CHAPTER 1

Candida albicans: A cellular and molecular perspective

1.1. Introduction:

The polymorphic yeast *Candida albicans*, like many pathogens, has both a benign and pathogenic association with its host. Candida forms a part of the commensal flora where healthy humans are estimated to have carriage rates of almost 50% in the gastrointestinal tract and between 10 and 20% in the oral cavity, anorectal tract, and vagina. In its pathogenic state, it causes a wide variety of diseases, including common mucosal manifestations such as oropharyngeal thrush and vaginitis (Lorenz *et al.*, 2004). In hospitalized patients, disseminated candidiasis is the fourth most common infection outpacing all gram-negative species and carries a mortality rate in excess of 30%. The pathogenesis of *C. albicans* infection is postulated to involve adhesion to host epithelial and endothelial cells and morphologic switching of yeast cells from the ellipsoid blastospore to various filamentous forms: germ tubes, pseudohyphae, and hyphae (Odds, 1988). Since there are limited treatment options, development of the next generation of antifungal agents will require a further understanding of the biology of infection. Recently, O-GlcNAc signaling is emerging as an integral system and viable target for biomedical investigation. This system may be a boundless source for insights into a variety of diseases and yield numerous opportunities for drug design.

Many human fungal pathogens undergo morphological transitions when they invade the human host. Changes in transcriptional regulation are important for generating phenotypic diversity among species, but the mechanisms underlying these regulatory changes are not well understood. Recent studies that measured nucleosome occupancy genome-wide have revealed strong associations between chromatin organization and gene expression (Lee, W. *et al.*, 2007). However, the relationship between evolutionary changes in DNA encoded nucleosome organization and expression divergence has not been examined.

Field *et al* (2009) studied the relationship between gene expression and the DNA-encoded nucleosome organization of promoters across two yeast species, the budding yeast *Saccharomyces cerevisiae* and the human pathogen *Candida albicans*, for which large compendia of gene expression data are available. These species show several phenotypic differences. Most notably, in high glucose, *C. albicans* grows by respiration and correspondingly activates transcription of genes required for the TCA cycle and
oxidative phosphorylation, whereas *S. cerevisiae* grows primarily by fermentation and correspondingly reduces transcription of respiration genes.

The study revealed that this large scale change in the expression of respiration genes is achieved, at least in part, through DNA sequence changes that alter the DNA-encoded nucleosome organization in their promoters. A strong support for this proposed mechanism comes out from the fact that these changes in nucleosome organization are also seen in a reconstitution of nucleosomes on purified DNA from *S. cerevisiae* and *C. albicans*. A system-level reprogramming of the yeast transcriptional network is associated with, and presumably achieved, in part, through evolution of intrinsic nucleosome organization encoded in the DNA sequence of promoters. This evolutionary mechanism for genetic change may also account for other types of phenotypic diversity observed across eukaryotic species. Yeast species exhibit a simple relationship between transcriptional programs and nucleosome organizations encoded in promoter sequences. Promoters of genes that are required for the typical mode of growth tend to encode relatively open nucleosome organizations, whereas promoters of genes that are not part of the typical growth pathways of the organism (for example, condition-specific and stress-response genes) tend to encode relatively closed nucleosome organizations. Notably, this relationship continues to hold even after the divergence of yeast into species that grow aerobically through pathways that involve cellular respiration and mitochondrial genes, and species that grow anaerobically through pathways that do not involve these genes.

1.2. Morphogenetic machinery:

*Candida albicans* is termed a dimorphic fungus because it proliferates in either a yeast form or a hyphal form. The switch between these forms is the result of a complex interplay of external and internal factors and the ability to switch between different growth forms appears to be linked to its capacity to establish and disseminate infections in the human host (Lo *et al.*, 1997; Gow *et al.*, 2002; Saville *et al.*, 2003; Zheng *et al.*, 2004).

This is coordinated in part by polarity-regulating proteins that are conserved among eukaryotic cells. However, yeast and hyphal cells are not the only morphological states of *C. albicans*. The opaque form required for mating, the pseudohyphal cell, and the chlamydospore represent distinct cell types that form in response to specific genetic or
environmental conditions. In addition, hyperextended buds can form as a result of various cell cycle-related stresses. Recent studies are beginning to shed light on some of the molecular controls regulating the various morphogenetic forms of this fascinating human pathogen (Fig: 1.1.).

1.2.1. Polarity Determinants:

It is believed that each form of growth provides critical functions required for the pathogenic lifestyle (Sudbery P, Gow N, Berman J. 2004). In both modes the cells exhibit polarized growth; the degree of polarity is more extreme in the hyphal state. In general, polarized growth is an important characteristic of cells, with intrinsic cues and extrinsic signals combining to determine both the shape of individual cells and their spatial organization in multicellular structures. Eukaryotic cells typically use small GTPases of the Rho superfamily, in particular members of the Cdc42/Rac group of enzymes, to regulate the overall process (Fukata M, Nakagawa M, Kaibuchi K. 2003, Jaffe AB, Hall A. 2005.); these GTPases appear implicated primarily in regulating actin polymerization and thus in controlling polarized growth and secretion through modulation of the actin cytoskeleton (Johnson DI. 1999.). Cdc42p is a central regulator of a complex of proteins required for proper polarized growth. This complex, initially identified in budding yeast and termed the polarisome (Sheu YJ, Barral Y, Snyder M. 2000), contains the formin Bni1p as well as the proteins Bud6p, Spa2p, and Pea2p. The polarisome is also implicated in polarized growth in C. albicans, as deletion of the homologs of Bni1, SPA2, and BUD6 results in similar phenotypes as in S.cerevisiae. Yeast-growth cells exhibit random budding and delocalized surface growth, leading to round cells with large bud necks; in hyphal growth conditions these mutants make abnormally thick hyphae. Although cells can still polarize in the absence of polarisome factors, polar growth cannot be maintained.

Unlike S. cerevisiae, but like higher cells, C. albicans also has a homolog of the Rac GTPase. This protein, termed Rac1p, plays a role distinct from that of Cdc42p, and is not needed for budding and proper actin organization, but it is needed for filamentation in matrix-embedded conditions (Bassilana M, Arkowitz RA. 2006.). Thus, there may be environment-specific polarity determining factors in hyphae. Components that directly interact with the actin cytoskeleton also play important roles in the control of C. albicans polarity.
Distinct morphological forms of *C. albicans*. In yeast-form growth a blastospore buds off a new cell, resulting in two discrete cells. The separated spindle pole bodies elaborate spindles that separate the chromosomes across the mother-daughter junction defined by a septin band (green). In pseudohyphal growth the nuclear division also crosses the mother-daughter junction defined by a septin band, and polarized growth is characterized by the polarisome. The cells themselves are more elongated than during yeast growth, and the cells remain attached after cytokinesis. Hyphal growth is defined by both a polarisome and a Spitzenkorper at the tip of the growing hyphae. Opaque-form cells are capable of responding to mating pheromone by elongating a mating projection, or shmoo. Chlamydospores are formed at the end of suspensor cells. They have a thicker cell wall and are larger than blastospores; the nucleus divides within the suspensor cell and then the daughter nucleus migrates into the chlamydospore across a septin structure. (Adapted from Malcolm Whiteway and Catherine Batchwitch. *Annu. Rev. Microbiol.* 2007.)
1.2.2. Spitzenkorper and hyphal development:

Polarised growth in filamentous fungi is much more extreme than in budding yeast (Hickey, 2001). A special structure, called the Spitzenkörper (apical body), which is located at or just behind the hyphal tip, is responsible for this dramatic polarisation of growth (Girbardt, 1957; Harris et al., 2005). The Spitzenkörper was originally recognised as a dark region in phase-contrast microscopy at the tip of actively growing hyphae (Girbardt, 1957). Subsequently, freeze-substitution electron microscopy revealed that it is rich in secretory vesicles (Grove and Bracker, 1970; Howard, 1981). More recently, it was shown that the amphiphilic styryl dye, FM4-64, labels the Spitzenkörper in numerous fungi and reveals it to be a 3D structure. (Fischer-Parton et al., 2000). The Spitzenkörper is thought to drive hyphal growth because changes in the direction of hyphal growth are anticipated by changes in the position of the Spitzenkörper (Reynaga Pena et al., 1997; Lopez-Franco, 1996) and is believed to have a function similar to that of the polarisome of the budding yeast (Harris SD, Read ND, Roberson RW, Shaw B, Seiler S, et al. 2005.). However, it does not appear that the switch from yeast to hyphal growth in C. albicans occurs by the transfer of regulatory control from a polarisome to a Spitzenkörper, as both elements have been identified in hyphal cells (Crampin H, Finley K, Gerami-Nejad M, Court H, Gale C, et al. 2005.) and, as noted, hyphal cells lacking polarisome components do not generate normal hyphae. Thus, the Spitzenkorper appears to be one of the unique characteristics of the hyphal growth pattern in C. albicans, and it may function in conjunction with, instead of in place of, the polarisome (Crampin H, Finley K, Gerami-Nejad M, Court H, Gale C, et al. 2005), (Fig:1.2.).

Because the Spitzenkorper is identified primarily as a vesicle-rich structure currently visualized in living cells through staining with dyes such as FM4–64, the protein/enzymatic components of the Spitzenkorper are as yet poorly characterized. In C. albicans the polarisome proteins Spa2p and Bud6p are generally not associated with the Spitzenkorper, whereas Bni1p and, when overexpressed, a fraction of Cdc42p are apparently associated with this structure. The Golgi structure of hyphal C. albicans cells is fundamentally relocalized to the growing tip of the cell, and this organellar redistribution is dependent on the formin Bni1p (Rida PC, Nishikawa A, Won GY, Dean N. 2006). It does not appear that this repositioned Golgi represents the Spitzenkorper, but the ability to identify cellular localization of membranes through
Fig:1.2. Colony morphologies of Candida albicans. A single strain can take on different colony morphologies on different media or as a consequence of PHENOTYPIC SWITCHING. a | Smooth colonies grown on salt-dextrose complete (SDC) medium; b | wrinkled colonies grown on spider medium; c | fuzzy colonies grown on milk-Tween agar; and d | embedded colonies suspended in a matrix of rich medium that contains sucrose. e | White-opaque phenotypic switching is seen here on SDC medium maintained at 23 °C. White cells (W) of the WO-1 strain were plated at 23°C for three days, and opaque colonies (O) and sectors appeared in the population. f,g | Cells in wrinked, embedded and fuzzy colonies are a mixture of yeast, pseudohyphal and true hyphal cells. A population of cells derived from different portions of wrinkled colonies is shown. (Adapted from Judith Berman and P.E.Sudbery. Nature Reviews Genetics 3, 3002).
staining with lipophilic dyes, and the ability to localize proteins through green fluorescent protein (GFP) tagging, will permit the identification of genes required for Spitzenkorper formation and positioning and the proteins that colocalize with the Spitzenkorper.

