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Integrated RNA- and protein profiling of fermentation and respiration in diploid budding yeast provides insight into nutrient control of cell growth and development

Emmanuelle Becker[a], *Yuchen Liu*[a,1,2], *Aurélie Lardenois*[a,2,3], *Thomas Walther*[a,2,4], *Joe Horecka*[b], *Igor Stuparevic*[a], *Michael J. Law*[c], *Régis Lavigne*[d], *Bertrand Evrard*[a], *Philippe Demougin*[e], *Michael Riffle*[f], *Randy Strich*[c], *Ronald W. Davis*[b,g], *Charles Pineau*[a,d], *Michael Primig*[a,*]

[a]*Inserm U1085 IRSET, Université de Rennes 1, 35042 Rennes, France*
[b]*Stanford Genome Technology Center, Palo Alto, CA 94304, USA*
[c]*Rowan University, School of Osteopathic Medicine, Stratford, NJ 08084, USA*
[d]*Inserm U1085 IRSET, Proteomics Core Facility Biogenouest, Université de Rennes 1, 35042 Rennes, France*
[e]*Biozentrum, University of Basel, Basel CH-4056, Switzerland*
[f]*Department of Biochemistry, University of Washington, Seattle, WA 98195, USA*
[g]*Department of Biochemistry, Stanford University, Stanford, CA 94305, USA*

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ABSTRACT

Diploid budding yeast undergoes rapid mitosis when it ferments glucose, and in the presence of a non-fermentable carbon source and the absence of a nitrogen source it triggers sporulation. Rich medium with acetate is a commonly used pre-sporulation medium, but our understanding of the molecular events underlying the acetate-driven transition from mitosis to meiosis is still incomplete. We identified 263 proteins for which mRNA and protein synthesis are linked or uncoupled in fermenting and respiring cells. Using motif predictions, interaction data and RNA profiling we find among them 28 likely targets for Ume6, a subunit of the conserved Rpd3/Sin3 histone deacetylase-complex regulating genes involved in metabolism, stress response and meiosis. Finally, we identify 14 genes for which both RNA and proteins are detected exclusively in respiring cells but not in fermenting cells in our sample set, including *CSM4*, *SPR1*, *SPS4* and *RIM4*, which were thought to be meiosis-specific. Our work reveals intertwined transcriptional and post-transcriptional control mechanisms acting when a *MAT***a**/α strain responds to nutritional signals, and provides molecular clues how the carbon source primes yeast cells for entering meiosis.

* *Corresponding author.*
E-mail address: michael.primig@inserm.fr (M. Primig).
[1] Present address: School of Medicine, Jianghan University, 430056 Wuhan, China.
[2] These authors made an equal contribution.
[3] Present address: INRA, UMR 703, ONIRIS, F-44307 Nantes, France.
[4] Present address: INSA, UMR CNRS 5504 & INRA 792, F-31077 Toulouse, France.

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Biological significance

Our integrated genomics study provides insight into the interplay between the transcriptome and the proteome in diploid yeast cells undergoing vegetative growth in the presence of glucose (fermentation) or acetate (respiration). Furthermore, it reveals novel target genes involved in these processes for Ume6, the DNA binding subunit of the conserved histone deacetylase Rpd3 and the co-repressor Sin3. We have combined data from an RNA profiling experiment using tiling arrays that cover the entire yeast genome, and a large-scale protein detection analysis based on mass spectrometry in diploid *MAT***a**/α cells. This distinguishes our study from most others in the field—which investigate haploid yeast strains—because only diploid cells can undergo meiotic development in the simultaneous absence of a non-fermentable carbon source and nitrogen. Indeed, we report molecular clues how respiration of acetate might prime diploid cells for efficient spore formation, a phenomenon that is well known but poorly understood.

1. Introduction

Budding yeast is an important model organism for biochemical studies, genetic analyses and genomic profiling of cell growth and division, and cell differentiation [1–3]. In addition, it is useful for the field of toxicogenomics, which investigates the cellular response to environmental stressors and drugs; [4,5], reviewed in [6].

