Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 10628–10632, November 1993
Biochemistry

Chromatin structure and regulation of the eukaryotic regulatory gene *GAL80*

(yeast/gene expression/*GAL* genes/nucleosomes)

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Communicated by K. E. van Holde, July 8, 1993 (received for review March 18, 1993)

ABSTRACT **The chromatin structure around the 5′ end of the yeast regulatory gene *GAL80* has been determined. The chromatin organization is very similar to that on the 5′ regions of the *GAL1-10* structural genes: a constitutive hypersensitive region containing the upstream activating sequence (UAS) element, and nucleosomes around this hypersensitive region. The downstream nucleosome, which is a positioned nucleosome, covers the TATA and transcription start sites. The nucleosome upstream of the hypersensitive region undergoes significant change when cells are grown in galactose, where *GAL80* gene expression is induced to maximal levels. The change may be related to the induction process. GAL4 protein binds strongly to the *GAL80* UAS in galactose-grown cells, less strongly in glycerol-grown cells, and not at all in glucose-grown cells. These data and published gene expression data are used to develop a model for the regulation of the *GAL80* regulatory gene.**

Transcription of many eukaryotic genes is highly regulated. How this occurs is the subject of active research in a variety of systems. Particular effort has gone into identifying and characterizing trans-acting regulatory factors. It will be interesting to learn how genes which encode such factors are regulated.

The *GAL* genes in yeast provide an opportunity to do this (cf. ref. 1). The major regulators (GAL4, GAL80, GAL3) of the *GAL* structural genes have been well characterized (2). GAL4 activates the high level of expression seen on the structural genes when cells are grown in galactose, through upstream activating sequence (UAS) elements located in the 5′ upstream regions of the various structural genes. GAL80 acts negatively, preventing expression of the structural genes when cells are grown in noninducing carbon sources such as glycerol and moderating the full level of induced structural gene expression when cells are grown in galactose (3). GAL3 acts in the galactose induction pathway (2).

All three *GAL* regulatory genes are expressed in cells grown in glycerol (2). Expression of *GAL80* and *GAL3* is induced 5- to 10-fold in galactose, in a process which depends on GAL4 (4, 5). Both genes have UAS elements. *GAL80* is also expressed in cells grown in glucose (4), which is uncommon for *GAL* genes. To study gene control, I have analyzed the chromatin structure on the 5′ region of the *GAL80* regulatory gene and compared this to the known (cf. ref. 6) chromatin structure on the 5′ regions of the *GAL1-10* structural genes.

MATERIALS AND METHODS

Yeast (*Saccharomyces cerevisiae*) cells were grown to early logarithmic phase in 1% yeast extract/2% bactopeptone plus 2% glucose/3% glycerol/2% ethanol ("glucose"), 2% galactose/3% glycerol/2% ethanol ("galactose"), or 3% glycerol/2% ethanol ("glycerol"). The isogenic series of strains used was described previously (3, 6). Spheroplasts were made and nuclei were isolated by method I of Lohr (7). Nuclei were digested with DNase I (EC 3.1.21.1), methidium-propyl-EDTA·Fe(II) [MPE·Fe(II)], or micrococcal nuclease (MNase; EC 3.1.31.1) as described (7). Chromatin cleavage sites were determined by indirect end label analysis (8, 9); DNA purified from nuclear digests was cut with *Eco*RI for DNase I hypersensitivity, MPE·Fe(II), and MNase studies or with *Taq* I for DNase I nuclear footprint analysis. This digested DNA was extracted, precipitated with ethanol, electrophoresed on 2.8% polyacrylamide/0.6% agarose nondenaturing gels [DNase I hypersensitivity/MPE·Fe(II)/MNase] or on 4.5–5.0% polyacrylamide/0.6% agarose/8 M urea denaturing gels (DNase I nuclear footprints), electrophoretically transferred to diazobenzyloxymethyl (DBM) paper, and hybridized as described previously (6) with (*i*) an ≈90-bp probe abutting the *Eco*RI site within the *GAL80* coding sequences (Fig. 1) for MNase, MPE·Fe(II), and DNase I hypersensitivity analysis or (*ii*) an ≈120-bp probe abutting the *Taq* I site lying well upstream of *GAL80* (Fig. 1) for DNase I footprint analysis. Probes were radiolabeled by random priming (11). The DBM paper was washed and exposed to XAR film (Kodak) as described (6). Features on the autoradiograms (band sizes, band widths, etc.) were quantified on a Zeineh laser densitometer. Note that autoradiographic intensity generally tends to decrease down the profile, unless there are specific nuclease-sensitive features, because probe hybridization efficiency decreases with decreasing DNA size (12).

