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A Gene-Specific Requirement for FACT during Transcription Is Related to the Chromatin Organization of the Transcribed Region▽

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The FACT complex stimulates transcription elongation on nucleosomal templates. In vivo experiments also involve FACT in the reassembly of nucleosomes traversed by RNA polymerase II. Since several features of chromatin organization vary throughout the genome, we wondered whether FACT is equally required for all genes. We show in this study that the in vivo depletion of Spt16, one of the subunits of *Saccharomyces cerevisiae* FACT, strongly affects transcription of three genes, *GAL1*, *PHO5*, and *Kluyveromyces lactis LAC4*, which exhibit positioned nucleosomes at their transcribed regions. In contrast, showing a random nucleosome structure, *YAT1* and *Escherichia coli lacZ* are only mildly influenced by Spt16 depletion. We also show that the effect of Spt16 depletion on *GAL1* expression is suppressed by a histone mutation and that the insertion of a *GAL1* fragment, which allows the positioning of two nucleosomes, at the 5′ end of *YAT1* makes the resulting transcription unit sensitive to Spt16 depletion. These results indicate that FACT requirement for transcription depends on the chromatin organization of the 5′ end of the transcribed region.

Organization of DNA into chromatin inhibits transcription in vitro at both initiation and elongation steps (24, 28). Conversely, transcription elongation in vivo is a very efficient process that is usually accompanied by the alteration of chromatin structure, indicating the high ability of RNA polymerases to overcome the nucleosomal barrier in the cell nucleus (59). Yeast genetics and in vitro experiments with animal cell extracts have defined a set of factors able to help RNA polymerase II (Pol II) to carry out transcription elongation in the chromatin context (22, 61). One of the main cellular functions allowing Pol II to transcribe chromatin is played by the FACT complex, which, so far, is the only known factor able to stimulate Pol II-dependent transcription elongation through chromatin in a highly purified system (42, 45).

The human FACT complex is composed of two proteins, p140 and SSRP1, closely homologous to the essential *Saccharomyces cerevisiae* proteins Spt16/Cdc68 (hereafter referred to as Spt16) and Pob3, respectively (43). Spt16 has been described elsewhere as a protein involved in transcription due to the Spt$^-$ phenotype (suppression of Ty insertions in yeast promoters) conferred by *spt16* alleles (34). In addition, Spt16 and Pob3 have also been involved in the transcriptional regulation of cell cycle progression and in replication (50, 52, 64). Although a direct role of Spt16 in transcription initiation has been shown (6), there are several lines of evidence that support a role of Spt16 in transcription elongation, including sensitivity of certain *spt16* alleles to 6-azauracil as well as physical and genetic interactions with known elongation factors (17). The in vivo association of FACT to elongating Pol II, both in *Drosophila melanogaster* and in yeast, also indicates a role in elongation (36, 51).

SPT16 belongs to the histone group of *SPT* genes. Other genes encoding transcription elongation factors also in this group are *SPT4*, *SPT5*, and *SPT6* (68). In addition to the physical interactions with Spt4-Spt5 (32) and the Paf complex (30, 58), yeast FACT (yFACT) has been reported to interact with cell elements related to chromatin remodeling, such as Chd1 (56) and the NuA3 histone acetyltransferase complex (25). Yeast FACT and the HMG box protein Nhp6 combine to form the nucleosome-binding factor SPN (18), which is able to reorganize nucleosomes in vitro (48). Human Spt16 itself binds to nucleosomes and to H2A/H2B dimers, whereas SSRP1 interacts with H3/H4 tetramers (3). On the one hand, these interactions allow FACT to destabilize nucleosomes during transcription by promoting a loss of one H2A/H2B dimer, as shown by in vitro experiments (reviewed in reference 4). On the other, mutations in the yFACT subunits are synthetically lethal, with mutations affecting chromatin assembly (19), and *spt16* mutations lead to the activation of cryptic transcription initiation sites within coding regions (26, 36). These lines of evidence suggest that FACT also plays a role in maintaining the integrity of chromatin structure during transcription by participating in the reassembly of those nucleosomes altered by Pol II transcription.

The two roles assigned to FACT during transcription elongation are related to chromatin structure. Since the features of chromatin organization (histone modifications, nucleosome spacing, and degree of nucleosome translational positioning) vary throughout the genome (63, 66, 69), we wondered whether the requirement of FACT during transcription elongation is the same for all genes. In order to answer this question, we decided to compare the influences of the in vivo depletion of Spt16 on the transcription of several genes, all of

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TABLE 1. Yeast strains used in this work

Strain	Genotype	Source or reference
BY4741	*MAT***a** *his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	7
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	7
Y24573	*MAT***a**/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/LYS2 MET15/met15Δ0 ura3Δ0/ura3Δ0 spt16*::*KanMX4/SPT16*	EUROSCARF
Y17202	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1*::*kanMX4*	EUROSCARF
Y13232	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 pho5*::*kanMX4*	EUROSCARF
Y10425	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 yat1*::*kanMX4*	EUROSCARF
SJY2-4C	*MATα his3Δ1 leu2Δ0 ura3Δ0 spt16*::*kanMX4* (pCM189SPT16)	This study
SJY6	*MATα his3Δ1 leu2Δ0 ura3Δ0 trp1*::*kanMX4 spt16*::*kanMX4* (pCM184SPT16)	This study
SJY25	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0 trp1*::*kanMX4 SPT16-myc*	This study
SJY29	*MATα his3Δ1 leu2Δ0 ura3Δ0 trp1*::*kanMX4 spt16*::*kanMX4* (pCM184SPT16myc)	This study
JJY26	*MAT***a** *ura3-52 leu2Δ1 trp1 his4-9126 lys2-1286 HO-lacZ hhf2-13*	46
M236-13A	*MATα leu2 ura3 trp1 lys2-1286 can1-100*	33

them sharing the same promoter to exclude differential influences at the initiation step. Our results showed that those genes, such as *GAL1* and *PHO5*, exhibiting translationally positioned nucleosomes at the 5′ end of the transcribed region, are clearly sensitive to the depletion of Spt16, whereas those genes showing a random nucleosome distribution are only mildly affected.

MATERIALS AND METHODS

Yeast strains, plasmids, and media. The yeast strains used in this study are described in Table 1 and are isogenic to the S288C derivative BY4741 (7). All SJY strains were constructed by standard genetic methods of tetrad analysis or transformation. SJY2-4C was constructed by sporulating the *spt16Δ/SPT16* heterozygote Y24573, previously transformed with the plasmid pCM189SPT16 (*URA3 CEN TEToff*::*SPT16*). As the addition of doxycycline did not completely switch off the expression of *SPT16* from this plasmid, we generated SJY6 by crossing SJY2-4C and Y17202 and by subsequent shuffling of pCM189SPT16 by pCM184SPT16 (*TRP1 CEN TEToff*::*SPT16*). In SJY6, *SPT16* expression was completely switched off by the addition of doxycycline. SJY25 was constructed by tagging genomic *SPT16* at its 3′ end with 18 Myc epitopes followed by *Kluyveromyces lactis TRP1*. It was made following a PCR-based strategy (29) using the plasmid GA2266 as the DNA template (kindly provided by Gustav Ammerer). SJY6 and SJY25 showed the same doubling time in the absence of doxycycline, and their time courses of growth inhibition in the presence of the drug were identical. The sequence of any primer used in this study is available upon request.

Plasmids p416GAL1-lacZ (*URA3 CEN GAL1*pr::*lacZ*), pSCh202 (*URA3 CEN GAL1*pr::*PHO5*), pSCh247 (*URA3 CEN GAL1*pr::*LAC4*), and pSCh255 (*URA3 CEN GAL1*pr::*YAT1*) were previously described (9, 11, 39). Plasmids pCM189SPT16 and pCM184SPT16 were constructed for this study by subcloning a PCR fragment containing the *SPT16* coding region into the BamHI site of either pCM189 or pCM184 (20). Plasmid pCM184SPT16myc was constructed by subcloning a PCR fragment of *SPT16myc*, amplified using genomic DNA from SJY25 as the DNA template, into the ClaI-NotI sites of pCM184SPT16. The proper functionality of these plasmids was confirmed by their ability to complement the temperature sensitivity of *spt16-197* in the absence of doxycycline but not in the presence of this drug. Plasmid pRS425hhf2-13 was constructed by subcloning a PstI fragment containing the *hhf2-13* allele into the PstI site of pRS425. The *hhf2-13* allele was obtained by gap repair from the JJY26 strain (kindly provided by José Pérez-Martín). Plasmid pFG20 was constructed by subcloning a 441-bp PCR fragment of the 5′ region of *GAL1* into the XbaI-SpeI sites of pSCh247.

