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# 1 From Commensal to Pathogen: *Candida albicans*

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## I. The Commensal School: Commensal and Pathogenic Attributes

Microbial infections are caused by bacteria, parasites or fungi that have distinct properties necessary for colonisation, survival and replication on or within their hosts, thereby causing damage and disease. They may have been acquired from the environment, from other hosts or from the host's own microbiota. However, not all hosts are equally susceptible to microbial infections and

even closely related fungal species can have very different ecologies and relationships with their hosts. **Thus, although virulence is due to microbial attributes, these attributes are only expressed in a susceptible host** (Casadevall and Pirofski 2007). This susceptibility can be defined by the host species, certain variants of a host species, the immune status of a host or the normal microbial flora of the host.

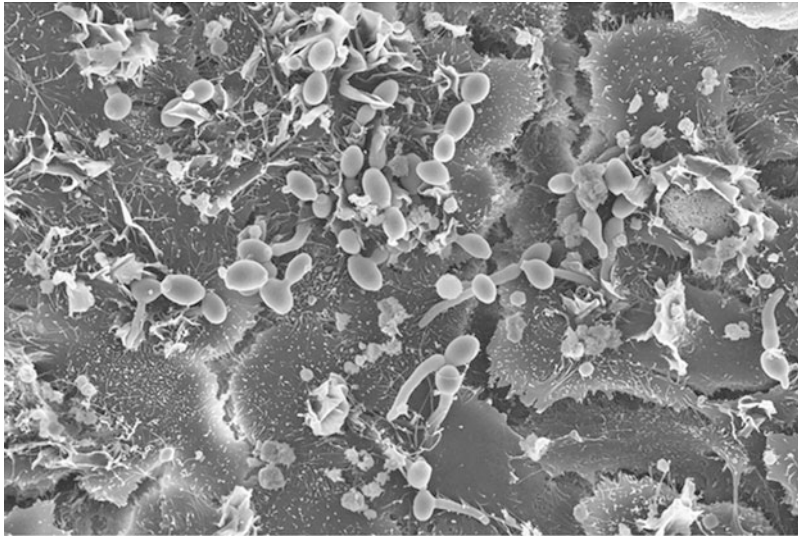
Most human pathogenic fungi, for example *Aspergillus fumigatus*, *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Paracoccidioides brasiliensis*, *Penicillium marneffe* and *Coccidioides immitis*, are environmental fungi, which normally live outside the human body and can cause exogenous infections. These fungi must have gained their pathogenic potential in certain environmental niches that resemble aspects of the human body. In these “environmental virulence schools” (Casadevall 2008), fungi must have been adapted to host-like environments during evolution and thus have acquired virulence attributes.

However, not all fungal pathogens of man come from the environment (or other non-human hosts). **In fact, the overwhelming proportion of infections caused by fungi are caused by organisms that are normally associated with human hosts.** These include *Candida*, *Malassezia* and *Pneumocystis* species and many dermatophytes (e.g. *Trichophyton rubrum*). With analogy to the environmental virulence schools, the virulence potential of these endogenous fungi can be explained by training in the “commensal virulence school” (Hube 2009). In the commensal environment, these fungi are constantly challenged by the host and other microbial inhabitants of the host and have evolved commensal factors, which can turn

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**Fig. 1.1.** Scanning electron micrograph of *C. albicans* growing on oral epithelial cell layer, in yeast, hyphal and pseudohyphal morphologies. Hyphae invade via

active penetration and induce endocytosis, indicated by membrane ruffling (Zakikhany K, Holland G, Özel M, Hube B, with permission)

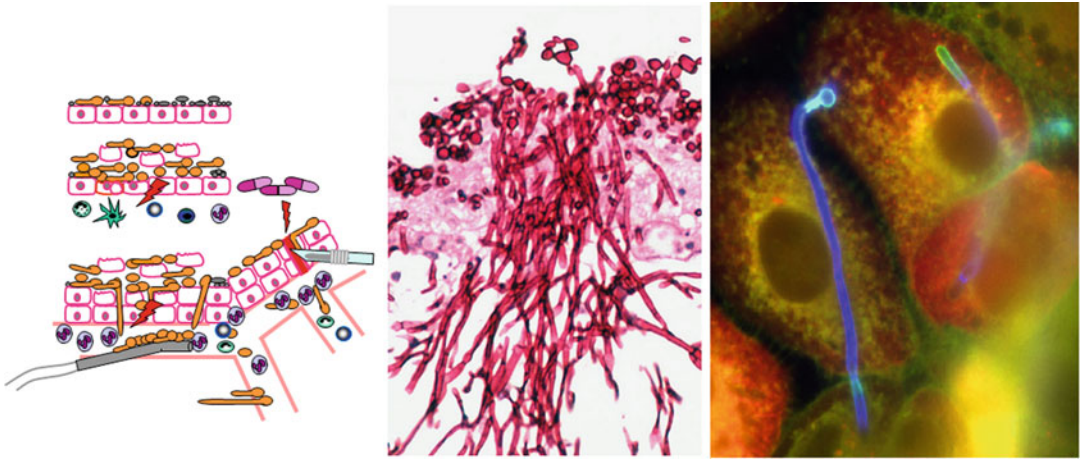
into virulence attributes once the conditions favour infection. In this chapter, we will describe some of the key commensal and virulence attributes of the most common pathogenic yeast, *C. albicans*.

