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MINIREVIEW

Histone deacetylase-mediated morphological transition in *Candida albicans*

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Candida albicans **is the most common opportunistic fungal pathogen, which switches its morphology from single-cell yeast to filament through the various signaling pathways responding to diverse environmental cues. Various transcriptional factors such as Nrg1, Efg1, Brg1, Ssn6, and Tup1 are the key components of these signaling pathways. Since *C. albicans* can regulate its transcriptional gene expressions using common eukaryotic regulatory systems, its morphological transition by these signaling pathways could be linked to the epigenetic regulation by chromatin structure modifiers. Histone proteins, which are critical components of eukaryotic chromatin structure, can regulate the eukaryotic chromatin structure through their own modifications such as acetylation, methylation, phosphorylation and ubiquitylation. Recent studies revealed that various histone modifications, especially histone acetylation and deacetylation, participate in morphological transition of *C. albicans* collaborating with well-known transcription factors in the signaling pathways. Here, we review recent studies about chromatin-mediated morphological transition of *C. albicans* focusing on the interaction between transcription factors in the signaling pathways and histone deacetylases.**

Keywords: *Candida albicans*, morphological transition, histone deacetylases (HDACs), transcription factor, chromatin structure

Introduction

Candida albicans is an opportunistic pathogenic fungus, which can cause diseases according to the host's condition. The pathogenic cues for *C. albicans* are mostly living environmental shift such as compromised immune system, a change of microbial flora from the treatment of antibiotics, or a medical device implant and organ transplantation (Shepherd *et al.*, 1985; Lim *et al.*, 2012). *Candida* species are reported to be the fourth-greatest cause of nosocomial bloodstream infections, and *C. albicans* is the most prevalent species in both mucosal and systemic infections (Calderone and Fonzi, 2001). Therefore, numerous researchers have focused on understanding how *C. albicans* becomes virulent in particular environmental conditions and defining the virulence factors of *C. albicans*.

The outstanding feature of *C. albicans* is its dynamic morphogenesis which is critical for virulence. *C. albicans* can grow in various morphological forms: from unicellular budding yeast to multicellular filamentous form including pseudohypha and true hypha (Kumamoto and Vinces, 2005). Pseudohypha is developed from budding cells which are not detached but elongated, so it has branched filament with constriction, while true hypha is a stretched structure without constriction and branch (Sudbery *et al.*, 2004). The yeast form of *C. albicans* facilitates its systemic infection in the host bloodstream, whereas its hyphal form can promote its invasive infection by penetrating host tissue and escaping from host immune cells. Given that *C. albicans* is an opportunistic pathogen, the dynamic morphological transition from yeast to filament is the most noticeable determinant for virulence of *C. albicans* rather than its static morphology, yeast or filamentous form, itself (Kumamoto and Vinces, 2005). Therefore, regulation of the dynamic morphogenesis from yeast to hypha is directly linked to control virulence of *C. albicans*.

Through intensive and long-term studies to understand how the morphological transition of *C. albicans* is regulated, the molecular signaling pathways beneath its morphogenesis have been defined along with the identification of various transcription factors (Sudbery, 2011). *C. albicans* induces its hyphal development, *in vitro*, responding to the extracellular factors such as serum, high temperatures of around 37°C, neutral pH, or a low nitrogen source (Taschdjian *et al.*, 1960; Lee *et al.*, 1975; Buffo *et al.*, 1984). Two major signaling pathways for the hyphal development in *C. albicans* are mitogen-activated protein (MAP) kinase pathway and cyclic AMP/protein kinase A (cAMP/PKA) pathway, which have Cph1 and Efg1 as its final downstream transcription factor (Liu *et al.*, 1994; Stoldt *et al.*, 1997). In addition, many other transcription factors are imported into these signaling pathways as downstream factors, including Flo8, Czf1, and Tup1 (Sudbery, 2011). Tup1 is a general transcriptional repressor, which always interacts with other transcription factors, such as Rfg1, Nrg1, or Ssn6 (Braun and Johnson, 2000; Hwang

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et al., 2003). The protruding feature of hyphal induction in *C. albicans* is the transcriptional induction of hypha-specific genes responding to outside environmental signals. The major products of these hypha-specific genes are cell wall proteins of *C. albicans* filaments, such as Hwp1, Als3, Rbt1, and Rbt3, which act to adhere into host epithelial cells (Kumamoto and Vinces, 2005). Interestingly, all of the defined signaling pathways for morphological transition in *C. albicans* are partly or mostly involved in regulating transcription for these hypha-specific genes by cascading signals from environmental factors.

