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Illuminating the Role of Protein Kinase A in Controlling Yeast Growth in Visible Light

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Abstract

Background: Because some yeasts have evolved a methylotrophic lifestyle, they can use the single-carbon molecule methanol as a source of carbon and energy. Pichia pastoris (also known as Komagataella sp.) is one of them and is commonly employed for the generation of heterologous proteins as well as a model organism for organelle research. Our present understanding of the methylotrophic lifestyle is primarily based on extensive biochemical investigations that discovered numerous important methanol utilisation enzymes and their localization to the peroxisomes, including alcohol oxidase and dihydroxyacetone synthase. The pentose phosphate pathway is thought to be involved in C1 assimilation, but the specifics of these events are not yet understood.

Results: In this study, we compared the development of P. pastoris on a medium containing equal amounts of methanol and glycerol and glucose, as well as the regulation patterns of 5,354 genes, 575 proteins, 141 metabolites, and fluxes through 39 processes. We discovered that the whole methanol absorption mechanism is restricted to peroxisomes as opposed to using a portion of the cytosolic pentose phosphate pathway for xylulose-5-phosphate regeneration, as was previously thought. P. pastoris (and perhaps other methylotrophic yeasts) have developed a duplicated set of methanol-inducible enzymes that are specific to peroxisomes for this purpose. Sedoheptulose-1,7-bisphosphate is used as an intermediary in this compartmentalised cyclic C1 assimilation mechanism known as the xylose-monophosphate cycle. The high demand for their respective cofactors, riboflavin, thiamine, nicotinamide, and heme, caused by the strong induction of alcohol oxidase, dihydroxyacetone synthase, formaldehyde and formate dehydrogenase, and catalase, is reflected in the strong up-regulation of the corresponding synthesis pathways on methanol. Because of the high outflow towards methanol metabolic enzymes and their cofactors, methanol-grown cells contain more protein but fewer free amino acids. This illustrates a higher flow towards amino acid and protein synthesis, which is also reflected in higher transcript levels, in conjunction with up-regulation of several amino acid biosynthesis genes or proteins.

Conclusions: When taken as a whole, our study demonstrates how coordinated analysis of data from different systems biology levels can help reveal as-yet-unknown cellular pathways and completely change how we think about cellular biology.

Keywords: Protein; Kinase; Yeast Growth; Visible Light

Introduction

Methylotrophic yeasts can take carbon from a variety of sources. Similar efficiency is achieved when using multicarbon sources like sugars and sugar alcohols like glucose, glycerol, or mannitol as opposed to decreased C1-compounds like methanol. In addition to the cells being properly equipped with the enzymes required for substrate metabolism, their coordinated expression is a requirement for [1-4] the efficient use of various carbon and energy sources. Several recombinant proteins are created using the methylotrophic yeast Pichia pastoris (syn. Komagataella sp.), and a growing number of biopharmaceuticals and industrial enzymes are also made using this method. Lately, P. pastoris has been used more frequently as a model organism for the growth of peroxisomes and secretory organelles. Its development has been primarily driven by the methylotrophic lifestyle, which includes peroxisomes—specialized organelles—as well as strong, tightly controlled promoters for the production of recombinant genes. Peroxisomes are described as intracellular organelles that house the enzyme catalase, which breaks down hydrogen peroxide (H2O2) and produces its own. Moreover, these organelles house the P. pastoris fatty acid beta-oxidation pathway. The key enzymes involved in the metabolism of a variety of unconventional carbon and nitrogen sources are yeast peroxisomal oxidases. In methylotrophic yeasts, peroxisomes are relatively abundant in methanol-grown cells but drastically drop in both number and volume upon catabolite repression. Peroxisomes include the first steps of the methanol utilisation pathway. Hansenula polymorpha, a different methylotrophic yeast, grows on glucose but only contains one tiny peroxisome, which can be used as a source for fission-based cell division when induction is initiated by switching the cells to methanol. The expression of genes associated to methanol utilisation is highly stimulated by methanol, in addition to genes encoding structural peroxisomal proteins. Alcohol oxidases are used in the initial stages of methanol absorption to turn methanol into formaldehyde, and a unique transketolase known as dihydroxyacetone synthase is used to create a C-C bond with the C1 molecule of formaldehyde. These two enzymes' reactions and their distribution in peroxisomes have been extensively studied. Pentose phosphate reactions are thought to be a part of the subsequent cycle of the absorption of methanol, but the specifics are not yet fully understood.

