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Preferential Accessibility of the Yeast *his3* Promoter Is Determined by a General Property of the DNA Sequence, Not by Specific Elements

XUHONG MAI, SUSANNA CHOU, AND KEVIN STRUHL*

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, Massachusetts 02115

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Yeast promoter regions are often more accessible to nuclear proteins than are nonpromoter regions. As assayed by *Hin*fI endonuclease cleavage in living yeast cells, *Hin*fI sites located in the promoters of all seven genes tested were 5- to 20-fold more accessible than sites in adjacent nonpromoter regions. *Hin*fI hypersensitivity within the *his3* promoter region is locally determined, since it was observed when this region was translocated to the middle of the *ade2* structural gene. Detailed analysis of the *his3* promoter indicated that preferential accessibility is not determined by specific elements such as the Gcn4 binding site, poly(dA-dT) sequences, TATA elements, or initiator elements or by transcriptional activity. However, progressive deletion of the promoter region in either direction resulted in a progressive loss of *Hin*fI accessibility. Preferential accessibility is independent of the Swi-Snf chromatin remodeling complex, Gcn5 histone acetylase complexes Ada and SAGA, and Rad6, which ubiquitinates histone H2B. These results suggest that preferential accessibility of the *his3* (and presumably other) promoter regions is determined by a general property of the DNA sequence (e.g., base composition or a related feature) rather than by defined sequence elements. The organization of the compact yeast genome into inherently distinct promoter and nonpromoter regions may ensure that transcription factors bind preferentially to appropriate sites in promoters rather than to the excess of irrelevant but equally high-affinity sites in nonpromoter regions.

In eukaryotic organisms, nucleosomes restrict access of activator proteins, TATA-binding protein (TBP), and the RNA polymerase II machinery to genomic DNA (9, 43). For purposes of economy and specificity, it is desirable for promoter regions to be preferentially accessible in comparison to nonpromoter regions. For example, the yeast genome contains approximately 6,000 Gcn4 binding sites, as defined by sequences with no more than one deviation from the optimal sequence RTGACTCAY (32), and they occur predominantly within structural genes. Because yeast cells contain considerably fewer than 6,000 Gcn4 molecules, and because binding of Gcn4 to many inappropriate sites is likely to be biologically catastrophic, the cell must possess a mechanism by which transcriptionally irrelevant Gcn4 binding sites are made relatively inaccessible in comparison to sites in Gcn4-dependent promoters.

Chromatin structure can be modified by nucleosome-remodeling complexes such as Swi-Snf and by histone acetylation (10, 49). Such perturbations increase access of proteins to nucleosomal templates in vitro, and they are important for transcription of many yeast genes. In some cases, chromatin remodeling is an activator-dependent event that is separate from the activation of transcription itself (1, 7, 29, 46). The Swi-Snf complex modifies the chromatin structure of the *SUC2* promoter region in a manner independent of transcriptional activity of the gene (12). Individual Reb1 or Cpf1 binding sites can affect chromatin structure, even though they support low levels of transcriptional activity (8, 18). Gcn4-dependent activation of the *his3* promoter is associated with localized histone acetylation mediated by Gcn5 histone acetylase (21), and targeted recruitment of the Sin3-Rpd3 histone deacetylase complex causes a highly localized domain of repressed chromatin structure (16, 17, 36). In these situations, it is demonstrated or presumed that proteins binding to specific promoter elements alter chromatin structure by recruiting nucleosome-modifying activities (42).

Several observations strongly suggest that in addition to undergoing chromatin changes mediated by DNA-binding activators and repressors, promoter regions are generally more accessible to nuclear proteins than are nonpromoter regions. First, yeast promoters often contain transcription-independent regions of nuclease hypersensitivity (6, 7, 18, 23, 25, 27, 33, 39, 46). Second, in several of these studies, micrococcal-nuclease mapping suggests that hypersensitivity reflects "nucleosome-free" regions, although it is unclear whether these regions are truly devoid of nucleosomes or have an alternative structure. Third, the Ty1 retrotransposon preferentially integrates in promoter regions rather than in protein coding sequences (5, 31, 47). Expression-independent hypersensitive sites in the *GAL1,10* and *HSP82* promoters are not determined by activator binding sites or TATA elements (23, 39), but the determinants that specify preferential accessibility of promoter regions in chromatin are unknown.

One sequence element that might cause a predisposition to promoter accessibility is poly(dA-dT), which is found broadly in yeast promoter regions and is required for wild-type transcriptional levels of many genes (40). Poly(dA-dT) is an unusual promoter element whose function depends on its intrinsic structure, not its interaction with activator proteins (15). Furthermore, poly(dA-dT) alters chromatin structure and increases protein accessibility over a distance that corresponds to an individual nucleosome (15, 50). However, the increased protein accessibility due to poly(dA-dT) sequences is quantitatively subtle, suggesting that these sequences may not be

* Corresponding author. Mailing address: Dept. of Biological Chemistry and Molecular Pharmacology, Harvard University, Boston, MA 02115. Phone: (617) 432-2104. Fax: (617) 432-2529. E-mail: kevin@hms.harvard.edu.

