Apr 27th, 3:00 PM - 4:00 PM

DLC-1 Over-Expression and Growth Inhibition in Saccharomyces Cerevisiae

Ella B. Baumgarten
University of Montana, Missoula, ella.baumgarten@umontana.edu

DLC-1 Over-Expression and Growth Inhibition in *Saccharomyces Cerevisiae*

Ella Baumgarten, Ekaterina Voronina Neuroscience, *Division of Biological Sciences*, University of Montana

1. Introduction

The activity and regulation of RNA-binding proteins (RBPs) is an important topic in studies of gene expression. RBPs have central roles in many cellular processes, such as cellular transport and localization. The regulation of RBPs includes interactions with cofactors.

One cofactor that our lab has identified is DLC-1, a small protein that prompts the association of RBPs with subcellular RNA granules (Wang et al,. 2016). Previous research on a protein, Pbp1, with similar effects as DLC-1 in *Saccharomyces cerevisiae* (yeast), shows that Pbp-1 over-expression leads to growth inhibition in yeast cells due to the promotion of excessive RNA granule formation (Swisher and Parker, 2010).

This finding has led to the central question: does over-expression of DLC-1 in yeast cells cause growth inhibition?

We hypothesize that due to similar effects between Pbp-1 and DLC-1, over-expression of DLC-1 in yeast cells will result in growth inhibition.

Yeast is used as our model organism because it is cost efficient, grows quickly, and has mammalian orthologs.

Understanding expression of DLC-1 relative to cell growth and RNA granule formation is important because abnormal RNA granule formation is linked to neurodegenerative diseases.

2. Previous Research

- Parker and Swisher (2010): Localization to, and Effects of Pbp1, Pbp4, Lsm12, Dhh1, and Pab1 on Stress Granules in *Saccharomyces cerevisiae*
- Findings: Pbp1 localizes to stress granules and promotes their formation. Pbp1 interacts with Lsm12, Pbp4, Pab1 and Dhh1. Pbp1, Pab1, and Dhh1 over-expression inhibits cell growth.
- Methods: Express proteins under control of galactose-inducible promoter in yeast, grow yeast on media that vary in concentrations of galactose and sucrose, incubate for 5 days at 30° C.

3. Methods

1. Plasmid Synthesis

Dyn2 (Yeast DLC-1), Dhh1 and Pab1 (positive controls), negative control

2. Yeast Transformation

Transfect plasmids into yeast (BY4741 parental strain)

3. Galactose Induction

Express proteins under the control of a galactose-inducible promoter on media containing varying amounts of galactose/glucose. Yeast grown on media with high galactose concentration will have the most protein expressed. Grow yeast on media for 3 days at 30° C.

4. Results

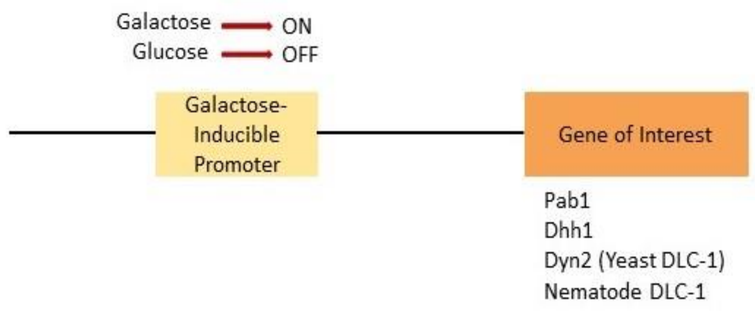

Figure 1. Diagram of plasmid that is over-expressed in yeast. Plasmid transfected into yeast includes galactose-inducible promotor attached to gene of interest. Galactose activates promoter and causes over-expression while glucose doesn't activate promoter.

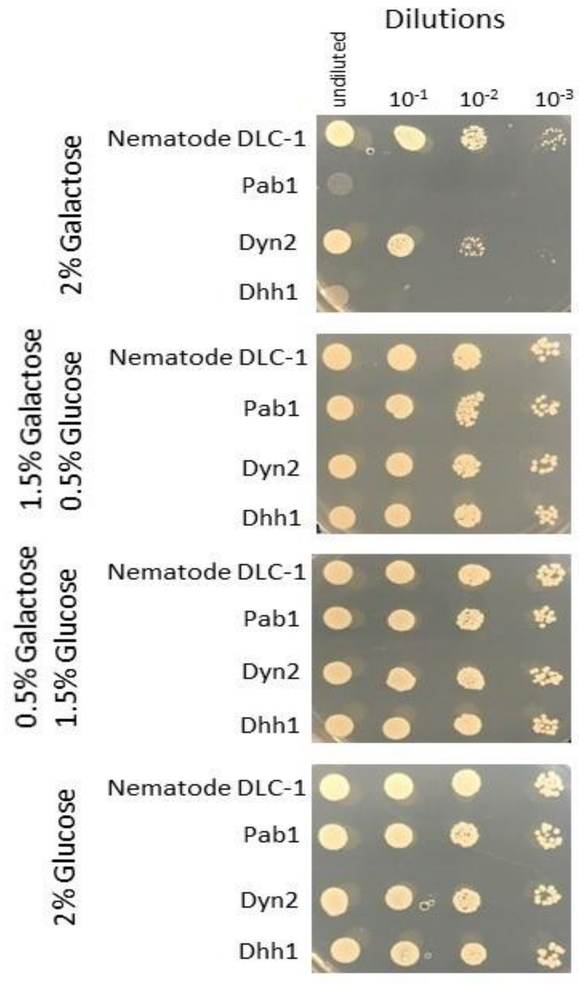

Figure 2. Dyn2 (yeast DLC-1) and Nematode DLC-1 over-expression causes growth inhibition in yeast cells. Proteins were expressed under the control of galactose-inducible promoters on media containing varying concentrations of galactose and glucose. The plates were then incubated for 30° C for 3 days. Dhh1 and Pab1 are positive controls.

5. Conclusions and Implications

- ❑ Over-expression of Dyn2 (yeast DLC-1) causes the growth inhibition of yeast cells
- ❑ Yeast DLC-1 may be important for formation of RNA granules
- ❑ Over-expression of DLC-1 and yeast Dyn2 may promote excessive stress granules

6. Future Directions

- ❑ Do galactose induction with liquid media that contains raffinose instead of glucose
- ❑ Make new negative control plasmid with GFP
- ❑ Verify Dyn2 mutation in the yeast knockout strain by PCR and test if this mutation alleviates growth inhibition
- ❑ Test whether over-expression of Dyn2 and worm DLC-1 promotes excessive RNA granule formation by using yeast strain with GFP-tagged stress granule component

Literature Cited

Swisher KD, Parker R (2010) Localization to, and Effects of Pbp1, Pbp4, Lsm12, Dhh1, and Pab1 on Stress Granules in Saccharomyces cerevisiae. PLoS ONE 5(4): e10006. https://doi.org/10.1371/journal.pone.0010006

Wang, X., Olson, J. R., Rasoloson, D., Ellenbecker, M., Bailey, J., & Voronina, E. (2016). Dynein light chain DLC-1 promotes localization and function of the PUF protein FBF-2 in germline progenitor cells. Development (Cambridge, England), 143(24), 4643–4653. http://doi.org/10.1242/dev.140921

Acknowledgments

Thank you to Sarah Hauser for the Nematode DLC-1 and the members of the Voronina lab for their support. Funding provided by NIH R01 GM109053 and CBSD CoBRE NIH P20 GM103546.

Developed with funding from the Howard Hughes Medical Institute