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The Evolutionary Rewiring of Ubiquitination Targets Has Reprogrammed the Regulation of Carbon Assimilation in the Pathogenic Yeast *Candida albicans*

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**ABSTRACT** Microbes must assimilate carbon to grow and colonize their niches. Transcript profiling has suggested that *Candida albicans*, a major pathogen of humans, regulates its carbon assimilation in an analogous fashion to the model yeast *Saccharomyces cerevisiae*, repressing metabolic pathways required for the use of alternative nonpreferred carbon sources when sugars are available. However, we show that there is significant dislocation between the proteome and transcriptome in *C. albicans*. Glucose triggers the degradation of the *ICL1* and *PCK1* transcripts in *C. albicans*, yet isocitrate lyase (Icl1) and phosphoenolpyruvate carboxykinase (Pck1) are stable and are retained. Indeed, numerous enzymes required for the assimilation of carboxylic and fatty acids are not degraded in response to glucose. However, when expressed in *C. albicans*, *S. cerevisiae* Icl1 (ScIcl1) is subjected to glucose-accelerated degradation, indicating that like *S. cerevisiae*, this pathogen has the molecular apparatus required to execute ubiquitin-dependent catabolite inactivation. *C. albicans* Icl1 (CaIcl1) lacks analogous ubiquitination sites and is stable under these conditions, but the addition of a ubiquitination site programs glucose-accelerated degradation of CaIcl1. Also, catabolite inactivation is slowed in *C. albicans ubi4* cells. Ubiquitination sites are present in gluconeogenic and glyoxylate cycle enzymes from *S. cerevisiae* but absent from their *C. albicans* homologues. We conclude that evolutionary rewiring of ubiquitination targets has meant that following glucose exposure, *C. albicans* retains key metabolic functions, allowing it to continue to assimilate alternative carbon sources. This metabolic flexibility may be critical during infection, facilitating the rapid colonization of dynamic host niches containing complex arrays of nutrients.

**IMPORTANCE** Pathogenic microbes must assimilate a range of carbon sources to grow and colonize their hosts. Current views about carbon assimilation in the pathogenic yeast *Candida albicans* are strongly influenced by the *Saccharomyces cerevisiae* paradigm in which cells faced with choices of nutrients first use energetically favorable sugars, degrading enzymes required for the assimilation of less favorable alternative carbon sources. We show that this is not the case in *C. albicans* because there has been significant evolutionary rewiring of the molecular signals that promote enzyme degradation in response to glucose. As a result, this major pathogen of humans retains enzymes required for the utilization of physiologically relevant carbon sources such as lactic acid and fatty acids, allowing it to continue to use these host nutrients even when glucose is available. This phenomenon probably enhances efficient colonization of host niches where sugars are only transiently available.

Carbon assimilation is fundamentally important for all organisms. When faced with choices of carbon source, microbes often assimilate preferred carbon sources to support the first phase of growth and then, having exhausted these carbon sources, turn to alternative energetically less favorable carbon sources to drive subsequent phases of diauxic growth. This selective carbon utilization is reflected in the differential regulation of genes and enzymes that support the uptake and catabolism of specific carbon sources. The *Escherichia coli lac* operon provides a classic example of this, mediating lactose utilization only after the preferred carbon source, glucose, is exhausted (1, 2). In *Saccharomyces cerevisiae*, glucose limits the assimilation of alternative carbon sources and represses respiration under aerobic conditions, promoting fermentative metabolism (the Crabtree effect [3, 4]).

*S. cerevisiae* is exquisitely sensitive to sugars: even glucose concentrations as low as 0.01% trigger the major redirection of cellular resources (5, 6). Glucose exerts its dramatic effects upon *S. cerevisiae* physiology via signaling pathways that include the glucose repression (Snf1 AMP kinase) pathway, cyclic AMP (cAMP)–protein kinase A signaling, and the sugar receptor repressor (Snf3–Rgt2) pathway (for reviews, see references 7 to 12). The cAMP-protein kinase A pathway activates ribosome biogenesis
and downregulates stress responses in response to glucose (13–16). The sugar receptor repressor pathway modulates the expression of hexose transporters (9, 17, 18). Meanwhile, the glucose repression pathway represses the transcription of genes involved in the assimilation of alternative carbon sources, such as galactose, ethanol, and fatty acids (7, 8).

These sugar signaling mechanisms comprise an interlinked network rather than parallel signaling pathways (8, 10, 12, 18, 19). Furthermore, glucose regulation is imposed at multiple levels in S. cerevisiae. They include transcriptional (7–9), posttranscriptional (5, 6, 20, 21), translational (22), and posttranslational mechanisms (23–28). For example, glucose represses the transcription of genes encoding the gluconeogenic enzymes fructose 1,6-bisphosphatase (FBP1) and phosphoenolpyruvate carboxykinase (PCK1) (29, 30) and triggers the accelerated degradation of the FBP1 and PCK1 mRNAs (5, 20). Furthermore, glucose triggers the phosphorylation and inactivation of fructose 1,6-bisphosphatase (FBP1) as well as its proteolytic degradation (31). The accelerated degradation of Fbp1 is mediated by vacuolar and ubiquitin-dependent mechanisms (28). Following glucose addition, Fbp1 is ubiquitinated and degraded via the proteasome (25, 28, 32). Like Fbp1, the glyoxylate cycle enzyme, isocitrate lyase (Icl1) is also subject to catabolite inactivation in S. cerevisiae (24).

