What do we know about the biology of the emerging fungal pathogen of humans *Candida auris*?

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ABSTRACT *Candida auris* is a worrisome fungal pathogen of humans which emerged merely about a decade ago. Ever since then the scientific community worked hard to understand clinically relevant traits, such as virulence factors, antifungal resistance mechanisms, and its ability to adhere to human skin and medical devices. Whole-genome sequencing of clinical isolates and epidemiological studies outlining the path of nosocomial outbreaks have been the focus of research into this pathogenic and multidrug-resistant yeast since its first description in 2009. More recently, work was started by several laboratories to explore the biology of *C. auris*. Here, we review the insights of studies characterizing the mechanisms underpinning antifungal drug resistance, biofilm formation, morphogenetic switching, cell aggregation, virulence, and pathogenicity of *C. auris*. We conclude that, although some progress has been made, there is still a long journey ahead of us, before we fully understand this novel pathogen. Critically important is the development of molecular tools for *C. auris* to make this fungus genetically tractable and traceable. This will allow an in-depth molecular dissection of the life cycle of *C. auris*, of its characteristics while interacting with the human host, and the mechanisms it employs to avoid being killed by antifungals and the immune system.

Key words: *Candida auris*, genetics, chromosomes, phenotype, morphogenetic switching, biofilm formation

1. INTRODUCTION

In the last decades, novel multidrug-resistant pathogens emerged as recurrent global threats to health care settings (Arastehfar et al., 2020). Among these species, *C. auris* became a major concern to medical mycology (Lone and Ahmad, 2019; Rhodes, 2019). This fungus is difficult to treat, has been frequently misidentified by commercially available systems, and its unique ability to colonize skin and smooth surfaces enabled its nosocomial spread (Chaabane et al., 2019; Kordalewska and Perlin, 2019; Rhodes and Fisher, 2019; Sabino et al., 2020). Especially, its ability to rapidly develop resistance or tolerance to antifungal drugs has garnered attention. There are only three main classes of clinically used antifungals (azoles, echinocandins, and polyenes); many *C. auris* isolates are resistant to multiple antifungals, often to compounds within two or even all three main classes (Chybowska et al., 2020; Lockhart, 2019; Sabino et al., 2020).

C. auris was named according to its first identification as an isolate from the ear canal of a Japanese patient about 10 years ago (*auris* being Latin for ear) (Satoh et al., 2009). The initial difficulties in identifying *C. auris* correctly triggered retrospective studies of strain collections which identified cases dating back to 1996 (Kwon et al., 2019; Sekizuka et al., 2019). Since then, *C. auris* has rapidly become a major healthcare threat causing outbreaks in hospital settings across all continents (Chybowska et al., 2020; Rhodes and Fisher, 2019; Sabino et al., 2020). Differences in

cellular, genetic, and molecular features of *C. auris* compared with other *Candida* species have become evident in many aspects of its biology. *C. auris* preferentially colonizes the skin rather than the gastrointestinal tract. Still, *C. auris* can disseminate to internal organs (liver, brain, lungs, bones, kidneys, and urinary tract) via a systemic bloodstream infection (candidemia); crude mortality of candidemia with *C. auris* ranges from about 30% to 70% (Lone and Ahmad, 2019; Sabino et al., 2020).

Due to the recent emergence of this pathogen, we are largely ignorant about its general biological traits. The lack of fundamental understanding about the origin and the life cycle of *C. auris* impedes our capacity to explain its sudden emergence, global spread, and unique phenotypic characteristics. Here, we review this worrisome emerging pathogen with special focus on its major biological traits.

2. PHYLOGENY AND GENOME ORGANIZATION

C. auris belongs to the phylum Ascomycota, and is part of the Saccharomycetes class; it is included in the CTG clade together with most of the *Candida* species (*Candida glabrata* being a notable exception) (Butler et al., 2009). The CTG clade is characterized by the unique translation of CUG codons as serine rather than leucine (Santos and Tuite, 1995). *C. auris* is placed with *C. haemulonii*, *C. duobushaemulonii*, and *C. pseudohaemulonii* to form a single clade of multidrug-resistant, human-pathogenic fungi; the *Candida haemulonii* species complex. The next extant relative to this species complex is *C. lusitaniae*, they all form a haploid group distantly related to more common diploid human pathogens *C. albicans*, *C. tropicalis* and *C. parapsilosis* (Chatterjee et al., 2015; Muñoz et al., 2018; Sharma et al., 2016).

Since the first draft genome of *C. auris* was obtained by whole-genome sequencing (WGS) (Chatterjee et al., 2015), hundreds of new whole-genome sequences of different isolates were generated (Chow et al., 2020; Lockhart et al., 2017; Muñoz et al., 2019, 2018; Sekizuka et al., 2019; Sharma et al., 2016). The size of the *C. auris* genome is 12-13 Mb with a GC content of ~45 % containing ~5,500 open reading frames on 5 – 7 chromosome-sized contigs (reviewed in Chybowska et al., 2020). Although initial studies indicated that *C. auris* could be diploid (Chatterjee et al., 2015; Sharma et al., 2016), generally its genome is haploid (Bravo Ruiz et al., 2019; Lockhart et al., 2017; Muñoz et al., 2018). The mitochondrial DNA of *C. auris* is ~27 kb in length and contains 15 coding sequences, 2 rRNA loci, and 32 tRNA loci; two mitochondrial genes, *NAD5* and *COB*, seem to contain introns (Sekizuka et al., 2019).

One striking signature of *C. auris* is its simultaneous and apparently independent emergence in four (maybe five) separate regions across the globe. The environmental reservoir of *C. auris* is unknown, and it is still enigmatic what facilitated its emergence as a multidrug-resistant human pathogen, although human activity – in the form of intensive agriculture and climate change – could have been a contributing factor (Casadevall et al., 2019; Fisher et al., 2020; Jackson et al., 2019). Initially, *C. auris* isolates were grouped into four unique geographical clades: clade I (South Asia), clade II (East Asia), clade III (South Africa), clade IV (South America) (Lockhart et al., 2017). More recently, a potential fifth clade from Iran was described (Chow et al., 2019). Isolates from different clades differ by tens of thousands of single nucleotide polymorphisms (SNPs), but the number of SNPs within each cluster is minimal, suggesting a series of clonal expansions (Chow et al., 2019; Lockhart et al., 2017; Muñoz et al., 2018). *C. auris* outbreaks reported in various countries, so far, seem to be caused by strains belonging to clades I – IV (Borman et al., 2017; Chow et al., 2020, 2018; Escandón et al., 2018; Eyre et al., 2018; Rhodes et al., 2018; Ruiz-Gaitán et al., 2018; Sekizuka et al., 2019; Sharma et al., 2016). Altogether, these results suggest multiple independent introductions of this fungus into different countries and clonal expansion within each outbreak.

The best assemblies of four strains (clades I-IV, one representative each) obtained to date suggest that the *C. auris* genome is organized in seven contigs (Muñoz et al., 2019, 2018). However, pulsed-field gel electrophoresis of *C. auris* clinical isolates, representing the four major clades, showed a plastic karyotype with five to seven chromosomes (Bravo Ruiz et al., 2019; Kwon et al., 2019; Oh et al., 2011). Interestingly, some karyotype variability is present

even between strains from the same clade which show minimal variation at the DNA level, suggesting that the karyotype heterogeneity is rapidly evolving (Bravo Ruiz et al., 2019). Large chromosomal rearrangements between isolates from different clades have also been observed in WGS data (Muñoz et al., 2019, 2018). Notably, isolates from clade II appear highly rearranged compared to strains from clades I, III, and IV; this is possibly a consequence of a naturally occurring loss-of-function mutation in the *DCC1* gene in clade II (Muñoz et al., 2019). Defective Dcc1 leads to genome instability in *S. cerevisiae* (Yuen et al., 2007), thus the mutation in *C. auris* clade II *DCC1* might result in a hypermutator phenotype. The gross chromosome rearrangements could be a barrier to genetic exchange via (para)sex between isolates from different clades, as they will likely result in inviable progeny.

