Candida albicans Als3, a Multifunctional Adhesin and Invasin

Yaoping Liu1 and Scott G. Filler1,2*

Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, 1 and David Geffen School of Medicine at UCLA, Los Angeles, California2

Candida albicans is part of the normal human flora, and it grows on mucosal surfaces in healthy individuals. In susceptible hosts, this organism can cause both mucosal and hematogenously disseminated disease. For C. albicans to persist in the host and induce disease, it must be able to adhere to biotic and abiotic surfaces, invade host cells, and obtain iron. The C. albicans hypha-specific surface protein Als3 is a member of the agglutinin-like sequence (Als) family of proteins and is important in all of these processes. Functioning as an adhesin, Als3 mediates attachment to epithelial cells, endothelial cells, and extracellular matrix proteins. It also plays an important role in biofilm formation on prosthetic surfaces, both alone and in mixed infection with Streptococcus gordonii. Als3 is one of two known C. albicans invasins. It binds to host cell receptors such as E-cadherin and N-cadherin and thereby induces host cells to endocytose the organism. Als3 also binds to host cell ferritin and enables C. albicans to utilize this protein as a source of iron. Because of its multiple functions and its high expression level in vivo, Als3 is a promising target for vaccines that induce protective cell-mediated and antibody responses. This review will summarize the multiple functions of this interesting and multifunctional protein.

Candida spp. are the fourth most common cause of nosocomial bloodstream infections, and Candida albicans accounts for approximately 50% of cases of candidemia (20, 67). This organism also causes at least 80% of cases of oropharyngeal and vulvovaginal candidiasis (57, 66). The predominance of C. albicans as a cause of both hematogenously disseminated and mucosal disease suggests that this organism possesses unique virulence factors compared to other species of Candida. One such factor, which is present only in C. albicans, is Als3. This protein plays a key role in multiple processes that are necessary for the organism to colonize the host and cause disease. These processes include adherence to host cells, biofilm formation, host cell invasion, and iron acquisition. Moreover, because Als3 is highly expressed in vivo, it is a promising target for therapeutic antibody and vaccine development. This review will discuss Als3 structure and function, as well as its utility as a therapeutic target.

GENETIC AND BIOCHEMICAL CHARACTERISTICS OF Als3

The ALS gene family. Als3 is encoded by the ALS3 gene, which is a member of the agglutinin-like sequence (ALS) gene family (23). This family was discovered by Hoyer et al. (24) based on its similarity to Saccharomyces cerevisiae SAG1, which codes for a agglutinin. The ALS gene family has eight members (ALS1 to ALS7 and ALS9) (Fig. 1). These genes encode cell surface proteins that are predicted to share the same overall structure (Fig. 2). At the N terminus is a signal peptide followed by a 300-amino-acid immunoglobulin-like domain and a 104-amino-acid threonine-rich domain that contains β-sheets (18, 22, 47, 55). Most Als proteins have adhesion function (18, 55, 72), and the binding domain for most substrates is located in the N terminus (31, 49, 55). The central domain of the Als proteins is composed of a variable number of 36-amino-acid tandem repeats. These repeats are rich in serine and threonine, exposed on the cell surface, and required for adherence function (31, 49). Because they are hydrophobic, the tandem repeats can directly mediate adherence to some substrates, such as polystyrene (16). There is significant strain-to-strain variation in the number of tandem repeats, and most strains possess two different-size alleles that specify different numbers of tandem repeats (24, 40, 71). In the case of Als3, a larger number of tandem repeats is associated with increased adherence when different-size alleles are expressed in C. albicans (40) but not when they are expressed in S. cerevisiae (35). The C terminus of Als proteins is serine and threonine rich and predicted to be heavily glycosylated. It contains a glycosylphosphatidylinositol anchorage sequence that is cleaved when the protein is covalently linked to the cell wall (18, 26).

Comparative genomic studies have revealed that most pathogenic Candida species, including Candida tropicalis, Candida parapsilosis, and Candida dubliniensis, contain multiple orthologs of the ALS genes (9, 25). However, none of these genes appear to be close orthologs of ALS3. Saccharomyces species, which rarely infect humans, do not contain ALS orthologs, suggesting that the products of this gene family may be uniquely important for fungal interactions with human cells.

Regulation of ALS3 expression. Als3 protein expression is regulated primarily at the transcriptional level. ALS3 is a hypha-specific gene that is expressed by C. albicans hyphae and pseudohyphae but not yeast-phase organisms (3, 23). Analysis of the ALS3 promoter using luciferase reporter constructs reveals that it contains two repression regions (R1 and R2) and two activation regions (A1 and A2) (Fig. 3). The hypha-specific repressors Tup1, Nrg1, and Rfg1 downregulate ALS3 transcription by binding to the two repression regions. The Efg1 and Cph1 transcription factors, which induce hyphal forma-

* Corresponding author. Mailing address: Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, 1124 W. Carson St., Torrance, CA. Phone: (310) 222-3813. Fax: (310) 782-2016. E-mail: sfiller@ucla.edu.

