Volume 13 Number 23 1985 Nucleic Acids Research

The relationship of regulatory proteins and DNase I hypersensitive sites in the yeast *GAL1-10* genes

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Received 9 September 1985; Revised and Accepted 13 November 1985

ABSTRACT

We have used yeast strains containing a disrupted positive (GAL4) and/or a disrupted negative (GAL80) regulatory gene to investigate the relationship of these regulatory proteins to the hypersensitive sites upstream of their target genes, GAL1-10. We find that neither of these regulatory proteins is required for the formation of the hypersensitive region. There is positive regulatory protein (dependent) binding to a portion of the hypersensitive region when GAL1 and 10 are expressed. However, similar binding can also occur under conditions in which the genes are not expressed. Thus, such binding is necessary but not sufficient for expression of GAL1 and 10 and control of GAL1-10 expression must also include processes which occur subsequent to GAL4/DNA binding. The negative regulatory protein GAL80 plays a significant role in these processes.

INTRODUCTION

DNase I hypersensitive sites are a general feature of the 5' upstream regions of eukaryotic genes (1). They appear to reflect functionally interesting regions of the chromosome (2) and often, but not always, are correlated with expression of the genes with which they are associated (3). Proteins have been shown to play a role in the generation of hypersensitive sites (4). The nature of the proteins involved is not known, although there are indications that they may be regulatory proteins for the associated gene (5). Hypersensitive sites can also be loci for the binding of proteins not involved in generating the hypersensitivity, again presumably a regulatory protein for the associated gene (6).

The recent observation of DNase I hypersensitive sites upstream of the GAL1-10 genes in yeast (7,8), together with the existence of defined regulatory gene mutations for this system, provides an excellent opportunity to examine the relationship between regulatory proteins and hypersensitive sites. GAL1 and GAL10 are two of the structural genes required for the utilization of galactose as a carbon source in yeast. They are divergently transcribed from an ~600 bp region of DNA (9). Their expression is carbon

source dependent: expressed in galactose; readily inducible, but not yet expressed, in glycerol/ethanol; totally repressed in glucose. When induced, these genes are very actively expressed, at least 1000-fold over the uninduced level (9). Although the hypersensitive sites associated with these genes are not expression dependent, i.e., are present in both the active and inactive states of the gene, the sites occur in a very interesting location (7), near the center of the Upstream Activator Sequence (UAS). This is the control element through which the induction of expression for GAL10 and GAL1 is mediated, by the action of the positive regulatory protein GAL4 (10,11). We will refer to this region as the Upstream Regulatory Sequence (URS) to denote the possibility that other regulatory proteins could also interact with this region.

The regulatory genes for the GAL system have been rather well characterized. GAL4 is a positive activator whose presence is absolutely required for expression (12). It exerts control at the level of transcription (13-15). GAL80 is a negative regulatory protein which prevents high level expression of the structural genes in the absence of inducer (16). Two other regulatory genes are involved in the wild-type GAL phenotype. GAL3 appears to be required for normal rapid induction (17) and GAL11 for full wild type levels of structural gene product (18). Little is presently known about GAL3 or GAL11. The GAL4 (19,20) and GAL80 (21) regulatory genes have been cloned, mutants in one or both of these genes created by gene disruption techniques (22) and the mutant regulatory genes replaced into their usual chromosomal context ((21); Johnston and Hopper, unpublished results). In this work, we use these mutants to investigate the role the regulatory proteins play in the chromatin structure of the GAL1- 10 intergenic region.

