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Quick guide

Candida albicans

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What is* Candida albicans*? *Candida albicans* is the best studied and most prevalent of the human fungal pathogens. *Candida* species are fungi that grow as yeasts and that are 'imperfect', meaning they apparently lack a complete sexual cycle; yet *C. albicans* and several related species clearly have the potential to engage in 'parasex' (described below). *C. albicans*, thought to be an obligate diploid, can form true filamentous hyphae in addition to the budding yeast and pseudohyphal (elongated yeast) cells seen in other *Candida* species and in the model yeast *Saccharomyces cerevisiae*.

What kinds of conditions cause* C. albicans *to become a pathogen? *C. albicans* is an opportunistic pathogen that resides as a harmless commensal in the gut, genito-urinary tract and skin. It becomes an opportunistic pathogen under a number of different host conditions, usually involving reduced immune competence or an imbalance of the competing bacterial microflora. Mucosal infections, such as oral thrush or vaginitis, are usually not life-threatening, but they can be the sentinel symptom of immune suppression, for example in patients infected with HIV. Much more serious are blood stream candidal infections, which are associated with high mortality rates. The limited arsenal of antifungal drugs and the ability of drug resistance to arise through multiple mechanisms, including the natural drug resistance of biofilms, contribute to the recalcitrance of candidal infections and their position as the third or fourth most common cause of nosocomial (hospital-acquired) infections.

What processes are most important for pathogenicity? The genetic and physiological changes accompanying the shift of *C. albicans* from a commensal to a pathogenic lifestyle are being investigated using clinical isolates, by animal studies (primarily with mice using oral and bloodstream infection models), and by tissue culture and other *in vitro* approaches (Figure 1). Next generation sequencing is expected to provide many insights into the genetic, genomic and transcriptomic changes involved, while studies of metabolism and protein composition should provide a clearer view of physiological changes that occur during this transition.

Another approach is to focus on gene families that have been amplified considerably in *C. albicans* relative to other, less pathogenic, *Candida* species. These include agglutinin-like sequence (*ALS*) genes, the products of which promote adhesion between individual cells, adhesion between cells and host tissues, and invasion of host tissue by stimulating endocytosis. Indeed, Als3 is being developed as an anti-fungal vaccine. Other amplified gene families encode secreted aspartyl proteases and lipases that can promote tissue invasion and ferric reductases, which facilitate iron-acquisition in the iron-limiting host environment. An intriguing gene family that also has been amplified considerably relative to less pathogenic *Candida* species is the telomere-associated *TLO* gene family, members of which appear to encode variant copies of a single component of the mediator complex, a major regulator of general transcription.

What is a parasexual cycle? While conventional meiosis has not been described, *C. albicans* does have two mating types (***a*** and ***alpha***) and orthologs of many genes involved in meiosis in *S. cerevisiae* and other eukaryotes. Most clinical isolates are diploid or near diploid, and a small proportion of them are homozygous for the mating-type locus, suggesting that they have the potential to mate. A physiogical/epigenetic switch from the standard 'white' phase to an 'opaque' state is a prerequisite for mating between diploids to form tetraploids (Figure 2). Under some stress conditions these tetraploids return to a near-diploid state, albeit with many of them carrying at least one extra (aneuploid) chromosome and some of them having undergone recombination events that require Spo11, a protein necessary for meiotic recombination in organisms with *bona fide* meiosis. This alteration of generations from diploids to tetraploids and then back to near-diploids without conventional meiosis is termed the 'parasexual cycle'. The aneuploid progeny generally carry from one to three aneuploid chromosomes. The ability to tolerate aneuploidy and other chromosomal rearrangements apparently provides *C. albicans* with a non-Mendelian type of genetic diversity that likely facilitates its adaptation to the broad range of harsh environments within the host.

