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Graphic by Laurent Bernard, cover: «Land ocean ice light» - Courtesy of NASA Visible Earth
Dear Colleagues, dear friends

Last year in Madrid where I announced our 6th Neuroprion meeting I had naively promised that it was going to be a superb meeting with excellent weather conditions. Luckily enough both my predictions were materialized and we were able to share the latest developments and ideas in the fascinating field of Prions in a terrific setting. I have to admit though that it was not the best period to organize a meeting, due to the world economical crisis and to health related issues while waiting for a new flu epidemic. I have to confess that many times I thought that the meeting was going to be postponed. However, this was a special occasion for me because last August I have completed 25 years in the fascinating field of Prion diseases and I considered the organization as a challenge.

Now, almost a month later and I continuously receive letters from participants expressing their appreciation. This is the best reward for all of our efforts to put this meeting together.

Although Prion diseases are not front page news anymore, they continue to be one of the most intriguing biological problems confronting us. We have learned a lot during the last two decades but there are still missing elements concerning the molecular nature of these pathogens, the pathogenesis process and the treatment of the related diseases. This challenge remains open for neuropathologists, neurologists, veterinarians and neuroscientists worldwide. In an era of rapid developments and innovations in neurodegeneration, there is an ever increasing need to regularly update our knowledge.

Prions once more have been proven that are extremely resistant to any circumstances and our meeting was held with over 500 participants from 30 countries from all over. The members of our International Scientific Program Committee have been asked to suggest the topics for the program; they have also proposed names for the plenary lecturers and subjects for the congress sessions.

Our program together with the excellent scientific presentations from the Prion field had also included few talks from other proteinopathies in an attempt to exchange information and establish a closer relation and with these fields.

Additionally, an exceptional panel for blood safety related issues was organized and finally in a round table with distinguished scientists was discussed the future of prion research worldwide.

We got approximately 300 abstract submissions and 40 of them have been proposed by the scientific committee for oral presentations at the top of our 22 invited prion and non prion protagonists. Few excellent papers with the most recent developments in the field of Prion research were also selected for hot topics presentations.

This year we had 9 awards for the poster submissions, one for each category including Protein misfolding, Diagnostics, Therapeutics and decontamination, Transmission and pathogenesis, Basic mechanisms of neurodegeneration, Function and cell biology, Genetics, Epidemiology and risk assessment and Natural and experimental strains. The posters were evaluated by members of the scientific committee and the award recipients gave a short presentation before the meeting closure.

At this point I would like once more to thank the members of the International Scientific Program Committee for their unlimited support everytime that I needed it. I would like to thank our sponsors, the personnel and the local meeting organizer Aethra travel, the members of Prion diseases at Aristotle University of Thessaloniki, the Center for Research and Technology Hellas and the Neuroprion Network of Excellence staff; finally, I would like to thank all of you for making possible an outstanding meeting.

I consider our annual Prion meetings as a success story and I strongly urge you to participate in the upcoming Prion 2010 meeting in Austria. I wish the organizers the best of luck for their effort.

I hope that all of you have only good memories from Porto Carras.

Theodoros Sklaviadis, Chairman of Prion2009
The physiological role of the prion protein (PrP\textsuperscript{C}) and its relation with the abnormal conformer (PrP\textsuperscript{Sc}) have been extensively studied in the last years, and several possible functions were attributed to PrP. However, some of the main questions in this field remain unanswered or poorly understood, such as the impact of the presence of PrP\textsuperscript{C} in individual cell types, the mechanisms that control the expression of PrP\textsuperscript{C}, and the mechanisms by which cells respond to prion infection and by which PrP\textsuperscript{Sc} is formed, propagated and accumulated within cells.

Rafael Linden and colleagues\cite{1} recently proposed that, instead of having a specific function, PrP\textsuperscript{C} would act in a variety of functional processes as a "dynamic cell surface platform for the assembly of signaling modules". Then, it would be now more than necessary to clearly define the interaction partners of PrP\textsuperscript{C} within the nervous system and others. Those questions and others have been well-covered in the "Functions & Cell Biology" topic at Prion2009 by 4 oral presentations and 21 posters.

The session began with a talk given by Cathryn Haigh describing the role of the N-terminal beta-cleavage product (N2) of PrP\textsuperscript{C} in a reactive oxygen species (ROS) response induced by serum deprivation. A synthetic N2 fragment encompassing murine amino acids 23-89 was shown to reduce ROS when pre-loaded with copper. Interestingly, an N-terminal peptide lacking the copper-binding octameric repeat domain (residues 23-50) also decreased the intracellular ROS. This effect was later shown to be dependent on cell-surface heparan-sulphate-containing proteoglycans associated with lipid-raft domains, and the redox-protective capacities of N2 and the peptide seems to depend on two proline residues within the N-terminal polybasic region of PrP\textsuperscript{C}.

In an attempt to characterize PrP\textsuperscript{C} molecular neighborhood, Gerold Schmitt-Ulms presented a quantitative investigation of the protein interactome. Using in-depth bioinformatic analyses, the author and his colleagues suggested direct interaction amongst PrP\textsuperscript{C} and its two paralogs, Doppel and Shadoo. Of note was the observation of a spatial proximity between PrP\textsuperscript{C} and two metal ion transporters of the ZIP family, ZIP6 and ZIP10. Further analyses led the authors to conclude that the prion protein gene family is phylogenetically derived from a ZIP-like ancestor molecule.

I have also spoken in this session, and presented data concerning a novel mechanism of neuroimmunoendocrine regulation of PrP\textsuperscript{C} expression. Acute systemic inflammation induced by lipopolysaccharide (LPS) led to massive up-regulation of PrP\textsuperscript{C} in mouse neutrophils, but not in other cell types. This effect was shown to be dependent on transforming growth factor (TGF)-\beta and glucocorticoids (GC), and it was also observed in restrained animals, a condition of behavioral stress that increases the serum levels of both TGF-\beta and GC. In vitro experiments provided evidence for an involvement of PrP\textsuperscript{C} in important neutrophil functions. These findings could have impact over both the physiological functions of PrP\textsuperscript{C} and the progression of prion-associated neurodegeneration.

The oral presentation session was well-concluded by Michel Dron, who demonstrated the effects of proteasome impairment on the biogenesis of PrP\textsuperscript{C} and the accumulation of PrP\textsuperscript{Sc} in murine derived neuronal cells. Treatment of infected and uninfected cells with proteasome inhibitors led to accumulation of a 26 kDa unglycosylated PrP species, and to an
intracellular redistribution of PrP\textsuperscript{C}, which became mainly localized within the Golgi complex. Of interest was the finding that prion infected cells treated with proteasome inhibitors presented intracellular accumulation of PrP\textsuperscript{Sc} within large aggresome-like structures. These findings point to a functional role of proteasomes not only for the control of correct expression of PrP\textsuperscript{C} but also for the reduction of PrP\textsuperscript{Sc} aggregation within cells.

The poster session covered several PrP cell biology concerns, including intracellular generation, metabolism and localization of PrP\textsuperscript{C} and PrP\textsuperscript{Sc}, role of PrP in oxidative stress and hypoxic condition, and involvement of PrP\textsuperscript{C} in cellular differentiation. Of special interest was the communication by the poster prize winner Yuzuru Taguchi, who presented a novel technique for imaging cell surface proteins in living cells. The most common subject was, however, the PrP\textsuperscript{C} interaction partners, which were some way (either by a novel technique, or by the identification of a putative PrP\textsuperscript{C} ligand) covered in almost half of the posters. It seems to me that the idea of PrP\textsuperscript{C} as a dynamic platform is leading scientists to look closer for the molecules that work together with PrP\textsuperscript{C} in an uncountable number of functional processes.

