Chromatin Accessibility Regulates White-Opaque Switching

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Candida albicans, a diploid polymorphic fungus, has evolved a unique heritable epigenetic program that enables reversible phenotypic switching between two cell types, referred to as “white” and “opaque”. These cell types are established and maintained by distinct transcriptional programs that lead to differences in metabolic preferences, mating competencies, cellular morphologies, responses to environmental signals, interactions with the host innate immune system, and expression of approximately 20% of genes in the genome. Transcription factors that regulate the establishment and heritable maintenance of the white and opaque cell types have been a primary focus of investigation in the field; however, other factors that impact chromatin accessibility, such as histone modifying enzymes, chromatin remodelers, and histone chaperone complexes, also modulate the dynamics of the white-opaque switch and have been
much less studied to date.

white-opaque switching Candida albicans

1. Introduction

Multicellular organisms are comprised of many phenotypically and functionally distinct cell types, the vast majority of which contain the same primary genomic sequence. How a single set of genomic “instructions” can reliably yield many distinct and heritable phenotypic states is a fundamental question in biology. We have begun to understand that a single genome can support many transcriptional programs, which in turn specify unique cell type specific patterns of gene expression, and ultimately establish distinct phenotypes. These cell types are often heritably maintained in an epigenetic manner following each cell division, and it has become increasingly apparent that chromatin structure and accessibility play important roles in the transcriptional regulation of cell type specificity.

Candida albicans, a unicellular polymorphic fungus, has evolved the ability to establish two transcriptional programs that give rise to two distinct cell types called “white” and “opaque” based on their appearance at the single colony level. The white and opaque cell types are heritably maintained in an epigenetic manner through thousands of cell divisions with no change to the primary sequence of the genome [1][2]. A growing body of literature has identified numerous similarities between the molecular mechanisms governing the *C. albicans* white-opaque switch and those that underlie heritable cell type differentiation in higher eukaryotes [3][4][5][6][7]. Since a similar heritable phenotypic switch is not observed in the classic model yeast *Saccharomyces cerevisiae*, *C. albicans* has emerged as a compelling “simple” and genetically tractable eukaryotic model system to study heritable transcriptional programs in higher eukaryotes.

The *C. albicans* white and opaque cell types are established and maintained by distinct transcriptional programs that lead to a wide range of phenotypic differences between the two cell types. These include differences in metabolic preferences, mating competencies, cellular morphologies, responses to environmental signals, interactions with the host innate immune system, and expression of ~20% of genes in the genome [3][4][8][9][10][11][12][13][14][15]. A variety of environmental cues have been identified that can bias the switch in favor of the white or opaque cell type. Growth in the presence of N-acetyl glucosamine, elevated CO_2 levels, acidic pH, anaerobic conditions, genotoxic or oxidative stress, and 25 °C all promote white to opaque switching, while 37 °C in the presence of glucose triggers en masse opaque to white switching [1][2][16][17][18][19][20][21]. The destabilizing effect of elevated temperature on opaque cells is not universal, however, and opaque cells can be heritably maintained at 37 °C when grown on alternative (i.e., non-glucose) carbon sources [22]. Under standard switch permissive laboratory growth conditions (25 °C on Lee's medium supplemented with 100 µg/mL uridine and 2% glucose, or other similarly comprised synthetic defined growth medium), phenotypic switching between the two cell types occurs stochastically at a frequency of approximately one switch event per 1000–10,000 cell divisions [16][17][18][19]. In other words, once established, each cell type is maintained through an epigenetic mechanism that is stably inherited over thousands of subsequent cell divisions.

2. Regulation of White-Opaque Switching by Chromatin Remodeling Complexes

Chromatin remodeling enzyme complexes modulate chromatin accessibility through the function of their ATPase-translocase domains. We can classify chromatin remodeling enzymes into four subfamilies, each of which carries out specialized functions [23]. ISWI and CHD complex subfamilies preferentially reduce chromatin accessibility by regulating the assembly and organization of nucleosomes. The SWI/SNF complex subfamily remodels chromatin by sliding or evicting nucleosomes, which generally increases chromatin accessibility [23]. The INO80 complex subfamily modulates chromatin accessibility by replacing canonical histones with histone variants, specifically targeting nucleosomes that flank transcription start sites. The SWR1 complex, a member of the Ino80 subfamily, is the only known regulator of this class that regulates white-opaque switching in *C. albicans* [5] and is discussed in more detail below.

