Curr Genet (2018) 64:81–86
https://doi.org/10.1007/s00294-017-0726-3

REVIEW

# The evolution of a G1/S transcriptional network in yeasts

**Adi Hendler[1] · Edgar M. Medina[2,3] · Nicolas E. Buchler[2,3] · Robertus A. M. de Bruin[4] · Amir Aharoni[1]**

Received: 3 July 2017 / Revised: 11 July 2017 / Accepted: 17 July 2017 / Published online: 25 July 2017


**Abstract** The G1-to-S cell cycle transition is promoted by the periodic expression of a large set of genes. In *Saccharomyces cerevisiae* G1/S gene expression is regulated by two transcription factor (TF) complexes, the MBF and SBF, which bind to specific DNA sequences, the MCB and SCB, respectively. Despite extensive research little is known regarding the evolution of the G1/S transcription regulation including the co-evolution of the DNA binding domains with their respective DNA binding sequences. We have recently examined the co-evolution of the G1/S TF specificity through the systematic generation and examination of chimeric Mbp1/Swi4 TFs containing different orthologue DNA binding domains in *S. cerevisiae* (Hendler et al. in PLoS Genet 13:e1006778. doi:10.1371/journal.pgen.1006778, 2017). Here, we review the co-evolution of G1/S transcriptional network and discuss the evolutionary dynamics and specificity of the MBF–MCB and SBF–SCB interactions in different fungal species.

Communicated by M. Kupiec.

✉ Nicolas E. Buchler
nicolas.buchler@duke.edu

✉ Robertus A. M. de Bruin
r.debruin@ucl.ac.uk

✉ Amir Aharoni
aaharoni@bgu.ac.il

[1] Department of Life Sciences and the National Institute for Biotechnology in the Negev, Ben-Gurion University of the Negev, 84105 Beersheba, Israel

[2] Department of Biology, Duke University, Durham, NC, USA

[3] Center for Genomic and Computational Biology, Duke University, Durham, NC, USA

[4] MRC Laboratory for Molecular Cell Biology, University College London, London WC1E 6BT, UK

**Keywords** Cell cycle · Evolution · Gene expression · G1-to-S transition · MBF and SBF

In eukaryotes, different steps of the cell cycle are promoted by waves of expression of large sets of genes (Benanti 2016; Granovskaia et al. 2010; Spellman et al. 1998; Whitfield et al. 2002). Co-regulated genes whose expression peaks at the G1-to-S transition promotes entry into S phase and enables the initiation (Start) of a new cell cycle. Although G1-to-S gene expression is regulated in many eukaryotes (Bahler 2005; Bar-Joseph et al. 2008; Bertoli et al. 2013; Côte et al. 2009), some regulators of the G1-to-S transition (e.g., transcription factors) are not conserved between Fungi and Metazoans (Cross et al. 2002; Medina et al. 2016). Currently, little is known regarding the evolution and specificity of the key TFs promoting G1/S gene expression in Fungi. In *Saccharomyces cerevisiae* budding yeast, MBF and SBF are the two protein complexes regulating G1/S transcription program (Amon et al. 1993; Bean et al. 2005; de Bruin et al. 2006). The MBF and SBF complexes contain a common Swi6 protein and the Mbp1 and Swi4 DNA binding proteins, respectively. In *Candida albicans*, both MBF and SBF complexes were identified, however, the mechanism and control of the G1/S transcription program are different from those in *S. cerevisiae* (see below for details) (Côte et al. 2009; Hussein et al. 2011; Ofir et al. 2012). Finally, in the *S. pombe* fission yeast, a related tetrameric complex containing the Cdc10 subunits with Res1 and Res2 DNA binding proteins regulates G1/S gene expression (Bahler 2005).

