COMMENTARY
Host-Microbe Biology

mSphere of Influence: Decoding Transcriptional Regulatory Networks To Illuminate the Mechanisms of Microbial Pathogenicity

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ABSTRACT Sadri Znaidi works in the field of molecular mycology with a focus on functional genomics in *Candida albicans*. In this mSphere of Influence article, he reflects on how the paper "An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis" by Chen et al. (C. Chen, K. Pande, S. D. French, B. B. Tuch, and S. M. Noble, Cell Host Microbe 10:118–135, 2011, https://doi.org/10.1016/j.chom.2011.07.005) made an impact on his research on how transcriptional regulatory networks function to control *C. albicans*' ability to efficiently interact with the host environment.

KEYWORDS *Candida albicans*, genomics, host-pathogen interactions, iron metabolism, transcription factors

The advent of functional genomics and systems biology has equipped us with the tools allowing us to decipher the biological circuitries that govern the expression of traits enabling microbial pathogens to efficiently thrive in the host environment. The armamentarium of virulence mechanisms used by pathogens to infect the human body is extensive, ranging from toxin release and tissue invasion (1, 2) to deprivation of host nutrients (3) and hijacking of immune cells (4). The opportunistic yeast *Candida albicans* adopts two lifestyles, a commensal lifestyle whereby it acts as a bystander, colonizing body organs without causing harmful effects, and a "pathogenic identity" in which it fully expresses virulence traits to cause disease. This duality was elegantly featured in the paper "An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis," by Chen et al. in 2011 (5), where the authors decode the transcriptional network that controls the ability of *C. albicans* to acquire iron (Sef1 regulon) while thriving as a pathogen in the iron-scarce environment of the bloodstream and map the circuitries that protect *C. albicans* from toxicity of high iron levels (Sfu1 regulon) encountered in the gastrointestinal (GI) tract, where *C. albicans* can thrive as a commensal. This work suggested that the *C. albicans* genome encodes two main genetic switches, i.e., transcription factors (TFs) Sfu1 and Sef1, allowing *C. albicans* to perfectly adapt to constraints imposed by iron availability in the GI tract and in the bloodstream, respectively. Such a *modus operandi* exemplifies how the evolutionary process remodeled the behavior of microbes that transition from a commensal to a pathogenic state and highlights the impact of decision-making proteins like TFs on their remarkable adaptation to disparate host niches. This study by the Suzanne Noble group was a source of inspiration to my research, prompting me to further investigate the consequences of genetically perturbing TFs on *C. albicans*' phenotypic traits pertaining to pathogenicity and commensalism and comprehensively map the associated transcriptional circuitries using genome-wide expression and location technologies. Future perspectives in this particular field, and in microbial systems

Citation Znaidi S. 2020. mSphere of Influence: decoding transcriptional regulatory networks to illuminate the mechanisms of microbial pathogenicity. mSphere 5:e00917-19. https://doi.org/10.1128/mSphere.00917-19.

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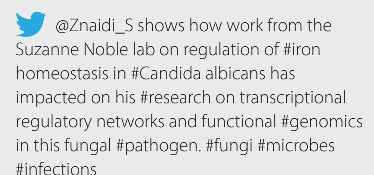
@Znaidi_S shows how work from the Suzanne Noble lab on regulation of #iron homeostasis in #Candida albicans has impacted on his #research on transcriptional regulatory networks and functional #genomics in this fungal #pathogen. #fungi #microbes #infections

Published 8 January 2020

biology in general, will certainly focus on generating a global landscape of TF networks that impact on key fitness attributes underlying the pathogenic and commensal facets of opportunistic microbes.

The Chen et al. paper (5) built on previous findings where *SEF1* showed up among a subset of genes whose deletion altered *C. albicans* infectivity in a murine model of systemic infection (6) and its ability to grow on iron-depleted medium (7). The fact that *SEF1* encodes a TF suggested that it may act as a transcriptional regulator of iron uptake; however, the prevailing model based on the rich literature from *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* was in favor of transcriptional control through positive (*S. cerevisiae* AFT-type regulators Aft1/Aft2) or negative (*S. pombe* GATA-type regulator Fep1) regulation (8, 9). The *C. albicans* genome encodes one homolog of each TF, respectively, named Aft2 and Sfu1 (10, 11). However, while Sfu1 is clearly involved in transcriptional repression of iron uptake genes (10), the role of Aft2 appears to be somewhat pleiotropic (12), and its function in *C. albicans* could have been modified. The authors combined chromatin immunoprecipitation (ChIP) and transcript profiling to decipher the regulatory network of Sef1 and Sfu1, together with Hap43, a conserved regulator of iron utilization genes (13). They found that the three TFs form a tightly knit circuitry, in which Sef1 clearly acts as an activator of iron uptake genes, whereas Sfu1 and Hap43 act mainly as negative regulators of iron uptake and iron utilization genes, respectively (5). These findings are striking, as they point to the occurrence of evolutionary remodeling of the circuitry controlling iron homeostasis in *C. albicans*, a process also termed "transcriptional rewiring" (14). The authors further emphasized the functional importance of the rewired circuitry. They showed that the *sef1*$^{-/-}$ and *sfu1*$^{-/-}$ mutants failed to transcriptionally induce and repress iron uptake genes in human plasma and in the GI tract and were inefficient at causing disease in a systemic infection model and at colonizing the murine gut, respectively, pointing to functional specificities associated with the two host niches. The results from animal experiments are fascinating, clearly demonstrating the specialization of the two genetic switches in the expression of pathogenic (iron acquisition from the host) and commensal (protection from iron toxicity) traits of *C. albicans* when it thrives in the bloodstream and in the gut.

