The Journal of Microbiology (2011) Vol. 49, No. 2, pp. 171-177
DOI 10.1007/s12275-011-1064-7

REVIEW

Candida albicans, a Major Human Fungal Pathogen

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(Received February 10, 2011 / Accepted March 21, 2011)

Candida albicans **is the most common human fungal pathogen (Beck-Sague and Jarvis, 1993). It is normally a harmless commensal organism. However, it is a opportunistic pathogen for some immunologically weak and immunocompromised people. It is responsible for painful mucosal infections such as the vaginitis in women and oral-pharangeal thrush in AIDS patients. In certain groups of vulnerable patients it causes severe, life-threatening bloodstream infections and it causes severe, life-threatening bloodstream infections and subsequent infections in the internal organs. There are various fascinating features of the *C. albicans* life cycle and biology that have made the pathogen the subject of extensive research, including its ability to grow in unicellular yeast, psudohyphal, and hyphal forms (Fig. 1A); its ability to switch between different but stable phenotypic states, and the way that it retains the ability to mate but apparently loses the ability to go through meiosis to complete the sexual cycle. This research has been greatly facilitated by the derivation of the complete *C. albicans* genome sequence (Braun *et al.*, 2005), the development of a variety of molecular tools for gene manipulation, and a store of underpinning knowledge of cell biology borrowed from the distantly related model yeast *Saccharomyces cerevisiae* (Berman and Sudbery, 2002; Noble and Johnson, 2007). This review will provide a brief overview of the importance of *C. albicans* as a public health issue, the experimental tools developed to study its fascinating biology, and some examples of how these have been applied.**

Keywords: *Candida albicans*, fungal pathogen, candidiasis, candidemia, morphological change

Diseases caused by *Candida albicans*

Mucosal infections

Candida albicans is a commensal organism that can be isolated from the gastrointestinal tract, and oral and vaginal mucosa of many, if not all, healthy individuals. It is not normally a problem, however it can cause symptomatic infections of mucosal membranes (Odds, 1988). The best known of these is commonly called thrush, characterized by white spots that can be readily removed to reveal an area of inflammation in the underlying membrane. The medical term for this condition is pseudomembranous candidiasis. Such infections commonly affect vaginal, oral-pharangeal, esophageal, and gastrointestinal mucosae.

Vulvo Vaginal Candidiasis (VVC) is a common infection and may affect up to 75% of women at least once in their life time (Sobel, 1997). Some women experience repeated episodes, a condition known as Recurrent Vulvo Vaginal Candidiasis (RVVC). Risk factors for VVC include the use of reproductive hormones, antibiotic treatment, use of oral contraceptives, and diabetes. The primary defense against mucosal infections is believed to normally be cell-mediated immunity; however, it is now thought that the innate immunity of the vaginal mucosa is more important and the symptoms of VVC in fact are caused by an overaggressive inflammatory response (Fidel, 2007). This inflammatory response may be triggered by a lower fungal burden in women suffering from RVVC. Azole antifungal agents, such as fluconazole and albaconazole, are used to treat VVC which normally responds readily to such treatments.

In contrast to the vaginal mucosa, cell-mediated mechanisms are important in the defense against *C. albicans* in the oral-pharangeal and esophagus mucosa. Oral-pharangeal candidiasis (OPC) is so common among AIDS patients that its appearance is considered to be marker of the development of AIDS in HIV-positive individuals (Klein *et al.*, 1984). OPC is also commonly associated with oral cancers, the use of dentures, and terminally ill patients failing to produce sufficient saliva (Runke, 2002).