Reference:

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colleagues, sought to detect PrP\textsuperscript{res} in blood from sheep infected with scrapie or bovine spongiform encephalopathy (BSE). Peripheral blood mononuclear cells (PBMC’s) were isolated from infected sheep during the clinical phase of the disease as well as from 3 months of age to the clinical endpoint. Using direct immunoassay, PBMC’s were tested for PrP\textsuperscript{res} and were further differentiated by cell type using cell surface markers and magnetic bead separation. PrP\textsuperscript{res} was detected in 54% of scrapie infected sheep and 71% of BSE infected sheep during the clinical phase. The detection of PrP\textsuperscript{res} increased throughout the course of the disease and during the second half of the incubation period. Finally, PrP\textsuperscript{res} detection was associated with non-recirculating B cells found primarily in the spleen, raising the possibility that these cells may be associated with the clearance of circulating PrP\textsuperscript{res}.

In addition to the oral presentations, there were over 70 poster presentations on the topic of diagnostics, therapeutics, and decontamination. Two posters (P.2.4 and P.2.5) presented data on detecting prions without the use of proteinase K digestion, but by using single particle counting to detect PrP\textsuperscript{res} aggregates. This approach uses surface fluorescence intensity distribution analysis. Prion particles are immobilized using capture antibodies to a chip surface. Following fluorescent antibody labeling, the chip surface is scanned with two laser beams that allows for the visualization of prion particles. The posters presented data on single particle counting of prion particles from scrapie infected sheep blood and brain homogenates from individuals with sCJD. Many more posters were presented on a variety of subjects including using brain MRI to diagnose sCJD and the results of the United States quinacrine trial for the treatment of sCJD.

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Basic Mechanisms of Neurodegeneration & Pathology
Prion2009, Thessaloniki - Chalkidiki
By Yannick Bailly

Severe loss of neurons is a key feature underlying the clinical symptoms of prion diseases. Despite extensive research, the cellular pathways leading to neuronal loss in TSEs still remain unclear. Although this cardinal question is far from being resolved, some of the data presented at Prion2009 on this topic merit to be mentioned. Reviewing our current knowledge about the molecular background of phenotypic variability of TSEs, Herbert Budka reminded us that tissue damage may result from several parallel, interacting or subsequent pathways that involve cellular systems associated with synapses, protein processing, oxidative stress, autophagy, and apoptosis. The data presented by Magdalena Larska and co-workers for BSE and Ignazio Cali et al. for sCJD implicating variations of gene expression of pathogenic factors between prion diseases confirm that the pathogenesis or source of disease may vary between TSE types.

The repertoire of pathways that lead to neuronal death is, however, limited (Sikorska et al. 2007). In all cases, both neuronal metabolism and circuits are probably disrupted and this generates a pro-apoptotic signal for neurons. In TSEs, apoptosis has become the most popular theory of cell death although its role has not been convincingly documented until now. Autophagy has been reported in TSEs, but its role in prion disease pathology is not well established (Libersky et al., 2008). However, the extensive synaptic autophagy observed in prion diseases (Sikorska et al., 2004) has been proposed to contribute to overall synaptic degeneration, a major feature of brain pathology leading to neuronal death in TSEs. Nevertheless, synaptolysis as an initiating event of neurodegeneration may be due to a reduction of depolarization-induced calcium transients which leads to a progressive impairment.
of glutamate release as reported by Assunta Senatore in Roberto Chiesa’s group using synaptosomes and cerebellar granule cells from the Tg(PG14) mice with inherited prion disease. The data presented by Deborah Brown and Jean Manson’s group are not in agreement with this concept. Although cytoskeletal disruption in dendritic spines plays a major role in neuronal dysfunction, they did not observe changes in postsynaptic densities or the presynaptic compartment nor disruption of afferent innervation before the end stages of the disease indicating that the neuronal loss is not the result of deafferentation.

Autophagy and prion diseases

Autophagy has been investigated in several cases of prion-related diseases including TSEs as exemplified in Prion2009. In TSEs, Hermann Schätzl reports that the induction of autophagy can have beneficial effects on infection by reducing the cellular load of PrPsc and preliminary data indicate that the cellular level of autophagy can modify the susceptibility to prion infection. Yasmine Aguib and collaborators show that manipulation of autophagy acts on acute and persistent infections by enhancing cell clearance of PrPsc and by prolonging survival in these animals. The data obtained so far indicate a general role for autophagy in the prion disease scenario although the biological function of autophagy per se in prion infection is still not resolved. In these diseases, as in other diseases characterized by accumulation of protein aggregates, autophagy could be a therapeutic target because of its potential protective role. In prion protein-deficient Purkinje cells of the Ngsk transgenic mouse, Stéphane Heitz and co-workers report that early induction of autophagy (probably resulting from toxicity of abnormal Doppel overexpression) does not protect neurons which nevertheless die and display signs of autophagic blockade reminiscent of what happens in Alzheimer’s disease-affected neurons. Jin-Kyu Choi et al. provide more insight into the molecular pathologic mechanisms involved in the death of the Ngsk Purkinje cells suggesting that functional deficit and death of the neurons is caused by a decrease in IP3R1 gene expression which interferes with the normal function of PrPc in Ca2+ regulation and synaptic activity. Whether modifications of IP3R1 gene expression occur in other Prnp KO lines without neuronal Doppel expression remains unknown.

Prions and inflammation

Prion replication is known to sensitize neurons to pro-inflammatory cytokines but how PrPsc replication potentiates neuronal sensitivity to inflammatory cytokines is still unclear. Benoît Schneider and co-workers in the Odile Kellermann’s group have made recent advances using 1C11 precursors and progenic neuronal cells. These cells are more vulnerable to the pro-inflammatory cytokine TNFα when infected with scrapie strains. This increased sensitivity is related to an increased expression of the death receptor TNFR1 at the surface of infected cells which results from the upregulation of TNFR1 transcripts and inhibition of metallo-proteinase-dependent TNFR1 shedding. Loss of the PrPc function on TNFR1 regulation underlies the increased vulnerability of infected neurons to inflammatory cytokines and is likely to exacerbate prion-induced pathogenesis.

MicroRNA as potential actors of neurodegeneration in prion diseases

Dysregulation of CREB-regulated microRNAs have been identified in prion-diseased brain tissue. CREB is a transcription factor that plays an important role in neuron survival. Overexpression of CREB in scrapie infected neurons affects the transcription of miRNAs, in particular mi132. The targets of mi132 are involved in synapse activity and display either downregulation (RICS) or upregulation (NMDAR). The expression of other miRNAs regulating synapse function, inflammation and cell death are also altered. Investigating the specific role of these miRNA in pathogenesis is a new and promising field of research which may contribute to our understanding of neurodegenerative diseases including prion diseases.

Species-specific neurotoxicity of mammalian prion proteins in the fly nervous system

Pedro Fernandez-Funez and co-workers presented data from very fascinating experiments using flies expressing HaPrP, MoPrP and RbPrP. The different PrP species caused distinct behaviours in flies. HaPrP induced severe spongiform pathology and locomotor dysfunction with formation of PrP

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aggregates and PrP isoforms with pathological conformation. MoPrP seemed to provoke an intermediate phenotype, but RbPrP did not. The authors interpret these results as indicating that protein misfolding and neurotoxicity are strongly influenced by PrP primary sequences. An alternative interpretation proposed by Hubert Laude suggests that the neuropathological effects of HaPrP in flies are due to the known intrinsic ability of the peptide signal sequence of HaPrP (and to a lesser extent MoPrP) to produce a truncated peptide. This prevents translocation of PrP which leads to the accumulation of the neurotoxic form in the cytosol (Juanes et al. J Biol Chem 2009). Since RaPrP can not produce truncated PrP, it does not have toxic effects in the fly brain.