In many yeast species, chromosome rearrangements, aneuploidy (abnormal number of single chromosomes), and polyploidy (whole chromosome sets present in more than two copies) have been described as drivers of genetic diversity and as an important trait for adaptation to environmental stresses and host niches; this includes the development of resistance to antifungal drugs (Bennett et al., 2014; Gerstein et al., 2017; Selmecki et al., 2009; Todd et al., 2017; Wertheimer et al., 2016). These changes in the genome structure could happen erroneously during mitosis or meiosis (chromosome segregation mistakes), or occur during concerted chromosome loss as part of parasexual reproduction. In microevolution assays under stress conditions, *C. auris* can undergo marked karyotype alterations within a short time (Bravo Ruiz et al., 2019). In some cases, these alterations were associated with fitness benefits, although other karyotype modifications seem to be stochastic and did not confer an obvious advantage. Strikingly, a case of acquired resistance to caspofungin without direct exposure to the drug was found: Isolates evolved under osmotic stress (2% sorbose) showed growth improvement in the presence of both, sorbose and caspofungin (Bravo Ruiz et al., 2019). Notably, under heat stress some strains reduced the number of chromosomes from seven to six, four or three chromosomes. These strains were perfectly viable and showed no growth deficiencies under standard laboratory conditions (Bravo Ruiz et al., 2019). This demonstrates that drastic modifications of the genome structure do not necessarily impinge on the viability of *C. auris* but might provide opportunities for general fitness adaptations. Further studies will be necessary to elucidate how karyotype variation is driven in *C. auris*; so far, (para)sexual reproduction has not been reported. The observed variation would most likely be due to gross chromosome rearrangements, and/or copy number variation (CNV) events of chromosomal sections, but diploidisation could play a role as well (Fan et al., 2020). Indeed, large subtelomeric regions have been lost from 10 out of 14 chromosomes ends in clade II isolates (Muñoz et al., 2019). In other *C. auris* clades, these subtelomeric regions contain genes encoding Hyr/Iff-family adhesins, which are likely important for virulence (Muñoz et al., 2019). Isolates from different clades had CNVs of genes potentially playing a role in adaptation to host stresses (Chow et al., 2020). Further CNVs related to antifungal resistance found in *C. auris* are discussed below (section 3.).

In general, repetitive regions, such as transposons, telomeres, and rRNA gene repeats, are known drivers of CNVs and gross chromosome rearrangements in yeast species (Argueso et al., 2008; Gordon et al., 2011; Mieczkowski et al., 2006; Rachidi et al., 1999). rRNA gene arrays have been described as recombinogenic, undergoing fluctuations in copy number and being the source of gross chromosome rearrangements. Indeed, in *C. albicans* the chromosome R, harbouring the rDNA region, has been described as the most unstable (Rustchenko et al., 1993). Due to their repetitiveness, rRNA gene clusters are difficult to accurately position on chromosomes in WGS data. However, rDNA has been identified in multiple numbers and on various chromosomes in whole genome assemblies (Chatterjee et al., 2015; Muñoz et al., 2018; Rhodes et al., 2018; Sekizuka et al., 2019; Sharma et al., 2016). Using Southern blotting on whole-chromosome pulsed-field gels, we observed a differential rDNA distribution between 26 *C. auris* isolates, rDNA repeats were found on up to four different chromosomes within a single strain (Bravo Ruiz et al., 2019). The rDNA region has also been related to the loss of global chromosomal stability, especially during senescence in fungi (Ganley and Kobayashi,

2014; Pal et al., 2018). Notably, aging *C. auris* cells apparently harbour transient gene duplications which disappeared when they replicate into a younger cell population (Bhattacharya et al., 2019). Whether rDNA or other repetitive elements have a role in *C. auris* genome stability or senescence needs further investigation.

3. ANTIFUNGAL RESISTANCE

Antifungal treatment is part of the primary healthcare response against fungal infections. Unfortunately, the antifungals available are limited and only three major classes of antifungals are routinely used in the clinic: azoles, polyenes and echinocandins. Occasionally, allylamines and the nucleoside analog 5-flucytosine are used as well, especially in combination therapy (de Oliveira Santos et al., 2018). Although for most *Candida* species antifungal resistance is exceptional, in the last decades incidences of multidrug resistant fungi have risen, this includes *C. auris*, and its close relatives *C. haemulonii*, *C. duobushaemulonii*, *C. pseudohaemulonii*, and *C. lusitaniae* (Muñoz et al., 2018). In a recent study analysing ~300 *C. auris* isolates, 80% were resistant to fluconazole, 23% to amphotericin B, and 7% to micafungin, and among them, 24% tested as resistant to at least two classes of antifungals, and 1% to antifungals of all three classes (Chow et al., 2020). The antifungal response of *C. auris* has recently been reviewed several times in detail (Chaabane et al., 2019; Chybowska et al., 2020; Lockhart, 2019). Hence, we only offer a short summary of known cellular mechanisms of antifungal resistance in *C. auris* (Figs. 1-2).

Gene prediction from WGS indicated that most of the genes associated with drug resistance in *C. albicans* are conserved in *C. auris* (Muñoz et al., 2018), and the mechanisms observed in *C. auris* to become drug-resistant are likely similar to those in other *Candida* species (de Oliveira Santos et al., 2018). Four basic mechanisms have been described (I) mutation of the drug target, (II) limiting the drug intake or favouring its efflux, (III) overexpression of the drug target, and (IV) biofilm formation (Figs. 1-2). However, other mechanisms involving different cellular pathways have also been described.

3.1. Mutations of antifungal target genes

Ergosterol is a key component of the fungal membrane, azoles inhibit its synthesis and thus prevent cell growth (de Oliveira Santos et al., 2018). Azoles specifically attack the active site of Lanosterol 14-α-demethylase which converts lanosterol to ergosterol (Fig. 1A); Lanosterol 14-α-demethylase is encoded by the *ERG11* gene. Thus, mutations affecting the active site of Erg11 potentially offer azole resistance (Berkow and Lockhart, 2017). Indeed, in *C. albicans* more than 140 SNPs have been identified in *ERG11* (Debnath and Addya, 2014). In azole-resistant *C. auris* strains, substitution mutations resulting in F126T, Y132F or K143R amino acid residue changes have been described (Figs. 1A, 2B); these mutations are strongly associated with geographic clades (Chow et al., 2020; Chowdhary et al., 2018; Kwon et al., 2019; Lockhart et al., 2017; Muñoz et al., 2018; Rhodes et al., 2018). Although no notable overexpression of Erg11 has been observed in azole-resistant isolates (Chowdhary et al., 2018), interestingly, the introduction of some of the mutated *ERG11* version from *C. auris* into *S. cerevisiae* resulted in elevated fluconazole resistance (Healey et al., 2018) suggesting that certain, but not all, mutations observed in *C. auris ERG11* offer protection from azole treatment.

The presence of echinocandins targets the production of glucan, a major component of the fungal cell wall, via inhibition of β-D glucan synthase. This impairment of the structural integrity of the cell wall mimics osmotic stress (Sucher et al., 2009). The subunits of β-D glucan synthase are encoded by the *FKS* genes (de Oliveira Santos et al., 2018). In many *Candida* species mutation in any of the two Hot Spot regions identified in the *FKS* genes is sufficient to confer echinocandin resistance (Perlin, 2015). In *C. auris*, three different *FKS1* substitution mutations (S639F, S639P or S652Y) in Hot Spot region I have been related to echinocandin resistance (Figs. 1B, 2B) (Berkow and Lockhart, 2018; Biagi et al., 2019; Chow et al., 2020; Chowdhary et al., 2018; Kordalewska et al., 2018; Rhodes et al., 2018). The relation of echinocandin and osmotic stress was further highlighted when we found a case of resistance to caspofungin in a clade II isolate grown in 2% sorbose

(osmotic stress) for a prolonged time (Bravo Ruiz et al., 2019).

Amphotericin B is a polyene which sequesters membrane-bound ergosterol, causing leakage through fungal membranes (Anderson et al., 2014); this results in cell death because essential small molecules escape from the cells. Therefore, alterations in cell membrane sterol composition can be a source of amphotericin resistance (Taff et al., 2013). To date, a causative mutation for amphotericin resistance has not been reported in *C. auris*, but a point mutation in *FLO8* has been observed in a resistant isolate (Fig. 1B) (Escandón et al., 2018). However, how mutation of *FLO8* would result in amphotericin resistance in *C. auris* is unclear, because in *C. albicans* the transcription factor Flo8 only indirectly protects cells from amphotericin-caused cell death by promoting hyphae formation (Laprade et al., 2016); hyphae are more resistant to amphotericin than yeast cells. *C. auris* does not seem to be able to form true hyphae (see section 4.2.), and the role of Flo8 in *C. auris* cell morphogenesis, if any, is not known. Hypothetically, it could modulate biofilm formation and/or cell aggregation in *C. auris*, both processes which might indirectly influence amphotericin susceptibility.