The Chen et al. paper (5) brought me straight back to my M.Sc. and Ph.D. years. In 2004, while preparing to move to Montreal, Canada, to start working with Martine Raymond on transcriptional regulation in *C. albicans*, I deposited the final version of my Master's thesis on the characterization of the iron regulator Fep1 (15); the last two paragraphs of my thesis commented on the following question: how close is transcriptional regulation of iron uptake in fission yeast to that in *C. albicans*? This question was based on the relatively high level of homology that we observed between the deduced amino acid sequences of *fep1*$^{+}$ and *SFU1*. Later on, the Labbé lab found that heterologous expression of *C. albicans* Sfu1 in *S. pombe* produced a functional protein that was able to turn off iron uptake genes in fission yeast (16). This study could have been a great starting point for comparative analyses of the transcriptional circuits that control iron homeostasis in the two yeast species, but the technologies allowing us to perform them were not routinely implemented yet. At that time, the *C. albicans* genome sequence had just been published (17), and many members of the *Candida* community started contributing to its annotation (18, 19). The excitement for doing functional genomics in *C. albicans* was mind-blowing at that time, and many labs—including my Ph.D. lab—started using and combining the ChIP-chip (ChIP with microarray technology) and transcriptomics technologies to map transcriptional regulatory networks (20–22). The Chen et al. paper naturally influenced my thinking for the following reasons. (i) It elegantly addressed one of the questions I was eager to answer during my early years in research. (ii) It exemplified the powerful use of genome-wide expression and location technologies to explain how transcriptional circuits are built, connected, and remodeled in nonpathogenic versus pathogenic yeast species. (iii) It fostered looking deeper into conditions that mimic the host niches in which *C. albicans* thrives. (iv) It used relevant animal models to learn more about the fascinating behavior of this

opportunistic yeast. This paper definitely laid the groundwork for implementing future large-scale approaches, such as systematic genetic perturbation of TFs combined with high-throughput phenotyping and ChIP-sequencing (ChIP-Seq) or transcriptome sequencing (RNA-Seq), providing a bird's eye view of the transcriptional circuitries operating in microbial species that alternate between commensal and pathogenic lifestyles.

The literature has been quite rich in discoveries since publication of the Chen et al. study (5). We currently know that Sef1 exerts its function through iron-dependent nuclear localization, a process orchestrated by its physical association with Sfu1 in the cytoplasm, when iron is abundant, and phosphorylation by Ssn3 cyclin-dependent kinase and nuclear localization, when iron is scarce (23). More recently, the Aberdeen Fungal Group showed that *SEF1* acts as a determinant of *C. albicans*' immune evasion capacity by strongly masking β-glucan (24), consistent with its role in *C. albicans*' virulence. Now, what happens in other *Candida* species? Work by the Hube lab provided further evidence that the evolution of iron sensing/uptake regulatory networks is quite flexible from one species to another (25). While *C. albicans* intercalated Sef1 into an ancient circuit (i.e., employed by *S. pombe*), *C. glabrata* apparently combined the systems used in *C. albicans* (Sef1) and *S. cerevisiae* (Aft1) to control iron homeostasis (25). Still, like in *S. cerevisiae*, Aft1 acts as the major regulator of iron uptake in *C. glabrata*, whereas Sef1 appears to control iron consumption processes (25). Clearly, future work will reveal quite surprising discoveries in the field, further illuminating the fascinating strategies used by microbes in the arms race for nutrient iron during host-pathogen interactions.

