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Regulators of commensal and pathogenic life-styles of an opportunistic fungus - *Candida albicans*

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Abstract

The yeast *Candida albicans* is primarily a commensal of humans that colonizes the mucosal surfaces of the gastrointestinal and genital tracts. Yet, *C. albicans* can under certain circumstances undergo a shift from commensalism to pathogenicity. This transition is governed by fungal factors such as morphological transitions, environmental cues for instance relationships with gut microbiota and the host immune system. *C. albicans* utilizes distinct sets of regulatory programs to colonize or infect its host and to evade the host defense systems. Moreover, an orchestrated iron acquisition mechanism operates to adapt to specific niches with variable iron availability. Studies on regulatory networks and morphogenesis of these two distinct modes of *C. albicans* growth, suggest that both yeast and hyphal forms exist in both growth patterns and the regulatory circuits are inter-connected. Here, we summarize current knowledge about *C. albicans* commensal-to-pathogen shift, its regulatory elements and their contribution to human disease.

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Take Aways

1. Both yeast and hyphal cells co-exist during pathogenic or commensal life of *C. albicans*.
2. Hyphal-specific virulence factors decide the shift from commensal to pathogenic growth of *C. albicans*.
3. An integrated regulatory circuit of *C. albicans* pathogenicity and commensalism evolved to respond to niche-specific signals.

Introduction

Host-microbe symbiosis is widely distributed within eukaryotes. However, mammals harbor the most diverse and dense microbial communities. Most of these microbes are harmless and even beneficial to their host (Abt and Pamer, 2014; Ezenwa et al., 2012; Round and Mazmanian, 2009) but a few, including *Candida albicans*, are able to change their fate and can penetrate tissues and organs. This fungus is associated with humans and is capable of causing superficial or disseminated infections (de Pauw, 2011).

C. albicans is a major opportunistic fungal pathogen responsible for an increasing number of life-threatening systemic infections. This yeast commonly lives as a commensal inside the gut of healthy individuals, but breakage of epithelial barriers and dysfunction of the host immune system favor the transition from commensalism to pathogenicity (Perlroth et al., 2007). During the commensal-to-pathogen shift, *C. albicans* undergoes transcriptional reprogramming and cellular differentiation inside the mammalian host to adapt to new niches that differ in micro-nutrient availability, CO₂ level, O₂ level and pH (Noble et al., 2017). *C. albicans* polymorphic nature has a major impact on virulence and is considered as essential during the transition from a commensal to a pathogenic mode of growth (Calderone and Fonzi, 2001; Mayer et al., 2013). Generally, the yeast-to-hypha transition plays a central role during *C. albicans* infection. Apart from morphological switches, adaptation to different niches such as variable ions concentrations also decides the fate of *C. albicans* growth. A well-orchestrated transcriptional network

regulating morphogenesis and adaption to variable iron conditions has been well characterized (Bohm et al., 2017; Chen et al., 2011). However, new factors contributing to *C. albicans* ability to adapt to different niches and undergo morphological changes have since been identified (Witchley et al., 2019; Znaidi et al., 2018).

