PRION STRUCTURES AND THE ULTRASENSITIVE DETECTION OF MISFOLDED PROTEIN AGGREGATES BY SEEDED FIBRILLIZATION

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The 3D structures of TSE prions (e.g. PrP\textsuperscript{Sc}) and the basis for their faithful propagation as distinct infectious agent strains remain unclear. Prevalent structural models have features that are inconsistent with major empirical observations. Thus, we have built alternative models based on the parallel in-register intermolecular β-sheet architecture of synthetic prion protein (PrP) amyloids. These models are consistent with many key features of PrP\textsuperscript{Sc}. In addition, our modeling has suggested structural features that might strongly influence PrP\textsuperscript{Sc}-like folding toward the N-terminus of the protease-resistant amyloid core; namely prolines 102 and 105, mutations of which are linked to genetic human prion diseases, and a nearby cluster of 4 lysines within residues 101–110. We have now shown empirically that substitution of these residues with alanines or asparagines results in recombinant PrP amyloid fibrils with extended proteinase-K resistant β-sheet cores and infrared spectra that are more reminiscent of \textit{bona fide} PrP\textsuperscript{Sc}. These results suggest these proline and lysine residues are key modulators of the conversion of normal PrP into pathological multimers. We and others have exploited the ability of prions to seed the fibrillation of recombinant PrP develop practical and ultrasensitive RT-QuIC assays for a wide variety of prions in many types of samples. Notably, RT-QuIC assays of CSF and nasal brushings from human Creutzfeldt-Jakob disease patients can provide diagnoses with >95% sensitivity and nearly 100% specificity. Our latest assays provide results in a matter of hours rather than days. Appropriate selection of substrates and conditions have allowed us to sensitively detect all types of human and animal prions that we have tested so far (n =28). Moreover, by comparing seeding activities with different substrates, prion strains can be discriminated. Similar approaches may be helpful in detecting other pathological protein amyloids and diagnosing the associated diseases.

\textbf{Keywords:} prion, structure, diagnosis