Oligomerization regulates phase separation of endocytic proteins

Steven J. Del Signore, Michelle F. Marchan, ShiYu Wang, and Avital A. Rodal

Department of Biology, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham MA

Brandeis bioinspired MRSEC

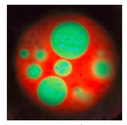
Abstract

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

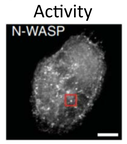
Biological context: Liquid liquid phase separation of biomolecules regulates diverse cellular processes and can form structures (condensates) of regular and limited size:

Nucleolus: Compartmentalization



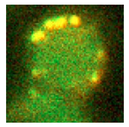
Feric et al 2016

Signaling clusters: Activity



Case et al 2019

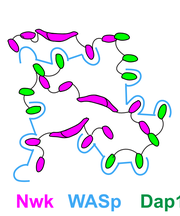
Endocytic spots: Activity, physical force

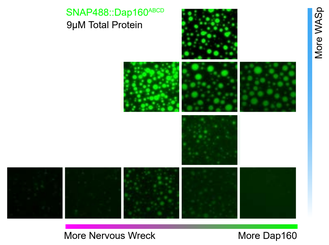


Sun et al 2017

Model system: Purified endocytic proteins.

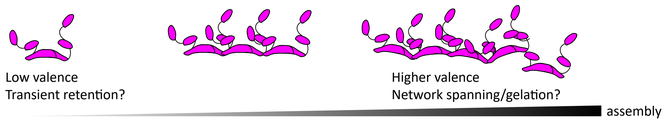
We use a minimal set of three endocytic proteins: Nervous Wreck, WASp, & Dap160
These proteins mutually interact & form condensates in vitro.
Nervous Wreck (Nwk) can oligomerize.





Role of assembly in phase separation:

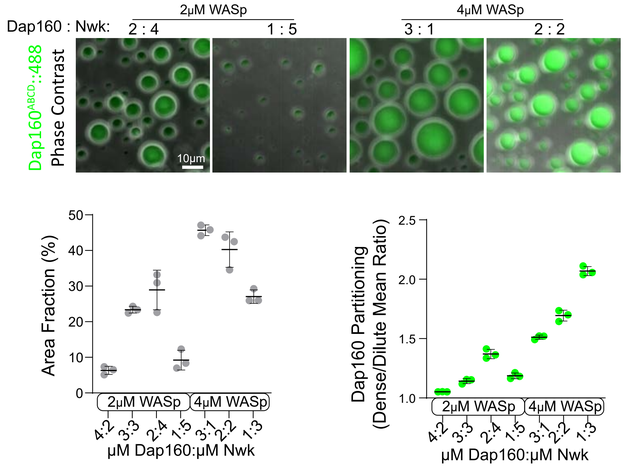
Assembly typically promotes phase separation by increasing valence and avidity of interactions and by lowering entropic cost of phase separation.
Nervous wreck assembles by oligomerization of its FBAR domain, and FBAR oligomerization is key to its biological activity of membrane binding and deformation



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?

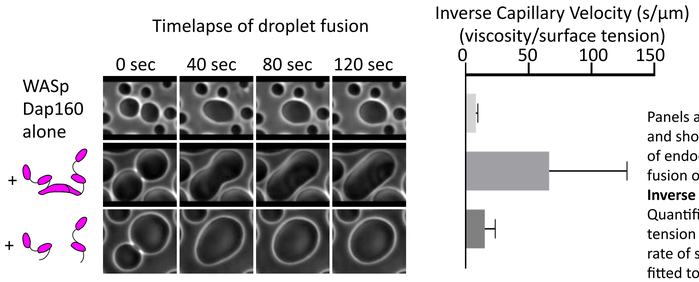
Nervous Wreck regulates endocytic condensate properties



