

CELL BIOLOGY

Yeast Physiology

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Technologies

The cellular responses to nutrients and stressful conditions represent one of the central themes for the Department of Physiology. The metabolism and transport of low-molecular-weight compounds are important objects in these studies. The primary tool in the forefront of these investigations is genetics, for which yeast is extremely well suited.

The group has strong expertise in both classical and molecular genetics. This includes the tools necessary for any directed genetic change of the yeast that does not kill the organism, e.g. deletion of non-essential genes, singly or in combinations, or substitution of a given wild-type gene with a version mutated to encode a given amino acid residue at a given site. This freedom of design is invaluable in breeding of industrial yeasts and in setting up highly controlled experiments for revealing physiological interactions and biochemical mechanisms. Equally important is the extensive experience in designing microbial selection screens for randomly or semi-randomly generated mutants, the identities of which give new information on metabolic or signalling pathways. The group also possesses know-how on genetic handling of hybrid industrial yeasts.

Projects

Investigations on nutrient uptake have in recent years focused on how some nutrients induce their own uptake through a sensing mechanism at the cell surface.

Investigations on the metabolism and transport of glycerol have been aimed at designing strains that yield more alcohol and the identification of a protein in the plasma membrane that is able to import glycerol even if the glycerol concentration in the yeast cell is higher than outside. This protein is presumed to have an important role in protection against osmotic stress in nature.

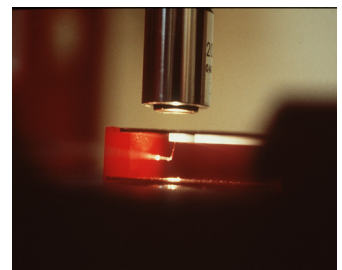
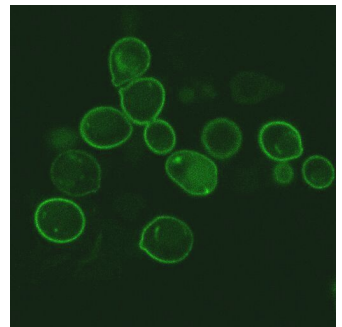
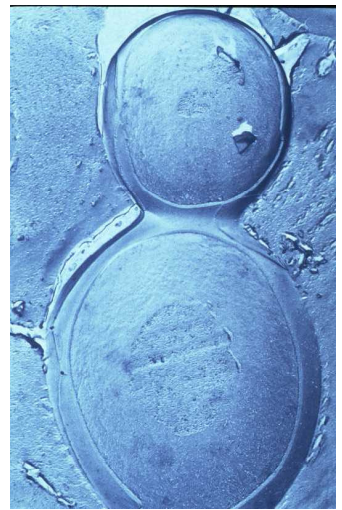
A recent project, which has been supported by a research contract with EU, is aimed at creating yeast mutant strains resistant to various stress factors, including combinations of stress factors typical for brewery fermentations.

Results

The group has discovered that yeast cells sense extracellular amino acids by a plasma membrane protein with high similarity to amino acid permeases. During evolution, a protein originally working as a transporter appears to have lost transport efficiency to undetectable levels while retaining its nutrient interaction to allow it to develop into a sensor. Important aspects of the sensing mechanism are being unveiled.

A method for increasing ethanol yield by decreasing production of glycerol and biomass has been developed in a collaboration with DTU. Carlsberg decided to not participate in the patenting but can freely use the technology in-house. The group recently identified the protein that can import and concentrate glycerol in the cell.

In both the project area on amino acids and the project area on glycerol, many genes were functionally and molecularly identified and physiological and mechanistic relationships have been revealed.



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Selected overviews:

Hansen, J. & M.C. Kielland-Brandt: Brewer's yeast: genetic structure and targets for improvement. In: J.H. de Winde (ed.) *Functional Genetics of Industrial Yeasts. Topics in Current Genetics, Vol. 2.* Springer-Verlag Berlin Heidelberg 2003, pp. 143-170.

