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Organization of the *GAL1–GAL10* intergenic control region chromatin

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ABSTRACT

A defined, "far upstream" promoter element, the Upstream Activator Sequence (UAS), which mediates the galactose dependent induction of expression of the GAL10 gene in yeast, is the locus of an anomalous, mainly expression independent chromatin structure. The UAS chromatin shows three symmetrical DNase I hypersensitive sites in brief digests, a loss of the 10 bp DNase I ladder pattern in more extensive digests and an enhanced staphylococcal nuclease sensitivity. This anomalous structure is confined to a small region of the UAS. The surrounding chromatin, including the TATA box regions shows a more typical, but expression dependent nucleoprotein, probably nucleosomal, organization. Such an arrangement may be a common feature of eukaryotic genes.

INTRODUCTION

A number of studies have suggested that the regions immediately upstream from genes have anomalous chromatin structure. For example, the upstream regions often contain DNase I hypersensitive sites (1) and S1 nuclease sensitive sites (2) and in fact can exhibit enhanced sensitivities to several nonspecific nucleases (3) and to restriction endonucleases (3,4). However, the structural causes for these sensitivities and details of the chromatin structure of any upstream (control) region remain largely unknown. Suggestions about the structure of a ribosomal upstream region have been made (5,6), but the presence of inactive genes in this repeated set makes the structural information ambiguous. The structure in gene upstream regions is of interest because these regions contain DNA sequences thought to be involved in controlling gene expression and the chromatin structure may be an aspect of that control.

The genes coding for the enzymes needed for utilization of galactose as a carbon source in yeast (7-9) present an attractive system for chromatin structure/function studies. The genes can be readily turned on and off by choice of carbon source. In galactose, they are very actively expressed,

for example each enzyme constitutes ~ 1% of the yeast soluble protein (10). In dextrose, the genes are repressed (11,12) to zero level of expression (12); in ethanol or glycerol they are still not expressed, but are no longer repressed, and can readily be induced to expression from this state. The genes are also single copy. Thus, in haploid yeast, which were used in these studies, the genes must be expressed for the cells to grow in galactose. For these reasons and because of the precise control of the gene, the active and inactive states of chromatin are exceptionally well defined. This presents some important advantages in chromatin structural analysis (13).

Two of the structural genes, GAL1 and GAL10, are transcribed divergently from an ~ 600 bp intergenic region (14). A promoter element mediating the galactose dependent induction of expression, in conjunction with the GAL4 positive regulatory gene, has been identified to lie within a 365 bp fragment of the intergenic GAL1 - GAL10 DNA (15). The identification of this element, capable of acting in vivo in a homologous system and under proper regulatory gene control, provides one of the clearest known examples of a gene control element. This paper describes experiments analyzing the chromatin structure of this genetically defined control element, operating in various states of gene expression.

MATERIALS AND METHODS

Yeast cells (strain D585-11c) were grown to early log (5 x 10^7 per ml) or stationary (20-30 x 10^7 per ml) in YEPD, YEPG or YEPE (1 percent yeast extract, 2 percent Bactopeptone, 2 percent dextrose, galactose or ethanol), nuclei were isolated and digested with staphylococcal nuclease or DNase I and the DNA isolated as described previously (16). Naked DNA digestions were performed as for the nuclear chromatin except at lower enzyme concentrations. DNA was electrophoresed on 2.5% polyacrylamide/0.5 % agarose nondenaturing gels or 3.5% - 5.4% polyacrylamide/0.4% agarose/7 M urea denaturing gels as described (13). DNA was electrophoretically transferred (17) to diazobenzyloxymethyl (DBM) paper (18). These DBM papers were treated and hybridized as described in (18).

Probes for hybridization were made from various fragments of the cloned yeast galactokinase gene. Plasmids were grown in L broth + tetracycline, chloramphenicol amplified and isolated by gentle lysis followed by CsCl/ethidium bromide equilibrium gradient centrifugation as described in (19). The yeast insert was removed by restriction endonuclease digestion,

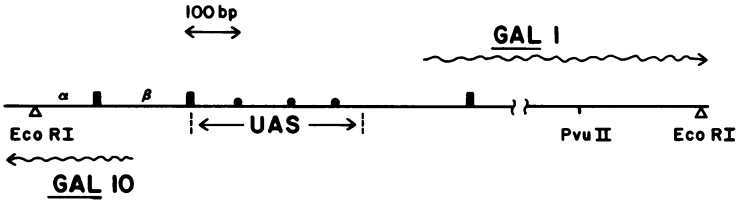

Figure 1. Restriction Map of the GAL1-GAL10 Region

The ~ 1.9 kb of DNA between two Eco RI sites ("Δ") in the GAL1-GAL10 region is shown. The approximate positions of the GAL1 and GAL10 transcripts and the UAS region are shown. Some of the restriction enzyme sites used in this work are also located: Dde I ("▮"); Hinf1 ("●"); Pvu II ("|"). Two of the Dde I fragments used in this work are labelled ("α", "β").

followed by isolation of the fragments by the method of Maxam and Gilbert (20). Hybridization probes were made from this DNA by repairing ends of restriction fragments with overlapping termini (Eco RI, Hinf1, Dde I) using [α^{32}-P] dTTP and [α-^{32}P] dATP as described in (21). Labelled fragments were isolated on 5.5 - 7% polyacrylamide gels by the methods described in (20). Autoradiograms were exposed to X-OMAT X-ray film at -70°C with intensifying screens present.

