Translational control of fatty acid synthesis controls nuclear division during the cell cycle

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Background

- Lipid synthesis during mitosis is essential for both human cells and yeasts. From our recent mass spec study on budding yeast, we found that among all other metabolites, the abundance of lipids is the highest late in the cell cycle. However, how the lipid homeostasis is maintained during the cell cycle is not understood.
- Given the involvement of lipids in cancers and other diseases such as cardiovascular disease, obesity, diabetes, etc., it is important to study the regulation of lipids during the cell cycle. Moreover, higher levels of lipogenic enzymes are indicative of poor prognosis of different cancers.
- Fatty acids are the building blocks of lipids. A genome-wide study performed in budding yeast in our lab showed that the translational efficiency of mRNAs encoding two major fatty acid synthesis enzymes, acetyl CoA carboxylase (*ACC1*) and fatty acid synthase (*FAS1*) peaks late in the cell cycle. Here, we show that the higher translational efficiency of *ACC1* and *FAS1* not only alters the abundance of different lipid species but also significantly promotes nuclear division during mitosis.
- Taken together, our data link core metabolic functions of lipogenic enzymes (Acc1p and Fas1p) to the morphological changes of the nucleus during cell division.

Major lipids peak late in the cell cycle

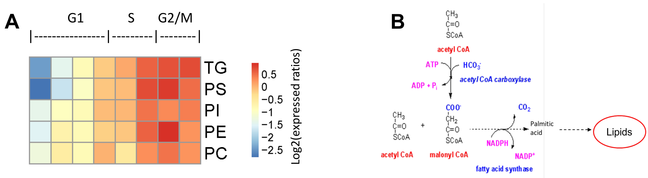


Figure 1: Abundance of lipids is highest late in the cell cycle (Blank et al MboC, 2020)

A. Heatmap of major lipid species during the cell cycle in a synchronous cell culture of wild-type, diploid cells (BY4743 background). The data were hierarchically clustered and displayed with the *pheatmap* R package.

B. Fatty acids are the building blocks of most lipids. Acc1p catalyzes the first committed step of the fatty acid biosynthesis pathway. Further reactions in the pathway to produce fatty acids are catalyzed by the Fas1,2p complex.

Lipogenic enzymes are under translational control in the cell cycle

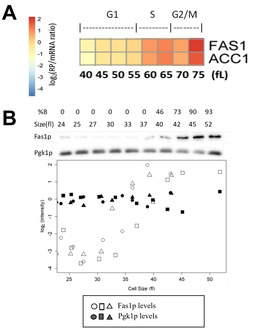


Figure 2: *ACC1* and *FAS1* are under periodic translational control during the cell cycle

A. Major lipogenic enzymes (*ACC1* and *FAS1)* are under periodic translational control in synchronous elutriated cultures of wild-type, diploid cells (BY4743 background). The x-axis shows the cell sizes (fl) at different time point in the cell cycle. The data were hierarchically clustered and displayed with the *pheatmap* R package.

B. Abundance of FAS1p during cell cycle is queried by immunoblotting, starting at the indicated cell sizes (fl) and budding index (% B). The graphs at the bottom quantify the band intensities for each independent experiment (indicated with different open symbols plotted on the *y*-axis as the $\log_2$ values of their expressed ratios against the corresponding cell size (*x*-axis). Experiment-matched loading controls (filled symbols) were also quantified.

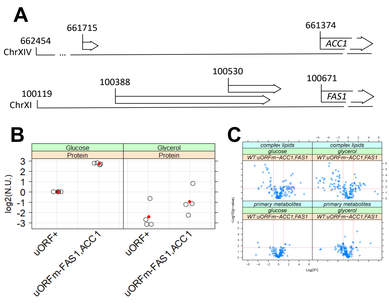
De-repressing translation of *ACC1* and *FAS1* alters the abundance of lipids

Figure 3: uORFs mediate translational control of *ACC1* and *FAS1*

A. Representation of the uORFs present in the 5' leader of *ACC1* and *FAS1*.

B. Strip plot of the protein levels of *FAS1-TAP* in asynchronous culture of wild-type cells and cells lacking uORFs, quantified from four independent experiments. The protein levels were normalized for loading against the Pgk1p levels and the log-2 transformed values were plotted on the y-axis for the mentioned strains.