What is morphological switching? *C. albicans* grows with several different morphologies: by budding to form oval yeast and elongated pseudohyphae; or with exclusively polarized growth to form uninucleate true hyphae, which resemble the true hyphae of filamentous fungi. Phenotypic switching of mating-type homozygous oval white cells to the oblong 'opaque' form involves a switch to mating competence as well as a change in cell-wall properties thought to make the cells more permeable to mating pheromones.

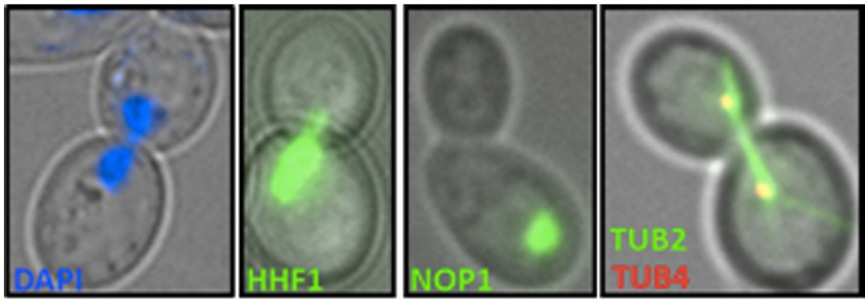

Figure 1. Fluorescent protein fusions in *Candida albicans*.
Useful fluorescent markers for tracking subcellular structures in *C. albicans* include: DAPI (DNA); Nop1-GFP (green fluorescent protein, localizing to the nucleolus); Hhf1-GFP (chromatin); Tub2-GFP (microtubules, green); and Tub4-mCherry fluorescent protein (spindle pole bodies, red). Micrographs courtesy of Shelly Applen and Benjamin Harrison.

Another morphological switch leads to formation of enlarged, round 'chlamydospores' at the end of an elongated stalk cell in response to specific signals and nutritional conditions.

All of the different morphological states are associated with different gene expression patterns and specific changes in cell cycle progression patterns: for example, yeast, pseudohyphae, and true hyphae exhibit different degrees of integration between the cell growth aspect of cell cycle progression and the nuclear and spindle cycles.

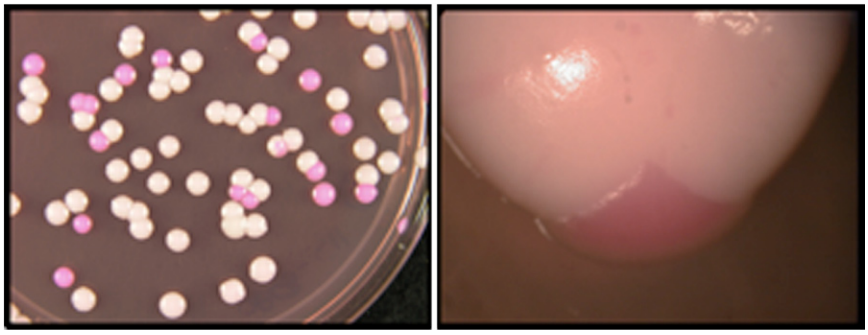

Figure 2. Phenotypic switching in *Candida albicans.*
Growth of *C. albicans* cells on phloxine plates detects colonies of cells that are either white (white colonies) or opaque (pink colonies) (left panel). A subset of cells in a mostly white colony switched to the opaque state, forming a sector in the colony (right panel). Photos courtesy of Meleah Hickman.

What are biofilms? Biofilms are multicellular communities growing on the surface of tissues or indwelling medical devices, such as catheters or dental prostheses. Cells in biofilms exhibit drug resistance relative to genetically identical planktonic cells (unattached cells growing in a liquid culture), which is due to heterogeneity of the morphology (yeast, pseudohyphae, hyphae, white and opaque cells) and of the metabolic state (quiescent and actively growing, level of pheromone responsiveness) in the population. In addition, the extracellular matrix material that is characteristic of biofilms can sequester some antifungal drugs, reducing their effective concentration. Biofilm communities also engage in signaling processes, incuding quorum sensing, a response to cell density that affects growth, morphogenesis and that likely promotes survival in the host.

