High resolution genome-wide occupancy in *Candida spp.* using ChEC-seq

Faiza Tebbji\textsuperscript{a}, Inès Khemiri\textsuperscript{a,b}, Adnane Sellam\textsuperscript{a,b#}

\textsuperscript{a} Montreal Heart Institute, Université de Montréal, Montréal, QC, Canada

\textsuperscript{b} Department of Microbiology, Infectious Diseases and Immunology, Faculty of Medicine, Université de Montréal, Montréal, QC, Canada

# Address correspondence to Adnane Sellam, adnane.sellam@umontreal.ca

Running Head: ChEC-seq genome-wide occupancy analysis in fungal pathogens
Abstract

To persist in their hostile and dynamic human host environments, fungal pathogens has to sense and adapt by modulating their gene expression to fulfil their cellular needs. Understanding transcriptional regulation on a global scale would uncover cellular processes linked to persistence and virulence mechanisms that could be targeted for antifungal therapeutics. Infections associated with the yeast Candida albicans, a highly prevalent fungal pathogen, and the multi-resistant related species C. auris are becoming a serious public health threat. To define the set of a gene regulated by a transcriptional regulator in C. albicans, Chromatin Immuno-Precipitation (ChIP) based techniques including ChIP-chip or ChIP-seq has been widely used. Here, we describe a new set of PCR-based MNase-tagging plasmids for C. albicans and other Candida spp. to determine genome-wide location of any transcriptional regulator of interest using Chromatin endogenous cleavage (ChEC) coupled to high-throughput sequencing (ChEC-seq). The ChEC procedure does not require protein-DNA crosslinking or sonication avoiding thus artefacts related to epitope masking or the hyper-ChIPable euchromatic phenomenon. In a proof-of-concept application of ChEC-seq, we provided a high-resolution binding map of the SWI/SNF chromatin remodeling complex, a master regulator of fungal fitness in C. albicans in addition to the transcription factor NsiI that is an ortholog of the DNA-binding protein Reb1 for which genome-wide occupancy were previously established in Saccharomyces cerevisiae. The ChEC-seq procedure described here will allow a high-resolution genomic location definition which will enable a better understanding of transcriptional regulatory circuits that govern fungal fitness and drug resistance in these medically important fungi.

Importance

Systemic fungal infections caused by Candida albicans and the ‘superbug’ C. auris are becoming a serious public health threat. The ability of these yeasts to cause disease is linked to their faculty to modulate the expression of genes that mediate their escape from the immune surveillance and their persistence in the different unfavourable niches within the host. Comprehensive knowledge on gene expression control of fungal fitness is consequently an interesting framework for the identification of essential infection processes that could be hindered by chemicals as potential therapeutics. Here, we expanded the use of ChEC-seq, a technique that was initially developed in the yeast model Saccharomyces cerevisiae to identify genes that are modulated by a transcriptional regulator, to the pathogenic yeasts C. albicans and C. auris. This robust technique will allow a better characterization of key gene expression regulators and their contribution to virulence and antifungal resistance in these pathogenic yeasts.

Keywords

ChEC-seq, Transcriptional regulatory network, Genome-wide occupancy, Candida albicans, Candida auris
Introduction

Candida species, in particular Candida albicans, are major components of the disease burden caused by fungi and are frequent cause of life-threatening invasive infections especially in immunocompromised patients. The emergent C. auris was the first fungal pathogen considered as an urgent public health threat due to its multidrug resistance, high transmissibility among patients in health-care facilities and elevated crude mortality (1). Other Candida species such as C. parapsilosis, C. tropicalis, C. guilliermondii and the azole-resistant yeasts C. glabrata and C. krusei are also frequent cause of Candidiasis and vulvovaginal infections (2–4). Current anti-Candida therapeutics suffer from diverse limitations including toxicity, resistance and interactions with other commonly prescribed drugs. This has led to increasing interest in studying mechanisms underlying resistance and virulence of Candida species with the ultimate goal to identify potential drug targets for novel antifungal therapeutic intervention. However, the diploid nature and the absence of a complete sexual cycle in most of Candida species limit the use of classical genetic approaches to dissect mechanisms controlling fungal fitness and antifungal resistance. Alternatively, applying genome-wide transcriptional methods such as those determining gene expression changes (DNA microarrays and RNA-seq) or genomic occupancy (ChIP-chip and ChIP-seq) in Candida species had significantly contributed to uncovering different facets of fungal biology that are critical for both opportunistic and commensal lifestyles, in addition to antifungal tolerance and resistance (5–17). These approaches had also helped to uncover a surprising extent of evolutionary plasticity of transcriptional regulatory circuits in these species as compared to the model yeast S. cerevisiae (18, 19).

While ChIP-chip and ChIP-seq have been traditionally used to unbiasedly map the binding of a transcriptional regulator (TR), this tool has some limitations that are attributed mainly to TR-DNA crosslinking and DNA shearing by sonication (20). Formaldehyde is commonly used for Protein-DNA crosslinking, however, this chemical preferentially generates protein-protein crosslinks which can cause epitope masking and consequently alters the efficiency of the immunoprecipitation procedure and led to increased signal background noise. Furthermore, DNA fragmentation by sonication can disrupt weak or transient TR-DNA or TR-histone interactions and generate DNA fragments with heterogenous sizes and thus, impede the refinement of binding site identification (21).

