Regulation of an intergenic transcript controls adjacent gene transcription in *Saccharomyces cerevisiae*

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Recent studies have revealed that transcription of noncoding, intergenic DNA is abundant among eukaryotes. However, the functions of this transcription are poorly understood. We have previously shown that in *Saccharomyces cerevisiae*, expression of an intergenic transcript, *SRG1*, represses the transcription of the adjacent gene, *SER3*, by transcription interference. We now show that *SRG1* transcription is regulated by serine, thereby conferring regulation of *SER3*, a serine biosynthetic gene. This regulation requires Cha4, a serine-dependent activator that binds to the *SRG1* promoter and is required for *SRG1* induction in the presence of serine. Furthermore, two coactivator complexes, SAGA and Swi/Snf, are also directly required for activation of *SRG1* and transcription interference of *SER3*. Taken together, our results elucidate a physiological role for intergenic transcription in the regulation of *SER3*. Moreover, our results demonstrate a mechanism by which intergenic transcription allows activators to act indirectly as repressors.

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The analysis of genome-wide transcription in many organisms, including bacteria, yeast, *Drosophila*, *Arabidopsis*, mouse, and human, has yielded a common, yet unexpected feature. In addition to the transcription of protein-coding sequences, there is also widespread transcription across non-protein-coding regions (for reviews, see Mattick 2003; Morey and Avner 2004; Huttenhofer et al. 2005; Johnson et al. 2005). In humans, such noncoding transcripts have been detected from intergenic DNA, from introns, and from antisense transcription of exons (Chen et al. 2002; Kapranov et al. 2002; Saha et al. 2002; Bertone et al. 2004; Kampa et al. 2004; Cheng et al. 2005). Additional analysis suggests that much of this transcription is regulated and, thus, may itself play regulatory roles (Cawley et al. 2004; Kim et al. 2005). Recent experiments in the yeast *Saccharomyces cerevisiae* have also provided evidence for extensive transcription of noncoding regions (Hurowitz and Brown 2003; Havilio et al. 2005; Wyers et al. 2005).

One large and broad class of noncoding RNAs that has been studied in prokaryotes and eukaryotes plays direct roles in the regulation of gene expression (for reviews, see Bernstein and Allis 2005; Storz et al. 2005). These regulatory noncoding RNAs have been shown to function at many different levels of gene expression. In *Escherichia coli*, >50 small noncoding RNA regulators have been identified (for reviews, see Gottesman 2004; Storz et al. 2005). In eukaryotes, one intensively studied group, short interfering RNAs (siRNAs), has been shown to control both chromatin structure and mRNA stability (for reviews, see Meister and Tuschl 2004; Bernstein and Allis 2005). Other well studied eukaryotic categories include the *Xist* and *Tsix* RNAs that are involved in X-inactivation in mammals, and the *roX1* and *roX2* RNAs that are required for dosage compensation in *Drosophila* (for reviews, see Andersen and Panning 2003; Bernstein and Allis 2005). In addition to these examples, noncoding RNAs have been shown to regulate virtually all known steps of gene expression from transcription initiation to mRNA translation.

Several studies have suggested a second class of noncoding transcription that plays an important role in transcription regulation. In this second class, it is the act of transcription that confers activation, rather than the RNA product itself (for review, see Morey and Avner 2004). One example of this type of regulation occurs in the *Drosophila* bithorax complex (BX-C). Several early studies demonstrated that transcription occurs across noncoding regions at BX-C, suggesting a possible role in

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mediating repression by the Polycomb group (PcG) complexes (Lipshitz et al. 1987; Sanchez-Herrero and Akam 1989; Cumberledge et al. 1990). More recent studies have shown a correlation between intergenic transcription across PcG response elements (PREs) at the BX-C locus and the relief of silencing by PcG complexes (Bender and Fitzgerald 2002; Hogga and Karch 2002; Rank et al. 2002). Most recently, studies using a transgenic reporter system have demonstrated that transcription in either direction across a PRE blocks repression by PcG complexes and that this activated state is inheritable (Schmitt et al. 2005). A second example occurs at the 50-kb human β-globin locus, where developmentally regulated intergenic transcription occurs in three large chromatin subdomains and is required for the proper developmental regulation of the five globin genes within this locus (Gribnau et al. 2000). The domains of intergenic transcription correlate with the regions of DNase I sensitivity, again strongly suggesting that transcription confers chromatin changes.

In contrast to cases in which intergenic transcription activates gene expression, we recently reported a case in *S. cerevisiae* in which intergenic transcription represses gene expression (Martens et al. 2004). Our work identified a noncoding RNA, *SRG1*, which is transcribed from intergenic DNA, and showed that transcription of *SRG1* across the promoter of the adjacent *SER3* gene represses *SER3* transcription. We provided evidence for a model in which transcription of *SRG1* represses *SER3* expression by transcription interference. However, the role of *SRG1* in normal *SER3* regulation was not addressed.

In this work, we use the *S. cerevisiae SRG1/SER3* system to provide new insights into the physiological role in regulation by an intergenic transcript. First, we show that *SRG1* transcription is induced by high serine levels, resulting in repression of *SER3*. Second, we identify the serine-responsive activator Cha4 as acting directly in *SRG1* induction, thereby indirectly repressing *SER3*. Third, we show that Cha4 recruits the SAGA and Swi/Snf coactivator complexes to the *SRG1* promoter in a serine-dependent manner. Finally, we show that both SAGA and Swi/Snf are required for *SER3* repression by facilitating Cha4-dependent activation of *SRG1*. Taken together, our results show that a physiological response to serine, repression of *SER3* transcription, occurs by activating *SRG1* intergenic transcription. Based on these results and previous work that implicates Cha4 as a direct activator of the serine catabolism *CHA1* gene (Holmberg and Schjerling 1996; Sabet et al. 2003), intergenic transcription provides a mechanism for a single protein to simultaneously activate and repress opposing pathways.