ACKNOWLEDGMENTS

I thank Martine Raymond, David P. Rogers, Bernard Turcotte, Aaron P. Mitchell, Marie-Elisabeth Bougnoux, Christophe d'Enfert, Erij Messadi, Helmi Mardassi, and Hechmi Louzir for helpful discussions and continuous support. I am indebted to all members of the Unité Biologie et Pathogénicité Fongiques (BPF unit) and the Laboratoire de Microbiologie Moléculaire, Vaccinologie et Développement Biotechnologique (LMMVDB) for their insights and help in my research.

My work at the Pasteur Institutes in Tunis, Tunisia, and Paris, France, is supported by grants/funds from the LMMVDB, the BPF unit, the Institut Pasteur de Tunis Programmes Collaboratifs Internes (grants PCI22 and PCI32), the Ministry of Higher Education and Scientific Research (Young Investigator Grant Award 19PEJC06-02), and the Institut Pasteur Division of International Affairs, Paris, France (Institut Pasteur International Network Affiliate Program Fellowship).

REFERENCES

1. Moyes DL, Wilson D, Richardson JP, Mogavero S, Tang SX, Wernecke J, Hofs S, Gratacap RL, Robbins J, Runglall M, Murciano C, Blagojevic M, Thavaraj S, Forster TM, Hebecker B, Kasper L, Vizcay G, Iancu SI, Kichik N, Hader A, Kurzai O, Luo T, Kruger T, Kniemeyer O, Cota E, Bader O, Wheeler RT, Gutsmann T, Hube B, Naglik JR. 2016. Candidalysin is a fungal peptide toxin critical for mucosal infection. Nature 532:64–68. https://doi.org/10.1038/nature17625.
2. Poole J, Day CJ, von Itzstein M, Paton JC, Jennings MP. 2018. Glycointeractions in bacterial pathogenesis. Nat Rev Microbiol 16:440–452. https://doi.org/10.1038/s41579-018-0007-2.
3. Lopez CA, Skaar EP. 2018. The impact of dietary transition metals on host-bacterial interactions. Cell Host Microbe 23:737–748. https://doi.org/10.1016/j.chom.2018.05.008.
4. Larsson M. 2005. HIV-1 and the hijacking of dendritic cells: a tug of war. Springer Semin Immunopathol 26:309–328. https://doi.org/10.1007/s00281-004-0178-y.
5. Chen C, Pande K, French SD, Tuch BB, Noble SM. 2011. An iron homeostasis regulatory circuit with reciprocal roles in *Candida albicans* commensalism and pathogenesis. Cell Host Microbe 10:118–135. https://doi.org/10.1016/j.chom.2011.07.005.
6. Noble SM, French S, Kohn LA, Chen V, Johnson AD. 2010. Systematic screens of a *Candida albicans* homozygous deletion library decouple morphogenetic switching and pathogenicity. Nat Genet 42:590–598. https://doi.org/10.1038/ng.605.
7. Homann OR, Dea J, Noble SM, Johnson AD. 2009. A phenotypic profile of the *Candida albicans* regulatory network. PLoS Genet 5:e1000783. https://doi.org/10.1371/journal.pgen.1000783.
8. Labbe S, Khan MG, Jacques JF. 2013. Iron uptake and regulation in *Schizosaccharomyces pombe*. Curr Opin Microbiol 16:669–676. https://doi.org/10.1016/j.mib.2013.07.007.
9. Outten CE, Albetel AN. 2013. Iron sensing and regulation in *Saccharomyces cerevisiae*: ironing out the mechanistic details. Curr Opin Microbiol 16:662–668. https://doi.org/10.1016/j.mib.2013.07.020.
10. Lan CY, Rodarte G, Murillo LA, Jones T, Davis RW, Dungan J, Newport G, Agabian N. 2004. Regulatory networks affected by iron availability in *Candida albicans*. Mol Microbiol 53:1451–1469. https://doi.org/10.1111/j.1365-2958.2004.04214.x.
11. Liang Y, Wei D, Wang H, Xu N, Zhang B, Xing L, Li M. 2010. Role of *Candida albicans* Aft2p transcription factor in ferric reductase activity, morphogenesis and virulence. Microbiology 156:2912–2919. https://doi.org/10.1099/mic.0.037978-0.
12. Xu N, Cheng X, Yu Q, Qian K, Ding X, Liu R, Zhang B, Xing L, Li M. 2013.

Aft2, a novel transcription regulator, is required for iron metabolism, oxidative stress, surface adhesion and hyphal development in *Candida albicans*. PLoS One 8:e62367. https://doi.org/10.1371/journal.pone.0062367.