Overall, growth of *C. albicans* in different niches is regulated by distinct sets of transcriptional and epigenetic regulatory mechanisms as well as signaling pathways which help in evading and escaping the host immune system (Anderson et al., 2016; Huang, 2012; Rai et al., 2018). During the last decades, research carried out on *C. albicans* was mostly focused on transcriptional regulators of morphogenesis, its adaptation to different niches and molecules involved during the shift from commensalism to pathogenicity. Until recently, it was thought that yeast growth is predominant during commensalism whereas hyphal growth supports *C. albicans* pathogenicity. However, recent discoveries indicate the existence of both yeast and hyphae throughout the gut when this fungus grows as commensal (Witchley et al., 2019). This indicates that it is not the hyphal morphology itself, but rather the expression of hyphae-specific virulence factors, which are the determinants of the shift from commensal to pathogenic state of *C. albicans* (Witchley et al., 2019). Therefore, based on recent studies, we speculate that hyphal induction is indispensable for *C. albicans* adaptation inside the host irrespective of the niches, even though the role of hyphal cells during gut colonization is not fully understood. We also surmise that transcription circuits regulating pathogenesis and commensalism are developed in an interconnected manner. A well-coordinated crosstalk evolved to enhance *C. albicans* fitness to adapt in different niches. In this review, we present a comparative analysis of transcription factors which regulate *C. albicans* pathogenesis and commensalism. This analysis reveals that key regulatory networks controlling these two distinct growth patterns of *C. albicans* are indeed interconnected and are essential during the host-specific environmental changes (**Figure 1**). This review covers recent findings highlighting the role of transcription factors contributing to morphogenesis, adaptation to different niches and regulatory circuits, which are found to be required to support both commensal and pathogenic growth modes of *C. albicans*.

Regulators of morphogenesis and their role in *C. albicans* colonization

As stated above, *C. albicans* is primarily a commensal of the human gut. However, this fungus exhibits distinct morphological forms and thus it is important to understand the connection

between a morphological state of the fungus and its commensal vs. pathogenic behavior. Previous studies have indicated the predominance of cells harboring yeast morphology in the commensal population in monocolonized mice (approximately 90%) (Bohm et al., 2017; White et al., 2007). Indeed, regulators that favor yeast over hyphal growth in the GI tract, such as Zcf8, Zfu2 and Try4, provide more commensal fitness (Bohm et al., 2017), since cells lacking these regulators are hyper-filamentous, and less efficient colonizers of the GI tract of germ-free mice. Similarly, Hms1 and Cph2 promote yeast growth over filaments under anaerobic conditions and null mutants of these regulators display impaired colonization (Perez et al., 2013; Rosenbach et al., 2010).

The predominance of yeast cells in the GI tract is also supported by key regulators of yeast to hyphal transition; indeed, deleting positive regulators of hyphal growth, namely *EFG1* and *UME6*, conferred enhanced fitness in the gut over the wild type (Pande et al., 2013; Pierce and Kumamoto, 2012). Moreover, even the doses of *EFG1* are important in determining cell differentiation as *EFG1* hemizygous cells are hypercompetitive for commensalism (Liang et al., 2019).

Experimental evolution of *C. albicans* in the GI tract of antibiotic-treated mice revealed an adaptation of cells via loss of function mutations in transcriptional activators of hyphal morphogenesis (Tso et al., 2018). These hyper-fit selected strains lost their ability to respond to hyphal-inducing stimuli. This work suggested that *C. albicans* growth in the gut is submitted to fitness selection. However, this was not observed in the absence of antibiotics indicating that the bacterial population limits the adaptation of *C. albicans* in the mouse GI tract. Therefore, pre-existing microbiota of the gut may compete with incoming species by occupying the same niche and inhibiting the growth of pathogens by activating the host immunity.

Interestingly, Tso *et al.* (2018) showed that 22 out of the 28 hyper-fit strains selected from the GI tract of mice carry mutations leading to a premature stop codon or frameshifts in the coding region of *FLO8*, encoding a transcription regulator which is essential for *C. albicans* hyphal development and virulence (Cao et al., 2006). However, in the absence of antibiotic pressure these *flo8* mutant strains were outnumbered by commensal bacteria. This suggests that *C. albicans* cannot afford to lose virulence factors which possibly participate in the initial stage of colonization and could be required for competition with other species occupying the same niche.