† Published ahead of print on 29 November 2010.
tion, bind to the two activation regions and upregulate ALS3 transcription. Tec1, another transcription factor that induces hyphal formation, does not activate ALS3 expression directly but instead functions through the zinc finger transcription factor Bcr1 (3, 37). Recently, Bastidas et al. (5) found that expression of ALS3 is inhibited under conditions of high nutrient availability. This inhibition occurs mainly through the protein kinase Tor1, which induces the expression of Nrg1 and Tup1 while downregulating expression of Efg1 and Bcr1 (5). ALS3 is also a target of the Rim101 alkaline response transcription factor (39). However, it is not yet known whether Rim101 binds directly to the ALS3 promoter or induces the expression of this gene indirectly.

**Als3 FUNCTION**

Als3 mediates adherence to diverse host substrates. Adherence to host constituents is necessary for *C. albicans* to colonize mucosal surfaces and subsequently cause disease. *C. albicans* possesses multiple adhesins that mediate binding to a variety of different host substrates (reviewed in reference 64). Many of these adhesins are encoded by the ALS gene family. Als3, like Als1 and Als5, has broad substrate specificity and thus mediates adherence to a variety of host constituents (55). Studies in which *C. albicans* Als3 was heterologously expressed in the normally nonadherent *S. cerevisiae* indicate that this protein mediates adherence to endothelial cells, oral epithelial cells, gelatin, fibronectin, fibrinogen, type IV collagen, laminin, and salivary pellicle (35, 55). Consistent with these results, an als3Δ/Δ null mutant strain of *C. albicans* has reduced adherence to endothelial cells and buccal epithelial cells (70). However, this mutant has normal adherence to fibronectin, possibly due to the compensatory effects of other Als proteins, such as Als1, that also bind to this extracellular matrix protein. As expected, both full-length monoclonal antibodies and single-chain variable fragments of human antibodies against Als3 block adherence to both endothelial and oral epithelial cells (6, 14, 28). These antibodies are directed against the N-terminal region of Als3, consistent with the model that this region contains the substrate binding domain.

Another murine monoclonal antibody, C7, has been found to bind to Als3 and inhibit adherence to host cells (8). Interestingly, this monoclonal antibody also reduces *C. albicans* germination, is fungicidal, and protects mice from disseminated candidiasis (33, 54). However, C7 binds to other antigens in addition to Als3, such as enolase and nucleoporin Nup88 (41). Also, other antibodies that bind only to Als3 do not inhibit germination or reduce *C. albicans* viability. Therefore, it is probable that many of the beneficial properties of C7 are mediated by its recognition of antigens other than Als3.

**Als3 plays a key role in biofilm formation.** A specialized form of adherence is biofilm formation. Biofilms are structured microbial communities that are attached to solid surfaces. *C. albicans* biofilm formation on dentures is associated with denture stomatitis. More importantly, biofilm formation on intra-vascular catheters plays a key role in the development of hematogenously disseminated candidiasis. Indeed, almost 80% of patients with candidemia have a central venous catheter in place at the time of diagnosis (20).

When *C. albicans* forms a biofilm, the initial basal layer consists of yeast-phase cells that are adherent to the substrate. On top of these cells is a mixture of pseudohyphae and hyphae (11, 37). Als3 plays a key role in biofilm formation. *C. albicans* mutants that lack Als3 produce scant, disorganized biofilms on catheter material in vitro (36, 69). Also, a bcr1Δ/Δ mutant, which has reduced expression of Als3 and other adhesins, has defective biofilm formation both in vitro and in the rat venous catheter model. Although an als3Δ/Δ mutant forms a normal biofilm in vivo, overexpression of ALS3 in the bcr1Δ/Δ mutant rescues its biofilm defects (36). These results indicate that, while multiple adhesins participate in biofilm formation in vivo, Als3 has a central role in this process. Other *C. albicans* adhesins that contribute to biofilm formation are Hwp1 and Als1 (36). Interestingly, a mixture of biofilm-defective hwp1Δ/Δ and als1Δ/Δ als3Δ/Δ mutants can form a hybrid biofilm both in vitro and...
and in the rat catheter infection model (38). These data suggest that the adherence of *C. albicans* cells to one another in a biofilm is mediated by the complementary binding of Hwp1 to Als1 and Als3.