RESULTS

A Constitutive "Hypersensitive" Region (HR). DNase I digests of chromatin show a distinctive strongly cleaved HR upstream of the *GAL80* gene (H in Fig. 2*A*, track 2). The region lies between 465 and 610 bp on the map in Fig. 1, which places its downstream end ≈60 bp upstream of the closest *GAL80* transcription start site (10). This HR is easily the strongest feature in these profiles.

Although there is some specific naked DNA cleavage in the *GAL80* 5′ region (Fig. 2*A*, track 1), the HR must reflect chromatin features. First, most of the HR, including the very intense part near the center, is not cleaved at all in naked DNA digests. Second, DNA outside the HR, for example the *GAL80* TATA, is readily cleaved in naked DNA digests but not in chromatin (Fig. 2*A*, track 2). Third, other reagents pick out the same HR (see below).

The *GAL80* HR is constitutive: it is present in cells grown in glucose, galactose (Fig. 2*A*, tracks 2 and 3), or glycerol (not

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Abbreviations: UAS, upstream activating sequence; MPE·Fe(II), methidium-propyl-EDTA·Fe(II); MNase, micrococcal nuclease; DBM, diazobenzyloxymethyl; HR, hypersensitive region.

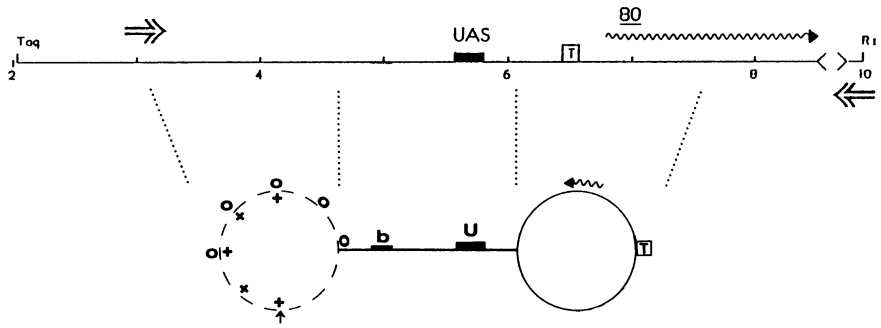

FIG. 1. DNA sequence and chromatin organization on the *GAL80* gene. The DNA sequence features are shown above the line: the UAS, the TATA box (T), and the *GAL80* transcript (wavy line). The *Taq* I site (Taq) and the *Eco*RI site (RI) used for indirect end-label mapping are identified. The tips of the large arrows above (Taq) or below (RI) the line locate the downstream ends of the probes used. The numbers below the line are hundreds of nucleotides. Zero is an *Mbo* II site, ≈665 bp from the (most upstream) transcription site (10). Note the compression between 800 and 1000 bp. The chromatin organization determined from this work is shown below the sequence map. Vertical dotted lines align the two maps. The hypersensitive region (solid line), containing the UAS (U) and the TC polypyrimidine tract (b), lies between a downstream nucleosome (solid circle), containing the TATA (boxed T) and transcription start sites, and an upstream nucleosome (dashed circle), wherein the galactose induction-dependent changes (+) and the GAL80-dependent changes (o) occur. The view is down onto the top of the nucleosome disc. DNA enters at the bottom (furthest away from the reader) of the upstream nucleosome (arrow in) and at the top (closest to the reader) of the downstream nucleosome. This is an arbitrary but convenient choice (see text). Features within the circle lie on DNA below the dyad in the nucleosome; features outside the circle are located on DNA above the dyad.

