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Chromatin-Mediated *Candida albicans* Virulence

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1. Introduction

Single cell eukaryotic pathogens must adapt to various environmental stimuli to successfully propagate in the mammalian host, evade attacks from its immune system and ensure future transmission to the next host. These processes often require dramatic morphological changes, expression of virulence-associated genes, and repair of DNA damaged by the hostile host environment. Many of these events involve chromatin alterations that are crucial for virulence. There are several thorough and recent reviews on chromatin modification of eukaryotic single cell pathogens such as *Plasmodium falciparum* [1,2], *Trypanosoma brucei* [1,3] and *Toxoplasma gondii* [4]. This review will focus on chromatin-regulated virulence factors in the fungal pathogen, *Candida albicans*, and in other *Candida* species.

C. albicans is the most prevalent human fungal pathogen [5]. In healthy individuals, *C. albicans* adheres to and colonizes the mucosal lining of the human gut as a commensal organism. If the ecological balance is disturbed through the use of antibiotics or severe immuno-deficiency, *C. albicans* can overgrow and cause a benign but irksome mucosal infection such as vaginitis, diaper rash, or oral thrush. However, *Candida* species can also cause more serious infections. As ubiquitous opportunistic pathogens, *Candida* species are the fourth leading cause of hospital-acquired infections [5], often resulting from implanted medical devices or organ transplants [6]. Fungal cells can spread into the circulatory system, leading to systemic candidiasis (candidemia). Although very rare, the mortality rate for systemic candidiasis is as high as 49%, despite anti-fungal treatment [7]. Furthermore, resistance to the currently available anti-fungal medications is rising [8,9]. This situation makes fungal infections a significant public health concern, and thus novel anti-fungal drug targets are of great interest. Most anti-*Candida* drugs target cell membrane or cell wall synthesis, so identification of alternative physiological pathways that affect pathogenicity is an important goal for biomedical research.

2. Chromatin, the platform for genome repair, transcription and maintenance

In recent years, chromatin proteins required for fungal virulence have been recognized as promising antifungal targets. Chromatin is the nucleoprotein complex that houses the genetic information in eukaryotes. The repeating chromatin building block is the

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nucleosome, 146 bp of DNA wrapped around a histone protein octamer comprised of two histone H2A-H2B dimers and one histone (H3-H4)₂ tetramer [10]. Histones have a primary role in packaging large genomes into small nuclei, and are generally inhibitory to biological processes because of their extensive contacts with the DNA. Several general molecular mechanisms are used to counter this inhibition and generate access to DNA in chromatin. First, histones undergo a wide variety of covalent modifications to modulate their biological activity during processes including nucleosome assembly, transcription, formation of silenced heterochromatin, and DNA repair (reviewed in [11], [12–14]). Post-translational modifications (PTMs) on histones include acetylation, methylation, phosphorylation, ubiquitylation and sumoylation. These modifications often provide binding sites for trans-acting proteins (reviewed in [15]). In the case of histone H4-K16 acetylation, the modification also directly alters the higher-order folding properties of chromatin [16]. Second, nucleosomes are moved along the DNA fiber, and in some cases evicted, by ATPase “remodeling” enzymes, which thereby regulate DNA accessibility (reviewed in [17]). Together, increased DNA accessibility and protein recruitment facilitate all biological processes related to chromatin, including DNA repair mechanisms (reviewed in [12,18]). Chromatin also contributes directly to genome stability as the platform for formation of kinetochores, the chromosomal structures that attach to microtubules for segregation of chromosomes during mitosis (reviewed in [19]). Therefore, the multiple contributions of chromatin to genome stability suggest that it may contain valuable targets for anti-fungal agents.

