Time-course proteomic profile of *Candida albicans* during adaptation to a fetal serum

Wataru Aoki1,2, Tomomi Ueda2, Yohei Tatsukami2, Nao Kitahara2, Hironobu Morisaka2, Kouichi Kuroda2 & Mitsuyoshi Ueda2

1 Japan Society for the Promotion of Science, Sakyo-ku, Kyoto, Japan
2 Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto, Japan

This work uses state-of-the-art proteomics methodology (LC-MS/MS) to examine the growth of the important pathogen, *Candida albicans*, as it adapts to fetal serum. They identify several proteins that may be candidate virulence factors.

**Keywords**
*Candida albicans*; proteomics; hyphae; yeast; serum; time-course proteomics.

**Correspondence:** Mitsuyoshi Ueda, Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606-8502, Japan.
Tel.: +81 (0)75 753 6110
fax: +81 (0)75 753 6112
e-mail: miueda@kais.kyoto-u.ac.jp

Received: 2 July 2012; revised 31 August 2012; accepted 31 August 2012. Final version published online 20 November 2012.

doi:10.1111/2049-632X.12003

**Editor:** Peter Timmis

**Abstract**
*Candida albicans* is a commensal organism; however, it causes fatal diseases if the host immunity is compromised. The mortality rate is very high due to the lack of effective treatment, leading to ceaseless demand for novel pharmaceuticals. In this study, time-course proteomics of *C. albicans* during adaptation to fetal bovine serum (FBS) was described. Time-course proteomics is a promising way to understand the exact process of adapting in dynamically changing environments. *Candida albicans* was cultivated in yeast nitrogen base (YNB) ± FBS media, and we identified 1418 proteins in the endpoint samples incubated for 0 or 60 min by a LC-MS/MS system with a long monolithic silica capillary column. Next, we carried out time-course proteomics of the YNB + FBS samples to identify top-priority proteins for adaption to FBS. We identified 16 proteins as nascent/newly synthesized proteins, and they were recognized as candidates of important virulent factors. Gene ontology analysis revealed that transport-related proteins were enriched in the 16 proteins, indicating that *C. albicans* probably put priority in time on the acquisition of essential elements. Time-course proteomics of *C. albicans* revealed the order of priority to adapt to FBS. Depicting time-course dynamics will lead to profound understandings of virulence of *C. albicans*.

**Introduction**
*Candida albicans* is a commensal organism and can be detected in almost all of the human population (Naglik *et al.*, 2003; Noble & Johnson, 2007). However, *C. albicans* could opportunistically cause recurrent superficial or systemic candidiasis if the host immunity is compromised by AIDS, cancer chemotherapies, or immunosuppressive drugs. The mortality rate of systemic candidiasis is notably high due to the lack of effective diagnoses and treatment (Goffeau, 2008). Hence, there is an urgent need to elucidate virulent mechanisms of *C. albicans* and development of novel pharmaceuticals.

The representative virulent attributes of *C. albicans* include the secreted aspartic protease family (Schaller *et al.*, 2005; Aoki *et al.*, 2011, 2012a, b), the agglutinin-like sequence family (Phan *et al.*, 2007; Almeida *et al.*, 2008; Hoyer *et al.*, 2008; Aoki *et al.*, 2012a, b), and yeast-to-hyphal transition (Lo *et al.*, 1997; Whiteway & Bachewich, 2007). Using these attributes, *C. albicans* binds to mammalian cells including human cells, invades epithelial tissues, and disseminates via bloodstream. In addition, adaptation to sera has absorbed considerable attention because it has been recognized as an integral part of the overall virulence. Adaptation to sera is indispensable for fatal disseminated candidiasis. However, the factors described above cannot clearly explain overall adaptation processes of *C. albicans*, and the existence of hidden virulent factors is anticipated. Thus, minute characterization of *C. albicans* adapting to sera has been a major research subject.

