AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews®

REVIEW

Candida parapsilosis: from Genes to the Bedside

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SUMMARY Patients with suppressed immunity are at the highest risk for hospital-acquired infections. Among these, invasive candidiasis is the most prevalent systemic fungal nosocomial infection. Over recent decades, the combined prevalence of non-*albicans Candida* species outranked *Candida albicans* infections in several geographical regions worldwide, highlighting the need to understand their pathobiology in order to develop effective treatment and to prevent future outbreaks. *Candida parapsilosis* is the second or third most frequently isolated *Candida* species from patients. Besides being highly prevalent, its biology differs markedly from that

Citation Tóth R, Nosek J, Mora-Montes HM, Gabaldon T, Bliss JM, Nosanchuk JD, Turner SA, Butler G, Vágvölgyi C, Gácser A. 2019. *Candida parapsilosis*: from genes to the bedside. Clin Microbiol Rev 32:e00111-18. https://doi.org/10.1128/CMR.00111-18.

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Published 27 February 2019

of *C. albicans*, which may be associated with *C. parapsilosis*' increased incidence. Differences in virulence, regulatory and antifungal drug resistance mechanisms, and the patient groups at risk indicate that conclusions drawn from *C. albicans* pathobiology cannot be simply extrapolated to *C. parapsilosis*. Such species-specific characteristics may also influence their recognition and elimination by the host and the efficacy of antifungal drugs. Due to the availability of high-throughput, state-of-the-art experimental tools and molecular genetic methods adapted to *C. parapsilosis*, genome and transcriptome studies are now available that greatly contribute to our understanding of what makes this species a threat. In this review, we summarize 10 years of findings on *C. parapsilosis* pathogenesis, including the species' genetic properties, transcriptome studies, host responses, and molecular mechanisms of virulence. Antifungal susceptibility studies and clinician perspectives are discussed. We also present regional incidence reports in order to provide an updated worldwide epidemiology summary.

KEYWORDS *Candida parapsilosis*, antifungal, epidemiology, experimental tools, genome, host response, pathogenicity, treatment, virulence

INTRODUCTION

Candida species account for the majority of fungal systemic bloodstream infections in intensive care units (ICUs) worldwide (1–3). Although the most prevalent and invasive species is *Candida albicans*, during the last two decades, its dominance has decreased as the numbers of invasive infections by non-*albicans Candida* (NAC) species have risen (4, 5). Of these, *Candida parapsilosis* is of particular importance, as it is able to form tenacious biofilms on central venous catheters (CVCs) and other medically implanted devices, thus threatening patients who have undergone invasive medical interventions. *C. parapsilosis* likewise grows rapidly in total parenteral nutrition administered to ICU patients, thereby placing undernourished children and low-birth-weight neonates (5–8) at increased risk. Although *C. parapsilosis* infections generally result in lower morbidity and mortality rates than *C. albicans* infections, several clinical isolates of this species have been reported to be less susceptible to echinocandins, and in some regions, resistance to azole treatment has also been noted, which complicates the choice of empirical antifungal drug therapy (9–12). Nosocomial outbreaks have also been reported in various geographical regions (13–15). Horizontal transmission is another feature of *C. parapsilosis*, in contrast to the prior-colonization-dependent vertical transmission of *C. albicans* (16). Due to these species-specific characteristics, *C. parapsilosis* is often the second or third most frequently isolated *Candida* species in ICUs.

Since the development of an effective system to specifically disrupt genes was reported in 2007 (17, 18), our understanding of *C. parapsilosis* biology has significantly advanced, and the number of investigations has increased, as this pathogen has continued to rise in clinical importance. Hence, this review aims to provide an up-to-date worldwide epidemiology of *C. parapsilosis*-driven candidemia and to summarize the latest findings on its pathogenesis, including a discussion of the species' genetic properties and molecular mechanisms of virulence. Transcriptome studies, potential antifungal drug resistance mechanisms, host immune responses, and clinicians' perspectives are also discussed.

INCIDENCE AND DISTRIBUTION TRENDS

Since the early 2000s, NAC species have significantly increased and currently often surpass *C. albicans* as the most prevalent causes of invasive *Candida* infections, depending on the geographical region (6, 19–22). For example, according to a recent national surveillance study in Japan by Kakeya et al. (21), *C. albicans* accounted for 58.2% of all candidemia episodes in 2003 yet only 30% of cases by 2014. Another recent study, by Pfaller et al. (23), highlighted that at 62% of the examined sites in North America, NAC species were responsible for more than half of all candidemia cases.

Similarly, Xiao et al. (19) reported that in Chinese hospitals, only 32% of *Candida* blood infections could be attributed to *C. albicans*.

Several studies also associate the increasing prevalence of NAC species with decreased susceptibility to commonly used antifungal drugs (12, 19, 24, 25). Of the commonly isolated NAC species, *C. parapsilosis* is often the second or third most prevalent, depending on the patient group as well as geographical regions. Although regional epidemiology studies are available, we lack an up-to-date view of the worldwide distribution of *C. parapsilosis* prevalence. We therefore collected data from national studies of *Candida* epidemiology from the last decade from each continent (Table 1). Reports were collected from 2009 to early 2018 and were filtered into multicenter/multihospital studies of invasive candidiasis, possibly representing entire countries. If nationwide studies were not available, data from reports involving multiple regions of a country were gathered. The data from regional studies collected cover epidemiological data from 2000 to 2015. Although *C. albicans* is the major cause of invasive candidiasis worldwide, *C. parapsilosis* is the second most commonly isolated species in southern Europe, some regions of Asia, and Latin America, in accordance with data from previous global epidemiology studies (26, 27). Regarding the Mediterranean regions of Europe, *C. parapsilosis* invasive infections account for 20 to 25% of episodes due to *Candida* species in Greece, Portugal, Italy, and Spain (28–33). Furthermore, a recent study by Arsić Arsenijević et al. highlights the increasing prevalence of *C. parapsilosis* infections in Serbia, where it is responsible for 46% of cases of invasive candidiasis in adults, which is similar to the frequency of *C. albicans* (34). In central Europe and Scandinavia, *Candida glabrata* is the most common NAC species, although *C. parapsilosis* follows closely behind (35–43). The same can be observed in Australia, where *C. parapsilosis* accounts for 16.5% of *Candida* bloodstream infections (44). In Asia and North America, the prevalence of *C. parapsilosis* is also region dependent. For instance, it is the second most commonly identified species in the hospital environment in Japan and China, being responsible for 20 to 23% of all *Candida* bloodstream infections, while it is the third most frequently identified *Candida* species in India and other tropical regions of Asia (19, 21, 45, 46). In North America, *C. glabrata* and *C. parapsilosis* equally account for cases of NAC-driven invasive candidiasis; however, the increasing prevalence of *C. parapsilosis* in Latin America is undebatable (47, 48). Besides being the most frequently isolated NAC species in South America, *C. parapsilosis* even outranks *C. albicans* infections in regions such as Colombia (38.5% to 36.7%) and Venezuela (39% to 26.8%) (49–53). Interestingly, a recent study by Govender et al. also highlights the increased incidence of *C. parapsilosis* in South Africa, where this species accounts for 35% of invasive *Candida* infections in the public sector (second most common) and >50% in private-sector hospitals, thus outranking *C. albicans* (12). In addition, only 37% of the isolated *C. parapsilosis* strains were shown to be susceptible to azole derivates, and cross-resistance was also reported, raising concern regarding the treatment of these infections (12). It is unclear whether the increased incidence of *C. parapsilosis* in South African regions is a recent trend or an already existing phenomenon, due to the limited number of available epidemiology studies from this region. This further underscores the need for up-to-date, regionwide surveys. Nevertheless, the empirical antifungal practice in these countries might be contributing to *C. parapsilosis*' increased prevalence.

In addition to threatening adult patients in ICUs, *C. parapsilosis* poses a well-recognized serious threat to newborns, especially those born prematurely and with low birth weight. A recent meta-analysis of cases of neonatal candidiasis by Pammi et al. revealed that *C. parapsilosis* is responsible for 33% of all invasive *Candida* diseases in newborns and accounts for approximately 80% of NAC-driven invasive infections (7). Furthermore, neonatal *Candida* disease due to this species is especially common in North America (33.8%) as well as in Australia (35.8%) (7). A nationwide study provided by Pfaller et al. further showed that among NAC species, *C. parapsilosis* accounts for most of the invasive candidiasis cases in children (<9 years old) and in neonates in North America (23). Furthermore, a national surveillance study of neonatal intensive

TABLE 1 Update on candidemia episodes caused by *C. parapsilosis*[a]

Region	% *C. parapsilosis* incidence (ranking)	% *C. albicans* incidence (ranking)	Yr(s)	No. of hospitals included	Reference
Europe					
Southern region					
Spain	24.9 (2nd)	45.3 (1st)	2010–2011	29 hospitals, nationwide	30
Italy	22 (4th)	73.4 (1st)	Undefined	3 hospitals in southern Italy	31
	14.8 (3rd, Lombardy)	52.1 (1st, Lombardy)	2009	34 centers, nationwide	32
	23.5 (2nd, other areas)	45.2 (1st, other areas)			
	20 (2nd)	59 (1st)	2012–2013	39 hospitals, northern Italy	33
Portugal	23 (2nd)	40.4 (1st)	2011–2012	10 hospitals, nationwide	29
Greece	22.7 (2nd)	45.4 (1st)	2005–2009	PICU only, nationwide	28
Serbia	46	46	2014–2015	5 adult ICUs, nationwide	34
Middle/northern regions					
Finland	5 (3rd)	67	2004–2007	5 regions, nationwide	35
Austria	8.7 (3rd)	52.2 (1st)	2007–2008	9 centers	36
France	7.5 (3rd)	57 (1st)	2005–2006	180 ICUs, nationwide	37
United Kingdom	10.3 (3rd)	52 (1st)	2008	3 centers in Scotland, 2 centers in Wales	38
Switzerland	5.4 (4th)	61.9 (1st)	2004–2009	17 hospitals	39
Denmark	3.7 (3rd in females, 5th in males)	57.1 (1st)	2004–2009	6 hospitals	40
Norway	4.3 (4th)	67.7 (1st)	2004–2012	National surveillance study	41
Sweden	9 (3rd)	61 (1st)	2005–2006	Undefined, nationwide	42
Iceland	5 (5th)	56 (1st)	2000–2011	14 hospitals	43
America					
South America					
Continental study	26.5 (all episodes)	37.6 (all episodes)	2008–2010	21 hospitals from 7 countries	53
Argentina	23.9 (2nd)	42.5 (1st)			
Brazil	25.8 (2nd)	40.5 (1st)			
Chile	28.9 (2nd)	42.1 (1st)			
Colombia	38.5 (1st)	36.7 (2nd)	2008–2010	21 hospitals from 7 countries	53
Ecuador	30.4 (2nd)	52.2 (1st)			
Honduras	14.1 (4th)	27.4 (1st)			
Venezuela	39 (1st)	26.8 (2nd)			
Peru	25.3 (2nd)	27.8 (1st)	2013–2015	3 hospitals, Lima-Callao	50
	28.1 (1st)	39.9 (1st)	2009–2011	9 hospitals, Lima	49
Argentina	22 (2nd)	44 (1st)	2010–2012	5 institutions	51
Brazil	24.1 (2nd)	34.3 (1st)	2007–2010	16 hospitals, 5 regions, nationwide	52
North America					
Continental study	12.2 (3rd)	49.5 (1st)	2004–2008	23 centers in USA, 2 in Canada	24
USA	17 (3rd)	38 (1st)	2008–2011	17 hospitals (Baltimore, MD), 24 hospitals (Atlanta, GA)	47
	17.4 (2nd)	50.7 (1st)	1998–2006	52 hospitals, nationwide	48
Canada	21 (2nd)	59 (1st)	2003–2013	Nationwide NICU surveillance	54
Asia					
Continental study	12.1 (4th)	41.3 (1st)	2010–2011	25 hospitals across Asia	46
Japan	23.3 (2nd)	39.5 (1st)	2003–2014	10 university hospitals, nationwide	21
China	20.0 (2nd)	44.9 (1st)	2009–2014	65 general hospitals from 27 provinces	19
India	10.9 (3rd)	20.9 (2nd)	2011–2012	27 ICUs, nationwide	45
Oceania					
Australia	16.5 (3rd)	44.4 (1st)	2014–2015	Nationwide surveillance	44
Africa					
South Africa	35 (public hospitals) (2nd), >50 (private hospitals) (1st)	46 (1st)	2009–2010	Hospitals in 11 public sectors, >85 private sectors	12

[a]ICU, intensive care unit; PICU, pediatric intensive care unit.

care units (NICUs) in Canada revealed that although *C. albicans* remains the major cause of neonatal invasive *Candida* disease (59%), *C. parapsilosis* is also highly prevalent (21%) (54). Recently, cases from European countries were also reported. For instance, according to a recent surveillance over 22 years in Norway, *C. parapsilosis* was the second most

common cause of neonatal candidiasis (41). Similarly, the United Kingdom neonatal surveillance network also reported that during a 6-year period, *C. parapsilosis* was the second most common cause of neonatal invasive *Candida* disease, accounting for 20% of cases in England (55).

