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Roles of SWI/SNF and HATs throughout the dynamic transcription of a yeast glucose-repressible gene

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Eucaryotic gene expression requires chromatin-remodeling activities. We show by time-course studies that transcriptional induction of the yeast glucose-regulated *SUC2* gene is rapid and shows a striking biphasic pattern, the first phase of which is partly mediated by the general stress transcription factors Msn2p/Msn4p. The SWI/SNF ATP-dependent chromatin-remodeling complex associates with the promoter in a similar biphasic manner and is essential for both phases of transcription. Two different histone acetyltransferases, Gcn5p and Esa1p, enhance the binding of SWI/SNF to the promoter during early transcription and are required for optimal *SUC2* induction. Gcn5p is recruited to *SUC2* simultaneously with SWI/SNF, whereas Esa1p associates constitutively with the promoter. This study reveals an unusual transcription pattern of a metabolic gene and suggests a novel strategy by which distinct chromatin remodelers cooperate for the dynamic activation of transcription.

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Introduction

Programming the expression of the genome is essential for the cellular response to a variety of signals that regulate metabolism, cell growth, differentiation, and development. Defined sets of genes are induced at specific developmental stages or upon environmental changes. The transcription of such genes must start at the right time and continuously integrate both extracellular stimuli and the cellular outputs in response to these stimuli. Accordingly, transcription of some genes occurs immediately, while transcription of others occurs days after exposure to a stimulus. The duration of transcription may be short or prolonged, and the transcription pattern may be constant or cyclical.

Transcription by RNA polymerase II (pol II) requires the concerted action of a large number of proteins that must be recruited to the target promoter. Each of the steps leading to formation of a transcript must contend with the repressive structure of chromatin, the basic unit of which is the nucleosome, which consists of 146 bp of DNA wrapped around an octamer of the four histones (Luger *et al*, 1997). The conformation of nucleosomes and the modification state of histones, both of which impact higher-order chromatin structure, are believed to be major determinants of localized chromatin structure. Importantly, the activity of a gene is largely dictated by the chromatin structure in which it resides (Wolffe, 1998), which can be modulated by enzymes that reversibly remodel chromatin.

Chromatin-remodeling enzymes, which are often part of large protein complexes, have been grouped into two major categories—ATP-dependent chromatin remodelers and covalent histone modifiers. ATPase remodelers, such as the yeast SWI/SNF complex, induce conformational changes in nucleosomes by altering DNA–histone interactions (Vignali *et al*, 2000; Martens and Winston, 2003); histone modifiers catalyze post-translational modifications of histones. Histone acetylation, the first modification shown to correlate strongly with transcriptional competence (Allfrey *et al*, 1964; Struhl, 1998), is controlled by the antagonistic activities of histone acetyltransferases (HATs) and deacetylases (HDACs) (Khochbin *et al*, 2001; Roth *et al*, 2001).

Local increases in chromatin accessibility can be achieved by the recruitment of chromatin-remodeling complexes by gene-specific transcription factors or components of the general transcription apparatus (Peterson and Workman, 2000; Vignali *et al*, 2000; Cosma, 2002; Sharma *et al*, 2003). Gene transcription often requires both ATP-dependent chromatin-remodeling complexes and HATs, and recent studies have underscored the critical importance of the interplay between these two types of activities in the regulation of transcription. For example, yeast SWI/SNF is required globally for transcription-associated histone acetylation during mitosis when chromatin is condensed (Krebs *et al*, 2000); conversely, histone acetylation may facilitate the affinity with which SWI/SNF binds to chromatin (Hassan *et al*, 2001, 2002; Agalioti *et al*, 2002). Studies of several promoters that are induced during differentiation or development have revealed that individual ATP-dependent and HAT remodeling enzymes are recruited temporally and that the order can vary at different promoters (Cosma *et al*, 1999; Krebs *et al*, 1999; Agalioti *et al*, 2000; Fry and Peterson, 2001; Reinke *et al*, 2001; Cosma, 2002; Soutoglou and Talianidis, 2002). Nevertheless, similar kinetic studies have not yet been carried out in genes whose induction occurs more rapidly (e.g., genes involved in stress response or metabolism); such genes may employ different strategies to establish active chromatin structure.

Here, we analyze by time course the transcriptional induction of the yeast glucose-regulated *SUC2* gene in response to acute glucose limitation. We discovered that induction of *SUC2* is rapid and, unexpectedly, proceeds in two distinct phases, the first of which is a stress response. SWI/SNF is essential for both phases of gene induction and associates

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with the *SUC2* promoter in a biphasic manner. Moreover, both the Gcn5p and Esa1p HATs facilitate the association of SWI/SNF with the promoter for optimal *SUC2* induction. Gcn5p is recruited to the promoter concurrently with SWI/SNF, whereas Esa1p associates constitutively with *SUC2*. Our study suggests a novel strategy by which distinct chromatin remodelers cooperate in activation of dynamic transcription.

Results

Acute glucose limitation induces biphasic transcription of a glucose-repressible gene

When limiting, the preferred carbon sources glucose and fructose can be derived from hydrolysis of other sugars (e.g., raffinose) by secreted invertase, which is encoded by the *SUC2* gene. Transcription of *SUC2* is therefore repressed by glucose and fructose and induced when glucose and fructose fall below certain threshold levels (Carlson, 1999; Herwig *et al*, 2001).

To better understand the transcriptional regulation of *SUC2*, we have studied its induction by an abrupt change of carbon source. We first monitored *SUC2* RNA levels in a time course following a rapid shift of yeast cells from glucose to raffinose (containing low glucose) medium. To our surprise, *SUC2* RNA accumulated rapidly and in two distinct phases: a short first phase in which transcript levels peaked at 45 min after induction and a prolonged second phase in which RNA reached steady-state levels at 2 h (Figure 1A). Shifting to a medium containing only low glucose (no raffinose) resulted in a similar biphasic pattern, except that *SUC2* RNA levels dropped to noninducing levels by 4 h (Figure 1A; data not shown). These results suggest that the biphasic transcription of *SUC2* is primarily a response to low glucose and that raffinose is required for the maintenance of *SUC2* transcription, as was observed previously (Ozcan *et al*, 1997; Recht and Osley, 1999; Geng *et al*, 2001).

To correlate the *SUC2* mRNA levels with active *SUC2* transcription, we measured the amount of pol II present at the *SUC2* promoter in chromatin immunoprecipitation (ChIP) experiments. The level of pol II crosslinking at the *SUC2* promoter (TATA box) increased four-fold immediately following the carbon source shift, and its presence at the promoter also showed a biphasic pattern that slightly preceded that of the *SUC2* RNA (Figure 1B). As a control, the levels of pol II associated with the *ACT1* promoter did not vary significantly during *SUC2* induction.

To probe the more general cellular response to acute glucose limitation, we determined the fraction of unbudded cells (cells in G1) in a time course following the carbon source shift. The transition from G1 to S phase is tightly controlled by the availability of carbon source, and glucose deprivation causes G1 arrest (Alberghina *et al*, 1998; Newcomb *et al*, 2003). Remarkably, the pattern of cells in G1 following the carbon source shift was also biphasic (Figure 1C). The beginning of each phase correlated with the binding of pol II to the *SUC2* promoter (Figures 1C and B). We interpret the accumulation of cells in G1 during limiting glucose as an indication that cells are experiencing a shortage of metabolic glucose. The biphasic accumulation of cells in G1 following acute glucose limitation suggests a dynamic change in cellular glucose metabolism, which likely underlies the biphasic induction of *SUC2*.

