Compartmentalization of metabolic pathways in Candida albicans: a matter of transport
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Summary

The yeast *Candida albicans* is a human commensal that resides in the gastro-intestinal tract, but is also the most common cause of human fungal infections. These infections range from relatively harmless superficial mucosal infections to life-threatening systemic infections that mainly occur in immunocompromised patients. *C. albicans* is a dimorphic fungus that is able to grow in the yeast or in the filamentous form and switching between both forms is essential for virulence. Phagocytic cells of the immune system, macrophages and neutrophils, play an important role in prevention of infection in healthy hosts. Upon recognition, macrophages and neutrophils initiate phagocytosis of *C. albicans* and employ antifungal mechanisms in an attempt to kill the fungus. One of these antifungal mechanisms is the respiratory burst of reactive oxygen species (ROS) that are released to attack the yeast within the phagolysosomal compartment. The transcriptional response of *C. albicans* at different time points after phagocytosis has been studied by microarray analysis. Three noticeable transcriptional changes were observed in the fungus: hyphal induction, oxidative stress defense and metabolic adaptation to a glucose-poor environment. The latter response is characterized by down-regulation of genes involved in glycolysis and up-regulation of genes involved in gluconeogenesis, β-oxidation of fatty acids and the glyoxylate cycle. In this thesis we have employed molecular and biochemical techniques to study central carbon metabolism and oxidative stress defense of the versatile fungus *C. albicans*.

Central carbon metabolism of eukaryotic cells is characterized by compartmentalization of metabolic pathways. The mitochondria harbor the tricarboxylic acid (TCA) cycle and the respiratory chain, which are both essential for generation of ATP. The β-oxidation of fatty acids is associated with peroxisomes in most eukaryotes and in yeasts like *C. albicans*. Acyl-CoAs of different lengths are completely metabolized to acetyl-CoA units in the peroxisomal matrix. Subsequently, acetyl-CoA (C2) is fed into the peroxisomal glyoxylate cycle to enable generation of malate (C4) units for gluconeogenesis and is transported to the mitochondrial TCA cycle where it is degraded to CO₂ and H₂O, thereby assisting in the production of ATP. Transport requirements differ between growth conditions, because the site of acetyl-CoA production depends on the carbon source. During growth on fatty acids the peroxisomal acetyl-CoA can immediately enter the glyoxylate cycle, but transport to the mitochondrial matrix is required for breakdown of acetyl-CoA in the TCA cycle. By contrast, during growth on ethanol or acetate acetyl-CoA is produced in the cytosol and has to be transported to both peroxisomes and mitochondria to enter the glyoxylate and TCA cycle, respectively.

The main finding that we describe in Chapters 2, 3 and 4 is the importance of carnitine in central carbon metabolism in *C. albicans*. We describe the role of carnitine acetyltransferases and the carrier carnitine in transport of acetyl units between compartments in *C. albicans*. In Chapter 2 we investigate the function of the carnitine acetyltransferase Cat2 and show that this enzyme has a dual localization to peroxisomes and mitochondria. In contrast with *Saccharomyces cerevisiae*, *C. albicans* lacks a peroxisomal citrate synthase and is completely dependent on Cat2 for growth on fatty acids, ethanol and acetate. We show that Cat2 does not contribute to virulence
of *C. albicans* in the mouse model of infection, but that the protein does play a role in biofilm formation of *C. albicans*. However, the exact contribution of Cat2 to this complicated mechanism remains to be investigated. The function of the peroxisomal and mitochondrial Cat2 isozymes is studied in more detail in Chapter 3. We use mutant strains that lack either the peroxisomal (the mitCAT2 strain) or mitochondrial (the perCAT2 strain) isoenzyme and study their phenotypes during growth on fatty acids, ethanol and acetate. During growth on these non-fermentable carbon sources, acetyl-CoA supply to both the mitochondrial TCA cycle and the peroxisomal glyoxylate cycle is essential. We show that the mitochondrial Cat2 is essential under all conditions tested, as the perCAT2 strain has the same growth defects as a cat2 null strain. However, the function of the peroxisomal Cat2 is less clear, because the mitCAT2 strain is still able to grow on ethanol and acetate and β-oxidize fatty acids. We discuss the implications for acetyl unit transport between compartments using the model presented in this chapter. The role of the other two putative carnitine acetyl-transferases, Yat1 and Yat2, is discussed in the Addendum to Chapter 3. We show that these proteins localize to the cytosol, a localization that seems to be consistent with their putative role in transport of acetyl units produced in the cytosol during growth on ethanol or acetate. However, the experiments presented in the addendum do not support a role for Yat1 and/or Yat2 as carnitine acetyl-transferases and additional data are necessary to unravel the function of these proteins. The characterization of the carnitine biosynthesis pathway in *C. albicans* is the subject of Chapter 4. In this chapter we identify the genes that encode the four enzymes of the pathway and study their function individually. Disruption strains for the genes encoding TMLD, TMABADH and BBD are analyzed phenotypically and we show that the strains lack their respective enzymatic activities. We show that the second enzyme of the pathway, HTMLA, is a member of the threonine aldolase family of proteins. Phenotypic analysis of constructed disruption strains revealed its specific function in carnitine biosynthesis in *C. albicans*.