3. Evading and repairing DNA damage are crucial for *Candida* pathogenicity

In collaboration with the receptor-mediated immune system, the innate immune system plays an integral part in clearing mammals of pathogens. Phagocytes, such as macrophages and neutrophils, engulf antibody- or complement-coated microbes into a membrane-bound compartment called a phagosome and eventually subject them to various toxic molecules [20,21]. These toxins include reactive oxygen species (ROS) such as hydroxyl radicals ($\bullet\text{OH}$), superoxide anions (O_2^-) and hydrogen peroxide (H_2O_2) that are generated through oxidative burst by the membrane-bound NADPH-oxidase enzyme (reviewed in [22]). *In vitro*, generation of ROS by phagocytes promotes *C. albicans* killing [23–29]. *In vivo*, mice that are deficient in the NADPH-oxidase enzyme as a model for chronic granulomatous disease, an illness that leads to elevated susceptibility to mycosal infections, are more susceptible to pulmonary and systemic candidiasis [30,31]. Accordingly, *C. albicans* mutant cells that lack the superoxide dismutase or catalase enzymes to neutralize these toxic molecules (*sod5*^{-/-} and *cta1*^{-/-} mutants, respectively) are less resistant to macrophages and less pathogenic in murine systemic candidiasis [28,32,33]. Together, these types of investigations established that proteins required for fungi to withstand ROS-mediated DNA damage are likely to be important for pathogenesis.

Subsequent work has shown that multiple DNA repair pathways are crucial for *C. albicans* pathogenesis. *rad52*^{-/-} mutants, defective in homologous recombination, and *lig4*^{-/-} mutants, deficient in non-homologous end joining, are avirulent in murine systemic candidiasis [34]. Further, multiple DNA repair genes along with oxidative stress genes are up-regulated when *C. albicans* comes into contact with macrophages *in vitro* [35]. However, there are some counterexamples to this trend; *C. albicans* mutants that lack base excision repair (BER) proteins (*apn1*^{-/-}, *ntg1*^{-/-} and *ogg1*^{-/-}) are not hypersensitive to H_2O_2 or exposure to macrophages [36]. One interpretation for the latter results is that overlapping functionality with other proteins results in minimal phenotypes in cells lacking a single BER protein [36]. In any case, these results emphasize the need to experimentally confirm the role of genes in damage sensitivity and fungal pathogenesis.

To evade killing in the phagosome, *C. albicans* changes morphology from budded cells to hyphal or pseudohyphal filaments, thereby rupturing out of the phagocyte (reviewed in [37], [38] and [39]). Various environmental stimuli can induce filamentation *in vitro*, including genotoxic stress caused by DNA damage or DNA replication blocks [40–42]. Filamentation from replication stress or DNA damage depends on the checkpoint proteins Rad53 and Rad9 [41], linking the ability to escape phagocytes to signaling pathways that sense and respond to DNA damage [41]. In sum, the mechanisms for sensing DNA damage, escaping the phagosome, and repairing DNA damage generated by host cell ROS are interconnected in *C. albicans* and are imperative for successful pathogenesis.

4. Aspects of chromatin that promote pathogenicity through genome stability

4.1. Rtt109 and histone H3K56 acetylation

Histone post-translation modifications are frequently studied as marks associated with gene expression. However, their contributions to DNA damage checkpoint signaling and DNA repair are also substantial [13,14]. A notable example is acetylation of histone H3 lysine 56 (H3K56ac), synthesized by the histone acetyltransferase termed Rtt109 (Repressor of Ty-1 Transposition 109) [43–45]. In fungi, acetylated lysine 56 quantitatively marks newly synthesized H3 histones [46–48] and is therefore very abundant in diverse yeast species [49–51]. Genome-scale views across the budding yeast cell cycle detects H3K56 acetylation in waves following DNA replication forks and also at sites of replication-independent histone exchange, demonstrating that this is a short-lived mark of new histone incorporation [46,48]. The rapid decay of this mark depends on sirtuin (Sir2 family) NAD⁺-dependent histone deacetylase (HDAC) enzymes, Hst3 and Hst4 in *S. cerevisiae* [47,50]. Point mutation of the H3 lysine 56 residue into an amino acid that cannot be acetylated or that mimics constitutive acetylation leads to hypersensitivity to genotoxic stress and DNA damaging agents, including endogenous DNA damage [46,49,52]. Accordingly, the enzymes that synthesize and hydrolyze H3K56ac are required for genotoxic stress resistance in *S. cerevisiae*, *Schizosaccharomyces pombe* and *C. albicans* [29,43,44,51,53]. In *S. cerevisiae*, deletion of *HST3* and *HST4* also leads to high rates of chromosome loss [50], demonstrating that it is important for H3K56 acetylation on chromatin to be transient. How H3K56 acetylation contributes to fungal genome stability is not fully understood. However, acetylation of H3K56 promotes histone deposition onto DNA at the replication fork during replication, at double strand breaks after DNA repair, and at promoters during transcriptional induction [54–56].