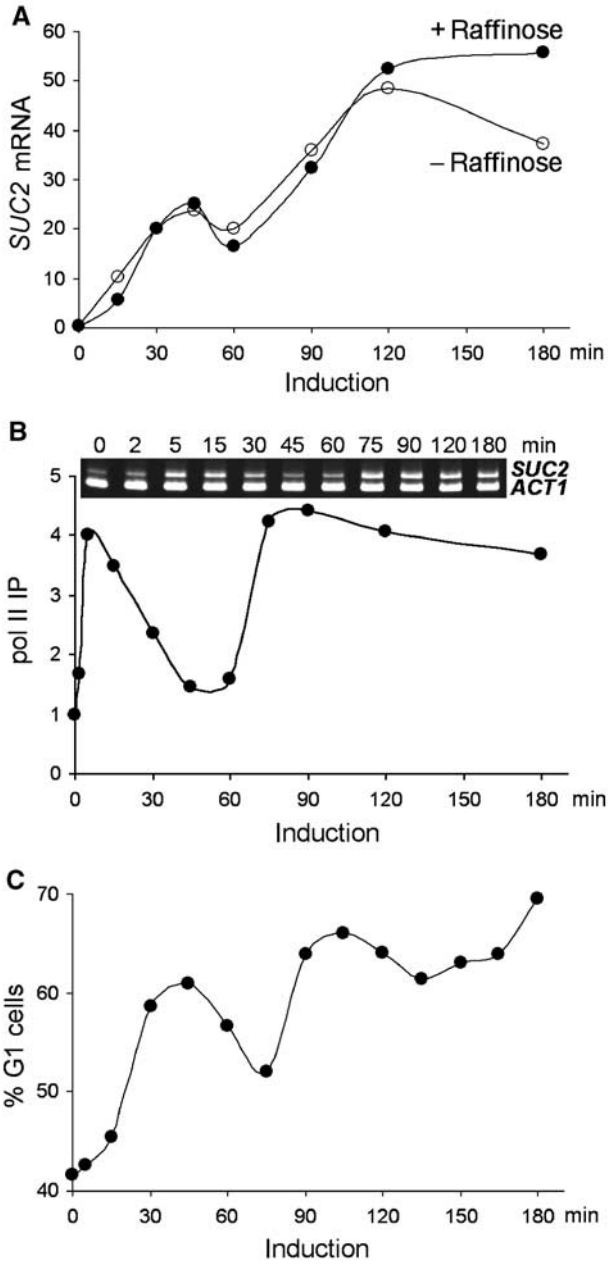

Figure 1 Biphasic induction of *SUC2*. Wild-type (BLY1) cells were grown in glucose (2%) medium to early logarithmic phase, and then quickly shifted to low-glucose (0.05%) medium containing raffinose (2%) unless noted. (**A**) Biphasic accumulation of *SUC2* mRNA. *SUC2* transcripts were quantified by real-time PCR in glucose-repressed cells (time 0) and cells induced in low-glucose media with (+ raffinose) or without (− raffinose) raffinose for the indicated times. The levels of *SUC2* transcripts were presented as percentages of *ACT1* transcripts. (**B**) Dynamic recruitment of pol II to *SUC2*. ChIP analysis using anti-pol II CTD antibody was performed on crosslinked chromatin prepared from glucose-repressed or raffinose-induced cells. IP efficiencies of the *SUC2* UAS sequence (nucleotides −154 to +45 relative to the translational start site) at different time points were determined by real-time PCR quantitation and presented as the fold increases relative to that at time 0. A semiquantitative multiplex PCR analysis of the precipitated DNA (upper panel) is also shown. A sequence of the *ACT1* promoter was co-amplified as an internal control. The PCR products were separated on an 8% polyacrylamide gel and stained with ethidium bromide. (**C**) Fluctuation of the G1 (unbudded) cell fractions. Cells were withdrawn from a raffinose-induced culture and fixed immediately in 3.7% formaldehyde. The mitotic index was determined microscopically and presented as the percentages of G1 cells.

Distinct chromatin-remodeling activities associate with the SUC2 promoter

Under glucose-repressing conditions, the *SUC2* promoter is packaged into an array of evenly positioned nucleosomes, which are remodeled in an SWI/SNF-dependent manner upon gene induction (Hirschhorn *et al*, 1992; Matallana *et al*, 1992; Gavin and Simpson, 1997; Wu and Winston, 1997; Geng *et al*, 2001). SWI/SNF is essential not only for the initiation but also for the maintenance of *SUC2* transcription (Biggar and Crabtree, 1999; Sudarsanam *et al*, 1999). Gcn5p, a histone H3/H2B acetyltransferase and component of both the SAGA and ADA complexes (Vignali *et al*, 2000), has also been implicated in maximally inducing *SUC2* (Pollard and Peterson, 1997; Biggar and Crabtree, 1999; Recht and Osley, 1999; Sudarsanam *et al*, 1999), but the mechanism by which Gcn5p stimulates *SUC2* transcription has not been elucidated.

Since transcription can be regulated at multiple steps, we tested whether the biphasic induction of *SUC2* results from the temporally regulated recruitment of SWI/SNF and/or Gcn5p. In parallel, we also assessed the role of a second histone acetyltransferase, Esa1p, which specifically acetylates histones H4 and H2A and is the catalytic subunit of the NuA4 HAT complex (Allard *et al*, 1999; Vogelauer *et al*, 2000; Suka *et al*, 2001). Esa1p is essential for cell cycle progression and is required for transcription of ribosomal protein genes and several other genes (Allard *et al*, 1999; Clarke *et al*, 1999; Galarneau *et al*, 2000; Reid *et al*, 2001). We measured the crosslinking of Snf5p (a core subunit of SWI/SNF), Gcn5p, and Esa1p to the *SUC2* promoter in ChIP experiments using specific polyclonal antibodies against each protein. Crosslinking of both Snf5p and Gcn5p to the *SUC2* upstream activating sequence (UAS) increased seven- and six-fold, respectively, within 5 min following the carbon source shift (Figure 2A, upper panel) and displayed biphasic patterns similar to that of pol II, although slightly less Snf5p and Gcn5p were crosslinked to *SUC2* during the second phase. In contrast, the binding of Esa1p increased two-fold within the first 5 min but decreased quickly to preinduction levels.

To confirm that preinduction levels of Esa1p were significantly higher than background at *SUC2*, we repeated ChIP assays in a strain expressing HA-tagged Esa1p (Figure 2A, lower panel). The *SUC2* UAS and flanking sequences were precipitated by anti-HA antibody much more efficiently from tagged than from untagged strains under glucose-repressing conditions (compare lanes 1 and 2; see also Figure 3C, lower panel). In addition, ChIP signals for *SUC2* increased slightly 5 min following the shift to low glucose (compare lanes 2 and 3). Esa1p also associated strongly with the promoters of two ribosome protein genes, *RPL2B* and *RPS11B*, consistent with a previous report (Reid *et al*, 2001). Importantly, similar patterns of Esa1p association with *SUC2*, *RPL2B*, and *RPS11B* were observed in ChIP experiments carried out with anti-Esa1p antibody (compare lanes 2 and 3 with lanes 4 and 5), thus validating the use of anti-Esa1p antibody in this study. We conclude that a significant amount of Esa1p associates constitutively with the *SUC2* promoter.

