Group: Membrane Enzymology

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Project title: Experimental evolution of substrate specificity in amino acid transporters.

Description

Cells need the capacity to acquire nutrients from the outside. These nutrients therefore have to cross the lipophilic cell membrane. To facilitate this, organisms have evolved a multitude of dedicated transport proteins, which can transport almost anything from small ions to large polypeptides.

One of the most important groups of nutrients are amino acids. As there are 20 proteogenic amino acids, one organism typically produces many transporters for different substrates. In yeast and other fungi, these transporters are mainly from the YAT family: appropriately named Yeast Amino acid Transporters. These proteins are secondary active transporters; they use the natural proton gradient and membrane potential to accumulate amino acids to high concentrations inside the cell.

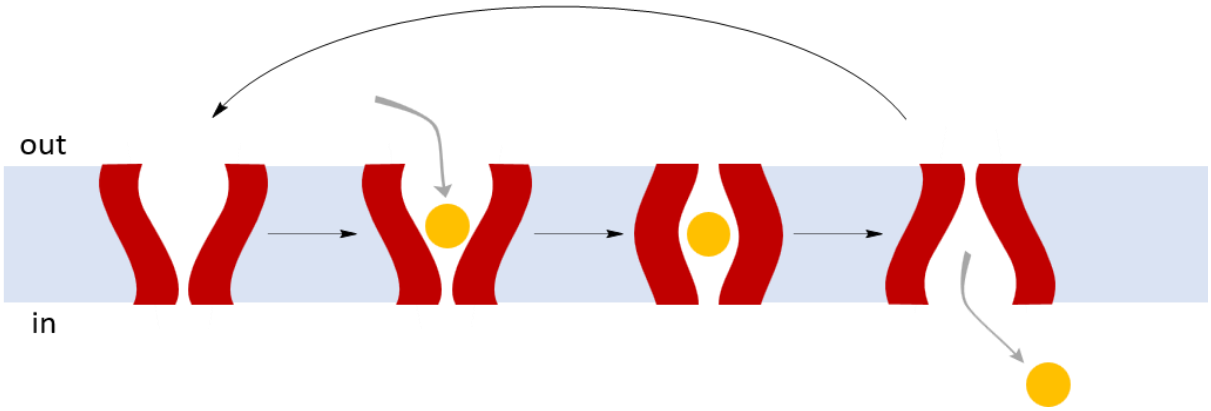

Figure 1. General reaction cycle of the alternating access mechanism of many transporters.

Each member of the YAT family has its own profile of transported substrates; some are very specific and transport only one amino acid, while others are promiscuous. Accordingly, the yeast *Saccharomyces cerevisiae* produces 18 different members of the YAT family, covering all proteinogenic amino acids. All these 18 transporters are related to each other, which means they have evolved from the same ancestor by mutation. The question is: which of these mutations actually leads to a switch of substrate specificity?

In this project, you will help set up a platform for in-vivo evolution of YAT members. The in-vivo approach works by specifically mutating a gene of interest while it is actively expressed in a host cell. This is a radically different approach to classical experimental evolution, where the mutagenesis is carried out in vitro (e.g., by error-prone PCR).

You will be using the OrthoRep platform, where the gene of interest is kept on a special yeast plasmid that is replicated with a high error rate. The yeast therefore accumulates random mutations in the gene, while the rest of the genome is not affected. Using this approach, we will mutate YAT genes to evolve the substrate specificity. For example, can we generate a

phenylalanine transporter from a lysine transporter? Or can we evolve a promiscuous transporter to become specific for one amino acid? The answers will be important for understanding the substrate specificity of transporters, and also provide a model for molecular evolution of one protein under a multitude of selective pressures. You can participate in all aspects of the process, and your exact role will be tailored to your interests and background.

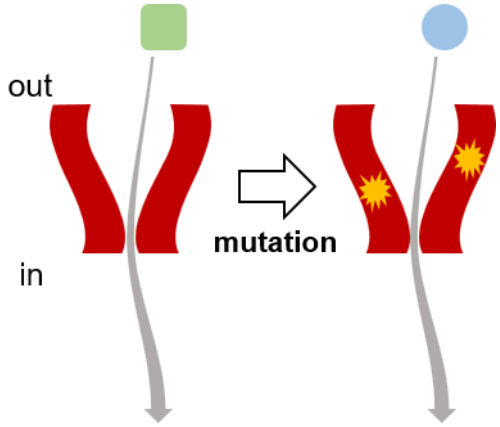

Figure 2. Schematic representation of substrate switch by mutation.

Techniques you can learn

- *Saccharomyces cerevisiae* culture
- Molecular biology techniques such as cloning and chromosomal editing
- Protein/gene sequence analysis
- Membrane protein expression and purification
- Liposome reconstitution and radiolabeled uptake assays

References

Bianchi, Frans, Joury S. van't Klooster, Stephanie J. Ruiz, and Bert Poolman. "Regulation of Amino Acid Transport in Saccharomyces Cerevisiae." *Microbiology and Molecular Biology Reviews* 83, no. 4 (November 20, 2019). https://doi.org/10.1128/MMBR.00024-19.

Bianchi, Frans, Joury S. van 't Klooster, Stephanie J. Ruiz, Katja Luck, Tjeerd Pols, Ina L. Urbatsch, and Bert Poolman. "Asymmetry in Inward- and Outward-Affinity Constant of Transport Explain Unidirectional Lysine Flux in Saccharomyces Cerevisiae." *Scientific Reports* 6, no. 1 (August 23, 2016): 31443. https://doi.org/10.1038/srep31443.