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Agrégation des protéines et maladies conformationnelles associées au vieillissement

Protein Misfolding and Aggregation in Ageing & Disease

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Rapport sur la Conférence

Conference Report

RESUME DU RAPPORT

Conférence Jacques Monod intitulée : Agrégation des protéines et maladies conformationnelles associées au vieillissement

Organisée par Mick Tuite, Président et Ronald Melki, vice-Président.

Plus de 20 maladies conformationnelles entraînant des dégénérescences cellulaires chez l'homme sont dues au mépliement et l'agrégation de protéines. Certaines de ces pathologies sont familiales et rares, à l'origine d'une réduction dramatique de la qualité et de l'espérance de vie. Si les protéines incriminées dans diverses maladies conformationnelles sont dissemblables par leurs structures primaires, elles forment toutes des oligomères toxiques présentant des similitudes structurales et fonctionnelles capables d'interagir avec les membranes cellulaires.

La conférence a permis a 30 scientifiques de renommée mondiale de présenter des travaux récents non publiés. En outre, 14 jeunes scientifiques, sélectionnés à partir des résumés soumis, ont été invités à présenter leurs travaux sous forme de communications orales courtes. Les autres participants ont présenté leurs travaux sous la forme de 60 affiches lors de deux sessions de deux heures. Globalement, 107 chercheurs de 14 pays ont participé à la Conférence. Les échanges ont étés nombreux et particulièrement fructueux et amicaux. Il est par ailleurs à noter que de nombreuses collaborations scientifiques ont été établies lors de la conférence qui a joué un véritable rôle de catalyseur.

Même si les principes fondamentaux du mépliement des protéines et leur agrégation sont intensément étudiés, ils sont loin d'êtres compris. Les conférenciers ont présenté tout ce que l'on sait à ce jour des évènements moléculaires et cellulaires à l'origine de l'agrégation des protéines. Des structures à haute résolution d'intermédiaires préfibrillaires et fibrillaires obtenues par cristallographie des protéines, RMN du solide, cryo-microscopie électronique, mutagenèse dirigée et spectrométrie de masse couplée aux échanges hydrogène/deutérium ont été présentées. Des algorithmes permettant de prédire la tendance de polypeptides à s'agréger ont aussi été présentés. La réponse cellulaire à l'agrégation des protéines, les mécanismes de neutralisation et d'élimination des agrégats toxiques et l'interaction de ces agrégats avec les différents compartiments et machineries cellulaires à l'origine de leur toxicité ont été abordés. Des modèles animaux allant de la drosophile (mouche du vinaigre) à la souris permettant d'étudier les conséquences du mépliement et de l'agrégation des protéines dans les maladies d'Alzheimer, de Huntington, de Creutzfeldt-Jacob, de l'ataxie cerebrocerebellaire et des serpinopathies ont étés présentés et et leur apport scientifique discuté. Enfin, des approches thérapeutiques destinées à empêcher l'agrégation des protéines ou à neutraliser l'effet délétère de ce processus ont été présentées.

Cette conférence a permis de faire évoluer notre façon de considérer les maladies conformationnelles. Certaines de ces maladies sont la conséquence de perte de fonctions cellulaires comme dans la maladie de Gaucher et la fibrose cystique alors que d'autres miment un gain de fonction toxique comme dans les maladies d'Alzheimer, de Huntington et de Creutzfeldt-Jacob. La conférence a permis aussi de dégager un consensus sur la nature des oligomères protéiques toxique. Il apparaît que ce sont des oligomères solubles qui sont les plus toxiques et non les larges oligomères fibrillaires. Enfin, de nouvelles stratégies thérapeutiques basées sur l'utilisation de petites molécules ayant pour cible les machineries de repliement ou de dégradation cellulaires, d'une part, de peptides dérivés des protéines dont l'agrégation est étroitement liée à la maladie, capables de perturber l'agrégation, d'autre part, ont été présentées lors de la conférence.

