What's With That JUNQ?: Protein Sequestration and Aggregation in Yeast

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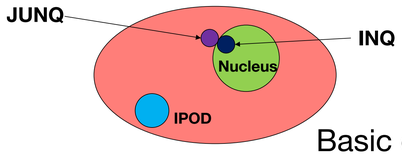
Introduction

What are we doing and why?

- Protein misfolding and the presence of sequestration sites has been linked to many neurodegenerative disorders (Alzheimer's, Huntington's, Parkinson's)
- What's pathogenic? The sites themselves or does the presence of the sites already indicate pathogenicity?
- Understanding how two specific misfolded protein sequestration sites interact may allow us to relate this to neurodegenerative diseases in general



Basic cell

Inspirations and Past Conclusions

- Eukaryotic cells sort their misfolded proteins into distinct compartments – soluble proteins to the JUNQ and insoluble to the IPOD (Kaganovich et al, 2008)[1]
- JUNQ inclusion bodies are asymmetrically inherited, so daughter cells do not get misfolded proteins (Ogrodnik et al, 2014)[2]
- The JUNQ and INQ home to each other but are not physically connected (Sontag, unpublished)

Terms to Know

- *juxtanuclear quality control compartment (JUNQ):* body of aggregated misfolded proteins localized just outside the nucleus
- *intranuclear quality control compartment (INQ):* body of aggregated misfolded proteins located inside the nucleus
- *nuclear localization signal (NLS):* localization tag that forces a protein to hone to the nucleus
- *nuclear export signal (NES):* localization tag that forces a protein out of the nucleus
- *luciferase TS (luci):* protein engineered to misfold above 37ºC
- *Von Hippel Lindau (VHL):* protein that will misfold in yeast

Results

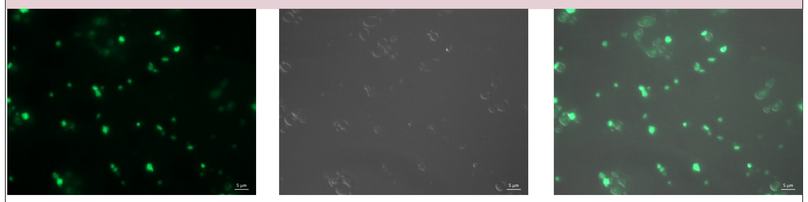
W303 + NLS-GFP-Luci


GFP | Transmitted Light | GFP + Transmitted Light

W303 + NLS-GFP-VHL


GFP | Transmitted Light | GFP + Transmitted Light

Y7092 + NLS-GFP-Luci


GFP | Transmitted Light | GFP + Transmitted Light

Conclusions

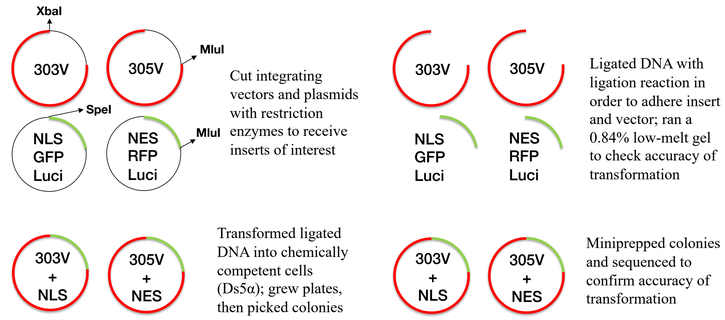
- Successful creation of integrating vectors pAG303-NLS-GFP-Luci, pAG303-NLS-GFP-VHL, pAG305-NES-RFP-Luci, pAG305-NES-RFP-VHL
- Successful integration of NLS-GFP-Luci into yeast strains W303 and Y7092 (evidenced by the imaging, where GFP marks the cell nuclei) and NLS-GFP-VHL into W303

Next Steps

- Integrating the NES-RFP-Luci and NES-RFP-VHL into the NLS-integrated W303 and Y7092, and doing a library screen of deletion collection with the Gitler lab to study JUNQ/INQ
- Creating a singular integrating vector with NLS and NES linked by a self-cleaving T2A peptide
- Reattempting the Y7092 + NLS-GFP-VHL integration

Methods

NLS-GFP-Luci and NES-RFP-Luci + Vectors



Cut integrating vectors and plasmids with restriction enzymes to receive inserts of interest

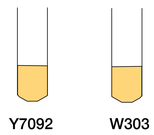
Ligated DNA with ligation reaction in order to adhere insert and vector; ran a 0.84% low-melt gel to check accuracy of transformation

Transformed ligated DNA into chemically competent cells (Ds5α); grew plates, then picked colonies

Miniprepped colonies and sequenced to confirm accuracy of transformation

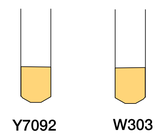
Integrating into Y7092 and W303

Day 0



Grow yeast cultures overnight in 30ºC shaker

Day 1

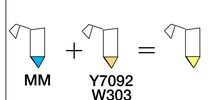


Dilute 250 ul in 5 mL YPD and let grow for three hours in 30ºC shaker

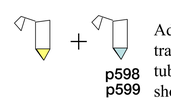
Linearize DNA of interest with 3ul NheI-HF, 5ul CutSmart, 42ul DNA and incubate for an hour at 37ºC

Spin down and discard supernatant; resuspend yeast in 1mL dH2O

Create transformation master mix, with 100 ul OneStep buffer, 10 ul Dithiothreitol (DTT), 5 ul salmon sperm DNA (SSDNA)



Spin down and discard supernatant; resuspend yeast in master mix



Add yeast and transformation mix to tubes with DNA; heat shock


Resuspend mix in 1.5 mL YPD; recover for 5 hours at 30ºC, then plate on selective plates

References

1: Kaganovich, Daniel et al. "Misfolded proteins partition between two distinct quality control compartments." *Nature* vol. 454,7208 (2008): 1088-95. doi:10.1038/nature07195
2: Ogrodnik M, et al. "Dynamic JUNQ inclusion bodies are asymmetrically inherited in mammalian cell lines through the asymmetric partitioning of vimentin". Proceedings of the National Academy of Sciences of the United States of America. 111 (22) (2014): 8049–54. doi:10.1073/pnas1324035111.

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