HSP90/70 chaperones are required for rapid nucleosome removal upon induction of the GAL genes of yeast

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Induction of transcription of the GAL genes of yeast by galactose is a multistep process: Galactose frees the activator Gal4 of its inhibitor, Gal80, allowing Gal4 to recruit proteins required to transcribe the GAL genes. Here, we show that deletion of components of either the HSP90 or the HSP70 chaperone machinery delays this induction. This delay remains when the galactose-signaling pathway is bypassed, and it cannot be explained by a chaperone requirement for DNA binding by Gal4. Removal of promoter-bound nucleosomes is delayed in a chaperone mutant, and our findings suggest an involvement of HSP90 and HSP70 in this early step in gene induction.

transcription | Gal4 | activation | recruitment | promoter

HSP90, HSP70, and their cochaperones (often referred to as chaperone machineries) are required for an array of processes during normal cell growth, in addition to their roles in preventing protein aggregation during heat shock and cell stress (1–5). In a few cases, it has been possible to pinpoint a specific step in a pathway at which the chaperones work. For example, the binding of hormones to receptors that activate gene transcription in mammalian cells (e.g., glucocortoid receptor and estrogen receptor) depends on HSP90 (6). It also has been suggested that HSP90 and various cochaperones play a role in transcriptional activation subsequent to hormone binding by promoting the cycling of receptor complexes on and off DNA (7, 8). In yeast, binding of heme to the transcriptional activator, Hap1 (a step required for activation by Hap1), is facilitated by HSP90 (9), and it has been proposed that HSP90 also may be involved in a step subsequent to heme binding (10). HSP90 is believed to work, in some cases at least, in conjunction with members of the HSP70 class of chaperones (6, 11), and a HSP90/HSP70 complex has been described (12).

The GAL genes of yeast (e.g., *GAL1*) are induced by addition of galactose to cells growing in culture. Galactose signaling frees DNA-bound Gal4 from the inhibitor Gal80, which results in recruitment by Gal4 of the various proteins required to transcribe the gene. Here, we show that rapid induction of the GAL genes requires components of both the HSP90 and HSP70 chaperone machineries, and we attempt to ascertain the earliest event in gene activation that is facilitated by these chaperones. We induce Gal4-mediated transcription in two ways that do not require transmission of the galactose signal to Gal80 and find that, nevertheless, induction is delayed in strains lacking one or more chaperone machinery components. DNA binding by Gal4 is not affected by loss of the chaperones, but the time course of recruitment of the transcriptional machinery to the *GAL1* promoter by Gal4 (previously determined in ref. 13) is delayed. We then turn to a recently developed nucleosome-positioning assay (G.O.B., V. Prabhu, M.F., D. Spagna, D. Schreiber, and M.P., unpublished data), that has allowed us to determine the dynamics of nucleosome binding at the *GAL1* promoter and of their removal upon induction. For the work presented here, the important finding of that unpublished study is that, upon induction in a wild-type strain, nucleosomes are quickly removed from the *GAL1* promoter. Here, we find that promoter nucleosome removal is delayed in a chaperone mutant, and we suggest that chaperones are involved in this early step of gene activation.

Results

The HSP90 and HSP70 Chaperone Machineries Are Required for Rapid *GAL1* Induction. Fig. 1*A* shows that induction of transcription of *GAL1* (by the addition of galactose to cells growing in raffinose) was delayed by the deletion of *hsc82*, a member of the HSP90 family. Full induction, which was reached within 20–30 min in wild-type cells, was reached only after ≈2 h in the mutant cells. We observed (Fig. 1*B*) a more dramatic delay in induction of *GAL1* with a strain deleted for *hsc*82 and expressing a point-mutant form of *hsp82* (14), the other member of the HSP90 family. We also assayed induction in a strain expressing a temperature-sensitive mutant of *hsp82* (14). As shown in Fig. 1*C*, we found that *GAL1* mRNA production was delayed when the temperature was increased before induction. Sti1 has been reported to be a cochaperone for HSP90 and HSP70 separately (15, 16) and also to link HSP90 and HSP70 in a complex (12, 17, 18). Fig. 1*D* shows that deletion of *sti1* also affected *GAL1* induction. Moreover, as shown in Fig. 1 *E* and *F*, induction was delayed when members of the HSP70 chaperone machinery were deleted. These members include Ssa1 and Ssa2 (Fig. 1*E*) and (the strongest effect) Ydj1 (Fig. 1*F*). Control genes (including *ACT1*) were expressed at wild-type levels in these various mutant strains, indicating that the mutations did not lead to a general impairment of transcription.

