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Pathologies du mépliement des protéines : processus moléculaires et perspectives thérapeutiques

Protein misfolding in disease: molecular processes and translational research toward therapy

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

La conférence « Pathologies du mépliement des protéines : processus moléculaires et perspectives thérapeutiques » s'est déroulée du 13 au 17 avril 2013 à Roscoff. Il s'agissait de la troisième conférence d'une série débutée en 2007. Les aspects de biologie structurale prédominants dans la première conférence se sont d'abord élargis aux aspects cellulaires en 2010 et aux modèles animaux en 2013. Le mépliement des protéines a été abordé à travers de nombreuses maladies neurodégénératives (Alzheimer, Huntington, Parkinson…) et certaines amyloïdoses périphériques. Cette conférence était centrée sur le mépliement, la transmission, la propagation des protéines modifiées dans ces pathologies et aussi les développements thérapeutiques.

Parmi les 101 participants venant de différents pays (Allemagne, Belgique, Bulgarie, Canada, Etats-Unis, France, Israël, Pays-Bas, Royaume-Uni, Russie, Suisse), 26 conférenciers de renommée internationale étaient invités auxquels se sont ajoutées 12 orateurs sélectionnés parmi les résumés soumis. De l'avis de tous, les communications affichées étaient également excellentes.

La communauté a encore apprécié les aspects multidisciplinaires de la conférence permettant des rencontres inhabituelles et innovantes. Les exposés ont couvert les champs de la biologie structurale et cellulaire mais aussi le développement de modèles animaux (drosophile, nématode, rongeurs et primates) avec des approches de transgenèse ou vectorisation virale.

Les discussions animées qui ont suivi les présentations ont fait apparaître de nombreuses interrogations : quelle est la nature des espèces toxiques dans ces pathologies ? Fibres, oligomères, autres… Que doit-on inclure dans une propagation de type prion ? Uniquement les protéines prions ou faut-il définir un nouveau terme tel que prionide pour le peptide Aß, l'alpha-synucléine, l'huntingtine et Tau. La protéostasie (homéostasie protéique), c'est-à-dire comment un organisme va équilibrer synthèse et dégradation des protéines avec une balance protéasome et protéines chaperonnes, a été un fil conducteur intéressant. Enfin, il y a une diversité d'idées parfois contradictoires pour les perspectives thérapeutiques de ces pathologies.

Au total, la conférence a répondu à de nombreuses questions mais d'autres ont été soulevées indiquant un intérêt croissant pour cette problématique.

CONFERENCE REPORT

Overview

The conference was focused on protein misfolding diseases including Alzheimer's, Parkinson's, Huntington's and prion diseases as well as related Amyloidoses. This meeting followed on from the series of three previous meetings on protein misfolding and aggregation and, this time, centred around transmission, spreading and potential for therapies. This field has evolved and expanded and this meeting was generally agreed to be particularly timely and exciting. As in the previous meetings, the talks were multidisciplinary and spanned between protein chemistry and structure and cell biology. The programme included talks in which the research utilized model organisms and also those focused on *in vitro* research. The presentations generated very lively discussions regarding the nature of the toxic species, the definition of "prion-like" transmission and potential targets for novel therapies. Proteostasis was a central focus of discussion; in particular, the investigation of how organisms maintain their protein synthesis degradation balance via the proteasome and chaperone machinery.

We invited 26 internationally world-renowned speakers from diverse countries including Belgium, France, UK, Germany, the Netherlands, Switzerland and the USA. All the speakers we invited accepted enthusiastically. We also selected 12 talks from the submitted abstracts and accepted a total of 101 participants. We were delighted to accept participants from Russia, Israel and Bulgaria as well as having an excellent representation from European countries and North America.
The meeting was composed of six titled sessions and two poster sessions with all participants either presenting a talk or poster.

The sessions were entitled

I. Structure and assembly of toxic oligomers and fibrils
II. Intrinsic mechanisms of cell toxicity
III. The state of the organism: ageing and proteostasis.
IV. Clinical implications and anatomopathology of protein aggregation
***V.* Physiological and pathological responses to protein aggregation**
VI. Diagnostic and therapeutic approaches to protein misfolding disorders.

Summary of Lectures and associated discussion

The following provides a brief overview of the science discussed in the oral presentations.

The conference was opened by a plenary lecture from Dr Michel Goedert (Cambridge, UK) who gave an excellent introduction to tauopathies including Alzheimer's disease (AD). Exciting new data was presented showing propagation of isolated tau from specific tauopathies into transgenic (tg) Alz17 mice. This work underlines the importance of spreading in AD by Braak staging. In tg mice, the tau deposition spreads from the injection site and "breeds true".

