads along neurons following the paths of neuron circuitry within the CNS; however, less is known about the process of initial brain infection. Here we studied spread of disease-associated PrPres in brain of C57BL mice from 30 minutes to 40 d after microinjection (0.5 μl) of scrapie infectivity into the striatum. After microinjection of 22L scrapie, PrPres was visible in the needle track and around nearby blood vessels at 30 min in both C57BL and Prnp-null (KO) control mice. This rapid initial spread to vessels appeared to be via brain interstitial fluid (ISF) flow in perivascular and periaxonal regions. At 3 dpi in KO mice, inoculated PrPres was mostly gone. Remarkably, at 3 and 7 dpi in C57BL mice, generation of new PrPres was detectable by immunohistochemistry, immunoblot and RT-QUIC assay. Again, PrPres was associated with perivascular sites of ISF drainage, and PrPres associated mainly with perivascular astroglia, and only minimally with neurons, microglia and oligodendroglia. By 20–40 dpi, PrPres had spread to ipsilateral thalamus and cerebral cortex at locations 1.5–2.5 mm distant from the injection site. Both the ipsilateral PrPres distribution and the rapid transit time suggested that spread from striatum was via neuronal circuitry. Thus early 22L scrapie spread involved both neurons and ISF flow, however PrPres mainly accumulated in astroglia. In experiments using scrapie strain ME7, similar spreading mechanisms were observed, but PrPres accumulated primarily in association with neuronal cell bodies and neuropil, which was not seen with strain 22L.

O.04: A single coding polymorphism in the PRNP gene significantly alters the transmission dynamics of blood-borne prions

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Sheep experimentally infected with bovine spongiform encephalopathy (BSE) have provided a useful model in which to study the risks of transmission of prion disease by transfusion of blood components. We have previously shown that several components relevant to human clinical practice (red cell concentrate, platelets, plasma) are infectious, and that leukodepletion of these components does not completely prevent disease transmission.
Further analysis of the data has identified factors which distinguish donor sheep that transmitted infection via blood components (“transmitters”), from those that did not (“non-transmitters”). The codon 141 polymorphism of the sheep PRNP gene (L → F) was strongly associated with the probability of transmitting infection, with the majority of transmitters having a 141LL genotype, while the majority of non-transmitters were 141LF. Since codon 141 genotype has also been associated with variation in incubation period, this finding may reflect the stage of the incubation period reached by the donor at the time when blood was collected, because titres of infectivity in blood tend to increase as animals progress toward the clinical phase of infection. Another factor that appears to differ between transmitting and non-transmitting donors is the extent of PrP deposition in lymphoid tissues, with transmitters having a higher proportion of positive lymphoid tissues than non-transmitters, regardless of their codon 141 genotype. This suggests that a single nucleotide polymorphism of PRNP, and/or the extent of prion replication in lymphoid tissues, may influence titres of blood-borne infectivity and thus the risk of disease transmission by transfusion of blood components.

O.05: Transmission of prions to primates after extended silent incubation periods: Implications for BSE and scrapie risk assessment in human populations

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Prion diseases (PD) are the unique neurodegenerative proteinopathies reputed to be transmissible under field conditions since decades. The transmission of Bovine Spongiform Encephalopathy (BSE) to humans evidenced that an animal PD might be zoonotic under appropriate conditions. Contrarily, in the absence of obvious (epidemiological or experimental) elements supporting a transmission or genetic predispositions, PD, like the other proteinopathies, are reputed to occur spontaneously (atypical animal prion strains, sporadic CJD summing 80% of human prion cases).

Non-human primate models provided the first evidences supporting the transmissibility of human prion strains and the zoonotic potential of BSE. Among them, cynomolgus macaques brought major information for BSE risk assessment for human health (Chen, 2014), according to their phylogenetic proximity to humans and extended lifetime. We used this model to assess the zoonotic potential of other animal PD from bovine, ovine and cervid origins even after very long silent incubation periods.

We recently observed the direct transmission of a natural classical scrapie isolate to macaque after a 10-year silent incubation period, with features similar to some reported for human cases of sporadic CJD, albeit requiring fourfold longer incubation than BSE. Scrapie, as recently evoked in humanized mice (Cassard, 2014), is the third potentially zoonotic PD (with BSE and L-type BSE), thus questioning the origin of human sporadic cases. We will present an updated panorama of our different transmission studies and discuss the implications of such extended incubation periods on risk assessment of animal PD for human health.