Rtt109 could not initially be identified as a HAT based on primary sequence homology to other HAT enzymes. It was implicated in H3K56 acetylation via an immunoblotting survey of the yeast genome deletion collection [57]. Rtt109 has several distinguishing properties; it has no close sequence homologues outside of the fungal kingdom and requires one of two histone chaperones, Vps75 or Asf1, to stimulate its catalytic activity [58–61]. Only with detailed bioinformatic analysis [62], and most clearly with high-resolution crystal structures [63–65], did it become clear that Rtt109 is a highly diverged relative of mammalian HAT p300, sharing a similar three-dimensional fold and a very small number of conserved residues. However, the chemistry of their active sites [66] and the catalytic mechanisms of Rtt109 and p300 are distinct [60,61,63,67,68]. Furthermore, in contrast to its role as a mark on all new H3 molecules in fungi, H3K56ac is much less abundant in higher eukaryotes [69,70], and the biological function for this mark in non-fungal organisms remains controversial. Rtt109 has favorable characteristics for a potential therapeutic target because it is dissimilar to mammalian enzymes, is required for fungi to resist genotoxic stress, is

important to aspects of fungal pathogenesis and acts in a biological process that appears to be specific to fungi.

This potential was directly tested. Our laboratory was first to demonstrate that loss of H3K56 acetylation via deletion of *RTT109* in *Candida albicans* strikingly reduces mortality in mice subjected to systemic candidiasis [29]. Furthermore, *rtt109*^{-/-} cells are incapable of efficiently colonizing the kidneys during acute (24 hours) or prolonged (20 days) infections [29,53]. Notably, we demonstrated that the poor pathogenicity of *rtt109*^{-/-} cells correlates with an inability to withstand phagocyte-generated ROS [29]. We showed that *rtt109*^{-/-} cells are significantly more susceptible to macrophages *in vitro* than wild-type cells [29]. However, preventing macrophage oxidative burst through inhibition of NADPH oxidase renders *rtt109*^{-/-} mutants equally resistant to macrophages as wild-type cells [29]. In addition, we observed that *C. albicans rtt109*^{-/-} cells are constitutively filamentous, have elevated levels of H2A serine 129 phosphorylation (γ H2A), and display constitutive induction of DNA repair genes [29], indicating spontaneous DNA damage. Constitutive filamentation is also observed in *rad52*^{-/-} cells [71] and upon treatment of wild-type cells with DNA damaging agents [41,42], indicating that this morphological phenotype serves as a sensitive indicator of endogenous genotoxic stress. These data demonstrate that the genotoxic stress protection provided by Rtt109 and H3K56ac is important for *C. albicans* to survive phagocytosis by macrophages, providing a mechanistic explanation for the pathogenesis data.

In *C. albicans*, *HST3* is an essential gene and encodes the sole NAD⁺-dependent sirtuin HDAC responsible for hydrolyzing H3K56ac [53]. Deletion of both *RTT109* alleles restores viability to cells lacking Hst3, indicating that hyperaccumulation of H3K56ac is indeed the reason for the lethality of *hst3*^{-/-} cells [53]. Further, heterozygous *hst3*^{+/-} mutants are hypersensitive to genotoxic stress and conditional *hst3*^{-/-} mutants are less well able to colonize organs such as the kidneys during systemic murine candidiasis [53]. Notably, a variety of other human pathogenic fungi including several other *Candida* species and *Aspergillus fumigatus* are sensitive to exposure to nicotinamide (NAM), an inhibitor of NAD⁺-dependent HDACs [53]. Rtt109 affects multiple processes in pathogenic fungi, because it is also required for epigenetically-regulated morphological “switching” events in *Candida albicans* [72], a process implicated in pathogenesis (see section 7). Together, these data suggest that metabolism of H3K56ac is a particularly good target for anti-fungal drug development.

4.2. Genome plasticity and the spindle assembly checkpoint

A major function of chromatin modification and remodeling is to ensure genome stability, that is, the proper inheritance of genomic information in macro-structural units called chromosomes. *C. albicans*, however, is notorious for generating aneuploidy in cultured strains [73,74]. In fact, genome instability is beneficial to *C. albicans* in some cases. For example, *C. albicans* readily loses a copy of chromosome 5 in order to survive on alternative carbon sources such as sorbose [75,76]. Also, the generation and stable perpetuation of two extra arms of Chr5L flanked by a centromere (5 i(5L)) provides resistance to fluconazole by providing additional copies of ergosterol synthesis genes [77]. Another remarkable example is that *Candida* centromeres can be moved to neighboring locations under the appropriate genetic selection [78]. These easily-selected chromosomal alterations in *C. albicans* raise the question of how this organism has evolved to allow for such extensive genomic instability.