I - Structure and assembly of toxic oligomers and fibrils

This session was separated into two sessions, A and B. In session A, Sheena Radford (Leeds, UK) described her work showing a comparision between catalysis and inhibition of aggregation by formation of alternative beta-2Microglobulin (B2M) complexes. These alternative complexes were shown to have different structures. Isabelle Landrieu (Lille, France) discussed her groups work to examine the structure of tau assemblies using solid state nuclear magnetic resonance (ssNMR) and suggested a role for heparin within the structure. Wei-Feng Xue (Kent, UK) gave a selected talk on the use of atomic force microscopy (AFM) to characterise stability of amyloid fibrils and their susceptibility to fragmentation and the relationship to the toxic species.

In session B, Louise Serpell described their work to characterise the structure of amyloid fibrils and the toxic effect of Aβ assemblies. John Viles (London, UK) described their work on Aβ interactions with human serum albumin, prion and copper II and the potential importance of these complexes for AD.

II - Intrinsic mechanisms of cell toxicity

Bart de Strooper (Leuven, Belgium) highlighted the importance of the β– and γ–secretases in the generation of Aβ and as potential drug targets. In particular, it was made clear that targetting particular γ–secretases could avoid potential side effects on notch signalling. Olga Corti (Paris, France) discussed the central role for mitochondrial proteins Pink1 and Parkin in Parkinson's disease (PD) for mitophagy. Anne Bertolotti (Cambridge, UK) showed that the proteasome has an important role in regeneration of amino acids for degrading proteins and that intervention to either enhance proteasomal function, providing essential amino acids, or pausing protein synthesis has potential for rescuing cells from degeneration. Andrea LeBlanc (Montreal, Canada) gave a selected abstract talk describing the phosphorylation of PrP by CDK5 which may enhance assembly. Another selected abstract talk was given by Helen Vignaud (Bordeaux, France) showing a yeast model system for studying Aβ aggregation to examine potential for cellular toxicity. Simon Ebbinghaus (Bochum, Germany) presented ground breaking methods development using fast relaxation imaging to follow protein folding using FRET data showing the association of labelled huntingtin (htt) exon 1, demonstrating the exciting potential of this method for monitoring protein folding and assembly in living cells.

III - The state of the organism: ageing and proteostasis.

Day 2 was opened with a talk by Rick Morimoto (Northwestern, USA) who uses *c.elegans* as a model system to study proteostasis and protein misfolding. He highlighted chaperone competition playing a role in the outcome of protein misfolding and also described cell to cell communication providing organism wide maintenance of stress responses. Frederic Saudou (Orsay, France) described a possible physiological role for htt in axonal vesicle transport via interactions with glyceraldehyde 6-phosphate dehydrogenase (GAPDH) using microfluidic chambers and imaging.

Ellen Nollen (Groningen, The Netherlands) followed the assembly of YFP-alpha-synuclein in *c.elegans* and revealed that this can lead to a reduction in tryptophan and that the phenotype may be rescued by adding Trp to the diet implicating the Trp degradation pathway in ageing and aggregation. Ina Vorberg (Bonn, Germany) gave a selected abstract talk showing the assembly and toxicity of yeast prion in mammalian cells and showing potential for transmission between adjacent cells and within brain slices. A selected abstract talk by Wouter Peelearts (Leuven, Belgium) described a new AAV-mediated animal model for PD.

IV - Clinical implications and anatomopathology of protein aggregation

Adriano Aguzzi (Zurich, Switzerland) questioned the use of the word prion-like and described his work showing toxicity of antibodies to PrP. Emmanuel Brouillet (Fontenay aux Roses, France) examined the differential vunerability of brain regions to htt aggregation. Maria Grazia Spillantini described the pathology of synucleinopathies and their results showing that α-synuclein can impair exocytosis at the synapse.

V - Physiological and pathological responses to protein aggregation

Session A was opened by Tricia Serio (Arizona, USA) who gave a presentation describing how Sup35 can be influenced by NatA highlighting the importance of aggregation size for cytotoxicity and the effect on cellular quality control pathways. Luc Bousset (Gif Sur Yvette, France) gave a selected abstract talk showing structural investigation of two structural polymorphs formed by α-synuclein giving alternative arrangements ofβ–strands within the fibres. Marie Alexander Albaret (Lyon, France) also gave a selected abstract talk introducing the term “abortosis” used by Herpes virus to maintain survival of host cells and showed an influence on Aβ42 aggregation. Byron Caughey (Montana, USA) showed transformation of PrPc to PrPsc removes the majority of α-helix and produces a β-sheet rich structure within the fibre. He also showed development of prion detection methods allowing detection down to picomolar levels.

