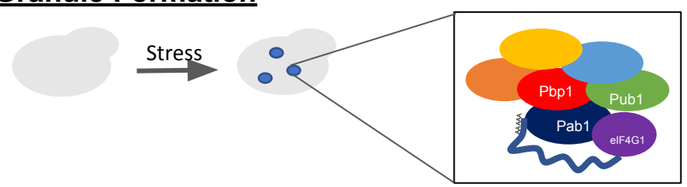
Determining the effects of Pab1 acetylation at K131 on stress granule dynamics in *Saccharomyces cerevisiae*

Sangavi Sivananthan, Sylvain Huard, Kristin Baetz
Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, uOttawa

Introduction

Does the acetylation of Pab1-K131 affect stress granule formation?

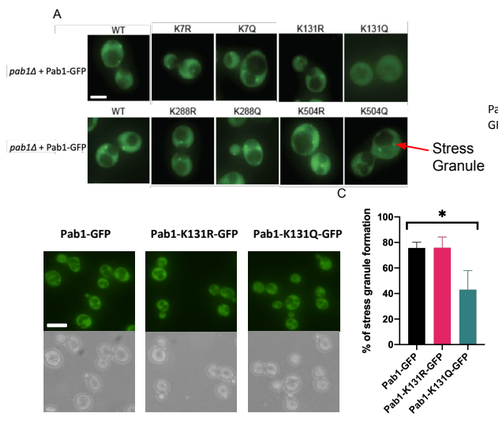
Stress Granule Formation



- Stress granules: cytoplasmic aggregates of repressed translational complexes
- Pab1: essential protein that protects the mRNA; needed to form stress granules
- KAT: lysine acetyltransferase that adds an acetyl group to lysine
- KDAC: lysine deacetyltransferase that removes an acetyl group from lysine
- Acetylation studies show hyperacetylated sites found on Pab1 are K7, K131, K288, and K504

Hypothesis: Acetylation of Pab1 inhibits the formation of glucose deprived stress granule formation. KAT mutants would increase glucose-deprived stress granules, whereas, KDAC mutants would suppress glucose-deprived stress granules.

Acetylation of Pab1K131 reduces glucose deprived stress granules



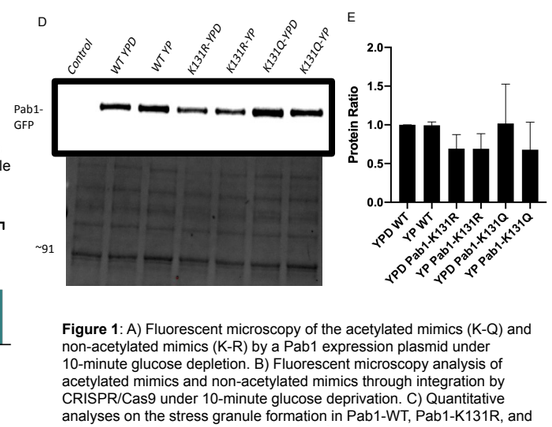


Figure 1: A) Fluorescent microscopy of the acetylated mimics (K-Q) and non-acetylated mimics (K-R) by a Pab1 expression plasmid under 10-minute glucose depletion. B) Fluorescent microscopy analysis of acetylated mimics and non-acetylated mimics through integration by CRISPR/Cas9 under 10-minute glucose deprivation. C) Quantitative analyses on the stress granule formation in Pab1-WT, Pab1-K131R, and Pab1-K131Q. *p<0.05 D) Western blot of glucose replete/deplete conditions by integration via CRISPR/Cas9. K131 mutant does not impact protein level of Pab1-GFP. E) Quantitative analysis of three trials.

