Deletion of the C-terminal part of helix alpha 2 does not prevent the prion conversion

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P.42: Deletion of the C-terminal part of helix alpha 2 does not prevent the prion conversion

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The mechanistic insights of prion conversion remain highly controversial. Several regions of PrP have been postulated as critical in this process; however, few attempts have been done to determine to what extent we can modify PrP sequences maintaining its properties. Recently, our laboratory progressed on these issues by showing that a PrP with eight extra amino acids inserted in the middle of the helix-2-helix-3 domain remains convertible into prion. These insertion mutants displayed no significant loss of the alpha helix content suggesting a replacement by the amino acids inserted. To determine whether a full size of H2 is required for prion conversion we performed series of deletions by site-directed mutagenesis in the ovine PrP sequence (VRQ haplotype). Mutants of PrP were then stably transfected in RK13 cells where protein expression and distribution of the mutants were monitored. The secondary structure of the recombinant proteins with similar deletions was analyzed by circular dichroism, showing no significant differences. The efficiency of infection was then tested through determination of the amount of protease resistant PrP accumulated in cells several passages after infection. Using 3 different prion strains for infection, we demonstrated that deletion of two turns of the H2 end does not prevent prion conversion indicating that this portion of the protein is not critically involved in the conformational change. We also probed that the susceptibility to infection was not altered in the mutants when compared to the wild type protein. The cells did propagate bona fide prions, infectious not only for naive homologous cell cultures but also for wt-expressing cells and for tg338 mice expressing the ovine PrP. This work demonstrates that the end of H2 is dispensable for prions and extends our previous conclusions that the amino acid specificity of this region was not required for the conversion.

To our best knowledge, most deletions in the protease-resistant domain that were reported so far remained unconvertible into prions with the exception of PrP106 produced by two large deletions in the first moiety of PrP^{C}. Our work demonstrates the possibility to produce prions with short internal deletions inside the protease-resistant core.

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P.43: Cloning the prion and the Shadoo proteins: Restriction enzyme Body Doubles and PCR cloning

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Here we demonstrate a procedure that solves a frequent problem in genetic engineering experiments that we faced working with the cDNA of the Prion and Shadoo proteins. This procedure allows the cloning of PCR fragments containing a recognition site of the restriction endonuclease (Type IIP) used for cloning in the sequence of the insert. A Type IIS endonuclease - a Body Double of the Type IIP enzyme - is used to generate the same protruding palindrome. Thus, the insert can be cloned to the Type IIP site of the vector without digesting the PCR product with the same Type IIP enzyme. We achieve this by incorporating the recognition site of a Type IIS restriction enzyme that cleaves the DNA outside of its recognition site in the PCR primer in such a way that the cutting positions straddle the desired overhang sequence. Digestion of the PCR product by the Body Double generates the required overhang. Hitherto the use of Type IIS restriction enzymes in cloning reactions has only been used for special applications, the approach presented here makes Type IIS enzymes as useful as Type IIP enzymes for general cloning purposes. To assist in finding Body Double enzymes, we summarised the available Type IIS enzymes which are potentially useful for Body Double cloning and created an online program (http://group.szbk.u-szeged.hu/welkergr/body_double/index.html) for the selection of suitable Body Double enzymes and the design of the appropriate primers. We routinely use this procedure to bypass this frequently arising problem.

P.44: Conformational properties of prion strains can be transmitted to recombinant prion protein fibrils in real-time quaking-induced conversion

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The phenomenon of prion strains with distinct biological characteristics has been hypothesized to be involved in the diverse structures of abnormal prion protein (PrP^{Sc}). However, the molecular basis of the strain diversity, including how to transmit the strain properties, remains uncertain. Real-time quaking-induced conversion (RT-QUIC) is a cell-free system that uses *E. coli*-derived recombinant PrP (rPrP) for the sensitive detection of PrP^{Sc}. To investigate whether properties of various prion strains can be transmitted to amyloid fibrils consisting of