Electron micrograph of autophagic membrane wrapping in a Purkinje cell presynaptic axon terminal (black star) making a synapse on a dendrite of a deep cerebellar neuron (white star) in the cerebellum of the Nagasaki PrP-deficient mouse (x18000).

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Enigmatic N2?
Functions & Cell Biology of PrP
Prion2009, Thessaloniki - Chalkidiki
By Cathryn Haigh

\[\text{PrP}^\text{C} \text{ undergoes various post-translational modifications that are likely to be linked to its function. One such modification is cleavage around the C-terminal end of the octarepeat copper-binding region, termed beta-cleavage. This event has been shown to be caused by reactive oxygen species (ROS) and has been linked with protection against detrimentally increased ROS. The cleavage event produces two fragments; an N-terminal fragment (N2) approximately corresponding to amino acids 23-89 and the partner C-terminal fragment (C2), approximating residues 90-231. Since the N-terminal fragment contains two well characterised domains, the aforementioned copper-binding octarepeats and at the far N-terminus (amino acids 23-28) a polybasic region show to be a glycosaminoglycan binding site, we hypothesized that this fragment could function to transduce a protective signal against a ROS insult.}

The location of the beta-cleavage event is not yet fully established but indications are that it can happen at the cell surface; therefore we tested if and how exogenously applied PrP fragments corresponding to N2 or regions within N2 could protect against one source of detrimental cellular stress, serum deprivation. Within a PrP null cell background the N2 peptide reduced intracellular ROS only when applied pre-mixed with two or more copper equivalents. The protective effect was not mediated, however, by the octarepeat region alone but further required interaction of the N-terminal polybasic region with heparan sulphate containing proteoglycans located in or associating with cholesterol rich lipid raft domains.

Mutational analysis of the proline residues within the polybasic domain showed that not only was the charge of this domain important for its function but
also the structure imposed by the steric hindrance of the rigid proline pyrrolidine ring. Mutation of the prolines in this region also dramatically increased the propensity of the mutant N2 peptide to aggregate. Copper co-ordination of the octarepeats reduced but did not prevent this aggregation. By studying cell surface interactions of the mutant peptide compared to the wild type we found that the polybasic region exerts a dominant effect over any specific association of the copper-saturated octarepeats with a cell surface binding partner, suggesting both regions must be correctly engaged with their binding partners at the cell membrane to ensure successful transduction of the protective signal.

The beta-cleavage event is seen to increase in disease, markers of ROS are also found to correlate with the appearance of PrP\textsuperscript{Sc} in mouse brains and the C2 fragment comprises most of the core of PrP\textsuperscript{Sc}. This has lead to the theory that beta cleavage is a toxic event. However, we and others argue that beta-cleavage may initiate a PrP-mediated cellular protective event both for non-disease related stress and to help protect cells against the disease-associated ROS insult.

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Building a Global Prion Disease Support Community, by Florence Kranitz and Suzanne Solvyns, on behalf of the CJD International Support Alliance

The CJD International Support Alliance (CJDISA), made up of not for profit prion disease support organizations around the world, was privileged to address the audience of distinguished prion disease researchers in attendance at Prion2009, Chalkidiki Greece on September 25th.

The Alliance presentation offered insight into its growing role, pointing out that this growth is due, in many respects, to the support and encouragement of the prion disease research community. Since its inception three years ago the CJDISA has developed a strong network which now has the ability to refer families affected by prion disease to medical professionals in almost every country, provide information and connect families to support mechanisms all over the world. These connections provide the building blocks for a global prion disease support community.

During the presentation the CJDISA expressed its deep gratitude to the scientific community and to Alliance Bio-Secure and Dr. Valerie De Broglie for sponsoring their attendance at this important meeting. They ended with a brief video showing patients and families affected by a prion disease which brought the human face of this tragic category of diseases directly to those who work daily to find treatments and, hopefully soon, a cure.
Altered expression of CRMPs in the brain of bovine spongiform encephalopathy-infected mice during disease progression

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Neurodegeneration is a progressive process, which could be initiated by an impairment of the mechanisms of neuroplasticity, leading to early neuronal dysfunction. We tested this hypothesis in transmissible spongiform encephalopathy (TSE) pathogenesis, at the molecular level, by investigating the possible participation of a family of proteins involved in neurite outgrowth and neuronal plasticity, the collapsin response mediator proteins CRMPs.

The CRMPs belong to a family of neuronal phosphoproteins [1], that consists of five isoforms, CRMP1-5. Highly expressed in the developing central nervous system, these signal transduction proteins have been directly attributed to the formation, outgrowth and guidance of neuritis, and participate in the modulation of process development in oligodendrocytes too (for review see [2]). CRMP2 has been shown to be phosphorylated by cyclin-dependent kinase 5 (cdk5), glycogen synthase kinase 3β (GSK3β) and by Rho kinase II (ROCKII), all of which can mediate neurite retraction.
In the adulthood, CRMP expression is maintained in brain areas retaining high plasticity [3]; as an example, CRMP1 is crucial for spatial learning and hippocampal plasticity [4]. Under pathological conditions, CRMPs have been proposed to play a role in neuronal death and neurodegenerative disorders: hyperphosphorylation of CRMP2 could be involved in the pathological aggregation of microtubule-associated proteins during Alzheimer disease [5, 6].

While recent studies suggest that synaptic alterations are first events in the mechanisms of prion-mediated neurodegeneration little is known on the identity of the neuronal plasticity-related genes potentially concerned. Thus in the present study we evaluated the possible implication of these CRMPs. The expression of 4 CRMPs was studied in the brain of C57Bl/6 mice infected with the BSE strain of prion agent. Using RT-PCR and Western-blot methods, CRMP-1, -2, -4 and -5 were analysed quantitatively in C57Bl6 mouse brains at mid-course (90 days post inoculation (d.p.i.)) and at the terminal stage of the disease (180 d.p.i.) induced by BSE strain of agent injected by intra cerebral route.

At the terminal stage of the disease, gene expression of each CRMP had decreased, that most probably reflects a participation in a generalized response to severe damage within the brain. In contrast to the overall disturbance in CRMP expression, only CRMP-4 mRNA levels were significantly modified in the brainstem at the mid-satge of the disease. Interestingly, this increase was concomitant with higher levels of CRMP-4 proteins. This specific up-regulation of CRMP-4 expression pointed to clearer participation of this protein in prion pathogenesis since at that time the only neuropathological abnormalities detected was the limited PrP<sup>Sc</sup> deposition. This over-expression may support the prion-initiated neurite disorganization or might promote the selective remove of damaged neuritis. Altogether our findings picked out originally CRMPs and especially CRMP-4 as credible actors in prion-induced neurodegenerative processes and already opens new insights into molecular mechanisms of prion pathogenesis.

This work was recently published in Brain research [7].

References

Degradation of scrapie infected brain homogenate by a novel bacterial keratinase

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The resilient nature of infective prion has meant that they cannot be destroyed by common proteases or conventional sterilisation practices. Their affinity for and stability on metal surfaces has critical health implications for reusable surgical and dentistry instruments.

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Effective methods for prion decontamination have become increasingly desirable in order to manage the risks of prion infectivity and transmissibility. Incineration and alkaline hydrolysis are currently the most widely used means for destruction of the infective agent but these methods have obvious limitations of environmental acceptability, application compatibility, cost and loss of reusable materials. Most chemical and physical methods are harsh and inadequate for practical large scale application.

