The Regulatory Protein GAL80 Is a Determinant of the Chromatin Structure of the Yeast GAL1–10 Control Region*

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Chromatin in the regions between the upstream activator sequence and the 5' ends of the yeast GAL1 and GAL10 genes has been analyzed by DNase I chromosomal footprinting and micrococcal nuclease digestion using the indirect end-labeling approach. Comparison of wild type chromatin digests to naked DNA digests shows that there are specific regions of these upstream sequences which are strongly protected in chromatin. Comparison to chromatin digests from cells disrupted for the positive regulatory gene, GAL4, or the negative regulatory gene, GAL80, and thus lacking GAL4 or GAL80 function, shows that these regions of protection in wild type chromatin are GAL80-dependent but not GAL4-dependent. The protected regions include DNA lying on (GAL10) or near (GAL1) the respective TATA boxes. These protections are present in both noninduced and induced cells. Both DNA strands are equally protected. Upstream of GAL1 there is a second protected region. This protection shows considerable expression and strand dependence. These observations provide the first evidence that the GAL80 function influences chromatin structure and suggest possible mechanisms by which GAL80 modulates the GAL1 and 10 promoters in induced cells. Micrococcal nuclease digests also suggest a role for GAL80 in a distinctive higher order organization of the intergenic region, perhaps involving multiprotein complexes.

A complete understanding of the control of expression of any eukaryotic gene will require a knowledge of how the transacting regulatory factors interact with the cis-acting control DNA sequences. One powerful way to analyze these DNA-protein interactions involves in vitro reconstituted systems, using specific transcription as a criterion for function. In amenable systems one can carry out such an analysis in vivo by comparing the effects of regulatory mutations on levels of gene expression and on DNA-protein interactions at the target gene. We (Lohr and Hopper, 1985) and others (Giniger et al., 1985) have been using this approach to study the yeast GAL1–10 genes. The GAL system was initially described several years ago by classical genetic approaches (cf. Oshima, 1982) and modern techniques of molecular genetics have been applied more recently (St. John and Davis, 1981; Guarente et al., 1982; Johnston and Davis, 1984; Brent and Ptashne, 1985; Johnston et al., 1986; Torchia and Hopper, 1986).

Some of the biochemical features of GAL regulation have already been described. The positive regulatory protein GAL4 binds to the major GAL1–10 regulatory DNA sequence, the upstream activator sequence or UAS* (Guarente et al., 1982), when GAL1 and 10 genes are expressed (cells grown in galactose) but not when the genes are repressed (cells grown in glucose). This is based on results from in vivo methylation (Giniger et al., 1985) and nuclear DNase I hypersensitivity and chromosomal footprinting (Lohr and Hopper, 1986). Factors in the cell extract that protect a similar region of DNA from DNase I in vitro (Bram and Kernberg, 1985).

GAL4 can also bind to the same region of the UAS, producing a similar DNase I protection pattern, under conditions in which GAL1–10 genes are not expressed (cells grown in glycerol/ethanol) or even not inducible (Lohr and Hopper, 1985). Thus GAL4 binding is only one step in the process of GAL1–10 expression. Other steps must be involved. Genetic data suggests that the GAL80 negative regulatory protein is involved in the control processes which occur subsequent to GAL4 binding (Torchia et al., 1984).

Since in vivo GAL4/UAS interactions (Giniger et al., 1985) appear to be maintained in nuclei (Lohr and Hopper, 1985), we have used this system to probe the rest of the GAL1–10 control region for other DNA-regulatory protein interactions. This is done by comparing DNase I chromosomal footprints from wild type and various regulatory protein mutants in different states of gene expression (cells grown in various carbon sources). The DNase I profiles produced are quite complex. However, there are several consistent and striking differences which suggest new information about the role of GAL80 in the chromosome structure of the regions 5' of GAL1 and GAL10. Micrococcal nuclease digests confirm and extend the conclusions from the DNase I footprints.

