

**REPORT ON COEN WORKSHOP: ANIMAL MODELS OF HUMAN  
NEURODEGENERATIVE DISEASES**

**Cambridge, 25<sup>th</sup> & 26<sup>th</sup> November 2010**

**Michel Goedert**

**Introduction**

Alzheimer's disease and Parkinson's disease are the most common neurodegenerative diseases. They are characterised by the abnormal assembly of a small number of proteins (A $\beta$ , tau and  $\alpha$ -synuclein) into filaments. Misfolding of tau or  $\alpha$ -synuclein is also central to a number of additional neurodegenerative diseases. Over the past 30 years, a direct correspondence between inclusion formation and the degenerative process has been firmly established.

This was made possible by the coming together of two independent lines of research. On the one hand, the biochemical study of the neuropathological lesions resulted in the discovery of their major molecular components. On the other hand, the study of rare, familial forms of Alzheimer's disease, frontotemporal dementia and Parkinson's disease led to the identification of gene defects that cause disease. Remarkably, the defective genes were found to encode or increase the expression of the main components of the neuropathological lesions. It therefore appears that a toxic property conferred by these mutations causes disease. A similar toxic property may also underlie the sporadic forms of disease.

Besides their conceptual importance, these findings made it possible to produce experimental animal models of the neuropathological features that define human neurodegenerative diseases. This has in turn led to the identification of some disease mechanisms. It is widely believed that this type of work will result in the development of the so far elusive mechanism-based therapies. In conjunction with the ability to identify at-risk individuals, these therapies constitute a major goal for research into neurodegenerative diseases.

**The state of the science**

Existing animal models of neurodegenerative diseases do in general not reproduce all aspects of the human diseases. However, they do faithfully model many of the disease-defining neuropathological lesions. Most animal models rely on the overexpression of human disease proteins carrying causative mutations, consistent with a gain-of-toxic function mechanism of disease. The most commonly used species are mouse, zebrafish, *Drosophila melanogaster*, *Caenorhabditis elegans* and yeast. Experimental models continue to contribute to

the elucidation of disease mechanisms and the identification of novel diagnostic and therapeutic strategies. They have played an important part in moving the field forward. Specific examples include: the discovery of neuronal intranuclear inclusions in a transgenic mouse model of Huntington's disease; the finding that A $\beta$  deposition can be prevented and existing deposits removed following immunisation of mice transgenic for human mutant amyloid precursor protein; the finding that tau inclusion formation is promoted in the presence of A $\beta$  or Danish amyloid aggregates; the identification of mechanistic similarities between prion diseases and more common neurodegenerative diseases. Besides facilitating the discovery of disease mechanisms, animal models continue to make it possible to identify genetic and pharmacological modifiers of neurodegeneration, especially in invertebrate models.

### **Future opportunities**

Considering that the basic framework of research into neurodegeneration is now solidly established, this is a good time to look forward. It will be important to standardise existing animal models by taking their genetic backgrounds into account. It will be equally important to understand how overexpression of disease proteins in animal models relates to the mechanisms operating in human diseases with a long preclinical phase and without disease protein overexpression. This may lead to the development of more complete animal models of human disease. Elucidation of the role played by pathways operating during human brain ageing will be essential for understanding neurodegeneration. Much needs to be learned about cellular mechanisms that can protect against the deleterious effects of aggregation-prone proteins. This may open up new therapeutic avenues and increase understanding of the relative importance of cell-autonomous and cell-nonautonomous mechanisms. Overall, there is a need for techniques that make it possible to follow neurodegenerative processes in animal models in a non-invasive manner using the imaging and biochemical tools that are being developed for the human conditions.

### **Barriers to progress**

Understanding neurodegeneration is an interdisciplinary undertaking. A major barrier to progress lies in the insufficient standardisation of existing animal models and their incomplete characterisation, which makes it difficult to relate individual findings to what is going on in diseased human brain with confidence. There is a need to cross-validate animal models and to investigate modifiers in multiple models. More often than not, a gulf exists between work on vertebrate and invertebrate models of disease. Pharmacological modification of

neurodegeneration constitutes a major goal, with animal models being essential for the testing of compounds. Animal models also play an important role in the identification of biochemical targets. However, the high-throughput screens needed to identify relevant compounds are often beyond those who would like to test them in their animal models.

### **Recommendations for action**

Progress in science cannot be predicted and has been said to depend “on new techniques, new discoveries and new ideas, probably in that order” (S. Brenner). However, it can be facilitated, especially in a field as mature as this one. The interdisciplinary nature of the work and the need for cross-validation and standardisation call for collaborations involving groups with complementary skills and shared objectives.