Recruitment of the Transcriptional Machinery by Gal4 Is Delayed in the *hsc82* Mutant. Fig. 2 shows the effect of the deletion of *hsc82* on the recruitment of the transcriptional machinery as measured by ChIP analysis. Fig. 2 *A–D* shows that the transcriptional machinery (including SAGA, mediator, TFIIE, and Pol II) was recruited more slowly to the *GAL1* promoter when these cells were induced by galactose. We examined the levels of two of these proteins (Spt20, a component of SAGA; and Rpb1, the large subunit of Pol II) and found that they were not affected by the deletion of *hsc82* (data not shown). As shown in Fig. 2 *E* and *F*, we found that the Hsc82 and Ssa1 proteins were recruited to the *GAL1* promoter (a 4- and 2-fold increase, respectively) upon induction. A more pronounced effect (a 6- and 3-fold increase, respectively) was seen in the coding region of *GAL1*.

Delayed *GAL1* Induction Is Not Due to Defects in Galactose Signaling or DNA Binding by Gal4. In attempting to explain the delay in induction and the recruitment of the transcriptional machinery,

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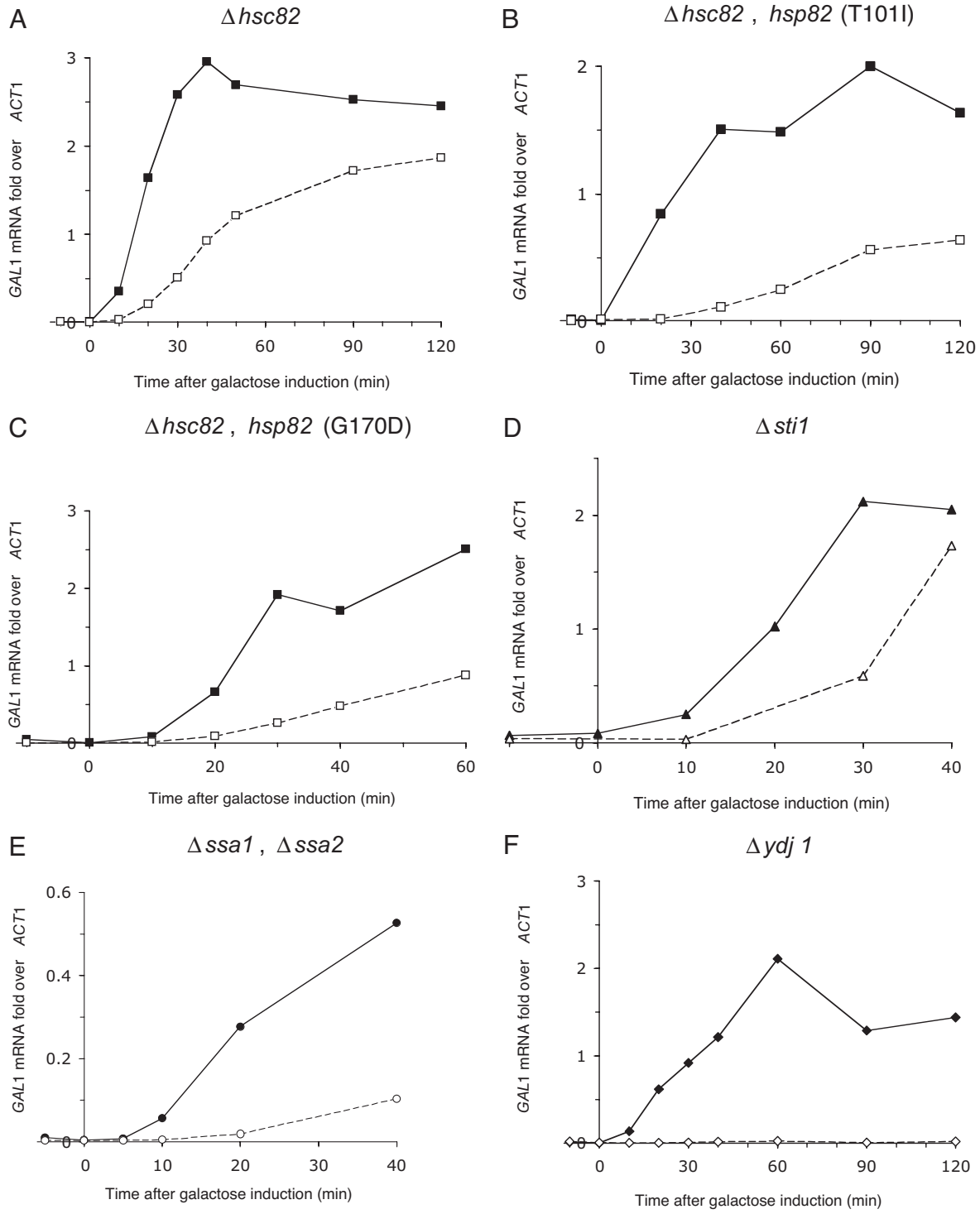