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

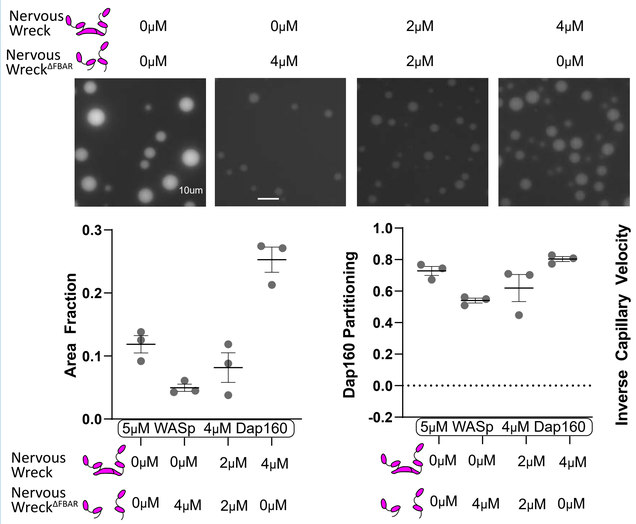
Area Fraction: Percent of imaged area occupied by droplets
Partitioning: Ratio of Dap160 fluorescence inside droplet to outside droplet

* At 2uM WASp, moderate Nwk concentrations maximize area fraction and Dap160 recruitment
* At 4uM WASp, Nwk decreases Area fraction but increases Dap160 recruitment
* Higher WASp concentrations promote LLPS



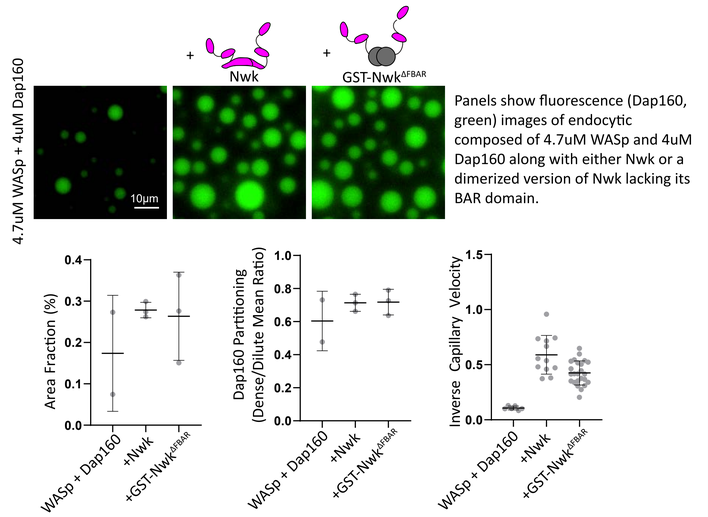
Panels are from timelapse movies and show phase contrast images of endocytic droplets during the fusion of two adjacent droplets.
Inverse capillary velocity: Quantifies viscosity/surface tension and measured as the rate of shape change of an ellipse fitted to the fusing droplets normalized to droplet size.

* Addition of Nwk (4uM) dramatically slows droplet fusion
* Addition of Nwk without its FBAR domain blocks the slowing of droplet fusion



Panels show fluorescence (Dap160, green) images of endocytic composed of 4uM WASp and 4uM Dap160 along with varying fractions of Nwk and Nwk lacking its oligomerizing BAR domain.

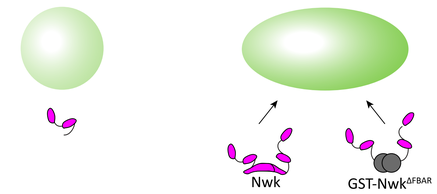
Dimerization of Nwk SH3 domains recapitulates effect of Nwk on droplets



Panels show fluorescence (Dap160, green) images of endocytic composed of 4.7uM WASp and 4uM Dap160 along with either Nwk or a dimerized version of Nwk lacking its BAR domain.

* Note - these are preliminary data
* Nwk increases inverse capillary velocity, as show earlier
* A dimerized variant of NwkΔFBAR is sufficient to recapitulate the effect of Nwk
* Suggests that either dimerization is sufficient for the effect of Nwk, or that Nwk does not extensively oligomerize under these conditions

Summary & next steps



Free SH3ab domains:
* Inhibit phase separation
* Block Dap160 recruitment
* Block multivalent WASp-Dap160 interactions?

Nwk SH3ab dimers:
* Enhance Dap160 recruitment
* Slow droplet fusion
* Enhance or inhibit phase separation depending on concentration of other ligands (eg WASp)

Next Steps:

Distinguish between effects due to WASp-Dap160 stoichiometry and Nwk
Test range of assembly sizes
Test regulataion of oligomerization by membrane charge
Size control by adapter proteins?