To circumvent these limitations, crosslinking- and sonication-free alternative methods has been developed recently (20, 22–25). In one such method termed chromatin endogenous cleavage (ChEC) (26), the TR of interest is fused to the micrococcal nuclease (MNase) in order to fragment unprotected neighboring chromatin upon MNase-activation by calcium (26). ChEC coupled to high-throughput sequencing (ChEC-seq) was efficiently used to map bindings of the generalist transcription factors Reb1, Abf1 and Rap1 in the budding yeast and has provided a high-resolution occupancy with more binding events as compared to ChIP-based tools (20). Additionally, temporal analysis of ChEC-seq data uncovered that TR can have two distinct binding behaviors; a fast binding uncovered by rapid MNase cleavage at locus with robust bona fide TR-binding motif and a second slow cleavage with low-scoring motifs that are preferentially sampled by a given TR. ChEC-seq has been successfully used to define genomic occupancy of the chromatin remodeler RSC complex (Rsc8 subunit) as ChIP procedure was less efficient (27,
Several recent investigations took advantage of ChEC-seq to study the role of different core components of the general transcriptional machinery such as mediators, SAGA, histone acetyltransferases and chromatin “pushers”, on global gene expression control and promoter nucleosome architecture in eukaryotes (29–32).

In this work, we describe a new set of PCR-based MNase-tagging plasmids for *C. albicans* and other *Candida* species to determine genome-wide location of any TR of interest by ChEC-seq. In a proof-of-concept application of ChEC-seq in *C. albicans*, we have selected NsiI that is an ortholog of the DNA-binding protein Reb1 for which genome-wide occupancy were previously established by ChEC-seq in *S. cerevisiae* (20). As our previous effort on mapping occupancy of the *C. albicans* chromatin remodeling complex SWI/SNF by ChIP-tiling arrays had led to a substantial background noise (7), we have used the ChEC-seq assay to obtain a high-resolution binding map of this master regulator of fungal fitness (6). The ChEC-seq procedure described here will allow a high-resolution genomic location definition which will enable a better understanding of transcriptional regulatory circuits that govern fungal fitness and drug resistance in these medically important fungi.

**Methods**

**Strains and media**

*C. albicans* was routinely maintained at 30°C on YPD (1% yeast extract, 2% peptone, 2% dextrose, with 50 mg/ml uridine). The *C. albicans* WT strain SN148 (*his1/his1, leu2/leu2, arg4/arg4, ura3/ura3::imm434, IRO1/iro1::imm434*) (33) used in this study derives from the SC5314 clinical strain. For *C. auris*, the clinical B8441 strain (34) was used for SAT1-MNase-tagging. For spot dilution assays, overnight cultures of both *C. albicans* and *C. auris* were diluted to an OD600 of 1 and fivefold serial dilutions were prepared in distilled water. A total of 4 µl of each dilution were spotted on YPD-agar plates for 1 days at different temperatures (30°C, 37°C and 40°C) and imaged using the SP-imager system.

**Construction of the pFA-MNase plasmids and the “MNase free” control strain**

The pFA-MNase-CaURA3, pFA-MNase-CaHIS1 and pFA-MNase-CaARG4 plasmids were constructed as following: DNA of the 3x FLAG epitope-MNase module was synthesized by Biobasic, with codon optimized for *C. albicans* (a total of eleven CTG codons of the MNase were changed to TTA or TTG). The *PacI*-*AscI* 3x FLAG-MNase fragment was cloned in the *PacI*-*AscI* digested pFA-TAP-CaURA3, pFA-TAP-CaHIS1 and pFA-TAP-CaARG4 (35). For pFA-MNase-SAT1, the pFA-MNase-CaURA3 was double digested with *AscI* and *SacI* restriction enzymes to remove the *URA3* auxotrophy marker. SAT1 marker was amplified from pFA-SAT1 (36) with primers (Table S1) containing restriction sites *AscI*-SacI and cloned into the *AscI*-SacI digested pFA-MNase. The resulting pFA-MNase-SAT1 was sequenced to confirm the integrity of the Sat1 dominant marker.