shown) and in cells lacking the GAL4 regulatory protein (Fig. 2*A*, track 4). The *GAL80* HR contains the UAS but not the TATA box (Fig. 2*A*). Constitutivity, UAS inclusion, and TATA exclusion are also characteristics of the HR on the *GAL1-10* 5′ region (13). The strong and uniform hypersensitivity of the *GAL80* HR suggests that it is a nonnucleosomal region, like the *GAL1-10* HR. Even the levels of *GAL80* and *GAL1-10* HR hypersensitivity are similar (Fig. 2*A*, tracks 2 and a). HR hypersensitivity is decreased in galactose-grown cells when GAL4 is present (Fig. 2*A*, tracks 2–4). This is also seen on *GAL1-10* (ref. 13; Fig. 2*A*, track b).

The chemical cleavage agent, MPE·Fe(II), detects a constitutive uniformly sensitive HR (Fig. 2*B*, tracks 2–4) in the same location as the DNase I HR. The chromatin nature of the HR is especially clear in these digests, for MPE·Fe(II) cleaves naked DNA uniformly (Fig. 2*B*, track 1). Again, DNA around the HR is strongly protected in chromatin (Fig. 2*B*, tracks 2 and 3).

Nucleosomes Surround the HR. Downstream from the HR (toward the gene), ≈140 bp of DNA is completely protected from MPE·Fe(II) cleavage. This suggests nucleosome protection. It is probably a positioned nucleosome; the band (A in Fig. 2*B*, track 2) downstream of the nucleosomal protection is narrower than the internucleosomal bands produced by MPE·Fe(II) digestion on *GAL1-10* (not shown), where nucleosomes are positioned (14, 15). This nucleosome covers the TATA and the *GAL80* transcription start sites. In galactose-grown cells, band A is less prominent (Fig. 2*B*, track 3). However, it is still cut and the region between the HR and site A remains protected.

The size of the chromatin-protected region upstream of the HR varies. In cells grown in nongalactose carbon sources or