This tight control of central carbon metabolism is thought to reflect the evolution of this model yeast under conditions of “feast or famine” and to enhance the competitiveness of S. cerevisiae in sugar-rich niches containing complex microflora (9).

S. cerevisiae is often viewed as a paradigm for other yeasts (33). However, yeast species inhabit diverse niches and have evolved under contrasting selective pressures leading to differing strategies of carbon utilization (34). For example, the major systemic fungal pathogen of humans, Candida albicans, inhabits niches that contain complex mixtures of carbon sources. During commensalism and mucosal infection, C. albicans colonizes the oral cavity and the gastrointestinal and urogenital tract, and during systemic infection, this pathogen can thrive in the bloodstream and most internal organs (35, 36). Few of these niches are rich in sugar. Blood glucose levels range from 4 to 7 mM (0.07 to 0.13%), whereas concentrations of about 111 mM (2%) are often used to impose glucose repression in in vitro experiments. Many niches are rich in alternative carbon sources, such as lactate, fatty acids, and amino acids. For example, lactic acid is found in ingested foods, is produced by host metabolic activity and by lactic acid bacteria in the gastrointestinal and urogenital tracts (37), and is essential for the proliferation of Candida glabrata in the intestinal tract (38). Also, glyoxylate cycle and fatty acid β-oxidation genes are expressed in the host and are required for the full virulence of C. albicans during systemic infections (39–42).

Although C. albicans occupies contrasting niches from S. cerevisiae, analogous sugar signaling pathways are thought to exist in these yeasts (33). Although there has been considerable rewiring of the regulatory circuitry that controls carbon metabolism (43–45), microarray experiments have revealed that C. albicans genes involved in the assimilation of alternative carbon sources are exquisitely sensitive to low concentrations of glucose (46, 47), like their orthologs in S. cerevisiae (6). However, unlike S. cerevisiae, C. albicans continues to respire in the presence of glucose, leading to its classification as a Crabtree-negative yeast (48).

These observations create an interesting conundrum relating to the carbon assimilation and pathogenicity of C. albicans: how can this yeast rapidly colonize niches that contain small amounts of glucose if many of the metabolic genes required for efficient growth in these niches are repressed by glucose? Could glucose regulation be relaxed at a posttranscriptional level in C. albicans, thereby facilitating simultaneous assimilation of sugars and alternative carbon sources in vivo? We have addressed these questions first by performing proteomic screens to identify proteins that are regulated in response to physiologically relevant carbon sources, revealing that gluconeogenic and glyoxylate cycle enzymes remain at high levels hours after glucose exposure. We then showed that C. albicans Pck1 and Icl1 are not destabilized by glucose, in contrast to S. cerevisiae Pck1 and Icl1, even though C. albicans has retained the molecular apparatus to program the accelerated, ubiquitin-mediated degradation of target proteins following glucose exposure. C. albicans Icl1 escapes degradation following glucose addition, because this enzyme lacks key ubiquitination sites required to target it for accelerated degradation. Our data show that there has been significant posttranscriptional rewiring during the evolution of this pathogen, thereby allowing C. albicans to continue to assimilate alternative carbon sources in the presence of glucose.

RESULTS
Carbon source has a major impact on the C. albicans proteome. Microarray studies have shown that the C. albicans transcriptome is exquisitely sensitive to glucose and that genes involved in the assimilation of alternative carbon sources are subject to glucose repression (46, 47). Our first aim was to establish whether this transcriptional regulation was reflected in the C. albicans proteome. To test this, we grew prototrophic C. albicans NYG152 cells (see Table S1 in the supplemental material) for 20 h in minimal medium containing glucose, lactate, oleate, or amino acids as the sole carbon source, harvested them in mid-exponential phase, prepared protein extracts, and subjected them to two-dimensional (2D) gel electrophoresis (Materials and Methods). Principal component analysis confirmed the reproducibility of the 2D gels from the independent replicate experiments and showed that the carbon source had a significant impact upon the C. albicans proteome (see Fig. S1A in the supplemental material). Proteins that displayed statistically significant changes in level on the basis of three independent experiments (Fig. 1A) were identified by tryptic digestion and matrix-assisted laser desorption ionization–time of flight (MALDI-ToF) mass spectrometry. Positive identifications were obtained for 206 2D gel features, representing 152 different C. albicans proteins, some distinct features representing isoforms of the same protein. The list of C. albicans proteins identified is presented in Table S2 and submitted to the PRIDE (proteomics identifications database) proteomic data repository (http://www.ebi.ac.uk/pride/) (accession numbers 3186 to 3192).

The network of proteins that displayed statistically significant changes in response to carbon source mainly comprised metabolic enzymes (Fig. 1B). Significant overlap was observed between the sets of proteins that were regulated in response to growth on lactate, oleate, or amino acids compared to the glucose condition. During growth on these organic acids, glycolytic enzymes were downregulated, and enzymes involved in gluconeogenesis, the glyoxylate cycle, the tricarboxylic acid (TCA) cycle and pathways involved in the assimilation of alternative carbon sources were
upregulated (Fig. 1B and 2B; see Fig. S2 in the supplemental material). For example, gluconeogenic (Pck1 and Fbp1) and tricarboxylic acid cycle enzymes (Aco1, Kgd1, and Mdh11) displayed reduced levels, and the glycolysis-specific enzyme pyruvate kinase (Cdc19) was at elevated levels (see Fig. S2 and Table S2 in the supplemental material) during growth on glucose compared to lactate-, oleic acid-, and amino acid-grown cells. During growth on oleate, fatty acid β-oxidation (Faa21/23/24, Fox2, Pot11, Pox4, and Tes1), glyoxylate cycle (Icl1), and additional TCA cycle enzymes (Cit1 and Fum11) were present at elevated levels compared to the levels in glucose-grown cells (Fig. 2; Table S2). Enzymes on many amino acid biosynthetic pathways were downregulated during growth on amino acids (Arg5,6, Bat21, Cpa2, His1, Hom6, Ilv5, Lys12, and Met6/14/15), and this was also the case during