1.2.3. **Nuclear Positioning and Division:**
In addition to the presence of the Spitzenkorper, other characteristics also distinguish a *C. albicans* cell initiating a bud from those initiating a germ tube that elaborate into hyphae. A critical difference is the arrangement of the septum and the division of the chromosomes. In yeast cells, as in cells of *S. cerevisiae*, the dividing nucleus is positioned to the neck between the mother and daughter cells, and the nuclear division partitions one chromosomal complement into the mother cell and the other into the daughter cell. This division takes place across the septum structure, which ultimately defines the site of cytokines (Sudbery P, Gow N, Berman J. 2004.). A molecularly similar event occurs in pseudohyphal cells—the nuclear division also occurs between the mother and daughter cell, and although the dividing cells are elongated relative to the yeast cells, the behavior of the dividing nucleus and the organization of the septum are similar.

The cellular behavior of the nucleus is distinct in unbudded yeast cells that are elaborating a germ tube while in the G1 phase of the cell cycle. In these cells the nuclear division and the position of the initial septum take place in the elongating germ tube rather than at the junction between the mother cell and the polarizing structure (Finley KR, Berman J. 2005.). The nucleus migrates into the extending germ tube, divides across the site of the incipient septum, and then one nucleus migrates back into the mother cell and the other moves out toward the tip of the elongating germ tube. This complex movement of the nucleus during the initial steps of hyphal development is orchestrated by microtubules. The dividing cell appears to be committed to elaborate a germ tube or a bud prior to any morphological distinction in the emerging polarized structure. However, the cell fate commitment appears to be reversible. Yeast cells in later cell cycle stages can be induced to form hyphae from their established buds (Finley KR, Berman J. 2005.). In these cells, the first nuclear division occurs across the bud neck, where there is also a constriction due to the initial bud emergence from the yeast cell. Thus, defining the initiation signals and how those signals are regulated by
external conditions will be an important goal of the next period of investigation of the yeast-to-hyphal transition.

1.2.4. Signaling Pathways:
The focus of many hundreds of research articles in the last ten years has revealed the complex and multiple signaling pathways and regulatory elements that collectively 'hard-wire' the yeast to hypha transition in *C. albicans* to environmental responses. These environmental conditions can vary considerably, but a standard trigger of hyphal development is the combined addition of serum to rich growth medium and the elevation of the growth temperature to 37°C (Whiteway M, Oberholzer U. 2004). However, nutrient-poor media such as Lee's or more chemically defined inducers such as N-acetyl-glucosamine, together with a rise in temperature to 37°C, are also suitable for inducing a high frequency of hyphal growth.

The initial observation that the combined loss of the Cph1p and Efg1p transcription regulators blocked the hyphal transition in most tested conditions, and led to reduced virulence, focused interest on these two transcription factors and their controlling networks. Subsequently, a large number of transcription regulators have been reported to play roles, either positive or negative, in the yeast-to-hyphal transition. Morphogenetic differentiation is accompanied by changes in gene expression of state-specific factors, many of which are also required for virulence. For example, comprehensive sets of hyphal, opaque and white specific genes have been identified through transcriptional profiling (Nantel A, Dignard D, Bachewich C, Harcus D, Marcil A, Bouin AP, Sensen CW, Hогues H, van het Hoog M, Gordon P et al., 2002; Lan CY, Newport G, Murillo LA, Jones T, Scherer S, Davis RW, Agabian N, 2002). Signal transduction pathways that may mediate the transcriptional changes associated with the morphological states have been identified (Berman J, Sudbery PE, 2002; Liu H, 2002; Dhillon NK, Sharma S, Khuller GK, 2003)(Fig :1.3.).

1.2.4.1. MAP Kinase Pathway:
Like *S. cerevisiae*, a mitogen-activated protein kinase (MAPK) pathway is involved in filamentation in *C. albicans*. The cascade consists of the kinases Cst20 (homologous to the p21-activated kinase [PAK] kinase Ste20), CaSte7/Hst7 (homologous to the MAP kinase kinase Ste7), and Cek1 (homologous to the Fus3 and Kss1 MAP kinases) (Clark et al., 1995; Kohler and Fink, 1996; Leberer et al., 1996; Singh et al., 1997; Whiteway
Fig: 1.3. **Signal transduction pathways that regulate morphogenesis.** At least four positive (arrowheads) and two negative (bars) pathways control morphological transitions in *Candida albicans*. The pathways that promote the switch from yeast to pseudohyphal and hyphal growth are shown as follows: MAP-kinase pathway in pink, cAMP pathway in green, Cph2 pathway in grey, Rim101 pH response pathway in blue and Czf1 matrix pathway in orange. Pathways that inhibit this switch are the Tup1–Nrg1–Rpg1 pathway in red and the Rbf1 pathway in purple. HSGs, hyphal-specific genes. *(Adapted from Liu et al., Curr. Opin in Microbiol, 2001).*
A transcription factor, Acpr/Cph1, which is homologous to the Ste12 that regulates mating and pseudohyphal growth in *S. cerevisiae*, has been identified (Malathi et al., 1994; Liu et al., 1994). Null mutations in any of the genes in the MAP kinase cascade (Cst20, Hst7, or Cek1) or the transcription factor Cph1 confer a hyphal defect on solid medium in response to many inducing conditions; however, all of these mutants filament normally in response to serum (Csank et al., 1998; Kohler and Fink, 1996; Leberer et al., 1996). Interestingly, although a cek1 MAP kinase mutant strain forms morphologically normal filaments in response to serum, it has a minor growth defect on serum-containing medium (Csank et al., 1998). The cek1 mutant strain also has a virulence defect that may be attributable to this growth defect (Csank et al., 1998, Guhad et al., 1998b). This indicates that the Cek1 MAP kinase may function in more than one pathway or that deletion of the gene causes aberrant cross talk between distinct MAP kinase cascades, similar to the altered signaling that occurs in a fus3 mutant of *S. cerevisiae*. The other elements of the pathway have small but varied effects on virulence. cst20 mutant strains have a modest virulence defect in a mouse model of systemic candidiasis (Leberer et al., 1996). However, hst7 and cph1 mutant strains are able to cause lethal infection in mice at rates comparable to wild-type strains (Leberer et al., 1996; Lo et al., 1997). In addition to these components, a MAP kinase phosphatase, Cpp1, has been identified which regulates filamentous growth in *C. albicans* (Csank et al., 1997). Disruption of both alleles of the CPP1 gene derepresses hyphal production and results in a hyperfilamentous phenotype. This hyperfilamentation is suppressed by deletion of the MAP kinase Cek1 (Csank et al., 1997). cpp1 mutant strains are also reduced for virulence in both systemic and localized models of candidiasis (Csank et al., 1997; Guhad et al., 1998a). Recently a G-protein α subunit homologue has been identified from *C. albicans*. Deletion of both the alleles of *GPA2* causes invitro defects in filamentation formation in Spider and SLAD media and in embedded conditions but not in serum containing media. These defects cannot be reversed by exogenous addition of cyclic AMP. However over expression of *HST7* can complement the filamentation defect showing that *GPA2* is required for MAP kinase signaling pathway (Sanchez-Martinez and Perez-Martin, 2002).

### 1.2.4.2. cAMP-PKA Pathway:

The cAMP-dependent protein kinase A (PKA) pathway plays a very important role in filamentation in *S. cerevisiae*, *C. albicans* and other fungi (Sonneborn et al., 2000). In


**Introduction**

*C. albicans*, an increase in cAMP level accompanies the yeast to hyphal transition, and inhibition of the cAMP phosphodiesterase induces this transition (Sabie and Gadd, 1992). Moreover, two cell-permeating PKA inhibitors, myristoylated protein kinase inhibitor (myrPKI) amide and the small-molecule PKA inhibitor H-89, both block hyphal growth induced by N-acetylglucosamine, but not in response to serum (Castilla et al., 1998).

The phosphodiesterase Pde2 that modulates the levels of cAMP is required for morphogenesis and virulence (Bahn YS, Staab J, Sundstrom P, 2003; Jung WH, Stateva LI., 2003). The regulatory subunit of the cAMP-dependent protein kinase, Bcy1, is required for localization of the catalytic subunit Tpk1 to the nucleus and is apparently essential (Cassola A, Parrot M, Silberstein S, Magee BB, Passeron S, Giasson L, Cantore ML., 2004). In addition, the G protein homolog Gpa2 appears to function upstream of the CEK1 MAP kinase pathway, and is required for hyphal development under multiple conditions (Sanchez-Martinez C, Perez-Martin J., 2002).

These signal transduction pathways form a complex and interconnected network that integrates environmental cues to control morphogenesis and transcription appropriately. The existence of integration within this network is well illustrated by the APSES protein Efg1, which receives information through multiple pathways and is involved in different programs such as hyphal development, chlamydospore formation, white-opaque switching and assembly of biofilms (Sonneborn A, Bockmuhl DP, Ernst JF., 1999; Stoldt VR, Sonneborn A, Leuker CE, Ernst JF., 1997; Tripathi G, Wiltshire C, Macaskill S, Tournu H, Budge S, Brown AJ., 2002). The specificity of the response may be achieved by phase-specific activation of EFG1, by modulation of EFG1 levels (Tripathi G, Wiltshire C, Macaskill S, Tournu H, Budge S, Brown AJ., 2002,) by post-translational modifications of Efg1 (Bockmuhl DP, Ernst JF., 2001), or by other transcription factors of the network that function synergistically with or antagonistically to Efg1. The use of whole genome profiling to study transcriptional networks should enhance our ability to unravel these complex webs of regulation.