The COEN Workshop on “Animal Models of Human Neurodegenerative Diseases” (Cambridge, 25<sup>th</sup>-26<sup>th</sup> November 2010) was designed to establish the state of the science, to identify future opportunities and to discuss how barriers to progress can be overcome. It consisted of 18 talks followed by discussion ([Annex I](#)). The presentations were provided by experts from the 3 founding COEN partners (UK, Germany and Canada), in addition to leading scientists from Europe, USA and Australia. Abstracts of talks can be found at [Annex II](#). In total 65 invited scientists from the 6 COEN partners and across Europe, took part in the Workshop. A list of participants can be found at [Annex III](#).

A number of topics were identified over the course of the meeting as being important areas of opportunity or barriers to progress :

- **New pathogenic mechanisms.**

Opinion was divided on the validity of many mouse models of neurodegenerative disease and their translational relevance to clinical studies. Some delegates viewed current models as sufficient to explore pathology, whereas others considered models to be incomplete but useful for mechanistic study. Delegates were in agreement that a programme to develop and make available new mechanistic models to explore pathogenic mechanisms, for example addressing chronicity and ageing, would be of great value to the community.

- **Role of genetic background and effects of multiple mutations.**

Following on from the debate about the predictive nature of animal models, it was acknowledged that consideration of the genetic background of the mouse strains used by different laboratories was important, and there needed to be validation of phenotypes across multiple backgrounds, in particular with regard to behavioural readouts; a programme which back-crossed models on different genetic backgrounds was proposed.

- **Novel biomarkers detecting early changes.**

Translation from animal models should allow the identification of potential physiological and behavioural markers associated with the preclinical state in humans.

- **Commonalities between acute and chronic animal models.**

Understanding such commonalities would provide exceptional insights into mechanisms. It would require modelling of the progression of disease *in vivo* with longitudinal evaluation of pathology and molecular changes, combined with identification of new biomarkers and production of appropriate transgenic models for real-time imaging in acute and chronic states.

- **Long-term *in vivo* imaging.**

Bioluminescence provides the potential for long term *in vivo* imaging. Further development of this technology will be vital to enable study of acute and chronic disease and the monitoring of transgene expression.

- **New animal models based on the findings from genome-wide association studies.**

With an increasing number of candidate genes identified through genome-wide association studies, there is a need to generate and validate new animal models based on emerging risk alleles as well as copy number variants. This may also require the use of other model systems, or combinations of physiological and behavioural readouts. Either

collaborative networks or a dedicated facility to coordinate such efforts would facilitate rapid assessment of new targets.

- **Disease mechanisms in different species.**

Fly and worm models are important systems for high-throughput drug screening, identification of chemical hits and reprofiling of existing drugs. The genetic tractability and existence of RNAi knockdown libraries in these systems was also seen as an advantage; however, lack of homology to human genes and the requirement to validate in higher systems were viewed as limitations. It was noted that vertebrate models amenable to high-throughput screening were emerging through the development of zinc finger knockout technology. Overall delegates agreed that it would be important to consider the best model for the question posed and that collaborative work across model platforms would be the most effective means to progress research and overcome barriers.

- **Antibody repository and core production facility.**

To provide standardised and cost-effective antibodies against different forms of protein aggregates for defining toxic species.

- **Mouse models based on the grafting of differentiated induced pluripotent stem cells.**

Induced pluripotent stem cells are being derived from skin biopsies of individuals with inherited neurodegenerative disease, followed by their differentiation into defined nerve cell types. Grafting of these nerve cells into mouse brain may give rise to novel models of neurodegeneration, which will be especially valuable for the study of the spreading of misfolded disease proteins.

- **Additional topics.**

A number of additional topics were considered to be key for progress in the field. They include:

- i. A $\beta$  – Tau interactions.
- ii. Mouse embryonic stem cell core facility.
- iii. Novel, mechanism-based treatments (potentially hitting biomolecular pathways at more than one point).

- iv. Understanding neuroprotective pathways to provide tractable targets for pharmacological intervention across a neurodegenerative disease.