Results

Serine-dependent regulation of SER3 *requires the expression of* SRG1 *from intergenic DNA*

We have previously shown that transcription of *SER3* is strongly repressed in YPD, a serine-containing medium, by transcription of *SRG1* from intergenic DNA (Fig. 1A; Martens et al. 2004) To investigate a physiological role for this repression mechanism, we performed Northern analysis to assay the response of *SER3* and *SRG1* RNA levels to changes in serine levels. First, when wild-type cells were grown in minimal medium with serine (SD + ser) and then shifted to minimal medium without serine (SD), there was a rapid but transient increase in *SER3* mRNA levels (Fig. 1B, lanes 1–9). Conversely, *SRG1* RNA levels, initially high in the presence of serine, underwent a rapid but transient decrease when the cells were shifted from SD + ser to SD medium. When the complementary shift was performed, from SD to SD + ser medium, the opposite effect was observed, as *SER3* mRNA levels decreased, while *SRG1* RNA levels were induced to a significantly greater level (Fig. 1C, lanes 1–9). These results demonstrate that expression of *SER3* and *SRG1* are tightly but oppositely regulated by the availability of serine.

Next we tested whether transcription of *SRG1* is re-

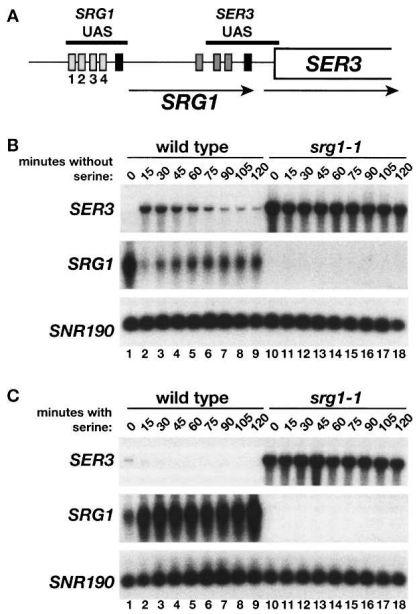

Figure 1. Effect of serine on *SER3* and *SRG1* expression. (*A*) A schematic of *SRG1* and *SER3*, showing TATA and putative UAS sequences conserved between *S. cerevisiae* and four related yeast strains. TATA elements are represented by black boxes, the putative *SRG1* UAS elements by light-gray boxes 5′ of the *SRG1* TATA (labeled 1–4), and the putative *SER3* UAS elements by dark-gray boxes 5′ of *SER3* TATA. The horizontal black bars mark the *SRG1* and *SER3* UAS regions that were amplified by PCR in ChIP experiments described later. The arrows indicate the orientation and positions of the *SRG1* and *SER3* transcripts. (*B*) Northern analysis of *SER3* and *SRG1* was performed on wild-type (FY2472) and *srg1-1* (FY2471; contains a mutation of the *SRG1* TATA) strains after a shift from SD + ser to SD medium. *SNR190* served as a loading control. Total RNA was isolated at 15-min intervals. These data are representative of three independent experiments. (*C*) Northern analysis was performed on the strains described in *B* after the opposite shift, from SD to SD + ser. These data are representative of three independent experiments.

quired for the serine-dependent regulation of *SER3*. To do this we examined RNA levels in an *srg1-1* mutant in which mutation of the *SRG1* TATA sequence greatly reduces *SRG1* RNA levels. In the *srg1-1* mutant, *SER3* was expressed at high levels independently of the presence or absence of serine (Fig. 1B [lanes 10–18], C [lanes 10–18]). Therefore, serine-dependent regulation of *SER3* occurs indirectly through the serine-dependent control of *SRG1* transcription.

Cha4 binds to the SRG1 *promoter to control serine-dependent transcription of* SRG1

To test for a serine-dependent response element within the *SRG1* promoter, we constructed mutations that delete four *SRG1* promoter sequences that are highly conserved between *S. cerevisiae* and closely-related yeasts (labeled 1–4 in Fig. 1A; Martens et al. 2004). These mutants were then tested for *SRG1* and *SER3* mRNA levels in the presence or absence of serine. A deletion that encompasses all four regions (*srg1-20*) abolished *SRG1* expression and caused *SER3* derepression in SD + ser medium (Fig. 2A, cf. lanes 1 and 3). Deletion of three of the four individual conserved sequences (UAS1, UAS2, and UAS4) caused no effect on serine-dependent expression of *SRG1* or *SER3* (Fig. 2A). In contrast, deletion of UAS3 (*srg1-23*) resulted in significant decreases in the induction of *SRG1* and repression of *SER3* in SD + ser medium (Fig. 2B, cf. lanes 1 and 9).

Further sequence inspection revealed that the UAS3 region overlaps a putative binding site (TGGAGATA CATCTCCA) for Cha4, an *S. cerevisiae* activator. Cha4 has been previously shown to be a serine-responsive activator of the *CHA1* gene, which encodes an enzyme involved in serine catabolism (Holmberg and Schjerling 1996; Sabet et al. 2003). To determine whether Cha4 plays a role in activating *SRG1* transcription we constructed and tested three mutations: a deletion of *CHA4* (*cha4*Δ), and both a deletion (*srg1-25*) and a multiple-point mutant (*srg1-26*) of the putative Cha4-binding site. By Northern analysis, we observed greatly decreased levels of *SRG1* RNA in all three mutants as compared with a wild-type strain when these strains were grown in SD + ser (Fig. 2B, cf. lanes 3,5,7 and 1). As expected, *SER3* repression is also abolished in these three mutants (Fig. 2B).