Proteome analysis is an important approach for comprehensive characterization of dynamic variations. Though proteome studies have been carried out so far, these...
researches were mostly endpoint analyses (de Groot et al., 2004; Pitarch et al., 2004; Thomas et al., 2009) with the exception of Kusch’s studies on *Candida* (Kusch et al., 2007). These endpoint studies have provided a lot of beneficial findings, depicting reference maps of two different conditions. Such studies have discerned complex differences between *C. albicans* cells and led to integration of preceding observations. However, we could not understand the exact process that how *C. albicans* adapt to drastic change of surrounding environments. To solve the problem, time-course proteomics is a promising way. It will lead to profound understandings of the detail processes and findings of novel pharmaceutical targets. Grasping the dynamic process could be a complement to the endpoint analyses. Transcriptome analysis is an alternative approach to reveal dynamic variations (Kadosh & Johnson, 2005; Bruno et al., 2010); however, a number of studies have indicated that the correlation between transcriptome and proteome in eukaryotic cells is weak (Gygi et al., 1999; Yin et al., 2004). Thus, proteome analysis that directly reflects the abundance of proteins has been recognized to be important.

Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) has been broadly used for proteome analysis of *C. albicans*. While 2D-PAGE has been widely used for protein separation for proteome analysis, it is associated with some problems including limitations of separation factors, isoelectric points, and molecular mass. Moreover, the low-throughput associated with 2D-PAGE could become a drawback for time-course analysis that needs many samples to be analyzed. The shotgun approach is a well-known alternative method of proteome analysis, which consists of liquid chromatography and tandem mass spectrometry. Using the method, we could identify a large number of proteins including low-abundant proteins in a high-throughput manner. In addition, construction of a system with ultra-performance of chromatographic separation showed excellent performance (Morisaka et al., 2012a). For example, a system with a long monolithic silica capillary column (350 cm) performed successful identification of 2602 proteins produced in *Escherichia coli* cells in one-shot (Iwasaki et al., 2010). Thus, we applied the LC-MS/MS system for proteome analysis in this study.

This study describes time-course variations of *C. albicans* proteome through recognition of a blood serum. Especially, we focused on the early stage of adaptation to the serum by LC-MS/MS system with a long monolithic silica capillary column. Such a condition could partially reflect the behavior of *C. albicans* during initial systemic candidiasis, that is, a condition could partially reflect the behavior of *C. albicans* during initial systemic candidiasis, in which *C. albicans* recognizes blood stream, adapts to the condition, and metastasizes to organs. Depicting time-course proteome dynamics will lead to profound understandings of *C. albicans* virulence.

**Materials and methods**

**Strains and media**

*Candida albicans* strain SC5314 (American Type Culture Collection) was used for isolation of proteins. *Candida albicans* was maintained in YPD media [1% (w/v) yeast extract, 2% (w/v) glucose, and 2% (w/v) peptone]. For adaptation to a serum, *C. albicans* was grown in yeast nitrogen base (YNB) + fetal bovine serum (FBS) media [0.67% (w/v) YNB without amino acids and ammonium sulfate (Becton, Dickinson and Company, NJ), 1.1 \times 10^{-2}% (w/v) leucine, 5.5 \times 10^{-3}% (w/v) tyrosine, 5.5 \times 10^{-2}% (w/v) tryptophan, and 10% (v/v) FBS (Life Technologies, CA)]. As a control, *C. albicans* was grown in YNB–FBS media [0.67% (w/v) YNB without amino acids and ammonium sulfate (Becton, Dickinson and Company), 1.1 \times 10^{-2}% (w/v) leucine, 5.5 \times 10^{-3}% (w/v) tyrosine, and 5.5 \times 10^{-2}% (w/v) tryptophan]. We chose a bovine serum but not a human serum because of availability, reproducibility, and comparability with previous results.

**Induction of hyphae**

*Candida albicans* was precultivated in 10 mL YPD media for 24 h. The cultured cells were collected by centrifugation at 4000 g for 5 min, and cells (1 g wet weight) were resuspended in 300 mL YNB + FBS media prewarmed at 37 °C. The media were immediately shaken for each time (0, 10, 20, 40, or 60 min). As a control, the *C. albicans* cells were resuspended in 300 mL YNB – FBS media shaking at 37 °C for 60 min. Then, each sample was instantly cooled by ice-cold water and centrifuged at 4000 g. The collected cells were frozen quickly using liquid nitrogen and preserved at −80 °C until required. Morphology of the cells was observed by a phase–contrast microscopy, and the length of hyphae was calculated using IMAGEJ (Abramoff et al., 2004).