In all eukaryotes including *C. albicans*, both transcriptional repression and activation are directly influenced by particular chromatin structures, of which dynamic change is mainly assessed by posttranslational histone modifications (Berger, 2007), then, the transcriptional control for morphological transition of *C. albicans* is closely linked to its chromatin structure regulated by posttranslational histone modifications.

The chromatin structure consists of histone octamer, which is composed of each two copies of H2A, H2B, H3, and H4, wrapped by 146 base pair DNA in the nucleus (Kornberg and Lorch, 1999). Because of the nucleosome's structural feature that histones' tails in the nucleosome are protruded outward from the nucleosome, histone proteins are easily targeted for the post-translational modifications such as acetylation, phosphorylation, methylation, and ubiquitylation. These chemical modifications of histone residues regulate chromatin structure by collaborating with their own modifying enzymes or other chromatin modifying factors, such as nucleosome remodeling factors, and thus make transcription factors act properly (Petty and Pillus, 2013). The regulation by modifying chromatin structure is an important epigenetic regulation, and now the epigenetic regulation is crucial in explaining the transcriptional regulation, and various histone modifying enzymes are defined to be involved in transcriptional regulation.

Among these various histone modifications, the representative modification of histone for regulating the chromatin structure is acetylation. Increased acetylation of histone lysine residues could be a hallmark of active chromatin because histone acetylation weakens inter-nucleosomal interactions and the binding of the nucleosomal components and thus leads to the chromatin relaxation for transcription (Wang and Hayes, 2008). The importance of histone acetylation for the transcriptional regulation lies in its reversible property depending on various histone acetylases and histone deacetylases (HDACs) (Cress and Seto, 2000).

Since the structural feature of acetylated histone loosens the chromatin structure, HDACs generally have been thought to lead the transcriptional repression. However, now we know that the equilibrium between histone acetylation and deacetylation is a critical factor for the fine-tuning transcription and various HDACs are important for the proper transcription (Bulger, 2005; Carrozza *et al.*, 2005).

In *C. albicans*, HDACs interacting with transcription factors play important roles in various biological phenomena such as drug resistance, biofilms formation in host (Nobile *et al.*, 2014), white-opaque phenotypic switching (Klar *et al.*, 2001; Hnisz *et al.*, 2009), and morphological transition from yeast to hypha (Hnisz *et al.*, 2010, 2012; Lu *et al.*, 2012; Lee *et al.*, 2015). Among these biological phenomena affected by HDACs, morphological transition of *C. albicans* from yeast to filament is well studied in its related signaling pathways involving various transcription factors.

Recently, our group identified that one of the transcription factors regulating the morphological transition in *C. albicans*, Ssn6, regulates hyphal development by interacting with Rpd31, orthologue of *S. cerevisiae* Rpd3, which is a histone deacetylase known as a regulatory factor of transcription initiation and elongation (Lee *et al.*, 2015). Here, we introduce histone deacetylases of *C. albicans* and several reports about how various transcription factors regulate the morphological transition of *C. albicans* by interacting with one group of chromatin modifiers, HDACs.

Histone deacetylases in *C. albicans*

In *C. albicans*, various histone deacetylases have been identified and they are all orthologues of *S. cerevisiae* histone deacetylases (Fig. 1) (Klar *et al.*, 2001; Hnisz *et al.*, 2010). In *S. cerevisiae*, HDACs are classified into classical HDACs, which use zinc ions as their cofactors, and non-classical HDACs, which are NAD^+-dependent. Classical HDACs are subdivided into the Class I Rpd3 family and Class II Hda1 family by sequence homology and phylogenetic analysis. In Fig. 1, we categorized HDACs in *C. albicans* according to the criteria of *S. cerevisiae* HDACs and displayed the conserved domains of *C. albicans* HDACs by using the conserved HDAC gene sequences from Candida Genome Database (http://www.candidagenome.org/) and by searching conserved domains from NCBI Search for Conserved Domains (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Class I HDACs include Rpd31 and Rpd32, which are orthologues of *S. cerevisiae* Rpd3, and Hos1 and Hos2, whereas Hda1 and Hos3 are classified as Class II HDACs (Yang and Grégoire, 2005; Li *et al.*, 2015).