One of the regulatory circuits controlling Efg1 function includes adenylyl cyclase and cAMP. Although the presence of cAMP is essential for morphogenetic switches such as the yeast-hyphal transition, it has not been conclusively established that cAMP levels serve as a metric for morphogenesis regulation. Exogenous addition of dibutyril cAMP enhances germ tube formation in wildtype cells, and rescues morphological defects associated with mutations in the cAMP-Ras pathway. Recently, the disruption of the
cAMP phosphodiesterase Pde2 (Bahn YS, Staab J, Sundstrom P., 2003, has been observed to raise basal cAMP levels. However, these two studies found different phenotypes for the pde2 mutants and measured different cAMP levels during a time course for germ tube formation. Further experimentation will be important to clarify whether cAMP is just permissive for morphogenetic differentiation, or whether it serves as a true signaling molecule.

A single Ras homolog, Ras1, has been identified in C. albicans, which is not essential for survival (Feng et al., 1999). The ras1 mutant strains have a severe defect in hyphal growth in response to serum and other conditions (Feng et al., 1999). In addition, while a dominant negative Ras1 mutation (RaslA16) caused a defect in filamentation, a dominant active Ras1 mutation (RaslV13) enhanced the formation of hyphae (Feng et al., 1999). The in vitro defects in morphological transition were reversed by either supplementing the growth media with cAMP or over expressing components of the filament inducing MAP Kinase cascade, demonstrating that it functions upstream of both cyclic AMP as well as MAP Kinase pathway (Leberer et al., 2001).

The Ras1 protein is likely to activate two protein kinase A (PKA) isoforms Tpk1p and Tpk2p (Bockmühl et al., 2001; Cloutier et al., 2003). tpk1 mutants are defective in hyphal formation on solid media but not so much in liquid (Sonneborn et al., 2000). Hyphal formation in tpk2 mutants, on the other hand, is partially affected on solid media but is blocked in liquid. A strain mutated for TPK2 containing a single allele of TPK1 under a regulatable promoter is unable to grow properly at low expression levels.

### 1.2.4.3. Other Signaling pathways and Regulators involved in Dimorphism

#### a) External pH:

The external pH is one of many signals important for regulating the yeast-to-hyphal transition. Many of the players in a pathway transmitting the pH signal to the intracellular machinery are homologs of the components identified in S. cerevisiae that also regulate response to pH. The main transcription factor is Rim101p (Davis D, Wilson RB, Mitchell AP. 2000), which is proteolytically activated by the Rim13p protease (Li M, Martin SJ, Bruno VM, Mitchell AP, Davis DA. 2004); loss of Rim101p function blocks alkaline-induced hyphal development. Rim20p and Rim8p act upstream of the proteolytic step (Davis D, Wilson RB, Mitchell AP. 2000), as do several membrane proteins, including Dfg16p, that may act as pH sensors (Barwell KJ, Boysen
JH, Xu W, Mitchell AP. 2005). The processing and activation of Rim101p also require members of the endosomal complex required for transport (ESCRT) protein module (Cornet M, Bidard F, Schwarz P, Da Costa G, Blanchin-Roland S, et al. 2005, Xu W, Smith FJ Jr, Subaran R, Mitchell AP. 2004). A large number of genes are under control of the Rim101p factor (Bensen ES, Martin SJ, Li M, Berman J, Davis DA. 2004), and connecting the expression of these genes to the morphological regulatory system is necessary to understand the link between pH sensing and morphogenesis.

b) Calcium signaling:
Calcium signaling has been implicated in the yeast-to-hypha transition. The A subunit of calcineurin, which is regulated by Ca2+ bound calmodulin (CaM) is necessary for proper hyphal development (Sanglard D, Ischer F, Marchetti O, Entenza J, Bille J, 2003) and a Ca2+/CaM-dependent protein kinase has been identified in C. albicans (Dhillon NK, Sharma S, Khuller GK, 2003).

c) Ume6:
Recently, CaUME6, a homolog of the Saccharomyces cerevisiae UME6 gene, has been shown to be required for hyphal elongation. The C. albicans ume6Delta strain showed a complete defect in hyphae formation under all the growth conditions tested. Wild-type UME6 expression depended on each hyphal regulator tested, and ectopic UME6 expression in efg1Delta, cph1Delta and ras1Delta cells rescued the hyphal defects of these mutants under some hyphal growth conditions. At low UME6 levels a particular subset of filament-specific genes is expressed that specifies growth largely in the pseudohyphal form. As UME6 levels rise, this same set of genes is expressed at a higher level and additional subset(s) of filament-specific genes are also expressed at increasing levels, causing cells to grow in the hyphal morphology. Thus, UME6 is a common downstream target of regulators promoting hyphal development.

d) Tec1:
Tec1 has recently been shown to regulate hyphal development and virulence in C. albicans (Schweizer et al., 2000). In S. cerevisiae, TEC1 transcription is regulated by Ste12, and cooperation between Tec1 and Ste12 is important for pseudohyphal growth (Madhani and Fink, 1997). In C. albicans, however, TEC1 transcription is not regulated by CPH1 (Lane et al., 2001a). tec1/tec1 mutants exhibit suppressed
filamentation in liquid serum-containing media. EFG1 overexpression does not suppress the morphological defect of the tec1/tec1 mutant, whereas TEC1 overexpression has a partial phenotype in the efg1/efg1 mutant (Schweizer et al., 2000). These results, coupled with the fact that efg1/efg1 strains have a more severe defect in hyphal development than do tec1/tec1 strains, suggest that TEC1 is one of the downstream effectors of Efg1.

e) Cph2:
Cph2, a myc family bHLH protein, has been found to regulate hyphal development in C. albicans (Lane et al., 2001b). cph2/cph2 mutant strains are impaired in hyphal development and in the induction of hypha-specific genes in liquid Lee’s media, and Cph2 is necessary for the transcriptional induction of TEC1. Cph2 binds directly to two sterol-regulatory-element-1-like elements upstream of TEC1. Furthermore, the ectopic expression of TEC1 expression suppresses the defect of cph2/cph2 in hyphal development. The function of Cph2 in hyphal transcription is therefore mediated, in part, through Tec1.

f) Bmh1:
Recently a 14-3-3 gene, BMH1 has been isolated from C.albicans. In S.cerevisiae, there are two such genes BMH1 and BMH2, which are essential for normal pseudohyphal induction and normal bud cell development. In C.albicans, however, there is just one copy of this gene and is essential for survival gene. The heterozygous mutant is defective in growth and morphogenesis (Cognetti et al., 2002).

g) Czf1:
An Efg1p-independent pathway of filamentation regulated by Czf1p which is an autoinhibited gene operates under microaerophilic/embedded conditions (Sonneborn et al., 1999a; Riggle et al., 1999; Giusani et al., 2002). The putative transcription factor Czf1 is probably an important element of the alternative pathway of filamentation in C. albicans (Brown et al., 1999). Homozygous czf1 null mutants filament normally under standard induction conditions, but they are defective in hyphal development when embedded in agar. Hyperfilamentation of efg1 and efg1 cph1 double mutants suggests that Efg1p is a negative modulator of the Czf1p pathway under microaerophilic/embedded conditions (Giusani et al., 2002). Separating the roles of the
physical matrix conditions from the changes in aeration associated with embedded hyphal development is important to establish a clear picture of this regulatory circuit.

**h) Flo8:**
The Flo8p transcription factor, which is essential for serum-induced hyphal development, controls a subset of Efg1p-regulated genes (Cao F, Lane S, Raniga PP, Lu Y, Zhou Z, *et al.* 2006). The regulation of Efg1p function through this pathway is suggested to be through direct phosphorylation by the cAMP dependent protein kinase (Bockmuhl DP, Ernst JF. 2001), and interaction with other regulators such as Flo8p and Czf1p may explain the ability of Efg1p to act both as an activator and as a repressor, depending on the conditions. As yet the direct relationships between the changes in gene expression and the changes in cell shape have not been established, and this will be an important area of future studies. Both Czf1p and Flo8p may physically interact with Efg1p, and Czf1p serves to permit hyphal growth during embedded growth by relieving the inhibitory action of Efg1p under these conditions.

**i) Cdc5:**
CaCDC5, a cell cycle regulator polo-like kinase in *C. albicans* has been identified. Cells lacking this gene are blocked early in nuclear division. The cell cycle defects are accompanied by the formation of hyphal like filaments under yeast growth conditions. The filaments resembled serum induced hyphae. Filament formation is not dependent on Cph1 or Efg1 but requires Cdc35 (Bachewich *et al.*, 2003).

**j) Mcm1:**
*C. albicans* homologue of *MCM1* was identified while screening for genes which could activate FLO11::lacZ expression in *S. cerevisiae*. Both over expression and repression of this gene led to the induction of hyphae. A hyphal specific gene *HWP1* was induced by repression of *CaMCM1*. But the change in expression was not dependent on *NRG1* or *TUP1* (Rottmann *et al.*, 2003). Thus *CaMCM1* is a component of a hitherto unknown regulatory mechanism of hyphal growth.

**k) Cdc42 and Cdc24:**
The function of different polarity establishment proteins in dimorphism was studied. Ectopic expression of a Rho G-protein, CaCDC42 (Ushinsky *et al.*, 2002; Hazan and
or it’s exchange factor Cdc24 were unable to form invasive hyphal filaments and germ tubes in response to serum or elevated temperature and yet could grow normally as a budding yeast. Further these mutants were avirulent in a mouse model for systemic infection. These results suggest that these proteins are required for invasive hyphal growth and pathogenicity of *C. albicans* (Bassilana et al. 2003).

1) **Csy1:**

The Csy1 amino acid sensor is important for activation of amino acid uptake and filamentation (Brega E, Zufferey R, Mamoun CB, 2004).