Fig. 1. Gene expression is delayed in yeast cells deleted or mutant for components of the HSP90 and HSP70 chaperone machineries. Cells growing in raffinose were induced with galactose as described in *Materials and Methods*. *GAL1* mRNA is depicted as fold over *ACT1* mRNA, which remained constant over the course of an induction. Filled symbols show measurements for wild-type cells, and open symbols show cells deleted for *hsc82* (*A*), deleted for *hsc82* and *hsp82* and expressing a mutant version of *hsp82* (T101I) (*B*), deleted for both *hsc82* and *hsp82* and expressing the temperature-sensitive mutant *hsp82* (G170D) (*C*), deleted for *sti1* (*D*), deleted for both *ssa1* and *ssa2* (two members of the SSA class of HSP70 chaperones) (29) (*E*), and deleted for *ydj1* (*F*), which acts as an HSP40 for the SSA proteins (30, 31). Cells were grown at 30°C except for the experiment shown in *C*, in which cells were shifted to 36°C for 1 h before galactose addition. mRNA was isolated and reverse transcribed as described in *Materials and Methods*. The resulting cDNA in the experiments described in this and the following figures was measured in quadruplicates, with a standard deviation <20%.

we considered and eliminated two possibilities: Chaperones might affect the transmission of the galactose signal to Gal80 or Gal4 binding to DNA. Fig. 3 *A* and *B* shows two examples of Gal4-mediated transcription that do not require galactose signaling. Yet in both cases, transcription was delayed in a chaperone mutant. In the experiment of Fig. 3*A*, Gal4-mediated