FINAL REPORT

Jacques Monod Conference: "*Protein Misfolding and Aggregation in Ageing & Disease*" Roscoff, Brittany, France, *April 11th-15th, 2007*

Prepared by: Mick Tuite (President); Ronald Melki (Vice-President)

Background

The conference focused on the role of protein misfolding and aggregation in disease and ageing in a wide range of unicellular and multicellular systems. In all species, proteins that do not remain correctly folded or that have failed to fold correctly are usually destroyed. In higher organisms, including humans, when this quality control process fails to remove the incorrectly folded proteins, this can result in a wide range of diseases. For example, we already know that the misfolding and subsequent aggregation of a specific protein is at the origin of over 20 different human diseases and in many cases these 'conformational diseases' are also associated with ageing. Neurodegenerative diseases such as Alzheimer's disease (AD) and Creutzfeldt Jakob disease (CJD) perfectly illustrate the debilitating consequences of misfolding diseases; they are associated with high societal costs and result in an extensive reduction in life expectancy and a dramatic diminution in the quality of life. This conference brought together leading scientists actively researching into such 'conformational diseases', together with a cohort of young scientists entering this field, in order to review what progress has been made and what the future directions for this research field should be.

We were delighted to be able to attract 30 world-leading scientists from across the globe to Roscoff to present their recent research. In choosing the speakers we wanted to include speakers working at the very forefront of research into the structural and molecular basis for protein misfolding and aggregation in a variety of cellular systems and contexts. Although actively studied, the fundamental mechanism of the misfolding of a variety of polypeptides leading to their aggregation and associated diseases is far from understood. The invited speakers provided a comprehensive description, at both the molecular and cellular level, of what we currently know about the early steps of aggregation, the high-resolution structures of pre-fibrillar and fibrillar amyloid states, and our ability to predict the aggregation propensity of polypeptides, all of which are crucial for the rational design of therapeutics preventing diseases associated with protein deposits. The scientific programme was developed to ensure that an integrated view of this important field was provided and also to set it into a wider clinical framework.

The meeting dealt with five main areas: the molecular events in protein aggregation, cellular factors important for protein folding & aggregation, the structure of amyloids, disease implications of protein aggregation and finally therapeutic approaches to protein misfolding disorders. 14 conference attendees were also invited to give short (15 min) presentations on their work and these talks were interspersed amongst the six sessions giving a total of 42 oral presentations. Two of the invited speakers (Eisenberg, Jackson) unfortunately withdrew at the last minute due to health or other reasons. All other attendees presented 60 posters on their work and these posters were viewed at two separate sessions which provided ample opportunity for participants to fully engage with all the science presented at the meeting. Overall, 107 senior and junior scientists from 14 countries attended the meeting.

In addition to the generous financial and administrative support of the Centre National de la Recherche Scientifique (CNRS), financial support was also obtained from Sanofi-Aventis and Landes Biosciences. The meeting was also used by the publisher Landes Biosciences to launch

their new journal *Prion* and complementary copies of the first issue were given to all attendees at the meeting. This first issue also contained 8 mini-reviews written by invited speakers at the Roscoff meeting and provided the attendees with background information on the talks they heard. EMBO sponsored the lecture of Anne Bertolotti through their Young Investigators Programme (EMBO-YIP).

Summary of Lectures and Associated Discussions

The following provides a brief overview of the science discussed in the oral presentations and, in so doing, we have broken this down by session.