Cells were grown in yeast extract-peptone medium or in complete synthetic medium, with 2% glucose, 2% galactose, or 3% ethanol, at 30°C (49). To support growth in ethanol as a carbon source, galactose was added at a concentration of 0.1%. For copper induction, 0.1 mM of copper sulfate was added.

Western blot analyses. Laemmli boiled crude extracts were run on a 10% sodium dodecyl sulfate (SDS)-polyacrylamide gel and transferred to nylon membranes (Hybond-ECL). After being blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% milk, proteins were detected using antibodies against Myc (monoclonal; Santa Cruz) and Rps8 (polyclonal) with peroxidase-conjugated goat anti-mouse and anti-rabbit immunoglobulin G, respectively. Blots were washed with Tris-buffered saline and 0.1% Tween 20 and developed by enhanced chemiluminescence reactions (Pierce). Signals were detected with Hyperfilms ECL (Amersham), with exposure from 15 s to 5 min, or were quantified in a FujiFilm phosphorimager.

Northern and transcription run-on analyses. Northern analysis was carried out as described elsewhere (9). Run-on analysis was performed according to previous protocols (13, 44) with the modifications described in reference 5. Eight or 12 h after the addition of 5 μg/ml doxycycline, cells were washed in 5 ml cold TMN buffer (10 mM Tris-HCl at pH 7.4, 5 mM $MgCl_2$, 10 mM NaCl) and the cell pellet was resuspended in 900 μl of sterile cold water (final volume, 950 μl). Then, the cell suspension was transferred to a fresh microcentrifuge tube, 50 μl of 10% *N*-lauryl sarcosine sodium sulfate (sarcosyl) was added, and cells were incubated for 20 min on ice. After the permeabilization step, cells were recovered by centrifugation and the supernatant was removed. In vivo transcription was performed by resuspending cells in 300 μl of reaction solution containing 50 mM Tris-HCl, pH 7.9, 100 mM KCl, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM dithiothreitol, 0.5 mM each of ATP, GTP, and CTP, and 100 μCi [α-^{33}P]UTP (3,000 Ci/mmol). The mix was incubated for 2 min at 30°C to allow transcription elongation. The reaction was stopped by the addition of 1 ml of cold TMN to the mix. Cells were recovered by centrifugation to remove the nonincorporated radioactive nucleotides. Total RNA was isolated using the hot acid phenol method. Cell pellets were resuspended in 400 μl TES [*N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid] and 400 μl acid phenol, mixed, and incubated for 60 min at 65°C. Contaminants were removed by successive extractions with phenol and chloroform. Labeled RNA was precipitated by adding 0.1 volume of 3 M NaAc and 2.5 volumes of cold ethanol overnight at −20°C. After centrifugation at maximum speed for 15 min, labeled RNA was washed once with 70% ethanol and dried. Before hybridization, RNA was fragmented with 0.04 N NaOH for 5 min on ice and then neutralized with an equal amount of HCl. All of the in vivo-labeled RNA was used for hybridization (0.35×10^7 to 3.5×10^7 dpm).

Ten micrograms of denatured DNA from each *PHO5*, *GAL1*, or *YAT1* PCR fragment was immobilized on Hybond N^+ filters with a pR600 slot blot (Hoefer). After nylon membranes were prehybridized for 1 h in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhart's solution, and 0.5% SDS, hybridizations were performed with 2 ml of the same solution containing labeled RNA for 36 to 40 h in a roller oven at 65°C. After hybridization, filters were washed once in 2× SSC and 0.1% SDS for 20 min and twice in 0.2× SSC and 0.1% SDS for 30 min. We performed each experiment at least three times, swapping the filters in each replicate among the different type of samples. Filters were exposed for 4 days to an imaging plate (BAS-MP; FujiFilm) that was read at 100-μm resolution in a phosphorimager scanner (FLA-3000; FujiFilm). Values were corrected for probe amounts by hybridization with labeled genomic DNA and normalized with the signal given by a ribosomal DNA probe also immobilized on the filter.

Chromatin immunoprecipitation assays. For chromatin immunoprecipitation (ChIP) analysis of Pol II or Spt16-myc, the SJY6 or SJY29 yeast strain was transformed with pSCh202, pSCh247, pSCh255, or p416GAL1lacZ plasmid. Strains were grown to mid-log phase in synthetic complete medium lacking uracil (SC-Ura) with either 2% glucose or 2% galactose. For cross-linking, cells were treated with 1% formaldehyde for 15 min at room temperature. Chromatin immunoprecipitation assays were performed as described previously (2) with 8WG16 or c-Myc (9E10) antibody. We amplified a 300-bp-long region of each open reading frame in different positions (5′, middle, and 3′). As a nontran-

scribed control, we amplified a region adjacent to *FUS1*. Primer mixes were empirically adjusted for balanced signals. PCR signals were quantified by a phosphorimager. Immunoprecipitation was defined as the ratio of each gene-specific product in relation to that of the nontranscribed region, always after normalization with the signal of its corresponding whole-cell extract. Several dilutions of the whole-cell extract were tested to make sure that the assays were in the linear range.

Nucleosome mapping. Yeast spheroplasts and micrococcal nuclease digestions were performed as described previously (14) with the modifications described in reference 10. Spheroplasts were prepared from mid-log-phase cultures grown in SC-Ura with 2% glucose. Cells were lysed and immediately digested with 7.5 to 125 mU of micrococcal nuclease. For naked DNA controls, genomic DNA was extracted as previously described and digested with 0.003 to 0.2 mU of micrococcal nuclease under the same conditions. Micrococcal nuclease-cleaved genomic DNA was digested with SalI [for *YAT1*, *GAL1*pr::*YAT1*, and *GAL1*pr::*GAL1(5′)-YAT1*], BamHI (for *GAL1*pr::*LAC4*), PvuII (for *GAL1*pr::*PHO5*), or HindIII (for *GAL1*) and resolved in 1.5% agarose without ethidium bromide. For the analysis of *YAT1*, *GAL1*pr::*YAT1*, and *GAL1*pr::*GAL1(5′)-YAT1*, the probe used was the 200-bp PCR fragment immediately upstream of the SalI site present in *YAT1*. For the analysis of *GAL*::*LAC4*, the probe used was the 205-bp PCR fragment immediately upstream of the BamHI site present in *LAC4*. For the analysis of *GAL*::*PHO5*, the probe used was the 195-bp PCR fragment immediately upstream of the PvuII site present in pRS416, close to the *GAL1* promoter. For the analysis of *GAL1*, the probe used was the 198-bp PCR fragment immediately upstream of the HindIII site.

RESULTS

Gene-specific effect of Spt16 depletion on mRNA levels. To investigate whether all genes are equally dependent on FACT for transcription elongation, we decided to compare several transcription units, which shared the same promoter but differed in the transcribed region, when depleting the in vivo levels of Spt16. Instead of using a degron approach, which would provoke a rapid destruction of the target protein after a heat shock, we decided to produce a slow reduction of Spt16 so as to lay out the conditions under which the protein could become limiting, but the cell physiology was not dramatically changed yet. In order to do so, we constructed the SJY6 strain, containing a Tet-controlled *SPT16* gene. Doxycycline, the tetracycline analogue, did not affect the growth rate of a wild-type strain (not shown). In the absence of doxycycline, the SJY6 strain showed a wild-type growth (Fig. 1A). Although in the absence of doxycycline, the abundance of Spt16 was higher in SJY6 than in the wild type (Fig. 1B), the Tet::*SPT16* construct did not produce an Spt$^-$ phenotype (not shown), suggesting that the excess of Spt16 was not sufficient to alter the correct function of Pol II machinery. Eight hours after 5 μg/ml doxycycline was added to SJY6 cells, the amount of Spt16 decreased below the wild-type levels (Fig. 1B). Two hours later, cells began to slow down their growth (Fig. 1A), but only after 12 h in the presence of doxycycline, cells began to accumulate in G_1 (not shown) and cell viability started to diminish (Fig. 1A). Although in this kind of in vivo study it is formally impossible to rule out the involvement of additional elements between the input (FACT depletion) and the monitored output (changes in gene expression), we believe that the results obtained after the start of Spt16 limitation (8 h in doxycycline) and before the decrease in cell viability (12 h in doxycycline) are likely the direct consequences of FACT depletion on gene expression. According to this, for the following studies we compared samples taken 6, 8, 10, and 12 h after adding doxycycline.

