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NOTES

DNase I-Hypersensitive Sites in the Galactose Gene Cluster of *Saccharomyces cerevisiae*

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Five DNase I-hypersensitive regions were associated with the *Saccharomyces cerevisiae* galactose gene cluster during both galactose induction and glucose repression of transcription. Four hypersensitive regions were located in areas flanking the *GAL* cluster genes, and one site occurred within *GAL10*. A DNase I-hypersensitive region located between the 5′ ends of divergently transcribed *GAL10* and *GAL1* contained sequences essential for the transcription of both genes.

Transcription of the galactose cluster genes of *Saccharomyces cerevisiae* is induced about 1,000-fold by the presence of galactose (17). This coordinate control is mediated by the protein products of a positive regulatory locus, *GAL4*, and a negative regulatory locus, *GAL80* (reviewed in reference 12). Mutations in a third regulatory gene, *GAL3*, result in slower adaptation to growth on galactose (22). In addition to induction by galactose, the genes of the galactose cluster are also subject to glucose repression (1).

lacZ fusion experiments utilizing the 365-base-pair (bp) *Dde*I-*Sau*3A DNA fragment from the region between the 5′ ends of *GAL10* and *GAL1* (Fig. 1B) have demonstrated that this region contains DNA sequences required for *GAL4* and *GAL80* regulation (5). This regulation may involve regulatory protein binding within this region. Recently, after the work reported here was completed, it was demonstrated by deletion analysis that a 108-bp guanine-plus-cytosine (G + C)-rich segment within the *Dde*I-*Sau*3A fragment is required for expression of both *GAL10* and *GAL1* (7).

DNase I-hypersensitive sites appear to be discontinuities in the normal repeated nucleosomal chromatin structure and have often been identified at or near DNA sequences known to be important in gene regulation (11, 14, 15). Such DNA-regulatory sequences are thought to act by binding regulatory proteins (3, 24, 25).

To identify potential regions of regulatory protein interaction, this study describes the DNase I-hypersensitive sites present within a 12-kilobase (kb) region containing the galactose gene cluster.

DNase I-hypersensitive sites were located by the method of indirect end labeling (23). The region first examined for DNase I-hypersensitive sites was that within the *Hin*dIII fragment that bridges *GAL10* and *GAL1*, as this area potentially contains upstream regulatory sequences for both genes. Strain D585-11C (GAL) was grown to mid-log phase on YP medium (1% yeast extract, 2% peptone) containing 2% galactose. Nuclei were then isolated as described previously (6), except that the cells were first pretreated with 1 M sorbitol, 1% (vol/vol) 2-mercaptoethanol, and 25 mM EDTA, pH 8, for 15 min at 34°C. Before DNase I digestion, the nuclei were washed once in DNase I digestion buffer (1 M sorbitol, 10 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid), pH 6.5], 10 mM $MgCl_2$, and 1 mM $CaCl_2$). After incubating the nuclei at a concentration of 0.2 to 0.4 mg of DNA per ml with DNase I, the resultant purified (2) DNA fragments were digested with *Hin*dIII. The DNA was then electrophoresed on a 1.5% agarose gel and transferred to nitrocellulose paper (13), and the Southern blot was hybridized with a probe containing the segment of *GAL10* indicated in Fig. 1A. Below the 2.5-kb parent *Hin*dIII fragment, two separate bands comigrated near the 939-bp DNA size marker (Fig. 2, lane 3). On the autoradiograms produced in some experiments, the broader upper band appeared to be a closely spaced doublet. The size of each DNase I-generated fragment corresponded to the distance from the *Hin*dIII terminus of the probe sequence to a DNase I-hypersensitive cutting site. The region containing the three sites extended for about 190 bp from ca. 150 bp upstream of the *GAL10* transcription initiation point to 270 bp upstream of the start of *GAL1* transcription (7) (Fig. 1B). Contained within this region was the 108-bp G + C-rich sequence important for both *GAL10* and *GAL1* transcription. The centers of the three cutting sites within this zone were ca. 190, 260, and 300 bp from the *GAL10* transcription initiation point.

A naked DNA control experiment demonstrated some sequence selection by DNase I (Fig. 2, lane 5); however, very little cutting occurred in the area between *GAL10* and *GAL1* which was hypersensitive to DNase I in the nuclei. Thus, the nuclear sites are hypersensitive as the result of chromatin conformation. The DNA used in this experiment was first isolated as described by Sherman et al. (13) and then subjected to pronase digestion and phenol extraction.

To extend the search for hypersensitive sites beyond the *GAL1* *Hin*dIII site, restriction enzyme digestion was performed with *Kpn*I rather than *Hin*dIII. Again the hypersensitive region between *GAL10* and *GAL1* was seen (Fig. 3A), but it was less well resolved here due to the lower percentage of agarose used in the gel. An additional DNase I band was observed whose 3.4-kb size corresponded to a hypersensitive area near the 3′ end of *GAL1* (Fig. 1A). DNase I digestion of isolated DNA (Fig. 3A, lanes 5 and 6) produced little hybridizing material in the areas corresponding to the chromosomal hypersensitive sites.

