426s Peptide Microarray-Based Mapping of Prion Transmission Barriers

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Prions are unique proteins; they influence cellular phenotypes in an epigenetic, reversible manner. This is possible since prion proteins can exist in at least two conformations (non-prion and prion), and conversion to the prion conformation typically involves assembly into oligomeric structures that change the activity of the corresponding proteins. Importantly, prions are unlike other aggregation-prone proteins since they are infectous and the prion state can be passed from one cell to another.

Specific prion proteins from different organisms (e.g., human vs. cow) often have similar protein sequences, and in some cases transmission of the prion state between species is possible (e.g., mad cow disease). The underlying mechanism appears to be largely based on the ability of a given protein in its prion state to physically recruit a related protein into its prion state.

We seek to provide insight into the physical basis of prion transmission barriers by using peptide microarrays to profile the specificity of a model prion protein, Sup35p, which is found in several yeast species, including S. *cerevisiae* (SC) and C. *albicans* (CA). We are employing arrays of 20mer peptides derived from the prion domains of SC and CA Sup35 and we find that a limited number of such peptides, although chemically similar, are specifically recognized by their corresponding prion protein. This finding is consistent with observations that SC and CA Sup35p do not affect each other's assembly kinetics *in vitro* or frequency of prion induction *in vivo*. Further, we and others have identified residues involved in the intermolecular contacts formed when both prions assemble into amyloid fibers using fluorescence energy transfer methods, and we find that peptides encoding such sequences are selectively recognized by each respective protein. We are currently analyzing a large number of mutations in the sequences of peptides corresponding to the intermolecular contacts of these proteins to search for mutations that would relax the specificity between SC and CA Sup35p and enable passage of their transmission barrier. These results are being verified both *in vitro* (assembly kinetics) and *in vivo* (prion induction frequency).