**Isolation of proteins**

Collected cells were resuspended with 1 mL lysis buffer [4% (w/v) 3-(3-cholamidepropyl)dimethylammonio-1-propanesulfonate (CHAPS), 1% (w/v) dithiothreitol, 1% (v/v) protease inhibitor cocktail (Sigma-Aldrich, MO), 7 M urea, and 2 M thiourea]. The solution was mixed with an equal volume of 0.5 mm glass beads (Tomy Seiko, Tokyo, Japan). Then, the cells were disrupted mechanically using BeadSmash 12® (Wakenyaku, Kyoto, Japan) at 4 °C, 4000 oscillations per minute for 1 min 10 times. The solution was centrifuged at 3000 g for 5 min, and the supernatant was collected.

**Trypsin digestion**

One mL of the supernatant was mixed with 135 μL of 200 mM triethyl ammonium bicarbonate (TEAB), 165 μL of distilled water, and 15 μL of 200 mM tris-(2-carboxyethyl) phosphine. The solution was incubated at 55 °C for 1 h. After the reaction, 15 μL of 375 mM iodoacetamide was added to the solution and incubated for 30 min. The reactant was mixed with 3 mL of ice-cold acetone and incubated at −20 °C for 2 h to precipitate proteins. Precipitated proteins were resuspended with 100 μL of TEAB and mixed with 2 μL of 1 μg μL⁻¹ sequencing grade modified trypsin (Promega, WI) at 37 °C overnight. Peptides (tryptic digests) were purified using Pierce detergent removal spin columns.
(Thermo Fisher Scientific, MA). Peptide concentration of the purified extracts was measured using Protein Assay Bicinchoninate Kit (Nacalai tesque, Kyoto, Japan).

**LC-MS/MS analysis**

Proteome analyses were performed by a liquid chromatography (Prominence nano flow system (Shimadzu, Kyoto, Japan))/mass spectrometry [LTQ Velos orbitrap mass spectrometer (Thermo Fisher Scientific)] system. Tryptic digests (20 μL) were injected and separated by reversed-phase chromatography using a long monolithic silica capillary column, prepared from a mixture of tetramethoxysilane and methyltrimethoxysilane (200 cm long, 0.1 mm ID) as described in the previous study (Motokawa et al., 2002), at a flow rate of 500 nL min⁻¹. The gradient was provided by changing the mixing ratio of the two eluents; A, 0.1% (v/v) formic acid, and B, acetonitrile containing 0.1% (v/v) formic acid. The gradient was started with 5% B, increased to 45% B for 600 min, further increased to 95% B to wash the column, then returned to the initial condition, and held for reequilibration. The separated analytes were detected on a mass spectrometer with full scan range 350–1500 m/z. For data-dependent acquisition, the method was set to automatically analyze the top 10 most intense ions observed in the MS scan. An ESI voltage of 2.4 kV was applied directly to the LC buffer distal to the chromatography column using a micrOem. The ion transfer tube temperature on the LTQ Velos ion trap was set to 300 °C.

**Protein identification**

All samples were subjected to duplicate LC-MS/MS analysis, and combined spectrometry data were used for protein identification. Protein identification was performed using MASCOT (Matrix Science, London, UK) against the assembly 21 protein database at Candida genome database (CGD) (Arnaud et al., 2005) containing 6198 sequences with a precursor mass tolerance of 20 p.p.m., and strict specificity allowing for up to one missed cleavage. For trypsin digestion, carbamidomethylation of cysteine was set as a fixed modification. The data were then filtered at a q-value ≤ 0.01 corresponding to 1% false discovery rate on a spectral level. Two independent experiments for each sampling point were performed, and proteins identified in both experiments with a MASCOT score (> 100) were accepted and listed in Table S1, Supporting Information. The gene ontology (GO) slim mapper at the CGD database was used to find functional categories of identified proteins (Costanzo et al., 2006).