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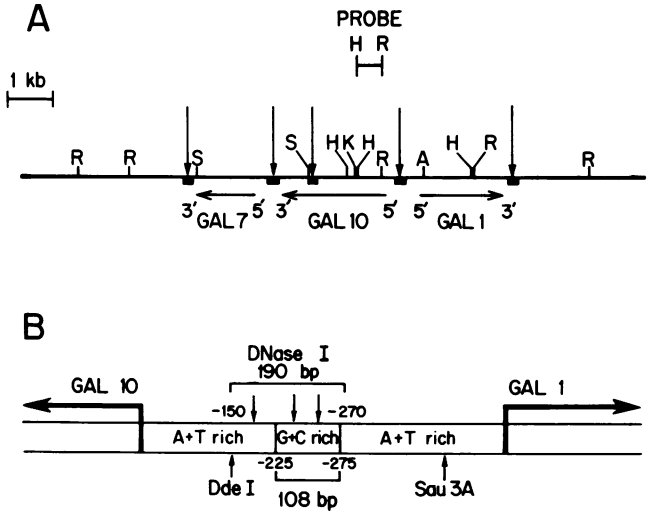

FIG. 1. (A) Locations of the DNase I-hypersensitive sites within the galactose gene cluster. Arrangement and orientation of genes are as described by St. John et al. (17). Downward arrows indicate centers of DNase I-hypersensitive regions. Also shown is the position of the probe sequence within the cluster. Abbreviations: R, *Eco*RI; H, *Hin*dIII; S, *Sal*I; K, *Kpn*I; A, *Ava*I. (B) Area between *GAL10* and *GAL1*, showing position of the 190-bp DNase I-hypersensitive region. Downward arrows represent centers of DNase I cutting. Positions of transcripts (large horizontal arrows) and nucleotide composition of DNA regions are as described by Johnston and Davis (7). Upward arrows show positions of *Dde*I and *Sau*3A restriction sites.

DNase I-hypersensitive sites leftward of the probe sequence were located as described above, but the restriction step was performed with *Eco*RI (Fig. 3B, lanes 2 through 4). The three hypersensitive regions identified occurred near the 3′ end of *GAL7* (4.3 kb), between the 5′ end of *GAL7* and the 3′ end of *GAL10* (2.4 kb), and within the *GAL10* structural gene near the *Sal*I site (1.5 kb) (Fig. 1A). None of these three regions was hypersensitive when the isolated DNA was digested with DNase I (Fig. 3B, lanes 5 and 6).

To investigate whether the galactose cluster hypersensitive sites existed before galactose induction, a mapping experiment was carried out with nuclei from strain D585-11C grown on YP medium with 2% glucose. A mapping experiment was conducted rightward from the *GAL10 Kpn*I site, and another was conducted with the *GAL10 Eco*RI site as its origin (Fig. 3C). DNase I-hypersensitive bands were seen at the same positions as in Fig. 3A and B. Thus, nuclei from these glucose-repressed cells contained all five regions of hypersensitivity seen during galactose induction of transcription.

S. cerevisiae YNN70 (19) carries a deletion of chromosome II that extends from the *Eco*RI site within *GAL10* to the first *Eco*RI site beyond the 3′ end of *GAL1* (Fig. 1A) and is incapable of transcribing *GAL10*. There was a dramatic reduction in the strain YNN70 *Kpn*I fragment size relative to that of strain D585-11C (Fig. 3C, lanes 3 and 1, respectively), which confirmed that a large part of the region rightward of the probe had been deleted. All three of the undeleted hypersensitive sites were observed in a mapping experiment utilizing *Eco*RI cleavage with strain YNN70 (Fig. 3C, lane 4). Therefore, the hypersensitive site within *GAL10* can exist independently of both upstream regulatory effect and transcription, as it is retained when part of the *GAL10* 5′ coding region, transcription initiation point, and upstream regulatory sequences are deleted.

Five expression-independent regions of DNase I hypersensitivity were identified within a 12-kb region containing the *S. cerevisiae* galactose gene cluster. The DNase I hypersensitivity observed between *GAL10* and *GAL1* represents an altered chromatin structure which contains the G + C-rich sequence required for transcription of both *GAL10* and *GAL1*. Similarly, sequences at the *GAL7* 5′ hypersensitive region may also identify sequences that are essential for transcription of that gene, although a deletion analysis of that region has not yet been reported. It is less clear what importance, if any, the hypersensitive sites observed flanking the 3′ ends of *GAL7* and *GAL10* might have. DNase I-hypersensitive sites have been observed in the 3′ flanking region of other genes (9, 21), but the possible function of sequences at these sites is unclear. It is possible that the 3′ flanking sites detected here might be associated with transcriptional units flanking the *GAL* cluster (18). The importance of the hypersensitive region within the *GAL10* structural gene, an unusual location for a hypersensitive site, is also unknown.

