Polyglutamine conformation in GST-polyglutamine fusion proteins

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The nuclear-encoded Sup35p protein is responsible for the transmissible prion-like [PSI⁺] determinant of yeast with Sup35p existing largely as a high molecular weight aggregate in [PSI⁺] strains. We have developed a simple plasmid shuffling assay to determine the consequences of mutations within the N-terminal prion-forming domain (PrD) of Sup35p on [PSI⁺] propagation. The human PrP octapeptide (PHGGGWGQ) is able to functionally replace a Sup35p oligonucleotide repeat to allow [PSI⁺] propagation in vitro. Replacement of the entire Sup35p-PrD with polyGln can also give rise to a stable [PSI⁺] determinant if more than 75 Gln residues are added. In addition, replacement of some or all of the Sup35p-PrD with the human α2C peptide or with other putative amyloid-forming peptides gives rise to a high level of mitotic instability but these sequences are nevertheless able to partially support propagation of the [PSI⁺] determinant. These results indicate that a range of peptides with amyloidogenic potential can functionally replace the critical oligopeptide repeat region necessary for propagation of the transmissible [PSI⁺] determinant in yeast.

PrP gene expression regulation in scrapie

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Scrapie is a naturally occurring transmissible spongiform encephalopathy (TSE) of sheep. The hallmark of the TSEs is the aggregation into partially protease resistant fibrils of a pathological isoform (PrPSc) of a normal cellular protein designated PrP. The expression of PrP is of critical importance to TSE development. Polymorphisms in the ovine PrP gene ORF are implicated with TSE susceptibility but cannot fully explain the varying responses of all sheep breeds to scrapie infection. The focus shifts therefore to expression regulation. For example alternative polyadenylation of the ovine PrP gene produces two mRNA transcripts (4.6 kb and 2.1 kb), both are found in ovine peripheral tissues whilst the 2.1 kb species is also present in neuronal tissue. Transfection assays of murine neuroblastoma cells with constructs expressing different regions of ovine PrP mRNA revealed the presence of sequences in the 3’UTR that modulate protein synthesis. In a new approach cell lines derived from both ovine neuronal and peripheral tissues will be transfected with PrP expression constructs with specific deletions in the 3’UTR. In order to enhance the detection of recombinant PrP against a background of endogenous PrP the recombinant PrP has been tagged with the 3X FLAG epitope. It is expected that these deletions will reveal the mechanisms underlying PrP gene expression.