The nucleoside analog 5-flucytosine is an inhibitor of RNA and DNA synthesis. Inside the cell, 5-flucytosine is converted into the toxic compound 5-fluoro-uridine-5'-monophosphate by Fur1 (Fig. 2B) (Hope et al., 2004). Mutations in *FUR1* have been associated with flucytosine resistance in *C. albicans* (Dodgson et al., 2004). In *C. auris*, an isolate resistant to 5-flucytosine carrying a SNP in *FUR1* which leads to an F211I residue change was reported (Fig. 2B) (Rhodes et al., 2018).

3.2. Exporting antifungals from the cell

Pathogenic *Candida* species develop resistance to clinical fungicides through active drug export mediated by multidrug efflux pumps. Therefore, the increase of the efflux pumps activity, either by higher expression or expansion of gene families, is a major drug resistance mechanism (Morschhäuser et al., 2007; Schuetzer-Muehlbauer et al., 2003). Especially, two families of efflux pumps have been involved in decreased susceptibility to antifungal drugs, the ATP-Binding Cassette (ABC) and the Major Facilitator Superfamily (MFS) transporters (de Oliveira Santos et al., 2018); both families are conserved in *C. auris* (Fig. 2A) (Chatterjee et al., 2015; Muñoz et al., 2018; Wasi et al., 2019). However, some subfamilies of ABC transporters are underrepresented and others expanded in *C. auris* compared to other *Candida* species (Wasi et al., 2019); this suggests a differential evolutionary pattern among fungal ABC transporters.

ABC transporters show high efflux activity in resistant *C. auris* isolates (Ben-Ami et al., 2017). Among the ABC transporter, Cdr1 is the transporter with the highest basal expression level. Cdr1 showed a consistently higher expression in multidrug-resistant *C. auris* strains compared to susceptible ones (Fig. 2A) (Rybak et al., 2019; Wasi et al., 2019; Zamith-Miranda et al., 2019). Furthermore, exposure to amphotericin B, but not to fluconazole, triggered an increase in ABC transporter expression, especially of Cdr1, Cdr4, Cdr6 and Snq2 in an antifungal-sensitive strain (Fig. 2A) (Wasi et al., 2019). Additionally, Ste6, Pxa1 and several members of the MRP subfamily were transcribed at a higher level in resistant strains compared to sensitive ones (Fig. 2A) (Wasi et al., 2019). The role of Cdr1 in drug resistance was experimentally confirmed in two independent studies where the *CDR1* gene was deleted from azole-resistant and susceptible isolates; in both cases, azole-susceptibility increased (Kim et al., 2019; Rybak et al., 2019). Furthermore, Mdr1, an MFS pump also related to drug resistance in fungi (de Oliveira Santos et al., 2018), showed increased expression in resistant *C. auris* strains, although *MDR1* deletion only caused a 2-fold decrease of itraconazole minimal inhibitory concentration (MIC) (Fig. 2A) (Rybak et al., 2019). Also, an uncharacterized member of the Dha1 family of the drug:proton antiporters was significantly higher expressed in an azole-resistant *C. auris* isolate (Fig. 2A) (Zamith-Miranda et al., 2019).

The regulation of Cdr1 and Cdr2 transporters is driven by the zinc-cluster transcription factor Tac1 in *C. albicans* (Coste et al., 2004). This transcription factor is present in two tandem copies in all representatives of the *C. haemulonii* species complex (Muñoz et al., 2018). Interestingly, after *in vitro* evolution in the presence of fluconazole, a clade I *C. auris* isolate

acquired fluconazole resistance rapidly. All the derivative strains had gain-of-function mutations in one of the *TAC1* paralogs, *TAC1B*, with an associated increase in Cdr1 expression (Fig. 2A-B) (Rybak et al., 2020). Indeed, many fluconazole-resistant clinical isolates have SNPs in *TAC1B* (Rybak et al., 2020). Particular *TAC1B* SNPs were found in various isolates (Fig. 2B) and, strikingly, these were often associated with a specific corresponding *ERG11* SNP. However, some substitutions (K247E, M653V, A651T, A15T, S195C, or P595L/H) in *TAC1B* apparently occur on their own (Rybak et al., 2020). The role of *TAC1B*, especially of the A640V variant, in mediating fluconazole resistance was corroborated experimentally by introducing the $TAC1B^{A640V}$ mutation into a sensitive isolate, which led to increased fluconazole resistance. Reciprocally, replacing the $TAC1B^{V640}$ variant in a resistant clade I isolate with the sensitive *TAC1B* allele results in the loss of the resistance (Rybak et al., 2020).

Deletion of *TAC1B*, but not of its paralog *TAC1A*, caused a decrease in fluconazole and voriconazole resistance in clade III and clade IV isolates without conspicuously dysregulating the expression of Cdr1 (Mayr et al., 2020). This suggests that *CDR1* expression is independent of Tac1B in clade III and IV strains, which is consistent with *TAC1B* in clade III isolates being devoid of non-synonymous SNPs (Rybak et al., 2020). This also might explain why Tac1B is not required for basal *CDR1* expression levels, whereas gain-of-function mutations have a role in *CDR1* overexpression as described for *C. albicans* (Coste et al., 2004). Further studies will be necessary to elucidate the exact role of Tac1B in *C. auris* azole resistance and if there are genuine differences in azole resistance mechanisms between clades.

3.3. Over-expression of the antifungal target

The existence of *C. auris* isolates, in which the drug resistance cannot be explained by mutations in target genes or by alteration of efflux pump expression, suggests other mechanisms for drug resistance/tolerance. For example, factors involved in ergosterol biosynthesis, including Erg11, are more abundant in resistant *C. auris* isolates than in sensitive ones (Fig. 1A) (Zamith-Miranda et al., 2019). Mutations in *UPC2*, encoding a transcription factor which regulates the expression of genes in the ergosterol pathway, have also been related to fluconazole resistance in *C. albicans* (Flowers et al., 2012). Furthermore, *C. auris* strains resistant to amphotericin B showed increased expression of *ERG1*, *ERG2*, *ERG6* and *ERG13* (Fig. 1A) (Muñoz et al., 2018).

Increased resistance to antifungals has been associated with the appearance of CNVs in *C. albicans* (Selmecki et al., 2006). Also in *C. auris*, large duplications and triplications encompassing the *ERG11* gene have been found in fluconazole-resistant isolates from different clades (Fig. 2B) (Chow et al., 2020; Muñoz et al., 2018). Interestingly, aging has been linked to drug tolerance for all the antifungal classes in *C. auris*. *CDR1* and *ERG11* are apparently duplicated in old cells (>10 generations); these duplications disappear when the cell population rejuvenates, (Bhattacharya et al., 2019). Old cells thus overexpress Cdr1 and Erg11 and exhibit higher tolerance to fluconazole, micafungin, 5-flucytosine and amphotericin B compared to younger cells (0-3 generation) (Figs. 1A, 2A) (Bhattacharya et al., 2019).

In addition, in clade II isolates a cytochrome P450 and a phospholipid-translocating P-type ATPase (flippase) were duplicated, these genes might contribute to fluconazole susceptibility via homeostasis of ergosterol biosynthesis regulated by the cytochrome p450 family, and via structural cell membrane stability modulated by the flippase (Figs. 1A, B) (Sekizuka et al., 2019).

3.4. Biofilms as a mechanism of antifungal resistance and tolerance

Biofilms are structured communities mediated by cell-cell adherence. Biofilm formation has been described as a key pathogenicity trait in *Candida* species also providing enhanced antimicrobial resistance/tolerance. Furthermore, biofilms likely play a role in the ability of a fungus to persist on various surfaces (Fanning and Mitchell, 2012). For mechanistic details on biofilm formation see section 4.4..

The formation of biofilm helps to resist and tolerate exposure to antifungals and disinfectants (Fig. 1C).

Biofilm maturation correlates with decreased susceptibility to various antifungal drugs (Dominguez et al., 2019; Kean et al., 2018a; Romera et al., 2019; Singh et al., 2019; Srivastava and Ahmad, 2020). For example, fluconazole being retained in the extracellular matrix of biofilms is a key determinant of fluconazole susceptibility in *C. auris* (Dominguez et al., 2019). Importantly, *C. auris* in biofilms is also less sensitive to disinfectants, such as chlorhexidine, povidone-iodine, hydrogen peroxide or sodium hypochlorite (Kean et al., 2018b; Sherry et al., 2017; Short et al., 2019).

