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Annotated Bibliography

Cherry JM, Hong EL, Amundsen C, Balakrishnan R, Binkley G, Chan ET, Christie KR, Costanzo MC, Dwight SS, Engel SR, Fisk DG, Hirschman JE, Hitz BC, Karra K, Krieger CJ, Miyasato SR, Nash RS, Park J, Skrzypek MS, Simison M, Weng S, Wong ED (2012) Saccharomyces Genome Database: the genomics resource of budding yeast. Nucleic Acids Res. Jan;40(Database issue):D700-5. [PMID:22110037]

Sequencing of some of the genome of Saccharomyces Cerevisiae was completed in this study. The structural prediction of Pho88 that was generated included 51.60% hydrophobic, 28.72% hydrophilic, and 19.68% neutral amino acids. This structural prediction was strong evidence that PHO88 is a membrane-bound protein.

Eisenberg D. (1984) Three-dimensional structure of membrane and surface proteins. Annu Rev Biochem 53 : 595-623

In this study, Eisenberg revealed the three dimensional structure of many membrane and surface proteins in yeast. This review focussed on two techniques: (a) diffraction studies at the molecular level, and (b) correlations and predictions from amino acid sequences of aspects of the three dimensional structure. Using techniques outlined by Eisenberg in 1984, researchers Yompakdee et al. generated and analyzed the hydrophobicity plots of Pho88p and Pho86p. They found that the predicted hydrophobicity plot of Pho88 suggested that, like Pho86p, it is also also likely a membrane bound protein.

Feldmann, H., Aigle, M., Aljinovic, G., André, B., Baclet, M. C., Barthe, C., … Boles, E. (1994). Complete DNA sequence of yeast chromosome II. The EMBO Journal, 13(24), 5795–5809.

Sequencing of the complete genome of Saccharomyces Cerevisiae was completed in this study. When the sequencing of Pho88 and other phosphate metabolism proteins was completed, Pho88 was found to be a 21kDa protein consisting of 188 amino acid residues and various hydrophobic and hydrophilic domains (1994).

Galanie, S., Thodey, K., Trenchard, I. J., Interrante, M. F., & Smolke, C. D. (2015). Complete biosynthesis of opioids in yeast. Science, 349(6252), 1095-1100. doi:10.1126/science.aac9373

This research paper presents a new synthetic biology technique discovered by the Smolke lab at Stanford University. Here, the researchers present a genetically engineered yeast strain that can convert sugar into thebaine, the key opiate precursor to morphine and other strong analgesics. These researchers suggest that tweaking the yeast pathways may allow medical chemists to produce more effective, less addictive painkillers. This new discovery provides

motivation and significance for uncovering the mechanisms of yeast metabolic pathways that aren't yet known in detail. Understanding each molecule and step involved in yeast metabolism may allow medical chemists to exploit these pathways for the development of better drugs.

Golemis E. (2002) Protein-protein interactions : A molecular cloning manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press. ix, 682.

A basic co-immunoprecipitation protocol is described in this source. This experiment is commonly used to reveal protein-protein interactions when one target protein is known. I planned to utilize this technique to discover protein-protein interactions between Pho88 and other phosphate transport proteins.

Hurto, R. L., Tong, A. H. Y., Boone, C., & Hopper, A. K. "Inorganic Phosphate Deprivation Causes tRNA Nuclear Accumulation Via Retrograde Transport in Saccharomyces Cerevisiae." Genetics 176.2, 2007 pp. 841-852. We obtained a plasmid encoding an N-terminal GST-tagged Pho88 (Martzen et al. 1999) and found that GST-Pho88 was located in the endoplasmic reticulum.

Although this study does not focus on the Pho88 mutant or PHO88 protein, they use the Pho88 mutant in a small portion of their experiments on tRNA retrograde transport. They discovered that deletion of PHO88 caused impaired P_i uptake and slowed growth.

Oshima, Y. "The Phosphatase System in Saccharomyces Cerevisiae." Genes & Genetic Systems, 72.6, 1997. pp. 323-334.

This was a key paper for the understanding of the phosphate transport pathway in yeast. Oshima *et al.* outline the role of all proteins known to be involved in uptake, and regulation. For example, regulatory activity on the phosphate uptake pathway is largely controlled by the protein product of pho81. Pho81 is a primary receptor for detecting inorganic phosphate (Pi) signals from the environment and functions as an inhibitor under low phosphate conditions. It was mentioned that Pho88's exact role is unknown in the pathway.

Mouillon, J., Persson, B. L. "New Aspects on Phosphate Sensing and Signalling in Saccharomyces Cerevisiae." FEMS Yeast Research, 6.2, 2006. pp. 171-176.

Mouillon and Persson report that although the function of Pho88 is unknown, the *pho88* mutant yeast are phenotypically similar to *pho86* mutant yeast. It is known that Pho86 is required for trafficking of Pho84 to the plasma membrane, and yeast with mutations in *PHO84* display a similar phenotype to *pho86* yeast. These relationships suggest that Pho88 and Pho86 may function by binding Pho84 to promote its maturation or trafficking. This paper suggests that two other genes, Pho87 and Pho91, may be chaperoned by Pho88 to reach the plasma membrane in a manner similar to the interaction of Pho86 and Pho84.

Yompakdee, C., Ogawa, N., Harashima, S., & Oshima, Y. "Molecular & General Genetics: A Putative Membrane Protein, Pho88p, Involved in Inorganic Phosphate Transport in Saccharomyces Cerevisiae." Springer, 251.7, 1996.

To confirm that Pho88p exists in the membrane, Yompakdee et al. conducted an immunoblotting analysis of cell fractions and confirmed cellular localization of Pho88p around the membrane of the endoplasmic reticulum. Through transformation experiments where extra copies of PHO86 and PHO88 were added to *S. cerevisiae,* it was found that the genes have additive, but independent functions in phosphate uptake. Genetic evidence indicates that PHO88, along with PHO86, modulates Pho81p activity in response to Pi signals.

Toh-E, A., & Oshima, Y. (1974). Characterization of a dominant, constitutive mutation, PHOO, for the repressible acid phosphatase synthesis in Saccharomyces cerevisiae. Journal of bacteriology, 120(2), 608-617.

The methods for cultivation of cells and assay of the repressible phosphatase enzyme activity are described in this paper.