Targeting of Swi/Snf to the yeast *GAL1* UAS_G requires the Mediator, TAF_{II}s, and RNA polymerase II

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The chromatin remodeling activity of the Swi/Snf complex is essential for the expression of several yeast genes. Previous studies have suggested that recruitment of Swi/Snf requires the action of transcriptional activators. However, reports in metazoans and in yeast have provided evidence of interactions between Swi/Snf and the RNA polymerase II holoenzyme/Mediator complex. Here we show that recruitment of Swi/Snf to the galactose-inducible gene *GAL1* cannot be fully achieved without the integrity of the Mediator complex, TAF_{II}s, and RNA polymerase II. Moreover, artificial recruitment of Mediator is sufficient to tether both Swi/Snf and SAGA to the *GAL1* UAS_G. We further demonstrate that Swi/Snf recruitment at *GAL1* does not require acetylation of chromatin by Gcn5 nor the presence of SAGA. Based on these results, we conclude that interactions between the Gal4 activator and Swi/Snf are not sufficient to recruit the latter to the *GAL1* UAS_G, since interactions with the Mediator, TAF_{II}s, and RNA polymerase II are also important.

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Introduction

Current views on mechanisms of gene transcription suggest that chromatin structure presents an important barrier to this fundamental cellular process. It is generally accepted that chromatin must be remodeled in order to allow transcription initiation to occur. Several mechanisms have been proposed to account for the process of chromatin remodeling. The first protein machinery to be exemplified as such, the Swi/Snf complex, hydrolyzes ATP as a source of energy to increase the accessibility of the transcriptional machinery to nucleosomal DNA (Vignali *et al*, 2000; Havas *et al*, 2001; Becker and Hörz, 2002). The Swi/Snf complex is composed of 11 tightly associated subunits, one of which is the DNA-dependent ATPase Swi2/Snf2 (Becker and Hörz, 2002). Swi/Snf has been shown

to bind DNA and nucleosomes but without any known sequence specificity (Quinn *et al*, 1996; Côté *et al*, 1998). A second way designed to overcome the chromatin barrier is to covalently modify histone tails by machines such as the SAGA complex (Roth *et al*, 2001). SAGA possesses histone acetyltransferase (HAT) activity catalyzed by the Gcn5 subunit that preferentially targets histone H3 (Hampsey, 1997; Grant *et al*, 1998; Brown *et al*, 2000). The complex also performs other transcription-related activities, like facilitation of TATA-binding protein (TBP) binding (Dudley *et al*, 1999). The Ada1, Spt7, and Spt20 subunits are essential for SAGA integrity, whereas the other components are not (Grant *et al*, 1997; Sterner *et al*, 1999). A third way that has been suggested to account for chromatin remodeling is histone variants. In this case, incorporation of such variants into chromatin would either directly or indirectly make it more permissive to transcription. For example, in differentiated cells, the *Drosophila* histone variant H3.3 gradually replaces H3 in a replication-independent fashion upon exiting the cell cycle (Ahmad and Henikoff, 2002). This replacement forces the remodeling of nucleosomes in order to rapidly induce genes that were previously repressed (Ahmad and Henikoff, 2002). H2A.Z is another histone variant that could potentially create such specialized chromatin. H2A.Z is required for the proper expression of a subset of genes in yeast (Santisteban *et al*, 2000; Adam *et al*, 2001; Larochelle and Gaudreau, 2003), and has been shown to antagonize the spread of heterochromatin silencing (Meneghini *et al*, 2003).

How chromatin remodeling complexes are recruited to genes is a topic of intense research. Different mechanisms have been proposed to explain how Swi/Snf is targeted to specific genes. First, interactions with the general transcription machinery have been suggested to be involved in targeting Swi/Snf to promoters, as yeast and human Swi/Snf were shown to be associated with forms of the RNA polymerase II (polII) holoenzyme (Wilson *et al*, 1996; Cho *et al*, 1998; Neish *et al*, 1998), and artificial recruitment of the RNA polII holoenzyme, thus the Mediator complex, is sufficient to remodel chromatin at the *PHO5* promoter *in vivo* (Gaudreau *et al*, 1997). Two recent reports propose a pathway in which the general transcription machinery mediates association of Swi/Snf with promoters (Sharma *et al*, 2003; Yoon *et al*, 2003). Second, it has been reported that ATP-dependent chromatin remodeling complexes and HAT complexes are targeted to promoters through interactions with gene-specific transcriptional activators. In the case of Swi/Snf, various studies have shown that it directly interacts with the acidic activation regions of Gcn4, Hap4, Gal4, Pho4, Swi5, and VP16 (Natarajan *et al*, 1999; Neely *et al*, 1999, 2002; Yudkovsky *et al*, 1999; Prochasson *et al*, 2003). Furthermore, an activating region is required for *in vitro* transcriptional stimulation of chromatin templates by Swi/Snf (Neely *et al*, 1999). Another study has demonstrated,

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by an *in vitro* assay, that the activating region of activators is required for the recruitment of Swi/Snf to a target gene (Yudkovsky *et al.*, 1999). Two partially redundant activator-interaction domains have been identified in Swi/Snf that are important for targeting of the complex *in vivo* (Prochasson *et al.*, 2003). Targeting has also been proposed to be achieved through a bromodomain that functions as an acetyl-lysine-binding domain. A bromodomain is found in the Swi2/Snf2 subunit of Swi/Snf (Haynes *et al.*, 1992) and in nearly all nuclear HATs (Jeanmougin *et al.*, 1997). Interestingly, several *in vitro* and *in vivo* studies suggest that histone H3 acetylation by Gcn5 is a prerequisite for recruitment and maintenance of the Swi/Snf complex at a promoter (Syntichaki *et al.*, 2000; Hassan *et al.*, 2001).

