Phase separation induces significant structure in the N-terminal region of the mouse prion protein

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Understanding the link between phase separation (PS) of disease-linked proteins to form condensates, and their aggregation, requires insights into the conformational changes the proteins undergo inside the condensates. Here, structural changes undergone by the mouse prion protein (moPrP) inside PS-induced condensate have been characterized. Hydrogen-deuterium exchange in conjunction with mass spectrometry shows that the N-terminal region (NTR), which is unstructured in monomeric native moPrP, gains significant stable structure as the condensate ages. The structured C-terminal domain remains native-like, but subtle changes are seen. Conformational change initiates in the native monomer inside the condensate, with different regions undergoing rapid, slow or no conformational change as it ages. The b1-a1 loop undergoes rapid conformational change to lose stability, while the NTR gains structure slowly, concomitantly with conformational change at the C-terminal end of α3. Infrared spectroscopy reveals an increase in b-sheet structure at the expense of disorder, and circular dichroism spectroscopy-monitored change occurs as fast as that of the b1-a1 loop. Dynamic light scattering measurements show that the protein assembles into a large oligomer as the condensate ages, which is shown to be responsible for the fraction of protein present as mobile monomer decreasing with time of aging. The study reveals how conformational change in the NTR of protein sequestered inside the condensate leads to a change in its material properties. Importantly, the conformational change reduces the capacity of PrP to form amyloid fibrils, as well as to engage in the various binding activities that give rise to PrP function.