Candidemia and disseminated candidiasis

Blood stream infections of *C. albicans* are known as candidemia. In normal healthy individuals, adequate protection against such infections is provided by the action of neutrophils. Candidemia can develop in patients who have abnormally low numbers of neutrophils as a result of certain blood cancers or immunosuppressant therapy. Additional risk factors are surgery that results in breaches in the gastrointestinal (GI) tract allowing the spread of *C. albicans* that reside in the GI

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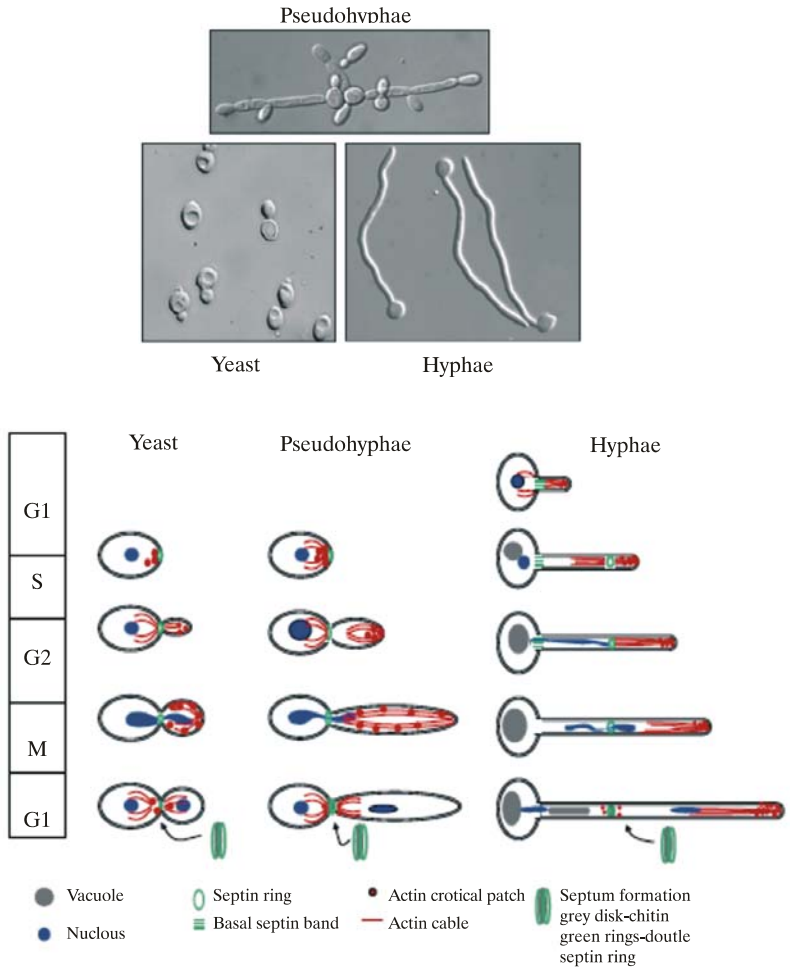

Fig. 1. Different growth morphologies of *C. albicans.* (A) Differential interference contrast (DIC) images of cells with hyphae, pseudohyphae, and yeast morphologies. (B) Diagramatic representation of the cell cycle of yeast and of the first cycle after induction of pseudohyphae and hyphae (reproduced from Sudbery *et al*., 2004).

tract, and the use of catheters in intensive care units that result in biofilm formation (discussed below). Candidemia can lead to the colonization of internal organs, a condition known as disseminated candidiasis. Candidemia and disseminated candidiasis are extremely serious medical conditions. Mortality depends on the exact cause and underlying risk factors: in different surveys, of mortality rates between 30% and 50% have been observed (Pfaller *et al.*, 1998; Kibbler *et al.*, 2003). The symptoms of candidemia are similar to bacterial septicemia, and the diagnosis of candidemia may come too late to allow antifungal treatments to work or to even be applied before death of the patient. For this reason, antifungal treatments are often administered prophylactically after bone marrow transplants or abdominal surgery. *Candida* infections have been documented as the fourth most common hospital-acquired infections, and the second most common cause of death from such infections in the United States. The majority of these are caused by *C. albicans* (Pfaller *et al.*, 1998).

