Meeting report

Unraveling prion structures and biological functions

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A report on a Joint Cold Spring Harbor Laboratory/ Wellcome Trust Conference on 'Prion Biology', Hinxton, UK, 7-11 September 2005.

While most recent prion meetings have focused on either mammals or fungi, the conference on prion biology held near Cambridge this September stood out as an attempt to represent research on mammalian and fungal prions equally, in order to provoke discussion on fundamental questions of prion structure, biogenesis, variability and biological role.

Prions of lower eukaryotes

Over the past decade several infective proteins, or prions, have been discovered in genetically tractable lower eukaryotes, where they act like cytoplasmically inherited genetic determinants. The opening talk of the meeting was delivered by Reed Wickner (National Institutes of Health, Bethesda, USA), who was the first to suggest 11 years ago that the non-chromosomal genetic determinants known as [*URE3*] and [*PSI*$^+$] in the yeast *Saccharomyces cerevisiae* were in fact prion proteins (enclosure in square brackets is the conventional nomenclature for cytoplasmically inherited genetic determinants in fungi). The proteins that correspond to [*URE3*] and [*PSI*$^+$], regulator of nitrogen metabolism Ure2 and translation termination factor Sup35, respectively, have carboxy-terminal domains that carry out a cellular function and auxiliary amino-terminal prion domains, which can adopt an abnormal 'prion' conformation. The prion domains of both these proteins are rich in glutamine (Q) and asparagine (N), but only that of Sup35 contains oligopeptide repeats, which are presumably required for [*PSI*$^+$] replication. Previously, Wickner's group had shown that random shuffling of amino acids in the Ure2 prion domain, a procedure named scrambling, usually does not impair the prion-forming capacity of the protein. At this meeting, Wickner described how randomization of the Sup35 prion domain, including the repeat region, also does not block prion formation, and concluded that unusual amino-acid composition, rather than specific sequences, determines prion-forming ability. According to Wickner, these experiments argue for an in-register parallel β-sheet structure for the prion fibrils, as scrambling would disrupt the correspondence of amino acids in any other β-strand structure.

Susan Lindquist (Whitehead Institute, Cambridge, USA) described elegant Sup35 cross-linking experiments that revealed that Sup35 monomers in amyloid fibrils are arranged in a 'head-to-head, tail-to-tail' fashion. Amyloid is the general name given to the fibrillar protein aggregate formed by prions and some other proteins. Such amyloid structure also implies parallel in-register arrangement of β strands in the prion fibrils. Lindquist proposed that these considerations, combined with the β-helical nanotube structure of the Sup35 fibrils, suggested a new structural model for prions, which may have broad implications for amyloids.

Prions come in different variants or 'strains'. In mammals, whose prions are infectious agents causing a set of fatal neurodegenerative diseases, different prion strains are defined by specific incubation times, distribution of vacuolar lesions in the brain, and patterns of accumulation. For yeast [*PSI*$^+$], strain differences can be revealed by differences in phenotypic manifestation (nonsense suppression caused by the aggregation-dependent inactivation of the translation termination factor Sup35) and stability of maintenance.

Generally, 'weak' [*PSI*$^+$] manifest less stable inheritance and worse phenotypic manifestation than 'strong' [*PSI*$^+$]. From her results, Lindquist suggested a structural basis for [*PSI*$^+$] variants: in 'weak' [*PSI*$^+$] variants a longer Sup35 fragment is incorporated into the amyloid core. The physical basis of prion strain differences was also considered by Jonathan Weissman (University of California, San Francisco, USA). His group had previously shown that Sup35 fibrils obtained *in vitro* at 4°C and 37°C transform yeast cells to strong [*PSI*$^+$] variants, and weak [*PSI*$^+$], respectively. Atomic force microscopy revealed two distinctions between the 4°C (Sc4) and 37°C (Sc37) fibrils. Sc4 fibrils polymerized more slowly than Sc37, but were more fragile and therefore smaller and more numerous, which ensured their efficient polymerization. Correlated with the strong phenotype of Sc4 fibrils is the fact that they are more susceptible to fragmentation *in vivo* than Sc37, presumably as the result of the activity of chaperone proteins. Thus, the efficiency of fibril severing by chaperones correlates with the mechanical strength of the fibril.