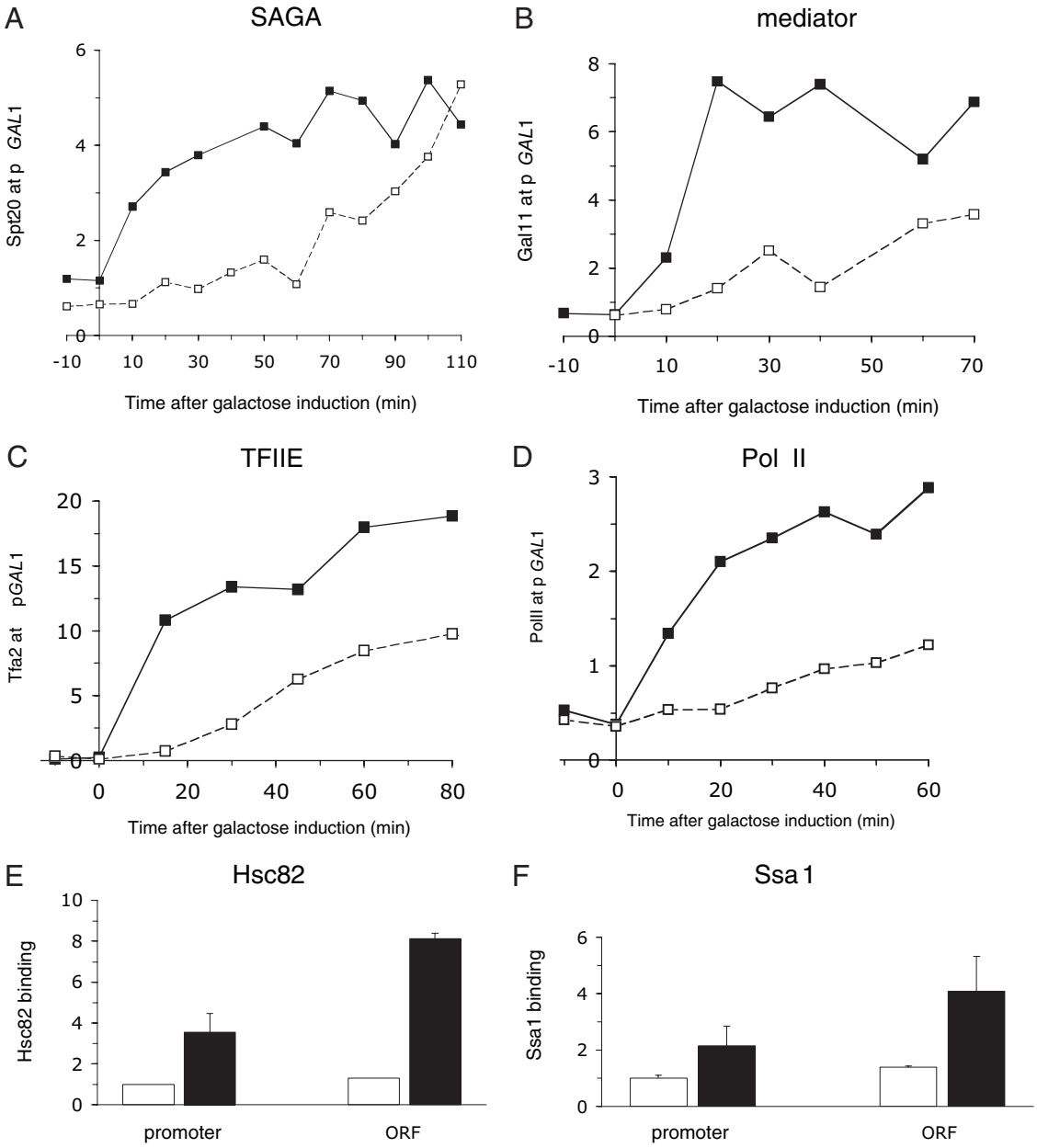

Fig. 2. Recruitment of the transcriptional machinery is delayed in cells deleted for *hsc82*, and Hsc82 and Ssa1 are directly recruited to *GAL1* in wild-type cells. (*A–D*) Cells that were either wild type (filled symbols) or deleted for *hsc82* (open symbols) were induced with galactose, and ChIP experiments were performed as described in *Materials and Methods*. Values are depicted as the ratio of immunoprecipitated DNA at the *GAL1* promoter over that precipitated at the promoter of *ACT1*. ChIP experiments were performed with antibodies against the HA-epitope in cells in which the endogenous *SPT20* gene (a component of the SAGA complex) was fused to the HA-epitope (*A*), Gal11 (a mediator component) (*B*), Tfa2 (a subunit of TFIIE) (*C*), and Pol II (*D*). (*E* and *F*) ChIP experiments were performed with antibodies against Hsc82 (*E*) and Ssa1 (*F*), with cells grown in raffinose (open bars) or cells that had been induced by galactose addition for 80 min (filled bars). The average of two samples each taken before and after induction is shown. Chaperone binding was measured at the *GAL1* promoter or in the *GAL1* ORF, values were normalized to the promoter of *ACT1*, and the fold increase in binding is depicted. Binding at the *GAL1* promoter before induction was arbitrarily set to 1.

transcription in a *gal80*-deleted strain was repressed by extended growth in glucose, an effect that was then relieved by transferring the cells to medium lacking glucose. The figure shows that this form of induction was significantly delayed in cells deleted for *hsc82* compared with wild type. Fig. 3*B* describes an experiment in which the fusion protein TetR-Gal4 activated a reporter gene, and here again in the chaperone mutant, in this case the cells were deleted for *ydj1*, induction was delayed.

The experiment of Fig. 3*C* shows that Gal4 bound to DNA efficiently in the absence of a chaperone. Thus, deletion of *hsc82* did not affect the levels of Gal4 bound to the *UASg* (upstream activating sequence galactose), as measured by a ChIP experiment, in cells grown in either raffinose or in raffinose plus galactose. Similar results were seen in cells deleted for the other chaperones of Fig. 1 (data not shown). We did not detect cycling of Gal4 on and off DNA in wild-type cells when Gal4 DNA binding was analyzed at 30-s intervals after the addition of galactose (G.O.B. and M.P., unpublished data), a result supported by previously reported findings (19). We examined the turnover of total Gal4 protein in yeast cells and found that it was