Both classes of HDACs, including Class I and II, contain HDAC catalytic domain consisting of conserved 390 amino acids, which removes acetyl group from ε-amino group of lysine residue and uses zinc ion as its cofactor because it has zinc ion binding site (Finnin *et al.*, 1999; de Ruijter *et al.*, 2003). Since Class I HDACs are usually smaller than Class II HDACs, their major parts are occupied by HDAC domain and Class I HDACs have short C-terminal tails (Fig. 1) (Yang and Seto, 2008).

Non-classical Sirtuin (Sir2-like protein) family is NAD^+-dependent HDACs and doesn't use zinc ion as a cofactor (Yang and Seto, 2008). In *C. albicans*, Hst1, Hst2 (Hnisz *et al.*, 2009), Hst3 (Wurtele *et al.*, 2010; Stevenson and Liu, 2011), and Sir2 (Pérez-Martín *et al.*, 1999) are conserved as non-classical Sirtuin family.

All classes of HDACs usually perform their various roles by forming huge complexes interacting with other proteins (Yang and Seto, 2008). Some components of these huge HDAC complexes contain the particular domain to bind the chromatin depending on the chromatin structure. Thus, HDAC complexes interact with a certain chromatin structure and have functions for the transcription of a specific gene

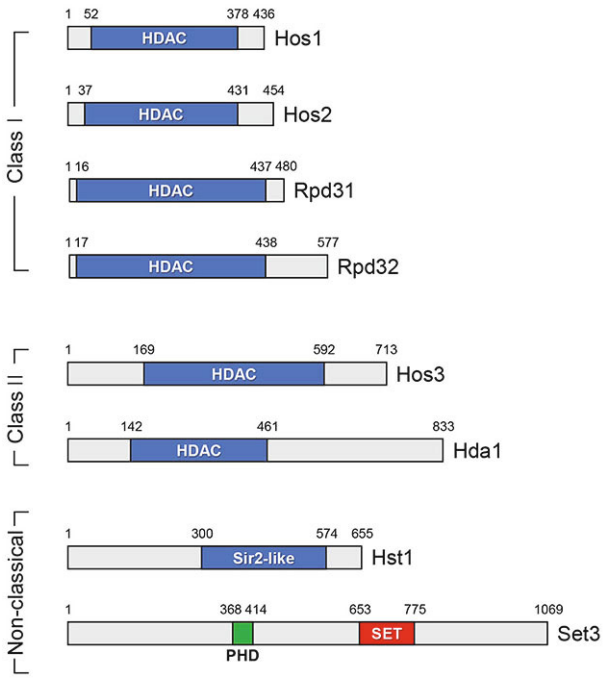

Fig. 1. Histone deacetylases in *C. albicans*. Five classical histone deacetylases in *Saccharomyces cerevisiae* are well conserved in *C. albicans*. Hos1, Hos2, Rpd31, and Rpd32 are class I histone deacetylases. *C. albicans* has two orthologues of *S. cerevisiae* Rpd3, which are Rpd31 and Rpd32. Class I histone deacetylases are usually small proteins of which the major part is HDAC domain. Hos3 and Hda1 are classified into Class II histone deacetylases and the size of Class II is usually bigger than that of Class I. Hst1, a component of Set3 complex, is a non-classical histone deacetylase which is similar with *S. cerevisiae* Sir2 NAD^+-dependent histone deacetylase. Set3, a main component of Set3 complex, contains PHD domain, which recognizes methylated lysine residue, and SET domain (Pijnappel *et al.*, 2001). These sequences are obtained from Candida Genome Database (http://www.candidagenome.org/) and the information about each domain is obtained in the NCBI search for conserved domains (http://www.ncbi.nlm.nih.gov/Strcture/cdd/wrpsb.cgi).

interacting with a certain transcription factor. Therefore, the studies about how HDACs regulate transcriptionally the hypha-specific genes in *C. albicans* are very important for understanding the mechanisms of the morphological transition in *C. albicans*.

