Roscoff (France), 5-9 juin 2010

Mépliement et agrégation des protéines dans les maladies associées au vieillissement

Protein Misfolding and Aggregation in Ageing & Disease

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RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Mépliement et agrégation des protéines dans les maladies associées au vieillissement

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Le mépliement des protéines et leur agrégation dans la cellule et les tissus entraînent des dégénérescences cellulaires chez l'homme. Les rôles du mépliement des protéines et leur agrégation dans le vieillissement et les situations pathologiques, plus précisément dans le cas des maladies d'Alzheimer, de Huntington, de Parkinson et des maladies à prions a été examiné lors de la conférence.

Pour cette seconde conférence de la série consacrée aux maladies conformationnelles, la première s'étant tenue en 2007, nous avons invité des scientifiques qui s'intéressent au vieillissement cellulaire et aux processus d'agrégation des protéines. Les présentations ont porté sur les maladies d'Alzheimer, de Parkinson, de Huntington et sur des maladies affectant les moto-neurones. Les spécialités représentées allaient de la biophysique à la biologie cellulaire.

Les organisateurs ont reçus 127 candidatures avant la date limite d'inscription et plus de 7 après cette date. Ils ont du sélectionner 81 candidats parmi les 127 pour maintenir la taille de la conférence dans des limites permettant des interactions intenses et amicales, d'une part, pour tenir compte des capacités maximales de la salle de conférence du centre de Roscoff.

Sur les 34 scientifiques de renommée mondiale, qui ont tous accepté avec enthousiasme notre invitation, 32 ont pu venir à Roscoff. Ces chercheurs et 13 scientifiques, jeunes pour l'essentiel, sélectionnés à partir des résumés soumis, s'intéressant aux mépliement des protéines et leur agrégation et au vieillissement et les pathologies qui y sont liées ont pu présenter des travaux récents non publiés et ont fortement interagi entre eux et avec l'audience. Nous nous sommes tout particulièrement attaché à identifier de nouvelles cibles thérapeutiques, à comprendre les causes des pathologies et leur progression. Nous nous sommes aussi attaché, avec les autres participants qui ont pu présenter leurs travaux sous la forme de 69 affiches lors de deux sessions de deux heures, dans une atmosphère studieuse, imaginative et amicale, à identifier de nouvelles questions/problématiques.

Le programme que nous avons assemblé a permis de couvrir des problèmes ayant rapport avec le vieillissement, la structure des protéines mépliées, les mécanismes à l'origine de leur mépliement et les voies de mépliement des protéines, la protéostase cellulaire et le rôle des chaperons moléculaires et de la machinerie cellulaire en charge de l'élimination ou « clearance » des protéines mépliées. Ce programme a aussi permis de traiter des problèmes de signalisation cellulaire et de réponse inflammatoire accompagnant le mépliement des protéines et leur agrégation dans les cellules et organismes. La conférence a comporté 9 sessions :

- Évènements moléculaires dans l'agrégation des protéines
- Bases moléculaires de l'assemblage des protéines en structures oligomériques et fibrillaires toxiques

- Les conséquences pathologiques de l'agrégation des protéines
- Diversité des agrégats protéiques
- Facteurs cellulaires qui régulent le repliement des protéines et leur agrégation
- La signalisation, la neurodégénérescence, la plasticité neuronale et la réponse inflammatoire
- La réponse cellulaire à l'agrégation des protéines
- Élimination des agrégats protéiques
- Approches génétiques et thérapeutiques des désordres dûs au mépliement des protéines

Dans l'ensemble, 115 chercheurs de 16 pays différents (Allemagne, Australie, Belgique, Espagne, Etats-Unis d'Amérique, France, Grèce, Italie, Israel, Japon, Pays-Bas, Royaume-Uni, Russie, Singapour, Suède, Suisse) ont participé à la Conférence. Les échanges ont étés intenses, nombreux et particulièrement fructueux et amicaux présageant de nombreuses futures collaborations.

