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PrP Biology and Function

PO-194 Alpha-cleavage levels of ovine prion protein in brain are associated with PRNP genotypes

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The cellular prion protein (PrP^{c}) is a determining factor in TSE pathogenesis, acting as substrate to the formation of abnormal, protease resistant PrP^{Sc}. PrP^{c}, which is widely expressed throughout the brain is encoded by the PRNP gene. It has long been accepted that polymorphisms occurring in the open reading frame specifically at positions 136, 154, 171 confer resistance/susceptibility to scrapie infection in sheep and influence incubation times of the disease.

'Normal' cellular processing of PrP^{c} includes a well-documented α-cleavage event, occurring at 115 (Val) of PrP (ovine) interrupting the so called neuro-toxic region. The truncated forms of PrP produced via this mechanism, known as N1 and C1 may play a role in neuro-protection. In sheep, C1 often displays as the prevailing form of PrP^{c} representing between 10–80% of total PrP in the brain. We report a novel association between the ovine PrP polymorphism at codon 171 and levels of α cleavage in the brain, indicating that presence of C1 at higher levels may decrease susceptibility to disease. This hypothesized inhibitory effect was further investigated in fibrilization assays by mixing of recombinant full length PrP and C1 fragments representing resistant and susceptible PrP variants.

PO-195 Distribution of the Shadoo protein in sheep

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SPRN is a member of the PRNP gene family, coding for the Shadoo protein (Sho) which has several structural similarities with PrP. Sho is a neuronally expressed glycoprotein potentially involved in the pathogenesis of prion diseases. In order to unravel its presently unknown function, its distribution was assessed. In a previous study, a polyclonal rabbit antibody against ovine Sho was developed.[1] In the current study, this antibody was used for immunohistochemistry (IHC) to localize Sho in the tissues along the prion pathway, starting with the oral uptake and ending in the brain. Therefore, samples were taken of the tonsils, lymph nodes (retropharyngeal and mesenteric), spleen, intestines, ganglia (prevertebral, vertebral and spinal), spinal cord, obex, cerebrum and cerebellum of a seven months old Bleu Du Maine female sheep. An overall distribution of the Shadoo protein was seen in every tissue the IHC was performed on. Especially nerve cells and intestinal epithelium- and muscle layers seem to be highly positive for Sho.

To confirm the specificity of the antibody used for this IHC, western blot and RT-PCR were performed on the same tissues. Samples of liver, kidney and muscle were also included. According to the Western Blot experiment, the Shadoo protein is present in every tissue.

In addition, RT-PCR showed that the mRNA for Sho is expressed in every tissue that was included in this experiment. Consequently, the results of the western blot and RT-PCR were in agreement with the outcome of the IHC experiment. However, in mice, LacZ staining showed Sprn-LacZ expression only in the male and female gonads. RT-PCR on the endogenous Sprn gene also showed a tissue-specific expression in the gonads.[2] Future studies will aim at determining the exact localization of Sho inside the cell through electron microscopy. Therefore, an immunogold-labeling technique will be applied.

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PO-196 TSE induced alterations of the host glycoproteome

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Previously, we have demonstrated that in response to BSE infection a fraction of the Î²-subunits of clusterin become modified by the addition of N-linked glycans of complex structure. The differential abundance of these particular isoforms of clusterin in urine enabled us to discriminate between BSE infected and age-matched Fleckvieh-Simmental cattle. Similarly, in response to infection strain specific glycosylation of PrP has been described in detail. The relative intensity and electrophoretic mobility of the three isoforms are characteristic of different TSE strains and suspected to represent the molecular basis of the distinct disease characteristics of the different strains. These two observations caused us to speculate that the general glycosylation machinery might be affected by TSE infection. This hypothesis predicted that TSE infection would alter both the proteoglycan profile

and the expression or activity of the enzymes responsible for the generation of proteoglycans. In this study 2D-DIGE was used to perform an unbiased screen of the proteoglycans found in scrapie-infected mouse brain and age matched controls. In addition, focused PCR-arrays were run to on the same samples to screen for the differential expression of glycosylation associated enzymes. These analyses demonstrated that specific perturbations of both the glycoproteome and glycosylation machinery were associated with disease progression. Thus the glycoproteome and associated enzymes expand the inventory of molecules to be mined for a TSE biomarker that is both sensitive and disease specific.

PO-197: Penetrant null alleles of the *Sprn* gene do not produce embryonic lethality in combination with PrP^{c}-deficiency

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The *Sprn* gene encodes Shadoo (Sho), a central nervous system (CNS) glycoprotein with biochemical properties similar to the unstructured region of PrP^{C}, and a candidate for the hypothetical protein that maintains PrP^{C} functions in *Prnp*$^{0/0}$ mice. To understand these relationships better we probed the cell biology of Sho and created knockout mice. Besides full-length and C1 C-terminal fragment, we report here that Sho also produces a 6kDa N-terminal neuropeptide, "N1," which is present in membrane-enriched subcellular fractions of wt and Tg.*Sprn* mice. In genetic studies *Sprn* null alleles were produced that delete all protein coding sequences yet spare the *Mtg1* gene transcription unit that overlaps the *Sprn* 3′ untranslated region (3′ UTR). The resulting mice bred to homozygosity were both viable and fertile, and had no overt perturbations in the expression of genes located immediately 5′ and 3′. However, *Sprn*$^{0/0}$ mice maintained in two distinct genetic backgrounds weighed less than wt mice. Contrasting with lethality reported for *Sprn* knockdown in *Prnp*$^{0/0}$ embryos using lentiviruses targeted against the *Sprn* 3′ UTR,[1] we established that double knockout *Sprn*$^{0/0}$ + *Prnp*$^{0/0}$ mice deficient for CNS expression of Sho and PrP^{C} are fertile, and viable up to 500 d of age. While divergent results likely reflect the alternative strategies of targeting the *Sprn* 3′ UTR rather than ablating the coding region, our data with penetrant *Sprn* null alleles rigorously exclude a hypothesis wherein expression of both PrP^{C} and Sho is required for completion of embryogenesis. Rather, in accord with some reports for PrP^{C}, Sho may contribute to trophic pathways that are active postnatally.

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PO-198: Responses of cultured cells to prion in the prolonged incubation and the transmission of prion from them to neural cells

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Abstract. An abnormal form of the normal cellular prion (PrP^{C}) called PrP^{Sc} is believed to be the infectious agent of prion diseases. However, the processes of uptake, intercellular spread and transmission of PrP^{Sc} to neural cells are not clearly defined yet. Much concern has been directed towards follicular dendritic cells and dendritic cells as main players in the transfer of PrP^{Sc} to the neural cells. However, the role of other cells such as macrophages, intestinal, mast cells or peripheral neural cells remains unclear. In order to get more understanding about these processes and about possible roles of these cell types in the accumulation and/or transfer of PrP^{Sc}, the cellular responses to PrP^{Sc} exposure were investigated in cell culture models during short (5 days) and prolonged (28 days) incubation periods in-vitro. Fifteen different cell lines of immune (RAW, J447, P388-D1, P388-D1(IL-1), J588L, P1.HTR and EL4), neural (N2a-3, NB41A3, GT1-7, RT4-D6P2T and TR6Bc1) intestinal (IEC-18) and other (NIH-3T3 and HeLa) cell types were used for that aim. The cellular characters that may be related to these cellular responses to PrP^{Sc} were also investigated. In addition, co-culturing the long incubated cells with neuroblastroma cell line to study the possibility of PrP^{Sc} transmission from these cells to neural cells was also investigated. After transient degradation phases, some immune, intestinal and neural cells accumulated PrP^{Sc} of Chandler or Obihiro strains for up to 28 days post-exposure. When the PrP^{Sc}-loaded cells were co-cultured with the N2a-3/EGFP neuroblastoma cells for 4 days followed by split, the transfer of infection from several cell lines were successful. Cell lines showed also accumulation of PrP^{Sc} along the 28 days of incubation when investigated with Obihiro PrP^{Sc} and successful transfer of PrP^{Sc} to Neural cells. Our results suggest that in-vivo, some cells may act as a reservoir for keeping the PrP^{Sc} particles till get the opportunity to transfer to other cells including neural cell. Moreover, the accumulation of PrP^{Sc} in the recipient cells may be an in vitro evidence on possible involvement of variable cell types including immune and intestinal cells in sustaining, accumulating and transferring PrP^{Sc} toward the CNS in vivo.