What causes antifungal drug resistance? The identification of drug targets and development of effective antifungal drugs is complicated by the similarity between the eukaryotic fungi and their hosts. There are only three major classes of antifungal drugs used to treat candidal infections. Resistance to the most widely used of these, the azoles, is increasing, especially in patients, such as those with HIV, who are given azoles prophylactically. The echinocandins were introduced in the last decade to target cell-wall biosynthesis and reports of echinocandin resistance and tolerance are becoming more frequent.

Drug resistance arises within existing commensal strains, and not via the horizontal transfer mechanisms typically seen in prokaryotic pathogens. Rather, antifungal drug resistance appears through several independent, and potentially synergistic mechanisms, including: altered activity or regulation of drug efflux transporters; new point mutations; homozygosis of existing mutations or increased copy number of genes encoding the drug targets (such as *ERG11*, which encodes lanosterol 140alpha-demethylase, the target of azole drugs involved in sterol biosynthesis); alterations in regulatory factors that regulate the drug efflux pumps or targeted biochemical pathways (such as the ergosterol biosynthesis pathway).

Some chromosomal rearrangements or amplifications have further effects on one or more of these mechanisms, thereby causing increased drug resistance. While aneuploidy is found in half of all drug-resistant isolates, the increased copy number of specific genes with canonical roles in drug resistance is responsible for at least some of the resistance seen in lab as well as clinical isolates, suggesting that the number of copies of specific genes or combinations of genes provides a selective advantage to some aneuploid isolates.

What molecular tools are available for working with* C. albicans *and other* Candida *species? In general, genetic studies of *C. albicans* have been slowed by the lack of a meiotic cycle, a haploid phase and stable autonomously maintained plasmids (most integrate very rapidly). Thus, most work has exploited genomics approaches that received a great boost with the 2004 publication of the first *C. albicans* genome sequence and subsequent assembly of this sequence into a chromosome-based genome map. The publication of sequences for other *Candida* species, in 2009, greatly facilitates work in these CUG clade members as well. Because of their native levels of drug resistance and/or their ability to become resistant rapidly, species such as *Candida krusei* and *Candida glabrata* are becoming more prevalent in the clinic.

Genomic studies are facilitated by several genome websites, including the Candida Genome Database, the Candida DB, the Broad Fungal Genome initiative, the Sanger institute, and Genolevures (see URL links below). Several useful collections of deletion strains, conditional repression strains and a soon-to-be available overexpression strain collection permit screening of a substantial proportion of known genes for their roles in specific phenotypes.

How similar is* C. albicans *to the model yeast* S. cerevisiae*? *C. albicans* and *S. cerevisiae* are thought to have diverged approximately 140–850 million years ago. While the two yeasts resemble each other in many ways, *C. albicans* is not simply a baker's yeast that lost meiosis and acquired virulence traits. *C. albicans* survives in a much broader range of ecological niches and undergoes a parasexual cycle involving a physiological switch to 'opaque' cells that is involved in regulating virulence in a broad range of pathogenic fungi and is not seen in *S. cerevisiae*. In addition, since their last common ancestor, the two

yeasts have rewired many regulatory networks, such that processes as diverse as ribosomal protein gene regulation, galactose metabolism and mating-type switching, which appear to be very similar at the physiological level, are regulated by different transcription factors that bind to different regulatory sequences.

Despite its evolutionary distance to *S. cerevisiae* being *relatively* short, *C albicans*, like other members of the 'CUG' clade, has a genetic code with a non-standard codon — CUG encodes serine rather than the conventional leucine — obviating the direct use of many genetic markers from heterologous organisms such as *S. cerevisiae*. This obstacle has been overcome by the development of codon-optimized markers, fluorescent proteins and other epitope tags, which can be inserted into the genome by several approaches all ultimately involving homology-based recombination. These tools have led to many of the important advances in our understanding of the molecular mechanisms specifying mating, white-opaque switching, biofilm formation and other processes important for pathogenicity and virulence. There are clearly many more to come.