DNase I-hypersensitive sites may result from disruption of the normal repeated nucleosomal chromatin structure by the

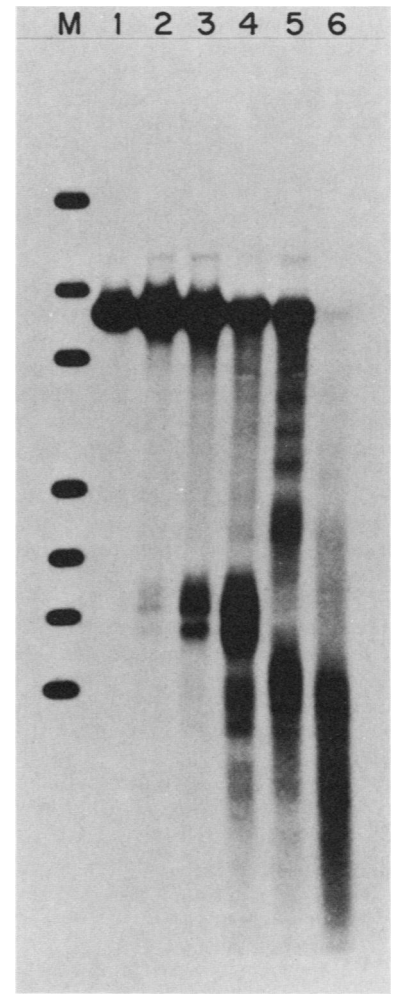

FIG. 2. Mapping hypersensitive sites between *GAL10* and *GAL1*. Southern blot of a 1.5% agarose gel containing 10 μg of *Hin*dIII-restricted DNA per lane was hybridized with the probe shown in Fig. 1A. Lanes: 1, strain D585-11C DNA; 2 through 4, nuclei from cells grown on galactose digested with 0.1, 0.4, and 1.6 U of DNase I per ml, respectively; 5 and 6, DNA digested with 0.1 and 0.3 U of DNase I per ml, respectively; M, size markers derived from pBR322 (bottom to top: 754, 939, 1,129, 1,404, 2,178, 2,819 and 4,362 bp).

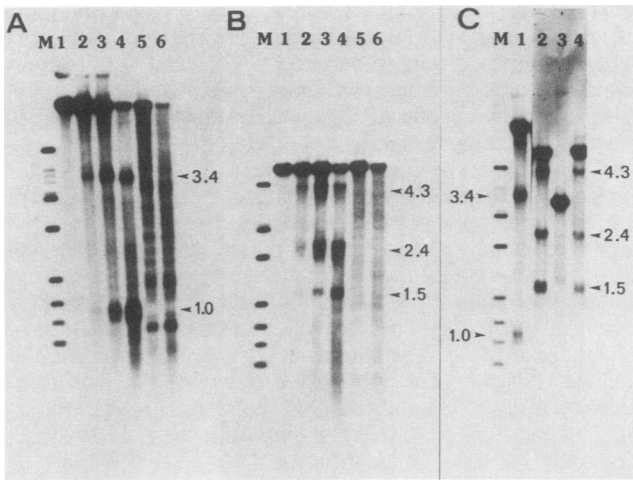

FIG. 3. DNase I-hypersensitive sites outside the *GAL10-GAL1* intergenic region. The DNA size markers (lanes M) and probe are described in the legend to Fig. 2. (A and B) Mapping hypersensitive regions from the *GAL10 Kpn*I and *Eco*RI sites. Southern blot of a 0.8% agarose gel containing 5 μg of DNA per lane. All DNA samples were restricted with *Kpn*I (A) or *Eco*RI (B). Lanes: 1, strain D585-11C DNA; 2 through 4, nuclei from cells grown on galactose incubated with 0.1, 0.4, and 1.6 U of DNase I per ml, respectively; 5 and 6, DNA digested with 0.05 and 0.1 U of DNase I per ml, respectively. (C) DNase I-hypersensitive regions in strains D585-11C and YNN70 grown on glucose medium. Southern blot of 0.8% agarose gel containing 5 μg of DNA per lane from nuclei digested with 0.4 U of DNase I per ml. Lanes: 1 and 2, strain D585-11C; 3 and 4, strain YNN70; 1 and 3, *Kpn*I restriction; 2 and 4, *Eco*RI restriction.

binding of sequence-specific binding proteins, as has been suggested by the chromatin reconstitution work of Emerson and Felsenfeld (4). At least for the hypersensitive sites between *GAL10* and *GAL1* and upstream of *GAL7*, binding by one or more of the galactose regulatory proteins may result in the hypersensitivity observed. As the *GAL* cluster hypersensitive sites are present during both galactose induction and glucose repression of transcription, whatever factors produce these sites must act under both transcriptional states. The existence of DNase I-hypersensitive sites before transcriptional activation has been observed previously for the *Drosophila melanogaster* heat shock genes (23) and for the *S. cerevisiae* alcohol dehydrogenase gene *ADR2* (16). By using the recently constructed deletion mutants for *GAL80* (20) and *GAL4* (8), it is now possible to test whether the protein products of these regulatory genes play a role in producing the DNase I-hypersensitive sites within the galactose gene cluster.

After this work was submitted for publication, similar results were reported for the hypersensitive region between *GAL10* and *GAL1* (10).

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