Étant donné l'importance du sujet abordé et les évolutions attendues dans un futur proche, les participants ont, à l'unanimité, demandé à ce que cette série continue. Ils ont désigné Luc Buée pour assister, comme vice-Président, Louise Serpell dans l'organisation de la future conférence de cette série. Luc Buée a accepté cette désignation,

Les organisateurs et les conférenciers remercient chaleureusement l'Institut Sciences biologiques et l'Institut écologie et environnement du CNRS qui ont soutenu financièrement la conférence et permis son organisation. Ils remercient aussi le soutien financier des sociétés Sanofi-Aventis et Farfield. Nous remercions Madame Lidoreau et son équipe pour l'organisation parfaite de la conférence et l'accueil chaleureux et efficace. Nous tenons enfin à remercier Mr Paoli pour l'intendance parfaite.

CONFERENCE REPORT

Final report from the Jacques-Monod Conference entitled: Protein Misfolding and Aggregation in Ageing & Disease

Roscoff, June 5-9, 2010

Background

The conference centred around discussion and exploration of the role of protein misfolding and abnormal aggregation in ageing and disease. This is a highly topical research area that addresses distressing diseases including Alzheimer's, Huntington's, Parkinson's disease and the transmissible "prion" diseases. This meeting followed from a very successful previous meeting held in 2007. For this second meeting in the series, we included scientists working on ageing that complemented those working in the protein aggregation field. The presentations spanned Alzheimer's disease, prions to Huntington's disease and motor neuron disease. The research specialists were from biophysics to cell biology. This series of conferences aims to bring together leading scientists with an interest in protein misfolding, aggregation, ageing and disease with younger scientists working in this area to discuss wide ranging issues regarding specifics of new therapeutic targets and understanding disease causes and progression and to identify new questions. The compact nature of the conference was particularly successful for generating lively discussions and for resulting in new collaborations.

We invited 33 world-leading scientists from all over the world and all accepted with enthusiasm. We put together a broad ranging, but focussed programme with leading scientists in the ageing field alongside those examining the structures of misfolded proteins, the mechanisms of protein misfolding, the role of cellular proteostasis, in particular that of molecular chaperones and the machinery in charge of misfolded protein clearance. The signalling pathways and inflammatory response accompanying protein misfolding were also evoked. The meeting was composed of 9 sessions with two poster sessions.

- Molecular events in protein aggregation
- Molecular basis of protein assembly into toxic oligomeric and fibrillar structures
- Disease implications
- Diversity of protein aggregates
- Cellular factors important for the regulation of protein folding and aggregation
- Signalling, neurodegeneration, brain plasticity and the inflammatory response
- Cellular responses to protein aggregation
- Clearance of protein aggregates
- Genetic and therapeutic approaches to protein misfolding disorders

13 short talks were invited from selected poster abstracts and these were placed within relevant sessions. All attendees presented posters.

The meeting obtained over 127 applications for places to attend the conference and only 81 places were available. The oversubscription of the meeting highlights the high regard for this series of meetings in the community and led to a selection of the very highest caliber attendees at the meeting. We carefully selected abstracts from young students and postdocs and the poster sessions were agreed to be of an excellent standard. Feedback regarding the talks also suggested a very high standard of presentations.

Summary of Lectures and Associated Discussions

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

Molecular events in protein aggregation - I. (Chairs Louise Serpell and Ulrich Hartl)

The meeting was opened by a Keynote lecture. This was an overview of the research field by Prof. Sheena Radford (Leeds, UK) who highlighted the challenges that still remain in the field for understanding proteins self-assembly and put the field in the context of the historical achievements. Her cutting edge research into the intermediate states of B2 microglobulin were also discussed. This was followed by a talk by Rosemary Staniforth (Sheffield, UK) showing her elegant model structure of cystatin amyloid fibrils formation involving domain swapping. David Teplow (UCLA, USA) described the influence of the slower aggregation, less toxic isoform Abeta 40 on Abeta 42 and described molecular dynamics simulations revealing a specific structural intermediate of Abeta 42 that may be relevant to the "toxic oligomer".