**FIG 1** Impact of carbon source on the *C. albicans* proteome. (A) Replicate 2D gels showing that Pox4 is more abundant during growth on oleic acid than on glucose and that Pox4 is retained after 2 h of exposure to glucose. (B) Network of *C. albicans* proteins regulated in response to carbon source. Nodes are connected by edges to the one or more conditions under which it was identified: upregulated ≥2-fold relative to growth on glucose (upward arrowhead); downregulated ≥2-fold (downward arrowhead). Functions that were differentially regulated on different carbon sources (rounded rectangle) have color-coded connecting lines: green for upregulated and red for downregulated. Functions regulated under all three conditions lie in three blocks in the center of the network interactions.
growth on lactate (Arg5,6, Bat21, His1, and Met6) or oleic acid (Arg5,6, Bat21, His1, and Met6) (Fig. 1; Table S2). These data largely confirmed expectations based on our understanding of metabolism in other yeasts and the limited experimental data on C. albicans metabolic regulation (35, 49). The observed changes in Pck1 and Icl1 levels were validated by Western blot analysis of these proteins following Myc tagging in C. albicans (Fig. 2A). Note that Icl1 levels were below the limits of detection in the lactate proteome (Table S2).

The levels of other types of protein were modulated in response to carbon source. These proteins included proteins involved in growth and cell polarity (Arp2, Cap2, Pfy1, Cof1, Crn1, and Rbp1), nuclear transport (Ntf2), DNA repair (Rad23), and protein folding (Pdi1 and Cyp51) (see Table S2 in the supplemental material). Interestingly, proteins involved in drug resistance (Pdr13 and Erg13) were also affected by carbon source, which is consistent with the recent finding that carbon source affects the antifungal drug resistance of C. albicans (50). Also, a number of stress functions were affected by carbon source (Table S2). The levels of Hsp70 family members (Hsp70/Ssa4, Ssa1, and Kar2) were differentially regulated in response to carbon source. The Trx1 thioredoxin was also dramatically upregulated during growth on lactate (Arg5,6, Bat21, His1, and Met6) or oleic acid (Arg5,6, Bat21, His1, and Met6) (Fig. 1; Table S2). These data largely confirmed expectations based on our understanding of metabolism in other yeasts and the limited experimental data on C. albicans metabolic regulation (35, 49). The observed changes in Pck1 and Icl1 levels were validated by Western blot analysis of these proteins following Myc tagging in C. albicans (Fig. 2A). Note that Icl1 levels were below the limits of detection in the lactate proteome (Table S2).

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growth on lactate, oleic acid, or amino acids, while the glutaredoxin-like protein Grx3 was downregulated on lactate and oleic acid. The levels of catalase glutathione peroxidize (Gpx1) were also upregulated on lactate. These data were consistent with the observation that host carbon source affects stress resistance in C. albicans (50).

Transcript profiling data are available for the effects of glucose on lactate-grown C. albicans cells (47). We found the correlation between these transcript profiling data and our proteomic data for lactate- and glucose-grown cells to be modest at best (correlation coefficient = 0.25) (Fig. 3A). No doubt differential protein stabilities, alterations in posttranslational modifications, and the existence of multiple 2D gel features for some proteins contributed to this. Nevertheless, this was consistent with other comparisons of transcriptomic and proteomic data sets, which vary considerably in their degree of correlation (51–54).

Differential effects of glucose on the C. albicans proteome in the short term and longer term. Having tested the impact on the C. albicans proteome of growth on different carbon sources, we examined the effects of glucose addition to cells growing on alter-
These data recapitulate earlier observations (23, 24) and confirm that these gluconeogenic and glyoxylate cycle enzymes are subject to catabolite inactivation in *S. cerevisiae*. Furthermore, the ICL1 and PCK1 transcripts were rapidly degraded in *S. cerevisiae* following glucose addition (Fig. 4C), confirming earlier reports to this effect (5, 6, 20). Therefore, *C. albicans* differs significantly from *S. cerevisiae* in that Icl1 and Pck1 are not subject to catabolite inactivation in response to glucose in the pathogenic yeast.

*C. albicans* has retained the apparatus for catabolite inactivation. In principle, the lack of glucose-accelerated degradation of Icl1 and Pck1 in *C. albicans* could have been due to the evolutionary loss of the catabolite inactivation apparatus in this pathogen. To test this, we asked whether *S. cerevisiae* Icl1 is subject to glucose-accelerated degradation in *C. albicans*.