The MNase-free control strain was constructed as following: The *C. albicans* codon-optimized DNA of the 3xFLAG-Mnase- nuclear localization signal (SV40) construct delimited by *NheI* and *MluI* restriction sites was synthesized. The *Nhel*-*MluI* digested 3xFLAG-Mnase-SV40 fragment was then cloned into Clp-pACT1-CYC vector (37) to ensure constitutive expression of MNase in *C. albicans*. The Clp-pACT1-3xFLAG-Mnase-SV40-CYC plasmid was linearized by *StuI* restriction enzyme and integrated at the RPS1 locus of the SN148 WT strain.
For the *C. auris* MNase control strain, the URA3 auxotrophy marker of the Clp-pACT1-3xFLAG-Mnase-SV40-CYC was replaced by SAT1 as follow: SAT1 marker was amplified from pFA-SAT1 with primers containing restriction sites NotI-NheI (Table S1) and cloned into the NotI-NheI digested Clp-pACT1-3xFLAG-Mnase-SV40-CYC. To allow the integration of the Clp-pACT1-3xFLAG-Mnase-SV40-CYC in the *C. auris* genome, the *C. albicans* RPS1 integrative locus was replaced by a short 900-bp *C. auris* intergenic region CauNI (*C. auris* Neutral Intergenic; PEKT0200001: 871,442-872,342) as following: CauNI region was amplified from the *C. auris* B8441 genomic DNA with primers containing restriction sites NotI-NheI (Table S1) and cloned into the NotI-NheI digested CIp-pACT1-3xFLAG-Mnase-SV40-CYC- SAT1 plasmid. The resulting plasmid was linearized by StuI restriction enzyme and integrated at the CauNI locus of the *C. auris* B8441 strain. The correct integration of the Clp-pACT1-3xFLAG-Mnase-SV40-CYC-SAT1 cassette was verified by PCR. Integration of any exogenous DNA or the Clp-pACT1-3xFLAG-Mnase-SV40-CYC-SAT1 at the CauNI has no impact on the in vitro growth of *C. auris*.

**PCR-based tagging of endogenous loci in *C. albicans* and *C. auris***

*SNF2* (C2_02100W_A) and *NSI1* (C6_03550C_A) were Mnase-tagged in vivo with the Mnase cassette PCR products following the protocol described by Lavoie et al. (35). The MNase cassettes were amplified using 120-bp primer pair with 20 bp of vector sequences (Forward: GGTGACCGATCCTCCGGGT and Reverse: TCGATGATCCAGCTCGT) and 100 bp from *SNF2* (C2_02100W_A) and *NSI1* (C6_03550C_A) (Table S1). PCR reactions were performed in 50 µl volumes with 1 ng pFA-MNase plasmid and the Q5 high fidelity polymerase (New England Biolabs). PCR thermocycling was executed as following: initial denaturing, 98°C for 3 min; 35 cycles using 98°C for 10 sec, 56°C for 30 sec and 72°C for 3 min. PCR products were used directly to transform the WT strain SN148 using lithium acetate transformation protocol (38). Transformants were selected on selective plates and positive colonies were analyzed by PCR to confirm the correct integration of the MNase-tag. For *C. auris*, CauSNF2 (B9J08_001192) and CauNSI1 (B9J08_003000) were both Mnase-tagged in vivo with the Mnase-SAT1 cassette as described for *C. albicans* with the exception that the 20 bp of the reverse vector sequence was: TCTGATATCATCGATGAATTCGAG.

**ChEC-seq procedure**

For each ChEC experiment, saturated overnight cultures of *C. albicans* Mnase tagged and Mnase free strains were diluted to a starting OD<sub>600</sub> of 0.1 in 50 ml YPD medium. Cells were grown at 30°C to an OD<sub>600</sub> of 0.7–0.8. Cells were pelleted at 3,000 g during 5 min and washed three times with 1 ml Buffer A (15 mM Tris pH 7.5, 80 mM KCl, 0.1 mM EGTA, 0.2 mM spermine, 0.5 mM spermidine, one tablet Roche cOmplete EDTA-free mini protease inhibitors, 1 mM PMSF). Cells were then resuspended in 800 µl Buffer A containing 0.1% digitonin (Sigma) and permeabilized for 10 min at 30 °C under shaking. MNase digestions were performed by adding CaCl₂ at final concentration of 5 mM and incubated at the indicated time at 30 °C. At each time point, a total of 200 µl aliquots of the ChEC digestions were transferred to a tube containing 50 µl of 250 mM EGTA to quench MNase digestions. For each factor analyzed, the timepoint “0” corresponds to a condition where MNase were not activated by CaCl₂. Nucleic acids were extracted using MasterPure yeast DNA purification kit (Epicentre, MPY80200) according the manufacturer’ instructions, and resuspended in 50 µl 0.1 X Tris-HCl buffer, pH 8.0. RNAs were
digested with 10 µg RNase A at 37 °C for 20 min. To assess MNase activity, 5 µl of digested DNA of each ChEC time-point (time after CaCl2 addition) was loaded on 1.5% agarose gel. ChEC DNA was subjected to size selection using the Pippin Prep (SageScience) size-selection system with 2% agarose gel cassette allowing the removal of multi-kilobase genomic DNA fragments and the enrichment of 100-400 bp DNA fragments.

**Library preparation, NGS sequencing and peak calling**

The NEBNext Ultra II DNA Library Prep Kit for Illumina was used to construct the ChEC-seq library following the manufacturer’s instruction. The quality, quantity and the size distribution of the libraries were determined using an Agilent Bioanalyzer. A 50-bp paired-end sequencing of DNAs were performed using an Illumina HiSeq 4000 sequencing system. Sequence were trimmed to remove adapters using TRIMMOMATIC (39). Read ends were considered to be MNase cuts and were mapped to the *C. albicans* genome (*Candida albicans* SC5314 assembly 22) (40) using Bowtie2 (41). ChEC alignment and track visualization using bedgraph files were performed as previously described (20, 42). Peaks were determined from the normalized ChEC ratio using MACS2 algorithm (43) with a window size of 60 bp. Cis-regulatory motif enrichment was assessed in the top high scoring 1000 peaks for both Nsi1 and Snf2 using MEME-ChIP software (44).