**Results**

**Induction of hyphae**

*Candida albicans* was incubated in a YPD medium and transferred to a YNB ± FBS. This experiment could be recognized as a very simple model for early systemic candidiasis in which *C. albicans* detects blood stream and adapts its metabolism to the condition. *Candida albicans* cells were collected at each time point (0–60 min). In the experiment, the 0 min cells were defined as the cells cultured in YPD for 24 h but not resuspended in YNB-based media. To confirm the process of serum-dependent hyphal extension (Xu et al., 2008), cell morphology was observed by phase–contrast microscopy. First, we observed morphology of endpoint *C. albicans*. *Candida albicans* showed a yeast form at 0 min and extended hyphae up to 7.23 and 6.95 μm in the sample of 60 min YNB ± FBS and 60 min YNB – FBS, respectively (Fig. 1a). Thus, we thought that we could separate the factor of the serum from that of hyphal development using the YNB – FBS sample. Next, we observed morphological change of *C. albicans* over time-course in the YNB + FBS sample. As a result, we found that *C. albicans* maintained its yeast form at 0–10 min, and started hyphal development at 20 min (Fig. 1b). Quantified by ImageJ, the average hyphal length at each time point (YNB + FBS) was 0, 0.06, 0.61, 3.22, or 7.23 μm at 0, 10, 20, 40, or 60 min, respectively (Fig. 1c). In the previous study, *C. albicans* in contact with Caco-2 cells for 120 min extended hyphae to 36.91 μm (Dalle et al., 2009), and this length was comparatively longer than 7.23 μm in our study. Thus, we speculated that *C. albicans*...
incubated in YNB ± FBS media for 0–60 min could be a good model to investigate dynamics of proteome variations in the early stage.

**Preparation and identification of total proteins**

Collected cells were mechanically disrupted to extract the total protein using a buffer containing protease inhibitors, urea, and CHAPS. The buffer enables to analyze total proteome including membrane proteins through protection against proteases and solubilization of hydrophobic proteins. The total proteins were subjected to trypsin digestion and preparation using a detergent removal spin column. The column removed most of CHAPS and minimized ion suppression effects in LC-MS/MS analysis. In fact, MS/MS analysis of peptides with treatment of the column identified the remarkable number of proteins compared to that of samples without removal of surfactants (data not shown). After preparation of peptides, its concentration was measured by BCA assay (Table 1). There were little differences in protein concentrations between the samples, indicating that the total amount of proteins did not largely alter during hyphal development for a short time.

The prepared peptides were subjected to LC-MS/MS analysis using a long monolithic silica capillary column (Morisaka et al., 2012b), and a lot of proteins were successfully identified by efficient separation. As a result, we identified 1130, 1012, and 701 proteins for the samples of 0, 60 min YNB – FBS, and 60 min YNB + FBS. In addition, 1034, 933, 868 proteins were identified at the time-course samples of 10, 20, and 40 min of YNB + FBS, respectively (Table 1). Descriptions of identified proteins were listed in Table S1. To grasp overall differences, the Venn diagram was depicted between the endpoint samples of 0, 60 min YNB – FBS, and 60 min YNB + FBS. The analysis revealed that 1418 unique proteins were identified in total. Among them, 517 proteins were found in all three samples and identified as a core set of *C. albicans* proteome. In the following section, we subtracted the proteins identified in the YNB – FBS sample from the proteins identified in the YNB + FBS sample. This manipulation enabled to focus on proteins induced by the serum, removing other factors.

**Newly produced proteins during hyphal development**

The term ‘newly produced proteins’ was defined as proteins (1) that were not found in the samples of 60 min YNB – FBS and 0 min and (2) that continued to exist until 60 min YNB + FBS once identified at a certain time point. Such proteins should be recognized as important effectors that positively contributed to maintenance of cell integrity in the serum. Analyzing the data, we revealed that four proteins were first identified at 10 min, and continuously found at the latter time points. In the same way, four proteins were found to be newly produced at each time point, respectively (20, 40, or 60 min) (Fig. 2b). In total, 16 proteins were classified as the newly produced proteins. To understand the functions and the biological processes, these proteins were subjected to the GO slim mapper in CGD (Table 2). As a result, proteins involved in the transport process or the lipid metabolic process occupied a large portion of nascent synthesized proteins. Especially, the trait was noticeable at 10 min. In the time point (10 min), three among four newly produced proteins were recognized as transport-related proteins (Hgt1, orf19.3767, and Atp16) involved in glucose and ATP acquisition. Proteins that respond to stresses, drugs, and chemical stimulus such as Rhr2, Erg6, and orf19.4123 were found in overall times. Interestingly, *C. albicans* started production of an apoptosis-related protein (orf19.713, an ortholog of human PDCD5) at 20 min.