All these available data underscore the importance of *C. parapsilosis* and urge us to enhance the investigation of this species' pathogenesis.

GENOMIC ANALYSIS

Genome Organization and Properties

Since 2009, the complete genome sequence of *C. parapsilosis* strain CDC317 has been available, along with those of five other species of the *Candida* clade (56). The genome size is about 13 Mbp. *C. parapsilosis* is diploid, with eight chromosome pairs whose sizes range from 0.9 to 3.0 Mbp. The chromosomes terminate at both ends with tandem arrays of telomeric repeats of a 23-nucleotide-long motif (5′-GGTCCGGATGTT GATTATACTGA-3′) that was originally identified by analysis of the RNA subunit of telomerase TER1 (57). In *Candida orthopsilosis* and *C. metapsilosis*, classified in the *C. parapsilosis sensu lato* complex, telomeric sequences differ from this motif at 6 and 8 nucleotide positions, respectively.

Initial analyses of the *C. parapsilosis* genome confirmed previous findings that the mating-type locus MTLa2 is a pseudogene (58) and also revealed a strikingly low level of heterozygosity, with only 1 heterozygous single nucleotide polymorphism (SNP) per 15,553 bases. The level of heterozygosity was 25- to 70-fold lower than that found in other diploid *Candida* species. Characterization of the genetic variability among strains using standard techniques, such as amplification fragment length polymorphism (AFLP) analysis, revealed low levels of polymorphisms across clinical isolates from different regions (59). Furthermore, analyses of more than 200 clinical isolates revealed that all of them had only the MTLa idiomorph (i.e. no strain of the alpha type was found) and confirmed that MTLa2 is a pseudogene (60). Altogether, such low genetic variability suggested a recent global expansion of a virulent clone with a defective mating system (60).

In the early 2010s, the significant drop in sequencing costs brought about by next-generation sequencing (NGS) enabled the sequencing of additional clinical and environmental strains (61). A comparison of four fully sequenced genomes of *C. parapsilosis* isolates from Europe and North America further confirmed extremely low levels of heterozygosity and low levels of genetic variation. The examined strains contained a certain level of variation in gene content, which included genes encoding lysophospholipases, efflux pumps, and peptidases. Notably, however, significant differences were found in the agglutinin-like sequence (*ALS*) gene family, which encodes large cell surface glycoproteins that play a role in host-pathogen interactions (62). In addition, the amount of copy number variations (CNVs) was considerable, with 40 such events being detected among the four strains. Importantly, one such variation affected the same gene, a putative arsenite transporter, with different genomic boundaries and copy numbers, indicating its independent occurrence. This finding indicated a shared strong selective pressure, which possibly occurred in the environment, as arsenite levels are normally exceedingly low in the human body. This analysis suggested that clinical isolates likely had recent, independent origins from the environment. In addition, nonmonophyletic patterns of shared CNVs and the presence of clustered regions with higher SNP density suggested the possibility of recombination among different *C. parapsilosis* strains.

In 2005, Tavanti et al. confirmed by multilocus sequence typing that *C. parapsilosis* isolates represent a species complex. This complex is now subdivided into three distinct species: *C. parapsilosis sensu stricto* (previously group I), *C. orthopsilosis* (previously group II), and *C. metapsilosis* (previously group III) (63). The first genome sequence of a *C. orthopsilosis* strain was reported in 2012, from a highly homozygous isolate (64). However, later sequencing of additional clinical strains obtained from the United States and Singapore highlighted the existence of hybrids in this species. The hybrid genomes

consisted of highly heterozygous blocks interspersed with highly homozygous blocks, indicating that loss-of-heterozygosity events had occurred since the hybridization event (65). It was subsequently proposed that the bulk of *C. orthopsilosis* isolates are hybrids, resulting from at least four independent events of hybridization between the same parental species, which diverge by approximately 5% at the nucleotide level (66). Whole-genome sequencing of 11 clinical isolates of *C. metapsilosis* revealed that this is also a hybrid species (67). All of the analyzed *C. metapsilosis* strains obtained from globally distributed locations are the result of the same hybridization event between two as-yet-unknown nonpathogenic parental lineages. Hence, the *C. parapsilosis* clade is thought to consist of at least five lineages, of which two pairs can form hybrids. Interestingly, both *C. metapsilosis* and *C. orthopsilosis* hybrids seem to have higher virulence potential in humans than their parents, because the homozygous *C. metapsilosis* parent strains have not been isolated from clinical samples, and only one of the homozygous parents of *C. orthopsilosis* was found in the clinical environment. This raises the possibility that virulent species can emerge from the hybridization of nonvirulent (or less-virulent) species (68).

Today, the genome sequence of *C. parapsilosis* is accessible at the Candida Genome Database (69), with up-to-date annotations, references, supplementations from genome-wide or transcriptomic studies, and additional sources of both coding and noncoding genomic regions (70). Its availability greatly contributes to advances in *C. parapsilosis*-related investigations. The Candida Gene Order Browser (71) provides syntenic information from comparisons of several *Candida* species, including *C. parapsilosis*. A comprehensive evolutionary analysis of every gene of *C. parapsilosis* along with precomputed and browsable gene phylogenies are provided by PhylomeDB (72). In addition, MetaPhORs provides phylogeny-based predictions of ortholog and paralog relationships between *C. parapsilosis* genes and those of hundreds of other species, including most other sequenced *Candida* species (73).

Unlike *Saccharomyces cerevisiae*, *C. albicans*, and most other yeast species, *C. parapsilosis* possesses a mitochondrial genome with an unusual molecular architecture. The mitochondrial genome consists of 30.9-kbp-long linear DNA molecules terminating on both sides with specific telomeric structures that have little resemblance to telomeres at the ends of eukaryotic nuclear chromosomes (74). The mitochondrial telomeres consist of inverted repeats with a subterminal repeat followed by tandem arrays of a 738-bp-long unit. These linear molecules include the genes for conserved mitochondrial proteins (i.e., seven subunits of NADH:ubiquinone oxidoreductase, three subunits of cytochrome *c* oxidase, apocytochrome *b*, and three subunits of ATP synthase), two rRNAs, and a set of 24 tRNAs (75, 76). In addition, *C. parapsilosis* mitochondria also contain extragenomic circular DNAs, dubbed telomeric circles (t-circles), that are composed exclusively of multimers of the telomeric sequence. The t-circles replicate autonomously via the rolling-circle mechanism, thus generating arrays of telomeric repeats that eventually recombine with the ends of linear DNA molecules (77, 78).

Essentially the same molecular architecture of the mitochondrial genome has also been found in several closely related species from the *Lodderomyces* clade, such as *C. metapsilosis*, *C. orthopsilosis*, and *C. theae* (79, 80) (E. Hegedűsová, B. Brejová, and J. Nosek, unpublished results). Because these species differ in the sequences of their mitochondrial telomeres, the telomeric motifs were proposed as potential molecular markers for clinical diagnostics (81). Interestingly, the mitochondria of several isolates of *C. metapsilosis* and *C. orthopsilosis* contain a mutant form of the genome. These mutants lack the t-circles, and their mitochondrial genomes have circularized via end-to-end fusions, further supporting the key role of t-circles in telomere maintenance (67, 79, 80, 82). Importantly, the t-circle-dependent maintenance of mitochondrial telomeres may have medical implications, as it parallels the alternative (telomerase-independent) lengthening of telomeres (ALT) at the ends of eukaryotic nuclear chromosomes (83). Moreover, as human cells possess a circular mitochondrial genome, the replication strategy of linear mitochondrial DNA in *C. parapsilosis* has been proposed as a promising molecular target for therapeutic intervention (84).

The linear mitochondrial genome and/or the corresponding DNA replication machinery seems to be responsible for the tolerance of *C. parapsilosis* to high doses of intercalating agents, such as ethidium bromide and acridine orange (85). On the other hand, compounds interfering with the splicing of a group II intron occurring in the mitochondrial *cox1* gene inhibit the growth of *C. parapsilosis* cells and represent potent antifungal drugs (86). Moreover, other mitochondrial functions such as the intricate electron transport pathways consisting of a conventional respiratory chain with all three phosphorylation coupling sites, a parallel respiratory chain, and alternative oxidase were shown to play a role in the susceptibility of *C. parapsilosis* cells to a range of drugs (87–89).

In the following sections, we focus on *C. parapsilosis* group I isolates only. The history, epidemiology, genomics, and virulence of the *Candida psilosis* complex have recently been reviewed by Németh et al. (90).

Transcriptome Studies

Similar to whole-genome sequencing, NGS approaches enable the unbiased interrogation of the transcriptome under various conditions. Massive sequencing of cDNA libraries (RNAseq) has gradually replaced the use of microarray-based technologies in transcriptome analyses of pathogenic species (91, 92). Standard, easy-to-use protocols are available for RNAseq to determine differential gene expression profiles in yeasts (93), which have been extensively applied in research of fungal pathogens. Furthermore, transcriptome sequencing technologies offer a plethora of additional possibilities such as the refinement of genome annotation, including the determination of the noncoding gene repertoire (94), assessing the structure of RNA transcripts (95, 96), and the dual interrogation of host and pathogen transcriptomes (97).

The first comprehensive transcriptomic study of *C. parapsilosis* by RNAseq was performed in 2011 after exposing this yeast to several conditions, including different media, temperatures, and oxygen concentrations (98). The detection of differentially expressed genes under these conditions enabled not only the identification of regulators of the hypoxia response in this species but also a comprehensive reannotation of the *C. parapsilosis* reference genome. Indeed, neither of the two previous versions of *C. parapsilosis* gene annotations (using CPAG and CPAR as gene name prefixes, respectively) included prediction introns in their gene models. RNAseq analyses of *C. parapsilosis* cells under a diverse set of conditions enabled the detection of transcripts for over 90% of the annotated protein-coding genes, enabling the identification of 422 introns, the annotation of 5′ and 3′ untranslated regions (UTRs), and the removal of more than 300 unsupported and the correction of approximately 900 gene models. RNAseq analysis of *C. parapsilosis* therefore facilitated a much-improved annotation of the reference genome (the refined version uses CPAR2 as the gene name prefix).

Subsequent studies in *C. parapsilosis* have exploited RNAseq to identify genes involved in specific pathways. One recent example is the identification of enzymes and transporters involved in the metabolism of hydroxyderivatives of benzene and benzoic acid in this species (as discussed below) (99, 100). In these studies, RNAseq, together with phylogenetic and synteny analyses, was instrumental in pinpointing the right candidates among paralogous members of several gene families, including 138 paralogs of the major facilitator superfamily of transporters (MFS), by identifying those expressed in the presence of the substrate. Finally, the identification of *C. parapsilosis* transcripts expressed upon exposure to human macrophages was used to prioritize the construction of knockout mutants to study host-pathogen interactions (101). Notably, 84% of the 19 constructed knockout mutants showed a phenotype different from that of the wild type under conditions related to virulence and interaction with the human host. The use of increased expression upon exposure to human macrophages as a selection criterion likely facilitated the high ratio of deletion mutants exhibiting a phenotype. It is expected that future studies will further exploit the possibilities of RNAseq in *C. parapsilosis*, particularly with respect to the relevant interactions with the host. In this context, the use of RNAseq for the identification of long noncoding RNAs

(lncRNAs) and for the dual assessment of expression by the host and pathogen has the potential to reveal novel pathways and interactions (97).