To confirm a direct role for Cha4 in serine-dependent activation of *SRG1*, we assayed Cha4 physical association with both the *SRG1* and *SER3* promoters by chromatin immunoprecipitation (ChIP). Our results show that when cells are grown in SD + ser, Cha4 strongly associates with the UAS region of the *SRG1* promoter (Fig. 3A [lanes 9–12], B, top panel); this association is lost in *srg1-25*, in which the putative Cha4-binding site in the *SRG1* promoter is deleted (Fig. 3A [lanes 17–20], B). We also observed a weak association of Cha4 with the *SER3* UAS region (Fig. 3A [lanes 9–12], B). However, this signal is likely caused by the close proximity of the *SRG1* and *SER3* UAS regions (221 base pairs (bp) between probes), as the *SER3* ChIP signal is lost in the *srg1-25*

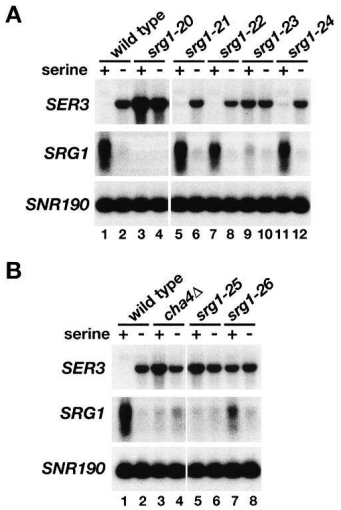

Figure 2. Identification of a serine response element in the *SRG1* promoter. (*A*) Northern analysis of *SER3* and *SRG1* on a wild-type strain (FY2460) and on a series of *srg1* promoter mutants. The *srg1-20* mutant (FY2467) has the entire *SRG1* UAS region deleted (Fig. 1A, regions 1–4). The *srg1-21* (FY2464), *srg1-22* (FY2465), *srg1-23* (FY2466), and *srg1-24* (FY2461) mutants are deletions of *SRG1* UAS sequences 1, 2, 3, and 4, respectively (Fig. 1A). Total RNA was isolated from cells grown in SD + ser medium and from cells that had then been shifted to SD medium for 25 min. *SNR190* served as a loading control. These data are representative of three independent experiments. (*B*) Northern analysis of *SER3* and *SRG1* was performed on wild-type (FY2460), *cha4*Δ *(FY2459), srg1-25* (FY2463), and *srg1-26* (FY2462) strains that were grown in SD + ser and SD medium as described in *A*. The *srg1-25* mutant is a deletion of a putative Cha4-binding site in the *SRG1* promoter and the *srg1-26* mutant has a triple-point mutation within this putative Cha4-binding site. These data are representative of three independent experiments.

mutant (Fig. 3A [lanes 17–20], B). Taken together, our results show that Cha4 binds to a single site within the *SRG1* UAS to activate *SRG1*, thereby repressing *SER3* transcription in serine-rich medium.

To test if Cha4 binding to *SRG1* is serine dependent, we compared Cha4 binding in minimal media with or without serine. Cha4 binding was only mildly decreased after shifting cells to SD medium for 25 min (Fig. 3A [lanes 13–16], B [bottom panel]), a condition in which *SRG1* transcription is at a low level (Fig. 1B). This result suggests that Cha4 binding is not strongly controlled by serine levels; rather, induction of *SRG1* in response to serine involves an event subsequent to Cha4 binding. This finding is consistent with previous studies that have characterized Cha4 activation at *CHA1* (Sabet et al. 2003).

The Spt3 and Spt8 subunits of SAGA are required to repress SER3

Whole-genome expression analyses originally highlighted *SER3* as a gene with interesting regulation, as

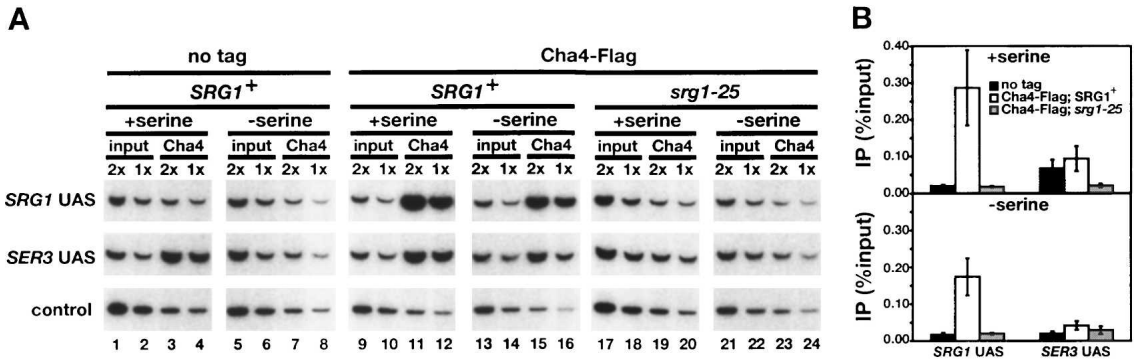

Figure 3. ChIP analysis of Cha4 association with the *SRG1* promoter. (*A*) ChIP analysis of Cha4 was performed on wild-type (FY2470) and *srg1-25* (FY2468) strains expressing Cha4-Flag and on an untagged control strain (FY1350). Cha4-Flag was immunoprecipitated with anti-Flag antibody from cells grown in SD + ser medium (+serine) and from cells that had been shifted from SD + ser to SD medium for 25 min (–serine). A representative set of PCR reactions that amplify the *SRG1* UAS and *SER3* UAS regions (see diagram in Fig. 1A) from twofold dilutions of chromatin is shown. The control primer set amplifies a region of chromosome V that lacks open reading frames (Komarnitsky et al. 2000). (*B*) Quantitation of ChIP analysis. The amount of *SRG1* UAS or *SER3* UAS that was amplified from immunoprecipitated DNA is expressed as a percentage of the amount of input DNA. Each bar represents the average and standard error from three independent experiments.