13. Hsu PC, Yang CY, Lan CY. 2011. *Candida albicans* Hap43 is a repressor induced under low-iron conditions and is essential for iron-responsive transcriptional regulation and virulence. Eukaryot Cell 10:207–225. https://doi.org/10.1128/EC.00158-10.
14. Whiteway M, Tebung WA, Choudhury BI, Rodriguez-Ortiz R. 2015. Metabolic regulation in model ascomycetes–adjusting similar genomes to different lifestyles. Trends Genet 31:445–453. https://doi.org/10.1016/j.tig.2015.05.002.
15. Znaidi S, Pelletier B, Mukai Y, Labbe S. 2004. The *Schizosaccharomyces pombe* corepressor Tup11 interacts with the iron-responsive transcription factor Fep1. J Biol Chem 279:9462–9474. https://doi.org/10.1074/jbc.M312787200.
16. Pelletier B, Mercier A, Durand M, Peter C, Jbel M, Beaudoin J, Labbe S. 2007. Expression of *Candida albicans* Sfu1 in fission yeast complements the loss of the iron-regulatory transcription factor Fep1 and requires Tup co-repressors. Yeast 24:883–900. https://doi.org/10.1002/yea.1539.
17. Jones T, Federspiel NA, Chibana H, Dungan J, Kalman S, Magee BB, Newport G, Thorstenson YR, Agabian N, Magee PT, Davis RW, Scherer S. 2004. The diploid genome sequence of *Candida albicans*. Proc Natl Acad Sci U S A 101:7329–7334. https://doi.org/10.1073/pnas.0401648101.
18. Braun BR, van Het Hoog M, d'Enfert C, Martchenko M, Dungan J, Kuo A, Inglis DO, Uhl MA, Hogues H, Berriman M, Lorenz M, Levitin A, Oberholzer U, Bachewich C, Harcus D, Marcil A, Dignard D, Iouk T, Zito R, Frangeul L, Tekaia F, Rutherford K, Wang E, Munro CA, Bates S, Gow NA, Hoyer LL, Köhler G, Morschhäuser J, Newport G, Znaidi S, Raymond M, Turcotte B, Sherlock G, Costanzo M, Ihmels J, Berman J, Sanglard D, Agabian N, Mitchell AP, Johnson AD, Whiteway M, Nantel A. 2005. A human-curated annotation of the *Candida albicans* genome. PLoS Genet 1:36–57. https://doi.org/10.1371/journal.pgen.0010001.
19. d'Enfert C, Goyard S, Rodriguez-Arnaveilhe S, Frangeul L, Jones L, Tekaia F, Bader O, Albrecht A, Castillo L, Dominguez A, Ernst JF, Fradin C, Gaillardin C, Garcia-Sanchez S, de Groot P, Hube B, Klis FM, Krishnamurthy S, Kunze D, Lopez MC, Mavor A, Martin N, Moszer I, Onesime D, Perez Martin J, Sentandreu R, Valentin E, Brown AJ. 2005. *Candida*DB: a genome database for *Candida albicans* pathogenomics. Nucleic Acids Res 33:D353–D357.
20. Hogues H, Lavoie H, Sellam A, Mangos M, Roemer T, Purisima E, Nantel A, Whiteway M. 2008. Transcription factor substitution during the evolution of fungal ribosome regulation. Mol Cell 29:552–562. https://doi.org/10.1016/j.molcel.2008.02.006.
21. Liu TT, Znaidi S, Barker KS, Xu L, Homayouni R, Saidane S, Morschhauser J, Nantel A, Raymond M, Rogers PD. 2007. Genome-wide expression and location analyses of the *Candida albicans* Tac1p regulon. Eukaryot Cell 6:2122–2138. https://doi.org/10.1128/EC.00327-07.
22. Srikantha T, Borneman AR, Daniels KJ, Pujol C, Wu W, Seringhaus MR, Gerstein M, Yi S, Snyder M, Soll DR. 2006. TOS9 regulates white-opaque switching in *Candida albicans*. Eukaryot Cell 5:1674–1687. https://doi.org/10.1128/EC.00252-06.
23. Chen C, Noble SM. 2012. Post-transcriptional regulation of the Sef1 transcription factor controls the virulence of *Candida albicans* in its mammalian host. PLoS Pathog 8:e1002956. https://doi.org/10.1371/journal.ppat.1002956.
24. Pradhan A, Avelar GM, Bain JM, Childers D, Pelletier C, Larcombe DE, Shekhova E, Netea MG, Brown GD, Erwig L, Gow NAR, Brown AJP. 2019. Non-canonical signalling mediates changes in fungal cell wall PAMPs that drive immune evasion. Nat Commun 10:5315. https://doi.org/10.1038/s41467-019-13298-9.
25. Gerwien F, Safyan A, Wisgott S, Hille F, Kaemmer P, Linde J, Brunke S, Kasper L, Hube B. 2016. A novel hybrid iron regulation network combines features from pathogenic and nonpathogenic yeasts. mBio 7:e01782-16. https://doi.org/10.1128/mBio.01782-16.