Genetic Toolbox

Use of reporter genes. Alternative codon usage in CTG clade species such as *C. parapsilosis* (which translate CTG as serine rather than leucine) causes difficulties in the design of reporter systems as well as the choice of selectable markers (102). Codon-modified versions of green fluorescent protein (yeast-enhanced green fluorescent protein [yeGFP]) suitable for use in *C. albicans* were first described by Cormack et al. (103) and Morschhauser et al. (104). Gerami-Nejad et al. (105) used site-directed mutagenesis to generate yellow fluorescent protein (yeYFP) and cyan fluorescent protein (yeCFP) variants designed to integrate at the C terminus and later at the N terminus (106) of target proteins. Red versions (red fluorescent protein [RFP] and yemCherry) and a new *C. albicans* GFP (CaGFP) were added later (107–109).

GFP fusions were first used in *C. parapsilosis* by Kosa et al. (110). Those authors constructed a series of replicating plasmids based on the CpARS7 origin of replication and using *MET2*, *LYS4*, *GAL1*, *URA3*, or *IMH3* as a selectable marker. Some of the plasmids can be used to express heterologous genes from the *CpGAL1* promoter. The introduction of eGFP by Cormack et al. (103) facilitated the tagging and localization of mitochondrial carriers, mitochondrial telomere binding proteins, metabolic enzymes, as well as plasma membrane transporters in *C. parapsilosis* (99, 100, 111–113). Constructs suitable for PCR-mediated C-terminal tagging of target genes with green, yellow, or mCherry fluorescent proteins using *NAT1* as the selectable marker are also available (114). GFP-labeled strains of *C. parapsilosis* have already been used to study phagocytosis of macrophages (115).

Recently, Defosse et al. (116) described a comprehensive toolkit for labeling *C. parapsilosis* and other yeasts of the CTG clade. Sets of plasmids express yeGFP, yeYFP, yeCFP, or yemCherry from promoters selected to function in many CTG species. A series of selectable markers is available, including *SAT1*, *HPH*, and *IMH3*, also expressed from species-specific promoters. The plasmids integrate randomly into the target species. Those authors also incorporated other reporter genes, including luciferase (*gLUC59*) and beta-galactosidase (*StlacZ*). It is likely that these plasmids will prove to be extremely useful for future studies in *C. parapsilosis*.

Gene disruption methods. Deleting or editing genes in *C. parapsilosis* faces the same problems encountered in many *Candida* species. The genome is diploid; thus, two alleles of each gene must be targeted, and the noncanonical translation of the CTG codon (102) means that many selectable markers cannot be directly used. However, transformation methods have been available for several decades.

Early attempts using "*Candida*" species showed that *Candida utilis* and *Candida* (*Yarrowia*) *lipolytica*, which do not belong to the CTG clade, can be transformed (117). By 1985, true CTG clade species (*Candida maltosa* and *Pichia guilliermondii*) were transformed with a plasmid expressing *ARG4* from *S. cerevisiae*, which returned auxotrophic strains to prototrophy (118). *ADE2* auxotrophs of *C. albicans* were complemented by transformation in 1986 (119). Transformation procedures were rapidly adapted for generating gene disruptions. Kelly et al. (120) used a *C. albicans ADE2* gene flanked by regions from *URA3* to disrupt one allele of *URA3* in *C. albicans* by homologous recombination. "URA blaster" methods remain popular methods for generating gene disruptions in *C. albicans*. However, there are several concerns associated with using *ura3* auxotrophic strains. First, in some backgrounds (such as *C. albicans* strains derived from CAI4), a proportion of the adjacent *IRO1* gene involved in iron utilization was removed when *URA3* was disrupted (121). Second, expression of *URA3* at ectopic positions can affect virulence phenotypes (122). Generating strains with multiple auxotrophies (e.g., *C. albicans* BWP17 *his1 arg4 ura3*) made several selectable markers available (123).

Early gene disruption strategies for *Candida* species relied on having auxotrophic strains, which somewhat limited the potential applications. Dominant drug-resistance-

selectable markers were therefore developed by modifying the coding sequence of existing systems to replace CTG codons.

The *SAT1* (streptothricin acetyltransferase) gene, which confers resistance to nourseothricin, is probably the most commonly used dominant selectable marker in *Candida* species. The first codon-modified versions were generated by replacing CTG codons with CTT or CTC and expressing the gene from the *C. albicans ACT1* promoter (124, 125). Reuss et al. (124) combined the marker with a regulatable *FLP* recombinase and surrounded the entire region with FLP recombination target (FRT) sites. This *SAT1* flipper cassette can be used to disrupt one allele of a target gene by homologous recombination (directed by sequences outside the cassette). *SAT1* and the rest of the cassette are recycled by inducing the expression of the FLP recombinase by growth on maltose, and the transformation step is repeated by using the same or a similar cassette to target the second allele. The *SAT1* flipper cassette was the first gene disruption method that was adapted for use in *C. parapsilosis*. Gácser et al. (17) applied the *C. albicans* constructs directly to *C. parapsilosis*, whereas Ding and Butler (18) adapted it by replacing the promoters driving the expression of *SAT1* and *FLP* with the orthologous sequences from *C. parapsilosis*. The *SAT1* flipper cassette has since been used to delete several genes in *C. parapsilosis* (60, 126–133).

The *SAT1* cassette has numerous advantages, in that it can be used in any isolate and more than one gene can be targeted. However, the system is relatively slow. Homologous sequences are usually added to the end of the cassette by cloning, and the cassette must be recycled from the first allele of each target gene before the second allele is disrupted. Some of these issues were addressed for *C. albicans*, when Noble and Johnson (134, 135) developed a system using a strain auxotrophic for three markers, *leu2*, *arg4*, and *his1*. Each allele of a target gene is disrupted using a different marker: *LEU2* derived from *C. maltosa* or *HIS1* or *ARG4* from *C. dubliniensis*. Sequences derived from the target gene are added by PCR, in a rapid and efficient process. This approach was used to delete several hundred regulatory genes in *C. albicans* (134). The system was adapted for *C. parapsilosis*, first by using the *SAT1* cassette to disrupt *LEU2* and *HIS1*. This facilitated the generation of a collection of $>$100 strains deleted for transcription factors and protein kinases (127). Later studies used the same method for the generation of smaller mutant sets (101, 136, 137).

Another alternative for gene deletion in yeast is via the newly introduced clustered regularly interspersed short palindromic repeat (CRISPR)-Cas9 system.

Introduction of CRISPR-Cas9 gene editing. Gene editing using CRISPR together with Cas9 and other endonucleases is gradually gathering momentum in yeasts (reviewed in references 138 and 139). The tools have many advantages, including the ability to target almost any sequence using carefully designed short guide RNAs (sgRNA) and the fact that in diploid genomes, both alleles are usually edited at the same time. In many yeasts, homology-directed repair (HR) is at least as efficient as, or more efficient than, nonhomologous end joining (NHEJ). A double-stranded break introduced by Cas9 can therefore easily be repaired using a donor DNA (repair template) that introduces stop codons or specific mutations or that results in gene deletions.

CRISPR-based systems have three main requirements: (i) expression of *CAS9* and targeting to the nucleus, (ii) expression of an sgRNA, and (iii) provision of a repair template. CRISPR was first used for gene editing in *C. albicans* by Vyas et al. (140). A codon-modified version of *CAS9* with a nuclear localization sequence from simian virus 40 (SV40) and a *SAT1* flipper cassette is targeted to the *ENO1* gene by selection for nourseothricin resistance. The *SAT1* cassette can be excised by recombination, allowing the marker to be reused to facilitate the integration of the sgRNA at a different site (*RP10* locus). Alternatively, both *CAS9* and sgRNA are integrated at *ENO1*. The sgRNA is expressed from a polymerase III (Pol III) promoter (*SNR52*). The repair template (constructed by overlapping PCR) is transformed into the cells at the same time as the integration cassettes. The system is very efficient, leading to editing rates of up to 80%. In early versions of the method, *CAS9* remained in the genome. The approach also

requires that both *CAS9* and the sgRNA be integrated into the genome, which can be slow and makes sequential editing of several genes difficult. Later, improved versions of CRISPR-Cas9-based genome-editing methods were further developed by exploiting recent observations of *C. albicans* genetic properties (141–146). These methods now allow single- or double-gene deletions, tagging, as well as open reading frame (ORF) reconstitutions.

The CRISPR-Cas9 system has been adapted for other *Candida* species, including *C. lusitaniae* (147). The authors of that study found that the efficiency of homology-directed repair in this species was increased by deleting KU70 and DNA ligase 4, parts of the NHEJ pathway. CRISPR has also been used successfully in *C. lusitaniae* and *Candida auris* by providing purified Cas9 protein (148). The CRISPR-Cas9 system developed for *C. parapsilosis* differs in many respects from systems used for other *Candida* species (149). One significant difference is that most of the components are carried on an autonomously replicating plasmid, originally described by the Nosek group (110, 150). The origin of replication (CpARS7) in the plasmid is artificial, in that it is derived from two different places on the *C. parapsilosis* genome. However, it is maintained under selection and is easily lost when selection is removed. A codon-adapted version of *CAS9* is expressed from the *C. parapsilosis TEF1* promoter, and *SAT1* is expressed from the *C. albicans ACT1* promoter. The guide RNA is present on the same plasmid, expressed from a polymerase II promoter (*C. parapsilosis* glyceraldehyde-3-phosphate dehydrogenase gene [*GAPDH*]) and surrounded by Hammerhead and hepatitis delta virus (HDV) ribozymes. The repair template is cotransformed with the plasmid. One significant advantage of the plasmid system is that it can be transformed into any isolate of *C. parapsilosis*: Lombardi et al. (149) used it to edit genes in 20 different clinical strains. Design of the repair template facilitates gene editing (introduction of stop codons or specific mutations), gene deletion, or the addition of specific tags or epitopes. Any number of genes can be sequentially edited in the same strain.

Autonomously replicating plasmids are not generally used in *Candida* species. However, they have been described for *Candida maltosa* (151, 152) and *Candida tropicalis* (153). It may therefore be possible to develop plasmid-based CRISPR systems for other species in the CTG clade.

MOLECULAR MECHANISMS OF *C. PARAPSILOSIS* VIRULENCE

Adhesion

Adhesion to various biotic and abiotic surfaces is a key preliminary stage of host colonization. These surfaces include indwelling medical devices, such as catheters and prosthetic devices made of polyvinyl chloride (PVC), polyurethane, or silicone, as well as host epithelial tissues. *C. parapsilosis* is particularly known for its ability to cause systemic infections through the colonization of implanted medical devices and high-glucose-containing parenteral nutrition. Therefore, the species' adhesive abilities, a critical step preceding biofilm formation, have been explored (154, 155). Interestingly, during these studies, an unusually high intraspecies variation in adhesion has been identified among *C. parapsilosis* clinical isolates compared to other *Candida* species (156). A correlation between the site of isolation and the rate of adhesion has also been observed, as *C. parapsilosis* mucocutaneous isolates express a higher-adhesion profile (156). Furthermore, cell surface hydrophobicity strongly correlates with adhesion to both polystyrene surfaces and buccal epithelial cells (ECs) (154, 156).

The molecular mechanisms for *C. parapsilosis* adhesion have also been investigated. Several proteins have been suggested to influence the adherence of this species to both abiotic and biotic surfaces. For example, either *C. parapsilosis* cell wall proteins (CWPs) that are covalently attached to the fungal cell wall, known as "true" CWPs, or cytoplasm-derived, temporary "atypical" CWPs effectively bind host cell extracellular matrix (ECM) proteins such as fibronectin, vitronectin, and laminin (157). These include true Als-, Hwp-, or Hyr-like proteins as well as transitory proteins required for various metabolic processes. The glycolysis and gluconeogenesis regulatory proteins Pgk

(phosphoglycerate kinase) and Eno1 (enolase 1) are required for the adhesion of *C. parapsilosis* to silicone-made materials (158).

Als-like proteins are present on the surface of *C. parapsilosis* pseudohyphae (157). The *C. parapsilosis* ortholog of *CaALS7* is a key determinant for adhesion to host epithelial cells, as gene disruption led to a decreased ability to adhere to human buccal epithelial cells and also resulted in decreased virulence *in vivo* (129). *CpALS7* is also necessary for *C. parapsilosis* to adhere to host ECM proteins under shear force (159). Additionally, *C. parapsilosis* cells require the presence of calcium, glutamic acid, and proline for adherence under shear flow conditions. A study by Butler et al. predicted that the *C. parapsilosis* genome encodes 5 Als-like proteins (56). Pryszcz et al. subsequently found a wide degree of variability among *ALS* genes, ranging from 1 to 5 gene copy numbers per isolate (61). However, whether an increase in copy number contributes to a different degree of virulence has not yet been confirmed. Orthologs of CFEMs (common in fungal extracellular membranes), another set of proteins influencing adhesion and biofilm formation in *C. albicans*, are also present in the *C. parapsilosis* genome, although their exact role in adhesion is not yet known (160, 161).

