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Meeting Report

The methylotrophic yeast *Hansenula polymorpha*: its use in fundamental research and as a cell factory

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Introduction

During the last decade methylotrophic yeasts have gained increasing interest, both for fundamental (van der Klei and Veenhuis, 1996) and applied purposes (van Dijk *et al.*, 2000; Gellissen, 2000). The special value of these yeasts (mainly *Pichia pastoris* and *Hansenula polymorpha*) in fundamental research is undoubtedly related to studies on the principles of peroxisome homeostasis (biogenesis vs. degradation). In particular, the morphological features of peroxisome development and selective degradation are more strongly pronounced than in *Saccharomyces cerevisiae*, which makes it possible to induce and follow the details in mutants more easily. In addition, peroxisome induction in baker's yeast is restricted to growth on oleate (and thus β -oxidation), whereas in the methylotrophic yeasts various peroxisomal metabolic pathways may occur, depending on the composition of the growth medium. These organisms also display the strongest induction rates that may rise to a level at which up to 80% of the total cell volume is occupied by peroxisomes.

The extremely high expression rates necessary to achieve this drew the attention of biotechnologists, who studied the use of these organisms as host for the production of valuable, heterologous proteins. Initially, *P. pastoris* was predominantly used; now *H. polymorpha* is gaining more and more attention.

The *H. polymorpha* system was shown to provide a remarkably versatile technology platform for heterologous gene expression. The *Hansenula* toolbox now includes a variety of host strains, new integration sites and several novel strong promoters beyond those derived from methanol metabolism genes. In the first *H. polymorpha* worldwide network (HPWN) conference, the recent developments in the use of this organism were discussed in a lively fashion. This report highlights several superb presentations of important, interesting and novel research.

H. polymorpha as a model system in fundamental research

In her keynote address, **Ida van der Klei** (Groningen) summarized the recent developments on peroxisome homeostasis in *H. polymorpha* (Figure 1). These studies are facilitated by the fact that detailed information is now available on the physiology, biochemistry and ultrastructure of the organisms, allowing the precise manipulation of peroxisome development, function and selective turnover of the organelles and their enzyme content (van der Klei and Veenhuis, 1997). Compartmentalization of specific functions into organelles is believed to be advantageous in the control of various types of cellular processes, e.g. by the

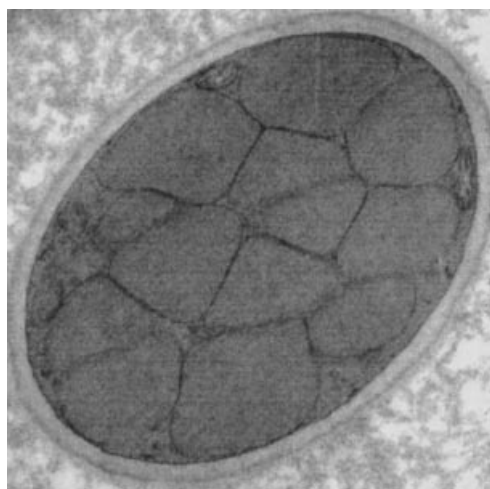


Figure 1. *H. polymorpha* cell, grown in a methanol-limited chemostat, crowded with peroxisomes

creation of unique microenvironments with specific (bio)chemical properties. The reasons for the cell to compartmentalise part of the key enzymes of methanol metabolism are now understood. This has shown to be essential to: (a) regulate the partition of formaldehyde produced from methanol over assimilatory and dissimilatory pathways; and (b) to ensure that H_2O_2 , produced as a side product from methanol oxidation, is metabolized by peroxisomal catalase. Leakage of H_2O_2 from the organelles is not lethal (as is often believed) but its metabolism outside the peroxisome leads to severe energetic disadvantages that would prevent growth on methanol. As with other organelles, extremely complex regulatory mechanisms for peroxisome generation and maintenance have evolved. Apart from this, the function of the organelles should be continuously and rapidly adaptable to ensure that the cell directs its resources optimally towards specific functions. One of the important ways to achieve this is to precisely control the abundance of the organelles. This may be achieved by tightly controlling two contradictory processes: the development of the organelles and their selective degradation under conditions that these organelles are no longer required for growth. Evidence was presented that these two processes converge at Pex14p, a protein which was initially described to be essential for peroxisome biogenesis. This control machinery enables the organism to maintain at least one small peroxisome after a shift of cells to

non-methylotrophic conditions. The physiological advantage of this mechanism is immediately clear, since it allows the cell to adapt rapidly to a new environment that requires new peroxisome functions.

José Siverio gave a summary report on aspects of nitrate metabolism in *H. polymorpha* (González *et al.*, 1999). **S. Krappmann** and **G. Braus** described the first gene and gene product involved in the biosynthesis of aromatic amino acids, the *HARO7* gene, encoding chorismate mutase (Krappmann *et al.*, 2000).

***H. polymorpha* as a cell factory**

A strong focus of the conference was the biotechnological application of *Hansenula polymorpha*. Several contributors to the conference described new elements for the further improvement of the platform technology based on this yeast. The range of elements presented included new promoters, integration sites and auxotrophy complementation markers. Applied examples included production of recombinant gelatins, HSA, technical enzymes and a report about the production processes for *H. polymorpha*-derived hepatitis B vaccines that were launched a few years ago.

C. P. Hollenberg from Düsseldorf and **H. A. Kang** from S.K. Rhee's group in Korea provided keynote addresses describing the platform technology based on this yeast species. Integration into *HARS* sequences and methods for the integration of heterologous DNA in anticipated copy numbers are especially attractive tools (Sohn *et al.*, 1999a, b). Several novel promoter elements were presented: **P. Sudbery** (Sheffield) reported on the strong constitutive expression of sequences fused to the *PMA1*-promoter (Cox *et al.*, 2000). **M. Suckow** (Rhein Biotech GmbH) described a novel promoter derived from the heat-inducible *TPS1* (trehalose-6-phosphate synthase) gene that appears to be even stronger than the traditional methanol-inducible *FMD* promoter (Amuel *et al.*, 2000). New hosts based on strains with a disrupted *ARO7* gene exhibiting a tyr^- phenotype were also described (Krappmann *et al.*, 2000).

Product and strain developments revealed interesting new features of the *H. polymorpha* system. **E. de Bruin** and **F. de Wolf** reported on their feasibility study for the production of recom-

binant collagens in this yeast. Collagens are derived from gelatins by post-translational prolyl-4 hydroxylation. This modification could be accomplished in other yeast systems by co-expression of a respective gene. The surprising finding of native collagen-like *H. polymorpha* proteins containing 4-hydroxyproline suggests the existence of a suitable endogenous hydroxylase gene, which could make the production of heterologous collagens more efficient (de Bruin *et al.*, 2000).

T. McMullin (BTR, USA) presented his group's efforts in the development of strains for the production of a fungal galactose oxidase. The data demonstrated a complex relationship of gene copy number and the accumulation of the gene product, suggesting that using gene copy number as a predictor of product accumulation may not always be accurate.

S.-J. Ahn (GCVC, Korea) presented a detailed report on the production process for the *H. polymorpha*-derived hepatitis B vaccine. GCVC received the status of a pre-qualified supplier for this vaccine from the World Health Organization, making *H. polymorpha* the only eukaryotic microbial expression system beside *S. cerevisiae* to be applied for the production of a launched pharmaceutical (Schaefer *et al.*, 2000).

The conference was also the initiation of the *H. polymorpha* Worldwide Network, aimed at providing a platform for information exchange between scientists working with this yeast. The next conference is scheduled for 2002, and a web page is currently in preparation.

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