Effects of Aggregation-Promoting Mutations on Stress Granule Dynamics

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Abstract

Mutations in a number of stress granule-associated proteins have been linked to various neurodegenerative diseases. Several of these mutations are found in aggregation-prone intrinsically disordered domains (IDRs) of these proteins. My studies have focused on two IDR-containing yeast stress granule proteins, Pab1 and Pbp1. I have introduced mutations designed to enhance aggregation of these proteins and observed effects on stress granule dynamics. Results suggest that these mutations affect IDR localization in the context of overexpression, but do not affect stress granule dynamics in an endogenous system. This has led to questions about how the proteostasis machinery affects stress granule dynamics.

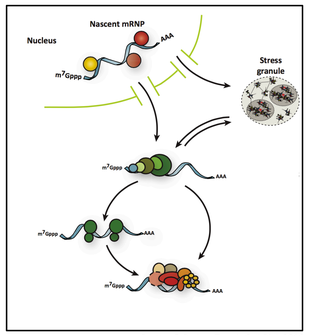
Stress Granules

What are stress granules?

- Cytoplasmic, membraneless organelles
- Composed of mRNA and translation initiation factors

What is their function?

- Form in response to stress
- Thought to protect certain mRNAs from degradation during stress



Protter & Parker. *Trends Cell Biol.* 2016

How does this process work?

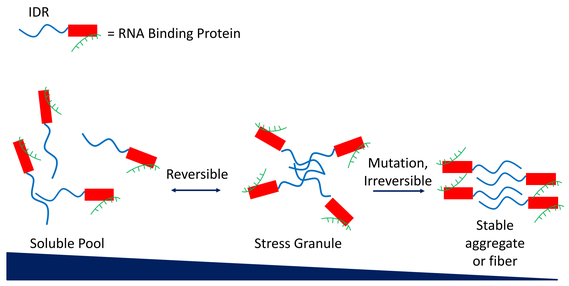
- Poorly understood mechanism
- Many constituent proteins contain aggregation-prone **intrinsically disordered regions (IDRs)**

  IDR=Unstructured region of low amino acid sequence complexity
- **Do the IDRs drive this reversible aggregation mechanism?**

Link to Disease

- Mutations in several stress granule proteins linked to ALS
- Pathological hallmark is cytoplasmic inclusions containing these proteins
- Normal aggregation process becomes irreversible
- Disease-relevant mutations occur in **intrinsically disordered regions (IDRs)**

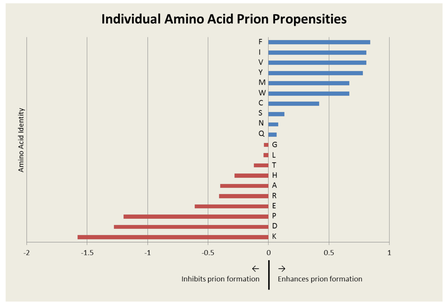
How do stress granule proteins get to this irreversibly aggregated state?



Predicting Protein Aggregation

PAPA

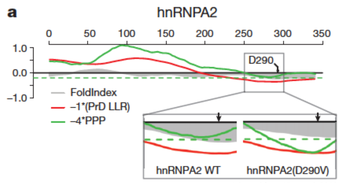
- PAPA (Prion Aggregation Prediction Algorithm)
- Scores each amino acid based on its ability to promote prion formation within a prion domain
- Use individual scores to determine prion propensity of entire sequence
- Aromatic and hydrophobic residues increase prion propensity
- Charged residues and proline inhibit prion forming ability



Adapted from Toombs et al. 2010.

hnRNPA2

- PAPA successfully predicted aggregation of hnRNPA2
- Human protein involved in stress granule formation
- D290V mutation in the prion-like domain implicated in a type of ALS (Kim et al. 2013)
- PAPA successfully predicted aggregation-promoting effect of this mutation



Adapted from Kim et al. 2013.

Key Question

Can we use PAPA to design aggregation-promoting mutations in known stress granule proteins that disturb stress granule dynamics?

Pbp1

- Yeast stress granule protein
- Homolog of ALS-associated human ATAXIN-2
- Contains a proline-rich intrinsically disordered region (IDR) at the C-terminus
- Prolines are generally considered aggregation-inhibiting

Pbp1 IDR

QTRFQQRQLNSMGNAV**P**GMN**P**AMGMNMGGMMGF**P**MGG**P**SAS**P**N**P**MMN GFAAGSMGMYM**P**FQ**P**Q**P**MFYH**P**SM**P**QMM**P**VMGSNGAEEGGGNIS**P**HV**P**AG FMAAG**P**GA**P**MGAFGY**P**GGI**P**FQGMMGSG**P**SGM**P**ANGSAMHSHGHSRNYHQ TSHHGHHNSSTSGHK

Protein	PAPA Score
Pbp1	-0.05 (-)
Pbp1 ΔPro	0.06 (+)

Hypothesis

Deleting the proline residues from the Pbp1 IDR will increase its aggregation propensity.