Hda1: The establisher of hyphal development inhibiting the Nrg1 recruitment

There are many studies that define the molecular signaling pathways for hyphal development of *C. albicans* under various hypha-inducing conditions. Representatively, Efg1 transcription factor is activated through the cAMP/PKA pathway and facilitates to express the hypha-specific genes for hyphal morphogenesis (Sudbery, 2011). In yeast state of *C. albicans*, Efg1 interacts directly with the promoters of hypha-specific genes. The histone acetyltransferase, NuA4 complex, is recruited to the promoters of hypha-specific genes by Efg1. However, Nrg1-Tup1 complex, a representative negative regulator of hyphal formation, inhibits the expression of hypha-specific genes without any hypha-inducing factors because the complex constantly interacts with the promoters of hypha-specific genes in yeast state (Lu *et al.*, 2008).

Therefore, Liu group suggested that the two temporally changed chromatin structures of hypha-specific gene promoters are required for the hyphal development of *C. albicans* (Lu *et al.*, 2011). The first phase leads to initiate hyphal induction, in which Nrg1 disappears temporally from the promoters of hypha-specific genes because Nrg1-Tup1 complex is a general repressor of hypha-specific genes. The other phase is for maintenance of hyphal state, in which some factors should inhibit the recruitment of Nrg1 to the promoters of hypha-specific genes. The chromatin structure changed by histone acetylation and deacetylation played a critical role for regulating the initiation and maintenance of hyphal development. Especially, Hda1 is recruited to the promoters of hypha-specific genes by another transcription factor, Brg1, and regulates hyphal development (Lu *et al.*, 2012).

During hyphal induction, the activated cAMP/PKA pathway leads to transiently down-regulated expression of *NRG1* gene and causes the disappearance of Nrg1 from the promoters of hypha-specific genes. Also NuA4 acetylates these promoter regions and causes Swi/Snf complex, a chromatin remodeling complex, to be recruited to these regions. This chromatin remodeling factor changes chromatin structure and then activates hypha-specific genes (Lu *et al.*, 2008). After initiation of hyphal induction, Nrg1 is synthesized in normal amount, but Brg1, a GATA transcription factor, interacts with these promoter regions and then recruits Hda1. This recruitment of Hda1 can change the chromatin structure of these regions and inhibits the binding of Nrg1 (Lu *et al.*, 2011, 2012).

NuA4 complex is a huge complex composed of 12 subunits including three components containing histone acetyltransferase (HAT) activity. The binding of NuA4 complex to the promoters of hypha-specific genes with Efg1 is partly dependent on auto-acetylated Yng2 which is one component of NuA4 complex and has HAT activity. Hda1 removes NuA4 complex from these promoter regions by deacetylation of Yng2 in NuA4 complex. This removal of NuA4 complex sequentially causes reassembly of nucleosome, strengthening the inhibition of Nrg1 binding to these promoter regions, and maintenance of hyphal development (Lu *et al.*, 2011). Hda1-null mutant showing short hyphal forms indicates that the recruitment of Hda1 is critical for the maintenance of hyphal state by changing the chromatin structure of these regions (Lu *et al.*, 2011).

Set3 complex: The transcription kinetics regulator of transcription factors for morphogenesis

The Set3 exists as a complex which is composed of seven polypeptides. The Set3 complex (Set3C) contains Set3, Hos2, Snt1, and Sif2, as its core components and other three proteins including Hos4, Hst1, and Cpr1. The Hos2 and Hst1 among components of Set3C have histone deacetylase activity. Hos2 is a classical HDAC like the Rpd3/Hda1 family and Hst1 is a NAD^+-dependent HDAC (Pijnappel *et al.*,

2001). The Set3C is recruited to the chromatin through the PHD finger domain of Set3, which recognizes the di-methylation of the fourth lysine of histone H3 in yeast (Fig. 1; Kim *et al.*, 2012). This Set3C is well conserved in *C. albicans* (Hnisz *et al.*, 2009, 2010) and has histone deacetylase activity *in vivo* (Hnisz *et al.*, 2012).