*C. albicans* strains were constructed in which the *S. cerevisiae* ICL1 (ScICL1) open reading frame (ORF) was expressed from the native ICL1 locus. The functionality of three-Myc (Myc3)-tagged *S. cerevisiae* Icl1 (ScIcl1) in *C. albicans* was first confirmed in DSCO2 cells (icll1/ScICL1) (see Table S1 in the supplemental material), the ScICL1-Myc3 gene suppressing the growth defect of *C. albicans* icll1 cells on lactate, oleic acid, pyruvate, and acetate as the sole carbon source (Fig. 5A). The stability of the Myc3-tagged ScIcl1 was then examined in lactate-grown *C. albicans* DSCO1 cells (ICL1/ScICL1-Myc3). ScIcl1 was stable in *C. albicans* in the absence of glucose but was rapidly degraded following glucose addition (Fig. 5B). These observations were reproducible, and analogous observations were made in oleic acid-grown *C. albicans* DSCO1 cells (see Fig. S4 in the supplemental material). We conclude that ScIcl1 is subject to glucose-accelerated degradation in *C. albicans*, and hence that during evolution *C. albicans* has retained the apparatus for catabolite inactivation.

In this case, has Calcl1 lost the signal that triggers glucose-accelerated degradation? The stability of the Calcl1-Myc3 protein was assayed in the *S. cerevisiae* strain D54-Y40 in which the Myc3-tagged Calcl1 open reading frame was expressed from the ScICL1 promoter at the native ICL1 locus (Fig. 5A). The stability of the Calcl1-Myc3 protein was stable in lactate-grown DS4-Y40 cells and was not destabilized by glucose addition relative to internal loading controls (Fig. 5B). Once again, these observations were reproducible, and analogous observations were made for oleic acid-grown *S. cerevisiae* DS4-Y40 cells (see Fig. S4 in the supplemental material). Therefore, Calcl1 appears to have lost the signal that triggers glucose-accelerated degradation.

Ubiquitination contributes to glucose-accelerated protein degradation in *C. albicans*. Ubiquitination contributes to glucose-mediated protein destabilization in *S. cerevisiae* (28, 32). Therefore, we tested whether ubiquitination contributes to glucose-accelerated degradation of ScIcl1 in *C. albicans*. The ScICL1-Myc3 cassette was integrated at the native ICL1 locus in a *C. albicans* ubi4 mutant that lacks polyubiquitin. Ubiquitination is significantly reduced in this ubi4 mutant, residual ubiquitin being expressed only from the UBI13 locus (55). The stability of the ScIcl1-Myc3 protein in these *C. albicans* DSCO3 cells (ubi4/ubi4 ICL1/ScICL1-Myc3) (see Table S1 in the supplemental material) was compared to ScIcl1-Myc3 stability in DSCO1 cells (UBI4/UBI4 ICL1/ScICL1-Myc3) and the stability of the Calcl1-Myc3 protein in CA1395 cells (UBI4/UBI4 ICL1/ICL1-Myc3). ScIcl1-Myc3 and Calcl1-Myc3 were stable in these strains during growth.
on lactate in the absence of glucose (not shown). Once again, ScICL1-Myc$_3$ was destabilized following glucose addition to wild-type DSCO1 cells (Fig. 7). However, ScICL1-Myc$_3$ was partially stabilized in the polyubiquitin mutant, suggesting that glucose-accelerated degradation of ScICL1 in C. albicans is at least partially dependent upon ubiquitination. This observation was reproducible in independent experiments, and furthermore, similar observations were made when the same strains were grown on oleic acid rather than lactate (see Fig. S5 in the supplemental material). The residual ScICL1-Myc$_3$ destabilization observed in lactate- or oleic acid-grown $ubi4$ cells could have been due either to the involvement of a second degradation pathway in this response (28) or to the residual ubiquitination that is observed in the C. albicans polyubiquitin mutant (55). We conclude that ubiquitination contributes to glucose-accelerated protein degradation in C. albicans.

FIG 5 ScICL1 is functional in C. albicans and rapidly degraded in response to glucose. (A) Expression of ScICL1 in C. albicans suppresses the carbon source conditional phenotypes of an icl1 mutant: ICL1, C. albicans CAS10 (ICL1/icl1) (see Table S1 in the supplemental material); icl1, C. albicans CA517 (icl1/icl1); ScICL1, C. albicans DSCO1 (ScICL1/icl1). (B) Impact of glucose on the levels of the ScICL1-Myc protein expressed in lactate-grown C. albicans DSCO1 cells relative to the abundance at time zero. (Top) Western blotting of ScICL1-Myc levels. (Bottom) Quantification of ScICL1-Myc levels expressed as a percentage of the abundance at time zero (100%): closed symbols, plus glucose; open symbols, control cells lacking glucose. Similar data were obtained from two independent replicate experiments.

FIG 6 CalCl1 is stable in S. cerevisiae following the addition of glucose. CalICL1-Myc$_3$ was expressed in S. cerevisiae DS4-Y40 cells (see Table S1 in the supplemental material) grown on lactate, and CalICL1-Myc$_3$ protein levels were assayed by Western blotting after glucose addition. CalICL1-Myc$_3$ protein levels are expressed as a percentage of the abundance at time zero (which was set at 100%): closed symbols, plus glucose; open symbols, control cells lacking glucose. Similar data were obtained from two independent replicate experiments.