O.06: Prion properties of the Alzheimer’s disease associated proteins in the yeast model

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Amyloid formation is implicated in various human diseases, and many amyloids are
suspected to possess transmissible (prion) properties. However, molecular mechanisms of amyloid formation and propagation are difficult to investigate in vivo due to complexity of the human organism. We have established a yeast model for studying the prion properties of mammalian (including human) proteins. Our model employs chimeric constructs, containing the mammalian amyloidogenic proteins (or domains) fused to various fragments of the yeast prion protein Sup35. Phenotypic and biochemical detection assays, previously developed for the Sup35 prion, enable us to detect prion nucleation and propagation by mammalian proteins. By using this approach, we have investigated prion properties of Abeta and tau proteins. Oligomerization and aggregation of Abeta and tau is known to be associated with Alzheimer’s disease in humans. We have shown that both proteins confer prion characteristics to the chimeric constructs in yeast. Effects of known pro-aggregation and anti-aggregation mutations in these proteins on prion formation in yeast generally correspond to their effects on the disease development in humans. For example, the D23N substitution in Abeta increases prion nucleation in the yeast system. New mutations with predicted effects on amyloidogenesis are being generated and tested. Formation of different prion “strains” by chimeric proteins has been detected in yeast. Assays for studying interactions between different amyloidogenic proteins in the yeast cell have been developed. Overall, yeast model enables us to perform genetic dissection of molecular processes leading to the initiation of Alzheimer’s disease.

Topics in Animal Prions

O.07: 2-aminothiazole treatment of chronic wasting disease in transgenic mice expressing elk PrP

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Treatment with the 2-aminothiazole IND24 extended the survival of wild-type mice infected with mouse-passaged RML scrapie prions, but also resulted in the emergence of a drug-resistant prion strain. IND24 treatment was also efficacious against Tg mice expressing ElkPrP and infected with chronic wasting disease (CWD) prions, but the impact of treatment on the properties of CWD prions was not determined. Here, we assessed whether IND24 treatment extended the survival of additional natural isolates in Tg mice infected with sheep scrapie or CWD prions using 2 isolates for each disease. Multiple IND24 treatment regimens doubled the incubation times for CWD-infected mice, but IND24 treatment had no effect on the survival of the ovine scrapie-infected mice. Biochemical, neuropathologic, and cell culture analyses were used to characterize the prion strain properties following treatment, and indicated that the CWD prions were not altered by IND24 treatment regardless of survival extension. A second passage in the absence of treatment reproduced the original survival time, and cells infected with prions from animals that were treated with IND24 were as susceptible to IND24 treatment as CWD prions that were never before exposed to IND24. These results suggest that IND24 may be a viable candidate for treating CWD in infected captive cervid populations, and raise questions about why some strains acquire resistance upon treatment whereas others do not.
O.08: H-type bovine spongiform encephalopathy associated with E211K prion protein polymorphism: Clinical and pathologic features in wild-type and E211K cattle following intracranial inoculation

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In 2006 an H-type bovine spongiform encephalopathy (BSE) case was reported in an animal with an unusual polymorphism (E211K) in the prion protein gene. Although the prevalence of this polymorphism is low, cattle carrying the K211 allele are predisposed to rapid onset of H-type BSE when exposed. The purpose of this study was to investigate the phenotype of this BSE strain in wild-type (E211E) and E211K heterozygous cattle.

One calf carrying the wild-type allele and one E211K calf were inoculated intracranially with H-type BSE brain homogenate from the US 2006 case that also carried one K211 allele. In addition, one wild-type calf and one E211K calf were inoculated intracranially with brain homogenate from a US 2003 classical BSE case. All animals succumbed to clinical disease. Survival times for E211K H-type BSE inoculated cattle (10 and 18 months) were shorter than the classical BSE inoculated cattle (both 26 months). Significant changes in retinal function were observed in H-type BSE challenged cattle only. Animals challenged with the same inoculum showed similar severity and neuroanatomical distribution of vacuolation and disease-associated prion protein deposition in the brain, though differences in neuropathology were observed between E211K H-type BSE and classical BSE inoculated animals. Western blot results for brain tissue from challenged animals were consistent with the inoculum strains.

This study demonstrates that the phenotype of E211K H-type BSE remains stable when transmitted to cattle without the E211K polymorphism, and exhibits a number of features that differ from classical BSE in both wild-type and E211K cattle.