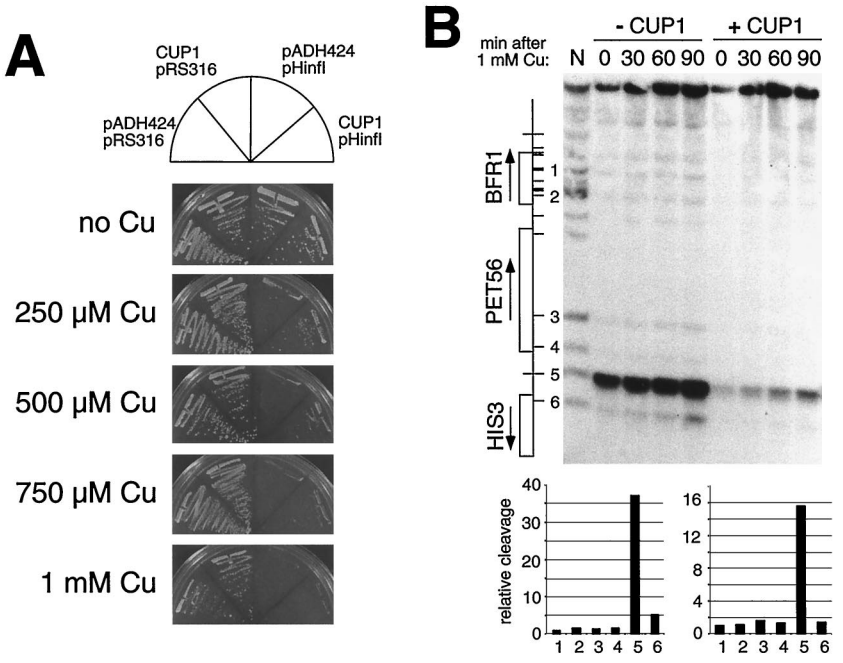

FIG. 1. Growth phenotypes and *Hin*fI cleavage in vivo in yeast strains containing p*Hin*fI. (A) Strain ySH104 transformed with the indicated plasmids was grown on synthetic complete solid medium lacking uracil and tryptophan and containing the indicated concentrations of $CuSO_4$. (B) Southern blots of genomic DNAs from strain ySH104, transformed with p*Hin*fI and either vector or the *CUP1*-overexpressing plasmid pSH212, grown in selective liquid medium, and induced with 1 mM $CuSO_4$ for the indicated time periods. As a control, genomic DNA from untransformed ySH104 cells was also partially digested with *Hin*fI in vitro (N). Open boxes indicate coding regions, arrows indicate the 5′-to-3′ orientation of genes, long transecting lines indicate Gcn4 consensus or near-consensus binding sites, and short lines indicate other *Hin*fI restriction sites. Relative *Hin*fI cleavage at the numbered genomic sites was calculated based on quantitation of band intensity by using ImageGauge (Fujimax) and normalized to the lowest-intensity band.

sufficient to distinguish promoter regions from nonpromoter regions.

In previous work, we developed a novel probe of chromatin structure involving the rapid induction of *Hin*fI endonuclease in yeast cells (15). In this approach, chromatin structure is determined in living cells under physiological conditions, in contrast to typical analyses that are performed on isolated nuclei. Moreover, because the endonuclease is expressed for only a short period of time prior to harvesting of cells, the results provide a snapshot of chromatin structure rather than an average steady-state structure as occurs in assays involving constitutively expressed methylases (19). In the present study, we utilized *Hin*fI cleavage in vivo to analyze the chromatin structure of multiple genomic regions, the DNA sequence determinants of selective accessibility of the *his3* promoter, and the effect of chromatin-modifying activities on the preferential accessibility of the *his3* promoter. Our results strongly suggest a novel mechanism of preferential accessibility that does not involve specific sequence elements but rather an overall structural characteristic(s) common to promoter regions.

MATERIALS AND METHODS

DNAs and strains. The *his3* alleles used in this work (see Fig. 3) were subcloned as *Sph*I-*Kpn*I or *Eco*RI-*Kpn*I fragments into Sc7321, an allele lacking the sequence between nucleotides −447 and −105 and containing an optimal Gcn4 site flanked by *Eco*RI sites adjacent to a short polylinker distal to the TATA region (14). These alleles have been described previously as follows: internal deletions of the *his3-pet56* divergent promoter region (Sc2883, Sc2884, Sc3121, Sc3110, Sc3621, Sc3268, and Sc3619) (reference 38 and unpublished data), derivatives lacking *his3* sequence between positions −447 and −105 and containing perturbations of the *his3* TATA region (14), and the *his3-151* and *his3-161* alleles, which contain modifications near the Gcn4 binding site (11). The *his3Δp* allele was derived from *his3-161* by replacing the *Sac*I-*Kpn*I fragment with a PCR-amplified product that contains a *Sac*I site at nucleotide +30 (+6 relative to start codon). Additional *his3* alleles (see Fig. 5) were generated by subcloning the desired *Sph*I-*Eco*RI, *Sph*I-*Sac*I, or *Sac*I-*Kpn*I fragments generated by PCR into the *his3*Δp allele. Yeast strains were generated by introducing the various *his3* derivatives by gene replacement into the normal chromosomal locus of ySH103 (*his3-Δ200 ura3-52 trp1-Δ161 lys2-Δ202 leu2-Δ1::PET56 gcn4Δ1*), a *gcn4-Δ1 leu2-Δ1::PET56* derivative of FY833 (48).

The *ade2::his3p* allele, which contains an insertion of the *his3* promoter region (nucleotides −120 to +30) between nucleotides 900 and 901 relative to the *ade2* start codon, was generated by subcloning three PCR fragments (an *Eco*RI site was generated at the border of +900 of *ade2* and −120 of *his3*, and a *Sac*I site was generated at the border of +30 of *his3* and +901 of *ade2*). The resulting allele was introduced into the *ade2* chromosomal locus of strain ySH103 by two-step gene replacement. The *rad6Δ::LEU2* molecule was generated by replacing the *rad6* coding region (between *Eco*RI sites at nucleotides −49 and +2715 relative to the start codon) with the *leu2* gene. The *ahc1Δ::hisG* allele, which replaces the entire *ahc1* coding region with the *Escherichia coli hisG* gene, was generated by cloning two PCR fragments of *ahc1* (a *Xho*I-*Bam*HI fragment containing nucleotides −655 to −100 relative to the start codon and a *Spe*I-*Xba*I fragment containing nucleotides +1805 to +2504 of *ahc1* downstream sequences) into the corresponding sites of pBS*hisG* (kindly provided by Jutta Deckert). DNAs encoding the *snf2Δ::LEU2* and *gcn5Δ::LEU2* alleles were kindly provided by Fred Winston. Strains containing the above-described *rad6*, *ahc1*, *snf2*, and *gcn5* alleles were generated by gene replacement of strain BY105 (*ura3-52 trp1-Δ161 lys2-Δ202 gcn4-Δ1*), which was kindly provided by Mark Benson.

To generate p*Hin*fI, the plasmid permitting copper-inducible expression of *Hin*fI endonuclease, the *Hin*fI coding sequence was amplified by PCR (sequence information kindly provided by Keith Lunnen) and subcloned into a *URA3* centromeric plasmid under the control of an Ace1-dependent *his3* promoter (20). To overexpress the copper metallothionein gene for sequestration of trace cupric ion, the *CUP1* coding sequence was amplified by PCR and subcloned into p424-*ADH1*, a *TRP1* 2μm plasmid containing the strong *ADH1* promoter (30), to generate pSH212.

Induction of *Hin*fI cleavage in vivo. Yeast strains containing p*Hin*fI without pSH212 were grown in synthetic complete medium lacking uracil to an A_{600} of 0.3 to 0.6. *Hin*fI expression was induced by the addition of $CuSO_4$ to a final concentration of 1 mM. Strains containing p*Hin*fI and pSH212 were generated by transforming the parent strains with pSH212, purifying colonies on selective synthetic complete medium, and then freshly transforming them with p*Hin*fI. Strains were freshly transformed with p*Hin*fI and utilized immediately for each experiment because p*Hin*fI-containing strains grow slowly and are prone to genetic alterations that eliminate endonuclease activity. The doubly transformed