A remarkable discovery from Witchley and colleagues confirmed the inhibitory role of hyphal regulatory network on *C. albicans* fitness inside the mammalian gut. These authors showed that disruption of transcription factors which favor hyphal formation and virulence namely Efg1, Brg1, Rob1, and Tec1, results in cells hyper-fit for gut colonization (Witchley et al., 2019). Therefore, these results support an inhibitory function for the hyphal inducing regulators on *C. albicans* ability to colonize in the mammalian gut. Interestingly, these authors also indicated a role for hyphal cells in the gut by showing their presence together with yeast cells throughout the mammalian gut, from the stomach to the large intestine (Witchley et al., 2019). They further showed the presence of hyphal cells inside the mammalian GI tract even in the absence of the key hyphal regulator *UME6*, although these cells are defective in their ability to form hyphae *in vitro*. The authors suggested that the inhibitory role of these regulators in gut colonization is independent of hyphal formation but occurs via the activation of the hyphal-specific pro-inflammatory secretory protease Sap6, and the hyphal cell surface adhesin Hyr1. Deletion of *SAP6* produces enhanced GI fitness similar to that of the *ume6* mutant and over-expression of *SAP6* results in an opposite phenotype (Witchley et al., 2019). Altogether, these observations suggest that filamentous cells could play a role during competition with other microbial species such as bacteria sharing the same niche, but strikingly with only a subset of hyphal genes being activated. Identifying the regulators of hyphal induction in the GI tract with restricted gene activation will be of future interest.

Iron homeostasis and regulatory circuits in commensal and pathogenic modes of *C. albicans* growth

C. albicans usually resides in the GI tract of the human host as a harmless commensal. In this niche, dietary iron is in excess, exposing *C. albicans* to a potential risk of iron toxicity. Conversely, during systemic infection, the fungus can be found in the bloodstream where iron is depleted. To maintain its iron homeostasis, *C. albicans* has developed a robust iron acquisition mechanism which operates depending on availability of iron in its environment (Almeida et al., 2009; Noble, 2013). During the commensal mode of growth in the GI tract, in iron-rich environment, Sfu1, a GATA family transcription factor, represses the siderophore transporter gene *SITI* as well as genes involved in iron uptake (including components of hemoglobin uptake) and in reductive iron transport. Sfu1 also inhibits the iron uptake by repressing *SEF1*,

that encodes a Zn2-Cys6 transcription factor, regulator of genes involved in iron uptake (Chen and Noble, 2012; Chen et al., 2011). Thus, Sfu1 prevents iron toxicity in the GI tract by limiting iron uptake in iron-replete environments (Noble, 2013).

During systemic infection iron availability is low in the bloodstream, and Sef1 directly activates the transcription factor encoding gene *HAP43* and genes participating in iron uptake. Activation of *HAP43* directly represses *SFU1* and genes involved in iron utilization and participates in activating iron uptake genes (Hsu et al., 2011; Singh et al., 2011). Besides, *SEF1* up-regulation in the bloodstream leads to the expression of virulence-associated genes and thus favors *C. albicans* pathogenic growth (Chen et al., 2011). Rim101, a zinc finger transcription factor, is required for the activation of iron uptake genes during iron starvation in the bloodstream under neutral or alkaline pH (Davis et al., 2000a; Davis et al., 2000b). These results indicate that two distinct growth patterns of *C. albicans* in variable iron condition are tightly controlled by different sets of specialized regulators (Bohm et al., 2017).

Besides regulators for iron homeostasis, a set of other factors also contributes to support *C. albicans* growth in different niches. Pande et al. identified a novel morphological state of *C. albicans*, named Gastrointestinally Induced Transition (GUT), in cells over-expressing *WOR1*, a master regulator of white-opaque switching (Pande et al., 2013). *WOR1*-over-expressing cells undergo a developmental change during a passage through the mice GI tract resulting in an elongated morphology as compared to bloodstream oval-shaped yeast cells. GUT cells display a specific transcriptome as well as metabolic activity and exhibit an enhanced commensal fitness as compared to the cells harboring the yeast morphology. Although GUT cells are morphologically close to the mating competent opaque cells, they lack pimples on their surfaces, and contain prominent vacuoles. Moreover, they are heterozygous at the mating type locus, thus being unable to respond to the mating pheromones (Pande et al., 2013). However, these cells were only isolated from *C. albicans* cells over-expressing *WOR1* and their presence in naturally growing *C. albicans* has not been observed. Therefore, GUT cells and their relevance during the *C. albicans* gut colonization need to be further investigated.