C. Metabolites that changed in abundance in wild type vs. *uORFm-ACC1,FAS1* cells were identified based on the magnitude of the difference (x-axis; Log2-fold change) and statistical significance (y-axis), indicated by the red lines.



De-repressing translation of *ACC1* and *FAS1* promotes nuclear division

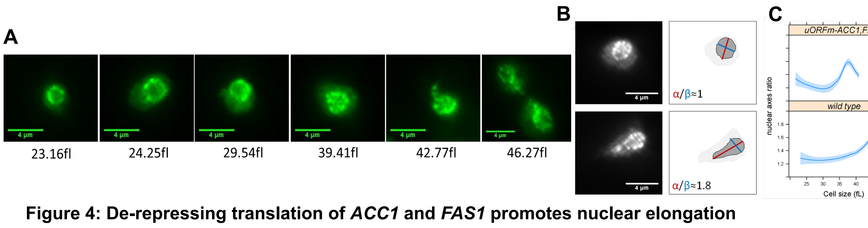


Figure 4: De-repressing translation of *ACC1* and *FAS1* promotes nuclear elongation

A. Representative images of nuclear envelope remodeling during cell cycle with the Nsp1p signal decorating the nuclear envelope. The x-axis has the cell sizes at different time points in a synchronous culture.

B. Schematic representation of the measurement of the sphericity of the nucleus. The red line represents the long axis and the blue line represents the short axis of the sphere. The ratio of the axes depict sphericity.

C. Changes in sphericity of the nuclear envelope during the cell cycle for both the uORF mutant and the wild type were quantified by plotting the nuclear axes ratio on the y-axis and the cell sizes on x-axis. > 2000 cells were quantified for each strains. Loess curves and the std errors at a 0.95 level are shown.

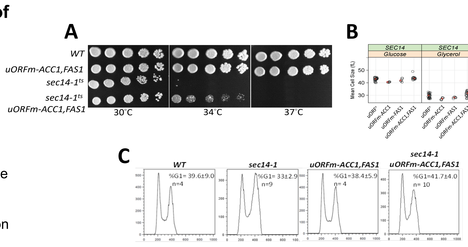
De-repressing translation of *ACC1* and *FAS1* suppresses the phenotypic defects of *sec14-1^{ts}*

Figure 5: De-repressing translation of *ACC1* and *FAS1* promotes nuclear elongation

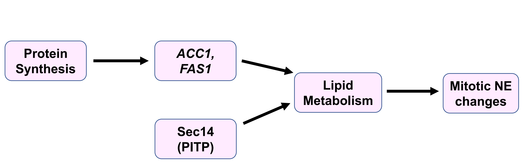
A. Serial dilutions (5-fold) of cultures of the indicated strains were spotted on solid media (YPD) and grown at the temperatures shown.

B. The mean size (y-axis) was measured from cultures of the indicated strains and media. The average in each case is shown in red.

C. Flow cytometry histograms, with cell number on the y-axis and fluorescence per cell on the x-axis.



Working Model



Conclusion

- The translational efficiency of lipogenic enzymes peaks late in the cell cycle, raising the levels of acetyl-CoA carboxylase and fatty acid synthase.
- Removing inhibitory uORFs from *ACC1* and *FAS1* increases the protein levels and alters the abundance of many lipid species.
- The uORF mutant promotes nuclear division.
- Translational control of lipid synthesis links lipid metabolism with the Sec14p-mediated pathways of membrane trafficking.

Acknowledgement

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Contact

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