3.5. Other mechanisms of resistance

The enrichment of proteins in the tricarboxylic acid (TCA) cycle seems to be a mechanism of fluconazole resistance in *C. auris* similar to *C. albicans* (Fig. 2C) (Guo et al., 2017; Zamith-Miranda et al., 2019). Depletion and inhibition of the heat shock regulator protein Hsp90 affect azole tolerance in some *C. auris* isolates (Fig. 2C) (Kim et al., 2019). Modifications of the cell wall structure in a strain lacking the stress-activated protein kinase (SAPK) Hog1 apparently cause caspofungin resistance (Fig. 1B) (Day et al., 2018). Exposure to voriconazole and amphotericin B upregulates a series of pathways involved in cell wall and cell membrane maintenance, and transport across membranes in *C. albicans* and *C. auris* (Liu et al., 2005; Muñoz et al., 2018), again indicating that cell walls and membranes are key players in general drug resistance and tolerance.

4. PHENOTYPE

As a newly discovered species, very little is known about the fundamental biological traits of *C. auris*. More studies will be necessary to fully understand this multidrug-resistant human pathogen, and appreciate its traits determining virulence, antifungal resistance, and environmental persistence. Interestingly, phenotypic observations in various strains often contradict each other, sometimes this can be explained by strains belonging to different clades. However, it also suggests that the observed phenotype differences depend on the particular isolate being studied. It is thus of the utmost importance to conduct analyses on multiple different strains ideally representing all clades for a detailed understanding of this newly discovered species as a whole.

4.1. Genome and transcriptomic analysis of metabolism and cell wall components

As a fungus included in the CTG clade, it shares many common characteristics with other members of this clade, but *C. auris* also sports unique characteristics (Table 1). Genome analyses taught us that central pathways in nutrient assimilation and metabolisms seem to be conserved in *C. auris* (Chatterjee et al., 2015; Muñoz et al., 2018). However, major differences in the central carbon metabolism between *C. auris* and *C. albicans* suggest that *C. auris* favours respiration by increasing ATP production and reducing oxidative stress, this potentially results in a better overall fitness (Guo et al., 2017; Zamith-Miranda et al., 2019). Accordingly, *C. auris* shows different carbon source assimilation abilities from closely related species, and appears to be unable to ferment (Satoh et al., 2009) (Table 1).

Potentially, there are also considerable differences in the *C. auris* cell wall structure and composition in comparison to other *Candida* species (Table 1). The agglutinin-like sequence (ALS) family genes which encode large cell-surface glycoproteins implicated in adhesion to host surfaces, is conspicuously expanded in *C. albicans*, but curiously underrepresented in *C. auris* (Chatterjee et al., 2015; Muñoz et al., 2018). Expression of the protein kinase C Pkc1, which regulates cell wall composition in *C. albicans* (Munro et al., 2007; Walker et al., 2008), was not detected in *C. auris*. Furthermore, cell wall remodelling enzymes were generally more abundant in *C. albicans* compared to *C. auris*, although with some exceptions (Table 1) (Zamith-Miranda et al., 2019). Altogether, this suggests that *C. auris* could employ distinct mechanisms for cell adhesion and cell wall integrity.

Extracellular hydrolytic enzymes act as important virulence factors helping in adherence to and invasion of host cells, thus causing tissue damage (Naglik et al., 2003). Compared to *C. albicans*, lipid metabolism was enhanced in *C. auris*, especially in structural glycerophospholipids and lysophospholipids suggesting a higher phospholipase activity (Table 1) (Semreen et al., 2019;

Zamith-Miranda et al., 2019). Indeed, phospholipase, proteinase and hemolysin activities have also been shown experimentally in *C. auris*, albeit with seemingly strain-specific degrees of activity (Kumar et al., 2015; Larkin et al., 2017; Wang et al., 2018).

4.2. Morphogenetic switching

C. auris usually grows as yeast cells with spherical to oval shape, with a size of 2-3 × 2.5-5 μm (Figs. 3A, B) (Ben-Ami et al., 2017; Borman et al., 2016; Bravo Ruiz et al., 2020; Pathirana et al., 2018; Satoh et al., 2009), although it produces pseudohyphae under certain conditions (Figs. 3C-E) (Bravo Ruiz et al., 2020; Kim et al., 2019; Yue et al., 2018). The optimum growth temperature for *C. auris* is 37–40 °C (Satoh et al., 2009) and it reaches stationary phase in ~20 hours, with doubling times of around 60 min in young cells and up to ~150 min in cells older than 30 generations (Bhattacharya et al., 2019; Larkin et al., 2017).

Morphogenetic switching enables many fungi to change from growing as unicellular yeasts to filaments (pseudohyphae or true hyphae). Filamentous growth allows the exploration of new environments and is considered a virulence trait in pathogenic fungi (Noble et al., 2017; Sudbery, 2011). The switch is triggered by a multitude of environmental factors, such as nutrient limitation, temperature, and pH changes. Signalling through the mitogen-activated protein kinase (MAPK) and the fungal cyclic AMP (cAMP)-protein kinase A (PKA) pathways regulates this switch from yeast to filamentous growth. However, *C. auris* fails to form filaments when exposed to triggers that stimulate yeast-filament transitions in *C. albicans* (Bravo Ruiz et al., 2020; Kim et al., 2019; Pathirana et al., 2018; Wang et al., 2018). Accordingly, *C. auris* differs from *C. albicans* regarding the presence and organisation of hyphae formation factors, such as the absence of important hyphal-specific genes and essential regulators of true hyphal growth from the *C. auris* genome (Fig. 3F). Furthermore, important regulatory determinants of filamentation either show conspicuous differences in domain organisation (Ume6) (Fig. 3F) (Bravo Ruiz et al., 2020; Chatterjee et al., 2015; Muñoz et al., 2018), or are not transcribed (Efg1) in *C. auris* (Fig. 3E) (Zamith-Miranda et al., 2019). Deletion of a key transcriptional repressor of filamentous growth, *TUP1*, triggers constitutive filamentation in *C. albicans* (Braun et al., 2000). In contrast, a *C. auris tup1*Δ strain does not show a filamentation phenotype (Fig. 3E), but grows in strings of yeast cells suggesting a cell separation defect (Bravo Ruiz et al., 2020). *C. auris* also secretes several filament-inhibiting metabolites, some of which are known from other *Candida* species, whereas some are hitherto undetected (Semreen et al., 2019). Altogether, these observations suggest that the yeast stage is the preferred growth form of *C. auris*.

We and others have shown that *C. auris* can form pseudohyphae when DNA is damaged or DNA replication is perturbed (Figs. 3C-E). Filaments are observed in many, but not all, *C. auris* isolates when exposed to various genotoxins, including the clinically relevant fungistatic 5-fluorocytosine (Bravo Ruiz et al., 2020; Kim et al., 2019) or in the presence of high concentrations of sodium chloride (10 %) (Wang et al., 2018) likely also in relation with DNA damage (Dmitrieva and Burg, 2005). Interestingly, the ability to generate pseudohyphae and the degree of filamentation are strain-specific, but do not seem to correlate with clade affiliation (Bravo Ruiz et al., 2020). Pseudohyphal growth in response to genotoxic stress (S phase checkpoint activation) has also been described in *C. albicans* and *S. cerevisiae* (Fig. 3E) (Chen et al., 2018; Jiang and Kang, 2003). Exposure to genotoxic stress activates the Rad53 kinase via the S phase checkpoint, which arrests the cell cycle temporarily. Once the issue is resolved, the cell cycle resumes. S phase activation also involves other components such as the sensor kinase Mec1 and the mediator proteins Rad9 and Mrc1 (Fig. 3E) (Pardo et al., 2017). In *C. albicans* and *S. cerevisiae rad53*- and *mec1*-defective strains genotoxin-induced filamentation is strongly reduced (Jiang and Kang, 2003; Shi et al., 2007). Interestingly, filamentation triggered by genotoxic stress involves, at least partially, different mechanisms than the formation of true hyphae, since hyphal-specific genes or important key regulators such as Efg1 or Ume6 do not affect genotoxin-induced pseudohyphae formation in *C. albicans* (Bachewich et al., 2003; Chen et al., 2018). This agrees with the observation that several of these key hyphal-specific genes are missing in *C. auris*, when it still forms pseudohyphae under these conditions. *C.*

auris strains deleted for the mediators *MRC1* and *RAD9* show perturbations of the filamentation pattern compared to the wild type (Bravo Ruiz et al., 2020). Unfortunately, *RAD53* and *MEC1* could not be deleted, which suggests that these genes might be essential in *C. auris* (Bravo Ruiz et al., 2020). The homologous recombination pathway has a key role in the deactivation of the S phase checkpoint once the DNA damage is repaired (Fig. 3E) (Prado, 2018). Mutants of the homologous recombination factors *RAD51* and *RAD57* grow as pseudohyphae in unperturbed conditions in *C. auris* (Bravo Ruiz et al., 2020). Our results in the *C. auris mrc1*, *rad9*, *rad51*, and *rad57* deletion mutants, were similar to those observed in the corresponding *C. albicans* mutants (García-Prieto et al., 2010; Shi et al., 2007).

