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Ordered Recruitment of Transcription and Chromatin Remodeling Factors to a Cell Cycle– and Developmentally Regulated Promoter

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Summary

Gene activation in eukaryotes requires chromatin remodeling complexes like Swi/Snf and histone acetylases like SAGA. How these factors are recruited to promoters is not yet understood. Using CHIP, we measured recruitment of Swi/Snf, SAGA, the repressor Ash1p, and transcription factors Swi5p and SBF to the *HO* endonuclease promoter as cells progress through the yeast cell cycle. Swi5p's entry into nuclei at the end of anaphase recruits Swi/Snf, which then recruits SAGA. These two factors then facilitate SBF's binding. Ash1p, which only accumulates in daughter cell nuclei, binds to *HO* soon after Swi5p and aborts recruitment of Swi/Snf, SAGA, and SBF. Swi5p remains at *HO* for only 5 min. Swi/Snf's and SAGA's subsequent persistence at *HO* is self sustaining and constitutes an "epigenetic memory" of *HO*'s transient interaction with Swi5p.

Introduction

Differential gene expression lies at the heart of cell specialization in multicellular organisms. Many, if not most, genes in the human genome are turned on in only a small subset of our cells. This exquisite specificity arises because the initiation of transcription from genes packaged into nucleosomes requires a combination of transcription factors, many of which are themselves cell type specific. Such combinatorial control is only possible if different transcription factors perform very different functions within promoters. It was hard to see how this could readily occur, let alone rapidly evolve, using a transcriptional apparatus as simple as that of bacteria. Recent discoveries unearthing the complexity of the equivalent apparatus in eukaryotic cells make this much easier to envision. The "Holo" RNA polymerase for protein-encoding genes (POLII) contains at least 50 components, whereas the general transcription factor TFIID contains at least a dozen. Furthermore, their activity frequently requires ATP-dependent multisubunit chromatin remodeling complexes such as Swi/Snf or histone acetyltransferases such as SAGA (*S*pt-*A*da-*G*cn5-*a*cetyltransferase) (Brownell and Allis, 1996; Kingston et al., 1996; Owen-Hughes et al., 1996; Peterson, 1996; Pugh, 1996; Grant et al., 1997; Kadonaga, 1998; Mizzen and Allis, 1998; Struhl, 1998).

The extraordinary, and at first sight excessive, complexity of the eukaryotic transcription apparatus solves the combinatorial control problem, but it represents a huge challenge to understand how so many different proteins cooperate in gene activation. How are chromatin remodeling factors and acetyltransferases recruited to specific promoters? Do Swi/Snf and SAGA cooperate in altering nucleosomes, and if so, how? What are the immediate consequences of nucleosome remodeling, and how stable is the remodeled state when the signal that set it up in the first place disappears? In what order do these different factors act, and are there instances during development when one event clearly precedes other events?

Fortunately, for our understanding of these complicated processes, the mechanisms by which genes are activated and repressed are highly conserved between fungi and animals. The study of transcriptional regulatory mechanisms has therefore benefited from genetic studies of the budding yeast *Saccharomyces cerevisiae*. One of the clearest and earliest examples of a gene with complex combinatorial control is the *HO* gene in yeast. *HO* encodes an endonuclease that generates a double-strand break at the yeast mating type locus, whose repair using silent mating type genes situated elsewhere in the genome results in switching between **a** and α mating types and vice versa (Oshima and Takano, 1971; Strathern et al., 1982). The purpose of this switching is to produce, from a single haploid spore, cell progeny with different mating types that then conjugate to form a nonmating diploid cell.

Mating type switching has a very precise pedigree, which accelerates the diploidization process and is determined exclusively by the pattern of *HO* transcription. *HO* is not expressed throughout the first cell cycle following spore germination, and as a consequence both progeny of the first division inherit the same mating type as the starting spore. Because yeast cells divide by budding, one of the two progeny is a "mother" cell, whereas the other corresponding to the now detached bud is a so-called "daughter" cell. Remarkably, *HO* is expressed very transiently during late G1 in the mother cell cycle but at no stage during that of the daughter cell (Nasmyth, 1983). As a consequence, mother cells switch their mating types and produce two new progeny with a changed mating type, whereas daughter cells divide to produce two progeny retaining the mating type of the original spore. The result is that the progeny of mother and daughter cells conjugate with each other and thereby produce with high frequency a pair of zygotes after only two cell divisions (Nasmyth, 1982).

HO transcription depends on two cell cycle–dependent transcription factors. The first of these, Swi5p, accumulates in the cytoplasm from S phase until the onset

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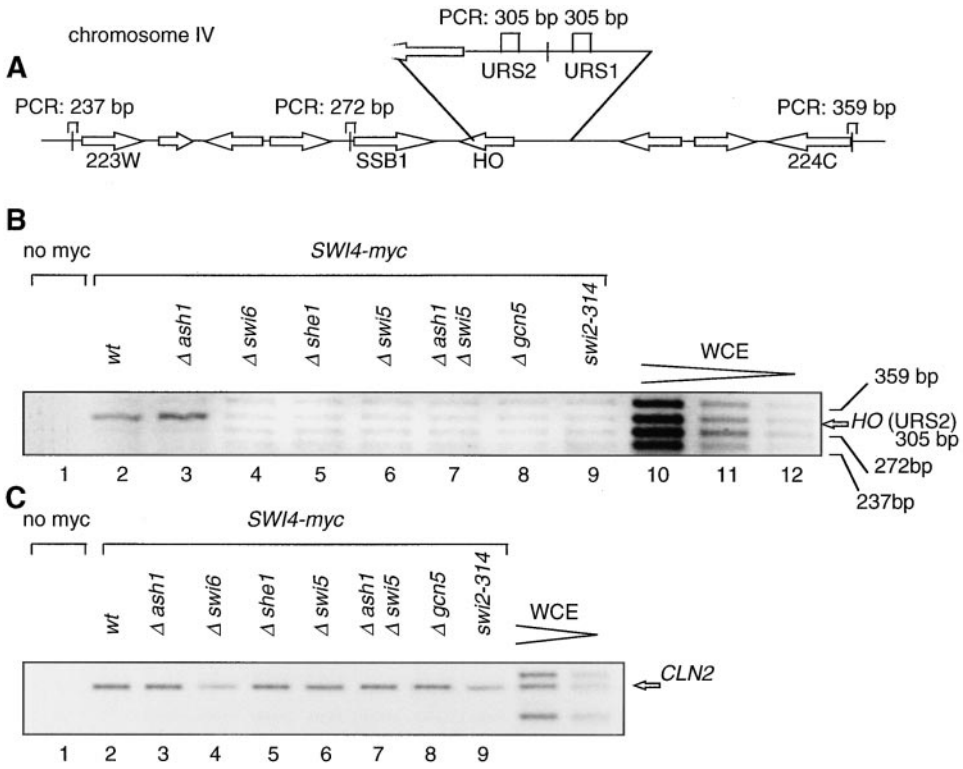

Figure 1. In Vivo Association of Swi4p with the *HO* and *CLN2* Promoters

(A) Scheme of PCR products on chromosome IV. Following cross-linking and immunoprecipitation, DNA was amplified by PCR. One pair of primers spanning the Swi4p-binding sites (URS2) or the Swi5p-binding site (URS1) was combined with three pairs of reference primers spanning promoter regions of flanking genes. The resulting size of each product is indicated. 223W and 224C are the ORFs YDL223W and YDL224C, respectively.

(B) Association of Swi4-Myc18 with the URS2 region of *HO*. PCR was performed with the primer pairs shown in (A) either on DNA fragments isolated after cross-linking and immunoprecipitation (lanes 1–9) or from 3-fold serial dilutions of whole-cell extract (WCE) (lanes 10–12). The samples were prepared from untagged cells (lane 1), *SWI4-myc18* cells (wt, lane 2) (K8115), *SWI4-myc18* Δ*ash1* (K8116), *SWI4-myc18* Δ*swi6* (K8117), *SWI4-myc18* Δ*she1* (K8118), *SWI4-myc18* Δ*swi5* (K8119), *SWI4-myc18* Δ*ash1* Δ*swi5* (K8120), *SWI4-myc18* Δ*gcn5* (K8121), and *SWI4-myc18 swi2-314* (K8122) (lanes 3–9, respectively).

(C) Association of Swi4-Myc with *CLN2* promoter. The same samples used in (B) were tested for the association on *CLN2* promoter with three primer pairs spanning either *CLN2* or promoter regions of two flanking genes.

of chromosome segregation due in part to phosphorylation of its nuclear localization sequence by cyclinB/Cdk1 kinases (Moll et al., 1991; Tebb et al., 1993). Swi5p's dephosphorylation by the Cdc14 phosphatase during late anaphase triggers its sudden accumulation within both mother and daughter nuclei, whereupon its synthesis ceases and it is rapidly degraded (Visintin et al., 1998). Thus, high levels of Swi5p only exist within nuclei for a short period as cells undergo cell division. Several genes, including that for the daughter cell–specific repressor Ash1p, are activated by the high levels of Swi5p during late anaphase (Bobola et al., 1996). *HO*, however, is not turned on until late in G1 of the next cycle, by which time the bulk of Swi5p has largely if not completely disappeared. To explain this conundrum, it has been proposed that Swi5p might form a stable complex at the *HO* promoter (Tebb et al., 1993).