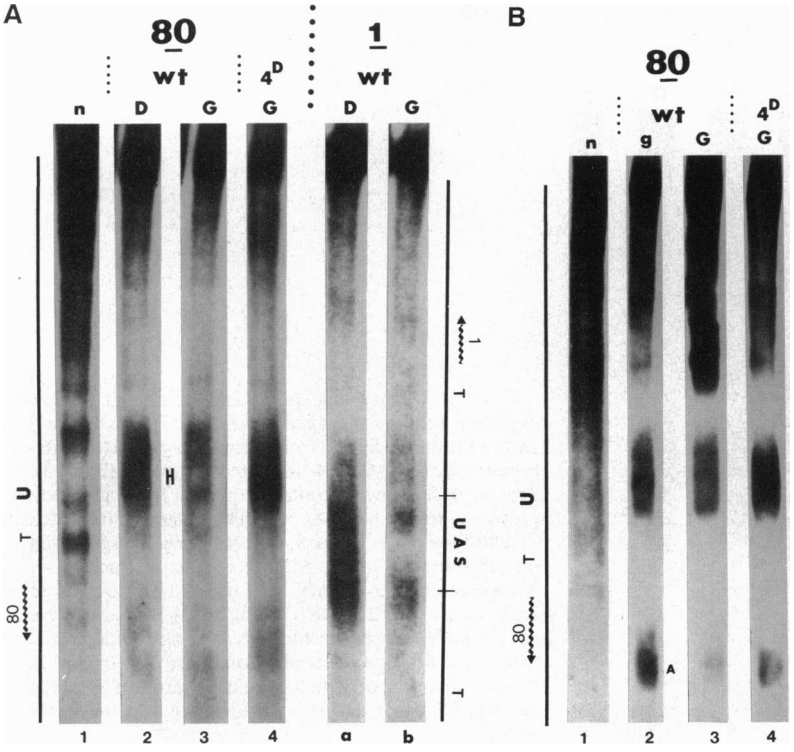

FIG. 2. DNase I and MPE·Fe(II) hypersensitivity. (*A*) DNase I digests. (*B*) MPE·Fe(II) digests. The UAS (U), TATA (T), and *GAL80* transcript (wavy line) are located to scale to the left of each autoradiogram. Naked DNA control digests (n) are shown in track 1 of *A* and *B*. In *A*, tracks 2 and 3 show chromatin digests from glucose-grown (D) and galactose-grown (G) wild-type (wt) cells. Track 4 shows a chromatin digest from galactose-grown cells lacking GAL4 (4^D). Tracks a and b are from the same piece of DBM paper as tracks 2 and 3 but hybridized with a *GAL1-10* probe. The *GAL1-10* UAS, TATA (T), and the *GAL1* transcript are located to scale to the right of tracks a and b. In *B*, tracks 2 and 3 show chromatin digests from wild-type (wt) glycerol-grown (g) and galactose-grown (G) cells and track 4, a digest of chromatin from galactose-grown cells lacking GAL4 (4^D).

in cells lacking GAL4, the protection extends for ≈140 bp (Fig. 2*B*, tracks 2 and 4), again suggesting nucleosome protection. In wild-type galactose-grown cells, the protected region shrinks, to ≈100 bp (Fig. 2*B*, track 3). If the nucleosome were lost under these conditions, then the entire region should become readily cleavable by MPE·Fe(II), as it is in naked DNA. However, although smaller, the region remains strongly protected. Thus, it seems likely that the change reflects a structural alteration or a loss of some components rather than complete nucleosome loss. Another possibility is replacement of the nucleosome with a smaller protein structure, such as a regulatory protein complex. This seems less likely because the rest of the protecting structure remains completely unchanged in galactose, by several criteria (see below). The chromatin pattern upstream of this first nucleosomal protection shows poorly resolved bands, even in inactive cells (Fig. 2*B*, tracks 2 and 4), due probably to heterogeneous structure in this region. In agreement, DNase I often shows a cutting site downstream of the HR, corresponding to A (Fig. 2*A*, track 4), but never one upstream of the HR.

MNase digestions confirm the DNase I and MPE·Fe(II) results. MNase cleaves the DNA sequence specifically at five major locations in this region (a–e), at various digestion extents (Fig. 3, tracks 1 and 4). MNase also produces chromatin-specific bands (A–C in Fig. 3, track 2). In chromatin digests, cleavage at B, b, and c predominates; all these sites lie within the hypersensitive region noted above (Fig. 3, tracks 7 and 8). In contrast, a and e, which are suggested to lie within nucleosomal regions, are not prominent in chromatin digests. Band d, which lies in the HR near its upstream border, is also never prominent in chromatin digests. Results below suggest an interaction in this region which could protect d and explain its low cleavage.

Chromatin-dependent band A lies ≈160 bp below the downstream border of the HR (Fig. 3, track 2). It is clearly a chromatin feature, for there is no cutting here in naked DNA. This band overlaps (Fig. 3, tracks 7 and 8) the MPE·Fe(II) band suggested to demarcate a nucleosome and thus confirms the location of this nucleosome. MNase digests also confirm the continued presence of this nucleosome in galactose-grown cells (Fig. 3, tracks 2 and 7).

Chromatin band C lies ≈120 bp above the upstream border of the HR, within the chromatin region protected from MPE·Fe(II) cleavage in wild-type cells grown on nongalactose carbon sources but exposed in galactose-grown cells. In agreement with the MPE·Fe(II) results, band C can become more prominent in galactose-grown cells (Fig. 3, tracks 6 and 7). However, its prominence varies (Fig. 3, track 2), as if MNase is less sensitive to the change that occurs in galactose than MPE·Fe(II) is.

DNase I Footprints. The DNase I nuclear footprint technique was used to look in detail at the HR and the anomalous nucleosome upstream of it. This approach uses denaturing gels and can detect DNA–protein interactions like the GAL4–UAS interaction (cf. ref. 13).