MATERIALS AND METHODS

This work involves comparisons of wild type 21R and several regulatory mutant strains: 4D, a strain disrupted for GAL4; 80D, a strain disrupted for GAL4; 80D; a strain disrupted for GAL80, 80D; a super repressor strain. These strains were constructed as described previously (Johnston and Hopper, 1982; Torchia et al., 1984; Johnston et al., 1986). The wild type 4D and 80D strains are congenic with each other: the 80D strain is closely related to the other three.

Nuclear chromatin cleavage patterns were analyzed by the indirect end-label approach (Nedospasov and Georgiev, 1980; Wu, 1980). Yeast cells were grown, nuclei were isolated, and chromosomal footprinting (Lohr, 1987). The deproteinized and purified DNA was cleaved to completion with a restriction endonuclease, repurified, electropho-

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¶ The abbreviations used are: UAS, upstream activator sequence; bp, base pair; DBM, diazobenzylloxymethyl.
RESULTS

There are three different gene activity states for GALI and GAL10 in wild type cells: expressed in galactose; inducible, glucose repressed; not expressed and not inducible, glucose repressed. In practice, cells are actually grown in either glycerol/ethanol, galactose plus glycerol/ethanol, or glucose plus glycerol/ethanol. Galactose plus glycerol/ethanol as a carbon source is necessary for the growth of cells like 40 or 80 which are functionally gal- and could not grow on galactose as a sole carbon source. For comparison purposes, we grow all cells on these complex carbon sources. Thus, in the text, reference to cells grown in glucose or galactose really means cells grown in glucose/glycerol/ethanol or galactose/glycerol/ethanol.

Three regulatory mutants are used in this work. The 40 strain lacks GAL4 function. Thus, GALI and 10 cannot be induced in any carbon source in this strain. 80 lacks GAL80 function. In these cells, GALI and 10 are still repressed in glucose but are induced at high level in glycerol/ethanol as well as in galactose containing media (Torchia et al., 1984). The 80 strain is constitutively uninducible.

DNase I Chromosomal Footprinting

DNase I chromosomal footprints were analyzed on low resolution denaturing gels consisting of 60% polyacrylamide, 60% agarose composite gels. Cleavage sites were mapped from an EcoRI site lying at -170 bp in GAL10 (Johnston and Davis, 1984; Yocum et al., 1984), the other in the coding sequence of GAL10. Radiolabeled 4X-HindII marker fragments run in the gels were used to determine size.

Hybridization probes were used by repair synthesis of EcoRI restriction sites with [32P]dATP and [32P]dTTP using reverse transcriptase (Goodman, 1982). The TaqI site in GAL10 is virtually coincident with the RI site. Thus, the RI-Rol fragment of GAL10 (115 bp) can be used as a probe to map DNase I cleavage sites on the coding strand 5' of the GAL10 gene. In the subclone of the GAL10-10 intergenic region used in this work (plasmid pSC481 described initially by St. John and Davis, 1981), an EcoRI site has replaced an Aat site at -900 bp. Thus, this new RI site lies within the 5' end of the coding sequence of GAL10. Repair of this RI site and cleavage with Ral produces a 50-bp probe fragment which can be used to map DNase I cleavage sites on the coding strand 5' of the GALI gene. The adjacent downstream coding sequences of the GALI gene were subcloned from a -1000-bp fragment with an EcoRI site inserted at the left (Fig. 1) end (St. John and Davis, 1981). Repair of this RI site and restriction by TaqI produces a probe (~200 bp) which can be used to map cleavage sites on the noncoding strand 5' of the GALI gene. The location of these probes are shown in Fig. 1. All probes were purified from gels (Maxam and Gilbert, 1980) before use in hybridization. Use of single strand-specific M13 probes produced very high backgrounds in these hybridizations. Thus we were unable to use such probes.

Hybridizations were carried out in formamide at 42 °C and papers were washed as described by Alwine et al. (1979). Autoradiography with precached XAR-5 film and intensifying screens was carried out at -80 °C. Films were scanned on a Beckmann DU spectrophotometer with gel scanning attachment.