Regulation of White-Opaque Switching by the SWR1 Chromatin Remodeling Complex

SWR1 encodes a chromatin remodeling enzyme that is responsible for the deposition of the histone variant H2AZ. The SWR1 complex, which is an ortholog of the human SRCAP complex, is a multiprotein complex responsible for replacing canonical histone H2A-H2B dimers with the histone variant H2A.Z-H2B dimers without disassembling the H3/H4 tetramer from DNA [24][25]. H2A.Z is a highly conserved variant of H2A that is found throughout all eukaryotes [26]. Developmentally regulated genomic loci show increased enrichment of H2A.Z relative to non-developmentally regulated loci [27]. H2A.Z is deposited specifically into the two nucleosomes that flank transcription start sites [28], and is essential in several higher eukaryotic organisms, but not in fungi [29][30]. In *C. albicans*, H2A.Z is enriched in white cells, relative to opaque cells, within the upstream intergenic region of *WOR1* [5]. The complex

responsible for depositing this histone variant appears to play a role in stabilizing the white cell type and destabilizing the opaque cell type, as deletion of *SWR1* causes a significant increase in the white to opaque switch frequency and in the heritable maintenance of opaque cells [5]. Since H2A.Z variant enriched sites have been shown to correlate with slightly increased chromatin accessibility relative to canonical histones [31], it is conceivable that higher levels of H2A.Z inhibit expression of *WOR1* by facilitating the binding of a repressor protein within the upstream intergenic region of *WOR1*.

A similar phenotype is observed upon disruption of the NuA4 complex, which is known to recruit and/or promote chromatin-related enzymatic activities of the SWR1 complex [32][33]. Therefore, it is likely that NuA4 regulates the white-opaque switch by modulating the recruitment or enzymatic activity of Swr1, which in turn results in decreased H2A.Z deposition throughout the genome. The nucleosome editing function of the SWR1 complex is also controlled through H3K56 acetylation, which is catalyzed by Rtt109. High levels of H3K56 acetylation led to decreased levels of H2A.Z deposition genome-wide [211]. This is notable as H3K56 acetylation itself has been implicated in altering histone turnover rates [212], which consequently alters genome-wide chromatin accessibility. It remains an open question whether H3K56 acetylation regulates the white-opaque switch by modulating the enzymatic activity of the SWR1 complex, or whether H3K56 acetylation directly regulates the white-opaque switch by modulating histone turnover rates.

3. Regulation of White-Opaque Switching by Histone Chaperone Complexes

The highly basic amino acid composition of histones makes them predisposed to aggregation and promiscuous histone-DNA interactions, thus necessitating a diverse network of histone chaperones to orchestrate the assembly and integration of histones into chromatin [34][35][36]. Below, we focus our discussion on the evolutionarily conserved histone chaperone complexes HIR (HIRA in humans) and CAF-1, and their roles in regulating the white-opaque switch in *C. albicans*. CAF-1 primarily assembles nucleosomes in a replication dependent manner [37][38], whereas HIR functions independent of replication [39][40]. Importantly, the replication coupled nucleosome assembly function of CAF-1 is conserved in humans [37][38]. Both chaperone complexes are essential in higher eukaryotes [41][42], which has complicated efforts to investigate their functions in cell type formation and maintenance. The *C. albicans* white-opaque switch provides a unique and robust alternative system to investigate the functions of these highly conserved chaperone complexes in higher eukaryotes.