The Set3C can inhibit the hypha-specific gene expression in yeast phase, through suppressing the cAMP/PKA pathway and its downstream transcription factor, Efg1. Thus, the Set3-null mutants or Hos2-null mutants grow as hyperfilamentous forms even though they are grown in YPD at 37°C, insufficient hypha-inducing condition (Hnisz *et al.*, 2010).

Kuchler group, in 2012, identified that Set3C regulates gene expression kinetics of various transcription factors about morphogenesis by using chromatin immunoprecipitation-sequencing (ChIP-seq) and whole transcriptome sequencing (RNA-seq). Set3C is distributed to highly transcribed regions in yeast phase of normal condition. Furthermore, from the comparison between ChIP-seq and RNA-seq data, in normal condition or hyphal inducing condition, they found that Set3C-binding genes in yeast form are highly expressed in yeast state, whereas Set3C-binding genes in hyphal form are highly expressed in hyphal state (Hnisz *et al.*, 2012). Although the Set3C is recruited to the actively transcribed gene regions specifically, the global pattern of gene expression in Set3-null mutant is almost equal to that in its control strain. However, from the measurement of RNA levels on several time points after hyphal induction, they found that the transcriptional kinetics in Set3-null mutant is different from its control strain. That is, in Set3-null mutant, there are lower transcriptional levels of *EFG1* and *NRG1*, which are transcription factors highly expressed in yeast state and transiently down-regulated after hyphal induction. On the other hand, in Set3-null mutant, there are higher levels of *BRG1* and *TEC1*, which are transcription factors transiently up-regulated under hyphal induction. These findings indicated that the chromatin remodeling is not only limited to regulation of hypha-specific genes but also extended to regulation of transcription kinetics in *C. albicans*. Their conclusions correlate with the role of Set3C for transcriptional regulation in *S. cerevisiae*. In *S. cerevisiae*, Set3C can regulate the transcriptional kinetics of several transcription factors for the adaptation to novel carbon source (Kim *et al.*, 2012). Therefore, the hyperfilamentous phenotype in the absence of Set3 is linked to the changed transcriptional kinetics caused from the abortion of chromatin remodeling by Set3 complex.

Rpd31: The repressor of hyphal induction and the activator of hyphal extension interacting with Ssn6

In *S. cerevisiae*, Class I HDAC, Rpd3, acts in two types of complex, Rpd3 small (Rpd3S) and Rpd3 large (Rpd3L) complex, depending on the interacting proteins (Carrozza *et al.*, 2005; Keogh *et al.*, 2005). Rpd3L complex is enriched in the promoter regions encoding specific transcription factors, whereas Rpd3S complex is highly distributed on the coding regions and has a role to inhibit intragenic antisense transcription (Kadosh and Struhl, 1998; Carrozza *et al.*, 2005; Keogh *et al.*, 2005; Alejandro-Osorio *et al.*, 2009; Yeheskely-Hayon *et al.*, 2013).

In *C. albicans*, there are two proteins as the orthologue of *S. cerevisiae* Rpd3: Rpd31 (orf19.6901, orf19.14093) and Rpd32 (orf19.2834, orf19.10352) (Hnisz *et al.*, 2009). Both Rpd31 and Rpd32, which have HDAC domain, suppress white to opaque transition (Srikantha *et al.*, 2001; Hnisz *et al.*, 2009). However, their exact correlation with yeast-to-hypha transition is not well understood.