HO's eventual activation in late G1 is part of a large transcriptional program mediated by a pair of related transcription factors called SBF (Swi4-Swi6 cell cycle box, CACGAAA) and MBF (MluI cell cycle box, ACGCG TNA), which share a common subunit, Swi6p, and have different but related site-specific DNA-binding proteins—Swi4p in the case of SBF, and Mbp1p in the case of MBF (Andrews and Herskowitz, 1989; Primig et al., 1992; Koch and Nasmyth, 1994). *HO* expression requires SBF and not MBF and is therefore dependent on Swi4p and Swi6p. Swi5p activates *HO* by binding to two sites between 1200 and 1400 bp from the start of transcription (a region called URS1), whereas SBF binds to a series of sites between 100 and 700 bp (a region called URS2) from the start of transcription (Stillman et al., 1988; Taba et al., 1991; Tebb et al., 1993). In addition to Swi5p and SBF, *HO* activation depends on Swi/Snf and on the SAGA histone acetyltransferase (Stern et al., 1984; Breeden and Nasmyth, 1987; Pollard and Peterson, 1997; Perez-Martin and Johnson, 1998). The discovery that mutations in histone genes could partially reverse defective *HO* transcription in *swi* mutants was the first hint that Swi/Snf's role might be to regulate chromatin structure (Kruger and Herskowitz, 1991; Hirschhorn et al., 1992; Winston and Carlson, 1992; Kruger et al., 1995; Pollard and Peterson, 1997). In vitro, Swi/Snf can alter nucleosome structure in a manner that facilitates the

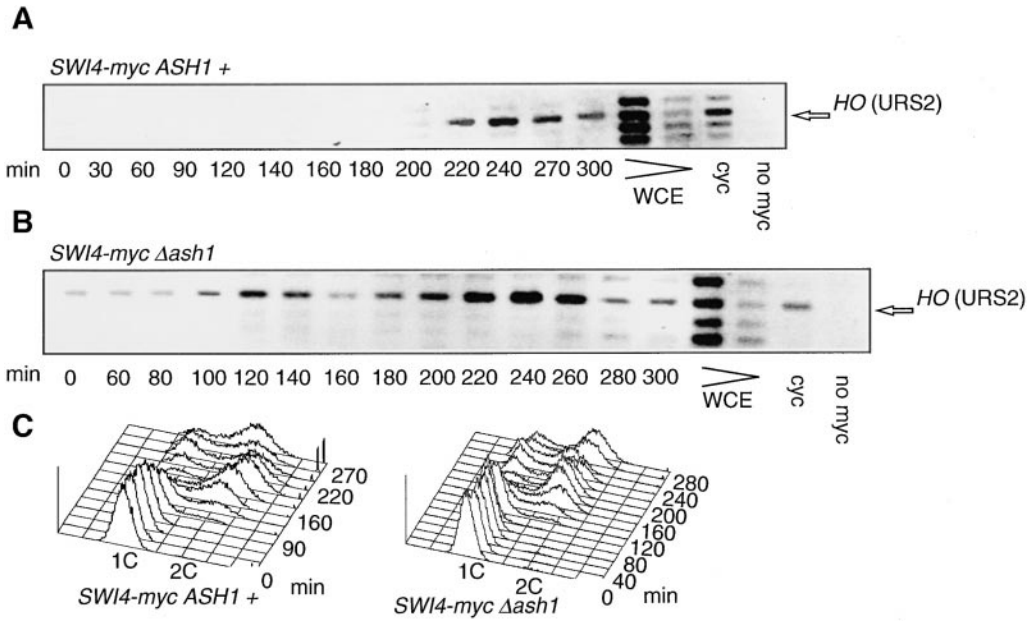

Figure 2. The Association of Swi4-Myc with *HO* Is Cell Cycle Regulated and Is Prevented by Ash1p in the First Cell Cycle of Daughter Cells

(A) In *ASH1*$^+$ cells, Swi4-Myc associates with URS2 region only in the second cell cycle. Small G1 daughter cells of the *SWI4-myc18* strain (K8115) were collected by elutriation and then incubated at 25°C in YEPR. Samples were taken every 20 or 30 min. PCR was performed on DNA fragments derived from the same volume of cross-linked cells for each time point or from whole-cell extract (WCE). DNA was also purified in parallel after cross-linking and immunoprecipitation of asynchronous cultures of the *SWI4-myc18* strain (cyc) or of untagged cells (no myc).

(B) Swi4-Myc18 associates with URS2 in the first cell cycle in the *SWI4-myc18 Δash1* strain (K8116). After elutriation of *SWI4-myc18 Δash1* strain, the same amount of the cells was collected for each time point. The samples were treated as in (A). PCR on purified DNA from the whole-cell extract (WCE), from cycling cells (cyc), and from untagged cells (no myc) is also shown.

(C) DNA content was measured by FACS from the samples collected in (A) and (B).

binding of transcription factors (Cote et al., 1994; Imbalzano et al., 1994; Kwon et al., 1994; Burns and Peterson, 1997; Schnitzler et al., 1998). Its role in vivo is less clear. Swi/Snf clearly affects, either directly or indirectly, the nuclease sensitivity pattern of chromatin from certain promoters, but whether it really promotes transcription by facilitating transcription factor recruitment is not known. Swi/Snf's highly conserved ATPase is encoded by the *SWI2* gene (Peterson, 1996; Pazin and Kadonaga, 1997; Pollard and Peterson, 1998). The SAGA complex is known to acetylate histone H3 within nucleosomes in vitro and in vivo. Its acetyltransferase subunit is encoded by *GCN5* (*SWI9*) (Georgakopoulos and Thireos, 1992; Marcus et al., 1994; Brownell et al., 1996; Grant et al., 1997; Ruiz-Garcia et al., 1997).

HO's expression only in mother cells is due to Ash1p's asymmetric accumulation (Bobola et al., 1996; Sil and Herskowitz, 1996). *ASH1* mRNA made during anaphase is transported along actin cables through the bud neck and across the bud by a type V myosin (She1/Myo4) and is anchored at the distal tip of buds (Jansen et al., 1996; Long et al., 1997; Takizawa et al., 1997; Bertrand et al., 1998; Münchow et al., 1999). The bulk of the newly synthesized Ash1p is therefore produced at the distal tip of buds and accumulates preferentially in the daughter cell nucleus. Ash1p has GATA1-like zinc fingers (Bobola et al., 1996; Sil and Herskowitz, 1996) and could therefore be a site-specific DNA-binding protein, but it has not yet been shown to bind to specific sequences in the *HO* promoter.

The *HO* promoter is remarkable, not only because it requires multiple transcription factors, but also because events needed for its activation clearly occur at defined stages of the cell cycle. It therefore presents a unique opportunity to dissect the order of events that lead to transcription. Enormous progress has been made in defining factors needed for transcription, in measuring their activity in vitro, and in discovering what they are capable of when artificially tethered to DNA sequences in vivo. However, we know rather little about the order of events that actually occur at real promoters in vivo. This knowledge will be essential for directing future in vitro studies.

In this paper, we used chromatin immunoprecipitation (CHIP) to measure the time and interdependence of transcription and chromatin remodeling factor recruitment to the *HO* promoter as cells exit from mitosis. Our data suggest that Swi5p's arrival during late anaphase recruits Swi/Snf, which then recruits the SAGA complex. Both Swi/Snf and SAGA are required for the subsequent recruitment of SBF. Thus, Swi/Snf both recruits transcription factors (in this case, SBF) and is recruited by transcription factors (in this case, Swi5p). We also show that Ash1p arrives at the *HO* promoter in daughter cells 5 min after Swi5p and aborts the recruitment of Swi/Snf and SAGA. Remarkably, Swi/Snf and SAGA remain bound to *HO* throughout the G1 period subsequent to mitotic exit, whereas Swi5p disappears within 5 min of its arrival. This suggests that by recruiting Swi/Snf and SAGA, Swi5p switches the *HO* promoter chromatin from

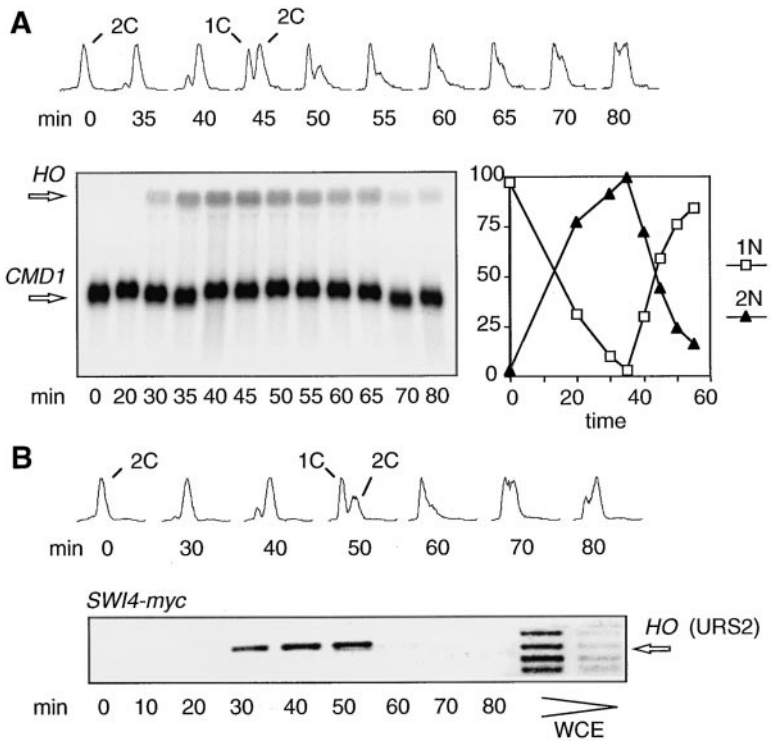

Figure 3. *HO* Expression and Association of Swi4-Myc with URS2 after Release from Metaphase Arrest

(A) *ho*mRNA was detected by Northern blot analysis. RNA was prepared from a *GAL-CDC20* Δ*cdc20* strain (K7428). Cells growing asynchronously in YEPRG were arrested in metaphase for 2 hr by galactose depletion. The cells were then released at 25°C after readdition of galactose, and samples were taken at the indicated times. As a control of the amount of loaded RNAs, the membrane was hybridized with a calmodulin probe (*CMD1*). The graph shows the percentage of mononucleate (1N) and binucleate cells (2N) counted after PI staining.

(B) The association of Swi4-Myc18 with the URS2 after release from metaphase arrest. The association of Swi4-Myc18 with the URS2 region of *HO* was analyzed using the *SWI4-myc18 GAL-CDC20* Δ*cdc20* strain (K8123) after synchronization in medium lacking galactose and subsequent release at 25°C. PCR was performed on DNA isolated from the cross-linked samples or purified from the whole-cell extract (WCE). DNA content was determined by FACS analysis. The 0 time point corresponds to the arrested sample taken after 2 hr of galactose depletion.

an inactive to an active state, which can be maintained for long periods even after the switching signal (Swi5p) has disappeared. This is an excellent example of an "epigenetic memory" (albeit one that cannot be transmitted in dividing cells) at a real promoter in wild-type cells.