The starting hypothesis that acetyl unit transport may contribute to virulence of *C. albicans* has not been confirmed in our studies. We show that Cat2 does not contribute to *C. albicans* virulence in the mouse model of infection, in which the yeast is injected in the bloodstream. This result confirms earlier findings showing that the β-oxidation of fatty acids does not contribute to virulence in this model. However, the mouse model of systemic candidiasis might not be very suitable to test alternative carbon metabolism, because it is blind to the establishment of infection and because the blood is rich in glucose. Alternative models should be employed that take carbon-limitation in account, for example during phagocytosis by macrophages and neutrophils.

Chapters 5, 6 and 7 of this thesis also deal with compartmentalization, but focus on proteins that contribute to oxidative stress defense. To survive the reactive oxygen species (ROS) produced by a macrophage or neutrophil after phagocytosis, *C. albicans* requires a functional defense mechanism. Additionally, ROS like hydrogen peroxide (**H**₂**O**₂) are produced intracellularly as by products of many metabolic pathways in aerobically living cells. The respiratory chain in mitochondria and oxidases in the peroxisomal matrix are the main sources of intracellular H₂O₂. All ROS either produced
intracellularly or entering the cell from the environment, need to be decomposed to prevent irreversible damage to proteins, DNA or lipids. Each organelle contains its own ROS-decomposing enzymes, but we especially focus on oxidative stress defense in the oxidative environment of the peroxisomal matrix. We investigate the subcellular localization of the following enzymes in *C. albicans*: the catalase (Cta1), the glutathione reductase (Glr1) and the NADPH-producing dehydrogenases of the pentose phosphate pathway, the main source of NADPH in the cell. NADPH plays an essential role as electron donor in redox reactions. **Chapter 5** describes our finding that the dehydrogenases of the pentose phosphate pathway, glucose-6-phosphate dehydrogenase (Zwf1) and 6-phosphogluconate dehydrogenase (Gnd1), have a dual localization to the cytosol and peroxisomes. We show that the Zwf1 and Gnd1 employ different mechanisms to achieve their dual targeting and we discuss the putative function of the NADPH production in peroxisomes. One of these functions could be to provide NADPH for the protection of peroxisomal catalase (Cta1) of *C. albicans*. In **Chapter 6** we study the contribution of Cta1 to oxidative stress defense in general and to peroxisomal oxidative stress defense specifically. We hypothesize that the enzymes of the peroxisomal glyoxylate cycle are particularly sensitive to oxidation and that catalase may protect these enzymes against oxidative damage. Finally we investigate the localization of the single Glr1 of *C. albicans* in **Chapter 7**. Reduced glutathione plays an important role in maintaining the redox state of the cell and is used as a cofactor by a variety of glutathione-dependent enzymes in different compartments. We show that Glr1 contains both a mitochondrial and a peroxisomal targeting signal (MTS and PTS2) in its N-terminus and localizes to the cytosol, mitochondria and possibly peroxisomes. Disruption of the *GLR1* gene revealed that its activity is essential for normal growth on all carbon sources.