Session 1: Molecular events in protein aggregation I

The meeting and the first session of the conference opened appropriately with a talk by Michel Goldberg who joined Jacques Monod's group in 1962 and was associated with Monod and his work for some 45 years. In his talk, which represented a fitting tribute to his mentor, Goldberg presented Monod's original ideas on protein folding and aggregation and his own contribution to these seminal studies. In the 1960's Monod became interested in protein folding and suggested that it represented a "second translation of the genetic message". Goldberg and Monod considered whether such a code would allow them to predict the 3D structure of a protein from its amino acid composition. This lead them to highlight the critical role that the experimental conditions, in particular ionic strength, play in correct protein folding. Later, Monod proposed that the intermolecular interactions leading to a polypeptide's ordered aggregation are specific to each polypeptide and this can account for the observation that the ordered aggregation of a given polypeptide does not affect that of another polypeptide. These concepts lie at the heart of the prion concept and also that of prion strains which represent alternatively (mis)folded conformers of the same protein, PrP. Thus Monod and Goldberg's contribution to the field of protein folding was to recognize the problem and to set in motion the research that was the focus of this conference. Such an historical context provided a fitting start to the conference.

The remainder of this session focused on current research activities and this begun with **Chris Dobson (Cambridge)** presenting an overview of his group's research on protein misfolding and aggregation *in vitro, in silico* and *in vivo.* At the outset Dobson stressed that misfolding is the default pathway for a newly synthesised protein and thus more 'typical' than its correct folding. Dobson showed that protein folding intermediates can be displaced from a productive assembly pathway by interacting with ligands as exemplified by his studies on the lysozyme/clusterin interaction. He also described his group's work on the physical properties of amyloid fibrils using Atomic Force Microscopy (AFM) noting that the amyloid fibrils that are found in many of the debilitating conformational diseases are significantly more rigid than other 'rigid' cellular structures such as actin filaments and microtubules. Indeed amyloid fibrils show slightly higher rigidity than steel wires with a comparable thickness.

Sheena Radford (Leeds) described her *in vitro* studies on the aggregation of a specific 99 residue protein ß2 microglobulin (ß2m) and fragments thereof of, in order to better understand how different proteins or protein fragments assemble into ordered, insoluble aggregates associated with conformational diseases. Using ß2m as a model, her group have identified rare aggregation-prone sequences and begun to define the sequence or structural properties that impose this amyloidogenic behaviour role played by different sequence determinants. ß2m assembles under physiological conditions into native-like fibrils that look like amyloids after populating a set of folding intermediates. At low pH (2.5) these amyloid fibrils form much faster but at neutral pH ß2m does not aggregate. At lower pHs different aggregates of ß2m form because at unfolding intermediates form that are not detected at neutral pH. Residues 62, 66, 67 and 70 of ß2m were shown to be required for aggregation thus defining a region that is critical for assembly. **Ehud Gazit (Israel)** reported on his group's studies on the peptide NFGAIL (Asn-Phe-Gly-Ala-Ile-Leu) that is found in the AD-

associated protein Aß. This peptide loses its capacity to assemble into amyloid fibrils when the Phe residue is mutated to Tyr whereas aggregates can still form if changed to Trp. This finding suggests that the packing of aromatic residues is critical for the initiation of assembly of a protein into an amyloid and Gazit went on to suggest that such aromatic residues could be used as targets for anti-assembly therapeutic strategies.

Fabrizio Chiti (Florence) reported that the aggregation propensity of a polypeptide can be predicted to some extent by sequence comparison and when taking into account the hydrophobicity of amino acid residues within a sequence. To facilitate such an analysis Chiti's group have taken an *in silico* approach, developing an algorithm that allows them to predict with some accuracy amyloid-forming regions in a polypeptide. Using this algorithm they identified key residues in two amyloidogenic proteins, the mammalian Tau protein and the yeast prion protein Sup35p. Using horse heart apomyoglobin as a model his group have also shown that when a given polypeptide sequence is scrambled the overall aggregation propensity of the protein does not change. What does change though is the kinetics of assembly and this might be due to conformational differences and/or the relative concentration of assembly competent states. I*n silico* approaches have also been developed by **Frédéric Rousseau (Brussels)** who described Waltz, a development of the widely used amyloidogenic prediction statistical mechanics algorithm Tango. Such *in silico* analyses are complicated by the fact that overlapping but different amino acid sequences appear to be critical for both amorphous (non-amyloid) aggregates and amyloidogenic peptides. Using the Waltz algorithm to screen 28 different proteomes Rousseau found that typically less than 5% of proteins for a given proteome do not contain at least one aggregation propensity sequence. Rousseau also demonstrated that evolution drives the loss of strongly aggregating sequences and where such sequences remain, in order not to aggregate, proteins have evolved to include charged residues (especially Arg, Pro and Lys) flanking the hydrophobic, aggregation prone residues and therefore act as 'gatekeepers' that efficiently oppose aggregation. Such positively charged gatekeepers may also be recognized by the chaperone machinery and may also provide a degree of substrate specificity in such recognition.