In budding yeast, the SBF and MBF were shown to regulate distinct branches of the G1/S transcriptional network where SBF promotes the expression of genes involved in morphogenesis including budding and MBF promotes the expression of genes involved in DNA replication and

repair (Bean et al. 2005; Ferrezuelo et al. 2010; Wittenberg and Reed 2005). The MBF complex can bind promoter sequences containing the MCB (*MluI* Cell-cycle Box) recognition sequence *ACGCGT* that is conserved across many fungal species including *C. albicans* (Côte et al. 2009) and *S. pombe* (Rustici et al. 2004). In contrast, the SCB (Swi4 Cell-cycle Box) recognition sequence *CRCGAAA*, bound by the SBF complex, is only found in budding yeasts including *S. cerevisiae*. Thus, it is generally assumed that ancestral Res (the progenitor of Swi4 and Mbp1 in Hemiascomycetes) bound an MCB-like motif (which we will call RCB) and that SCB is the more specialized DNA binding motif that emerged after Res duplication. This scenario represents a classic case of neofunctionalization after gene duplication, where one of the paralogs (Swi4) evolves a new function and DNA binding specificity (SCB) to regulate old and new G1/S target genes (Voordeckers et al. 2015).

## New insights regarding MBF and SBF evolution

Despite extensive studies in different organisms, relatively little was known regarding how Swi4 and Mbp1 DNA binding domains (DBDs) co-evolved to recognize the SCB and MCB DNA binding sequences, respectively, to synchronize the expression of a large set of genes during the G1-to-S transition. To address these questions, we recently generated and examined the function of different chimeric Mbp1 and Swi4 TFs in *S. cerevisiae* (Hendler et al. 2017). Specifically, we generated 16 different chimeric TFs by systematic replacements of native *S. cerevisiae* DBD in Mbp1 and Swi4 with orthologs from different fungal species of different clades. Examination of these chimeric TFs revealed that all TFs containing the DBD of orthologs of distant Hemiascomycetes and other fungi fused to *S. cerevisiae* Mbp1 activation domain (AD) were unable to complement the *S. cerevisiae* Mbp1 suggesting that the Mbp1 regulator in *S. cerevisiae* evolved relatively recently. In contrast, we found that chimeric TFs containing the DBD of distant orthologs fused to *S. cerevisiae* Swi4 AD can complement the native *S. cerevisiae* Swi4. Detailed examination of the phenotype of *S. cerevisiae* strains expressing the different chimeric TFs lacking the endogenous Mbp1 and Swi4 showed different levels of complementation. We found that while chimeric TFs containing closely related DBDs (e.g., from *K. lactis*, *C. albicans*) did not lead to significant phenotypic defects, chimeric TFs containing distantly related DBDs (e.g., from *Y. lipolytica*, *N. crassa*, *S. pombe*) led to slow growth rate and severe morphological defects upon cell growth, budding and division (Hendler et al. 2017).

Using genome wide expression analysis, we found that these chimeric TFs lead to the expression of a progressively limited subset of SBF-dependent target genes (Fig. 1). Interestingly, bioinformatics analysis of these transcription programs showed that the subset of SBF-targets regulated by the chimeric TFs contain motifs more closely related to MCB consistent with a Res-like ancestor found in *S. pombe*. These findings suggest that Swi4 network expansion took place by expanding the ancestral SBF regulon, which contained MCB motifs, via inclusion of the modern SCB motif (Figs. 1, 2). Further support for the functional division of the SBF regulon to "modern" genes containing SCB motifs and "ancient" genes containing MCB motifs came from chromatin