Andersen, H.A., R.F. Gaber, P.L. Madsen, P.S. Nielsen, K. Ottow & M.C. Kielland-Brandt: Amino acid uptake and sensing in yeast. *Proc. 28th EBC Congress, Budapest 2001*, pp. 417-426.

Selected research papers:

Poulsen, P., B. Wu, R.F. Gaber and M.C. Kielland-Brandt: Constitutive signal transduction by mutant Ssy5p and Ptr3p components of the SPS amino acid sensor system in *Saccharomyces cerevisiae*. *Eukaryot. Cell* 4, 1116-1124, 2005.

Poulsen, P., B. Wu, R.F. Gaber, K. Ottow, H.A. Andersen and M.C. Kielland-Brandt: Amino acid sensing by Ssy1. *Biochem. Soc. Trans.* 33, 261-264, 2005.

Ferreira, C., F. van Voorst, A. Martins, L. Neves, R. Oliveira, M.C. Kielland-Brandt, C. Lucas and A. Brandt: A member of the sugar transporter family, Stl1p is the glycerol/H⁺ symporter in *Saccharomyces cerevisiae*. *Mol. Biol. Cell* 16, 2068-2076, 2005.

Eckert-Boulet, N., P.S. Nielsen, C. Friis, M.M. dos Santos, J. Nielsen, M.C. Kielland-Brandt and B. Regenber: Transcriptional profiling of extracellular amino acid sensing in *Saccharomyces cerevisiae* and the role of Stp1p and Stp2p. *Yeast* 21, 635-648, 2004.

Gaber, R.F., K. Ottow, H.A. Andersen & M.C. Kielland-Brandt: Constitutive and hyperresponsive signaling by mutant forms of *Saccharomyces cerevisiae* amino acid sensor Ssy1. *Eukaryotic Cell* 2, 922-929, 2003.

Nielsen, P.S., B. van den Hazel, T. Didion, M. de Boer, M. Jørgensen, R.J. Planta, M.C. Kielland-Brandt & H.A. Andersen: Transcriptional regulation of the *Saccharomyces cerevisiae* amino acid permease gene *BAP2*. *Mol. Gen. Genet.* 264, 613-622, 2001.

Holst, B., C. Lunde, F. Lages, R. Oliveira, C. Lucas & M.C. Kielland-Brandt: *GUP1* and its close homologue *GUP2*, encoding multimembrane-spanning proteins involved in active glycerol uptake in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 37, 108-124, 2000.

Nissen, T.L., M.C. Kielland-Brandt, J. Nielsen & J. Villadsen: Optimization of ethanol production in *Saccharomyces cerevisiae* by metabolic engineering of the ammonium assimilation. *Metabol. Engin.* 2, 69-77, 2000.

Regenber, B., L. Düring-Olsen, M.C. Kielland-Brandt & S. Holmberg: Substrate specificity and gene expression of the amino-acid permeases in *Saccharomyces cerevisiae*. *Curr. Genet.* 36, 317-328, 1999.

Didion, T., B. Regenber, M.U. Jørgensen, M.C. Kielland-Brandt & H.A. Andersen: The permease homologue Ssy1p controls the expression of amino acid and peptide transporter genes in *Saccharomyces cerevisiae*. *Mol. Microbiol.* 27, 643-650, 1998.

Hansen, J. & M.C. Kielland-Brandt: Inactivation of *MET10* in brewer's yeast specifically increases SO₂ formation during beer production. *Nature Biotechnol.* 14, 1587-1591, 1996.

Selected patent applications:

Nielsen, J., T.L. Nissen & M.C. Kielland-Brandt: Metabolically engineered microbial cell with an altered metabolite production. Patent application, WO 00/03020, 2000.

Nielsen, J., T.L. Nissen & M.C. Kielland-Brandt: Metabolically engineered microbial cell comprising a modified redox activity. Patent application, WO 00/03021, 2000.