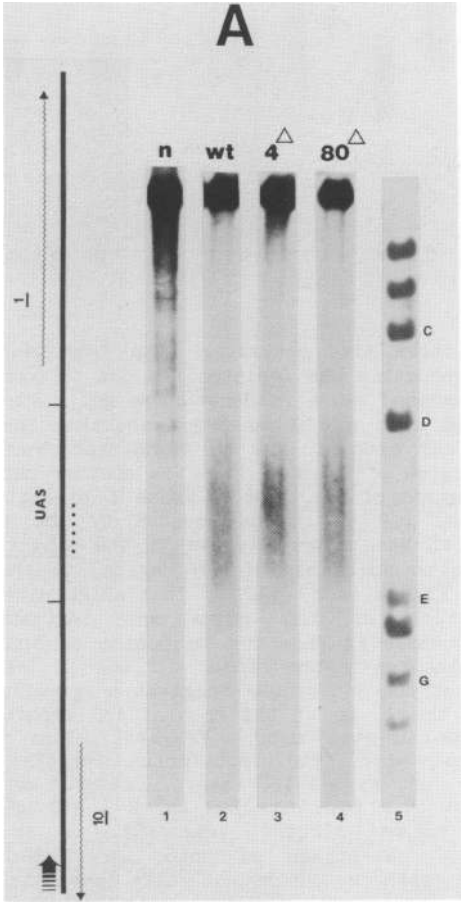
MATERIALS AND METHODS

Yeast strains were constructed as described previously (19,21). Strains were grown to log phase with shaking at 30°C in YEP (0.5% Yeast Extract, 1% Bactopeptone) supplemented with 1) 2% glucose/3% glycerol/2% ethanol, "D" or 2) 2% galactose/3% glycerol/2% ethanol, "G" or 3) 3% glycerol/2% ethanol, "g". Nuclei were isolated, DNase I digestions performed and DNA extracted as described previously (7). The DNA samples were then recut with EcoRI (for the hypersensitive site analysis) or Taq I (for the footprint analysis). Naked DNA DNase I digestions were performed at the same DNA concentrations as for nuclear digests but lower [DNase I], then isolated and recut with restriction enzymes as for the chromosomal samples. Na_2 EDTA was added to 10 mM, the DNA

extracted once with isoamyl alcohol/chloroform and the DNA precipitated in ethanol. DNA was redissolved and electrophoresed on nondenaturing 2.4% polyacrylamide/0.5% agarose composite gels or 4.8-5.5% polyacrylamide/0.6% agarose/7 M urea denaturing gels. DNA was transferred to DBM paper, hybridized with a 120 bp RI-Dde I or RI-Rsa I probe labelled by repair synthesis and exposed to x-ray film, all as described previously (7).

RESULTS AND DISCUSSION

Because the GAL1-10 hypersensitive region is present even when the genes are repressed by glucose, one can examine the requirements for generation of the hypersensitive region independently from effects involved in, or arising from, gene expression. This analysis is shown in Figure 1A. Lane 2 shows the