To correlate the presence of Gcn5p and Esa1p at *SUC2* with their histone acetyltransferase activities, we compared the histone acetylation levels in wild-type and *gcn5* or *esa1* mutant cells. In the wild type, the overall H3 acetylation at the *SUC2* UAS increased four-fold immediately following induction and correlated well with Gcn5p cross-linking at

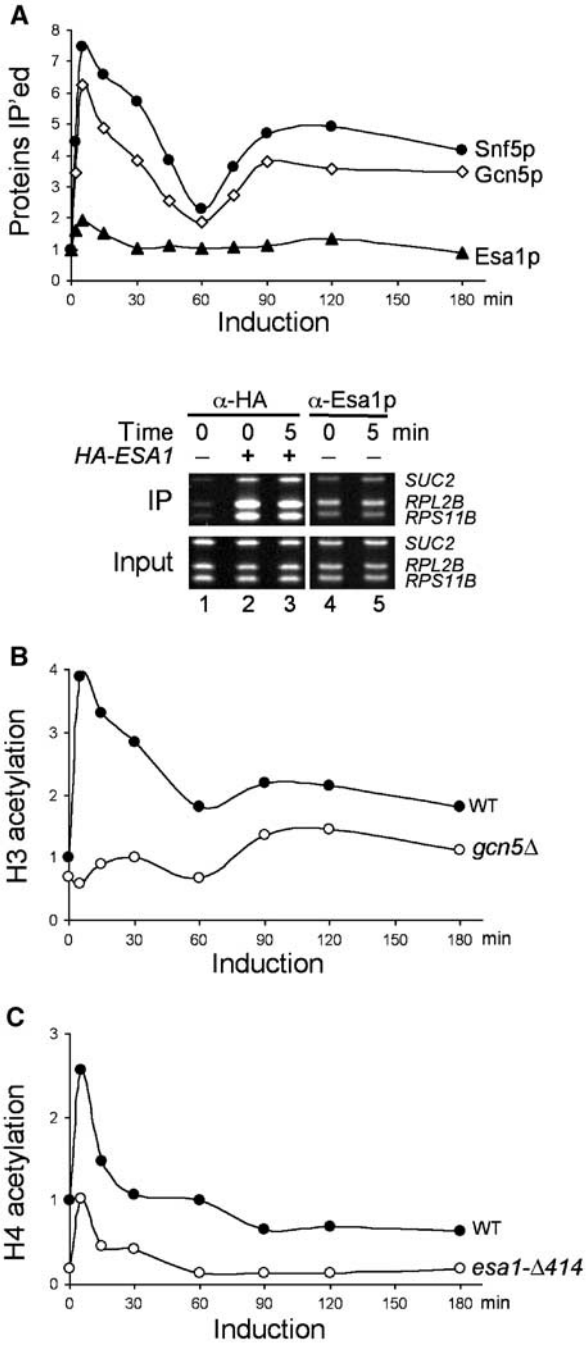

Figure 2 Snf5p and histone acetyltransferase activities associate with the *SUC2* promoter. (**A**) The crosslinking of Snf5p, Gcn5p, and Esa1p to the *SUC2* UAS DNA was analyzed by ChIP in raffinose-induced wild-type (BLY1) cells using polyclonal antibodies against each protein and the levels (IP efficiencies) presented as fold over preinduction levels (upper panel). ChIP experiments using anti-HA or anti-Esa1p antibodies were performed in *HA-ESA1* (BLY431) and *ESA1* (BLY1) cells that were glucose repressed (time 0) or derepressed for 5 min. The *SUC2* UAS and the promoter sequences of two ribosomal genes were amplified by PCR from the precipitated (IP) and input DNAs and stained with ethidium bromide after separation on 8% polyacrylamide gels (lower panel). (**B**) The acetylation of histone H3 at *SUC2* UAS in wild-type (BLY1) and *gcn5*Δ (BLY417) cells was compared by ChIP using an antibody against diacetylated H3 (K9 and K14). The levels of acetylation (IP efficiencies) were shown relative to wild-type preinduction levels. (**C**) The levels of acetylation of H4 at *SUC2* UAS in wild-type (BLY1) and *esa1-Δ414* (BLY457) cells were compared by ChIP as in (B) using an antibody against tetra-acetylated H4 (K5, K8, K12, and K16).

SUC2 throughout the experiment (Figure 2B). In *gcn5*Δ cells, the increase in H3 acetylation was largely abolished (Figure 2B), suggesting that Gcn5p is the major HAT responsible for H3 acetylation during *SUC2* activation.

The overall H4 acetylation at *SUC2* increased transiently following the carbon shift in the wild type (Figure 2C). In the conditional *esa1-Δ414* mutant, the basal levels of H4 acetylation and the induced increase in H4 acetylation were both significantly reduced compared to the wild type, suggesting that Esa1p contributes to both the basal and induced H4 acetylation at *SUC2*.

To address the specificity of the association of these chromatin-remodeling activities with *SUC2*, we assessed the crosslinking of Snf5p, Esa1p, and acetylated H3 and H4 at different DNA sequences along the *SUC2* locus. At 5 min (and at other time points) following induction, Snf5p crosslinking increased over the entire *SUC2* promoter region and peaked at the UAS sequence (Figure 3A; data not shown). Thus, Snf5p is specifically recruited to the *SUC2* promoter following the carbon source shift, presumably as SWI/SNF (Geng *et al*, 2001).

The highest increase in H3 acetylation occurred at the *SUC2* promoter, although significant acetylation was also detected at regions upstream and downstream of the *SUC2* promoter 5 min following induction (and at other time points) (Figure 3B; data not shown). These results suggest that Gcn5p is also specifically recruited to the *SUC2* promoter but appears to be distributed more widely than Snf5p.

The increase in H4 acetylation 5 min following *SUC2* induction was restricted to the same regions as H3 acetylation (Figure 3C, upper panel). Consistent with this finding, the parallel increase in Esa1p's association with *SUC2* also occurred primarily at the *SUC2* promoter region (Figure 3C, lower panel). Under repressing conditions, however, Esa1p appeared to associate globally with the *SUC2* locus, with a marginally higher affinity for the promoter region. In conclusion, SWI/SNF and Gcn5p were dynamically and specifically recruited to *SUC2* following gene induction, whereas Esa1p associated globally with *SUC2* under repressing conditions and was specifically but transiently recruited upon gene induction.

SWI/SNF, Gcn5p, and Esa1p differentially regulate the dynamic induction of SUC2

To investigate the roles of SWI/SNF, Gcn5p, and Esa1p in regulating the dynamic transcription of *SUC2*, we tested how loss-of-function mutations in the chromatin-remodeling factors affect *SUC2* mRNA levels at various times following induction. Little *SUC2* RNA was synthesized throughout induction in *snf5*Δ cells (Figure 4A), demonstrating that SWI/SNF is critical for both phases of *SUC2* induction.