Session 2: Molecular events in protein aggregation II

Key to developing further our understanding of the molecular events in the aggregation of proteins in disease is the development of ever more intrusive technologies. This was illustrated in the first talk in the second session given by **David Teplow (Los Angeles)** who described the multidisciplinary studies his group is undertaking on the folding and assembly of the amyloidogenic Aß peptide associated with AD. To document the assembly of the two key peptides, Aß40 and Aß42, Teplow's group have used ion mobility mass spectrometry (IMMS), a new and novel technique that allows one to document non-covalent interactions. The measurements made reveal differences in the oligomeric states of Aß40 and Aß42 that may account for the differences in their assembly properties. IMMS will allow for the determination of oligomeric species of Aß in solution and the geometry of these oligomers.

Aggregation of the microtubule-associated tau proteins into filaments is also a common feature in AD patients and in other tauopathies. **Luc Buée (Lille)** described how Tau phosphorylation is a prerequisite for such aggregation, but the molecular mechanism(s) that lead to changes in Tau phosphorylation are poorly understood. Buée's group have now shown that beside being phosphorylation dependent, Tau aggregation is also strongly dependent on the regulator prolyl cis/trans isomerase Pin1 and suggested that such regulators of Tau phosphorylation are a viable target for therapeutic intervention.

Paul Muchowski (San Fransico) further developed the identification of new drug targets but for treatment of Huntington's Disease (HD). Muchowski described the use of a yeast-based screen to

identify genes associated with the toxicity of a mutant huntingtin fragment (htt) which led to the finding that deletion of the yeast gene *BNA4* that encodes kynurenine 3-mono oxygenase (KMO) alleviated toxicity. KMO is a mitochondrial enzyme implicated in the kynureinine pathway of tryptophan degradation and is also found exclusively in the microglia in the central nervous system (CNS) suggesting that mutant htt induces a transcriptional defect that activates the kynureinine pathway in the CNS and that this contributes to the neurodegeneration seen in HD patients. Human KMO may therefore represent a novel 'druggable' target since its inhibition might reduce the detrimental effect of htt in the CNS.

Two further talks in this session dealt with studies on the toxicity associated with the aggregation of htt in HD; **Philippe Djian (Paris**) described his group's research into the aggregation of htt carrying polyQ expansions in the HD brain while **Anne Bertolotti (Cambridge)** described her recent studies on modulators of PolyQ aggregation. Djian reported that the most toxic htt aggregates appear to be those located in the nucleus as cytoplasmic aggregates with the same size as the nucleus, have no effect on neuron viability. Djian suggested that small intranuclear aggregates of htt fragments are the toxic species in HD. Bertolotti's used cultured mammalian cells and yeast to probe the cellular factors that are important for htt aggregation and associated toxicity and showed that aggregation seems to be a property of the nucleo-cytoplasmic compartment which in turn depends mainly on proteasome activity. Pro residues that flank the expanded polyQ region were found to act as cis-active modulators of toxicity thus highlighting the importance of neighboring amino acids in modulating htt aggregation and toxicity.