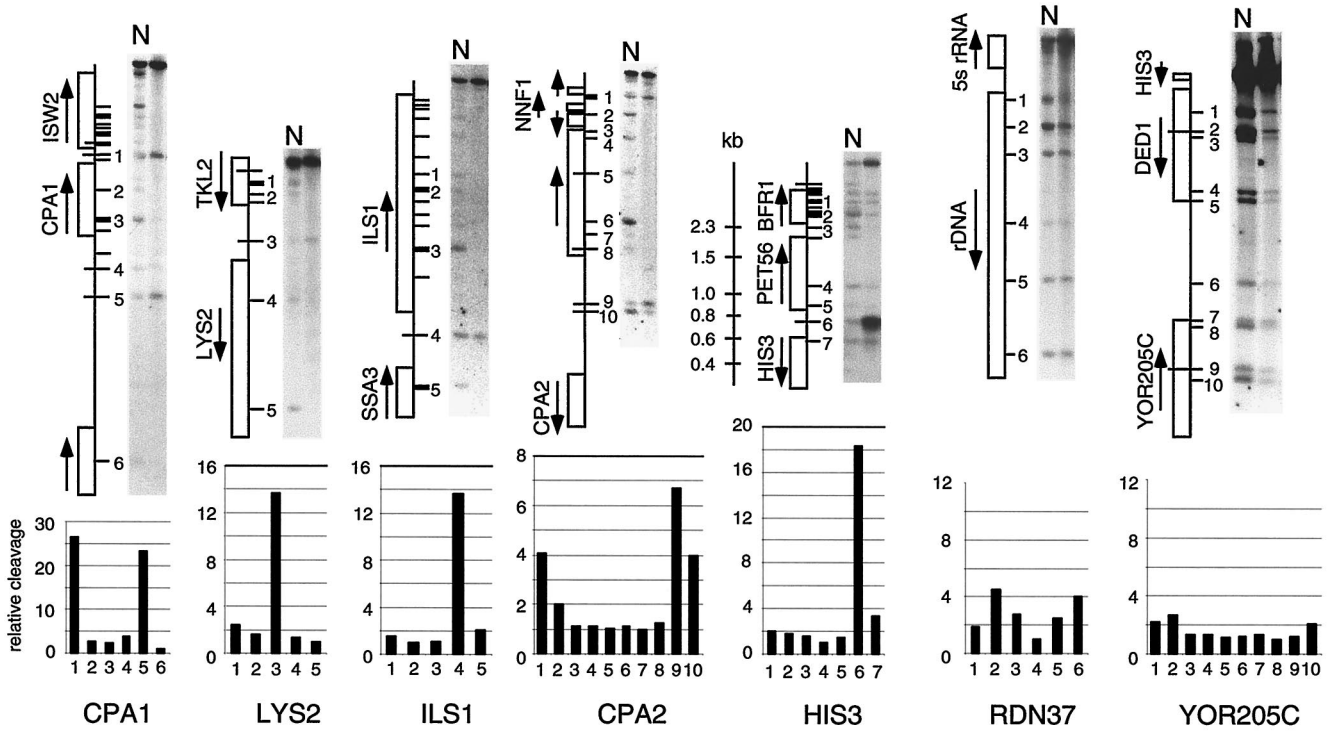

FIG. 2. *Hin*fI cleaves preferentially in promoter regions in vivo. Gcn4-dependent genes (CPA1, LYS2, ILS1, CPA2, and HIS3), Gcn4-independent genes (RDN37 and YOR205C), and adjacent genomic regions were analyzed. Genomic DNA was prepared from strain ySH104, containing pSH212 and p*Hin*fI, that was induced with 1.5 mM $CuSO_4$ for 90 min, and the DNA was hybridized to the indicated probes. As a control, genomic DNA from untransformed ySH104 cells was also partially digested with *Hin*fI in vitro (N). Schematics are as described in the legend to Fig. 1B. Relative *Hin*fI cleavage at the numbered genomic sites was calculated based on quantitation of band intensity by using ImageGauge (Fujimax) and normalized to the lowest-intensity band.

strains were grown in synthetic complete medium lacking uracil and tryptophan to an A_{600} of 0.3 to 0.6, and *Hin*fI expression was induced by the addition of $CuSO_4$ to a final concentration of 1.5 mM. The increased level of $CuSO_4$ was based on the difference in copper sensitivity observed between p*Hin*fI-bearing strains with and without pSH212. In both cases, 50-ml aliquots of cells were harvested for preparation of genomic DNA at various time points (up to 90 min) after induction of *Hin*fI expression with copper.

Southern blot analysis. Genomic DNAs (2 μg, as estimated by agarose gel electrophoresis) from copper-induced cells were digested to completion with *Kpn*I, *Eco*RV, or *Psh*AI. Southern blot analysis was performed by standard procedures, with fractionation of DNA on a 1.5% agarose gel and hybridization to randomly primed ^{32}P-labeled probes. Control genomic DNAs were also isolated from the isogenic strains lacking the p*Hin*fI plasmid; digested to completion with *Kpn*I, *Eco*RV, or *Psh*AI; and then partially digested with *Hin*fI (2 U for 5 min at 37°C). The extent of relative *Hin*fI cleavage at various sites was quantitated by phosphorimager analysis using ImageGauge software (Fujix) and normalized to *Hin*fI cleavage in adjacent genomic sites or the rRNA gene (rDNA) locus. The efficiency of *Hin*fI cleavage in vivo at the Gcn4 binding site within the *his3* promoter (i.e., the hypersensitive site) was 1%. DNA probes were generated by PCR amplification or restriction enzyme digestion of the following gene fragments (relative to ATG): *CPA1* (nucleotides −1336 to −1144 for the *Kpn*I blot), *CPA2* (+475 to +373 for the *Eco*RV blot), *HIS3* (+495 to +305 for the *Kpn*I blot), *ILS1* (−758 to −576 for the *Eco*RV blot), *LYS2* (+1141 to +774 for the *Eco*RV blot), rDNA (+1378 to +948 for the *Kpn*I blot), YOR205C (+1084 to +1429 for the *Kpn*I blot), and *ADE2* (+901 to +1302 for the *Psh*AI blot).

RESULTS

The Gcn4 binding site in the *his3* promoter is hypersensitive to *Hin*fI cleavage in vivo. The *Hin*fI recognition sequence, GANTC, is contained within the core of the consensus Gcn4 binding site (RTGACTCAY) (11, 32). Thus, Gcn4 binding sites in promoter and nonpromoter regions are a subset of all *Hin*fI sites, and the extent to which they are cleaved provides an assay of their relative accessibility in vivo. The parent yeast strain for all strains used in these experiments contained a *gcn4* deletion so that effects of Gcn4 binding would not confound the analysis.