A final challenge will be to determine how the signaling pathways regulate the morphogenesis machinery. In the case of hyphal development, a promising target would be Cdc42 and the components of the polarisome, as Cdc42 was found localized at the hyphal tip throughout apical elongation (Hazan I, Liu H., 2002). Whether the polarisome is a direct target of signaling pathways, whether Cdc42p activity or localization is regulated through the transmission of external hyphae-inducing conditions, or whether the Spitzenkorper forms in direct response to the environment must be addressed. Molecular motors such as Myo5 are also localized to the hyphal tip (Oberholzer U, Marcil A, Leberer E, Thomas DY, Whiteway M., 2002) and we have much less information on this regulation of matrix-embedded growth (Cao F, Lane S, Raniga PP, Lu Y, Zhou Z, et al. 2006).

Overall, it is critical that researchers establish how these signaling pathways control the machinery directing polarized growth. Ultimately the link between signaling and morphogenesis must be made at the level of the growth pattern itself, not solely at the spectrum of gene expression correlated with this pattern.

1.2.5. **Roles of Cyclins and Cyclin-Dependent Kinases in Morphological Control:**

The central cell cycle regulatory kinase, as well as a group of regulatory cyclin molecules, has been identified in *C. albicans*, and several of these molecules appear to have specific roles in morphogenetic control. The initial identification of functional cyclins and the cyclin-dependent kinase was through analysis of *C. albicans* gene functions in *S. cerevisiae* (Sherlock G, Bahman AM, Mahal A, Shieh JC, Ferreira M, Rosamond J. 1994., Sheu YJ, Barral Y, Snyder M. 2000), but genome-sequencing
efforts have now allowed the comprehensive identification of the cyclin/kinase gene families in the fungal pathogen (Berman J. 2006).

As in the model yeast *S. cerevisiae* there is a single cyclin dependent kinase that is the key regulator of cell cycle progression, and this kinase interacts with a variety of cyclins to control the G1, S, and mitotic transitions, as well as aspects of morphogenesis (Mendenhall MD, Hodge AE. 1998).

Ccn1p is a member of the G1 class of cyclins on the basis of both structural and expression pattern similarity to G1 cyclins of yeast and is essential for the maintenance, but not initiation, of hyphal growth under specific nutrient conditions (Loeb JD, Sepulveda-Becerra M, Hazan I, Liu H. 1999).

The cyclin Hgc1p also shows structural similarity to the G1 cyclin class but was expressed only in hyphal cells. Hgc1p is required for hyphal development under a variety of tested conditions (Zheng X, Wang Y, Wang Y. 2004), and Hgc1p expression is limited to the apical, growing cell of the developing hyphae (Wang A, Lane S, Tian Z, Sharon A, Hazan I, Liu H. 2007).

Another G1 class cyclin, Cln3p is required for budding but not initiation of hyphal growth, as regulated shut-off of Cln3p expression leads to cell enlargement followed by initiation of germ tube extension (Bachewich C, Whiteway M. 2005). Currently the data linking cyclins and cell morphogenesis have focused primarily on the role of cell cycle regulators in the transition between the yeast and hyphal states. It is important to extend these observations to other aspects of morphogenesis. For example, cell cycle regulation is coupled to the development of the mating projection during mating, and the control of cyclin function is likely important for this process. Similarly, cell cycle control in the development of the chlamydospore is a critical component of the process. Overall the link between the cyclin-regulated function of the cyclin-dependent protein kinase and cellular morphogenesis is expected to be strong, and the challenge is to make this link in each of the unique morphological forms of *C. albicans* and then to establish the details of the regulatory circuit.

### 1.2.6. The White-opaque switch:

Although the dramatic change between the yeast and hyphal state is a well-studied hallmark of *C. albicans*, phenotypic switching in particular, the white-opaque
transition, has also been an area of extensive investigation. The white-opaque switch
was initially identified as a cellular and colony morphology transition that was limited
to specific strains. The switch was epigenetic—each state was stable but capable of
transitioning to the other state at a frequency that was higher than the standard mutation
rate, and the frequency could be modified by external conditions such as temperature
cells are of the classic ovoid shape characteristic of budding yeasts such as S. cerevisiae,
and they form domed colonies that are a creamy color. Opaque-form cells
are elongated and have a cell wall that has frequent pits (Anderson JM, Soll DR. 1987),
unlike the relatively smooth cell wall surface of white-form cells. Opaque-form cells
generate colonies that are flattened and more gray than the colonies generated by white-
form cells (Slutsky B, Staebell M, Anderson J, Risen L, Pfaller M, Soll DR. 1987). The
viability of opaque-form cells is reduced relative to white-form cells under many
growth conditions, and this allows the opaque-form cells to be distinguished as forming
colonies that can be more readily stained by vital dyes such as phloxine B.

Recent evidence has established that this white-opaque transition is intimately coupled
to the sexual mating process in C. albicans. The ability to switch to the opaque state
depends on whether the cells are homozygous for the MTL locus that controls cell type;
most C. albicans cells are heterozygous for the MTL locus (MTLa/MTLα) and thus are
unable to switch (Miller MG, Johnson AD. 2002). The inability of MTL homozygous
strains to switch is regulated by the α1/α2 repressor. This heterodimeric repressor is
derived from the α1 protein encoded by the MTLa locus, and the α2 protein encoded by
the MTLα locus, and thus can only be generated in heterozygous strains. A key
regulatory role of this repressor is to control the expression of another transcription
factor, the product of the WOR1 gene (Huang G, Wang H, Chou S, Nie X, Chen J, Liu
H. 2006, 136). TheWor1p transcription factor (Table 1) appears to be a primary
controller of the opaque state; ectopic expression ofWor1p efficiently induces the
opaque state in MTL homozygotes, and a pseudo-opaque state can be triggered by
activating Wor1p expression even in MTL heterozygotes (Zordan RE, Galgoczy DJ,
Johnson AD. 2006.). The regulation ofWor1p explains the epigenetic characteristics of
the opaque state. Wor1p is autoregulated; chromatin immunoprecipitation experiments
suggest as many as five binding sites for Wor1p in its own promoter region. The
Table 1

Transcriptional regulators of morphogenesis:

<table>
<thead>
<tr>
<th>State</th>
<th>Factor</th>
<th>Loss-of-phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Opaque state</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State</td>
<td>Factor</td>
<td>Loss-of-phenotype</td>
</tr>
<tr>
<td>Opaque state</td>
<td>Worlp</td>
<td>Blocks opaque-state</td>
</tr>
<tr>
<td></td>
<td>Efglp</td>
<td>Activates subset of functions</td>
</tr>
<tr>
<td></td>
<td>Tuplp</td>
<td>Modulates subset of functions</td>
</tr>
<tr>
<td><strong>Hyphal state</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyphal state</td>
<td>Rim10lp</td>
<td>Block in alkali-induced hyphae</td>
</tr>
<tr>
<td></td>
<td>Czflp</td>
<td>Block in matrix-induced hyphae</td>
</tr>
<tr>
<td></td>
<td>Flo8lp</td>
<td>Block in serum-induced hyphae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Derepresses embedded hyphae</td>
</tr>
<tr>
<td></td>
<td>Efglp</td>
<td>Block in serum-induced hyphae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Derepresses embedded hyphae</td>
</tr>
<tr>
<td></td>
<td>Hap5lp</td>
<td>Block in hyphae on Spider medium</td>
</tr>
<tr>
<td></td>
<td>Efhlp</td>
<td>Synergizes with efglp</td>
</tr>
<tr>
<td></td>
<td>Ace2lp</td>
<td>Defect in hypoxia-triggered hyphae</td>
</tr>
<tr>
<td></td>
<td>Mcm1lp</td>
<td>Activates hyphae</td>
</tr>
<tr>
<td></td>
<td>Ashlp</td>
<td>Reduces filaments on Spider medium</td>
</tr>
<tr>
<td></td>
<td>Cph2lp</td>
<td>Moderate inhibition of hyphae</td>
</tr>
<tr>
<td></td>
<td>Tec1lp</td>
<td>Supresses serum induced hyphae</td>
</tr>
<tr>
<td><strong>Chlamydosposes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydosposes</td>
<td>Rim10lp</td>
<td>Affects proper timing</td>
</tr>
<tr>
<td></td>
<td>Nrglp</td>
<td>Derepresses formation on Staib Agar</td>
</tr>
<tr>
<td></td>
<td>Efglp</td>
<td>Blocks chlamydospose formation</td>
</tr>
<tr>
<td><strong>Pseudohyphal state</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudohyphal state</td>
<td>Tuplp</td>
<td>Constitutive pseudohyphae</td>
</tr>
<tr>
<td></td>
<td>Nrglp</td>
<td>Constitutive pseudohyphae</td>
</tr>
<tr>
<td></td>
<td>Fkh2lp</td>
<td>Constitutive pseudohyphae</td>
</tr>
<tr>
<td></td>
<td>Tec1lp</td>
<td>Constitutive pseudohyphae</td>
</tr>
<tr>
<td></td>
<td>Rfglp</td>
<td>Constitutive pseudohyphae</td>
</tr>
<tr>
<td></td>
<td>Ssn6p</td>
<td>Moderately pseudohyphal</td>
</tr>
<tr>
<td></td>
<td>Ace2lp</td>
<td>Inappropriate pseudohyphae</td>
</tr>
<tr>
<td></td>
<td>Rap1lp</td>
<td>Inappropriate pseudohyphae</td>
</tr>
<tr>
<td><strong>Mating projections</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mating projections</td>
<td>Cph1lp</td>
<td>Mating defective</td>
</tr>
</tbody>
</table>
current data suggest that once Worlp expression is established, it tends to remain on, with a positive-feedback loop keeping the cell in the opaque state.