An abstract selected short talk by Motomasa Tanaka (Japan) replaced a cancellation by Jonathan Weissman and described structural insights using small angle X-ray scattering into two conformations of Sup35NM fragment assemblies, highlighting the importance of strains.

Molecular events in protein aggregation - II. Chairs Rosemary Staniforth and David Teplow.

Joost Schymkowitz (Brussels, Belgium) opened the session with a selected abstract talk describing a new algorithm designed to identify sequence determinants of amyloidogenicity, specifically involving spacial and structural knowledge. Eva Mandelkow (Hamburg, Germany) described the assembly of tau in Alzheimer's disease and suggested that "toxic tau" can poison native talk suggesting a prion like mechanism of templated aggregation. This was followed by a talk by Philippe Derreumaux (Paris, France) who discussed his work involving coarse-grained molecular dynamics simulations to follow assembly of the Sup35 fragment GNNQQNY showing specific cross-beta like structural intermediates on the assembly pathway.

Molecular basis of protein assembly into toxic oligomers and fibrillar structures. Chairs Sheena Radford

This session focussed particularly on advancements in the structure determination of amyloid fibrils, with X-ray fibre diffraction and biophysical studies revealing insights into side chain associations by Louise Serpell (Sussex, UK) and solid state NMR showing the elucidation of new structures of prion fibrils by Anja Bockman (Lyon, France) and Beat Meier (Zurich, Switzerland). Frederic Rousseau (Brussels, Belgium) revealed exciting new work showing that p53 assembles to form aggregates associated with tumour formation in cancers.

Disease implications of protein aggregation - I. Chairs Eva Mandelkow and Antoine Triller

The session was opened by Massimo Stefani (Florence, Italy), describing that HypF aggregates made under different growth conditions possess different toxic characteristics. In addition, he showed toxicity is related to membrane permeability and that the membrane composition is important. George Bloom (Virginia, USA) gave a selected short talk and described his work showing a connection between the two major AD pathologies and the interplay between Abeta oligomers and tau assembly and the effect on microtubules. A new protein, fyn was been described and the importance of caveoli in Abeta production. Luc Buee (Lille, France) showed work following the development of AD neurofibrillary tangles and the role for tau in AD pathology and disease progression. He raised a critical question that is not often dealt with: why neuronal death occurs several years after protein aggregation onset. He also discussed the therapeutic role for immunotherapy against tau.

Vittorio Bellotti (Pavia, Italy) described the clinical setting of dialysis related amyloidosis involving deposition of B2M and described possible therapeutic targets. Maya Schuldiner (Rehovot, Israel) described her innovative high-throughput screening system making use of GFP tagged proteins/peptides and yeast cells for examining the consequences of protein localization, expression and cell growth conditions on protein aggregation. She highlighted in particular the use of the tools she is developing in systems biology approaches.

Disease implications of protein aggregation - II. Chairs Alfred Goldberg and Luc Buée

David Lomas (Cambridge, UK) talked about his seminal work on the Serpin diseases, focussing particularly on deposition in liver and loss of function in lungs of alpha1-antitrypsin. He showed that polymers are toxic in fly brain and that aggregation can be prevented by short designed peptides. Exciting work was presented showing that skin cell from patients could be induced to form hepatocytes as a model for disease and potential therapy. By changing the expression levels of PrP, Ina Vorberg (Munich, Germany) showed that the infectivity of PrP aggregates is affected. Surprisingly, the overexpression appears to correlate with reduced infectious prion levels suggesting different pathways for PrP aggregation with a partitioning between infectious particles and non-infectious aggregates. Patrik Brundin (Lund, Sweden) brought in the clinical context of Parkinson's disease and described follow up of patients who were grafted embryonic dopaminergic neurons and showed that Lewy bodies develop in the grafted tissue thirteen year later which points to the possibility of disease transmission from cell to cell and spreading of disease throughout the brain. He then presented work suggesting this indeed occurs.