**Als3 is bound by *Streptococcus gordonii*.** When *C. albicans* grows on mucosal surfaces, it adheres to the normal bacterial flora in addition to host cells. It can also form a mixed-species biofilm with oral bacteria on prosthetic materials such as dentures. One bacterium with which *C. albicans* can form a biofilm is *Streptococcus gordonii*. This bacterium enhances *C. albicans* biofilm formation by abrogating the *C. albicans* farnesol-based quorum-sensing mechanism and thereby stimulating hyphal growth (4). *S. gordonii* cells bind to *C. albicans* hyphae in an Als3-dependent manner. Furthermore, *S. gordonii* adheres avidly to *S. cerevisiae* expressing *C. albicans* Als3 but not to a control strain of *S. cerevisiae* that does not express this protein. The attachment of *S. gordonii* to Als3 is mediated by the multifunctional polypeptide adhesins SspA and SspB (56). *S. gordonii* also binds to *C. albicans* Als5 (27). Other Gram-positive cocci, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Streptococcus pyogenes*, also adhere to *C. albicans* (44). Whether Als3 mediates these adhesive interactions is not yet known. On the other hand, the Gram-negative bacterium *Pseudomonas aeruginosa* binds to *C. albicans* hyphae and subsequently kills the fungus (21). Hyphae of a *C. albicans* als3ΔΔ mutant have wild-type susceptibility to killing by *P. aeruginosa*. Thus, Als3 is not essential for the attachment of this bacterium to *C. albicans*, and other fungal ligands for *P. aeruginosa* must exist (7).

**Als3 is an invasin.** After *C. albicans* adheres to either oral epithelial cells or vascular endothelial cells, it can invade these cells. *C. albicans* invasion into oral epithelial cells is a key feature of oropharyngeal candidiasis (10, 17, 32, 48, 53). Also, during hematogenously disseminated candidiasis, blood-borne organisms must invade the endothelial cell lining of the vasculature to reach the deep tissues (19). *C. albicans* must invade oral epithelial cells and endothelial cells in order to damage these cells in vitro (15, 43). Moreover, *C. albicans* mutants with impaired capacity to invade and damage host cells in vitro frequently have attenuated virulence in murine models of oropharyngeal and hematogenously disseminated candidiasis (12, 39, 43, 51, 61). These data suggest that the capacity of *C. albicans* to invade and damage host cells is an important virulence attribute.

One mechanism by which *C. albicans* can invade both oral epithelial cells and endothelial cells is by inducing its own endocytosis. This process occurs by a zipper-like mechanism whereby the host cell is induced to produce pseudopods that progressively surround the organism and pull it into the cell (43, 50, 68). *C. albicans* hyphae are endocytosed much more readily than are yeast-phase organisms, suggesting that hyphae express specific invasin-like molecules on their surface that bind to one or more host cell receptors and induce endocytosis. Als3 is one of these invasins. A *C. albicans* als3ΔΔ mutant is endocytosed poorly by both oral epithelial cell lines and vascular endothelial cells in vitro. Moreover, latex beads coated with the recombinant N terminus of Als3 (rAls3-N) are efficiently endocytosed by these host cells, whereas control beads coated with bovine serum albumin (BSA) are not (47). Similarly, a strain of *S. cerevisiae* that expresses *C. albicans* Als3 is avidly endocytosed by endothelial cells, in contrast to control strains of *S. cerevisiae* that do not express Als3 (55). Due to their defect in inducing host cell endocytosis, strains of *C. albicans* with either reduced or absent expression of ALS3 have significantly reduced capacity to damage epithelial cells and endothelial cells in vitro (39, 47, 70). Although *C. albicans* must invade host cells to some extent to damage these cells, invasion by itself is not sufficient to induce damage. For instance, killed *C. albicans* cells are endocytosed by both endothelial and epithelial cells, but they do not cause detectable damage to these cells (15, 43). Thus, a factor(s) produced by viable organisms is required to cause host cell damage after invasion occurs.

Some of the host cell receptors for Als3 have been identified. Two of these receptors are E-cadherin on epithelial cells and N-cadherin on endothelial cells (47). Binding of Als3 to these receptors is sufficient to induce endocytosis because latex beads coated with rAls3-N are endocytosed by Chinese hamster ovary cells that heterologously express either E- or N-cadherin. Computer-assisted molecular modeling of the interactions of Als3 with either E- or N-cadherin suggests that the immunoglobulin domains in the N-terminal region of Als3 bind to the extracellular immunoglobulin domains of the cadherins (Fig. 4). Intriguingly, the binding parameters of the Als3–E-cadherin interaction are similar to those of one E-cadherin molecule binding to another E-cadherin molecule. Thus, *C. albicans* Als3 functions as a molecular mimic of mammalian E-cadherin (47).