There are many genes whose expression switches between cells in the white form and cells in the opaque form (Lan CY, Newport G, Murillo LA, Jones T, Scherer S, et al. 2002.). Many of these genes are likely to control the distinct physiologies of the two different cell types: white-form cells express genes characteristic of a fermentative life style, whereas opaque form cells show characteristics of an oxidative metabolism. However, many genes regulated differently between the two cell types presumably define the different growth patterns and cell surface structures exhibited by the white- and opaque-form cells. Analysis of opaque-specific genes for the controllers of morphological patterning is an important step in the analysis of the opaque-phase cells. The Efg1p transcription regulator that is a critical positive controller of the yeast-to hyphal transition and the Tupilp negative regulator of pseudohyphal development also play roles in the white-opaque switch (Table 1). Efg1p is highly expressed in white-form cells but not in opaque-form cells (Sonneborn A, Bockmuhl DP, Ernst JF. 1999.); this expression is driven from a strong, white-phase specific promoter (Lachke SA, Srikantha T, Soll DR. 2003). Loss of EFG1 expression causes otherwise white-form cells to, take on some of the morphologies of opaque form cells, in particular the elongated cell shape, and to express some opaque-specific genes (Sonneborn A, Bockmuhl DP, Ernst JF. 1999, Srikantha T, Tsai LK, Daniels K, Soll DR. 2000.). Ectopic overproduction of Efg1p shifts opaque-form cells to the white morphology (Sonneborn A, Tebarth B, Ernst JF. 1999.), and thus repression of EFG1 expression appears necessary to establish the opaque state. However, certain structural characteristics of the opaque state, in particular the surface pimples, are not found in the efg1-null strains. Therefore, it appears that Efg1p may regulate a subset of the genes involved in establishing the opaque state.

The Tup1p transcriptional regulator also influences the opaque state. Loss of Tup1p, which creates constitutive pseudohyphae like state in white-form cells (Braun BR, Johnson AD. 1997), also dramatically affects the morphology of opaque-form cells (Park YN, Morschhauser J. 2005). Loss of tup1 deregulates expression of some phase-specific genes but still permits the establishment of a mating-competent cell type.
These experiments have identified a number of transcriptional regulators that play critical roles in the establishment of the opaque state; however, we do not know the details of how these regulators control morphology. Some early experiments characterized the actin cytoskeleton in opaque-phase cells, but the spatial and temporal regulation of the polarity machinery has not been extensively examined. The ability to form the surface pimples characteristic of the opaque state must be under regulatory control, but this process is currently enigmatic.

1.2.7. Pheromone-Mediated Morphological Changes:
In addition to directing the morphology of the opaque-form cell itself, the establishment of the opaque state is a prerequisite for the induction of morphological modifications due to the action of mating pheromones. These pheromones are diffusible peptide and lipopeptide molecules that are produced by cells of one mating type and act on cells of the other mating type. The action of the pheromones initiates a spectrum of physiological, morphological, and cell cycle changes that prepare the potential mating partners for cell and nuclear fusion (Bardwell L. 2005).

Both *C. albicans* and *S. cerevisiae* produce structurally distinct pheromones from each of the two mating types. MATα cells of the budding yeast and MTLα cells from *C. albicans* produce simple peptide pheromones (Bennett RJ, Uhl MA, Miller MG, Johnson AD. 2003, Lockhart SR, Zhao R, Daniels KJ, Soll DR. 2003., Naider F, Becker JM. 2004., Panwar SL, Legrand M, Dignard D, Whiteway M, Magee PT. 2003), and the MATa and MTLa cells from the two species produce pheromones that are either proven or predicted to be lipid modified peptides (Caldwell GA, Naider F, Becker JM. 1995, Dignard D, El-Naggar AL, Logue ME, Butler G, Whiteway M. 2007). The identification of the single gene encoding the *C. albicans* α-factor peptide has allowed for the chemical synthesis of the pheromone and the analysis of the behavior of cells in the presence of this factor. *C. albicans* cells treated with pheromone modify their gene expression profiles (Lockhart SR, Zhao R, Daniels KJ, Soll DR. 2003), their cell cycle progression, and their cellular morphology. In keeping with the terminology established for the modified morphology developed by pheromone-treated cells in *S. cerevisiae*, *C. albicans* MTLa cells treated with α-factor have been termed shmoo. The establishment of the polarized growth of the shmoo without the constriction inherent in the dividing bud of a *S. cerevisiae* cell has led some researchers

The observation that a subset of the pheromone-induced genes overlap with the hyphae-induced genes (Bennett RJ, Uhl MA, Miller MG, Johnson AD. 2003) is consistent with the possible involvement of extensively elongated structures in the mating process. Pheromones also appear to coordinate with nutrient signals in the arrest of opaque-form cells during mating; therefore, developing the link between mating-pheromone-induced signaling, nutritional status, and cell cycle regulation. This is necessary to fully understand pheromone-mediated morphological response.

1.2.8. Chlamydospore formation:

Chlamydospores of *C. albicans* superficially resemble a stable, resting cell and are distinct from the proliferative forms characterized by the yeast or hyphal states, but there is currently no strong evidence that long term viability is exhibited by these cells. Initial studies on chlamydospores focused primarily on their structure and the conditions that generated their formation. These investigations established that environments low in oxygen, light, temperature, and nutrients were most conducive to the development of chlamydospores, and that the cells themselves were large, with thick walls (Jansons VK, Nickerson WJ. 1970) and a high lipid (Miller SE, Spurlock BO, Michaels GE. 1974) and carbohydrate content.

Chlamydospores have been observed to germinate under certain conditions, and protocols have been developed to purify significant numbers of these spores. Media consisting of rice or cornmeal agar supplemented with the detergent Tween 80 are standard conditions for the induction of chlamydospores. These chlamydospores themselves form at the ends of branched filaments or suspensor cells that form under the inducing conditions. The formation of chlamydospores under specific conditions is one of the most effective ways to distinguish *C. albicans* from its close relative, *C. dubliniensis* (Staib P, Morschhauser J. 1999.). Current studies are providing a more detailed picture of the regulatory circuits controlling the production of chlamydospores in *C. albicans*. The transcriptional regulator Efg1p (Sonneborn A, Bockmuhl DP, Ernst JF. 1999) and the MAP kinase Hog1p are required for chlamydospore formation. In
both cases, filamentation occurred during the microaerophilic conditions, but production of the chlamydospores themselves was blocked.

A more general search for functions required for chlamydospore formation was undertaken with a collection of insertion mutants. This approach did not demand the preselection of candidate genes but rather directly screened for defects in chlamydospore formation with a set of 217 genes inactivated through a transposon mutagenesis and strategy (Nobile CJ, Bruno VM, Richard ML, Davis DA, Mitchell AP. 2003). This study identified the SUV3, SCH9, and ISW2 genes as essential contributors to the formation of chlamydospores, and RIM11, RIM101, and MSD3 were needed for proper timing of their production.

1.2.9. Other Growth Modes:

Pseudohyphal Growth

*C. albicans* cells exhibit several patterns of growth that involve extensive cell elongation but do not involve the formation of true hyphae. The pseudohyphal pattern is the best studied of these filamentous growth modes. Pseudohyphal growth involves elongated cells that remain in chains but are separated by true constrictions, and not by septa. It has been controversial whether pseudohyphal growth is an intermediate stage in the yeast to-hypha transition, but current work suggests that pseudohyphal cells represent a discrete cell growth pattern (Sudbery P, Gow N, Berman J. 2004). The absence of a Spitzenkorper, and the positioning of nuclear division across the mother-daughter junction, suggest that pseudohyphal growth is closer to yeast growth than to hyphal growth.

Major distinctions from the yeast form are the extended period of polarized growth exhibited by the pseudohyphal cells and the tendency for the cells to remain attached even though proper septa have formed between them. Some environmental conditions, such as medium rich in phosphate (Hornby JM, Dumitru R, Nickerson KW. 2004) or in alkanes (Sorkhoh NA, Ghannoum MA, Ibrahim AS, Stretton RJ, Radwan SS. 1990), have been reported to stimulate the pseudohyphal pattern of division. In addition, a number of mutants lead to constitutive pseudohyphal growth. Several of these mutations are in genes involved in cell cycle regulation, implicating this process in control of this morphogenetic state. This involvement is logical because the switch
from yeast growth to pseudohyphal growth arises from a subtle modulation in the length of time the cell spends in the polarized growth mode relative to the isotropic growth mode. A panel of factors involved in this mode of growth is enlisted below:

a) Fkh2:
Inactivation of the Fkh2p transcription factor (Table 1) implicated in the regulation of mitotic cyclins, or of the Fkh2p target cyclin Clb4p, generates cells that remain pseudohyphal under both hyphae-inducing and yeast-growth conditions. Cph1 and Efg1 were not required for pseudohyphal morphology of fkh2 mutants, implying that it acts in pathways downstream or parallel to them. Cells lacking Fkh2p were unable to damage human epithelial and endothelial cells in vitro, suggesting that Fkh2p contributing to C. albicans virulence (Bensen et al., 2002).

b) Grl1:
Loss of Grl1p, an F box protein involved in the degradation of the G1 cyclins Ccn1p and Cln3p, leads to a pseudohyphal growth state (Li WJ, Wang YM, Zheng XD, Shi QM, Zhang TT, et al. 2006). Perhaps extension of the G1 phase by stabilizing the G1 cyclins or reducing G2 cyclins can trigger the pseudohyphal state.

c) Cdc4:
Another F box protein, Cdc4p, is implicated in morphogenetic control. Loss of Cdc4p generates cells that are even more hyphal than those created by loss of Grl1p, suggesting that a critical target of the Cdc4p version of the SCF (Skp1-Cullin-1/Cdc53-F-box protein) complex is necessary to block hyphal growth (Atir-Lande A, Gildor T, Kornitzer D. 2005).

d) Nim1 related kinases:
In C. albicans the Nim1p-related kinases Gin4p and Hsl1p function in the regulation of the pseudohyphal state; mutation of either kinase leads to constitutive pseudohyphae (Wightman R, Bates S, Amornrattanapan P, Sudbery P. 2004).

Hyperpolarized Buds:
A second occasion in which C. albicans cells exhibit a filamentous growth pattern distinct from hyphal growth occurs under certain cellular stresses or in the absence of
certain gene products that influence the cell cycle. Treatment of yeast-form cells with the DNA synthesis inhibitor hydroxyurea (HU) resulted in a growth mode in which the bud continued to elongate in the absence of further DNA replication (Bachewich C, Thomas DY, Whiteway M. 2003). Treatment of *C. albicans* cells with nocodazole, another chemical that blocks cell cycle progression, also causes elongation of the bud in the absence of further DNA replication.