**Data availability**

The sequences of plasmids pFA-MNase-CaHIS1, pFA-MNase-CaARG4, pFA-MNanse-CaURA3 and pFA-MNase-SAT1 have been submitted to GenBank and have been assigned the following accession numbers: MT181237, MT181238, MT181239 and MT223485. All ChEC-seq data generated in this study were submitted to GEO database under the accession number GSE150063.

**Results and discussion**

**Plasmid toolbox for MNase tagging in *C. albicans* and non-*albicans* Candida species**

We have previously constructed a series of pFA plasmids for C-terminal HA-, TAP- and MYC-tagging in *C. albicans* with the *URA3*, *HIS1* and *ARG4* autotrophy markers (35). Here, we have used these plasmids as a starting point to build a new pFAs plasmids that allow a C-terminal tagging of any protein of interest at its native chromosomal location with the MNase. We synthesized a DNA construct encoding a 3x FLAG epitope and MNase that have been codon-optimized for *C. albicans*. This construct was used to replace the DNA sequence of the TAP-tag in the pFA-TAP-CaURA3, pFA-TAP-CaHIS1 and pFA-TAP-CaARG4 to generate the pFA-MNanse-CaURA3, pFA-MNase-CaHIS1 and pFA-MNase-CaARG4, respectively. These plasmids allow the use of a single 120-bp primer pair (20 bp of vector sequences and 100 bp from the gene to be tagged) for PCR-based tagging of endogenous loci in *C. albicans* (**Figure 1A**). These primers are compatible with the pFA-TAP/HA/MYC (35) and the pFA-XFP tagging systems (36, 45).

We have also constructed the pFA-Mnase-SAT1 plasmid with the dominant selectable marker *SAT1* that confers resistance to the antibiotic nourseothricin for Mnase-tagging in clinical strains of *C. albicans* and non-*albicans* Candida species such as the superbug *C. auris*.

**ChEC-seq experimental procedure**
ChEC-seq has been initially used in *S. cerevisiae* to map the genomic occupancy of canonical general regulatory factors such as the RNA polymerase I Enhancer Binding protein ScReb1 (20). Here, we have selected the Nsi1 (C6_03550C_A), that is the ScReb1 ortholog in *C. albicans*, to perform ChEC-seq. Additionally, we were also interested in the catalytic subunit of the SWI/SNF complex, Snf2, to explore the potential of ChEC-seq in mapping genomic occupancy of chromatin remodeling complexes. We have previously mapped the genomic location of the *C. albicans* Snf6 that is a fungus specific SWI/SNF subunit using ChIP coupled to high-density tiling arrays (7). Thus, the SWI/SNF genome-wide binding data generated by ChEC-seq can be compared to those of ChIP-chip to assess the sensitivity of each technique. We also generated an MNase control strain (“free MNase”) with a 3xFLAG-tagged MNase module fused to an SV40 nuclear localization signal under the control of ACT1 and integrated at the *RPS1* locus. Constitutive expression of MNase (Free MNase) or MNase-tagging of Nsi1 or Snf2 had no perceptible effect on the growth of *C. albicans* at different temperatures (Figure 1B).

Both *C. auris* Snf2 and Nsi1 orthologs were also MNase-tagged using PCR cassettes generated from the pFA-Mnase-SAT1 plasmid. For the MNase control strain, 3xFLAG-tagged MNase module was inserted into the neutral intergenic locus *CauNI* where integration has no effect on the *in vitro* fitness of *C. auris* (Figure 1B). As for *C. albicans*, MNase-tagging of CauNsi1 and CauSnf2 does not affect the growth of *C. auris* (Figure 1B).

To provide a proof of principle for using ChEC in *Candida* spp., we focused our effort on *C. albicans*. The *S. cerevisiae* ChEC procedure described by Zentner et al. (20) (Figure 1C) was followed with some modifications. *C. albicans* cells were permeabilized with digitonin for 10 min prior to MNase activation with 5mM CaCl₂. We presumed that the treatment of permeabilized cells with calcium would engender both specific and non-specific cleavages. We therefore made a size selection of the ChEC DNA before preparing the sequencing library to enrich small fragments less than 400 bp. Prior to size selection and for each transcriptional regulator and the free MNase strain, we analyzed the kinetic of DNA digestions by agarose gel electrophoresis. Analysis of minute-scale timepoints revealed notable smearing of genomic DNA of all TF–MNase fusions by the 5 min time point. This pattern increased over time until 60 min. In contrast, digestion in the free MNase strain yielded earlier a smearing by 30 s (Figure 1D). The 5, 20 and 60 min digestion times were selected for both Snf2 and Nsi1 ChEC-seq experiments. Size selection was performed using the Pippin Prep size-selection system with 2% agarose gel cassette. The goal of this stage is to remove multi-kilobase fragments of genomic DNA and enriched a small fragment. The 2% Agarose gel cassettes allows enrichment of DNA fragments below 100-400 bp. Alternatively, size selection could be performed using the paramagnetic beads for size selection and buffer exchange steps such as the AMPure XP cleanup kit (Beckman coulter) (20, 46).