**Disappeared proteins during hyphal development**

The term ‘disappeared proteins’ was defined as proteins (1) that continuously existed from 0 min to a certain time, (2) that were never identified at the latter time points once disappeared at the certain time, and (3) that were not identified in the sample of 60 min YNB – FBS. While these proteins have possible advantage in a nutrition-rich condition, they might have disadvantage in a harsh environment and be recognized as needless proteins. Analyzing the data, 217 proteins were identified as the disappeared proteins (Fig. 2c and Table S2). *Candida albicans* might give a high priority to their very survival in a harsh environment by down-regulation of extra processes and focusing limited energy to the indispensable processes. While the number of proteins identified at the 60 min YNB – FBS sample (1012 proteins) was fewer than that at 0 min (1130 proteins), YNB + FBS caused more radical reduction (701 proteins at 60 min). These results indicated that radical down-regulation of extra processes was serum-dependent.

**Discussion**

In almost previous reports, end points of cells incubated for several hours were used as proteome samples (Pitarch et al., 2002; Hernandez et al., 2004; Martinez-Gomariz et al., 2012b), and a lot of proteins were

<table>
<thead>
<tr>
<th>Sample name</th>
<th>YNB – FBS</th>
<th>YNB + FBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein concentration (mg mL(^{-1}))</td>
<td>3.21</td>
<td>3.80</td>
</tr>
<tr>
<td>The number of identified proteins</td>
<td>1130</td>
<td>1012</td>
</tr>
</tbody>
</table>
et al., 2009). In contrast, this study describes time-course proteomics of *C. albicans* at the early stage. Thus, this study could be recognized as a complementary approach, and reveal the most basic, and the top-priority alteration for adaptation to the serum. In an attempt to carry out time-course proteomics, a high-throughput system is essential to measure many samples. In this study, we used a LC-MS/MS system with a long monolithic silica capillary column and performed high-throughput proteome analysis.

We identified 1130 proteins at 0 min using the LC-MS/MS system, and observed that the number of identified proteins in the both YNB ± FBS samples decreased over the course of time (Table 1). This phenomenon could be partially caused by inoculation of *C. albicans* cells into the fresh media. However, the downward trend was rather predominant for the YNB + FBS sample than the YNB /C0 FBS sample. This observation suggested that *C. albicans* down-regulated various cell processes during adaptation to the serum and hyphal development, and it might be important to utilize limited energy in a hostile environment. In the previous study, cytoplasmic and surface proteins from yeast cells, hyphae, and biofilm of *C. albicans* were separated using 2-dimensional difference gel electrophoresis (2D-DIGE), and differentially abundant proteins were comprehensively analyzed (Martinez-Gomariz et al., 2009). Such an analysis led to a finding that 67 of the 82 significantly affected processes were less abundant in hyphae and only 10 more abundant in hyphae. This result supported our observation that *C. albicans* decreased the variation of its proteome.

Early studies using a 2D-PAGE failed to find dynamic differences between reference maps (Niimi et al., 1996; Choi et al., 2003; Rupp, 2004; Thomas et al., 2006). These results were due to a bias toward abundant proteins, sample complexity, and low dynamic range of 2D-PAGE. In contrast, we used a LC-MS/MS-based technique that can identify proteins with less abundance and difficult biochemical properties. Thus, we succeeded in finding dynamic variations of proteome including disappearance and nascent synthesis of proteins (Fig. 2a). Analyzing the Venn diagram, we identified 251 nascent proteins in the YNB − FBS sample and 20 proteins in the YNB + FBS sample. This result indicated that our experimental approach successfully revealed large fluctuation in *Candida* proteome.

It was important to verify validity of our approach based on previous reports. Thus, we tried to compare our results of identified proteins to a recent representative article. Monteliva et al. (2011) carried out quantitative profiling of *C. albicans* yeast-to-hyphae transition. In the study, they analyzed endpoint cytoplasmic proteins of yeast and hyphal cells using 2D-DIGE and detected 106 protein spots with significant variation in abundance. Among them, 61 spots were excised out and identified to be 46 unique proteins. These proteins were produced by both yeast and hyphal cells, but showed different abundance. In our study, such proteins should be identified in all time-course samples (0–60 min).

