

FIGURE 8.1 Molecular basis of $[PSI^+]$ prion propagation. Isogenic *Saccharomyces cerevisiae* in the (A) $[psi^-]$ and (B) $[PSI^+]$ states [3]. The protein determinant of $[PSI^+]$, Sup35, is (C) soluble and complexed to Sup45 in the $[psi^-]$ state and (D) insoluble and inactive in the $[PSI^+]$ state. The inactivation of Sup35 causes read-through of stop codons and large phenotypic changes, some of which are beneficial. [9,10,42]. (E) The assembly of Sup35 into amyloid fibers occurs via a nucleated conformational conversion process. This involves a nucleation step where soluble Sup35 oligomeric intermediates form and mature into amyloid nuclei. (From [59], with permission of AAAS.) (F) This assembly process produces a lag phase in which the oligomers form and mature, and then a rapid assembly phase in which mature nuclei template monomeric or oligomeric Sup35 into amyloids. The lag phase is eliminated by adding preformed Sup35 amyloids to soluble Sup35. (G) Transmission electron microscopy image of amyloids of a portion of the Sup35 protein (the N-terminal and middle domains, NM). Spherical NM oligomeric intermediates are associated with the ends of the amyloids, suggesting their relevance to amyloid assembly as well as amyloid nucleation. The width of the NM fibers is approximately 10 nm. (From [59], with permission of AAAS.)

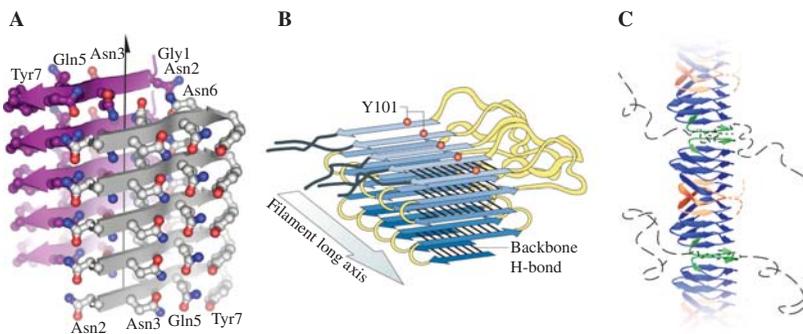


FIGURE 8.2 Amyloid structures of Sup35 peptide and protein fragments. (A) Crystal structure of ${}^7\text{GNNQQNY}^{13}$, a 7-mer peptide from the N-terminus of Sup35. The crystal structure reveals a high degree of geometric complementarity between opposing strands, which leads to exclusion of water at this interface and explains the stability of these amyloids. (B) In register, parallel β -sheet model of NM amyloid structure based on solid-state NMR results. (From [99], with permission of Macmillan Publishers Ltd.) This model proposes that most of the residues in the N domain and some residues in the M domain self-stack (such as indicated residue, Y101). (From [7], with permission of Macmillan Publishers Ltd.) (C) B-helix model of NM amyloid structure. This model proposes that two amino acid segments in the N domain are in intermolecular contact, whereas the intervening region is in intramolecular contact. (From [69].)

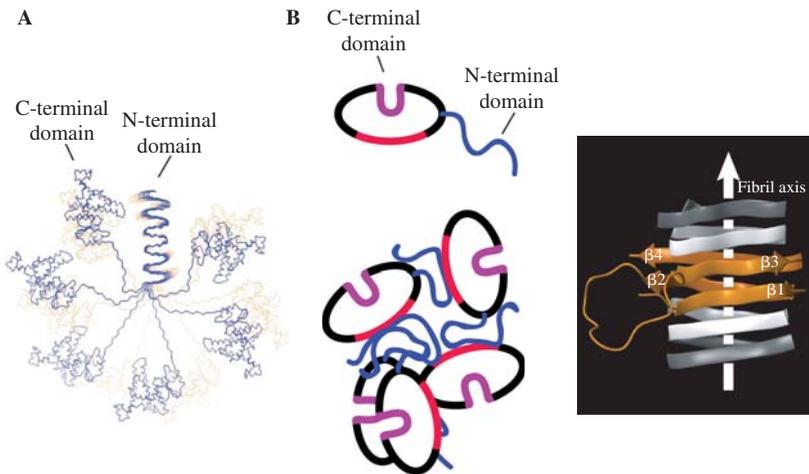


FIGURE 8.3 Amyloid structures of Ure2 and Het-s. (A) In register, parallel β -sheet model of Ure2 amyloid structure [7,112]. This model proposes that most of the residues in the N-terminal domain self-stack on each other, while the C-terminal displayed on the surface. (From [103], with permission. Copyright © 2004 National Academy of Sciences, U.S.A.) (B) Helical model of Ure2 fiber structure. This model proposes that the Ure2 amyloid conformation is a helical assembly of Ure2 monomers in a near native conformation where the N- and C-terminal domains are in contact. (From [111], with permission). Copyright © 2005 American Society of Biochemistry and Molecular Biology.) (C) β -Solenoid model of Het-s amyloid structure. This model proposes that the amyloid conformation involves two β -strand–turn– β -strand motifs. (From [120], with permission of Macmillan Publishers Ltd.)