In conclusion, while a number of themes and approaches were debated, three particular areas were endorsed most enthusiastically as those which would benefit from collaborative approaches and provide input into technological development and the provision of shared infrastructure for the community:

- i) Identification of novel pathogenic mechanisms in animal models that are relevant to human neurodegenerative diseases.**
- ii) Identification of early biomarkers in animal models that are relevant to human neurodegenerative diseases.**
- iii) Production and characterization of improved animal models of human neurodegenerative diseases.**

**Annex I**

**COEN WORKSHOP ON ANIMAL MODELS OF  
HUMAN NEURODEGENERATIVE DISEASES**

**MRC Laboratory of Molecular Biology  
Cambridge, UK**

**25<sup>th</sup> – 26<sup>th</sup> November 2010**

**Thursday 25<sup>th</sup> November**

12.00 LUNCH Foyer

13.00	Michel Goedert	<b>Introduction</b>
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Chair: Michel Goedert		<b>The value of model organisms</b>
13.15	David Sattelle	<i>C. elegans</i>
13.35	Mel Feany	<i>D. melanogaster</i>
14.05	Christian Haass	Zebrafish
14.25	Mathias Jucker	Mouse
14.55	Claudio Cuello	Rat

15.15 REFRESHMENTS Foyer

Chair: Eva Maria Mandelkow		<b>Disease proteins</b>
15.45	David Westaway	Prions
16.05	Giovanna Mallucci	Prions
16.25	Sarah Lloyd	Prions
16.45	Lennart Mucke	A $\beta$ and Tau
17.10	Jürgen Götz	A $\beta$ and Tau
17.40	Wolfgang Wurst	European conditional mouse mutagenesis programme

19.30 DINNER University Arms Hotel, Cambridge

**COEN WORKSHOP ON ANIMAL MODELS OF  
HUMAN NEURODEGENERATIVE DISEASES**

**Friday 26<sup>th</sup> November**

Chair: Pierrette Gaudreau		<b>Disease proteins Monitoring neurodegeneration</b>
09.00	Chris Shaw	TDP-43 and FUS
09.25	Jochen Herms	Two-photon imaging
09.45	Kurt Giles	Bioluminescence imaging

10.05 REFRESHMENTS Foyer

Chair: Markus Tolnay		<b>Mechanisms and therapeutic approaches</b>
10.40	Patrick Aebischer	Using viruses to study disease
11.10	David Rubinsztein	The relevance of autophagy
11.40	Jean-Pierre Julien	The usefulness of immunotherapy

12.10	Robin Buckle	The COEN initiative
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12.25 LUNCH Foyer

13.30	Chairs:	<b>Discussion</b>
	Bart De Strooper (Model organisms)	
	Pierluigi Nicotera (Disease proteins)	
	Paul Matthews (Monitoring neurodegeneration)	
	Michel Goedert (Mechanisms and therapeutic approaches)	

15.30 End of Workshop

## Annex II

### Summaries – COEN Workshop talks

#### Patrick Aebischer

The discovery of gene mutations leading to familial forms of Parkinson's disease has vastly improved our understanding of the cause of neuronal degeneration. However, the discovery of effective neuroprotective treatments has been hampered by the lack of mammalian models that faithfully replicate cardinal disease features. To complement existing transgenic animals, we have developed viral vectors to express, in adult dopaminergic neurons, mutated proteins implicated in autosomal dominant inherited Parkinson's disease. Following intranigral injection of adenoviral vectors, the expression of human  $\alpha$ -synuclein leads to motor impairment, a phenotype initially due to impaired vesicular release prior to overt neurodegeneration. We show that the induced lack of dopamine release crucially depends on the ability of  $\alpha$ -synuclein to bind lipid membranes, highlighting a critical role in the homeostasis of organelles involved in neurotransmission. Similarly, we show that adenoviral vectors can adapt the large cDNA encoding human LRRK2, and retrogradely transduce rat nigral neurons from their striatal terminals. This mode of gene delivery leads to an acute loss of dopaminergic neurons in response to the dominant G2019S LRRK2 mutation. Neurons expressing LRRK2 show transient signs of endogenous tau phosphorylation, confirming the upstream role of LRRK2 on pathogenic proteins. As illustrated by these results, viral vectors open new research avenues to elucidate how Parkinson's disease-associated proteins lead to disease in the adult mammalian central nervous system.