Enzymatic digestion of prions is considered as a mild, environmentally friendly, economically sound and safe method for the decontamination of prion infected materials, and was one of the science objectives and targets in UK Transmissible Spongiform Encephalopathy Directorate 2003-2006 Science Strategy [1]. Enzymatic degradation of prions provides a viable alternative for decontaminating animal carcasses, specified risk materials, medical instruments and laboratory equipments. A number of research effort on enzymatic degradation, inactivation and decontamination of infective prions have reported different levels of success under various experimental conditions; most of these methods required augmentation by preheating of prion contaminated tissue, addition of chemical surfactant/detergent and oxidizing agents, high alkaline pH, high temperature and long digestion time [2-6]. A stand-alone enzymatic alternative for prion degradation that is environmentally safe, compatible for use on sensitive materials, and suitable for practical use is highly desirable.

Our approach was based on the hypothesis that the structural similarity of feather keratin and infective prion in terms of their β-sheet proteic content may enable feather degrading bacteria to degrade prions. Proteolytic and keratinolytic microorganisms were isolated from farmyard waste and sewage sludge on feather meal agar. Of thirty-two isolates, one was selected on the basis of casein-agar hydrolysis assay, keratinase assay and Proteinase K equivalent curve for its ability to produce keratinase. The isolate was identified and designated as Bacillus licheniformis N22. Keratinase of this bacterium was purified by a step-wise feedback approach using the HiTrap Blue column (GE Healthcare), and the molecular weight determined as ≈ 28KDa by SDS-PAGE. This novel keratinase demonstrated significant activity on keratin azure (11 U/mL) and completely degraded melanised feather within 48h at 50°C (Fig.1). Scrapie infected mouse (ME7) brain homogenate was digested with this keratinase at 50°C for 2h in the absence of chemical surfactant/detergents and without preheating the prion sample. PrPsc signal of the digested sample was reduced to a level undetectable by western blotting analysis (Fig. 2). Further optimisation of incubation conditions is currently ongoing, and future work includes degradation of BSE prions and evaluation of residual infectivity by animal bioassay.

![Fig. 1: Degradation of melanised feather](image1)

![Fig. 2: Scrapie prion degradation analysed by western blotting.](image2)

**References**


Serial Passage of sCJD in Humanised Transgenic Mice Indicates a Minimum of Two Major Transmission Strains of Infectious Agent

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Questions remain about the aetiology of sporadic CJD and whether phenotypic variation is solely controlled by factors such as PRNP codon 129 genotype and biochemistry of PrPSc. Variation in infective strain has not been clearly demonstrated in sCJD as there are difficulties in transmission to wild-type and some transgenic mice lines. By serial passage of sCJD in humanised transgenic mice we aimed to understand which dominant factors determine the strain transmission characteristics for the three most commonly observed phenotypes of sCJD (MM1, MV2, and VV2; >95% of all cases).

Mice used for this study were gene targeted transgenic lines expressing the human PRNP open reading frame with Met/Val variation at codon 129. We performed intracerebral inoculation with brain homogenates derived from these mice previously inoculated with frontal cortex from sCJD patients. Mice were assessed for clinical signs of TSE and all mice were scored for the level of TSE associated vacuolation in 12 brain regions (lesion profiling), and mean scores used for Hierarchical Cluster Analysis. The resulting dendrogram or tree structure showed groups of mice with a similar distribution of TSE vacuolation in the brain. The similarities within each group can show which factors are determining the vacuolation patterns seen.

Primary passage of sCJD subgroups MM1, MV2, and VV2 shows that with regards to incubation period (time to clinical phase of disease) and overall susceptibility of each genotype mice to infection, MM1 sCJD data are different to MV2 and VV2. Transmission properties of the latter two subgroups appear very similar. Cluster analysis of the lesion profile data however does not show this separation; instead the grouping is dependent primarily on the PrPSc found in the mice, and secondarily on the mouse codon 129 genotype. These factors must therefore be more dominant strain parameters than the original sCJD subgroup.

Serial passage shows that host PrPSc type and codon 129 genotype again are the major determinants of secondary transmission properties. There is a strong association between type 2 PrPSc and homozygosity for C129-Val, and type 1 in all genotype hosts seen by the split in the cluster analysis dendrogram that separates the data into two clear groups. In some second passage mice from the MV2 and VV2 strains, there has been a reduction in incubation period to a level similar to that seen in sCJD type MM1 transmission, indicating a common thread between type 1 and the vacuolar pathology seen.

This suggests that there are at least two major sCJD transmission strains within the three most common clinico-pathological phenotypes.

Funding from NeuroPrion (HUMTRANS project) has facilitated the breeding of these unique transgenic mice in additional institutes in Italy and Germany which means that we are now able to investigate transmission properties of many more forms of human and animal TSE.

Characterization of the molecular heterogeneity of mutant prion proteins

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One striking feature of inherited prion diseases is their phenotypic heterogeneity. PrP mutations are associated with defined clinical and neuropathological phenotypes: Creutzfeldt-Jakob disease (CJD), Gerstmann-Sträussler-Scheinker (GSS) syndrome or fatal familial insomnia (FFI). An
important source of phenotypic variation is the polymorphism at codon 129 of PrP, where either methionine or valine can be encoded. How sequence variants of the PrP gene may encode the information to specify distinct disease phenotypes is a central question in prion biology. It is postulated that each mutation, in combination with the 129 polymorphism, produces folding variants of the PrP polypeptide that are selectively toxic to specific neuronal populations. As yet, however, there is no direct experimental support to this hypothesis.

The goal of our project is to define the correlation between the folding of mutant PrP molecules and their selective toxicity. We generated transgenic mouse lines expressing the murine PrP homologues of the nine-octapeptide repeats insertion associated with a mixed CJD-GSS phenotype (referred to as Tg(PG14) mice), and the D178N/V129 and D178N/M129 mutations linked, respectively, to CJD and FFI (referred to as Tg(CJD) and Tg(FFI) mice) (1-3). Mutant PrP in the brains of these mice can be distinguished from PrP\textsuperscript{C} by several biochemical properties, including propensity to form detergent-insoluble aggregates, and reactivity to the 15B3 antibody, which recognizes a variety of misfolded and aggregated forms of PrP (4).

In order to characterize the conformation of the mutant PrP aggregates, we developed a procedure for purifying these fractions from the brain of Tg mice using sequential centrifugations and immunoprecipitation with 15B3 (5). Mutant PrP aggregates obtained with this method are highly pure, and retain their biological properties, including toxicity to cultured neurons. To characterize the structure of the aggregates we are using a panel of biochemical and biophysical tests, including a new conformation assay called lysine-tagging mass spectrometry (LT-MS). This procedure is based on the fact that guanidination of proteins converts solvent-exposed lysines to homoarginines, resulting in a mass increase of 42.0218 Da per residue. By comparing the profile of guanidination of the different aggregates using mass spectrometry, we will map the solvent accessibility of the lysines, obtaining information on the quaternary structure of the aggregates.

We recently demonstrated that in the Tg mouse brains a subpopulation of mutant PrP molecules remains soluble and shares many biochemical properties with PrP\textsuperscript{C} (6). The soluble molecules most likely represent the precursors of the mutant PrP aggregates, and knowing their conformation may help deciphering the pathway of oligomerization of the different mutants. To characterize the conformation of the soluble forms of mutant PrP we have developed a conformation-dependent immunoassay based on surface plasmon resonance (CDI-SPR). This assay compares the reactivity of the different mutants to a panel of monoclonal antibodies directed against defined regions of PrP.

In contrast to previous work focused on the characterization of recombinant mutant PrPs expressed in bacteria, our approach offers the opportunity of defining the structural features of PrP molecules isolated from a mammalian source.