Studies in both *S. cerevisiae* and human HeLa cells have revealed that the HIR and CAF-1 complexes modulate nucleosome dynamics [43], which in turn affect chromatin accessibility. Other than their replication dependent functions, these two enzymes have also been shown to have several overlapping functions that are unrelated to replication. Recent work in *C. albicans* has shown that they function similarly to their orthologs in *S. cerevisiae*. Deletion of *C. albicans HIR1,* a subunit of the HIR complex, had no effect on white-opaque switching, while deletion of CAC2, a subunit of CAF-1 complex, resulted in an overall increase in switching in both directions [44]. On the other hand, deletion of a subunit of both chaperone complexes in *C. albicans* has been shown to lead to reduced opaque cell stability, as evidenced by wildtype levels of white to opaque switching and a sixfold increase in

opaque to white switching [44]. These results alone do not definitively point to a specific chaperone complex responsible for regulating opaque cell stability; however, they do reveal that nucleosome dynamics can significantly affect cell type maintenance in the context of the white-opaque switch. Modulating nucleosome dynamics has a significant effect on chromatin accessibility [45], and recent studies have acknowledged the impact of chromatin accessibility on cell type specification and maintenance [46][47][48][49][50]. It is possible that opaque cells, more so than white cells, depend on increased chromatin accessibility to maintain their cell type specific transcriptional program, which could explain why deleting subunits of the HIR and CAF-1 complexes have dramatic effects on opaque cell stability.

References

1. Zordan, R.E.; Miller, M.G.; Galgoczy, D.J.; Tuch, B.B.; Johnson, A.D. Interlocking transcriptional feedback loops control white-opaque switching in Candida albicans. PLoS Biol. 2007, 5, 2166–2176, doi:10.1371/journal.pbio.0050256.
2. Zordan, R.E.; Galgoczy, D.J.; Johnson, A.D. Epigenetic properties of white-opaque switching in Candida albicans are based on a self-sustaining transcriptional feedback loop. Proc. Natl. Acad. Sci. USA 2006, 103, 12807–12812, doi:10.1073/pnas.0605138103.
3. Tuch, B.B.; Mitrovich, Q.M.; Homann, O.R.; Hernday, A.D.; Monighetti, C.K.; de La Vega, F.M.; Johnson, A.D. The transcrip-tomes of two heritable cell types illuminate the circuit governing their differentiation. PLoS Genet. 2010, 6, e1001070, doi:10.1371/journal.pgen.1001070.
4. Hernday, A.D.; Lohse, M.B.; Fordyce, P.M.; Nobile, C.J.; Derisi, J.L.; Johnson, A.D. Structure of the transcriptional network controlling white-opaque switching in Candida albicans. Mol. Microbiol. 2013, 90, 22–35, doi:10.1111/mmi.12329.
5. Guan, Z.; Liu, H. Overlapping functions between SWR1 deletion and H3K56 acetylation in Candida albicans. Eukaryot. Cell 2015, 14, 578–587, doi:10.1128/ec.00002-15.
6. Anderson, M.Z.; Porman, A.M.; Wang, N.; Mancera, E.; Huang, D.; Cuomo, C.A.; Bennett, R.J. A multistate toggle switch defines fungal cell fates and is regulated by synergistic genetic cues. PLoS Genet. 2016, 12, e1006353, doi:10.1371/journal.pgen.1006353.
7. Frazer, C.; Staples, M.I.; Kim, Y.; Hirakawa, M.; Dowell, M.A.; Johnson, N.V.; Hernday, A.D.; Ryan, V.H.; Fawzi, N.L.; Finkelstein, I.J.; et al. Epigenetic cell fate in Candida albicans is controlled by transcription factor condensates acting at su-per-enhancer-like elements. Nat. Microbiol. 2020, 5, 1374–1389, doi:10.1038/s41564-020-0760-7.
8. Takagi, J.; Singh-Babak, S.D.; Lohse, M.B.; Dalal, C.K.; Johnson, A.D. Candida albicans white and opaque cells exhibit distinct spectra of organ colonization in mouse models of infection. PLoS

ONE 2019, 14, e0218037, doi:10.1371/journal.pone.0218037.