As a result of comparative analysis, we found that almost all of the 46 unique proteins were included in all time-course samples, except for three proteins (Rib5, Thi4, and Thi13) (Table S1). Rib5 was identified at 0–40 min, but 60 min. There was a possibility that Rib5 might be produced at endpoint cells, but down-regulated through initial hyphal development in the serum. In contrast, Thi4 and Thi13 were not identified in any samples. Slight differences between hyphae-inducing media could cause the result that only Thi4 and Thi13, involved in a thiamine biosynthetic process, were not produced by *C. albicans*. In fact, it is known that

![Fig. 2 Overview of proteins identified in this study.](https://academic.oup.com/femspd/article-abstract/67/1/67/2367396)
differences in proteome could be derived from the types of media (Rupp, 2004). In any case, we concluded that our approach was validated based on consistency in the representative previous report.

In our study, we identified 16 nascent/newly synthesized proteins (Fig. 2b and Table 2). They might be associated with virulence and could be promising pharmaceutical targets. First, we found that proteins associated with the transport process were enriched in the sample of 10 min. Among four newly produced proteins at 10 min, three proteins were classified as transport-related proteins (Hgt1, orf19.3767, and Atp16) involved in acquisition of energy resources in the nutrition-poor serum. The reason of the enrichment at 10 min (Hgt1, orf19.3767, and Atp16) involved in acquisition of essential elements. The speculation led to a hypothesis that C. albicans probably put priority in time on the acquisition of energy resources in the nutrition-poor serum. C. albicans probably put priority in time on the acquisition of essential elements. The speculation led to a hypothesis that C. albicans might lose its viability without these transport-related proteins. In fact, Hgt1, a high-affinity glucose transporter, is known as an essential factor (Davis et al., 2002). These results support a possibility that the proteins identified at the beginning stage of adaptation to the serum (10 min) in this study might be a cluster of important virulent attributes and possible drug targets. In the previous study, Kusch et al. (2007) showed that dynamic proteome fluctuation could occur in 15 min by quantitation of in vivo protein synthesis after pulse labeling of the proteins with radioactive L-[35S]-methionine. Refinement of our LC-MS/MS approach using quantitative analysis might enable us to extend these results.

Already known virulent attributes were gradually produced by C. albicans after the 20 min time point (Table S1). These proteins included Als3 (20 min), Rbt1 (40 min), and Dur1,2 (60 min). Als3 and Rbt1 are cell surface proteins associated with invasive growth (Berman & Sudbery, 2002), adhesion to host cells (Hoyer et al., 1998; Martin et al., 2011), and induction of endocytosis (Phan et al., 2007). These proteins were thought to be produced to adhere to endothelial cells and establish a foothold for biofilm development. Dur1,2, an urea amidolyase that was needed for urea utilization as nitrogen source, was produced at 60 min. Microorganisms could maintain their life without nitrogen sources for a long time but carbon sources, because they are able to recycle nitrogen by degradation of intracellular proteins (Onodera & Ohsumi, 2005). The appearance of Dur1,2 at the later time point might reflect a relatively low priority of nitrogen acquisition from urea. These proteins, Als3, Rbt1, and Dur1,2 were also identified at the YNB – FBS sample, indicating that they were also necessary in YNB – FBS and not specific for survival in the serum.