PO-199: Prion protein expression dictates kinetics of behavioral defects in prion infected mice

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It is hypothesized that prion diseases are caused by the recruitment of the cellular prion protein, PrP^{C}, into an infectious protease resistant isomer, PrP^{Res}. PrP^{C} knockout mice ($PrP^{-/-}$) are known to be resistant to disease compared with wildtype mice; however, the function of PrP^{C} and its relationship to behavior and cognition remain relatively unknown. In a pilot study comparing the behavior and cognition skills of $PrP^{-/-}$ mice to wildtype mice, we found that $PrP^{-/-}$ mice have a significant cognitive deficiency in odor-guided tasks ($p < 0.05$), but not spatial guided tasks as compared with the wild-type. We are now conducting a longitudinal study using behavioral and cognitive tests with $PrP^{-/-}$, wildtype, and PrP^{C} overexpressing mice to determine the effects of mouse adapted prion strains on behavior and cognition. Behavioral tests include burrowing and nesting, and cognitive tests include novel scent and cookie finding. We hypothesize that mice that overexpress PrP^{C} should show more behavioral and cognitive deficits as the mouse adapted prion strains deplete or sequester PrP^{C} as compared with wildtype mice. Thus, PrP^{C} overexpressing mice inoculated with a mouse adapted prion strain should resemble the $PrP^{-/-}$ phenotype in behavior and cognition. It appears that control PrP^{C} overexpressing mice resemble a wildtype phenotype more than the $PrP^{-/-}$ phenotype in cognitive tasks; whereas, the phenotype of the inoculated PrP^{C} overexpressing mice does not resemble the cognitive phenotype of either the wildtype or $PrP^{-/-}$ mice three months post inoculation. So far we have only seen behavioral differences in the PrP^{C} overexpressing mice at the onset of clinical signs; whereas, the inoculated wildtype mice show behavioral differences before the onset of clinical signs of prion disease. Therefore, the overexpression of PrP^{C} seems to impair the ability of PrP^{Res} to affect behavior functions in mice inoculated with a mouse adapted prion strain, but does not rescue the animal from clinical disease. This may be due to more PrP^{C} remaining available to aid normal behavioral functions in infected overexpressing mice compared with PrP^{C} depletion affecting behavior during prion disease in wildtype mice. This pilot study of the behavioral and cognitive differences between control and inoculated $PrP^{-/-}$, wildtype, and PrP^{C} overexpressing mice could potentially lead to a pre-clinical behavioral test of prion diseases in mice.

PO-200: Nanobody-stabilize the crystal structure of full-length human PrP

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Prions are fatal neurodegenerative transmissible agents causing several diseases such as Creutzfeldt-Jakob disease in human, spongiform encephalopathy in bovine, also known as mad cow disease, and scrapie in sheep. Camelidae antibodies or Nanobodies are a class of immunoglobulin that lacks the light chains and are composed of a single domain which possess all characteristics of conventional antibodies. Using a selective Nanobody (Nb_PrP_01), we obtained a high-resolution crystal structure of hPrP23–231/Nb_PrP_01 (1.5…). The structure reveals that the full-length structure contains a new structural feature. Our results illustrate the potential application of nanobodies in improving the crystallization of highly flexible proteins.

PO-201: Neuritogenesis—The prion protein controls beta1 integrin signaling activity

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Background. The sprouting of neurites represents a very early stage in the implementation of the neuronal phenotype and relies on localized actin network reorganization and focal adhesion turnover. While increasing data indicate that PrP^{C} takes part to neurite outgrowth, the underlying mechanisms remain elusive.

Objectives. Our goal was (1) to assess the impact of PrP^{C} silencing in neuronal progenitors on actin and focal adhesion structure and neuritogenesis and (2) to define how PrP^{C} contributes to the onset of neuronal polarity.

Methods. We take advantage of two cellular models: the neuroectodermal 1C11 cell line able to differentiate into serotonergic or noradrenergic neuronal cells and the PC12 cell line. 1C11 precursors, and their neuronal progenies, as well as PC12 cells endogenously express PrP^{C}. Using a siRNA-based strategy, we established clones of 1C11 and PC12 cells constitutively silenced for PrP^{C} (PrP^{null} cells).

Results. PrP^{C} depletion in 1C11 precursor and PC12 cells impairs neuritogenesis. This relates to actin disorganization, increased actin microfilaments stability and altered focal adhesion turnover. PrP^{C} silencing promotes clustering and activation of beta1 integrins. This triggers the overactivation of the RhoA-Rock-LimK-Cofilin signaling pathway, which hampers localized actin reorganization and the initial sprouting of neurites along

neuronal differentiation. All these events are self-sustained by an over secretion of fibronectin in the surrounding milieu of PrP^{null}-1C11 cells. Finally, inhibition of Rho Kinases unlocks neurites sprouting in PrP^{C} depleted 1C11 cells, thus highlighting the dominant negative role of Rho Kinases on neuritogenesis.

Discussion. We substantiate that PrP^{C} contributes to the sprouting of neurites along neuronal differentiation of 1C11 precursor cells. By modulating beta1 integrin interactions with fibronectin, PrP^{C} controls actin architecture and focal adhesion dynamics necessary to neuritogenesis.

Figure 1: http://www.eventure-online.com/parthen-uploads/6/12PRI/img1_185025.jpg

Caption 1: Impact of PrPC depletion on neuritogenesis

PO-202: KV4.2 channel complex modulation by cellular prion protein (PrP^{c})

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Although misfolded forms of cellular prion protein (PrP^{C}) are key elements in the pathogenesis of transmissible spongiform encephalopathy, emerging data suggests that this protein may have multiple physiological functions including control of neuronal excitability. Activation of Kv4 channel complex mediates the neuronal A-type currents that play an important role in somatodendritic signal integration, including neuronal excitability. The Kv4 complex is composed of four α-subunits and at least two classes of auxiliary β-subunits, KChIPs and DPLPs. PrP^{C} is reported to be physically associated with Dipeptidyl-Peptidase-Like-Protein 6 (DPP6, also known as DPPX) but its impact upon the action of the channel complex is not known. We performed whole-cell recordings and analysis of functional properties of A-type currents in HEK293T cells transiently expressing recombinant Kv4.2 complexes (Kv4.2/KChIP2/DPPX-S) and PrP^{C}. In these cells, voltage-dependence of activation and steady-state prepulse inactivation of A-type currents was significantly shifted to a more positive membrane potential, and the time course of activation as well as inactivation was also significantly slower compared with cells expressing Kv4.2 channel complexes in the absence of PrP^{C}. However, PrP^{C} failed to demonstrate any effects on the functional properties of A-type currents in the absence of DPPX. We conclude that PrP^{C} modulation of intrinsic properties of Kv4.2 channel complex requires the presence of DPPX and our data suggests that PrP^{C}-DPPX complexes may regulate excitability of neurons.