FIG 7 Inactivation of polyubiquitin inhibits glucose-accelerated ScICL1 degradation in C. albicans. ScICL1-Myc$_3$ was expressed in C. albicans DSCO3 ($ubi4/ubi4$) (see Table S1 in the supplemental material) and DSCO1 (wild type [wt]) (UBI4/UBI4) cells grown on lactate, and then ScICL1-Myc$_3$ protein levels were assayed after the addition of glucose by Western blotting. For a control, CalICL1-Myc$_3$ levels were assayed in C. albicans DSCO3 ($ubi4/ubi4$) cells grown on lactate and following glucose addition. ScICL1-Myc$_3$ and CalICL1-Myc$_3$ levels are expressed as a percentage of their abundance at time zero (which was set at 100%). Similar data were obtained from two independent replicate experiments.
C. albicans Icl1 lacks ubiquitination sites that trigger glucose-accelerated protein degradation. Why does Calcl1 escape glucose-accelerated ubiquitin-mediated protein degradation? We screened for consensus ubiquitination target sites in Calcl1 and Sccl1 using UbpPred (predictor of protein ubiquitination sites) (http://www.ubpred.org/index.html) (56). The UbpPred software, which was trained on 272 ubiquitination sites in S. cerevisiae, predicts ubiquitination sites based on numerous properties of the target amino acid sequence, including the net and total charge, aromatic content, charge/hydrophobicity ratio, sequence complexity, flexibility, amphipathic moment, and intrinsic disorder (56). The Sccl1 and Calcl1 proteins display strong intrinsic disorder (56). The Sccl1 and Calcl1 proteins display strong amino acid sequence similarity (78% similarity and 67% identity). Yet while Sccl1 contains two strong consensus ubiquitination sites at residues 158 and 551 according to UbpPred, Calcl1 carries no high-confidence ubiquitination targets (Fig. 8A). Therefore, we reasoned that the lack of such a ubiquitination site protected Calcl1 from glucose-accelerated degradation.

To test this, we fused the carboxy-terminal ubiquitination site from Sccl1 onto the carboxy terminus of Calcl1 along with a Myc3 epitope tag and then compared the stability of this Calcl1-Ubi-Myc3 protein with control Calcl1-Myc3 and Sccl1-Myc3 proteins in C. albicans. The stabilities of these proteins were first measured in mid-exponential C. albicans CA1395 (ICL1/ICL1-Myc3), DSCO1 (ICL1/ScICL1-Myc3), and DSCO4 (ICL1/ICL1-Ubi-Myc3) cells grown on lactate. All of the proteins were stable under these conditions (not shown). Once again, Sccl1-Myc3 was destabilized following glucose addition, whereas Calcl1-Myc3 remained stable (Fig. 8B). Interestingly, the Calcl1-Ubi-Myc3 protein was reproducibly destabilized following glucose addition. Furthermore, analogous observations were made in C. albicans CA1395, DSCO1, and DSCO4 cells grown on oleic acid (see Fig. S6 in the supplemental material). Therefore, the addition of a ubiquitination site was sufficient to trigger glucose-accelerated degradation of the normally stable Calcl1-Myc3 protein in C. albicans. These observations reinforced the view that C. albicans has retained the apparatus for glucose-accelerated protein degradation and suggested that the lack of an appropriate ubiquitination site in Calcl1 prevents this protein from entering this degradation pathway.

C. albicans continues to assimilate alternative carbon sources following glucose exposure. Is this evolutionary rewiring of ubiquitination targets between C. albicans and S. cerevisiae reflected in the metabolic activities of these yeasts? To test this, we compared [14C]lactate assimilation by mid-exponential C. albicans RM1000 and S. cerevisiae W303-1B cells that were grown on lactate in the presence and absence of glucose (Fig. 9). As expected, the presence of glucose inhibited the assimilation of [14C]lactate by S. cerevisiae. However, glucose did not inhibit [14C]lactate by C. albicans over the 4-h period examined, which was consistent with the retention by this pathogen of glyoxylate cycle and glucogenogenic enzymes after glucose addition. These reproducible observations were reinforced by analogous data on oleic acid assimilation by C. albicans and S. cerevisiae cells. Glucose inhibited [3H]oleic acid assimilation in S. cerevisiae W303-1B cells, but not in C. albicans RM1000 cells (see Fig. S7 in the supplemental material). These data indicate that, unlike S. cerevisiae, C. albicans continues to assimilate alternative carbon sources after exposure to glucose.

FIG 8 Addition of a consensus ubiquitin site stimulates glucose-accelerated degradation of Calcl1 in C. albicans. (A) Schematic representation illustrating the existence of high-confidence ubiquitination sites in Sccl1 and the lack of such sites in Calcl1 as predicted by UbpPred (http://www.ubpred.org/index.html) (56). (B) The carboxy-terminal ubiquitination site from Sccl1 was fused to Calcl1 to create Calcl1-Ubi-Myc3 in C. albicans DSCO4 (see Table S1 in the supplemental material). These cells were grown on lactate, and the levels of Calcl1-Ubi-Myc3 were assayed by Western blotting after the addition of glucose. For controls, the stabilities of Calcl1-Myc (CA1395) and Sccl1-Myc (DSCO1) (gray squares) in C. albicans were compared under equivalent conditions. Calcl1-Ubi-Myc, Sccl1-Myc, and Calcl1-Myc levels are expressed as a percentage of their abundance at time zero (which was set at 100%). Similar data were obtained from two independent replicate experiments.