The mechanisms involved in pseudohyphal growth in response to S phase checkpoint activation are not well understood, and further studies will be necessary to fully elucidate their role. However, this potentially could be explained by the constitutive activation of the Clb2-Cdc28 complex by Rad53 through the Polo kinase Cdc5 in response to genotoxic stress (Fig. 3E) (Simpson-Lavy and Brandeis, 2010; Zhang et al., 2009). The activation of Clb2-Cdc28 prevents the entry into mitosis and the associated switch from polarized to isotropic growth (Lew, 2003); cells would be stuck in the apical growth phase, thus forming filaments. Furthermore, the cAMP and MAPK pathways have been implicated in pseudohyphal growth in response to genotoxic stress via downstream regulators (Chen et al., 2018).

In *C. auris*, a morphogenetic transition from yeast to pseudohyphal growth is also caused by depletion of *HSP90* or pharmacologically inhibiting it with geldanamycin (Fig. 3E) (Kim et al., 2019). Hsp90 is a heat shock family protein which acts as a chaperone and influences a diverse range of signal transducers. Hsp90 impairment resulted in the transcriptional upregulation of factors predicted to be involved in filamentous growth. These genes are partially different to those regulated by Hsp90 in *C. albicans*, the regulation of cell surface-associated genes during filamentous growth appears to be conserved in both species though (Kim et al., 2019). Interestingly, in *C. albicans* inhibition of Hsp90 induces pseudohyphal growth via cAMP-PKA signalling and regulation of Cdc28 in an Efg1-independent way, as well as by additional still uncharacterized mechanisms (Shapiro et al., 2011; Shapiro and Cowen, 2010). Notably, direct inhibition of Rad53 by Hsp90 has been observed in *S. cerevisiae* (Khurana et al., 2016), potentially tying the S phase checkpoint and the heat shock response to each other.

There potentially are also other pathways by which filamentation can be induced. Clade I strains can produce pseudohyphae on cornmeal agar (Dalmau cultures) (Szekely et al., 2019). Furthermore, a strain (likely clade I) which presented a filamentation-competent phenotype with a propensity to grow as pseudohyphae at low temperatures (20-25 °C) was isolated from an infected mouse (Wang et al., 2018; Yue et al., 2018). Compared with the original yeast form, the filamentation-competent strain differentially expressed genes modulating basic cellular functions, such as transcriptional regulation, cell cycle control, and cell component organization. Compared to yeast cells, filamentation-competent cells expressed homologs of the *C. albicans* hyphal regulators and genes associated with iron metabolism more strongly, but filamentous growth inhibitors were downregulated. Interestingly, *EFG1*, a transcription factor important for hyphal growth in *C. albicans*, was also downregulated in filamentation-competent cells in *C. auris*, reinforcing the idea of an Efg1-independent filamentation in *C. auris* (Fig. 3E). Likewise, genes encoding histone proteins or histone modifiers were present at lower levels in filamentous cells, implying that epigenetic regulation may be involved in filamentation competency (Yue et al., 2018). However, 13 clinical isolates from systemic infection in human patients were unable to grow as filaments at low temperatures (Bravo Ruiz et al., 2020), therefore the observed phenotype by Yue and collaborators seems to be strain-specific and the molecular determinants which drive this different phenotype need to be identified.

There currently is not enough data available on *C. auris* infections to fully appreciate the potential role of morphogenetic switching during pathogenesis. However, during infection, cells may encounter various conditions generated by the host or by other microorganisms cohabiting a given niche, that lead to cell cycle arrest. Switching to filamentous growth

might be advantageous in certain situations. No obvious differences between the filamentation-competent strain and the original yeast form were observed in a mouse bloodstream infection model, although fungal burdens of filamentous cells in the brain and lung compared to typical yeast cells were significantly higher (Yue et al., 2018). Furthermore, in a mouse skin colonization model, typical yeast cells were found to predominately grow on the skin surface, whereas filamentous cells often invaded the epidermal layer (Yue et al., 2018). In any case, the varying capacity of different strains to form filaments suggests that independent clinical isolates could use morphogenetic switching during different phases of pathogenesis, and further investigation is necessary to elucidate the role of filamentation during pathogenesis.

4.3. Stress response

C. auris has an exceptional capacity to colonize and persist on surfaces, being more resistant than other *Candida* species to disinfection procedures (Chaabane et al., 2019; Kean et al., 2018b). The unique environmental stress resistance profile of *C. auris* could explain this unusual trait (summarized in Table 2).

Compared to other *Candida* species, *C. auris* can grow at higher temperatures (Ben-Ami et al., 2017; Bravo Ruiz et al., 2019; Kumar et al., 2015; Satoh et al., 2009; Wang et al., 2018), and is more resistant to oxidative stress by hydrogen peroxide, cationic stress, and cell wall stress (Table 2) (Day et al., 2018; Heaney et al., 2020; Pathirana et al., 2018; Satoh et al., 2009; Wang et al., 2018; Welsh et al., 2019). The latter reinforces the notion that *C. auris* differs from *C. albicans* in its cell wall composition. However, *C. auris* is more sensitive to oxidative stress by superoxide and peroxide (Table 2) (Day et al., 2018; Pathirana et al., 2018) and high temperatures seem to sensitize *C. auris* to copper sulfate (Wang et al., 2018). Also, *C. auris* shows a preference for alkaline over acidic environments (Table 2) (Day et al., 2018; Heaney et al., 2020) which, in combination with favouring respiration over fermentation (Zamith-Miranda et al., 2019) and the inability to grow in anaerobic conditions (Day et al., 2018), indicates that *C. auris* is not well-equipped to live in the human gut. Many of these characteristics seem to be strain-specific (Table 2).The Hog1 pathway is among the most-conserved stress-sensing and signalling mechanisms across fungi (Nikolaou et al., 2009). As in *C. albicans*, the *C. auris hog1*Δ mutant strain showed differences in stress resistance, cell morphology, cell aggregation, and virulence with respect to the parental strain (Alonso-Monge et al., 1999; Day et al., 2018). Regarding stress tolerance, *hog1* was more sensitive to cationic, oxidative, osmotic stresses and to highly acidic environments, as well as detergent (sodium dodecyl sulfate, SDS) stress (Day et al., 2018). Indeed, in the wild-type parental strain Hog1 was activated by phosphorylation in response to cationic, oxidative, and detergent stress (Day et al., 2018). The *hog1* mutant grew as elongated yeast cells which clump together, this would indicate a role for Hog1 in cell wall organization and made the *hog1* mutant resistant to cell wall stress. All, or some, of these *hog1* mutant phenotypes likely cause its reduced virulence (Day et al., 2018).

4.4. Biofilm formation and aggregation

Overall, the capability of *C. auris* to form biofilms seems similar to those of other *Candida* species with a tendency to produce less-developed biofilms than *C. albicans in vitro* (Dominguez et al., 2019; Kean et al., 2018b; Larkin et al., 2017; Romera et al., 2019; Sherry et al., 2017; Singh et al., 2019; Srivastava and Ahmad, 2020). *C. auris* also demonstrates a high capacity for biofilm formation in synthetic sweat medium designed to mimic axillary skin conditions (Horton et al., 2020). Colonization models (rat-catheter and porcine skin) indicate that *in vivo C. auris* actually form more consistent biofilms with a higher biomass than *C. albicans* (Dominguez et al., 2019; Horton et al., 2020). Differences in the extent of biofilms formed by *C. auris* isolates suggest that strain- or clade-specific factors influence this trait. *C. albicans* biofilms are formed by a mix of yeast cells, hyphae and pseudohyphae, whereas *C. auris* biofilms solely consist of yeast cells and present a smaller amount of extracellular matrix (Larkin et al., 2017; Romera et al., 2019). The biofilm matrix of *C. auris* is rich in mannan-glucan polysaccharides (Table 3) (Dominguez et al., 2019). As biofilms mature, they become thicker and the metabolic activity decreases (Table 3) (Srivastava and Ahmad, 2020). Cells in biofilms differ in their

transcriptional programme from planktonic cells (Table 3). In biofilms, adhesins, glycosylphosphatidylinositol (GPI)-anchored cell wall factors, and proteins involved in biofilm formation in *C. albicans* are upregulated (Table 3) (Kean et al., 2018a; Short et al., 2019; Wasi et al., 2019). The GPI-anchored factors are also expanded in *C. auris*, with unique genes only found in the *C. auris* genome (Muñoz et al., 2018), pointing to potentially interesting and specialized roles of biofilm formation in antifungal drug resistance (Fig. 1C). During biofilm formation also efflux pumps from the ABC and MFS transporter families are more highly expressed (Table 3) (Kean et al., 2018a; Short et al., 2019). The high expression of efflux pumps in biofilms might explain the increased antifungal resistance of *C. auris* biofilms (Fig. 2A) (see also section 3.4.). Strikingly, clade II strains are weaker biofilm formers (Oh et al., 2011), in line with their genomes lacking adhesins of the Hyr/Iff family (Muñoz et al., 2019).