PO-203: An N-terminal cleavage fragment of the prion protein binds to specific assemblies of the amyloid-β peptide and blocks their neurotoxic effects

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Alzheimer disease (AD) is associated with progressive dementia and accumulation in the brain of the amyloid-β (Aβ) peptide, a cleavage product of the amyloid precursor protein (APP).[1] Compelling evidence suggest that soluble, oligomeric assemblies of Aβ are primarily responsible for the synaptic dysfunction underlying the cognitive decline in AD.[2] So far, the identity of the cellular receptors to which these oligomers bind to exert their neurotoxic effects has remained enigmatic. Recently, the cellular prion protein (PrP^{C}) has emerged as a novel and unexpected candidate receptor for Aβ oligomers.[3] Several reports also suggest that PrP^{C} could directly mediate the synaptotoxic effects of Aβ oligomers, although this evidence is still controversial.[4–6] Interestingly, the two putative binding sites for Aβ oligomers identified in PrP^{C} (residues 23–27 and 95–110) are both encompassed within the flexible, N-terminal tail of the molecule (residues 23–111).[7] This region is proteolytically released as part of the normal, cellular processing of PrP^{C}, to produce a soluble fragment called N1.[8] An artificial, secreted form of PrP^{C} was previously reported to suppress cognitive impairment in a mouse model of AD.[9] Therefore, regardless of whether PrP^{C} mediates the neurotoxicity of Aβ oligomers, soluble forms of PrP^{C} such as the N1 fragment could sequester oligomers in the extracellular space and show therapeutic benefit in AD.

Here, by applying several in vitro and in vivo assays, we show that N1 binds to specific assemblies of Aβ oligomers with nanomolar affinity. We also report that N1 fully abrogates the synaptotoxic effects of Aβ oligomers in cultured hippocampal neurons and in a novel toxicity assay using the nematode *C. elegans*.

Collectively, these data provide strong experimental evidence supporting the idea that N1, or small peptides derived from it, could be potent inhibitors of Aβ oligomer toxicity and represent an entirely new class of therapeutic agents for AD.

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PO-204: The effect of a C-terminal membrane anchor on prion protein interactions with membranes

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Transmissible spongiform encephalopathies (TSEs) are infectious neurodegenerative disorders that occur in a broad range of animals (e.g., scrapie in sheep, BSE in cattle and CJD in humans). The hallmark of these disorders is the conversion of cellular prion protein, PrP^{C}, to a protease-resistant form PrP^{Res}.[1–3] PrP^{C} is typically attached to cellular membranes via a glycosylphosphatidylinositol (GPI) anchor. Although the physiological role of PrP^{C} located on the outer leaflet of cellular membranes is not yet fully understood, membrane attachment seems to play a key role during the conversion of PrP^{C} into PrP^{Res}.[2, 4] However, studies with membrane-associated PrP variants have been severely limited by the challenging access to sufficient amounts of homogeneous posttranslationally modified PrP. We have previously established a semisynthetic method based on expressed protein ligation (EPL) of recombinant prion protein and chemically synthesized membrane-anchor peptides to create lipidated PrP variants.[5, 6] Based on this strategy, two lipidated PrP constructs [full length PrP(23-231) and N-terminally truncated PrP(90-231)] were successfully prepared and refolded into predominantly alphα-helical structures, typically found for PrP^{C}. These lipidated PrP constructs were analyzed with respect to interaction with liposomes consisting of different phospholipids via floatation assays and fluorescence.

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PO-205: Prion and albumin complex as a biomarker in blood

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Misfolding of prion protein (PrP to PrP^{Sc}) can cause neurodegenerative prion diseases. As a glycosylphosphatidylinositol (GPI)-anchored membrane protein, the normal form of PrP (PrP^{c}) could function as a receptor for amyloid-β in the extracellular space. PrP^{c} was suggested to be involved in memory, synaptic neuronal communication, and anti-oxidation as a neuroprotective agent. Still, more investigations should clarify the normal physiological functions of PrP^{c} and its clearance. On the other hand, the biggest medical concerns are the contamination of PrP^{Sc} in human-derived materials, such as gonadotrophins, growth hormone, and dura mater. These materials are considered to cause iatrogenic Creutzfeldt-Jakob disease. From our previous study, we suggested PrP^{c} could bind to human serum albumin (HSA). We identified binding epitopes in PrP^{c} using epitope mapping enzyme-linked immunosorbent assay (EpiMap ELISA). In the plasma, a simple and powerful technique, called differential ELISA (dELISA), could detect PrP^{c}/albumin complex from mouse and human plasmas. The basic concept of dELISA relied on sandwich ELISA techniques using capturing PrP^{c} antibody and capturing HSA antibody conjugated with horseradish peroxidase. We collected mouse plasmas using various anticoagulants to quantitate the PrP^{c}/albumin complex. Various factors seemed to affect the PrP^{c}/albumin complexes, like retinoic acid in vivo and detergents in vitro. Diseased human plasmas were collected and assayed in comparison to normal plasmas for assessing the potential of PrP^{c}/albumin complex, as a possible biomarker. Higher PrP^{c}/albumin complex concentrations in plasma were observed from diseased plasmas in comparison to normal plasmas. More detailed results will be presented in the poster. Physiological functions of the PrP^{c}
/albumin interaction are not well understood, but the human-derived albumin supplements should be reconsidered for screening.

PO-206: Developmental prion protein expression in eutherian and methaterian mammalian central nervous system

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In the past 20 years, the expression of PrP^{C} in the placental mammals central nervous system (CNS) has been intensely studied. A role for the PrP gene in mouse embryogenesis has been proposed. In addition, the expression of PrP^{C} in adult chickens has also been investigated. However, the expression of PrP^{C} in the CNS of

marsupial mammals has not been previously explored. Here, we show for the first time the expression pattern profile of PrP^C during post-natal development of a methaterian marsupial: the South-American short-tailed opossum (Monodelphis domestica). Like in other mammals the expression of the protein is developmentally regulated. In the newly born opossum PrP^C signal is detected from P15 onward in the hippocampus, the thalamus and the cortex. Differently from the mouse the PrP^C signal is not present in the white matter fiber bundles of the septo and of the thalamolimbic neurocircuitry.

Prion diseases have been so far reported in eutherian mammals but not in methaterian. The different PrP^C localization pattern observed in the opossum might argue for different function of the protein in addition to diverse susceptibility to these maladies.

PO-207: Shadoo is found in nuclear speckles, has high affinity for quadruplex DNA and induces DNA conformational change

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Several studies have established a functional interaction between prion protein (Prp) and its homolog shadoo: Shadoo has a neuroprotective effect in murine prion disease; and in sheep brain the Prp and shadoo expression levels are correlated. Prp and shadoo interact[1] via their hydrophobic tracts.[2]

Here we identify DNA-binding activity in the N-terminal domain of shadoo. N-terminal shadoo-DNA interactions were measured by surface plasmon resonance (SPR) and circular dichroism (CD) responses of DNA probes to the peptide "shadoo26-48": GGRGGARGSARGGVRGGARGASR which contains the tetra-repeat sequences. The DNA probes were designed to yield various secondary structures:

Structure Probe Sequence

B-DNA
AGCTGACTAGCTAAGCTGACTAGCTAAGCTGACTAGCTA
TCGACTGATCGATTCGACTGATCGATTCGACTGATCGAT

B-ZDNA Junction

GCGCGCGCGCGTAGCTGACTAGCTAAGCTGACTAGCTA
CGCGCGCGCGCATCGACTGATCGATTCGACTGATCGAT
Z-DNA
GCGCGCGCGCGCGCGCGCGCTATATA
CGCGCGCGCGCGCGCGCGCGATATAT

Quadruplex (Parallel)

CATGTACAGGGGGGTACGTCAC
CATGTACAGGGGGGTACGTCAC
CATGTACAGGGGGGTACGTCAC
CATGTACAGGGGGGTACGTCAC

In SPR studies shadoo26–48 displayed highest affinity for quadruplex DNA. Shadoo26–48 also bound duplex DNA but at lower affinity and as a function of secondary structure. CD spectral changes revealed that shadoo26–48 induced conformational changes in the Z-DNA containing probes, which were consistent with B-Z transition. We also present immunofluorescent microscopy data showing shadoo and Z-DNA epitopes in the nuclear speckles of both rabbit and human synovial fibroblasts.

We conclude that the N-terminal domain of shadoo has DNA-binding activity which is selective for particular secondary structures, specifically quadruplex DNA. Shadoo is located in nuclear speckles in rabbit and human synovial fibroblasts, which indicates a functional aspect to its DNA-binding capacity and speckle localization. We hypothesize that one aspect of shadoo function is the formation and stabilization of Z-DNA, which is induced on binding of shadoo26–48 to Z-forming dsDNA.