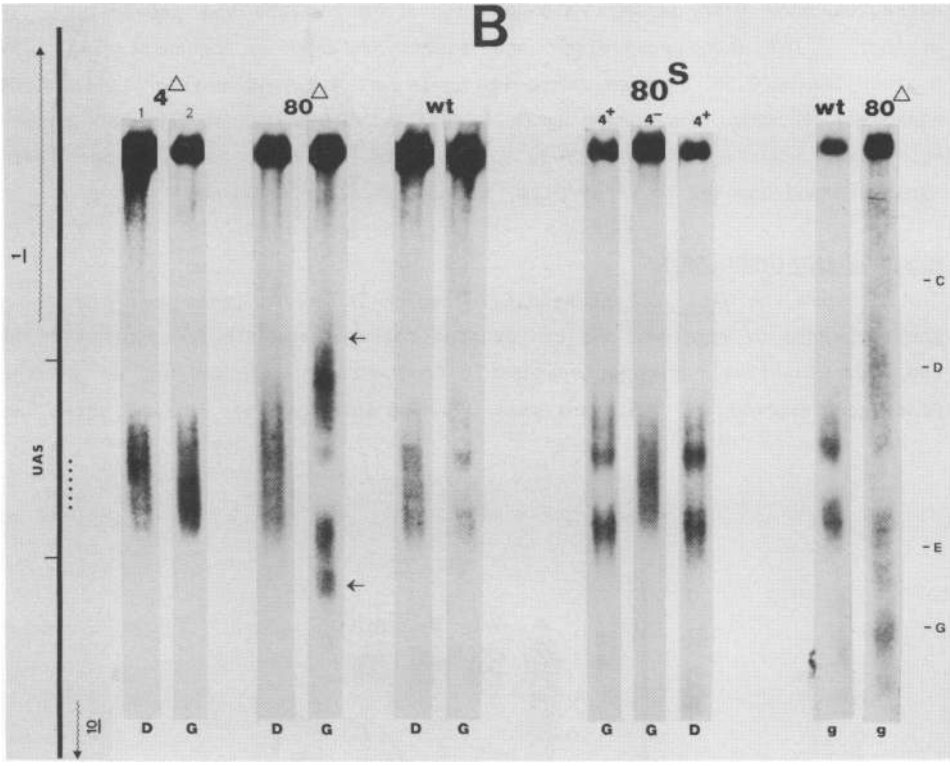


Figure 1 Hypersensitive site analysis: DNA from DNase I digested yeast nuclear chromatin was isolated and cut to completion with Eco RI, electrophoresed on a nondenaturing gel, transferred to DBM and hybridized with a 120 bp probe abutting the RI site in GAL10. This Eco RI site is ~200 bp downstream from the 5' end of the coding region of GAL10. There is another near the 3' end of the coding region of GAL1 (9). DNase I digestion sites are mapped within the 1.9 kb region bounded by the two RI sites. The location of the probe relative to the GAL1-10 genes is shown in the drawn to scale map of the region, to the left of the first track in Figure 1A ("▮▮▶"). Initiation sites and directions of transcription of the genes are indicated by " ⟶ ". Electrophoresis is from top to bottom so that smaller DNA sizes locate cleavage sites closer to the RI site in GAL10. The location of the 365 bp DNA fragment originally defined as the UAS (10) and the 75 bp of DNA crucial for induction (30) "...." are also shown in the map. Fragment sizes were determined by comparison with the mobilities of ϕX-Hae III restriction fragments, cf. Figure 1A, lane 5. Several of these fragments are identified (C = 872, D = 603 bp, E = 310 bp, G = 234 bp). The various mutant strains are: "4^{Δ}", a strain disrupted in GAL4; "80^{Δ}", a strain disrupted in GAL80; "$80^{S},4^{+}$", a super repressor with a functional GAL4 present; "$80^{S},4^{-}$", a super repressor strain with a mutant gal4 protein. Only 80^{Δ} and wild

type cells ("wt") can exhibit high levels of GAL1-10 expression and only in the appropriate medium (see text). A) The hypersensitive region from several strains, all grown in glucose. The strains are identified at the top of the track. All samples were electropheresed on the same gel. A digestion profile from naked DNA is shown in lane 1. B) The analysis of carbon source dependence. Again, strains are identified at the top. The carbon source used in growth is noted below the track. In the text, carbon source is in parenthesis, i.e. strain gal4$^{\Delta}$ grown in glucose is 4$^{\Delta}$ (D). Samples in lanes 1-10 are from the same gel. Lanes 1 and 2 are numbered, for reference. Again the mobilities of several ϕX-Hae III restriction fragments run on these gels are shown (C = 872 bp; D = 603 bp, E = 310 bp, G = 234 bp).

DNase I hypersensitive region from a wild type strain (with respect to the GAL regulon) which is isogenic with the regulatory gene disruption mutants used in this study and closely related to two other regulatory mutants used (see Table I). Both the location (near the center of the URS) and the size (~150 nucleotides) of the hypersensitive region are very similar to that reported previously (7) for the GAL1-10 hypersensitive region in another wild-type yeast strain.

As reported previously (7), the hypersensitive region is a chromatin feature, since the region is not hypersensitive in naked DNA digests (Figure 1A, lane 1). The brief digest shown in lane 1 is closely matched in digestion extent with chromatin samples in lanes 2-4 but shows no evidence of URS hypersensitivity. With increasing digestion, intensity spreads uniformly throughout the naked DNA profile, with no preferential URS sensitivity (not shown). Lane 1 was obtained from the wild type strain SJ21R (Table 1). Since the mutants used in this work involve changes at regulatory loci, not at the structural gene locus (GAL1-10), this control is appropriate for all strains which will be analyzed.

Surprisingly, the absence of one or both regulatory proteins has little effect on the hypersensitivity. In a strain in which GAL4 has been disrupted and in which no wild type GAL4 mRNA can be detected (Johnston, S. and Hopper, J. E., submitted for publication), the hypersensitive region is present and is not detectably different from the wild-type hypersensitive region (Figure 1A, wt vs 4$^{\Delta}$). Thus, the hypersensitive region does not appear to depend on the presence of the positive regulatory gene GAL4 for its existence. However, because of the way in which the strain was constructed, it is possible that a 7 kD portion of the amino terminus of GAL4 could still be made in these cells. Even though such a fragment has not been detected, it remains formally possible that this fragment is present and that it is responsible for the

Table I S. cerevisiae Strains

Strain	Comments	Relevant Genotype	GAL1-10 Expression	Source
*SJ21R	wt=wild type	GAL4GAL80	induced in galactose	ref (19)
*SJ21R-4$^{\Delta}$	4$^{\Delta}$=GAL4 disruption	gal4GAL80	uninducible	Johnston and Hopper, (unpublished)
*TT21R-80$^{\Delta}$	80$^{\Delta}$=GAL80 disruption	GAL4gal80	constitutive	ref (21)
SJB80^{S}	80^{S},4^{+}=super-repressor	GAL4GAL80^{S}	uninducible	ref (19)
SJF80^{S}	80^{S},4^{-}=super-repressor mutant GAL4	gal4GAL80^{S}	uninducible	ref (19)

A brief description of the strains used is given above. The strains shown by "*" are isogenic with one another. These strains are closely related to the 80^{S} strains (coefficient of kinship = 0.35 (19)). Note that the glucose repression system is still in operation in strain 80$^{\Delta}$. Thus, GAL1-10 is expressed with or without galactose but expression is repressed by glucose.

hypersensitive region noted in 4$^{\Delta}$ cells. We think this is unlikely because, as will be shown below, we can detect a GAL4 dependent binding event (in other strains under other conditions) but these 4$^{\Delta}$ cells never show any evidence of the striking features associated with that event.