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Materials and Methods

While numerous research have examined the cellular responses of P. pastoris to methanol induction in the context of the generation of recombinant proteins, it is mostly unknown how non-recombinant strains would react to the various carbon sources. Hence, we made the decision to look into P's biological reactions. The methanol cultures were co-fed with glycerol to enable the same chemostat-controlled constant specific growth rates for direct comparison. A number of transcriptome regulatory studies of P. pastoris, examining the effects of growth rate, unfolded protein response (UPR) activation, oxygen availability, osmotic stress, or heterologous protein production, were possible due to the availability of whole genome sequences. Further information about the traits of P. pastoris cultivated at various temperatures, osmolarity, UPR induction, and oxygen supply was revealed by analyses of the host proteome. More recently, using 2D-DIGE and [6-11] subsequent mass spectrometry identification of differentially abundant proteins, P. pastoris strains expressing an insulin precursor were examined for alterations in the cellular proteome as adaptive response to methanol induction during fed batch growth. Enzymes involved in the dissimilatory methanol metabolism and UPR induction were found in high abundance. Changes in metabolic fluxes brought on by the regulation of cellular enzyme concentrations will eventually modify the concentrations of free metabolites. Understanding metabolic networks requires quantitative measurement of intracellular fluxes. Initial P. pastoris genome-scale metabolic network models and flux distributions of central carbon metabolism suggest that methanol (co-)assimilation is a growth rate-related process, with potential consequences for the pentose phosphate pathway. The current study uses steady-state cultures of non-producing P. pastoris with a uniform specific growth rate and the carbon supply as the examined variable for transcriptomics, proteomics, metabolomics, and fluxomics investigations. This integrated systems level study made it possible to identify cellular functions like vitamin biosynthesis and amino acid metabolism that are co-regulated with methanol metabolism. These co-regulation patterns also served as the basis for deciphering the as-yet-unknown processes of sugar phosphate rearrangements recycling xylulose-5-phosphate for methanol fixation. Here, we offer a novel theory that describes the assimilation of methanol as a distinct, highly controlled mechanism that results from the duplication of the relevant genes.

Findings and analysis

P. pastoris CBS7435 was grown in chemostats at a set specific growth rate of 0.1 h-1, or around 60% of the maximum growth rate on glucose. To prevent growth rate-dependent impacts during genome-scale analysis, constant growth is a must. A mixed feed strategy with glycerol-methanol co-feeding was used because the highest specific growth rate on pure methanol as a carbon source would be substantially lower and intracellular carbon fluxes could not be evaluated on methanol alone.

Chemostat cultivation and strains

The 400 mL working volume of a 1.4-L bioreactor (DASGIP Parallel Bioreactor System, Germany) was used for the chemostat cultivations. In a nutshell, P. pastoris CBS7435 cryostock was inoculated into 100 mL of pre-culture media (10 g yeast extract, 20 g peptone, and 10 g glycerol per litre) and cultured at 28 °C at 150 rpm overnight. The bioreactor was inoculated with this culture at an optical density (OD600) of 1.0. The cells were cultivated in carbon-limited chemostats with a dilution rate of 0.1 h1 for at least seven residence periods after a batch phase of about 24 hours before samples were taken.

Proteomics

Preparation of the sample and cell lysis

In a 100 mM triethylammonium bicarbonate (TEAB) solution containing 30 mM tris(2-carboxyethyl)phosphine hydrochloride and 2% SDS, cells were lysed using glass beads in accordance with Dragosits' instructions. Cellular proteins were extracted with chloroform/methanol, dried, dissolved in TEAB buffer, and trypsinized after being incubated for 45 min at 56 °C (to minimise cysteine bridges). Tandem Mass Tag (Thermo Scientific) labelling was carried out in accordance with Pichler's instructions and the manufacturer's guidelines.

Protein and peptide identification

By comparing the obtained spectra with a database containing distinct P. pastoris protein sequences, the programme Mascot was utilised to identify the peptides and proteins. Mascot employs the MOWSE (MOlecular Weight SEarch) score, where the greater the peptide value, the more matches there are. Peptide scores are added together to create protein scores. The matching of at least two distinct peptides with a score of greater than 25 is necessary for protein identification.

Conclusions

Methylotrophy is a special ability of microbes to survive on C1 molecules and requires effective routes to generate C-C bonds and oxidise C1 compounds via hazardous intermediates. The methylotrophic yeast P. pastoris has unique regulatory and metabolic characteristics, which are thoroughly explained by this systems level analysis. We were able to precisely pinpoint the probable pathway for XYL5P regeneration during methanol ingestion by co-regulating enzymes with AOX and DAS at the mRNA and protein level. Instead of the PPP proteins, which are essentially not transcriptionally or translationally regulated in this study, we showed that the xylulose-monophosphate cycle uses a unique set of methanol-induced enzymes found in the peroxisome.

Author Contributions

The diagnosis and treatment of this cat were handled exclusively by Jennifer Weng and Harry Cridge. This report was written by Jennifer Weng, and Harry Cridge gave it a critical appraisal. The final draught of the manuscript has received the approval of both Jennifer Weng and Harry Cridge.

Conflict of Interest

According to the authors, there are no conflicts of interest that might be thought to compromise the objectivity of the research presented.

Ethics Statement

The case described in this report was handled as part of the regular clinical caseload at the university teaching hospital; an IACUC or other ethical approval was not necessary. All facets of this patient's care had the owner's consent.

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