## II. Commensal Growth

During commensal growth on mucosal surfaces, *C. albicans* has to get hold of the mucus or adhere directly to epithelial cells (Fig. 1.1). Therefore, the fungus must express adhesion factors. Although yeast cells of *C. albicans* express adhesive proteins on their surface (Zupancic and Cormack 2007), some of the most dominant adhesins are known to be associated specifically with hyphal morphology in vitro. However, it is not clear whether yeast cells or hyphae or both are characteristic of commensal colonisation. Because the expression of hyphal-associated genes has been detected during asymptomatic carriage, it is possible that commensal cells include hyphae (Mochon et al. 2010; Naglik et al. 2006, 2003). However, it has also been shown that genes normally hyphal-associated can also be expressed by yeast cells under certain conditions (Andaluz et al. 2006; Fradin et al. 2005; Rosenbach et al. 2010; Sosinska et al. 2008; White et al. 2007).

This includes the in vivo expression of certain hyphal-associated genes by *C. albicans* yeast cells colonising the murine gastrointestinal tract (after removal of the bacterial flora), a niche where the dominant morphology has been shown to be yeast cells (Rosenbach et al. 2010; White et al. 2007).

In the commensal phase, *C. albicans* co-exists and interacts with bacteria of the microflora (Piispanen and Hogan 2012). These probiotic bacteria generally contribute to a balanced and protective immunity and can produce molecules such as fatty acids, thereby directly inhibiting fungal growth, blocking epithelial binding sites and competing for nutrients. Although nutrients are relatively rich in the gut, they are quickly absorbed by the microbial flora and epithelial cells, forcing colonising *C. albicans* cells to **use nutrient acquisition mechanisms efficiently and to be metabolically flexible**. Changing conditions (e.g. pH, osmolarity) in different host niches probably require stress adaptation mechanisms to be deployed for growth to be permissible. It also seems feasible that commensal microbes, such as *C. albicans*, have developed immune evasion strategies to avoid recognition because the trigger of immune responses or inflammation would cause additional stress. Nevertheless, occasionally invading hyphae may cause mild local



**Fig. 1.2.** *Left:* Cartoon of the various states of *C. albicans* from commensalism on the epithelial surface to invasion and pathogenicity due to insult via immunosuppression, injury and introduction as a biofilm on foreign bodies. *Middle:* Invasive properties of hyphae of *C. albicans* on model epithelium from chick chorioallantoic membrane

(Gow NAR, with permission). *Right:* *C. albicans* inter-epithelial invasion of hyphae through one oral epithelial cell and invasion into an adjacent cell. Dark blue parts of hyphae are intracellular and bright blue parts are extracellular (Almeida R, Hube B, with permission)

damage, thereby attracting phagocytes. These transient confrontations with the immune system may select for fungal attributes that enable fungal cells to cope with situations beyond the commensal environment. This may include the refinement of transcriptional programs that prepare invading cells for challenges associated with the transition from commensalism to pathogenicity – a phenomenon that can be described as “predictive adaptation”. One of these programs is the transition from yeast to hyphal growth and the expression of hyphal-associated genes. These genes encode adhesins, invasins, iron acquisition factors, hydrolases, damaging factors and enzymes that neutralise reactive oxygen species.

In the invasive phase, fungal hyphae, not yeast cells, invade into and through epithelial cell layers, damage their membranes and exploit their cellular content as nutrients. At this stage, fungal cells are recognised, the immune system is activated and armed, and phagocytes (monocytes, macrophages, dendritic cells, neutrophils) are attracted, establishing a local battlefield. If the fungus succeeds and invades into deeper tissue or gains access to blood vessels via damaged barriers, fungal cells may enter the blood stream and escape through the endothelial layers to colonise the major organs, causing life-

threatening systemic infections (Fig. 1.2). Attributes that enable the fungus to invade, cause damage, counteract the immune system, cope with changing stresses, exploit host molecules as nutrients, and colonise non-commensal niches are true pathogenicity attributes and are described in this chapter.