Results

SBF Associates with *HO* during G1 in Mother but Not Daughter Cells

To detect the association of *HO* with its regulatory factors, we introduced multiple Myc epitopes at their C termini and used a Myc-specific monoclonal antibody to immunoprecipitate DNA cross-linked to Myc-tagged proteins after treatment of live cells with formaldehyde (Hecht et al., 1996; Tanaka et al., 1997). We then measured the abundance of specific DNA sequences within these immunoprecipitates using the polymerase chain reaction (PCR). The chromosomal location of our PCR fragments (237, 272, 305-URS1, 305-URS2, and 359 bp) is shown in Figure 1A. Each reaction contained four sets of primers, which enabled us to measure simultaneously the relative abundance of four different DNA fragments in our immunoprecipitates. Figure 1B shows that URS2 sequences, which contain the *HO* promoter's known SBF-binding sites, are preferentially immunoprecipitated when cross-linked DNA is prepared from cells expressing Swi4-Myc18p (Figure 1B, lane 2). As expected, association between Swi4p and *HO* was dependent on Swi6p (Figure 1B, lane 4). Our chromatin immunoprecipitation (CHIP) assay showed that Swi4-Myc binds selectively to URS2 but not to promoter sequences upstream of URS2, which are known as URS1 (data not shown).

To address whether the binding of Swi4p to *HO* differs between mother and daughter cells, we measured association between Swi4-Myc and URS2 as unbudded G1 daughter cells isolated by centrifugal elutriation progress through the cell cycle. Swi4p was absent from *HO* throughout the daughter cell cycle but appeared at the onset of cytokinesis as daughter cells gave birth to their first bud (Figure 2A). This implies that Swi4p associates with *HO* on mother but not daughter cell chromosomes as cells embark on a new cell cycle. We repeated the experiment with daughter cells from an *ash1* mutant strain, in which *HO* is expressed in both mother and daughter cells (Bobola et al., 1996; Sil and Herskowitz, 1996). In this case, Swi4-Myc was associated with URS2 throughout the daughter cell G1 period. Its abundance at *HO* increased somewhat during late G1, then declined as cells completed DNA replication, and increased again at the onset of cell division (Figure 2B). These data suggest that Ash1p blocks Swi4p's recruitment to *HO*. As predicted by this hypothesis, the selective immunoprecipitation of URS2 (from asynchronous cells) was abolished by deletion of *SHE1*, which causes Ash1p to accumulate to high levels in mother cells as well as daughter cells (Jansen et al., 1996) (Figure 1B, lane 5). Furthermore, deletion of *ASH1* approximately doubled the amount of URS2 immunoprecipitated with Swi4-Myc from asynchronous cells (Figure 1B, lanes 2 and 3).

The CHIP analysis of elutriated *ash1* mutant cells suggested that Swi4p's association with *HO* might be regulated by the cell cycle as well as by Ash1p. However, the poor synchrony of elutriated cells did not permit us to determine precisely when Swi4p arrives at and disappears from *HO*. To do this, we used cells whose *CDC20* gene was replaced by a copy under control of the *GAL1-10* promoter. *CDC20* encodes an unstable

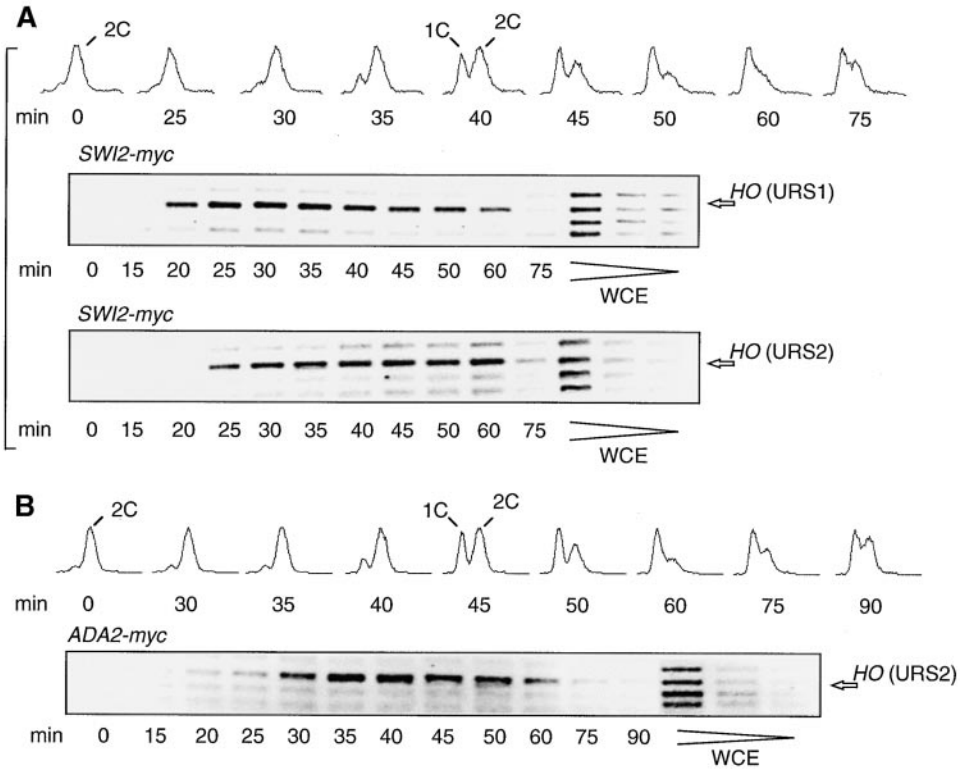

Figure 4. The Association of Swi2-Myc and Ada2-Myc Is Cell Cycle Regulated In Vivo

(A) Swi2-Myc association with the URS1 and URS2 region of *HO* was analyzed by testing the binding of Swi2-Myc18 using a *SWI2-myc18 Δash1 GAL-CDC20 Δcdc20* strain (K8124). The culture was arrested by incubation in YEPR in metaphase for 2 hr and then released into galactose containing medium at 25°C. Cell samples taken every 5 or 10 min were cross-linked and analyzed by PCR after immunoprecipitation. (B) Ada2-Myc binding to URS2 was investigated by determining the association of Ada2-Myc18. The asynchronous *ADA2-myc18 Δash1 GAL-CDC20 Δcdc20* (K8125) culture was arrested in metaphase and then released at 25°C. Samples were analyzed as in (A).
The FACS data of both experiments are also shown.

activator of the anaphase-promoting complex (APC), whose activity destroys Pds1p, an inhibitor of the sister chromatid–separating protein Esp1p (Cohen-Fix et al., 1996; Visintin et al., 1997; Ciosk et al., 1998; Lim et al., 1998; Shirayama et al., 1998). Asynchronous cultures of *GAL-CDC20* cells can be uniformly arrested in metaphase by growth for one generation in medium lacking galactose and then stimulated to undergo nuclear division highly synchronously by readdition of galactose. *GAL-CDC20* cells separate sister chromatids 10–15 min after galactose readdition, they produce *HO* mRNAs after 30–35 min, and undergo cell separation after 40–45 min (Figure 3A). Swi4-Myc arrived at *HO* 20 min after sister chromatid separation, remained there for much of the subsequent G1 period, and disappeared suddenly at the onset of DNA replication (Figure 3B). These data suggest that SBF arrives at *HO* around the same time as transcription initiation when cells are released from a *cdc20* arrest. In nonperturbed cells, there may normally be a gap between SBF's arrival and *HO* transcription.

SBF's Binding to *HO* Depends on Swi5p, Swi/Snf, and SAGA

We next addressed whether Swi4p's association with *HO* depends on Swi5p, on Swi/Snf, or on the SAGA complex. The selective association between Swi4-Myc and URS2 detected by the CHIP assay was absent in *swi5* and in *swi5 ash1* double mutants. It was also absent in *gcn5* (*swi9*) and *swi2-314* mutants (Figure 1B, lanes 6–9). Using the very same samples, we measured association between Swi4-Myc and the *CLN2* promoter, whose transcription is independent of Swi5p, Ash1p, Swi/Snf, and SAGA (Pollard and Peterson, 1998). Binding was reduced but not eliminated (as is transcription) (Breeden and Mikesell, 1991; Dirick et al., 1992; Nasmyth, 1993) by a *swi6* mutation but unaffected by *she1*, *swi2*, *swi5*, or *gcn5* mutations (Figure 1C). We conclude that the binding of Swi4-Myc to *HO* as cells enter G1 depends on Swi5p, Swi/Snf, and on the Gcn5p histone acetyltransferase.