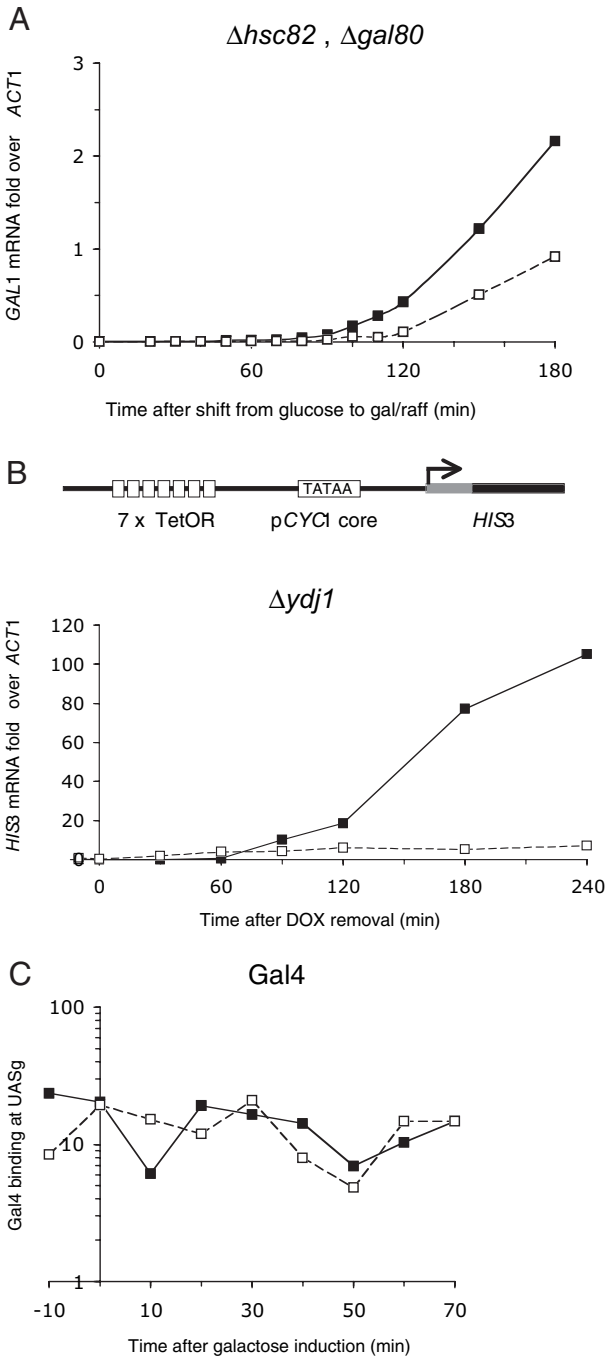

Fig. 3. The effect of chaperones on *GAL1* expression is downstream of galactose signaling and Gal4 DNA binding. (*A*) Yeast cells that were deleted for *gal80* and *gal4* contained a shortened Gal4 activator and were either wild type (filled symbols) or deleted for *hsc82* (open symbols) and were grown in SC media containing 2% glucose. *GAL1* expression was induced by shifting the cells to media containing 2% galactose and 2% raffinose. *GAL1* mRNA is depicted as fold over *ACT1*. (*B*) A schematic of the tetracycline-inducible reporter is shown above the figure. Cells bearing the Tet-reporter and also expressing a fusion of TetR to an activation domain of Gal4 were either wild type (filled squares) or deleted for *ydj1* (open squares). Cells were grown in SC media containing 2% glucose and 2 μg/ml DOX, and reporter gene expression was induced by removal of the antibiotic. Reporter *HIS3* mRNA is depicted as fold over *ACT1*. (*C*) ChIP experiments with an antibody against the DNA-binding domain of Gal4 were performed as described in *Materials and Methods* for cells grown in raffinose with or without galactose. Immunoprecipitated DNA was measured at the *UASg*, and values are depicted as the ratio of immunoprecipitated DNA at the promoter of *ACT1*. Squares are as in *A*.