The genome of *C. albicans*

C. albicans belongs to the *Saccharomycetaceae* family of ascomycete fungi. It is distantly related to the budding yeast *S. cerevisiae* which has been extensively used as a model to investigate fundamental aspects of cell biology and genetics. A distinctive feature of the *C. albicans* genetic make-up is an abnormality in its genetic code in which the codon CUG encodes serine rather than leucine (Santos and Tuite, 1995). It shares this property with a group of related species that are collectively known as the CUG clade (Butler *et al.*, 2009) This clade includes other pathogenic yeast such as *Candida paropsilosis* and *Candida tropicalis. C. albicans* and the other pathogenic yeasts are diploid organisms with eight chromosomes. The size of *C. albicans* genome is 13.3-13.4 Mb encoding 6,100-6,200 genes; the exact statistics are slightly different in two different strains that have been sequenced (Braun *et al.*, 2005; Butler *et al.*, 2009). Compared to the *S. cerevisiae* genome, a total of 21 gene families have expanded among the CUG clade of pathogenic yeasts which include lipases, adhesions, oligopeptide transporters, cell wall mannoproteins, transcription factors, and ferric reductases (Butler *et al.*, 2009).

The genus name *Candida* was originally used for yeast in which no sexual cycle had been observed. To date, no com-

plete sexual cycle has been observed in *C. albicans* despite the demonstration that an elaborate mechanism for mating is still operational (discussed below). In order to generate genetic variability in the absence of sexual recombination, the *C. albicans* genome has evolved to show plasticity. A striking example of this is the way that azole antifungal agents has led to the appearance of an isochromosome V which results in an extra copy of the chromosome arm carrying *ERG11* that encodes lanosterol-14a-demethylase, the enzyme targeted by azoles, and TAC1 that encodes the transcription factor which upregulates the expression of *MDR1*, a drug efflux pump (Selmecki *et al.*, 2006). A survey of the population structures of *C. albicans* strains using a technique called Multi-Locus Sequence Typing (MLST) revealed that isolates can be assigned to 17 different clades which tend to cluster within the geographical area from which the isolate was obtained (Odds and Jacobsen, 2008). These clades show differences with respect to antifungal agent resistance and the structure of cell wall adhesion proteins which contain internal repeats of which the number varies within clades.

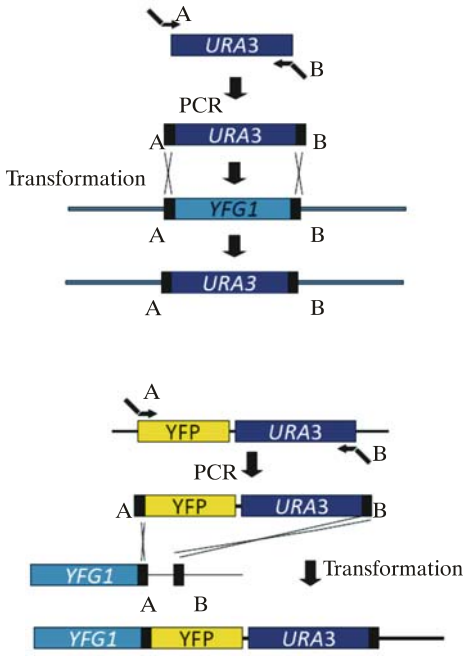

Fig. 2. Techniques of gene manipulation in *C. albicans.* (A) Gene replacement by the generation of a linear DNA cassette. PCR primers are used that anneal to a common template containing a selectable marker. These primers have 70-100 nucleotide (nt) 5′ extensions (A and B) which are identical to chromosomal sequences either side of the gene to be deleted (*YFG=Your Favorite Gene)*. In the resulting linear DNA cassette, the selectable marker is flanked by these sequences which can then recombine with the corresponding chromosomal sequence resulting in the deletion of the intervening gene. (B) Generation of a gene fused to a fluorescent protein. A linear cassette is generated containing a selectable marker and a sequence encoding a fluorescent protein, in this case YFP, a variant of GFP. The linear cassette is flanked by 70-100 nt sequences (A and B) that correspond to the C-terminus of the target gene and a downstream sequence, respectively.