Yeast cells transformed with the inducible *Hin*fI expression plasmid grew normally on medium lacking copper, but they displayed a slow-growth phenotype on medium containing 250 μM $CuSO_4$ and were inviable on medium containing 500 μM $CuSO_4$ (Fig. 1A), presumably due to increased *Hin*fI activity. Southern blot analysis of genomic DNA isolated after induction of early-log-phase cells with 1 mM $CuSO_4$ (Fig. 1B) showed *Hin*fI hypersensitivity of the Gcn4 binding site in the *his3* promoter, as noted previously (15). However, in this strain background, there was significant *Hin*fI cleavage prior to induction by copper (this was also observed to some extent in the strain previously tested). This problem could be minimized by overexpressing the *CUP1* metallothionein gene in order to chelate trace amounts of copper and suppress *Hin*fI expression in the absence of exogenous copper. The resulting strain grew slowly in the presence of 750 μM $CuSO_4$ and became inviable in medium containing 1 mM $CuSO_4$, and genomic analysis revealed that there was little *Hin*fI cleavage prior to addition of copper. To amplify *Hin*fI cleavage, cells were induced with 1.5 mM $CuSO_4$, which enhanced the signal without qualitatively changing the cleavage pattern (see Fig. 2).

The *Hin*fI site corresponding to the Gcn4 binding site in the wild-type *HIS3* promoter was cleaved approximately 10- to 20-fold more efficiently than any of the neighboring 13 sites located 0.2 to 1 kb away in the *his3* and *pet56* coding regions (Fig. 1B and 2). This *Hin*fI hypersensitivity clearly reflects some aspect of chromatin structure, because the same *Hin*fI sites in purified DNA were cleaved with equal efficiency. Furthermore, *Hin*fI hypersensitivity occurred in a region with in-

creased accessibility to other probes of chromatin structure (15, 27, 38, 45).

Differential *Hin*fI sensitivity at Gcn4 binding sites in promoter versus nonpromoter regions. To extend these observations, we examined the *Hin*fI sensitivity patterns of several Gcn4-activated genes (*HIS3*, *ILS1*, *LYS2*, *CPA1*, and *CPA2*), all of which contain Gcn4 binding sites 100 to 500 bp upstream of the start codon. Some of these genes contain consensus or near-consensus Gcn4 sites within their own or neighboring coding regions. The *Hin*fI sensitivity of these Gcn4 sites, transcriptionally relevant and irrelevant, can thus be directly compared in a single experiment. We also tested two Gcn4-independent genes, *DED1* and *YOR205C*, each of which contains a consensus Gcn4 binding site within its coding region. As additional internal controls for *Hin*fI cleavage in vivo, we examined sites within nonpromoter regions of the rRNA precursor and 5S RNA locus, which occur as 100 to 200 tandem copies.

All of the promoter regions tested showed strikingly enhanced (5- to 20-fold) *Hin*fI cleavage at Gcn4 binding sites compared to neighboring *Hin*fI sites in nonpromoter regions (Fig. 2). An additional region of *Hin*fI hypersensitivity was observed downstream of the *cpa1* gene; it corresponds to sites (including one near-consensus Gcn4 site) immediately upstream of the *isw2* coding region. Furthermore, a *Hin*fI site which is not a Gcn4 site in the distal *cpa1* promoter was also preferentially cleaved, although less strongly (fourfold). Conversely, *Hin*fI cleavage at Gcn4 sites in nonpromoter regions in *ded1* and *YOR205C* was comparable to that of other *Hin*fI sites in nonpromoter regions (Fig. 2), indicating that consensus Gcn4 sites do not, per se, confer *Hin*fI hypersensitivity. Thus, *Hin*fI cleavage in promoter regions is generally increased relative to that in adjoining genomic sites, confirming that increased accessibility is a common characteristic of promoter regions.

***Hin*fI hypersensitivity of the Gcn4 binding site in the *his3* promoter is not determined by any of the previously defined promoter elements.** Detailed mutational analysis of the *his3* promoter region has identified the following promoter elements: initiator elements that specify the +1 and +13 mRNA start sites (2); a consensus TATA element (nucleotides −45 to −40), T_R, that is responsible for +13 transcription (3); a collection of nonconsensus TATA elements (nucleotides −80 to −53), T_C, that is responsible for +1 initiation (14, 28); a Gcn4 binding site located between nucleotides −100 and −91 and an adjacent tract of 9 dA-dT residues (11); a poly(dA-dT) element (nucleotides −130 to −115) that is important for Gcn4-independent transcription of *his3* as well as the divergently transcribed *pet56* (15, 40); and a poorly characterized sequence around nucleotide −140 which makes a minor contribution to maximal induced levels of transcription (44). In addition, there is a nonconsensus TATA element (nucleotide −150) that is responsible for *pet56* transcription, which is initiated in the direction opposite from a position 191 bp upstream of *his3* +1 (40).

To determine which, if any, of these promoter elements are important for the *Hin*fI hypersensitivity of the Gcn4 binding site, we examined a set of promoter derivatives whose transcriptional properties had been previously characterized. In one set of derivatives (Fig. 3A), sequences upstream of an optimal Gcn4 site were deleted and the TATA region was perturbed in a variety of ways (14). In these cases, the *pet56* promoter and the initial part of the *pet56* structural gene were deleted, and the level of *his3* transcription was extremely low due to the absence of Gcn4 and (in some cases) because of poorly functioning TATA elements. Another set of derivatives (Fig. 3B) lacked the *pet56* promoter region and contained

