Changes in Protein Structure and Distribution Observed at Pre-Clinical Stages during Scrapie Pathogenesis

Beamline: U10b

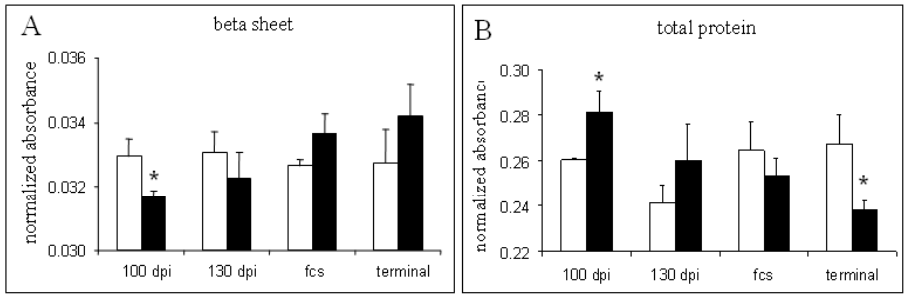
Technique: Infrared microspectroscopy

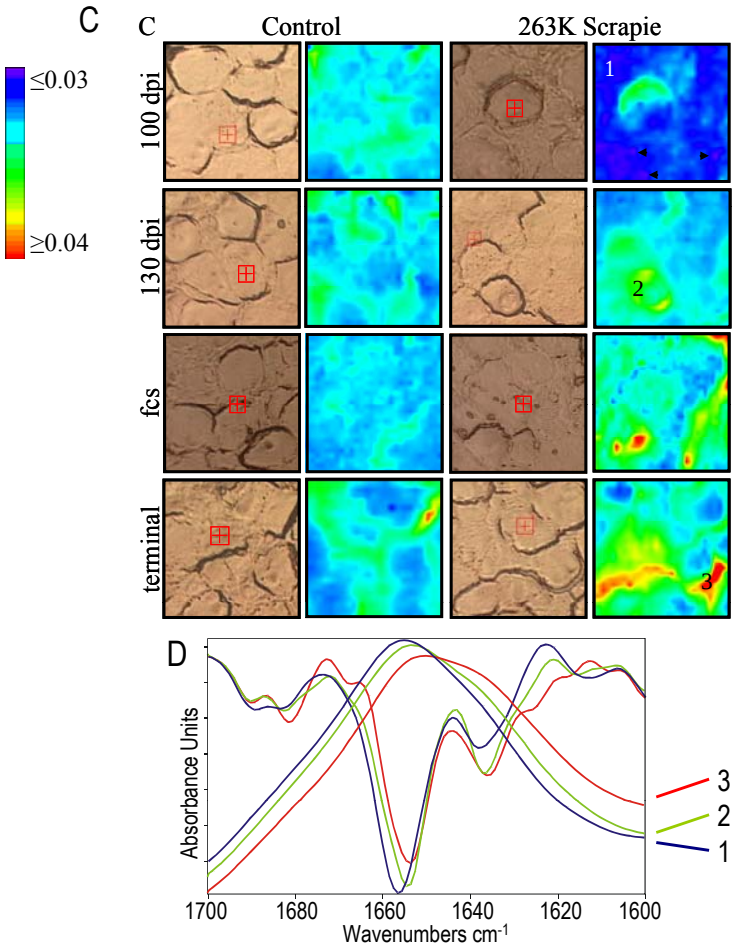
Researchers:
L.M. Miller, A. Kretlow, Q. Wang (BNL), M. Beekes, D. Naumann (Robert Koch-Inst., Berlin)

Publications:

1) A.Kretlow, Q. Wang, M. Beekes, D. Naumann, L.M. Miller. *Anal.Bioanal.Chem.* submitted

2) A. Kretlow, Q. Wang, J. Kneipp, P. Lasch, M. Beekes, L.M. Miller, D. Naumann. *Biochim. Biophys. Acta* 1758: 948-59 (2006).





Motivation: Transmissible spongiform encephalopathies, such as scrapie, mad cow disease and Creutzfeldt-Jakob disease are fatal neurodegenerative disorders of the CNS and are characterized by the conversion of the α-helical rich normal prion protein into aggregates of its β-sheet rich conformer. To analyze the disease-related protein structural changes during scrapie pathogenesis directly in the tissue we investigated dorsal root ganglia of scrapie infected hamsters at different time points of the disease.

Results: Results showed clear changes in protein content, composition, and distribution as the disease progressed. At pre-clinical time points, the scrapie-infected animals exhibited a significant increase in protein expression, yet the β-sheet protein content was significantly lower than in controls. As the disease progressed, the β-sheet protein content increased near the cell membrane and in the cytoplasm of infected neurons, above the amount attributable to PrP^{Sc}. Subsequent immunostaining confirmed that this increase was at least partly due to the formation of PrP^{Sc}. At the terminal stage, the relative protein expression declined significantly, possibly due to degeneration and death of neurons. Based on these findings, we suggest that the pre-clinical stages of scrapie are characterized by an overexpression of proteins low in β-sheet content. As the disease progresses, PrP^{C} is converted to PrP^{Sc}, along with the conversion or replacement of other α-helical-rich proteins by β-sheet proteins. The dramatic changes in protein content and structure at pre-clinical time points emphasizes the need for identifying protein changes involved in early pathogenesis, which are important for understanding the disease and may provide a mechanism for early TSE diagnosis and treatment.

Mean values + average deviation for the (A) β-sheet and (B) total protein content for infected (black bars) and control (white bars) hamster dorsal root ganglia at the four investigated time points. Asterisks mark significant differences ($p<0.05$) between scrapie and control. (C) Photomicrographs of unstained cryo-sections (1st and 3rd columns) and corresponding FTIRM images (2nd and 4th columns) of the β-sheet distribution for control (left) and infected (right) ganglia at different time points. Areas exhibiting extremely low β-sheet at 100 dpi are indicated by arrowheads. (D) Original and 2nd derivative spectra of areas indicated by numbers in the chemical maps. Red square in photomicrographs: 10x10 μm.