Mechanisms of Disease

O.09: Mutant prion proteins related to genetic prion diseases impair intracellular trafficking

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Fatal familial insomnia (FFI), genetic Creutzfeldt-Jakob disease (gCJD) and Gerstmann-Sträussler-Scheinker (GSS) syndrome are neurodegenerative disorders linked to mutations in the prion protein (PrP) gene. The mechanism of neurotoxicity of mutant PrP is not clear, but misfolding and intracellular accumulation may contribute to the pathogenic process. We previously found that mouse (mo) PrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.¹ To further characterize the trafficking defect induced by the FFI mutation, and test whether other mutants had similar effects, we analyzed the efficiency of secretory transport in cells expressing moPrP D177N/M128, homologous to the D178N/M129 mutation associated with FFI, accumulates in the Golgi of neuronal cells, impairing post-Golgi trafficking.
trafficking system, we monitored the intracellular transport of the temperature-sensitive vesicular stomatite virus glycoprotein (VSVG), a well-established cargo reporter. We observed marked alterations in secretory transport, with VSVG accumulating mainly in the Golgi complex. Our results indicate that different pathogenic mutations share the property of impairing intracellular trafficking. This suggests that defective intracellular transport may be a general mechanism of neurotoxicity in genetic prion diseases.

1. Massignan T, Biasini E, Lauranzano E, Vegliandese P, Pignataro M, Fioriti L, Harris DA, Salmona M, Chiesa R, Bonetto V. Mutant prion protein expression is associated with an alteration of the Rab GDP dissociation inhibitor alpha (GDI)/Rab11 pathway. Mol Cell Proteomics 2010; 9(4):611–622; PMID: 19996123; http://dx.doi.org/10.1074/mcp.M900271-MCP200

O.10: The sheddase ADAM10 significantly impacts on prion disease

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Proteolytic processing of key proteins, be it deleterious or protective, is relevant in many neurodegenerative proteinopathies such as Alzheimer’s or prion disease. With regard to the latter, while α-cleavage in the middle of the cellular prion protein (PrP\textsuperscript{C}) has been shown to impair misfolding into the pathogenic isoform (PrP\textsuperscript{Sc}) and thus to be protective against prion disease, the role of another physiological cleavage (i.e., shedding in close proximity to the GPI-anchor of PrP\textsuperscript{C}) remained largely unknown. We and others have identified ADAM10 as the physiologically relevant sheddase of PrP\textsuperscript{C} regulating its membrane homeostasis.

Using a novel mouse model, we show that depletion of ADAM10 in forebrain neurons leads to posttranslational increase of PrP\textsuperscript{C} levels. When infected with prions, these mice present with drastically shortened incubation times, increased PrP\textsuperscript{Sc} formation and upregulation of calpain. Our spatiotemporal analyses also suggest that absence of shedding impairs spread of prion pathology within the brain. Moreover, our mouse model provides some interesting insights into central issues discussed in the prion field, such as (i) an inhibitory effect of anchorless PrP versions on the conversion process, (ii) mechanisms of prion-associated neurotoxicity, and (iii) a likely disparity between PrP\textsuperscript{Sc} amounts and prion infectivity.

Taken together, ADAM10-mediated shedding seems to have a dual role in prion diseases thus emphasizing the relevance of proteolytic processing in these conditions. Given the suggested role of PrP\textsuperscript{C} as a receptor for toxic protein oligomers in more common proteinopathies our findings might impact on these devastating conditions as well.

Structure/Function

O.11: Structural determinants of phenotypic diversity and replication rate of human prions

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The infectious pathogen responsible for prion diseases is the misfolded, aggregated form of the prion protein, PrP\textsuperscript{Sc}. In contrast to recent progress in studies of laboratory rodent-adapted prions, current understanding of the
molecular basis of human prion diseases and, especially, their vast phenotypic diversity is very limited. Here, we have purified proteinase resistant PrPSc aggregates from two major phenotypes of sporadic Creutzfeldt-Jakob disease (sCJD), determined their conformational stability and replication tempo in vitro, as well as characterized structural organization using recently emerged approaches based on hydrogen/deuterium (H/D) exchange coupled with mass spectrometry. Our data clearly demonstrate that these phenotypically distant prions differ in a major way with regard to their structural organization, both at the level of the polypeptide backbone (as indicated by backbone H/D exchange data) as well as the quaternary packing arrangements (as indicated by H/D exchange kinetics for histidine side chains). Furthermore, these data indicate that, in contrast to previous observations on yeast and some murine prion strains, the replication rate of sCJD prions is primarily determined not by conformational stability but by specific structural features that control the growth rate of prion protein aggregates.