Acetylation of Pab1-K131 does not impact stress granule formation on heat shock nor other stressors

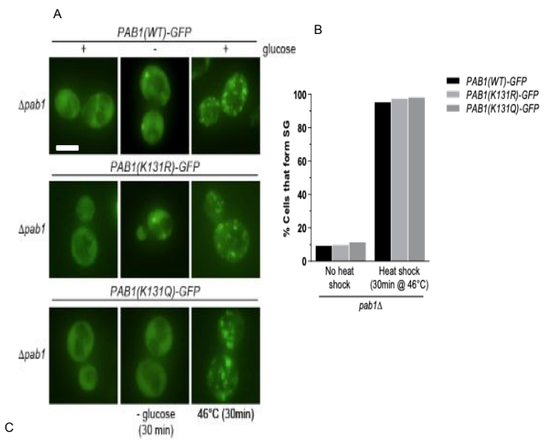
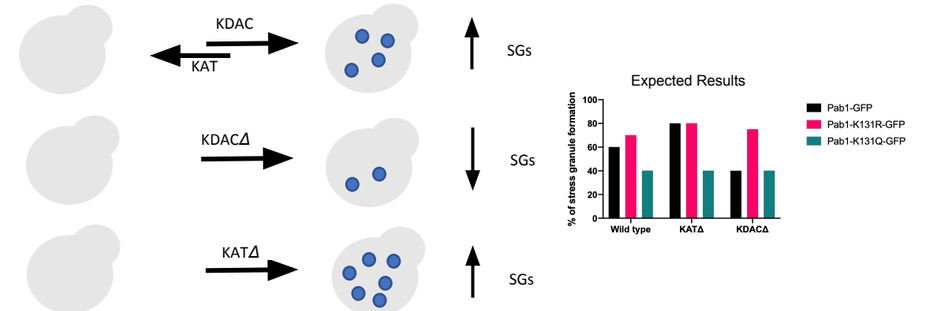


Figure 2: A) Fluorescent microscopy of acetylated mimics (K-Q) and non-acetylated mimics (K-R) under heat shock. B) Quantification analysis on stress granule formation of Pab1-GFP, Pab1-K131R-GFP, and Pab1-K131Q-GFP on heat shock. C) Dot assay of acetylation site of K131 amongst various stressors did not show growth defects.

Genetic screening to identify KAT/KDAC regulating Pab1 acetylation state



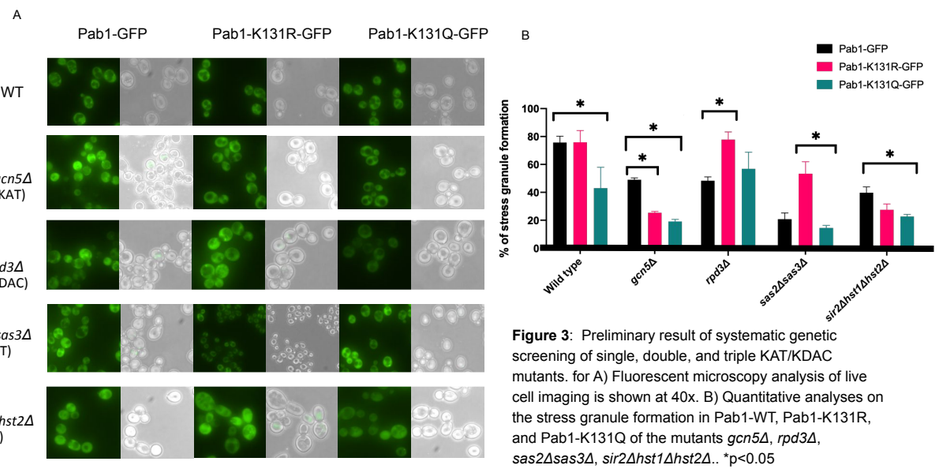


Figure 3: Preliminary result of systematic genetic screening of single, double, and triple KAT/KDAC mutants. for A) Fluorescent microscopy analysis of live cell imaging is shown at 40x. B) Quantitative analyses on the stress granule formation in Pab1-WT, Pab1-K131R, and Pab1-K131Q of the mutants *gcn5Δ*, *rpd3Δ*, *sas2Δsas3Δ*, *sir2Δhst1Δhst2Δ*.. *p<0.05

Future directions

- Decipher the molecular mechanisms by which Pab1-K131 acetylation impacts glucose- deprived stress granule formation
  - Assess the ability to assemble Poly(A)-binding from the complex
  - Assess the ability to assemble eIF4G from the complex
- Use biochemical genetics to identify KAT/KDAC responsible for Pab1-K131

CIHR IRSC
Canadian Institutes of Health Research
Instituts de recherche en santé du Canada