Morphology

Although various methods of molecular diagnostics are available to identify clinical isolates, species identification through microbiological phenotyping is still a commonly applied method. Laffey and Butler described four heritable colony morphologies of *C. parapsilosis*, including smooth and crater-like or concentric and crepe phenotypes, which are either white or creamy (162). Whereas the former two contain many yeast cells, the latter have more pseudohyphae (162). Later, Nosek et al. described additional colony phenotypes, such as wrinkled (white), stalk, and superwrinkled (creamy) (163).

To date, there is no record of true hyphae formed by *C. parapsilosis*. Environmental stimuli that are thought to influence morphology transition are stress-inducing conditions, including elevated temperature (37°C), the presence of serum, starvation, and high CO_2 levels and low O_2 levels that are present within the host (164–166). Although the exact inducers of the yeast-to-pseudohypha switch are poorly investigated, a specific subset of amino acids promotes this behavior (167). Furthermore, morphological transformation is usually associated with alterations in the cell wall structure. For example, during the yeast-to-filamentous growth transition, pseudohypha-specific cell surface proteins and adhesins (e.g., Als-like sequences) appear on the external layer of the elongating yeast's cell wall (157).

C. parapsilosis pseudohyphae contribute to virulence. Although in terms of uptake, host phagocytic cells do not differentiate between yeast and pseudohyphal forms, *C. parapsilosis* pseudohyphae are more resistant to killing by macrophages and induce higher levels of host cell damage *in vitro*, thus contributing to the species' pathogenicity (115, 168). Therefore, the underlying molecular mechanisms regulating morphology transition have also been investigated. Recent studies have identified several transcriptional regulators responsible for morphology regulation. These include transcriptional factors and kinases that are also involved in adhesion and biofilm regulation.

One such regulator is the ortholog of *C. albicans EFG1*, a transcriptional factor inhibiting the yeast-to-filamentous growth transition, that plays a similar role in controlling *C. parapsilosis* morphology (126). The transcriptional factor *UME6* also induces filamentous growth in *C. parapsilosis*, which is similar to its reported function in *C. albicans* (127, 169). Orthologs of *C. albicans CPH2* and *CZF1* in *C. parapsilosis* also positively regulate the yeast-to-pseudohyphal switch, as their removal results in reduced colony wrinkling and biofilms of the corresponding strain with the mutants growing mainly as yeast cells (127). *C. parapsilosis OCH1* also determines morphology, as deletion of this gene significantly decreases the amount of pseudohyphae under filamentous-growth-inducing conditions (170). Additional regulators of *C. parapsilosis* pseudohyphal growth include the ortholog of *CaSPT3* (*CPAR2_200390*), which negatively regulates filamentous growth, whereas *CPAR2_501400*, equivalent to *CaCWH41*, is essential for pseudohyphal formation (101, 171, 172). Notably, *CpEFG1*, *CpOCH1*, and

CpSPT3 are important factors in virulence (101, 126, 170). Due to their pleiotropic effects as signaling regulators, the specific key points at which these genes contribute to morphology status are still unclear.

Biofilm Formation

Adhered cells set the stage for biofilm formation on both biotic and abiotic surfaces. Following attachment, fungal cells rapidly proliferate to colonize the base material, thereby forming a monolayer. Following initiation, yeast cells undergo morphology transition, and filamentous forms appear, enhancing multilayer formation. Besides establishing a mixed population, during maturation, fungal cells also produce and secrete extracellular matrix elements to provide both structural and functional stability. Once the complex and stable multistructural biofilm stage is achieved, fungal cells are protected and await beneficial environmental conditions to initiate dispersal (173). The generated mature biofilm is a threat to the host, as it provides protection against both antifungal reagents as well as immune recognition (174, 175).

The structure and composition of biofilms formed by *Candida* species are highly species dependent. Compared to *C. albicans*, *C. parapsilosis* forms less-complex and thin biofilms. However, the presence of *C. parapsilosis* biofilms on plastic medically implanted devices is still the major source of infections. In terms of structure, biofilms formed by this species mainly consist of aggregated blastospores, pseudohyphae, and large amounts of extracellular carbohydrates with low levels of proteins (176, 177). Large variation in the overall biofilm-forming abilities among different clinical isolates was reported (176, 178). In the presence of high-glucose or lipid-rich media, however, *C. parapsilosis* biofilms are readily formed (179), which correlates with the increased incidence of *C. parapsilosis*-driven candidemia in patients receiving parenteral nutrition (163).

Previous studies also reported the resistance of *Candida* biofilms to various antifungal drugs. Nett et al. used radiolabeled fluconazole to show that beta-glucans in the ECM of *C. albicans* biofilms effectively sequester the drug (180). Subsequent work showed that matrix beta-glucans sequester multiple classes of antifungals in biofilms formed by *C. albicans*, *C. parapsilosis*, and other *Candida* species (181–183). The activity of efflux pumps was also shown to contribute to antifungal resistance in early-stage biofilm production by *C. albicans*. Mukherjee et al. showed that deletion of drug efflux pumps (encoded by *CDR1*, *CDR2*, and *MDR1*) led to increased azole sensitivity of this species within the first 6 h of biofilm formation (184). The role of these families in the drug resistance of *C. parapsilosis* biofilms is currently unknown. Nevertheless, resistance to amphotericin B, terbinafine, fluconazole, voriconazole, ravuconazole, and posaconazole was recorded in various cases of *C. parapsilosis* biofilms (185–187).

Due to the necessity and complexity of the various stages of biofilm formation, the process requires an extensive regulatory network. Although the molecular background of *C. albicans* biofilm formation is the most extensively studied to date, advances have been made in understanding *C. parapsilosis* biofilms. The described biofilm regulatory network in *C. albicans* is different from that of *C. parapsilosis*. Holland et al. compared the transcriptional profiles of biofilms formed by the two species and found that the expression of genes involved in metabolism and hyphal growth was increased in biofilms of both species (127). A cohort of *C. parapsilosis*-specific genes was further identified, including many predicted membrane transporters as well as a large number of genes with as-yet-unknown functions. In addition, the transcriptomic changes revealed during biofilm formation are similar to transcriptomic responses to hypoxia in this species (188). Many of the genes upregulated under both conditions encode proteins involved in fatty acid (FA) biosynthesis or glycolysis. Hydrolytic lipase secretion as well as trehalose metabolism also influence biofilm development (17, 132).

Although *C. parapsilosis*' biofilm regulatory network has not yet been entirely defined, major differences have been observed in biofilm development regulation relative to that of *C. albicans*. For instance, *BRG1* and *TEC1* have key roles in *C. albicans*

biofilm formation; however, no such role has been confirmed for the orthologous genes in *C. parapsilosis* (127). Although *CZF1*, *UME6*, *GZF3*, and *CPH2* were highlighted as key contributors in *C. parapsilosis*, all these genes are negligible in *C. albicans* (127). *ACE2*, *BCR1*, and *EFG1* are further required for biofilm development, similar to *C. albicans* (126, 127, 161). Additionally, *RBT1*, a cell surface protein, was confirmed to promote *C. parapsilosis* biofilm formation (188). *MKC1* was also suggested to be involved in the formation of *C. parapsilosis* biofilms (127). CFEM proteins, such as Rbt5, Pga10, and Csa1, were proposed to be required for *C. albicans* biofilms (189). Although the CFEM family has undergone expansion in *C. parapsilosis* (7 genes), these proteins appear not to be required for biofilm formation (161). Likewise, four members of the *C. albicans* biofilm regulatory network (*TEC1*, *ROB1*, *FLO8*, and *BRG1*) have no role in *C. parapsilosis* (127, 190). Furthermore, although the role of *NDT80* and *NRG1* in *C. albicans* biofilm development is evident, their function in *C. parapsilosis* may be altered, as deletion of the corresponding genes results in a severe growth deficiency (127, 191). All these recent findings confirm that biofilm formation in the two species is regulated in different ways.

Hydrolytic Enzyme Production

During invasion, pathogenic fungi secrete various hydrolytic enzymes in order to facilitate host entry. Secreted hydrolytic enzymes disrupt host cell membranes, degrade extracellular matrix elements, and, thus, damage host tissues. Besides impairing host barrier function, fungal hydrolytic enzymes may also promote cell adhesion, biofilm formation, or intracellular survival. Such secreted fungal enzymes are divided into three major groups: secreted aspartyl proteases, lipases, and phospholipases. To date, among *C. parapsilosis* secreted hydrolytic enzymes, aspartyl proteases and lipases are the best characterized.

Previously, Ramos et al. showed that 15 out of 16 *C. parapsilosis* clinical isolates, derived from cases of cutaneous candidiasis, actively secreted aspartyl proteases (Saps) (192). Similarly, additional studies reported that >88% of *C. parapsilosis* clinical isolates actively secreted these enzymes (168, 193–195). On the other hand, others reported a lower number (<37%) of clinical strains producing hydrolytic proteases (196–199). Although there is wide variability among *C. parapsilosis* isolates in terms of Sap production, it is commonly agreed that isolates derived from host surfaces (e.g., skin or vaginal mucosa) are more likely to produce and secrete these enzymes than those obtained from systemic infections or from the environment. This could explain why surface isolates are more invasive. The molecular background of *C. parapsilosis* Sap production has been rigorously investigated. To date, three aspartyl protease-encoding genes (*SAPP1* to *SAPP3*) have been identified. Out of these, *SAPP1* is responsible for the majority of Sapp production. Interestingly, this gene is duplicated in the *C. parapsilosis* genome, generating *SAPP1a* and *SAPP1b* loci (131). Studies with a ΔΔ*sapp1a*-ΔΔ*sapp1b* deletion mutant strain confirmed that *C. parapsilosis* secreted proteases enhance yeast cell survival in human serum, promote resistance to phagocytosis and killing by the host, and also facilitate intracellular survival and host cell damage (131). *SAPP* genes are further suggested to contribute to host invasion by degrading proteins involved in host defense, such as complement and antimicrobial proteins, or antibodies to evade humoral immune responses. They are also thought to degrade various additional proteins (e.g., extracellular matrix and cell surface proteins) in order to enhance nutrient acquisition during invasion. Although Sapp1 greatly determines the virulence of *C. parapsilosis*, it is not solely responsible for protease production. Horvath et al. previously showed that in the absence of *SAPP1*, the expression of an additional secreted-protease-encoding gene (*SAPP2*) is significantly elevated, revealing a compensatory effect (131). Sapp2 acts on substrates different from those cleaved by Sapp1, which suggests that there are altered activation mechanisms for the two proteins (200). *SAPP3* may also translate to an active protein, as Silva et al. reported elevated *SAPP3* expression levels during infection of reconstituted human oral epithelium (201). Although these studies confirmed the activation of Sapp2 and Sapp3 under

the applied conditions, their direct role in *C. parapsilosis* virulence has yet to be determined. In addition to the disruption of *SAPP1*, the protease inhibitor pepstatin A greatly decreases the destruction of host epithelial and epidermal surfaces by this species (202, 203). Hence, *C. parapsilosis* secreted proteases are some of the most potent pathogenicity-enhancing factors.

Lipases secreted by *C. parapsilosis* are also considered key virulence determinants. If present, their secretion varies greatly between isolates (36% to 80%), and they significantly enhance pathogenicity (168, 197). To date, four secreted-lipase-encoding genes have been identified in the genome of *C. parapsilosis*, although only two (*LIP1* and *LIP2*) have been confirmed to encode functionally active proteins (17). Deletion of *CpLIP1* and *CpLIP2* revealed their role in mechanisms required for successful host invasion, including nutrient acquisition and sustainability in a lipid-rich environment, enhancement of adhesion and biofilm formation, suppression of both cellular and humoral immune responses during infection, promotion of survival after phagocytosis, and progression of host tissue invasion (17, 115, 204). Furthermore, the lipase inhibitor ebelactone B and aspirin also protect reconstituted human tissue (RHT) in the setting of *C. parapsilosis* infection, which supports a role for secreted lipases in pathogenesis (203, 205). For a comprehensive review of *C. parapsilosis* secreted lipases as potent pathogenicity determinants, see our previous minireview (206).

