

# STRESS RESPONSIVE DEGRADATION OF MRNA ENCODING MITOCHONDRIAL PROTEINS

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## Background

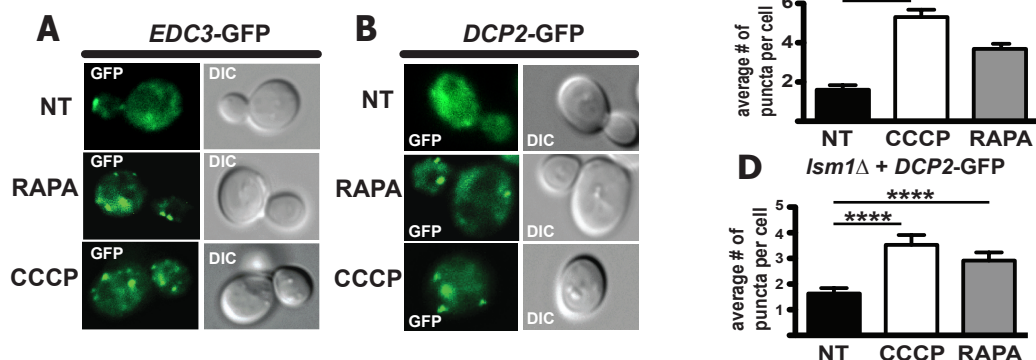
The degradation of cytosolic mRNA is crucial to the regulation of gene expression and can be induced by numerous cellular perturbations. Although many stressors are known, cytoplasmic mRNA degradation has never been studied in the context of organellar stress. Recent studies have demonstrated that mitochondrial protein import stress activates a cytosolic response that leads to the degradation of proteins bound for the mitochondria. However, in the context of a similar stress, there remains nothing known regarding the fate of cytoplasmic mRNA encoding mitochondrial proteins. Thus, we sought to study mitochondrial stress induced mRNA degradation. In order to assay the induction of mRNA degradation, we engineered several *Saccharomyces cerevisiae* strains in which GFP was fused to proteins involved in mRNA decapping and degradation. The accumulation of these proteins in subcellular structures termed p-bodies is commonly used to assay active mRNA degradation. We hypothesized that the accumulation of p-bodies would increase in response to mitochondrial stress. In order to induce stress, we chose two methods. First, we treated the cells with small molecules that uncouple the mitochondrial membrane and disable mitochondrial protein import. Second, we used FASII mutant strains, thereby creating a genetic model of mitochondrial stress. In order to assess p-body accumulation, we have employed fluorescent microscopy. Furthermore, we will perform an RNA-seq assay and measure the amount of mRNA encoding a subset of mitochondrial proteins that exists in a cell under normal conditions and compare the results to the amount of said mRNA present when mitochondrial stress is induced.

## Methods

In the first method, wild type cells were treated for 3 hours with 10  $\mu$ M CCCP or 200 ng/ml Rapamycin (RAPA) in order to induce cellular stress. CCCP uncouples the mitochondrial membrane and arrests protein import. RAPA inhibits TOR. Fluorescent microscopy was used to detect Edc3-GFP or Dcp2-GFP in cells treated with rapamycin or CCCP or with no treatment prior to imaging. For the second method, we engineered *oar1 $\Delta$  and *lsm1* $\Delta$ *oar1* $\Delta$  mutants that expressed *EDC3*-GFP. Fluorescent microscopy was used to detect Edc3-GFP in WT cells and in *oar1* $\Delta$  and *lsm1* $\Delta$ *oar1* $\Delta$  mutant cells.*