In a disruption mutant of the negative regulatory protein GAL80, in which no wild type GAL80 mRNA can be detected (21), the hypersensitive region also remains present (Figure 1A, 80$^{\Delta}$ vs wt). Again, its location is the same, near the center of the URS. The hypersensitive region often does appear to be slightly larger in gal80$^{\Delta}$ digests but the significance of this is unknown. Thus, the hypersensitive region does not depend on the negative regulatory protein GAL80 for its existence either. Using a strain which is disrupted for both regulatory genes, we also examined the unlikely possibility that the hypersensitive region requires only the presence of either GAL4 or GAL80 and can thus exist in the absence of either protein. Again, the hypersensitive region remains present (not shown). Thus, the positive and negative regulatory proteins, GAL4 and GAL80, appear to play little or no role in generating the GAL1-10 URS hypersensitive region.

We then looked at the effects of these regulatory proteins on the hypersensitive region in nuclei from cells grown in the presence of galactose. Galactose induces expression of GAL1-10 in the wild type. Since functional GAL4 is required for expression of the GAL1-10 genes, gal4^{-} cells cannot grow with galactose as a sole carbon source. Therefore, all comparisons in this study use the following carbon sources: glucose/glycerol/ethanol (repression

conditions); galactose/glycerol/ethanol (expression conditions); glycerol/ethanol (inducible conditions). This allows us to analyze the effect of the presence of galactose in the medium, even in genotypically noninducible cells. In the strain in which GAL4 is disrupted, there is no carbon source dependence; the hypersensitive region is the same when glucose or galactose is present (Figure 1B, 4^{Δ}(D vs G)). The slight intensity distribution differences between the hypersensitive regions in the two 4^{Δ} tracks in Figure 1B are not reproducibly present. In the strain in which GAL80 is disrupted, there is a striking carbon source dependent change in the hypersensitive region (Figure 1B, 80^{Δ}(D vs G)). In nuclei from galactose grown cells, a large portion of the central part of the normally hypersensitive region loses its DNase I sensitivity entirely. (For convenience, we will refer to a region which loses hypersensitivity as a coldspot). There remains some intensity in the distal portions of the normally hypersensitive region. However, most of the DNase I hypersensitivity now resides in regions which are not hypersensitive in wild type or 4^{Δ} cells in any carbon source, or in 80^{Δ} cells in glucose. One of the newly hypersensitive regions lies in a TATAA box region for GAL10 and another lies very near a TATAA box region for GAL1 (" ← ", Figure 1B, 80^{Δ}(G)). This remarkable pattern of hypersensitivity is highly reproducible and is not dependent on digestion extent, within the range of digestion useful for hypersensitive site analysis.

The pattern from the wild-type strain isogenic with this set of mutants shows some of these carbon source dependent changes. From cells grown in galactose, the URS region is still hypersensitive but there is a loss of intensity near the center of the region, as in the 80^{Δ} cells. However, the coldspot in the wild type strain is somewhat smaller than that observed for gal80^{Δ} cells (Figure 1B, wt(G) vs 80^{Δ}(G)) and hypersensitivity is definitely not shifted to new regions as in the 80^{Δ} cells.

Previous work (7) showed much less striking carbon source dependent differences in the URS region than we see in strain 21R, although the two strains show qualitatively similar behavior. One of us (Lohr) would like to point out that some lanes in Figure 1 of that publication (7) were mislabelled: lane 4 (Figure 1, reference 7), which shows a very small, coldspot-like feature, is from galactose grown cells; lanes 2-3 (Figure 1, reference 7) with no coldspot, are from glucose grown cells. We have confirmed the apparent quantitative differences in coldspot size between the two strains by direct comparison (on the same gel) of the hypersensitive regions from both strains grown in galactose (data not shown). The cause of

these strain differences are not known. They may be related to the fact that the GAL4 in the strain used here was selected as a phenotypic wild type revertant of a $gal4^-$ mutant (19). However, Johnston and Hopper have shown by enzymatic assay of GAL proteins that this revertant behaves like an authentic GAL wild type (19). Although these wild type strain differences may be interesting, they are not pertinent to this work since the strain 21R, shown above in Figure 1B, is the comparable one for the mutants used here.

The GAL4 dependent induction of GAL1-10 is mediated through the DNA in this region (10,11) and it has recently been shown, from methylation protection studies, that GAL4 binds here (23). Protein binding would certainly be expected to decrease the DNase I accessibility of the bound DNA sequences and thus could explain the loss of hypersensitivity noted above. The presence of the coldspot in the wild-type but not in the 4^Δ cells (Figure 1B, 4^Δ(G) vs. wt(G)) shows clearly that the coldspot is GAL4 dependent, since these two strains differ only in the presence of GAL4.