Several mutants generate similar bud elongation; inactivation of the CDC5 gene encoding the *C. albicans* polo kinase (Bachewich C, Thomas DY, Whiteway M. 2003) and shutoff of the repair gene RAD52 (2) cause extensive bud growth. Blocking mitotic cyclin degradation by eliminating the destruction boxes of Clb2p or Clb4p traps cells in mitosis and also triggers elongated bud growth (Bensen ES, Clemente-Blanco A, Finley KR, Correa-Bordes J, Berman J. 2005), as does deletion or overproduction of a stabilized form of Sol1p (5). Regulated repression of the cyclin-dependent kinase Cdc28p (Umeyama T, Kaneko A, Niimi M, Uehara Y. 2006.) and the essential B-type cyclin Clb2p (Bensen ES, Clemente-Blanco A, Finley KR, Correa-Bordes J, Berman J. 2005) also generates extended bud elongation. In contrast to deletion of other cell cycle genes that cause typical pseudohyphal growth, the elongated bud phenotype seems to be specific for depletion of essential cell cycle genes. The elongated buds resemble true hyphae in that they maintain polarized growth at the tip and do not show periodic constricted growth as do pseudohyphae. They also demonstrate nuclear movement out of the mother yeast cell and into the filament. Similar to pseudohyphae, a constriction is present at the junction between the yeast cell and elongated bud, which is consistent with the bud forming prior to the cell cycle arrest and the elongated growth mode. Because the organism can grow in such a morphology that eventually expresses hyphal and virulence-specific factors, an act that requires a large amount of resources, this growth mode may be important for pathogenicity and/or survival in the host.
1.2.10. Negative regulatory circuit:

a) Tup1 and Ssn6:

In addition to cell cycle perturbations that lead to constitutive pseudohyphae, defects in the Tup1p regulatory circuit lead to this phenotype (Table 1). Tup1p and the associated protein Ssn6p play important regulatory roles in *S. cerevisiae*; they interact with different DNA binding partners to provide a generic gene repression function (Smith RL, Johnson AD. 2000). For specific transcription modules the functional consequences of loss of Tup1p or Ssn6p can be different; therefore Tup1p and Ssn6p do not provide completely overlapping functions. In *C. albicans* Tup1p inactivation causes cells to proliferate in a pseudohyphal growth mode. However, the ssn6 deletion mutant does not trigger equivalent pseudohyphal development in *C. albicans* (Garcia-Sanchez S, Mavor AL, Russell CL, Argimon S, Dennison P, *et al.* 2005), although normal morphology is perturbed. The mutant lacking Ssn6 displayed a stubby pseudohyphal growth pattern, derepressed filament specific genes in response to increased temperature and failure to develop true hyphae. The global transcriptional consequences of the tup1 and ssn6 mutations are different (Garcia-Sanchez S, Mavor AL, Russell CL, Argimon S, Dennison P, *et al.* 2005), suggesting that the Ssn6p corepressor is not a critical component of the Tup1p regulatory circuit that represses the pseudohyphal growth pattern.

b) Tcc1:

An alternative tetratricopeptide repeat protein, Tccp1p, may play the co repressor role with Tup1p in the repression of pseudohyphal growth, as loss of Tccp1p creates similar phenotypes and affects patterns of gene expression similar to loss of Tup1p.

c) Nrg1:

It appears that Nrg1p DNA binding protein targets theTup1p(Tcc1p) co repressor to a variety of promoters involved in regulation of morphogenesis, as loss of Nrg1p function leads to constitutive pseudohyphal growth (Garcia-Sanchez S, Mavor AL, Russell CL, Argimon S, Dennison P, *et al.* 2005), and microarray analysis suggests that Tup1p and Nrg1p influence distinct but overlapping gene sets.

d) Rfg1:

A HMG protein, Rfg1p (Kadosh D, Johnson AD. 2005), appears to function as a targeting element for Tup1p(Tcc1p); loss of Rfg1p leads to a similar constitutive
pseudohyphal phenotype, but the networks of gene expression modulated by Nrg1p and Rfg1p are different. NRG1 is repressed by hyphal inducing conditions, and Rfg1p may be shut off posttranslationally (Kadosh D, Johnson AD. 2001).

e) Rap1:
In *S. cerevisiae*, the Rap1 protein acts as both a transcriptional silencer and a structural protein at telomeres by binding to a sequence designated the RPG box (Drazinic *et al.*, 1996). A *C. albicans* homologue of Rap1 has been identified which is not essential for survival. The rap1 mutants formed budding as well as pseudohyphal cells under conditions that promote budding yeast growth in wild type strain. The phenotype was reverted upon reintroduction of a functional copy. Thus this gene is required to repress pseudohyphae formation under conditions favouring growth as budding yeast (Biswas *et al.*, 2003).

f) Rbf1:
A *C. albicans* protein, Rbf1p, was identified which is not homologous to Rap1p, but binds to the RPG box of *S. cerevisiae* (Ishii *et al.*, 1997). Rbf1p contains two glutamine-rich regions embedding a region with weak similarity to bHLH domains, which binds to RPG sequences. Homozygous *rbf1* null mutants grew in filamentous form in all media tested; the filaments formed had the characteristics of pseudohyphae rather than true hyphae (Ishii *et al.*, 1997; N. Ishii, M. Watanabe and Y. Aoki, unpublished). Thus, Rbf1p seems to be involved exclusively in pseudohyphal, but not true hyphal growth.

g) Sir2:
The Sir2 protein represses hyphal formation, which is consistent with the role of Sir2p as a repressor in *S. cerevisiae* (although it is unrelated to pseudohyphal growth in this species) (Perez-Martin *et al.*, 1999).

h) Rad6:
Another repressing factor is the Rad6 protein, which besides contributing to UV protection, represses hyphal growth under inducing conditions by an unknown pathway; its deficiency under non-inducing conditions generates a pseudohyphal phenotype.
Introduction

(Leng et al., 2000. Thus inactivation of this negative regulatory circuit is a critical component of morphogenetic control in C. albicans.

1.3. Arsenal of the Pathogen:
The recent completion of the C. albicans genome sequence (http://sequence-www.stanford.edu/group/candida/index.html; http://candida.bri.nrc.ca/candida) enables genomic analysis directly in C. albicans, which promises to be a valuable tool in the study of this asexual diploid yeast. Genomic studies of morphogenesis and responses to stress, blood, and neutrophils have begun to elucidate the complex transcriptional programs that C. albicans uses to survive in vivo. The last decade has seen the identification of a number of potential virulence factors. These affect adherence to host tissues, secretion of specific enzymes, synthesis of the cell wall, metabolic pathways and most notably the ability of fungal pathogens to switch readily between different types of morphologies. Virulence of the opportunistic pathogen, Candida albicans, relies on an assemblage of the following attributes:

1.3.1. Thigmotropism/Chemotropism:
Hyphae of the human pathogenic fungus Candida albicans exhibit thigmotropism behaviour in vitro, in common with phytopathogenic and saprotrophic fungi. Deeper penetration of keratinized epithelia is assisted by hypha formation, and C. albicans hyphae may use contact sensing (thigmotropism) as a guiding mechanism. It is proposed that while thigmotropism may be a plausible mechanism to invade tissue invaginations, chemotropism can explain C. albicans hyphal invasion patterns of both endothelium and epithelium (Davies et al., 1999).

1.3.2. Hydrolytic enzymes:
Most notable among the repertoire of virulence determinants expressed by C. albicans are a family of 10 proteolytic enzymes known as the secreted aspartic proteases (Saps) (Naglik JR, Albrecht A, Bader O, Hube B 2004; Naglik JR, Challacombe SJ, Hube B). The SAP gene family has been shown to be differentially expressed under a variety of laboratory growth conditions and during experimental C. albicans infections in vitro and in vivo. The contribution of the Saps to mucosal and systemic infections and their involvement in adherence, tissue damage and evasion of host immune responses was

Morschhauser et al used a recombination-based genetic reporter system to detect the induction of the SAP1-SAP6 genes during infection of reconstituted human vaginal epithelium. Only SAP5, but none of the other tested SAP genes, was detectably activated in this in vitro infection model. To directly address the importance of the SAP1-SAP6 genes for invasion of reconstituted human epithelia (RHE), a set of mutants were created in the wild-type *C. albicans* model strain SC5314 in which either single or multiple SAP genes were specifically deleted. Even mutants lacking all of the SAP1-SAP3 or the SAP4-SAP6 genes displayed the same capacity to invade and damage both oral and vaginal RHE as their wild-type parental strain, in contrast to a nonfilamentous efg1Delta mutant that was avirulent under these conditions. It was, therefore, concluded from these results that the secreted aspartic proteases Sap1p-Sap6p are not required for invasion of RHE by *C. albicans*.

*Candida albicans* Saps have been shown to degrade a variety of host defense proteins such as lactoferrin and immunoglobulins (Hube B 1996). A recent study by Villar et al. (2007) demonstrated that *C. albicans* is able to degrade E-cadherin, the major protein in epithelial cell junction and that the degradation was mediated by Sap5. Therefore, the Sap isoenzymes appear to have a variety of functions in vivo which are probably called upon at different stages and in different types of *C. albicans* infections (Albrecht A, et al. 2006). Although the majority of the Sap enzymes are secreted by the fungus, the most recently identified members, Sap9 and Sap10 are cell surface glycosylphosphatidylinositol (GPI)-anchored proteases located in the fungal cell membrane and cell wall that have been described to be similar to the *S. cerevisiae* yaspin Yps1 (Albrecht A, et al. 2006; Gagnon-Arsenault I, Pairise L, Tremblay J, Bourbonnais Y.,2008).

The work by Meiller et al, (2009) directly implicated the Sap9 protease as the main isoenzyme responsible for the degradation of Hst-5(Histatin-5) by *C. albicans* cells, corroborating the previous indications that the active proteolytic substance is cell-associated rather than secreted. Furthermore, a recent study demonstrated that among the SAP genes, SAP9 is the most highly and consistently expressed gene during oral infections in vivo (Naglik JR, et al. 2008). These findings seem to ascribe an important
role for Sap9 in oral candidiasis, as the increased expression of a proteolytic enzyme capable of efficiently degrading Hst-5 may be a key event in the transition from commensal to pathogenic growth or may further exacerbate the infection process.