Techniques for gene manipulation

The absence of a sexual cycle, obligate diploid state of the genome, lower frequency of homologous recombination, and non-standard genetic code all make genetic manipulation more difficult in *C. albicans* than the *S. cerevisiae* model. However, sequencing of the genome and techniques for transformation have allowed a workable set of tools to be developed (Berman and Sudbery, 2002; Noble and Johnson, 2007). Most techniques of genetic manipulation are based on amplification by linear DNA cassettes containing a selectable marker. A commonly used *C. albicans* host strain called BWP17 has three auxotrophies caused by deletions of the *ARG4*, *HIS1*, and *URA3* genes. This allows three successive rounds of transformation using DNA cassettes containing the appropriate wild-type gene. In addition, the *ura3* marker can be recycled by using positive selection for loss of *URA3* by resistance to 5-fluro-orotic acid (5-FOA). However, one should be careful when using the *URA3* marker because ectopic expression of *URA3* can influence the virulence phenotypes (Staab and Sundstrom, 2003; Brand *et al*., 2004). Deletion of a particular gene strain requires two successive transformations to delete both copies of the gene (Fig. 2A). Visualization of a protein *in situ* is achieved by fusion of the encoding sequence to GFP or its derivatives (Fig. 2B). Conditional gene expression can be engineered by promoter substitution with a promoter such as *MET3,* which is repressed by methionine and cysteine in the growth medium (Care *et al.*, 1999).

Morphological changes

Yeast hyphae and pseudohyphae

A distinctive characteristic of *C. albicans* is its ability to grow with three distinct morphologies – yeast, pseudohyphae, and true hyphae (Fig. 1). For a review of this, see Sudbery *et al.* (2004). Unicellular yeast cells grow by budding. In pseudohyphal cells, the buds elongate and fail to separate from the mother cell, producing filaments of elongated buds but retaining constrictions at the septal junctions. True hyphae consist of chains of tube-like cells with no constrictions at the septal junctions. The extent of bud elongation in pseudohyphal cells is highly variable. Pseudohyphal filaments can consist of cells which are so elongated that they resemble hyphae. However, there are a number of fundamental differences in the mode of growth and organization of the cell cycle between hyphae and pseudohyphae (Fig. 1B). Hyphal growth, which is believed to be one of the important virulence factors, is promoted by a variety of environmental conditions such as a 37°C growth temperature, the presence of serum, neutral pH, high CO_2, growth in embedded conditions, and N-acetylglucosamine. Yeast form growth is favored by a 30°C growth temperature and acidic pH (pH 4.0). Pseudohyphal growth occurs as conditions shift towards those favoring hyphal growth such as 35°C or pH 5.5. Formation of hyphae from yeast mother cells is regulated by a quorum-sensing mechanism in which yeast cells produce small molecules such as the alcohol farnesol that inhibit the formation of hyphae (Hornby *et al.*, 2001).

The fact that pseudohyphal morphology and conditions promoting its formation are both intermediate between those of yeast and hyphae has led to the common view that pseudohyphae are a transitional stage. This view is strengthened by the observation that manipulating the expression of the tran-