A 4X-HindII digest of the GALI-10 intergenic region and 5' ends of GALI and 10 has been sequenced (Johnston and Davis, 1984; Yocum et al., 1984). Potential TATA box regions and transcription initiation sites have been located within this sequence. These were used in this work. However, the TaqI site in GALI lies beyond this sequenced DNA. Thus, to locate cleavage sites mapped from this TaqI site on the sequenced region, we needed to locate it precisely. To do this, we performed a Southern analysis using electrophoretic transfer from a high resolution acrylamide gel. The TaqI site lies at ~130 bp from the GAL10-10 EcoRI site (not shown).

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are quantified in the densitometer scans in Fig. 4A. Note that the just above the protected region is again quite accessible in 80D digests. We cannot interpret its partial protection in profiles from wild type induced cells.

DNA in regions a and b are also DNase I accessible in digests from 80D cells grown in glucose (Fig. 2, track 7), conditions in which GAL10 is not induced. Thus, the exposure of these regions does not require high levels of gene expression.

Regions a and b remain protected in chromatin digests from cells disrupted for the GAL4 protein (Fig. 2, track 8) or from 80D super repressed cells (Fig. 2, track 5). These results allow us to generalize the wild type results. As long as 80 protein is present in the cell, regions a and b are protected in the
FIG. 3. DNase I chromosomal footprints in the chromatin region upstream from GAL1. DNA from nuclear DNase I digests was electrophoresed on denaturing gels, electroblotted to DBM, and probed as described under "Materials and Methods." Naked DNA profiles are marked with an n. Profiles from the coding strand have a dot beneath them. Cell type (wild type (wt), 4D, 80D, or 80S) is shown in bold letters. Carbon source (D = glucose, G = galactose) is indicated immediately above each track. The boundary of the GALA/UAS interaction region is marked by a |. Prominent bands in the naked DNA profile are labeled with small alphabetical letters. Their locations (from the GAL10 RI site) on the DNA are as follows: a, 510 bp; b, 555–570 bp; c, 630–655 bp; d, 700–750 bp; e, 760–780 bp. The mobilities of radiolabeled φX-HaeIII restriction fragments used for size determinations in the gels are identified with capital letters: D, 603 bp; E, 310 bp; F, 281 bp; G, 271 bp. Fragments E–G were present and used for size determinations in the autoradiogram shown in A but lie below the bottom of the print shown here. The transcription initiation site and direction of GAL1 transcription are shown with a wavy arrow. The location of the TATA box is shown by T. Other symbols are identified in the text. Electrophoresis is from top to bottom. The gel used to produce A resolved DNA in the size range ~250–650 bp. The tracks show from left to right: wild type cells grown in glucose (track 1) or galactose (track 2); track 3, a naked DNA digest; 80D cells grown in galactose (track 4) or glucose (track 5); track 6, a repeat of track 1, wild type cells grown in glucose noncoding strand, (for comparison purposes); wild type cells grown in glucose, coding strand (track 7); wild type cells grown in galactose, coding strand (track 8). The skewing in the pattern near the very tops of tracks 2, 4, and 8 are due to
chromatin; protection does not require a GAL+ cell or a functional GAL4.

There are several possible TATA box sequences 5' of GAL10. The best consensus (TATAA) is one at 265 bp. Thus, the most strongly GAL80-dependent protected region (~270 bp) is essentially coincident with the best consensus TATA.

B) 5' of GAL1—Fig. 3 shows chromosomal footprints of the region upstream of GAL1. As for GAL10, there is high intensity (O) immediately below the UAS/GALA boundary (I) and near the 5' end of GAL1 in profiles from repressed chromatin (Fig. 3A, track 1). There is another high intensity region at the GAL1 TATA (T). There are two regions of very low intensity in these profiles (xxxxx and xxxx, Fig. 3A). These lie ~550-610 and ~700-750 bp from the GAL10 RI site.

Profiles differ somewhat when the gene is expressed (Fig. 3A, track 2). There is significantly more intensity in the upper low intensity region (xxxxx, Fig. 3A), less sensitivity at 680 bp, and slightly higher intensity in the lower low intensity region (xxxxx, Fig. 3A). These differences are quantified in densitometer scans (Fig. 4B).