Various genetic studies in yeast suggest that the Swi/Snf chromatin remodeling complex functions together with the Gcn5 HAT to counteract the repressive effects of chromatin. Importantly, yeast genes regulated by Swi/Snf largely overlap those controlled by Gcn5 (Holstege *et al.*, 1998). Moreover, combination of *swi/snf* and *ada/gcn5* mutations has synthetic phenotypes suggesting that these two complexes can perform overlapping functions (Pollard and Peterson, 1997; Roberts and Winston, 1997). Recruitment of different types of chromatin remodeling complexes has been shown to be highly ordered at some promoters. For example, *PHO8* induction first requires histone acetylation by Gcn5 and then ATP-dependent chromatin remodeling by Swi/Snf (Gregory *et al.*, 1999). In addition, acetylation also precedes Swi/Snf action for the activation of the interferon- β (*IFN- β*) gene and activation by the retinoic acid receptor/retinoid X receptor (RAR/RXR) in human cells (Agalioti *et al.*, 2000; Dilworth *et al.*, 2000). On the other hand, in yeast, expression of the *HO* gene and expression of many genes in late mitosis require remodeling by Swi/Snf for the subsequent recruitment of SAGA (Cosma *et al.*, 1999; Krebs *et al.*, 1999).

The expression of the *GAL1* gene in asynchronous *Saccharomyces cerevisiae* cells is generally independent of Swi/Snf (Burns and Peterson, 1997; Gaudreau *et al.*, 1997; Krebs *et al.*, 2000). However, certain conditions, such as a weakened Gal4 target promoter (Burns and Peterson, 1997; Gaudreau *et al.*, 1997) or a more condensed chromatin structure during mitosis (Krebs *et al.*, 2000), impose a requirement for Swi/Snf to fully induce the *GAL1* gene. A conceptually similar result has been obtained by Santisteban *et al.* (2000), which shows that either Swi/Snf or Gcn5 become essential at *GAL1* and *PHO5* when *HTZ1*, the H2A.Z-encoding gene, is deleted. These results raise the possibility that Swi/Snf and Gcn5, and perhaps other chromatin remodeling complexes, perform redundant functions at certain promoters that become essential only under conditions where chromatin is less permissive to transcription.

In order to investigate Swi/Snf targeting mechanisms, we performed chromatin immunoprecipitation (ChIP) at the *GAL1* and *GAL7* promoters. Our results show that Swi/Snf is recruited to the *GAL1* and *GAL7* UAS_G elements in asynchronous yeast cells even though the complex is not required for transcription of these genes. Moreover, Swi/Snf and SAGA are recruited with similar kinetics upon induction of the *GAL* genes and have a similar distribution over the *GAL1-10* locus, being largely enriched at the *GAL1* UAS_G region. Our results also show that neither histone acetylation by Gcn5 nor SAGA subunits are required for Swi/Snf recruitment at the *GAL1*

and *GAL7* UAS_G elements. Furthermore, recruitment of the Swi/Snf and SAGA complexes can be achieved by artificially tethering the Mediator to *GAL1* using a Gal4(DBD)-Gal11 fusion. Importantly, disruption of either the Mediator complex, TAF_{II} subunits, or RNA polymerase II dramatically reduces Swi/Snf recruitment to the *GAL1* UAS_G.

Results

Swi/Snf is recruited to the GAL1 and GAL7 UAS_G elements upon galactose induction

Generally independent of Swi/Snf action, *GAL1* gene expression can become strongly Swi/Snf-dependent either by changing promoter strength or chromatin architecture (Burns and Peterson, 1997; Gaudreau *et al.*, 1997; Krebs *et al.*, 2000). This raises the question of how and under which conditions Swi/Snf is targeted to the *GAL1* promoter. Either the Swi/Snf complex is only recruited when transcription of *GAL1* undergoes conditions adverse to the achievement of full gene induction (e.g. during mitosis) or Swi/Snf is always recruited upon *GAL1* induction independently of the requirement for its activity. In order to distinguish between these two possibilities, we measured the binding of Swi/Snf to the *GAL1* promoter under conditions not requiring Swi/Snf. Two representative subunits of the complex, Swi1 and Snf5, were Myc tagged and used in ChIP experiments, followed by quantitative PCR analyses. Cells were grown in the presence of raffinose, and galactose was then added to induce *GAL* gene expression. Aliquots for primer extension and ChIP assays were taken before, and 15, 30, 60, and 120 min following addition of galactose. A schematic representation of the *GAL7* and *GAL1-10* loci and the regions analyzed by PCR in the ChIP assays are depicted in the upper panel of Figure 1A. The bottom part of Figure 1A shows a representative titration of the input and immunoprecipitated material used for PCR to demonstrate linearity of the reactions. PCR amplifications corresponding to the *ARN1* promoter region were also carried out in most of our ChIP assays as an internal control to normalize the assays.

Figure 1B first shows that Swi/Snf subunits, Swi1-Myc and Snf5-Myc, are efficiently recruited to the *GAL1* UAS_G. However, recruitment of both subunits—and thus the Swi/Snf complex—is abolished when the ChIP assay is carried out in a strain lacking the Gal4 activator. Since Gal4 regulates expression of the *GAL7* gene, association of Swi1-Myc and Snf5-Myc to the *GAL7* UAS_G was therefore analyzed. Swi/Snf was also efficiently crosslinked to the *GAL7* UAS_G in a wild-type (WT) strain but not in the *gal4 Δ* strain (Figure 1B and D). Measurement of Swi/Snf binding at different time points following galactose induction demonstrates that the Swi/Snf complex is present and maintained at the *GAL1* and *GAL7* UAS_G elements throughout induction. In addition, Swi/Snf is rapidly recruited, reaching highest levels after 30 min of induction, and is then maintained at a constant level (Figure 1C and D). This is consistent with previous studies suggesting that the Swi/Snf complex is required for maintenance of activated transcription *in vivo* (Biggar and Crabtree, 1999; Sudarsanam *et al.*, 1999). Figure 1E shows that, as previously reported (Burns and Peterson, 1997; Gaudreau *et al.*, 1997), the *GAL1* and *GAL7* genes can be fully induced in a strain lacking *SWI1*. Taken together, these results indicate that the Swi/Snf complex is efficiently

