Repurposing the Yeast Peroxisome for Compartmentalizing Multi-enzyme Pathways

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Project Goals

Our long-term project goal is to establish the peroxisome as a synthetic organelle that can compartmentalize multiple enzymes and be made selectively permeable to substrates and cofactors to achieve high flux for this engineered metabolic pathway. Success would enable pathways to function with minimal crosstalk with cytosolic factors as well as engineer environments distinct from the cytosol (e.g., redox state or pH could potentially be altered in this subcompartment). Towards this overall goal, we are striving to repurpose the peroxisome in *S. cerevisiae* and are addressing multiple challenges that will be discussed herein: 1. efficiently targeting heterologous cargo into the peroxisome lumen, 2. determining the rules for natural metabolite permeability through this organelle's lipid bilayer, 3. testing the efficacy of compartmentalizing a model pathway, and 4. genetically engineering the peroxisome membrane to have reduced permeability.

Abstract

Eukaryotic organisms have evolved organelles for spatially separating certain biochemical reactions from others as well as the creation of distinct chemical environments. Similarly, it would be valuable to have synthetic control over an organelle such that the identity of the imported enzymes can be determined to catalyze desired reactions and avoid undesired reactions that may occur in the cytosol. We have decided to proceed towards this grand aim by repurposing an existing organelle - the peroxisome. The choice of this organelle were several-fold. First, the peroxisome is not required for *S. cerevisiae* viability provided long chain fatty acids are not used as a sole carbon source. Second, this organelle is induced to impressive sizes in some methanol utilizing yeasts such as *Pichia pastoris* and *Hansenula polymorpha*, sometimes greater than 70 percent of total cell volume. Third, the peroxisome has been naturally specialized by several organisms to alter the composition of cargo protein for new functions.

Our recent work has determined a size-dependent (sieve-like) leakiness to small molecules smaller than approximately 400-730 Daltons (Deloache, Russ, & Dueber, 2016). These measurements were made using an enzyme sequestration assay we developed for *in vivo* determination of permeability, removing the need for organelle purification that has complicated previous studies in the field; as such, our work provides the strongest evidence yet on this long-standing debate. However, the permeability of the peroxisome membrane in *S. cerevisiae* is a substantial limitation for repurposing this organelle as a generalizable compartment for multi-enzyme pathways. As such, we are faced with the challenge of engineering the peroxisome for reduced permeability to small molecules. We hypothesize that the protein

importomer (the complex enabling translocation of protein from the cytosol into the peroxisome lumen) is the major source of this leakiness. However, knocking out the importomer sets up a "chicken and egg" conundrum: how can proteins be trafficked into the peroxisome lumen in the absence of the protein importomer?

To determine whether the importomer is the main determinant for peroxisome permeability we have developed a strategy to target heterologous membrane proteins to the peroxisome, that have been re-routed from the ER. The ER provides the hub for sorting and trafficking membrane proteins to many organelles, including the plasma membrane, Golgi apparatus, vacuole, and peroxisomes. We have shown that the *Neurospora crassa* cellodextrin transporter 1 (CDT1) can be targeted to the peroxisome membrane and that CDT1 is functional at the peroxisome membrane, observed by transport of X-glucoside substrates into the peroxisome lumen. Going forward, we aim to use this strategy to 1) traffic soluble protein to the peroxisome that has firstly been directed to the ER lumen using a heterologous membrane protein scaffold and 2) direct orthogonal protein transporters from systems with tight regulation of small molecule transport across the membrane, such as the TAT translocon from the plasma membrane of *E. coli.* These systems can be screened in cells with the native protein import machinery knocked-out in order to determine the genes/factors responsible for peroxisome permeability using our *in vivo* assay (Deloache, Russ, & Dueber, 2016), in a systematic way. Ultimately, engineering robust methods to target soluble protein to the peroxisome lumen, in the absence of the native machinery, requires sophisticated understanding of both cell biology and engineering disciplines to address these challenges.

References

1. Deloache, W. C., Russ, Z. N., & Dueber, J. (2016). Towards repurposing the yeast peroxisome for compartmentalizing heterologous metabolic pathways. Nature Communications, 7, 11152. http://doi.org/10.1038/ncomms11152

Funding statement.

The funding for this project is from DOE BER Award DE-SC0008084.

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