In naked DNA digests from this region, there are several intense bands or band clusters which are useful indicators of DNase I protection levels in chromatin (a-e, Fig. 3A, track 3). Locations of these bands are given in the legend to Fig. 3. Three of the strongly cleaved clusters, b, c, and d, fall within regions of much lower intensity in the repressed chromatin profiles. Thus, the low intensity of these regions in the chromatin profiles must reflect strong protection by chromosomal proteins. In contrast, DNA around a is very strongly cleaved in chromatin. Thus, this region is not significantly protected by chromosomal proteins. Chromatin around e shows an intermediate level of protection. The intensity is less than in the naked DNA profiles but it is quite significant. In chromatin from cells expressing GAL1, protection around b and c is significantly relieved while that around d is only somewhat decreased. Thus, even under conditions of maximal gene expression in wild type cells, there is considerable chromatin-dependent protection in the d region. Other regions (a and e) are not strongly affected by gene expression.

Removal of GAL80 function exposes the DNA in regions b-d to DNase I digestion (Fig. 3A, tracks 4 and 5). There is little change around a. Around e, there is a loss of one band (<, Fig. 3A, track 4) and some smearing of bands. Thus, there is a major effect upon 80 removal in chromatin regions b-d (~550-750 bp from the GAL10 RI site). The protection observed in these regions of wild type chromatin must therefore be dependent on GAL80. Again, these results cannot determine whether there is actual binding of the GAL80 protein to the regions. These observations are quantified in the densitometer scans shown in Fig. 4.

There is a strong increase in the exposure of these upstream regions in both glucose and galactose grown 80D cells but it is somewhat greater in galactose grown cells (Fig. 3A, tracks 4 and 5). Track 5 was near the edge of the paper and did not all transfer. Nevertheless, the accessibility changes in the upstream regions are apparent. Similar results can be seen in Fig. 3B (tracks 3 and 4). Thus, exposure of these 5' regions in 80D cells does not require high levels of gene expression. However, increased gene expression does increase their DNase I sensitivity.

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Fig. 4. Densitometer scans of DNase I chromosomal footprints 5' of GAL10 and GAL1. Part A shows densitometer scans from the autoradiogram which is printed in Fig. 2. Direction of increasing mobility in the gel is from right to left in the scan. Naked DNA bands a and b and the glucose dependent protection at ~215 bp (-----) are located in the scan. The scans are from wild type repressed chromatin (-----), wild type induced chromatin (-----), and 80D chromatin from galactose grown cells (-----). Part B shows densitometer scans from the autoradiogram which is shown in Fig. 3A. Direction of increasing gel mobility is from right to left in the scan. Regions b, c, x,...x, and the TATA region are located in the scan. The scans are from wild type repressed chromatin (-----), wild type induced chromatin (-----), and 80D chromatin from galactose grown cells (-----).
Removal of GAL4 function or super repression in \textsuperscript{80\textdegree} cells does not cause exposure of either protected region (Fig. 3B, tracks 2 and 6). Thus again, so long as there is GAL80 in the cell, the protections are present. The system need not have GAL4 or be GAL\textsuperscript{80}. There is a single TATA box region upstream of \textit{GAL1}, at 680 bp. The GAL80-dependent protections lie around this TATA, on both sides in glucose grown cells but mainly downstream in galactose grown cells (Fig. 3A). The TATA itself is exposed. Although the two differ in their precise relation to the TATA, the \textit{GAL10} and the lower \textit{GAL1}-protected regions may be analogous. In both, protection extends for some distance toward the gene from the TATA and the protection is not strongly gene expression dependent. There is nothing 5' of \textit{GAL10} which resembles the upper protected region 5' of \textit{GAL1}.

The \textit{GAL1} TATA is not strongly cleaved in naked DNA digests (cf. Fig. 3A, track 3), so its hypersensitivity in glucose repressed profiles must be chromatin dependent. Hypersensitivity at this site is also observed in cells in which \textit{GAL1} is not inducible due to regulatory mutations. For example, cells disrupted in \textit{gal4} (Fig. 3B, track 2) or super repressed cells (80\textsuperscript{5}) (Fig. 3B, track 6) show this hypersensitivity. In the mutants, the hypersensitivity is present in any carbon source (cf. Fig. 3B). The hypersensitivity is absent in wild type cells grown in galactose (Fig. 3A, track 2) or glycerol/ethanol (not shown) or in cells lacking the GAL80 protein, even in glucose (Fig. 3B, track 3). Thus, hypersensitivity of the \textit{GAL1} TATA is associated with noninducibility of \textit{GAL1} and depends on GAL80. This hypersensitivity may be a result of the surrounding protected regions.