C. parapsilosis also secretes phospholipases albeit with wide intraspecies variation, with studies reporting that 9 to 90% of clinical isolates produce the enzyme (193, 196, 198, 199). However, their contribution to pathogenicity is still a matter of debate, as there is no direct link between their expression and the degree of virulence. It is hypothesized that fungal phospholipases may enhance virulence by disrupting host cell membranes (207, 208). Unfortunately, the molecular background of phospholipase production is as yet unexplored in *C. parapsilosis*.

Cell Wall Assembly

Given that the first contact of the host-pathogen interaction takes place between the fungal cell wall and host cell surface receptors, studying the nature of this interaction is indispensable in order to explore host immune responses. However, the dynamically changing nature of fungal cell walls makes their investigation rather challenging. Such behavior is due to the changes in the environmental conditions between specific niches that these cells encounter.

Regardless of environmental stress conditions, in general, fungal cell walls contain four major components in different layers. Ranging from interior to exterior, the cell wall of *Candida* species consists of a basal chitin layer, coated by β-1,3- and β-1,6-glucans, that is penetrated by highly glycosylated mannoproteins and covered by a relatively rich layer of mannans (209). Depending on the site of mannosylation, one can differentiate between *N*-linked (oligosaccharides attached to asparagine) and *O*-linked (oligosaccharides binding to serine/threonine) mannans that influence cell adhesion and also cell wall integrity (170, 210). The compositions of *C. albicans* and *C. parapsilosis* cell walls are quite similar; however, the proportions and organizations of the different components vary. For example, while *C. albicans* chitin and β-1,3-glucans are mostly found close to the plasma membrane and are therefore masked by mannans (211, 212), both polysaccharides are significantly more exposed on the surface of *C. parapsilosis* cells (213). This observation is consistent with the high cell wall porosity found in *C. parapsilosis* (213). The obtained data also suggest that *C. parapsilosis N*-linked mannans are shorter than those of *C. albicans*, generating a porous wall surface, and this feature could help to expose inner wall polysaccharides on the cell surface (213, 214). Furthermore, the amount of glycan under the mannan layer of *C. parapsilosis* is larger than in *C. albicans*, which may function to strengthen/stabilize the cell wall (213, 215). In contrast to the *C. albicans* cell wall, *C. parapsilosis O*-linked mannans are naturally masked by *N*-linked mannans, which may affect interactions with immune cells (170). These data demonstrate that despite having similar components, *C. albicans* and *C.*

parapsilosis cell walls have subtle but relevant differences in both composition and organization.

To identify specific proteins present on the surface of *C. parapsilosis* cells, surfaceomics and shotgun proteomics studies have been performed. In addition to classic CWPs (e.g., Als adhesins and Hwp), atypical proteins (glyceraldehyde-3-phosphate dehydrogenase, Hsp70 chaperones, enolase 1, and endo-1,3-beta-glucanase) are also present on the surface, as mentioned above (157). Fungal CWPs not only play a structural role in maintaining the shape and strength of this cellular component but also have diverse functions during fungal adaptation to different niches. For example, transcriptional regulation of proteins involved in glycolysis (Eno1, Pgk, and Gapdh), general metabolic enzymes (Pdc1 and Pdc11), and heat shock proteins (Ssa1) is markedly altered under oxidative stress (216). Similarly, a CFEM family member (cell surface adhesins in *C. albicans*) and two orthologs of *C. albicans* classic cell wall proteins, Pir1 and Ecm33, are differentially regulated in the presence of human sera (217). Under the same conditions, human serum proteins attached to the surface of *C. parapsilosis* cells were also examined, among which complement components, apolipoproteins, fibrinogen, vitronectin, and albumin were identified (217). Interacting partners of laminin, fibronectin, and vitronectin as major extracellular matrix proteins were subsequently identified, as described above. Fungal proteins with possible human plasminogen and high-molecular-mass kininogen binding roles were also later identified. These include the heat shock protein Ssa2, the above-mentioned Als-like proteins, and 6-phosphogluconate dehydrogenase 1 (217, 218). This could represent a mechanism to disguise the fungal cells, avoiding recognition by elements of the immune system.

Immunogenic proteins associated with the cell wall also modify host-pathogen interactions. Among the most prevalent immunogenic proteins found in the *C. parapsilosis* cell wall are the translation initiation factor eIF4A subunit (Tif1), ATP synthase subunit beta (Atp2), enolase (Eno1), glyceraldehyde-3-phosphate dehydrogenase (Gap1), heat shock protein 70 (Ssb1p), pyruvate decarboxylase (Pdc11), ATP synthase subunit alpha (Atp1), phosphoglycerate kinase (Pgk1), alcohol dehydrogenase (Adh1), fructose-bisphosphate aldolase (Fba1), isocitrate dehydrogenase (Idh2), and guanine nucleotide binding protein subunit beta-like protein (Bel1) (219). These cell wall moonlighting proteins have also been identified as being immunogenic in *C. albicans*, with the exception of Idh2 (219). Other CWPs could have a role in nutrient acquisition or interaction with host components, such as the cell wall adsorbed aspartic proteinase 1 (Aspp1) or an ectophosphatase, as they influence adhesion to epithelial cells (220, 221).

Thus far, there are no functional studies to demonstrate how *C. parapsilosis* cell wall components are synthesized, but bioinformatic analyses of the genome indicate that this organism possesses the basic components to elaborate glucans and mannans, as described for *C. albicans* (56). The α1,6-mannosyltransferase Och1 is one of the few proteins characterized so far in *C. parapsilosis*, and as in *C. albicans*, it provides the addition of the first mannose residue of the *N*-linked glycan outer chain, allowing the further extension of the α1,6-polymannose backbone that is further decorated by lateral mannooligosaccharides (170). Possibly via regulating cell wall homeostasis, *C. parapsilosis* Och1 also regulates virulence, similarly to that of *C. albicans* (170).

Although no mechanistic studies have been conducted, orthologs of *C. albicans CHS1*, *CHS2*, *CHS3*, and *CHS8* (encoding chitin synthases) and *CHT1*, *CHT2*, *CHT3*, and *CHT4* (encoding chitinases) have been identified within the *C. parapsilosis* genome (101). This suggests that similar machineries are present for cell wall biosynthesis and chitin homeostasis regulation in both species. Notably, however, a recent study demonstrated the role of *C. parapsilosis* Spt3, a filamentous growth regulatory transcriptional factor, in cell wall homeostasis maintenance, which is a function not associated with the orthologous gene in *C. albicans*, and this finding might suggest the presence of alternative routes for cell wall homeostasis regulation (101).

Among the targets currently explored for detection of this species in the clinical setting, hyphal wall protein 1 (*HWP1*), a hypha-specific cell wall protein with adhesive properties, has been suggested to be a good marker for species identification (222). The

cell wall β-1,3-glucan content has been used to discriminate fungal cells growing in a planktonic stage from those forming biofilms. The level of this cell wall component increased 10-fold in both *in vivo* and *in vitro* biofilms generated by *Candida* cells, including those from *C. parapsilosis* (223). Therefore, it has been proposed that the β-1,3-glucan levels in cells isolated from indwelling devices be evaluated to assess the possible formation of fungal biofilms (223). In contrast, other cell wall components were not effective markers of *C. parapsilosis* infections. For example, detection of mannan antigen with the Platelia Candida Ag Plus assay showed a relatively low sensitivity in clinical samples (224).

Although our knowledge has recently significantly expanded regarding the cell wall structure of *C. parapsilosis*, there are major unexplored areas that are yet to be investigated.

Fatty Acid Metabolism

Fatty acids, as key building components of lipids, play an indispensable role in the organization and maintenance of cell membranes and various intracellular compartments. Both saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) determine membrane flexibility, regulate metabolic processes, and serve as precursors for a wide variety of complex macromolecules; thus, they are essential for viability. Recently, it was also suggested that FAs influence the virulence of pathogenic species; however, whether this is a direct or an indirect effect needs to be further investigated.

Pathogenic species acquire fatty acids either via *de novo* synthesis or through the degradation of external sources. *De novo* fatty acid synthesis requires both fatty acid synthases (chain elongation) as well as fatty acid desaturases (double-bond introduction), while secreted hydrolytic lipases contribute to the utilization of fatty acid from external lipids. A defect in either route will not affect fungal viability on its own, although if they malfunction simultaneously, the result is lethal (225).

Fatty acid synthases along with elongases are required for long-chain SFA biosynthesis, a preliminary step for later UFA production. *CpFAS1* is essential for the growth of *C. parapsilosis* in the absence of exogenous (saturated) fatty acids, as disruptant cells are severely restricted in UFA production (130). Furthermore, subsequent gene deletion affects not only the yeast's fatty acid metabolism but also its pathogenicity, as Δ/Δ*fas2* cells were sensitive to the presence of human serum and less virulent both *in vitro* and *in vivo* (130). Nguyen et al. showed that the fatty acid desaturase Ole1, which introduces the first double bond to saturated fatty acids, is also essential in *C. parapsilosis*, as Δ/Δ*ole1* cells were unable to produce unsaturated FAs in the absence of exogenous fatty acids. Furthermore, *CpOLE1* also influences virulence, as Δ/Δ*ole1* cells were sensitive to human serum and less virulent both *in vitro* and *in vivo*, similarly to Δ/Δ*fas2* cells (128).

In order to provide protection against gluco- and lipotoxicity, *C. parapsilosis* cells form lipid droplets (LDs) to prevent the accumulation of fatty acids and free glucose in toxic concentrations within the cytoplasm. In *C. parapsilosis*, *FIT2* regulates the formation of such triacylglycerol-containing cytoplasmic compartments serving as free fatty acid and lipid precursor reservoirs. Besides regulating fungal viability, *FIT2* also affects *C. parapsilosis*' virulence, possibly in an indirect manner (226). Interestingly, it has been suggested that *FAS2* and *OLE1* are also required for protection against gluco- and lipotoxicity via enhancing the formation of LDs. This was confirmed by the impaired LD formation in both the Δ/Δ*fas2* and Δ/Δ*ole1* strains (227).

Ole2, another fatty acid desaturase with hypothetical delta-9 desaturase activity, is involved in the processing of palmitoleic and oleic acids in *C. parapsilosis* (136). Interestingly, the amount of monounsaturated FAs increased markedly in the Δ/Δ*ole2* strain, thereby calling into question Ole2's presumed delta-9 desaturase activity (136). Enhanced phagocytosis and killing of Δ/Δ*ole2* cells and the induced increased levels of interleukin-10 (IL-10) suggested that *CpOLE2* may also be involved in virulence regulation (136). Although functional Δ12/Δ15- and Δ15-fatty acid desaturases (*FAD2* and

FAD3) have also been identified in this species, their influence on virulence has not yet been investigated (228).

Prostaglandin Production

FAs also serve as precursors for the synthesis of complex macromolecules, including immunomodulatory prostaglandins. In the host, prostaglandins are physiologically active compounds acting as potent regulators of diverse mechanisms, including inflammation. Several genera of pathogenic fungi are capable of producing prostaglandin-like molecules (136, 229–233), and these macromolecules enhance fungal pathogenesis by hijacking host immune responses (233). *C. parapsilosis*, similarly to *C. albicans*, is able to produce prostaglandins from exogenous arachidonic acids. This includes the biosynthesis of prostaglandin E_2 (PGE_2) and PGD_2, although other subsets of prostanoids have also been identified in smaller amounts (e.g., PGA_2 and PGB_2) (136). To date, only a few attempts have been made to identify the molecular bases of their biogenesis.

Due to the presence of a cytochrome *b* domain, it has been suggested that besides regulating fatty acid metabolism, Ole2 is also involved in the regulation of prostaglandin production, particularly PGE_2. However, while Ole2 greatly determines PGE_2 production in *C. albicans*, it cannot be associated with such functions in *C. parapsilosis* (136, 230). Recently, Chakraborty et al. identified genes potentially involved in *C. parapsilosis* prostaglandin production. These include the orthologous genes of *CaFET3*, *ScPOX1-3*, and *ScPOT1*, affecting the production of PGE_2, 15-keto-PGE_2, and PGD_2 (137). Besides regulating eicosanoid biosynthesis, they also influence fungal pathogenicity, as their removal results in enhanced phagocytosis and killing by human macrophages and also alters cytokine responses *in vitro*. Furthermore, disruption of *CpFET3* and *CpPOT1* resulted in attenuated virulence *in vivo*.