**Genome-wide binding of Nsi1 and Snf2 by ChEC-seq**

To assess the ChEC-seq performance in *C. albicans*, we have chosen to map the genomic occupancy of Nsi1 that is an ortholog of the DNA-binding protein Reb1 for which the genome-wide occupancy were previously established by ChEC-seq in *S. cerevisiae* (20). We detected 2548, 4771 and 4523 Nsi1 peaks upon 5, 20 and 60 min MNase activation, respectively (Table S2 and Figure 2A). *De novo* motif analysis of intergenic bound Nsi1 regions showed a significant enrichment of the *bona fide* Reb1/Nsi1 site (TTACCCGG) at 5 min while a non-
specific long AC/TG-rich sequence was the most enriched at 20 and 60 min (Figure 2B). This suggest that the 5 min MNase cleavage mapped the Nsi1 fast class binding events while 20 and 60 min correspond to the slow class binding that lack robust consensus motif. Thus, as for S. cerevisiae, our ChEC-seq data recapitulated the time-dependent binding behavior of transcriptional regulators and can be used to map early high-affinity interactions with consensus motifs and sequence that are preferentially sampled by a given protein (20). Our genome-wide occupancy data recapitulated the overall functions of either Nsi1 or Reb1 in S. cerevisiae as reflected by Nsi1 binding to the promoter of ribosome biogenesis and rRNA genes (Figure 2C-F) (47–49).

ChEC-seq of Snf2 identified 4145, 6446 and 6215 peaks at 5, 20 and 60 min MNase cleavage, respectively, which is 10-fold higher than the number of peaks detected under similar growth conditions by ChIP-tiling array of the SWI/SNF subunit, Snf6 (7) (Figure 3A-B and Table S3). As for Nsi1, the 20 and 60 min ChEC-seq data were similar and might capture the slow sites. The Snf2 fast bound promoters were enriched mainly in carbohydrate metabolism mirroring the previously characterized role of the SWI/SNF complex in C. albicans (Figure 3C) (6, 7). Snf2 occupied promoters of hexose transport and carbon utilization genes (galactolysis) that were shown to be modulated by the SWI/SNF subunit Snf5 (6) (Figure 3D).

**Conclusion**

We have constructed a new set of PCR-based MNase-tagging plasmids to map genomic occupancy of different transcriptional regulators in the human pathogenic yeast C. albicans and other non-albicans Candida species. Compared the other ChIP-based techniques, the ChEC procedure relies on total DNA extraction instead of chromatin solubilization and does not require protein-DNA crosslinking or sonication avoiding thus artefacts related to epitope masking or the hyper-ChIPable euchromatic phenomenon (50, 51). So far, ChEC has been exclusively used in the model yeast S. cerevisiae to map chromatin occupancy of general transcriptional regulators (42), chromatin remodelers (27, 30, 52) and histone modifiers (31, 32) in addition to transcription factors (53, 54). As many transcriptional regulators and chromatin remodelers are key virulence and drug resistance factors in C. albicans and other fungi (6, 13, 17, 55–57), the ChEC-seq represents thus an attracting tool to unbiasedly decipher transcriptional regulatory networks of fungal fitness.

**Acknowledgment**

We thank Benjamin Albert and David Shore (University of Geneva) for sharing reagents and their technical guidance regarding the ChEC protocol. This work was supported by funds from the Canadian Institutes for Health Research project grant (CIHR, grant IC118460) and the start-up funds from the Montréal Heart Institute (MHI) to AS. AS is a recipient of the Fonds de Recherche du Québec-Santé (FRQS) J2 salary award.