In a genetic screen of 77 *C. albicans* knockout transcription factors, Perez et al. revealed the role of eight transcriptional regulators (TR) in GI tract colonization, systemic infection, or both, in mouse models. Transcription factors such as *TYE7*, *ORF19.3625*, and *LYS144* show a predominant role in GI tract colonization, whereas *RTG1*, *RTG3*, *ZCF21*, *LYS14* and *HMS1* are

important for both systemic infection as well as gut colonization (Perez and Johnson, 2013; Perez et al., 2013) (**Table 1**). Therefore, Perez et al. suggested the development of integrated and overlapping circuits between commensalism and pathogenicity to colonize different niches. A different approach was used by Znaidi et al. who screened 572 signature-tagged conditional over-expression strains in a murine gut colonization assay and identified *CRZ2* (a C2H2 transcription factor) as a regulator of *C. albicans* colonization. *CRZ2* over-expression increases early colonization, while a null mutant shows impaired GI-tract colonization by modulating cell wall functions and adaptation to extracellular changes in pH (Znaidi et al., 2018) (**Figure 1**).

Genes and molecules involved in breaching the host tissue barrier

In the first two sections, we addressed networks regulating commensalism and pathogenicity of *C. albicans* in different niches. However, research carried out on genes involved in various vital pathways of *C. albicans* is mainly focused on pathogenic traits. Yet, *C. albicans* predominantly thrives as a commensal. For instance, the function of hyphal-related determinants essential during pathogenesis of *C. albicans* (such as invasins Als3 and Ssa1, and candidalysin, described below) has been thoroughly investigated. As stated previously, hyphae induction also occurs during commensal growth, yet hyphal-associated determinants required for *C. albicans* commensal growth are unknown. In this section, we will describe genes and molecules identified as hyphal-associated determinants and their importance during pathogenicity of *C. albicans*. *C. albicans* transition from commensalism to pathogenic state can occur upon host immune dysfunction, dysbiosis of resident microbiota, or damage of the mucosal intestinal barriers (Pappas et al., 2018; Vergidis et al., 2016; Zhai et al., 2020). *C. albicans* can invade the host epithelial cells, either by induced endocytosis or active penetration (Naglik et al., 2011). Induced endocytosis is mainly achieved by two invasins, Als3 and Ssa1, through binding to the N-cadherin and E-cadherin of host endothelial and epithelial cells (Phan et al., 2007; Sun et al., 2010). However, during the active penetration, the cAMP/PKA signaling pathway activates the expression of hyphae-associated genes through Efg1, a process considered as essential for active penetration and epithelial damage.

In a screen of more than 2000 deletion mutants of *C. albicans*, Allert et al. identified genes involved in cellular damage and translocation across enterocytes *in vitro*. In this screen, 172 knockout mutants conferred less damage, whereas 102 mutants showed increased damage as