FIG 9 Glucose does not inhibit lactate assimilation by C. albicans. Exponential C. albicans RM1000 and S. cerevisiae W303-1B cells (see Table S1 in the supplemental material) grown on lactate were suspended in fresh medium containing [14C]lactate and 2% glucose (gray bars) or with no glucose (black bars). The assimilation of radiolabeled lactate by cells was assayed at various times thereafter (Materials and Methods). Similar data were obtained from two independent replicate experiments.
DISCUSSION

The prevailing view is that C. albicans fits the S. cerevisiae paradigm with regard to the impact of glucose upon central carbon metabolism. This influences current thinking about nutrient adaptation during infection and host-fungus interactions (46, 47, 57). However, this view does not resonate well with the evolution of these yeasts in contrasting niches that differ significantly with regard to carbon source availability (40) and with an early report that C. albicans is a Crabtree-negative yeast (48). We now show that, despite the similar effects that glucose exerts on the C. albicans and S. cerevisiae transcriptomes (46, 47), these yeasts display fundamental differences in the effects of glucose on central metabolic functions and carbon assimilation. The levels of many gluconeogenic, glyoxylate cycle, and fatty acid β-oxidation enzymes do decline in C. albicans after protracted growth on glucose, and the changes in the proteomic network (Fig. 1) reflect those observed previously during a comprehensive comparison of exponential- and stationary-phase C. albicans cells (58). However, these enzymes are stable during short-term exposure to glucose (Fig. 2 and 4; see Table S2 in the supplemental material), and this allows C. albicans cells to continue to assimilate alternative carbon sources even in the presence of glucose, unlike S. cerevisiae (Fig. 9).

Our analyses of Icl1 and Pck1 turnover have suggested that enzymes involved in the assimilation of alternative carbon sources are retained in C. albicans following glucose addition because these enzymes remain stable while their transcripts are degraded (Fig. 4). We show that this contrasts with S. cerevisiae cells that degrade these enzymes following glucose addition and their transcripts (Fig. 4). It is conceivable that carboxy-terminal tagging of Calcl1 and ScIcl1 affected their localization and stability. Nevertheless, our data were consistent with the study of López-Boado et al. (24) who reported that Icl1 is subject to catabolite inactivation in S. cerevisiae. We then showed that, although Calcl1 and CaPck1 are not subjected to glucose-accelerated protein degradation in C. albicans, this pathogen has retained the molecular apparatus to execute this function, as ScIcl1 is degraded following glucose addition when expressed as a functional enzyme in C. albicans (Fig. 5). Rather, Calcl1 lacks the signal that triggers glucose-accelerated protein degradation in S. cerevisiae (Fig. 6).

Ubiquitin-dependent mechanisms contribute to the catabolite inactivation of Fbp1 in S. cerevisiae (28), and protein ubiquitination is known to be important for nutrient adaptation as well as growth, morphogenesis, and stress responses in C. albicans (55, 59–64). Therefore, we reasoned that ubiquitination might also contribute to glucose-accelerated protein degradation in C. albicans and that Calcl1 might lack critical ubiquitination sites required to target this protein for catabolite inactivation. Three observations support this hypothesis. First, the inactivation of polyubiquitin (Ubi4) lowered the rate of ScIcl1 degradation in C. albicans (Fig. 7). ScIcl1 degradation was not completely blocked because the UBI3 gene would provide residual ubiquitin in C. albicans ubi4 cells (55, 65). Second, in silico analyses of the Scl1 and Calcl1 sequences revealed that while Scl1 contains two high-confidence ubiquitination sites, Calcl1 contains none (Fig. 8A) despite these proteins displaying a high degree of overall sequence identity (67%). Third, the addition of a carboxy-terminal ubiquitination site to the Scl1 protein was sufficient to program this protein for glucose-accelerated protein degradation in C. albicans (Fig. 8). Therefore, during the evolution of C. albicans, this pathogen has retained the molecular apparatus that mediates glucose-accelerated protein degradation, and Calcl1 has evolved to escape this process.

Our proteomic analyses indicate that other C. albicans enzymes involved in the assimilation of alternative carbon sources lack ubiquitination sites are retained following glucose exposure (Fig. 3B; see Table S1 in the supplemental material). Furthermore, our in silico comparisons have suggested that enzymes specific for gluconeogenesis and the glyoxylate cycle lack the requisite ubiquitination sites required for glucose-accelerated protein degradation. S. cerevisiae malate synthase (ScMls1) contains three low-confidence ubiquitination sites according to UbPred (http://www.ubpred.org/index.html) (56), whereas CaMls1 contains no putative ubiquitination sites. UbPred also predicts that both ScFbp1 and ScPck1 contain high-confidence ubiquitination sites, whereas CaFbp1 and CaPck1 do not. This was consistent with our observation that, following glucose addition, ScPck1 is degraded in S. cerevisiae, but CaPck1 is not degraded in C. albicans (Fig. 4). Meanwhile, our previous work has indicated that a number of glycolytic enzymes are ubiquitinated in C. albicans (55). Therefore, significant evolutionary rewiring of ubiquitination targets appears to have occurred in this pathogen, allowing it to assimilate alternative carbon sources in the presence of glucose, unlike S. cerevisiae (Fig. 9).

These observations have several important implications for our understanding of C. albicans as a pathogen. First, our data indicate that under some conditions there is significant dislocation between the transcriptome and proteome. Microarray studies have contributed significantly to our understanding of C. albicans pathobiology (43, 44, 46, 66–68), and it is frequently presumed that changes in the transcriptome are reflected in corresponding changes in the C. albicans proteome and physiology. In some cases, there is a good correlation between the transcriptome and proteome, for example with regard to the induction of glycolytic enzymes in response to glucose (Fig. 3) and during amino acid starvation (54). However, this is not always the case (69), and this study demonstrates clearly that caution is required in making assumptions about the proteome based on the transcriptome. Indeed, this explains the conundrum as to why C. albicans is a Crabtree-negative yeast (48) even though its transcriptome is exquisitely sensitive to glucose (47). Thankfully, many studies provide experimental confirmation of working hypotheses on the basis of their microarray observations.