Increasing Aggregation Propensity of a Stress Granule Protein

Key Question

Does deletion of the proline residues from the Pbp1 IDR result in aggregation of the IDRs upon overexpression?

Hypotheses

1. Deleting prolines from the Pbp1 IDR will result in formation of SDS-resistant oligomers in response to overexpression, whereas the wild-type IDR will remain soluble in SDS.
2. Deletion of the proline residues from the Pbp1 IDR will result in foci formation upon IDR overexpression *in vivo*.

1. SDD-AGE (Semi-Denaturing Detergent Agarose Gel Electrophoresis)

Experiment

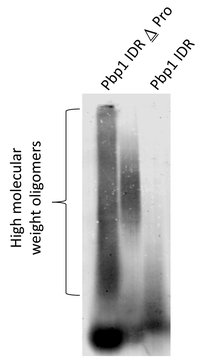
- Overexpress (increase concentration of) the Pbp1 IDR with (wild-type) or without (Pbp1 IDR△Pro) proline residues for 24hrs

Predicted Result

- More aggregation prone IDR (Pbp1 IDR△Pro) will form high molecular weight oligomers
- Wild-type Pbp1 IDR will remain soluble in detergent

Results

- Pbp1 IDR △Pro forms high molecular weight species, characteristic of aggregation.
- Wild-type Pbp1 IDR remains soluble.



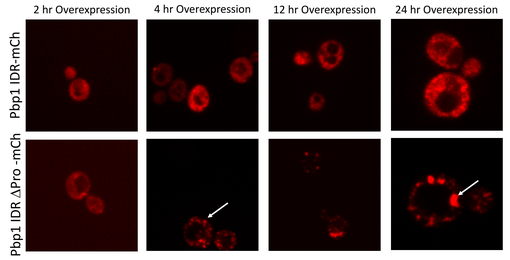
2. Fluorescence Microscopy

Experiment

- Fuse the Pbp1 IDR and Pbp1 IDR △Pro to the fluorescent protein mCherry.
- Overexpress the Pbp1 IDR with or without proline residues for 2, 4, 12, and 24hrs

Predicted Result

- More aggregation prone IDR (Pbp1 IDR△Pro) will begin to aggregate at earlier timepoints than the wild-type Pbp1 IDR.
- This is observed through localization of protein as visualized with fluorescent mCherry (denoted with white arrows below).



Results

- Pbp1 IDR △Pro forms aggregates in the cell after a shorter period of overexpression than the wild-type Pbp1 IDR.

Key Question

Are stress granule (SG) dynamics altered upon deletion of the proline residues from the Pbp1 IDR?

Hypotheses

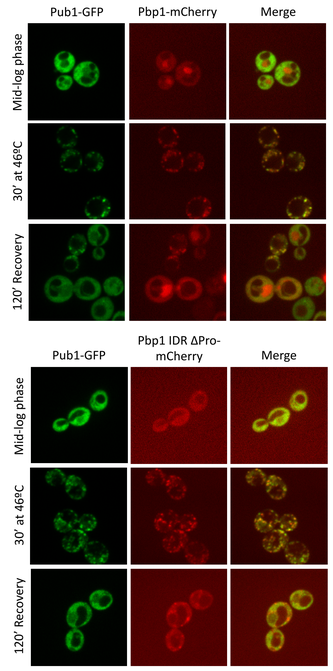
Deletion of the proline residues from the core SG proteins Pab1 and Pbp1 will result in slower SG disassembly rates or SG persistence.

Experiment

- Full-length Pbp1 and Pbp1 IDR ΔPro endogenously tagged with mCherry
- Pub1 tagged with GFP as a stress granule marker.
- Cells exposed to heat shock at 46ºC to induce stress granule formation.

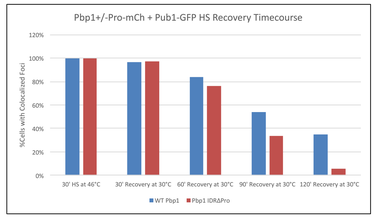
Predicted Result

- Pbp1 IDR ΔPro stress granules will form stress granules that will disassemble slower than wild-type Pbp1 stress granules.



Results

- Pbp1 IDR ΔPro stress granules appear to disassemble faster than wild-type stress granules.
- Making Pbp1 more aggregation-prone does not cause stress granules to persist.



Future Directions

Outstanding Questions

- How are stress granules able to resolve themselves when core proteins are made much more aggregation-prone?
- Are there different disassembly pathways for normal stress granules and stress granules containing proteins that are mutated?

Next Steps

- Investigate the role of the disaggregase machinery in stress granule disassembly
- Introduce different aggregation-promoting mutations into Pbp1
- Investigate the effects of aggregation-promoting mutations on other stress granule proteins.

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