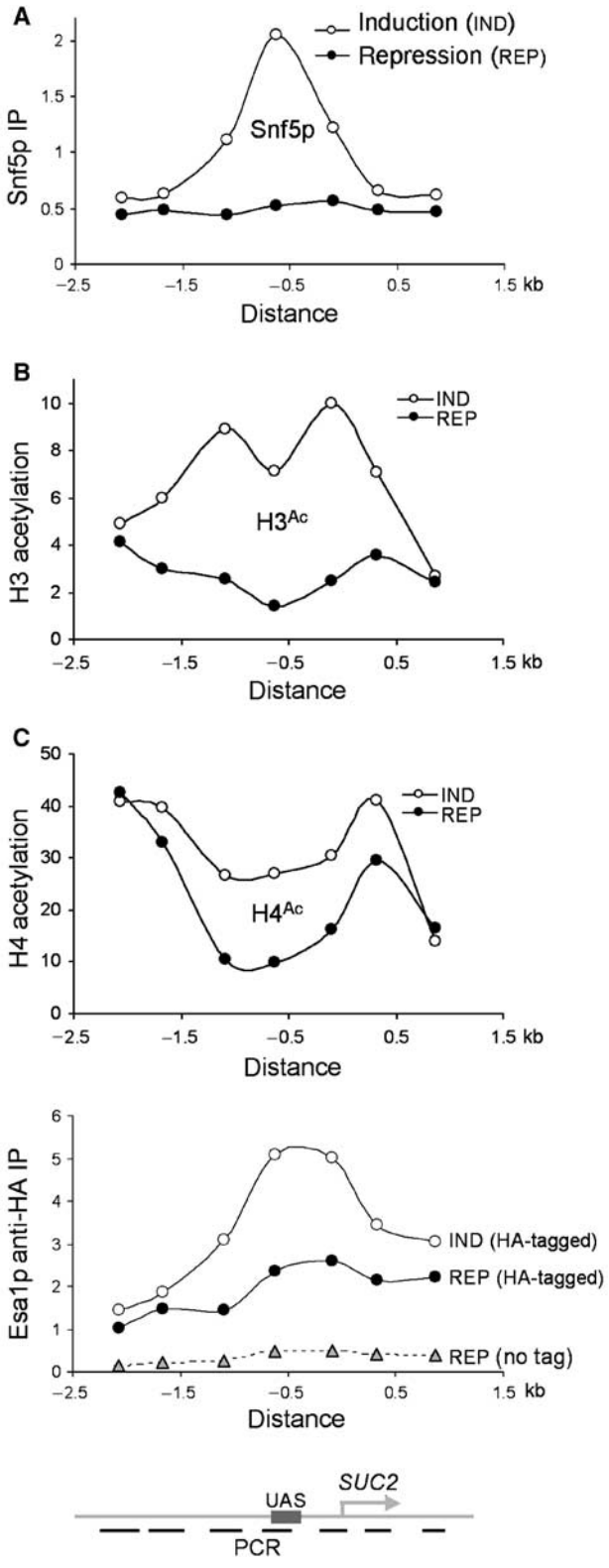

Figure 3 *SUC2* promoter-specific association of chromatin remodelers or remodeling activities. (**A**) Crosslinking of Snf5p to the different *SUC2* sequences (shown schematically below (C)) was determined by measuring [α-^{32}P]-dCTP-labeled multiplex PCR products of the precipitated and input DNAs from the same experiment for Figure 2A. IP efficiencies of *SUC2* sequences were shown relative to that of a subtelomeric sequence (Vogelauer *et al*, 2000). The distribution pattern of Snf5p, 5 min following induction, is shown. The distances are relative to the translational start site. The binding of Snf5p to *SUC2* following gene induction is under-represented for the sequences tested, as the levels of Snf5p crosslinking to the subtelomeric sequence following *SUC2* induction also increased slightly. The distribution of H3 (**B**) and H4 (**C**, upper panel) acetylation at *SUC2* was determined and presented as in (A). The distribution of Esa1p (C, lower panel) was similarly assayed from anti-HA ChIP experiments, except that IP efficiencies were shown in arbitrary units for comparison between HA-tagged and untagged (no tag) strains.

In *gcn5*Δ cells, *SUC2* transcript levels reached 75% of the wild-type levels after 3 h of induction, consistent with previous studies (Biggar and Crabtree, 1999; Sudarsanam *et al*, 1999). However, only 25–35% of the wild-type levels of *SUC2* mRNA were achieved during the first 2 h of induction in the *gcn5* mutant (Figure 4A), indicating that Gcn5p plays a more important role in activating *SUC2* transcription than previously appreciated (Biggar and Crabtree, 1999; Recht and Osley, 1999; Sudarsanam *et al*, 1999). These results demonstrate that Gcn5p accelerates the kinetics of *SUC2* induction. A similar role for Gcn5p has been observed at *PHO5* (Barbaric *et al*, 2001).

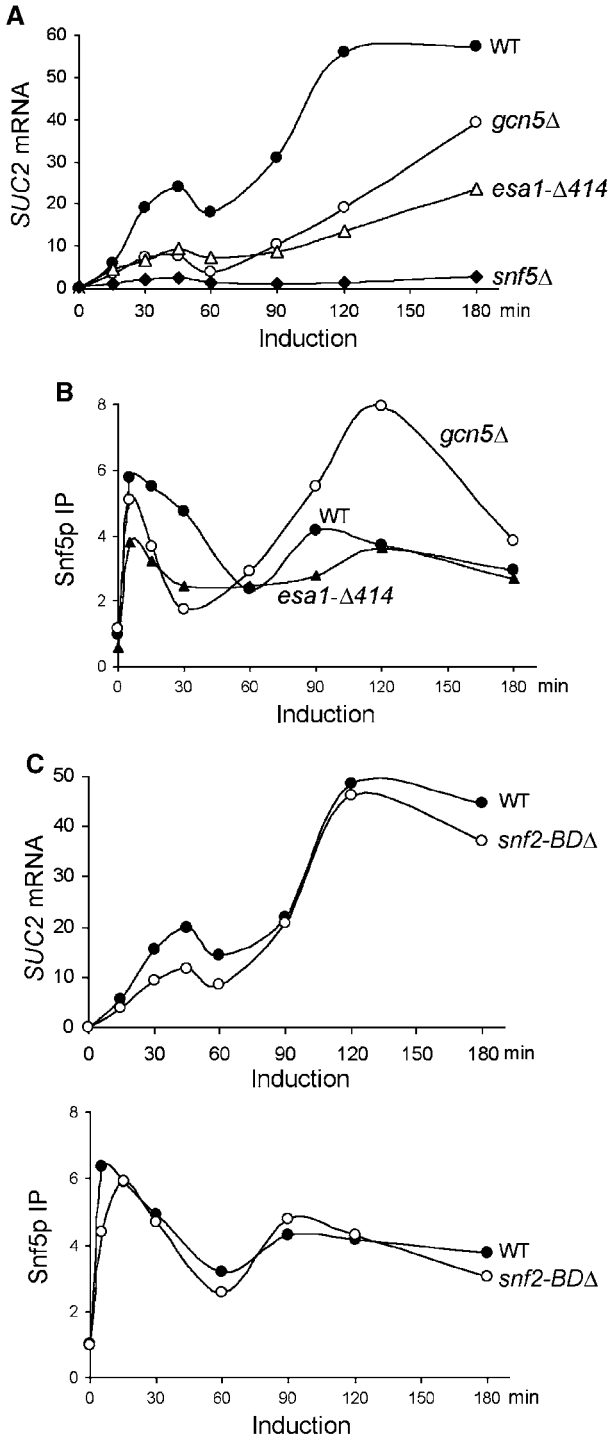

Figure 4 Snf5p, Gcn5p, and Esa1p differentially control *SUC2* transcription. (**A**) The levels of *SUC2* mRNA in wild-type (BLY1), *gcn5*Δ (BLY417), *esa1-Δ414* (BLY457), and *snf5*Δ (BLY3) cells were assayed as in Figure 1A. (**B**) The crosslinking of Snf5p to the *SUC2* promoter was assayed as in Figure 2A. IP efficiencies were shown relative to the wild-type preinduction levels. (**C**) The levels of *SUC2* mRNA (upper panel) and the crosslinking of Snf5p to the *SUC2* promoter (lower panel) in wild-type (BLY1) and *snf2-BD*Δ (BLY663) cells were assayed as in (A) and (B), respectively. These data are representative of three independent time courses.