#### Claudio Cuello

The importance of investigating the early Alzheimer's-like amyloid pathology was stressed, as the initial "preclinical" stages could give us the opportunity of finding new therapeutic targets and much needed biomarkers. The importance of elucidating the complex molecular preclinical events and behavioural consequences are highlighted by the dramatic (and costly) failure of therapeutics administered after the onset of clinical disease. I made the case that such investigations can be best conducted in transgenic models of evolved species and illustrated the merits of using rat transgenic models. This was exemplified by the current studies with the McGill-R-Thy1-APP rat transgenic model, which carries only one transgene [human mutant amyloid precursor protein (APP)] copy per allele, as opposed to more "genetically aggressive" models. Rats have richer cognition and a more complex social behaviour than mice. They have also a more evolved central nervous system (CNS), with postnatal CNS maturation resembling that of humans. This model has shown that the A $\beta$  oligomer burden provokes cognitive impairment and suffices to disrupt key metabolic (e.g. ERK-CREB-CRE-regulated gene expression; NGF) pathways, the cortical and hippocampal proteomic patterns, and results in diminished context discrimination of hippocampal place neurons. The model also allows the repeated sampling of cerebrospinal fluid for the search of new biomarkers for early Alzheimer's disease.

#### Mel Feany

The powerful genetic tools available in the simple model organism *Drosophila* have long made the fruit fly a favourite experimental system for investigating basic biological processes. More recently, *Drosophila* has also emerged as a

powerful tool for the study of human neurodegenerative diseases. A number of fly models of human neurodegenerative disorders have been created, including models relevant to Alzheimer's disease and Parkinson's disease. These models often show remarkable similarity to their human counterparts from the behavioural, biochemical, and neuropathological perspectives. Fly models of human neurological diseases have been used productively to perform forward genetic screens, test specific hypotheses, and screen drug collections. More recently, *Drosophila* models have emerged as a useful system in which to investigate increasing numbers of genes emerging from genome-wide association studies. While confirmatory studies in vertebrate models and the human disease itself are needed to validate the insights that emerge from fly models, *Drosophila* models of neurodegenerative disease provide a useful complement to disease models in other organisms.

### **Kurt Giles**

Bioluminescence imaging offers a new paradigm for research in neurodegenerative diseases. Previously, the only way to determine disease progression was to euthanize the animal and study the brain pathology or biochemistry. Using transgenic mice expressing a luciferase reporter under the control of the glial fibrillary acidic protein promoter, we have shown that prion disease can be measured, in live mice, in less than half the time compared to onset of clinical signs. Applying this to drug studies, we have demonstrated that bioluminescence imaging predicts drug efficacy. We then went on to show that the same principle enables *in vivo* monitoring of disease progression in models for Alzheimer's disease.

### **Jürgen Götz**

The interaction between A $\beta$  and tau was assessed, starting with the key pathologies displayed by wild-type and mutant tau transgenic mouse models. Using iTRAQ quantitative proteomics and functional assays, evidence was presented that A $\beta$  and tau both separately and synergistically impair mitochondrial functions. A novel dendritic function of tau in mediating A $\beta$  toxicity was also presented. Tau targets the src kinase Fyn to the dendritic spine, where it phosphorylates the NR2b subunit of the NMDA receptor, leading to stabilization of the NMDAR-PSD95 complex. Disrupting this complex pharmacologically or by crossing A $\beta$ -producing mice onto a tau null background or with mice overexpressing a truncated form of tau (that is excluded from dendrites) abrogates the toxicity that characterizes A $\beta$ -producing mice.

### **Christian Haass**

Zebrafish was presented as a model system for the functional analysis of genes associated with neurodegeneration. As an example, the morpholino-mediated knockdown of BACE1 was presented, which leads to a dramatically reduced escape response. Similar findings were observed upon treatment with BACE inhibitors. Moreover, a driver- and responder-based expression system for the efficient generation of transgenic zebrafish lines was described (tau transgenic zebrafish lines were generated). Tau transgenic fish were used to screen *in vivo* for kinase inhibitors and to evaluate the potential of methylene blue. Zinc finger technology allowed the specific mutation of both TDP-43-like genes and resulted in a phenotype with reduced blood flow. Moreover, zebrafish was used to image tau-dependent neuronal cell death and mitochondrial transport *in vivo*. Thus, zebrafish is perfectly suited to the functional analysis of genes related to

neurodegeneration, to drug screening even in a semi high-throughput format and to *in vivo* imaging.