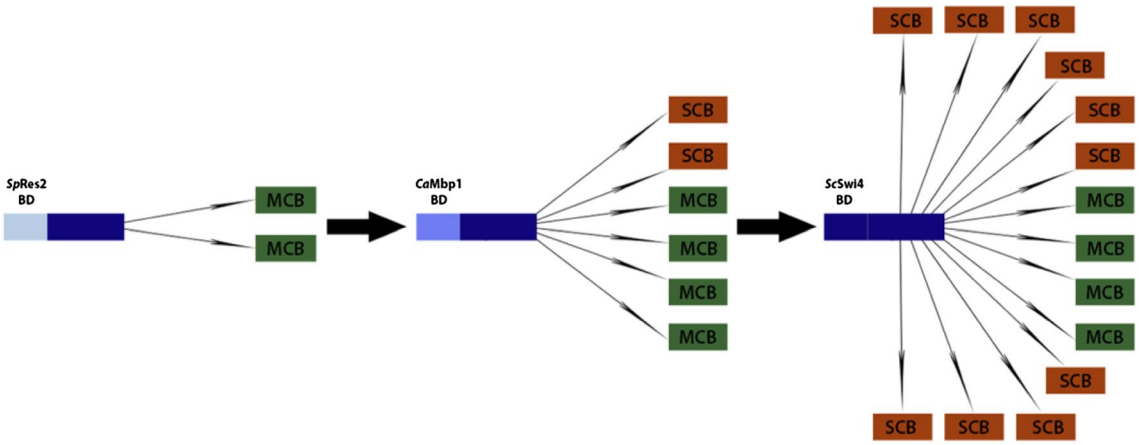


**Fig. 1** Schematic representation of genome wide transcription analysis in *S. cerevisiae* of chimeric Swi4 TFs containing orthologs DBDs from different fungal species (Hendler et al. 2017). The chimeric TF containing orthologue DBD from *S. pombe* (Res2) leads to the expression of ~11% of SBF-dependent target genes while in *S. cerevisiae* chimeric TF containing Mbp1 DBD from *C. albicans* leads to the expression of ~%40 of SBF-dependent target genes. These subsets of genes are enriched with motifs that are more closely related to MCB consistent with a Res-like ancestor found in *S. pombe*. The expression of a smaller subset of genes, in some chimeric TFs, leads to phenotypic defects including slow growth rate and morphological abnormalities (Hendler et al. 2017). A small number of genes containing MCB or SCB motifs that are expressed in *S. cerevisiae* by the chimeric TF are shown for illustration