In chromatin from glycerol-grown cells (Fig. 4, track 2), there is some protection of DNA at the position of the UAS. Digests of naked DNA show no UAS protection (Fig. 4, track 1). In galactose-grown cells, the level of UAS protection increases significantly over that observed in glycerol-grown cells (Fig. 4, tracks 3 and 4). Tracks 2 and 5 and 3 and 4 compare extent of digestion for each carbon source; results are unaffected. The UAS protection in galactose (or glycerol) depends on GAL4 (Fig. 4, tracks 8 and 9) and thus probably reflects GAL4 binding to the UAS. *GAL80* gene expression is 5- to 10-fold higher in galactose than in glycerol (4). However, these expression differences cannot explain the protection differences because UAS protection on *GAL1-10* is indistinguishably strong (Fig. 4, tracks a and b) in glycerol (no expression) or in galactose (>1000-fold induction). The *GAL80* and *GAL1-10* footprints in Fig. 4 come from the same piece of DBM paper. Such comparisons are not subject to experimental variations or other artifactual explanations and thus are extremely reliable. In glucose, there is no detectable

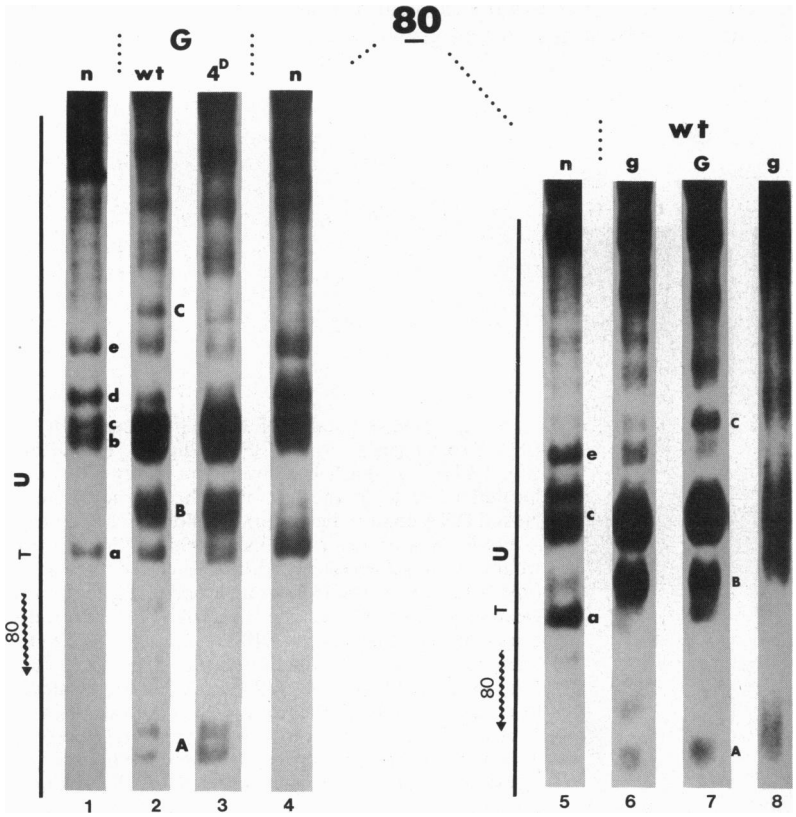

FIG. 3. MNase digests. Tracks 1, 4, and 5 show digestion profiles of naked DNA (n). Tracks 2 and 3 show chromatin digests from galactose-grown (G) wild-type (track 2) or GAL4-disrupted (4^D) cells (track 3). Tracks 6–8 show chromatin digests from glycerol-grown (g, tracks 6 and 8) or galactose-grown (G, track 7) wild-type cells. Track 8 is an MPE·Fe(II) digest, run on the same gel as the MNase digests in tracks 5–7. Sequence features (UAS, TATA, etc.) are located to scale as in Fig. 2. *Hae* III fragments of radioactive ϕX174 DNA present in the gels (and autoradiograms) were used for size determinations here and in Fig. 2. Band a in track 6, which is usually present in these digests, is absent due to a small bubble on the DBM paper.