Nutrient Competition

During invasion, pathogenic fungi require carbon and nitrogen sources as well as trace elements for survival within the host. Such species are in constant competition with the host, along with the residual commensal microbes. In order to acquire essential nutrients under such restrictive environmental conditions, pathogenic species have evolved different acquisition mechanisms. For instance, several pathogenic fungi secrete various hydrolytic enzymes to degrade complex macromolecules in their close environment, which, besides enhancing deep invasion, also provide a wide range of carbon as well as nitrogen sources ready for uptake. Such enzymes include the above-mentioned fungal secreted lipases and proteases. Trace elements are likewise important for both viability as well as pathogenicity, due to the diverse roles of metalloproteins. These proteins require zinc, copper, or iron cofactors for proper functioning. *HAP5*, a subunit of the core binding factor (CBF) transcription regulatory complex, is required for the uptake of accessible iron in *C. parapsilosis*, similar to its role in *C. albicans* (101, 234). It has also been suggested that *CpHAP5* influences the activity of cytochrome proteins (hemeproteins) of the respiratory chain, possibly in an indirect manner. *C. parapsilosis HAP5* also enhances virulence, as null mutant strains were less virulent both *in vitro* and *in vivo* (101). Unfortunately, to date, we lack information about zinc and copper acquisition regulatory factors that could influence uptake mechanisms in this species. Previous studies revealed that *C. parapsilosis* cells tolerate the presence of potassium (KCl), sodium (NaCl), and lithium cations (LiCa) at high concentrations (235–237). However, whether this feature directly contributes to pathogenesis is yet to be determined.

Survival Strategies

In order to survive the restrictive environmental conditions present in the host, several pathogenic fungi have evolved different escape mechanisms (238–244). Besides inducing a delayed, tolerogenic immune response (discussed below), other survival mechanisms have been observed during *C. parapsilosis* infections. For example, similar to other pathogenic yeasts, *C. parapsilosis* cells are able to survive within phagocytes

and endothelial cells (115, 245). Intracellular budding, pseudohypha formation, and induced exocytosis were also observed. Furthermore, engulfed yeast cells inhibited the host cells' attempt to complete mitosis (115). In endothelial cells, internalized yeasts resist acidification by the host cell and are protected from neutrophil killing *in vitro* (246). Although the underlying molecular mechanisms regulating the above-mentioned phenomena are largely unknown, it has been suggested that the species' secreted lipases and extracellular proteases might be involved in survival within maturing phagosomes. For instance, removal of *CpSAPP1* as well as *CpLIP1-LIP2* resulted in delayed phagolysosome maturation following uptake by human macrophages (131, 247).

Metabolism of Hydroxyderivatives of Benzene and Benzoic Acid

Hydroxyderivatives of benzene and benzoic acid are toxic compounds exhibiting antimicrobial activities. For example, salicylate (2-hydroxybenzoate), aspirin (acetylsalicylate), phenol, and catechol are highly toxic to *C. parapsilosis* cells (113, 248). However, this yeast is able to assimilate a range of hydroxybenzenes (hydroquinone and resorcinol) and hydroxybenzoates (3-hydroxybenzoate, 4-hydroxybenzoate, 2,5-dihydroxybenzoate [gentisate], 2,4-dihydroxybenzoate, and 3,4-dihydroxybenzoate [protocatechuate]). Functional analyses have demonstrated that these compounds are metabolized via the 3-oxoadipate and gentisate pathways, and most of the genes coding for the corresponding enzymes are organized into metabolic gene clusters (MGCs). The gentisate pathway gene cluster is located in the subtelomeric region of chromosome V and comprises the genes for four enzymes catalyzing biochemical reactions in this pathway (3-hydroxybenzoate 6-hydroxylase, gentisate 1,2-dioxygenase, a putative maleylpyruvate isomerase, and fumarylpyruvate hydrolase), a transporter belonging to the major facilitator superfamily (MFS) involved in hydroxybenzoate transport across the plasma membrane, and a binuclear zinc cluster (Zn_2Cys_6) transcription factor controlling gene expression. The 3-oxoadipate pathway gene cluster contains genes for three metabolic enzymes (hydroxyquinol 1,2-dioxygenase, a putative maleylacetate reductase, and 3-oxoadipate CoA transferase) and a zinc cluster transcription factor. The remaining enzymes of this pathway (i.e., 4-hydroxybenzoate 1-hydroxylase, hydroquinone hydroxylase, and 3-oxoadipyl-CoA thiolase) and the corresponding plasma membrane transporters are encoded by genes located outside this cluster. Both MGCs are regulated independently in a substrate-specific manner. They are highly expressed in cells assimilating hydroxyaromatic substrates but repressed in cells grown in glucose-containing media (99, 100, 113; A. Cillingová, R. Tóth, A. Gácser, and J. Nosek, unpublished data).

Although both hydroxybenzoates and hydroxybenzenes can be degraded via the 3-oxoadipate pathway, there is an important physiological difference in the degradation of these compounds. The conversion of 4-hydroxybenzoate to hydroquinone includes a decarboxylation step, releasing carbon dioxide that is readily converted by a carbonic anhydrase into bicarbonate anion. In fungi, bicarbonate activates intracellular signaling via adenylyl cyclase, thus affecting various processes, including filamentation and virulence (249). These features illustrate another difference between *C. parapsilosis* and *C. albicans*, as the latter assimilates hydroxybenzenes but does not metabolize hydroxybenzoates. The *C. albicans* genome contains two MGCs coding for the catechol and hydroxyhydroquinone branch of the 3-oxoadipate pathway, but it lacks the gene for decarboxylating monooxygenase (4-hydroxybenzoate 1-hydroxylase), orthologs of hydroxybenzoate transporters, as well as the gentisate pathway cluster. Hence, it grows in media containing phenol, catechol, hydroquinone, or resorcinol as a sole carbon source (250).

In addition to hydroxybenzenes and hydroxybenzoates, other compounds can be channeled into these pathways. For example, *C. parapsilosis* cells overexpressing monooxygenases that catalyze the first reaction in both pathways (i.e., 3-hydroxybenzoate 6-hydroxylase and 4-hydroxybenzoate 1-hydroxylase) exhibit increased tolerance to terbinafine (Lamisil) (113). This observation is consistent with the

finding that salicylate 1-monooxygenase from *Aspergillus nidulans* also confers resistance to this antifungal drug (251). Esters of phthalic acid (phthalates) are additional putative substrates of the 3-oxoadipate pathway (e.g., terephthalate can be decarboxylated to 4-hydroxybenzoate or hydroquinone) (A. Cillingová and J. Nosek, unpublished data). These compounds are commonly used as additives to increase the flexibility and durability of plastic materials, and the ability of *C. parapsilosis* to utilize phthalates as carbon sources may, in part, explain its affinity for surfaces of medical plastics.

ANTIFUNGAL SUSCEPTIBILITY AND RESISTANCE

Although an extensive discussion of pharmacotherapy for candidiasis is beyond the scope of this review, a discussion of some points relevant to infections caused by *C. parapsilosis* is warranted. Unlike *C. glabrata*, *C. parapsilosis* has maintained high rates of susceptibility to the azole antifungals. Although testing for azole susceptibility is recommended, these agents have largely retained their usefulness for *C. parapsilosis* infections. Echinocandins (caspofungin, micafungin, and anidulafungin) are currently recommended as first-line empirical therapy for patients with suspected or proven disseminated candidiasis in many clinical settings (252). *C. parapsilosis* has been reported to have higher MICs for the echinocandins than other *Candida* species (9, 253). However, patients with *C. parapsilosis* candidemia who were initially treated with echinocandins have not been noted to have worse outcomes (254), and no clinical studies have demonstrated the superiority of another class of antifungals over echinocandins for *C. parapsilosis* (252).

Susceptibility testing is recommended for all *Candida* isolates associated with invasive disease. In particular, azole testing is recommended to provide an oral therapeutic option. Especially for patients previously receiving an echinocandin, echinocandin susceptibility testing of *C. parapsilosis* isolates should be performed (252). With the exception of endocarditis, amphotericin B is no longer commonly used in adults for invasive candidiasis due to toxicities, albeit the lipid formulations have reduced nephrotoxicities (255–257). Notably, amphotericin B is relatively well tolerated in neonates (258, 259). Susceptibility testing for *C. parapsilosis* has been well validated by methodologies from both the Clinical and Laboratory Standards Institute (CLSI) (standard M27-A4) (260) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (10, 261).

Azole resistance is well documented in *C. parapsilosis*. Despite the concern for a genetic predisposition to resistance to echinocandins, surveys of *Candida* collections show that azole resistance occurred more commonly than echinocandin nonsusceptibility. For example, out of 122 *C. parapsilosis* strains, 9.8% were resistant to fluconazole, while 3.2% were resistant to echinocandins (10). In a prospectively collected series of *C. parapsilosis* isolates, 4.8% were resistant to fluconazole and 0.6% were resistant to echinocandins (11). The global rates of fluconazole resistance range between 2 and 5% (262, 263), and a recent review assessed 6,023 isolates and revealed an overall rate of resistance to fluconazole of 3.4% (25).

Azole resistance has been well associated with prior or current fluconazole use (264–267), and resistance can develop even with exposure to systemic antibiotics (268, 269). Surveys of isolate collections reveal the rate of fluconazole resistance to be 0 to 4.6% (270–274). The average MIC_{50} values of fluconazole for *C. parapsilosis* are from 0.5 to 1 μg/ml (186, 200, 271, 275–277). Rates of resistance to itraconazole range from 1.5 to 4% (270, 271). Given the low MIC_{50} ($\leq$0.03 μg/ml) of voriconazole, the rate of resistance is less than 2% (272, 278). Cross-resistance to azoles can occur, including in strains responsible for outbreaks (279). For example, one large study found that only 37% of fluconazole-resistant isolates were susceptible to voriconazole (272). Voriconazole resistance can also develop in patients during exposure to fluconazole (280).

During recent years, advances have been achieved in terms of understanding the molecular bases of decreased antifungal drug susceptibility. For example, alterations in ergosterol biosynthetic mechanisms and upregulation of multidrug transporters in the

cell wall are the two most common pathways of development of azole resistance (281–283). In *C. albicans*, three major regulators are commonly highlighted to regulate azole resistance, two multidrug transporters (Cdr1 and Mdr1) and an enzyme involved in ergosterol biosynthesis (Erg11), as all three are overexpressed in the presence of azole derivates.

Among the members of the ERG family (genes encoding proteins involved in the ergosterol biosynthesis pathway) in *C. albicans*, as well as in *C. parapsilosis*, overexpression of *ERG11* (encoding a lanosterol 14-α-demethylase) in the presence of azoles has been highlighted by multiple studies (25, 283–285). In *C. albicans*, Upc2, the key transcriptional regulator of *ERG11*, has also been identified, as gain-of-function mutations in the corresponding gene's sequence led to a significant increase in *ERG11* expression (286). The Y132F substitution in *ERG11* is exclusively found in azole-resistant isolates of *C. albicans* (25, 285). In *C. parapsilosis*, the same substitution event also correlates with azole resistance (281, 287). However, the transcriptional regulator Upc2 does not regulate *ERG11* (overexpresses independently from *UPC2*) in this species but is required for the expression of 13 other ergosterol biosynthesis genes (281, 288). Besides Upc2, another transcriptional factor, Ndt80, is also suggested to regulate ergosterol synthesis in *C. parapsilosis* and thus determines azole resistance, similarly to *C. albicans* (288, 289).

