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Proteome analysis of aerobically and anaerobically grown *Saccharomyces cerevisiae* cells

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ABSTRACT

The yeast *Saccharomyces cerevisiae* is able to grow under aerobic as well as anaerobic conditions. We and others previously found that transcription levels of approximately 500 genes differed more than two-fold when cells from anaerobic and aerobic conditions were compared. Here, we addressed the effect of anaerobic growth at the post-transcriptional level by comparing the proteomes of cells isolated from steady-state glucose-limited anaerobic and aerobic cultures. Following two-dimensional gel electrophoresis and mass spectrometry we identified 110 protein spots, corresponding to 75 unique proteins, of which the levels differed more than two-fold between aerobically and anaerobically-grown cells. For 21 of the 110 spots, the intensities decreased more than two-fold whereas the corresponding mRNA levels increased or did not change significantly under anaerobic conditions. The intensities of the other 89 spots changed in the same direction as the mRNA levels of the corresponding genes, although to different extents. For some genes of glycolysis a small increase in mRNA levels, 1.5–2 fold, corresponded to a 5–10 fold increase in protein levels. Extrapolation of our results suggests that transcriptional regulation is the major but not exclusive mechanism for adaptation of *S. cerevisiae* to anaerobic growth conditions.

1. Introduction

The yeast *Saccharomyces cerevisiae* is one of the few eukaryotic organisms that can grow well under aerobic and anaerobic conditions. To cope with these different environmental conditions this organism needs mechanisms to adapt its metabolism. Molecular oxygen is not only essential for respiration, but is also required in several biosynthetic pathways, like those for heme, sterols, unsaturated fatty acids, pyrimidines and deoxyribonucleotides [1]. Transcription analyses have shown that the expression levels of approximately 500 genes differ significantly when aerobic and anaerobic cultures are compared [2–5]. Several factors for transcriptional regulation of anaerobic metabolism have been proposed. One of those is the upregulation of aerobic genes by a homodimer of Hap1p, bound to heme [6] and the derepression by Hap1p when heme is absent [7]. ROX1 is one of the targets of Hap1 and Rox1p represses hypoxic genes in the presence of oxygen.

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Other genes have been implicated in anaerobic regulation because of their heme-dependency, such as SUT1, ORD1 [8] and HAP2/3/4/5 [9]. In another regulatory system UPC2 and ECM22 are implicated in a dual role in the induction of anaerobic sterol import [10–12]. Recently, it has been reported that the histone deacetylase Rpd3 is required for the expression of anaerobic genes [13]. All of these genes together regulate the transcriptional response to oxygen availability in a complex way.

The contribution of posttranscriptional regulation to the adaptation of *S. cerevisiae* to anaerobic growth conditions is much less understood. In this study we used two-dimensional gel electrophoresis and mass spectrometry to analyze the effect of anaerobic growth conditions on the *S. cerevisiae* proteome. Using these techniques we identified 75 proteins of which the levels differed more than two-fold when aerobically and anaerobically-grown cells were compared. Comparison of the effects of anaerobic growth at the proteomic level with that at the transcriptional level indicates a prominent role for transcriptional regulation. Nevertheless, the levels of a significant number of proteins are regulated posttranscriptionally.

2. Materials and methods

2.1. Strains and culture conditions

The *S. cerevisiae* strain CEN.PK113-7D (MATa; P. Kötter, Göttingen, Germany) was used in this study. Chemostat cultivation under aerobic and anaerobic conditions was performed at 30 °C in 1-Liter working volume laboratory fermentors (Applicon Schiedam, The Netherlands) with glucose-limitation at a dilution rate of 0.1 h^{-1} as described previously [5]. Anaerobic conditions were maintained by flushing the medium reservoir and the fermentor with pure nitrogen gas (0.5 l min^{-1}). In addition, norprene tubing and butyl septa were used to minimize oxygen diffusion into the anaerobic cultures.