Figure 1 *In vivo* binding of Swi/Snf to the *GAL1* and *GAL7* *UAS_G* elements. **(A)** Top: Representation of the *GAL7* and *GAL1-10* loci. Transcriptional initiation sites (arrows with +1), Gal4 binding sites (*UAS_G*) (black crossbars), and open reading frames (open shaded rectangles) are represented. Regions amplified by PCR in the ChIP experiments are identified by a line. Bottom: PCR titration of input and immunoprecipitated material for Snf5-Myc at the *GAL1* *UAS_G*. **(B)** ChIP analysis of the binding of Swi1-Myc and Snf5-Myc to the *GAL1* and *GAL7* promoters. Binding of Swi1-Myc and Snf5-Myc over time after galactose induction is shown for both the WT (KLY012 and KLY014) and *gal4Δ* strains (KLY015 and KLY016). **(C, D)** Binding of Swi/Snf to the *GAL1* and *GAL7* promoters, respectively. **(E)** Ability of *swi1Δ* cells to induce the *GAL1* and *GAL7* genes. *GAL1* primer extension analyses from WT (CY258) and *swi1Δ* cells (CY340) grown either in the presence of glucose (Glu) or induced by galactose for 3 h (Gal).

recruited to the *GAL1* and *GAL7* genes upon addition of galactose, and that recruitment is dependent on Gal4. Furthermore, Swi/Snf is recruited and maintained at the *GAL1* promoter under activated conditions independently of its requirement for full gene activity.

Swi/Snf and SAGA are recruited to the same region at the *GAL1-10* locus

Previous studies have demonstrated that the SAGA complex is recruited to the *GAL1* *UAS_G* by the Gal4 activator (Bhaumik and Green, 2001; Larschan and Winston, 2001; Bhaumik *et al*, 2004). In order to compare the recruitment kinetics of SAGA with that of Swi/Snf, we measured binding of the Myc-tagged Gcn5 HAT subunit to both *GAL1* and *GAL7* *UAS_G* elements at different time points following induction. Gcn5-Myc is efficiently and rapidly recruited to both the *GAL1* and *GAL7* *UAS_G* elements upon galactose induction (Figure 2A and B). A quantification of these results is presented in Figure 2B. The results further show that the Gcn5 subunit of SAGA is recruited to the *GAL1* and *GAL7* *UAS_G* elements with binding kinetics similar to those of the Swi/Snf complex (compare Figures 1C, D and 2B).

We next investigated the distribution of the Swi/Snf and SAGA complexes over the *GAL1-10* locus in order to compare their distribution patterns. Figure 2C first depicts a cartoon of the locus as well as the regions analyzed by PCR. A ChIP assay was carried out using yeast strains bearing Myc-tagged versions of Gcn5 or Snf5, and the *GAL1-10* genes were induced by the addition of galactose. The bottom part of the figure shows a representative ChIP experiment, and Figure 2D shows a quantification of these experiments. The distribution of Swi/Snf is identical to that of SAGA, and both are enriched only over the *UAS_G* region.

Histone acetylation and SAGA are not required for Swi/Snf targeting and maintenance at *GAL1* and *GAL7*

The experiments of Figure 2 demonstrate that Swi/Snf and SAGA are specifically recruited to the *GAL1* and *GAL7* *UAS_G* elements with similar kinetics. Since Swi2/Snf2 possesses a bromodomain, it is conceivable that histone acetylation by Gcn5 is involved in Swi/Snf recruitment and/or maintenance. To directly test this possibility, we measured the binding of Swi1-Myc at both *UAS_G* elements in a strain lacking the Gcn5 HAT. Figure 3A shows PCR analyses of the Swi1 ChIP at both the *GAL1* and *GAL7* *UAS_G*'s in a *gcn5Δ* strain. Quantification of Swi1-Myc binding reveals that Swi/Snf recruitment in *gcn5Δ* cells is comparable to its recruitment in WT cells (Figure 3B). Similar results were obtained using Snf5-Myc (data not shown). These results suggest that an acetylation mark by Gcn5 is not essential to recruit and maintain the Swi/Snf complex at the *GAL1* and *GAL7* *UAS_G* elements.

We also tested whether Swi/Snf could be recruited to *GAL* genes in the absence of the entire SAGA complex, which is important for *GAL1* gene induction (Marcus *et al*, 1994; Dudley *et al*, 1999; Bhaumik and Green, 2001). ChIP experiments were performed in *spt20Δ* cells (Figure 3C) since Spt20 is essential for the integrity of the SAGA complex (Grant *et al*, 1997; Sterner *et al*, 1999). Quantification of these results demonstrates that Swi1-Myc is efficiently recruited to the *GAL1* and *GAL7* *UAS_G* elements in the absence of SAGA (Figure 3D). Similar results were also obtained when testing the binding of Snf5-Myc (data not shown). We have also performed primer extension experiments to test the effect of either a *GCN5* or *SPT20* deletion on *GAL1* induction in the Myc-tagged Swi1 or Snf5 yeast strains. As previously reported by others (Marcus *et al*, 1994; Dudley *et al*, 1999), *GCN5* deletion did not affect *GAL1* induction whereas *SPT20* deletion significantly impaired *GAL1* induction (Figure 3E). Taken

Figure 2 Distribution of Swi/Snf and SAGA at the *GAL1-10* locus. (A) ChIP analysis of the binding of Gcn5-Myc (KLY031) to the *GAL1* and *GAL7* UAS_G during galactose induction. (B) Quantification of experiments as illustrated in (A). (C) Top: Representation of the *GAL1-10* locus. Regions amplified in the *GAL10* open reading frame (10 O-A), the *GAL1-10* promoter region (10 P-A, UAS_{G1-10}, and 1 P-A) and in the *GAL1* open reading frame (1 O-A to 1 O-C) are represented. Bottom: Distribution of Snf5-Myc and Gcn5-Myc over the *GAL1-10* locus. ChIP analysis of the binding of Snf5-Myc and Gcn5-Myc to the *GAL1-10* locus after 30 min addition of galactose. Data of input and immunoprecipitated material are shown for each region investigated. (D) Quantification of experiments as illustrated in (C).

together, these results show that neither histone acetylation by SAGA nor SAGA integrity is required for Swi/Snf recruitment at both the *GAL1* and *GAL7* UAS_G elements at least in the presence of the activator.