Cell Cycle–Dependent Recruitment of Swi/Snf and SAGA to *HO*

Different subsets of yeast genes are regulated by the Swi/Snf and SAGA complexes (Holstege et al., 1998). *HO* along with several other genes requires both. It is conceivable that both Swi/Snf and SAGA are present throughout yeast chromosomes but are only required for altering chromatin structure at some genes. Alternatively, these two complexes might be recruited very specifically to those promoters that are dependent on their

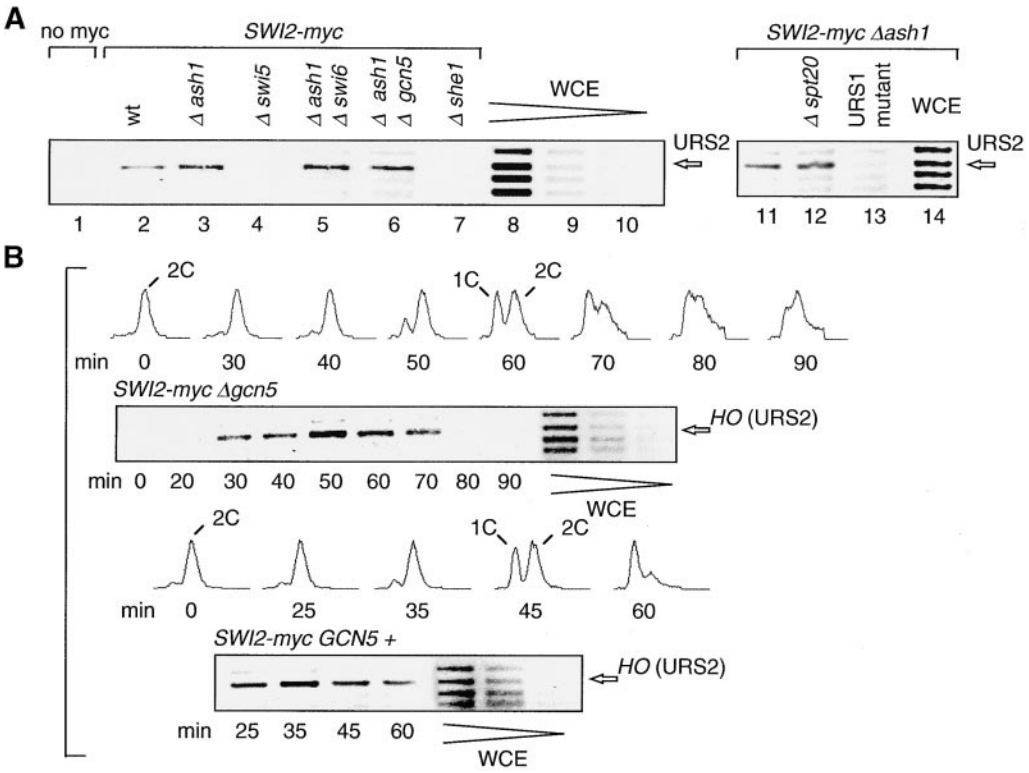

Figure 5. Swi2-Myc In Vivo Association with *HO* Is Dependent on Swi5p, on She1p, and on a URS1 Element but Is Independent of Swi6p, Gcn5p, and Spt20p

(A) Association of Swi2-Myc with URS2 in various mutants. *SWI2-myc18* wild-type (K8126), *SWI2-myc18* Δ*ash1* (K8127), *SWI2-myc18* Δ*swi5* (K8128), *SWI2-myc18* Δ*ash1* Δ*swi6* (K8129), *SWI2-myc18* Δ*ash1* Δ*gcn5* (K8130), and *SWI2-myc18* Δ*she1* (K8131) asynchronous cultures (lanes 2–7, respectively) and the untagged wild-type strain (lane 1) were grown in YEPR at 25°C. The association with URS2 was then analyzed by PCR. The amplification of the DNA from the whole-cell extract (WCE) is also shown (lanes 8–10 and 14). The strains *SWI2-myc18* Δ*ash1* (K8127), *SWI2-myc18* Δ*ash1* Δ*spt20* (K8132), and *SWI2-myc18* Δ*ash1* URS1 mutant (K8133) were cultured in YEPR and treated as described above (lanes 11–13).

(B) The association of Swi2-Myc is independent of Gcn5p. The association of Swi2-Myc was investigated in the *Swi2-myc18* Δ*ash1* Δ*gcn5 GAL-CDC20* Δ*cdc20* (K8134) strain after arrest in metaphase followed by release into galactose. The binding of Swi2-Myc to URS1 was in parallel analyzed using the *GCN5*$^+$ (K8124) correspondent strain. The FACS data are also shown.

activity. We used the CHIP assay to measure association between Swi2-Myc (a subunit of Swi/Snf) (Pollard and Peterson, 1998) and Ada2-Myc (a subunit of SAGA) (Berger et al., 1992; Grant et al., 1998). Both URS1 (data not shown) and URS2 (Figure 5A, lane 2 and Figure 6A, lane 2) were selectively immunoprecipitated by Swi2-Myc and by Ada2-Myc. A subset of SAGA's subunits, including Ada2p, are also part of a smaller ADA complex. SAGA contains additional subunits, including Spt20p, which is required for SAGA's integrity (Grant et al., 1997, 1998; Sterner et al., 1999). We found that Ada2-Myc's association with *HO* was dependent on *SPT20* (Figure 6A, lanes 12 and 13), which implies that Ada2p binds *HO* as part of the SAGA complex. Our data show that Swi/Snf and SAGA are nonrandomly distributed on yeast chromatin.

We next measured the association of Swi2p and Ada2p with URS2 during the arrest and release of *GAL-CDC20* cells. Neither Swi2-Myc nor Ada2-Myc was bound to URS2 during metaphase. Swi2-Myc appeared at URS2 25 min after addition of galactose (Figure 4A), and Ada2-Myc appeared around the same time (Figure 4B). Both proteins remained at *HO* for the next 30–35 min and disappeared around the onset of S phase. Using the same samples, we detected Swi2p's association with URS1 5 min before its association with URS2 (Figure 4A). These data suggest that Swi/Snf might arrive at *HO* shortly before SBF.

Recruitment of Swi/Snf Depends on Swi5p and Is Blocked by Ash1p

Deletion of the *ASH1* gene roughly doubled the amount of URS2 associated with Swi2-Myc in asynchronous cultures, whereas deletion of *SHE1* or *SWI5* abolished the selective immunoprecipitation (Figure 5A, lanes 2–4 and 7). Figure 5A also shows that Swi2-Myc's association with URS2 is abolished by double point mutations (lane 13) that destroy URS1's two Swi5-binding sites (Tebb et al., 1993). This confirms that the effect of mutating *SWI5* is a direct one and is not due to pleiotropic effects on mitotic exit. In contrast, deletions of *SWI6*, *SPT20*, or *GCN5* had no effect (Figure 5A, lanes 5, 6, and 12). We investigated the dependence of Swi2p's association on *GCN5* in further detail, because it has recently been proposed that histone acetyltransferases might stimulate recruitment of Swi/Snf to promoters

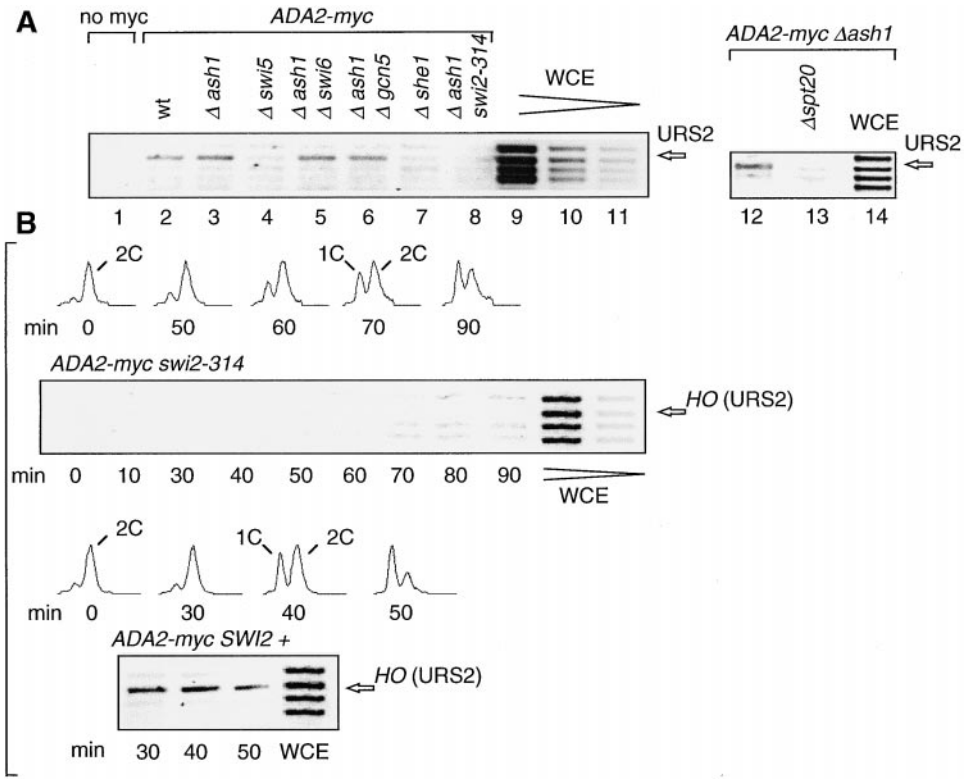

Figure 6. Ada2-Myc Binds to *HO* In Vivo, and Its Association Is Dependent on Swi5p, She1p, Swi2p, and Spt20p but Independent of Swi6p and Gcn5p

(A) Association of Ada2-Myc with URS2 in various mutants. PCR on DNA fragments purified after cross-linking and immunoprecipitation of *ADA2-myc18* cells (K8135) (lane 2, wt) and of *ADA2-myc18* Δ*ash1* (K8136), *ADA2-myc18* Δ*swi5* (K8137), *ADA2-myc18* Δ*ash1* Δ*swi6* (K8138), *ADA2-myc18* Δ*ash1* Δ*gcn5* (K8139), *ADA2-myc18* Δ*she1* (K8140), and *ADA2-myc18* Δ*ash1 swi2-314* (K8141) (lanes 3–8, respectively) was performed to detect the association of Ada2-Myc with URS2. PCR analysis was also done on a sample from untagged cells (no myc) (lane 1) and on the DNA purified from the whole-cell extract (WCE) (lanes 9–11 and 14). The strains *ADA2-myc18* Δ*ash1* (K8136) and *ADA2-myc18* Δ*ash1* Δ*spt20* (K8142) were also cultured in YEPR and analyzed after cross-linking and immunoprecipitation (lanes 12–13).

(B) The association of Ada2-Myc is dependent on Swi2p. *Ada2-myc* Δ*ash1 swi2-314 GAL-CDC20* Δ*cdc20* (K8143) and the correspondent *SWI2*$^{+}$ strain (K8125) were processed in parallel to investigate the dependence of Swi2p on the association of Ada2-Myc with URS2. The binding to URS2 of Swi2-Myc was investigated after arrest in metaphase followed by release into galactose containing medium. PCR was performed on the purified DNA samples after cross-linking and immunoprecipitation. The FACS data are also shown.

(Pollard and Peterson, 1998). Figure 5B shows that the time course of Swi2p's association with URS2 as *GAL-CDC20* cells exit the cell cycle synchronously was unaffected by deletion of *GCN5*. These data imply that the recruitment of Swi/Snf to the *HO* promoter is independent of SAGA and SBF but is dependent on Swi5p and is inhibited by Ash1p.