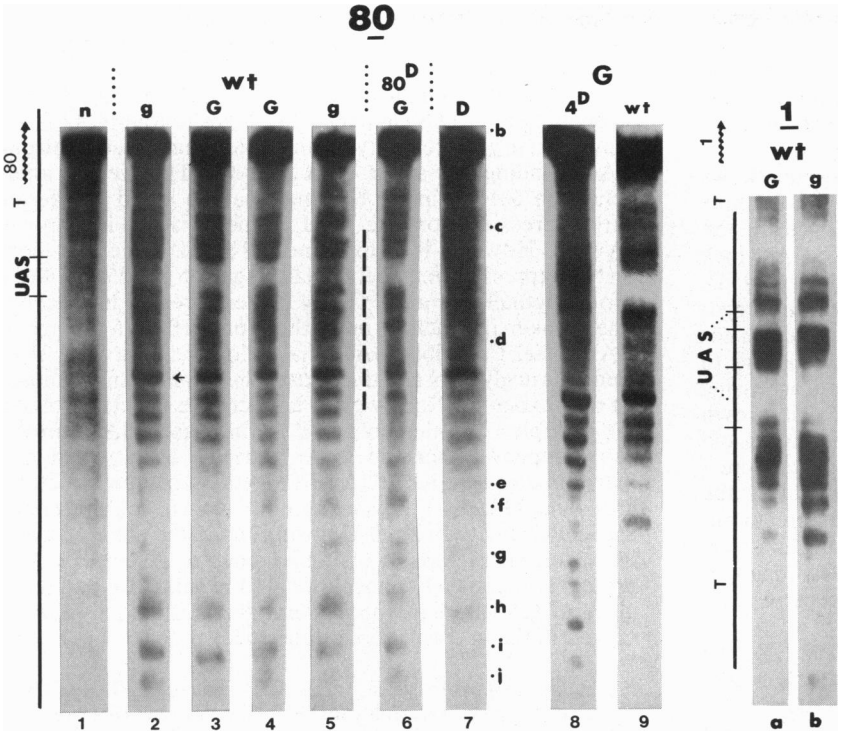

FIG. 4. DNase I footprint analysis. These denaturing gel profiles show a naked DNA digest (track 1) and chromatin digests from glycerol-grown (g, tracks 2 and 5); galactose-grown (G, tracks 3 and 4), or glucose-grown (D, track 7) wild-type cells or from galactose-grown (G) 80^D (lacking GAL80) cells (track 6). *GAL80* sequence features (UAS, TATA) are located to scale to the left of track 1. Tracks 8 and 9 show chromatin digests from galactose-grown 4^D or wild-type cells, run on another gel. The *Msp* I fragments of pBR322 used to determine sizes in these profiles are located to the right of track 7: b = 527 bp; c = 404 bp; d = 307 bp; e = 242 bp; f = 238 bp; g = 217 bp; h = 201 bp; i = 190 bp; and j = 180 bp. In these profiles, cleavage sites are mapped from an upstream *Taq* I site (at 206 bp), so further upstream of the gene corresponds to lower in the autoradiogram, the opposite to the directionality in Figs. 2 and 3. For reference, the hypersensitive region (determined from the lower resolution data in Figs. 2 and 3) is shown to the right of track 5 (dashed line). The arrow next to track 2 locates the ≈490-bp band (see text). Tracks a and b show *GAL1-10* profiles taken from the same piece of DBM paper as tracks 2 and 3.

UAS protection (Fig. 4, track 7). Thus, GAL4 does not bind to the *GAL80* UAS in glucose.