The SJY6 strain was transformed with plasmids containing the following genes driven by the *GAL1* promoter: *PHO5* (pSCh202), *YAT1* (pSCh247), *Escherichia coli lacZ* (p416GAL1-lacZ), and *K. lactis LAC4* (pSCh255). We cultured these transformants in selective galactose medium both in the presence of 5 μg/ml doxycycline and in the absence of the drug. The mRNA levels of the mentioned genes and the endogenous *GAL1* gene were then analyzed by Northern blotting. In the absence of doxycycline, the Northern conditions and time of exposure required to detect all hybridization signals were the same, suggesting that similar levels of mRNA accumulation were occurring in the cell for the five tested genes.

The depletion of Spt16 strongly affected the mRNA levels of the *GAL1* gene. Ten hours after the addition of doxycycline, the mRNA levels of *GAL1* were one-half of the untreated control, and 2 hours later, they hardly reached 25% (Fig. 1C). No expression of the endogenous *PHO5* gene is detected in this high-phosphate medium due to the strong repressive nucleosomal organization of the *PHO5* promoter (60). However, when *PHO5* was transcribed from the *GAL1* promoter, its mRNA levels were also severely affected by the depletion of Spt16. Just 8 h after the addition of doxycycline, a significant decrease in the *PHO5* mRNA levels was detected compared to those of the control; 4 hours later, they were less than one-third of the control levels (Fig. 1C). In contrast, the levels of *lacZ* mRNA were only mildly affected by Spt16 depletion when *lacZ* mRNA was transcribed from the *GAL1* promoter. No significant differences with the control were observed until 12 h after the addition of doxycycline, and at this time, the levels of *lacZ* mRNA still reached 60% of the control level (Fig. 1C).

To exclude the possibility that this difference between *GAL1* and *PHO5* on the one hand, and *lacZ* on the other, was due to the larger length of *lacZ* mRNA (*GAL1*, 1.7 kb; *PHO5*, 1.5 kb; *lacZ*, 3.1 kb), we investigated the effect of Spt16 depletion on *YAT1*, a 2-kbp-long yeast gene that showed transcriptional behavior similar to that of *lacZ* in other mutants affected in gene expression (11). Since the mRNA levels of the endogenous *YAT1* gene were undetectable under our culture conditions (not shown), the Northern results reflected the influence of Spt16 depletion on the *GAL1pr*::*YAT1* transcription unit. As shown in Fig. 1C, *YAT1* mRNA was not affected by the depletion of Spt16 even 12 h after the addition of doxycycline. To further confirm that the length of the transcription unit was not related to the sensitivity to Spt16 depletion, we analyzed the mRNA levels of *LAC4* (3.1 kb), a eukaryotic homologue of *lacZ* isolated from the yeast *Kluyveromyces lactis*. When *LAC4* mRNA was transcribed from the *GAL1* promoter, its accumulation was clearly affected by Spt16 depletion. Ten hours after the addition of doxycycline, the mRNA levels were already significantly far from the control, and 2 hours later, they reached only one-third of the control levels (Fig. 1C). Altogether, the results shown in Fig. 1C indicate a differential effect of Spt16 depletion on the mRNA levels of the tested transcription units. Since all shared the same promoter, we inferred that the detected differences should be related to the transcribed sequences.

It has been reported that *spt16* mutations and in vivo depletion of Spt16 activate transcription initiation from cryptic promoters within coding regions (26, 36). As can be seen in Fig. 1D, after 12 h in the presence of doxycycline, the cryptic pro-

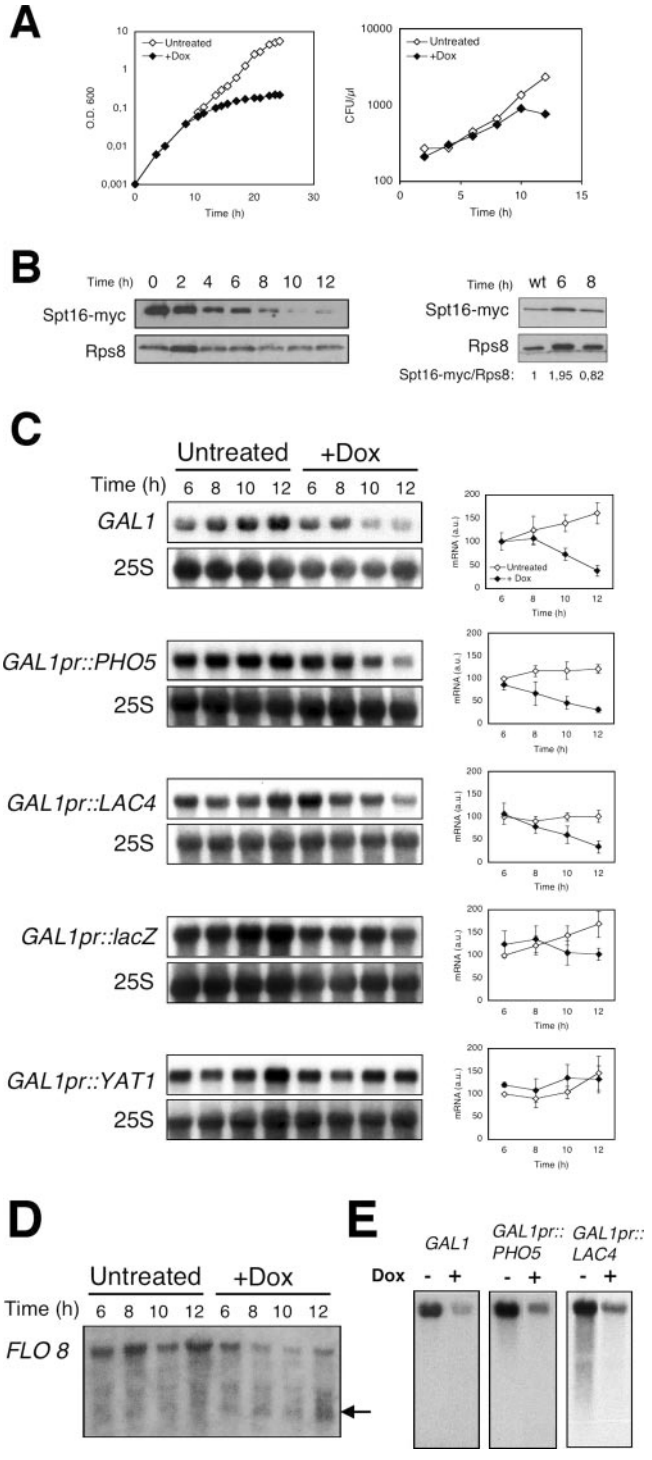

FIG. 1. The in vivo depletion of Spt16 differentially affects the mRNA levels of five transcription units. (A) Growth profile of strain SJY6, containing a doxycycline (Dox)-repressible *SPT16* allele, in galactose medium with or without 5 μg/ml doxycycline. Culture absorbance at 600 nm (O.D. 600) and the number of CFU per μl of culture (CFU/μl) are represented. (B) Levels of Spt16-myc in SJY29 cells along its in vivo depletion, followed by Western blotting, and comparison with the levels of Spt16-myc in the nondepleted wild-type SJY25 strain. Ribosomal protein Rps8, followed by specific antibodies, was used as the internal loading reference. (C) Northern analysis of the mRNA levels of the indicated transcription units during Spt16 depletion (strain SJY6). The results of a typical experiment are shown on the left, and the averages from at least three independent experiments are shown on the right. (D) Northern analysis of *FLO8* during Spt16 depletion. (E) Full-lane pictures of some of the Northern experiments whose results are shown in panel C (12 h with or without Dox). a.u., arbitrary units.

moter located in *FLO8* also became slightly activated. However, no additional transcripts were detected in any of the cases where Spt16 depletion led to a marked decrease of the full-length transcript. As shown in Fig. 1E, neither *GAL1* nor *PHO5* or *LAC4* produced secondary transcripts 12 h after the addition of doxycycline. We concluded that the detected declines in full-length transcripts were not mediated by the activation of cryptic initiation sites but rather were related to a postinitiation event.