The *esa1-Δ414* mutation reduced *SUC2* transcription to the same extent as the *gcn5*Δ mutation in the first phase of induction and caused a greater reduction in *SUC2* transcription during the second phase (Figure 4A). These results suggest that Esa1p is important for *SUC2* transcription throughout induction and thus provide physiological significance for the constitutive presence of Esa1p and its HAT activity at *SUC2* (Figure 2A and C).

The important, but nonessential, roles of Gcn5p and Esa1p in the transcriptional activation of *SUC2* prompted us to test whether Gcn5p or Esa1p stimulates *SUC2* transcription by facilitating SWI/SNF chromosomal binding. In *gcn5*Δ cells, Snf5p was crosslinked to the *SUC2* promoter as well as in the wild type 5 min following induction, demonstrating that the initial SWI/SNF association was independent of Gcn5p (Figure 4B). However, the efficient binding of SWI/SNF to the *SUC2* promoter during the first phase of induction was lost soon thereafter, as indicated by the rapid decrease in crosslinking of Snf5p between 5 and 30 min. Interestingly, during the second phase of induction, SWI/SNF associated with *SUC2* even more efficiently in *gcn5* cells than in wild-type cells (Figure 4B). Higher-than-wild-type binding of SWI/SNF to the *RNR3* promoter was also observed in *gcn5* cells 2 h after induction by DNA damage, although its association with chromatin was not tested at earlier time points (Sharma *et al*, 2003). Snf5p crosslinking to *SUC2* was also significantly reduced in the *esa1-Δ414* mutant during early gene induction (0–60 min), but reached wild-type levels by 2 h (Figure 4B). These results show that both Gcn5p and Esa1p are necessary for maximal binding of SWI/SNF to the *SUC2* promoter early during gene induction but not at later stages of transcription, suggesting compensation by other mechanisms (see Discussion). Conversely, we found that Gcn5p and Esa1p associate with *SUC2* independently of SWI/SNF (data not shown). We conclude that Gcn5p and Esa1 stimulate *SUC2* transcription partly by facilitating the association of SWI/SNF with the promoter.

The bromodomain is a conserved protein motif that recognizes and binds to acetylated histones (Zeng and Zhou, 2002). To test the role of the Snf2p bromodomain (the only bromodomain in SWI/SNF) in SWI/SNF recruitment to *SUC2*, we measured *SUC2* mRNA levels and Snf5p's association with *SUC2* in cells lacking the Snf2p bromodomain. In contrast to *gcn5*Δ and *esa1-Δ414* mutations, deletion of the Snf2p bromodomain primarily affected the first phase of *SUC2* transcription, reducing *SUC2* RNA levels by less than two-fold (compare Figure 4A and C, upper panel). Importantly, Snf5p's association with *SUC2* was only minimally affected in the *snf2-BD*Δ mutant (Figure 4C, lower panel). These results suggest that the Snf2p bromodomain plays little role in mediating the HAT-facilitated SWI/SNF association with *SUC2*. The more dramatic effect of an Snf2p bromodomain deletion on SWI/SNF binding to *SUC2* reported previously

(Hassan *et al*, 2002) may reflect differences in experimental reagents or conditions.

AMPK/Snf1 and cAPK co-regulate SWI/SNF and Gcn5p occupancy and SUC2 transcription

Two major signaling pathways transmit the glucose signal in yeast cells. One pathway involves the Snf1 kinase, the yeast homolog of the mammalian AMP-dependent protein kinase (AMPK). Snf1p is activated by low glucose, and is essential for the transcriptional induction of glucose-repressed genes, including *SUC2* (Carlson, 1999). The other pathway involves the cAMP-dependent protein kinase (cAPK), which controls cell growth in response to nutrient availability and acts antagonistically to Snf1 in nutrient response (Cannon and Tatchell, 1987; Thompson-Jaeger *et al*, 1991; Thevelein and de Winde, 1999), although its role in regulating *SUC2* transcription is unresolved (Hubbard *et al*, 1992).

To test the potential roles of Snf1 and cAPK in regulating the dynamic recruitment of SWI/SNF and Gcn5p to the *SUC2* promoter, we monitored Snf5p and Gcn5p promoter occupancy in the *snf1K84R* and *bcy1*Δ mutants. The *snf1K84R* mutation specifically abolishes the Snf1 kinase activity (Celenza and Carlson, 1989), and deletion of the *BCY1* gene, which encodes the regulatory subunit of cAPK, causes constitutive cAPK activity (Cannon and Tatchell, 1987; Toda *et al*, 1987). *snf1K84R* completely abolished the limiting glucose-induced increase in crosslinking of both Snf5p and Gcn5p (Figure 5A (upper panel) and B). We conclude that the *snf1K84R* mutation's abrogation of both Snf5p and Gcn5p recruitment is a selective rather than a general event, as Snf5p and Gcn5p recruitment to another target promoter, *RNR3* (Sharma *et al*, 2003), was not dramatically affected by *snf1K84R* (Figure 5A, lower panel; data not shown).

Deletion of *BCY1* delayed the promoter association of both Snf5p and Gcn5p in the first phase of *SUC2* induction, so that the crosslinking of both proteins peaked at 30 min rather than at 5 min following induction (Figure 5A (upper panel) and B). Notably, at later time points, higher levels of crosslinking of both proteins were detected in *bcy1*Δ cells compared to wild-type cells. These results demonstrate that Snf1 kinase activity is essential for the recruitment of SWI/SNF and Gcn5p to *SUC2* in response to limiting glucose, and suggest that down-regulation of cAPK activity is important for their early recruitment.

Gcn5p-dependent H3 acetylation at the *INO1* and *HO* promoters requires phosphorylation of H3 serine 10. Moreover, Snf1 is required for H3 phosphorylation at *INO1* (Lo *et al*, 2001). To test whether Snf1 similarly controls Gcn5p's HAT activity at *SUC2*, we measured *SUC2* H3