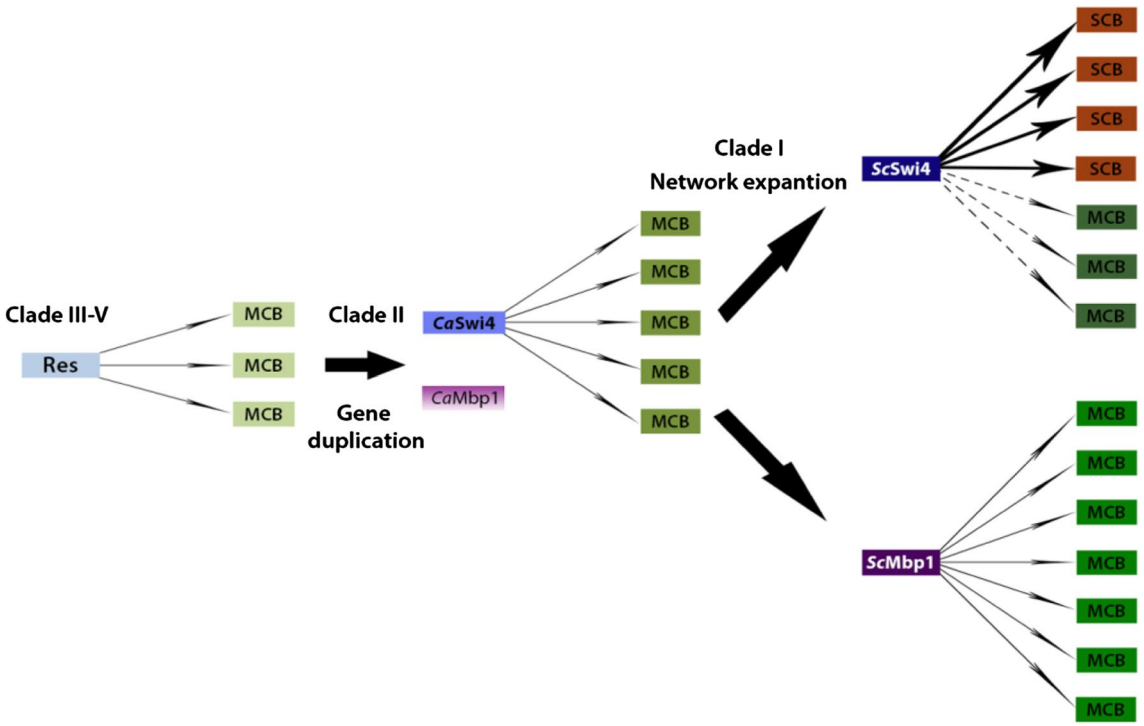


**Fig. 2** The evolution of Swi4 and Mbp1 in different fungi species. Res TFs (*light blue*) from clades III–V promote the expression of a smaller set of genes containing MCB motif, relative to *S. cerevisiae* G1/S targets (Rustici et al. 2004). Swi4 (*blue*) in *C. albicans* was shown to mediate the expression of G1/S genes containing MCB motif in their promoter while Mbp1 (*pink*) was shown to be non-functional (Hussein et al. 2011). In clade I both Swi4 (*dark blue*) and Mbp1 (*purple*) are functional mediating the expression of genes containing SCB and MCB, respectively, in their promoter. Analysis of Swi4/Mbp1 chimeric TFs in *S. cerevisiae* (Hendler et al. 2017) indicates that the Swi4 regulon contains both SCB and MCB motifs where the SCB motifs are optimized for binding to Swi4 (*bold arrows*)

immunoprecipitation (ChIP) experiments. We found that *S. cerevisiae* Swi4 exhibits much higher affinity for the SCB motif relative to the MCB-like motif while the chimeric TFs containing distantly related DBDs can only bind the MCB-like motif (Hendler et al. 2017). These result suggest that the *S. cerevisiae* Swi4 evolved for optimized binding to the SCB motif to enable normal cell growth and morphogenesis. In general, these results reveal that transcription network expansion can depend on gradual co-evolution of the DBD with diverse promoters to include genes containing new regulatory motifs for optimizing cellular fitness.

## Conservation of Swi4 function and regulation

In *S. cerevisiae*, the SBF and MBF complexes exhibit high functional overlap. It was shown that a single *swi4*- or *mbp1*-deletion leads to moderate phenotypic effects, however, the double *swi4*- and *mbp1*-deletion leads to non-viability. Despite the high functional overlap between SBF and MBF complexes, extensive research in the past decade revealed significantly different mechanism of regulation between the two complexes (Costanzo et al. 2004; de Bruin et al. 2004, 2006). While SBF is a transcriptional activator required to activate G1/S transcription during G1, MBF is a transcriptional repressor that inhibits transcription outside of G1. In accordance, inactivation of SBF inhibits the expression of G1/S targets, while inactivation of MBF leads to constitutively high levels of its G1/S targets. In *S. cerevisiae*, two repressors, Whi5 and Nrm1, were previously shown to regulate SBF and MBF transcription, respectively (de Bruin et al. 2004, 2006). Whi5 was shown to bind and repress SBF activity in G1 and transcription is activated by G1-cyclin/CDK phosphorylation of Whi5, which shuttles it out of the nucleus (Costanzo et al. 2004; de Bruin et al. 2004). Upon S phase entry SBF-dependent transcription is inactivated via Clb/CDK phosphorylation of Swi4, which disrupts promoter-binding. MBF-dependent transcription is inactivated by Nrm1 via an auto-regulatory negative feedback loop that is present in both *S. cerevisiae* and *S. pombe* (de Bruin et al. 2006). Nrm1, a G1/S target itself, is a co-repressor that accumulates upon S phase entry and binds MBF to repress transcription. Nrm1/Whi5 homologues are also identified in *C. albicans* and have been shown to complement the *whi5*- and *nrm1*-deletion in *S. cerevisiae* (Ofir et al. 2012). However, functional analysis indicated