Differential effect of Spt16 depletion on transcription. Assuming that the activity of *GAL1* was not differentially influenced by the coding sequences inserted downstream, the differential effect of Spt16 depletion on the mRNA levels could be due either to a direct, but distinctive, influence of the Spt16 shortage on transcription elongation by Pol II or to an indirect effect of Spt16 scarcity on the stability of the different transcripts. To distinguish between these two possibilities, we determined Pol II processivity along the five tested genes 10 h after the addition of doxycycline. In order to do so, we measured the level of Pol II occupancy at the five transcription units. It has been reported for several genes and for the hybrid transcription unit *GAL1*pr::*YLR454* that the level of Pol II association is constant throughout coding regions (35, 36). However, after Spt16 depletion, Pol II occupancy in the middle and at the 3′ end of *GAL1* decreased to 0.6 and 0.7, respectively, in relation to the occupancy at the 5′ end (Fig. 2A). Similar drops in Pol II occupancy were observed in the middle and at the 3′ end of *GAL1*pr::*PHO5* and, to a lesser extent, at *GAL1*pr::*LAC4* (Fig. 2A). However, no effect of Spt16 depletion on Pol II occupancy was observed at *GAL1*pr::*lacZ* and at *GAL1*pr::*YAT1* (Fig. 2A). The alteration in Pol II occupancy was limited to the three transcription units that also showed reduced levels of mRNA accumulation, and in all three cases, Pol II occupancy did not decrease further when being transcribed from the middle of the genes to the 3′ ends. Taking into account that this technique detects only major defects in Pol II processivity (35, 38), we consider that these modest decreases in Pol II occupancy are significant enough and compatible with a processivity defect of Pol II at the 5′ regions caused by Spt16 depletion. However, since Pol II molecules sitting at the promoter might also contribute to the 5′ ChIP signals, a defect in the initiation-to-elongation transition cannot be completely excluded.

We also performed run-on assays with the endogenous *GAL1* gene and *GAL1*pr::*PHO5*, as representatives of the transcription units sensitive to Spt16 depletion, and *GAL1*pr::*YAT1*, as the least sensitive to Spt16 shortage. We used 5′ and 3′ probes to measure the density of elongating polymerases at both ends of the transcribed regions. Eight hours after the addition of doxycycline, the three tested genes started to show a decline in their run-on signals that, only in the cases of the 3′ ends of *GAL1* and *GAL1*pr::*PHO5*, was significantly different

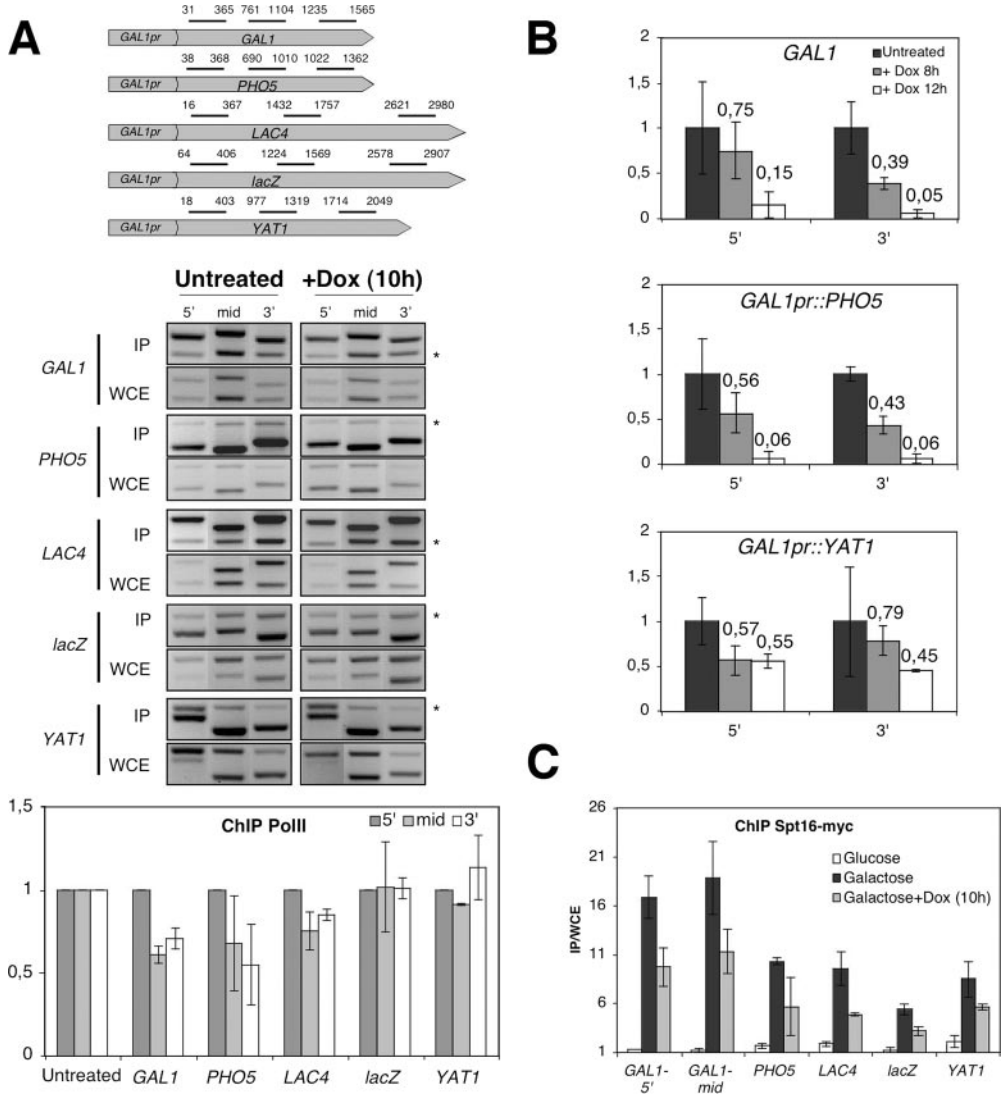

FIG. 2. Distribution of Pol II and occupancy of Spt16 during Spt16 depletion. (A) ChIP analysis of Pol II on the five indicated transcription units. Transformants of the SJY6 strain were grown is SC-Ura galactose medium in the presence of doxycycline (Dox) for 10 h. Cross-linked chromatin was immunoprecipitated with the monoclonal 8WG16 anti-Pol II antibody. PCR was conducted on two dilutions of whole-cell extract (WCE) and two different amounts of immunoprecipitated DNA (IP) (only the most diluted are shown). PCR primers flank 300-bp segments located in the 5′, central (mid), and 3′ regions of each gene. Diagrams at the top indicate the position of the PCR amplicons relative to the beginning of the coding regions. A nontranscribed region adjacent to *FUS1* was used as a control (marked with an asterisk). The results of a typical experiment for each gene are shown in the middle, and the averages from three independent experiments are shown at the bottom. Following the study by Mason and Struhl (35), data are expressed as the ratio (distribution) of Pol II occupancy in a given gene relative to the 5′-most position, followed by normalization of each value to the corresponding position at the same gene in the untreated strain, which was defined as "1." For economy, "untreated" represents any of the five genes in the untreated condition. (B) Results of the run-on analyses of *GAL1*, *GAL1*pr::*PHO5*, and *GAL1*pr::*YAT1* in SJY6, cultured in galactose for 8 or 12 h in the presence of doxycycline (+Dox). Signals from different experiments were averaged after correction for probe amounts (hybridizing with labeled genomic DNA) and after normalization with the signal given by a ribosomal DNA probe. To facilitate the interpretation of the results, the data are presented in relation to the values of the same strain cultured during 8 h in the absence of the drug. The 5′/3′ ratios of run-on signals in this condition were 2.77 for *GAL1*, 0.52 for *PHO5*, and 0.46 for *YAT1*. DNA segments (200 bp long) from the 5′ and 3′ regions of each open reading frame were used as probes. The averages from at least three independent experiments are shown. (C) ChIP analysis of Spt16 on the five indicated transcription units in the wild type and in an Spt16-depleted strain. Transformants of the indicated Spt16-myc-expressing strains were grown for 10 h in SC-Ura medium with either glucose or galactose (SJY25) or in galactose plus doxycycline (SJY29). Cross-linked chromatin was immunoprecipitated with anti-Myc antibody. A PCR was conducted on two dilutions of whole-cell extract and two different amounts of immunoprecipitated DNA. PCR primers flank 300-bp segments located in the central region of each gene and in the 5′ end of the *GAL1* coding region (GAL1-5′). A nontranscribed region adjacent to *FUS1* was used as a control. The averages from three independent experiments are shown.

from that of the untreated control (Fig. 2B). Furthermore, the results showed that 12 h after the addition of doxycycline, the density of elongating Pol II at both ends of *GAL1* was around 10% of the control values (Fig. 2B). Very similar results were obtained when the run-on analysis was performed with the *GAL1*pr::*PHO5* transcription unit (Fig. 2B). In contrast, at the same time of depletion, *GAL1*pr::*YAT1* showed run-on signals five times higher at both ends of *YAT1* (Fig. 2B).