The second important implication relates to fungal nutrient assimilation during colonization and disease progression. C. albicans occupies dynamic host niches that contain complex mixtures of carbon sources that change over time through a combination of host and fungal metabolic activities. Many niches contain minimal sugar concentrations (e.g., the urogenital tract), while in others, C. albicans is transiently exposed to sugars (e.g., the oral cavity and gastrointestinal tract). This opportunistic pathogen probably evolved in niches such as these. Also, the evidence suggests that a proportion of C. albicans cells that infect internal organs actively assimilate alternative carbon sources rather than sugars (40, 70). Furthermore, lactate assimilation is essential for the proliferation of C. glabrata in the intestine (38). Our data now suggest that transient exposure to sugar does not prejudice the assimilation of alternative carbon sources and, as a result, the growth of the fungus in these niches. This explains why both gluconeogenic and glycolytic functions can be expressed in C. albicans cells infecting...
the kidney (40) and why mutations that block gluconeogenesis or the glyoxylate cycle partially attenuate the virulence of *C. albicans* (39–42). Clearly, *C. albicans* does not conform to the *S. cerevisiae* paradigm of glucose regulation, and this is important for the pathogenicity of this yeast.

**MATERIALS AND METHODS**

**Strains and growth conditions.** *C. albicans* and *S. cerevisiae* strains (see Table S1 in the supplemental material) were grown at 30°C in minimal medium (0.67% yeast nitrogen base) containing glucose (2%) (SD), lactic acid (2%), oleic acid (0.2%), or mixed amino acids (2%) as the sole carbon source (71).

**Strain construction.** *C. albicans* strains expressing Icl1-Myc3 were made by PCR amplification of a Myc3-URA3 cassette and transforming these products into *C. albicans* strains RM1000 (see Table S1 in the supplemental material). Insertion at the correct locus was confirmed by diagnostic PCR, and expression of Icl1-RM1000 (see Table S1 in the supplemental material). Controls were performed for all the following strain constructions.

To replace a CaICL1 allele in *C. albicans* with a Myc3-tagged ScICL1 open reading frame (ORF) from *S. cerevisiae*, the ScICL1 locus was first Myc3 tagged by PCR amplifying the Myc3-URA3 cassette with primers DS_3F (F stands for forward) and DS_3R (R stands for reverse) (see Table S3 in the supplemental material) and transforming this cassette into *S. cerevisiae* BY4743 to create strain DS3-Y30 (Table S1). The new ScICL1-Myc3-URA3 locus was then PCR amplified using primers DS_5F and DS_5R, and this cassette was then transformed into *C. albicans* strains RM1000 to create strain DSCO1 (CaICL1/CaICL1p-ScICL1-MYC3-URA3) (Table S1) and also transformed into *C. albicans* CA510 to create strain DSCO1 (CaICL1/CaICL1p-ScICL1-MYC3-URA3) (Table S1). To introduce the CaICL1-ScICL1-Myc3-URA3 allele into the *C. albicans* DSCO cassette and transform strain DSCO3 (ubl4/ubl4 background (55), the CaICL1-ScICL1-Myc3-URA3 cassette was PCR amplified using the DS_5F and DS_5R primers (see Table S3 in the supplemental material) and transformed into *C. albicans* DSCO to create strain DSCO3 (ubl4/ubl4 CaICL1/CaICL1p-ScICL1-Myc3-URA3) (Table S1).

*C. albicans* DSCO4 expresses CaICL1 with the carboxy-terminal ubiquitination site from ScICL1 at its carboxy terminus (CaICL1/CaICL1p-UBI-Myc3-URA3) (see Table S1 in the supplemental material). This was achieved by PCR amplifying the 3′ end of the ScICL1 ORF along with the conjoined MYC3-URA3 sequences from *S. cerevisiae* DSCO3 cells using the DS_6F and DS_6R primers (Table S3) and transforming this CaICL1-UBI-Myc3-URA3 cassette into *C. albicans* RM1000.

The ScICL1 and ScPCK1 loci were epitope tagged in *S. cerevisiae*. ScICL1 was tagged with Myc3, at its 3′ end by PCR amplification of the MYC3-NAT cassette in pYM21 (72) with primers DS_1F and DS_1R (see Table S3 in the supplemental material) and transformation of *S. cerevisiae* BY4743 to generate strain DS1-W10 (ScICL1-MYC3-NAT) (Table S1). ScPCK1 was tagged with hemagglutinin (a six-hemagglutinin tag [HA6]) by PCR amplification of the HA6-KTRP1 (KTRP1 stands for Kluyveromyces lactis TRP1) cassette in pYM3 (73) with primers DS_2F and DS_2R (Table S3) and transformation of *S. cerevisiae* W303-1B to create strain DS1-W20 (ScPCK1-HA6-KTRP1) (Table S1).

The ScICL1 ORF in *S. cerevisiae* BY4743 was replaced with CaICL1-Myc3 to create the S. cerevisiae strain DS4-Y40 (ScICL1-CaICL1-Myc3-URA3) (see Table S1 in the supplemental material). The CaICL1-Myc3-URA3 ORF was PCR amplified from *C. albicans* CA1395 (Table S1) using the DS_4F and DS_4R primers (Table S3).