To demonstrate that the coldspot reflects protein binding, we applied the DNase I footprinting technique (24), which has demonstrated ability to detect DNA/protein interactions. We used an indirect end-labelling approach (25,26) and have analyzed low resolution footprints from nuclear chromatin digests. The $gal80^\Delta$ cells grown in glucose, which do not show a coldspot, show a continuous pattern of intensity over the URS region (Figure 2, 80^Δ(D)). This pattern is similar, but not identical to, the pattern from a naked DNA digest (Figure 2, lane 1). When the 80^Δ strain is grown in galactose, conditions which produce a coldspot, sites in two regions of the footprint become strongly protected from DNase I ("]", Figure 2, 80^Δ (G)). Both of these regions show prominent cleavage in a naked DNA digest (Figure 2, lane 1) and in the glucose grown chromatin digests (Figure 2, 80^Δ (D)). The protected regions are absent in cells lacking GAL4 protein, even when grown in the presence of galactose (Figure 2, 4^Δ(G)). These cells also show no coldspot (Figure 1B, 4^Δ(G)). Thus, the coldspot near the center of the hypersensitive region must result from the binding of protein, presumably GAL4 protein. If it is not GAL4, it is a protein whose binding to this region is GAL4 dependent.

These GAL4 dependent protected regions in 80^Δ(G) lie approximately 350-440 nucleotides and 460-485 nucleotides from the RI site in GAL10 and thus map to the same general region of DNA as the coldspot. Fragment sizes (and thus locations) were determined by comparison with ϕx 174-Hae III marker restriction fragments. Because of the possibility of sequence specific

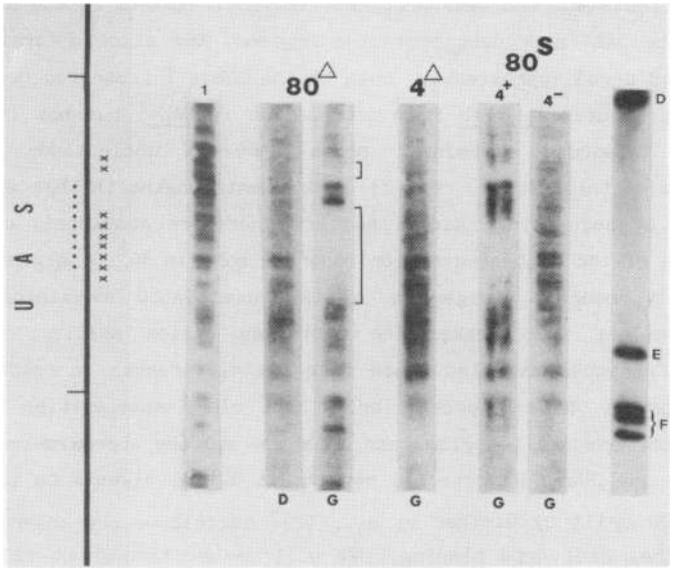


Figure 2 Footprint Analysis: Nuclear DNase I digestion samples, obtained as in Figure 1, were recut with Taq I, isolated and electrophoresed on a denaturing gel, transferred to DBM and hybridized with a probe abutting the Taq I site in GAL10, all as described previously (7). One Taq I site is virtually coincident with the RI site in GAL10 while the other is ~300 bp downstream from the 5' end of the GAL1 coding sequences. Cleavage sites are mapped within this 1100 nucleotide region. Electrophoresis is from top to bottom so smaller DNA sizes locate sites closer to GAL10. These are low resolution footprints because the gels used are not able to resolve single nucleotides, although they do cover more extensive ranges than higher resolution gels. A drawn to scale map of the URS region showing the 75 bp of DNA which are crucial for induction (30) "...." and the regions protected from methylation in GAL4$^+$ cells grown in galactose (23) "x" are shown in the map to the left of the first track. Lane 1, which is numbered for reference, shows a naked DNA profile from this region. Sizes were determined by comparison with end labelled ϕX174-Hae III restriction fragments present on the gels. Several of these (D = 603 bp, E = 302 bp, F = 281/271 bp) are shown and identified to the right. Note that individual strands of the smaller fragments are resolved on these gels so that there are 4 bands for the F region, which consists of 2 fragments (281 and 271 bp). Again, strains are identified at the top and carbon source used in growth is noted below the track. A repaired RI-Rsa probe was used to obtain these profiles. Thus, this data maps digestion sites only on the GAL10 coding strand or the "top" strand in the nomenclature of Giniger et al. (23). A more detailed footprint analysis of this region, including noncoding strand maps and a discussion of other interesting features noted, will be presented elsewhere (Lohr and Hopper, work in progress).