Diversity of protein aggregates. Chair Anja Bockman and David Lomas

Tricia Serio (Providence, USA) talked about her elegant studies of yeast prions propagation and the difference between strain phenotypes highlighting the possible involvement of yeast prion aggregates size. Ronald Melki (Gif-Sur-Yvette, France) described studies on the Ure2p yeast prion indentifying important regions within the natively folded domain of Ure2p that are essential for assembly and propagation. This was followed by a selected short talk by Ngai-Shing Mok (Leeds, UK) that showed the cryo-electron microscopy structure of the Ure2p fibril revealing clear large globular repeats along the fibre axis consistent with the Ure2p molecule retaining the native structure. Thomas Jahn (Cambridge, UK) gave a selected short talk on the toxicity of Abeta in a Drosophila model system showing that Abeta variants expressed in transgenic flies show AD like phenotypes.

Cellular factors important for the regulation of protein folding and aggregation. Chairs Tricia Serio and Rick Morimoto

Anne Bertolotti (Cambridge, UK) brought up the importance of the proteasome in protein degradation and described her focus on amylotrophic lateral sclerosis (ALS) and the Cu/Zn superoxide dismutase variant related to hereditary disease. She showed that mutants expose hydrophobic regions on the surface that may be related to increased propensity for aggregation and she suggested that aggregation could be mediated by the 19S proteasome.

Ellen Nollen (Groningen, The Netherlands), an expert working on polyglutamine aggregation in *C. elegans* identified modifiers of protein aggregation (moags). She described some potential moags she had identified and their human orthologs. Ulrich Hartl (Martinsreid, Germany) talked about the innovative work performed in his group aimed at exploring the cytotoxicity of a de-novo designed beta-sheet forming peptide and showed that the aggregates of this non-disease related peptide form amyloid-like aggregates that can be identified using the oligomer conformational antibody, A11. Proteome analysis highlighted upregulation of various proteins including mitochondrial and cytoskeletal proteins. Andrew Dillin (La Jolla, USA) is an expert on the unfolded protein response in mitochondria and he particularly focussed on ageing and longevity, contributing valuable knowledge to the meeting. This talk was followed by Antoine Triller (Paris, France) who talked about the development of a system using tagging Abeta oligomers with quantum dots to follow in a dynamic manner Abeta oligomers binding to the membrane and internalisation and calcium imaging to follow effects. He particularly highlighted the involvement of mGluR5 in the membrane and its role in toxicity.

Signalling, neurodegeneration, brain plasticity and the inflammatory response. Chairs Andrew Dillin and Ron Kopito

Peter Walter is an expert on protein homeostasis. He described the unfolded protein response commenting that this makes the life and death decisions between homeostasis and apoptosis. He highlighted the importance of the ER resident kinase Ire1 and presented a crystal structure showing possible assembly in the membrane. Elodie Angot-Duboruf (Lund, Sweden) gave a selected short talk showing that monomers, oligomers and fibrils of alpha-synuclein are all uptaken and internalised into cortical neurons. Joel Buxbaum (Scripps, USA) described the interaction between transthyretin and Abeta showing that TTR is found in brain and that it affects Abeta aggregation. He showed in particular that TTR knockout mice are more severely affected by APP over-expression.