Recruitment of SAGA to *HO* Depends on Swi/Snf

The association of Ada2-Myc with URS2 was also increased by deletion of *ASH1* and abolished by deletion of *SWI5*, *SHE1*, or *SPT20* (Figure 6A, lanes 3–4, 7, and 13). It was also unaffected by deletion of *SWI6* (Figure 6A, lane 5). In this regard, Ada2p's recruitment to *HO* resembles that of Swi2p. However, Ada2-Myc's association with URS2 did not occur in *swi2* mutant cells even when *ASH1* was deleted (Figure 6A, lane 8). To confirm this result, we measured the association between Ada2-Myc and URS2 in *ash1 swi2 GAL-CDC20* cells upon release from their metaphase arrest. *Swi2* mutants grow more slowly than wild type and pass from metaphase to G1 more slowly. However, at no time during this period could we detect any selective association between Ada2-Myc and URS2 (Figure 6B). The lack of Ada2-Myc binding to *HO* in *swi2* mutants is unlikely to be due simply to the slow growth of these cells because Ada2-Myc's binding to *HO* was unaffected by deletion of *GCN5*, which also causes slow growth (Figure 6A, lane 6). It has recently been shown that the SAGA complex remains largely intact in *gcn5* mutants (Sterner et al., 1999). Though Gcn5p is essential for *HO* transcription, it is not required for SAGA's recruitment to the *HO* promoter (Figure 6A). These data, along with the observation that Swi2p arrives at *HO* marginally earlier than Ada2p, suggest that Swi5p recruits Swi/Snf to *HO* and that Swi/Snf then promotes recruitment of SAGA. Changes in chromatin induced by SAGA (and possibly Swi/Snf) are then required for SBF's recruitment.

Swi5p and Ash1p Bind *HO* Only Transiently

The foregoing data suggest that by recruiting Swi/Snf to *HO*, Swi5p sets in motion at the end of mitosis a complex series of events needed for *HO*'s eventual activation in late G1. To investigate whether Swi5p remains

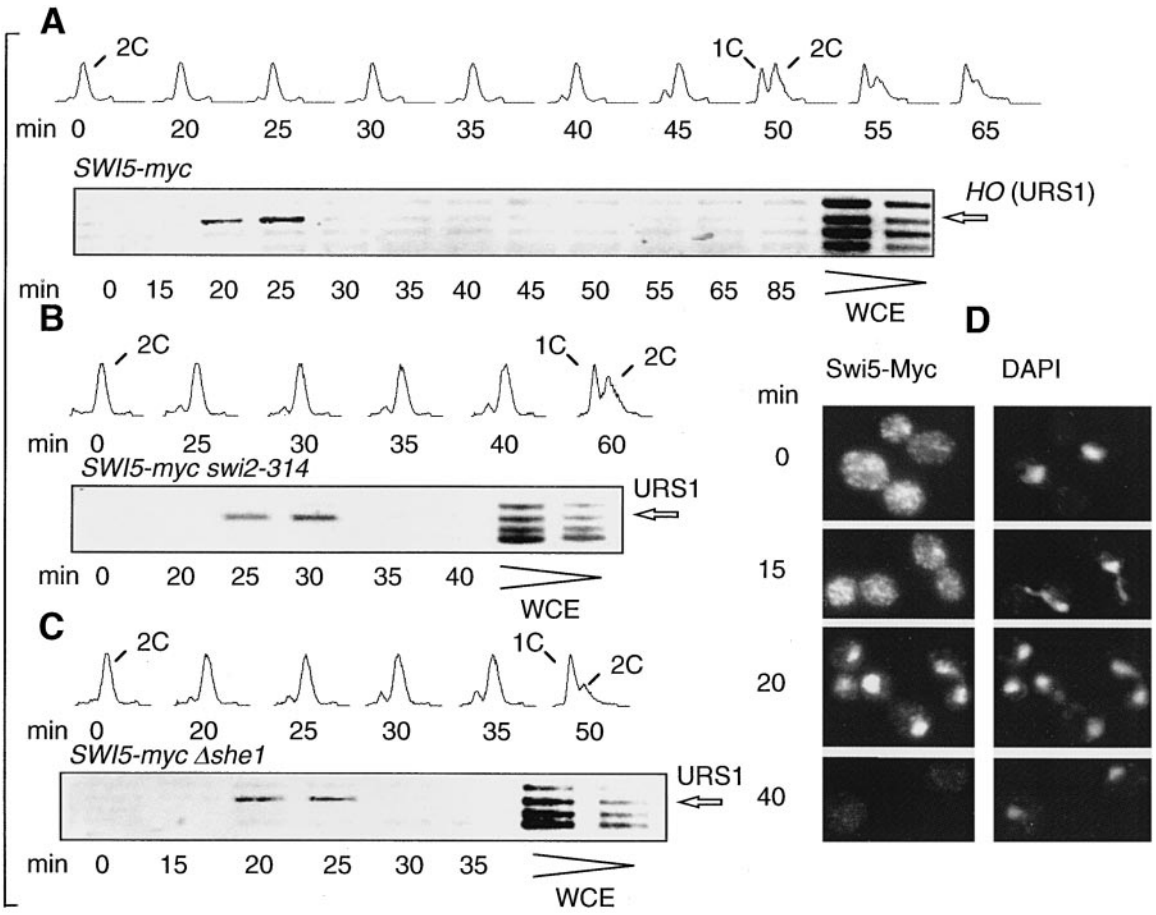

Figure 7. Swi5-Myc Binds to the URS1 Region of *HO* Transiently at Late Anaphase, and Its Association Is Independent of Swi2p and of She1p

(A–C) *Swi5-myc* Δ*ash1 GAL-CDC20* Δ*cdc20* (K8144), *Swi5-myc* Δ*ash1 GAL-CDC20* Δ*cdc20* Δ*she1* (K8146), and *Swi5-myc* Δ*ash1 GAL-CDC20* Δ*cdc20 swi2-314* (K8145) were analyzed in parallel to test the association of Swi5-Myc with the URS1 region of *HO*. The strains K8144, K8145, and K8146 were grown in YEPRG and arrested in metaphase by depletion of galactose. After release by readding galactose, samples were taken at indicated time points. PCR was performed on purified DNA fragments, and DNA content was measured by FACS analysis.

(D) Indirect immunofluorescence of Swi5-Myc after release from metaphase arrest. Cells of the indicated time points from the same sample used in (A) were fixed for indirect immunofluorescence. Staining with an anti-Myc antibody and diaminophenylindole (DAPI) are shown.

bound to *HO* during G1, we initially tried to detect association between Swi5-Myc and URS1 in asynchronous cultures but failed to do so. This failure suggests that Swi5p might bind *HO* only very transiently. To improve the chances of detecting a fleeting association between Swi5p and *HO*, we looked for association during the highly synchronous exit from M phase of *GAL-CDC20* cells. By this means, we detected a transient association between Swi5-Myc and URS1, starting around 20 min after galactose addition and lasting for no more than 5 min (Figure 7A). There are three reasons for believing that Swi5p's rapid disappearance is genuine and not due to epitope masking. First, we used a tagged Swi5 protein that contained nine tandem Myc epitopes at its C terminus. Second, Swi5p's appearance at *HO* coincided with its accumulation within nuclei, whereas its subsequent disappearance from *HO* coincided with Swi5p degradation. For example, Swi5p accumulated to high levels within nuclei in 70% of cells by 20 min but had already disappeared in most if not all cells by 40 min (Figure 7D). Third, other genes known to be regulated by Swi5p are activated only transiently as cells undergo cell division and are not expressed during the subsequent G1 period (Koch and Nasmyth, 1994). Our data suggest that Swi5p arrives at *HO* about 5 min before Swi/Snf and then recruits it to the promoter. They also suggest that having recruited Swi/Snf and thereby SAGA to the *HO* promoter, Swi5p is rapidly degraded and is not required to maintain the association of both chromatin remodeling factors with *HO* during the subsequent G1 period.

Our data suggest that accumulation of Ash1p within nuclei aborts recruitment of Swi/Snf by Swi5p. Ash1p normally only accumulates to high levels in daughter cell nuclei, but the delocalization of *ASH1* mRNA in *she1* mutants causes it to accumulate to high levels in mother and daughter nuclei. Swi5p's association with URS1 was unaffected by a *she1* mutation (Figure 7C), which suggests that Ash1's arrival within nuclei has little or no effect on Swi5's occupancy of the *HO* promoter. Swi5-Myc's association with URS1 was also unaffected by a *swi2* mutation (Figure 7B). This emphasizes that Swi/

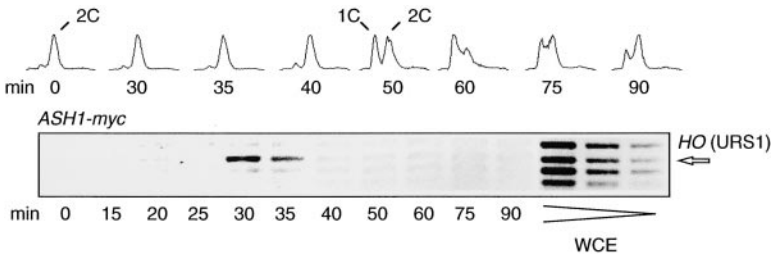

Figure 8. Ash1-Myc Associates Transiently with the URS1 Region of *HO* Promoter

Association of Ash1-Myc with URS1 after release from metaphase arrest. The purified DNA fragments from *ASH1-myc18 Δshe1 GAL-CDC20 Δcdc20* strain (K8147) were analyzed to detect the association of Ash1-Myc with the URS1 *HO* promoter region. The FACS data are also shown.

Snf's role in recruiting transcription factors to *HO* is very specific. It is needed for SBF's, but not Swi5's, recruitment.

To investigate whether Ash1p acts directly on the *HO* promoter, we used CHIP to measure its association with URS1 as *she1 GAL-CDC20* cells exit M phase (Figure 8). Ash1-Myc selectively associated with URS1 30 min after addition of galactose, but like Swi5p it soon thereafter disappeared. The arrival of Ash1p at *HO* 5–10 min after Swi5p is consistent with the observation that *ASH1* transcription is dependent on Swi5p and the related transcription factor Ace2p (Bobola et al., 1996).