We also compared coding and noncoding strand profiles on this region. Strand independent features include the protection around region \textit{d} in wild type chromatin, from either carbon source (Fig. 3A, tracks 2 and 6–8) and the accessibilities of the 5' end of \textit{GAL1} (region \textit{e}) and of region \textit{a}. The greatest strand-dependent differences involve the upper protected region in glucose grown cells, (××××, Fig. 3A). In coding strand profiles, the intensity at the TATA is diminished and bands toward the UAS become more prominent (Fig. 3A, tracks 6 and 7). As a result, the upper protected region is much smaller and there is much less difference between profiles from glucose and galactose grown cells in this region on this strand. The constitutively uninducible 4\textsuperscript{5} (Fig. 3B, tracks 1 and 2) and 80\textsuperscript{0} (Fig. 3B, tracks 6 and 7) show the same strand-specific protections in this region as glucose repressed wild type cells. 80\textsuperscript{0} profiles show little or no strand dependence (not shown).

**Micrococcal Nuclease Digests**

Micrococcal nuclease digestion of chromatin from wild type cells produces a distinctive pattern of bands (\textit{a}, \textit{b}, \textit{c}) across the \textit{GAL1–10} intergenic region. DNA from micrococcal nuclease digestion was restricted with EcoRI, electrophoresed on nondenaturing gels, electroblotted to DBM, and probed as described under “Materials and Methods.” Naked DNA profiles are marked with \textit{n}. The sequences essential (West et al., 1984) for UAS function (‒‒‒), TATA boxes (7), and transcription start sites (−−−) are located. Cell type (wild type (\textit{wt}), 4\textsuperscript{5}, or 80\textsuperscript{0}) is shown in bold letters. Carbon source (\textit{D} = glucose; \textit{G} = galactose) is indicated immediately above each track. Some of the \textit{φX}-HaeIII marker fragments used for size determinations are shown (C, 872 bp; D, 603 bp; E, 310 bp; H, 234 bp; I, 194 bp). Other symbols are identified in the text. Electrophoresis is from top to bottom. In \textbf{A} the tracks are, from left to right: wild type grown in galactose (\textit{track 1}), 4\textsuperscript{5} cells grown in galactose (\textit{track 2}), 80\textsuperscript{0} cells grown in glucose (\textit{track 3}) or galactose (\textit{track 4}), naked DNA (\textit{track 5}). \textit{Tracks 6–9} show a darker print of \textit{tracks 1} and 3–5, respectively, focusing on the intergenic region. \textit{Track 6} is overprinted as much as \textit{tracks 7–9} but the bands at \textit{a}, \textit{b}, and \textit{c} are already so dark in \textit{track 1} that they cannot get much darker. However, if there was intensity between \textit{b} and \textit{c} in this track that would become visible in the overprint. In \textbf{B}, \textit{tracks 1–3} are from more extensive digests than in \textbf{A}, showing wild type cells grown in galactose (\textit{track 1}), a naked DNA digest (\textit{track 2}), and 80\textsuperscript{0} cells grown in galactose (\textit{track 3}). \textit{Tracks 4–6} show another extensive set of digests, restricted with TaqI, run on a higher resolution gel and showing more intensity in the intergenic region: a naked DNA digest (\textit{track 4}), wild type (\textit{track 5}), and an 80\textsuperscript{0} (\textit{track 6}) digest from cells grown in galactose.
be in Fig. 5A because smaller DNA produces a less intense signal than higher molecular weight DNA in these hybridizations (Lohr, 1981). Thus, in wild type chromatin the GAL10 intergenic region is quite hypersensitive to micrococcal nuclease. The same type of intergenic region pattern is also present in digests from 4D chromatin (Fig. 5A, track 2), from 80D chromatin (not shown), or from wild type glucose-repressed chromatin (not shown).