One possibility could have been a relaxed spindle assembly checkpoint (SAC) in this organism; however, that doesn't appear to be the case. The spindle assembly checkpoint ensures that all kinetochores are properly attached to a spindle prior to anaphase, promoting accurate segregation of chromosomes to the daughter cells (reviewed in [79]). Bai et al.

hypothesized that because pathogenic fungi face extensive chromosomal damage from phagocyte ROS, the spindle assembly checkpoint pathway would be imperative for pathogenicity [40]. Indeed, homozygous deletion of *MAD2*, a protein essential for SAC function, renders *C. albicans* hypersensitive to H₂O₂ and results in zero mortality in systemic candidiasis [40]. Therefore, like the DNA damage checkpoint [41], the spindle assembly checkpoint in *C. albicans* functions to promote pathogenesis.

5. Response to stimuli via differential transcription regulation mediates *C. albicans* pathogenicity

Like many pathogens, *C. albicans* uses transcriptional responses to external stimuli to survive in different environments and to perpetuate infections. These transcriptional responses are specifically tailored to each situation. For example, the family of secreted aspartic proteinase (*SAP1-6*) genes are not expressed in normal laboratory growth conditions but are induced differentially during the course of an infection in mice [80]. For example, *SAP2* expression is stronger in yeast cells colonizing the liver than the kidneys [80]. Likewise, different sets of *C. albicans* genes are induced upon *in vitro* incubation with epithelia cells versus endothelial cells, mimicking an oral or bloodstream infection, respectively; different responses are also observed upon incubation with neutrophils versus monocytes [81,82]. In addition to tissue specificities, there are kinetic regulations as well, because *C. albicans*' transcriptional profile changes as the course of the interaction with phagocytes proceeds [35]. Data from genome-wide transcriptional profiles of *C. albicans* in relation to pathogenicity have been reviewed previously [83–85]. The extensive transcriptional networks that are activated during pathogenesis indicate that multiple chromatin regulatory events are in play; a few of the best-studied examples are outlined below.

6. Aspects of chromatin that promote pathogenicity through transcription regulation

6.1 Set1 Methyltransferase

The first account of a histone modifying enzyme affecting *C. albicans* pathogenicity was discovered in an interesting fashion. Cheng et al. performed an immunological screen for proteins expressed from a *C. albicans* genomic library that would be recognized by sera from HIV-positive patients suffering from oral candidiasis [86]. The histone methyltransferase (HMT), Set1, was one of three immunogens that were identified. Set1 is a well-conserved HMT that targets histone H3 lysine 4 [87]. A unique species-specific 208 amino acid N-terminus domain proved to be immunogenic in humans, allowing for this discovery [88].

Set1 and methylation of H3K4 are generally associated with active gene transcription [89], but are also involved in DNA damage resistance [90]. H3K4 can be mono-, di-, or tri-methylated by Set1. Although *C. albicans set1*^{-/-} mutants lose all detectable H3K4 methylation, these cells exhibit few morphological phenotypes except for hyperfilamentation when embedded in agar [88]. Strikingly, *set1*^{-/-} mutants are significantly less successful at adhering to mammalian cells *in vitro*, at colonizing organs in mice, and at causing mortality in murine systemic candidiasis [88].

6.2. Gcn5-containing complexes

Gcn5 is a well-conserved histone acetyl transferase that provides HAT activity to several transcription activator complexes such as SAGA and ADA [91]. Gcn5-mediated acetylation

also promotes genome stability by catalyzing PTMs related to nucleosome assembly [92]. In *C. albicans*, inactivation of the SAGA/ADA complexes, by eliminating core subunit Ada2, results in decreased H3K9-acetylation (a mark of active transcription for many inducible loci), increased sensitivity to oxidative stress and fluconazole, and a defective response to *in vitro* filamentation stimuli [93,94]. Ada2 occupies the promoter of many oxidative stress- and fluconazole-induced genes, and accordingly *ada2*^{-/-} cells display reduced transcription of these genes. As a result, these mutants cause less mortality in mice and *Caenorhabditis elegans* infection models [93,94].