### **Jochen Herms**

The usefulness of long-term *in vivo* two-photon imaging for understanding the mechanisms of amyloid plaque growth and nerve cell loss, as well as the kinetics of spine loss and dendritic destruction throughout the progression of Alzheimer's disease, was presented. Individual methoxy-X04-stained amyloid plaques were imaged over a period of 6 weeks in 12- and 18-month-old Tg2576 mice. In 12-month-old mice, newly appearing amyloid plaques were initially small in volume and subsequently grew over time. The growth rate of plaques was inversely proportional to their volume, thus amyloid plaques that were already present at the first imaging time point grew over time, but more slowly compared to new plaques. In 18-month-old Tg2576 mice, newly appearing plaques were never observed, nor was there a significant growth of preexisting plaques measured during the 6 weeks of imaging. Moreover, data were presented showing that dendritic spine loss in triple transgenic (3xTG)-AD mice (mutant APP, mutant presenilin-1, mutant tau) occurs independently of A $\beta$  plaque formation. At 4-6 months of age, spine loss was found to be the consequence of layer III nerve cell loss, whereas spine loss in old 3xTG-AD mice was due to dendritic pathology. Nerve cell loss depended on the expression of the fractalkine receptor on the surface of microglia, whereas dendritic pathology in old 3xTG-AD mice was due to the accumulation of hyperphosphorylated tau. We conclude that spine loss in transgenic animal models for Alzheimer's disease, at least in the cerebral cortex, is not a primary event caused by A $\beta$  oligomers, but is secondary to nerve cell loss or dendritic failure.

### **Mathias Jucker**

Age-related neurodegenerative diseases are largely limited to humans and only rarely occur spontaneously in animals. Genetically engineered mouse models recapitulate aspects of the human diseases and are instrumental for studying disease mechanisms and for testing therapeutic strategies. If considered within the range of their validity, the models have been predictive of clinical outcome. Translational failure is less the result of the incomplete nature of the models than of inadequate preclinical studies and misinterpretation of the models. There is a need for large preclinical mouse studies with clinically relevant biomarkers. A promising avenue to improve the predictive value of mouse models for clinical studies would be to adapt aspects of the design, quality control and transparency of clinical studies in humans. Most importantly, the outcome measures of preclinical studies should focus on parameters and biomarkers with proven relevance in clinical settings, or which can be tested in the clinic. For example, A $\beta$ -imaging and cerebrospinal fluid (CSF) measurement of A $\beta$  and tau have become valuable biomarkers for the diagnosis and progression of Alzheimer's disease. However, few mouse studies have analyzed A $\beta$  and tau in CSF, despite the prospect that such studies could help to clarify the mechanisms governing changes in levels of the proteins in human CSF. Rather, a primary endpoint of most mouse studies has so far been *post mortem* neuropathology, which is arguably not very useful for translational purposes.

### **Jean-Pierre Julien**

The finding of a secretion pathway and toxicity for mutant superoxide dismutase-1 (SOD1) raised the possibility of using immunization approaches to reduce or

neutralize the burden of toxic SOD1 species in the nervous system. In order to develop a passive immunization approach, mouse monoclonal antibodies against misfolded forms of SOD1 were produced. Immunodetection of misfolded SOD1 occurred solely in motor neurons in the spinal cord of G93A-SOD1 mice. Intracerebroventricular infusion of antibodies against SOD1 resulted in a significant extension of life span of G93A-SOD1 mice. These results suggest that passive immunization strategies should be considered as a potential treatment for familial amyotrophic lateral sclerosis caused by SOD1 mutations. The use of bioluminescence for imaging neuroinflammation and neuronal damage in mouse models for amyotrophic lateral sclerosis expressing either mutant SOD1 or genomic fragments encoding wild-type or mutant TDP-43 was also described.

### **Sarah Lloyd**

This talk gave an overview of the breadth of the mouse modelling work carried out at the MRC Prion Unit. Through the use of human PrP transgenic mice, bovine spongiform encephalopathy (BSE) was considered to be transmissible to humans. Such mouse lines are also able to evaluate the risk from emerging diseases and infected tissues. Disease arrest and recovery in conditional knockout mice provided proof of principle that targeting PrP<sup>c</sup> is therapeutically valuable and this is now being pursued through immunotherapy and small molecule screening strategies. For prion diseases, the mouse is not truly an experimental model, as wild-type animals are naturally susceptible to disease. This provides an ideal opportunity to exploit the diversity available to look for disease modifier genes and provides a test bed for therapeutic approaches to target common mechanisms of neurodegeneration.

### **Giovanna Malluci**

Prion diseases are rare neurodegenerative diseases, but have great advantages for providing mechanistic insights. Firstly, the central pathogenic mechanism is clear and easily accessible; secondly, prion-infected mice are a key model system for understanding fundamental mechanisms of neurodegeneration. We showed that mice with prion infection can be cured at the stage of early synaptic dysfunction, when they have reversible impairments at neurophysiological, behavioural and morphological levels. Reversing this early stage of dysfunction allows long-term neuronal survival and clinically cures the animals, both in transgenic mice and using lentivirally-mediated RNA inhibition. Synaptic dysfunction is a key early phase in all neurodegenerative disorders and we hypothesise that common mechanisms underlie this dysfunction, irrespective of the specific disease entity, representing a window for intervention and access to pathways for therapy across the spectrum of these disorders.