mobility effects and the dearth of restriction fragments in the exact DNA size range of the GAL4 dependent protected regions, the sizes determined here can only be considered approximate. Both of the DNase I protected regions contain sequences protected *in vivo* from methylation in *GAL4*$^+$ but not in *gal4*$^-$ cells grown in galactose containing media (369-406 nucleotides and at 470 nucleotides on this strand, ref. 23). Fragment lengths in that work (23) were determined by sequencing ladders and are therefore absolutely correct. The larger size of the protected region reported here in 80$^\Delta$(G) digests arises, at least partly, because Giniger et. al (23) used GAL80 containing strains and the presence of GAL80 makes the protected region smaller, as discussed below. Differences may also arise from the differences in features assessed (methylation vs DNase I protection). The close similarities in form (one large region, one small region) and location and the approximate similarities in size of our DNase I protected regions in 80$^\Delta$(G) digests to those observed in GAL4GAL80 cells by Giniger et al., (23) suggest we are observing the same event as they did, GAL4 binding. We will assume throughout this paper that all the GAL4 *dependent* binding events we observe actually reflect GAL4 protein binding, based on the analogy of our results to the results of Giniger et al. (23). The sequences protected from DNase I in nuclear chromatin correspond approximately to the sequences protected from DNase I *in vitro* by a purified protein extract containing a URS binding activity (370-415 and 470-505 nucleotides, ref. 27). Sizes in the latter work were also determined by comparison with ΦX-Hae III marker restriction fragments (27).

Other workers have also detected small, protein bound regions located within larger hypersensitive areas and speculated that the binding proteins involved might be regulatory molecules for the associated structural genes (5,6). The similar observations made here with a defined regulatory protein certainly strengthen those suggestions. However, results with the other gene sets are not totally analogous to *GAL1-10*. In both the heat shock and β-globin genes, multiple factors appear to be involved. Furthermore, in the β-globin work, the only proteins which have so far been shown to bind within the hypersensitive region are also the factors responsible for making the region hypersensitive (5). This is not the case for *GAL1-10* since neither GAL4 nor GAL80 protein is required for the existence of the hypersensitive region. *GAL1-10* appears to be more similar to the Drosophila heat shock genes, where at least one of the (regulatory) protein factors binds to an already existing hypersensitive region under conditions of gene expression (6). Although the factors responsible for the formation of the hypersensitive

region are still not known in either gene set, our work suggests the possibility that the factors responsible for the hypersensitive region in GAL1-10 may not be GAL1-10 specific. In agreement with this, we have preliminary evidence that another GAL1-10 associated regulatory protein, GAL3, plays no role in setting up the hypersensitive region (Lohr, Torchia and Hopper, unpublished results).

We also examined the effect of GAL4 in two super-repressing mutants of the GAL80 gene ($GAL80^S$). These strains are closely related to the others used in this study (Table 1). The gal4GAL80^S strain is not a disruption but arose from a naturally occuring mutation in GAL4. Even though GAL4 GAL80^S cells cannot express GAL1-10 in the presence of galactose, they show a coldspot near the center of the hypersensitive region (Figure 1B, $80^S,4^+$ (G)). The pattern resembles the pattern from wild-type cells grown in galactose (Figure 1B, wt(G)). However, the gal4$^-$ strain carrying the same allele of GAL80^S shows an intact hypersensitive region (Figure 1B, $80^S,4^-$(G)), showing that the coldspot is a functioning GAL4 dependent phenomenon. GAL4GAL80^S also shows a protected region in the DNase I footprint while gal4GAL80^S does not (Figure 2, $80^S,4^+$ vs. 4^-). Therefore, this coldspot in the uninducible GAL4GAL80^S strain must reflect GAL4 dependent protein binding. This shows that GAL4 dependent binding to the URS can be uncoupled from induction of the GAL1-10 genes.

Surprisingly, we also detect a coldspot in the GAL80^S strain grown in glucose (Figure 1B, $80^S,4^+$(D)). This coldspot is GAL4 dependent because it is absent in $80^S,4^-$ cells grown in any carbon source (cf Figure 1B, $80^S,4^-$(G)). $80^S,4^+$ cells grown in glucose also show two protected regions in the DNase I footprint (not shown). We have no certain explanation for the apparent GAL4 binding to the URS under these conditions. It is possible that there is very low level expression from these genes in 80^S cells in glucose. For example, another GAL structural gene, MEL1, is expressed at low level in glucose grown cells containing this allele of 80^S (Post-Beitenmiller, M. and Hopper, J., unpublished observations). However, if there is expression of GAL1 and 10 under these conditions, we would expect it to be quite low since expression of GAL7 is below the limits of detection by enzymatic assay (19) in glucose grown cells containing this allele of 80^S. GAL7 is another structural GAL gene, lying downstream of GAL10, which is presumed to be regulated like GAL1 and 10.