Discussion

Ordered Recruitment of Factors to the *HO* Promoter

Genetic and biochemical studies over the past decade or more have made it abundantly clear that transcriptional activation in eukaryotic cells is a highly complex process. Genetics excels in identifying factors and enzymes required for transcription in vivo, whereas biochemistry excels in testing what they are capable of in vitro. However, neither discipline can tell us definitively the order of events that actually occur on real promoters in vivo. This sort of knowledge is essential for directing future biochemical studies and can only be obtained by measurements on live cells.

Using chromatin immunoprecipitation on formaldehyde fixed cells and a powerful new technique for synchronizing cells at the end of the budding yeast cell cycle, we have been able to measure with some accuracy the association with *HO* promoter sequences of five key factors that regulate its transcription. These are SBF, Swi5p, Ash1p, Swi/Snf, and SAGA. Remarkably, the association of all five factors with *HO* is tightly cell cycle regulated. They arrive at the promoter soon after cells initiate anaphase and depart either soon thereafter, as in the case of Swi5p and Ash1p, or shortly before they enter S phase, as in the case of SBF, Swi/Snf, and SAGA. Furthermore, due to the asymmetric accumulation of the Ash1 repressor, SBF, Swi/Snf, and SAGA only associate with the *HO* promoter of chromosomes that segregate into mother cells. By comparing the timing and interdependence of these factors' association with *HO*, we are able to piece together a unique picture of how an active promoter is created on mother cell chromosomes and how an inactive one is created on daughter cell chromosomes (see Figure 9).

The first factor to arrive at *HO* is the zinc finger transcription factor Swi5p, which enters both mother and daughter nuclei upon activation of the Cdc14 phosphatase 5–10 min after the separation of sister chromatids and immediately binds to sequences within URS1 over 1 kb upstream of the transcription start site (Tebb et al., 1993; Visintin et al., 1998). As far as *HO* is concerned, Swi5p has two important effects. The first is that it leads to recruitment of the Swi/Snf complex to URS1 and URS2. The second is that it activates *ASH1* transcription, presumably by binding to the *ASH1* promoter (Bobola et al., 1996). *ASH1* mRNA is transported from both mother and daughter nuclei to the distal tip of the bud, where it is anchored and translated (Long et al., 1997; Takizawa et al., 1997; Bertrand et al., 1998; Gonzalez et al., 1999). The bulk of Ash1p synthesized at bud tips then enters daughter cell nuclei and arrives at the *HO* promoter 5–10 min after Swi5p. Ash1p's arrival at *HO* aborts recruitment of Swi/Snf by Swi5p. Swi/Snf is therefore only recruited to *HO* on mother cell chromosomes. It is unclear from our data what exactly happens during the interval between the arrival of Swi5p and Ash1p. Swi/Snf might, for example, bind transiently to daughter cell chromosomes during this period. Our data suggest that the full recruitment of Swi/Snf to *HO* does not occur immediately after Swi5p's binding. Swi/Snf associates with URS1 very soon after Swi5's arrival but does not associate with URS2 until 5 min later. There appears to be a 5–10 min interval between the arrival of Swi5p and that of Swi/Snf at URS2, during which Ash1p has time to arrive on daughter cell chromosomes and abort Swi/Snf recruitment. Our finding that Swi/Snf binds to both URS1 and to URS2 suggests that Swi/Snf's recruitment to *HO* is a complex and possibly cooperative process involving several Swi/Snf complexes. Swi5p's arrival clearly triggers this process, but it may take several minutes to complete. One thing is clear: neither Swi5p nor Ash1p lingers long on the promoter. Both proteins disappear within 5–10 min of their arrival. Remarkably, Swi5p disappears at around the same time as Swi/Snf appears.

The arrival of Swi/Snf has two consequences on mother chromosomes. It recruits the SAGA complex whose histone acetyltransferase activity associated with Gcn5p (Swi9p), then facilitates the binding of SBF, without which *HO* cannot be transcribed. This is as far as we have thus far been able to track events. SBF's arrival is not, however, the end of the story, because *HO* transcription awaits activation of Cdk1 kinase by G1 cyclins (probably Cln3p). In an attempt to test whether G1 cyclins might be required to trigger the recruitment by SBF of TBP to the *HO* promoter, we tried to measure the binding of TBP to *HO* using CHIP but have so far been unsuccessful. It will clearly be of interest to determine which general transcription factors are recruited directly by SBF's arrival at *HO* and which ones require the subsequent activation of Cln/Cdk1 kinases.

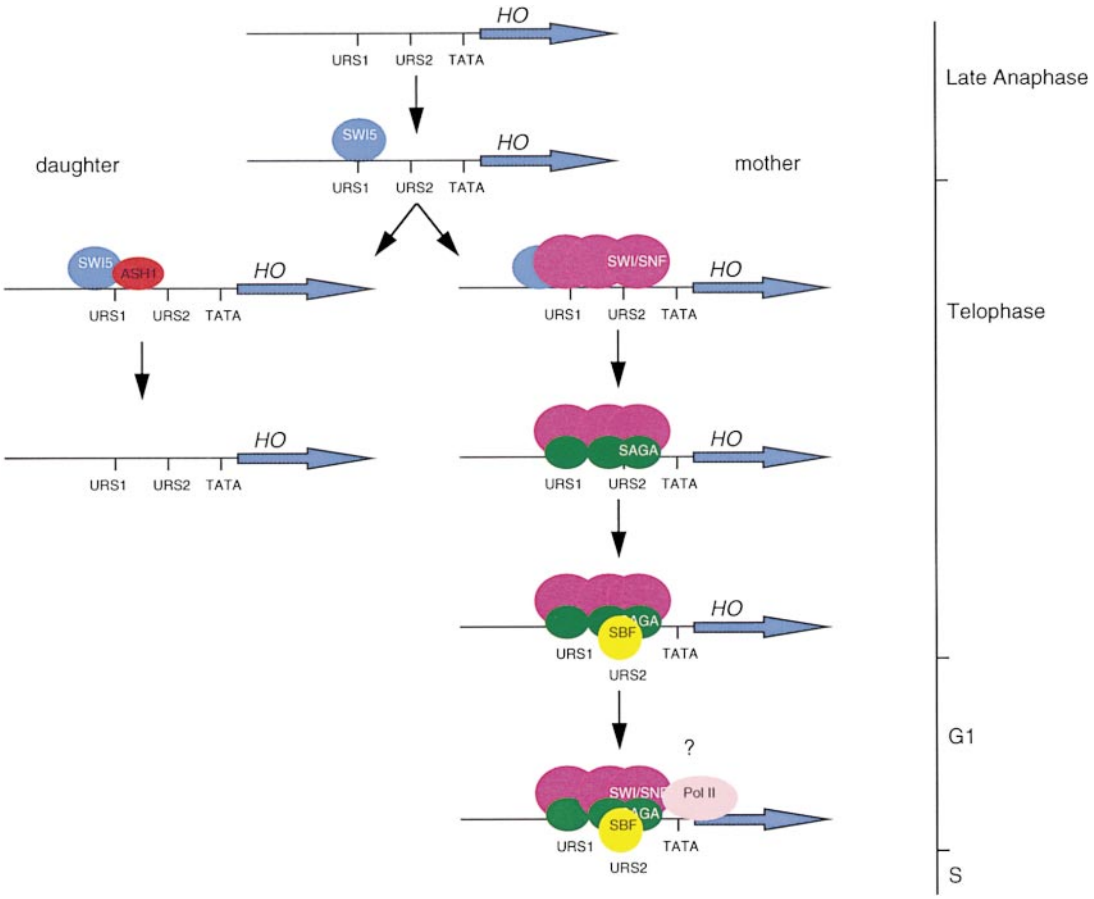

Figure 9. Ordered Recruitment of Transcription and Chromatin Remodeling Factors to the *HO* Promoter

The association of Swi5p, Ash1p, Swi/SNF, SAGA, and SBF is cell cycle regulated. The first factor to arrive at *HO* is Swi5p, which enters both mother and daughter nuclei, binds to sequences within URS1, and activates *Ash1* transcription. In daughter cells, Ash1p accumulates in nuclei, and its transient association with the *HO* promoter prevents recruitment of the Swi/Snf complex by Swi5p. In mother cells, Swi5p recruits Swi/Snf complexes to URS1 and URS2. Swi/Snf then proceeds to recruit SAGA complexes. Although Swi5p association is very transient, Swi/Snf and SAGA persist at the *HO* promoter during G1 phase. Swi/Snf and SAGA then facilitate the binding of SBF to URS2 at telophase, which is required for *HO* transcription. Activation of Cdk1 by G1 cyclins leads to *HO* transcription in late G1, when PolII holoenzyme might be recruited to the *HO* promoter. See details in the text.

Transcription Factors and Chromatin Remodeling Factors: Who Recruits Whom?

In vitro, Swi/Snf alters nucleosome structure in a manner that permits the binding of site-specific transcription factors or TBP to weak or moderately strong binding sites on DNA packaged into nucleosome arrays (Burns and Peterson, 1997). Thus far, there has been little evidence that Swi/Snf contributes to transcriptional activation at real promoters in vivo by recruiting transcription factors. Its recruitment of Gal4p to moderate or weak binding sites in vivo is of uncertain physiological relevance because induction of the *GAL1-10* promoter is largely independent of Swi/Snf. It has long been recognized that Swi/Snf is needed at only some promoters (Holstege et al., 1998), but there has been no evidence whether and how it is recruited only to these promoters. It has been somewhat of a conundrum whether the same factors that recruit Swi/Snf are themselves aided in their binding to nucleosomes by Swi/Snf.