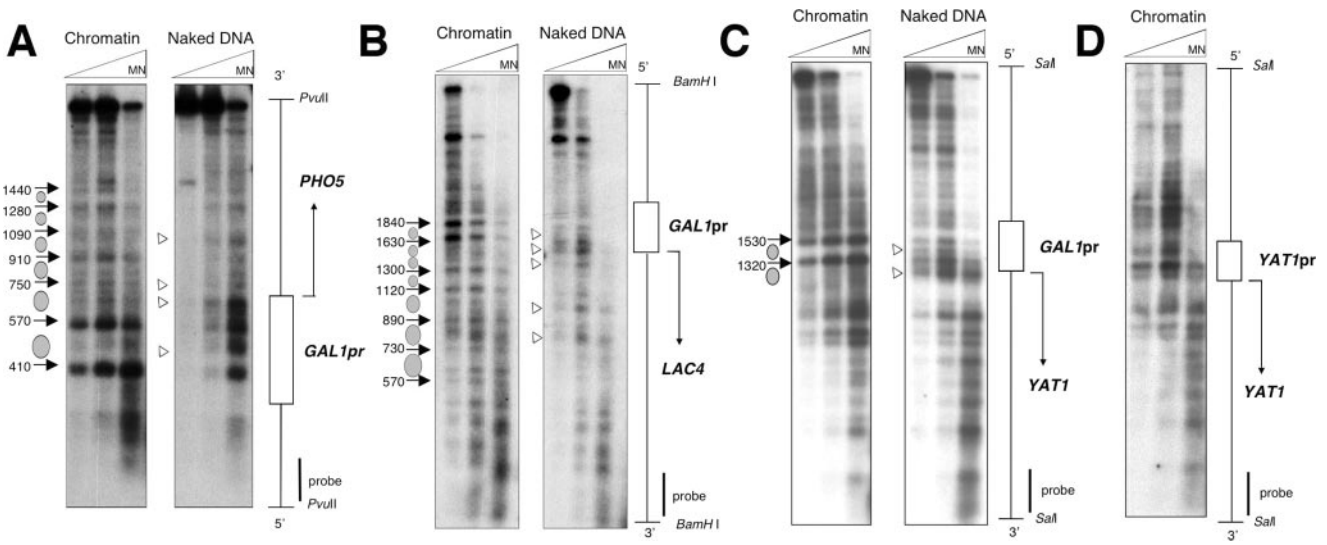

FIG. 3. *GAL1pr*::*PHO5* and *GAL1pr*::*LAC4*, but not *GAL1pr*::*YAT1*, exhibit translationally positioned nucleosomes at their transcribed regions. Chromatin and naked DNA samples of Y13232(pSCh202) (A), BY4742(pSCh247) (B), Y10425(pSCh255) (C), and BY4742 (D) were treated with micrococcal nuclease (MN), digested with the indicated restriction enzymes, resolved in agarose gels, and hybridized with the noted DNA probes to map nucleosomes at *GAL1*pr::*PHO5* (A), *GAL1*pr::*LAC4* (B), *GAL1*pr::*YAT1* (C), and chromosomal *YAT1* (D). Horizontal arrows indicate chromatin-dependent hypersensitive sites for micrococcal nuclease; white triangles indicate protections of micrococcal nuclease cuts present at the naked DNA lanes. Ovals show the positions of the mapped nucleosomes.

Considering the results of Pol II distribution by ChIP and run-on, we conclude that the different levels of mRNA accumulation measured after we added doxycycline were due to a differential effect of Spt16 depletion on transcription. We conclude that this effect is likely to take place at the elongation phase or at the initiation-to-elongation transition. The similar half-lives of *GAL1* and *GAL1*pr::*lacZ* mRNAs during Spt16 depletion rule out changes in mRNA stability as the main explanation for the detected gene-specific effect of Spt16 shortage, although minor effects at this level cannot be completely excluded (not shown).

As the FACT complex travels with Pol II during elongation (36, 51), we considered whether the gene-specific differences detected were related to an unequal presence of FACT at the transcribed regions before Spt16 depletion. To answer this question, we carried out ChIP analysis of Spt16 under nondepleting conditions. To avoid influencing the ChIP analyses by possible Spt16 present at the *GAL1* promoter, the PCR oligonucleotides selected corresponded to the central region of each gene and they were at least 700 bp away from the start codon. As shown in Fig. 2C, a transcription-dependent ChIP signal for Spt16 was detected in the five genes studied. These results indicated that Spt16 is present even in those genes, such as *YAT1* and *lacZ*, whose transcription is least influenced by the depletion of Spt16. We also performed ChIP analysis of the effect of Spt16 depletion on the amounts of this protein associated with the five genes tested; in all cases, the association of Spt16 became significantly diminished (Fig. 2C). We also estimated the presence of Spt16 at the 5′ end of the *GAL1* coding region. Spt16 was also strongly recruited to this sequence during transcription, and as expected, its depletion caused a decrease in the level of protein present (Fig. 2C). According to these results, it is difficult to imagine that the local amounts of FACT present at the transcription units, either before or during Spt16 depletion, might explain the degree of sensitivity to Spt16 shortage. Instead, we envisaged some intrinsic feature of the transcribed region to be the element that explains the differential response of the five tested genes to Spt16 depletion.

Correlation between nucleosomal organization and sensitivity to Spt16 depletion. We have shown elsewhere that, when introduced in the yeast genome, *lacZ* does not exhibit translationally positioned nucleosomes, even when it is fused to the *GAL1* promoter (11). In contrast, the *GAL1* gene shows an ordered array of nucleosomes on the transcribed region (11). Taking into account these previous results and the solid in vitro data assigning a chromatin-remodeling role to FACT in transcription elongation (42, 48), we decided to investigate the chromatin organization of the other three transcription units so far studied in this work.

Indirect end-labeling experiments of chromatin preparations, partially digested with micrococcal nuclease, showed clear patterns compatible with positioned nucleosomes at the transcribed regions of *GAL1*pr::*PHO5* and *GAL1*pr::*LAC4* but not at the transcribed region of *GAL1*pr::*YAT1* (Fig. 3A to C). In the *GAL1*pr::*PHO5* transcription unit, the comparison of the chromatin lanes to the ones of naked DNA gave a combination of hypersensitive sites and protections that predicted an array of at least six nucleosomes covering the *GAL1* promoter and the first half of the *PHO5* gene (Fig. 3A). Additional hypersensitive sites are visible in the second half of the *PHO5* gene, suggesting that the nucleosomal array might extend further. A similar picture was obtained from the *GAL1*pr::*LAC4* transcription unit (Fig. 3B). The intensity of the bands corresponding to the predicted linker regions was not always the same, but the differences between the lanes corresponding to chromatin and naked DNA are clear enough to conclude that chromatin is not positioned at random at the transcribed re-

gions of *GAL1*pr::*PHO5* and *GAL1*pr::*LAC4* (Fig. 3A and B). In contrast, no positioned nucleosomes can be located on the *YAT1* gene in the context of the *GAL1*pr::*YAT1* transcription unit. Although the two characteristic nucleosomes of the *GAL1* promoter are clearly visible, there is no pattern in the transcribed region pointing to a stable positioning of nucleosomes on *YAT1* (Fig. 3C). In order to investigate whether this random nucleosomal structure was related to the plasmidic nature of the *GAL1*pr::*YAT1* construct, we tried to map nucleosomes on the genomic *YAT1* gene. The very same pattern of micrococcal nuclease cuts already shown on the transcribed region of *GAL1*pr::*YAT1* was detected on the transcribed region of the chromosomal *YAT1* gene. These results rule out a possible episomal artifact and confirm that the presence of positioned nucleosomes in a promoter is not enough to transmit this positioning to any adjacent chromatin region, just as we have previously found with *GAL1*pr::*lacZ* (11). The DNA sequence of the transcribed region seems therefore to be essential for nucleosome positioning downstream of the *GAL1* promoter.

Considering the previously published data and the results shown in Fig. 3, we can establish a good correlation between the sensitivity of a transcription unit to Spt16 shortage and the occurrence of translationally positioned nucleosomes on its transcribed region. This is the case for the endogenous *GAL1* gene and the *GAL1*pr::*PHO5* and *GAL1*pr::*LAC4* transcription units. Vice versa, *GAL1*pr::*lacZ* and *GAL1*pr::*YAT1*, the two least Spt16-dependent transcription units, exhibit randomly positioned nucleosomes at their transcribed regions. Although the degree of nucleosome positioning is not exactly the same in all cases, hereafter we call "positioned" all genes showing a nonrandom pattern of chromatin and "nonpositioned" those genes whose micrococcal nuclease pattern is the same in both the chromatin and the naked DNA samples.

Since all transcription units so far analyzed were controlled by the *GAL1* promoter, we wondered whether the correlations between nucleosome positioning and Spt16 dependency could be extended to other genes. We first measured the effect of Spt16 depletion on the mRNA levels of the chromosomal *YAT1* gene, transcribed in ethanol-containing medium. As shown in Fig. 4A, Spt16 depletion did not cause a negative effect on *YAT1* mRNA levels but rather an increase that might be caused by the up-regulation of the *YAT1* promoter under these conditions (ethanol, absence of glucose, and limitation of Spt16).