those studies suggested that both Swi/Snf (Holstege et al. 1998; Sudarsanam et al. 2000) and SAGA (Holstege et al. 1998) serve as repressors of *SER3*. Our more recent studies have examined the role of Swi/Snf in *SER3* repression (Martens and Winston 2002; Martens et al. 2004). Now, to further investigate the role of SAGA in *SER3* repression, we measured *SER3* mRNA levels in eleven mutants, each lacking a different SAGA subunit and impairing different SAGA actitivies. Our results show that only particular classes of SAGA components are required for *SER3* repression. Two SAGA mutants believed to impair TBP recruitment, *spt3*Δ and *spt8*Δ, both had dramatically increased levels of *SER3* mRNA, similar to that previously observed for a deletion of *SNF2*, which encodes the catalytic subunit of the Swi/Snf chromatin remodeling complex (Fig. 4A, lanes 1–4). *SER3* mRNA levels were also greatly derepressed in three other SAGA mutants, *ada1*Δ, *spt20*Δ, and *spt7*Δ although not quite to the extent observed for *spt3*Δ and *spt8*Δ (Fig. 4A, lanes 5–7). Ada1, Spt20, and Spt7 are required for the structural integrity of SAGA (Grant et al. 1997; Sterner et al. 1999). In contrast, none of the other SAGA mutants tested, including *gcn5*Δ and *ubp8*Δ, had any effect on *SER3* repression (Fig. 4A, lanes 8–11). These results strongly suggest that the Spt3/Spt8 TBP recruitment activity of SAGA (Dudley et al. 1999b; Warfield et al. 2004) is required for *SER3* repression, while the Gcn5 histone acetyltransferase activity (Grant et al. 1997) and the Ubp8 deubiquitylation activity (Henry et al. 2003; Daniel et al. 2004) of SAGA play little, if any, role.

To address the roles of both SAGA and Swi/Snf in activating *SRG1* transcription, we assayed *SRG1* RNA levels in both *spt3*Δ and *snf2*Δ mutants. To look under conditions maximally inducing for *SRG1*, cells were grown in SD + ser medium. Northern analysis shows that the serine-induced levels of *SRG1* were decreased, albeit modestly, in both mutants compared with wild type. In the *snf2*Δ mutant, induction of *SRG1* was reduced 1.5-fold to twofold (Fig. 4B, lanes 2,3) consistent with both our previous Northern analysis (Martens et al. 2004) and with ChIP experiments in which we found a twofold decrease in the association of the Rpb3 subunit of RNA polymerase II to *SRG1* in a *snf2*Δ strain (data not shown). In the *spt3*Δ mutant, induction was reduced two- to threefold (Fig. 4B, lanes 4,5). Therefore, our results show that both SAGA and Swi/Snf are required for full induction of *SRG1* in serine-rich medium.

Association of SAGA and Swi/Snf to the SRG1 *promoter is dependent on Cha4 and serine*

Our results show that Cha4, Spt3, and Snf2 are required for full induction of *SRG1* in medium with serine, suggesting that Cha4 recruits SAGA and Swi/Snf to the *SRG1* UAS. To test this possibility, we performed ChIP experiments on Swi2 of Swi/Snf (Fig. 5A) and Ada1 of SAGA (Fig. 5B). In wild-type strains, we detected a strong association of both proteins with the *SRG1* UAS, with a lower level of association over the adjacent *SER3* UAS (Fig. 5, top panels). Significantly, the association of both Snf2 and Ada1 with these regions was reduced to background levels when either *CHA4* (*cha4*Δ) or the Cha4-binding site in the *SRG1* UAS (*srg1-25*) was deleted. These results strongly suggest that both Swi/Snf and SAGA are recruited to the *SRG1* UAS by Cha4.

Serine-dependent activation by Cha4 appears to involve a step that occurs subsequent to Cha4 binding (Fig. 3B; Sabet et al. 2003; our results). To test whether recruitment of SAGA and Swi/Snf by Cha4 are affected by serine levels, we examined the association of Snf2 and Ada1 to the *SRG1* UAS after shifting cells from SD + ser to SD medium. In contrast to Cha4, which remained associated with the *SRG1* UAS in the absence of serine, there was no significant recruitment of either Snf2 or Ada1 when cells were grown in SD medium (Fig. 5A,B;

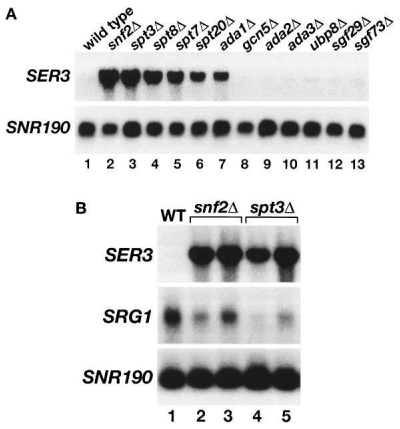

Figure 4. Repression of *SER3* is dependent on the Spt3 and Spt8 subunits of SAGA. (*A*) Northern analysis of *SER3*. Total RNA was isolated from wild-type (FY3), *snf2*Δ (FY1360), *spt3*Δ (FY294), *spt8*Δ (FY50), *spt7*Δ (FY963), *spt20*Δ (FY1098), *ada1*Δ (FY1560), *gcn5*Δ (FY1600), *ada2*Δ (FY1548), *ada3*Δ (FY1596), *ubp8*Δ (FY2473), *sgf29*Δ (FY2474), and *sgf73*Δ (FY2475) strains that were grown in YPD. *SNR190* served as a loading control. These data are representative of three independent experiments. (*B*) Northern analysis of *SER3* and *SRG1*. Total RNA was isolated from wild-type (FY4), two *snf2*Δ (FY2150 and FY2151), and two *spt3*Δ (FY930 and FY2142) strains that were grown in SD + ser medium. RNA levels were averaged for at least three independent experiments. *SER3* mRNA levels are 31.4 ± 2.7 and 27.5 ± 2.3 in *snf2*Δ and *spt3*Δ strains, respectively, as compared with wild type. *SRG1* RNA levels are 0.59 ± 0.06 and 0.37 ± 0.07 in *snf2*Δ and *spt3*Δ strains, respectively, as compared with wild type.

bottom panels). Therefore, Cha4 recruits SAGA and Swi/Snf to the *SRG1* UAS in a serine-dependent manner.