Greek National Scrapie Surveillance programme from 2002 up 2008 – A review

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Introduction

Slaughtered animals for human consumption and dead animal over 18 months of age in accordance with a sample size, (1) as well as the entire clinical suspect are tested. In case of Scrapie suspicion, movement restrictions are obligatory. In positive sheep flocks the measures are, either culling, or fully genotyped. In this case genetically sensitive animals are removed from the herd and resistant animals are selected.

Results

During the implementation of the Greek National Scrapie surveillance programme from 2002 up to 2008, a total of 141,197 samples originated from small ruminants were checked. 98,326 coming from sheep population and 43,591 from goat population. From the above samples 103,224 were healthy slaughtered and 37,973 of risk population. 2146 samples were found positive to Scrapie (1911 sheep and 235 goat), isolated from 223 infected flocks.

Table 1

<table>
<thead>
<tr>
<th>Target group</th>
<th>Sheep</th>
<th>Goat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Positive</td>
</tr>
<tr>
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<td>169</td>
</tr>
<tr>
<td>Fallen stock</td>
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</tr>
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<td>1899</td>
<td>596</td>
</tr>
<tr>
<td>Total</td>
<td>98326</td>
<td>1911</td>
</tr>
</tbody>
</table>

In 2002 all positive cases were index cases with a prevalence up to 0.31%. From 2003 secondary cases were increased progressively with the prevalence reaching 2.61% in 2008. As it concern the primary cases (index cases), there was an average of 67 cases per year up to 2008.

Positive flocks

Classical Scrapie was diagnosed first time in Greece in 1986. (2) From 1986 up to 2001 9 positive flocks were found with 45 positive animals in all over the country. All were as a result of the passive surveillance.

In 2002 the small ruminants active surveillance programme began. From this year and then the positives flocks were increased year by year coming up to 223 in 2008 by an average of 33 new positive flocks per year up to 2008. The most positive flocks are located in North and Central Greece.

The poster prize winners

16
Atypical Scrapie

The first case was detected in 2006 through active surveillance from slaughtered sheep. The profile of the protein in TeSeE Western Blot (Biorad) was those of the Atypical Scrapie (11-12 KD).

Until the end of 2008 five more cases of Atypical Scrapie were found in five different flocks from different regions. All samples were detected from slaughtered sheep. No clinical symptoms were noted in any sheep from each flock. Any other animals which tested from the same flocks found negative. The genotype was ARR/AHQ in four samples, AHQ/AHQ and ARH/ARQ.

Genotyping

In accordance to 999/2001, genotyping was done to:

- each positive TSE case in sheep
- a number of healthy sheep (600 samples every year)
- all the animals of positive folks. (Genetically sensitive animals are removed from the herd)

Genotyping in positive sheep

From 2002 up to 2008 1336 positive sheep samples were analyzed with either an RFLP(3) or a Real Time PCR method. From them was found:

- VRQ haplotype at a percentage of 7.63% either as homozygote or as heterozygote, usually in combination to ARQ haplotype.
- AHQ and ARH haplotype as homozygote or heterozygote at a percentage of 16.10%
- ARK haplotype in combination to ARQ haplotype in six cases
- ARR haplotype in combination to ARQ haplotype in 5 classical Scrapie samples and in combination to AHQ haplotype in 4 atypical Scrapie samples.
- No ARR/ARR genotype was found.

ARQ/ARQ genotype is the most frequency genotype in Greek positive to Scrapie sheep at a percentage of 75.58%.

Genotype distribution in healthy sheep

From 2005 up to 2008, 973 samples coming from healthy sheep, were analyzed. From them and according to British NSP system the distribution was: ARR/ARR: 5.44%, ARR/…:29.18%, ARQ/ARQ:49.33%, AHQ/AHQ,AHQ/…ARH/ARH,ARH/…: 9.76%, ARR/VRQ: 1.23%, VRQ/…: 5.03%

Average genotype distribution in positive flocks

From 2006 up to 2008, 30.601 animals coming from 120 positive flocks were fully genotyped resulting to:

ARR/ARR: 5.80% (rams only 97) ARR/…:30.88%, (rams 389) ARQ/…AHQ/……54.72% ARR/VRQ: 0.63% VRQ/……: 2.60%

Conclusions

Greece is the second European country after Cyprus in positive cases and flocks with a prevalence of 2.6%. Most of the cases are secondary cases with ARQ/ARQ being the dominant genotype, pointing out that when VRQ/genotypes are in low percentage, then ARQ/ARQ genotypes are the most susceptible.
The progressively increase of the positive cases within the flocks or within the same region underline the significance of the breeding schemes based on genetic selection.

References

Recycling of PrPSc via retrograde transport pathway from endosome to TGN in Neuro2a mouse neuroblastoma cells
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Prion diseases are fatal neurodegenerative diseases characterized by the accumulation of an abnormal isoform of prion protein (PrPSc) in the central nervous system. Although the propagation of prion in neurons is believed to be tightly associated with neuronal degeneration, the molecular mechanisms of prion propagation in cells have not been fully understood yet. To clarify the mechanisms of prion propagation in cells, we tried to analyze the intracellular localization of PrPSc in Neuro2a mouse neuroblastoma cells persistently infected with prion 22L strain by indirect immunofluorescence assay (IFA). PrPSc could be detected in prion infected cells by IFA with the pretreatment of chaotropic reagent as previously reported, however, a cellular isoform of prion protein (PrPc) was also detected in uninfected cells, depending on the cell condition and anti-PrP antibodies used for the detection. We found that PrPSc could be specifically detected by using mouse monoclonal antibody (mAb) 132 that recognizes the region adjacent to the most amyloidogenic region of PrP (aa112-119). As a result of the double staining with mAb 132 and organelle markers, the majority of the granular staining of PrPSc was found at peri-nuclear regions juxtaposed to trans-Golgi network (TGN). PrPSc at peri-nuclear regions was co-localized with internalized Shiga toxin B subunit that is known to be transported from endosome to TGN via retrograde transport pathway. When the cells were cultured at 20°C to prevent the retrograde transport, the PrPSc was dispersed to cytoplasm, however, PrPSc signals returned to peri-nuclear regions 15min after transferring the cells to 37°C. Interestingly, the returned peri-nuclear PrPSc signals disappeared after the additional 15 min incubation at 37°C, and then appeared again after the additional 60 min incubation. This observation suggests that PrPSc is dynamically transported from endosome to peri-nuclear regions and recycled to plasma membrane. PrPSc at peri-nuclear regions was well co-localized with clathrin heavy chain (CHC), Rab9, Rab11 and components of retromer, which are factors associated with retrograde transports from endosomes to TGN. After fractionation of the post nuclear supernatant (PNS) from prion infected cells by density gradient centrifugation, PrP was detected in the fractions containing CHC. Furthermore, PrP was co-immunoprecipitated with CHC when the PNS from prion infected cells was immunoprecipitated by using anti-CHC antibody but PrPSc was slightly co-immunoprecipitated with CHC in the case of uninfected cells, suggesting that PrPSc in prion infected cells is associated with clathrin coated structure. In conclusion, our findings propose an idea that PrPSc is associated with the retrograde transport from endosomes to peri-nuclear regions including TGN via clathrin coated structure and the retrograde transport from endosomes to TGN is involved in the recycling of PrPSc.

Optical Imaging of Apoptosis in Organs of Prion-Infected Mice
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Apoptosis has been identified as an important cell death mechanism in the brain of patients with Creutzfeldt-Jacob disease and in mouse-adapted
models of prion disease (1,2). Caspases play a key role in effecting apoptosis. We have determined the extent of caspase activation in the brain and peripheral organs of mice infected with the M1000 strain of mouse-adapted prions (derived from the Fukuoka-1 strain originating from a patient who died from Gerstmann-Straussler-Scheinker syndrome (3,4)).