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PO-208: Deficits of Prnp0/0 mice in associative learning increases during aging

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Introduction. Previous studies had already shown that the cellular prion protein (PrP^c) is a multifunctional protein that plays a role in cellular adhesion, neurite outgrowth, excitability and maintenance, differentiation and synaptic plasticity. Mouse models, in which PrP^c gene (Prnp) was disrupted, have been used in several behavioral studies to investigated the role of PrP^c. Although, first observations on PrP^c knockout mice (Prnp 0/0) did not reveal any important behavioral abnormalities,[1] later studies had pointed out differences between Prnp 0/0 and wildtype animals (WT). Prnp 0/0 mice exhibit an altered circadian rhythm and alterations in sleep.[2] Another important role of PrPC lies within the processing of sensory information by the olfactory system[3] and for the thermal nociception.[4] However, the precise physiological function of PrPC is still poorly understood.

Material and Methods. For each experiment at least 10 adult male Prnp 0/0 mice and wild type (WT) mice aged between 3 and 15 mo were used. The genetic background of both Prnp 0/0 and WT mice was derived from both 129/Sv and C57BL/6. Prnp 0/0 mice are homozygous for the disrupted PrPC gene (Zurich I), produced as previously described by Bueler et al.[2]. Both groups were subjected to the fear conditioning behavioral task. In this test mice learn to predict an aversive event such as an electrical foot shock. After that they will be able to remember the neutral context (chamber) and a neutral stimulus (tone) which they heard before.

Results. In the present behavioral study we investigated whether PrPC has an influence on the associative learning and if PrPC may play a protective role during aging. Interestingly, we found that Prnp0/0 mice were markedly more affected on age-related impairments in associative learning than WT animals.

Moderate differences could be even observed in 3-mo old Prnp0/0 mice and increase during aging.

Conclusion. Our findings providing evidence that Prnp0/0 mice are impaired in associative learning processes and Prnp0/0 mice were more susceptible to age-related alterations.

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PO-209: Cellular prion protein: Role in excitotoxicity and in metal ions homeostasis

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The physiological function of the cellular prion protein (PrP^C) is still elusive. In order to unveil physiological role of PrP, knockout (KO) murine models have been developed. KO mice showed impairments in synaptic functionality, as well as increased excitotoxicity susceptibility. To understand the molecular mechanisms underlying PrP^C neuroprotective role, we are characterizing different aspects of glutamatergic synapses in PrP wild-type (WT) and KO mice. In particular, we compared the expression of proteins involved in excitotoxic mechanism in PrP WT and KO hippocampi at different ages encompassing P1 to P365: NMDA receptors, neuronal nitric oxide synthase (nNOS), plasma membrane Ca^{2+} ATPase (PMCA), sarco(endo)plasmic reticulum Ca^{2+} ATPase (SERCA), Na^+/ Ca^{2+} exchanger (NCX). We found that PrP KO mice show altered expression levels of NMDA receptors, PMCA and SERCA. Once excitotoxicity is induced, the caspase cascade is activated, driving the neuron to apoptosis. Therefore, we compared also caspase-3 expression between PrP WT and KO hippocampi and we found that it is higher in PrP KO P365 mice. A key role in glutamatergic synapses functionality, as well as in the control of mechanisms leading to neurodegeneration, is played by metals. In fact, essential metals are crucial both in neurodevelopment, exerting pleiotropic, metal-specific and often cell-specific effects on morphogenesis, growth and differentiation, and in the pathogenesis of neurodegenerative diseases. Essential metals are fundamental players in the control of oxidative and nitrosative stresses which, if not properly balanced, are responsible for neurodegeneration. Cu and Zn are involved in the regulation of NMDA receptors activity, functioning as allosteric inhibitors and preventing their over-activation. In addition, PrP^C is a metal binding protein showing high affinity for Cu and lower affinity for Zn and Mn. To characterize metal ion metabolism, Ca, Cu, Fe, Zn and Mn concentrations have been measured in hippocampus, emisphere and liver of PrP WT and KO mice at the different ages. Moreover, the expression of metal-binding proteins involved in essential metal homeostasis, e.g., transferrin, transferrin receptor 1, ferritin H and L, ceruloplasmin, ATP7a, copper transporter 1 and 2, have been determined. These findings may help to understand the physiological role of PrP^C in the central nervous system and in glutamatergic synapses.

PO-210: Nuclear localization and transport of Shadoo and prion proteins

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Prion protein (PrP) that is known for its central role in transmissible spongiform encephalopathies has been reported to possess two nuclear localization signals (NLS) and localize in the nuclei of certain cells in various forms. We identified a nuclear localization signal in Shadoo protein (Sho)—newest member of the Prion protein family—that shares structural similarities and functional analogies with PrP. The NLS is located in the N-terminal, positively charged region of Shadoo—from aa. 25 to aa. 61—and seems to mediate the nuclear and nucleolar enhancement of Sho even in the presence of an ER-targeting signal sequence.

We aim to identify the narrowest region, the relevance of the $(RXXX)_8$ motif, the crucial amino acids and posttranslational modification sites required for the nuclear localization, and characterize the nature of the nuclear transport mediated by the NLS of either Sho or PrP.

We found that Sho's NLS was not able to direct those fusion constructs which cannot diffuse freely through the nuclear pore complex, to the nucleus. After energy depletion the nuclear enhancement of a Sho-GFP constructs (that are able to diffuse freely into the nucleus) was abolished, but the nucleolar signal remained unchanged suggesting a binding partner of Sho in the nucleolus.

PO-211: Prions as a biological probe to examine the role ofPrP^cin β-cell biology and diabetes in BSE infected macaques

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In the pancreas, cellular prion protein (PrP^C) is specifically and abundantly expressed in islets. Its functions in insulin producing β-cells are not known. We have shown recently that PrP^C expression in rat β-cells is glucose dependent and that PrP^C knockout mice have impaired glucoregulation. These observations suggest a close relationship between PrP^C and β-cell function as well as glucose metabolism.

Prions, the major compound of which is PrP^{Sc} (Sc, scrapie), are the transmissible pathogenic agent associated with neurodegenerative diseases. Studies in rodents have shown that infection with prions leads to disruption of endocrine homeostasis including impaired glucose tolerance, obesity, hypoglycemia, and hyperinsulinemia as well as histopathological changes in islets. However, the molecular basis of these metabolic dysfunctions is not known. Cynomolgus monkeys (*Macaca fascicularis*) are used as non-human primate (NHP) models for diabetes as well as for variant Creutzfeldt-Jakob disease (vCJD) research since this NHP species develops spontaneous type 2 diabetes during aging and simian vCJD years after oral bovine spongiform encephalopathy (BSE) uptake, respectively.

In the present longitudinal BSE-in-macaque study, we collected plasma samples during incubation time and post mortem tissues from orally BSE-infected female cynomolgus monkeys (n = 12; age = 5 y) and non-infected age-/sex-matched controls (n = 9). Fasting blood glucose levels were determined at regular intervals in freshly obtained whole blood samples. Proinsulin, insulin, C-peptide, and glucagon concentrations were retrospectively evaluated using commercially available ELISAs. In addition, islet morphology and expression of insulin, glucagon and PrP^C in islets were investigated using immunohistochemistry.

MOCK controls became obese 3 y after the start of the study and 4 out of 9 developed diabetes. Islet morphology of diabetic animals showed pronounced amyloid deposits similar to those in human type 2 diabetes (T2D). There was no significant increase in plasma insulin concentrations during aging. BSE-infection caused simian vCJD 4–6 y after infection. Intriguingly, BSE-infected animals developed obesity within 12 mo of the study and 3 out of 12 developed diabetes. The infection was also associated with elevated plasma insulin concentrations.

In summary, oral BSE infection modified the course of T2D in a NHP model years before the disease-inducing agent entered the brain. This indicates that prions could exert a direct effect on PrP^C in insulin-producing β cells. Furthermore, our observations of metabolic abnormalities in BSE infected NHP are in line with findings in prion infected rodents. Understanding the role of PrP^C in β-cell biology will provide novel information and new insights for glucoregulation in health and disease.