Als3-induced endocytosis is mediated by the clathrin-dependent endocytic machinery, which requires dynamin and cortactin as well as clathrin (34). The cadherins can activate this pathway. Other proteins expressed on the host cell surface can also activate this pathway, and it is probable that some of them function as additional host cell receptors for Als3. As evidence that cadherins are not the only host cell receptor for *C. albicans*, small interfering RNA (siRNA) knockdown of endothelial cell N-cadherin by 90% reduces the endocytosis of this organism only by 34% (46). Recently, it has been reported that the epidermal growth factor receptor and HER2 are additional host cell receptors that are bound by Als3 and induce endocytosis (73). The relationship among these receptors and the cadherins during the endocytosis of *C. albicans* is currently under investigation.

Another *C. albicans* invasin is Ssa1, which is a member of the HSP70 family of heat shock proteins and is expressed on the surface of hyphae. An *ssa1ΔΔ* mutant is defective in binding to E-cadherin and N-cadherin and is endocytosed poorly by oral epithelial cells and vascular endothelial cells in vitro (61). Latex beads coated with recombinant Ssa1 are avidly endocytosed by epithelial and endothelial cells, demonstrating that this protein functions as an invasin. Importantly, the *ssa1ΔΔ* mutant has significantly attenuated virulence in mouse models of oropharyngeal and hematogenously disseminated candidiasis. Interestingly, the endocytosis defect of an *ssa1ΔΔ als3ΔΔ* double mutant is similar to that of an *als3ΔΔ* single mutant (61). A likely explanation for this result is that Ssa1 and Als3 bind to the same host cell receptors. It is even possible that Ssa1 and Als3 may form a multiprotein receptor complex, analogous to the integrins in mammalian cells.

**Als3 is a receptor for ferritin and mediates iron acquisition from the host.** In order for a microorganism to survive in the
host, it must be able to obtain iron. In mammals, virtually all iron is complexed with host cell proteins, and 30% of total iron is bound to ferritin (2). C. albicans, unlike S. cerevisiae, can utilize ferritin as the sole source of iron. Almeida et al. (1) discovered that C. albicans hyphae, but not yeast-phase cells, can bind to both purified ferritin and ferritin contained within epithelial cells. Most intriguingly, hyphae of an als3Δ/Δ mutant are unable to bind ferritin and thus grow poorly on media containing ferritin as the sole source of iron. Heterologous expression of C. albicans ALS3 in S. cerevisiae enables this organism to bind ferritin. These observations demonstrate that Als3 functions as a ferritin receptor and facilitates the capacity of C. albicans to obtain iron from the host.

Role of Als3 in virulence. As expected from the multifunctional nature of Als3, mutant strains of C. albicans that lack this protein have prominent defects in assays of host cell interaction, biofilm formation, and iron acquisition in vitro. In addition, Als3 is strongly expressed by hyphae in the kidneys of mice with disseminated candidiasis (14), and high levels of ALS3 mRNA are present in oral scrapings of patients with oropharyngeal candidiasis (68). Thus, one would expect that an als3Δ/Δ mutant would have significantly attenuated virulence in experimental animal models of candidiasis. We have found that an als3Δ/Δ mutant has wild-type virulence when inoculated into immunocompetent adult mice via the tail vein. In contrast, Tsai et al. (65) found that an als3Δ/Δ mutant has significantly attenuated virulence when inoculated intraperitoneally into 2-day-old mouse pups. Thus, Als3 appears to play a variable role in virulence, depending on the route of inoculation and the age and immune status of the host.

There are at least two possible explanations for why als3Δ/Δ mutants have little or no virulence defects in some mouse models of candidiasis. One is that the functional redundancy of other Als proteins and additional adhesins and invasins masks the effects of the absence of Als3. Another possibility is that there is compensatory upregulation of other adhesins and invasins when Als3 is absent. This compensatory upregulation may occur to a variable extent, depending on the microenvironment of the host to which the organism is exposed. These issues of functional redundancy and compensatory upregulation illustrate the complexity of studying proteins such as Als3 that are members of a larger protein family.

A key difference between many in vitro models of C. albicans-host cell interaction and in vivo virulence studies is the effects of hyphal formation. Mutants of C. albicans with defects in hyphal formation have severe host cell interaction defects in vitro. For example, they are unable to damage and escape from macrophages (30), and they have markedly reduced capacity to invade and damage both epithelial cells and endothelial cells (43, 45). Although C. albicans mutants that are locked in the yeast phase have significantly reduced lethality when inoculated into adult mice via the tail vein, they are able to escape from the bloodstream and invade and persist in the target organs (30, 52). Thus, in experimental animal models, these yeast-phase organisms are somehow able to adhere to and invade host cells and obtain iron from the host in the absence of Als3, which is expressed only by hyphae. How yeast-phase organisms are able to carry out these processes is currently unknown, but it is likely that they express one or more functional equivalents of Als3.