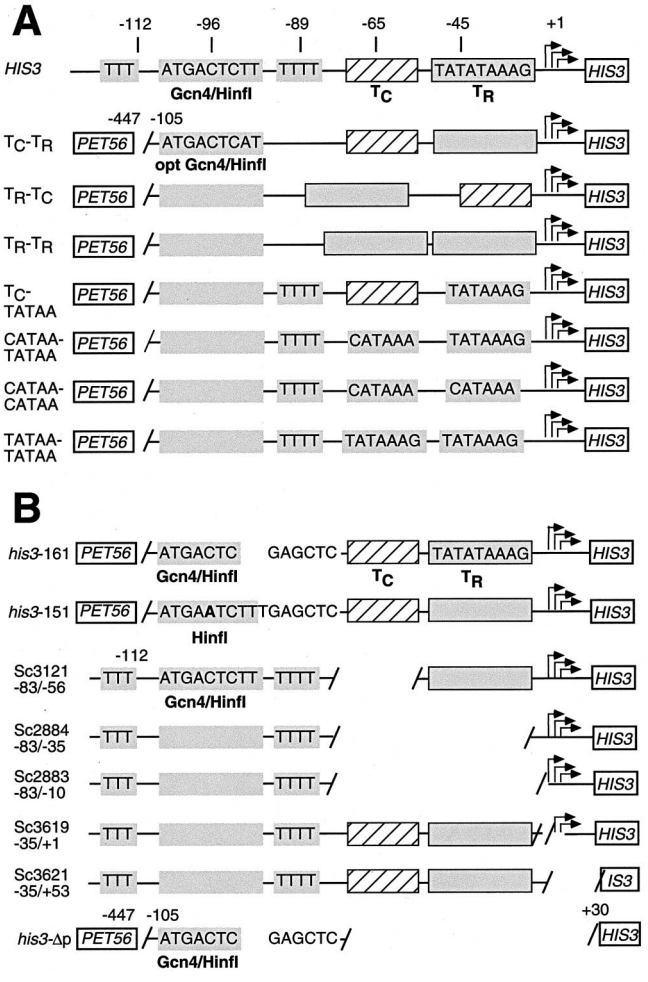

FIG. 3. Structures of *his3* promoter derivatives. (A) *his3* promoter derivatives containing an optimal Gcn4 site with various TATA element combinations in addition to a deletion of the *pet56* promoter (upstream deletion) (14). (B) *his3* promoter derivatives containing a deletion of the *pet56* promoter (upstream deletion), the short proximal T tract (*his3-161*), or a nonfunctional Gcn4 site in addition to *his3-151* (11); derivatives containing wild-type upstream sequence of the *pet56* promoter and deletions of various portions of the core promoter region (38; K. Struhl, unpublished data); and the *his3-Δp* derivative constructed here. Arrows represent transcriptional start sites, open boxes indicate the *HIS3* coding region, and shaded boxes represent promoter elements as indicated. Numbering is relative to the +1 transcriptional start site of *HIS3*. The drawing is not to scale.

mutations that inactivated the Gcn4 binding site (but not the *Hin*fI recognition sequence) and the short dA-dT tract downstream (11). We also examined derivatives with variously sized internal deletions that removed one or more of the *his3* promoter elements described above, although the *pet56* promoter remained essentially intact (2, 38). Finally, a double deletion removing the entire *his3-pet56* promoter region, such that the Gcn4 binding site was flanked by portions of the respective protein coding regions, was generated. In all experiments, *Hin*fI cleavage at the *his3* promoter and flanking regions was normalized to the averaged cleavage at neighboring *Hin*fI sites in the *his3-pet56* locus and also compared to cleavage at sites within in the rDNA locus.

Surprisingly, all of the extensively modified derivatives that had retained some promoter sequence showed *Hin*fI hypersensitivity at the Gcn4 binding site at a level comparable to that observed in the wild-type locus (Fig. 4). The preservation of

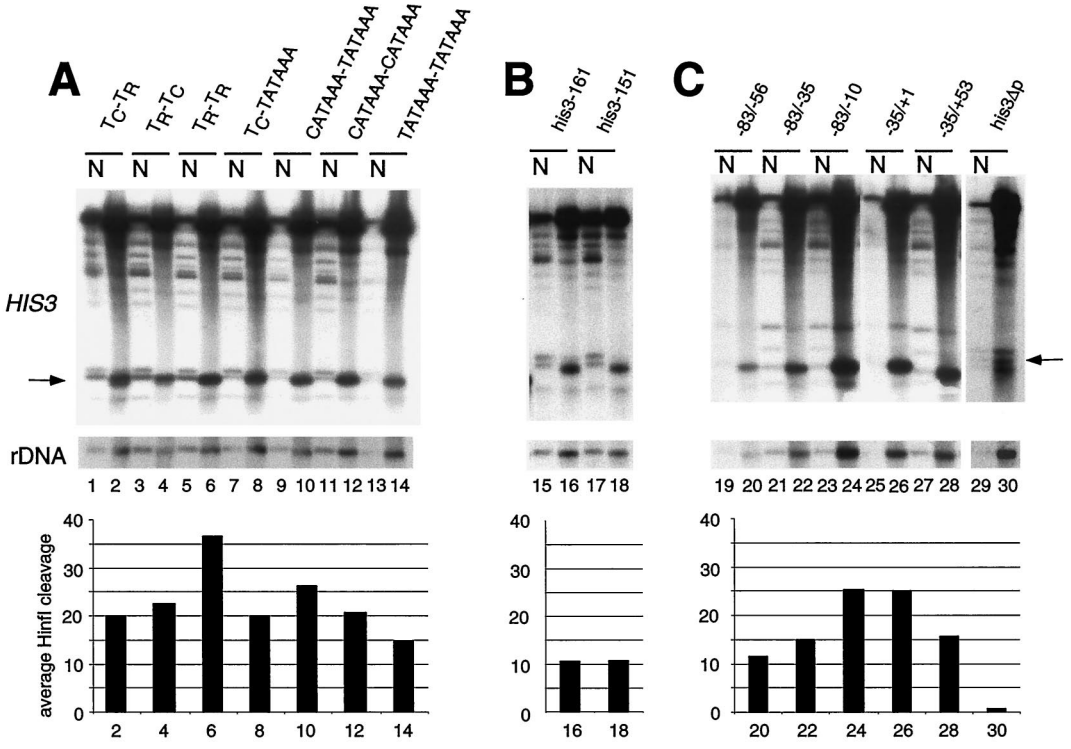

FIG. 4. *Hin*fI hypersensitivity at the *his3* Gcn4 binding site is unaffected by any of the previously defined promoter elements. Shown are Southern blots of DNA subjected to in vivo *Hin*fI cleavage at *his3* promoter derivatives with TATA element combinations (see Fig. 3A) and rDNA controls (A), *his3* promoter deletions (see Fig. 4B) (B), and *his3* alleles −161 (proximal T tract deletion) and −151 (nonfunctional Gcn4 site) (see panel B) (C). Genomic DNA was prepared from the corresponding strains, containing pSH212 and p*Hin*fI, that were induced with 1.5 mM $CuSO_4$ for 90 min, and the DNA was hybridized to the indicated probes. As a control, genomic DNA from untransformed cells was also partially digested with *Hin*fI in vitro (N). *Hin*fI cleavage at the Gcn4 binding site (indicated with arrows) and five adjacent genomic sites was quantitated by phosphorimager analysis (Fujimax) and normalized to the lowest-intensity site, and the averaged cleavage of the five adjacent *Hin*fI sites was used to calculate the relative *Hin*fI preference for the Gcn4 binding site (averaged *Hin*fI preference). The standard deviation of normalized cleavage at the adjacent sites ranged between 0.6 and 1.4.

the hypersensitive site is thus independent of poly(dA-dT) sequences, a functional Gcn4 binding site, functional TATA or initiator elements, and transcription of the *his3* and/or *pet56* gene. However, hypersensitivity was abolished in the derivative in which the Gcn4 binding site was immediately flanked on both sides by protein coding sequences. These observations argue that preferential accessibility of the Gcn4 binding site depends on the presence of a promoter region, but not on transcriptional activity per se or a specific sequence element.