### **Lennart Mucke**

To be useful, experimental models do not necessarily have to simulate all aspects of human disease. In fact, their power may lie more in the dissection than in the recapitulation of the complexity encountered in dementing conditions, such as Alzheimer's disease. Human amyloid precursor protein (hAPP) transgenic mice are good models, not only of amyloid deposition, but also of synaptic and cognitive dysfunction caused by pathogenic A $\beta$  oligomers. Compound transgenic mice have been used to identify molecules that modulate this dysfunction, including the tyrosine kinases Fyn and EphB2, the group IVA isoform of phospholipase A<sub>2</sub>, the microtubule-associated protein tau and apolipoprotein E.

Notably, all of these factors also modulate aberrant excitatory neuronal activity, which appears to contribute critically to network dysfunction in hAPP mice and, possibly, also in Alzheimer's disease. To explore the therapeutic potential of these and other leads, the establishment of a centre for the comprehensive, independent analysis of experimental animal models in rigorously designed preclinical trials was recommended.

### **David Rubinsztein**

(Macro) autophagy is a bulk degradation process that mediates the clearance of long-lived proteins and organelles. Autophagy is initiated by double-membraned structures, which engulf portions of the cytoplasm. The resulting autophagosomes fuse with lysosomes, where their contents are degraded. This process is a key regulator of the levels of intracytoplasmic aggregation-prone proteins that cause neurodegenerative diseases, including mutant huntingtin (in Huntington's disease), mutant  $\alpha$ -synuclein (in forms of Parkinson's disease), and wild-type and mutant forms of tau (in various dementias). The clearance of such substrates is retarded when autophagy is compromised. Autophagy-upregulating drugs enhance the clearance of mutant huntingtin, mutant ataxin 3 (that causes a form of spinocerebellar ataxia), and mutant and wild-type forms of tau in a range of animal models (flies, zebrafish and mice) and attenuate the toxicities of these proteins *in vivo*. When we initiated our studies, the only known pharmacological way of inducing autophagy chronically was with rapamycin. Although rapamycin is designed for long-term use, it has side effects which may make it unattractive to patients who may need to take the drug for decades. As far as we are aware, these side effects are unrelated to rapamycin's autophagy-inducing properties. Thus, we have embarked on a series of studies to identify novel autophagy-upregulating compounds and have discovered pathways that are independent of the target of rapamycin. We have shown that drugs acting on such pathways are protective in fly and zebrafish models of Huntington's disease and have recently shown benefits for one of these drugs in a mouse model. We have also tried to understand more about the machinery regulating mammalian autophagy. Our initial motivation for these studies was that this would lead us to identify novel specific therapeutic targets, and this has been the case. These studies have also led to important insights about the roles of autophagy in a range of diseases, including Parkinson's disease associated with  $\alpha$ -synuclein overexpression, forms of motor neuron disease caused by mutations in the dynein machinery and lysosomal storage diseases.

### **David Sattelle**

The model organism *C. elegans* provides ready access to forward and reverse genetics and is increasingly used to model aspects of human nervous system and neuromuscular disorders. Transgenic lines expressing human proteins involved in neurodegenerative disorders, such as tau and A $\beta$ , and mutants in orthologues of proteins which underlie human familial neurodegenerative disorders are also deployed. New developments in automated behavioural phenotyping are facilitating large-scale genetic screens and chemical library screens in the search for genetic and chemical suppressors of adverse pathologies. *C. elegans* can also help leverage our investment in genome-wide association studies to yield insights into the roles of candidate risk factors and their potential as drug targets. Whilst recognising the limitations of invertebrate models, the speed, low-cost and high-throughput of such *in vivo* screens are attractive in functional follow-up of findings from genome-wide association studies, as well as in accelerating towards the clinic new chemistry and drug re-use.