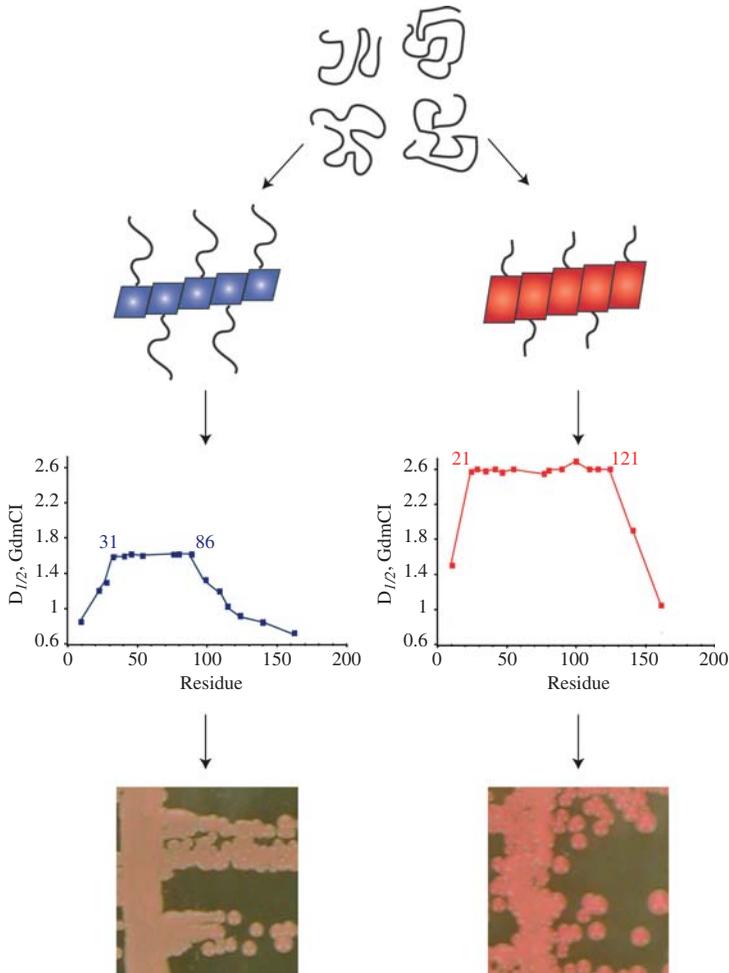


FIGURE 8.4 Sup35 strains have different-size amyloid cores. Different Sup35 strains can be formed *in vitro* by assembling the protein into amyloids at different temperatures. Fibers formed at 4°C have a smaller number of residues in their core than those formed at 37°C [69,70]. To determine the size of the amyloid cores, single-cysteine mutants were introduced into the NM portion of Sup35 [69]. The single-cysteine mutants were then labeled with a fluorophore sensitive to solvent exposure (acrylodan) and assembled into fibers. By disassembling the fibers with increasing amounts of guanidine hydrochloride, the midpoint of unfolding was determined using site-specific acrylodan fluorescence measurements. For fibers formed at 4°C, residues 31 to 86 formed a contiguous core of solvent shield residues, while residues 21 to 121 formed the amyloid core for fibers formed at 37°C. Transformation of unlabeled [13,69] or labeled (Krishnan and Lindquist, unpublished results) fibers formed at 4°C into yeast yielded strong [PSI^+] phenotypes; the corresponding transformations with fibers formed at 37°C produced weak [PSI^+] phenotypes.

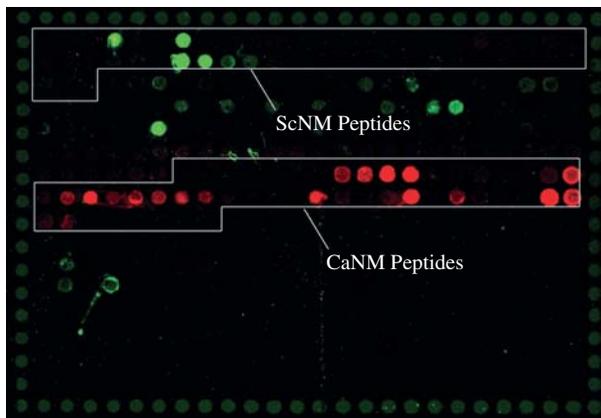


FIGURE 8.5 Figure 5 Peptide microarray analysis of yeast prion species barriers. Fluorescently labeled NM domains of Sup35 from *S. cerevisiae* (ScNM) and *C. albicans* (CaNM) were incubated with overlapping peptide libraries (20 residues per peptide) derived from a sequence of each NM protein [79]. Both ScNM and CaNM bound selectively to a small subset of their own peptides but did not cross-react with peptides from the other species.