Progressive deletion of the *his3* promoter region in either direction causes a progressive loss of *Hin*fI accessibility. All of above-described derivatives that displayed normal *Hin*fI hypersensitivity contained either the intact *his3* or *pet56* promoter region, leaving open the possibility that preferential accessibility is due to redundant elements in these two promoters. To investigate this possibility and to determine the minimal region necessary to confer *Hin*fI hypersensitivity, we analyzed additional strains in which one promoter region was removed and the other promoter region was successively deleted (Fig. 5). In the first set of derivatives (Fig. 5A), *his3* sequences downstream of the Gcn4 site were deleted and the *pet56* promoter region was successively deleted from Gcn4 binding site. The second set of derivatives (Fig. 5B) lacked *pet56* promoter sequences upstream of the Gcn4 site and contained a successively deleted *his3* promoter region (with deletions starting from the downstream end). The third set of derivatives (Fig. 5C) was comparable to the second set, except that the *his3* deletion series started from the upstream end. Analysis of genomic DNAs purified from these strains indicated that all *Hin*fI sites were cleaved to comparable extents (Fig. 6).

Analysis of *Hin*fI cleavage in vivo indicated that the *his3* region is considerably more important than the *pet56* region with respect to preferential accessibility. *Hin*fI hypersensitivity at the Gcn4 binding site was drastically reduced in all cases in which the *his3* promoter region was completely deleted (Fig. 7A), indicating that the *pet56* promoter region is insufficient to confer preferentially accessibility. However, the *pet56* region contributes to accessibility, because the least-deleted derivatives showed approximately threefold-higher cleavage of the Gcn4 site. In contrast, several deletion mutants lacking the entire *pet56* region displayed preferential *Hin*fI cleavage that was comparable to that of the wild-type promoter.

The most interesting observation was that in the absence of the *pet56* promoter region, successive deletion of the *his3* promoter region from either direction resulted in a progressive loss of *Hin*fI hypersensitivity (Fig. 7B and C). As a consequence, nonoverlapping segments of the *his3* promoter region conferred equivalent levels of *Hin*fI hypersensitivity, and preferential accessibility was related to the length of the *his3* promoter region. This observation indicates that there are multiple determinants of preferential accessibility within the *his3* promoter. The present deletion analysis suggests that there are at least five such determinants that contribute in a cumulative (although not necessarily a quantitatively equivalent) fashion. The observation that progressive deletion in either direction

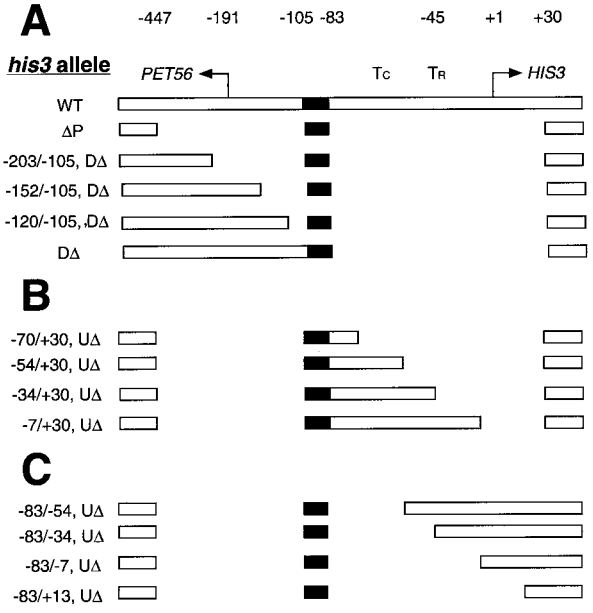

FIG. 5. Structures of *his3* promoter derivatives. (A) *his3* promoter derivatives containing a downstream deletion (DΔ) of positions −83 to +30 and the indicated deletions upstream of the Gcn4 binding site. (B) *his3* promoter derivatives containing an upstream deletion (UΔ) of positions −447 to −105 and the indicated deletions downstream of the Gcn4 binding site originating from the 3′ end. (C) *his3* promoter derivatives containing an upstream deletion (UΔ) of positions −447 to −105 and the indicated deletions downstream of the Gcn4 binding site originating from the 5′ end. Arrows represent transcriptional start sites of *his3* and *pet56*. Numbering is relative to the +1 transcriptional start site of *HIS3*. Open boxes represent the sequence, and black boxes represent the Gcn4 binding site. The drawing is not to scale. WT, wild type.

resulted in a gradual loss of function is analogous to the situation with acidic activation domains (13).

The *his3* promoter region is sufficient to confer accessibility when placed in the middle of the *ade2* structural gene. To determine whether the *his3* promoter region is sufficient for conferring preferential accessibility, we examined *Hin*fI cleavage of a strain in which a 150-bp segment of the *his3* promoter region (positions −120 to +30) was inserted in the middle of the *ade2* structural gene (Fig. 8). Cleavage of the Gcn4 binding site in the inserted *his3* promoter (band 6, which is absent in the wild-type strain) was ninefold more efficient than any of the neighboring four sites located in the *ade2* coding region. The level of preferential accessibility is comparable to the observed 12-fold effect on the Gcn4 binding site within the native *ade2* promoter region (band 2). Thus, the *his3* promoter region is sufficient to confer preferential accessibility, even when it is translocated to the middle of an otherwise inaccessible structural gene.

***Hin*fI sensitivity at Gcn4 binding sites in the *his3* promoter is not determined by the Swi-Snf, SAGA, Ada, or Rad6 chromatin-modifying activities.** To investigate the effect of the Swi-Snf chromatin-remodeling complex, the SAGA and ADA histone acetylase complexes, and Rad6, which ubiquitinates histone H2B (35), we analyzed *Hin*fI cleavage in isogenic *snf2*, *gcn5*, *ahc1*, and *rad6* deletion strains. These strains were derived from BY105, which displays greater preferential *Hin*fI cleavage at the Gcn4 binding site in the *his3* promoter (30-fold increase) than in the strain background of the previous experiments. As shown in Fig. 9, the *Hin*fI hypersensitivity of the Gcn4 binding site in each of the mutant strains was comparable to that observed in the wild-type strain, indicating that these chromatin-modifying activities are not required for preferential accessibility of the *his3* promoter region.

DISCUSSION

A general property of the *his3-pet56* promoter region is responsible for preferential accessibility in vivo. Using inducible *Hin*fI cleavage to measure accessibility of nuclear proteins to chromatin in living yeast cells, we showed that *Hin*fI sites in a variety of promoter regions are cleaved 5- to 20-fold more efficiently than sites in nonpromoter regions. The hypersensitivity of the *Hin*fI site within the *his3-pet56* promoter region is locally determined, because preferential cleavage was abolished when promoter sequences flanking both sides of the *Hin*fI site were removed yet was retained when this region was translocated to the middle of the *ade2* structural gene. In several derivatives, transcription from both the *his3* and *pet56* promoters was virtually eliminated yet *Hin*fI cleavage at the Gcn4 site occurred at a level comparable to that of the wild-type chromosomal locus. In a similar vein, Swi-Snf- or activator-dependent alterations of chromatin structure (1, 7, 12, 29, 33, 46) or nuclease hypersensitivity (23, 39) can occur in the absence of a functional TATA element and transcriptional activity of the promoter.

