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SUMMARY of
2018 RESEARCH RESULTS REPORT
For International Collaborative Research with IPR, Osaka University

Research Title		Mechanism of amyloid fibril formation of α-synuclein
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Summary

Background: Although the cellular interior is crowded with various biological macromolecules, the distribution of these macromolecules is highly inhomogeneous. Eukaryotic cells contain numerous proteinaceous membrane-less organelles (PMLOs), which are condensed liquid droplets formed as a result of the reversible and highly controlled liquid-liquid phase transitions [Curr. Opin. Struct. Biol. 44, 18-30 (2017)]. On the other hand, amyloid fibrils involved in various diseases are formed by a nucleation-growth mechanism, similar to the crystallization of solutes from solution. Solubility and supersaturation are two of the most important factors determining crystallization of solutes. Moreover, crystallization competes with glass formation in which solutes collapse into amorphous aggregates. Recent studies on the formation of amyloid fibrils and amorphous aggregates indicate that the partition between distinct types of aggregates can be explained by a kinetic and thermodynamic competition between them [Curr. Opin. Struct. Biol. 36, 32-39 (2016)].

Specific Aims: The role of supersaturation in forming PMLOs is far from unclear. It has been observed that PMLOs have the propensity to mature, changing their properties from liquid-like to solid-like [Nature Reviews Mol. Cell Biol. 18, 285-298 (2017)]. Initially, the components in the condensed phase exhibit only transient interactions and lack appreciable order. Thus, the molecules freely rearrange (and exchange with the surrounding solution) and the molecular dynamics can be described as that of a liquid. Over time, the phase becomes more solid-like amyloid fibrils.

Methods: With α-synuclein, an intrinsically disorder protein associated with Parkinson's disease, we study the liquid-to-solid transitions focusing on the role of supersaturation and phase transition. We will use a *C. elegance* system expressing α-synuclein with yellow fluorescence protein.

Results and Discussion: The experiments showed the ultrasonication-accelerated fibril formation of α-synuclein. The formation of rigid fibrils was confirmed by TEM imaging of cell lysate. It is possible that α-synuclein first formed amorphous aggregates and then amorphous aggregates were converted to amyloid fibrils. However, at this stage, it was not possible to distinguish amorphous aggregates and amyloid fibrils directly inside cells. If we could use sophisticated methodologies distinguishing the clustered amorphous aggregates and rigid and elongated amyloid fibrils, we may be able to observe a kind of phase transition from droplet-like amorphous aggregates to amyloid fibrils as observed for FUS protein (Cell 162, 1066-1077 (2015). One possibility is to use a high resolution fluorescence imaging system monitoring the process of aggregation.