The search for novel prion proteins goes on, as their discovery might enable new prion-related processes in nature to be uncovered and the importance of prions to be estimated. Pascale Beauregard (Université de Montreal, Canada) reported convincing genetic and biochemical data for the existence of a prion in *Schizosaccharomyces pombe*, the first to be found in a yeast other than *S. cerevisiae*. This prion, [*cif1*], allows cell survival in the absence of the essential chaperone, calnexin. Jessica Brown (Massachusetts Institute of Technology, Cambridge, USA) described in her poster a novel prion-like determinant of *S. cerevisiae*, named [*GAR*$^+$], which determines resistance to the non-hydrolyzable glucose analog D-(+)-glucosamine. In contrast to known yeast prions, [*GAR*$^+$] is not cured by deletion of the heat-shock protein gene *HSP104,* but is cured by simultaneous deletion of the *SSA1* and *SSA2* genes encoding Hsp70 heat-shock proteins. Ludmila Mironova (St Petersburg University, Russia) described a search for proteins underlying [*ISP*$^+$], another Hsp104-independent prion-like determinant causing anti-suppression, a phenotype opposite to that of [*PSI*$^+$]. A likely candidate for the [*ISP*$^+$] prion protein is the transcriptional factor Sfp1. One of us (I.D.) presented biochemical and microscopic evidence for the prion nature of the Lsm4 protein, one of several candidate prions which were identified previously in a genetic screen for the [*PIN*$^+$] protein.

Although relatively few investigators study the [Het-s] prion of the filamentous fungus *Podospora anserina*, their results make a significant contribution to the prion field. Indeed, [Het-s] is the only prion with a confirmed biological function: fusion of a [Het-s] mycelium with one expressing the non-prionizable *het-S* allele triggers the heterokaryon incompatibility reaction, which leads to the death of the hybrid mycelium. Recent progress in understanding the molecular basis of this incompatibility reaction was reported by Sven Saupe (Institute de Biochemie et de Génétique Cellulaire, Bordeaux, France), who has shown that the carboxy-terminal domain of HET-S is prionizable, but prion formation is blocked by the functional amino-terminal domain. Presumably, HET-S can co-polymerize with the HET-s protein, and their oligomers trigger the incompatibility reaction. Ronald Riek (The Salk Institute, La Jolla, USA), Cristiane Ritter (The Salk Institute) and Ansgar Siemer (ETH Zurich, Switzerland) consecutively presented their excellent collaborative structural studies of [Het-s], which have particularly broad significance. The normally flexible carboxy-terminal tail of the HET-s protein can undergo a spontaneous conformational transition into amyloid fibrils. The fold of these fibrils comprises four β strands made up of two pseudo-repeat sequences, each forming a β-strand-turn-β-strand motif. Structure-based mutagenesis revealed that this conformation is the functional and infectious entity of the HET-s prion.

Several speakers focused on the mechanisms underlying the *de novo* appearance of yeast prions. It is known that the prion form of the Rnq1 protein, [*PIN*$^+$], promotes the *de novo* appearance of [*PSI*$^+$] and [*URE3*], apparently by directly seeding QN-rich prion aggregates. Susan Liebman (University of Illinois, Chicago, USA) presented further studies on the interaction between [*PIN*$^+$], [*PSI*$^+$] and an artificial prion, [*CHI*$^+$]. [*PIN*$^+$] efficiently seeded [*CHI*$^+$], while [*PSI*$^+$] stimulated the appearance of [*PIN*$^+$]. While it appears overall that all QN-rich prions can stimulate each other's appearance, evidence suggesting that similar interactions may occur with non-QN-rich prions was also presented. Mick Tuite and colleagues (University of Kent, Canterbury, UK) have studied the appearance of [*PSI*$^+$] at natural Sup35 levels. The appearances of [*PSI*$^+$] were not related to any alterations in the gene *SUP35*, and they were not affected by chemical agents that cause protein misfolding. The study of proteins associated with Sup35 revealed the presence in [*PIN*$^+$] [*psi*$^-$] cells ([*psi*$^-$] denotes the absence of [*PSI*$^+$]) of a small oligomeric complex insoluble in the detergent SDS, and containing both Sup35 and Rnq1 proteins. This finding is important because hybrid particles may represent an intermediate step leading to the appearance of [*PSI*$^+$].

The biological importance of prions was discussed by Kim Allen (Columbia University, New York, USA). Earlier studies suggested that the prion-like behavior of the translational regulator protein CPEB may underlie memory formation in the mollusc *Aplysia*. Allen showed that several mouse CPEB homologs also form prion-like aggregates in yeast, and that aggregate size, number and distribution are affected by the expression of chaperones. Aggregate formation by mouse full-length CPEB-3 and CPEB-4 proteins was also shown in neuroblastoma cells. The amino-terminal domain of mouse CPEB-3 is rich in glutamine, similar to yeast prions, whereas the amino-terminal domain of CPEB-4 is rich in proline and harbors sequence motifs similar to those implicated in amyloid formation by the mammalian prion protein PrP.