RESULTS

Cleavage sites in the Intergenic Region. For an overall view of the structure of the chromatin in this region, staphylococcal nuclease and DNase I cleavage sites within a 1.9 kb (kilobase pair) region, including the 5' end of the coding sequence of GAL10, the GAL1 - GAL10 intergenic region and much of the coding sequence of GAL1 (cf. Figure 1), were mapped in nuclear digests by the "indirect end label" technique (22,23). In this approach, nuclear chromatin is briefly digested with the nonspecific nuclease (MNase or DI), the DNA is isolated and then recut to completion with a restriction endonuclease. The DNA is electrophoresed, transferred to a suitable support and hybridized with a specific probe, one end of which abuts the restriction site used. The sizes of the bands in the resulting autoradiogram locate cleavage sites of the nonspecific nuclease.

Mapping DNase I cleavage sites from the RI site in GAL10 shows three hypersensitive bands (Figure 2, trs. 2-4). These are present in very brief digests (Figure 2, tr. 3) and continue to dominate the pattern as digestion continues (Figure 2, tr. 2). These cleavage sites are located 380, 440 and

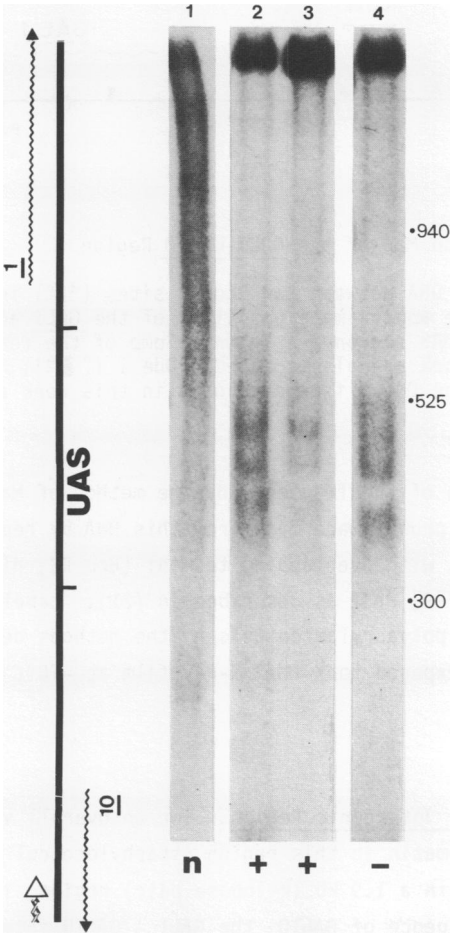

Figure 2. DNase I Cleavage Sites on the GAL1-GAL10 Region

DNA from brief DNase I digests of yeast nuclei or deproteinized DNA was isolated and recut with Eco RI. This DNA was electrophoresed, transferred to DBM paper and hybridized with a 120 bp RI-Dde I probe from the GAL10 coding sequences (tracks 1-4). The accompanying map shows, to scale, the sites of initiation and the direction of transcription of GAL1 and GAL10 transcripts (⟿▶) and the position of the UAS element defined by Guarente et al. (15). The mobilities of several DNA restriction fragments run on the same gel are shown to the right. The probe is shown with its end point located to scale (⋈▷) and the arrow pointing in the direction of larger DNA sizes (i.e., cleavage sites). Electrophoresis is from top to bottom. The tracks are identified as to the state of gene activity of the chromatin from which the DNA was isolated: "+", expressed (galactose); "-", inactive (dextrose). "n" refers to the pattern from naked DNA. Data contained in

cleavage site maps will be referred to as "patterns" to be distinguishable from data contained in "profiles" obtained by using only the nonspecific nuclease (cf. Figures 4,5).

Track	Carbon Source	Probe
1	-	RI-Dde I in GAL10 ("α")
2	log/galactose	"
3	" / "	"
4	" /dextrose	"

495 bp (base pairs) from the RI site and thus lie precisely within the UAS control element identified by Guarente et al. (15). Naked DNA control digests show a fairly uniform smear of DNA, with no digestion preference for the UAS region at any extent of digestion (cf. Figure 2, tr. 1). Mapping from the RI site in GAL1 shows the same results, uniform sensitivities in the coding sequences and hypersensitive sites in the UAS (not shown).

The presence of this hypersensitivity in the UAS region chromatin is not expression dependent for the chromatin patterns are similar from cells grown in galactose (expressed), dextrose (repressed) (Figure 2, trs. 2 vs. 4), or from cells grown to stationary phase in galactose (not shown). The latter conditions cause a loss of the active chromatin features associated with this gene (13).