The session and first day was completed by a talk from **Marie-Lise Maddelein (Bordeaux)** on the fungal prion HET-s that controls vegetative incompatibility in *Podospora anserina*. The [Het-s] prion phenotype correlates with the aggregation state of the HET-s protein and her recent NMR-based structural studies of the prion form of the HET-s protein, done in collaboration with **Ritter (Braunschweig)** who spoke in a later in the conference, have described what appears to be the 'infectious fold' of the HET-s protein. Their model suggests that an amyloidogenic nucleus could be formed by a single molecule of HET-s. A full elucidation of the link between the structure and infectivity of such proteins is going to be crucial in order to fully understand the mechanism by which the infectious form is propagated.

Session 3: Cellular factors important for protein folding & aggregation
Molecular chaperones play a significant role in ensuring not only that polypeptide chains are correctly folded, but also come into play where protein folding goes wrong and the polypeptide chain aggregates. In this session studies carried out both *in vivo* and *in vitro* exemplified the key role of cellular factors in both protein folding and aggregation.

Ronald Melki (Gif-sur-Yvette) described his *in vitro* studies on the roles played by various yeast molecular chaperones in the assembly of the Gln/Asn-rich yeast prion proteins Ure2p and Sup35p. Several families of molecular chaperone modulate the assembly of Ure2p and Sup35p into fibrils *in vitro*; in particular the Hsp70 and Hsp40 family members sequester the Gln/Asn-rich yeast prions in an assembly incompetent state while the ClpB-related chaperone Hsp104p favours nucleation and assembly. Melki proposed that these functional differences among the different chaperones modulate the propagation of the prion traits through a fine tuning of the oligomeric state of prion proteins *in vivo*. Later in the session **Mick Tuite (Canterbury)** described analogous *in vivo* studies on the role played in particular by Hsp104p in the propagation of the prion form of Sup35p (known as [*PSI*$^{+}$]). Reversibly inhibiting the ATPase activity of this chaperone *in vivo* using guanidine hydrochloride, has allowed his group to probe the role of the chaperone directly in both prion propagation and *de novo* formation of the [*PSI*$^{+}$] prion. By applying a complex stochastic model to

these data, an indirect means of establishing the number of prion seeds ('propagons') necessary for continued propagation of the prion state has been developed.

Until now, with the exception of prions, amyloids associated with 'conformational diseases' were thought to be non-infectious. This view must now change as **Ron Kopito (Stanford)** reported that his group has observed prion-like propagation of polyQ aggregates in mammalian cells. Amyloid-like fibrillar polyglutamine peptide aggregates can be internalized by cultured mammalian cells and once in the cytoplasm, these polyQ aggregates become sequestered in aggresome-like inclusion bodies together with the cytoplasmic chaperone Hsp70 and components of the ubiquitin-proteasome system. These aggregates are then able to recruit soluble cytoplasmic proteins providing they share homologous amyloidogenic sequences and this recruitment leads to a change in heritable phenotype. Although the rate of propagation was low this could be the consequence of the active transport of these aggregates to the centrosome.

Ineke Braakman (Utrecht) turned the attention of the meeting to folding events in the endoplasmic reticulum (ER) by describing how the ER handles the large increase in folding demand when resting B lymphocytes differentiate into plasma cells that need to correctly fold and secrete thousands of antibody (specifically IgM) molecules per second. During such differentiation the IgM accumulates in striated crystals as assembly intermediates. Using a proteomics approach her group was able to show that the levels of all the of the molecular chaperones resident in the ER, with the exception of Grp94, increase dramatically. One novel chaperone, SmErp1 a protein involved in Fab folding is particularly overexpressed and together with the Hsp70-related chaperone BIP, is associated in the ER with the inert and crystalline form of stable and off pathway folding intermediates of the heavy chain of IgMs.