**Als3 AS A VACCINE TARGET**

**Vaccination with rAls3-N protects mice against candidiasis.** The risk factors for developing both hematogenously disseminated and mucosal candidiasis are well defined (reviewed in reference 42). Thus, a vaccine that prevented these diseases could be targeted to patients who either have the appropriate risk factors or are likely to develop them. The fact that Als3 is highly expressed on the C. albicans surface in vivo makes it a good target for a vaccine. Spellberg et al. (60) found that vaccination with rAls3-N protects immunocompetent mice from both vaginal candidiasis and lethal disseminated candidiasis. It also significantly reduces oral fungal burden in the corticosteroid-treated mouse model of oropharyngeal candidiasis. Interestingly, the same rAls3-N vaccine is also protective in the mouse model of methicillin-resistant Staphylococcus aureus bacteremia, probably due to antigenic cross-reactivity (59). The vaccine both improves survival and reduces organ bacterial burden in this model.

Adoptive transfer experiments and studies with different strains of knockout mice, including gamma interferon (IFN-γ)-/-, interleukin 17 (IL-17)-/-, and gp91phox-/- strains, indicate that the mechanism of rAls3-N vaccine-induced protec-
tion against both C. albicans and S. aureus bloodstream infection is the induction of a Th1 and Th17 immune response (29, 58–60). Antibodies are neither necessary nor sufficient for the efficacy of this vaccine. Vaccination with rAls3-N primes Th1, Th17, and Th1/Th17 lymphocytes to produce high levels of IFN-γ and IL-17A, as well as the chemokines KC and MIP-1α. These proinflammatory cytokines enhance the capacity of phagocytes to kill both pathogens and thereby prevent disease (29). Human trials of the rAls3-N vaccine are in final preparation.

Vaccination with β-1,3-glucan induces antibodies against Als3. It has also been found that vaccination of mice with laminarin (purified β-1,3-glucan) conjugated with genetically inactivated diphtheria toxin protects mice from infections caused by both C. albicans and Aspergillus fumigatus (62). The mechanism of this protection is the induction of anti-β-1,3-glucan antibodies. The protective effects of this vaccine against C. albicans infection can be mimicked by a monoclonal antibody directed against β-1,3-glucan (63). Although this monoclonal antibody has several targets, one of them is Als3, which is a glucan-linked glycosylphosphatidylinositol-anchored protein. Therefore, Als3 is a promising target for two different types of vaccines.

**FUTURE DIRECTIONS**

Although the role of Als3 in many key aspects of C. albicans biology has been investigated, a number of questions about this protein remain unanswered. For example, none of the Als proteins has been successfully analyzed by X-ray crystallography. Thus, the exact three-dimensional structure of Als3 has not been definitively determined. Furthermore, detailed structure-function analyses of this protein have not yet been done. Also, it is highly probable that there are additional host cell targets of Als3 that have not been discovered to date. Finally, the list of C. albicans proteins that function similarly to Als3 and can compensate for its absence is currently incomplete.

Answering these questions will provide important new information about how C. albicans adheres to host and bacterial substrates, forms biofilms, and invades host cells. This information also holds promise to the development of new approaches to prevent and treat candidal infections.

**ACKNOWLEDGMENTS**

This work was supported in part by NIH grants R01AI054928 and R01DE017088 to S.G.F.