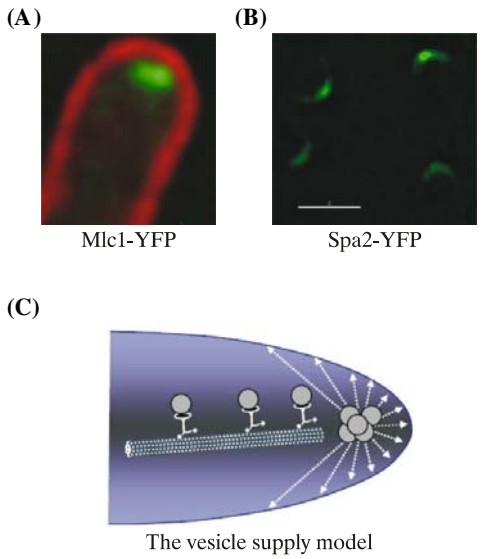

Fig. 3. Apical structures that drive polarized growth of hyphae. (A) Mlc1-YFP localizes to a subapical spot representing the Spitzenkörper. (B) Spa2-YFP localizes to a surface crescent representing the polarisome. (C) The vesicle supply model of hyphal growth. Long-distance transport of secretory vesicles to the tip is mediated by cytoskeletal elements such as microtubules. At the tip, vesicles accumulate in the Spitzenkörper before they are transported onward to the surface. As shown in the diagram, if the vesicles travel out in equal directions from the Spitzenkörper, the tip will receive a greater density of vesicles thereby driving tip growth. [Panels (A) and (B) are reproduced from Crampin *et al.* (2005)]

scription factor *UME6* controls the morphological outcome: low-level expression of *UME6* results in pseudohyphal growth while high-level expression results in hyphal growth (Banerjee *et al.*, 2008; Carlisle *et al.*, 2009). However, there are clear qualitative differences between hyphae and pseudohyphae. Indeed, the mechanism of polarized growth, cell cycle organisation, and hydrolysis of the primary septum after cytokinesis all suggest that pseudohyphae are far more similar to yeast than hyphae (Crampin *et al.*, 2005).

Modes of polarized growth

Extensive research in the budding yeast *S. cerevisiae* has shown that polarized growth occurs by secretory vesicles being transported along actin filaments to the site of polarized growth (Sudbery and Court, 2007). The site of polarized growth is marked by cortical markers produced during the action of bud site selection pathways. As a result, a Rho-type GTPase called Cdc42 is converted to its active GTP-bound state. Activated Cdc42 results in the formation of three multi-protein complexes: the polarisome, the exocyst, and a septin ring. The septin ring organizes the formation of the primary and secondary septa between the mother and daughter cells. The polarisome initiates the formation of actin filaments along which post-Golgi secretory vesicles are delivered to the sites of polarized growth where they dock with the exocyst before fusing with the plasma membrane.

In *C. albicans*, a similar mechanism operates to promote polarized growth in yeast and pseudohyphae. Polarized growth is limited to the first part of the cell cycle although it continues for a longer period in the buds of pseudohyphae morphology compared to yeast morphology. However, in hyphae the mode of polarized growth is different. A structure called a Spitzenkörper (Fig. 3A) forms at the apex (Crampin *et al.*, 2005; Jones and Sudbery, 2010). The Spitzenkörper is rich in secretory vesicles which can by observed by the spot-like localization of vesicle-associated proteins such as Mlc1 visualized by fusion with GFP. In contrast, proteins of the polarisome and exocyst form a surface crescent (Fig. 3B). A vesicle supply model for hyphal growth postulates that vesicles accumulate in the Spitzenkörper before radiating out to the cell surface. Since the Spitzenkörper is close to the tip (Fig. 3C), a higher number of vesicles reaches the tip than the hyphal walls distal to the tip, resulting in tip growth (Bartnicki-Garcia *et al.*, 1989). The presence of polarisome and exocyst components in the surface crescent suggests that the original version of the vesicle supply model should be modified by these components acting to focus the supply of vesicles from the Spitzenkörper to the tip. Another difference of yeast and pseudohyphae compared to hyphae is that hyphae growth is continuous from the tip for entire duration of the cell cycle.