Ulrich Hartl (Martinsried) again raised the issue of cellular toxicity associated with expression of htt with an expanded polyQ tract and described how polyQ–associated toxicity is modulated by molecular chaperones. For example, Hsp70 in conjunction with its co-chaperones from the Hsp40 family affect polyQ aggregation and suppress its toxicity while the eukaryotic cytoplasmic chaperonin TRiC/CCT acts synergistically with Hsp70 in this process. Sequence context of the polyQ also modulated htt toxicity (as also reported by Bertolotti). Interestingly, studies on htt toxicity in yeast revealed that overexpression of the ClpB-related chaperone Hsp104p drives the toxic polyQ proteins into larger inclusions with shorter fibrils and reduced toxicity.

Cellular factors other than chaperones that affect protein aggregation and its associated toxicity were also discussed. **Cristina Cecchi (Florence)** described her studies on how membrane cholesterol modulates β-amyloid cytotoxicity and showed that membranes enriched in cholesterol incorporate smaller amounts of the toxic prefibrillar Aß42 oligomers and this led to the suggestion that cholesterol may be used to protect against neurodegeneration. **Lev Osherovich (San Francisco**) reported his studies on how the highly conserved insulin/IGF-1 signalling pathway influences age-related aggregation and toxicity of proteins with expanded polyQ tracts in the worm *C. elegans.* Using an RNAi-based strategy he was able to show that the daf-2 insulin receptor plays a general role in modulating the cellular responses to misfolded proteins; for example, loss of daf-2 function rescues the paralysis caused by expression of Aß42 in the worm.

Further insights into the role of cellular factors were provided by **Tricia Serio (Rhode Island) who** described her *in vivo* studies of the dynamics of the aggregation of the yeast prion protein Sup35p in yeast cells followed in real time. Her studies showed that yeast prions are propagated via three discrete dynamic transitions in the structure of existing protein and she was able to dissect the role of Hsp104p in these transitions. Using Sup35p fused to various fluorescent tags (e.g. GFP, DsRed etc) Serio's work revealed that the existing soluble Sup35p is almost immediately converted to an

insoluble form when a [*PSI*$^+$] cell is mated to a [*psi*$^-$] cell and that this remodeling is independent of Hsp104p and its associated ATPase activity. Serio concluded that the primary role of Hsp104p is in fragmenting Sup35p aggregates, but that in addition it has a secondary role in nucleation as discussed earlier in the session by Melki.

Session 4: Structure of amyloids

Amyloid fibrils associated with a variety of proteins and different fatal diseases contain a common structural feature, namely a cross-β spine. **Louise Serpell (Sussex)** described what we have learnt about the architecture of these fibrils from X-ray fiber diffraction images, electron microscopy and solid-state NMR. Using short designed peptides Serpell's group have been able to explore how primary sequence influences the amyloid structure. Using a new computer program Serpell's group are able to simulate the diffraction patterns and the packing of peptides within fibrils and crystals and this has allowed them to assess whether the various models for amyloid structure fit the experimental data. In a subsequent talk from **Philippe Derreumaux (Paris)** further demonstrated the value of such computer simulations, this time in furthering our understanding of the early steps of polypeptide aggregation. In particular he focused on our current understanding of the dynamics and free energy surface of the assembly of amyloid-forming peptides that has emerged from coarse-grained protein simulations. Such computer simulations will also allow the modeling of the assembly of polypeptides such as PrP. **Alfonso De Simone (Naples)** illustrated how replica exchange molecular dynamics can be used to study the structural basis of amyloid fibre formation and stabilization using the amyloid-forming peptide GNNQQNY. **Riccardo Pellarin (Zurich)** reported how molecular dynamics simulations of a coarse-grained polypeptide model lead them to propose an *in silico* designed model for how proteins assemble into amyloid fibrils. The model reveals a helical assembly and shows that the fibrils are bundles of 4 protofibrils.