compared to the wild type (Allert et al., 2018). Many of the genes whose deletion results in less damage are also involved in morphogenesis, pathogenesis and stress responses. However, eight hypo-damaging deletion mutants, either highly- (*TEA1*, *ORF19.3335*, *PEP1*, and *ECE1*), or moderately-impaired for epithelial damage (*NPR2*, *ORF19.2797*, *AAF1* and *HMA1*) did not associate with induction of filamentation. All but *ECE1* are impaired in adhesion, invasion or in hyphal length. Therefore, *ECE1* is not required for hyphal induction, but to maintain the structure of *C. albicans* hyphae. Candidalysin, a product of *ECE1*, is a 31 amino acid peptide toxin essential for mucosal infection. The authors suggested that candidalysin damages epithelial cell plasma membranes and stimulates the activating protein A (AP-1) transcription factor c-Fos and the MAPK phosphatase MKP1, which trigger and regulate proinflammatory cytokine responses (Allert et al., 2018; Basmaciyan et al., 2019; Moyes et al., 2016). Candidalysin production and function have not been studied when *C. albicans* grows as a commensal in a healthy host. Therefore, investigating the candidalysin function inside the host in the presence of the gut microbiota during commensal growth would shed new light on the role of this toxin. In the study of Allert et al. (2018), cells deleted for the transcription factor *TEA1* were shown to cause less damage to epithelial cells. Further study on *TEA1* would undoubtedly bring more understanding to the regulation of the shift from commensal to pathogenic growth.

Conclusions and perspectives

The opportunistic fungus *C. albicans* is able to adapt to variable environmental changes within the host, and can experience distinct life-styles, as a commensal or a pathogen. Although many fungal species are known as human opportunistic pathogens, the commensal relationship between host and fungi is not as well understood as the pathogenic relationship. The niches for commensalism and pathogenicity are generally distinct and thus fungi have developed specialized regulatory mechanisms for these two different life-styles. However, comparative analysis of regulators required for pathogenicity and commensalism of *C. albicans* indicates that both these life forms of *C. albicans* are also controlled by a common set of regulators. This reveals that *C. albicans* regulatory networks are integrated and perhaps required during the niche changes. Thus, the circuits regulating these two distinct growth patterns of *C. albicans* are tightly knit and a well-coordinated crosstalk occurs during the environmental changes (**Figure 1**).

So far, the majority of the experiments aimed at studying *C. albicans* gut colonization have been performed in the presence of antibiotics, which limits the growth of other microbial populations including bacterial species. At this stage, it is important to characterize the role of the different bacterial species from the microbiota with specific impact on fungal gut colonization. As discussed, iron availability is important in deciding the fate of *C. albicans* growth, but the role of other ions (such as zinc) during the shift from commensalism to pathogenicity needs to be addressed. Finally, the involvement of *C. albicans* hyphal cells during gut colonization is poorly understood; thus, studies on molecular differences between hyphae of commensal and pathogenic states are required to understand the participation of key molecules during transition from commensalism to pathogenicity.

Conflict of Interest No conflict of interest

Author Contributions

L.S.R, L.V.W, S.B. and C. D. Conceived the idea. L.S.R, L.V.W, M. E, S.B, and C. D. wrote the manuscript.

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Table 1. Transcriptional regulators with a role in GI tract colonization, establishment of systemic infection or both

