Human optic atrophy associated *OPA1* gene induces mitochondrial dysfunction in *Saccharomyces cerevisiae*

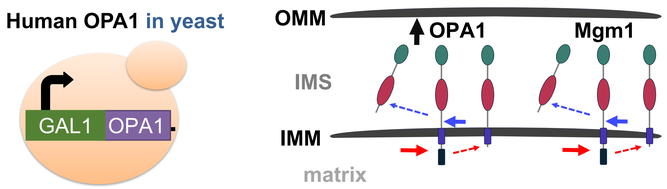
Annabel Vivian P. Almazan, Shuzhen Chen, Shulin Ju, Quan Zhong

Department of Biological Sciences, Wright State University, 3640 Colonel Glenn Hwy., Dayton, Ohio

INTRODUCTION

- Mitochondria play essential roles in cell death and cell survival. Fusion and fission of mitochondria are regulated by a conserved network of genes and are required for mitochondria morphology and function[2,3].
- OPA1 encodes a dynamin-like GTPase that regulates the fusion of the mitochondrial inner membrane and the integrity of mitochondrial cristae[2]. OPA1 is genetically linked to Dominant Optic Atrophy, an optic neuropathy occurring in 1 in 50,000 individuals[6].
- OPA1 and its yeast homolog, Mgm1, are proteolytically processed by mitochondrial proteases, producing both long and short forms of the proteins. Perturbing the expression level or the balance of long and short forms of OPA1/Mgm1 leads to mitochondrial defects[1,2,4,7, 9,10,12,13].
- Human OPA1 is processed into long and short forms in yeast[8]. However, heterologous expression of OPA1 in yeast leads to overproduction of the short form of the protein[8].
- Only by fusing the N-terminal proteolytic region of yeast Mgm1 to human OPA1 can the protein complement Δ*mgm1* deletion mutant[14].

OBJECTIVES

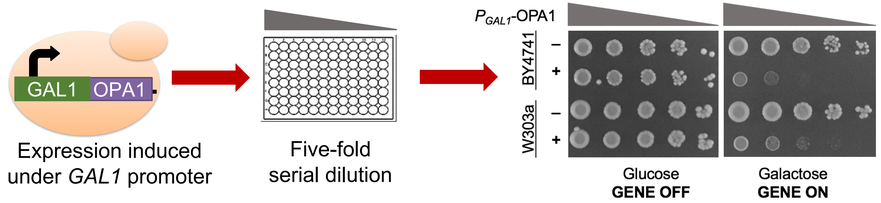


- We hypothesize that yeast can be used as a model system to test the effects of the abnormal level and imbalanced long/short forms of OPA1 on mitochondrial function and cell fitness.
- We aim to identify sequences and molecular domains of the OPA1 protein that are involved in mis-regulation of mitochondrial structure and function upon overexpression.
- We design genetic approaches to search for yeast and human genes that suppress cellular defects induced by OPA1 overexpression.

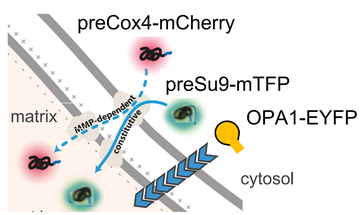
APPROACHES

Examine effect of OPA1 on yeast

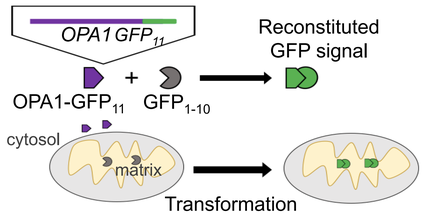
➢ ***Characterize the effect of OPA1 overexpression on cellular fitness:***



➢ ***Observe OPA1-EYFP localization and mitochondria health:***



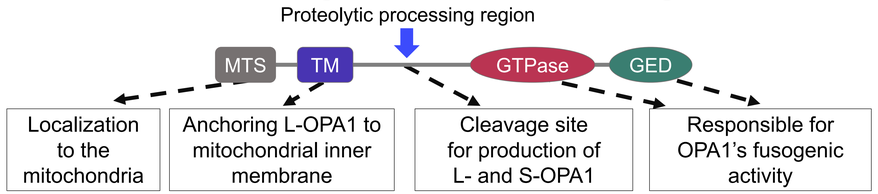
Re-engineered MitoLoc[16] tags mitochondria structure (mTFP) and membrane potential (mCherry), while EYFP tags OPA1 localization.