The naked DNA digestion pattern is quite different. First, the intergenic region is not hypersensitive (Fig. 5A, track 5). Intensity is distributed more uniformly among the bands throughout the profile. There are also more bands in the intergenic region in naked DNA digests (Fig. 5A). In track 5, bands between \( \beta \) and \( \gamma \) can be seen and in the darker exposure in track 9 bands below \( \alpha \) can be seen (Fig. 5A). Some of the sites which are not cleaved in wild type chromatin are stronger in naked DNA sites than sites which are cleaved (cf. the site between \( \beta \) and \( \gamma \) versus \( \beta \) and the site below \( \alpha \) versus \( \alpha \), track 9). Thus, the DNA not cleaved in wild type chromatin must be protected by chromosomal proteins. These protected sequences lie at 200-270 and 570-630 bp and thus include DNA intergenic region in naked DNA digests (Fig. 5A). In the wild type chromatin profile. This region had run off the higher resolution gel in Fig. 5B (tracks 1 and 2).

The sequences of the lower GALI DNase I-protected region (700-750 bp) may also be protected from micrococcal nuclease digestion in wild type chromatin (cf. Fig. 5B, tracks 1 and 2). However, we cannot unambiguously assess this because these sequences lie very close to the strong \( \gamma \) site in wild type chromatin.

When GAL80 function is removed, the digestion pattern becomes very much like the naked DNA pattern, both in cleavage site utilization and lack of intergenic region hypersensitivity (Fig. 5A, track 4). This similarity is present at all extents of digestion analyzed (cf. Fig. 5B, tracks 2 and 3 or 4 and 6). Thus, the micrococcal nuclease hypersensitivity and protection of DNA from 200 to 270 and 570 to 630 bp in wild type chromatin are GAL80-dependent features.

The very different character of the intergenic region in the presence and absence of GAL80 is shown most clearly in the higher resolution gel in Fig. 5B (tracks 5 and 6). While cleavage in wild type chromatin occurs almost exclusively at the \( \alpha \), \( \beta \), and \( \gamma \) positions, cleavage at the sites below \( \alpha \) is stronger than cleavage at \( \alpha \) and cleavage between \( \beta \) and \( \gamma \) is comparable to cleavage at \( \beta \) in 80D digests. This gel also shows a micrococcal nuclease doublet near the 5' end of GAL10 in the wild type chromatin profile. This region had run off the gel in the other cases shown above.

In the 80D profiles, there is strong cleavage at a site which is not a naked DNA site, at the GAL10 TATA box (Fig. 5B, track 6). In wild type chromatin there are also some cleavages at sites which are not strong naked DNA sites. However, there is no cleavage at the GAL10 TATA (Fig. 5B, track 5). Thus, exposure of this TATA depends on removal of GAL80. Similar results were obtained with DNase I.

In digests from 80D cells grown in glucose, the pattern is intermediate between the wild type and 80D patterns from galactose-grown cells; there is a partial exposure of sites between \( \beta \) and \( \gamma \) (C, Fig. 5A, track 3 or the darker exposure in track 7) but still significant intensity at \( \beta \) and \( \gamma \). A similar overexposure of the wild type chromatin profile shows only the faintest trace of intensity between \( \beta \) and \( \gamma \) (Fig. 5A, track 6). Compared to wild type, there is also less intensity at \( \alpha \) (and more intensity below \( \alpha \)) in profiles from 80D glucose grown cells. The intensity below \( \alpha \) is again only a fraction of that seen in 80D galactose grown profiles (not shown). Although the levels of DNA between \( \beta \) and \( \gamma \) and below \( \alpha \) are modest in these 80D glucose grown digests, their presence at this level is a highly repeatable observation. The same type of pattern is also seen in more extensive digests from 80D glucose grown cells (not shown).