Hyphal-specific gene expression and signal transduction pathways

Hyphal growth is characterized by expression of several hundred genes including one encoding cell wall proteins, adhesions, and extracellular proteases called secreted aspartyl proteases or SAPs (Brown, 2002). Environmental cues inducing hyphal morphogenesis are transduced by a complex network of signal transduction pathways that activate transcription factors such as Efg1 and Ume6, while negative regulation is provided by a heterodimer consisting of Tup1 and Nrg1 (Biswas *et al.*, 2007; Brown *et al.*, 2007). One of the main pathways for the hyphal growth is based on cAMP which activates two cyclic-AMP-dependent protein kinases, Tpk1 and Tpk2, which in turn activate the downstream transcription factor Efg1. A key component of this pathway is adenylate cyclase which responds directly to various environmental signals such as CO_2 (Klengel *et al.*, 2005), the quorum-sensing agent farnesol (vis-Hanna *et al.*, 2008), and an active component of serum recently shown to be bacterial peptidoglycan (Xu *et al.*, 2008). Recently, it was found that hyphal growth requires phosphorylation of Sec2 by Cdc28-Ccn1/Hgc1 kinase (Lane *et al*., 2010), and that some ribosomal proteins such as Asc1 are also involved in hyphae formation and virulence (Kim *et al*., 2010; Liu *et al*., 2010).

Biofilms

C. albicans is able to form a biofilm when free-living cells, called planktonic cells, are streamed over a solid surface (Blankenship and Mitchell, 2006; Nobile and Mitchell, 2006). At first, yeast cells adhere to the solid surface. They then undergo morphogenesis to produce a dense layer of cells of mixed morphology embedded in a extracellular matrix rich in the secreted cell wall polymer 1,3 glucan. *In vivo*, the biofilm protects cells against host defenses. Biofilms are a serious problem in medical practice because they can form on the inside wall of intravenous catheters where they continually shed cells that are directly injected into the bloodstream of the

patient. Biofilms can also form in other medical devices such as artificial heart valves and are also a problem in dentures. Genetic screens have identified a number of genes required for biofilm formation (Nobile and Mitchell, 2006). Some of these are one which have already been identified as being required for hyphal formation such as the transcription factor Efg1 and the kinase Yak1. Others are apparently related specifically to biofilm formation. The zinc finger transcription factor Zap1 is thought to regulate the production of matrix polysaccharide components. Another zinc finger transcription factor called Bcr1 regulates the formation of adhesions that allow yeast cells to stick to the solid surface. A set of alcohol dehydrogenases may regulate the level of quorum-sensing alcohols (Nobile *et al.*, 2009).

Phenotypic switching and mating

A long-standing observation of certain *C. albicans* strains is that they undergo a phenomenon called phenotypic switching (Slutsky *et al.*, 1985). There are a number of different classes of such switching; one of the most well-characterized is white-opaque switching (Slutsky *et al.*, 1987). The most common form of *C. albicans* forms colonies that are smooth, white, and dome-shaped. In yeast- morphology promoting conditions, the cells within these colonies will be oval-shaped. This form is called "white". When a suspension of white phase cells are plated out, about 1 in 1,000 colonies will have a different appearance being flatten and having a grey color. Within these colonies, the cells are larger and have an oblong rather than opaque appearance. Scanning electron micrographs show that the surface of these cells is covered in pimples. These cells are said to be in the "opaque" phase. Opaque cells will switch back to white again with a frequency of about 1:1,000 colonies. Thus, the white or opaque phase is stable from generation to generation, but cells switch between phases at a rate which is too high to be due to genetic mutation, so the process is believed to be under epigenetic control.