Our recent findings revealed that Rpd31 also intimately connected to morphogenesis through the interaction with Ssn6 in *C. albicans*. Ssn6 is a general transcriptional repressor interacting with Tup1, which inhibits the morphological transition from yeast to hypha in *C. albicans* (Hwang *et al.*, 2003). Ssn6-null mutant can be a filamentous form by just increasing the temperature to 37°C. Also in this condition, hypha-specific genes are highly expressed in Ssn6-null mutant (Hwang *et al.*, 2003; García-Sánchez *et al.*, 2005). However, it is suggested that Ssn6 has a Tup1-independent role as well as a transcriptional repressor interacting with Tup1 for the hyphal formation through the comparison between phenotypes of Ssn6-null mutant and Tup1-null mutant. Recently, we defined that Ssn6 interacts with Rpd31 regardless of Tup1's presence by co-purification experiment (Lee *et al.*, 2015). Both loss of Ssn6 and Rpd31 causes filamentous forms of *C. albicans* and highly expressed hypha-specific genes at 28°C, a normal growth temperature for unicellular yeast state. Therefore, we concluded that Rpd31 could act as a negative regulator interacting with Ssn6 for the hyphal formation. However, these hyperfilamentous phenotype in a double mutant of Ssn6 and Rpd31 showed unusual filamentous forms which are short hyphae with irregular width.

We identified that this defective extension of hyphae is from *UME6* expression regulated by Rpd31 (Lee *et al.*, 2015). Ume6 of *C. albicans* has been identified as a filament-specific transcriptional regulator, and it is a critical factor for hyphal extension and virulence of *C. albicans* (Banerjee *et al.*, 2008).

We found that *UME6* expression level significantly decreased in the absence of Rpd31, Ssn6, or both. Also chromatin immunoprecipitation (ChIP) assay data indicated the recruitment of Rpd31 to the promoter of *UME6* gene required Ssn6 and the recruitment of Ssn6 to the promoter of *UME6* required Rpd31. These data suggested Rpd31 interacting with Ssn6 regulates hyphal induction and extension in different manners respectively (Lee *et al.*, 2015). While *C. albicans* maintains its unicellular yeast state, Rpd31 acts as a repressor by interaction with the Ssn6-Tup1 complex and thereby removes acetyl group of histone lysine residues in the promoter regions of some hypha-specific genes, such as *HWP1* and *ECE1*. Once *C. albicans* initiates its hyphal formation responding to environmental cues, Rpd31 induces the expression of *UME6* as an activator interacting with Ssn6 and thus regulates proper hyphal extension of *C. albicans*. These findings lead us to the expansion of knowledge by understanding complicated but tightly regulated chromatin remodeling correlated with morphogenesis in *C. albicans*.

Conclusion

We discussed recent discoveries about histone deacetylases affecting the morphological transition of *C. albicans* by interacting with related transcription factors. The HDACs in *C. albicans* have their functional roles for both the dynamic morphological transition and the maintenance of yeast or hyphal state.

We summarized the transcriptional regulation of hypha-related genes or transcription factor-encoding genes by various HDACs for morphological transition in *C. albicans* (Fig. 2). When *C. albicans* grows in unicellular yeast state, Efg1 cannot induce hypha-specific genes because Rpd3 (or Rpd31) or other HDACs block Efg1's function of transcriptional activation by interacting Nrg1-Tup1 or Ssn6-Tup1 repressor complex (Fig. 2A) (Lu *et al.*, 2011; Lee *et al.*, 2015). When *C. albicans* is exposed to extracellular signals for hyphal-development, HDACs move away from the promoter regions of hypha-specific genes, and block the repressive functions of Nrg1-Tup1 or Ssn6-Tup1 complexes, while, Efg1 can activate the transcription of hypha-specific genes by collaborating with NuA4, histone acetyltransferase (Fig. 2B) (Lu *et al.*, 2011; Lee *et al.*, 2015). In some cases, Nrg1 repressor can be regulated by reducing its transcriptional level (Lu *et al.*, 2011). Once the hyphal development of *C. albicans* initiates, the HDACs maintain the hyphal state or extend the hyphal form by regulating the expression of hypha-specific genes or transcription factor-encoding genes (Fig. 2C). Hda1 is recruited to the promoter regions of hypha-specific genes by interacting with Brg1, and blocks Nrg1 binding to these regions (Lu *et al.*, 2012). Furthermore, Set3C, a complex containing HDAC activity, regulates transcription kinetics of transcription factors involved in the signaling pathways of morphological transition in *C. albicans* (Hnisz *et al.*, 2012). Rpd31, an orthologue of *S. cerevisiae* Rpd3, activate *UME6* gene, a transcription factor for the hyphal extension and virulence, by interacting with Ssn6 (Lee *et al.*, 2015).