Likewise, in the fungal pathogen *Cryptococcus neoformans*, Gcn5 is essential for pathogenicity in the murine inhalation model of cryptococcosis [95]. *Gcn5* mutant strains display increased sensitivity to H₂O₂ and elevated temperatures, but not to other stresses such as starvation or high salt [95]. Most interestingly, *gcn5* mutants are deficient in capsule formation, the mechanism by which this organism protects itself from being phagocytosed [95].

6.3 Sirtuin deacetylases in heterochromatin silencing

A major reoccurring theme in unicellular eukaryotic pathogens is the regulation of genes encoding variant surface proteins located in heterochromatin, that is, regions that are stably silenced because of a heritable chromatin structure [96]. Certain pathogens, such as *Plasmodium falciparum* and *Trypanosoma brucei*, evade the immune system by expressing only one of numerous variants of a single coat protein gene, via a mechanism termed allelic exclusion (reviewed in [1] and [97]). In both these organisms, the regulated loci are sub-telomeric and regulation is dependent on the formation of heterochromatin. In *P. falciparum*, this allelic exclusion requires the Sir2 sirtuin-family histone deacetylase and in *T. brucei*, the Rap1 telomeric DNA binding protein is required [98–101]. In *C. albicans*, loss of heterochromatin silencing through *SIR2* homozygous deletion causes unstable inheritance of colony morphology [102]. Although the sirtuin inhibitor NAM is toxic to *C. albicans* cells, this lethality likely results from effects on the Hst3 sirtuin [53], and it is not known whether there is a role for Sir2-mediated heterochromatin silencing in *C. albicans* pathogenicity.

Candida glabrata, another fungal pathogen, possesses two sub-telomeric gene clusters that code for seven Epithelia Adhesin (*EPA*) membrane proteins [103,104][103,105]. *EPA1* is the only *EPA* gene expressed in laboratory cultured cells and is essential for adhesion to mammalian cells *in vitro*. However, *epa1*^{-/-} cells are not deficient in pathogenicity [106]. Other *EPA* genes (*EPA2-6*) are silent in cultured cells but can be de-repressed upon loss of telomeric heterochromatin silencing by deleting *SIR2*, *SIR3* or *RAP1* [103,105]. Interestingly, silencing of the *EPA6* genes is determined by the amount of nicotinic acid (NA) in the environment [105]. *C. glabrata* is auxotrophic for NA and requires available NA to synthesize NAD⁺ and allow NAD⁺-dependent sirtuin deacetylases, like Sir2, to function [105]. *EPA6* is selectively expressed during the mouse model for *C. glabrata* urinary tract infection, but not during systemic candidiasis or in culture [105]. Tissue culture media provides sufficient NA, however, urine does not. This explains why *EPA6* is selectively expressed in NA depleted environment and why deletion of the *EPA* cluster leads to significant avirulence in the case of urinary tract infections. In conclusion, there is a direct link between de-repression of sirtuin-mediated silenced virulence genes with the appropriate environmental cues in *C. glabrata*.

6.4 SWI/SNF and Chromatin remodeling

Chromatin maintenance not only involves histone modification, but it also involves chromatin remodeling to change local nucleosome density. The multi-subunit complex, SWI/SNF promotes transcription of inducible genes by sliding nucleosomes on DNA,

providing access for transcriptional machinery [107,108]. The SWI/SNF chromatin remodeler complex plays a crucial role in *C. albicans* virulence. Deletion of either *SWI1* or *SNF2*, encoding DNA binding/transcription co-activator and ATPase subunits, respectively, results in complete loss of pathogenicity in murine systemic candidiasis [109]. In culture, these mutants cannot express hyphae (filamentation)-specific genes in response to stimuli such as serum or nutrient starvation, and as a result do not filament [109]. Upon exposure to serum or nutrient starvation, Snf2 is recruited to hyphae-specific genes (*HWP1*, *ALS3*, *ECE1*) by the combined action of the NuA4 HAT complex and the hyphae-specific transcription factor Efg1 [110]. Via ChIP assays, both Efg1 and NuA4 can be found at these hyphae-specific genes regardless of cellular morphology [110]. However, NuA4 requires Efg1 to reside at these promoters, indicating that Efg1 recruits NuA4 and that this in turn recruits SWI/SNF to allow transcription of hyphae-specific genes [110]. Therefore, the hyphae-specific transcription factor Efg1, which promotes pathogenicity in murine systemic candidiasis [111], harnesses both a chromatin modifying enzyme and a remodeling complex to transcribe filament-specific genes. It is likely that a multitude of chromatin proteins contribute to virulence-related gene expression by similar mechanisms.