Swi/Snf and SAGA can be recruited to the *GAL1* UAS_G by artificial recruitment of the Mediator complex

Since it was previously demonstrated that Swi/Snf copurifies with the Mediator (Wilson *et al*, 1996), and since recruitment of the Mediator is sufficient to remodel nucleosomes at the *PHO5* promoter (Gaudreau *et al*, 1997), we wanted to test whether tethering the Mediator complex to the *GAL1* UAS_G would be sufficient to recruit Swi/Snf in the absence of the Gal4 activator. In order to address this issue, we made use of a fusion protein bearing the Gal4 DNA-binding domain (DBD) fused to the C-terminal end of the Gal11 Mediator component. When appropriately targeted to a promoter region, this fusion protein directly recruits the transcriptional machinery to the promoter and thereby bypasses the requirement for a transcriptional activator to induce a target gene. Such bypass experiments have been extensively documented (Ptashne and Gann, 2002). Figure 4A shows that expression of the Gal4

DBD alone—in a strain deleted for *GAL4* and bearing a Myc-tagged version of Snf5—does not induce *GAL1* expression even when galactose is added. The figure further demonstrates that the Gal4-Gal11 fusion can efficiently induce *GAL1* as previously reported (Farrell *et al*, 1996; Gaudreau *et al*, 1997). Moreover, the fusion protein is insensitive to galactose induction since it is not subjected to inhibition by Gal80 (Ma and Ptashne, 1987). PCR analyses of a ChIP assay using the same strains as in Figure 4A and quantification of the results are presented in Figure 4B and C. As expected, we observe no significant binding of Swi/Snf when the Gal4 DBD alone is expressed, whereas we observe efficient Swi/Snf recruitment when Gal4-Gal11 is expressed. Interestingly, we observe that Swi/Snf is constitutively recruited to the *GAL1* UAS_G when using this fusion protein, a result consistent with that of Figure 4A. Similar results were also obtained when using other Mediator components fused to a DBD such as Gal4-Srb2, targeted to *GAL1*, or LexA-Srb2 and LexA-Srb6, targeted to a synthetic *lacZ* reporter gene construct (data not shown) (Farrell *et al*, 1996; Gaudreau *et al*, 1999).

We next tested if artificial recruitment of the Mediator would also be able to recruit SAGA to the *GAL1* UAS_G. The

Figure 3 SAGA is not required for Swi/Snf recruitment at the *GAL1* and *GAL7* UAS_G elements. (A) ChIP analysis of Swi1-Myc binding to the *GAL1* and *GAL7* UAS_G in WT (KLY022) and *gcn5Δ* (KLY024) strains upon galactose induction. (B) Quantification of the experiment illustrated in (A). (C, D) Same experiment as in (A, B) with the exception that the Swi1-Myc binding was analyzed in WT (KLY020) and *spt20Δ* (KLY028) cells. (E) Ability of Swi1-Myc- and Snf5-Myc-expressing yeast cells to induce the *GAL1* gene. Primer extension assays were carried out with RNA purified from WT (KLY020, KLY021, KLY022, and KLY023), *gcn5Δ* (KLY024 and KLY025), and *spt20Δ* cells (KLY028 and KLY029) induced with galactose for 3 h.

Figure 4 Swi/Snf and SAGA are recruited to the *GAL1* UAS_G by artificial recruitment of the Mediator. (A) Artificial recruitment of the Mediator bypasses the requirement for a transcriptional activator. A strain deleted for *GAL4* and bearing a Myc-tagged version of Snf5 was transformed by a plasmid expressing the Gal4 DBD alone (KLY019) or fused to the C-terminal end of Gal11 (KLY018). Primer extension analyses were carried out with purified RNA from both strains. Yeast cells were grown in the presence of raffinose (–) and then galactose (+) was added in order to induce *GAL1* gene expression. (B) ChIP analysis of the binding of Swi/Snf to the *GAL1* UAS_G in yeast cells expressing Gal4-Gal11. Binding of Snf5-Myc in the strains used in (A). (C) Quantification of experiments as illustrated in (B) is shown. (D, E) Same experiment as in (B, D) with the exception that the binding of Gcn5-Myc (KLY044 and KLY043) was assessed. (F) ChIP analysis of the binding of Srb4-Myc and Rpb1 to the *GAL1* UAS_G in yeast cells expressing a Gal4-Gal11 fusion (KLY091 and KLY092). (G) Quantification of experiments as illustrated in (F).

assay presented above was repeated with Myc-tagged Gcn5 instead of Myc-tagged Snf5 and, surprisingly, similar results were obtained. Expression of the Gal4-DBD alone did not allow binding of Gcn5-Myc, while significant recruitment of Gcn5-Myc was achieved when expressing the Gal4-Gal11 fusion (Figure 4D). Quantification of the results shows that Gcn5-Myc is also efficiently recruited to the *GAL1* UAS_G in a constitutive fashion (Figure 4E). Thus, even though a direct interaction between the Gal4 activator and the Tra1 subunit of SAGA has been demonstrated *in vivo* (Bhaumik *et al*, 2004), direct or indirect interactions with the Mediator are sufficient to target SAGA, as well as Swi/Snf, to the *GAL1* UAS_G.