that the *C. albicans* Nrm1 is more similar to *S. cerevisiae* Whi5 due to its direct binding to *C. albicans* Swi4 (Ofir et al. 2012). In addition, the intracellular localization of *C. albicans* Nrm1 oscillates through the cell cycle similar to *S. cerevisiae* Whi5. Additional studies have revealed the functional importance of SBF in *C. albicans* by examining the phenotypes of *mbp1*- and *swi4*-deletion strains. This study showed that while *swi4*-deletion leads to significant phenotypic defects *mbp1*-deletion had mild phenotypic defects (Hussein et al. 2011). Overall, these studies highlight the importance of SBF complex in *C. albicans* and highlight the plasticity of G1/S regulation within hemiascomycetes.

## MBF and SBF specificity

The functional overlap between MBF and SBF complexes in *S. cerevisiae* as well as the small differences in sequence of the MCB and SCB motifs (Bean et al. 2005) highlight the difficulty in understanding the promoter specificity of these complexes. Whilst all yeast species contain MCB motifs in their genome it is unclear whether *S. cerevisiae* MBF is more similar to the ancestral TF complex. Our findings that the SBF regulon in *S. cerevisiae* contains a subset of targets containing MCB-like sequences and that chimeric TFs containing distantly related DBDs bind MCB-like motifs in *S. cerevisiae* (Fig. 1) suggest that SBF is more closely related to the ancestral TF complex and that MCB-like sequences are likely the ancestral MCB/SCB motifs (RCB). Examination of SBF binding to different promoters in *S. cerevisiae* using ChIP revealed that the binding affinity of *S. cerevisiae* SBF to SCB motifs is much higher than to RCB motifs (Hendler et al. 2017) showing that the *S. cerevisiae* SBF must co-evolved with the SCB to enable high binding affinity.

Previous biochemical studies, examining the binding of Mbp1 and Swi4 DBDs to oligonucleotide duplexes containing one copy of MCB and SCB sequences, showed similar binding affinities of Mbp1 and Swi4 for both sequences (Taylor et al. 2000). These results suggest that the highly conserved core *CGCG* recognition sequence found in yeast and mammalian cells and is present in both MCB and SCB motifs contributes significantly to DBDs binding affinity. This motif is probably an essential prerequisite for MBF and SBF binding, but is not sufficient for achieving MBF and SBF specificity in yeast. Thus, other factors may contribute to specificity in the context of the yeast chromosomes. In addition, natural evolutionary changes in the DBDs protein sequence and promoter sequence may significantly influence MBF and SBF binding specificity in different organisms. It is possible that the accumulated effects of natural mutations in non-conserved residues of Mbp1 and Swi4 DBDs can affect promoter-binding specificity; however, these changes are very difficult to identify. Previous biochemical studies using protein Nuclear Magnetic Resonance (NMR) allowed the identification of residues in Mbp1 and Swi4 DBDs that change their conformation upon DNA binding (Taylor et al. 2000). This study showed that the majority of residues that participate in DNA binding are conserved between Mbp1 and Swi4 DBDs except for K60 in Mbp1 where aspartic acid occupies the equivalent position in Swi4. Such residues can contribute to the degree of affinity for MBF and SBF with specific DNA binding sequences. Additional residues that affect binding specificity can be identified by structural and sequence alignment analysis. The recently solved structure of the DBD of PCG2, the orthologue of Mbp1 from *Magnaporthe oryzae*, bound to MCB can shed new light on DNA binding specificity (Liu et al. 2015). Sequence and structural analysis of PCG2-DNA complex allowed us to identify two residues, T21 and Y85, located near the PCG2 binding pocket that are conserved in most fungi but change to lysine and phenylalanine, respectively, in the ancestor of *S. cerevisiae* and *K. lactis* (Fig. 3). To examine whether these residues might affect the specificity of Mbp1, we inserted the Y85F and T21 K mutations into Mbp1 DBD orthologues and examined the function of the mutated chimeric TFs in *S. cerevisiae*. Unfortunately, we found that these mutations do not affect the chimeric TF function in *S. cerevisiae* (data not shown) suggesting that changes in specificity may be dictated by the contribution of multiple and yet unidentified residues in the Mbp1/Swi4 DBDs.