PO-212: ADAM8 is the primary protease for α-cleavage ofPrP^c in muscles

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The cellular prion protein (PrP^C) is subjected to the physiological α-cleavage at a region critical for both PrP toxicity and the conversion of PrP^C to its pathogenic prion form (PrP^{Sc}), generating the C1 and N1 fragments. ADAM10, ADAM17 and ADAM9 were originally implicated in the α-cleavage of PrP^C, but recent reports indicate otherwise and suggest that an unidentified protease is responsible for the α-cleavage of PrP^C. Here we show that (1) ADAM8 can directly cleave PrP^C to generate C1 in vitro, (2) PrP C1/full length ratio is greatly decreased in the skeletal muscles of ADAM8 knockout mice, and (3) the PrP C1/full length ratio is linearly correlated with ADAM8 protein level in myoblast cell line C2C12 and in skeletal muscle tissues of transgenic mice. These results indicate that ADAM8 is primarily responsible for the α-cleavage of PrP^C in muscle cells.

PO-213: Biochemical interactions between the cellular prion protein and DPP6

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Dipeptidyl aminopeptidase-like protein 6, DPP6, was identified by 13 tryptic peptides in a PrPC interactome study using time-controlled transcardiac perfusion cross-linking of mouse brain.[1] DPP6, also known as DPPX, is a type II transmembrane (TM) protein with a large β-propeller ectodomain; it is known to be a critical component of neuronal A-type K^+ currents.[2] Interestingly, studies by Alier et al. have demonstrated modulation of A-type currents in cholinergic neurons of the basal forebrain by application of PrP central region peptides, and by oligomeric assemblies of Aβ1–42 peptide. Strikingly, the latter effects were absent when reversed Aβ42–1 peptide was applied, and were abrogated by genetic deletion of PrP or by application of the anti-PrP antibody Sha31. Here we have undertaken further studies to understand the biochemical mechanisms underlying these effects, and the possible ramifications for pathogenesis of prion and Alzheimer

Disease. Following transfection of plasmid constructs into recipient cells (RK-13, N2a, HEK293T or a sub-line of HEK293T with reduced endogenous PrPC levels), formaldehyde cross-linking was used to capture high molecular weight complexes. These interaction studies were performed using a deletion series of PrP and DPP6 alleles, as well as familial prion disease associated point mutations in PrP. In each instance complexes were separated from monomeric PrPC "input" by SDS-PAGE, and then visualized by western blotting. For DPP6, peptide antisera against distinct regions of the molecule as well as HA-affinity tags were used for detection or co-immunoprecipation. These experiments have allowed a delineation of the interaction domains of the respective proteins, and illustrate that DPP6 is able to form complexes with two other members of the prion protein family, but not with other TM proteins such as APP. To assess electrophysiological outputs, a further series of experiments have employed bigenic expression vectors to allow the synthesis of additional protein components, Kv4.2 and KChIP2, required for functional reconstitution of A-type currents. These transfected cells were assessed in the presence and absence of wt and mutant PrP and subsequent to exposure to different types of human Aβ assemblies. Biochemical analyses of these studies will be presented here, with electrophysiological outputs to be presented by Ma et al.

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PO-214: Glutamate excitotoxicity in transgenic mice with a deletion of residues 105–125 of the prion protein

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Introduction. To evaluate the importance of the central region of the prion protein (PrP) with respect to neurotoxic and/or neuroprotective effects, our laboratory previously engineered transgenic mice expressing PrP with a deletion of amino acid residues 105–125, also referred to as PrPΔCR.[1] These animals develop severe ataxia within the first weeks of life, depending on co-expression of wild type PrP, and show a drastically reduced life span. The most prominent neuropathological finding in TgΔCR mice expressing one allele of wild type PrP is a severe loss of cerebellar granule neurons, starting from postnatal day 13, and progressing until death, which occurs at 25 d of age on average. Morphological studies suggest that an excitotoxic mechanism may be involved in these events.[2] We chose to further experimentally dissect the mode of granule cell death in TgΔCR mice in a cerebellar slice culture model, where the three-dimensional cytoarchitecture is maximally preserved.

Materials and Methods. Cerebellar slices of TgΔCR mice and controls were prepared between postnatal days 10 and 12 and kept in culture for at least 14 d. Cell death was evaluated by propidium iodide uptake in untreated slices and after addition of various substances including glutamate receptor agonists for 24 h on postnatal day 25. Some of the slices were processed for transmission electron microscopy. Poly(ADP-ribose) (PAR) was quantified in cerebellar homogenates obtained from mice at different ages using a commercial sandwich ELISA.

Results. Granule neurons in TgΔCR cerebellar slice cultures are more susceptible to glutamate-, NMDA-, and kainate-, but not AMPA-, induced toxicity as compared with controls. Electron micrographs of the granule layer in TgΔCR cerebellar slice cultures show darkening nuclei which are similar to those previously shown in paraffin sections of TgΔCR mouse cerebella, and are morphologically reminiscent of excitotoxic cell death. Cerebellar PAR-levels were significantly elevated in TgΔCR mice on postnatal day 15, but not on postnatal days 10, 21 or 24.

Conclusions. In accordance with previous morphological findings, these results are highly suggestive of an involvement of excitotoxic mechanisms in cerebellar granule cell death in TgΔCR mice. Cerebellar PAR-levels are significantly elevated at the time around the onset of granule cell death. TgΔCR neurons are significantly more sensitive to the cytotoxic effects of glutamate as compared with controls. This enhanced cytotoxicity seems to be mediated by NMDA and kainate, but not AMPA, responsive subtypes of gutamate receptors. These results are consistent with other studies[3] which show that ΔCR PrP induces spontaneous ion channel activity in a variety of cell types, including neurons. We hypothesize that, in the in vivo situation, normal glutamate-induced synaptic activity combines with ΔCR PrP-induced channel activity to precipitate an excitotoxic form of neuronal death.

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PO-215: Prion protein interacts with the RNA-induced silencing complex (RISC) and modulates micrornas

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Introduction. Small RNAs associate with the RNA-induced silencing complex (RISC) in order to regulate gene expression and control a large array of biological functions. RISC complexes localize in different RNA granules, including P-Bodies and stress granules, as well as with endo-lysosomal compartments called multivesicular bodies (MVBs). Argonaute proteins (AGO) and the GW182 protein are core components of RISC, and their interaction is mediated by GW/WG motifs. Interestingly, the N-terminal domain of PrP^C contains four GW/WG motifs. Here, we investigate the possible connection between PrP^C and RISC.

Objectives. (1) to determine if PrP^C colocalizes and interacts with the RISC complex, and if it modulates micro RNA (miRNA) and small interfering RNA (siRNA) activities; (2) To determine the role of PrP^C familial mutants associated with Creutzfeldt-Jakob Disease (PrPD178N, E200K), Gerstmann-Straussler-Scheinker syndrome (P102L, 145stop, 160stop) on miRNAs and siRNAs activities; and (3) To determine the effect of familial mutants on the assembly of stress granules and P-bodies.

Materials and Methods. The interaction between PrP^C and the RISC complex was determined by co-immunoprecipitation with PrP^C, AGO2 and GW182 antibodies. The co-localization of PrP^C and RISC were confirmed by immunofluorescence and confocal microscopy. MiRNA activity were determined using a renilla luciferase target mRNA with let-7a complementary binding sites in its 3"[2] UTR and siRNA activity were determined using a siRNA target the 3¢UTR of firefly luciferase mRNA. The assembly of stress granules induced after a heat shock or an oxidative stress and of P-bodies was detected by fluorescence in situ hybridization (FISH) and immunofluorescence, respectively.