High resolution NMR approaches have also been applied to the study of a range of other amyloids. **Guy Lippens (Lille)** showed how NMR and fluorescence spectroscopy have been used to study the role of Tau phosphorylation in its aggregation and interactions with microtubules. Tau binds to microtubules in a given conformation and a number of resonances observed in by NMR for soluble Tau, disappear when the protein is bound to microtubules. This structure-based approach has allowed them to dissect the role of individual phosphorylation sites on the structure and function of Tau. **Anja Böckman (Lyon)** studies the *Bacillus subtilis* protein Crh using solid-state NMR. The Crh protein retains a significant fraction of native structures upon aggregation under physiological conditions into amorphous aggregates and fibrils. Böckman showed that conformational changes can occur within these aggregates upon increase of the temperature a partially unfolded intermediate state is reached which is then converted into β-sheet rich structures.

The final part of this session **Jonathan Weissman (San Francisco)** dealt with his recent analysis of the yeast prion protein Sup35p and the structural basis of yeast prion variants. Using a combination of solution NMR and amide hydrogen/deuterium (H/D) exchange his group found that two different [*PSI*$^+$] prion variants show major structural differences. Specifically, it seems that amino acid residues spanning from position 4 to 35 of the Sup35p protein are involved in a core structure while the residues in the region 36-68 exchange or not depending on which prion variant is being examined i.e. the core of the fibrils formed by the two different strains have different sizes in different prion variants. This structural difference could account for the propensity of different prion variants to propagate more or less efficiently.

Session 5: Disease implications of protein aggregation

The deposition of proteins of aberrant conformation is the hallmark of several neurodegenerative diseases such as AD, HD and prion diseases such as CJD. In this session the focus moved more towards what we know about the molecular basis of the underlying pathology associated with

amyloid aggregates in a range of diseases. **Fabrice Klein (Strasbourg)** for example, outlined the current debate about the nature of the toxic form of htt proteins carrying various expanded polyQ tracts. Klein's group has constructed two such proteins: a non-pathogenic polyQ22 and a pathogenic polyQ41 and looked at the kinetics with which they form aggregates and the stability of such aggregates. His group has made use of the 1C2 antibody that recognizes the soluble form of both polyQ tracts and which inhibits polyQ-driven aggregation. Klein proposed that polyQ22 and polyQ41 both share a common structure. **Aphrodite Kapurniotu (Aachen)** suggested that there is a molecular link between two other conformational diseases, AD and type 2 diabetes. Kapurniotu noted that the corresponding amyloid-forming proteins, Aß and IAPP respectively share 50% similarity, 25% identity and both contain the peptide NF/KGAIL. The addition of an assembly incompetent form of IAPP to both peptides inhibits their assembly suggesting a common assembly mechanism and some form of link between these two very different diseases.

Several speakers described the use of the fruit fly (*Drosophila melanogaster*) as model in which to explore the pathogenicity of protein aggregates. **Leila Luheshi (Cambridge)** reported how the expression of variant human Aß in transgenic fruit flies causes neuronal alterations that have effect on the mobility and longevity with a good correlation between the propensity to form protofibrils and toxicity. There was however a poor correlation between toxicity and the propensity to form fibril mature fibrils. Thus the fruit fly provides a good model in which to better understand the pathogenicity of Aß. **Christelle Lasbeiz (Paris)** also showed that the fruit fly can be used as a conditional model of spinocereballar ataxia 7 (SCA7).

David Lomas (Cambridge, UK) described how α1-antitrypsin aggregates are at the origin of a set of diseases known as serpinopathies. The neuronal form (neuroserpin) is found associated with Alzheimer's plaques while the Aß-neuroserpin stoichiometry within these plaques is 1:1 which raises the issue of whether or not there is co-assembly of unrelated polypeptides within these plaques. Using a fruit fly model Lomas described how his group had shown that oxidative stress is the most effective event leading to Aß and serpin aggregation while ferritin protects against aggregation.

Significant attention is still being paid to the spongiform encephalopathies which arise primarily either by infection, stochastically or in association with mutations in the gene encoding the PrP protein. **Christina Sigurdson (Zurich)** from Adriano Aguzzi's laboratory reported studies with novel transgenic mouse models for probing the link between prion plaque formation in the brain and the disease.