Our finding that Swi/Snf is recruited to *HO* by a transcription factor, Swi5p, whose association with *HO* clearly does not require Swi/Snf, resolves this problem. Swi/Snf recruitment to *HO* by Swi5p is analogous to the recruitment of the Sin3-Rpd3 histone deacetylase by the transcription factor Ume6p (Kadosh and Struhl, 1997, 1998; Rundlett et al., 1998). Meanwhile, our observation that once recruited by Swi5p, Swi/Snf proceeds to recruit SAGA and SBF is evidence that Swi/Snf promotes transcription initiation at a real promoter by facilitating the recruitment of specific transcription factors. Interestingly, Swi/Snf's arrival at *HO* is clearly not sufficient for recruiting SBF, because this also depends on histone acetylation mediated by SAGA.

Cooperation between Swi/Snf and SAGA

Many of the genes whose transcription is dependent on Swi/Snf are also dependent on SAGA (Holstege et al., 1998). *HO*'s extreme dependence on both factors makes it a particularly good candidate for studying how they cooperate to promote transcription. Our data showing that Swi/Snf recruits SAGA to the *HO* promoter at the end of mitosis is consistent with the finding that histone

H3 acetylation at *HO* commencing at this stage of the cell cycle requires *SWI2* (J. Krebs et al., submitted). Swi/Snf's recruitment to *HO* is, in contrast, completely independent of SAGA. This implies that nucleosome remodeling at *HO* takes place in two stages. We propose that Swi/Snf alters nucleosomes in a manner that permits their association with SAGA, which then acetylates the N-terminal lysines of histones H3 and H4. Both steps are clearly required for SBF binding. The changes in nucleosome structure induced by Swi/Snf are quite stable in vitro, but it is nevertheless possible that histone hyperacetylation is needed in vivo to stabilize the "open" or "active" nucleosome state produced by Swi/Snf. It is clear that the effects of Swi/Snf on SAGA activity in vitro should now be investigated as should be the effects of SAGA on nucleosomes treated with Swi/Snf. Swi/Snf might facilitate SAGA activity at other promoters. Furthermore, other types of histone hyperacetylation might depend on different Swi/Snf-like factors.

How Does Swi5p Recruit Swi/Snf to *HO*?

Swi5p could recruit Swi/Snf directly. Swi/Snf might, for example, bind to one of Swi5p's transactivation domains. Alternatively, Swi5p might instead recruit Swi/Snf by an indirect mechanism, by interfering with factors that hinder Swi/Snf's association. It may be relevant in this regard that *HO*'s requirement for Swi5p can be bypassed, albeit only partially, by loss of Sin3p, which associates with the Rpd3 histone deacetylase (Stillman et al., 1994). Whether Sin3p's role at *HO* is to promote histone deacetylation is, however, unclear because Rpd3 (Sdi2), though required to ensure Swi5 dependence of an *HO-LacZ* fusion, is not needed for a bona fide *HO* gene to be Swi5 dependent. These results nevertheless raise the possibility that Swi5p might recruit Swi/Snf to *HO* by interfering with Sin3p and its associated histone deacetylases. However, an alternative explanation for the bypass is that the state of histone acetylation at *HO* is due to a balance between acetyltransferases and deacetylases. Inactivation of Sin3p might tip the balance toward acetylation, which might facilitate recruitment of Swi/Snf, albeit inefficiently, in the absence of Swi5p. Swi5p is clearly not the only transcription factor capable of recruiting Swi/Snf to *HO*, because replacement of the Swi5-binding region URS1 by the *GAL1-10* UAS gives rise to a hybrid *GAL/HO* promoter that is still Swi/Snf dependent (Nasmyth, 1987). Swi/Snf is presumably recruited to the URS2 present in *GAL/HO* by Gal4p.

Combinatorial Control

Our results show that Swi5p and SBF have very different roles at the *HO* promoter. Swi5p recruits Swi/Snf and thereby indirectly recruits SBF. SBF has no role in recruiting either Swi/Snf or SAGA. It binds to sequences that are closer to *HO*'s TATA box and possibly, though this remains to be demonstrated, has some role in recruiting general transcription factors or even the RNA polymerase holoenzyme. If so, chromatin remodeling and polymerase recruitment must be quite distinct steps in promoter activation, which can be independently regulated. This gives rise to a novel type of combinatorial control that may be important for cell type–specific promoters in multicellular organisms.

Switching Factors and Epigenetic Memory

One of our more provocative findings is the discovery that Swi5p binds to the *HO* promoter only fleetingly. Our data suggest that Swi5p disappears from *HO* within minutes of Swi/Snf and SAGA recruitment during late anaphase. Remarkably, both Swi/Snf and SAGA remain stably associated with *HO* for most of the subsequent G1 period. In the experiments reported here, G1 was no more than 30 min. However, under other circumstances, G1 can extend for several hours or even days. Mother cells arrested in G1 for several hours by inactivating the Cdk1 (Cdc28) protein kinase still activate *HO* when Cdk1 activity is restored (Tebb et al., 1993). Even more remarkable, stationary phase mother cells can spend several days in G1 and still express *HO* when fresh nutrients stimulate cells to reenter the mitotic cell cycle (Nasmyth, 1985). We suggest that, by recruiting Swi/Snf, Swi5p triggers a change in the state of *HO* promoter chromatin that can be maintained for several days (as long as cells remain in G1) after Swi5p's disappearance. The nucleosomal structural transitions induced by Swi/Snf and SAGA might themselves facilitate their continued association and/or their continued recruitment without any further help from Swi5p. Swi5p therefore represents an example of a novel type of site-specific transcription factor that is required to change or "switch" a promoter's chromatin structure but is not required to maintain it. This particular type of epigenetic memory is clearly not transmissable through the cell cycle because it is most likely destroyed by the activation of Cdk1 kinases in late G1. However, similar types of epigenetic memories very possibly have important roles in maintaining stable patterns of gene expression in postmitotic differentiated cells, and their decay might conceivably contribute to aging.

Experimental Procedures

All strains were derived from W303 (Jansen et al., 1996). *SWI2*, *SWI4*, *SWI5*, *ASH1*, and *ADA2* were tagged at the C terminus with multiple Myc epitopes at their original chromosomal loci (W. Zachariae, unpublished data). *ASH1*, *SHE1*, *SWI5*, *SWI6*, *GCN5*, and *SPT20* were deleted by homologous recombination using PCR method (Jansen et al., 1996). The *swi2-314* mutant was reported previously (Breeden and Nasmyth, 1987). The *GALCDC20* Δ*cdc20* strain was kindly provided by U. Surana (Lim et al., 1998). Cell samples from the asynchronous cultures were collected from cultures at 25°C in YEP containing 2% raffinose (YEPR). For the release from metaphase arrest, strains containing *GALCDC20* Δ*cdc20* were grown at 25°C in YEP containing 2% raffinose and 2% galactose (YEPRG) until mid–log phase. Cells were collected by filtration, washed with YEPR, and grown for 2 hr in YEPR at 25°C. The metaphase-arrested cells were then released by readding 2% galactose and grown for another 60–90 min. Methods for CHIP, FACS, centrifugal elutriation, indirect immunofluorescence, and the Northern blot were described previously (Jansen et al., 1996; Tanaka et al., 1997). Formaldehyde treatment of cells for CHIP was done at room temperature for 20 min followed from 4°C incubation for 12–16 hr.

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References

Andrews, B.J., and Herskowitz, I. (1989). The yeast SW14 protein contains a motif present in developmental regulators and is part of a complex involved in cell-cycle-dependent transcription. Nature *342*, 830–833.

Berger, S.L., Pina, B., Silverman, N., Marcus, G.A., Agapite, J., Regier, J.L., Triezenberg, S.J., and Guarente, L. (1992). Genetic isolation of ADA2: a potential transcriptional adaptor required for function of certain acidic activation domains. Cell *70*, 251–265.

Bertrand, E., Chartrand, P., Schaefer, M., Shenoy, S.M., Singer, R.H., and Long, R.M. (1998). Localization of ASH1 mRNA particles in living yeast. Mol. Cell *2*, 437–445.

Bobola, N., Jansen, R.P., Shin, T.H., and Nasmyth, K. (1996). Asymmetric accumulation of Ash1p in postanaphase nuclei depends on a myosin and restricts yeast mating-type switching to mother cells. Cell *84*, 699–709.

Breeden, L., and Mikesell, G.E. (1991). Cell cycle-specific expression of the SWI4 transcription factor is required for the cell cycle regulation of HO transcription. Genes Dev. *5*, 1183–1190.

Breeden, L., and Nasmyth, K. (1987). Cell cycle control of the yeast HO gene: cis- and trans-acting regulators. Cell *48*, 389–397.

Brownell, J.E., and Allis, C.D. (1996). Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. Curr. Opin. Genet. Dev. *6*, 176–184.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D.G., Roth, S.Y., and Allis, C.D. (1996). Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell *84*, 843–851.

Burns, L.G., and Peterson, C.L. (1997). The yeast SWI-SNF complex facilitates binding of a transcriptional activator to nucleosomal sites in vivo. Mol. Cell Biol. *17*, 4811–4819.

Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M., and Nasmyth, K. (1998). An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. Cell *93*, 1067–1076.

Cohen-Fix, O., Peters, J.M., Kirschner, M.W., and Koshland, D. (1996). Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. Genes Dev. *10*, 3081–3093.

Cote, J., Quinn, J., Workman, J.L., and Peterson, C.L. (1994). Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. Science *265*, 53–60.

Dirick, L., Moll, T., Auer, H., and Nasmyth, K. (1992). A central role for SWI6 in modulating cell cycle Start-specific transcription in yeast. Nature *357*, 508–513.

Georgakopoulos, T., and Thireos, G. (1992). Two distinct yeast transcriptional activators require the function of the GCN5 protein to promote normal levels of transcription. EMBO J. *11*, 4145–4152.

Gonzalez, I., Buonomo, S.B.C., Nasmyth, K., and von Ahsen, U. (1999). *ASH1* mRNA localization in yeast involves multiple secondary structural elements and Ash1 protein translation. Curr. Biol., in press.

Grant, P.A., Duggan, L., Cote, J., Roberts, S.M., Brownell, J.E., Candau, R., Ohba, R., Owen-Hughes, T., Allis, C.D., Winston, F., Berger, S.L., and Workman, J.L. (1997). Yeast Gcn5 functions in two multisubunit complexes to acetylate nucleosomal histones: characterization of an Ada complex and the SAGA (Spt/Ada) complex. Genes Dev. *11*, 1640–1650.