On day 3, Session B was opened by Rémy Sadoul (Grenoble, France) who gave an excellent overview of the role for exosomes in intercellular communication particularly for neuronal cells and the potential for transmission of microRNAs as well as possibly other small molecules. Ronald Kopito (Stanford, USA) described his work showing the role for ubiquitination in the aggresome, with a particular focus on htt aggregation. He reiterated the view of previous speakers in highlighting the importance of chaperone competition in protein misfolding and the potential for protein misfolding in overwhelming the proteasome. Bart Dermaut (Lille, France) described a TDP43 model for Frontotemporal dementia-amylolateral sclerosis (FTD-ALS) in Drosophila. He showed that loss of TDP43 homologue in flies results defects resulting in wing deformation due to loss of specific neurons at the pupal stage.

Session C was opened by Gunilla Westermark (Uppsala, Sweden) showing interesting data showing the influence of Aβ on deposition of islet amyloid polypeptide (IAPP) in diabetes type 2 and coassembly of the two amyloidogenic proteins. Ana Maria Cuevo (New York, USA) is an expert on autophagosome formation and her talk focused on microautophagy in late endosomes

and showed that disease related variants of α-synuclein are unable to enter lysosomes and may assemble on the surface. Simon Dujardin (Lille, France) gave a selected abstract talk to show his new transgenic rat using lentivirus infection attached to human tau. This was able to show potential transmission of tau to distant regions. Ronald Melki described their new work showing that, if particle number is taken into account, then full-length, mature amyloid fibrils can be shown to permeabilise synthetic membrane vesicles and to be internalized into neuronal cells resulting in cell death. He showed that Hsc70 can prevent toxicity.

VI - Diagnostic and therapeutic approaches to protein misfolding disorders

In Session A, Fred Rousseau (Leuven, Belgium) gave the first talk of this session showing the groups' work showing the identification of aggregation prone peptides that can be found in tumour cells and can also be targeted to alter a particular cellular pathway resulting in phenotypic changes. He showed their work to develop peptides as antibiotic peptides. Luc Buée (Lille, France) described the tau isoforms in detail and means for targeted therapies in terms of associated effects such as cholesterol. Xinyi Li gave a selected abstract talk showing the synergistic effects of transthyretin (TTR) on Aβ and that Aβ is able to bind to TTR in the thyroxine binding site. Mark Dheinain (Fonternay-Aux-Roses, France) described their development of methods to enhance the detection of amyloid plaques in brain using Gadolininium to improve early diagnosis and also to enable evaluation of therapy efficacy.

On day four, Session B, Giovanna Mallucci (Leicester, UK) gave a selected abstract talk and described very exciting, recently published work showing the rescue of degenerative phenotypes in scrapie infected mice using drugs against PERK1 pathway. Priyanka Joshi (Cambridge, UK) gave a selected abstract talk that showed the development of computational methods to search for amyloidogenesis inhibitors using a model structure for Aβ. This exciting meeting was closed by Erich Wanker (Berlin, Germany) who highlighted the work on Huntington's disease using htt-Exon1 and the development of a series of conformational antibodies enabling the identification of different structural populations in the assembly pathway. This was used to evaluate potential aggregation inhibitors in terms of their potential to halt aggregation at particular points in the pathway. This was used to show the efficacy of a possible therapeutic called "O4" and was used to dissect the point of interaction in the pathway.

Conclusions and recommendations

This was the third meeting in a series of CJM meetings on protein misfolding and it was extremely successful. The content included the central theme of protein misfolding and the field has evolved towards issues regarding proteostasis, prion-like transmission and emerging therapies since the meeting in 2010. Major issues were discussed regarding cell to cell transmission, templated aggregation and the nature of the toxic species. The conference again attracted diverse participants from structural biologists and protein chemistry to cellular neuroscientists and clinicians.

It was generally considered that this was a highly successful, enjoyable and fruitful meeting resulting in excellent discussions regarding key issues in protein misfolding and yielding potential new collaborations. The participants were unanimous in their support for a fourth meeting and were excited about the prospect of this being held within 2 or 3 years. Prof. Luc Buee (Lille, France) will become Chair and he will be joined by Dr Ellen Nollen (Groningen, The Netherlands) as co-chair and they will submit an application to CJM for a follow up meeting in this series.

We were lucky enough to receive many highly complementary comments, one of which is quoted anonymously below

"just a short email to let you know that this was, honestly, ONE OF THE MOST EXCITING MEETINGS I have ever been to! Thank you so much for organizing it. Everything was perfect: talks, discussions, organization, interaction, food and even the weather. I had a wonderful time and came back very inspired with lots of new ideas."

Recommendations were made for the poster sessions to be longer (2 ½ hours) and for the posters to be housed within Gulf-stream hotel to allow a more permanent display of posters to generate discussion throughout the meeting.