Overall, the studies reported here propose the direction of future works for understanding the mechanisms of *C. albicans*' morphological transition and virulence related to the chromatin structures. However, we have just reviewed various studies focusing on the acetylation and deacetylation of histones. Some scientists reported other histone modifications including the fourth lysine methylation of histone H3 or histone H2B ubiquitylation (Leng *et al.*, 2000; Raman *et al.*, 2006). More information about various histone modifiers and modifications themselves is required to define the molecular mechanisms of *C. albicans*' morphological transition and virulence in the transcriptional level. These findings will also contribute to the field of epigenetic regulation involved in the cellular differentiation by using *C. albicans* as a model and to the application for anti-fungal drug development by focusing on HDACs as its target.

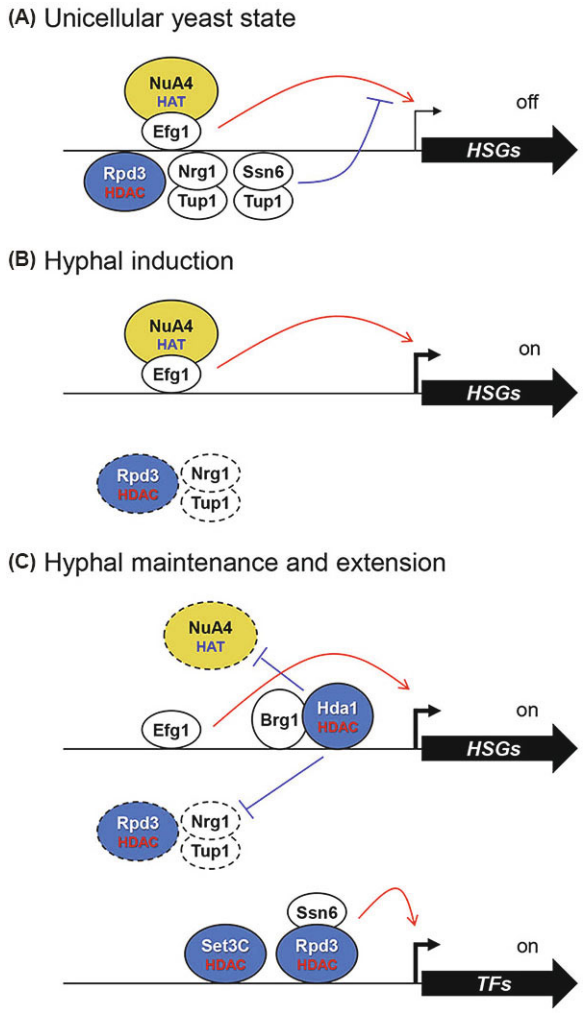

Fig. 2. The mechanisms of how HDACs regulate the transcription of hypha-related genes in *C. albicans*. (A) While *C. albicans* grows in yeast state, Efg1-NuA4 complex binds to the promoters of hypha-specific genes. However, hypha-specific genes cannot be expressed because Rpd3, Nrg1-Tup1, and Ssn6-Tup1 complex bind to the promoter regions of these genes as negative regulators. (B) When *C. albicans* meets a certain environmental factors for hyphal induction, Rpd3 and Nrg1-Tup1 are removed from the promoter regions of hypha-specific genes and then this removal facilitates the transcription of hypha-specific gene. (C) After the hyphal development initiates, Hda1 binds to the promoter regions of hypha-specific genes directly interacting with Brg1 for blocking the recruitment of Nrg1 in these regions and thus hyphal development maintains without Nrg1 repression. Rpd3-Ssn6 complex binds to the promoter of *UME6* gene, of which product is a regulator for hyphal extension and virulence in *C. albicans*, and activate the transcription of *UME6* gene. Set3 complex binds to the promoters of *BRG1* and *TEC1* genes, of which product are transcription factors for hyphal development, and regulates the transcription kinetics of these genes. Red arrows indicate transcriptional activation and blue lines indicate inhibiting functions. HSGs, hypha-specific genes; TFs, transcription factors.

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