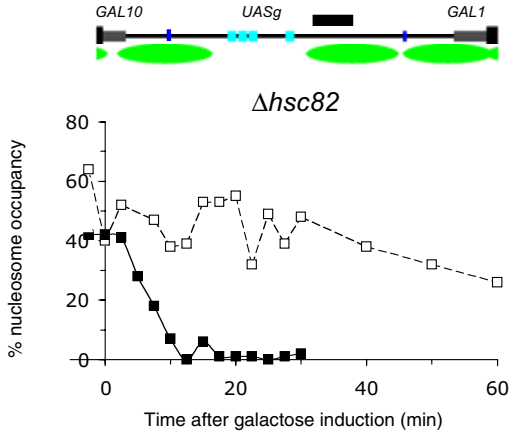

Fig. 4. Nucleosomes at the *GAL1* promoter are removed more slowly in the absence of *hsc82*. Nucleosome occupancy was measured by the method of G.O.B., V. Prabhu, M.F., D. Spagna, D. Schreiber, and M.P. (unpublished data). The nucleosomes are indicated in green in the schematic above the figure, and the location measured is indicated by the black bar. Gal4-binding sites are indicated in light blue, and TATAA elements are indicated in dark blue. The transcriptional and translational start sites of the flanking *GAL1* and *GAL10* genes are indicated in gray and black, respectively. Cells were either wild type (filled squares) or deleted for *hsc82* (open squares) and were induced by the addition of galactose.

not significantly affected by the deletion of *hsc82* or *ssa1* and *ssa2* (data not shown).

Promoter Nucleosome Removal Is Greatly Delayed in Cells Deleted for *hsc82*. The data presented thus far showed that the delay in *GAL1* gene induction and in the recruitment of the transcriptional machinery seen in strains deleted for components of the HSP90 and HSP70 chaperone machineries was not due to a defect in galactose signaling or in DNA binding by Gal4. An early process mediated by Gal4 upon induction of *GAL1* is the removal of promoter nucleosomes (G.O.B., V. Prabhu, M.F., D. Spagna, D. Schreiber, and M.P., unpublished data). To determine whether this process requires HSP90, we turned to the method developed in that study. Briefly, the method determines the sensitivity of specific DNA regions to micrococcal nuclease over a wide range of enzyme concentrations. This analysis allows us to determine the fraction of a given DNA segment in a population of cells that is protected by a nucleosome, and thus to follow the course of nucleosome removal upon induction. Fig. 4 shows that the removal of promoter nucleosomes at *GAL1*, upon induction, was greatly impaired in cells deleted for *hsc82*. The figure describes the removal of the single nucleosome positioned just to the right of the *UASg*, and the other two nucleosomes shown in the figure behaved essentially identically. Thus, whereas in wild-type cells nucleosome removal was complete by 12 min in the chaperone mutant nucleosome occupancy decreased by only ≈20% within the first hour of induction.

Discussion

Our findings suggest that removal of promoter nucleosomes at the GAL genes by DNA-bound Gal4 is facilitated by the HSP90/HSP70 chaperone machineries. Our findings that components of both HSP70 and HSP90 complexes, as well as the protein Sti1 [which can link the two chaperone complexes (12, 17, 18)], are all required for efficient nucleosome removal, and that both machineries are recruited to the *GAL1* promoter upon induction, suggest that these proteins work together in this early step of gene induction. Of the various complexes that affect nucleosome binding *in vitro* (e.g., SWI/SNF, RSC, and INO80), none has been shown to be required for induction of the GAL

genes *in vivo*. In contrast, it is reported that SWI/SNF, working with the so-called histone chaperone Asf1, is required for efficient induction of the *PHO5* gene (21, 22). There are reports of synthetic lethality generated by deletion of part of the HSP90/70 complexes and a so-called histone chaperone (Hir1) (23), on the one hand, and HSP90 and the INO80 and SWI/SNF remodelling complexes on the other (20). It remains to be seen what general rules, if any, might emerge governing nucleosome removal effected by protein complexes recruited by transcriptional activators.

Materials and Methods

***Saccharomyces cerevisiae* Strains.** We used strains derived from BY4741 (MATα, his3Δ1, leu2Δ0, met15Δ0, and ura3Δ0 S288C) deleted for *hsc82*, *sti1*, or *ydj1* obtained from the European Saccharomyces Cerevisiae Archive for Functional Analysis. We also disrupted the *GAL4* and *GAL80* genes in BY4741 and the *hsc82* deletion derivative by inserting a PCR fragment containing the *HIS5* gene from *Schizosaccharomyces pombe* in the coding region of *GAL4* and the *LEU2* gene at *GAL80*. Details on the primers used to create the respective PCR fragments can be provided on request. The strains thus created expressed a fusion of the minimal activation domain of Gal4 to its DNA-binding domain from a plasmid (see below). We also used an *hsc82* deletion strain derived from W303α (*can*1–100, *ade*2–1, *his*3–11,15, *trp*1–1, and *ura*3–1) provided by Susan Lindquist (Whitehead Institute for Biomedical Research, Cambridge, MA). Results obtained with this strain and the BY4741 derivative were indistinguishable. Point mutants *hsp*82 T101I and G170D have been described previously (14). A strain deleted for *hsc82* requires the expression of wild-type Hsp82 or one of these mutants for viability. The strain deleted for *ssa*1 and *ssa*2 was a derivative of strain W303α (*leu*2–3,112, *trp*1–1, *ura*3–1, *his*3–11,15, *lys*2Δ, *can*1–100, and *ade*2–1) and was provided by Elizabeth A. Craig (University of Wisconsin, Madison, WI). The strains used to detect SAGA recruitment were derivatives of BY4741 bearing a fusion of the endogenous *SPT*20 gene to the HA-epitope tag.