## **Chris Shaw**

The transactive repressor DNA-binding protein 43 (TDP-43) is the major protein constituent of detergent-resistant neuronal cytoplasmic inclusions in 90% of amyotrophic lateral sclerosis (ALS) and tau-ve frontotemporal lobar dementia cases. TDP-43 is a ubiquitously expressed protein that has two RNA binding domains (RRMs), nuclear localisation and export signals and a C-terminal glycine-rich domain that is involved in mediating protein-protein interactions. TDP-43 regulates RNA transcription, splicing and transport and is part of the large Drosha complex that regulates micro-RNA biogenesis. Mutations in the glycine-rich domain are linked to familial and sporadic amyotrophic lateral sclerosis and appear to increase the toxicity of TDP-43 in transfected chick spinal neuronal precursors. Deletion of the RRM1, RRM2 and C-terminal domain ameliorate or abolish toxicity in transduced yeast cells and motor neurons of *Drosophila* and chick. The RRM2 and C-terminal domains are essential for self-association and oligomer formation. Published mouse models overexpressing mutant (A315T) or wild-type TDP-43 demonstrate age-dependent neurodegeneration, but only partially replicate human pathology. Knock out and overexpression of TDP-43 greater than 3-fold over endogenous are embryonic lethal. In summary, the RNA binding and self-association properties of TDP-43 appear to be critical in initiating neurodegeneration. Knock-in transgenic mice may provide a better model for disease.

## **David Westaway**

Prion infections can be modeled in many animals without recourse to genetic engineering. The disease process gives hard endpoints, including overt nerve cell loss. However, in distinction to other neurodegenerative diseases, prion replication to generate PrP<sup>Sc</sup> - misfolded from PrP<sup>C</sup> - can be uncoupled from cellular pathogenesis, e.g. prion replication in peripheral tissues gives no pathology and mice with one copy of the PrP gene (*PRNP*) accumulate PrP<sup>Sc</sup> hundreds of days before clinical symptoms appear. Prion replication to generate PrP<sup>Sc</sup> can be recreated *in vitro*, in cell culture and in cell-free systems. Genetic prion diseases are caused by a plethora of human *PRNP* mutations, but only four have been modeled in transgenic mice, and these animals have had partial or controversial neurological phenotypes. Sporadic prion disease can be attributed to spontaneous misfolding of PrP<sup>C</sup> and can perhaps be modeled in cell culture and cell-free systems. One avenue of current research to understand PrP misfolding focuses on the transition metal binding capabilities of the PrP N-terminal domain: this capability may represent an ancient feature of PrPs inherited in an evolutionary descent from the ZIP family of zinc transporters.

## **Wulfgang Wurst**

This presentation introduced the efforts of the European conditional mouse mutagenesis program (EUCOMM), the international mouse knockout consortium (IKMC) and the EUCOMMTOOLS program. These consortia aim at generating mouse models for every gene and to make it possible to inactivate any gene in any cell at any point during the life of the animal. So far, EUCOMM/IKMC have generated more than 12,000 embryonic stem cell lines and 1,000 mouse knockout lines which are available to the scientific community. The usefulness of this approach was illustrated with the production of models of inherited Parkinson's disease.

## Annex III

**COEN Workshop on Animal Models of Human Neurodegenerative Diseases  
MRC Laboratory of Molecular Biology, Cambridge: 25th - 26th November 2010**

Last Name	First Name	Department / Institute / University
Aebischer*	Patrick	Ecole Polytechnique Fédérale, Lausanne, Switzerland
Attems	Johannes	Institute for Ageing and Health, Newcastle University, UK
Balling	Rudi	Centre for Systems Biomedicine, University of Luxembourg
Baulieu	Etienne	INSERM UMR788, University of Paris XI, France
Bertolotti	Anne	MRC Laboratory of Molecular Biology, Cambridge, UK
Blandini	Fabio	IRCCS Neurological Institute, University of Pavia, Italy
Bolam	Paul	MRC Anatomical Neuropharmacology Unit, Oxford, UK
Breen	Kieran	Parkinson's Disease Society, London, UK
Buchman	Vladimir	School of Biosciences, Cardiff University, UK
Buckle	Robin	MRC Head Office, London, UK
Buée	Luc	INSERM U837, University of Lille, France
Bujdoso	Raymond	Dept. of Veterinary Medicine, Cambridge University, UK
Bussey	Tim	Dept. of Experimental Psychology, Cambridge University, UK
Chambraud	Beatrice	INSERM UMR788, University of Paris XI, France
Ciana	Paolo	Department of Pharmacological Sciences, University of Milan, Italy
Coleman	Michael	Babraham Research Institute, Cambridge, UK
Collinge	John	MRC Prion Unit, University College London, UK
Collingridge	Graham	MRC Centre for Synaptic Plasticity, University of Bristol, UK
Compston	Alastair	Dept. of Clinical Neurosciences, Cambridge University, UK
Crowther	Damian	Dept. of Genetics, Cambridge University, UK
Crowther	Tony	MRC Laboratory of Molecular Biology, Cambridge, UK

