Electron microscopy studies of yeast prion Ure2p fibrils

Ngai Shing Mok and Neil Ranson

Introduction

Prions are protein molecules that can assume a stable misfolded conformation, which can autocatalytically induce the misfolding of the normal conformation of the protein. These misfolded forms are prone to aggregation, and typically an amyloid-like aggregate results. The mammalian protein PrP is the causative agent for neurodegenerative disorders such as Creutzfeldt-Jakob Disease (and its variants) in humans, Bovine Spongiform Encephalopathy (BSE) in cattle, and Scrapie in sheep. However the prion phenomenon is not confined to higher organisms, and several prion proteins are found in yeast. Direct studies of PrP are exceptionally difficult owing to the long incubation period of the disease, and the risk of iatrogenic infections. Yeast prions are harmless to humans and therefore easier to handle, and so make ideal model systems to study the prion phenomenon. One such yeast prion, Ure2p, forms ordered, twisted fibrils, which are thought to contain an amyloid-like structure. However, only low-resolution, 2-D structural data is currently available to support this model.

Current work

Low-resolution CCD images of unstained Ure2p fibrils in ice have been collected on a Tecnai F20 microscope in the Astbury Centre. Image averages of aligned fibril segments show that twisted Ure2p fibrils have a distinct punctate appearance and rather poor helical order. We are currently implementing an improved alignment scheme and assessing polarity of the segments before generating a preliminary 3-D model. We are also collecting a much larger, higher resolution dataset on film to extend the resolution of these preliminary 3-D models.

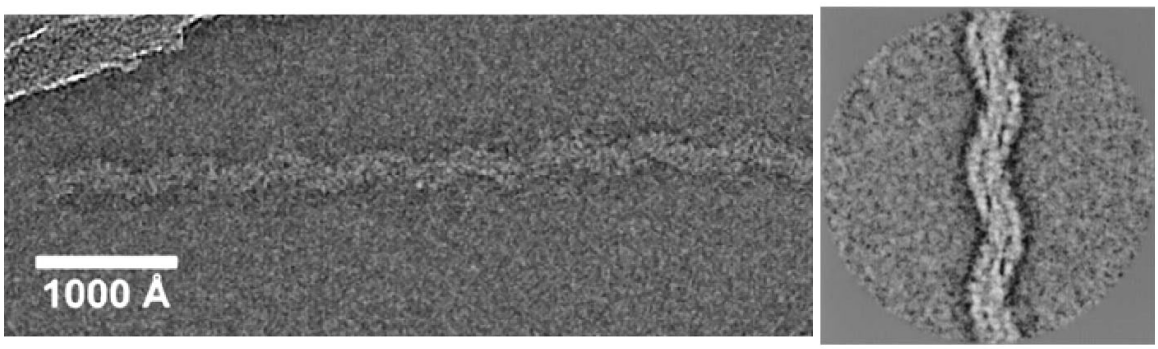

Figure 1. On the left, a raw cryo-EM image of an unstained, twisted Ure2p fibril embedded in vitreous ice. The fibril was grown from wild-type, full length Ure2p at physiological pH and ionic strength. On the right, an average of ~100 aligned Ure2p fibril segments shows the twisted, punctate appearance of the Ure2p fibril in greater detail.

Publications

Ranson, N., Stromer, T., Bousset, L., Melki, R. and Serpell, L. C. (2006). Insights into the architecture of the Ure2p yeast protein assemblies from helical twisted fibrils. *Protein Sci.* **15**, 2481-2487.

Funding

This work was supported by the BBSRC. NAR is a University of Leeds Research Fellow

Acknowledgements

This work is in collaboration Drs. Ronald Melki and Luc Bousset at the Laboratoire d'Enzymologie et Biochimie Structurales, CNRS, Gif-sur-Yvette, France.