Results. We show that PrP^C interacts with AGO2 and GW182, two core proteins of the RISC complex. PrP^C also colocalizes with RISC in the cytoplasm and in MVBs. Overexpression of PrP^C inhibits miRNA and siRNA activities while knockdown of PrP^C increases miRNA activity. PrP^C familial mutants associated with Creutzfeldt-Jakob disease (PrPD178N, E200K), and Gerstmann-Straussler-Scheinker syndrome (P102L, 145stop, 160stop) lost their capacity to inhibit the miRNA and siRNA activities compared with PrP^C. However, compared with empty vector, genetic mutants decreased the miRNA activity by alternative mechanisms which implicate a deregulation of endosomes-MVBs maturation and an inhibition of P-Bodies assemblies. In addition, these mutants interfere with the formation of stress granules and are more susceptible to apoptosis following cellular stress.

Conclusion. We found that PrP^C interacts with AGO2 and GW182 in MVBs. PrP^C is a negative regulator of miRNA and siRNA activities. Familial mutations lost this function of PrP^C and inhibit P-Bodies and stress granules formation and MVBs maturation, both implicated in RNA homeostasis and cell survival. Our results suggest that interference with RNA metabolism may be involved in neuronal degeneration during the pathogenesis of prion diseases.

PO-216: Examining C3 cleavage of the cellular prion protein

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The cellular prion protein PrP^C has two well-described endoproteolytic cleavage sites, producing the C-terminal fragments C1 and C2 and the corresponding N-terminal counterparts, which have unresolved biological significance. However, when we transiently transfected murine PrP^C containing a 10 residue c-myc epitope tag into N2a cells, another immunoreactive PrP^C fragment at approximately 7kDa was visible on SDS-PAGE and western blot analysis of whole cell lysates, indicating there may be a separate proteolytic processing site in the PrP^C C-terminus. This PrP-myc construct has been previously characterized as producing normal PrP^C, with unaltered behavior or function in vivo or in vitro, including glycosylation, trafficking, localization and incorporation into PrP^{Sc} aggregates.[1]

Extensive literature searching found that the most commonly utilized anti-PrP antibodies are directed to epitopes in the middle region of PrP^C, and furthermore published western blot images are often trimmed, therefore the presence of any small far-C-terminally truncated fragments may have been overlooked. Hence this cleavage event, producing 'C3', is almost completely uncharacterized. The only exception is the suggestion of the involvement of a cysteine protease in a far-C-terminal cleavage event, and C3 localization at the cell surface as described by Taguchi et al. (2009).[2] Bioinformatics database searching (MEROPS) identified a potential caspase cleavage site at codon 177 of murine PrP, and it is interesting to note that the surrounding residues (V175, D177, C178, V179) are highly conserved in mammals, with 100% identity in various species including humans, monkeys, dolphins and whales, deer and antelope, mice, cattle, sheep, cats, pigs and horses.

We found that C3 was also present in HEK, SHSY5Y and RK13 cells after overexpression of PrP-myc, indicating that cells of neuronal and non-neuronal origin, and cells from various species, all contain the necessary protease/s required for this cleavage event. Furthermore, SHSY5Y cells expressing a mutant PrP^C which is membrane-tethered at the N-terminus as well as GPI-anchored at the C-terminus,[3] contained a fragment with a molecular weight consistent with N3. Treating cells with PNGaseF and PIPLC indicated the C3 fragment is GPI-anchored, but not glycosylated, perhaps signifying that this fragment is cleaved only from an unglycosylated substrate. The incidence and relevance of this cleavage event to normal PrP^C cellular biology and to prion infection is still under investigation.

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PO-217: PrP and Shadoo are required for trophoblastic development

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We have recently suggested the requirement of either the prion or Shadoo protein for early mouse embryogenesis.[1] Indeed, knockdown of Sprn by RNA interference in FVB/N PrnpKO embryos led to early embryonic lethality whereas no such phenotype was observed in FVB/N PrnpWT embryos.

Using an identical approach on a recently developed Prnp transgenic mouse line that express at physiological levels the ovine PrPARR allele under the transcriptional regulation of the mouse Prnp promoter (phgPrP vector), we could demonstrate the strict relationship between the lethal phenotype and the absence of both PrP and Shadoo. This result goes against the hypothesis of an Sprn- and Prnp-unrelated susceptibility of the FVB/N PrnpKO to such manipulation.

Histological analysis reveals developmental defect of the ectoplacental cone and important hemorrhage surrounding the Prnp-knockout-Sprn-knockdown E7.5 embryos. This observation was suggestive of an extra-embryonic origin of the lethal phenotype and was compatible with our recent observation of a specific expression of Sprn in such extra-embryonic tissues.[2] To analyze further this hypothesis, we performed a trophoblastic cell lineage-restricted RNA interference, as previously described.[3] The results allowed us to conclude that the observed lethal phenotype could be attributed to the sole role of these proteins in this trophectoderm-derived compartment.

RNaseq analysis was then performed on early embryos of various Prnp and Sprn genotypes. It indicated that these two proteins share overlapping biological functions in major developmental processes, as expected, but also that their invalidation affects the expression of ectoplacental-specific genes and inflammatory pathways, sustaining the histological observations and the results of the targeted-lentiviral infections.

Overall, our data provide biological clues in favor of a crucial and complementary role of the prion protein family during trophoblastic development. It explains ours previously reported data on the embryonic lethality of FVB/N PrnpKO, SprnKD embryos. Whether expression of these genes is also required for the development of the embryo itself remains an open question.

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PO-218: Expression of hamsterPrP^{c} in *Pichia pastoris*

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Up to now recombinant prion protein (PrP^C) has been primarily expressed in bacterial or mammalian expression systems. PrP^C derived from bacteria lacks posttranslational modifications and is stored as inclusion bodies,[1, 2] which have to be refolded. To enable appropriate posttranslational modifications, a eukaryotic expression system can be used. In case of expression in mammalian cell lines, PrP^C was shown to be posttranslationally modified, however, the yield is low. To overcome these limitations the yeast expression system can be employed.[3,4] Yeast cells are already proven for large-scale expression of eukaryotic proteins due to their signal sequence processing and growth to high cell densities. Only a few groups reported the expression of different PrP^C constructs using the yeasts *P. pastoris* and *S. cerevisiae*.[5-7] However, these constructs differentiate from native PrP^C protein sequences.

In this study, we describe a *Pichia pastoris* X-33 based expression system in order to obtain fully posttranslational modified PrP^C. PrP^C derived from Syrian golden hamster sequence is expressed with native signal sequences enabling posttranslational modifications e.g., attachment of the GPI-anchor and glycosylations.

PrP^C expressed in *Pichia pastoris* was analyzed by Western Blotting and showed the typical three-band pattern corresponding to the unglycosylated, monoglycosylated and diglycosylated PrP^C. The glycosylations could be proved using enzymatic treatment with amidase PNGaseF resulting in only one remaining band with a molecular weight of the unglycosylated PrP^C. Immunofluorescence microscopy analysis of the cells using anti-PrP antibodies as well as membrane preparation by ultracentrifugation were used to prove, that the recombinant PrP^C is localized in the cell membrane indicating the presence of the GPI-anchor.

This expression system using *Pichia pastoris* cells provides a suitable alternative to obtain fully posttranslational modified

PrP^C for purification in high amounts for biochemical and structural studies.

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PO-219: PrP^cknockdown impairs stem cell homeostasis by cancelling notch signaling

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While the cellular prion protein PrP^C has been the focus of intense research for its involvement in Transmissible Spongiform Encephalopathies, much less attention has been devoted to its physiological function. This notably relates to the lack of obvious abnormalities in PrP^C knockout mice. This apparently normal phenotype however contrasts with the very high degree of conservation of the prion protein gene (Prnp) in mammalian species and the ubiquitous expression of PrP^C, further suggesting a major physiological function.

Several lines of evidence indicate that PrP^C is involved in the self-renewal of various embryonic and adult stem cells. However, the accurate contribution of PrP^C to stem cell homeostasis remains unclear.

