Success Story
COMET

FFG

acib innovations from nature

acib
Austrian Centre of Industrial Biotechnology
Programme: COMET – Competence Centers for Excellent Technologies
Programme line: K2-Centres
COMET subproject, duration and type of project:
Engineering of protein secretion in Pichia pastoris, [01/2010 – 12/2015], multi-firm

[Novel Metabolic Pathway in Biotech Yeast Discovered]

*Pichia pastoris (*syn. *Komagataella sp.*) is able to utilize the small single carbon compound methanol as carbon and energy source. The utilization of methanol necessitates the coordinated expression of a subset of genes. Everything seemed to be known about this pathway until researchers of acib and BOKU discovered a novel pathway for methanol assimilation, which revolutionized the understanding of *P. pastoris* cell biology. It was found that *P. pastoris* is evolutionary on the safe side having duplicate copies of genes for breaking down methanol. Furthermore, it could be shown that used models and assumptions used the last 30 years were wrong.

Introduction

Over the last decades yeast became an important cell factory for industrial biotechnology. Valuable products ranging from enzymes to biopharmaceuticals are produce with yeast. Especially, the methylotrophic yeast *Pichia pastoris (*syn. *Komagataella sp.*) turned out to be an indispensable host organism for the production of such products.

Beside its industrial relevance, *P. pastoris* is used as model for studying cellular organelles (such as peroxisomes). The peroxisome is essential for methylotrophic yeast such as *P. pastoris*, because they are predominantly involved in the metabolism of unusual carbon sources (like methanol). The peroxisome proliferation changes with the used carbon source, and is for example induced when methanol is used as carbon source (Fig.1).

Several key enzymes for the utilization of methanol as carbon source are known. Furthermore, assimilation of small molecules containing one carbon atom was supposed to involve metabolites from other metabolic pathways. Everything seemed to be known until researchers from the Austrian Centre of Industrial Biotechnology (ACIB) and the University of Natural Resources and Life Sciences (BOKU) discovered a novel pathway for methanol assimilation in *P. pastoris*. Furthermore, they could show that models and assumptions, which have been used the past 30 years were wrong.

Methanol assimilation in *P. pastoris* by a yet unkown metabolic pathway

The newly identified pathway describes how methanol is used as substrate for biomass formation. This pathway uses a specialized set of genes, which were found through analysis of unique systems biological data, which obtained in this work. This second set of specialized genes is only induced during growth on methanol and is located within the peroxisome, where methanol assimilation happens.

Furthermore, the researchers found amazing similarities for this metabolic pathway with

plants. In plants the greenhouse gas carbon dioxide (CO_2) is used as a nutrient for biomass formation. The whole process takes place within the chloroplasts and is referred as the Calvin cycle. For *P. pastoris* it is similar. Here methanol is converted, which is also a one carbon compound like CO_2, in the peroxisome. The common grounds for both pathways are consecutive steps of formation of chemical bonds between carbon compounds with a final rearrangement to sugar phosphates and other compounds needed for biomass formation (Fig.2).

Furthermore, it was shown that the mentioned rearrangement reactions specifically take place within the peroxisome, which wasn´t known up to now. The novel metabolic pathway was named xylulose-monophosphate cycle.

In this respect these findings make *P. pastoris* unique among the methylotrophic yeasts.

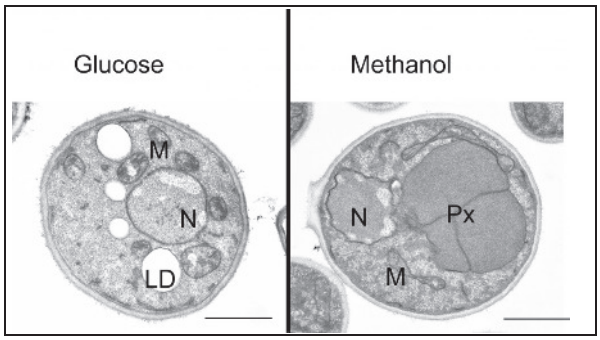

Fig. 1: Electron microscopy of *P. pastoris* grown on glucose or methanol. N, Nucleus; M, Mitochondria; LD, Lipid droplet; Px, Peroxisome. Scale bar: 1 µm

Impact and effects

This work shows how interpretation of multi levels system biology data can contribute to elucidation of yet unkown cellular pathways and revolutionized the understanding of cellular biology of *P. pastoris*.

The gained knowledge about the methanol assimilation pathway will also have a major impact on the understanding and evolution of methylotrophic yeast.

Methylotrophic yeast, like *P. pastoris*, play an important role as host for industrial recombinant protein production. For the production of a recombinant protein, promotors with different expression levels are necessary to enable productivity. The novel genes identified in this work show different expression patterns (induced during growth on methanol) and therefore can be used for expression of a recombinant protein. Furthermore, the promotors with different regulatory strength can be used for cell and metabolic engineering.

The work was recently published in the prestigious journal BMC Biology.

Systems-level organisation of yeast methylotrophic lifestyle, Rußmayer et al. 2015. BMC Biology 13:80

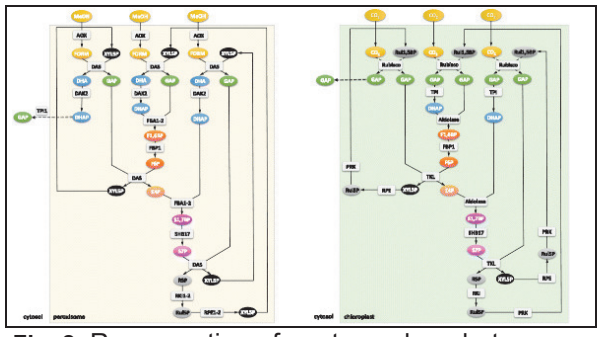

Fig. 2: Regeneration of pentose phosphates. Left: Methanol assimilation through the xylulose-monophosphate cycle, Right: Rearrangement reactions of the Calvin cycle

Contact and information
K2-Centre acib, Muthgasse 11, 1190 Wien, www.acib.at
Department of Biotechnology, BOKU-University of Natural Resources and Life Sciences Vienna
Project coordinator: Diethard Mattanovich, diethard.mattanovich@boku.ac.at

Project partners

Organisation	Country
Biocrates Life Sciences AG	Austria
Biomin Holding GmbH	Austria
Boehringer Ingelheim RCV	Austria
Lonza AG	Switzerland
Sandoz GmbH	Austria
VTU GmbH	Austria
TU Graz	Austria