A curious phenotype of some *C. auris* isolates is their propensity to form cellular aggregates under certain growth conditions; this feature is apparently quite common in clade III strains (Borman et al., 2016; Brown et al., 2020). It is still somewhat unclear whether aggregating strains are better at forming biofilms than non-aggregating *C. auris* isolates, although a trend seems to point in that direction (Brown et al., 2020; Singh et al., 2019). This tendency of aggregating strains being more efficient at forming biofilms would be supported by the finding, that adhesins (including *ALS* genes) and various cell wall components are upregulated in biofilms of an aggregating clade III isolate compared to a non-aggregating clade I strain; the same aggregating isolate also caused a greater pro-inflammatory response in an artificial wound model (Brown et al., 2020). However, it is a bit counterintuitive that non-aggregating isolates are more virulent in a *Galleria mellonella* and a neutropenic mouse infection model, although in *Galleria* this is dependent on the cell number in the inoculum (Borman et al., 2016; Forgács et al., 2020; Sherry et al., 2017). The current analysis of this cellular behaviour potentially is compounded by strain- and clade-specific effects other than aggregation itself. Therefore, careful genetic dissection of the aggregation phenotype is required to elucidate the mechanism(s) underpinning it, and to understand the specific contribution of aggregation to biofilm formation and virulence.

5. VIRULENCE AND PATHOGENICITY

As a newly emerged pathogen with barely a decade of research aiming at understanding its biological traits, there remain numerous unanswered questions about the virulence and pathogenicity of, as well as the immunological host reaction to *C. auris*. We only provide a short summary of the virulence traits of *C. auris* here, because this has recently been reviewed in detail (Kean et al., 2020).

C. auris shares virulence traits with other pathogenic *Candida* species, including oligopeptide transporters (OPTs), mannosyl transferases, siderophore-based iron transporters, secreted proteases and lipases, and determinants of biofilm formation (Chatterjee et al., 2015; Muñoz et al., 2018). All of these play a multitude of roles in colonization, invasion and micronutrient acquisition.

Pathogenicity of *C. auris* has been tested in different invertebrate and vertebrate infection models. As with other traits, *C. auris* strain-dependent characteristics likely play a role in defining virulence and pathogenicity. Generally, *C. auris* has been found to be of similar virulence as other *Candida* species. In the invertebrate *Galleria mellonella* infection model, *C. auris* was less virulent than *C. albicans*, and non-aggregating strains were more virulent than aggregating ones, as mentioned in section 4.4. (Borman et al., 2016; Sherry et al., 2017; Wang et al., 2018). In *Drosophila melanogaster*, *C. auris* was more virulent than *C. albicans*, and no difference in virulence was observed between aggregative or non-aggregative isolates (Wurster et al., 2019).

The vertebrate zebrafish system has been used to study the response of neutrophils to *C. auris* infection (Johnson et al., 2018). *C. auris* evades neutrophil detection, and neutrophils failed to form extracellular traps in the presence of *C. auris*, which normally ensnare microbial pathogens including *C. albicans* (Johnson et al., 2018). Studies with wild-type and neutropenic murine infection models also revealed strain-specific pathogenicity of *C. auris* (Ben-Ami et

al., 2017; Fakhim et al., 2018; Forgács et al., 2020; Torres et al., 2020; Wang et al., 2018; Xin et al., 2019). Interestingly, *C. auris* seems to accumulate preferentially in the kidneys, where they form cellular aggregates (Ben-Ami et al., 2017; Fakhim et al., 2018; Yue et al., 2018). However, a high fungal burden can also be observed in the heart and the brain as a result of *C. auris* disseminated disease (Torres et al., 2020). Notably, *C. auris* fails to induce a strong inflammatory response in human peripheral blood mononuclear cells (PBMCs) as other *Candida* species do (Navarro-Arias et al., 2019). The ability of human monocyte-derived macrophages to phagocytose *C. auris* is also less efficient compared to interactions with other *Candida* species (Navarro-Arias et al., 2019). Human neutrophils from healthy donors fail to attack and kill *C. auris* altogether (Johnson et al., 2018).

Overall, this paints a rather worrisome picture of *C. auris* being as pathogenic as *C. albicans* and probably more so than other *Candida* species, and at the same time evading important protection measures of immune systems.

6. GENETIC MANIPULATION

One key advance to obtain detailed insight into *C. auris* biology is to genetically manipulate it. This will allow the scientific community to elucidate which cellular processes and molecular mechanisms underpin its (multi)drug resistance, virulence, and pathogenicity.

C. auris can be transformed by electroporation or by making it chemically competent for the uptake of genome alteration cassettes (Bravo Ruiz et al., 2020; Defosse et al., 2018; Grahl et al., 2017; Mayr et al., 2020). Currently, there are three dominant drug resistance cassettes available to introduce and select for the desired constructs, these cassettes confer resistance to the antibiotics nourseothricin, hygromycin B, and mycophenolic acid (Defosse et al., 2018).

A CRISPR(clustered regularly interspaced short palindromic repeats)-Cas9 system was developed for non-*albicans Candida* species, which relies on the delivery of the genome editing tool as a ribonucleotide protein (Grahl et al., 2017). This system was successfully applied in *C. auris* to generate a tetracycline-repressible allele of *HSP90*, delete *CDR1* and *MDR1*, and introduce point mutations into *TAC1B* (Kim et al., 2019; Rybak et al., 2020, 2019).

Additionally, we and others have started to generate deletion mutants by introducing dominant drug resistance markers flanked by sequence homologies to the target locus (Bravo Ruiz et al., 2020; Day et al., 2018; Mayr et al., 2020). Apparently, *C. auris* is less efficient at homology-directed targeting of transformed DNA than the model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, and requires homologous flanking regions in excess of 1 kb for correct targeting of usable efficiency in most instances (Bähler et al., 1998; Baudin et al., 1993; Bravo Ruiz et al., 2020; Mayr et al., 2020). Short homologous sequences (100 bps) were sufficient to delete *HOG1* (Day et al., 2018), although this might have been an exceptional locus-dependent effect (our own observations; J. Quinn, personal communication).

Inactivation of non-homologous end joining has been a successful strategy to improve gene targeting in fungi with low homology-directed repair efficiency (e.g. Arras and Fraser, 2016; Cen et al., 2015; Liu et al., 2019; Tani et al., 2013; Villalba et al., 2008). We also pursued this strategy by deleting the *YKU80* ortholog of *C. auris* (our unpublished data); Yku80 is a central factor in non-homologous end joining. Unfortunately, the *C. auris yku80*Δ mutant displayed a strong growth defect, and did not improve targeting efficiency during genetic transformation (our unpublished observations). Also, the chemical inhibition of non-homologous end joining (Arras and Fraser, 2016) was unsuccessful. Low concentrations of the inhibitors, 2-chloro-10-(3-dimethylaminopropyl)phenothiazine hydrochloride (chlorpromazine) and N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide (W7), did not make a difference compared to the untreated control, whereas high concentrations would block cell growth (our unpublished observations).