O.12: Scrapie-specific C-terminal antibody reveals conformational differences between prion strains

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Misfolding of the cellular form of prion protein (PrPc) into the pathological PrP (PrPSc) in the brain can lead to transmissible spongiform encephalopathies or prion diseases in humans and animals. PrPSc is an alpha-helix-rich monomer, while PrPc forms beta-sheet-rich oligomers and amyloid fibrils. Prion strains have been classified based on different incubation times, neuropathological lesions and biochemical characteristics. However, the detailed structure of PrPSc and conformational differences between strains are poorly understood. In this study, we used antibodies to different epitopes to probe the structures of PrPSc isolated from brains of mice with either the Chandler or 22L strains or hamsters with 263K scrapie. Epitope mapping of PrPSc was performed under native or guanidine-denatured conditions by indirect-ELISA. Our results showed that only a small subset of antibodies recognized epitopes in the native structure of PrPSc. One of those antibodies, with a conformationally sensitive C-terminal epitope, had strongly differing reactivities to the native Chandler and 22L strains of PrPSc despite the fact that these murine prions share the same PrP primary structure. Although it has long been apparent that prion strains can differ conformationally near the N-termini of molecules forming the proteinase-K resistant core of PrPSc, our results show evidence for strain-dependent conformational differences near the C-terminal as well.

O.13: Misfolded wild-type SOD1 induced by pathological FUS or TDP-43 transmits intercellularly and is propagation-competent

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Clinically indistinguishable cases of amyotrophic lateral sclerosis (ALS) can be caused by either inheritable mutation in the genes encoding SOD1, TDP-43, FUS, among others, or can occur sporadically. Misfolded SOD1 has been detected in both familial and sporadic ALS patients, despite SOD1 mutations accounting for only ~2% of total cases. We previously reported that pathological FUS or TDP-43 kindles misfolding of human wild-type (wt) SOD1 in living cells. Here, we use human
cell cultures and mouse primary neural cultures expressing human wtSOD1, to establish that FUS or TDP-43-induced misfolded SOD1 can traverse between cells through the incubation of untransfected cells with conditioned media, triggering conversion of endogenous SOD1 in a prion-like fashion. This intercellular spread is arrested by pre-incubation of the conditioned media with misfolded SOD1-specific antibodies, demonstrating their therapeutic potential. We find that recipient cells pre-treated with SOD1-siRNA do not contain misfolded SOD1, indicating that endogenous SOD1 is required as substrate for active conversion. Our data also shows that conditioned media obtained from mutant TDP-43 and FUS transfected, or wild-type TDP-43 over-expressing, cells is cytotoxic to the recipient cells. Furthermore, transfection of TDP-43 into cells triggers its cleavage, mis-localization and hyperphosphorylation; these properties are not observed in untransfected cells incubated with conditioned media from pathological TDP-43 transfected cells, further confirming that the transmission of SOD1 mis-folding occurs independently of TDP-43.

**O.14: The architecture of recombinant prions is similar to that of brain-derived prions: Insights from limited proteolysis**

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Substantial evidence suggests that PrPSc is a 4-rung β-solenoid, and that individual PrPSc subunits stack to form amyloid fibers. We recently used limited proteolysis to map the β-strands and connecting loops that conform the PrPSc solenoid. Using high resolution SDS-PAGE followed by epitope mapping, and mass spectrometry, we identified positions ~117/119, 133–134, 152–153, 141, 162, 169 and 179 as PK cleavage sites in PrPSc. Such sites define loops and/or borders of β strands, and are helping us define the threading of the b-solenoid.

We have now extended this approach to recombinant PrPSc (recPrPSc). We apply the term recPrPSc to *bona fide* recombinant prions prepared by PMCA, exhibiting infectious properties with attack rates of 100%.

Limited proteolysis of a variety of mouse and bank vole recPrPSc species, prepared under slightly different conditions, yields the same N-terminally truncated PK-resistant fragments seen in brain-derived PrPSc, indicative of an overall similar architecture of both prion types. However, lower resistance to PK and a comparatively higher abundance of smaller fragments with respect to the “canonic” ~90–230 PK-resistant core, suggests higher flexibility and nuances in threading for recPrPSc. Furthermore, doubly N- and C-terminally truncated fragments, in particular ~90–152, are often detected; similar fragments are characteristic of atypical strains of brain-derived PrPSc.