Note that the wild type intergenic results differ from previous results using another strain of presumably wild type yeast, D585-11C (Lohr, 1984). In that strain, there is significant intensity between \( \beta \) and \( \gamma \) and below \( \alpha \) in digests from galactose grown cells. However, we do not see this with the wild type strain (21R) used in this work, in a number of digests or at any extent of digestion. Other intergenic region features like the form of the DNase I hypersensitive region and the expression dependent UAS coldspot (reflecting GAL4/UAS binding) also differ markedly between D585-11C and 21R (Lohr and Hopper, 1985). The significance of these differences is not known. The strain D585-11C is not well characterized. In contrast, the characteristics of the GAL system in 21R have been extensively analyzed, genetically and biochemically (Johnston and Hopper, 1982; Torchia et al., 1984). Thus, 21R is a much more reliable strain for these studies. Furthermore, the major conclusions from this work rely on comparisons of a series of strains congenic with 21R. Such a series provides the cleanest possible system for analysis, by varying one component of the GAL system at a time. Note that the expression-dependent changes within the GAL1 gene itself, noted in the previous analyses (Lohr, 1984), are also seen on GALI in 21R (not shown).

**DISCUSSION**

The negative regulatory protein GAL80 is involved to a significant extent in the structure of GAL1-10 control region chromatin. DNase I shows regions at ~240-280, ~550-610, and ~700-750 bp to be protected in chromatin from cells containing functional GAL80 (wild type, 4D, 80D) but exposed in chromatin from cells lacking GAL80 (80P). Micrococcal nuclease finds sequences from 200-270 and 570-630 bp to behave similarly. Thus, both nucleases detect GAL80-dependent protection in chromatin, in similar regions. Micrococcal nuclease shows that GAL80 is also responsible for an apparent higher order effect, micrococcal nuclease hypersensitivity of intergenic region chromatin.

Both nucleases find the same DNA to be highly nuclease accessible: around 170, 340, 510, and 680 bp for micrococcal nuclease; the 5' end of GAL10 (160-170 bp), between marker fragment E and the GAL4/UAS boundary (300-350 bp), around \( \alpha \) (510 bp), and at the GAL1 TATA (680 bp) for DNase I.

Patterns from the two nucleases differ somewhat in sizes of GAL80-protected regions and in certain cases, their extent of exposure in the absence of GAL80 (cf. 80D cells in glucose) or upon induction of gene expression (cf. wild type chromatin in the far upstream protected region of GAL1). However, GAL80 is probably only one determinant of the nucleoprotein structure on the intergenic region (see below). Some of the differences between DNase I and micrococcal nuclease digests of 80-containing versus 80-lacking chromatin may reflect different responses of the two nucleases to the rest of this complex structure. The two nucleases also differ in a number of enzymatic properties, cf. sequence specificity, preference for single and double strand templates, etc. Thus, it seems most significant, at least for this work, that GAL80 removal changes patterns from both nucleases in basically similar ways.

Expressed genes are more sensitive to nucleases (cf. Reeves, 1984). Do such effects contribute to these results? Comparison
of galactose grown profiles from 80D and wild type cells shows some very striking differences, which we suggest are due to GAL80 protections, in wild type chromatin. In both of these cell types, GAL1 and 10 are very strongly expressed under these conditions. However, expression is slightly higher (1.5-fold) in 80D. This small expression difference seems unlikely to explain the large profile differences, especially since in both cell types expression occurs at such a high level. Comparison of profiles from 80D glucose grown cells to profiles from wild type galactose grown cells provides a more compelling argument. All the protections present in the wild type are at least partially lost in the 80D chromatin, including both the TATA protections, as probed by DNase I and the protection far upstream of GAL1, as probed by DNase I and micrococcal nuclease. Yet, GAL1 is expressed at ~30-fold lower levels in the 80D cells. Thus, gene expression cannot explain the exposure of DNA in these 80D digests. A glucose effect cannot explain the exposure either because profiles from wild type glucose grown cells are, if anything, more protected in these regions than profiles from wild type galactose grown cells. Thus, the absence of GAL80 must be largely responsible for exposure of these sequences. However, at least for the GAL1 protections, there is a further exposure when the genes are expressed in 80D cells. Thus, absence and gene expression can both increase nuclease accessibility in this region. The wild type results are consistent with this view, induction (in the presence of GAL80) produces at best a partial exposure of protected regions. It may be important that 80 removal and gene expression both can produce similar effects in the upstream region regarding the possible mode of action of GAL80 as a negative regulatory factor.