The striking and unexpected finding of our study is that preferential accessibility of the *his3-pet56* promoter region does not depend on a specific sequence element. This accessibility does not depend on Gcn4 (which is absent from all of the yeast strains) or on any sequence or combination of sequences upstream of the *his3* core promoter region. Thus, the preferential sensitivity of promoter regions observed here is mechanistically distinct from the activator-dependent changes in chromatin structure. Furthermore, since deletion or modification of TATA elements does not affect *Hin*fI hypersensitivity, preferential accessibility does not result from the local

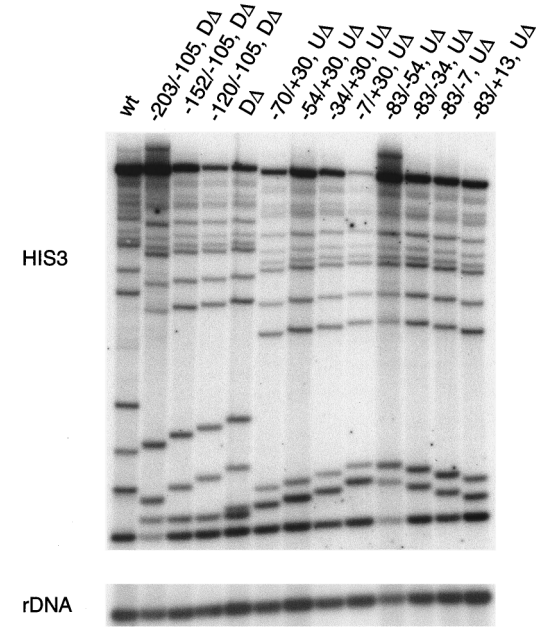

FIG. 6. *Hin*fI cleavage of purified genomic DNAs from strains containing *his3* promoter derivatives shown in Fig. 5. Southern blots containing 25% of the amount of DNA used in Fig. 7 were hybridized with *his3* (the second band from the bottom represents the *Hin*fI site within the Gcn4 binding sequence) and rDNA probes. wt, wild type.

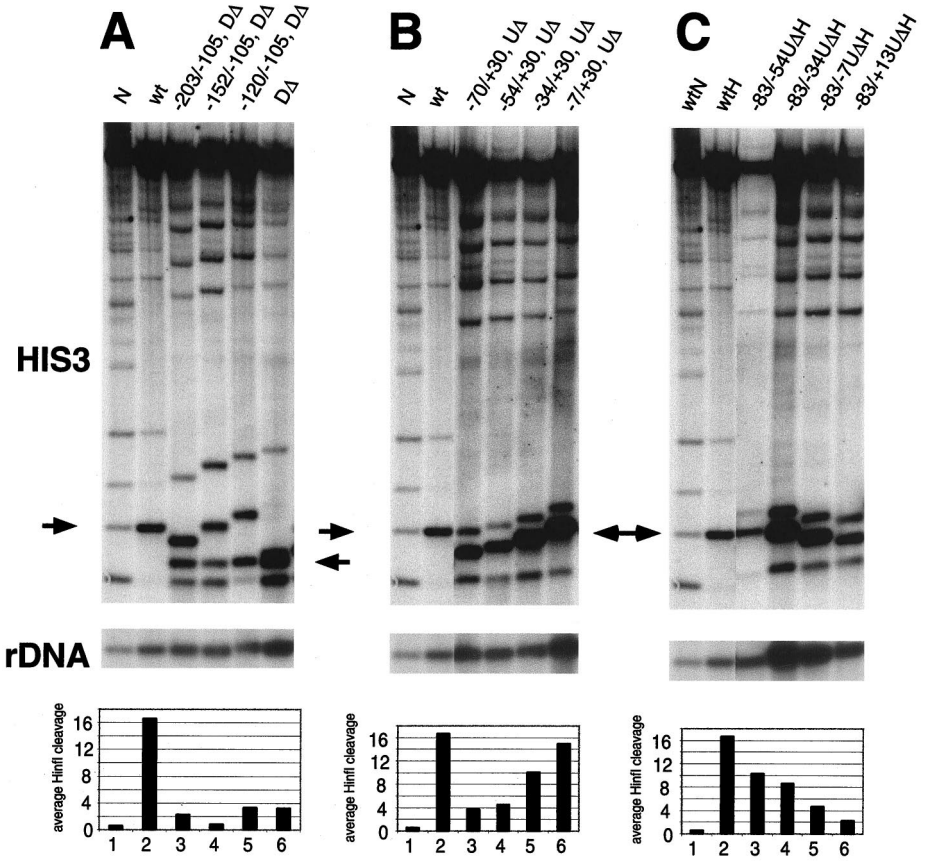

FIG. 7. Progressive deletion of the *his3* promoter region in either direction causes a progressive loss of *Hin*fI hypersensitivity. Shown are Southern blots of in vivo *Hin*fI-cleaved DNA from strains containing *his3* promoter derivatives lacking downstream sequence with various deletions of upstream sequence (see Fig. 5A) and rDNA controls (A), *his3* promoter derivatives lacking upstream sequence with various deletions of downstream sequence from the 3′ end (see Fig. 5B) and rDNA controls (B), and *his3* promoter derivatives lacking upstream sequence with various deletions of downstream from the 5′ end (see Fig. 5C) and rDNA controls (C). Genomic DNA was prepared from the corresponding strains, containing p*Hin*fI, that were induced with 1 mM $CuSO_4$ for 90 min, and the DNA was hybridized to the indicated probes. As a general control, genomic DNA from SH104 without p*Hin*fI was also partially digested with *Hin*fI in vitro (N). wt, wild type.

DNA distortion due to binding by TBP. In this regard, TBP is not generally associated with TATA elements in vivo under conditions of transcriptional inactivity (22, 24). Finally, poly (dA-dT) elements do not constitute a significant basis of preferential access to promoter regions. Although an artificially long dA-dT stretch (42 bp) can increase *Hin*fI cleavage by 70% (15), the magnitude of this effect is considerably less than the difference between *Hin*fI cleavage at sites in promoter versus nonpromoter regions. In the derivatives here, which involve dA-dT tracts of physiological length, there is no detectable effect on cleavage at an immediately adjacent *Hin*fI site.

The combined results of our deletion analysis indicate that multiple determinants within the *his3-pet56* promoter region contribute in an additive fashion to preferential accessibility. When the *pet56* promoter region was removed, progressive deletion of the *his3* promoter region from either direction resulted in a gradual loss of *Hin*fI hypersensitivity. Thus, the degree of preferential accessibility was related to the length, but not the precise sequence, of the *his3* promoter region that was present in the various derivatives. The simplest explanation for these results is that each observable decrease in *Hin*fI cleavage reflects the removal of at least one determinant of preferential accessibility; in this interpretation, the *his3* promoter region would contain at least four determinants. In addition, the *pet56* promoter region clearly contains determinants of preferential accessibility because some *Hin*fI hypersensitivity is observed when the *his3* promoter region is completely deleted and because similar *his3* derivatives that do or do not contain the *pet56* region have different levels of *Hin*fI cleavage. Thus, there appear to be at least five determinants in the *his3-pet56* promoter region that contribute to preferential accessibility. Since there is no obvious motif that is repeated within the *his3-pet56* promoter region or that is contained in other promoters displaying *Hin*fI hypersensitivity, our results suggest that preferential accessibility is due to a general property of the DNA sequence.