Ongoing comparison of specific digestion patterns (relative abundances of individual fragments) and incubation times will allow extracting conclusions on relationships between structure and biological properties of different recPrPSc species. Recombinant PrPSc offers exciting opportunities for structural studies not possible to date with brain-derived PrPSc.
O.15: In a mammalian model of epithelial-to-mesenchymal transition the prion protein mediates β-catenin-dependent transcriptional activation of a key EMT regulator

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The physiological function of the prion protein (PrP) has remained elusive despite its widely recognized role in neurodegenerative diseases and sustained efforts to understand its molecular biology. On the basis of its evolutionary relationship to ZIP zinc transporters and the characteristics of a gastrulation arrest phenotype in a PrP-deficient zebrafish model, we considered that PrP may contribute to the morphogenetic reprogramming of cells underlying epithelial-to-mesenchymal (EMT) transitions. We now report that consistent with this hypothesis, PrP levels can be observed to increase more than 5-fold during EMT, and its CRISPR-Cas9-mediated knockout interferes with EMT in NMuMG cells, a widely used mouse model for studying this cellular program. Subsequent endeavors to dissect the molecular underpinnings of this phenotype revealed that PrP-deficient cells fail to execute an essential step during EMT. Surprisingly, this impairment was caused by a failure of PrP-deficient cells to activate transcription of a critical EMT mediator. A subsequent comparative global proteome analysis of wild-type and PrP-deficient NMuMG cells pointed toward β-catenin as a transcriptional regulator that contributes to this deficiency of PrP-knockout cells. Indeed, pharmacological blockade or siRNA-based knockdown of β-catenin mimicked PrP-deficiency. By placing PrP in a signaling pathway that is essential for EMT in NMuMG cells, our study introduces an easily accessible model for studying signaling downstream of PrP, and provides a fresh angle for understanding the role of PrP in health and disease.

O.16: Effect of cellular prion on neurogenesis after acute injury and chronic prion infection

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The cellular prion protein (PrP\textsuperscript{C}) has been associated with varied biological processes including cell signaling and neuroprotection, yet its physiological function(s) remain ambiguous. The goal of this study is to determine the role of PrP\textsuperscript{C} in adult neurogenesis using the murine olfactory system model. Olfactory sensory neurons (OSNs) within the olfactory sensory epithelium (OSE) undergo continual neurogenesis, integration, and turnover throughout adulthood, making it a useful model to study neuronal development. Here we determine the effect of PrP\textsuperscript{C} level on neurodevelopment in two injury models: acute injury and prion-induced neurodegeneration.

Acute nasotoxic injury was induced by methimazole injection and results in synchronized OSN regeneration. To investigate the role of PrP\textsuperscript{C} in OSN proliferation, dividing cells in the OSE were quantified using BrdU incorporation. Gene expression indicative of OSN differentiation was assessed by quantitative real-time PCR. Analysis revealed subtle effects of PrP\textsuperscript{C} on OSN differentiation and these altered gene expression patterns offer potential pathways to investigate PrP\textsuperscript{C} function in OSE neurogenesis. During prion infection there was an increase in nascent/immature OSN differentiation markers and a reduction in mature OSN gene expression. Additionally, the number of neural progenitor cells was initially increased, but over time fewer of those cells remained in the OSE. These findings suggest a loss of mature OSNs during prion infection.
either by premature death or a deficiency in OSN maturation, and as a result, there is an increase in proliferation of neural progenitor cells to replenish the loss of mature OSNs.

O.17: Defining routes to neurodegeneration and the impact of the immune system

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In diseases such as Alzheimer’s disease or prion diseases, aberrant folding of host encoded proteins acts as seeds for ‘prion-like” propagation of normally folded protein to abnormal conformations. Misfolded proteins are regarded as causal factors of disease although their precise role in neurodegeneration remains unanswered. Activation of glial cells is an early pathological sign of disease, thus alterations in the status of these cells in the brain may impact on the disease process. Indeed viral infection has a controversial role in chronic neurodegeneration and has been implicated in both precipitating and driving the disease process.

Here we use prion models of neurodegeneration to demonstrate that prion disease can be both transmissible and non-transmissible. We examined the role of misfolded protein in the process of disease and in contrast to generally accepted selective spread of misfolded proteins, ‘prion seeds’ were widespread, distributed independently of neurodegeneration. Despite this, neurodegeneration and inflammatory responses were restricted to specific brain regions thus demonstrating that a misfolded protein seed is insufficient to initiate a neurodegenerative cascade.

We examined the role of glial cells and the impact of viral infection on the disease process and observe that a single co-infection event with a neurotropic viral agent has the ability to alter multiple aspects of disease, regional targeting, neuronal survival, inflammatory profile and biochemical properties of the misfolded prion protein. Such co-infection events can therefore have long term consequences for disease progression.

These data address the underlying mechanisms of neurodegeneration and identify new therapeutic targets for neurodegenerative diseases.