**References**

1. Sabino R, Veríssimo C, Pereira ÁA, Antunes F. 2020. Candida Auris, An Agent of Hospital-Associated Outbreaks: Which Challenging Issues Do We Need to Have in Mind? 2. Microorganisms 8:181.
2. Dufresne SF, Cole DC, Denning DW, Sheppard DC. 2017. Serious fungal infections in Canada. Eur J Clin Microbiol Infect Dis 36:987–992.
3. Gabaldón T, Naranjo-Ortíz MA, Marcet-Houben M. 2016. Evolutionary genomics of yeast pathogens in the Saccharomycotina. FEMS Yeast Res 16.
4. Turner SA, Butler G. 2014. The Candida Pathogenic Species Complex. Cold Spring Harb Perspect Med 4:a019778.
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.
6. Burgain A, Pic É, Markey L, Tebbji F, Kumamoto CA, Sellam A. 2019. A novel genetic circuitry governing hypoxic metabolic flexibility, commensalism and virulence in the fungal pathogen Candida albicans. PLOS Pathog 15:e1007823.
7. Tebbji F, Chen Y, Sellam A, Whiteway M. 2017. The Genomic Landscape of the Fungus-Specific SWI/SNF Complex Subunit, Snf6, in Candida albicans. mSphere 2:e00497-17.
8. Askew C, Sellam A, Epp E, Hogues H, Mullick A, Nantel A, Whiteway M. 2009. Transcriptional regulation of carbohydrate metabolism in the human pathogen Candida albicans. PLoS Pathog 5:e1000612.
9. Perez JC, Kumamoto CA, Johnson AD. 2013. Candida albicans commensalism and pathogenicity are intertwined traits directed by a tightly knit transcriptional regulatory circuit. PLoS Biol 11:e1001510.
10. Nobile CJ, Fox EP, Nett JE, Sorrells TR, Mitrovich QM, Hernday AD, Tuch BB, Andes DR, Johnson AD. 2012. A recently evolved transcriptional network controls biofilm development in Candida albicans. Cell 148:126–138.
11. Nobile CJ, Nett JE, Hernday AD, Homann OR, Deneault J-S, Nantel A, Andes DR, Johnson AD, Mitchell AP. 2009. Biofilm Matrix Regulation by Candida albicans Zap1. PLOS Biol 7:e1000133.
12. Tierney L, Linde J, Müller S, Brunke S, Molina J, Hube B, Schöck U, Guthke R, Kuchler K. 2012. An Interspecies Regulatory Network Inferred from Simultaneous RNA-seq of Candida albicans Invading Innate Immune Cells. Front Microbiol 3:85.
13. Price RJ, Weindling E, Berman J, Buscaino A. 2019. Chromatin Profiling of the Repetitive and Nonrepetitive Genomes of the Human Fungal Pathogen Candida albicans. mBio 10:e01376-19.
14. Tucey TM, Verma J, Harrison PF, Snelgrove SL, Lo TL, Scherer AK, Barughahare AA, Powell DR, Wheeler RT, Hickey MJ, Beilharz TH, Naderer T, Traven A. 2018. Glucose Homeostasis Is Important for Immune Cell Viability during Candida Challenge and Host Survival of Systemic Fungal Infection. Cell Metab 27:988-1006.e7.
15. 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.
16. Shapiro RS, Robbins N, Cowen LE. 2011. Regulatory circuitry governing fungal development, drug resistance, and disease. Microbiol Mol Biol Rev 75:213–267.
17. Villa S, Hamideh M, Weinstock A, Qasim MN, Hazbun TR, Sellam A, Hernday AD, Thangamani S. 2020. Transcriptional control of hyphal morphogenesis in Candida albicans. FEMS Yeast Res 20.
18. Lavoie H, Hogues H, Whiteway M. 2009. Rearrangements of the transcriptional regulatory networks of metabolic pathways in fungi. Curr Opin Microbiol 12:655–663.
19. Li H, Johnson AD. 2010. Evolution of transcription networks–lessons from yeasts. Curr Biol 20:R746–53.
20. Zentner GE, Kasinathan S, Xin B, Rohs R, Henikoff S. 2015. ChEC-seq kinetics discriminates transcription factor binding sites by DNA sequence and shape in vivo. Nat Commun 6:1–13.
21. Zentner GE, Henikoff S. 2014. High-resolution digital profiling of the epigenome. Nat Rev Genet 15:814–827.
22. Steensel B van, Henikoff S. 2000. Identification of in vivo DNA targets of chromatin proteins using tethered Dam methyltransferase. Nat Biotechnol 18:424–428.
23. Wang H, Mayhew D, Chen X, Johnston M, Mitra RD. 2012. “Calling cards” for DNA-binding proteins in mammalian cells. Genetics 2012/01/03. 190:941–949.
24. Kasinathan S, Orsi GA, Zentner GE, Ahmad K, Henikoff S. 2014. High-resolution mapping of transcription factor binding sites on native chromatin. Nat Methods 2013/12/15. 11:203–209.
25. Meers MP, Bryson TD, Henikoff JG, Henikoff S. 2019. Improved CUT&RUN chromatin profiling tools. eLife 8:e46314.
26. Schmid M, Durussel T, Laemmli UK. 2004. ChIC and ChEC: Genomic Mapping of Chromatin Proteins. Mol Cell 16:147–157.
27. Kubik S, O’Duibhir E, de Jonge WJ, Mattarocci S, Albert B, Falcone J-L, Bruzzone MJ, Holstege FCP, Shore D. 2018. Sequence-Directed Action of RSC Remodeler and General Regulatory Factors Modulates +1 Nucleosome Position to Facilitate Transcription. Mol Cell 71:89-102.e5.
28. Yen K, Vinayachandran V, Batta K, Koerber RT, Pugh BF. 2012. Genome-wide Nucleosome Specificity and Directionality of Chromatin Remodelers. Cell 149:1461–1473.
29. Tourigny JP, Saleh MM, Schumacher K, Devys D, Zentner GE. 2018. Mediator Is Essential for Small Nuclear and Nucleolar RNA Transcription in Yeast. Mol Cell Biol 38:e00296-18.