9. Lohse, M.B.; Ene, I.V.; Craik, V.B.; Hernday, A.D.; Mancera, E.; Morschhäuser, J.; Bennett, R.J.; Johnson, A.D. Systematic ge-netic screen for transcriptional regulators of the Candida albicans white-opaque switch. Genetics 2016, 203, 1679–1692, doi:10.1534/genetics.116.190645.
10. Solis, N.V.; Park, Y.-N.; Swidergall, M.; Daniels, K.J.; Filler, S.G.; Soll, D.R. Candida albicans white-opaque switching influ-ences virulence but not mating during oropharyngeal candidiasis. Infect. Immun. 2018, 86, 1–14, doi:10.1128/iai.00774-17.
11. Craik, V.B.; Johnson, A.D.; Lohse, M.B. Sensitivity of white and opaque Candida albicans cells to antifungal drugs. Antimicrob. Agents Chemother. 2017, 61, 8–11, doi:10.1128/aac.00166-17.
12. Pande, K.; Chen, C.; Noble, S.M. Passage through the mammalian gut triggers a phenotypic switch that promotes Candida albicans commensalism. Nat. Genet. 2013, 45, 1088–1091, doi:10.1038/ng.2710.
13. Lohse, M.B.; Johnson, A.D. Differential phagocytosis of white versus opaque Candida albicans by Drosophila and mouse phag-ocytes. PLoS ONE 2008, 3, e1473, doi:10.1371/journal.pone.0001473.
14. Miller, M.G.; Johnson, A.D. White-opaque switching in Candida albicans is controlled by mating-type locus homeodomain proteins and allows efficient mating. Cell 2002, 110, 293–302, doi:10.1016/S0092-8674(02)00837-1.
15. Sasse, C.; Hasenberg, M.; Weyler, M.; Gunzer, M.; Morschhäuser, J. White-opaque switching of Candida albicans allows im-mune evasion in an environment-dependent fashion. Eukaryot. Cell 2013, 12, 50–58, doi:10.1128/EC.00266-12.
16. Alby, K.; Bennett, R.J. Phenotypic switching is sensitive to multiple inputs in a pathogenic fungus. Mol. Biol. Cell 2009, 2, 509–511, doi:10.1091/mbc.E09-01-0040.In.
17. Huang, G.; Srikantha, T.; Sahni, N.; Yi, S.; Soll, D.R. CO2 regulates white-to-opaque switching in Candida albicans. Curr. Biol. 2009, 19, 330–334, doi:10.1016/j.cub.2009.01.018.
18. Huang, G.; Yi, S.; Sahni, N.; Daniels, K.J.; Srikantha, T.; Soll, D.R. N-acetylglucosamine induces white to opaque switching, a mating prerequisite in Candida albicans. PLoS Pathog. 2010, 6, e1000806, doi:10.1371/journal.ppat.1000806.
19. Morrow, B.; Anderson, J.; Wilson, J.; Soll, D.R. Bidirectional stimulation of the white-opaque transition of Candida albicans by ultraviolet irradiation. J. Gen. Microbiol. 1989, 135, 1201–1208, doi:10.1099/00221287-135-5-1201.
20. Ramírez-Zavala, B.; Reuß, O.; Park, Y.N.; Ohlsen, K.; Morschhäuser, J. Environmental induction of white-opaque switching in Candida albicans. PLoS Pathog. 2008, 4, e1000089, doi:10.1371/journal.ppat.1000089.

21. Alby, K.; Bennett, R.J. Stress-Induced Phenotypic Switching in Candida albicans. Mol. Biol. Cell 2009, 20, 3178–3191, doi:10.1091/mbc.e09-01-0040.

22. Lohse, M.B.; Hernday, A.D.; Fordyce, P.M.; Noiman, L.; Sorrells, T.R.; Hanson-Smith, V.; Nobile, C.J.; DeRisi, J.L.; Johnson, A.D. Identification and characterization of a previously undescribed family of sequence-specific DNA-binding domains. Proc. Natl. Acad. Sci. USA 2013, 110, 7660–7665, doi:10.1073/pnas.1221734110.

23. Clapier, C.R.; Iwasa, J.; Cairns, B.R.; Peterson, C.L. Mechanisms of action and regulation of ATP-dependent chroma-tin-remodelling complexes. Nat. Rev. Mol. Cell Biol. 2017, 18, 407–422, doi:10.1038/nrm.2017.26.

24. Mizuguchi, G.; Shen, X.; Landry, J.; Wu, W.H.; Sen, S.; Wu, C. ATP-driven exchange of histone h2az variant catalyzed by SWR1 chromatin remodeling complex. Science 2004, 303, 343–348, doi:10.1126/science.1090701.