We next wanted to confirm that Gal4-Gal11 would indeed be able to recruit the Mediator as well as RNA polII at *GAL1*. To test this, we used a Myc-tagged *Srb4* strain expressing either Gal4 (DBD) or Gal4-Gal11. Figure 4F thus shows a ChIP assay using anti-Myc antibodies to immunoprecipitate *Srb4*-containing complexes or anti-Rpb1 antibodies to immunoprecipitate RNA polII-containing complexes. The results of the figure show that both *Srb4* and Rpb1 can be efficiently recruited to the *GAL1* UAS_G by the Gal4-Gal11 fusion but not by Gal4(DBD). Quantification of these results is shown in Figure 4G.

The Mediator is required for Swi/Snf recruitment at the *GAL1* UAS_G

Efficient recruitment of Swi/Snf to *GAL1* by the Gal4-Gal11 fusion protein suggested that integrity of the Mediator might be important for the process. In order to address this issue, we performed ChIP experiments with a yeast strain bearing a thermosensitive (*ts*) allele of *Srb4* (Thompson and Young, 1995), as well as Myc-tagged Swi1. When shifting these cells to a nonpermissive temperature, *Srb4* is quickly inactivated and the integrity of the entire Mediator complex is compromised (Koh *et al.*, 1998; Kang *et al.*, 2001). Figure 5A shows that Swi1-Myc is efficiently recruited to *GAL1* upon galactose induction when cells are grown at permissive temperature (28°C), whereas Swi1-Myc recruitment is significantly reduced when the same cells are shifted to nonpermissive temperature (37°C) (Figure 5B). We next wanted to verify if shifting the *srb4-138* strain to 37°C would indeed compromise recruitment of the Mediator to *GAL1*. We thus Myc-tagged Gal11 in the *srb4-138* strain background and carried out a ChIP experiment. Figure 5C shows that Gal11-Myc is efficiently recruited at the *GAL1* UAS_G upon galactose induction when cells are grown at 28°C. However, when cells are grown at 37°C, Gal11-Myc recruitment at *GAL1* is severely affected, a result demonstrating that the integrity of the Mediator complex is severely compromised, although not completely, under our experimental conditions. Quantification of these results is shown in Figure 5D. It is interesting to note that the extent of Swi/Snf recruitment at *GAL1* when cells are shifted to 37°C is comparable to Mediator recruitment. This thus underscores the importance of Mediator integrity for the recruitment of Swi/Snf to *GAL1*. Taken together, the results presented here and in the previous figure suggest that the

Swi/Snf complex must interact, directly or indirectly, with the Mediator in order to be recruited to the *GAL1* gene. A similar result was also obtained at the *GAL7* gene thereby extending our results to another *GAL*-inducible gene (data not shown).

Figure 5 The Mediator is required for Swi/Snf recruitment to the *GAL1* UAS_G. (A) ChIP analysis of the binding of Swi1-Myc to the *GAL1* UAS_G in the absence of the Mediator. Temperature-sensitive *srb4-138* mutant (KLY042) containing Swi1-Myc was grown at 28°C and either shifted to 37°C for 45 min to inactivate *srb4* (37°C) or kept at the permissive temperature (28°C). Galactose was added to media as in previous experiments *GAL* gene expression. (B) Quantification of experiments as shown in (A). (C, D) Similar experiment as in (A, B) except that binding of Gal11-Myc was analyzed (KLY090). PCRs contain chromosome II telomere primers used here as an internal control to normalize signals for each lane. (E) Persistence of the Swi/Snf complex in the absence of the Mediator. KLY042 cells were grown at the permissive temperature and galactose was added to the media for 30 min to induced *GAL* gene expression. Cells were then shifted to inducing media at permissive (28°C) or nonpermissive temperature (37°C). Aliquots were taken 30, 60, 120, and 180 min after a shift to the nonpermissive temperature. PCRs contain chromosome II telomere primers as an internal control. (F) Quantification of experiments as illustrated in (E).

We wanted to determine whether Mediator was also necessary for Swi/Snf maintenance at the *GAL1* UAS_G after it has been fully recruited to this gene. To investigate this, we again made use of the Swi1-Myc *srb4-138* strain as described in Figure 5A and B. Cells were grown at 28°C, and Swi1-Myc binding was monitored after 30 min of *GAL1* induction. In this strain, we had observed that maximal Swi/Snf binding at *GAL1* could be achieved within 30 min (see Figure 5B). We next shifted the same cell culture to 37°C and monitored Swi1-Myc binding after 30, 60, 120, and 180 min after inactivation of the Mediator. The results show that Swi1-Myc binding is not significantly affected under these conditions, suggesting that the Mediator is not required to maintain the Swi/Snf complex at *GAL1* after it has been recruited.