Name	Phenotype	Function	References
CPH2	impaired in gut colonization ¹	contributes to morphogenesis ²	Rosenbach <i>et al.</i> , 2010 ¹ Lane <i>et al.</i> , 2001 ²
CRZ1	Impaired in kidney colonization ¹	calcineurin-regulated ² and some role in membrane integrity ³	Noble <i>et al.</i> , 2010 ¹ Santos and Larrinoa, 2005 ² Hameed <i>et al.</i> , 2011 ³
CRZ2	promotes commensalism in the GI tract ¹	involved in adaptive response to extracellular pH ¹	Znaidi <i>et al.</i> , 2018 ¹
EFG1	defective in virulence ¹ increased gut colonization ^{2,3,4}	major regulator of morphogenesis and metabolism; role in cell-wall regulation and adhesion ⁵	Lo <i>et al.</i> , 1997 ¹ Pierce <i>et al.</i> , 2013 ² Pande <i>et al.</i> , 2013 ³ Hirakawa <i>et al.</i> , 2015 ⁴ Doedt <i>et al.</i> , 2004 ⁵
EFH1	promotes commensalism in the GI tract ¹	contributes to morphogenesis	White <i>et al.</i> , 2007 ¹ Doedt <i>et al.</i> , 2004 ²
FLO8	defective in virulence ¹ increased gut colonization ²	major regulator of morphogenesis ^{1,2,3}	Cao <i>et al.</i> , 2006 ¹ Tso <i>et al.</i> , 2018 ² Du <i>et al.</i> , 2012 ³
HMS1	defective in kidney colonization ¹ impaired in gut colonization ^{1,2}	involved in acquisition of amino acids ¹ and contributes to morphogenesis ³	Perez <i>et al.</i> , 2013 ¹ Böhm <i>et al.</i> , 2017 ² Shapiro <i>et al.</i> , 2012 ³
LYS14	defective in kidney colonization ¹	regulation of lysine biosynthesis genes ²	Perez <i>et al.</i> , 2013 ¹ Priyadarshini <i>et al.</i> , 2016 ²
LYS144	impaired in gut colonization ¹	regulation of lysine biosynthesis genes ²	Perez <i>et al.</i> , 2013 ¹ Priyadarshini <i>et al.</i> , 2016 ²
RGTI	reduced kidney colonization ¹	transcriptional repressor involved in the regulation of glucose transporter genes ²	Vandeputte <i>et al.</i> , 2011 ¹ Brown <i>et al.</i> , 2006 ²
RTG1	reduced kidney colonization ¹ impaired in gut colonization ^{1,2}	involved in regulation of galactose catabolism genes ³ and sphingolipid homeostasis ⁴	Perez <i>et al.</i> , 2013 ¹ Böhm <i>et al.</i> , 2017 ² Dalal <i>et al.</i> , 2016 ³

			Moreno-Velasquez <i>et al.</i> , 2020 ⁴
RTG3	defective in kidney colonization ¹ impaired in gut colonization ^{2,3}	involved in regulation of galactose catabolism genes ⁴ and sphingolipid homeostasis ⁵	Noble <i>et al.</i> , 2010 ¹ Perez <i>et al.</i> , 2013 ² Böhm <i>et al.</i> , 2017 ³ Dalal <i>et al.</i> , 2016 ⁴ Moreno-Velasquez <i>et al.</i> , 2020 ⁵
SEFI	defective in kidney colonization ^{1,2} impaired in gut colonization ³	activator of both iron uptake and iron-utilization genes ³	Noble <i>et al.</i> , 2010 ¹ Vandeputte <i>et al.</i> , 2011 ² Chen <i>et al.</i> , 2011 ³
SFU1	promotes GI tract colonization ¹	iron regulation; restricts iron uptake ^{1,2}	Chen <i>et al.</i> , 2011 ¹ Lan <i>et al.</i> , 2004 ²
TEC1	defective in virulence ¹ impaired in gut colonization ²	contributes to biofilm formation and morphogenesis ^{1,2}	Schweizer <i>et al.</i> , 2000 ¹ Rosenbach <i>et al.</i> , 2010 ²
TRY4	impaired in gut colonization ¹	regulator of yeast cell adherence ^{1,2}	Böhm <i>et al.</i> , 2017 ¹ Finkel <i>et al.</i> , 2012 ²
TYE7	impaired in gut colonization ^{1,2}	regulates expression of glycolytic genes ³	Perez <i>et al.</i> , 2013 ¹ Böhm <i>et al.</i> , 2017 ² Askew <i>et al.</i> , 2009 ³
UPC2	increased kidney colonization ¹ but attenuated in virulence ²	regulator of ergosterol biosynthetic genes and sterol uptake ^{3,4}	Vandeputte <i>et al.</i> , 2011 ¹ Lohberger <i>et al.</i> , 2014 ² Silver <i>et al.</i> , 2004 ³ Hoot <i>et al.</i> , 2010 ⁴
WOR1	promotes commensalism in the GI tract ^{1,2}	required to establish and maintain the opaque state ³	Pande <i>et al.</i> , 2013 ¹ Prieto <i>et al.</i> , 2017 ² Huang <i>et al.</i> , 2006 ³
ZCF8	impaired in gut colonization ¹	regulator of yeast cell adherence ^{1,2}	Böhm <i>et al.</i> , 2017 ¹ Finkel <i>et al.</i> , 2012 ²
ZCF21	increased kidney colonization ¹ impaired in gut colonization ²	regulates expression of cell surface proteins and cell wall modifying proteins ^{3,4}	Vandeputte <i>et al.</i> , 2011 ¹ Perez <i>et al.</i> , 2013 ² Böhm <i>et al.</i> , 2016 ³ van-Wijlick <i>et al.</i> , 2016 ⁴
ZFU2	increased kidney colonization ¹ impaired in gut colonization ²	regulator of yeast cell adherence ^{1,2}	Vandeputte <i>et al.</i> , 2011 ¹ Böhm <i>et al.</i> , 2017 ²