To address this issue, we are exploiting the murine 1C11 cell line, which displays the hallmarks of a neuroepithelial progenitor and is endowed with the capacity to undergo neuronal differentiation upon induction.[1] 1C11 cells endogenously express PrP^C and have been instrumental in ascribing a signaling function to PrP^C.[2] We recently reported on the isolation of stably PrP^C-depleted clones from the 1C11 progenitor and that the depletion of PrP^C expression in these cells interferes with the onset of neuronal differentiation.[3]

Here, we document that the knockdown of PrP^C drastically impacts on Notch signaling, a critical pathway involved in stem cell maintenance. By cancelling the expression of the Notch ligand Jagged-1, PrP^C depletion is associated with a switch off of the Notch pathway.

In view of the overexpression of PrP^C in various cancers, identifying molecular targets and signaling pathways controlled by this protein may have physiopathological implications.

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PO-220: liv-1 ZIP ectodomain shedding in prion-infected mice resembles cellular response to transition metal starvation

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We recently documented the co-purification of members of the LIV-1 subfamily of ZIP zinc transporters (LZTs) with the cellular prion protein and, subsequently, established that the prion gene family descended from an ancestral LZT gene. Here we began to address whether the study of LZTs can shed light on the biology of prion proteins in health and disease. Starting from an observation of abnormal LZT immunoreactive bands in prion-infected mice, subsequent cell biological analyses uncovered a surprisingly coordinated biology of ZIP10 and prion proteins that involves alterations to N-glycosylation and endoproteolysis in response to manipulations to the extracellular divalent cation milieu. Starving cells of manganese or zinc, but not copper, causes shedding of disordered N-terminal domains of PrPC and ZIP10. For ZIP10 this posttranslational biology is influenced by an interaction between its prion protein-like ectodomain and a conserved metal coordination site within its C-terminal multi-spanning transmembrane domain. The transition metal starvation-induced cleavage of ZIP10 can be differentiated by an immature N-glycosylation signature from a constitutive cleavage targeting the same site. Data from this work provide a first glimpse into a hitherto neglected molecular biology that ties PrP to its LZT cousins and suggest that manganese or zinc starvation may contribute to the etiology of prion disease in mice.

PO-221: Structural characterization and biological implications of cellular prion in complex with stress inducible protein 1

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Conversion of the cellular prion protein (PrP^C) to a pathogenic misfolded conformer has traditionally been associated with neurodegeneration. Complex formation of PrP^C with various ligands modulates multiple cellular responses ascribing its endogenous function as a scaffold protein for cell surface signaling complexes. Notably, PrP^C complex formation with the secretable co-chaperone Hsp70/Hsp90 organizing protein/stress-inducible protein 1 (Hop/STI1) induces potent neurotrophic transmembrane signaling[1–2]. The binding interface of PrP^C-STI1 has been mapped to residues encompassing $STI1^{230–245}$ and $PrP^{115–128}$. $STI1^{230–245}$ falls within the TPR2A domain of STI1, which mediates Hsp90 binding during refolding of key eukaryotic regulatory proteins[3]. The study of neurotrophic signaling associated with STI1 has tremendous implications for our understanding of neurodegenerative diseases, warranting detailed structural study.

The current study aims to identify interfacing residues and induced conformational changes in PrP^C and STI1 upon binding by NMR and other biochemical techniques. Our studies indicated TPR2A as the primary domain mediating PrP^C-STI1 complex formation. Multiple biophysical techniques confirmed murine TPR2A to exist as a monomer in solution. Peptide array analysis revealed three distinct regions of PrP^C mediating TPR2A binding: 91–110, 169–188, as well as the previously reported STI1 binding site spanning residues 109–128. Our results provide new structural insights regarding the nature of PrP^C interaction with STI1.

PO-222: The consequence of Shadoo overexpression on the drug hypersensitivity caused by PrP Δcr

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Prion diseases or transmissible spongiform encephalopathies are spongiform degenerations of the brain with variable degree of amyloidal plaque formation, caused by the accumulation of an abnormal isoform of the cellular prion protein, referred as PrP^{Sc}. Prion protein constructs with deletions in their N-terminal flexible domains involving the Central Region (Δ105–125 or ΔCR) have a neurotoxic phenotype when expressed in PrP knockout transgenic mice. Although infectious matter did not form in these transgenic mice, the coexpression of wild-type PrP seems to eliminate the neurotoxic phenotype making these deletion constructs ideal to study how the physiological function of PrP^C might be subverted to produce neurotoxic effects. Expressing PrPΔ105–125, or other N-terminally truncated PrP constructs in mammalian cell lines was shown to cause hypersensitivity to certain antibiotics. This drug hypersensitive phenotype can be also rescued by co-expression of wild type PrP^C. The Shadow of Prion protein, or Shadoo is the newest member of the prion protein family. The physiological function of this protein is mostly unknown, but various models suggested that Shadoo may have overlapping functions with the prion protein. Like PrP^C it also ameliorated the excitotoxicity caused by glutamate, in cultured cells. Furthermore, it rescued cells from spontaneous apoptosis caused by a toxic deletion mutant of PrP in transiently transfected primary cell cultures as well as in various immortalized cell lines. Here we examined if the hypersensitivity to Zeocin caused by PrPΔ105–125 could also be rescued by the Shadoo protein.

PO-223: Identifying protective PrP residues with flies—Insights into the dog PrP-N158D substitution

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The central event in the pathogenesis of all forms of prion disease involves a conversion of the host-encoded cellular prion protein PrPc to its pathogenic conformer PrPsc. However, the molecular mechanisms that regulate this conformational conversion are mostly unknown. A clue to understanding the structure and conformational dynamics of PrP has come from the dog, a rare mammal resistant to prion diseases. A comparative study identified a charged amino acid (PrPD158) in dog PrP that is not conserved in other mammals susceptible to prion diseases (PrPN158). Unfortunately, little is known about how this residue affects PrP structure. We hypothesized that altering the charge of the loop connecting Helix 1 and the first β-sheet could affect the stability of the globular domain and, thus, the toxicity of PrP. To determine the stabilizing effect of Asp158, we compared transgenic flies expressing wild type (MoPrP) and mutant (MoPrP-N158D) mouse PrP. We first observed that the MoPrP-N158D protein is more stable than MoPrP since significantly lower levels of mRNA lead to comparable levels of protein, suggesting that MoPrP is actively degraded in flies. We have shown before that MoPrP accumulates disease-specific PrP isoforms by immunoprecipitation with the 15B3 conformational antibody. However, flies expressing MoPrP-N158D do not accumulate 15B3-specifc conformations, indicating its higher conformational stability. Finally, whereas expression of MoPrP in motor neurons induced aggressive locomotor dysfunction in climbing assays, flies expressing MoPrP-N158D were similar to control flies, supporting the lack of toxicity. These results demonstrate that Asp158 exerts a key stabilizing activity on PrP and prevents formation of disease-specific PrP isoforms. Thus, this PrP loop can be targeted for the development of anti-prion therapies.

PO-224: Interactive role of Arf1 and Alpha tubulin-1 protein during the intracellular transportation of PrP^c

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Cellular prion protein (PrP^C) is a highly conserved protein during the evolution of mammals. The accumulation of misfolded and aggregated forms of PrP^C (known as PrP^{Sc}) causes transmissible neurodegenerative diseases. However, the cellular function of this ubiquitous protein and its interaction with other cellular proteins is still not clear. In this study we aimed to reveal the interactive role of recently known proteins interacting with PrP^C.[1] Previously, we affinity purified Arf1 and Alpha tubulin-1protein by using STrEP-Tactin-chromatography, in-gel digestion, and identification by Q-TOF MS/MS analysis in prion protein-deficient murine hippocampus (HpL3–4)neuronal cells.[2] Here, we further investigated their interaction with PrP^C by using confocal laser scanning microscopy and reverse co-immunoprecipitation approach in different cell cultures. Reverse co-immunoprecipitation with anti-Arf1 and anti-Alpha tubulin-1 antibodies precipitated the PrP^C confirming their potential interaction. Confocal microscopy results in relation to Pearson's correlation coefficient showed a partial but significant colocalization of Arf1 and Alpha tubulin-1 to the PrP^C. Furthermore, Arf1 deactivation by brefeldin A treatment downregulated PrP^C expression and redistributed PrP^C into the cytosol. When cells were treated with nocodazole an increased PrP^C expression and redistributed into the cytosol was observed. The present study demonstrates that both Arf1 and Alpha tubulin-1 interact with PrP^C and disturbance in the Arf1 and Alpha tubulin-1 leads to the redistribution of PrP^C in the cells.