7. Regulation of white-opaque switching

The *C. albicans* clinical isolate WO-1 is termed a “switching strain”, because it can convert between two morphologies, white round colonies made of round cells or opaque flat colonies made of elongated cells [112]. (These morphological phenotypes are distinct from those described above that arise in *sir2*^{-/-} cells [102]). Increased pathogenicity is attributed to either white cells or opaque cells depending on the site of infection, hinting that phenotypic switching could be a virulence factor to better colonize distinct environments (reviewed in [113,114]). White-opaque switching is regulated by transcriptional feedback loops that can be stable over many generations (reviewed in [113,115]). This type of stability suggested that some aspects of chromatin regulation might be involved, and therefore several investigators have directly tested whether histone modifying enzymes affect white-opaque phenotypic switching [116–118]. Notably, destabilization of the SET3C HDAC complex, by deleting the genes encoding either core subunits Set3 or Hos2, decreases white-opaque switching in WO-1 strains. These *set3*^{-/-} and *hos2*^{-/-} cells also exhibit hypersensitivity to filamentation stimuli such as low nutrient agar (Lee’s media) or elevated growth temperature (37°C) [118,119]. Despite having a Set domain common to methyltransferase enzymes, Set3 has not been observed to have histone methyltransferase activity. In contrast, the SET3C complex possesses HDAC activity through its Hos2 and NAD⁺-dependent Hst1 subunits. In *S. cerevisiae*, this complex has a primary role in repressing genes involved in sporulation, a meiotic process that does not occur in *C. albicans* [120]. Nevertheless, *set3*^{-/-} *C. albicans* are hyperfilamentous and cause significantly less mortality during murine systemic candidiasis [119]. SET3C is believed to be the homologue of human HDAC3/SMRT, and it will be of great interest to determine the virulence-associated loci that are regulated by this complex.

8. Conclusions

The contributions of chromatin to fungal pathogenesis are just beginning to be elucidated. It is evident from the studies discussed above that repair of chromatin, transcriptional activation, and manipulation of genome plasticity are all used to promote fungal pathogenicity. In addition to the examples mentioned above, there are numerous observations that support chromatin proteins as potential targets for development of new anti-*Candida* drugs. In some cases, inhibitory compounds might work best in combination with existing therapeutics. For instance, *rtt109*^{-/-} *C. albicans* cells are sensitive to some but not all four classes of clinically-approved anti-fungal drugs, suggesting that compounds that

inhibit Rtt109 would be particularly effective in combination with particular drug classes. Specifically, *rtt109*^{-/-} cells are sensitive to 5-fluorocytosine [29], a member of the pyrimidine class of drugs, which disrupt nucleotide synthesis and lead to genotoxic stress [121]. *rtt109*^{-/-} cells are also sensitive to echinocandins (caspofungin and micafungin) [53] which interfere with cell wall β -glucan synthesis. However, *rtt109*^{-/-} mutants are not sensitive to azoles (fluconazole) and polyenes (amphotericin B) [29], both of which interfere at distinct points with cell membrane integrity [122]. As another example, in other *Candida* species that infect humans (*C. parapsilosis* and *C. tropicalis*), the class I HDAC inhibitor trichostatin A (TSA) increases sensitivity to azole-based antifungal drugs, such as fluconazole and intraconazole [123]. This synergy occurs because TSA prevents up-regulation of the genes that code for the targets of azoles involved in ergosterol synthesis (*ERG1* and *ERG11*) [123]. The multiplicity of possible chromatin-related targets provides many similar combinations to test via this type of synergistic approach.

With the exception of Rtt109 and histone H3K56 acetylation, all the enzymes and modifications so far identified as having an effect on virulence are conserved in mammals (Table 1). This complicates the ability to predict aspects of chromatin regulation that could serve as targets for anti-fungal agents. Nonetheless, because so many proteins involved in multiple aspects of chromatin biology affect pathogenicity, future work will determine which ones will provide the most likely targets for novel anti-fungal agents.

