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PROTEIN MISFOLDING IN DISEASE – TOXIC AGGREGATION-PRONE PROTEINS IN AGING AND AGE-RELATED DISEASES: FROM STRUCTURE TO PATHOLOGY AND SPREADING

MEPLIEMENT DES PROTEINES – VERS UNE AGREGATION TOXIQUE DES PROTEINES AU COURS DU VIEILLISSEMENT ET DES MALADIES LIEES A L'AGE : DE LA STRUCTURE A LA PATHOLOGIE ET SA PROPAGATION

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The influence of nonsense mutations in the SUP35 gene on the [PSI$^+$] prion propagation in yeast *Saccharomyces cerevisiae*

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Various changes in the protein structure can lead to its abnormal aggregation. Recently several cases of amyloidosis or new prion diseases caused by truncated proteins were found. Such non-functional protein fragments may arise by different mechanisms one of which is a preliminary termination of translation due to nonsense mutations in the corresponding gene. Therefore, the study of this phenomenon may be of great interest.
Yeast may offer a convenient model to investigate the mechanisms of prionization as about ten proteins with prion properties are described for this object. [*PSI*$^+$] is the most studied yeast prion. Its structural protein Sup35 (eRF3) belongs to a second class translation termination factors. In the presence of amyloid aggregates of this protein, as well as mutations in the essential *SUP35* gene, the efficiency of translation termination is reduced that promotes nonsense suppression. In our laboratory a collection of viable *sup35* nonsense mutations was obtained. These mutations lead to the appearance of C-terminally truncated proteins including Sup35 protein. We identified several *sup35* alleles that led to spontaneous [*PSI*$^+$] formation and appearing of Sup35p aggregates. This allowed to suggest that the truncated fragments produced in strains with *sup35* nonsense mutations can induce prionization, as has been shown before by *in vitro* systems.
According to our results truncated fragments can serve as seeds for the [*PSI*$^+$] formation. Therefore, they potentially may coaggregate with full length protein in [*PSI*$^+$] cells and affect prion propagation. We revealed different effects of nonsense-mutations on [*PSI*$^+$] propagation: several mutations led to prion loss, another ones changed its properties. In most dramatic cases combination of some mutations with [*PSI*$^+$] factor led to the cell death.
Effects of *sup35* nonsense mutations on [*PSI*$^+$] maintenance had never been studied before. For the first time we have shown that spontaneous nonsense mutations in prion structural gene may differently affect the [*PSI*$^+$] prion induction and maintenance. Since observed effects did not correlate with positions of mutations we propose existence of multiple mechanisms explaining all phenomena which are now under investigation.
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Investigating the link between primary sequence, amyloidogenicity and toxicity

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β- amyloid 1-42 ($A\beta_{1-42}$) is a self-assembling protein that goes through many structural changes before forming the extracellular plaques characteristic of Alzheimer's disease. The link between $A\beta_{1-42}$ structure and toxicity is a major topic of interest and it is widely accepted that the oligomeric species is neurotoxic. Research in this area, however, has been hindered by a lack of suitable peptide control. We have studied the conformational changes of the $A\beta_{1-42}$ peptide over time by combining a range of biophysical approaches including circular dichroism, and Thioflavin T fluorescence with transmission electron microscopy and compared this to a novel, rationally-designed, assembly- resistant $A\beta_{1-42}$ peptide variant ($vA\beta_{1-42}$). This $vA\beta_{1-42}$ differs in sequence by only two amino acids, however, does not self-assemble or form β-sheet structure. Furthermore, toxicity assays on both human neuroblastoma cells and hippocampal neurons confirmed that unlike $A\beta_{1-42}$, $vA\beta_{1-42}$ is not toxic. $vA\beta_{1-42}$ therefore serves as a sequence related peptide in $A\beta_{1-42}$ studies and further highlights the importance of sequence for a protein to self-assemble. The assembly of the two main $A\beta_{1-42}$ controls, $A\beta_{42-1}$ and Aβ scrambled, have also been characterised and shown to assemble into fibrils and the toxicity of the oligomeric and fibrillary species have been examined in cell culture. By comparing the sequences of toxic disease related amyloids, such as $A\beta_{1-42}$, with non-toxic synthetic amyloids, this work aims to elucidate the key characteristics for toxicity

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