In *C. albicans*, besides ERGs, upregulation of multidrug transporters (efflux pumps) has also been observed during exposure to azole derivates. Two such efflux pumps are encoded by *CDR1* and *MDR1* (290–293). The expression levels of both transporters are elevated if mutations occur in the corresponding transcriptional regulators' sequences. Of these, upon gain-of-function mutations in the transcriptional factor *MRR1*, expression levels of *MDR1* are elevated, whereas gain-of-function point mutations in *TAC1* enhance the expression of *CDR1* in *C. albicans* (294, 295). The upregulation of *CDR1* and *MDR1* orthologs was also confirmed in *C. parapsilosis* in the presence of azoles (281, 283). However, their regulation differs from what was observed in *C. albicans*. In *C. parapsilosis*, overexpression of *MDR1* and *CDR1* is less dependent on mutations in *MRR1* and *TAC1* (281). Further studies revealed that both Cdr1 and Mdr1 in this species are only partially required for decreased azole susceptibility, as disruption of the corresponding genes resulted in only a mild decrease in fluconazole MICs (281). These studies suggest that there is often a combination of molecular mechanisms, including sterol and efflux pump gene alterations, responsible for resistance in *C. parapsilosis* (133, 283, 296).

C. parapsilosis displays reduced susceptibility *in vitro* to echinocandins (297–299), and the yeast can cause infection in the setting of echinocandin administration (300–305). Notably, caspofungin MICs for *C. parapsilosis* are higher than for other *Candida* species, with MIC_{50} values typically ranging from 0.85 to 2 μg/ml (186, 271, 275, 306, 307). Similarly, micafungin has an average MIC_{50} of 1 μg/ml (271, 308), and anidulafungin has an average MIC_{50} of 2 μg/ml (271, 276, 309). Interestingly, pharmacodynamics evaluations reveal that echinocandin requirements for efficacy against *C. parapsilosis* by AUC/MIC (area under the concentration-time curve over 24 h in the steady state divided by the MIC) targeting were higher than for the other *Candida* species tested (310). Alterations in cell wall structure, reduced affinities for the glucan synthase protein complex, and variations in regulatory networks are thought to contribute to resistance (307). *C. parapsilosis* has more naturally occurring *fks* polymorphisms than most other pathogenic *Candida* strains (311). One such example is the naturally occurring amino acid substitution "P660A" in the "hot spot" 1 (HS1) region of *FKS1*, which might result in a decreased sensitivity of the corresponding glucan synthase to echinocandins (312, 313). It has also been suggested that substitutions in the HS regions of *FKS2* could have similar effects, although to date, no such alterations have been revealed (314). According to Martí-Carrizosa et al. (314), mutations outside the HS regions of *FKS* sequences could also contribute to such a response (304, 312, 315). Notably, *C. parapsilosis FKS1* and *FKS2* mutations are different from those located in HS regions of other *Candida* species (314). Interestingly, the mitochondrial respira-

tory pathways in *C. parapsilosis* are also linked to echinocandin susceptibilities (89). A concerning result was obtained in an experiment where susceptible *C. parapsilosis* isolates were subjected to serial exposure to echinocandins, which led to high rates of resistance to caspofungin, micafungin, and anidulafungin as well as cross-resistance to all three drugs. Furthermore, serial exposure to echinocandins also induced resistance to azoles and flucytosine (316). Remarkably, echinocandins can paradoxically promote the growth of some isolates of *C. parapsilosis* and other *Candida* species *in vitro* at concentrations above the MIC_{50} for the isolates (317). However, murine studies with caspofungin-resistant isolates have revealed that administration of caspofungin to mice infected with caspofungin-resistant isolates modified virulence, demonstrating that *in vitro* data may not clearly correlate with *in vivo* outcomes and suggesting that host immune responses may contribute to this effect (318).

Ongoing studies continue to expand our understanding of azole and echinocandin resistance. Interestingly, resistance to these agents can be linked to single pathways. For example, a recent publication reveals that C-5 desaturase activity regulates susceptibility to both azoles and echinocandins, as disruption of *ERG3* leads to high-level azole and intermediate- to high-level echinocandin resistance (319).

Amphotericin B "tolerance" in *C. parapsilosis* was first noted in 1983, when minimal fungicidal concentrations of several strains were noted to be ≥32-fold higher than their respective MICs (320). Moreover, resistance to amphotericin has been well documented (321–323), with surveys of clinical strains revealing a resistance rate of ~3% (271). However, a recent analysis of *C. parapsilosis* isolates from pediatric patients in Argentina reveals that 15% of the isolates displayed high amphotericin MIC_{50} values, with a range of 0.5 to 2 μg/ml and a geometric mean of 1 μg/ml (324). A review of data from a large collection of studies, however, shows that the typical MIC_{50} range of amphotericin B for *C. parapsilosis* is 0.13 to 1 μg/ml (186, 200, 271, 275–277).

Although infrequently utilized, flucytosine can be used in combination, typically with azoles or amphotericin B, for candidal meningitis (325, 326) or endocarditis (327). Flucytosine resistance rates generally range from 2% to 6.4%, although a 1975 study reported that 24% of *C. parapsilosis* isolates were resistant (271, 328).

The studies described above show that *in vitro* drug susceptibility results are not clearly correlated with patient outcomes. *C. parapsilosis* biofilms are a major factor in outcomes for certain patients (329), particularly in the setting of infected foreign materials. In particular, *C. parapsilosis* is frequently associated with central venous catheters (330–333). Biofilms can adversely impact the function of antifungals, primarily through binding the compounds or otherwise impairing their ability to reach yeast cells. *C. parapsilosis* biofilms have increased resistance to standard formulations of amphotericin B and azoles (185, 334), but amphotericin lipid formulations may retain activity (186), and echinocandins can reduce the metabolic activities of *C. parapsilosis* biofilms (185, 186, 335).

HOST IMMUNE RESPONSES

Our knowledge of antifungal immunity has expanded at a remarkable pace over the past decade. For example, new host recognition mechanisms with intracellular signaling pathways along with intercellular and humoral effects have been revealed. The control of *C. parapsilosis*-driven infections, similarly to other fungal species, relies on the proper activation of both innate and adaptive immune responses. Along with new knowledge about other pathogenic fungi, advances have also been achieved in terms of the exploitation of anti-*C. parapsilosis* immune responses.

Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) are two main classes of pathogen recognition receptors (PRRs) that commonly recognize fungal pathogen-associated molecular patterns (PAMPs). Among the TLRs, TLR2, TLR4, and TLR6 are involved in the recognition of *C. parapsilosis* cells by both gingival epithelial cells (ECs) and human macrophages (336, 337). Further studies suggest that among CLRs, Galectin-3 and Dectin-1 are required for *C. parapsilosis*-induced immune responses (338–340). According to Linden et al., Galectin-3 blockers added to neutrophils inhib-

ited *C. parapsilosis* phagocytosis, while the addition of exogenous Galectin-3 to the culturing medium enhanced yeast cell uptake (338). Additionally, gal3$^{-/-}$ mice were more susceptible to *C. parapsilosis* infection than wild-type mice (339). The same study further demonstrated that lower levels of Galectin-3 are present in human neonatal cord blood sera than in healthy adult sera, which might also explain the susceptibility of infants to this species. Decreased inflammatory cytokine levels after Dectin-1 inhibition also suggested the receptor's inclusion in *C. parapsilosis* recognition by human blood-derived macrophages (340). To date, we lack further information about the inclusion of other pattern recognition receptors in *C. parapsilosis* recognition.

In terms of the immune responses triggered following recognition, the activation and effector functions of innate and adaptive immune cells have also been examined.

Among myeloid cells, peripheral blood mononuclear cells (PBMCs), peripheral blood mononuclear cell-derived macrophages (PBMC-DMs), polymorphonuclear neutrophils (PMNs), dendritic cells (DCs), and bone marrow-derived macrophages (BMDMs) all participate in the innate immune responses triggered by *C. parapsilosis*. Out of these, effector functions and cytokine release of PBMC-DMs, PMNs, and PBMCs upon *C. parapsilosis* challenge are the most examined. Professional phagocytes actively phagocytose and kill *C. parapsilosis* cells (both yeast and pseudohyphae) to enhance clearance (115, 341–344). Complement activation and proinflammatory (IL-1β, IL-6, and tumor necrosis factor alpha [TNF-α]) and anti-inflammatory (IL-10) cytokine production were further observed in the case of PBMCs (340, 343). Upon *C. parapsilosis* infection, all three classical mitogen-activated protein kinases (MAPKs) (p38, extracellular signal-regulated kinase [ERK], and Jun N-terminal protein kinase [JNK]) are involved in cytokine production in mononuclear cells, although the induced cytokine responses vary from those induced by *C. albicans* (340). NF-κB activation was also confirmed during *C. parapsilosis* invasion, along with the formation of a granuloma-like structure by PBMCs, which was later infiltrated by PMNs and CD4$^+$ and CD8$^+$ lymphocytes, enhancing the early production of gamma interferon (IFN-γ) and control of the infection (344, 345). Besides phagocytosis, killing, and the production of proinflammatory cytokines (IL-1α, IL-6, TNF-α, and CXCL-8), DCs also form fungipods, pseudopodial protrusions observed especially in the presence of *C. parapsilosis* that are thought to promote fungal recognition (346, 347). On the contrary, BMDMs have been shown to release IL-27 in the presence of this fungus, which resulted in an anti-inflammatory response. This was supported by the observation that IL-27R$^{-/-}$ mice displayed enhanced fungal clearance (348). Thus, innate immune responses against *C. parapsilosis* greatly differ between the examined myeloid cell types.

Regarding host cell killing, *C. parapsilosis* causes relatively mild damage to PBMC-DMs compared to *C. albicans* (115). Host cell rupture due to pseudohyphal growth of the ingested cells has been reported (115). Additionally, this species induces macrophage death via inflammation-derived apoptosis (pyroptosis) similarly to *C. albicans*, although this event is possibly differently regulated by the two species (337).

ECs at barrier sites are also active participants in innate immune responses, as during colonization with a pathogenic species, they produce various cytokines and chemokines to attract professional immune cells to the site of invasion. For example, *C. albicans* infection triggers strong cytokine release from epithelial cells, including the secretion of neutrophil and monocyte attractants (349–351). In contrast, small amounts of cytokines are released during oral epithelial cell colonization by *C. parapsilosis* (352). This phenomenon is possibly due to the unresponsiveness of the MAPK/c-Fos signaling pathway, the primary invasion-alarming intracellular signaling route in epithelial cells for other yeasts (353). Besides maintaining a defined although still relatively low level of IL-1α induction, only mild host cell damage has been observed after coincubation of *C. parapsilosis* with oral squamous epithelial cells (352).

Nevertheless, by using an engineered human oral mucosa model, *C. parapsilosis* has been shown to be capable of forming biofilms and invading connective tissues (336). Interestingly, however, in this model, upregulation of TLR2, TLR4, and TLR6 was observed in human gingival epithelial cells, which was accompanied by the production

of IL-1β, TNF-α, IFN-γ, β-defensin-1, β-defensin-2, and β-defensin-3 (336), which is likely responsible for the growth inhibition of *C. parapsilosis* cells (336).

These results suggest that during the colonization of epithelial barriers, host inflammatory responses triggered by *C. parapsilosis* may be present; however, they are not comparable to those induced by *C. albicans*. As during *C. albicans* invasion, the presence of true hyphae is thought to be the major inducer of immune responses (352), *C. parapsilosis*' inability to form true hyphae may contribute to the lack of an effective inflammatory reaction. Previous studies also showed that *C. albicans* hyphae actively secrete candidalysin, a fungal toxin encoded by *ECE1* that also actively contributes to host cell damage (354). *In silico* data analyses suggest that the orthologous gene is not present in *C. parapsilosis*, which may further explain the ineffective induction of an adequate immune response.

In terms of adaptive immunity, T helper 1 (Th1) and T helper 17 (Th17) cells are the main T lymphocytes required for anti-*Candida albicans* responses (355, 356). Interestingly, *C. parapsilosis* is not able to induce significant levels of cytokines that are associated with Th1/Th17 responses; instead, host cells infected with this species produced elevated levels of IL-10, a cytokine that is characteristic of Th2 activation (340). Such results suggest that *C. parapsilosis* may cause a Th1-to-Th2 shift in T lymphocyte responses during infection, which might promote anti-inflammatory responses.