30. Kubik S, Bruzzone MJ, Challal D, Dreos R, Mattarocci S, Bucher P, Libri D, Shore D. 2019. Opposing chromatin remodelers control transcription initiation frequency and start site selection. Nat Struct Mol Biol 26:744–754.
31. Bruzzone MJ, Grünberg S, Kubik S, Zentner GE, Shore D. 2018. Distinct patterns of histone acetyltransferase and Mediator deployment at yeast protein-coding genes. Genes Dev.
32. Baptista T, Grünberg S, Minoungou N, Koster MJE, Timmers HTM, Hahn S, Devys D, Tora L. 2017. SAGA Is a General Cofactor for RNA Polymerase II Transcription. Mol Cell 68:130-143.e5.
33. Noble SM, Johnson AD. 2005. Strains and strategies for large-scale gene deletion studies of the diploid human fungal pathogen Candida albicans. Eukaryot Cell 4:298–309.
34. Lockhart SR, Etienne KA, Vallabhaneni S, Farooqi J, Chowdhary A, Govender NP, Colombo AL, Calvo B, Cuomo CA, Desjardins CA, Berkow EL, Castanheira M, Magobo RE, Jabeen K, Asghar RJ, Meis JF, Jackson B, Chiller T, Litvintseva AP. 2016. Simultaneous Emergence of Multidrug-Resistant Candida auris on 3 Continents Confirmed by Whole-Genome Sequencing and Epidemiological Analyses. Clin Infect Dis 64:134–140.
35. Lavoie H, Sellam A, Askew C, Nantel A, Whiteway M. 2008. A toolbox for epitope-tagging and genome-wide location analysis in Candida albicans. Bmc Genomics 9:578.
36. Schaub Y, Dunkler A, Walther A, Wendland J. 2006. New pFA-cassettes for PCR-based gene manipulation in Candida albicans. J Basic Microbiol 46:416–429.
37. Blackwell C, Russell CL, Argimon S, Brown AJ, Brown JD. 2003. Protein A-tagging for purification of native macromolecular complexes from Candida albicans. Yeast 20:1235–1241.
38. Hermday AD, Noble SM, Mitrovich QM, Johnson AD. 2010. Genetics and molecular biology in Candida albicans. Methods Enzymol 470:737–758.
39. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimer for Illumina sequence data. Bioinformatics 30:2114–2120.
40. Skrzypek MS, Binkley J, Binkley G, Miyasato SR, Simison M, Sherlock G. 2017. The Candida Genome Database (CGD): incorporation of Assembly 22, systematic identifiers and visualization of high throughput sequencing data. Nucleic Acids Res 45:D592–D596.
41. Langmead B, Salzberg SL. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9:357–359.
42. Grünberg S, Henikoff S, Hahn S, Zentner GE. 2016. Mediator binding to UASs is broadly uncoupled from transcription and cooperative with TFIID recruitment to promoters. EMBO J 35:2435–2446.
43. Zhang Y, Liu T, Meyer CA, Eeckhoute J, Johnson DS, Bernstein BE, Nusbaum C, Myers RM, Brown M, Li W, Liu XS. 2008. Model-based analysis of ChIP-Seq (MACS). Genome Biol 9:R137.
44. Machanick P, Bailey TL. 2011. MEME-ChIP: motif analysis of large DNA datasets. Bioinformatics 27:1696–1697.
45. Gola S, Martin R, Walther A, Dunkler A, Wendland J. 2003. New modules for PCR-based gene targeting in Candida albicans: rapid and efficient gene targeting using 100 bp of flanking homology region. Yeast 20:1339–1347.
46. Grünberg S, Zentner GE. 2017. Genome-wide Mapping of Protein-DNA Interactions with ChEC-seq in Saccharomyces cerevisiae. J Vis Exp JoVE 55836.
47. Bosio MC, Fermi B, Spagnoli G, Levati E, Rubbi L, Ferrari R, Pellegrini M, Dieci G. 2017. Abf1 and other general regulatory factors control ribosome biogenesis gene expression in budding yeast. Nucleic Acids Res 45:4493–4506.
48. The Reb1 homologue Ydr026c/Nsi1 is required for efficient RNA polymerase I termination in yeast | The EMBO Journal.
49. Lang WH, Reeder RH. 1993. The REB1 site is an essential component of a terminator for RNA polymerase I in Saccharomyces cerevisiae. Mol Cell Biol 13:649–658.
50. Teytelman L, Thurtle DM, Rine J, van Oudenaarden A. 2013. Highly expressed loci are vulnerable to misleading ChIP localization of multiple unrelated proteins. Proc Natl Acad Sci 110:18602.
51. Park D, Lee Y, Bhupindersingh G, Iyer VR. 2013. Widespread Misinterpretable ChIP-seq Bias in Yeast. PLOS ONE 8:e83506.
52. Biernat E, Kinney J, Dunlap K, Rizza C, Govind CK. 2020. The RSC complex remodels nucleosomes in transcribed coding sequences and promotes transcription in Saccharomyces cerevisiae. bioRxiv 2020.03.11.987974.
53. Albert B, Tomassetti S, Gloor Y, Diig D, Mattarocci S, Kubik S, Hafner L, Shore D. 2019. Sfp1 regulates transcriptional networks driving cell growth and division through multiple promoter-binding modes. Genes Dev 33:288–293.
54. Bar-Ziv R, Brodsky S, Chapal M, Barkai N. 2020. Transcription Factor Binding to Replicated DNA. Cell Rep 30:3989-3995.e4.
55. Nishikawa JL, Boeszoermenyi A, Vale-Silva LA, Torelli R, Posteraro B, Sohn Y-J, Ji F, Gelev V, Sanglard D, Sanguinetti M, Sadreyev RI, Mukherjee G, Bhryavabhotla J, Buhrlage SJ, Gray NS, Wagner G, Näär AM, Arthanari H. 2016. Inhibiting fungal multidrug resistance by disrupting an activator–Mediator interaction. Nature 530:485–489.
Figure legends