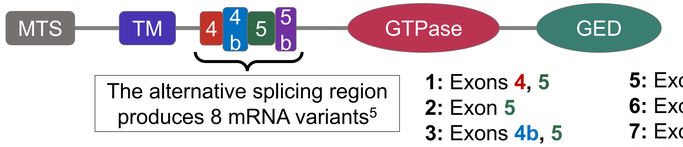
Using a Split GFP system[15], a reconstituted GFP signal indicates whether the protein is targeted into mitochondria.

➢ ***Characterize the effect of different forms of OPA1 on cellular fitness***

- **Generate truncations using PCR-based site-directed mutagenesis[11]:**



- **Analyze whether the OPA1 isoforms have differential impacts on fitness:**



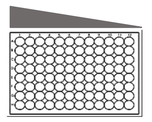
1: Exons **4**, **5**
2: Exon **5**
3: Exons **4b**, **5**
4: Exons **5**, **5b**
5: Exons **4**, **4b**, **5**
6: Exons **4b**, **5**, **5b**
7: Exons **4**, **5**, **5b**
8: Exons **4**, **4b**, **5**, **5b**

Identification of genetic suppressors of OPA1

➢ ***Search for yeast gene suppressors:***

Can deletion or overexpression of yeast genes associated with mitochondrial fission-fusion processes (e.g. Mgm1) alleviate OPA1-induced toxicity? Other regulatory genes include:
Pcp1 – cleaves Mgm1 into L- and S-Mgm1[10]
Yta10 – cleaves OPA1 in yeast[8]
Yta12 – cleaves OPA1 in yeast[8], etc.

Genetic screens

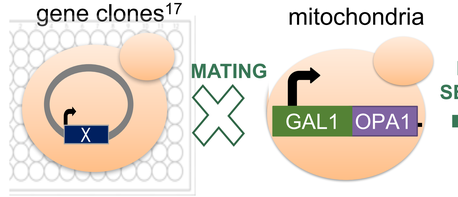


Differences in:
Cellular fitness?
Mitochondria health?

➢ ***Search for human gene suppressors by overexpression screen:***

Overexpression:
~15,000 human gene clones[17]

Poor growth & defective mitochondria



Can any human genes rescue OPA1-induced cellular defects?

CONCLUSIONS

OPA1 overexpression induces fitness and mitochondrial defects in yeast. Genetic analysis suggests that OPA1's mitochondrial targeting, proteolytic processing, and GTPase activity are required for the defects. Overexpression and deletion experiments allowed for the identification of human and yeast functional interaction partners of OPA1 in yeast. Overall, our findings support the use of yeast as a model system to study evolutionarily conserved human gene functions.

REFERENCES

1. Anand, R. et al. (2014) J Cell Biol. 204: 919-929.
2. Chan, D. (2012) Annu Rev Genet. 46, 265-287.
3. Chen, H., & D. Chan. (2009) Hum Mol Genet. 18(R2), R169-R176.
4. Del Dotto, V. et al. (2017) Cell Rep. 19(12), 2557-2571.
5. Del Dotto, V. et al. (2018) BBA. 1859(4), 263-269.
6. Delettre, C. et al. (2000) Nat Genet. 26(2), 207-210.
7. DeVay, R.M. et al. (2009) J Cell Biol. 186(6), 793-803.
8. Duvezin-Caubet, S. et al. (2007) Mol Biol Cell. 18:3582-3590.
9. Griparic, L. et al (2004) J Biol Chem. 279(18):18792-8.
10. Herlan, M. et al. (2003) J Biol Chem. 278(30), 27781-27788.
11. Kunkel, T.A. (1985) Proc Natl Acad Sci U.S.A. 82(2):488-492.
12. MacVicar, T. & T. Langer (2016) J Cell Sci. 129(12), 2297-2306.
13. Mishra, P. et al (2014) Cell metab. 19(4), 630-641.
14. Nolli, C. et al. (2015) Mitochondrion. 25, 38-48.
15. Ruan, L. et al. (2017). Nat. 543(7645), 443-446.
16. Vowinckel, J. et al. (2015). Mitochondrion. 24, 77-86.
17. Hayden, E. et al (2020) G3 (in Press)

ACKNOWLEDGMENTS

We are grateful for the human gene clone collections shared by the Center for Cancer Systems Biology (CCSB) for construction of the human gene library[17].