The data from the 80^S cells also illustrate the tight correlation that we observe between coldspot presence and protected regions in the footprint. In all cases in which we have seen the coldspot in the hypersensitive region, we have also observed evidence of protein binding to the same region in the

footprint. In the absence of a coldspot, we see no evidence for protein binding in the footprint. This correlation includes the wild-type strain (footprint not shown).

We also examined the GAL1-10 hypersensitive region under circumstances in which the genes are readily inducible but not actually expressed (i.e. in cells grown in glycerol/ethanol). In the wild type strain, chromatin from glycerol/ethanol grown cells shows a coldspot of the same size and at the same location as that from galactose grown cells (Figure 1B, wt(G) vs wt(g)). The intensity differences between these two tracks are a result of different digestion extents in the samples. The footprints from the two are also similar (not shown). This shows that there is GAL4 (dependent) protein binding to this region of DNA when the cells are in the inducible state, even though the genes are not expressed at detectable levels under these conditions. Therefore, this binding is not sufficient for gene expression. Such a conclusion is supported by the data from the $GAL80^{S}$ strain, which shows a wild type GAL4 (dependent) interaction with the URS, even though the GAL1 and 10 genes cannot even be induced in this strain. These observations allow us to distinguish two stages in GAL1 and GAL10 expression. One stage involves the GAL4 (dependent) protein binding to the URS region. The second stage includes the actual process of transcription. In wild type cells, the first stage occurs whenever functional GAL4 is present in nonrepressing carbon sources (i.e., galactose or glycerol). It is a necessary but not sufficient condition for expression.

The $gal80^{\Delta}$ cells provide information about the second stage of expression. In 80^{Δ} cells, the GAL genes are very actively expressed in glycerol/ethanol, in contrast to wild type cells (21). Since GAL4 is bound to this region of DNA under these conditions in both cell types (Figure 1B, wt(g) vs 80^{Δ}(g); footprints not shown), this difference indicates that the GAL80 negative regulatory protein plays a major role in the control processes of gene expression which occur subsequent to the binding of GAL4 to the URS. Thus, in the absence of GAL80, the binding of GAL4 is sufficient to trigger events required for expression and the control exerted at the second stage of expression is lost. Because the wt and 80^{Δ} strains are isogenic, they differ only in the presence of the GAL80 protein and the differences noted must reflect the action of GAL80.

The hypersensitivity and the footprint data both suggest that some aspects of GAL4 binding to the URS in $gal80^{\Delta}$ cells differ from GAL4/URS binding in other cell types. In the hypersensitive pattern, there is transfer

of hypersensitivity to DNA sequences outside of the usual hypersensitive region, upon GAL4 binding in the absence of GAL80 (Figure 1B, 80^{Δ} (G)). From galactose grown cells, the regions which become newly hypersensitive lie closer to the genes being expressed and include TATA box sequences. This newly acquired sensitivity may reflect a chromatin change which acts to aid RNA polymerase in locating these regions, much as the URS hypersensitivity could help GAL4 find its binding site. Or this new sensitivity could be the result of some aspect of the process by which GAL4 mediates gene expression, such as polymerase entry. The expression level of GAL genes in 80^{Δ} cells grown in galactose is about 1.5 times the level in wild type cells grown in galactose (21). This higher level of expression may be the reason we can detect these hypersensitivity changes in the 80^{Δ} cells. A similar process may be part of the second stage of gene expression in wild type cells and modulating this process one of the roles GAL80 plays in regulating the GAL structural genes. This is consistent with the suggestion that even the fully induced wild type expression level reflects a balance between the effects of GAL4 and GAL80 (and perhaps other regulatory proteins) and is not necessarily the maximum possible intrinsic to the promoter (19).

In chromatin digests from 80^{Δ} cells grown in glycerol, the hypersensitivity appears to be transferred to even more of the transcription unit (Figure 1B, 80^{Δ}(g)). The expression level of GAL1 in these cells is 3 times the expression level from wild type cells grown in galactose. This very high level of expression could be responsible for the enhanced hypersensitivity transfer. Thus, there appears to be a rough correlation between expression level and extent of hypersensitivity transfer (wt < 80^{Δ}(G) < 80^{Δ}(g)).