Potential molecular mechanisms. The general property of the *his3-pet56* (and presumably other) promoter regions inferred to be responsible for increased accessibility to nuclear proteins is unknown. However, it has long been observed that yeast promoter regions are relatively AT rich compared to the genome as a whole, and the promoter regions analyzed in this study have a 5.5% lower GC content than their adjacent coding regions. Thus, AT richness or some other feature of base composition (e.g., frequency of certain di- or trinucleotides) might serve to distinguish promoter from nonpromoter regions. In this regard, replacement of *HIS3* upstream promoter sequences by relatively GC-rich sequences from bacteriophage λ DNA eliminated micrococcal-nuclease hypersensitivity in the *HIS3* TATA region (41).

There are several classes of explanations for how a broad and rather crude feature such as overall AT richness might

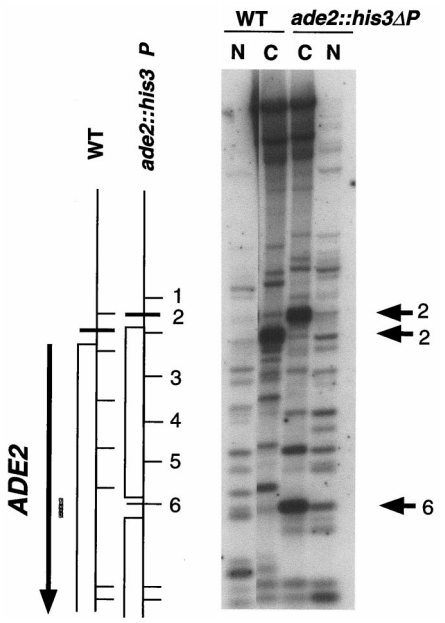

FIG. 8. The *his3* promoter region is sufficient to confer accessibility when located within the *ADE2* structural gene. Shown is a Southern blot of genomic DNAs from strains containing the wild-type (WT) *ADE2* or *ade2::his3p* alleles and p*Hin*fI that were induced for 90 min with 1 mM $CuSO_4$ (C) or untransformed cells partially digested with *Hin*fI in vitro (N). For the wild-type and mutant alleles, open boxes represent the *ADE2* coding region (the long arrow indicates the 5′-to-3′ orientation of *ADE2*), the vertical gray bar under the vertical long arrow indicates the inserted 150-bp *his3* promoter region, and short horizontal lines indicate *Hin*fI restriction sites. Band 6 corresponds to the Gcn4 binding site within the inserted *his3* promoter, and band 2 (whose mobility differs in the wild-type and mutant alleles) corresponds to the Gcn4 binding site within the *ade2* promoter region and serves as an internal control.

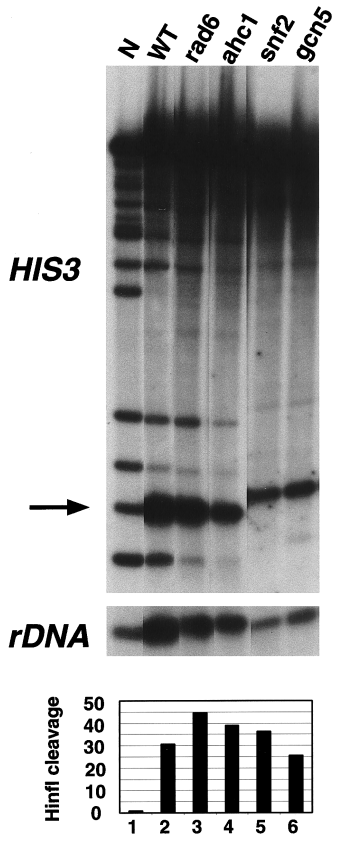

FIG. 9. Swi-Snf, SAGA, and Ada complexes and Rad6 are not responsible for the *Hin*fI hypersensitivity at the *his3* Gcn4 site. Wild-type (WT) and *rad6*, *ahc1*, *snf2*, or *gcn5* deletion strains that contain p*Hin*fI were induced with 1 mM $CuSO_4$ for 90 min, and genomic DNAs were analyzed by Southern blotting with *his3* and rDNA probes. As a control, genomic DNA from the wild-type strain was partially digested with *Hin*fI in vitro (N). Preferential *Hin*fI cleavage at the Gcn4 binding site (indicated by an arrow) was determined with respect to the average cleavage of three adjacent genomic sites.

lead to differential protein accessibility in physiological chromatin. First, nucleosomes might associate poorly with or be less stable on AT-rich DNA sequences, thereby providing a weaker barricade to proteins. In a related model, AT-rich sequences might be poor substrates for nonhistone proteins that render chromatin structure more inaccessible. Second, AT-rich regions might interact with nonhistone proteins that relax chromatin structure or inhibit nucleosome formation. Third, chromatin-modifying activities such as the Swi-Snf, Rsc, and related complexes might have untargeted genome-wide effects that are sensitive to overall base composition. The Swi-Snf and Rsc complexes directly interact with DNA (26, 34), and such interactions might have some sequence specificity. Fourth, positioning of nucleosome cores is significantly affected by an intrinsic preference for certain sequence periodicities that are related to DNA bending; e.g., the minor grooves of AAA and AAT face inward toward histones, whereas those of GGC and AGC face outward (4, 37). Such sequence-dependent effects on intrinsic nucleosome positioning might result in internucleosomal regions which display preferential accessibility to nuclear proteins. Whatever the molecular explanation, a key feature of all of these models is that the structural differences between promoter and nonpromoter regions extend over relatively long distances, thereby resulting in cooperative effects that significantly affect protein accessibility.

Biological significance. Our results suggest that the compact yeast genome is organized into structurally distinct promoter and nonpromoter regions that inherently differ in their accessibility to nuclear proteins. In principle, this genomic organization is useful for ensuring that transcription factors bind preferentially to appropriate sites in promoters rather than to the excess of irrelevant but equally high-affinity sites in nonpromoter regions. A generally increased accessibility of promoters regardless of their transcriptional activity is also useful given that many genes are required only in response to specific environmental or developmental cues. Distinguishing promoters of inactive genes from nonpromoter regions allows the cell to economize on regulatory factors by lowering the threshold for binding to a specific subset of the genomic DNA. This effectively decreases the concentration of competing, nonfunctional binding sites without stimulating transcription in the absence of the appropriate signal. Thus, we suggest that the general promoter accessibility we have observed provides a context in which further gene-specific regulation can occur.

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