Previous structural and biochemical studies focused on DBDs binding analysis with small stretches of DNA sequences (Taylor et al. 2000). However, binding affinity in vivo is likely to depend on extended sequences around the binding motif and local chromatin structure. In the context of the chromatin, binding specificity may be dictated by a much larger stretches of DNA sequences, the chromatin structure and the chromosomal location. It is possible that promoters containing MCB/SCB motifs are optimal for MBF/SBF binding at the native chromosomal location and changes in promoter location will result in alteration of binding specificity. In addition, chromatin environment might also dictate the role of SBF as a transcriptional activator and MBF as a transcriptional repressor. With recent advancement in CRISPR/CAS9 technologies in *S. cerevisiae* (DiCarlo et al. 2013; Ryan et al. 2014; Si et al. 2017), we can now more easily switch SBF and MBF promoters to examine the role of local chromatin in MBF/SBF binding and function.

In summary, research in the past three decades allowed the discovery of key players promoting G1/S transcription program. While significance advancements have been made in the characterization of MBF/SBF complexes and their respective Nrm1/Whi5 regulators, very little is

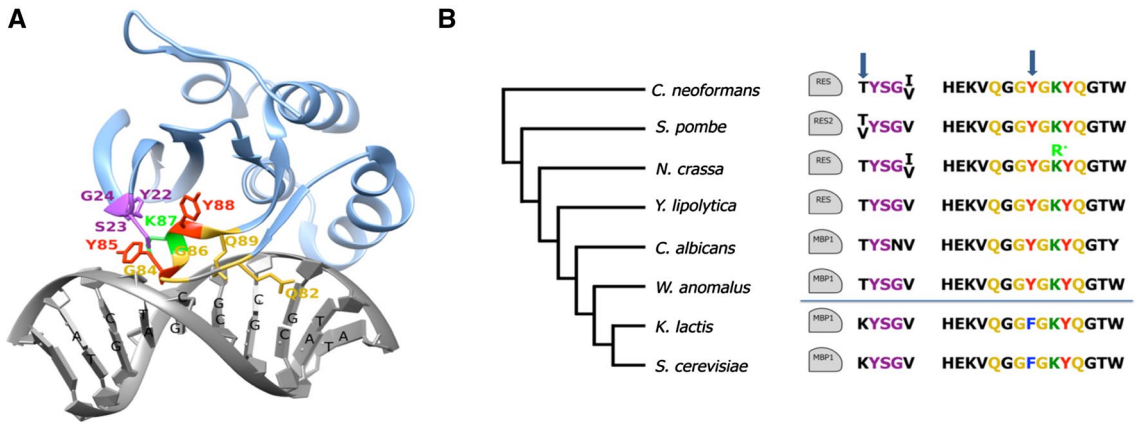


**Fig. 3** Structure and sequence analysis of PCG2 (Res) binding domain from the rice blast fungus (*Magnaporthe oryzae*) relative to Mbp1 orthologues in hemiascomycetes for the identification of functional residues that may affect DNA binding specificity. **a** Structural analysis of PCG2 bound to an MCB DNA binding motif (Liu et al. 2015) *highlights* key residues that are in direct contact or located in the close vicinity of the PCG2 DNA binding site (PDB:4UX5). **b** Sequence alignment of Mbp1 and Res from different fungal species focusing on key residues in PCG2 (*highlighted* in **a**). This analysis identifies two residues, T21 and Y85, which differ between *S. cerevisiae* and *K. lactis* Mbp1 belonging to clade 1 and more distantly related fungal species. These residues could affect the co-evolution of Mbp1-DNA interactions. T21 and Y85 are *highlighted* by *blue arrows*

known regarding the evolution of these proteins together with their target DNA binding sequence. Moreover, little is known regarding the specificity of MBF and SBF complexes and how each complex recognizes a subset of G1/S target genes. Thus, future studies addressing these questions can shed new light on the evolution and mechanism of G1/S transcription and reveal in more details on how such essential process is controlled with high precision allowing progression through the cell cycle.