TAF_{II}s and RNA polII are required for Swi/Snf recruitment at the *GAL1* UAS_G

A previous report had demonstrated that Swi/Snf recruitment to a stress-inducible gene, *RNR3*, required TAF_{II}s, the general transcription machinery, as well as the Mediator (Sharma *et al*, 2003). We imagined that *GAL1*, much like *RNR3*, could also require TAF_{II}s and the general transcription machinery in order to recruit Swi/Snf. However, in the case of *RNR3*, no activator seems to be required for Swi/Snf recruitment since this gene is predominantly regulated by a repression mechanism (Sharma *et al*, 2003). It is thus conceivable that the requirement of *RNR3* for TAF_{II}s and the general transcription machinery would be unique and not applicable for genes that are induced predominantly by the action of an activator. Thus, in order to test if recruitment of Swi/Snf at *GAL1* also required TAF_{II}s and the general transcription machinery, we made use of three yeast strains, each bearing a thermo-sensitive allele of either *RPB1* (*rpb1-1*), which encodes the large subunit of RNA polII, *TAF1* (*taf1-2*), and *TAF12* (*taf12-9*) (see Sharma *et al*, 2003). All three yeast strains bear a Myc-tagged version of Swi2/Snf2. ChIP experiments were carried out with all three yeast strains either at the permissive temperature (28°C) or at the nonpermissive temperature (37°C). Figure 6A, C, and E shows ChIP data for the *rpb1-1*, *taf1-2*, and *taf12-9* strains, respectively, whereas Figure 6B, D, and F shows quantifications of these data. The results show that in all three strains, as expected, Swi2-Myc recruitment to the *GAL1* UAS_G is efficient when cells are grown at the permissive temperature, whereas this binding is almost abolished when cells are grown at the nonpermissive temperature. Taken together, these results show that TAF_{II}s and RNA polII are also essential for Swi/Snf targeting to the *GAL1* UAS_G.

Discussion

In this study, we have shown that the Swi/Snf chromatin remodeling complex is recruited to both the *GAL1* and *GAL7*

UAS_G elements in asynchronous cells, a context that does not require the function of Swi/Snf for maximal gene activity. Furthermore, recruitment of Swi/Snf does not require action

Figure 6 RNA polII and TAF_{II}s are required for Swi/Snf recruitment to the *GAL1* UAS_G. (A) ChIP analysis of the binding of Swi2-Myc (YJR598) to the *GAL1* UAS_G in an *rpb1-1* yeast strain. The experiment was performed as described in Figure 5A. (B) Quantification of experiments as illustrated in (A). (C, D) Similar experiments as in (A, B) except that binding of Swi2-Myc was assessed in a *taf1-2* mutant (YJR595). (E, F) Analysis of the binding of Swi2-Myc in a *taf12-9* mutant (YJR592).

of SAGA. Importantly, targeting of both complexes to the *GAL1* UAS_G can be achieved by artificial recruitment of the Mediator complex, a condition that bypasses the requirement for a transcriptional activator. In agreement with these findings, Swi/Snf recruitment to the *GAL1* UAS_G in the absence of either the Mediator, TAF_{II}s, or RNA polII is strongly affected.

Since Swi/Snf is present at as few as 200 copies per yeast cell (Côté *et al*, 1994; Cairns *et al*, 1996), it may be assumed that it is recruited to target genes only if there is a strict requirement for the activity of the complex. Interestingly, we have shown by ChIP assays that Swi/Snf is recruited to the *GAL1* and *GAL7* UAS_G elements regardless of the level of requirement for that chromatin remodeling complex. Why then is Swi/Snf recruited to *GAL1*? It is conceivable that Swi/Snf facilitates chromatin remodeling in asynchronous yeast cells as well as during mitosis. However, in unsynchronized cells, chromatin remodeling by Swi/Snf is dispensable since Swi/Snf and SAGA can perform redundant functions (Biggar and Crabtree, 1999; Sudarsanam *et al*, 1999), but when gene expression conditions become more subject to chromatin-mediated repression, such as when yeast cells enter mitosis, Swi/Snf might have a rate determinant role (Krebs *et al*, 1999).

Different orders of recruitment schemes for the Swi/Snf and SAGA complexes have been described for different promoters (Cosma *et al*, 1999; Gregory *et al*, 1999; Krebs *et al*, 1999; Agaloti *et al*, 2000; Dilworth *et al*, 2000). Our results show that both complexes are recruited with similar kinetics upon galactose induction, suggesting that they are simultaneously recruited to the *GAL1* UAS_G. Moreover, the presence of Swi/Snf or SAGA is not a prerequisite for the recruitment of the other complex since the amount of Swi/Snf immunoprecipitated in *gcn5Δ* and *spt20Δ* mutant cells is similar to a WT strain. In addition, the Swi/Snf independence of *GAL1* in unsynchronized cells makes it unlikely to be important for the subsequent recruitment of the required SAGA complex (Marcus *et al*, 1994). Another argument in favor of the suggestion that Swi/Snf and SAGA recruitment are not interdependent is that histone H3 acetylation levels at the *GAL1* promoter are maintained when Swi/Snf is absent (Krebs *et al*, 2000). Based on these results, we propose that no special order of recruitment is necessary in promoter targeting for Swi/Snf and SAGA at *GAL1* and *GAL7*. However, there could be an order of action for these cellular regulators in cases where the chromatin architecture of the promoter is altered. This idea is supported by the fact that Swi/Snf is a prerequisite for acetylation by Gcn5 in late mitosis (Krebs *et al*, 2000).

Several models have been proposed to explain how chromatin remodeling complexes are recruited to their target genes, and among these, targeting by a transcriptional activator has been widely reported (Natarajan *et al*, 1999; Neely *et al*, 1999, 2002; Yudkovsky *et al*, 1999). In a recent study, Bhaumik *et al* (2004) identified a direct interaction between the Gal4 activator and the Tra1 subunit of SAGA *in vivo*. Our results are also consistent with the fact that the Gal4 transcriptional activator plays a determinant role in the recruitment of both the Swi/Snf and SAGA complexes to the *GAL1* and *GAL7* loci upon galactose induction. However, targeting of Swi/Snf and SAGA to *GAL1* is unlikely to be the consequence of their sole interaction with the acidic activator Gal4 since artificial recruitment of the Mediator can also recruit both complexes to *GAL1*. These results suggest that both

Swi/Snf and SAGA can be actively recruited to those *GAL* genes simply by direct or indirect interactions with a Gal11-bearing Mediator. Moreover and importantly, Swi/Snf recruitment to *GAL1* is strongly affected in the absence of the Mediator, but the latter is not necessary to maintain Swi/Snf at *GAL1* once it has been fully recruited. It is conceivable that once recruited, Swi/Snf is able to interact with either other components of the transcriptional machinery or with chromatin, thereby making interaction with the Mediator dispensable. In agreement with this last possibility, we find that TAF_{II}s and RNA polII are also required to recruit Swi/Snf to *GAL1*. While we anticipate functional interactions between Swi/Snf and the Mediator, it is likely that another important role of the Mediator in Swi/Snf recruitment would be to assemble a functional transcription initiation complex—as its recruitment to *GAL1* precedes that of the general transcription machinery (Bryant and Ptashne, 2003)—including polII and TFIID, thus allowing Swi/Snf to interact with the transcriptional machinery. This possibility remains to be tested.