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Highlights

- We review studies that address chromatin modifications and pathogenicity in *Candida albicans*.
- Resistance to DNA damage from reactive oxygen species is vital to *C. albicans* pathogenicity.
- We highlight RTT109 and H3K56 acetylation as major factors in promoting pathogenicity.
- Several other chromatin-regulating pathways are also critical for pathogenicity.
- We conclude that fungal-specific chromatin proteins should be targets for novel anti-fungal agents.

Table 1Summary of Chromatin maintenance proteins that affect pathogenicity of *C. albicans*

Gene	Human Homologue	Known Functions	<i>C. albicans</i> Mutant Phenotype
RTT109	None* *(Distant structural homologue of P300 PAT)	<ul style="list-style-type: none"> HAT (H3K56, H3K9 and H3K27) Required for resistance to DNA damage and replication fork blocks 	<ul style="list-style-type: none"> Increased sensitivity to genotoxic stress and certain antifungal agents (echinocandins and 5-FC) [29,53] Constitutive filamentation [29] Significant decrease in murine candidiasis mortality [29] Significant decrease in organ colonization in murine systemic candidiasis [29,53] Significant increase in susceptibility to macrophage killing via macrophage-derived ROS [29] Significant decrease in maintenance of opaque cell phenotype in MTLα/α cells [72]
HST3	SIRT1	<ul style="list-style-type: none"> NAD⁺-dependent HDAC (H3K56) 	<ul style="list-style-type: none"> Homozygous lethal [53] Conditional homozygous mutant causes significant decrease in organ colonization in murine systemic candidiasis [53]
MAD2	Mad2L1 and MAD2L2	<ul style="list-style-type: none"> Spindle Assembly checkpoint protein to monitor kinetochores integrity Binds CDC20-APC complex to arrest cells in metaphase 	<ul style="list-style-type: none"> Increased survival on sorbose media through loss of Chr5 [40] Increased sensitivity to H₂O₂ [40] Significant decrease in filamentation upon injection into mice peritoneal cavity [40] Significant decrease in murine candidiasis mortality [40]
SET1	SET1A	<ul style="list-style-type: none"> HMT subunit of COMPASS (H3K4) Promotes transcription 	<ul style="list-style-type: none"> Hyperfilamentous in embedded agar [88] Decreased adherence to mammalian cells [88] Increases sensitivity to H₂O₂ [88] Decreased mortality and organ colonization in murine candidiasis [88]

Gene	Human Homologue	Known Functions	<i>C. albicans</i> Mutant Phenotype
			<ul style="list-style-type: none"> Increased white-opaque switching in MTLa/a WO-1 strain [118]
ADA2	ADA2	<ul style="list-style-type: none"> Transcription co-activator sub-unit of SAGA/ADA and SILK HAT complexes Greatly enhances Gcn5 HAT activity <i>in vivo</i> 	<ul style="list-style-type: none"> Increased sensitivity and decreased transcriptional response to oxidative stress and fluconazole [93] Significant decrease in murine and <i>C. elegans</i> candidiasis mortality [93,94] Required for true hyphae formation [94]
SWI1 and SNF2	ARID1A and SMARCA4	<ul style="list-style-type: none"> Sub-units of the chromatin remodeling complex SWI/SNF involved in nucleosome repositioning Swi1—DNA binding protein involved in transcription activation Snf2—ATPase sub-unit required for integrity of SWI/SNF complex 	<ul style="list-style-type: none"> Required for true hyphae formation and expression hyphae-specific genes [109] Significant decrease in murine candidiasis mortality [109]
SET3 and HOS2	HDAC3/SMRT	<ul style="list-style-type: none"> Subunits of the SET3C HDAC complex involved in transcription repression Set3—non-enzymatic protein containing SET and PHD domain. Minimal core subunit of SET3C. Hos2—Class I HDAC. Minimal core subunit of SET3C. 	<ul style="list-style-type: none"> Hyper-responsive to filamentation stimuli [119] Decreased white-opaque switching in MTLa/a WO-1 strain [118] Significant decrease in murine candidiasis mortality (<i>set3^{-/-}</i>) [119]

Abbreviations: PAT (protein acetyltransferase), HAT (histone acetyltransferase), 5-FC (5-fluorocytosine), ROS (reactive oxygen species), HDAC (histone de-acetylase), HMT (histone methyltransferase)