Oligomerization regulates phase separation of endocytic proteins

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Abstract

Liquid-liquid phase separation of biomolecules is an important mechanism to spatially organize and regulate a wide variety of cellular processes. We and others have found that higher order assembly of constituent biomolecules is an important regulator of phase separation and the biochemical and biophysical properties of phase separated biomolecular condensates, though the relevant mechanisms remain poorly understood. Our goal is to understand the basic mechanisms that tune assembly to create biomolecular condensates with defined size, material properties, and biochemical activities. To this end, we use a model system that consists of three proteins – WASp, Nervous Wreck, and Dap160, which collectively regulate the endocytosis at the plasma membrane. These proteins mutually interact by known SH3 domain interactions and undergo liquid-liquid phase separation in vitro. We find that WASp and Dap160 are sufficient to form condensates. Interestingly, addition of Nervous Wreck (which can oligomerize) increases the viscosity and alters composition of the condensates. To test how oligomerization of Nervous Wreck controlled these properties, we compared normally oligomerizing Nervous Wreck to variants that existed exclusively as dimers or monomers. We found that loss of assembly progressively eliminated the effect of Nervous Wreck on endocytic condensate properties. Our next goals are to tune Nervous Wreck assembly to ‘program’ droplet properties, and to characterize the biochemical activities of these droplets.

Introduction

Biological context: Liquid liquid phase separation of biomolecules regulates diverse cellular processes and can form structures (condensates) of regular and limited size:

- Nuclear: Nuclear organization and transcription
- Endocytic spots: Endocytic sorting, signaling clusters, and detergent resistant nanodomains

Model system: Purified endocytic proteins

We use a minimal set of three endocytic proteins: Nervous Wreck, WASp, & Dap160. These proteins mutually interact & form condensates in vitro. Nervous Wreck (Nwk) can oligomerize.

Nervous Wreck regulates endocytic condensate properties

Panels show composite phase contrast (gray) and fluorescence (Dap160, green) images of endocytic droplets.

Role of assembly in phase separation:

- Assembly typically promotes phase separation by increasing valence and avidity of interactions and by lowering entropic cost of phase separation.
- Nervous Wreck assembly by oligomerization of its FBAR domain, and FBAR oligomerization is key to its biological activity of membrane binding and deformation.

Questions:

- How does assembly of endocytosis proteins control their phase separation?
- How can we control assembly to program condensate size/viscosity/activity?
- How does assembly enhance or inhibit phase separation by other protein interactions?

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Summary & next steps

Free SH3 domains:
- Enhance WASp-Dap160 recruitment
- Slow droplet fusion

Nervous Wreck SH3 domains:
- Inhibit phase separation
- Block Dap160 recruitment
- Block multivalent WASp-Dap160 interactions?