Chromatin digests also show a distinctive ladder of bands whose downstream (gene-proximal) terminus lies at ≈490 bp (← in Fig. 4, track 2). Naked DNA digests show some weak bands in the same region but their location and relative intensities differ from those in the chromatin profiles. This chromatin ladder pattern is not affected by carbon source. The ladder bands are generally spaced by 10–11 bp, like the bands produced from DNase I digestion within a nucleosome. Thus, the ladder probably arises from the nucleosome suggested to lie upstream of the HR. However, the ladder continues to ≈490 bp, beyond the nucleosome terminus at ≈465 bp, and thus overlaps into the HR (Fig. 4). A ladder pattern results from digestion of DNA lying on a protein surface, histone or nonhistone (16). Perhaps this ladder continues into the HR because the nucleosome interacts with the HR, or with proteins on the HR. For example, densitometer traces show that HR intensity trails off more gradually on this upstream edge of the HR than on the downstream edge (not shown). This suggested interaction could also explain the chromatin protection of MNase site d mentioned above. The ladder terminates in an interesting location within the HR, at the upstream end of a 19-residue TC polypyrimidine tract (b in Fig. 1).

The GAL80 protein may regulate its own expression (4). I looked for GAL80-dependent structural features on the *GAL80* gene upstream region by comparing DNase I patterns from isogenic wild-type and 80^D cells (Fig. 4, tracks 4 and 6). The latter lack a viable GAL80 but still contain an intact *GAL80* upstream region (3). Both strains show strong UAS protection. The 80^D footprint depends on GAL4; it is absent in $80^D/4^D$ cells (not shown). Thus, in galactose, GAL4 protein still interacts strongly with the *GAL80* UAS in the absence of GAL80 protein. However, GAL80 removal does cause changes within the chromatin ladder pattern; there is smearing and loss of intensity at bands 3–4 and enhancement of bands 5–7, counting the ≈490-bp band as band 1. Thus, the ladder pattern in wild-type cells depends on GAL80 protein. The bands affected by GAL80 removal lie within the upstream nucleosome (Fig. 1). Their GAL80 dependency may reflect an interaction of GAL80 with this nucleosome.

DISCUSSION

Gross Organization. All three cleavage agents used give the same picture of the gross chromatin organization on the *GAL80* 5′ region (Fig. 1). There is a constitutive, uniformly hypersensitive, nonnucleosomal region which contains the UAS element. This hypersensitive region is flanked by nucleosomes, one of which covers the TATA and transcription start sites. This chromatin organization is like that seen on the *GAL1-10* 5′ region. It is probably the combination of nucleosomes around a large open stretch of DNA which produces the hypersensitivity of both HRs (cf. ref. 14). The constitutive nature of these HRs means that nucleosome loss is not necessary for exposure of the major promoter in the *GAL* system, as in some gene systems (cf. ref. 17).

Nucleosome Involvement. The most striking chromatin response on the *GAL80* upstream region is the change which occurs on the distal end of the nucleosome upstream of the HR in galactose-grown cells (Figs. 2*B* and 3). The precise nature of this change is not clear. Because it results in a loss of protection on part of the region, it may involve a loss of some components from the nucleosome. The region affected is ≈30–40 bp near the nucleosome edge, so it could reflect some loss of histone H2A/H2B. H2A/H2B depletion is a characteristic of nucleosomes on transcribed sequences (cf. ref. 18). Alternatively, the *GAL80* gene is expressed in all carbon sources but the change occurs only in galactose. Thus, the change seems to be linked to induction and may reflect activity of *GAL*-specific expression or regulation machinery. Possibly GAL-specific factor(s) are "stored" on this upstream nucleosome under noninducing conditions and recruited from it during induction. Storage of factors near a promoter before induction could facilitate the assembly of a regulatory complex during a gene response. Loss of the stored factors might cause the exposure seen in galactose.

Storage might be a general function of nucleosomes. Factors could interact with nucleosomal DNA or with the histones. Binding of the glucocorticoid and progesterone

receptors to nucleosomes (19, 20) exemplifies the former. The activity of histone H4 tails in yeast repression and activation (cf. ref. 21) could be an example of the latter. H4 tails affect *GAL1* induction (21); they could play a role in the induction-linked changes on the *GAL80* upstream nucleosome.