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>ORF no.</th>
<th>Description</th>
<th>Candida GO slim process</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 min</td>
<td>orf19.5437</td>
<td>RHR2; glycerol 3-phosphatase</td>
<td>Response to stress; carbohydrate metabolic process; generation of precursor metabolites and energy</td>
</tr>
<tr>
<td></td>
<td>orf19.4527</td>
<td>HGT1; high-affinity glucose transporter</td>
<td>Transport; response to chemical stimulus; response to drug</td>
</tr>
<tr>
<td></td>
<td>orf19.3767</td>
<td>orf19.3767; ortholog(s) have role in protein targeting to vacuole</td>
<td>Transport; protein catabolic process</td>
</tr>
<tr>
<td></td>
<td>orf19.7678</td>
<td>ATP16; subunit of the mitochondrial F1F0 ATP synthase</td>
<td>Transport</td>
</tr>
<tr>
<td>20 min</td>
<td>orf19.713</td>
<td>orf19.713; ortholog(s) have role in apoptosis</td>
<td>Cannot be mapped to a GO slim term</td>
</tr>
<tr>
<td></td>
<td>orf19.3686</td>
<td>orf19.3686; ortholog(s) have role in mitochondrial proton-transporting ATP synthase complex assembly</td>
<td>Organelle organization</td>
</tr>
<tr>
<td></td>
<td>orf19.487</td>
<td>SPT14; putative DNA-binding transcription factor; predicted role in regulation of biogenesis of the cell wall</td>
<td>Lipid metabolic process; protein modification process</td>
</tr>
<tr>
<td></td>
<td>orf19.4825</td>
<td></td>
<td>Biological process unknown</td>
</tr>
<tr>
<td>40 min</td>
<td>orf19.1631</td>
<td>ERG6; Delta(24)-sterol C-methyltransferase</td>
<td>Response to chemical stimulus; lipid metabolic process; response to drug</td>
</tr>
<tr>
<td></td>
<td>orf19.4620</td>
<td>orf19.4620; ortholog(s) have role in protein import into mitochondrial inner membrane</td>
<td>Transport; organelle organization; cellular membrane organization</td>
</tr>
<tr>
<td></td>
<td>orf19.4594</td>
<td>orf19.4594; ortholog(s) have role in endocytosis</td>
<td>Transport; cellular membrane organization; vesicle-mediated transport</td>
</tr>
<tr>
<td></td>
<td>orf19.5342.2</td>
<td>orf19.5342.2; putative THO complex subunit</td>
<td>Biological process unknown</td>
</tr>
<tr>
<td>60 min</td>
<td>orf19.4123</td>
<td>orf19.4123; putative THO complex subunit</td>
<td>Transport; response to stress; RNA metabolic process; DNA metabolic process</td>
</tr>
<tr>
<td></td>
<td>orf19.2439</td>
<td>orf19.2439</td>
<td>Lipid metabolic process</td>
</tr>
<tr>
<td></td>
<td>orf19.6211</td>
<td>orf19.6211</td>
<td>Biological process unknown</td>
</tr>
<tr>
<td></td>
<td>orf19.2009</td>
<td>PEX12; ortholog(s) have role in protein import into peroxisome matrix</td>
<td>Transport; organelle organization</td>
</tr>
</tbody>
</table>
Potentially important processes at each time point

- Energy acquisition (Glucose and ATP)
- Lipid metabolism
- Response to stress
- Apoptosis
- Lipid metabolism
- Response to stress
- Lipid metabolism
- Response to chemical stimuli

Time

0 min 10 min 20 min 40 min 60 min

Adaptation to a scrum

Fig. 3 Schematic representation of the result of time-course proteomics. Candida albicans activates proteins involved in transport of energy resources, responding to YNB + FBS media. Next, C. albicans produces proteins involved in the lipid metabolism process, the stress-response process, and the apoptosis process.

Interestingly, a protein (orf19.713) possibly associated with the apoptosis process was identified at 20–60 min but only 0 min and the YNB – FBS sample. The protein has structural homology to Saccharomyces cerevisiae Ymr074cP and human PDCD5, recently characterized to be an apoptosis-promoting molecule (Wang et al., 2004; Li et al., 2007; Hong et al., 2009). However, it remains to be clarified that orf19.713 directly involves in the apoptosis process in C. albicans. Recent studies have revealed that unicellular organisms cause programmed cell death as multicellular behavior during, for example, biofilm development (Lewis, 2000; Engelberg-Kulka et al., 2006). Unicellular organisms often exhibit altruistic behavior (Frohlich & Madeo, 2000) and cause cell death to preserve cell populations in the face of nutrient insufficiency, ensuring survival of a few cells and propagation of the genome (Jin & Reed, 2002). Sensing the serum, C. albicans elongates hyphae, causes cell–cell aggregation, and develops biofilm. It is an interesting topic whether disruption of orf19.713 lead to alteration of multicellular behavior of C. albicans in systemic infections.

In conclusion, we succeeded in grasping dynamic variations of Candida proteome by the time-course analysis. The summary of our results was illustrated in Fig. 3. These results indicated that C. albicans is a sophisticated logic circuit processing many tasks in the order of priority. It was noticeable that our time-course approach could be complemented to conventional endpoint analyses and reveal the detail process of C. albicans morphological changes. Our approach will be refined using quantitative analysis that virtually ensures existence of dynamic variations of proteome. Such a research could find important virulent factors, leading to development of novel pharmaceuticals.

Acknowledgements

This work was supported by the Japan Society for the Promotion of Science (grant 22-101). The authors declare that they have no conflict of interest.

References


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Proteins identified in our study.
Table S2. Disappeared proteins during adaptation to the serum.

Please note: Wiley-Blackwell is not responsible for the content or functionality of any supporting materials supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.