Session 6: Therapeutic approaches to protein misfolding disorders

As exemplified by many talks in earlier sessions, a better understanding of the molecular basis of the various conformational disorders of man is beginning to emerge. As the human population ages so the frequency with which many of these diseases arise in the human population will increase rapidly. The final session of the conference therefore looked at progress in identifying therapeutic approaches to various such disorders.

Jeff Kelly (La Jolla) pointed out that the eukaryotic cell is packed with machinery devoted to dealing with polypeptides with aberrant folds. Such misfolding can lead to a loss of function - as in type 1 Gaucher disease, the most common of the lysosomal storage diseases - or to a gain of function – as in the amyloidoses. In Gaucher disease a glucocerebrosidase is not functional and Kelly proposed a novel way of treating such a disease which is due to the loss of function of an enzyme: using inhibitors of this enzyme that would stabilize a significant % of the enzyme molecules leading to the accumulation of the enzyme-inhibitor complex within the cells. At very high complex concentration a fraction of the enzyme is found unbound to its inhibitor because of the dissociation constant and this fraction should allow the cell to have a basal enzymatic activity and survive.

Significant progress was reported by **Erich Wanker (Berlin)** in identifying small molecules that inhibit polyQ aggregation in vitro. The most efficient product identified in this screen was epigallocatechin-gallate (EGCG) from green tea. EGCG inhibits polyQ compaction upon fibrillogenesis and when it crosses the cell membrane it can lead to a decrease in the toxicity due to polyQ72 aggregation in yeast cells. EGCG also inhibits Aß42 and α-synuclein aggregation in the fruit fly. Critically the 700 kDa oligomers formed in the presence of EGCG do not seed the assembly of the soluble forms of the proteins and are not toxic to cultured cells. EGCG (and perhaps other such drugs) may induce the conversion of amyloidogenic proteins into off-pathway and non-toxic oligomeric structures and thus represent a promising new therapeutic strategy to prevent or at least slow down the pathogenesis associated with amyloidoses.

In the final presentation of the conference, **Marc Blondel (Roscoff)** described the mechanism of action of a group of antiprion drugs using both yeast and mammalian cells. The cellular target of two of the most potent antiprion compounds, 6AP and GA is the ribosome although neither inhibit protein synthesis per se. Rather they seem to target a ribosome-associated “chaperone” activity associated with the heavy subunit of the ribosome (50S). Both compounds eliminate both fungal and mammalian prions and this provides the first real evidence for the universality of the process by which prions are propagated in fungi and humans.

Final Comments

As evidenced by the cutting edge and largely unpublished research described at the conference, a conceptual framework is beginning to emerge which allows us to better understand the way in which the unique folds found in proteins, encoded by specific amino acid sequences, are reached within a universal mechanism of protein folding. One of the great challenges we now face is to fully understand how the misfolding and/or mis-assembly of certain intracellular proteins leads to the gain of toxic function diseases including Huntington’s and Parkinson’s and loss of function diseases including Cystic Fibrosis and the lysosomal storage diseases. An increasing body of evidence indicates that soluble amyloid oligomers or β-sheet-rich protofibrils, rather than mature fibrils or amyloid plaques, are the major toxic species behind these diseases. The exact nature of the pathogenic molecules and the mechanisms by which they induce dysfunction and toxicity, however, was the subject of intensive debate throughout the meeting. By studying model systems including transgenic animals, yeast and the fruit fly, information on the nature of the toxic oligomers, cellular factors that modulate their toxicity and the identification of viable therapeutic strategies to override this toxicity are beginning to emerge. The application of in silico approaches to these problems is also beginning to bear fruit.

Perspectives

The participants felt there is a need for organizing another meeting on the issue in 2009. They elected on the last day of the Conference Dr Louise Serpell (University of Sussex) to act as a vice-president for the next conference. Ronald Melki and Louise Serpell are asked to apply for another Jacques Monod Conference on protein aggregation, ageing and disease.