**REFERENCES**

1. Almeida, R. S., et al. 2008. The hyphal-associated adhesin and invasin Als3 of Candida albicans mediates iron acquisition from host ferritin. PLoS Pathog. 4:e1000217.
2. Almeida, R. S., D. Wilson, and B. Hube. 2009. Candida albicans iron acquisition within the host. FEBS Lett. 582:1000–1012.
3. Argimon, S., et al. 2007. Developmental regulation of an adhesin gene during cellular morphogenesis in the fungal pathogen Candida albicans. Eur. Cells Mol. Biol. 5:682–692.
4. Bamford, C. V., et al. 2009. Streptococcus gordonii modulates Candida albicans biofilm formation through intergeneric communication. Infect. Immun. 77:3696–3704.
5. Bastidas, R. J., J. Heitman, and M. E. Cardenas. 2009. The protein kinase Tor1 regulates adhesin gene expression in Candida albicans. PLoS Pathog. 5:e1000294.
6. Bencher, B., et al. 2009. Recognition of Candida albicans Als3 by the germ tube-specific monoclonal antibody 3D9.3. FEMS Immunol. Med. Microbiol. 55:314–323.
7. Brand, A., J. D. Barnes, K. S. Mackenzie, F. C. Odds, and N. A. Gow. 2008. Cell wall glycans and soluble factors determine the interactions between the hyphae of Candida albicans and Pseudomonas aeruginosa. FEMS Microbiol. Lett. 287:46–55.
8. Brenn, R., et al. 2007. Fungicidal monoclonal antibody C7 binds to Candida albicans Als3. Infect. Immun. 75:3680–3682.
9. Butler, G., et al. 2009. Evolution of pathogenicity and sexual reproduction in eight Candida species. Nature 459:657–662.
10. Cawson, R. A., and K. C. Rajasingham. 1972. Ultrastructural features of the invasive phase of Candida albicans. Br. J. Dermatol. 87:435–443.
11. Chandra, J., et al. 2001. Biofilm formation by the fungal pathogen Candida albicans: development, architecture, and drug resistance. J. Bacteriol. 183:5385–5394.
12. Chiang, L. Y., et al. 2007. Candida albicans protein kinase CK2 governs virulence during oropharyngeal candidiasis. Cell. Microbiol. 9:233–245.
13. Cleary, L. A., et al. 2010. Als3p is not required for C. albicans virulence in the mouse model of disseminated candidiasis. abstr. 30B, p. 69. Abstr. 10th ASM Candida Meeting.
14. Coleman, D. A., et al. 2009. Monoclonal antibodies specific for Candida albicans Als3 that immobilize fungal cells in vitro and in vivo block adhesion to host surfaces. J. Microbiol. Methods 78:71–78.
15. Filser, S. G., J. N. Swerdlow, C. Hobbs, and P. M. Lucket. 1995. Penetration and damage of endothelial cells by Candida albicans. Infect. Immun. 63:976–983.
16. Frank, A. T., et al. 2010. Structure and function of glycosylated tandem repeats from Candida albicans ALS adhesins. Eukaryot. Cell 9:405–414.
17. Garcia-Tamayo, J., G. Castillo, and A. J. Martínez. 1982. Human genital candidiasis: histochemistry, scanning and transmission electron microscopy. Acta Cytol. 26:7–14.
18. Gaunt, N. K., and S. A. Klotz. 1997. Expression, cloning, and characterization of a Candida albicans gene, ALA1, that confers adherence properties upon Saccharomyces cerevisiae for extracellular matrix proteins. Infect. Immun. 65:5289–5294.
19. Grubb, E. S., et al. 2008. Candida albicans-endothelial cell interactions: a key step in the pathogenesis of systemic candidiasis. Infect. Immun. 76:4370–4377.
20. Hajjeh, R. A., et al. 2004. Incidence of bloodstream infections due to Candida species and in vitro susceptibilities of isolates collected from 1998 to 2000 in a population-based active surveillance program. J. Clin. Microbiol. 42:1519–1527.
21. Hogan, D. A., and R. Kolter. 2002. Pseudomonas-Candida interactions: an ecological role for virulence factors. Science 296:2229–2232.
22. Hoyer, L. L., and L. E. Hecht. 2001. The ALS5 gene of Candida albicans and analysis of the Als5p N-terminal domain. Yeast 18:49–60.
23. Hoyer, L. L., T. L. Payne, M. Bell, A. M. Myers, and S. Scherer. 1998. Candida albicans ALS3 and insights into the nature of the ALS gene family. Clin. Microbiol. Rev. 11:451–459.
24. Hoyer, L. L., S. Scherer, A. R. Shatzman, and G. P. Livi. 1995. Candida albicans ALS1: domains related to a Saccharomyces cerevisiae sexual agglutinin separated by a repeating motif. Mol. Microbiol. 15:59–54.
25. Jackson, A. P., et al. 2009. Comparative genomics of the fungal pathogens Candida dubliniensis and Candida albicans. Genome Res. 19:2231–2244.
26. Kaptayan, J. C., et al. 2000. The cell wall architecture of Candida albicans wild-type cells and cell wall-defective mutants. Mol. Microbiol. 36:601–611.
27. Klotz, S. A., et al. 2007. Candida albicans Als proteins mediate aggregation with bacteria and yeasts. Med. Mycol. 45:563–570.