Our nucleosome-mapping results could not be compared to the data obtained with tiled arrays for a substantial part of the *Saccharomyces cerevisiae* genome, as that study did not include the coding regions of *YAT1*, *PHO5*, or *GAL1* (69). We decided to measure the mRNA levels of two genes, highly expressed in YPD, whose chromatin organizations have been described in that study: *SRO9* and *CIT2* (69). The first one displays a translationally positioned nucleosome at the 5′ end of the transcribed region, whereas the second one lacks translationally positioned nucleosomes at its 5′ end, displaying, however, several of them on the second half of its coding region. As shown in Fig. 4B, the mRNA levels of *SRO9* were very sensitive to Spt16 depletion, while the amounts of *CIT2* mRNA were not negatively affected by the Spt16 shortage but, like those of *YAT1*, were rather upregulated.

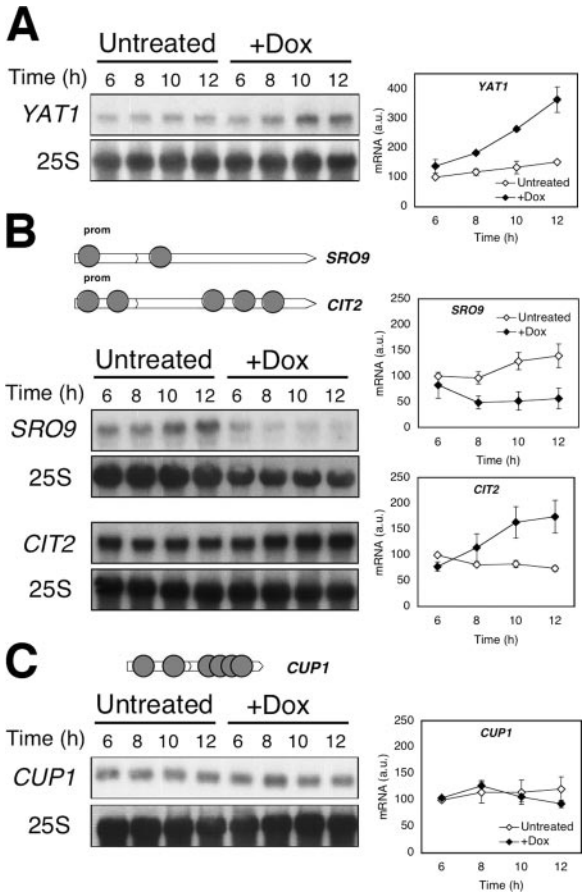

FIG. 4. Effect of Spt16 depletion on the expression levels of several genes driven by their own promoters. (A) Northern analysis of the mRNA levels of *YAT1* during Spt16 depletion. SJY6 cells were grown in SC with ethanol to induce *YAT1* expression. (B) Northern analyses of the mRNA levels of *SRO9* and *CIT2* during Spt16 depletion. SJY6 cells were grown in SC. Nucleosome positioning at the two genes is depicted as described by Yuan et al. (69). (C) Northern analysis of the mRNA levels of *CUP1* during Spt16 depletion. SJY6 cells were grown in SC plus copper. Overlapping phases of nucleosomes at the transcribed *CUP1* gene are depicted as described by Shen et al. (55). The results of a typical experiment and the quantification of three independent experiments are shown in each case. Dox, doxycycline; prom, promoter; a.u., arbitrary units.

We also examined the effect of Spt16 depletion on the mRNA levels of the methallothionein-coding *CUP1* gene, whose chromatin is not organized into a unique array of positioned nucleosomes when transcribed but into clusters of overlapping nucleosome positions (55). We measured the effect of Spt16 depletion on the accumulation of *CUP1* mRNA in cells growing in copper-containing medium. No significant effect was observed (Fig. 4C).

Altogether, the last set of results, obtained from genes transcribed from their own promoters, further suggest the role of Spt16 in transcription of genes exhibiting positioned nucleosomes, especially when they are located at the 5′ end of their transcribed regions. In order to test whether this correlation reflects a cause-and-effect relationship, we designed experiments to detect possible changes in the degree of Spt16 requirement for the analyzed genes in response to changes in

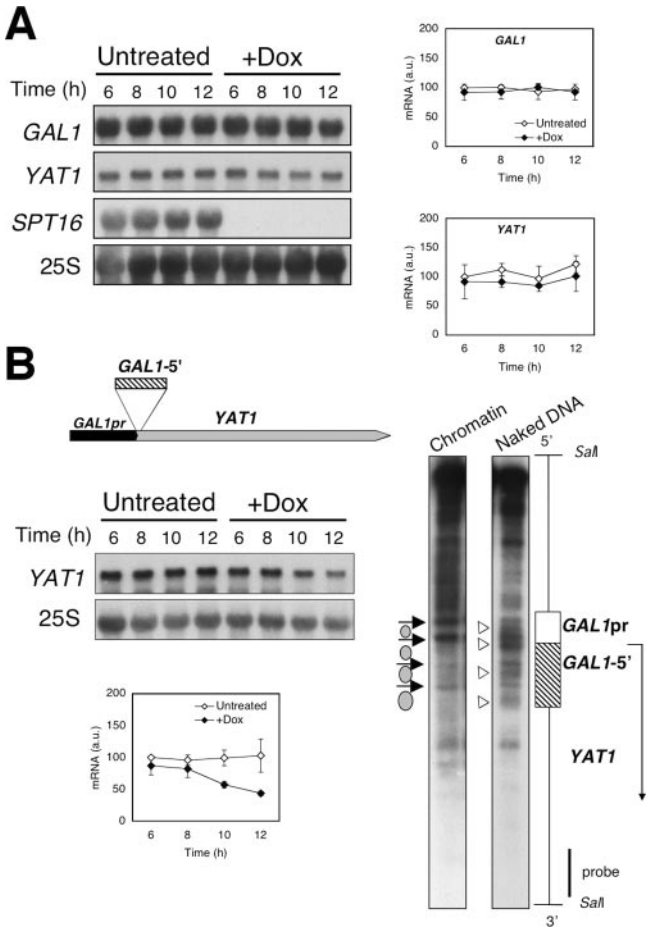

FIG. 5. Exchange of the expression patterns of *GAL1* and *YAT1* during Spt16 depletion by alteration of nucleosome positioning. (A) Northern analyses of the mRNA levels of *GAL1* and *YAT1* during Spt16 depletion in cells expressing the *hhf2-13* histone H4 allele. SJY6(pRS425hhf2-13) cells were grown in SC without Leu (galactose). The results of a typical experiment are shown on the left, and the averages from three independent experiments are shown on the right. Expression of *SPT16* was controlled by Northern analysis to exclude an effect of *hhf2-13* on the Tet promoter. (B) Northern analysis of the mRNA levels of *GAL1*pr::*GAL1(5′)-YAT1* during Spt16 depletion. The results of a typical experiment and the averages from three independent experiments are shown. A comparison of chromatin and naked DNA samples, treated with micrococcal nuclease and analyzed by indirect end labeling, is shown on the right. An explanation of nucleosome mapping appears in the legend to Fig. 3. Dox, doxycycline; a.u., arbitrary units.

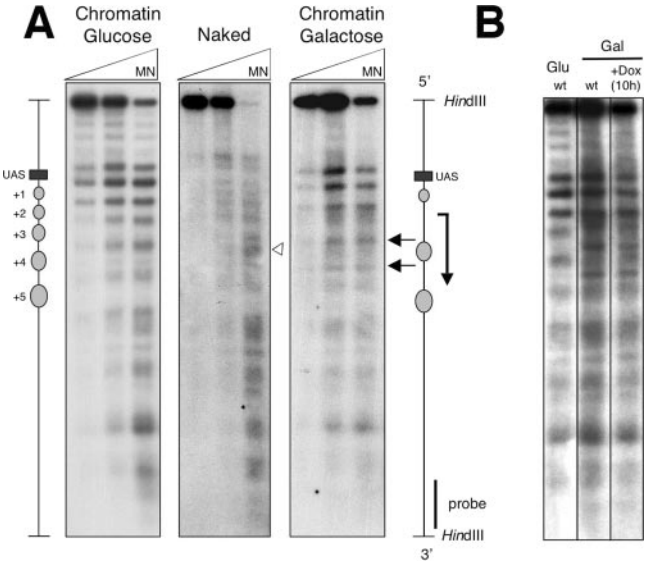

FIG. 6. Nucleosomal organization of *GAL1* under repressive and activating conditions. (A) Nucleosome mapping at *GAL1*, under conditions of repression (glucose) and transcription (galactose). Chromatin and naked DNA samples from BY4741 cells were treated with micrococcal nuclease (MN) and analyzed by indirect end labeling. An explanation of nucleosome mapping appears in the legend to Fig. 3. (B) Nucleosomal organization of *GAL1*, under activation conditions, during Spt16 depletion. Chromatin samples of BY4741 cells (wt), grown in glucose or galactose for 10 h, and SJY6 cells, grown in galactose and doxycycline (Dox) for 10 h, were treated with micrococcal nuclease and analyzed by indirect end labeling. UAS, upstream activation sequence.