The white-opaque switching has been shown to be an integral part of a mechanism that allows mating (for a review see Bennett and Johnson, 2005). When the *C. albicans* genome was first sequenced, it was apparent that genes required for mating were all present in the genome and potentially functional. Most *C. albicans* isolates are apparently heterozygous for a and α mating type loci, each of which contain two mating cistrons a1 and a2, and α1 and α2, respectively. The two heterozygous loci produce an a1/α2 heterodimer that represses genes required for mating. When strains homozygous or hemizygous for one mating type were generated by genetic manipulation, it was found that these could mate at a very low frequency with strains that were of the opposite mating type. Most *C. albicans* strains remain stably in the white phase. It turns out the strains which undergo phenotypic switching are rare strains that are homozygous for a mating type, and that the a1/α2 heterodimer represses switching to the opaque phase. However, opaque phase cells mate efficiently with cells of the opposite mating type in the opaque phase. Thus, compared to *S. cerevisiae* where cells of the opposite mating type able to mate, an additional layer of control is present in *C. albicans* (Fig. 4). Most strains are diploid and heterozygous for mating type. Rarely, they become homozygous for a mating type, possibly by first becoming haploid for chromosome 5 followed by duplication of the remaining monosomic chromosome. Such cells are then able to switch to the opaque phase where they can now mate with cells of the opposite mating type which are also in the opaque phase (Tsong *et al.*, 2003, 2006). An interesting elaboration to this scheme is that although white cells homozygous for mating type are not competent for mating, when exposed to the mating pheromone of the opposite mating type they form a biofilm. This apparently stabilizes the chemotropic gradients that allow the germ tubes of rare opaque cells of opposite mating type to orient their growth towards each other to facilitate mating (Daniels *et al.*, 2006; Tsong *et al.*, 2006). After germ tubes of opposite mating types meet, they fuse; this is followed by nuclear fusion called karyogamy (Lockhart *et al.*, 2003).

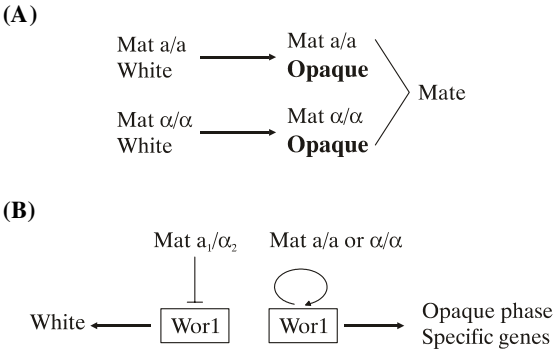

Fig. 4. Control of white-opaque phase switching. (A) White cells that are homozygous for mating type can switch to the opaque phase and are then mating competent. (B) The transcription factor Wor1 promotes its own transcription and that of opaque phase genes. In cells heterozygous for mating type, the heterodimeric gene regulator a_1/α_2 represses the expression of Wor1 so that cells are locked into the white phase and unable to mate.

Although white phase cells homozygous or hemizygous are able to switch to the opaque phase, they only do so at a low frequency. The elegant mechanism which controls this switch is now being uncovered (Fig. 4B). Opaque phase-specific transcription is driven by a transcription factor called Wor1, which is negatively regulated by the a_1/α_2 heterodimer. Thus, in a/α cells Wor1 is repressed and the cells are locked into the white phase. In cells that are homozygous or hemizygous for mating type, the repression of Wor1 is relieved. Wor1 promotes its own transcription so it can generate a self-sustaining feedback loop driving cells into the opaque phase. However, it is only present in white phase cells at concentrations that are normally too low to initiate this feedback loop. Only on rare occasions when the concentration transiently increases by chance can the feedback loop initiate and so flip the cell into a second stable state now in the opaque phase and allow them to become mating competent. Further levels of this regulation are now been uncovered involving more transcription factors such as Wor2 and Efg1, the latter already known to play a central role in regulating the yeast hyphal switch.

What is the biological role of such an elaborate mating mechanism? The zygote formed by the mating of two diploid cells is tetraploid. Meiosis has never been observed in *C. albicans* and its genome is missing *IME1*, the major regulator of

meiosis in *S. cerevisiae.* Tetraploid cells have been observed to regain the diploid state through chromosome loss during which recombination has been detected. Thus, the process of mating in *C. albicans* constitutes a parasexual rather than true sexual cycle but does provide a route for genetic exchange between different strains.

Acknowledgements

This work was supported in part by FPR08B1-230 NRF grant.

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