Mice in the terminal stages of prion disease were administered sulforhodamine B-Val-Ala-Asp(OMe)-fluoromethylketone (SR-VAD-fmk) via the tail vein shortly before euthanasia and excision of organs. This cell permeable pan-caspase inhibitor irreversibly binds to the catalytic site of active caspases, yielding a red fluorescent measure of apoptotic activity. Tissue from the brain, eye, heart, skeletal muscle, lung, spleen, kidneys, rectum, liver and ileum was examined using a combination of fluorescence imaging, microscopy, and spectroscopy, together with immunohistochemistry.

A significant increase in SR-VAD-fmk fluorescence was observed in the brain, lung and kidneys of M1000-infected mice (Figures 1 and 2). Wide-field fluorescence microscopy of brain sections further localised increased apoptotic activity to the thalamus, occipital lobe, hippocampus and pons. Immunohistochemical analysis indicated that pan-caspase activation was localised adjacent to regions of PrPSc deposition in the brain. Western blotting of whole brain homogenates revealed both caspase 3 and caspase 6 were increased in infected mice.

The findings show that optical imaging using a fluorescent marker of active caspases is a useful technique for detecting apoptotic activity in prion disease. The observation that apoptosis localises to organs (brain, lung and kidney) which have been shown to transmit disease (5) suggests a promising application to longitudinal studies of disease progression and therapy by employing in vivo fluorescence imaging.

Acknowledgement:
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References

Fig 1. Overlay of fluorescence and white-light images of ex vivo brains from M1000-infected and control mice, following tail vein injection of SR-VAD-fmk or physiological saline. Images were acquired with a FujiFilm LAS-3000 Imaging System ($\lambda_{ex} = 520$ nm LED, $\lambda_{em} = 580$ nm long-pass).

Fig 2. Fluorimetry of tissue homogenates from brain, lung and kidney of M1000-infected and control mice. SR-VAD-fmk fluorescence was normalised by total protein content of the homogenate. $p$ values correspond to a one-tailed unpaired t-test with Welch’s correction.

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Biarsenical labeling by IDEAL protocol provides new insights into effects of anti-prion drugs on PrP cell biology: Application to Dextran Sulfates and Pentosan Polysulfate

By Yuzuru Taguchi

Genetic tagging with the tetracysteine motif (TC; CCXXCC), which binds biarsenical dyes such as FlAsH, has been expected to be an alternative imaging probe to fluorescent proteins for live-cell imaging of proteins. Despite some impressive achievements including imaging of connexins, the original biarsenical labeling protocol often resulted in very intense background and was not applicable to extracellular TC motifs. Recently, we achieved very specific labeling of TC-tagged PrP (TC-PrP) on live cells by developing the “IDEAL” protocol (Instant with DTT, EDT and Low temperature). FlAsH-labeled proteins are amenable to SDS-PAGE analysis by in-gel detection on a laser scanner (fluorescent gel analysis). This prompted us to use IDEAL-labeling as a tool for biochemical investigations of PrP because it facilitates rapid, quantitative pulse-chase analyses. Pulse-chase analysis of PrP metabolism could benefit prion research in various ways. For example, it would reveal short-term effects of anti-prion reagents on PrP that are not appreciated in conventional inhibition assays, which represent cumulative effects over 2-3 days of incubation. In addition, short incubations enable examination of reagents that are cytotoxic in long incubations. We applied this approach to assessing the influences of representative anti-prion reagents, pentosan polysulfate (PPS) and dextran sulfates (DSs), on PrPsen and PrPres metabolism.

Cells expressing TC-PrP were pulselabeled with FlAsH and then chased for 5-6 hours in medium containing various concentrations (60-1,000 ng/ml) of PPS or DSs of different molecular sizes, namely DS5, DS20, DS100 and DS500. After the incubations, cells were harvested and subjected to fluorescent gel analysis to evaluate levels of FlAsH-labeled PrPres, full-length PrPsen (Full-PrPsen), and C3-fragments. Besides pulse-chase analyses, conventional inhibition assays were also performed to confirm the effects of each reagent on the cell line we used.

The results were rather dramatic. While conventional assays confirmed inhibitory effects with subtle differences between reagents, except for DS5, pulse-chase analyses exhibited clear differences. PPS showed similar results to DS20. Full-PrPsen levels were drastically reduced by DS100 and DS500 at concentrations ≥250 ng/ml, whereas DS20 and PPS showed only a moderate degree of reduction at concentrations ≥500 ng/ml. DS5 did not affect Full-PrP throughout the concentration range tested. C3 levels were significantly increased by DS100 or DS500, whereas DS5, DS20 or PPS had no effect. In live-cell confocal imaging studies, DS500 induced more rapid internalization and increased juxtanuclear accumulation of fluorescent PrP compared to PPS, suggesting active proteolysis of PrP in the DS500-treated cells. Comparison of PTA-precipitated fractions suggested a correlation between the amount of aggregated Full-PrP and the molecular sizes of DSs.

Thus, FlAsH-pulse-chase analyses characterized further details of the effects of DSs and PPS on PrP metabolism than conventional methods alone. Influences of DSs on PrPsen and PrPres varied depending on molecular sizes and concentrations. Interestingly, PPS gave similar results as DS20 at all points tested despite its small size (~4.2 kDa). Remarkably, the relationship between Full-PrP levels and PrPres levels after DS treatment implied the possibility of two general inhibition mechanisms that either are or are not accompanied by reduction of Full-PrP levels. These insights may aid the development of more efficacious anti-prion drugs. The present experiments demonstrated the usefulness of pulse-chase biarsenical labeling in determining the effects of compounds on PrP metabolism. Application of this technique to modifiers of cellular activities such as endocytosis inhibitors, protein-synthesis inhibitors and cytoskeleton-disrupting drugs is expected to unveil a detailed picture of the cell biology of PrPsen and PrPres.

References
Workshop «New developments in TSEs of domestic and wild animals»

The 2009 NeuroPrion/GoatBSE workshop on animal TSEs in Porto Carras: “New developments in TSEs of domestic and wild animals”.

This year a workshop was again organized on TSEs in target animals, the day before the Prion2009 Conference started. This was actually the second TSE workshop that research progress on cervids and other animals, including goats was presented. Even though the announcement for the workshop was delayed, the interest was overwhelming, since the meeting enjoyed the presence of 100 attendees. Within the limited time available there were 13 excellent presentations and a lively discussion about the significance of the presented data.

In the welcoming address, Mick Stack (VLA, Weybridge, UK) remembered the first BSE cases in 1986 and the excitement of finding the first scrapie-associated fibrils on their electron microscope, also thanks to the experiences on scrapie in Scotland and Germany. He also remembered going to many TSE conferences and was amazed to see the breadth of research expand during the years of the BSE epidemic. Although aware of reduced funding, he was optimistic that some recent progress had been made with regard to environmental aspects of the spread of TSEs and towards the development of possible ante mortem tests.

The first session was focused on bovine, ovine and caprine TSEs. Thierry Baron (AFSAA, Lyon, FR) illustrated the unusual TSE isolates of CH1641-like type in sheep and goats, and the differences towards L-type. This was apparent not only in biochemical analysis but also in bioassays with transgenic Tg4 mice. Further, bioassay results with bovine C-, H- and L-type BSE isolates in C57BI mice were discussed.

