Dynamic Prions Revealed by Magic

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Prion proteins can be propagated as amyloid fibrils with several different conformational variants. By providing structural information at atomic level for two such variants of a yeast prion, Frederick and colleagues, in this issue of *Chemistry & Biology*, reveal how conformational flexibility can generate phenotypic diversity.

Prions are a remarkable subclass of amyloid proteins associated with potentially infectious brain diseases in mammals, but they also act as novel epigenetic regulators of a range of cellular functions in yeast and other fungi. Fungal and mammalian prions share a common amyloid core consisting of the characteristic cross-β molecular architecture that forms long, unbranched filaments rich in β sheets. They also share the perplexing ability to exist as distinct, heritable conformations. In mammals, this conformational diversity leads to prion strain-specific neuropathologies, whereas, in the yeast Saccharomyces cerevisiae, the prion variants can have distinguishable and often beneficial impacts on the host. What has been missing until now is an insight into the structural differences at the atomic level underlying the prion strains (variants) and their biological impact. The elegant paper by Frederick et al. (2014) in this issue of *Chemistry & Biology*, addresses this by using two variants of the yeast [PSI+] prion.

Although near atomic resolution pictures of the structural organization of the amyloid core have been obtained for several types of amyloid fibrils, the link between amyloid structure and their varied biological properties and function has remained elusive (Toyama and Weissman, 2011). Some amyloid assemblies display a potential for toxic gain-of-function, whereas others are inert or tolerated and, as in the case of several different yeast prions, may even perform beneficial biological functions. This is also the case with conformational variants of a given amyloid that can also generate additional functional diversity (Newby and Lindquist, 2013). What emerges from the Frederick et al. (2014) study is a detailed picture not only of two distinct conformations of the amyloid form of Sup35, the underlying [PSI+] prion protein, but also the dynamic behavior of these structures. These findings lead the authors to propose a plausible mechanism that can explain why these distinct conformational forms of Sup35 behave so differently in vivo.

The groundbreaking studies by Weissman and his colleagues (Toyama et al., 2007) first demonstrated that two variants of the yeast [PSI+] prion could be distinguished by differences in the conformation of the amyloid core that involves the N terminus of Sup35 (Figure 1A). The Sup35 conformations studied were associated with a “weak” and a “strong” variant of [PSI+] (Figure 1B), so called because of the difference in the strength of the prion-associated nonsense suppression phenotype. Remarkably, once a prion variant conformation is established, it is faithfully propagated via a two stage process involving seeded growth of transmissible prion particles (propagons) through monomer addition at fibril ends and the subsequent fragmentation of the amyloid fibril by the combined action of three chaperones: Hsp104, Hsp70 (Ssa1), and Hsp40 (Sis1) (Figure 1C).

To explore the conformational distinctions between weak and strong prion fibrils, Frederick et al. (2014) use solid-state magic angle spinning nuclear magnetic resonance (MAS NMR). The outcome is the first detailed analysis of the dynamic properties of two different variants of the amyloid forms of the [PSI+] prion over a wide range of timescales. Although MAS NMR was first used over a half a century ago, Frederick et al. (2014) attractively demonstrate the applicability of this technique to deciphering amyloid properties, thereby enabling a comprehensive structural and dynamic study of these normally refractory molecules in the solid or semi-solid state with restricted molecular motion. Despite the global restricted molecular motion that is prevalent in amyloid fibers, their pioneering approach reveals critical intramolecular motion over a wide range of timescales that highlight rigid and flexible regions in the two different prion variants of Sup35.

Like the majority of studies to date, Frederick et al. (2014) focus on the Sup35NM form of the prion protein lacking the functional C domain (Figure 1A). The N-terminal region (N) resides within the amyloid core and is essential for [PSI+] formation and propagation, whereas the adjacent and highly charged region (M) contains a primary binding site for the Hsp104 chaperone (Helsen and Glover, 2012). One of their important findings is that the Sup35NM amyloid fibrils have both rigid and dynamic regions, with the hitherto relatively conformationally unexplored M region being highly dynamic in both strong and weak prion fibrils. Another recent study of the M region using solid-state NMR has also suggested that M is a highly flexible region (Luckgei et al., 2013), which contrasts the findings of Shewmaker et al. (2009), who reported that the M region forms part of the rigid amyloid core. Using 13C-13C INEPT-TOBSY NMR experiments, Frederick et al. (2014) reveal that the M region in the weak amyloid fibrils is 40% more dynamic than in the strong amyloid fibrils. Furthermore, they show for the first time that while the N region lies within distinct but partly overlapping regions of the rigid amyloid core in both weak and strong fibers as shown by others (Toyama et al., 2007; see Figure 1A), the amyloid core of the two
variant fibrils is also quite distinct at the conformational level.

The dynamic, variant-specific behavior of the Sup35 M region is intriguing. By showing that this behavior can modulate the association between Hsp104 and the Sup35 fibrils in vivo, Frederick et al. (2014) identify how the conformational variability of the amyloid fibril may influence prion behavior. In considering this, it is important to note that productive binding of Hsp104 to Sup35 fibrils in vivo (i.e., leading to fragmentation) requires the Hsp70 chaperone Ssa1 to recruit Hsp104 to the fibril (Winkler et al., 2012). The effects seen by Frederick et al. (2014) may therefore reflect modulation of Ssa1 binding. Surprisingly the weak fibrils contain a higher proportion of tightly bound Hsp104; but, rather than leading to increased fibril fragmentation, it apparently reduces it. The ability of Hsp104 to bind nonproductively to Sup35 fibrils via an additional Ssa1-independent, nonproductive binding mode has been recently reported (Winkler et al., 2012).

How faithfully do the structural data obtained with in vitro generated fibrils of Sup35NM mirror the conformations taken up by either full-length Sup35 or the conformations in the cell? This concern has been raised by several studies, e.g., (Luckgei et al., 2013), but Frederick et al. (2014) go some way to address this concern by studying the structure of Sup35NM fibrils generated in vitro using “natural” seeds extracted from cells. All other structural studies of [PSI+] variants have employed Sup35NM fibrils generated in vitro using different temperatures of incubation to generate the different variant-associated forms of Sup35 (Tanaka et al., 2004). It is also assumed that for a given [PSI+] variant, one is studying a homogenous collection of fibril conformations in the cell extracts used to seed the in vitro polymerization. However, recent genetic studies have indicated that the [PSI+] prion can potentially exist as a “cloud of variants” in vivo (Bateman and Wickner, 2013), and these may comprise Sup35 structures in addition to those characterized by Frederick et al. (2014). The existence of multiple conformational variants for a given prion would greatly expand the range of phenotypes a given prion may generate, be it in relation to disease states in mammals or the short-term or long-term acquisition of new and beneficial traits in fungi.

REFERENCES