## References


Amon A, Tyers M, Futcher B, Nasmyth K (1993) Mechanisms that help the yeast cell cycle clock tick: G2 cyclins transcriptionally activate G2 cyclins and repress G1 cyclins. Cell 74:993–1007

Bahler J (2005) Cell-cycle control of gene expression in budding and fission yeast. Annu Rev Genet 39:69–94. doi:10.1146/annurev.genet.39.110304.095808

Bar-Joseph Z, Siegfried Z, Brandeis M, Brors B, Lu Y, Eils R, Dynlacht BD, Simon I (2008) Genome-wide transcriptional analysis of the human cell cycle identifies genes differentially regulated in normal and cancer cells. Proc Natl Acad Sci USA 105:955–960. doi:10.1073/pnas.0704723105

Bean JM, Siggia ED, Cross FR (2005) High functional overlap between MluI cell-cycle box binding factor and Swi4/6 cell-cycle box binding factor in the G1/S transcriptional program in *Saccharomyces cerevisiae*. Genetics 171:49–61

Benanti JA (2016) Create, activate, destroy, repeat: Cdk1 controls proliferation by limiting transcription factor activity. Curr Genet 62:271–276. doi:10.1007/s00294-015-0535-5

Bertoli C, Skotheim JM, de Bruin RA (2013) Control of cell cycle transcription during G1 and S phases. Nat Rev Mol Cell Biol 14:518–528. doi:10.1038/nrm3629

Costanzo M, Nishikawa JL, Tang X, Millman JS, Schub O, Breitkreuz K, Dewar D, Rupes I, Andrews B, Tyers M (2004) CDK activity antagonizes Whi5, an inhibitor of G1/S transcription in yeast. Cell 117:899–913. doi:10.1016/j.cell.2004.05.024

Côte P, Hogues H, Whiteway M (2009) Transcriptional analysis of the *Candida albicans* cell cycle. Mol Biol Cell 20:3363–3373

Cross FR, Archambault V, Miller M, Klovstad M (2002) Testing a mathematical model of the yeast cell cycle. Mol Biol Cell 13:52–70. doi:10.1091/mbc.01-05-0265

de Bruin RA, McDonald WH, Kalashnikova TI, Yates J 3rd, Wittenberg C (2004) Cln3 activates G1-specific transcription via phosphorylation of the SBF bound repressor Whi5. Cell 117:887–898. doi:10.1016/j.cell.2004.05.025

de Bruin RA, Kalashnikova TI, Chahwan C, McDonald WH, Wohlschlegel J, Yates J 3rd, Russell P, Wittenberg C (2006) Constraining G1-specific transcription to late G1 phase: the MBF-associated corepressor Nrm1 acts via negative feedback. Mol Cell 23:483–496. doi:10.1016/j.molcel.2006.06.025

DiCarlo JE, Norville JE, Mali P, Rios X, Aach J, Church GM (2013) Genome engineering in *Saccharomyces cerevisiae* using CRISPR-Cas systems. Nucleic Acids Res 41:4336–4343. doi:10.1093/nar/gkt135

Ferrezuelo F, Colomina N, Futcher B, Aldea M (2010) The transcriptional network activated by Cln3 cyclin at the G1-to-S transition of the yeast cell cycle. Genome Biol 11:R67. doi:10.1186/gb-2010-11-6-r67

