Carbon Catabolite Control in *Candida albicans*: New Wrinkles in Metabolism

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ABSTRACT Most microorganisms maintain strict control of nutrient assimilation pathways to ensure that they preferentially use compounds that generate the most energy or are most efficiently catabolized. In doing so, they avoid potentially inefficient conflicts between parallel catabolic and metabolic pathways. The regulation of carbon source utilization in a wide array of bacterial and fungal species involves both transcriptional and posttranscriptional mechanisms, and while the details can vary significantly, carbon catabolite control is widely conserved. In many fungi, the posttranslational aspect (carbon catabolite inactivation [CCI]) involves the ubiquitin-mediated degradation of catabolic enzymes for poor carbon sources when a preferred one (glucose) becomes available. A recent article presents evidence for a surprising exception to CCI in the fungal pathogen *Candida albicans*, an organism that makes use of gluconeogenic carbon sources during infection (D. Sandai, Z. Yin, L. Selway, D. Stead, J. Walker, M. D. Leach, I. Bohovych, I. V. Ene, S. Kastora, S. Budge, C. A. Munro, F. C. Odds, N. A. Gow, and A. J. Brown, *mBio* 3[6]:e00495-12). *In vitro*, addition of glucose to cells grown in a poor carbon source rapidly represses transcripts encoding gluconeogenic and glyoxylate cycle enzymes, such as phosphoenolpyruvate carboxykinase (Pck1p) and isocitrate lyase (Icl1p), in both *C. albicans* and *Saccharomyces cerevisiae*. Yet, uniquely, the *C. albicans* proteins persist, permitting parallel assimilation of multiple carbon sources, likely because they lack consensus ubiquitination sites found in the yeast homologs. Indeed, the yeast proteins are rapidly degraded when expressed in *C. albicans*, indicating a conservation of the machinery needed for CCI. How this surprising metabolic twist contributes to fungal commensalism or pathogenesis remains an open question.

A core challenge for all organisms is the need to procure sufficient nutrients to generate both energy and the necessary macromolecules to drive cellular replication. The potential for rapid changes in nutrient availability is particularly acute for microorganisms, and most have responded by maintaining the ability to use a wide variety of compounds as the primary sources of carbon, nitrogen, phosphorous, and sulfur. At the same time, cellular metabolism is tuned to preferentially use nutrients that are most efficiently assimilated or that provide the most energy. For carbon, the preferred source for most organisms is glucose, and when it is present, cells generally do not express other carbon catabolic pathways, even for other sugars. While this deprives the cell of some metabolic flexibility, the expression of catabolic pathways, sometimes requiring entire organelles, for nutrients that will not be used is energetically wasteful. This regulatory concept—carbon catabolite control (CCC)—is broadly conserved in both prokaryotic and eukaryotic microbes, though the mechanisms differ between and within kingdoms (reviewed in references 1 and 2). In a recent article, Dr. Alistair Brown and colleagues at the University of Aberdeen identified a startling wrinkle in CCC in the fungal pathogen *Candida albicans* that suggests that this organism has retuned its metabolism to make use of both preferred and nonpreferred carbon sources simultaneously (3).

In fungi, CCC has been defined by the paradigm established in *Saccharomyces cerevisiae* (and to a lesser extent in *Aspergillus nidulans*), in which the addition of glucose to cells previously grown with a poor carbon source (lactate or oleate, for instance) rapidly changes both the transcriptome and proteome. Gluconeogenic genes are downregulated by the well-conserved Mig1p transcriptional repressor (carbon catabolite repression [CCR]) (4), while gluconeogenic enzymes are degraded in a ubiquitin-dependent manner (carbon catabolite inactivation [CCI]) (5). Surprisingly, the Brown group found that only the transcriptional part occurs in *C. albicans*—the genes are rapidly repressed, but their protein products are stable and apparently active for many hours (3). This disconnect between mRNA and protein abundance also provides a sobering reminder that one of the fundamental assumptions of genomics—that changes in mRNA abundance are a good proxy for downstream protein and phenotypic changes—is not universally true.

To make this discovery, Dr. Brown's group used a proteomics platform to compare cells grown long term (20 h) in alternative carbon sources (lactate, oleate, and amino acids) to those grown in glucose. Unsurprisingly, there were substantial differences related to glycolysis, gluconeogenesis, and a variety of catabolic pathways. They next grew cells in oleate for 20 h before adding glucose for 2 h. The proteome profiles for glycolytic enzymes showed high correlation between the short- and long-term glucose conditions; that is, proteins like Cdc19p (pyruvate kinase) and Eno1p (enolase) were abundant when glucose was present, regardless of the duration, relative to findings for oleate-grown cells, as expected. In contrast, the levels of gluconeogenic and glyoxylate enzymes, such as isocitrate lyase (Icl1p) and phosphoenolpyruvate carboxykinase (Pck1p), were poorly correlated between the two glucose experiments: they were both much more abundant after 2 h in glucose than would have been predicted from the *S. cerevisiae* paradigm.

To explain this observation, they hypothesized that these en-

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zymes are subject to transcriptional regulation by carbon source (CCR) but not the ubiquitin-mediated degradation (CCI). To test this, they used epitope-tagged alleles of *C. albicans ICL1* and *PCK1* and measured the levels of both mRNA and protein following readdition of glucose to lactate-grown cultures. As expected, transcript levels dropped rapidly, but the proteins were stable over many hours. In *S. cerevisiae*, in contrast, both mRNA and protein were rapidly lost after glucose readdition.

