Uncovering the Role of *EAF1* in the Regulation of **Lipid Synthesis** and **Membrane Composition** in *Saccharomyces cerevisiae*

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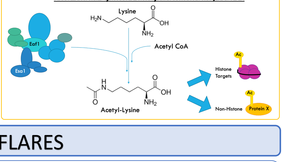
PROJECT OVERVIEW

We have identified **nuclear flares** upon **deletion of *EAF1***, the main scaffolding subunit of the **yeast lysine acetyltransferase, NuA4**. Upon further investigation we also found drastic defects in vacuole fusion.

Hypothesis: We propose that NuA4 is responsible for regulating lipid synthesis, and availability of lipids for membranes. This is potentially through NuA4-dependant **acetylation of Pah1**, a key regulator at the cross-section between **lipid droplet synthesis** and **phospholipid synthesis.**

INTRODUCTION

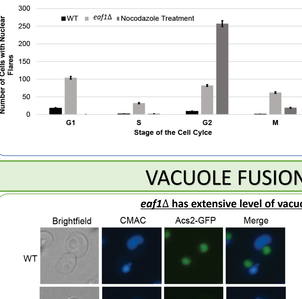
The Yeast Lysine Acetyltransferase, NuA4



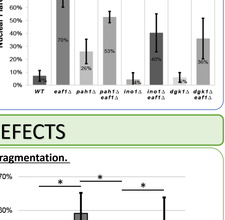
NUCLEAR FLARES

What are Nuclear Flares?

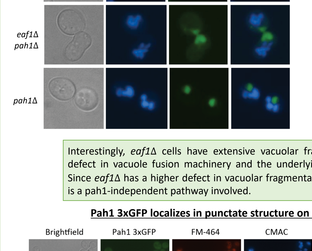
An extension of nuclear membrane due to mitotic arrest OR an underlying lipid dysregulation.



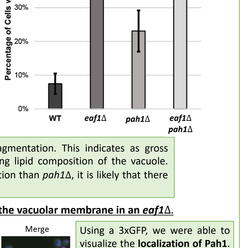
Nuclear Flares occur in an *eaf1Δ*



Nuclear flares in *eaf1Δ* are not due to mitotic arrest.

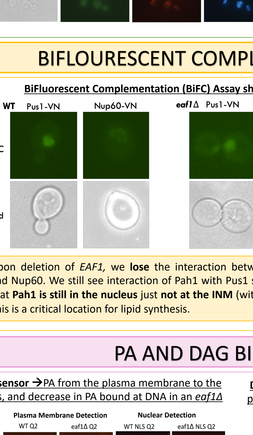


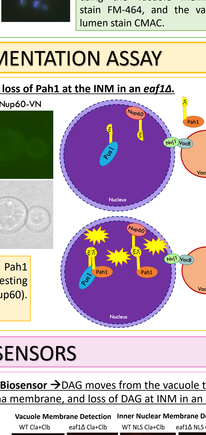
***eaf1Δ* has more nuclear flares than *pah1Δ*. Deletion of *INO1* and *DGK1* partially rescue nuclear flares in an *eaf1Δ*.**



VACUOLE FUSION DEFECTS

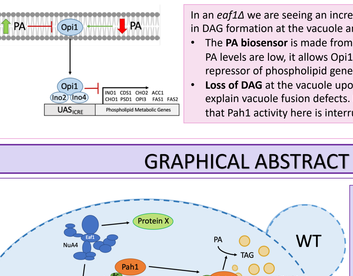
***eaf1Δ* has extensive level of vacuolar fragmentation.**





Interestingly, *eaf1Δ* cells have extensive vacuolar fragmentation. This indicates as gross defect in vacuole fusion machinery and the underlying lipid composition of the vacuole. Since *eaf1Δ* has a higher defect in vacuolar fragmentation than *pah1Δ*, it is likely that there is a pah1-independent pathway involved.

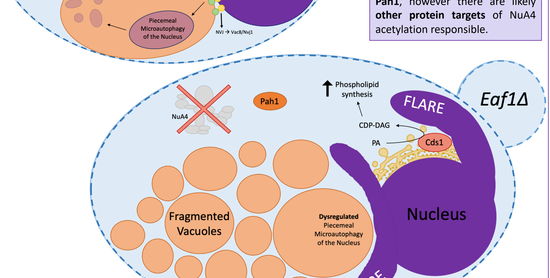
Pah1 3xGFP localizes in punctate structure on the vacuolar membrane in an *eaf1Δ*.



Using a 3xGFP, we were able to visualize the **localization of Pah1**. Under WT condition, Pah1 is diffusely expressed throughout the **cytosol**. However, upon deletion of *EAF1*, Pah1 appears in **punctate** structure around the **vacuolar membrane**, potentially at the NVJ. This was confirmed using the vacuole membrane stain FM-464, and the vacuole lumen stain CMAC.

BIFLOURESCENT COMPLIMENTATION ASSAY

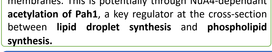
BiFluorescent Complementation (BiFC) Assay shows loss of Pah1 at the INM in an *eaf1Δ*.



Upon deletion of *EAF1*, we **lose** the interaction between Pah1 and Nup60. We still see interaction of Pah1 with Pus1 suggesting that **Pah1 is still in the nucleus** just **not at the INM** (with Nup60). This is a critical location for lipid synthesis.

PA AND DAG BIOSENSORS

PA Biosensor → PA from the plasma membrane to the nucleus, and decrease in PA bound at DNA in an *eaf1Δ*

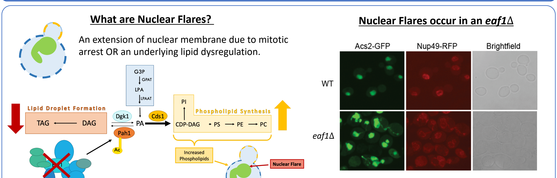


DAG Biosensor → DAG moves from the vacuole to the plasma membrane, and loss of DAG at INM in an *eaf1Δ*

In an *eaf1Δ* we are seeing an increase in PA pools and a reduction in DAG formation at the vacuole and the INM.

- The **PA biosensor** is made from the Opi1 PA binding site. When PA levels are low, it allows Opi1 to act as a transcriptional repressor of phospholipid genes, by revealing its NLS signal.
- **Loss of DAG** at the vacuole upon deletion of *EAF1* could further explain vacuole fusion defects. Loss of DAG at the INM confirms that Pah1 activity here is interrupted in an *eaf1Δ*.

GRAPHICAL ABSTRACT



In conclusion, we have shown evidence that there is a **disruption** of underlying **lipid synthesis** upon deletion of *EAF1*. This presents as **nuclear flares**, **vacuole fusion defects**, and even **increased cell size**. This suggests that NuA4 plays an important role in regulating the **balance** of lipid droplets and membrane phospholipids. We believe a percentage of these defects are due to NuA4's relationship with **Pah1**, however there are likely **other protein targets** of NuA4 acetylation responsible.

FUNDING

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