**Figure 1. ChEC-seq method in *C. albicans* and *Candida spp.***

(A) Plasmid constructs for *in vivo* TR-MNase tagging and the construction of the “free MNase” control strains in *C. albicans* and *C. auris*. (B) Phenotypic characterization of strains bearing the MNase-tagged Nsi1 and Snf2 TRs, and the “free MNase” control constructs in *C. albicans* and *C. auris*. For both species, WT, Snf2<sup>MNase</sup>, Nsi1<sup>MNase</sup>, the free MNase and the control (empty vector) strains were serially diluted, spotted on YPD and incubated for one day at different temperatures. (C) Schematic representation of the experimental setup of the ChEC-seq methodology. *Candida spp.* cells where a TR of interest is fused to MNase are permeabilized with digitonin prior to MNase activation with calcium. This will lead to the fragmentation of unprotected neighboring chromatin. The resulting fragmented DNA is purified and subjected to size selection prior to high-throughput sequencing. (D) Evaluation of genomic DNA fragmentation by agarose gel electrophoresis at 0, 0.5, 1, 2.5, 5, 10, 20 and 60 minutes of calcium exposure in the Snf2<sup>MNase</sup>, Nsi1<sup>MNase</sup> and the free MNase strains in *C. albicans*. L: DNA ladder.

**Figure 2. Genome-wide occupancy of the transcription factor Nsi1 with ChEC-seq.**

(A) Temporal analysis of Nsi11 binding events. Venn diagram showing the overlap of Nsi1 binding events at three distinct MNase activation times (5, 20 and 60 min). (B) Motif scores for Nsi1 bound promoters at 5, 20 and 60 min after MNase activation. The motif logos were generated using MEME-ChIP software on the 1000 high scoring peaks. (C-F) Snapshot of genomic regions showing the ChEC-seq signal for Nsi1<sup>MNase</sup> and the free MNase strains at 5, 20 and 60 min after MNase activation. The position of Nsi1 motifs are indicated by the dashed lines. Nsi1 occupies the promoter of *MLC1* (C), *AIM7* (D) and *ASM4* (E), in addition to many sites within the rDNA locus (F).

**Figure 3. Genome-wide occupancy of the chromatin remodeler Snf2 with ChEC-seq.**

(A) Venn diagram showing the overlap of Snf2 promoter bindings at 5, 20 and 60 min after MNase activation. (B) Comparison of SWI/SNF genomic occupancies by ChEC-seq and ChIP-chip method. Venn diagram of overlap between Snf2 ChEC-seq sites and Snf6 ChIP-chip using high-density tiling arrays. (C) Gene ontology of biological process associated with Snf2-bound promoters at 5 min after MNase activation. The *P* values were calculated using hypergeometric distribution as described in the GO Term Finder Tool website (http://candidagenome.org/cgi-bin/GO/goTermFinder). (D) Snapshot of genomic regions showing the ChEC-seq signal for Snf2<sup>MNase</sup> and the free MNase strains at 5, 20 and 60 min after MNase activation. Genome browser view of Snf2 ChEC-seq displaying promoter occupancies of carbohydrate metabolism genes including galactolysis (*GAL1, GAL10*) and hexose transport (*SHA3, HGT2, HGT1*).
Supplementary data.
Table S1. List of primers used in this study.
Table S2. List of Nsi1 binding peaks
Table S3. List of Snf2 binding peaks
**A**

MNase tagging

- **FLAG**
- MNase
- Ura3, His1, Arg4, Sat1

Marker

**in vivo “free MNase”**

- **pACT1**
- FLAG
- MNase-SV40
- CIC
- Ura3 or Sat1

Marker

Insertion at the *RPS1* or the CauNl locus

**B**

|         | 30°C | 37°C | 40°C |
|---------|------|------|------|
| WT(SN148)            |      |      |      |
| SNF2<sup>MNase</sup> |      |      |      |
| NSI1<sup>MNase</sup> |      |      |      |
| ClpAct-MNase          |      |      |      |
| ClpAct-URA3           |      |      |      |
| WT(B8441)            |      |      |      |
| CauSNF2<sup>MNase</sup> |      |      |      |
| CauNSI1<sup>MNase</sup> |      |      |      |
| ClpAct-MNase          |      |      |      |
| ClpAct-SAT1           |      |      |      |

C. albicans

C. auris

**C**

Cell permeabilization & MNase activation by CaCl<sub>2</sub>

**D**

Time after Ca<sup>2+</sup> addition (min)

- **L**
- 0
- 0.5
- 1
- 2.5
- 5
- 10
- 20
- 60

**DNA fragments**

DNA size selection & preparation of sequencing library

**MNase free**

**Nsi1-MNase**

**Snf2-MNase**