**Proteomics.** Replicate cultures of *C. albicans* NGY152 (CA14 containing Clp10 [74]) were grown in minimal medium containing glucose (2%), lactic acid (2%), oleic acid (0.2%), or mixed amino acids (2%) as the sole carbon source. Samples from the cultures were taken, then subcultured in the same medium, grown for a further 20 h, and harvested in exponential phase (optical density at 600 nm [OD600] of 0.8). Glucose (2%) was added to some cultures, and the cells were harvested after 2 h. Protein extracts were prepared, subjected to 2D gel electrophoresis, and stained with Coomassie blue as described previously (54). Independent triplicate experiments were done for each growth condition. Gel features were compared using Phoretix 2D Expression (Nonlinear Dynamics, Newcastle upon Tyne, United Kingdom) and GeneSpring (Silicon Genetics, San Carlos, CA). Principal component analysis scores were plotted in 3D (SIMCA-P version 11.0; Umetrics AB, Sweden). Proteins displaying statistically significant differences in mean spot volume (*P* ≥ 0.05) were identified by MALDI-ToF mass spectrometry of tryptic peptides (54) using MS-Fit and MASCOT (Matrix Science, Boston, MA). Protein annotations were from the *Candida* Genome Database (http://www.candidagenome.org/). The proteomic data set is available in the supplemental material (see Table S2 in the supplemental material) and at the PRIDE proteomic data repository (http://www.ebi.ac.uk/pride/) (accession numbers 3186 to 3192). The carbon source network was constructed using Cytoscape version (http://www.cytoscape.org/) (75).

**Western blotting.** Protein extracts were subjected to Western blotting as described previously (76). Membranes were probed with a mouse anti-Myc antibody (diluted 1:10,000) (Sigma). The secondary antibody was peroxidase-conjugated rabbit anti-mouse IgG. Signals were detected with an enhanced chemiluminescence (ECL) Western blotting kit (Amersham, United Kingdom) and quantified using a Fuji FLA-3000 imager.

**RNA analyses.** RNA was prepared (77, 78), yields were quantified using an RNA 6000 Nano assay, and RNA integrity was assessed using an Agilent 2100 bioanalyzer (79). ICL1 and PCK1 transcript levels were quantified to the internal ACT1 mRNA by qRT-PCR using primers (see Table S3 in the supplemental material). RNA samples (2 μg) were incubated in 20-μl reactions with DNase I (1.5 μl), RNase OUT (1.5 μl), and DNase I buffer (2 μl) (Invitrogen, United Kingdom) at room temperature for 15 min. cDNA was then made with SuperScript II reverse transcriptase (Invitrogen, United Kingdom) following the manufacturer’s protocols. Real-time RT-PCR SYBR green (Roche, United Kingdom) was performed using the manufacturer’s instructions with a LightCycler 480 real-time PCR system (Roche).

**Lactate and oleic acid assimilation.** Lactate and oleic acid assimilation was assayed by measuring the incorporation by *C. albicans* and *S. cerevisiae* of [14C]lactate and [3H]oleic acid into trichloroacetic acid-precipitable material. Yeast cells were grown on YPLactate (2% Bacto peptone and 1% yeast extract containing 2% lactate) or YPOleic acid (2% Bacto peptone and 1% yeast extract containing 0.2% oleic acid) at 30°C to an OD of 1, harvested, and resuspended in 1 ml fresh prewarmed YP-Lactate or YPOleic acid. Glucose (2%) was added to half of the cells, and no glucose was added to control cells. Then, 1.85 MBq of [14C]lactate and [3H]oleic acid was added at time zero, and samples were taken at various times thereafter. The samples were precipitated in 5% trichloroacetic acid at 0°C, washed at 0°C in a series of solutions (i) fresh 5% trichloroacetic acid containing 0.1% SDS, (ii) 50% ethanol, and (iii) 100% ethanol), dried, and subjected to scintillation counting (Packard BioScience). The results are means and standard deviations from triplicate assays. Similar results were obtained in triplicate independent experiments.

**SUPPLEMENTAL MATERIAL**

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl?doi=10.1128/mBio.00495-12/-/DCSupplemental.

Figure S1, PDF file, 0.1 MB. Figure S2, PDF file, 0.1 MB. Figure S3, PDF file, 0.1 MB. Figure S4, PDF file, 0.1 MB. Figure S5, PDF file, 0.1 MB. Figure S6, PDF file, 0.1 MB. Figure S7, PDF file, 0.1 MB. Table S1, PDF file, 0.1 MB. Table S2, PDF file, 0.1 MB. Table S3, PDF file, 0.1 MB.

Sando et al.
ACKNOWLEDGMENTS

We thank Esperanza Lopez-Franco for help with the construction of C. albicans Pck1-Myc and Icl1-Myc strains. We thank Phil Cash, Mike Lorenz, and Ben Distel for stimulating discussions and helpful advice. D.S. was supported by a scholarship from Universiti Sains, Malaysia. M.D.L. was the recipient of a Carnegie/Caledonian scholarship from the Carnegie Trust, and a Sir Henry Wellcome postdoctoral fellowship from the Wellcome Trust (096072). This work was also supported by the United Kingdom Biotechnology and Biological Research Council (BBS/B/06679 and BB/F00513X/1), the Wellcome Trust (080088 and 097377), and the European Commission (PITN-GA-2008-214004 and ERC-2009-AdG-249793).

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