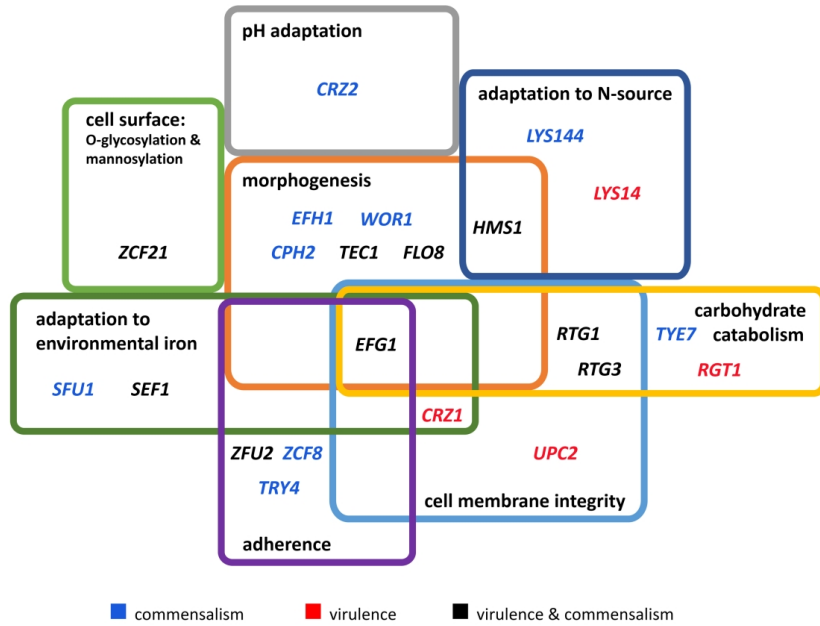


Figure 1: Transcriptional regulators involved in commensalism and virulence of *C. albicans*

A summary of *C. albicans* transcription regulators which have been identified to play a major role in commensal life (in blue: CRZ2, LYS144, EFH1, WOR1, CPH2, SFU1, TRY4, TYE7 and ZCF8), virulence (in red: CRZ1, LYS14, RGT1 and UPC2) or both (in black: EFG1, FLO8, HMS1, RTG1, RTG3, SEF1, TEC1 and ZFU2) and their functions in the cell: adaptation to pH (grey box), to N-source (dark blue box), or to external iron (dark green box), morphogenesis (orange box), carbohydrate catabolism (yellow box), cell membrane integrity (light blue box), adherence (purple box) and cell surface (light green box). For references, see Table 1. Functions supported by the identified regulators indicate that both commensalism and virulence require adaptation to available resources of nitrogen and carbohydrate as well as adaptation to iron, required to maintain energy metabolism but which in high concentrations can also be toxic to cells. Sensing and signalling changes in environmental pH appear to be vital for the fungus survival. Adaptation to the human host also presupposes that *C. albicans* is able to undergo morphological changes, that involve changes in the cell membrane and the cell surface. In line with this, adherence appears to be important to establish the commensal lifestyle in the gut.