Granovskaia MV, Jensen LJ, Ritchie ME, Toedling J, Ning Y, Bork P, Huber W, Steinmetz LM (2010) High-resolution transcription atlas of the mitotic cell cycle in budding yeast. Genome Biol 11:R24. doi:10.1186/gb-2010-11-3-r24

Hendler A, Medina EM, Kishkevich A, Abu-Qarn M, Klier S, Buchler NE, de Bruin RAM, Aharoni A (2017) Gene duplication and co-evolution of G1/S transcription factor specificity in fungi are essential for optimizing cell fitness. PLoS Genet 13:e1006778. doi:10.1371/journal.pgen.1006778

Hussein B, Huang H, Glory A, Osmani A, Kaminskyj S, Nantel A, Bachewich C (2011) G1/S transcription factor orthologues Swi4p and Swi6p are important but not essential for

cell proliferation and influence hyphal development in the fungal pathogen Candida albicans. Eukaryot Cell 10:384–397. doi:10.1128/EC.00278-10

Liu J, Huang J, Zhao Y, Liu H, Wang D, Yang J, Zhao W, Taylor IA, Peng YL (2015) Structural basis of DNA recognition by PCG2 reveals a novel DNA binding mode for winged helix-turn-helix domains. Nucleic Acids Res 43:1231–1240. doi:10.1093/nar/gku1351

Medina EM, Turner JJ, Gordan R, Skotheim JM, Buchler NE (2016) Punctuated evolution and transitional hybrid network in an ancestral cell cycle of fungi. eLife. doi:10.7554/eLife.09492

Ofir A, Hofmann K, Weindling E, Gildor T, Barker KS, Rogers PD, Kornitzer D (2012) Role of a *Candida albicans* Nrm1/Whi5 homologue in cell cycle gene expression and DNA replication stress response. Mol Microbiol 84:778–794. doi:10.1111/j.1365-2958.2012.08056.x

Rustici G, Mata J, Kivinen K, Lio P, Penkett CJ, Burns G, Hayles J, Brazma A, Nurse P, Bahler J (2004) Periodic gene expression program of the fission yeast cell cycle. Nat Genet 36:809–817. doi:10.1038/ng1377

Ryan OW, Skerker JM, Maurer MJ, Li X, Tsai JC, Poddar S, Lee ME, DeLoache W, Dueber JE, Arkin AP, Cate JH (2014) Selection of chromosomal DNA libraries using a multiplex CRISPR system. eLife. doi:10.7554/eLife.03703

Si T, Chao R, Min Y, Wu Y, Ren W, Zhao H (2017) Automated multiplex genome-scale engineering in yeast. Nat Commun 8:15187. doi:10.1038/ncomms15187

Spellman PT, Sherlock G, Zhang MQ, Iyer VR, Anders K, Eisen MB, Brown PO, Botstein D, Futcher B (1998) Comprehensive identification of cell cycle-regulated genes of the yeast *Saccharomyces cerevisiae* by microarray hybridization. Mol Biol Cell 9:3273–3297

Taylor IA, McIntosh PB, Pala P, Treiber MK, Howell S, Lane AN, Smerdon SJ (2000) Characterization of the DNA-binding domains from the yeast cell-cycle transcription factors Mbp1 and Swi4. Biochemistry 39:3943–3954

Voordeckers K, Pougach K, Verstrepen KJ (2015) How do regulatory networks evolve and expand throughout evolution? Curr Opin Biotechnol 34:180–188. doi:10.1016/j.copbio.2015.02.001

Whitfield ML, Sherlock G, Saldanha AJ, Murray JI, Ball CA, Alexander KE, Matese JC, Perou CM, Hurt MM, Brown PO, Botstein D (2002) Identification of genes periodically expressed in the human cell cycle and their expression in tumors. Mol Biol Cell 13:1977–2000. doi:10.1091/mbc.02-02-0030

Wittenberg C, Reed SI (2005) Cell cycle-dependent transcription in yeast: promoters, transcription factors, and transcriptomes. Oncogene 24:2746–2755. doi:10.1038/sj.onc.1208606