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PO-225: Gender andPrP^c dependent expression pattern of Tau, P-Tau and APP in the liver of PrP^c knockout mice

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Increasing evidence indicates considerable overlap between tauopathies; prionopathies, and other protein-misfolding diseases. In addition, often typical hallmarks of these disorders, synergistic effects of tau protein, amyloid β, and other pathologic proteins, suggest interactions of pathological proteins engaging common downstream pathways. Understanding the tissue-specific expression of Tau, P-Tau and APP is crucial considering that cells expressing PrP^C might bear a higher risk for neurodegenerative diseases. Recent reports also demonstrated the accumulation of PrP^{Sc} in the liver of sheep naturally infected with scrapie at both clinical and preclinical stages of the disease.[1] In the present study, we aimed to check the expression pattern of Tau, P-Tau and APP in the liver of 3, 9 and 20 mo old male and female PrP^C knockout ($PrP^{-/-}$) mice. The expression pattern was analyzed by quantitative western blot and Immunofluorescence in the liver tissue. Tau expression was significantly downregulated in female $PrP^{-/-}$ mice compared with males. Phosphorylation of Tau was not significantly regulated in the liver. In contrast, APP level was significantly PrP^C dependent and downregulated in both male and female $PrP^{-/-}$ mice. Immunofluorescence analysis verified the results. In conclusion, the present study demonstrates for the first time gender dependent expression of Tau protein, in contrast to APP which showed more PrP^C dependent expression. This study could provide the way to dissect the functional involvement of PrP^C and may contribute to the higher prevalence of neurodegenerative diseases in females.

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PO-226: Characterization of intracellular trafficking ofPrP^{Sc} for prion propagation in cells in the early stage of infection

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Introduction. While molecular mechanism of prion propagation has been analyzed with cells persistently infected with prion, events required for establishment of prion infection, especially on the early stage after prion inoculation, are poorly understood.

Material and Methods. PrP^{Sc} was purified from brains of mice infected with 22L prion strain. The purified PrP^{Sc} was inoculated into a Neuro2a subclone, N2a-3.In order to analyze the trafficking of inoculated PrP^{Sc}, purified PrP^{Sc} was labeled with amine reactive fluorescent dye, Alexa Fluor 555 succinimidyl ester.Newly generated PrP^{Sc} was analyzed by indirect immunofluorescence assay (IFA) using an anti-PrP antibody mAb 132. EGFP-tagged wild-type and dominant negative mutants of Rab GTPase; Rab4a, Rab5a, Rab7, Rab9, Rab11a or Rab22a were expressed in N2a-3 by transient transfection with Lipofectamine 2000.Alexa Fluor 488 labeled transferrin or LDL was used for a marker of endocytic recycling pathway or endo-lysosomal pathway.

Results and Discussion. Six hours after the uptake, the majority of the inoculated PrP^{Sc} was detected at late endosomes to which internalized LDL was transported.These results suggest that the most of inoculated PrP^{Sc} is directed to endo-lysosomal pathway of cell.We next analyzed the kinetics of localization of newly generated PrP^{Sc} by IFA. Two days after the inoculation, newly generated PrP^{Sc} as well as the inoculated PrP^{Sc} could be simultaneously detected in the same cell.In contrast to the localization of inoculated PrP^{Sc}, the newly generated PrP^{Sc} was localized at peri-nuclear regions of the cell, at least some of which were thought to be endocytic recycling compartments.Thereafter, newly generated PrP^{Sc} increased and was continuously detected at peri-nuclear regions similarly to persistently infected cells.To clarify if the endocytic pathway associated with generation of PrP^{Sc}, we analyzed de novo synthesis of PrP^{Sc} in cells which wild type or dominant negative mutant of some Rab GTPases was overexpressed.Overexpression of a dominant negative mutant of Rab7, as well as the overexpression of wild-type of Rab9, and a dominant negative mutant of Rab11a decreased the generation of PrP^{Sc}.These results suggest that the transfer of trafficking of inoculated or newly generated PrP^{Sc} from the endo-lysosomal pathway to the endocytic recycling pathway has an important role in propagation of prion on early stage of prion infection.

PO-227: Expression ofPrP^{c}in enteric glial cells of the domestic cat

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Introduction. Feline spongiform encephalopathy (FSE) is a progressive and fatal neurodegenerative disease affecting domestic and wild *Felidae*. It belongs to the group of transmissible spongiform encephalopathies (TSEs) which are known to occur in humans and in several animal species. FSE of domestic felines is thought to result from ingestion of bovine spongiform encephalopathy (BSE) contaminated food, whereas prion diseases of wild *Felidae* (for instance, cheetah, puma, ocelot, tiger, lion, etc.) is likely due to ingestion of BSE-infected cattle carcasses. TSEs are caused by pathological isoforms (PrP^{Sc}) of the host-encoded cellular prion protein (PrP^{C}). A crucial factor for the initiation of infection is PrP^{C} expression in the host cells. In acquired TSEs, the gastrointestinal tract (GIT) is the main prion entry site. Thus, in order to better understand the early pathogenesis of FSE prion infections, this study was aimed to charactetize the cell types which constitutively express PrP^{C} in the cat GIT.

Materials and Methods. Using indirect immunofluorescence, we performed an accurate analysis of the distribution of PrP^{C}-immunoreactivity (PrP^{C}-IR) in the neuro-muscular layer of the domestic cat GIT. Specifically, the colocalization of PrP^{C}-IR with glial cells and neurons were tested by applying appropriate antibodies targeting glial and pan-neuronal markers. Single and double labeling experiments were performed in cryosections from the cat duodenum to the ascending colon. PrP^{C} was identified using the mouse anti PrP^{C} monoclonal antibody (MAB1562, #NMM1769910, 1:500, Millipore); enteric neurons were identified with the two pan-neuronal markers rabbit anti-protein gene product 9.5 (PGP 9.5) (AB176, #NG1684683, 1:1000, Millipore) and mouse anti-human neuronal protein (Hu) (20064, #313003, 1:200, Invitrogen). Enteric glial cells were identified with a rabbit anti-fibrillary acidic protein (GFAP) (AB5804, #NG1758235, 1:300, Millipore) and a rabbit anti-S100 protein (Z0311, 1:400, Dakocytomation).

Results. PrP^{C}-IR was mainly observed in enteric glial cells co-expressing both GFAP and S100. PrP^{C}-IR enteric glial cells surroundedmyenteric and submucosal plexus neurons lacking PrP^{C} immunolabeling. Also, neural processes, running either singly or in small fascicles and giving off myenteric and submucosal neurons, were negative for PrP^{C}-IR. PrP^{C}-IR enteric glial cells were detected in ganglionated plexuses of any investigated gut segment of the domestic cat.

Conclusions. Our findings provide a solid morphological basis showing that enteric glial cells in the GIT of the domestic cat constitutively express PrP^{C}. This finding, which is in line with previous evidence in the mouse intestine, indicates that even in the cat enteric glial cells may be a potential target for infectious prions. Likely, enteric glial cells may be targeted by prions (PrP^{Sc}) not only via oral infection (as a first-step of neuroinvasion), but also as a result of a centrifugal spread of prions from central to enteric nervous system as demonstrated during experimental prion infection. Further data are awaited to shed light on prion infection and its pathways, from or to the gut, in FSE.

Figure 1: http://www.eventure-online.com/parthen-uploads/6/12PRI/img1_189745.jpg

Figure 2: http://www.eventure-online.com/parthen-uploads/6/12PRI/img2_189745.jpg