Both SAGA and Swi/Snf are required for SRG1*-mediated transcription interference*

Because *snf2*Δ and *spt3*Δ have only modest defects in *SRG1* transcription we performed experiments to determine the effects of these mutations on transcription interference by *SRG1*. Since the direct activators of *SER3* have not been identified, we integrated the *SRG1* promoter at the *GAL1* locus, 5′ of the *GAL1* UAS (Fig. 6A), in wild-type, *snf2*Δ, and *spt3*Δ strains. These strains were analyzed for both transcription from the *SRG1* promoter and Gal4 binding. (Note that in this experiment *GAL1* will not be transcribed as the cells are grown in glucose, a condition permissive for Gal4 binding, but repressive for *GAL1* transcription. These conditions were necessary to avoid secondary effects of *snf2*Δ and *spt3*Δ mutations on galactose-grown cells.) Using a probe to the *GAL1* UAS region we detected transcription from the *SRG1* promoter that appears to extend to the end of *GAL1*. Consistent with our previous results, transcription from the *SRG1* promoter is decreased two- to threefold in *snf2*Δ, and *spt3*Δ mutants and is undetectable in an *srg1-1* mutant, containing a mutated *SRG1* TATA (Fig. 6B; Martens et al. 2004). ChIP analysis demonstrates that, like the *srg1-1* mutant, *snf2*Δ and *spt3*Δ mutations relieve the inhibition of Gal4 binding observed with wild type *SRG1* (Fig. 6C). Unexpectedly, Gal4 binding to the *GAL1* UAS is three- to fourfold higher in the *snf2*Δ strain than in either the *spt3*Δ or *gal1*::*srg1-1p* strains (Fig. 6C). However, in a control experiment, we found that Gal4 binding to wild-type *GAL1* was similarly increased in a *snf2*Δ strain (Supplementary Fig. S2), which would account for this difference. The requirement for Swi/Snf and SAGA in transcription interference with Gal4 binding in this construct strongly suggests that Swi/Snf and SAGA are required for transcription interference of *SER3*. Although we cannot rule out the possibility that Swi/Snf and/or SAGA have additional functions in *SER3* repression, our results suggest that one function of SAGA and Swi/Snf in *SER3* repression is to facilitate transcription interference by activating transcription of *SRG1*.

Discussion

In this work we have elucidated a physiological role for an intergenic transcript in gene regulation. Our results have shown that the repression of the *S. cerevisiae SER3* gene in response to high levels of serine occurs by the induction of intergenic transcription of *SRG1*. Our results also show that the serine-dependent induction of

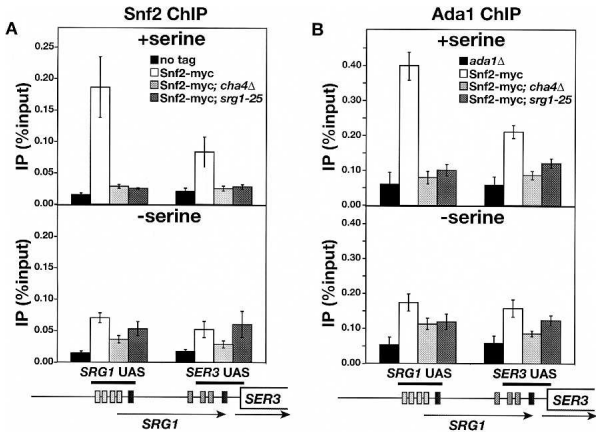

Figure 5. ChIP analysis of Snf2 and Ada1 association to the *SRG1* promoter. (*A*) ChIP analysis of Snf2 was performed on wild-type (FY2470), *cha4*Δ (FY2469), and *srg1-25* (FY2468) strains expressing Snf2-myc and an untagged control strain (FY1350). Snf2-myc was immunoprecipitated with anti-myc A14 antibody from chromatin isolated from cells that were grown in SD + ser medium (+serine) and from cells that were shifted from SD + ser to SD medium for 25 min (–serine). The amounts of *SRG1* UAS and *SER3* UAS that were amplified from immunoprecipitated DNA are expressed as percentages of the amounts of input DNA. Each bar represents the average and standard error from three independent experiments. (*B*) ChIP analysis of Ada1. Ada1 was immunoprecipitated with anti-Ada1 antibody from the same chromatin that is described in *A* with the exception of the untagged control strain, which was replaced by an *ada1*Δ control strain (FY1560). Each bar represents the average amount and standard error of three independent experiments.

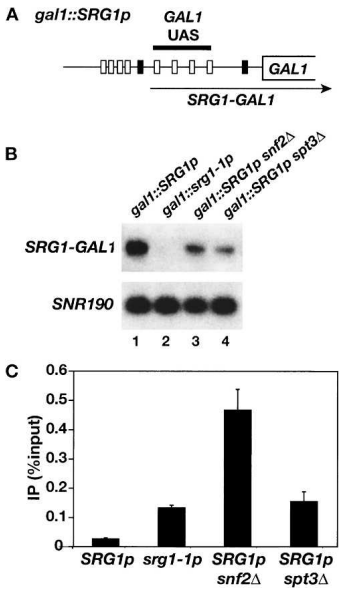

Figure 6. Effect of *snf2*Δ and *spt3*Δ on transcription interference by *SRG1*. (*A*) A schematic of *gal1*::*SRG1p*. The *SRG1* UAS (gray boxes) and TATA (left-most black box) sequences were integrated into the *GAL1* promoter, 5′ of the four Gal4-binding sites (white boxes). The arrow indicates the *srg1-GAL1* RNA that is transcribed as a result of the *SRG1* promoter insertion. The *gal1*::*srg1-1p* allele contains a similar insertion of the *srg1-1* TATA mutant promoter. Under the conditions of the experiment, with cells grown on glucose, there is no transcription from the normal *GAL1* initiation site; however, Gal4 is still bound under these growth conditions (Dudley et al. 1999b; Ren et al. 2000). (*B*) Northern analysis was performed on *gal1*::*SRG1p* (FY2476), *gal1*::*srg1-1p* (FY2477), *snf2*Δ *gal1*::*SRG1p* (FY2478), and *spt3*Δ *gal1*::*SRG1p* (FY2479) strains that were grown in YPD medium. Transcription from the *SRG1* promoter was detected using a probe to the *GAL1* UAS (*SRG1-GAL1*). *SNR190* RNA was measured as a loading control. (*C*) ChIP analysis of Gal4 was performed on the same strains described in *B*. The amount of *GAL1* UAS that was PCR-amplified from immunoprecipitated DNA was calculated relative to the amount amplified from input DNA. Each bar represents the average and standard error from three experiments.