7. OUTLOOK

C. auris began its "career" as a human pathogen not too long ago, and the last few years saw a tremendous effort by multiple research groups to make inroads

into understanding its epidemiology, genome evolution, virulence, and pathogenicity. Although there is a wide range of biochemical assays and cell biological methods available which can be readily applied to clinical isolates of *C. auris* in the laboratory, truly mechanistic insight can only be gained by making *C. auris* genetically tractable (see section 6.). It is thus of the utmost importance that molecular tools for genetic manipulation are developed. This is doubly difficult, firstly, because *C. auris* is a comparatively new organism to research, and, secondly, because it belongs to the CTG clade which means that tools developed for the model yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* are not usable. Over the last years, a lot of progress has been made in building such CTG clade-compatible tools, particularly for *C. albicans* (Gao et al., 2018; Legrand et al., 2018; Mielich et al., 2018; Vyas et al., 2015). However, in our experience these molecular genetic tools need adaptations to make them functional in *C. auris*. Another major issue is the rather random usage of *C. auris* strains. Moving forward with more mechanistic studies it will become necessary to restrict experiments to a single strain or a small set of isolates to enable straightforward inter-laboratory comparisons; also finances and the amount of laboratory bench labour become limiting when comparing many isolates. Ideal candidates for such *C. auris* lab strains should have the following features: (I) representative for the species or at least for their clade, (II) available fully annotated high-quality genome sequence, (III) not multidrug-resistant, and (IV) not refractory to genetic manipulation. For example, the type-strain CBS10913T (clade II) and the clade I strain VPCI479/P/13 would potentially be reasonable candidates fulfilling at least some of these criteria (Satoh et al., 2009; Sharma et al., 2015; Wasi et al., 2019). CBS10913T would be a representative for clade II strains, which generally behave very differently compared to isolates from other clades. VPCI479/P/13 is largely antifungal-sensitive, which allows *in vitro* evolution for resistant isolates. Genetic manipulation of VPCI479/P/13 has been successful multiple times in our hands (Bravo Ruiz et al., 2020). However, other strains with published and fully-assembled WGS, such as B8441 (clade I) and B11221 (clade III) (Muñoz et al., 2018), are viable alternatives. B8441 and B11221 are also genetically tractable (Mayr et al., 2020; Rybak et al., 2020). There is also a great resource available from the Centre of Disease Control and Prevention (CDC, Atlanta, GA, USA), where a set of strains covering the main clades will be provided upon request (Lutgring et al., 2017). Nevertheless, studying particular clinical isolates to understand the underlying causes of unique traits will remain a worthwhile objective.

These are exciting times, albeit for disconcerting reasons, to be a mycologist. Understanding the biology and life cycle of *C. auris* will keep the scientific community busy for decades to come.

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Table 1. Metabolism and cell wall characteristics of *C. auris*

Metabolic characteristics	**References**
Use of the respiration over fermentation	Satoh et al., 2009; Zamith-Miranda et al., 2019
Glycolysis/gluconeogenesis, ribosomes, and phagosomes downregulated[a]	Zamith-Miranda et al., 2019
Tricarboxylic acid cycle and amino acid metabolism upregulated[a]	Zamith-Miranda et al., 2019
Lipid metabolism enhanced, especially glycerophospholipids and lysophospholipids[a]	Semreen et al., 2019; Zamith-Miranda et al., 2019

Cell-wall related traits	**Reference**
Agglutinin-like sequence (ALS) genes underrepresented[a]	Chatterjee et al., 2015; Muñoz et al., 2018
Protein Kinase C (Pkc1) undetected	Zamith-Miranda et al., 2019
Chitin remodelling enzymes, 1,3-β-D-glucan synthase, and mannoprotein-remodelling enzymes underrepresented[a]	Zamith-Miranda et al., 2019
1,3-β-glucosidase Xog1 and the α-1,2-mannosyltransferase Mnn21 overrepresented[a]	Zamith-Miranda et al., 2019

[a]compared to *C. albicans*

Table 2. Stress response in *C. auris*

Condition	Details[a]	In comparison to *C. albicans*	References
High temperature	Growth up to 42°C	more resistant	Ben-Ami et al., 2017; Bravo Ruiz et al., 2019; Heaney et al., 2020; Kumar et al., 2015; Satoh et al., 2009; Wang et al., 2018
Oxidative stress			
Superoxide (menadione)	300 µM	less resistant	Day et al., 2018
Peroxide (tert-butyl hydroperoxide)	1.25 mM	less resistant	Day et al., 2018; Heaney et al., 2020
Hydrogen peroxide	8 mM	more resistant	Day et al., 2018; Heaney et al., 2020; Pathirana et al., 2018
Cell wall stress			
Congo red	300 µg/ml	generally more resistant[b]	Day et al., 2018; Heaney et al., 2020
Calcofluor	200 µg/ml	generally more resistant[b]	Day et al., 2018; Heaney et al., 2020
Cationic stress			
Sodium chloride	1.75 M	generally more resistant[b]	Day et al., 2018; Heaney et al., 2020; Satoh et al., 2009; Wang et al., 2018
Calcium chloride	1 M	more resistant	Day et al., 2018
Histatin 5	7.5 µM	generally less resistant[b]	Pathirana et al., 2018
pH stress			
Alkaline pH	pH13	more resistant	Day et al., 2018; Heaney et al., 2020
Acidic pH	pH2	less resistant	Day et al., 2018; Heaney et al., 2020

[a]Most extreme condition/concentration tested is indicated
[b]strain-specific response in *C. auris*

Table 3. Biofilm formation in *C. auris*

Characteristics in mature biofilms	References
Rich in mannan-glucan polysaccharides	Dominguez et al., 2019
Thicker when mature	Srivastava and Ahmad, 2020
Metabolic activity decreases	Srivastava and Ahmad, 2020
Efflux pump activity increases	Kean et al., 2018a; Srivastava and Ahmad, 2020
Antifungal resistance increases	Dominguez et al., 2019; Kean et al., 2018a; Romera et al., 2019; Singh et al., 2019; Srivastava and Ahmad, 2020

Upregulated in biofilms	Reference
Adhesins (e.g. Hyr3 and Als5)	Kean et al., 2018a; Short et al., 2019
Glycosylphosphatidylinositol (GPI)-anchored cell wall factors (e.g. Iff4, Csa1, Pga26, Pga52)	Kean et al., 2018a
Candidapepsin-5 (Sap5)	Kean et al., 2018a
ABC and MFS transporter families (e.g. Rdc3, Snq2, Cdr1, Mdr1, Mdr2 and YhdE)	Kean et al., 2018a; Short et al., 2019
Biofilm formation proteins (e.g. Kre6 and Exg)	Kean et al., 2018a; Short et al., 2019

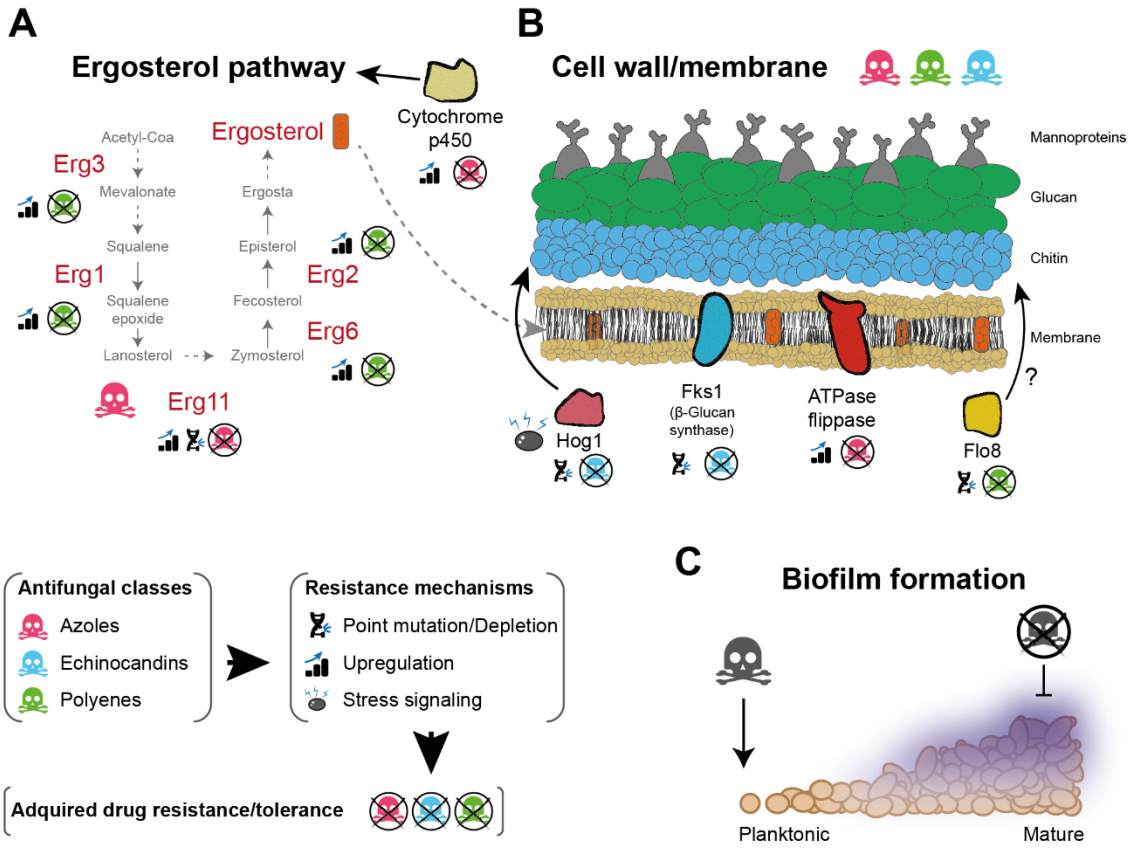

Figure 1. Schematic representations of mechanisms conferring antifungal resistance or tolerance in *C. auris*. **(A)** Ergosterol pathway, **(B)** cell wall and membrane, and **(C)** biofilm formation. See main text (section 3.) for details.

