

Introduction: Transmissible spongiform encephalopathies (TSEs) including Creutzfeldt-Jakob disease, fatal familial insomnia, bovine spongiform encephalopathy and scrapie are fatal, incurable neurodegenerative disorders. TSEs are caused by the infectious isoform of the prion protein (PrP^{Sc}) which is formed by a conformational conversion from the cellular prion protein (PrP^C). The prion protein family includes two additional members besides PrP^C, the Doppel and the newest prion protein, the Shadoo. In 2008 an evolutionary conserved RGG-box motif, which is known to be able to bind RNA, was identified in silico by Gready et al in the amino acid sequence of Shadoo.

Objectives: Here we study the nucleic acid binding features of mouse Shadoo and aim to map its nucleic acid binding region.

Materials & methods: Three deletion mutants of Shadoo were introduced: I deleted the predicted nuclear localization signal (delNLS), the RGG-box (delRGG), as well as the glycine and arginine rich (RXXX)₈ motif. Proteins were expressed fused to a mCherry fluorescent tag in *E. coli* and partially purified using Ni-NTA agarose beads. The nucleic acid binding was examined with a newly developed Agarose Gel Shift Assay (AGSA) method, which exploits the advantages of a fluorescent protein fusion tag.

Results: According to AGSAs, wild type Shadoo is capable of binding both RNA and single or double stranded DNA oligos but not plasmid DNA fragments. The three deletion mutants could not bind nucleic acid.

Conclusion: Mouse Shadoo can bind both RNA and DNA. Even the shortest N-terminal deletion prevents binding. Further experiments aim to finalize mapping the binding region, examine length and sequence specificity of the substrate.

P-M33:

EXAMINATION OF THE EFFECT OF A DOWNSTREAM TRANSLATION INITIATION SITE ON THE LOCALIZATION OF PROTEINS USING THE SECRETORY PATHWAY

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Introduction: Fluorescent proteins are often used for studying protein localization and trafficking. When they are fused to the N-terminus of proteins using the secretory pathway, they must be inserted after the secretion signal. It is a common practice to insert the fluorescent protein in a way which leaves the starting translation initiation (TI) site of the fluorescent protein intact. Our experiments revealed the formation of a truncated form of the fusion protein with an altered localisation.

Objectives: Our aim was to study the effect of the downstream TI-site which was likely responsible for the mislocalisation we detected. We study the possible mechanisms causing this dual localizations on both protein and mRNA level.

Materials & methods: We designed a model for this study, where it is easy to distinguish the full length and truncated proteins by targeting to either the plasma membrane or the nucleus, respectively, and can be visualized by confocal microscopy.

Results: In the presence of both TI-sites we observed nuclear localization in nearly 30% of the transfected cells, which was almost entirely eliminated when the downstream TI site was removed. This made our model

system ideal for the following studies, where we examined whether the effect of the 2nd TI-site occurred at the protein or at the RNA level.

Conclusion: Although such constructs are generally used with double TI-sites it is very important to eliminate the downstream one to avoid the formation of truncated and mislocalised proteins and to decrease the signal: background ratio in fluorescent imaging.

N1:

THE LATERALIZED BRAIN IN PARKINSON'S DISEASE

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Introduction: The onset of PD features starts on either the right or left side.

Objectives: The side of onset appears to determine the prognosis of the disorder and other features. Right side tremor dominant has a better prognosis in contrast to left side dominant bradykinesia-rigidity. In addition, left side onset of motor features is associated with cognitive decline.

Materials & methods: Therefore, an intricate relation appears to exist between the side of disease onset and progression/severity and other non-motor symptoms. Unilateral PD in turn corresponds to neuronal nigrostriatal degeneration in the contralateral hemisphere. Indeed positron emission tomography has demonstrated a positive correlation between symptom asymmetry and brain function (Hoorn et al, 2011), which, corresponds to a unilateral pattern of degeneration.

Results: This phenomenon appears to be exclusive to PD and is not found in other degenerative disorders. Additionally, the variation in motor symptom(s) dominance exhibited in the disorder, conforms with the notion that PD is a spectrum disease with many sub-groups.

Conclusion: Thus, clinical and post mortem studies on “lateralisation” may serve as a vital tool in understanding the mechanism(s) eliciting the characteristic destruction of the SN neurons. Additionally, it may be employed as a predictive indicator for the symptomology and prognosis of the illness thus allowing selective treatment strategies for the pronounced hemispheric degeneration.

N2:

MITOCHONDRIA AND THE PATHOGENESIS OF NEURODEGENERATIVE DISEASES

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Much evidence suggests that mitochondria have a central role in aging-related neurodegenerative diseases. Mitochondria are critical regulators of cell death, a key feature of neurodegeneration. Mutations in mitochondrial DNA and oxidative stress both contribute to aging, which is the greatest risk factor for neurodegenerative diseases. In all of the major neurodegenerative diseases, there is strong evidence that mitochondrial dysfunction occurs early and acts causally in disease pathogenesis. Recent studies have shown that mitochondrial dysfunction occurs early in the course of Alzheimer's Disease and that both beta-amyloid and tau protein oligomers act synergistically to impair mitochondrial function, contributing to synaptic deficits. In Parkinson's Disease (PD), the genetic causes of early onset PD are linked to mitochondrial dysfunction. The genes parkin and PINK1 are involved in mitophagy, and DJ1 stabilizes Nrf2, a critical genetic pathway involved in modulating oxidative damage. Recent work in Huntington's Disease confirms