We also used derivatives of the strain NLY2 (MATα, *ura*3–52, *his*3Δ200, *leu*2–1, *lys*-, *trp*1Δ63, *gal*4$-$, and *gal*80$-$) (24), which had a Tet-reporter integrated at the *URA3* locus. Derivatives of this strain that were deleted for *ydj1* were created by inserting a PCR fragment containing the *TRP1* gene into the coding region of *YDJ1*, which resulted in complete removal of the *YDJ1* ORF. The resulting strains expressed a fusion of an activation domain of Gal4 to the Tet repressor (TetR) from a plasmid (see below).

Plasmids. The plasmid bearing a fusion of an activation domain of Gal4 to its DNA-binding domain was constructed by inserting a PCR fragment containing the *GAL4* promoter and the DNA-binding domain of *GAL4* (442 bp upstream of the ATG to 441 bp downstream) into vector pRS316 as an EcoRI/HindIII fragment. Then a PCR fragment containing the minimal activation domain of Gal4 (amino acids 840–881) and the *GAL4* terminator (200 bp) was inserted into the resulting vector as a HindIII/KpnI fragment. Primers used to create the corresponding PCR fragments can be given on request. The tetracycline inducible reporter was constructed by modification of the reporter described previously (25). In brief, the cassette containing the TetOR sites, the *CYC1* promoter, and the lacZ gene were excised from vector pCM145 and inserted into pYIplac211 as an EcoRI/HindIII fragment. Subsequently, the lacZ gene was replaced with the *HIS3* gene from *S. cerevisiae*. The TetR-*GAL4* activation domain fusion was constructed by inserting a PCR fragment containing the activation domain of Gal4 (amino acids 768–881) into vector pCM240 as an NcoI/SalI fragment. Primers used to create the PCR fragment can be given on request.

Antibodies. We used antibodies against Gal4 (sc-577; Santa Cruz Biotechnology), HA-1 (sc-7392; Santa Cruz Biotechnology), and RNA polymerase II (8WG16; Covance). Antibodies against Gal11 and Tfa2 were synthesized in the laboratory and have been described previously (26). The antibody against Hsc82 was provided by Susan Lindquist and was generated against a C-terminal peptide of Hsc82. The antibody against Ssa1 was synthesized for this study against recombinantly expressed protein purified from *Escherichia coli*.

Purification of Recombinant Ssa1 for Antibody Generation. The *SSA1* gene from *S. cerevisiae* was amplified with primers 5′-TATGCTCGAGATGTCAAAAGCTGTC-3′ and 5′-AGACGGATCCTTAATCAACTTCTTC3-′ from genomic DNA. The resulting XhoI/BamHI fragment was inserted into vector pET15b (Novagen), creating an N-terminal 6xHIS-fusion protein. The protein was expressed in *E. coli* strain BL21-CodonPlus(DE3)-RIL (Stratagene). In brief, the cells were grown in LB media containing ampicillin to an OD_{600} of 0.6 at 37°C. Cells were induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside and grown for 20 h at 17°C. Cells were then harvested and resuspended in buffer A [30 mM K_2HPO_4/KH_2PO_4, 100 mM KCl, 6 mM imidazol, and protease inhibitor mixture (complete minus EDTA; Roche) (pH 8.5)]. Cells were lyzed in a French pressure cell, and the cell lysate was treated with DNAseI (Roche) in the presence of 5 mM $MgOAc_2$. Ssa1 protein was purified from the soluble fraction on a HIS-Trap column (GE Healthcare/Life Sciences) with a gradient from 6–600 mM imidazol. The fractions containing Ssa1 were pooled, and the purified protein was injected into rabbits for antibody production according to standard procedures (Cocalico Biologicals).