25. Luk, E.; Ranjan, A.; FitzGerald, P.C.; Mizuguchi, G.; Huang, Y.; Wei, D.; Wu, C. Stepwise histone replacement by SWR1 re-quires dual activation with histone H2A.Z and canonical nucleosome. Cell 2010, 143, 725–736, doi:10.1016/j.cell.2010.10.019.

26. Zlatanova, J.; Thakar, A. H2A.Z: View from the top. Structure 2008, 16, 166–179, doi:10.1016/j.str.2007.12.008.

27. Creyghton, M.P.; Markoulaki, S.; Levine, S.S.; Hanna, J.; Lodato, M.A.; Sha, K.; Young, R.A.; Jaenisch, R.; Boyer, L.A. H2AZ is enriched at polycomb complex target genes in es cells and is necessary for lineage commitment. Cell 2008, 135, 649–661, doi:10.1016/j.cell.2008.09.056.

28. Raisner, R.M.; Hartley, P.D.; Meneghini, M.D.; Bao, M.Z.; Liu, C.L.; Schreiber, S.L.; Rando, O.J.; Madhani, H.D. Histone vari-ant H2A.Z Marks the 5′ ends of both active and inactive genes in euchromatin. Cell 2005, 123, 233–248, doi:10.1016/j.cell.2005.10.002.

29. Jackson, J.D.; Gorovsky, M.A. Histone H2A.Z has a conserved function that is distinct from that of the major H2A sequence variants. Nucleic Acids Res. 2000, 28, 3811–3816, doi:10.1093/nar/28.19.3811.

30. Liu, X.; Li, B. GorovskyMA Essential and nonessential histone H2A variants in Tetrahymena thermophila. Mol. Cell. Biol. 1996, 16, 4305–4311, doi:10.1128/mcb.16.8.4305.

31. Brunelle, M.; Nordell Markovits, A.; Rodrigue, S.; Lupien, M.; Jacques, P.É.; Gévry, N. The histone variant H2A.Z is an im-portant regulator of enhancer activity. Nucleic Acids Res. 2015, 43, 9742–9756, doi:10.1093/nar/gkv825.

32. Jagannath, A.; Wood, M.J.A. Efg1-mediated recruitment of NuA4 to promoters is required for hypha-specific Swi/Snf bind-ing and activation in Candida albicans. Mol. Biol. Cell 2009, 20, 521–529, doi:10.1091/mbc.E08.

33. Altaf, M.; Auger, A.; Monnet-Saksouk, J.; Brodeur, J.; Piquet, S.; Cramet, M.; Bouchard, N.; Lacoste, N.; Utley, R.T.; Gaudreau, L.; et al. NuA4-dependent acetylation of nucleosomal histones H4 and H2A directly stimulates incorporation of H2A.Z by the SWR1 complex. J. Biol. Chem. 2010, 285, 15966–15977, doi:10.1074/jbc.M110.117069.

34. Gurard-Levin, Z.A.; Quivy, J.-P.; Almouzni, G. Histone chaperones: Assisting histone traffic and nucleosome dynamics. Annu. Rev. Biochem. 2014, 83, 487–517, doi:10.1146/annurev-biochem-060713-035536.

35. Amin, A.D.; Vishnoi, N.; Prochasson, P. A global requirement for the HIR complex in the assembly of chromatin. Biochim. Biophys. Acta Gene Regul. Mech. 2012, 1819, 264–276, doi:10.1016/j.bbagrm.2011.07.008.

36. Elsässer, S.J.; D'Arcy, S. Towards a mechanism for histone chaperones. Biochim. Biophys. Acta Gene Regul. Mech. 2012, 1819, 211–221, doi:10.1016/j.bbagrm.2011.07.007.

37. Kaufman, P.D.; Kobayashi, R.; Stillman, B. Ultraviolet radiation sensitivity and reduction of telomeric silencing in Saccha-romyces cerevisiae cells lacking chromatin assembly factor-I. Genes Dev. 1997, 11, 345–357, doi:10.1101/gad.11.3.345.

38. Stillman, B. Chromatin assembly during SV40 DNA replication in vitro. Cell 1986, 45, 555–565, doi:10.1016/0092-8674(86)90287-4.