Gabriele Vaccari (ISS, Roma, IT) presented the experimental oral infection studies of Cecilia Buccalosi in Italian Sarda sheep of a series of different genotypes, including K176, K143 and T137. Further results in studies by PMCA seem to reflect that the PrP polymorphism barrier is comparable to that seen in sheep infections, where the ease of transmission of the scrapie isolate is dependent of the PrP genotype: ARQ > AHQ > ARH >> ARR. This further illustrated the power of in vitro conversion studies for interpretations of transmission barriers.

Frederic Lantier (INRA, Tours, FR) showed data on mixed infections with BSE and scrapie (several isolates tested). Spleen and brain exhibited different signatures, being respectively BSE and scrapie like and this was further confirmed by inoculations in Tg338 (ovine V136PrP) and Tg110 (bovine PrP) mice.

Christine Hoffmann and Martin Eiden (FLI, Riems, GE) are studying Cypriot goats including about 50 cases of TSEs, all probably of scrapie type. Most cases except 2 were obex and LRS positive. The M142 and 146S/D polymorphisms might be protective. Progress on ongoing studies in Tg shARQIX mice and also PrP conversions by PMCA with recPrPs was presented.

Maria Carmen Garza (UNIZAR, Zaragoza, SP) is studying the placentas of biopsy positive ARQ/ARQ ewes (naturally infected) carrying lambs of ARQ and ARR PrP haplotypes. She could establish that even in presence of ARR haplotypic lambs the fetal side in the placenta could accumulate PrPSc positivity.
Oliver Bannach (HHU, Düsseldorf, GE) presented the original technique of confocal fluorescent detection on antibody coated glass surfaces, that can bind PrP aggregates from plasma of sheep in clinical stage of scrapie. An intensive processing pretreatment of the plasmas is probably required to accomplish this detection, and a mix of differently labelled antibodies is used for dual label detection of bound aggregates.

The second session was dedicated to CWD.

Aru Balanchandran (CFIA, Ottawa, CANADA) updated the occurrence and further spread of CWD in wild and farmed cervids in Canada. A postmortem study using IHC, ELISA and western blot on two white tailed-deer farms depopulated for CWD, showed that lymphnodes in the head (retropharyngeal LN and tonsils) are the best targets for diagnosis, while rectal mucosa associated lymphoid tissue (RAMALT) has an intermediate sensitivity. RAMALT can serve as a useful tissue for detecting CWD infection at flock level, including preclinical diagnosis.

Candace Mathiason presented data about the facile transmission of CWD in deer through fomites or contact exposure as well as blood transfused from presymptomatic/clinically affected deer. An novel type of observation was the CD41 and CD21 but not CD14 linkage for susceptibility in these blood transfusions.

Nick Haley extended studies on deer orally infected with excreta like urine and feces from subclinical CWD donor deer. He used serial PMCA - as inoculum served retropharyngeal lymph nodes and brain obex tissues from CWD deer, and as substratum served Tg[CerPrP] mouse brain - and Tg[cerPrP] mouse bioassay. The presence of infectivity in these excreta could now be proven.

Nathaniel Denkers showed that Tg[cerPrP] mice could be CWD infected by aerosol administration or nasal administration, but by oral route only when tongue was abraded.

Jason Bartz found that it takes a rather long time (days to weeks) for different soil types (clay or sand) to absorb PrP\textsuperscript{Sc} or recPrP, while the condition of the infectious material (purified or whole brain homogenate; hyper and drowsy) is an important determinant in the absorption process.

David Seelig performed a detailed and sensitive immunohistochemical study of prion spread after oral, intracerebral, intravenous and intraperitoneal inoculation of Tg[cerPrP] mice with CWD prions. The spread appears in the parasympathetic and sympathetic elements of the autonomic and enteric nervous systems. Unique was the localisation of PrP\textsuperscript{D} in enteric glia cells in intestinal villi, while no intralymphatic PrP staining was observed.

Mick Stack outlined the pioneering role of the NeuroPrion cervids group for the European CWD surveillance and the progress made by experimental BSE infections in red deer by Martin et all. who showed that principally BSE could be detected in cervids with the tools available.

The organisation was by Mick Stack (VLA, Weybridge, UK), Jan Langeveld (CVI-WUR, Lelystad, NL), and also for the first time by someone of the American continent, Edward Hoover (CSU, Fort Collins, CO). Next year we plan again to organize such meeting, in Salzburg, the day before the Prion2010 conference. We intend to consider the situation in goats, sheep, cervids and cattle for selecting presentations. Suggestions are welcome. Further information can be found in the workshop Programme and Abstracts brochure.

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The young researchers training

By Karen Dobie and Kayleigh Wagg (Roslin Institute and R(D)SVS, University of Edinburgh), two young researchers who have participated in the training.

A group of 31 young researchers arrived at the beautiful Athena Pallas resort for a three-day intensive course organised and funded by NeuroPrion which aimed to teach scientific communication to the young researchers. The group was split into two allowing two different sessions to be run.

The presentation skills course run by Dr Christian Dumpitak and Knut Hannemann (both University of Dusseldorf) taught the researchers about all aspects of presentation from body language and gestures, to slide design and pronunciation. This highly interactive session allowed the researchers to form friendships and gain confidence in public speaking with one of the hardest lessons being to accept your applause.

The other half of the group worked on their debating skills, with husband and wife duo, Prof Alun Williams and Marianna Fletcher Williams. The first debate was on a non-scientific topic to learn the debating technique and how to structure arguments before moving on to more scientific debates such as “Should prion funding be continued in the current economic recession?”

Each evening, different presentations took place with the students presenting at the Neuroprion conference given a chance to practice their talks and receive feedback as well a fantastic talk on how use PowerPoint as a presentation tool from Dr Valerie Sim. The group also received an enjoyable non-scientific talk by Anne-Charlotte Panissie on Greek mythology and Homer.

The researchers then all attended the Neuroprion conference, for many their first international conference where they were able to hear the latest science and use what they had learnt to point out the presentation flaws of the speakers to each other. Although being made to work a long day the researches still found time to socialise and form new friendships across Europe. The course was enjoyed by everyone and provided invaluable training that young researchers may not normally receive and will be highly recommended to future young researchers by all.
A thing of beauty is a joy forever (John Keats)
By Muhammad Khalid Farooq Salamat (INRA Jouy-en-Josas, France) a young researcher who has participated in the training.

Days are disappeared but the memories always remain alive in the hard drives of our minds and souls. Neuroprion Young Researcher’s Event (19-22 September) was such a promising event whose aroma will always strike upon our minds. I reached Athena Palace Village (Halkidiki, Greece) at 3am Sunday morning after a tiresome travel of 20 hours, “thanks" to Air France. After taking breakfast, we were asked to gather in the conference halls, located on the other side of that paradise. Dynamic and enthusiastic team of Dr Benjamin Scherr, event incharge (CEA, France), under supervision of Dr Jean-Philippe Deslys, Neuroprion coordinator and Head of CEA (France) prion research team, along with all event tutors gave us warm welcome and useful guidelines. After that they divided us into two groups comprising each of 15 people thus giving us chance of benefiting maximum from this event by intermingling with people from around the globe with their versatile experiences to share.