Cell Growth. Yeast cells were grown exponentially overnight at 30°C to an OD_{600} of 0.2–0.5 before induction. For galactose induction, cells were grown in SC media containing 2% raffinose, and then 2% galactose was added. For other experiments, cells were grown in media containing 2% glucose, followed by resuspension in media containing 2% raffinose and 2% galactose. For tetracycline induction, cells were grown in media containing 2% glucose and 2 μg/ml doxycycline (DOX). Cells were washed once with media lacking DOX, followed by resuspension of the cells in the same media.

mRNA Measurements. mRNA was extracted from cells by a modified version of the hot acid phenol extraction method (27). On average, mRNA of 10 ml of yeast cell culture was extracted. After extraction, the mRNA was reverse transcribed by AMV reverse transcriptase (Roche) according to the manufacturer's instructions, and the resulting cDNA was measured by quantitative real-time PCR with primers corresponding to fragments in the *GAL1*, *HIS3*, or *ACT1* loci as indicated in the figure legends. The final levels of *GAL1* mRNA showed some variability from experiment to experiment presumably due to differences in cell growth and in the individual reverse transcriptase reactions. For this reason, each individual experiment was performed with a mutant strain and its corresponding wild type side by side.

ChIP Experiments. ChIP experiments with antibodies against the HA-epitope, Gal4, Gal11, Tfa2, and PolII were performed as described previously (13). For the ChIP experiments with antibodies against Hsc82 and Ssa1, we used a modified version of the protocol described previously (28), the details of which can be given on request.

cDNA and DNA Measurements by Real-Time Quantitative PCR. cDNA and DNA was measured by real-time quantitative PCR on a 7900HT (ABI) or a Light Cycler 480/384 (Roche) as described previously (ref. 13 and G.O.B., V. Prabhu, M.F., D. Spagna, D. Schreiber, and M.P., unpublished data). For cDNA measurements, primers used were 5′-TGCTCGATCCTTCTTTTCCA-3′ and 5′-TTGCGAACACCCTTGTTGTA-3′ for detection of a fragment in the *GAL1* coding region 840 bp from the ATG; 5′-AACTGGGACGATATGGAAAA-3′ and 5′-GAAGGCTGGAACGTTGAAAG-3′ for a fragment in the *ACT1* coding region 622 bp downstream of the ATG; and 5′-TACGCAGTTGTCGAACTTGG-3′ and 5′-GCGAGGTGGCTTCTCTTATG-3′ for a fragment in the *HIS3* coding region 418 bp downstream of the ATG. DNA measurements of ChIP experiments were performed with primers 5′-TGTTCGGAGCAGTGCGGCGC-3′ and 5′-ACGCTTAACTGCTCATTGCT-3′ for detection of a fragment in the *UASg* (408 bp upstream of the ATG); 5′-TTATGAAGAGGAAAAATTGGCAGTA-3′ and 5′-TGGTTGTTAATTTGATTCGTTAATTTG-3′ in the *GAL1/10* promoter (266 bp upstream of the ATG); 5′-TTGAAACCAAACTCGCCTCT-3′ and 5′-ATTGGGAAGGAAAGGATCAAAC-3′ in the *ACT1* promoter (254 bp upstream of the ATG); and 5′-AATCATAAATTTAGTCTGTGCTAGTC-3′ and 5′-AAATGAATCGATACAACCTTGGCA-3′ in the *PHO5* promoter (251 bp upstream of the ATG).

Micrococcal Nuclease-Sensitivity Experiments. Micrococcal nuclease-sensitivity experiments were performed by using the method of G.O.B., V. Prabhu, M.F., D. Spagna, D. Schreiber, and M.P. (unpublished data). Briefly, cells were harvested and cross-linked with formaldehyde, and the cross-linked DNA was digested with micrococcal nuclease over a wide range of enzyme concentrations. The amount of DNA remaining after digestion was determined by quantitative PCR and plotted against the concentration of micrococcal nuclease. The data were fitted to a two-state decay curve, with a fast rate for the fraction of DNA that was very sensitive to digestion and a slower rate for the fraction that was highly protected against digestion. This analysis revealed the positioning of nucleosomes flanking the *UASg*. To determine promoter nucleosome removal upon induction of *GAL1* in the absence of *HSC82*, protection was measured 266 bp upstream of the *GAL1* translational start codon with primers 5′-TTATGAAGAGGAAAAATTGGCAGTA-3′ and 5′-TGGTTGTTAATTTGATTCGTTAATTTG-3′.

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