They next examined whether *C. albicans* had retained the capability for CCI through reciprocal experiments expressing either the *Candida* proteins in yeast or the yeast proteins in *Candida*. Using the same experimental design—adding glucose to lactate-grown cells—they demonstrated that ScIcl1p is unstable in *C. albicans* and CaIcl1p is stable in *S. cerevisiae* (Pck1p behaved similarly), and the instability of the heterologously expressed yeast proteins was ubiquitin dependent. Thus, *C. albicans* is capable of CCI, but at least several proteins are immune to this degradation. The bioinformatic prediction software tool UbPred suggested that the *C. albicans* proteins lack consensus ubiquitination sites found in the yeast homologs despite a high degree of overall similarity.

If the enzymes persist, does the cell retain the ability to assimilate the nonpreferred carbon source? To test this, the Brown group added glucose to cells grown in lactate or oleate, as before, but spiked in radiolabeled lactate or oleate at the same time. The radioactivity in trichloroacetic acid precipitates continued to increase during the 4-h time course, indicating that cells continued to assimilate these nonpreferred compounds even in the presence of glucose. This parallel utilization of fermentable and nonfermentable carbon sources had not previously been observed in fungi.

The extent of this regulatory rewiring can only be indirectly inferred but is likely to be on a large scale. The continued assimilation of the nonpreferred compounds implies that the entire catabolic pathways for both compounds remain present; in the case of oleate, this involves an entire organelle (peroxisomes). Thus, *C. albicans* has maintained the capability for nutrient-induced protein degradation but has specifically exempted large parts of carbon metabolism from a regulatory process that is widely conserved in prokaryotic and eukaryotic microbes.

Why might this be, and is it significant? *C. albicans* has evolved in the unique niche of the mammalian host, a rarity among the fungi—there are no more than a relative handful of host-adapted commensal or pathogenic species. An opportunistic pathogen such as *C. albicans* must successfully compete for nutrients, both with the host itself and with the microbiome. It has long been apparent that pathogens are subject to specific nutritional deficiencies in the host; for instance, nucleotide auxotrophs are among the most avirulent mutants in both bacterial and fungal pathogens. This has been both a boon and a scourge for researchers: the absolute avirulence of *purA* mutants made this gene the perfect marker in the original *Salmonella in vivo* promoter trap experiments (6), while the avirulence of *ura3* mutants has posed substantial technical problems in *C. albicans* (7). There is also a rich literature on the challenges of iron acquisition within the host.

The last decade has seen evidence from many organisms that carbon is also limiting in the host. Though glucose is present at a low but relatively constant concentration in the bloodstream, this is clearly not the case in all host niches. The most prevalent bacterial pathogen, *Mycobacterium tuberculosis*, activates alternative carbon assimilation pathways when resident within macrophages; mutation of these pathways attenuates virulence (8–10). In *C. albicans*, these same pathways are induced by contact with phagocytes and *in vivo* (11, 12). Attenuation of virulence is observed in strains lacking key carbon assimilation enzymes of gluconeogenesis, such as fructose-1,6-bisphosphatase (Fbp1p) and Pck1p and the glyoxylate enzyme Icl1p (11, 13). The only compelling explanation for these findings is that human pathogens experience carbon limitation during infection of at least some host niches, which they overcome by utilizing alternative carbon sources. While some pathogens, or some sites of infection, appear not to require alternative assimilation pathways, their conservation in microbes and absence from mammals makes them tantalizing (if unrealized) targets for antimicrobial development.

The continued assimilation of nonpreferred carbon sources in the presence of glucose poses an energetic problem. Readdition of glucose induces glycolysis without inactivating gluconeogenesis: Pck1p is one of the two rate-limiting steps of gluconeogenesis (Fbp1p is the other) and remains stable and apparently active for many hours. Does this mean the cell tolerates these reciprocal pathways being active at the same time? Synthesizing glucose only to subsequently degrade it constitutes a wasteful cycle. Indeed, these futile cycles of degradation and synthesis were observed in a yeast strain engineered to constitutively express *PCK1* and *FBP*, resulting in a growth rate 20% lower than that of control strains on glucose (14). Sandai et al. (3) did not directly test whether such futile cycles were occurring in *C. albicans* under these conditions, but the continued presence of the enzymes certainly makes that a possibility and raises the question of whether this species has additional posttranslational mechanisms to prevent the energy loss these metabolic-catabolic cycles would entail.

The overall conclusion from the work of Sandai et al. (3) is that *C. albicans* maintains its capacity to assimilate alternative carbon sources for some time after a preferred source becomes available. Assessing the evolutionary benefits of this is by definition speculative but likely returns to the idea of nutritional stress within the host. The best-characterized carbon source transition in fungi is undoubtedly the switch from glucose to galactose utilization in yeast. This relatively conservative change (one sugar for another) results in a temporary cessation of growth ("lag phase") that can last 2 to 4 h, more than a doubling time, as the needed enzymatic machinery is synthesized. For a pathogen transiting from one niche to another, exposed to a complex microbiota, and under constant threat of immunological attack, lengthy growth pauses may simply be untenable, and avoiding them is worth the temporary metabolic inefficiency. It is tempting to speculate based on the data from Sandai et al. that *C. albicans* would be more fit in an environment in which carbon availability was constantly changing in both quantity and quality than an organism like yeast in which CCI is fully active. Whether this is true, and how it might affect virulence, offers fertile ground for future studies. It appears there are a few more surprises yet to be uncovered in microbial metabolism.

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