Inflammasomes are cytoplasmic multiprotein complexes that are required for the regulation of proinflammatory cytokine production (IL-1β and IL-18) as well as for pyroptosis induction (357, 358). As a result of its function, the processed mature and released form of IL-1β activates acute-phase inflammatory responses as well as adaptive immune responses through inducing the differentiation of Th17 cells (359). However, for the activation of caspase-1, a member of the NLRP3 inflammasome required for pro-IL-1β maturation, a second signal (i.e., a danger signal) is required (360). For instance, NLRP3 inflammasomes activate rapidly upon *C. albicans* exposure, due to lysosome rupture, increased K^+ efflux, and intracellular reactive oxygen species (ROS) release (361). *C. parapsilosis* also induces NLRP3 inflammasome activation although only after 24 h of coincubation. The difference between the two species might be due to the lack of early-phase ROS release from mitochondria after engagement with *C. parapsilosis* and also to delayed lysosome rupture possibly as a result of slow phagocytosis (337). Consequently, the delayed activation of NLRP3 inflammasomes causes late-phase IL-1β release and, thus, a late antifungal immune response to *C. parapsilosis*.

It is therefore possible that instead of the early-phase acute immune activation commonly observed in the case of *C. albicans*, *C. parapsilosis* induces a prolonged and possibly tolerogenic host reaction that may promote the fungus' hiding as a potential survival strategy. These observations further highlight the differences between the biologies of *C. albicans* and *C. parapsilosis* infections.

Nevertheless, it has also been shown that human peripheral blood lymphocytes are capable of inducing the production of immunoglobulins against invasive *Candida* species to promote fungal clearance (362). During investigations of such responses, antibodies specific to the carboxyl fragment of *C. albicans* heat shock protein 90 (HSP90) have been identified (363, 364). The level of such antibodies was shown to correlate with disease outcome (365, 366). Given that the identified epitope also has homologs in other closely related species, such as *C. parapsilosis*, a recombinant human antibody fragment (Mycograb) was produced, and its efficiency and synergy with other anticandidal drugs were tested *in vitro* and later *in vivo* (367, 368). Mycograb alone reduced fungal burdens in the liver, kidney, and spleen of mice challenged with *C. parapsilosis* and reduced the number of positive biopsy samples. A certain level of synergy with amphotericin B but no synergistic effect with fluconazole was detected in mice infected with this species (368). Later, a recombinant human IgG1 antimannan antibody, whose epitope is found on the cell walls of several *Candida* species, including *C. parapsilosis*, was used to passively immunize mice before infection with lethal doses of fungal cells. Immunized animals were significantly more resistant to disseminated

infection by *C. albicans* than control animals and showed reductions of infection foci in kidneys (369). This protection involved the promotion of fungal uptake and killing by macrophages, demonstrating that a humoral response could lead to protective immunity against *Candida* cells (369).

CLINICAL PERSPECTIVES

Clinical features of human infections with *C. parapsilosis* in many ways mimic those associated with *Candida* infection in general. Although the signs and symptoms vary according to the characteristics of the patient population at risk, a common presentation is sepsis or septic shock, which can be indistinguishable from the same condition attributable to a bacterial infection (370). As such, a high index of suspicion must be maintained in appropriate clinical settings in order to consider and empirically treat a fungal etiology. For example, a disseminated fungal infection must be among the etiologies considered in a neutropenic bone marrow transplant patient with fever and/or other signs of sepsis, particularly if other risk factors are present, such as the presence of a central venous catheter or recent exposure to broad-spectrum antibiotics. Empirical antifungal therapy is warranted in these cases, as early recognition and treatment are associated with decreased mortality (371). The clinical features in neonates are likewise similar to those seen with bacterial infections. However, neonates who develop invasive candidiasis are more likely to be thrombocytopenic and to have hyperglycemia (372–374).

Despite the significant overlap in clinical aspects of candidiasis, some features that distinguish *C. parapsilosis* infection from that associated with *C. albicans* have been noted. Data from a population-based surveillance study in Spain compared 78 episodes of *C. parapsilosis* bloodstream infection with 175 controls with infection caused by *C. albicans*. Factors that independently predicted *C. parapsilosis* infection included being of a neonatal age, being a transplant recipient, having prior antifungal therapy (mainly fluconazole), and receiving parenteral nutrition. The mortality rate was lower in patients with *C. parapsilosis* than in those with *C. albicans* infections (375, 376). A lower mortality rate from *C. parapsilosis* infection was also noted in a prospective, population-based study among adults hospitalized in medical and surgical ICUs throughout Spain. The rate of death within 7 days for *C. parapsilosis* infection was 7%, versus 56% for *C. albicans* (odds ratio, −0.21), in this series (371).

The lower mortality rate seen with *C. parapsilosis* is consistent with its reduced virulence relative to *C. albicans*, as described above and as noted in animal models (339, 377, 378). A recent series of autopsy findings in premature infants with candidemia who died likewise supports the reduced virulence of this species (379). *C. albicans* infection is frequently widely disseminated in postmortem tissues and can also be associated with inflammatory and tissue-destructive features. In contrast, *C. parapsilosis*-infected tissues generally lacked severe inflammation, and the organism was not easily detected by routine histopathological staining. However, a heavy fungal burden was nonetheless present in blood, intestines, and lungs when assessed by targeted immunohistochemical assays.

CONCLUDING REMARKS

During recent years, a decrease in the frequency of *C. albicans*-driven invasive disease has been observed, while the number of *C. parapsilosis* infections has risen. Although invasive candidiasis caused by the two species manifests similarly, there are considerable differences between the pathobiology of *C. albicans* and that of *C. parapsilosis*. Such divergent behaviors might determine the patient groups at risk as well as the species' incidence.

In this review, we summarize factors that could help clinicians and infection biologists better understand the pathogenesis of *C. parapsilosis*, which may later set the groundwork for a different approach for treating such infections. *C. parapsilosis* is most common in Latin America and the Mediterranean regions of Europe; however, outbreaks have occurred in diverse geographical regions. The reason for the species' region

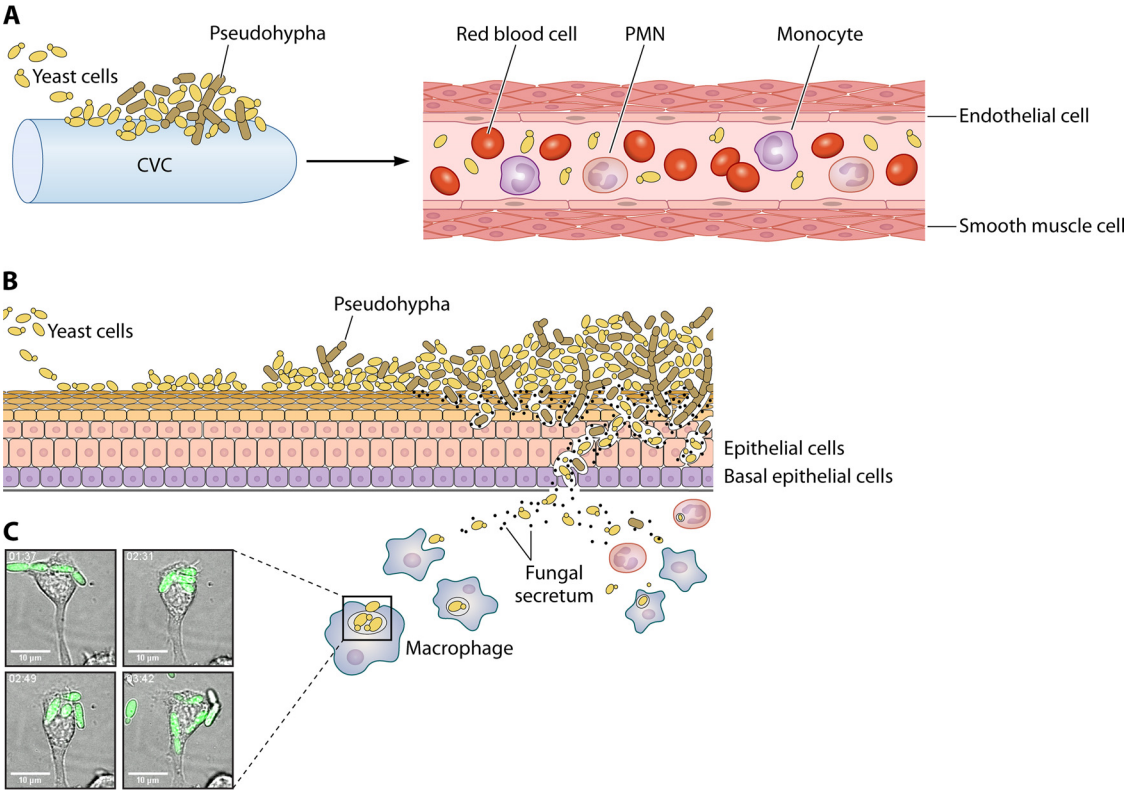

FIG 1 Transmission routes and pathogenesis of *C. parapsilosis*. (A) Central venous catheter (CVC) colonized by *C. parapsilosis* cells as the source of infection. Implantation of the contaminated device results in systemic dissemination. (B) Colonization and invasion of host epithelial surfaces. Invasion is supported by various virulence factors, including morphology transition and the release of fungal secretions such as hydrolytic enzymes. (C) Following phagocytosis, fungal cells not only survive but also may induce exocytosis or replicate within host cells. (Microscopic image series were taken by Csaba Papp.)

preference is yet unknown, although similarly to the cases of other pathogenic species, HLA polymorphism could be a potential factor that may determine prevalence. At this time, more is known about the preferred patient groups at risk: the increased prevalence among children and low-birth-weight neonates is possibly due to horizontal transmission of the fungus. Both transition from the hands of health care workers and prolonged use of total parenteral nutrition are suggested to serve as the origins of neonatal infections, while prolonged use of central venous catheters and other medically implanted devices serve as high-risk factors for infections among surgically treated patients.

Following the local colonization of abiotic surfaces, *C. parapsilosis* forms biofilms effectively, providing protection for planktonic cells that later serve as the source of infection (Fig. 1A). Following the rapid colonization of biotic surfaces, fungal cells may invade an impaired barrier that is promoted by morphology transition along with cell wall rearrangements, secretion of host cell/tissue-degrading enzymes, and the release of other host immunomodulatory compounds, e.g., fungal prostaglandins (Fig. 1B). Fungal cell wall rearrangements may further contribute to impairment of recognition and the host response. Notably, *C. parapsilosis* cells not only survive phagocytosis but also may induce exocytosis and actively proliferate within host cells (Fig. 1C).

Despite our increased understanding of *C. parapsilosis* biology through discoveries by clinical and basic science research over the past decade, several virulence and survival mechanisms are yet to be explored. For example, fungal metabolic pathways are altered during a progressing infection, although how this contributes to virulence in *C. parapsilosis* is not yet known. Furthermore, pathogens compete for available micronutrients and trace elements, but the strategy by which *C. parapsilosis* acquires these essential elements during interaction with the host is not known. Such mechanisms are yet to be revealed.

ACKNOWLEDGMENTS

H.M.M.-M. is supported by the Consejo Nacional de Ciencia y Tecnología, Mexico (reference numbers PDCPN2014-247109 and FC 2015-02-834), the Universidad de Guanajuato (reference number CIIC 95/2018), and the Red Temática Glicociencia en Salud (CONACYT-Mexico). G.B. is supported by the Science Foundation Ireland (12/IA/1343). J.M.B. is supported by an institutional development award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (NIH) (P30GM114750) and the Eunice Kennedy Shriver National Institute of Child Health and Human Development at the NIH (R21HD089278). J.N. is supported by the Slovak Research and Development Agency (APVV-14-0253) and the Slovak Grant Agency (VEGA 1/0333/15). A.G. is funded by NKFIH K 123952, GINOP2-3-2-15-2016-00015, and GINOP 2.3.3-15-2016-00006. A.G. is further supported by a momentum grant of the Hungarian Academy of Sciences (LP2018-15/2018) and by a grant of the Ministry of Human Capacities, Hungary (20391-3/2018/FEKUSTRAT).

We thank Csaba Papp for preparing the intracellular survival images.

We declare no competing financial interests.

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Geraldine Butler is a Professor of Genetics at the Conway Institute at University College Dublin, Ireland. Prof. Butler has worked on yeast since her Ph.D. studies in Trinity College Dublin in the 1980s, followed by postdoctoral training at the University of Michigan. She began to study *Candida parapsilosis* in the early 2000s, attracted by its significant role as a pathogen and the lack of associated molecular research. Prof. Butler's group carried out the first genome sequence survey of *C. parapsilosis*, followed by the first complete genome sequence in 2009. Her laboratory helped develop methods for molecular analysis, including generating large numbers of gene deletions and, together with Dr. Siobhán Turner, developing a CRISPR-based gene-editing system. Prof. Butler is particularly interested in genome evolution of fungi.

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