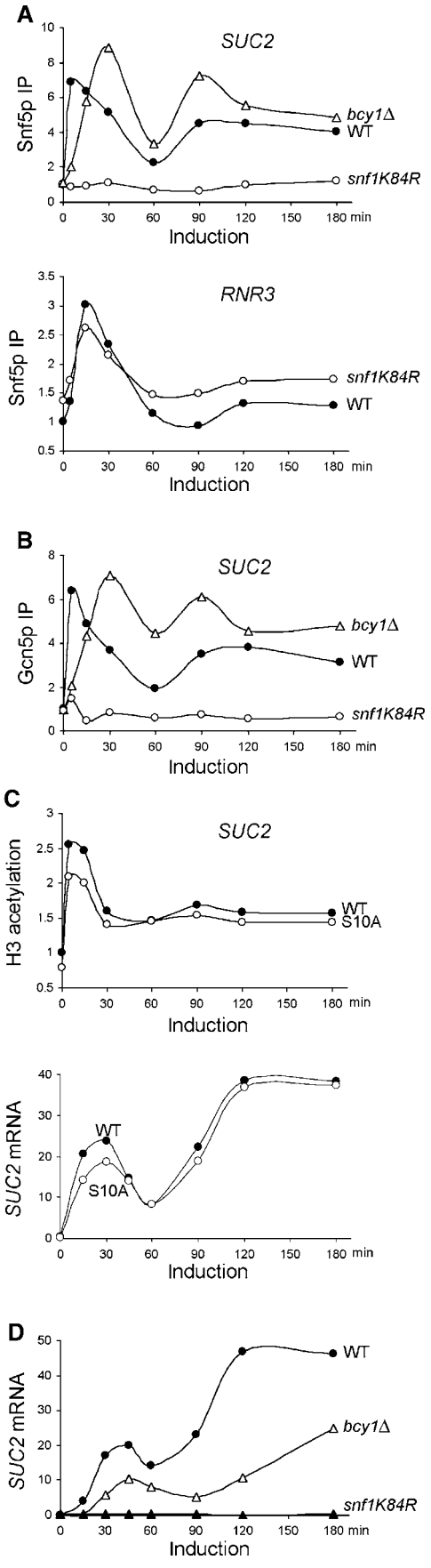

Figure 5 AMPK/Snf1 and cAPK regulate both recruitment of Snf5p and Gcn5p and transcription of *SUC2*. The crosslinking of Snf5p (**A**) and Gcn5p (**B**) to the *SUC2* or *RNR3* promoters and the levels of *SUC2* mRNA (**D**) were measured in wild-type (BLY1), *bcy1*Δ (BLY553), and *snf1K84R* (BLY463) cells as in Figure 4. (**C**) H3 acetylation (upper panel) and *SUC2* mRNA levels (lower panel) were assayed in wild-type (BLY484) and H3 S10A mutant (BLY485, in which serine 10 was changed to alanine) cells as in Figures 2B and 4A, respectively. These data are representative of at least two independent time courses each.

acetylation in the same mutant (H3 S10A) in which H3 acetylation at both *INO1* and *HO* promoters was diminished (Lo *et al*, 2001). By contrast, we found that the S10A mutation only slightly reduced the basal levels of H3 acetylation at the SUC2 UAS, but did not alter the magnitude of increase in H3 acetylation following induction (Figure 5C, upper panel), suggesting that S10 phosphorylation is not a prerequisite for H3 acetylation at *SUC2*. Moreover, the H3 S10A mutation only slightly reduced *SUC2* mRNA levels and Snf5p binding to *SUC2* during the early phase of transcription (Figure 5C, lower panel; data not shown). Therefore, it is unlikely that Snf1 plays a prominent role in regulating Gcn5p's H3-acetylating activity via phosphorylation of H3 serine 10 at *SUC2*.

Consistent with the essential role of Snf1 in controlling the recruitment of SWI/SNF and Gcn5p to *SUC2*, no *SUC2* transcripts were detected in the *snf1K84R* mutant throughout gene induction (Figure 5D). Despite the overall increased efficiency in SWI/SNF and Gcn5p crosslinking to *SUC2* in *bcy1*Δ cells compared to wild type, the *SUC2* mRNA levels in the mutant were actually reduced two-fold, suggesting that cAPK also negatively regulates *SUC2* transcription at steps other than promoter recruitment of SWI/SNF and Gcn5p.

Stress response transcription factors Msn2p/Msn4p function specifically in early SUC2 induction

Several lines of evidence suggest that Msn2p and Msn4p, two functionally redundant transcriptional factors involved in multiple stress responses, function in the dynamic induction of *SUC2*. First, the early induction of *SUC2* resembles a stress response in that SWI/SNF, Gcn5p, and pol II are recruited to the promoter within 2 min (Figures 2A and 1B). Second, acute glucose deprivation activates Msn2p/Msn4p transcriptional activity, presumably through downregulation of cAPK (Gorner *et al*, 2002). Third, *MSN2* was isolated as a multicopy suppressor of the *SUC2* expression defects in both *snf1* and *snf5* temperature-sensitive mutants (Estruch and Carlson, 1993; our unpublished data). Finally, Msn2p and Msn4p bind to the *SUC2* promoter DNA *in vitro* (Estruch and Carlson, 1993).

Like *bcy1*Δ, deletion of *MSN2/MSN4* delayed the recruitment of both Snf5p and Gcn5p to *SUC2* (Figure 6A and B). Moreover, *SUC2* mRNA levels in the *msn2*Δ *msn4*Δ mutants were two-fold lower than in the wild type in the first phase of gene induction. Interestingly, in the second phase of induction, *SUC2* mRNA accumulated more rapidly and reached higher levels in the mutant than in the wild type (Figure 6C), consistent with the parallel increases in promoter association of both Snf5p and Gcn5p (Figure 6A and B). We infer from these results that Msn2p/Msn4p act specifically in the early phase of *SUC*2 induction.

To substantiate the involvement of Msn2p/Msn4p in the first phase of *SUC2* transcription, the association of Msn2p with *SUC2* chromatin was measured by ChIP using a polyclonal anti-Msn2p antibody. We found that Msn2p crosslinking at the *SUC2* promoter increased more than three-fold 5 min following gene induction but quickly returned to repression levels within 30 min and did not increase further throughout the induction (Figure 6D). Additionally, the rapid recruitment of Msn2p to the *SUC2* promoter was essentially abolished in the *bcy1*Δ mutant (Figure 6D), consistent with the ability of cAPK to inhibit the nuclear localization of Msn2p (Gorner *et al*, 2002). Since the nuclear localization of Msn2p and Msn4p is regulated in the same fashion (Gorner *et al*, 2002), we assume that Msn4p associates with *SUC2* similarly.