Though I was much tired and was in dire need of rest, but the wonderful methodology of Professor Alun Williams and Marianna Fletcher Williams (UK) alongside Dr Christian Dumptak and Knut Hannemann (University of Dusseldorf, Germany) made me forget about any fatigue and we were completely lost in the charisma of this saga. This was the interest and attractions created by them that no one was absent during those three days even though all distractions in the form of pools, beach and a sunny weather were there to lure. Marianna and Alun taught the techniques how to make an effective debate/discussion and face the audience questions politely and patiently. The focus of other team lead by Christian and Knut was on presenting science in a comprehensive, competent and convincing way. Preparation and planning of scientific presentation alongwith analysis of aims and do’s and don’ts while preparing power point presentation were also areas of main focus. They cunningly used techniques of appearance before audience and convincing them without uttering a single word as the quotation goes “Well-timed silence hath more eloquence than speech” (Martin Fraquhar Tupper). The purpose was to improve the gesture and mimics, lessen stress and nervousness, appearance and presence before audience and winning them over. Rhetoric and physical exercises of voice and breath improve and tips to deal with stress were very useful. Warm exercises such as yawning, Ssssss, Prrrrr and “ba-ba-ba, bo-bo-bo, bu-bu-bu” were amusing. “Most difficult” but the funniest part was receiving applause while staying on the stage, every time we ran away before this.

At the end of first day, presentations “Brain reactions and communications” by Jean-Philippe Deslys and “My own experience of scientific presentation” by Dr Valerie Sim (Canada) were equally admired. A journey through Greek mythology and history with Anne-Charlotte Pannissié (Université Paris-I) was also pleasant and informative. Her beautiful depiction of Homer’s masterpieces Iliad and Odyssey was worth listening. Detailed introductions and fruitful discussions on different research areas in particular related to my work went side by side, during the coffee breaks, lunch and buffet dinner. The taste of delicious Greek cuisine with fresh salad, always with onions, still makes me think of that and my mouth starts watering.

In the beginning, we were communicated to bring a 10min oral PowerPoint presentation on research work, on the basis of which two would be selected for Prion2009. Due to large number of participants, we were asked to come forward and offer names voluntarily whosoever wanted to present. Only I and Simon Nicot (AFSSA, France) were “dare enough” to present our names and hence selected without any competition. For a brief moment I accounted it for as my foolishness but it was too late. Then I took it as a task and a rare chance that should never be missed.

In the evening before the dawn of D-day, we made a rehearsal of our presentations. The participants were requested to evaluate us whether we had used effectively all what we had been taught during this workshop. Self and external evaluation particularly
The young researchers training

New insights into « CH1641-like » natural scrapie

By Simon Nicot (AFSSA, Lyon, France), a young researcher who has participated in the training.

The fourth NeuroPrion Young Researchers Event, dedicated to training in scientific communication, was organized this year in Chalkidiki, Greece, and, for the first time, just before the Prion2009 conference. In this context, it was particularly appreciable for us to stay a full week with colleagues working on prion diseases.
and coming from different European countries.

We were directly welcomed at the Thessaloniki airport by Dr Jean-Philippe Deslys and Dr Benjamin Scherr, coordinators of Neuroprion and of the Young Researchers Event. The training took place in the beautiful Athena Pallas Village, near the Porto Carras Grand Resort where the Prion2009 conference was held. During the first day of the training, students were divided into two groups of 16 people, each group having a different work session. The first workshop in which I participated was dedicated to “Training in debating techniques” and was nicely conducted by Pr Alun Williams and Marianna Fletcher Williams. I appreciated the structure of this workshop, organized in a debate fashion and covering general topics as well as prion diseases topics. The main objective was to become able to support a motion and to be convincing towards others. For this, we learned that it was necessary to structure our talks, by pointing out different aspects or categories of a motion. After the first session of workshops, the two groups took turns. The second workshop entitled “Presenting science” was animated by Dr Christian Dumpitak and Dr Knut Hannemann, and was based on how to present Science in a comprehensive, competent and convincing way. For me this session appeared very interesting and useful, mostly because it was very concrete and that we could directly see our improvements. We learned a lot of things, from how to stand in front of the public, how to deal with nervousness, through preparation and planning of scientific presentations, etc... We also learned how to give and receive feedback after presentations. I thank these two teachers a lot, who were absolutely excellent. At the end of the training, two young researchers were selected to present a 10 min powerpoint presentation, based on their research work, during a plenary session of the Prion2009 conference. As we were numerous, the selections were made among volunteers. Only two students, I and my colleague Muhammad Khalid Farooq Salamat (INRA, Jouy-en-Josas, France), were volunteers and, as a consequence, selected.

I thus had the great opportunity to present recent findings from my lab, the institute AFSSA of Lyon in France. The topic of my presentation was about strain-specific molecular features of the prion protein in ruminants, and the title was “C-terminal prion fragments as a tool for the molecular discrimination of prion strains”. There were two main parts in the talk, in which I particularly focused on rare natural isolates of scrapie, called “CH1641-like”, that share some of their molecular features with BSE, and even more with atypical L-type BSE or BASE.

First I presented Western blot data on prion fragments, from a range of TSE sources from ruminants, after transmission into ovine transgenic mice (TgOvPrP4; A136R, R144Q, allele). From these mice, we isolated the brain prion protein, with or without protease digestion, before Western blot analyses (this work is now accepted for publication in the Journal of General Virology). Our main finding was the identification of a C-terminal PrP fragment of approximately 14 kDa in its unglycosylated form (PrPres#2 or CTF14), which was specifically detected with a C-terminal antibody (SAF84). This PrPres#2 was particularly abundant in “CH1641-like” scrapie isolates, but was absent in both classical and L-type BSEs. This finding thus provided an additional and useful method for the discrimination of prion strains of ruminants, through the identification of C-terminally cleaved PrP fragments.

The second part of the presentation was dedicated to the further characterization of the “CH1641-like” isolates of scrapie. We showed that these scrapie isolates had a peculiar behavior following transmission into ovine transgenic or wild-type mice, contrary to other TSE sources including the experimental scrapie isolate CH1641, natural classical scrapie isolates, and also classical and L-type BSEs from cattle. The differences observed were related to the splenotropism (demonstrated in the TgOvPrP4 mouse line) or to the ability of crossing a species barrier (demonstrated by primary transmissions into wild-type C57Bl/6 mice). Indeed, CH1641-like scrapie isolates were the only TSE sources characterized by a higher molecular mass of PrPres in the spleen than in the brain. Also, compared to the experimental CH1641 isolate, CH1641-like isolates were efficiently transmitted into wild-type mice, but with an alternative molecular phenotype (i.e. of high molecular mass), suggesting the emergence of another scrapie strain that pre-existed in the original isolate/host. These observations led us to speculate that “CH1641-like” scrapie isolates might actually be composed of a mixture of two distinct prion strains (or more accurately two distinct prion components): the CH1641 “stricto sensu” prion component, major, and another minor prion component similar to that of classical scrapie. The final proof that these two
prion components represent two distinct prion strains is still in progress, through a second passage from the spleen of “CH1641-like” scrapie in TgOvPrP4 mice. Interestingly, similar findings on the emergence of a new prion strain from a BASE isolate, upon transmission to bank voles, were presented during the Prion2009 conference (Dr Romolo Nonno). Also, Dr Umberto Agrimi, from the same institute, gave a talk on a case report of natural co-infection of a sheep with both classical and atypical (or Nor98) prion strains. In this regard, and thanks to a grant from Neuroprion, my team and I will soon have the great opportunity to share our findings and collaborate with our Italian colleagues from the Istituto Superiore di Sanità in Rome.

At the end of my presentation, two questions were kindly asked by Dr Romolo Nonno and Dr Jan Langeveld. I had some difficulties to hear the questions well, but globally, I correctly answered them, as my PhD director Dr Thierry Baron mentioned during a feedback session just after the presentation. I thank also Dr Christian Dumpitak for his very helpful feedback.

Altogether, this week in Greece was a very good experience for me. The training helped me in improving my English and my skills in presenting science, and it was also very interesting to talk with PhD colleagues working in the prion field. A very good atmosphere prevailed between organizers, teachers and students. I also enjoyed the Prion2009 conference, my first one, full of very interesting scientific topics.