Grant, P.A., Sterner, D.E., Duggan, L.J., Workman, J.L., and Berger, S.L. (1998). The SAGA unfolds: convergence of transcription regulators in chromatin-modifying complexes. Trends Cell Biol. *8*, 193–197.

Hecht, A., Strahl-Bolsinger, S., and Grunstein, M. (1996). Spreading of transcriptional repressor SIR3 from telomeric heterochromatin. Nature *383*, 92–96.

Hirschhorn, J.N., Brown, S.A., Clark, C.D., and Winston, F. (1992). Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. Genes Dev. *6*, 2288–2298.

Holstege, F.C., Jennings, E.G., Wyrick, J.J., Lee, T.I., Hengartner, C.J., Green, M.R., Golub, T.R., Lander, E.S., and Young, R.A. (1998). Dissecting the regulatory circuitry of a eukaryotic genome. Cell *95*, 717–728.

Imbalzano, A.N., Kwon, H., Green, M.R., and Kingston, R.E. (1994). Facilitated binding of TATA-binding protein to nucleosomal DNA. Nature *370*, 481–485.

Jansen, R.P., Dowzer, C., Michaelis, C., Galova, M., and Nasmyth, K. (1996). Mother cell-specific HO expression in budding yeast depends on the unconventional myosin Myo4p and other cytoplasmic proteins. Cell *84*, 687–697.

Kadonaga, J.T. (1998). Eukaryotic transcription: an interlaced network of transcription factors and chromatin-modifying machines. Cell *92*, 307–313.

Kadosh, D., and Struhl, K. (1997). Repression by Ume6 involves recruitment of a complex containing Sin3 corepressor and Rpd3 histone deacetylase to target promoters. Cell *89*, 365–371.

Kadosh, D., and Struhl, K. (1998). Targeted recruitment of the Sin3-Rpd3 histone deacetylase complex generates a highly localized domain of repressed chromatin in vivo. Mol. Cell Biol. *18*, 5121–5127.

Kingston, R.E., Bunker, C.A., and Imbalzano, A.N. (1996). Repression and activation by multiprotein complexes that alter chromatin structure. Genes Dev. *10*, 905–920.

Koch, C., and Nasmyth, K. (1994). Cell cycle regulated transcription in yeast. Curr. Opin. Cell Biol. *6*, 451–459.

Kruger, W., and Herskowitz, I. (1991). A negative regulator of HO transcription, SIN1 (SPT2), is a nonspecific DNA-binding protein related to HMG1. Mol. Cell Biol. *11*, 4135–4146.

Kruger, W., Peterson, C.L., Sil, A., Coburn, C., Arents, G., Moudrianakis, E.N., and Herskowitz, I. (1995). Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. Genes Dev. *9*, 2770–2779.

Kwon, H., Imbalzano, A.N., Khavari, P.A., Kingston, R.E., and Green, M.R. (1994). Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. Nature *370*, 477–481.

Lim, H.H., Goh, P.Y., and Surana, U. (1998). Cdc20 is essential for the cyclosome-mediated proteolysis of both Pds1 and Clb2 during M phase in budding yeast. Curr. Biol. *8*, 231–234.

Long, R.M., Singer, R.H., Meng, X., Gonzalez, I., Nasmyth, K., and Jansen, R.P. (1997). Mating type switching in yeast controlled by asymmetric localization of ASH1 mRNA. Science *277*, 383–387.

Marcus, G.A., Silverman, N., Berger, S.L., Horiuchi, J., and Guarente, L. (1994). Functional similarity and physical association between GCN5 and ADA2: putative transcriptional adaptors. EMBO J. *13*, 4807–4815.

Mizzen, C.A., and Allis, C.D. (1998). Linking histone acetylation to transcriptional regulation. Cell Mol. Life Sci. *54*, 6–20.

Moll, T., Tebb, G., Surana, U., Robitsch, H., and Nasmyth, K. (1991). The role of phosphorylation and the CDC28 protein kinase in cell cycle-regulated nuclear import of the *S. cerevisiae* transcription factor SWI5. Cell *66*, 743–758.

Münchow, S., Sauter, C., and Jansen, R.-P. (1999). Association of the class V myosin Myo4p with a localized messenger RNA in budding yeast depends on She proteins. J. Cell Sci. *112*, in press.

Nasmyth, K.A. (1982). Molecular genetics of yeast mating type. Annu. Rev. Genet. *16*, 439–500.

Nasmyth, K. (1983). Molecular analysis of a cell lineage. Nature *302*, 670–676.

Nasmyth, K. (1985). A repetitive DNA sequence that confers cell-cycle START (CDC28)-dependent transcription of the HO gene in yeast. Cell *42*, 225–235.

Nasmyth, K. (1987). The determination of mother cell-specific mating type switching in yeast by a specific regulator of *HO* transcription. EMBO J. *6*, 243–248.

Nasmyth, K. (1993). Regulating the HO endonuclease in yeast. Curr. Opin. Genet. Dev. *3*, 286–294.

Oshima, Y., and Takano, I. (1971). Mating types in *Saccharomyces*: their convertibility and homothallism. Genetics *67*, 327–335.

Owen-Hughes, T., Utley, R.T., Cote, J., Peterson, C.L., and Workman, J.L. (1996). Persistent site-specific remodeling of a nucleosome array by transient action of the SWI/SNF complex. Science *273*, 513–516.

Pazin, M.J., and Kadonaga, J.T. (1997). SWI2/SNF2 and related proteins: ATP-driven motors that disrupt protein-DNA interactions? Cell *88*, 737–740.

Perez-Martin, J., and Johnson, A.D. (1998). Mutations in chromatin components suppress a defect of Gcn5 protein in *Saccharomyces cerevisiae*. Mol. Cell Biol. *18*, 1049–1054.

Peterson, C.L. (1996). Multiple SWItches to turn on chromatin? Curr. Opin. Genet. Dev. *6*, 171–175.

Pollard, K.J., and Peterson, C.L. (1997). Role for ADA/GCN5 products in antagonizing chromatin-mediated transcriptional repression. Mol. Cell Biol. *17*, 6212–6222.

Pollard, K.J., and Peterson, C.L. (1998). Chromatin remodeling: a marriage between two families? Bioessays *20*, 771–780.

Primig, M., Sockanathan, S., Auer, H., and Nasmyth, K. (1992). Anatomy of a transcription factor important for the start of the cell cycle in *Saccharomyces cerevisiae*. Nature *358*, 593–597.

Pugh, B.F. (1996). Mechanisms of transcription complex assembly. Curr. Opin. Cell Biol. *8*, 303–311.

Ruiz-Garcia, A.B., Sendra, R., Pamblanco, M., and Tordera, V. (1997). Gcn5p is involved in the acetylation of histone H3 in nucleosomes. FEBS Lett. *403*, 186–190.

Rundlett, S.E., Carmen, A.A., Suka, N., Turner, B.M., and Grunstein, M. (1998). Transcriptional repression by UME6 involves deacetylation of lysine 5 of histone H4 by RPD3. Nature *392*, 831–835.

Schnitzler, G., Sif, S., and Kingston, R.E. (1998). Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. Cell *94*, 17–27.

Shirayama, M., Zachariae, W., Ciosk, R., and Nasmyth, K. (1998). The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. EMBO J. *17*, 1336–1349.

Sil, A., and Herskowitz, I. (1996). Identification of asymmetrically localized determinant, Ash1p, required for lineage-specific transcription of the yeast HO gene. Cell *84*, 711–722.

Stern, M., Jensen, R., and Herskowitz, I. (1984). Five SWI genes are required for expression of the HO gene in yeast. J. Mol. Biol. *178*, 853–868.

Sterner, D.E., Grant, P.A., Roberts, S.M., Duggan, L.J., Belotserkovskaya, R., Pacella, L.A., Winston, F., Workman, J.L., and Berger, S.L. (1999). Functional organization of the yeast SAGA complex: distinct components involved in structural integrity, nucleosome acetylation, and TATA-binding protein interaction. Mol. Cell Biol. *19*, 86–98.

Stillman, D.J., Bankier, A.T., Seddon, A., Groenhout, E.G., and Nasmyth, K.A. (1988). Characterization of a transcription factor involved in mother cell specific transcription of the yeast HO gene. EMBO J. *7*, 485–494.

Stillman, D.J., Dorland, S., and Yu, Y. (1994). Epistasis analysis of suppressor mutations that allow HO expression in the absence of the yeast SW15 transcriptional activator. Genetics *136*, 781–788.

Strathern, J.N., Klar, A.J., Hicks, J.B., Abraham, J.A., Ivy, J.M., Nasmyth, K.A., and McGill, C. (1982). Homothallic switching of yeast mating type cassettes is initiated by a double-stranded cut in the MAT locus. Cell *31*, 183–192.

Struhl, K. (1998). Histone acetylation and transcriptional regulatory mechanisms. Genes Dev. *12*, 599–606.

Taba, M.R., Muroff, I., Lydall, D., Tebb, G., and Nasmyth, K. (1991). Changes in a SWI4,6-DNA-binding complex occur at the time of HO gene activation in yeast. Genes Dev. *5*, 2000–2013.

Takizawa, P.A., Sil, A., Swedlow, J.R., Herskowitz, I., and Vale, R.D. (1997). Actin-dependent localization of an RNA encoding a cell-fate determinant in yeast. Nature *389*, 90–93.

Tanaka, T., Knapp, D., and Nasmyth, K. (1997). Loading of an Mcm protein onto DNA replication origins is regulated by Cdc6p and CDKs. Cell *90*, 649–660.

Tebb, G., Moll, T., Dowzer, C., and Nasmyth, K. (1993). SWI5 instability may be necessary but is not sufficient for asymmetric HO expression in yeast. Genes Dev. *7*, 517–528.

Visintin, R., Prinz, S., and Amon, A. (1997). CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. Science *278*, 460–463.

Visintin, R., Craig, K., Hwang, E.S., Prinz, S., Tyers, M., and Amon, A. (1998). The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. Mol. Cell *2*, 709–718.

Winston, F., and Carlson, M. (1992). Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. Trends Genet. *8*, 387–391.