39. Green, E.M.; Antczak, A.J.; Bailey, A.O.; Franco, A.A.; Wu, K.J.; Yates, J.R.; Kaufman, P.D. Replication-independent histone deposition by the HIR complex and Asf1. Curr. Biol. 2005, 15, 2044–2049, doi:10.1016/j.cub.2005.10.053.

40. Prochasson, P.; Florens, L.; Swanson, S.K.; Washburn, M.P.; Workman, J.L. The HIR corepressor complex binds to nucleo-somes generating a distinct protein/DNA complex resistant to remodeling by SWI/SNF. Genes Dev. 2005, 19, 2534–2539, doi:10.1101/gad.1341105.

41. Houlard, M.; Berlivet, S.; Probst, A.V.; Quivy, J.-P.; Héry, P.; Almouzni, G.; Gérard, M. CAF-1 is essential for heterochroma-tin organization in pluripotent embryonic cells. PLoS Genet. 2006, 2, e181, doi:10.1371/journal.pgen.0020181.

42. Roberts, C.; Sutherland, H.F.; Farmer, H.; Kimber, W.; Halford, S.; Carey, A.; Brickman, J.M.; Wynshaw-Boris, A.; Scambler, P.J. Targeted mutagenesis of the Hira gene results in gastrulation defects and patterning abnormalities of mesoendodermal derivatives prior to early embryonic lethality. Mol. Cell. Biol. 2002, 22, 2318–2328, doi:10.1128/mcb.22.7.2318-2328.2002.

43. Ray-Gallet, D.; Woolfe, A.; Vassias, I.; Pellentz, C.; Lacoste, N.; Puri, A.; Schultz, D.C.; Pchelintsev, N.A.; Adams, P.D.; Jansen, L.E.T.; et al. Dynamics of histone H3 deposition in vivo reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity. Mol. Cell 2011, 44, 928–941, doi:10.1016/j.molcel.2011.12.006.

44. Stevenson, J.S.; Liu, H. Nucleosome assembly factors CAF-1 and HIR modulate epigenetic switching frequencies in an H3K56 acetylation-associated manner in Candida albicans. Eukaryot. Cell 2013, 12, 591–603, doi:10.1128/EC.00334-12.

45. Klemm, S.L.; Shipony, Z.; Greenleaf, W.J. Chromatin accessibility and the regulatory epigenome. Nat. Rev. Genet. 2019, 20, 207–220, doi:10.1038/s41576-018-0089-8.

46. Hu, G.; Cui, K.; Northrup, D.; Liu, C.; Wang, C.; Tang, Q.; Ge, K.; Levens, D.; Crane-Robinson, C.; Zhao, K. H2A.Z facilitates access of active and repressive complexes to chromatin in embryonic stem cell self-renewal and differentiation. Cell Stem Cell 2013, 12, 180–192, doi:10.1016/j.stem.2012.11.003.

47. Serra-Cardona, A.; Zhang, Z. Replication-coupled nucleosome assembly in the passage of epigenetic information and cell identity. Trends Biochem. Sci. 2018, 43, 136–148, doi:10.1016/j.tibs.2017.12.003.

48. Zaret, K.S.; Mango, S.E. Pioneer transcription factors, chromatin dynamics, and cell fate control. Curr. Opin. Genet. Dev. 2016, 37, 76–81, doi:10.1016/j.gde.2015.12.003.

49. Schulz, V.P.; Yan, H.; Lezon-Geyda, K.; An, X.; Hale, J.; Hillyer, C.D.; Mohandas, N.; Gallagher, P.G. A unique epigenomic landscape defines human erythropoiesis. Cell Rep. 2019, 28, 2996–3009.e7, doi:10.1016/j.celrep.2019.08.020.

50. Li, D.; Liu, J.; Yang, X.; Zhou, C.; Guo, J.; Wu, C.; Qin, Y.; Guo, L.; He, J.; Yu, S.; et al. Chromatin accessibility dynamics dur-ing iPSC reprogramming. Cell Stem Cell 2017, 21, 819–833.e6, doi:10.1016/j.stem.2017.10.012.