SRG1 depends upon Cha4, which binds to the *SRG1* promoter, and that activation by Cha4 requires the SAGA and Swi/Snf coactivator complexes.

Taken together with previous studies (Holmberg and Schjerling 1996; Sabet et al. 2003), our results suggest a model in which the control of *SRG1* intergenic transcription is the primary mechanism that regulates *SER3* in response to changes in serine. In this model, *SRG1* transcription also allows Cha4 to serve as both an activator and repressor of transcription. When cells are exposed to high levels of serine, Cha4 directly activates the *CHA1* gene, required for serine catabolism, and indirectly represses the *SER3* gene via activation of *SRG1* (Fig. 7, top panel). The requirements for *CHA1* activation may be similar to those for *SRG1*, as *CHA1* activation is impaired in both *snf2*Δ and *spt3*Δ mutants, albeit only modestly in *snf2*Δ, and both Swi/Snf and SAGA are recruited to the *CHA1* promoter in a serine-dependent fashion (Supplementary Fig. S1; R. Morse, pers. comm.). In serine-starvation conditions, the opposite regulation occurs, as Cha4 no longer recruits SAGA and Swi/Snf, resulting in a reversal of the transcription states of *CHA1* and *SER3* (Fig. 7, bottom panel; Supplementary Fig. S1). Thus, *S. cerevisiae* can quickly adapt to changes in intracellular serine by coordinately regulating serine catabolism and biosynthesis using a single activator. This model also illustrates a mechanism by which intergenic transcription increases the potential functions of regulatory proteins as it allows activators to act indirectly as repressors.

In addition to the factors identified in this work that control *SRG1* transcription in response to serine levels, other physiological conditions and factors may play important roles in this regulatory system. Sequence conservation suggests that, in addition to the Cha4-binding site, other *SRG1* promoter sequences may be required for *SRG1* transcription under some conditions (Fig. 2A; Cliften et al. 2003; Kellis et al. 2003; Martens et al. 2004). These sequences may respond to other physiological signals that control serine levels, such as general nitrogen metabolism, one-carbon metabolism, and fatty acid biosynthesis. In addition, we have previously identified a region of the *SER3* promoter that is required for *SER3* activation (Martens et al. 2004); however, the factors that bind to this putative *SER3* UAS remain unknown and we have not found any evidence for a role for this region in response to serine. We also note that histone H3 mutants that cause defects in *SER3* repression have been identified (Sabet et al. 2003; Duina and Winston 2004). The analysis of these mutants, as well as the identification of other factors required for *SRG1* and *SER3* regulation, will provide further insight into the physiological relevance for regulation of *SER3* by intergenic transcription.

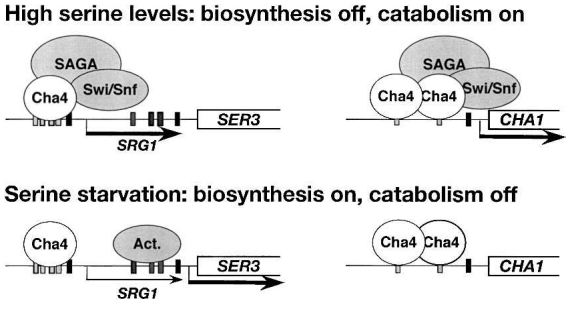

Figure 7. A model for the coordinated regulation of serine biosynthesis and catabolism by Cha4. (*Top*) In the presence of high serine levels, Cha4 indirectly represses the serine biosynthetic gene *SER3* via activation of *SRG1* and directly activates the serine catabolic gene *CHA1*. (*Bottome*) Under serine-starvation conditions, when Cha4 is no longer able to recruit SAGA and Swi/Snf, the expression states of *SER3* and *CHA1* are reversed. In this model, the expression of *SER3* also requires putative activators (Act.) that bind to the previously identified *SER3* UAS (Martens et al. 2004). Thus, Cha4 can act as both an activator and as a repressor in response to serine.

Another aspect of the *SER3/SRG1* regulatory system that remains to be determined is the role of serine in the Cha4-dependent recruitment of SAGA and Swi/Snf. Like many *S. cerevisiae* activators that respond to intracellular metabolites (for review, see Sellick and Reece 2005), Cha4 binding to DNA is not significantly affected by serine levels (our results; Sabet et al. 2003). Possibly, serine or an intermediate in the serine biosynthetic pathway directly modulates Cha4 activity by inducing a conformational change that facilitates recruitment of SAGA and Swi/Snf. A similar mechanism has been suggested for Put3, an activator of the proline-utilization pathway, as proline directly interacts with Put3 to cause conformational changes that induce the transcription activity of Put3 (Axelrod et al. 1991; Sellick and Reece 2003). Alternatively, serine may indirectly control Cha4 activity by controlling the interaction of Cha4 with an as-yet-unidentified second protein, similar to the galactose-mediated regulation of Gal4 by interaction with Gal80 (Sellick and Reece 2005).

A number of different mechanisms have been proposed for transcription interference (for review, see Shearwin et al. 2005) and other naturally occurring cases of transcription interference have been identified (for examples, see Hausler and Somerville 1979; Cullen et al. 1984; Proudfoot 1986; Corbin and Maniatis 1989; Boussadia et al. 1997; Moseley et al. 2002). Our data show that loss of either SAGA or Swi/Snf causes only a two- to threefold decrease in *SRG1* RNA levels, yet this modest decrease appears to be sufficient to abolish interference as it is accompanied by a 50-fold increase in *SER3* mRNA levels. One possibility is that there is a threshold level of *SRG1* transcription that is sufficient to confer transcription interference and that a modest decrease in that level allows activators full access to the *SER3* promoter. However, given the modest effects on *SRG1* RNA levels observed in the *snf2*Δ and *spt3*Δ mutants, SAGA and Swi/Snf may play additional roles to repress *SER3* besides *SRG1* activation. Consistent with this possibility, Snf2-dependent changes in chromatin structure extend to the *SER3* TATA sequence (Martens and Winston 2002). Therefore, there may be two roles for Swi/Snf in *SER3* repression, through activation of *SRG1* and by inhibition of factor binding to the *SER3* promoter.