Where can I find out more?

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Primer

Unfolded protein response

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In eukaryotic cells, the endoplasmic reticulum (ER) is a membrane-enclosed interconnected organelle responsible for the synthesis, folding, modification, and quality control of numerous secretory and membrane proteins. The processes of protein folding and maturation are highly assisted and scrutinized but are also sensitive to changes in ER homeostasis, such as Ca^{2+} depletion, oxidative stress, hypoxia, energy deprivation, metabolic stimulation, altered glycosylation, activation of inflammation, as well as increases in protein synthesis or the expression of misfolded proteins or unassembled protein subunits. Only properly folded proteins can traffic to the Golgi apparatus, whereas those that misfold are directed to ER-associated degradation (ERAD) or to autophagy. The accumulation of unfolded/misfolded proteins in the ER activates signaling events to orchestrate adaptive cellular responses. This unfolded protein response (UPR) increases the ER protein-folding capacity, reduces global protein synthesis, and enhances ERAD of misfolded proteins.

In mammals, the UPR is signaled through three ER transmembrane protein sensors (Figure 1): inositol-requiring kinase 1 (IRE1), pancreatic ER eIF2a kinase (PERK), and activating transcription factor 6 (ATF6). The luminal domain of each sensor responds to the level of unfolded/misfolded protein in the ER. If the cell cannot resolve the protein-folding defect, cell-death signaling pathways are activated. As our understanding of ER protein-folding pathways and the mechanisms of UPR signaling of adaptive and apoptotic responses has grown, so has the significance of their impact on the etiology of multiple human pathologies, including metabolic syndrome, neurodegenerative disorders, inflammatory disease and cancer. With this armamentarium of knowledge, it is now possible for rational design of therapeutics to target protein-folding pathways and UPR signaling to resolve protein misfolding in disease states in a manner that was not previously conceivable.

IRE1–XBP1

The most conserved branch of the UPR is mediated by IRE1, a type I transmembrane protein with both a serine/threonine kinase domain and an endoribonuclease (RNase) domain in its cytosolic portion. In yeast, Ire1p is the only identified ER stress sensor. There are two IRE1 homologues in mammals: IRE1α is expressed ubiquitously, whereas IRE1β expression is mostly restricted to the intestinal epithelium.

Two models have been proposed for the activation of IRE1/Ire1p. The first is a competition-binding model that posits unfolded and/or misfolded proteins in the ER lumen compete with Ire1p for binding to the chaperone BiP. Ire1p binding to BiP inhibits signaling, whereas Ire1p released from BiP forms homodimers and oligomers that promote *trans*-autophosphorylation and RNase activation. Alternatively, since the crystal structure of the yeast Ire1p luminal domain revealed the presence of a peptide-binding groove similar to that of major histocompatibility class I molecules, it was proposed that unfolded/misfolded proteins directly bind to the amino-terminal luminal domain of Ire1p to induce dimerization. A recent study showed that unfolded proteins and hydrophobic peptides bind to the core luminal domain of Ire1p, which then undergoes dimerization *in vitro*. In contrast, the peptide-binding cleft in the crystal structure of the luminal domain of human IRE1α is not solvent accessible and the luminal domain does not interact with unfolded proteins *in vitro*. A mutant form of IRE1α that cannot bind to BiP is able to spontaneously dimerize and activate kinase/RNase activities in the absence of ER stress, suggesting that mammalian IRE1α may be released from BiP but may not require peptide binding for activation.

The luminal domain of IRE1α forms homodimers in the plane of the ER membrane, juxtaposing the kinase domains for *trans*-autophosphorylation to stimulate the kinase and RNase activities. These activities initiate the removal of a 26 base intron from the mRNA encoding X-box-binding protein