Structural changes, interpreted as transcription-dependent, around the GAL1–10 TATA boxes have recently been reported in a UV photofootprinting study (Selleck and Majors, 1987). Most of their conclusions were based on comparisons of patterns from wild type and 80D cells grown in glycerol. Thus, around the GAL1–10 TATA boxes have recently been reported in this work are shown in Figure 6. Naked DNA sites cleaved in the wild type, expressed in 80D, there are differences due to the GAL80 protection in wild type chromatin. In both of these conditions. However, expression is slightly higher (1.5-fold) in 80D. This small expression difference seems unlikely to explain the large profile differences, especially since in both cell types expression occurs at such a high level. Comparison of profiles from 80D glucose grown cells to profiles from wild type galactose grown cells provides a more compelling argument. All the protections present in the wild type are at least partially lost in the 80D chromatin, including both the TATA protections, as probed by DNase I and the protection far upstream of GAL1, as probed by DNase I and micrococcal nuclease. Yet, GAL1 is expressed at ~30-fold lower levels in the 80D cells. Thus, gene expression cannot explain the exposure of DNA in these 80D digests. A glucose effect cannot explain the exposure either because profiles from wild type glucose grown cells are, if anything, more protected in these regions than profiles from wild type galactose grown cells. Thus, the absence of GAL80 must be largely responsible for exposure of these sequences. However, at least for the GAL1 protections, there is a further exposure when the genes are expressed in 80D cells. Thus, absence and gene expression can both increase nuclease accessibility in this region. The wild type results are consistent with this view, induction (in the presence of GAL80) produces at best a partial exposure of protected regions. It may be important that 80 removal and gene expression both can produce similar effects in the upstream region regarding the possible mode of action of GAL80 as a negative regulatory factor.

Structural changes, interpreted as transcription-dependent, around the GAL1–10 TATA boxes have recently been reported in a UV photofootprinting study (Selleck and Majors, 1987). Most of their conclusions were based on comparisons of patterns from wild type and 80D cells grown in glycerol. Thus, in addition to differences in expression (unexpressed in wild type, expressed in 80D), there are differences due to the presence or absence of GAL80. Evidently, these workers assumed there were no GAL80/DNA interactions. Many of the changes they detect coincide with features we note: changes exactly at the GAL10 TATA; eight changes in the 530–680-bp region, six of which are located between 530 and 630 bp and thus fall in or near the upper protected region 5′ of GAL1. Thus, many of the changes noted by Selleck and Majors (1987) have GAL80 dependence as well as expression dependence.

GAL80 is likely to be more than simply a DNA-binding protein on the GAL1–10 intergenic region. It may affect DNA indirectly, acting via other proteins, for example, TATA-binding proteins. GAL80 may also be part of a multiprotein regulation complex located on the intergenic region (cf. Echols, 1986).

One possibility is a GAL4-GAL80 complex, as has been previously suggested (Matsumoto et al., 1978; Perlman and Hopper, 1979). The DNA-protein interactions we observe (GAL4 and GAL80 dependent) certainly do not preclude such protein-protein interactions as well. We note that GAL4- and GAL80-dependent DNA binding domains are distinct, their closest boundaries are about 60 bp apart, and the DNA between these domains is quite accessible to nuclease. Thus, a GAL4-GAL80 complex must always lie far enough away from the DNA in these interdomain regions to allow ready nuclease access.

Nucleosomes are another possible type, or perhaps only another component, of intergenic region multiprotein complex involving GAL80. The spacing of micrococcal nuclease and DNase I accessible sites across the intergenic region is certainly suggestive of their presence. Nucleosome folding would compact the DNA in this region and thus could facilitate interactions among the various DNA elements. Note that the sequences protected from nuclease cleavage support nucleosome coverage for the 170–340-bp region and the 510–680-bp region but not for the UAS except by GAL4 (see above; also Lohr and Hopper, 1985). A map of nuclease protections and possible nucleosomes on this region is shown in Figure 6.

What kind of, or indeed whether, a multiprotein complex is present on the GAL1–10 intergenic region remains unknown. However, it is clear that GAL80 plays a significant role in the chromatin structure of this region. Thus, if such a complex is present, GAL80 is very likely to be a major component of it.

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