In conclusion, our work has demonstrated a mechanism by which the regulation of noncoding, intergenic transcription provides a physiologically important response in a metabolic signaling pathway. Studies in other systems, including the *Drosophila* BX-C complex and the human β-globin locus, have demonstrated other cases where regulation of noncoding, intergenic transcription plays important regulatory roles (Gribnau et al. 2000; Schmitt et al. 2005). Additional recent studies have shown that noncoding transcription is widespread (Mattick 2003; Morey and Avner 2004; Huttenhofer et al. 2005; Johnson et al. 2005) and that a significant fraction is regulated (Cawley et al. 2004; Kim et al. 2005). Taken together, these studies suggest that the control of noncoding, intergenic transcription may represent a broadly used mechanism for transcriptional regulation.

Materials and methods

S. cerevisiae *strains and media*

All *S. cerevisiae* strains used (Table 1) are isogenic with a *GAL2*$^+$ derivative of S288C (Winston et al. 1995). Strains were constructed by standard methods, either by crosses or by transformation (Ausubel et al. 1991). Details are available upon request. The *spt8*Δ*302*::*LEU2* (Eisenmann et al. 1994), *spt3-202* (Winston and Minehart 1986), *spt3*Δ*203*::*TRP1* (Happel and Winston 1992), *spt7*Δ*402*::*LEU2* (Gansheroff et al. 1995), *spt20*Δ*100*::*URA3* (Roberts and Winston 1996), *snf2*Δ::*LEU2* (Cairns et al. 1996), *snf2*Δ*1*::*HIS3* (Abrams et al. 1986), *gcn5*Δ::*HIS3* (Roberts and Winston 1997), *ser33*Δ*0*::*kanMX* (Martens and Winston 2002), *srg1-1* (Martens et al. 2004), *RPB3-HA*::*LEU2* (Kolodziej et al. 1990), *SNF2-C18myc*::*TRP1* (Martens and Winston 2002), and *cha4*Δ*0*::*kanMX* (Open Biosystems) alleles have been previously described. The *ada2*Δ::*HIS3*, *ada1*Δ::*HIS3*, and *ada3*Δ::*HIS3* mutations were constructed by replacing their open reading frames with *HIS3* (S. Roberts and F. Winston, unpubl.). The *ubp8*Δ*0*::*kanMX*, *sgf29*Δ*0*::*kanMX*, and *sgf73*Δ*0*::*kanMX* mutations were constructed by replacing their open reading frames with the *kanMX* marker (Brachmann et al. 1998). The *srg1-20* mutation replaces *SRG1* promoter sequences from –660 to –560 (relative to the *SER3* ATG) with three copies of the myc epitope. The *srg1-21*, *srg1-22*, *srg1-23*, *srg1-24*, and *srg1-25* mutations replace *SRG1* promoter sequences –659 to –646, –644 to –636, –611 to –604, –590 to –582, and –612 to –592 each with an AvrII site (CCTAGG). The *srg1-26* mutation contains three point mutations in the Cha4 consensus binding site within the *SRG1* promoter (TGGAGATACATCTCCA to ctGAGATACATCTCaA). This mutant sequence has been shown to be defective for Cha4 binding (Holmberg and Schjerling 1996). The *CHA4-Flag*::*kanMX* allele encodes one copy of the Flag epitope at the 3′ end of *CHA4*, which was constructed by PCR-mediated integration using plasmid pDM714 (kind gift from D. Moazed, Harvard Medical School, Boston, MA). The *gal1*::*SRG1p* allele contains the *SRG1* promoter and transcription initiation sites (base pairs –713 to –445 relative to the *SER3* ATG) at position –556 of *GAL1* (relative to *GAL1* ATG). The *gal1*::*srg1-1p* allele has an insertion of the same DNA except with the *srg1-1* promoter containing the TATA mutation. As indicated, strains were grown in either YPD (1% yeast extract, 2% peptone, and 2% glucose) or minimal media (0.145% yeast nitrogen base, 0.5% ammonium sulfate, 2% glucose, and supplemented with required amino acids) with 1 mM serine (SD + ser) or without serine (SD).

Northern analysis

Northern hybridization analysis was performed as previously described (Ausubel et al. 1991). Probes for *SER3*, *SRG1*, and *SNR190* were previously described (Martens and Winston 2002; Duina and Winston 2004; Martens et al. 2004). A probe specific to the *GAL1* 5′ UTR was generated by random labeling a PCR product, containing the *GAL1* sequence from –443 to –157 (relative to the *GAL1* ATG), amplified from genomic DNA. RNA levels were quantitated using a PhosphorImager (Molecular Dynamics) and normalized to *SNR190*, whose levels are unaffected by the mutations and growth conditions studied here.

ChIP analysis

ChIP analysis was performed as previously described (Dudley et al. 1999b; Martens and Winston 2002) with the following modifications: For the Cha4, Snf2, and Ada1 ChIPs, 200-mL cultures