In the DNase I footprint, the larger protected region is more extensive (by 20-30 nucleotides) in 80^{Δ} cells than in other cell types (cf Figure 2, 80^{Δ}(G) vs $80^{S},4^{+}$(G)), including wild type cells (not shown). As mentioned previously, this protected region is also larger than the region protected from methylation *in vivo* (23) or from DNase I *in vitro* (27) in GAL4GAL80 cells. The increased protection occurs at the high molecular weight end (i.e. distal to GAL10) of this protected region. The increased protection in 80^{Δ} cells suggests that GAL4 can interact more extensively with the URS region in the absence of GAL80. This increased interaction might be related to the increased expression of the associated structural genes in 80^{Δ} cells compared to wild type (21). Perhaps the more extensive binding is in itself more efficient for expression. Alternatively, the GAL80 protein might inhibit the

passage of other proteins such as RNA polymerase, resulting in freer movement of these proteins in its absence. These or other possibilities cannot currently be distinguished. The occurence of changes in the GAL4/DNA interaction in gal80$^-$ cells is quite consistent with the model suggesting that GAL4 and GAL80 proteins interact in the process of regulating the GAL structural genes (19,28,29). Thus, the chromatin structure one observes in wild type cells under normally expressing conditions probably results from the combination of the effects of GAL4 and GAL80, as does the expression level.

Both expression dependent and constitutively present hypersensitive sites have been noted (1). The GAL1-10 URS region is an example of the latter. It may be a general feature of this class of hypersensitive sites that they reflect preselected regions of the chromosome to which positive regulatory proteins bind, as one (necessary) step in the process of gene expression, as appears to be the case for GAL1-10. The preexpression binding of the positive activator may be a feature which facilitates the rapid induction of these genes. Work with this system should lead to further insights on how eukaryotic genes are controlled.

ACKNOWLEDGEMENTS

We thank J. Lopez for technical assistance, Anna Leon for patient typing of the manuscript, Drs. T. St. John and R. Davis for GAL clones and PHS grants GM27623 and a Research Career Development Award, CA00911, for funding support for the Lohr lab and PHS grant GM 27925 for funding support for the Hopper lab.

REFERENCES

1. Elgin, S. (1981) Cell 27, 413-15.
2. Jongstra, J., Reudelhuber, T., Oudet, P., Benoist, C., Chae C., Jeltsch, J., Mathis, D. and Chambon, P. (1984) Nature 307, 708-14.
3. Elgin, S. (1982) Nature 300, 402-3.
4. Emerson, B. and Felsenfeld, G. (1984) Proc. Natl. Acad. Sci., USA 81, 95-99.
5. Emerson, B., Lewis, C., and Felsenfeld, F. (1985) Cell 41, 21-30.
6. Wu, C. (1984) Nature 309, 229-234.
7. Lohr, D. (1984) Nuc. Acids Res. 12, 8457-74.
8. Proffitt, J. (1985) Mol. Cell. Biol. 5, 1522-4.
9. St. John, T. and Davis, R. (1981) J. Mol. Biol. 152, 285-315.
10. Guarente, L., Yocum, R. and Gifford, P. (1982) Proc. Natl. Acad. Sci., USA 79, 7410-14.
11. Johnston, M. and Davis, R. (1984) Mol. Cell. Biol. 4, 1440-48.
12. Douglas, H. and Hawthorne, D. (1966) Genetics 54, 911-6.
13. Hopper, J. Broach, J. and Rowe, L. (1978) Proc. Natl. Acad. Sci. USA 75, 2878-82.
14. Hopper, J. and Rowe, L. (1978) J. Biol. Chem. 253, 7566-69.
15. St. John, T. and Davis, R. (1979) Cell 16, 443-52.

16. Douglas, H. and Hawthorne, D. (1972) J. Bact. 109. 1139-43.
17. Winge, U. and Roberts, L. (1948) C. R. Trav. Lab Carlsberg Ser. Physiol. 24, 263-315.
18. Nogi, Y. and Fukusawa, T. (1980) Curr. Genetics 2, 115-120.
19. Johnston, S. and Hopper, J. (1982) Proc. Natl. Acad. Sci., USA 79, 6971-75.
20. Laughon, A. and Gesteland, R. (1982) Proc. Natl. Acad. Sci., USA 79, 6827-31.
21. Torchia, T., Hamilton, R., Cano, C. and Hopper, J. (1984) Mol. and Cell. Biol. 4, 1521-27.
22. Rothstein, R. (1983) Meth. Enzymol. 101, 202-11.
23. Giniger, E., Varnum, S. and Ptashne, M. (1985) Cell 40, 767-774.
24. Schmitz, A. and Galas, D. (1979) Nuc. Acids Res. 6, 111-137.
25. Nedospasov, S. and Georgiev, G. (1980) Biophys. Biochem. Res. Commun. 92, 532-39.
26. Wu, C.(1980) Nature 286, 854-60.
27. Bram, R. and Kornberg, R. (1985) Proc. Natl. Acad. Sci., USA 82, 43-7.
28. Matsumoto, K., Toh-E, A. and Oshima, Y. (1978) J. Bact. 134, 446-57.
29. Perlman, D. and Hopper, J. E. (1979) Cell 16, 89-95.
30. West, R., Yocum, R. and Ptashne, M. (1984) Mol. Cell Biol. 4, 2467-78.