Although Saps are secretory proteins, little is known about the intracellular trafficking and secretion of these proteins. An analysis of the *C. albicans* pre-vacuolar protein sorting gene VPS4, demonstrated that extracellular Sap2p is absent in the culture supernatants of the vps4delta null mutant. The *C. albicans* vps4delta mutant failed to produce extracellular Sap4-6p. Next, when tested in a mouse model of disseminated candidiasis, the vps4delta mutant was greatly attenuated in virulence. Histopathological analysis indicated that infection with the vps4delta mutant did not cause renal microabscess formation, in contrast to the wild-type strain. Lee et al (2009) demonstrated that VPS4 is required for extracellular secretion of Sap4-6p, and that *C. albicans* requires an intact pre-vacuolar secretory pathway for wild-type virulence in vivo.

1.3.3. Cell surface adhesins:

Coordinated cell–cell adhesion is an essential biological process widely employed by organisms throughout the tree of life. In metazoans, cellular adhesion is important for numerous processes ranging from establishment of body plans and maintenance of differentiated tissues to regulation of cancer progression. Bacterial and fungal species commonly rely on cellular adhesion during mating and conjugation and for maintenance of multicellular biofilms that function as anchored shields against foreign attack by antimicrobial agents.

In *C. albicans*, multiple adhesins mediate attachment to epithelium, endothelium, or platelets. In a landmark finding Gale et al (1998) have found a single gene Int1 linked to adhesion, filamentous growth and virulence in *Candida albicans*. Int1p is a cell surface protein with limited similarity to vertebral integrins. Another data indicate that talin, a cytoskeletal protein, might interact with Int1p to mediate morphogenesis by modulating the actin cytoskeleton (Alleson et al., 2001).

Members of another family of adhesions, the agglutinin-like-sequence (ALS), also contribute to virulence in *C. albicans* (Hoyer, 2001). Als1p of *C. albicans* is a member of a family of seven glycosylated proteins with homology to the *S. cerevisiae* α-agglutinin.
protein that is required for cell-cell recognition during mating. Ligand binding is thought to be associated with the amino-terminus of the protein. Both Als1p and Als5p appear to provide an adhesion function.

Strains of *C. albicans* deleted in the α-1,2-mannosyltransferase gene (MNT1) are less able to adhere in vitro and are avirulent (Burman *et al.*, 1998). Mnt1p is a type II membrane protein that is required for both O- and N- mannosylation in fungi. Also PMT1, a gene encoding an O-glycolysation mannosyltransferase, is required for adherence to an epithelial cell line (Timpel *et al.*, 1998).

1.3.4. **Biofilm formation:**

Adhesion also plays major roles in other virulence-associated traits of several fungal pathogens. In *Candida albicans*, the most pervasive human fungal pathogen, cellular adhesion is essential for biofilm development. *C. albicans* biofilms form on both biotic and abiotic surfaces (such as tissues, plastic prosthesis, dentures and catheters) (Kojic EM, Darouiche RO 2004) and function as reservoirs of infective cells that among immunocompromised individuals can cause deep seated and often fatal mycosis (Odds FC 1988). The typical architecture of a biofilm consists of mixed layers of intertwined yeast and hyphal cells stabilized by adhesive interactions among neighboring cells. These cell–cell adhesive interactions are mediated by a set of cell surface displayed adhesins, including the Als proteins and the cell wall protein Hwp1. The ALS genes ALS1 and ALS3, two of eight ALS family members, are required for adherent interactions during biofilm formation in both in-vitro and in-vivo models of catheter biofilm formation and appear to have redundant functions (Nobile CJ, Andes DR, Nett JE, Smith FJ, Yue F, *et al.* 2006). HWP1, which codes for a cell surface glycoprotein targeted by mammalian transglutaminase that links Hwp1 to proteins on the mammalian cell surface (Sundstrom P 1999), surprisingly is also required for cell adhesive interactions during biofilm formation (Nobile CJ, Andes DR, Nett JE, Smith FJ, Yue F, *et al.* 2006). Notably, Als1, Als3 and Hwp1 play complementary roles during biofilm formation suggesting that they might interact to promote adhesion between adjacent cellular surfaces (Nobile *et al.*, 2008).

Adhesin regulation in *C. albicans* occurs primarily at the transcriptional level. During biofilm formation, expression of ALS1, ALS3 and HWP1 is regulated by the
transcription factor Bcr1 (Nobile CJ, Mitchell AP 2005). Additional factors, such as the transcription factors Tec1, and the repressors Nrg1 and Tup1, have also been implicated in regulating adhesin expression.

In organisms ranging from yeasts to humans, the Tor1 signaling pathway responds to nutrient-derived signals and orchestrates cell growth. In the human fungal pathogen Candida albicans, Tor1 signaling also functions to promote growth. There is also a novel role for the Tor1 molecular pathway in promoting hyphal growth of C. albicans on semi-solid surfaces and in controlling cell–cell adherence. Gene expression analysis and genetic manipulations implicate the known cell surface adhesins Als1 and Als3 as mediators of Tor1-regulated cellular adhesion. Further genetic analysis identified the transcriptional regulators Bcr1, Efg1, Nrg1, and Tup1 that together with Tor1 compose a regulatory network governing adhesin gene expression and cellular adhesion. Given that the Tor pathway is the target of several small molecule inhibitors including rapamycin, a versatile pharmacological drug used in medicine, there is considerable interest in Tor signaling pathways and their function.

1.3.5. **Morphogenetic transition:**

This fungus’s dramatic and frequent switch between growth as ovoid yeast and as filamentous pseudohyphae and hyphae has long been implicated in its success as a pathogen. Work from many laboratories has elucidated regulatory mechanisms for the switch from yeast to filamentous growth, and the role of hyphal growth in virulence.

The *C. albicans* dimorphic yeast–hyphae transition is thought to underlie its success as a pathogen. Mutants locked as yeasts (lacking Cph1 and Efg1 transcription factors or cyclin Hgc1) or filaments (lacking the Tup1 repressor) are both avirulent, linking both forms to pathogenesis (Lo HJ, *et al.* 1997; Zheng X, *et al.* 2004; Braun BR, *et al.* 2000). Subsequently, a strain was engineered in which morphogenesis is controlled by regulated expression of a filamentation repressor, Nrg1, with the *tet* promoter (Saville SP, *et al.* 2003). Cells grown without doxycycline express Nrg1 and grow as yeast, whereas growth with doxycycline repressed Nrg1 and filamentous growth ensued. Animals infected with yeast remained healthy yet harbored a significant latent fungal burden in the kidney. Adding doxycycline to drinking water activated filamentation, with progression to lethal infection. These studies provide robust support
for the concept that dimorphic transitions underlie \textit{C. albicans} virulence, and they show that yeast can penetrate tissues, whereas hyphae are necessary for progression to lethal infection. In cultured macrophages \textit{S. cerevisiae} is killed after phagocytosis, whereas \textit{C. albicans} yeast switch to hyphae, killing and escaping macrophages (Lorenz MC, Fink GR 2002).

Little is known about the reverse switch, from filamentous to yeast growth, although mutants defective in the hypha-to-yeast switch show reduced virulence in each case in which this trait was examined. (Braun BR, Kadosh D, Johnson AD 2001; Murad AM, \textit{et al.} 2001) Shen \textit{et al} (PNAS, Dec, 2008) propose that the filament-to-yeast switch is a critical part of the life cycle of \textit{C. albicans}, because yeast cells fulfill specific roles during colonization and infection. Yeast possesses suitable dimensions and physical properties to provide the fungus facile access to the host’s bloodstream. So far, two genes are known to affect lateral yeast growth: mutants in \textit{PDE2} have decreased, and those in \textit{CAP1} have increased lateral yeast growth (Bahn YS, Staab J, Sundstrom P., 2003; Bahn YS, Sundstrom P 2001). Given the dearth of studies of the hypha-to-yeast switch, our goal was to identify novel regulators of this switch. This search identified a hyperfilamentous mutant with a transposon insertion in the promoter of the \textit{C. albicans} \textit{pescadillo} homolog, \textit{PES1}. In all organisms studied to date, \textit{pescadillo} homologs are essential. In contrast, we find that \textit{C. albicans} cells tolerate loss of the \textit{pescadillo} homolog under hyphae-inducing, but not under yeast-inducing conditions.

\textit{Pescadillo} homologs of yeast, mouse, and human act in proliferation control and ribosome biogenesis (Kinoshita Y, \textit{et al.} 2001, Lerch-Gaggl A, \textit{et al.} (2002)). It is not clear whether these distinct functions are based in distinct biochemical activities. \textit{PES1} is cotranscribed with genes involved in rRNA processing and ribosome biogenesis (Ihmels J, Bergmann S, Berman J, Barkai N 2005) (Candida Genome Database), suggesting that it may participate in these functions in \textit{C. albicans} as well. One possibility is that growth arrest during \textit{PES1} depletion is caused by disruption of rRNA processing, leading to activation of a “nucleolar stress” cell cycle checkpoint (Pestov DG, Strezoska Z, Lau LF 2001), or to depletion of ribosomes and slowing of translation. If this is the case, tolerance of hyphae for \textit{PES1} depletion might indicate that hyphal cells need less ribosomes to grow than yeast cells. Alternatively, Pesl may have separate roles in proliferation control and rRNA processing.
1.3.6. Super oxide dismutases:

In a recent report, by Frohner et al (2009) it was shown that yeast and hyphal forms of *C. albicans* rapidly induce ROS in primary innate immune cells such as macrophages and dendritic cells. It was further shown that the GPI-anchored Sod5 and Sod4 enzymes act to degrade extracellular ROS produced by innate immune cells. Strikingly, *C. albicans* strains lacking SODs Sod4 and Sod5 fail to counteract the host-derived oxidative burst and are thus hyper-susceptible to killing by primary BMDMs (bone marrow derived macrophages), suggesting a physiological role of cell surface SODs in the evasion of immune surveillance. The ROS induction is independent of morphology as both yeast and filamentous forms of *C. albicans* trigger ROS in BMDMs.