Saccharomyces cerevisiae was the first eukaryote for which the complete genome sequence was determined (see Saccharomyces Genome Database, SGD) [7,8]. The protein-coding part of the yeast genome includes *verified genes*, which are conserved open reading frames (ORFs) typically associated with a biological process, *uncharacterized genes*, which play no known roles, and *dubious genes*, which are assumed not to encode functional proteins [8]. The yeast transcriptome has been studied in numerous conditions using microarrays and RNA-Sequencing (RNA-Seq) [9–12]. Furthermore, protein-profiling experiments detected nearly all theoretically predicted yeast proteins in pre-fractionated extracts from growing cells [13,14]. Very recent feasibility studies reported vastly improved experimental protocols yielding quantitative information about protein concentrations in total cell extracts [15–17].

To produce the metabolite adenosine triphosphate (ATP), which is critical for storing chemical energy, yeast cells process fermentable and non-fermentable carbon sources by activating distinct metabolic pathways. Fermentation of sugars such as glucose and fructose is efficient and down-regulates enzymes required for metabolizing alternative carbon sources via a process called carbon catabolite repression; reviewed in [18]. Fermentation leads to the accumulation of glycerol and ethanol, which are further processed via oxidation. When yeast cells are cultured in rich medium in the presence of the two-carbon compound acetate, they use the tricarboxylic acid (TCA) cycle, and the glyoxylate cycle for energy production [19,20]. Previous analyses of yeast cell growth under highly controlled conditions limited for carbon, nitrogen, phosphorus and sulphur revealed that both transcriptional control and post-translational regulation mechanisms play roles in the response to nutrient starvation [21–23]. Fermentation and respiration are not only relevant for understanding yeast growth and development but also for human diseases; for example, the so-called Warburg effect has been observed in tumor cells that excessively ferment glucose into lactate [24].

Ume6 is the DNA binding subunit of two conserved repressor complexes including either the histone deacetylase (HDAC) Rpd3 and the co-repressor Sin3 [25,26], or the chromatin-remodeling factor Isw2 [27]. Ume6 was initially identified as a mitotic repressor for early meiosis-specific genes, and later shown to orchestrate metabolic functions, stress response, and the onset of meiotic differentiation [28–30]. Among others, Ume6 directly represses *SIP4*, which encodes a C6 zinc cluster transcriptional activator involved in gluconeogenesis, during growth in the presence of glucose [30,31]. Ume6 becomes unstable when vegetatively growing cells switch from metabolizing glucose to processing acetate, and is temporarily degraded during meiosis by the anaphase promoting complex/cyclosome (APC/C), before it accumulates again at later stages of spore formation [32,33].

In earlier work we have developed *Direct Iterative Protein Profiling* (DIPP) to comprehensively analyze the proteome of fermenting diploid yeast cells in a cost-effective manner by widely available standard mass spectrometry [34]. In the present study we report the changes in the proteome of diploid *MAT***a**/α yeast cells undergoing logarithmic growth in rich medium with fermentable glucose (YPD) or non-fermentable acetate (YPA) in the context of corresponding RNA-profiling data from wild-type cells and a *ume6* mutant strain. This approach enabled us to gain insight into molecular events that underlie the switch from fermentation to respiration, and that prime diploid *MAT***a**/α cells for the transition from mitotic growth to meiotic differentiation.

2. Materials and methods

2.1. Culture conditions for protein profiling

Samples from YPD (yeast extract, peptone and dextrose) were cultured as published [34]. In addition, cells grown in YPD were transferred into 100 ml YPA (rich medium with acetate) pre-warmed to 30 °C at a cell density of 2×10^6 cells/ml and cultured until they reached 3×10^7 cells/ml, before they were harvested in two 50 ml aliquots and washed with sterile water. The pellets were then snap-frozen in liquid nitrogen and stored at −80 °C.