Cellular reponse to protein aggregation. Chairs Peter Walter and Ronald Melki

Valerie Mezger (Paris, France) described her work on heat-shock factors in longevity and oncogenesis and her talk was followed by Leila Luheshi (Cambridge, UK) selected short talk. Leila discussed the development of camelid antibodies and affibodies to investigate particular epitopes involved in the formation of toxic Abeta assemblies. Jenny Russ (Berlin, Germany) gave a short selected talk on ALS disease pathways. She reported three hubs of protein networks involved in RNA processing, protein sorting, and cell development involved in ALS . Richard Morimoto (Evanston, USA), a world-leader in C. elegans transgenic model for degenerative diseases and proteostasis described how powerful this model system is to investigate proteostasis, longevity and the effect of protein aggregation. This work highlights the central role of the Heat Shock Factors, in particular HSF1, in suppressing misfolding and enhancing longevity. He underlined the tight interrelationship between metabolism, cell stress, proteostasis and cell health and the key role of sirtuins. He also described the complex

neuronal signalling and feedbacks critical for the response to stress and protein aggregation and cell survival.

Clearance of protein aggregates. Chairs Ellen Nollen and Patrick Brundin.

Ron Kopito (Stanford, USA) described the involvement of the proteasome in accumulation of polyQ aggregates in Huntington's disease, suggesting that polyQ aggregates are able to inhibit the proteasome via an ubiquitin-independent indirect effect involving chaperones. Elin Esbjorner (Cambridge, UK) gave a selected short talk showing the development of methods to follow tagged Abeta42 into cells using fluorescence and confocal microscopy and showing entry into lysosomes. Anna Von Mikecz (Dusseldorf, Germany) showed that nanoparticles of SiO2 can induce nuclear inclusions composed of polyQ containing proteins. Alfred Goldberg (Boston, USA) is an expert on the proteasome and he described an overview of proteasomal function and revealed new cryo-electron microscopy structures showing the proteasomal gate opening with changes in the conformation of the substrate polypeptide he uses. He also presented a ligand that may have therapeutic applications given that its binding to the proteasome maintain the gate "open". Finally, he highlighted the importance of ubiquitination of substrate polypeptide for binding/recognition by the proteasome. Maliha Shah (Dusseldorf, Germany) gave a short selected talk and described their screening approach for modifiers of alpha-synuclein aggregation related to Parkinson's disease. She identified a ribosomal binding protein as a modifier and potential therapeutic target. A short selected talk by Eric Hewitt (Leeds, UK) described work on fragmentation of mature amyloid fibrils to produce material that causes membrane permeation.

Genetic and therapeutic approaches to protein misfolding disorders. Chairs Louise Serpell and Ronald Melki.

Marc Blondel (Brest, France) began the final session by describing the development of therapeutics targeting ribosomal subunits. The experimental work he presented suggests that the ribosomal RNA has chaperone properties involved in the folding of proteins implicated in a number of neurodegenerative diseases. Damian Crowther (Cambridge, UK) described transgenic fly model of AD and showed that long-lived flies show an over-expression of ferritin. The work suggested an involvement in iron in oxidative stress and showed that clioquinol can reduce Abeta induced toxicity. In vitro experiments showed iron influences Abeta assembly. The conference closed with a talk by Mick Tuite (Kent, UK), the previous conference chair. He presented his work on yeast prion transmission. He highlighted the importance of the octapeptide repeats within the N-terminal sequence of Sup35 in the maintenance, strength and stability of the [PSI+] prion phenotype.

Summary

This was a meeting that involved participation of a broad range of expertise from clinicians to chemists, from those working on protein structure, to cell biologists with expertise in ageing, chaperones, protein aggregation and drug development. Experts in UPR, proteostasis and homeostasis and ageing were brought together with long-term participants in the protein misfolding field to discuss progress and key advances.

Beside the exchanges during the poster sessions, two general discussions were held. Critical issues were raised and comments were made. The participants unanimously thought that there is a need to pursue the series of conferences on protein misfolding and aggregation in ageing and disease. The participants feel that the next Jacques Monod Conference on this topic

should be held in 2012. They unanimously decided to ask Luc Buée (Lille, France) to assist Louise Serpell (vice-chair of the conference) to organise the future Jacques Monod Conference on this topic.

This was a highly successful meeting, generally accepted to be of a high standard, with exception presentations and informative posters. The small size of the meeting was ideal for exchange of ideas and encouraged participation of younger academics.

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