their chromatin organization. We first made use of the histone H4 allele *hhf2-13*, a dominant H4-R45H mutation that causes alterations of chromatin structure by disrupting essential DNA-histone interactions (40, 65) and favors nucleosome mobility in vitro (15). Overexpression of *hhf2-13* suppressed the negative effect of Spt16 depletion on the *GAL1* mRNA levels, indicating that the impairment of *GAL1* expression caused by Spt16 depletion was mediated by chromatin structure (Fig. 5A). The absence of *SPT16* mRNA in the doxycycline-treated *hhf2-13* cells indicated that the suppression was not due to a deficient repression of the Tet promoter (Fig. 5A). The levels of *GAL1*pr::*YAT1* mRNA remained unaffected. This rules out the suppression as a consequence of a general increase in either mRNA levels or *GAL1* promoter activity caused by *hhf2-13* (Fig. 5A).

We also engineered *GAL1*pr::*YAT1* to introduce positioned nucleosomes in its transcribed region. We inserted a 430-bp fragment from the 5′ end of the *GAL1* transcribed region into the *GAL1*pr::*YAT1* transcription unit, immediately downstream of the promoter. The resulting *GAL1*pr::*GAL1(5′)-YAT1* transcription unit became sensitive to Spt16 depletion, as shown in Fig. 5B. We checked that, as expected, the inserted *GAL1* fragment is able to position two nucleosomes (Fig. 5B), confirming that even in a plasmid, both the promoter and the transcribed region of *GAL1* keep their chromatin organization. Again in this transcription unit, a low number of positioned nucleosomes seems to be sufficient to make transcription Spt16 dependent, at least when they are located at the 5′ end of the transcribed region. We conclude that FACT seems to be required for transcription of the DNA sequences immediately downstream of the initiation site whenever they are organized into translationally positioned nucleosomes.

Since FACT seemed to be necessary for transcription when the 5′ end of the transcribed region was occupied by positioned nucleosomes, we decided to investigate possible chromatin reorganizations at that region of the *GAL1* gene after transcription induction. As shown in Fig. 6A, the first three nucleosomes that were clearly positioned at the *GAL1* transcribed region when wild-type cells were grown in glucose (+2, +3, and +4) became relocated when cells were grown in galactose.

In agreement with previous studies (14), nucleosome +2, occupying the region where the preinitiation complex (PIC) gets assembled, was substituted in galactose by a broad hypersensitive region. In addition, the space corresponding to nucleosomes +3 and +4 was occupied by a pattern of protections and hypersensitivities that are compatible with a single positioned nucleosome in the middle and two smaller structures at both sides (Fig. 6A). Further studies would be needed to clarify this aspect. In any case, this experiment shows that, although the 5′ end of the *GAL1* transcribed region suffers drastic chromatin reorganization after activation, the nucleosomal distribution of this region in galactose is not random but shows at least one positioned nucleosome. To test whether this nucleosomal pattern was still present during Spt16 depletion, we analyzed the nucleosomal organization of *GAL1* in SJY6 cells growing in galactose plus doxycycline. Ten hours after doxycycline was added, the pattern of micrococcal nuclease cuts was very similar to that of the wild type in galactose (Fig. 6B). We conclude that *GAL1*, and likely other genes displaying positioned nucleosomes immediately downstream of the initiation site, keeps a positioned nucleosomal organization during transcription, which seems to require FACT to be transcribed.

DISCUSSION

Depletion of FACT affects transcription of genes differentially. Pol II cannot carry out productive transcription of DNA templates organized in chromatin, both at initiation and at elongation phases, in the absence of additional factors. In vitro transcription experiments have demonstrated the ability of FACT to stimulate transcription elongation of chromatin templates by Pol II (42) in cooperation with H2B monoubiquitination (45). In those in vitro experiments, the activity of FACT as a chromatin-dependent elongation factor was demonstrated by comparing its effects on naked DNA and chromatin. The same kind of experiment is not possible in vivo since cell DNA is always organized in chromatin. However, the features of chromatin organization vary throughout the genome. In animal cells, some genomic regions show a higher tendency to establish translationally positioned nucleosomes than others (66). In *Saccharomyces cerevisiae*, 65% of the genome shows translationally positioned nucleosomes; promoter-proximal sequences within coding regions show a higher tendency toward nucleosome positioning, but coding regions almost entirely covered by nonpositioned nucleosomes are also found (69). Following an in vivo depletion strategy, we have tested the consequences of FACT scarcity on the expression of several genes. In this kind of in vivo experiment, it is formally impossible to exclude the involvement of additional elements. However, the simultaneity between the decrease of Spt16 below the wild-type level and the documented effects on mRNA levels, transcription rates, and Pol II occupancies indicates that our results describe direct effects of FACT depletion on gene transcription.

We have shown here that FACT is required for the transcription of three coding regions driven by the *GAL1* promoter (*GAL1*, *PHO5*, and *LAC4*), but it is significantly less necessary for the transcription of two others also driven by the *GAL1* promoter (*YAT1* and *lacZ*). We first showed that mRNA levels of these five genes were differentially affected by Spt16 depletion. We then showed by ChIP that increased amounts of Pol II were associated to the 5′ end of the transcribed regions of *GAL1*, *GAL1*pr::*PHO5*, and *GAL1*pr::*LAC4* in response to Spt16 depletion, whereas no significant change in Pol II distribution was found in *GAL1*pr::lacZ or *GAL1*pr::*YAT1*. Furthermore, the results of run-on experiments also reflected lower densities of elongating Pol II after Spt16 depletion at *GAL1* and *GAL1*pr::*PHO5* than at *GAL1*pr::*YAT1*. The phenomenon described in this article is not restricted to genes driven by the *GAL1* promoter. We have shown that the expression of the native *SR09* gene is negatively affected by Spt16 depletion. In contrast, the expression levels of *CIT2*, *CUP1*, and *YAT1*, driven by their own promoters, were not. Altogether, our results indicate that the effects of FACT depletion on transcription are gene specific.

We have found a good correlation in the set of analyzed genes between the sensitivity to Spt16 depletion and the translational positioning of nucleosomes at the 5′ end of the coding regions. A relationship between translational positioning and nucleosome stability, shown in vitro by challenging reconstituted nucleosomes with high salt concentrations or temperature, has been observed elsewhere (16, 47). At least in some cases, the specific interactions between the DNA sequence and the histone octamer determine both positioning and stability (62, 67). This provides the simplest explanation for the connection between nucleosome positioning and Spt16-dependent transcription. We find it reasonable that the translationally positioned nucleosomes that we have detected at the coding regions of the studied genes, or at least a subset of them, are more reluctant to slide or to be transferred than those nucleosomes occupying nonpositioned genes. These stable nucleosomes would require the octamer disassembly-reassembly activity of FACT.

The connection between positioning and nucleosome stability is also supported by the phenotype of some histone mutants which show defects in nucleosome positioning in vivo (65) and an increased nucleosome mobility in vitro (15), due to alterations of the histone-DNA interactions on the surface of the nucleosome (40). We have used one of these histone mutants (*hhf2-13*) to test our hypothesis, and we found that the impairment of *GAL1* transcription after Spt16 depletion was clearly suppressed by *hhf2-13*, making *GAL1* expression insensitive to Spt16. It is the central DNA wrap of the nucleosome which is affected by *hhf2-13* (40). The same region of nucleosomal DNA is also perturbed by FACT action, according to the in vitro studies of FACT-nucleosome interaction (48). We find it full of sense, therefore, that *hhf2-13* suppresses the absence of FACT at a gene displaying positioned nucleosomes. It is theoretically possible that this suppression is not caused by the histone mutation itself but by the increase in histone dosage produced by the introduction of extra copies of the H3 and H4 coding genes (12). We do not favor this interpretation, since it has been shown that histone imbalance does not affect *GAL1* chromatin organization and does not derepress *GAL1* transcription (41). But even if this were true, the results of this experiment would support that the gene-specific effect of FACT depletion is mediated by chromatin.