While this study does not directly prove the prion-related nature of memory in higher eukaryotes, it represents a significant step towards this.

Mammalian prions

Claudio Soto (University of Texas, Galveston, USA) presented impressive results on *in vitro* amplification of PrP^{Sc}, the infectious form of PrP, in the protein misfolding cyclic amplification system (PMCA). He demonstrated that PMCA is capable of amplifying prion infectivity with indefinite dilutions of minuscule amounts of initial PrP^{Sc} seeds. Soto emphasized the potential application of PMCA for detection of ultra-low levels of infectivity in blood. Surachai Supattapone (Dartmouth Medical School, Hanover, USA) presented the results of experiments in which PMCA was used to generate the protease-resistant conformer of the prion protein using PrP^{Sc} purified from scrapie brains and PrP^{C} (the normal conformer of PrP) purified from normal brains. Ongoing bioassay experiments with these *in vitro*-generated PrP^{Sc} produced in the presence of additional synthetic cofactors may eventually reveal all the molecular components required for the efficient replication of prions. While amplification of PrP^{Sc} using components extracted from normal and scrapie brains seems completely successful, reconstitution of prion infectivity *de novo* from synthetic components still remains puzzling.

In his presentation, Bruce Chesebro (Rocky Mountain Laboratories, Hamilton, USA) clearly demonstrated that prion toxicity could be separated from prion infectivity. He showed that the onset of typical clinical scrapie was substantially delayed in mice that expressed PrP without a glycosylphosphatidylinositol anchor. Remarkably, these mice were able to replicate prion infectivity and produced the protease-resistant conformer of PrP in the form of amyloid plaques, but failed to develop clinical symptoms of prion disease for a prolonged time. Byron Caughey (Rocky Mountain Laboratories, Hamilton, USA), on the other hand, took a biochemical approach to identifying the most infectious prion particles. Fractionation of PrP by size revealed that the highest level of infectivity per unit of mass belongs to particles with approximate molecular weights of only 300-600 kDa. A question of great interest is whether these highly infectious prion particles originate from fibril fragmentation or from distinct non-fibrillar species.

Neil Mabbott (Institute for Animal Health, Edinburgh, UK) discussed routes of prion migration between potential sites of exposure and the lymphoid tissues. He emphasized the possibility of acquiring infectious prions through the skin and the role of Langerhans cells (dendritic cells) in transporting prions to the lymphoid tissues. Adriano Aguzzi (University Hospital, Zurich, Switzerland) presented results that suggest a relatively high likelihood of prion transmission through urine, which could be one of the possible means of horizontal spread of prions in brain-wasting disease of elk and deer. Roger Morris (Wolfson Centre for Age-Related Disease, King's College London, London, UK) described his work on identifying the neuronal transmembrane receptor that is involved in the rapid recycling of PrP^{C} and the cellular uptake of PrP^{Sc}. He found that PrP^{Sc} bound to the surface of primary neurons was rapidly endocytosed. Internalization of PrP^{Sc} was in direct competition with internalization of PrP^{C}, implying that the same receptor was involved in both processes.

Edward Malaga-Trillo (University of Konstanz, Germany) presented a new evolutionary perspective on the possible function of PrP and the molecular mechanisms driving the diversification of PrP domains from fish to mammals. He reported the establishment of a novel genetic model for prion research, the zebrafish. Most notably, using the zebrafish model, Malaga-Trillo presented the first clear PrP loss-of-function phenotypes, which might be used to delineate a conserved function of vertebrate PrPs during early development.

In the closing lecture, Christopher Dobson (University of Cambridge, UK) considered general questions of amyloid formation. He presented evidence in support of the concept that the ability of proteins to form amyloid is generic. Many normally non-amyloidogenic proteins can form amyloid *in vitro* under conditions that destabilize their structure. The fact that very few proteins do form amyloid *in vivo* may be explained as a result of billions of years of protein evolution. This point of view predicts that, in general, proteins prone to convert to the prion state are not likely to carry a specific prion consensus sequence and are not likely to be identified by sequence analysis.

Probably, the most significant achievements reported at the conference related to prion structure, in both the sense of spatial structure and the role of the primary structure. Important questions for the future relate to the mechanisms of prion propagation, including the role of chaperones and possible curing mechanisms. The number and variety of known prion-like phenomena grows, but only the future will show the full picture of their occurrence and importance for living organisms.