Table 1. Saccharomyces cerevisiae *strains*

Name	Genotype
FY3	*MAT***a** *ura3-52*
FY4	*MAT***a** (prototroph)
FY50	*MAT***a** *ura3-52 leu2Δ1 spt8Δ302::LEU2 trp1Δ63 his4-917δ*
FY294	*MAT***a** *ura3-52 lys2-173R2 leu2Δ1 trp1Δ63 his4-917δ spt3-202*
FY930	*MAT***a** *ura3-52 lys2-173R2 his3Δ200 spt3-202*
FY963	*MAT***a** *ura3-52 leu2Δ1 spt7Δ402::LEU2 his4-917δ*
FY1098	*MAT***a** *ura3-52 spt20Δ100::URA3 leu2Δ1 his3Δ200*
FY1350	*MATα ura3Δ0 lys2Δ0 leu2Δ0*
FY1360	*MAT***a** *ura3-52 lys2-173R2 leu2Δ1 snf2Δ::LEU2 his3Δ200*
FY1548	*MAT***a** *ura3-52 lys2-128δ leu2Δ1 his3Δ200 ada2Δ::HIS3*
FY1560	*MAT***a** *ura3-52 lys2-173R2 his3Δ200 ada1Δ::HIS3*
FY1596	*MAT***a** *ura3-52 lys2-173R2 his3Δ200 ada3Δ::HIS3 his4-912δ*
FY1600	*MAT***a** *ura3-52 leu2Δ1 his3Δ200 gcn5Δ::HIS3 his4-917δ*
FY2142	*MATα spt3Δ0::kanMX*
FY2150	*MAT***a** *snf2Δ0::kanMX*
FY2151	*MAT***a** *snf2Δ0::kanMX*
FY2245	*MAT***a** *ura3-52 lys2-128δ leu2Δ1 RPB3-HA::LEU2 his3Δ200 trp1Δ63 SNF2-C18myc::TRP1 his4-912δ ser33Δ0::kanMX*
FY2459	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 cha4Δ0::kanMX*
FY2460	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 CHA4-Flag::kanMX*
FY2461	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 CHA4-Flag::kanMX srg1-24*
FY2462	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 CHA4-Flag::kanMX srg1-26*
FY2463	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 CHA4-Flag::kanMX srg1-25*
FY2464	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 CHA4-Flag::kanMX srg1-21*
FY2465	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 CHA4-Flag::kanMX srg1-22*
FY2466	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 CHA4-Flag::kanMX srg1-23*
FY2467	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 CHA4-Flag::kanMX srg1-20*
FY2468	*MATα ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 met15Δ0 SNF2-C18myc::TRP1 CHA4-Flag::kanMX srg1-25*
FY2469	*MATα ura3Δ0 lys2Δ0 leu2Δ0 SNF2-C18myc::TRP1 cha4Δ0::kanMX*
FY2470	*MATα ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 trp1Δ63 SNF2-C18myc::TRP1 CHA4-Flag::kanMX*
FY2471	*MAT***a** *ura3Δ0 lys2Δ0 his3Δ200 leu2Δ0 srg1-1*
FY2472	*MAT***a** *ura3Δ0 lys2Δ0 his3Δ200 leu2Δ0*
FY2473	*MATα ura3-52 lys2-173R2 leu2Δ1 arg4-12 ubp8Δ0::kanMX*
FY2474	*MAT***a** *ura3-52 lys2-173R2 leu2Δ1 arg4-12 sgf29Δ0::kanMX his4-917δ*
FY2475	*MATα ura3-52 lys2-173R2 leu2Δ1 arg4-12 sgf73Δ0::kanMX his4-917δ*
FY2476	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 ser33Δ0::kanMX gal1::SRG1p*
FY2477	*MAT***a** *ura3Δ0 lys2Δ0 leu2Δ0 his3Δ200 ser33Δ0::kanMX gal1::srg1-1p*
FY2478	*MATα ura3-52 lys2Δ0 leu2Δ1 snf2Δ::LEU2 his3Δ200 gal1::SRG1p*
FY2479	*MATα ura3Δ0 lys2Δ0 leu2Δ1 his3Δ200 his4-917δtrp1Δ63 spt3Δ203::TRP1 gal1::SRG1p*
FY2502	*MAT***a** *ura3-52 lys2-128δ leu2Δ1 RPB3-HA::LEU2 snf2Δ1::HIS3 his4-912δ ser33Δ0::kanMX*

were grown to a density of 0.8×10^7 to 1.0×10^7 cells/mL in SD + ser medium and then split into two equal volumes. One half was treated with buffered formaldehyde (11% formaldehyde, 0.1M NaCl, 1 mM EDTA, and 50 mM Hepes-KOH at pH 7.5) to a final concentration of 1%. Cells from the other half of the culture were harvested by centrifugation, resuspended in SD medium, incubated for 25 min at 30°C, and then treated with buffered formaldehyde to a final concentration of 1%. The following antibodies were used for ChIP from one-fifth of the total amount of cross-linked chromatin: 4 µL of mouse A14 anti-myc ascites (Santa Cruz Biotechnology), 2 µL of rabbit anti-Flag M2 serum (Sigma-Aldrich, Inc), and 1 µL of rabbit anti-Ada1 serum (a generous gift from L. Guarente, Massachusetts Institute of Technology, Cambridge, MA). Input DNA (0.002% and 0.001%) and immunoprecipitated DNA (4% and 2% for Cha4-Flag and Snf2-myc, 2% and 1% for Ada1) were subjected to quantitative radioactive PCR and the products were separated on an 8% nondenaturing polyacrylamide gel. For the Gal4 ChIPs, 200-mL cultures were grown to a density of 1×10^7 to 2×10^7 cells/mL in YPD and then 37% formaldehyde was added to a final concentration of 1%. Gal4 was immunoprecipitated from one-tenth of the total amount of cross-linked chromatin with 1 µL; of anti-Gal4 serum (Santa Cruz Biotechnology, Inc.). Input DNA (0.002% and 0.001%) and immunoprecipitated DNA (1.33% and 0.67%) were analyzed as described for the Cha4 ChIPs. The PCR primers amplify the following regions whose coordinates are given relative to the *SER3* ATG: *SRG1* UAS primers amplify a 259-bp product from –714 to –456 and *SER3* UAS primers amplify a 298-bp product from –234 to +65. Primers that amplify the *GAL1* UAS and the control region from chromosome V that lacks open reading frames have been previously described (Dudley et al. 1999a; Komarnitsky et al. 2000). All ChIP experiments were quantitated by PhosphorImager analysis (Molecular Dynamics). Association of factors to specific DNA sequences was calculated as a percentage of the amount of coimmunoprecipitated DNA relative to the input DNA.

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