Optical Induction of Membraneless Organelles

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Cells are composed of membraneless organelles that form through self-assembly of intrinsically disordered proteins (IDPs). These IDPs are thought to self-associate to form mesoscale protein condensates. Many strategies have been developed in order to induce protein phase separation, however, outside of protease-dependent systems, the field lacks the ability to quickly induce assembly in response to external triggers. To overcome these limitations, we sought to build a light-inducible system for protein phase separation that requires only short pulses of light to achieve organelle assembly. We used variants of the intrinsically disordered RGG domain from the LAF-1 protein fused to optochemical dimerization domains. Previous studies have shown that increased RGG valency raises its phase transition temperature. We sought to dimerize RGG multimers to form higher valency proteins that would inducibly phase separate into liquid droplets at room temperature. As a proof of concept, we first demonstrated that rapamycin (Rap) induces phase separation at room temperature by fusing FK506 binding protein (FKBP) and FKBP-Rapamycin binding domain (FRB) to RGG domains and dimerizing the RGG domains with Rap. Next, to test optical induction of phase separation, we used a photocaged version of the Rap molecule, dRap, which uncages upon 405 nm illumination. In the dark, proteins are miscible. Following a short pulse of laser light, we observed rapid formation of liquid droplets. These experiments demonstrate the performance of our approach for irreversible triggering of protein phase separation and is currently being implemented in S. Cerevisiae. In the future, our light-inducible system has important implications for regulating phase separation and developing synthetic organelles.

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