The fact that SAGA is not required for the recruitment of Swi/Snf to *GAL1* could imply that it is neither required for the recruitment of the Mediator, a prediction that is in agreement with the study of Bryant and Ptashne (2003). However, this result was recently challenged by Bhaumik *et al* (2004). We have thus carried out ChIP experiments aimed at measuring Mediator recruitment in the absence of SAGA under our experimental conditions and find that while there is a notable delay in Mediator recruitment, SAGA is not strictly essential for this process (see Supplementary Figure 1). Interestingly, it was also shown that the Mediator was not necessary for the recruitment of SAGA at the *GAL1* UAS_G (Bhaumik *et al*, 2004). Thus, it is likely that the interaction between Swi/Snf and the Mediator is more significant for its action than the interaction that exists between SAGA and the Mediator. In fact, Swi/Snf but not SAGA has previously been shown to copurify with RNA polII holoenzymes (Wilson *et al*, 1996). We surmise that even though the action of Gal4 alone may be sufficient to recruit Swi/Snf and SAGA to the *GAL1* and *GAL7* genes, the Mediator/transcriptional machinery can also independently fulfill this function. In contrast, the study of Yudkovsky *et al* (1999) has suggested that the Swi/Snf complex can be efficiently recruited to a chimeric *HIS4*-based promoter template from a yeast extract preparation bearing a deletion in the Mediator component *SRB2*. In a similar *in vitro* experiment, deletion of *SRB2* was shown to prevent recruitment of the Mediator and RNA polII to a promoter (Ranish *et al*, 1999). It is conceivable that at some promoters, the Mediator might not elicit interactions with Swi/Snf, or alternatively the discrepancy existing between our results and theirs might be due to the use of different experimental systems. The involvement of other types of interactions, such as those elicited by the bromodomain of Swi2/Snf2 and acetylated chromatin, remains to be assessed at the *GAL* genes. However, we consider the contribution of the Swi2/Snf2 bromodomain to be nonsignificant at *GAL1* since it was shown that Swi/Snf is still efficiently recruited in *gcn5Δ* cells to *GAL1*, and since Gcn5 is the primary H3 HAT at *GAL1* (Krebs *et al*, 2000). Furthermore, H4 was shown to be deacetylated upon galactose induction at *GAL1* (Deckert and Struhl, 2001). Thus, we imagine that activator and Mediator/transcriptional machinery-dependent interactions with certain chromatin remodeling/coactivator complexes could perform redundant

recruitment functions that would make up a system designed to quickly and efficiently induce certain genes. This mechanism could be particularly useful for genes that require quick and robust induction, such as the *GAL* genes, in order for an organism to adapt to a rapidly changing environment.

Materials and methods

Yeast strains and genetic methods

A list of yeast strains used in this study is provided in Table I. The Myc-tagged strains used in our studies were generated by homologous recombination using a PCR-directed method with a 9Myc-*URA3* cassette (Cosma *et al.*, 1999).

Gene induction and primer extension assays

GAL genes expression was induced by growing cells to OD₆₀₀ of 1.0 in yeast nitrogen base supplemented with the required amino acids and 2% raffinose. Galactose was then added to a final concentration of 5% for up to 60 min. In order to monitor *GAL* gene expression in an *swi1Δ* strain, cells were grown in yeast extract-peptone-2% dextrose to OD₆₀₀ of 1.0, and then the cells were washed three times with sterile water and resuspended in yeast extract-peptone-2% galactose. Cells were further incubated for 3 h. For primer extension assays, 20 μg of RNA was used, and primer extension analyses were carried out essentially as previously described (Ma and Ptashne, 1987). The sequences for the oligonucleotide primers used were as

follows: *GAL1*, CTCCTTGACGTTAAAGTATAGAGG; *GAL7*, GGATGG TAACGTCTATGGGAATGGC. For RNA loading controls, 10 μg of total RNA was loaded onto agarose-formaldehyde gels.

Chromatin immunoprecipitations

ChIP experiments were performed as described previously (Adam *et al.*, 2001; Laroche and Gaudreau, 2003) with some modifications. Briefly, cells were grown to an OD₆₀₀ of 0.8 in yeast nitrogen base supplemented with the required amino acids and 2% raffinose. Galactose was then added to a final concentration of 5% to induce *GAL* genes, and 50 ml samples were collected 0, 15, 30, 60, and 120 min after induction. For temperature-shift experiments, cells were either grown in yeast nitrogen base supplemented with the required amino acids and 2% raffinose or in yeast extract-peptone-2% raffinose at 28°C until OD₆₀₀ reached 0.8. Cells were then pelleted and resuspended in media prewarmed at 37°C. Following a 45-min preincubation period, galactose was added to induce *GAL* gene expression. To perform the Swi/Snf persistence experiment, cells were grown until OD₆₀₀ reached 0.8. *GAL* gene expression was induced by the addition of galactose (5% final concentration). After 30 min induction, cells were pelleted and resuspended in inducing media at permissive (28°C) or nonpermissive temperature (37°C). Samples (50 ml) were collected 0 and 30 min after induction before the temperature shift, and 30, 60, 120, and 180 min after shifting cells to nonpermissive temperature (37°C). All immunoprecipitations were performed using 500 μl of whole-cell extract incubated with either anti-Myc (9E10, Santa Cruz) or anti-CTD (8WG16, BabCo) antibodies coupled to magnetic beads (Dyna). In all, 1 or 3 μl of DNA was used for the PCRs. PCRs contained either *ARN1* or