28. Laforce-Neshbitt, S. S., M. A. Sullivan, L. L. Hoyer, and J. M. Bliss. 2008. Inhibition of Candida albicans adhesion by recombinant human antibody single-chain variable fragment specific for Als3p. FEMS Immunol. Med. Microbiol. 54:195–202.
29. Lin, L., et al. 2009. Th1-Th17 cells mediate protective adaptive immunity against Staphylococcus aureus and Candida albicans infection in mice. PLoS Pathog. 5:e1000703.
30. Lo, H. J., et al. 1997. Nonfilamentous C. albicans mutants are avirulent. Cell 90:539–549.
31. Loza, L., et al. 2004. Functional analysis of the Candida albicans ALS1 gene product. Yeast 21:473–482.
32. Montes, L. F., and W. H. Wilborn. 1968. Ultrastructural features of host-parasite relationship in oral candidiasis. J. Bacteriol. 96:1349–1356.
33. Moragues, M. D., et al. 2003. A monoclonal antibody directed against a Candida albicans cell wall mannoprotein exerts three anti-C. albicans activities. Infect. Immun. 71:5273–5279.
34. Moreno-Ruiz, E., et al. 2009. Candida albicans internalization by host cells is mediated by a clathrin-dependent mechanism. Cell. Microbiol. 11:1179–1187.
35. Nobbs, A. H., M. M. Vickerman, and H. F. Jenkinson. 2010. Heterologous expression of Candida albicans cell wall-associated adhesins in Saccharomyces cerevisiae reveals differential specificities in adherence and biofilm formation and in binding oral Streptococcus gordonii. Eukaryot. Cell 9:1622–1634.
36. Nobile, C. J., et al. 2006. Critical role of Bcr1-dependent adhesins in C. albicans biofilm formation in vitro and in vivo. PLoS Pathog. 2:e63.
37. Nobile, C. J., and A. P. Mitchell. 2005. Regulation of cell-surface genes and biofilm formation by the C. albicans transcription factor Bcr1p. Curr. Biol. 15:1150–1155.
38. Nobile, C. J., et al. 2008. Complementary adhesion function in C. albicans biofilm formation. Curr. Biol. 18:1017–1024.
39. Nobile, C. J., et al. 2008. Candida albicans transcription factor Rim101 mediates pathogenic interactions through cell wall functions. Cell. Microbiol. 10:2180–2196.
40. Oh, S. H., et al. 2005. Functional specificity of Candida albicans Als3p proteins and clade specificity of ALS3 alleles discriminated by the number of copies of the tandem repeat sequence in the central domain. Microbiology 151:673–681.
41. O’Brien, A., M. J., et al. 2005. Antifungal and antitumor activities of a monoclonal antibody directed against a stress mannoprotein of Candida albicans. Curr. Mol. Med. 5:393–401.
42. Pappas, P. G., et al. 2009. Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin. Infect. Dis. 48:503–535.
43. Park, H., et al. 2005. Role of the fungal Ras-protein kinase A pathway in governing epithelial cell interactions during oropharyngeal candidiasis. Cell. Microbiol. 7:499–510.
44. Peters, B. M., et al. 2010. Microbial interactions and differential protein expression in Staphylococcus aureus-Candida albicans dual-species biofilms. FEMS Immunol. Med. Microbiol. 59:293–305.
45. Phan, Q. T. Ph, T. Belanger, and S. G. Filler. 2000. Role of hyphal formation in interactions of Candida albicans with endothelial cells. Infect. Immun. 68:3485–3490.
46. Phan, Q. T., R. A. Fratti, N. V. Prasadaro, J. E. Edwards, Jr., and S. G. Filler. 2005. N-cadherin mediates endocytosis by Candida albicans by endothelial cells. J. Biol. Chem. 280:10455–10461.
47. Phan, Q. T., et al. 2007. Als3 is a Candida albicans invasive that binds to caderhins and induces endocytosis by host cells. PLoS Biol. 5:e64.
48. Rajasingham, K. C., S. J. Challacombe, and S. Torey. 1989. Ultrastructure and possible processes involved in the invasion of epithelial cells by Candida albicans in vaginal candidiasis. Cytobios 60:61–1274.
49. Rauchou, J. M., et al. 2006. Threonine-rich repeats increase fibronectin binding in the Candida albicans adhesin Als5p. Eukaryot. Cell 5:1664–1673.
50. Rottensteiner, D., et al. 1985. Adherence of Candida to cultured vascular endothelial cells: mechanisms of attachment and endothelial cell penetration. J. Infect. Dis. 152:1264–1274.
51. Sanchez, A. A., et al. 2004. Relationship between Candida albicans virulence during experimental hematogenously disseminated infection and endothelial cell damage in vitro. Infect. Immun. 72:596–601.
52. Saville, S. P., A. L. Lazzell, C. Montagudo, and J. L. Lopez-Ribot. 2003. Engineered control of cell morphology during infection of yeast and filamentous forms of Candida albicans during infection. Eukaryot. Cell 2:1053–1060.
53. Scherwitz, C. 1982. Ultrastructure of human cutaneous candidosis. J. Invest. Dermatol. 78:200–205.
