Engineering membrane transporters to increase the available cytosolic acetyl-CoA in *S. cerevisiae*

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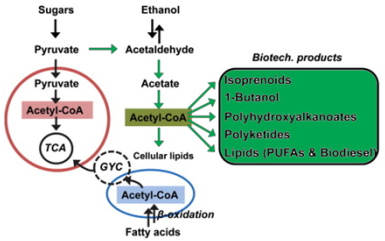
Abstract

Acetyl CoA (AcCoA) is an important starting molecule for the biosynthesis of many biotechnologically relevant metabolites, such as isoprenoids used as flavors and fragrances, biodiesels, and anticancer drugs. In the yeast *Saccharomyces cerevisiae*, AcCoA metabolism occurs in at least four subcellular compartments, yet no innate pathway exists for direct transport between compartments. Our research aims to increase cytosolic AcCoA levels by using protein transporters AT-1 and the yeast homologue YBR220C to move the molecule from the mitochondrial compartment into the cytosol. We investigate the insertion of targeting sequences for the mitochondrial inner membrane under constitutive promoters of varying strength to properly localize these protein transporters.

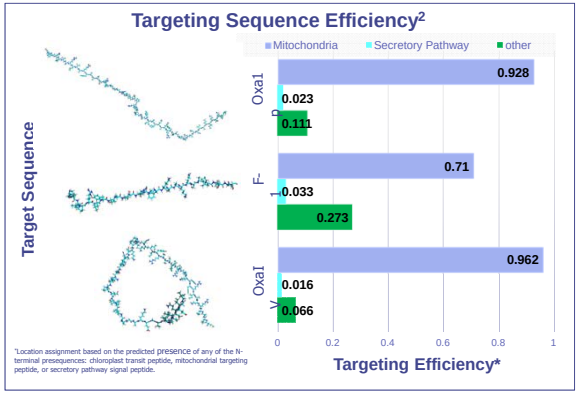
Figure 1. Outline of Acetyl CoA metabolism in *S. cerevisiae*. Increasing cytosolic AcCoA concentrations can improve production of bioproducts such as those in the green box.[1]



Targeting to the Mitochondria

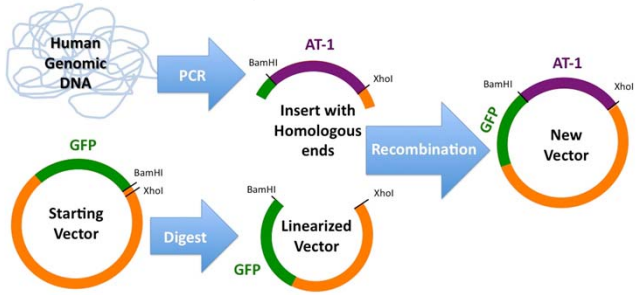
Figure 2. Targeting Sequences with high efficiencies are derived from mitochondrial proteins active in cellular metabolism. We utilized 3 targeting sequences:

- Mitochondrial Oxidase Assembly protein 1 (Oxa1p)
- ATPase F-1 β-subunit (F-1)
- Cytochrome Oxidase IV (OxaIV)



Plasmid Design

Figure 3. DNA sequences encoding protein transporters AT-1 and YBR220 are inserted into plasmid vectors using homologous recombination. AT-1 is sourced from the human genome and directly moves Acetyl-CoA from the cytosol to the ER lumen.



Localization of GFP-tagged Proteins

Figures 4, 5. The fluorescence microscope used for this study.

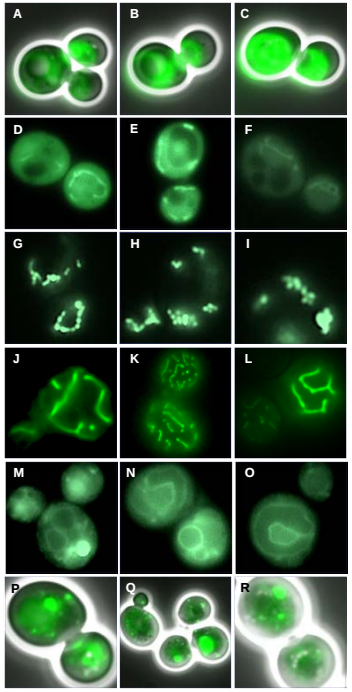
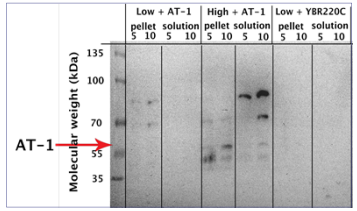


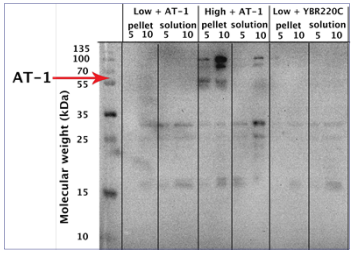
Fig. 6. Green Fluorescent Protein (GFP) is used to verify the correct localization of a targeting sequence or transport protein to the mitochondria.

Images were taken using 390 nm excitation and 510 nm emission filters and a 100X oil immersion lens. (**A-C**) GFP. (**D-F**) F-1 targeting sequence localizes GFP to the mitochondria with large amounts of cytosolic GFP. (**G-I**) Oxa1p and (**J-L**) OxaIV targeting sequences localize GFP to the mitochondria. (**M-O**) AT-1 protein transporter localizes to subcellular membranes. (**P-R**) YBR220C protein transporter aggregates in the cytosol.

Verifying Transporter Expression

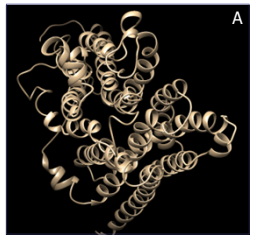
Figure 7. The protein transporters AT-1 and YBR220C successfully express within the yeast cell. Western blotting of pelletted recombinant cells and supernatant containing plasmids encoding AT-1 or YBR220C regulated by a high or low strength promoter confirmed successful expression. AT-1 has a molecular weight of 64.0 kDa and YBR220C has a molecular weight of 65.1 kDa. Bands were strongest for samples taken from the pellets, indicative of the insolubility of membrane proteins. Of the three plasmids, AT-1 under control of the high strength promoter showed the greatest expression.

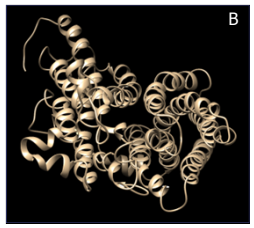




FUTURE DIRECTIONS

- Use the mitochondrial tags to localize the AT-1 (**A, B**)[3] and YBR220C protein transporters to the mitochondria.
- Determine whether transporters localized to the mitochondria are toxic to the cell.
- If AT-1 and YBR220C are not toxic in the mitochondrial inner membrane, Acetyl-CoA levels within the cell can be assessed to determine if amounts in the cytosol have increased.
- Determine the effect of the transporters and concomitant cytosolic AcCoA increase upon titers of bioproducts.





Citations

[1] Chen, *Metabolic Engineering*, 2012
[2] Emanuelsson, *Nature Protocols* 2007
[3] Kelley LA and Sternberg MJE, *Nature Protocols*, 2009

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