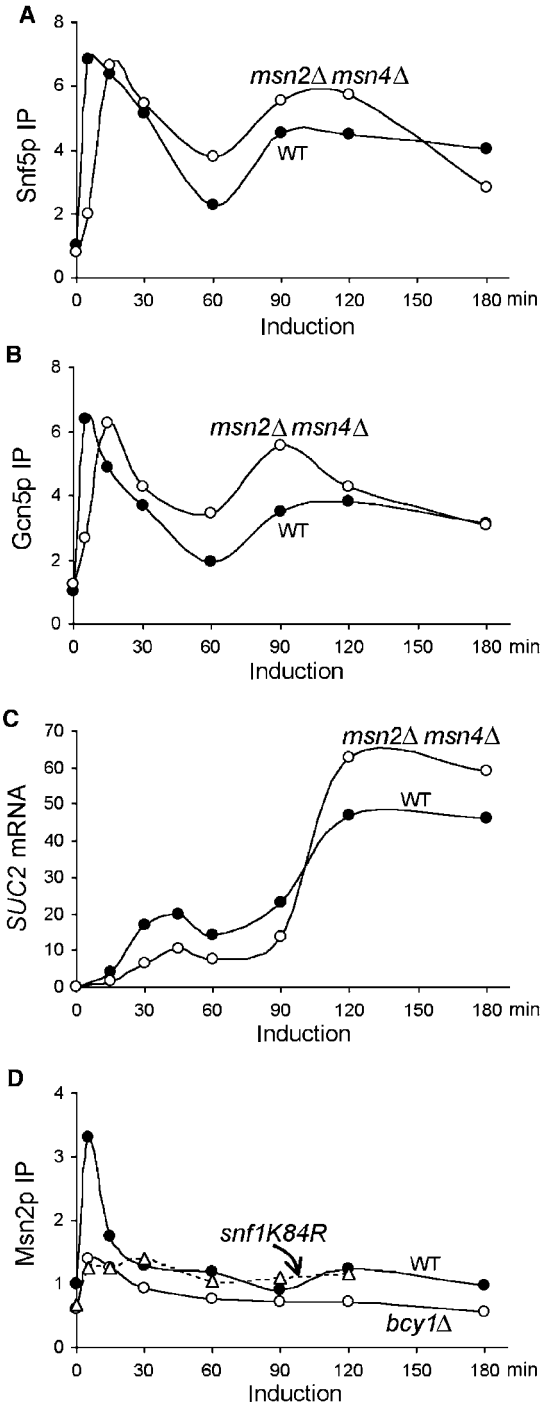

Figure 6 Msn2p/Msn4p are involved in early *SUC2* induction. The crosslinking of Snf5p (**A**) and Gcn5p (**B**) to the *SUC2* promoter and levels of *SUC2* mRNA (**C**) in the *msn2*Δ*msn4*Δ mutant (BLY559) were measured in the same experiments as in Figure 5. (**D**) The crosslinking of Msn2p to the *SUC2* UAS in wild-type (BLY1), *bcy1*Δ (BLY553), and *snf1K84R* (BLY463) cells was measured by ChIP using polyclonal anti-Msn2p antibody, and the levels (IP efficiencies) were shown relative to wild-type preinduction levels. The data in (A–C) are representative of three independent time courses, and those in (D) of two independent time courses.

Interestingly, further analysis of the association of Msn2p with the *SUC2* promoter in *snf5*Δ and *snf1K84R* mutants suggested that Msn2p binding to *SUC2* requires the presence of SWI/SNF and the Snf1 kinase activity. Like in the *bcy1*Δ mutant, the induced increase in Msn2p crosslinking to the *SUC2* promoter was largely abolished in the *snf1K84R* mutant (Figure 6D). Identical results were obtained in the *snf5*Δ mutant (data not shown). The effects of *snf5*Δ and *snf1K84R* on Msn2p association with the *SUC2* promoter are unlikely to be caused by defective expression of *MSN2* or the failure of Msn2p to enter the nucleus, as SWI/SNF does not regulate *MSN2* transcription (Holstege *et al*, 1998) and *snf1*Δ does not block the nuclear localization of Msn2p (Mayordomo *et al*, 2002). Together, these results suggest a role for Msn2p/Msn4p in the early induction of *SUC2*. Although we cannot rule out the possibility that Msn2p and Snf5p interact indirectly, we favor the idea that Msn2p and SWI/SNF function cooperatively for promoter association to initiate *SUC2* transcription.

Discussion

Here, we present evidence that *SUC2* transcription proceeds in a biphasic fashion. Different chromatin remodelers (SWI/SNF, Gcn5p, and Esa1p) play distinct roles and cooperatively activate *SUC2* transcription. Our study underscores the importance of applying time-course approaches in exploring the interplay of transcription factors in transcription.

Biphasic transcriptional induction in nutrient starvation

Although raffinose, a substrate of the *SUC2* gene product, is required for continual *SUC2* transcription, it does not appreciably affect the accumulation pattern of *SUC2* transcripts during the first 2 h of induction (Figure 1A). This result suggests that the biphasic pattern of *SUC2* transcription is not due to feedback from *SUC2* transcription itself, but rather reflects dynamic cellular changes in glucose metabolism. Therefore, it is likely that transcription of other glucose-regulated genes proceeds in a similar dynamic fashion, although this remains to be proven. Interestingly, we noticed that both nitrogen and amino-acid starvation also induce biphasic transcription from a large set of genes (Gasch *et al*, 2000). Thus, biphasic transcriptional induction could be a general response to the stress of nutrient starvation (Gasch *et al*, 2000; Causton *et al*, 2001; Gorner *et al*, 2002).

Why is the transcriptional induction biphasic? We suggest that the initial stress response triggers a transient repression of energy-consuming processes such as translation, transcription, and cell cycle progression (Martinez-Pastor and Estruch, 1996; Alberghina *et al*, 1998; Ashe *et al*, 2000; Gasch *et al*, 2000; Causton *et al*, 2001; Newcomb *et al*, 2003) (Figure 1C). As a result, the cell compensates for the sudden shortage of energy or other nutrients and quickly restores the balance between energy production and consumption. However, this balance is then upset when the cell resumes energy-consuming processes once it has corrected the stress-induced damages. The biphasic transcriptional repression that parallels transcriptional induction during amino-acid or nitrogen starvation (Gasch *et al*, 2000) further supports the idea that a general mechanism is responsible for the biphasic pattern.

The two phases of *SUC2* transcription may initiate independently, resembling the cyclical transcription of the estrogen-induced human cathespin D gene, in which the same group of transcription factors is recruited for each cycle (Shang *et al*, 2000). At *SUC2*, the two phases seem to communicate. For example, cells increase recruitment of SWI/SNF and Gcn5p to *SUC2* after early phase transcription is reduced by deletions of *BCY1* or *MSN2/MSN4*, and the second phase of recruitment of SWI/SNF increases in the *gcn5* mutant. We suggest that the cell senses the overall response in the first phase and adjusts the levels of the following responses accordingly. Nevertheless, it remains possible that factors recruited during the first phase, or stable modifications of histones (e.g., methylation) made in the first phase, persist and contribute to later phase(s) of transcription.

Distinct modes of recruitment of chromatin-remodeling enzymes

The data presented here strongly suggest, although do not prove, that SWI/SNF and Gcn5p are recruited to the *SUC2* promoter concurrently, rather than in a temporal order as has been defined at cell cycle-regulated or differentiation-induced promoters (Cosma *et al*, 1999; Shang *et al*, 2000; Agalioti *et al*, 2002; Soutoglou and Talianidis, 2002). A recent study suggests that the two remodeling factors are also recruited in parallel to osmotic stress-responsive promoters (Proft and Struhl, 2002). We hypothesize that different strategies for transcriptional regulation underlie the different modes of recruitment. For example, genes involved in stress response or metabolism respond to signals that sense different aspects of the cell's status simultaneously. The concurrent association of these chromatin-remodeling activities assures a rapid on–off switch for gene activity. In contrast, genes required for cell cycle progression, differentiation, or development sense and integrate various events that occur in sequence; the sequential recruitment of chromatin remodelers may function as checkpoints for subsequent transcriptional events to ensure that a gene will be activated at the right time.

Notably, Esa1p associates constitutively with *SUC2* to promote rapid and efficient transcriptional induction. In contrast to this mode of action, Reid *et al* (2001) have shown that Esa1p is specifically targeted to ribosomal protein genes to activate transcription, and that levels of promoter-associated Esa1p correlate with levels of gene transcription. We propose a new role for Esa1p, based on our results and by analogy to the role of nontargeted HDACs in rapidly turning off gene transcription (Vogelauer *et al*, 2000), in which Esa1p functions globally to turn on rapidly gene transcription by maintaining chromatin fluidity via acetylation of histone H4. Future studies examining the kinetics of transcription are needed to test this model.