The best-studied *C. albicans* SODs with respect to their role in pathogenesis are Sod1 and Sod5. (Fradin et al., 2005). Both appear to be required for virulence of *C. albicans* in invasive mouse models (Hwang et al., 2002). Further, fungal cells lacking Sod1 are sensitive to menadione and more sensitive to killing by macrophages than a wild-type strain (Hwang et al., 2002). *SOD5* is upregulated under osmotic and oxidative stress conditions, as well as during yeast to-hyphae transition (Martchenko et al., 2004). Moreover, transcriptional profiling indicates that *SOD5* expression is also upregulated by neutrophil contact, in presence of neutrophils and viability of a sod5DID mutant is reduced relative to the wild type. The surface location of Sod4, Sod5 and Sod6 prompted the notion that they may protect *C. albicans* against extracellular stress (Fradin et al., 2005; Gantner et al., 2005). Recently it was demonstrated that Sod5, and to a lesser extent Sod4, catalyses destruction of host-derived ROS. Interestingly, sod5DID and sod4DID sod5DID *C. albicans* show decreased viability in the presence of macrophages. Thus, it was identified that *SOD5* was as a novel *C. albicans* gene, mediating detoxification of host derived ROS. The results suggest a molecular mechanism whereby fungal pathogens can escape the immediate early immune response, namely the oxidative burst reaction.

1.3.7. Utilization of N-acetylglucosamine:

N-acetylglucosamine (GlcNAc) is an amino sugar that carries out important roles in a broad range of cells from bacteria to humans. One aspect of GlcNAc function is to mediate cellular signaling. In bacteria, GlcNAc induces components that are important for colonization of human hosts, including fimbrins that mediate adhesion to host cells.
Introduction

(Sohanpal et al., 2004), multidrug exporter genes (Hirakawa et al., 2006) and Curli fibers that promote biofilm formation (Barnhart et al., 2006). In mammals, GlcNAc is a key sensor of nutrient status that is involved in insulin signaling, cell cycle control, and other essential processes. Studies on two species of dental plaque forming bacteria, Streptococcus mutans and Streptococcus sobrinus have shown that though S. sobrinus is more acidogenic than S. mutans, still it is less frequently isolated from human population as it is incapable of utilizing GlcNAc. (Homer et al., 1993). This suggests that perhaps organisms capable of utilizing GlcNAc as carbohydrate source are better adapted to infect and persist within host. C. albicans is capable of taking up GlcNAc and using it as an energy source, whereas the nonpathogenic S. cerevisiae lacks the proteins needed to transport and catabolize GlcNAc (Kumar et al., 2000; Singh et al., 2001). Mutational analysis indicates that the enzymes needed to catabolize GlcNAc in C. albicans also contribute to virulence (Singh et al., 2001; Yamada-Okabe et al., 2001).

1.4. Host defense against Candida:
Epidermal and external epithelial surfaces are normally defended against microbial invasion by a range of factors. The factors which come into action during host defense against the pathogen are as follows:

1.4.1. Farnesol production:
Farnesol, a precursor in the isoprenoid/sterol pathway, was recently identified as a quorum sensing molecule produced by the fungal pathogen Candida albicans. Farnesol is involved in inhibition of germination and biofilm formation by C. albicans and can be cytotoxic at certain concentrations. In addition, we have shown that farnesol can trigger apoptosis in mammalian cells via the classical apoptotic pathways. In order to elucidate the mechanism behind farnesol cytotoxicity in C. albicans, the response to farnesol was investigated using proteomic analysis. Global protein expression profiles demonstrated significant changes in protein expression resulting from farnesol exposure. Among the down-regulated proteins were those involved in metabolism, glycolysis, protein synthesis and mitochondrial electron transport and respiratory chain, whereas proteins involved in folding, protection against environmental and oxidative stress, actin cytoskeleton reorganization and apoptosis were up-regulated. Cellular changes that accompany apoptosis (regulated cell death) were further analyzed using
Introduction

fluorescent microscopy and gene expression analysis. Results indicated ROS accumulation, mitochondrial degradation and positive TUNEL in the farnesol-exposed cells concurrent with increased expression of antioxidant-encoding and drug response genes.

1.4.2. Histatin secretion:
Most notable among the natural immune salivary antimicrobial peptides are the histatins, a family of low-molecular-weight, histidine-rich, cationic proteins produced and secreted by human parotid and submandibular-sublingual glands (Helmerhorst EJ, Troxler RF, Oppenheim FG 2001; Oppenheim FG, et al. (1988). Histatins show killing activities against numerous oral bacteria, as well as potent antifungal properties against pathogenic fungi including *C. albicans* (Jainkittivong A, Johnson DA, Yeh C-K 1998; Helmerhorst EJ, et al. 1999). Histatin-5 (Hst-5) specifically, a 24-amino acid member of the family, has the highest level of activity against *C. albicans* including strains resistant to antifungal agents, implicating a different mode of action than the commonly used drugs (Jang WS, Li XS, Sun JN, Edgerton M 2008).

Histatin-5 is believed to exert its anti-candidal effect through binding to receptor proteins (Ssa1 and Ssa2) on the fungal cell membrane (Li XS, Reddy MS, Baev D, Edgerton M 2003). Once internalized, Hst-5 inhibits mitochondrial respiration, thus inducing the formation of reactive oxygen species thereby leading to mitochondrial and cytoplasmic membrane damage, efflux of ATP and cell death (Koshlukova SE, Lloyd TL, Araujo MWB, Edgerton M 1999). Recently, expansive investigations by Mochon et al. (2008) provided direct evidence for a breach in plasma membrane as the initial damage by extracellular Hst-5 on *C. albicans* and a mechanism of its internalization into the cytoplasm.

Although not since substantiated, studies in the early 1990s had reported changes in salivary histatin concentrations in HIV+ individuals, the result of salivary gland dysfunction (Mandel ID, Barr CE, Turgeon L 1992). Given the important role of saliva in maintaining oral health, it is conceivable that alterations in salivary gland secretion and/or composition are liable to contribute to the markedly enhanced predisposition of this population to oral candidiasis. Yet studies confirming these important observations have been lacking, most likely due to the lack of feasible methods for measuring
salivary histatin concentrations. Recently, however, Meiller et al (2009) confirmed these observations in a study comparing the levels of salivary Hst-5 between a group of HIV+ and HIV2 individuals. Results from the investigation demonstrated significantly lower Hst-5 levels in the HIV+ group, concomitant with increased prevalence of *C. albicans* in the oral cavity, highlighting the involvement of host innate immunity in the protection against *C. albicans* colonization (Torres SR, Garzino-Demo A, Meeks V, Meiller TF, Jabra-Rizk MA 2008).

1.4.3. **PhospholipaseA(2):**
Phospholipase A (2) (PLA (2)) hydrolyzes the sn-2 position of cell membrane phospholipids to release fatty acids and lysophospholipids. It was previously reported that group V secretory PLA (2) (sPLA (2)) translocates from the Golgi and recycling endosomes of mouse peritoneal macrophages to newly formed phagosomes and regulates the phagocytosis of zymosan, suggesting a role in innate immunity in a model of systemic candidiasis, mice lacking group V sPLA(2) had an increased fungal burden in the kidney, liver, and spleen at day 7 postinfection and increased mortality.

1.4.4. **Toll-like receptors:**
Toll-like receptors (TLRs) constitute a family of pattern-recognition receptors (PRRs) that recognize molecular signatures of microbial pathogens and function as sensors for infection that induce the activation of the innate immune responses as well as the subsequent development of adaptive immune responses. It is well established that TLRs, mainly TLR2 and TLR4, are involved in the host interaction with *Candida albicans* and play a significant role in the development of host immune responses during candidiasis. Recognition of *C. albicans* by TLRs on the phagocytic cells activates intracellular signaling pathways that trigger production of proinflammatory cytokines that are critical for innate host defence and orchestrate the adaptive response. T helper (Th) cell reactivity plays a central role in regulating immune responses to *C. albicans*: Th1-response provides control of fungal infectivity, although this proinflammatory (Th1) host response needs to be counterbalanced through Th2 and regulatory T (Treg) cells to ensure an optimal, protective Th1 response. Recently, a new subset of Th cells, Th17, has been shown to play a role in antifungal immunity, and TLRs may also contribute to the polarization towards a proinflammatory Th17 response.
1.4.5 **Reactive oxygen species:**
Mammalian innate immune cells produce reactive oxygen species (ROS) in the oxidative burst reaction to destroy invading microbial pathogens. Using quantitative real-time ROS assays, a recent report shows that both yeast and filamentous forms of the opportunistic human fungal pathogen *Candida albicans* trigger ROS production in primary innate immune cells such as macrophages and dendritic cells.

1.4.6 **Histidine-rich-glycoproteins:**
That HRGs protect from systemic infection by *Candida* is illustrated further by an investigation, demonstrating a novel antifungal role of histidine-rich glycoprotein (HRG), an abundant and multimodular plasma protein. HRG bound to Candida cells, and induced breaks in the cell walls of the organisms. Correspondingly, HRG preferentially lysed ergosterol-containing liposomes but not cholesterol-containing ones, indicating a specificity for fungal versus other types of eukaryotic membranes. Both antifungal and membrane-rupturing activities of HRG were enhanced at low pH, and mapped to the histidine-rich region of the protein. Ex vivo, HRG-containing plasma as well as fibrin clots exerted antifungal effects. In vivo, Hrg (-/-) mice were susceptible to infection by *C. albicans*, in contrast to wild-type mice, which were highly resistant to infection. The results demonstrate a key and previously unknown antifungal role of HRG in innate immunity. (Rydengard *et al.*, 2008).

A recent study describes the interaction between two diverse human pathogens that reside within the gastrointestinal tract and shows that the prokaryote, *Salmonella typhimurium*, reduces the viability of the eukaryote, *C. albicans*. Identifying the molecular mechanisms of this interaction may provide more important insights into microbial pathogenesis.