### A. Nutrient Acquisition and Metabolic Flexibility

During the transition from commensal growth to pathogenicity, and during dissemination and colonisation of the different host organs and niches, the available local nutrients change dramatically. The fact that *C. albicans* can proliferate in all these niches indicates a high degree of metabolic flexibility and suggests that several host sources can be exploited as nutrients. During systemic infections, the fungus disseminates via the bloodstream, which is relatively rich in glucose (6–8 mM), the preferred carbon source of *C. albicans*. However, circulating phagocytes, in particular neutrophils and to a lesser extent monocytes, efficiently phagocytose *C. albicans*. Within the phagosome, the fungus is exposed to a nutrient-starved environment and **switches from glycolysis to gluconeogenesis and activates**

**the glyoxylate cycle.** In fact, key enzymes of the glyoxylate cycle, such as isocitrate lyase and citrate synthase seem to be required for full virulence of *C. albicans* (Fradin et al. 2005; Lorenz and Fink 2002; Miramón et al. 2012). Single-cell profiling and global transcriptional profiling (Barelle et al. 2006; Thewes et al. 2007; Zakikhany et al. 2007) of hyphae invading epithelial tissues or organs showed that genes encoding for enzymes of both the glycolysis and glyoxylate cycles are activated, possibly reflecting different subpopulation of cells. Secreted lipases (Lips) and phospholipases B (PLBs) of *C. albicans* can degrade host lipids and phospholipids to provide non-glucose carbon sources. Secreted aspartic proteases (Saps) are able to hydrolyse host proteins, thereby releasing peptides and amino acids that can serve as a source of carbon and nitrogen. Indeed, *C. albicans* can use all natural amino acids as nitrogen sources (our unpublished data).

Interestingly, growth on carbon sources available within the host other than glucose, such as lactate or amino acids, can render *C. albicans* more resistant to environmental stresses and can increase its virulence potential in vivo (Ene et al. 2012). A combination of carbon sources, or alternatives to glucose and fructose such as pyruvate, sorbitol, oleic acids and galactose, increase resistance to osmotic stress. With the exception of oleic acid, this corresponds to an increase in kidney burden in systemically infected mice (Ene et al. 2012). However, the impact of carbon source on virulence depends on the site of infection in mouse models, e.g. cells grown on oleic acid produce greater vaginal infection and low systemic infection, although systemic infection is enhanced by growth on lactate and amino acids (Ene et al. 2012). Additionally, unlike the non-pathogenic *Saccharomyces cerevisiae*, *C. albicans* is able to assimilate lactate and oleic acid in the presence of glucose (Sandai et al. 2012), allowing *C. albicans* to swiftly adapt to an environment in which nutrient availability can change as quickly as a phagocyte can engulf a cell. This metabolic flexibility and the consequent protective benefits under stress give *C. albicans* an advantage within various host environments, under either commensal or pathogenic conditions.

## B. Micronutrients

Iron is an essential element for both the host and *C. albicans*. However, in the host, iron is almost completely associated with host proteins, which prevents not only iron-dependent production of toxic free radicals, but also microbial growth ("nutritional immunity"). Like other commensals and many other pathogens, *C. albicans* **has developed several strategies for iron acquisition within the host** (Almeida et al. 2009). Although *C. albicans* cannot produce siderophores, the fungus can **exploit iron from siderophores produced by other microbes**, an iron acquisition strategy that may be particularly useful in the commensal stage. Siderophores and other iron complexes are taken up by the Sit1/Arn1 transporter (Heymann et al. 2002; Hu et al. 2002).

In addition to uptake of siderophores, *C. albicans* can **exploit iron from iron-containing host proteins**. For example, haemoglobin can be used as an iron source during the pathogenic stage. Haemoglobin iron utilisation may occur predominantly via hyphae, which can bind to erythrocytes (Moors et al. 1992). Following the release of haemoglobin by an unknown mechanism, specific receptors on the surface of *C. albicans* such as *RBT5*, *RBT51*, *WAP1/CSA1*, *CSA2* and *PGA7* mediate uptake (Weissman and Kornitzer 2004). This process also seems to be hyphal-associated because the expression of some haemoglobin receptors is co-regulated with hyphal formation (Braun et al. 2000). Once haemoglobin is internalised, it is hydrolysed or denatured and haem is released (Weissman et al. 2008). A haem oxygenase (Santos et al. 2003), encoded by *HXM1*, degrades haem intracellularly (Pendrak et al. 2004). Another host molecule that can serve as an iron source for *C. albicans*, at least in vitro, is transferrin. Although the transferrin receptor remains unknown, it was shown that binding is necessary for iron utilisation from transferrin (Knight et al. 2005). Furthermore, *C. albicans* is able to bind the main host iron storage protein ferritin on the surface of hyphae via the hyphal-associated adhesin and invasin Als (see below) (Almeida et al. 2008). Following binding, the iron content of ferritin must be released by a mechanism that probably includes acidification.