The analysis of the nucleosomal organization of *GAL1* in glucose and in galactose indicates a deep reorganization of the 5′ end of the coding region after activation, in agreement with

the inverse correlation between histone-DNA interactions (measured by ChIP) and transcriptional activity reported for *GAL* genes (54). It is worth mentioning that the transcription-dependent changes in chromatin structure that we have detected at the *GAL1* coding region were not observed in a previous analysis of *GAL1* chromatin (11). The main difference between the two analyses was the genetic background. In the previous study, we used W303-derived strains, whereas in the present study, all strains were isogenic to BY4741. Further studies would be needed to clarify this striking difference. In any case, in both BY4741 and W303 cells grown in galactose, the 5′ end of the *GAL1* coding region is not nucleosome free. At least one positioned nucleosome is present during transcription in that region, suggesting that the reported decrease in histone occupancy of *GAL* genes during transcription (54) does not involve a random nucleosomal distribution.

Finally, an important piece of evidence connecting FACT and positioned nucleosomes at the 5′ end of the coding region comes from the insertion of two positioned nucleosomes between the promoter and the transcribed region of the Spt16-independent *GAL1*pr::*YAT1* transcription unit. The resulting *GAL1*pr::*GAL1(5′)-YAT1* became sensitive to Spt16 depletion. Altogether, our results suggest that FACT is required for the transcription of those genes whose transcribed region is organized into positioned nucleosomes at the 5′ end.

It has been shown that FACT plays a role in preventing the activation of cryptic initiation sites by contributing to the proper reposition of nucleosomes after the passage of elongating Pol II (26, 36). We have indeed shown here the slight activation of a cryptic initiation site present in *FLO8* 12 h after adding doxycycline. However, 8 or 10 h after doxycycline was added, times chosen for the functional analyses of this study, the cryptic initiation site present in *FLO8* was not active yet, and the nucleosomal organization of *GAL1* was similar to that of the wild type. We have shown that the negative effect of Spt16 shortage on the accumulation of *GAL1*, *PHO5*, and *LAC4* mRNAs was not due to the activation of cryptic initiation sites within their coding regions. Moreover, the results of the run-on experiments show a lower density of elongating Pol II at *PHO5* and *GAL1* and do not support secondary transcripts emerging in these genes. Mason and Struhl (36) suggested that the overall negative effect of Spt16 depletion on transcription might be due to a competition between normal promoters and cryptic initiation sites for the transcriptional machinery. According to this hypothesis, the higher number of initiation sites originated in the cell by the depletion of Spt16 might affect the *GAL1* promoter due to a subsequent scarcity of general transcription factors. Since the five genes driven by the *GAL1* promoter do not behave the same, we can also exclude this explanation for the phenomenon described here, unless the sequences located downstream differentially affect the activity of the promoter (see below).

FACT has also been involved in PIC assembly by facilitating TATA-binding protein binding to the TATA box in the context of a nucleosome. According to this, FACT depletion might also affect transcription initiation in a promoter-specific manner. However, it is difficult to explain all of the results shown in this work in terms of transcription initiation. We have shown here that several transcription units driven by the same promoter (*GAL1*pr) exhibit different degrees of sensitivity to Spt16 depletion. The diverse nucleosomal distributions at the coding regions might differentially affect the chromatin organization of the *GAL1* promoter. However, we did not find such differences at the nucleosomal mapping that we have carried out, although subtle differences cannot be completely ruled out. We would then expect a decrease in Pol II recruitment. In contrast, we have found accumulation of Pol II at the 5′ end of the Spt16-dependent genes. We cannot exclude that a part of this accumulation corresponds to initiating Pol II due to the inherent inaccuracy of the ChIP technique, but in that case, the results would be compatible with a role of FACT in the transition from initiation to elongation and not in PIC assembly.

Although we cannot completely rule out an initiation component, the simplest interpretation of our results suggests that an involvement of FACT in transcription elongation may be immediately after initiation has occurred. According to this perspective, FACT would be required for facilitating transcription through those nucleosomes less prompt to slide or to be transferred. In the absence of FACT, Pol II would pause in front of such nucleosomes and would eventually become arrested. The comparison of the patterns of Pol II distribution after Spt16 depletion, obtained by ChIP and by run-on, detects a difference: in *GAL1* and *GAL1*pr::*PHO5*, the amounts of immunoprecipitated Pol II located at 5′ are higher than in the rest of the gene; in contrast, the densities of active Pol II are roughly similar at the 5′ and 3′ ends of both genes. The simplest explanation for this phenomenon would be that the excess of Pol II present at 5′ became arrested after suffering backtracking and therefore was undetectable by a run-on assay. This hypothesis is also in agreement with published results showing how nucleosomes induce Pol II arrest in vitro by stabilizing its backtracked conformation (27).

In *Drosophila* polytene chromosomes, Saunders et al. (51) have shown that FACT is not recruited to RNA polymerase III-dependent genes, which are known to undergo nucleosome transfer rather than disassembly during in vitro transcription elongation. It may be possible that FACT-dependent nucleosome disassembly/reassembly would be required only by Pol II to transcribe positioned nucleosomes, whereas those nucleosomes not exhibiting a fixed translational positioning might be more likely to transfer or slide during transcription elongation. We do not have data to distinguish which of the two proposed functions of FACT, disassembly or reassembly, is critical for transcription of *GAL1* and the other Spt16-dependent genes studied here. However, if we consider positioning as an indication of nucleosome stability, as discussed before, it seems more likely that transcription elongation of highly organized chromatin requires the nucleosome disassembly activity of FACT. If this interpretation is true, an explanation must be provided for the predominant requirement for FACT at the 5′ end of the coding regions. Either nucleosomes positioned at these regions are particularly stable, or the capability of Pol II machinery to interact with nucleosomes changes along the transcribed region by including perhaps other histone chaperones such as Asf1, also acting during transcription elongation (53). Alternatively, the accumulation of positive DNA supercoiling ahead of Pol II might facilitate nucleosome reorganization once genes have been transcribed to some extent, as it has been shown elsewhere (31), reducing the FACT requirement at these regions.

Does every gene require its own menu of factors after transcription initiation? Biochemical and genetic analyses during the last 15 years have described a numerous set of factors playing auxiliary roles in Pol II-dependent mRNA biogenesis after transcription initiation, favoring mainly processivity (35). However, little is known about the relative importance of each of these factors in terms of the number of genes that requires their function. Since they measure the combination of initiation, elongation, and mRNA stability, global transcriptome analyses have not been very useful in this respect. In this work, by comparing five genes under the control of the same promoter, we have shown that FACT is not equally required for all genes during transcription. It was recently reported that, although recruited to the transcribed region of the human p21 gene in a carboxyl-terminal domain (CTD) kinase-dependent manner when it becomes activated by p53, FACT is dispensable for p21 expression (21). In fact, p21 transcription does not require CTD phosphorylation at Ser2, indicating that the requirement of P-TEFb for transcription elongation is also gene specific (21). It is worth mentioning that *CUP1*, one of the genes whose expression is not affected by Spt16 depletion, can be transcribed by a mutant version of Pol II that lacks the CTD (37). Altogether, these elements suggest a relationship between the requirements for CTD phosphorylation and FACT. In this respect, it would be interesting to analyze the nucleosomal organization of p21 and other possible P-TEFb- and FACT-independent mammalian genes.

By using the same five transcription units driven by the *GAL1* promoter that have been analyzed in this work, we have shown elsewhere that the THO complex, involved in the connection between transcription elongation and mRNA transport, is also not uniformly needed for all of them (11). It is interesting that those transcription units whose elongation is highly dependent on FACT are not strongly affected by *tho* mutations; this is the case for *GAL1*, *GAL1*pr::*PHO5*, and *GAL1*pr::*LAC4*. However, *GAL1*pr::*lacZ* and *GAL1*pr::*YAT1*, dramatically affected by *tho* mutants, are only mildly affected by Spt16 depletion. According to the chromatin analysis presented here, the THO complex seems to be specially needed at genes with random chromatin organization. Since THO plays a role in preventing the formation of R loops by nascent mRNA (23), a contribution of positioned nucleosomes in preventing R loops can be suggested.

Another gene-specific factor involved in postinitiation events is TFIIS, an elongation factor dispensable for the expression of most genes, which plays a capital role in transcriptional activation of *Drosophila hsp70*. It does so by releasing promoter-proximal paused Pol II from arrest (1). Pol II pausing in *hsp70* at the transcription elongation step seems to be influenced by the nucleosomal organization of the promoter-proximal region (8, 57). Considering FACT, P-TEFb, THO, and TFIIS, the emerging picture is that the intrinsic properties of the transcribed region of a given gene determine the set of factors required for its proper mRNA biogenesis.

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