2.2. Proteome analysis

Protein extraction, two-dimensional gel electrophoresis, statistical analyses, in-gel tryptic digestion and mass spectrometry were done as described before [14]. Briefly, total protein extracts were prepared from cells resuspended in Lysis-Buffer (50 mM NaCl, 10 mM sodium phosphate, pH 7.4, 1 mM PMSF) containing Complete® Proteinase Inhibitor Cocktail (Roche) using a FastPrep instrument (Bio101). Membrane protein-enriched fractions were prepared using a commercial membrane protein extraction kit (Pierce), after disrupting the cells using the FastPrep instrument. For two-dimensional gel electrophoresis protein samples (125 μg of protein) were loaded onto 24 cm IPG strips 3–10 NL (Amersham Biosciences) followed by isoelectric focusing for 1 h at 500 V, 1 h at 1000 V and 15 h at 8000 V using a Protean® IEF Cell (Bio-Rad). After subsequent treatments with dithiothreitol and iodoactamide, strips were then loaded onto 11% SDS-polyacrylamide gels followed by electrophoresis in a Protean® Plus Dodeca Cell (Bio-Rad) at 12.5 mA per gel. Gels were fixed in 10% methanol/7% acetic acid for 1 h and stained overnight with SYPRO Ruby protein stain (Bio-Rad). After washing in 10% methanol/7% acetic acid for 1 h gels were kept in water until they were scanned using a Typhoon 9410 Variable Mode Imager (Amersham Biosciences). Spot detection, matching, quantification and statistical analysis were performed using the PDQuest 7.3.1 software package (Bio-Rad). Proteins present in the excised protein spots were digested using 50 ng of modified trypsin (Promega). For LC-MS analysis, samples were injected onto a nano-LC system (Ultimate, Dionex/LC Packings, Amsterdam, The Netherlands) equipped with a peptide trap column (Pepmap 100, 0.3 i.d.×1 mm, Dionex/LC Packings, Amsterdam, The Netherlands) and an analytical column (Pepmap 100 , 0.075 i.d×150 mm, Dionex/LC Packings). The mobile phases consisted of (A) 0.04% formic acid/0.4% acetonitrile and (B) 0.04% formic acid/90% acetonitrile. A 45 min linear gradient from 0 to 60% B was applied at a flow rate of 0.2 μl/min. The outlet of the LC system was coupled to an HCT ion-trap mass spectrometer (Bruker Daltonics, Bremen, Germany) using a nano-electrospray ionisation source. Eluting peptides were analyzed in the data dependent MS/MS mode over a 400–1600 m/z range. The five most abundant fragments in each MS spectrum were selected for MS/MS analysis by collision-induced dissociation. Mass spectra were evaluated using the DataAnalysis 3.1 software package (Bruker Daltonics). MS/MS spectra were searched against the *S. cerevisiae* NCBI database using the Mascot search algorithm (Matrix Science Ltd., London, UK) allowing one missed cleavage site. Carbamidomethylcysteine was taken as a fixed modification and oxidized methionine as a variable modification. When a protein was identified with an almost identical homolog in the *S. cerevisiae* proteome, the mass spectra were screened for selective peptides to enable discrimination between homologs.

2.3. Western blot analysis

Western blot analyses were performed as described before [14]. Anti-yeast alcohol dehydrogenase I, anti-yeast aldehyde

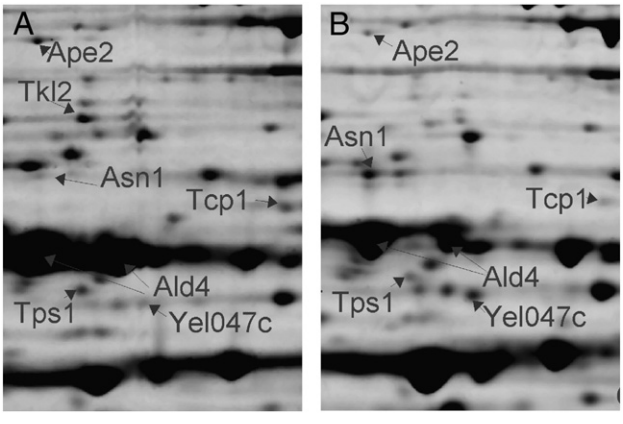

Fig. 1 – Part of a typical two-dimensional gel of protein extracts from aerobically (A) and anaerobically (B) grown CEN.PK113-7D cells. Left, lower isoelectric point; right, higher isoelectric point. Top, higher molecular weight; bottom, lower molecular weight. Some of the proteins identified by mass spectrometry are indicated.