In fact, *C. albicans* is only able to use ferritin as an iron source under conditions that permit acid production, suggesting that iron acquisition from ferritin by *C. albicans* is pH-mediated (Almeida et al. 2008).

To utilise iron from transferrin, from ferritin or from the environment, *C. albicans* uses the reductive pathway, which consists of several ferric reductases, multicopper oxidase(s) and iron permeases. The extracellular pH greatly influences the availability of iron because neutral and alkaline pH favours the oxidation from the soluble  $\text{Fe}^{2+}$  to the non-soluble  $\text{Fe}^{3+}$  ion. The *RIM101* pathway of *C. albicans* is required for an appropriate transcriptional response to the environmental pH by coordinating the upregulation of genes involved in the reductive pathway, for example by upregulation of ferric reductases and the ferritin receptor Als3 under alkaline conditions (Baek et al. 2008; Liang et al. 2009; Nobile et al. 2008).

In addition to iron, further **trace metals such as zinc, manganese and copper are also essential for growth and survival of *C. albicans*** in the commensal and pathogenic phases, suggesting that nutritional immunity is a much more extensive phenomenon than iron sequestration. Yet, the mechanisms of acquisition of metals other than iron are largely unknown. A novel mechanism for zinc acquisition by *C. albicans* was recently described (Citiulo et al. 2012) by which the fungus secretes the zinc-binding pH-regulated antigen 1 (Pra1). This binds extracellular zinc and re-associates with a Pra1 receptor and zinc transporter (Zrt1) on the cell surface. Because this system resembles iron uptake via siderophores, Pra1 was named a “zincophore”.

### III. Stress Response

Whether in the pathogenic phase or commensal phase of interaction with the host, *C. albicans* faces a number of environmental and immune-derived stresses. Each niche with which the fungus is associated presents unique environmental factors, from the varying pH of the gastrointestinal and urogenital tracts (Brown et al. 2012) to the osmotic pressures of the oral mucosa and kidneys. A multifaceted and robust stress response is crucial for commensal adaptation, but also for virulence (Brown et al. 2012).

Many environmental and stress signals in fungi are sensed via mitogen-activated protein kinase (MAPK) pathways. As with numerous classical signalling pathways, particularly MAPK pathways, signals are propagated as a series of phosphorylation events, each kinase phosphorylating the next in turn until transcription factors are activated. Three such MAPK pathways are activated in *C. albicans*, with each pathway distinguished by a specific kinase, Mkc1, Hog1 or Cek1 (Monge et al. 2006).

The Hog1 MAPK pathway is considered a core response and is activated after many types of stresses (Monge et al. 2006), including oxidative and osmotic stresses. Hog1 also plays a role in morphogenesis, cell wall formation and the response to thermal stress (Monge et al. 2006). However, Hog1 phosphorylation occurs at different times post stress in response to osmotic, oxidative and nitrosative stresses, even responding to osmotic stress from sodium chloride and sorbitol at distinct time points (Kaloriti et al. 2012). Activation of Hog1 in response to osmotic stress leads to accumulation of glycerol within the cell, which counters the loss of water due to chemical gradients (Monge et al. 2006). Also responding to oxidative and osmotic stress, the Mkc1 pathway is primarily noted for cell wall salvage and biogenesis as well as for maintaining cellular integrity (Monge et al. 2006; Munro et al. 2007), whereas the Cek1 pathway mediates filamentation and mating, and possibly responds to quorum sensing molecules (Mayer et al. 2012; Monge et al. 2006). Cells without Cek1 are unable to form hyphae in low-nitrogen media, although this can be overcome by the presence of serum (Monge et al. 2006). As would be anticipated, the ability to respond to the host battery of stresses is crucial for the success of *C. albicans* as a pathogen because deletion of the major stress response components Mkc1, Hog1 or Cek1 results in attenuated virulence in the mouse model (Alonso-Monge et al. 1999; Csank et al. 1998; Diez-Orejas et al. 1997).

Within the host, further environmental stressors are also produced by the immune system, specifically phagocytic cells capable of producing reactive oxygen species (ROS) and reactive nitrogen species (RNS). In its role as a pathogen, *C. albicans* copes with both of these stresses with a series of **detoxifying enzymes**. The oxidative stress response to ROS such as hydroxyl radicals and peroxide includes synthesis of antioxidants, detoxifying enzymes such as catalase (Cta1) and superoxide dismutases (Sod1-5), and repair of damaged proteins (Brown et al. 2012; Hwang et al. 2002; Martchenko et al. 2004; Wysong

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