Cuello*	Claudio	Dept. of Pharmacology, McGill University, Montreal, Canada
Davies	Megan	MRC Laboratory of Molecular Biology, Cambridge, UK
Davies	Stephen	Dept. of Cell and Developmental Biology, University College London, UK
De Strooper	Bart	VIB Dept. of Molecular and Developmental Genetics, University of Leuven, Belgium
Di Monte	Donato	DZNE, University of Bonn, Germany
Eberhart	Astrid	Canadian Institutes for Health Research, Toronto, Canada
Ermer	Veronika	DZNE, University of Bonn, Germany
Fawcett	James	Dept. of Clinical Neurosciences, Cambridge University, UK
Feany*	Mel	Dept. of Pathology, Harvard University, Boston, USA
Fisher	Elizabeth	Institute of Neurology, University College London, UK
Fraser	Paul	Centre for Research in Neurodegenerative Diseases, University of Toronto, Canada
Gaudreau	Pierrette	Dept. of Medicine, University of Montreal, Canada
Giese	Peter	Institute of Psychiatry, King's College London, UK
Giles	Katherine	MRC Head Office, London, UK
Giles*	Kurt	Institute for Neurodegenerative Diseases, University of California San Francisco, USA
Goedert	Michel	MRC Laboratory of Molecular Biology, Cambridge, UK
Götz*	Jürgen	Brain and Mind Research Institute, University of Sydney, Australia
Grant	Seth	Wellcome Trust Sanger Centre, Cambridge, UK
Haass*	Christian	DZNE, University of Munich, Germany
Hardy	John	Institute of Neurology, University College London, UK
Hastings	Michael	MRC Laboratory of Molecular Biology, Cambridge, UK
Heneka	Michael	Dept. of Neurology, University of Bonn, Germany
Herms*	Jochen	Centre for Neuropathology and Prion Research, University of Munich, Germany
Heuschling	Paul	Life Sciences Research Unit, University of Luxembourg
Höglinger	Günter	Biomedical Research Centre, University of Marburg, Germany
Holzer	Max	Paul Flechsig Institute of Brain Research, University of Leipzig, Germany

Houart	Corinne	MRC Centre for Developmental Neurobiology, King's College London, UK
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Sastre	Magdalena	Dept. of Medicine, Imperial College London, UK
Sattelle*	David	MRC Functional Genomics Unit, Oxford, UK
Shaw*	Christopher	MRC Centre for Neurodegeneration Research, King's College London, UK

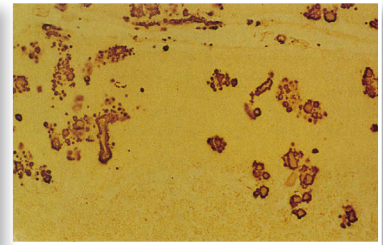
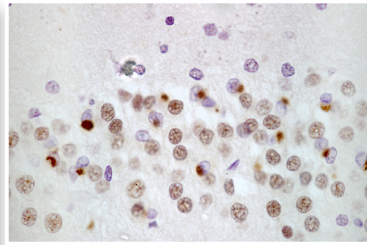
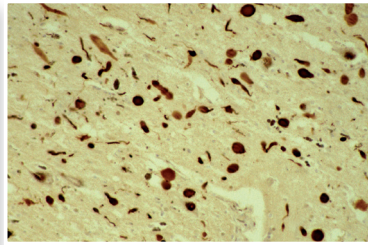
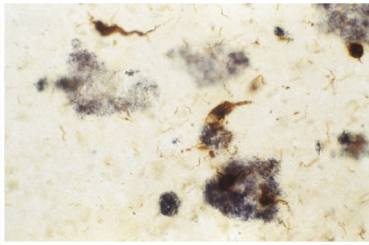
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# COEN Workshop on Animal Models of Human Neurodegenerative Diseases

MRC Laboratory of Molecular Biology, Cambridge, UK

25th - 26th November 2010



## Speakers

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M. Feany (Boston), K. Giles (San Francisco),  
J. Götz (Sydney), C. Haass (München),  
J. Herms (München), M. Jucker (Tübingen),  
J.P. Julien (Quebec), S. Lloyd (London),  
G. Mallucci (Leicester), L. Mucke (San Francisco),  
D. Rubinsztein (Cambridge), D. Sattelle (Oxford),  
C. Shaw (London), D. Westaway (Edmonton)**

## Participants

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