54. Sevilla, M. I., B. Robledo, A. Rementeria, M. D. Moragues, and J. Ponton. 2006. A fungicolous monoclonal antibody protects against murine invasive candidiasis. Infect. Immun. 74:3042–3045.
55. Sheppard, D. C., et al. 2004. Functional and structural diversity in the Als protein family of Candida albicans. J. Biol. Chem. 279:30840–30849.
56. Silverman, R. J., A. H. Nobbs, M. M. Vickerman, M. E. Barbour, and H. F. Jenkinson. 2010. Interaction of Candida albicans cell wall Als protein with Streptococcus gordonii SspB adhesin promotes development of mixed-species communities. Infect. Immun. 78:4644–4652.
57. Sobel, J. D. 1986. Recurrent vulvovaginal candidiasis. A prospective study of the efficacy of maintenance ketoconazole therapy. N. Engl. J. Med. 315:1455–1458.
58. Spellberg, B., et al. 2008. Antibody titer threshold predicts anti-candidal vaccine efficacy even though the mechanism of protection is induction of cell-mediated immunity. J. Infect. Dis. 198:256–260.
59. Spellberg, B., J., et al. 2006. Efficacy of the anti-Candida rAls3p-N or rAls1p-N vaccines against disseminated and mucosal candidiasis. J. Infect. Dis. 194:256–260.
60. Sun, J. N., et al. 2010. Host cell invasion and virulence mediated by Candida albicans Ssa1. PLoS Pathog. 6:e1000181.
61. Torosantucci, A., et al. 2005. A novel glyco-conjugate vaccine against fungal pathogens. J. Exp. Med. 202:597–606.
62. Torosantucci, A., et al. 2009. Protection by anti-beta-glucan antibodies is associated with restricted beta-1,3 glucan binding specificity and inhibition of fungal growth and adherence. PLoS One 4:e3592.
63. Tromchin, G., M. Pihet, L. M. Lopes-Bezerra, and J. P. Bouchara. 2008. Adherence mechanisms in human pathogenic fungi. Med. Mycol. 46:749–772.
64. Tsai, N. Y., S. S. Lafortne-Nesbitt, R. Tucker, and J. M. Bliss. 2010. A murine model for disseminated candidiasis in neonates. Pediatr. Res. [Epub ahead of print.] doi:10.1203/PDR.0b013e318206fd3e.
65. Vazquez, J. A., et al. 2006. A multicenter randomized trial evaluating posaconazole versus fluconazole for the treatment of oropharyngeal candidiasis in subjects with HIV/AIDS. Clin. Infect. Dis. 42:1179–1186.
66. Wispelinghoff, H., et al. 2004. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. Clin. Infect. Dis. 39:309–317.
67. Zakikhany, K., et al. 2007. In vivo transcript profiling of Candida albicans identifies a gene essential for interepithelial dissemination. Cell. Microbiol. 9:2938–2945.
68. Zhao, X., et al. 2006. Candida albicans Als3p is required for wild-type biofilm formation on silicone elastomer surfaces. Microbiology 152:2287–2299.
69. Zhao, X., et al. 2004. ALS3 and ALS8 represent a single locus that encodes a Candida albicans adhesin: functional comparisons between Als3p and Als8p. Microbiology 150:2415–2428.
70. Zhao, X., et al. 2007. Analysis of ALS5 and ALS6 allelic variability in a geographically diverse collection of Candida albicans isolates. Fungal Genet. Biol. 44:1298–1309.
71. Zhao, X., S. H. Oh, K. M. Yeater, and L. L. Hoyer. 2005. Analysis of the Candida albicans Als5p and Als6p adhesins suggests the potential for compensatory function within the Als family. Microbiology 151:1619–1630.
72. Zhu, W., P. Boontheung, J. A. Loo, and S. G. Filler. 2010. Roles of HER2 and EGFR signaling in host cell invasion by Candida albicans, abstr. 180B, p. 127. Abstr. 10th ASM Conf. Candida Candidiasis.

Yaoping Liu received a B.S. degree in biology from Shandong Normal University, Jinan, China, and a Ph.D. in microbiology from the Chinese Academy of Sciences, Shenyang, China. He performed postdoctoral work on antisenesce-mediated regulation of the EcoRI restriction-modification system in Escherichia coli at the Institute of Medical Sciences, University of Tokyo, Japan. He is currently a postdoctoral research associate in the Division of Infectious Disease at the Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center. His research focuses on discovering novel Candida albicans genes, the products of which govern the interactions of this organism with host cells.

Scott G. Filler received an A.B. degree in biology from Dartmouth College, Hanover, NH, and an M.D. from the David Geffen School of Medicine at the University of California, Los Angeles. As a postdoctoral fellow, he studied the pathogenesis of C. albicans infections with John E. Edwards, Jr., at Harbor-UCLA Medical Center. In 1991, he joined the faculty of the David Geffen School of Medicine at UCLA, where he is currently a professor of medicine. The focus of his research is the molecular mechanisms by which C. albicans and Aspergillus fumigatus invade and damage host cells and thereby cause disease.