Table I Yeast strains used in this study

Strain	Genotype	Source
BY4741	<i>MATa</i> , <i>his3Δ1</i> , <i>leu2Δ0</i> , <i>met1Δ50</i> , <i>ura3Δ0</i>	RESEGEN
CY258	<i>MATα swi1Δ::LEU2</i> , <i>his3-Δ200</i> , <i>ura3-Δ99</i> , <i>leu2-Δ1</i> , <i>ade2-101</i> , <i>lys2-801</i>	C Peterson (Univ. Mass. Medical School)
CY340	<i>MATα</i> , <i>his3-Δ200</i> , <i>ura3-Δ99</i> , <i>leu2-Δ1</i> , <i>lys2</i>	C Peterson (Univ. Mass. Medical School)
FY250	<i>MATα</i> , <i>his3-Δ200</i> , <i>ura3-52</i> , <i>leu2-Δ1</i> , <i>trp1Δ63</i>	F Winston (Harvard Medical School)
JPY9	<i>MATα</i> , <i>his3-Δ200</i> , <i>ura3-52</i> , <i>leu2-Δ1</i> , <i>trp1Δ63</i> , <i>gal4Δ11</i>	Barberis <i>et al.</i> (1995)
KLY012	FY250; <i>SWI1-9myc::URA3</i>	This study
KLY014	FY250; <i>SNF5-9myc::URA3</i>	This study
KLY015	JPY9; <i>SWI1-9myc::URA3</i>	This study
KLY016	JPY9; <i>SNF5-9myc::URA3</i>	This study
KLY018	KLY016; <i>pSO74::LEU2</i>	This study
KLY019	KLY016; <i>pLRG234::LEU2</i>	This study
KLY020	BY4741; <i>SWI1-9myc::URA3</i>	This study
KLY021	BY4741; <i>SNF5-9myc::URA3</i>	This study
KLY022	PSY316; <i>SWI1-9myc::URA3</i>	This study
KLY023	PSY316; <i>SNF5-9myc::URA3</i>	This study
KLY024	PSY316 <i>gcn5Δ</i> ; <i>SWI1-9myc::URA3</i>	This study
KLY025	PSY316 <i>gcn5Δ</i> ; <i>SNF5-9myc::URA3</i>	This study
KLY028	7390; <i>SWI1-9myc::URA3</i>	This study
KLY029	7390; <i>SNF5-9myc::URA3</i>	This study
KLY031	FY250; <i>GCN5-9myc::URA3</i>	This study
KLY033	JPY9; <i>GCN5-9myc::URA3</i>	This study
KLY042	Z628; <i>SWI1-9myc::URA3</i>	This study
KLY043	KLY033; <i>pSO74::LEU2</i>	This study
KLY044	KLY033; <i>pLRG234::LEU2</i>	This study
KLY053	BY4741; <i>GAL11-9myc::URA3</i>	This study
KLY054	7390; <i>GAL11-9myc::URA3</i>	This study
KLY055	BY4741; <i>SRB4-9myc::URA3</i>	This study
KLY056	7390; <i>SRB4-9myc::URA3</i>	This study
KLY079	JPY9; <i>SRB4-9myc::URA3</i> , <i>pSO74::LEU2</i>	This study
KLY080	JPY9; <i>SRB4-9myc::URA3</i> , <i>pLRG234::LEU2</i>	This study
KLY090	Z628; <i>GAL11-9myc::URA3</i>	This study
KLY091	JPY9; <i>SRB4-9myc::URA3</i> , <i>pSO74::LEU2</i>	This study
KLY092	JPY9; <i>SRB4-9myc::URA3</i> , <i>pLRG234::LEU2</i>	This study
MAY424	W303; <i>htz1Δ::hisg</i>	Adam <i>et al.</i> (2001)
PSY316	<i>MATα</i> , <i>ade2-101</i> , <i>his3-Δ200</i> , <i>leu2-3</i> , <i>112</i> , <i>lys2</i> , <i>ura3-53</i>	Candau <i>et al.</i> (1996)
PSY316 <i>gcn5Δ</i>	PSY316; <i>gcn5Δ::hisg</i>	Candau <i>et al.</i> (1996)
JR592	PH499; <i>taf12-9::HIS3</i> , <i>SNF2-13-myc::KanMX</i>	Sharma <i>et al.</i> (2003)
JR595	PH499; <i>taf1-2::TRP1</i> , <i>SNF2-13-myc::KanMX</i>	Sharma <i>et al.</i> (2003)
JR598	Z676; <i>rpb1-1</i> , <i>SNF2-13-myc::KanMX</i>	Sharma <i>et al.</i> (2003)
W303α	<i>MATα</i> , <i>ura3-1</i> , <i>leu2-3</i> , <i>112</i> , <i>ade2-1</i> , <i>his3-11</i> , <i>15</i> , <i>trp1-1</i> , <i>can1-100</i>	Primig <i>et al.</i> (2000)
Z628	<i>MATa</i> , <i>his3-Δ200</i> , <i>leu2-3</i> , <i>ura3-52</i> , <i>srb4Δ2::HIS3</i> , <i>pR42882::LEU2</i>	Thompson and Young (1995)
7390	BY4741; <i>spt20Δ::kanMX4</i>	RESEGEN

chromosome II telomere primers (*TEL*) used as an internal control to normalize signals for each lane. All the ChIP data were quantified by phosphorImaging (Phosphor Imager, Molecular Dynamics). Reactions without antibodies were also carried out for all experiments. The sequence of the oligonucleotides used for all of the experiments is available upon request. Quantification of results represents mean values and standard deviations from at least two independent experiments.

Supplementary data

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

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