Dynamic interplay of chromatin-remodeling activities

Despite differences in their temporal association with the *SUC2* promoter, Gcn5p and Esa1p are equally important for optimal *SUC2* transcription. This may be explained, in part, by our finding that both proteins facilitate SWI/SNF's binding to the *SUC2* promoter (Figure 4B). Our results further support the notion that HATs enhance the interaction of SWI/SNF with chromatin (Hassan *et al*, 2001, 2002; Agalioti *et al*, 2002), which could explain the ability of Gcn5p to increase nucleosome remodeling *in vivo* (Gregory *et al*, 1998, 1999; Syntichaki *et al*, 2000; Sharma *et al*, 2003). In addition, we

showed that the Snf2p bromodomain plays at most only a minor role in SWI/SNF's association with *SUC2* (Figure 4C). Therefore, other mechanism(s) must exist. One possibility is that the local HAT-modifiable structure of chromatin plays an important role in mediating HATs-facilitated SWI/SNF–chromatin association.

Interestingly, SWI/SNF binding to *SUC2* is impaired by *esa1* and *gcn5* mutations only in the first phase of induction; wild-type or even higher levels of SWI/SNF binding are achieved at later stages of induction. One explanation is that the HAT mutants compensate by generating stronger signals (e.g., a more active Snf1 kinase) to recruit more SWI/SNF to *SUC2* during later gene induction. This compensatory mechanism may also involve other factors that normally play redundant roles with the Gcn5p and Esa1p HATs. Regardless of the mechanism, an important finding from this study is that the effects of loss of HAT functions on the binding of SWI/SNF to chromatin *in vivo* differ temporally during the transcription of a single gene. Such roles for HATs could easily be missed in studies examining only steady-state transcription. Dynamic time-course studies therefore provide a more unambiguous examination of the interplay of these activities (and other factors) throughout the transcription of a gene.

Materials and methods

Yeast strains and media

Saccharomyces cerevisiae strains used in this study are in the S288c genetic background and are listed in Table I. Mutant strain constructions are described in Supplementary data.

YEP medium contains 1% yeast extract (Difco) and 2% bactopeptone (Difco). To induce the *SUC2* gene, early logarithmic phase (OD_{600} 0.5–0.6) cells grown in YPD medium (YEP + 2% dextrose) were collected onto a filter by filtration, washed with water, and resuspended immediately in YPR medium (YEP + 0.05% dextrose + 2% raffinose) unless noted. All cells were cultured at 30°C.

RNA analysis

Yeast cells were frozen on dry ice following collection. Total RNA was isolated with hot phenol (Ausubel *et al*, 1995) and reverse-transcribed with oligo $(dT)_{12-18}$ (Amersham) and Omniscript reverse transcriptase (Qiagen). The cDNA was quantified by real-time PCR analysis with primers amplifying *ACT1* (+323 to +613) or *SUC2* (−1 to +120) sequences. The *ACT1* transcript serves as a control. All the experiments were performed with at least two independent RNA preparations.

Chromatin immunoprecipitation

ChIP was carried out essentially as described previously (Geng *et al*, 2001), except that the IP buffer contains 50 mM HEPES (pH 7.5), 150 mM NaCl, 1 mM EDTA, 0.1% SDS, 1% Triton X-100, and 0.1% sodium deoxycholate. The following amounts of antibodies were used in a typical IP reaction: 3 µl anti-Snf5p (Geng *et al*, 2001), 2 µl anti-Pol II CTD (unphosphorylated; BabCO), 10 µl anti-Gcn5p or anti-Esa1p (Santa Cruz), 8 µl anti-HA (Santa Cruz), 6 µl anti-Msn2p (gift of F Estruch), or 1.5 µl anti-diacetyl-H3 (acetylated at K9 and K14) or anti-tetra-acetyl-H4 (acetylated at K8, K12, K14, and K16) (Upstate Biotechnology). The amounts of chromatin inputs varied with individual antibodies and were within the linear ranges in which the amount of DNA precipitated was proportional to the input. Inputs and precipitated DNAs were measured for specific sequences by real-time PCR or semiquantitative multiplex PCR. IP efficiencies were calculated by dividing IP signals by the corresponding input signals. All ChIP experiments were performed with at least two independent chromatin preparations. Primers were designed to amplify the following *SUC2* sequences: (−2239 to −1900), (−1817 to −1506), (−1228 to −960), UAS (−748 to −498), (−208 to 24), (−150 to +45), (+225 to +432), and (+780 to +960). Other primers amplify a 150-bp subtelomeric sequence (Vogelauer *et al*, 2000) or promoter sequences of *ACT1* (−290 to −117), *RPL2B* (−439 to −232), *RPS11B* (−421 to −253), or *RNR3* (−450 to −288).

Mitotic index

Yeast cells were fixed in 3.7% formaldehyde in the culture medium. After resuspension in phosphate-buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) and sonication, the single (unbudded), small-budded, and large-budded cells were determined microscopically. Experiments were carried out with three independent cultures.

PCR

Real-time PCR was performed with a DNA Engine Opticon system (MJ Research) and SYBR Green (Sigma). PCR reactions (15 µl) were set up in triplicate for each DNA sample with 0.5 U of HotStarTaq DNA polymerase (Qiagen). Relative quantitation was achieved from standard curves prepared from serial dilutions of genomic DNA. For most experiments, the cycle threshold ($c(t)$) was set at a fluorescence intensity of 0.0046 on baseline-subtracted data graphs (fluorescence versus cycle number). The values obtained from triplicate reactions were averaged, with errors <20%.

Multiplex PCR was conducted in reactions similar to those for real-time PCR except that they contained multiple primer pairs. After 27–29 cycles of amplification, the PCR products were separated on 8% polyacrylamide gels and stained with ethidium bromide; in some experiments, the PCR products were labeled by [α-^{32}P]-dCTP (Amersham) and quantified with a phosphorImager (Molecular Dynamics). PCR amplifications of both input DNA and ChIP-ed DNA were carried out in predetermined linear ranges.

Supplementary data

Supplementary data are available at *The EMBO Journal* Online.

Table I Yeast strains

Strain	Genotype	Source
BLY1 (=MCY829)	MATα *his3-Δ200 lys2-801 ura3-52*	This lab
BLY3	MATα *ade2-101 his3-Δ200 ura3-52 snf5-Δ2*	This lab
BLY417	MATα *his3-Δ200 lys2-801 gcn5::HIS3*	This study
BLY431	MATα *his3-Δ200 lys2-801 ura3-52 HA3-ESA1*	This study
BLY457	MATα *his3-Δ200 lys2-801 ura3-52 esa1-Δ414*	This study
BLY463 (=MCY2693)	MAT*a his3-Δ200 leu2-3,112 ura3-52 snf1K84R*	M Carlson
BLY553	MATα *his3-Δ200 lys2-801 ura3-52 bcy1::URA3*	This study
BLY484 (=JHY86)	MATα *ura3-52 leu2-3,112 trp1-289 his3Δ1 Δ(hht1 hhf1) Δ(hht2 hhf2)* pJH18[CEN ARS *TRP1 HHT2 HHF2*]	CD Allis
BLY485 (=JHY87)	MATα *ura3-52 leu2-3,112 trp1-289 his3Δ1 Δ(hht1 hhf1) Δ(hht2 hhf2)* pJH15[CEN ARS *TRP1 hht2-3*(S10A) *HHF2*]	CD Allis
BLY559	MATα *his3-Δ200 lys2-801 ura3-52 msn2::URA3 msn4::HIS3*	This study
BLY663	MATα *his3-Δ200 lys2-801 ura3-52 snf2-BDΔ*	This study

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