Another part of this same upstream nucleosome shows negative regulator (GAL80)-dependent structural features (Fig. 1). In one region, the induction-linked changes and the negative regulator-dependent changes involve DNA which lies adjacent (above/below) in the nucleosome fold (Fig. 1). This suggests an obvious model. In cells grown on nongalactose carbon sources, GAL80 protein acts directly (or indirectly) from (through) one part of this nucleosome to prevent GAL-specific factors on another part from accessing downstream DNA sequences or other factors. In cells grown on galactose, the inhibitory action of GAL80 is relaxed and the GAL factors interact with their targets to induce expression. Note that looping of the HR DNA could bring the induction- and GAL80-affected regions in the upstream nucleosome in contact with the TATA and transcription start sites in the downstream nucleosome. Thus these changes might actually take place within a large nucleoprotein complex at the 5′ end of the gene.

Like the nucleosome upstream of the *GAL80* HR, the nucleosomes located around the *GAL1-10* HR undergo structural changes in galactose (22) and show GAL80-dependent changes (6). The first nucleosome on the *GAL1* side of the HR is particularly affected. Deletion studies have shown that removal of this DNA can decrease expression of *GAL10*, the distal gene, without affecting expression of the proximal gene, *GAL1* (23). This nucleosome might act for *GAL10* like the nucleosome upstream of the *GAL80* HR.

***GAL80* Gene Regulation.** The UAS protection results can be combined with published gene expression data to suggest how the *GAL80* regulatory gene might be regulated. In glucose, there is no UAS protection. The low *GAL80* expression seen in glucose probably occurs via a basal promoter, which is not under *GAL* control. This avoids using the potent *GAL* expression apparatus to promote low-level expression.

The *GAL80* gene is expressed in glycerol, at the same level as in glucose. Expression in glycerol does not require GAL4 (4), so the weak GAL4–UAS interaction in glycerol cannot be a part of *GAL80* gene expression under these conditions. *GAL80* expression in glycerol is probably also driven by the basal promoter. This expression does require the *GAL80* upstream region (24).

In galactose, *GAL80* expression is induced 5- to 10-fold. Induced expression is regulated differently than basal expression; it is GAL4 dependent and is repressed by glucose (4). In galactose, there is a strong GAL4–UAS interaction. Induced *GAL80* gene expression must be UAS driven (see also ref. 24).

These patterns of regulation fit GAL80 function. GAL80 is needed in all carbon sources, to keep the *GAL* structural genes off (nongalactose) or to moderate their induced expression (galactose). A basal promoter can provide the low levels needed in nongalactose carbon sources. Apparently, higher levels are needed in galactose, perhaps to prevent runaway expression. To satisfy these needs, *GAL80* expression is increased. The increase is tied to the *GAL* system by using the traditional *GAL* elements, the UAS and GAL4.

GAL structural genes are not expressed during growth on nongalactose carbon sources. However, except in glucose, they are in a state from which they can be rapidly induced if galactose becomes available. The presence of GAL4 bound strongly to the UAS (cf. *GAL1-10* in glycerol) must aid this rapid inducibility.

UAS protection data show that in galactose, GAL4 binds strongly to one UAS element (*GAL80*) or to four (*GAL1-10*). However, in glycerol, only the multiple elements show strong GAL4 binding; the single UAS does not. This weaker interaction on *GAL80* might be functional, to avoid interfering with expression from the basal promoter which is active in glycerol. How can it be explained? The *GAL4* gene is more highly expressed in glycerol than in galactose (25), so there should actually be more GAL4 protein present in glycerol. The weaker interaction must therefore reflect a change in GAL4 itself; possibly GAL4 has a lower affinity for UAS elements in glycerol and this causes the weaker binding noted on the *GAL80* UAS. However, cooperative effects involving the multiple UAS elements (and the increased GAL4 levels) can compensate for the lower intrinsic affinity and still maintain strong GAL4/UAS interactions on the structural genes like *GAL1-10*, thus ensuring that their UAS elements are loaded tightly with GAL4 to produce the state of ready induction characteristic of these genes in glycerol. GAL4 protein is specifically phosphorylated in galactose but not in glycerol (26). Perhaps phosphorylation differences are involved in the carbon source differences noted.

I thank J. Hopper for yeast strains, P. Dervan for MPE·Fe(II), and M. Johnston and K. van Holde for useful discussions. This work was supported by National Institutes of Health Grant R01 GM37788.

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