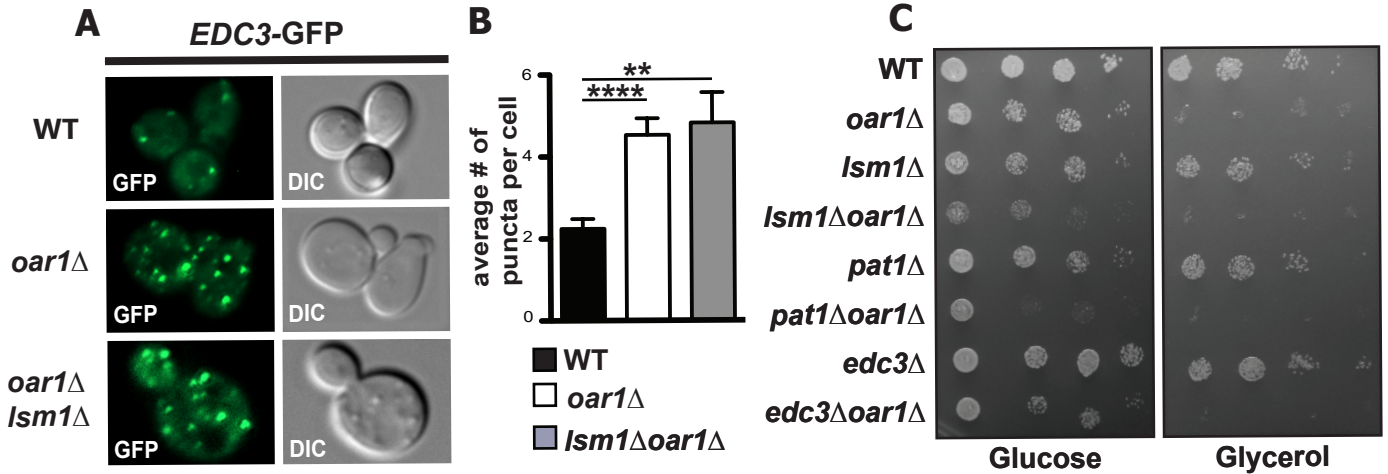
## Results

We observed that the average number of puncta per cell increased significantly after treatment with CCCP or Rapamycin when compared to non-treated cells. This leads us to believe that p-bodies do accumulate due to pharmacologically-induced mitochondrial stress. Additionally, the number of puncta per cell was significantly greater in *oar1* $\Delta$  and *lsm1* $\Delta$ *oar1* $\Delta$  mutants than in wild type cells. Thus, providing further evidence that mitochondrial stress leads to the accumulation of p-bodies.



## Figure 1

Fluorescent microscopy was used to detect Edc3-GFP (A) or Dcp2-GFP (B) in cells treated with rapamycin or CCCP for 3 hours prior to imaging. The average number of puncta per cell were counted in cells expressing *EDC3*-GFP (C) and *DCP2*-GFP (D). Error bars represent SEM. \*\*\*\* $p < .00005$ .



## Figure 2

FASII is required for electron transport chain (ETC) assembly, therefore by creating *oar1*Δ mutants, the ETC doesn't assemble and we are able to induce mitochondrial stress through genetic engineering. Fluorescent microscopy was used to detect Edc3-GFP (A) in *oar1*Δ and *lsm1*Δ *oar1*Δ mutants and WT cells. The average number of puncta per cell were counted in cells expressing *EDC3*-GFP (B). Error bars represent SEM. \*\*\*\* $p < .00005$  \*\* $p < .005$ . C. Growth of the indicated mutant strains was assayed on glucose and on glycerol.

## Conclusion

We set out to discover the fate of mRNA when mitochondrial stress is induced. The results of fluorescent microscopy show that both our pharmacological and genetic model of mitochondrial stress induction were successful in showing an increase in the formation and accumulation of p-bodies. This increase in formation of p-bodies leads us to believe that mRNA degradation also increases upon the onset of mitochondrial stress. Therefore, we feel confident in moving forward and performing an RNA-seq assay. With this assay, we will be able to detect if there is a significant decrease in the amount of mRNA present in the cell when mitochondrial stress is induced. Specifically, the amount of mRNA that encodes a subset of mitochondrial proteins. We hope that our discoveries will further our knowledge and understanding of cellular function and how mitochondrial stress can cause a myriad of responses in the cell.

## References

Decker, C. J., and R. Parker. "P-Bodies and Stress Granules: Possible Roles in the Control of Translation and MRNA Degradation." *Cold Spring Harbor Perspectives in Biology*, vol. 4, no. 9, Mar. 2012, doi:10.1101/cshperspect.a012286.