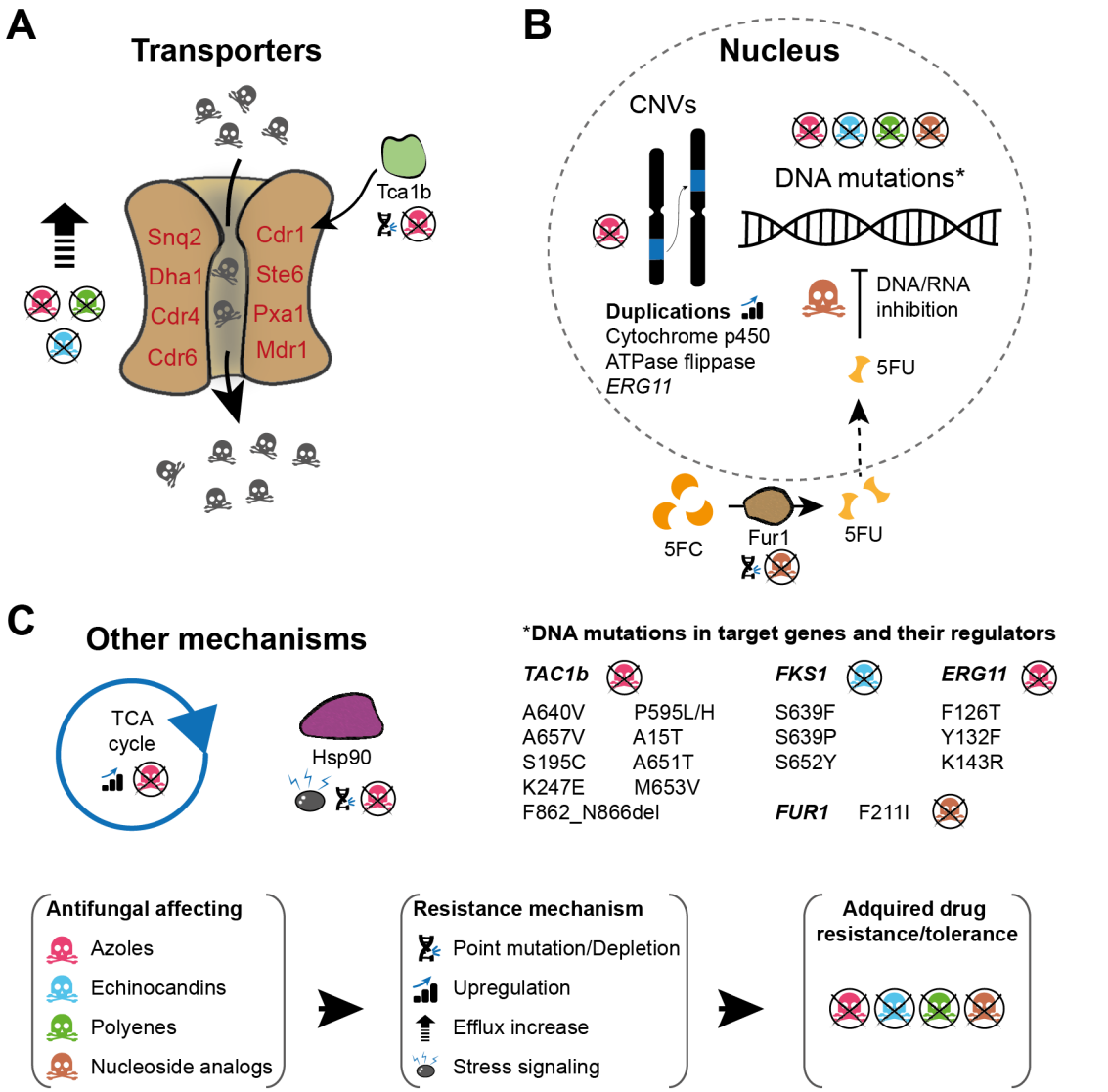

Figure 2. Schematic representations of mechanisms conferring antifungal resistance or tolerance in *C. auris*. **(A)** Transporters, **(B)** mutation and gene regulation, and **(C)** other mechanisms. CNVs = Copy number variations, 5FC = 5-Flucytosine, 5FU = 5-fluoro-uridine-5'-monophosphate, TCA = Tricarboxylic acid. See main text (section 3.) for details.

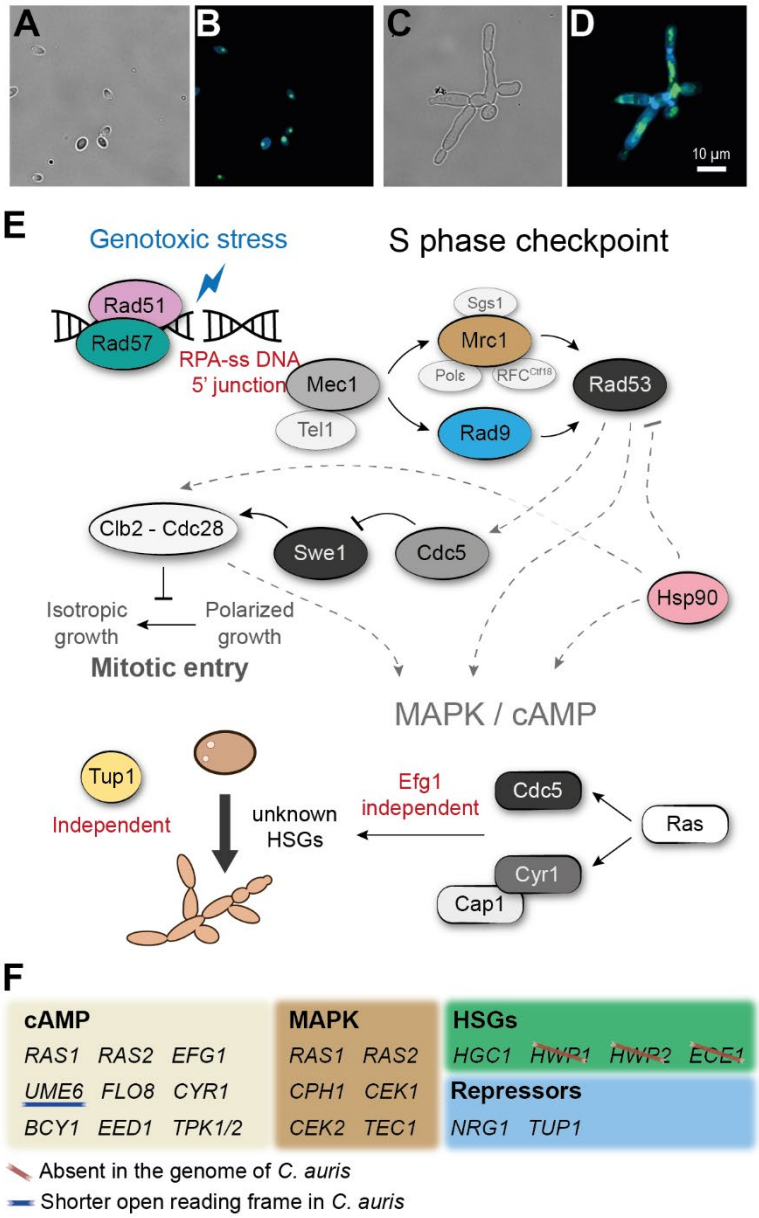

Figure 3. Morphogenetic switching in *C. auris*, see main text (section 4.2.) for details. **(A-D)** Microscopy images of strain B11109. **(A, B)** Yeast cells grown on standard yeast-peptone-dextrose medium (YPD), and **(C, D)** filaments grown on YPD containing 100 mM of hydroxyurea. A bright-field images (A, C) and merged fluorescent image (chitin stained by calcofluor white [blue] and DNA stained by SYBR green I [green]) (B, D) are shown. **(E)** Schematic representation of the likely regulation of the S phase checkpoint-related pseudohyphal growth of *C. auris*. Factors tested in *C. auris* are in full colour, pathways known in yeasts but still elusive in *C. auris*, are shown in grey shades. Potential downstream pathways related to filamentous growth are connected by dotted lines. **(F)** Important hyphae-related genes in *Candida albicans*. Genes absent or modified in the genome of *C. auris* are indicated; HSGs = hyphal-specific genes, MAPK = mitogen-activated protein kinase pathway, cAMP = cyclic AMP pathway.