The hypersensitive pattern is approximately symmetrical. Each of the two outside bands is equally spaced from the middle one (~ 60 bp) and also approximately equidistant (~ 200 bp) from the site of transcription initiation of GAL1 and GAL10, respectively. The possible significance, if any, of this arrangement is not clear but since both GAL1 and GAL10 are induced by galactose, this same element could mediate the induction process on both genes. The symmetry could be a reflection of this.

Staphylococcal nuclease cleavage site maps for this 1.9 kb region are shown in Figure 3. Digestion of the chromatin from cells grown in dextrose, where the genes are repressed, produces a distinctive band pattern corresponding to cleavage sites spaced at about yeast nucleosome intervals (~ 170 bp) through the intergenic region and GAL1 coding sequences (Figure 3, tr. 1). The cleavage site pattern of naked DNA in the same region shows a number of distinctive sites (Figure 3, tr. 3) but does not reproduce the chromatin pattern. For example, the strongest cleavages in naked DNA are just upstream of GAL10 and GAL1, and in the 5' coding region of GAL1, while the most pronounced cleavage in repressed chromatin occurs at bands 2 and 3 within the UAS region. One of the strongly cleaved chromatin sites in the

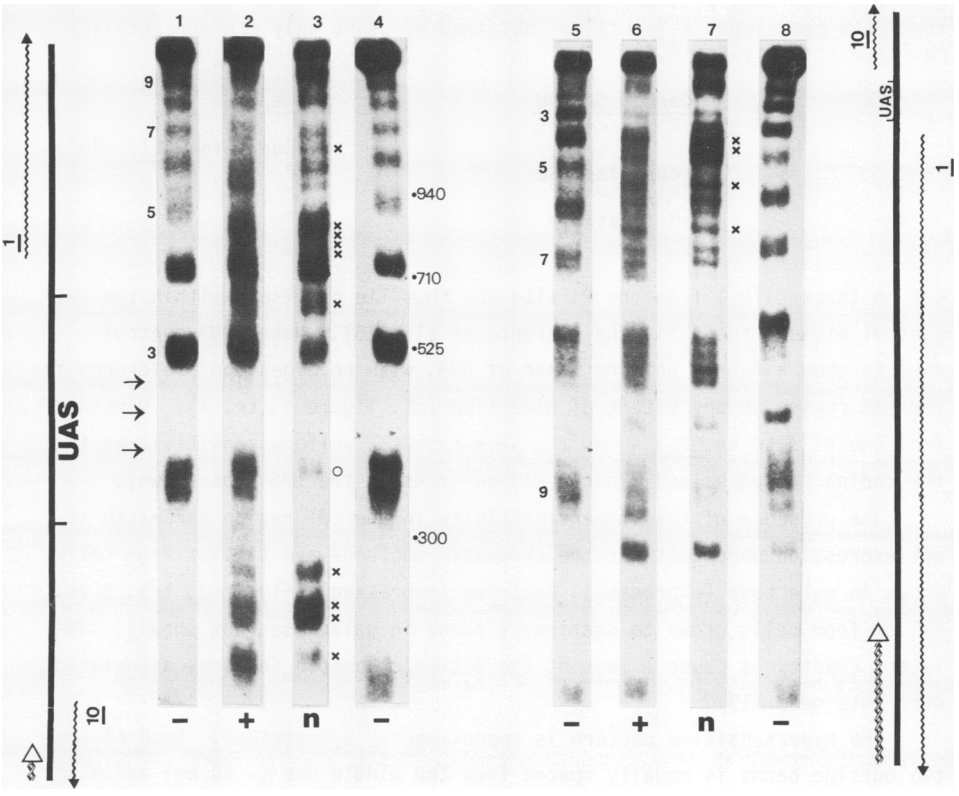

Figure 3. Staphylococcal Nuclease Cleavage Sites on the GAL1-GAL10 Region

DNA from brief staphylococcal nuclease digests of yeast nuclei or deproteinized DNA was treated as in Figure 2 and the resulting DBM paper was hybridized with a 120 bp RI-Dde I probe from the GAL10 coding sequences (tracks 1-4) or a 250 bp RI-Pvu II probe from the GAL1 coding sequences (tracks 5-8). The two probes thus look over the same 1.9 kb region but from different directions. The accompanying maps show, to scale, the sites of initiation and the direction of transcription of GAL1 and GAL10 transcripts (⟿▶) and the position of the UAS element defined by Guarente et al. (15). The mobilities of several restriction fragments run on the same gel are shown to the right of track 4. The probes are shown with their end points located to scale (⟿▷) and the arrow pointing in the direction of larger DNA sizes (i.e., cleavage sites). Electrophoresis is from top to bottom. The tracks are identified as to the state of gene activity of the chromatin from which the DNA was isolated: "+", expressed (galactose); "-", inactive (dextrose, stationary in galactose). "n" refers to patterns from naked DNA digests. The arrows to the left of track 1 locate the DNase I hypersensitive sites identified in Figure 2. For reference, every other band in track 1 is numbered (3, 5, 7, 9) and again in track 5. The various other symbols in the figure are identified in the text.

Track	Carbon Source	Probe
1	log/dextrose	RI-Dde I in GAL10
2	" /galactose	"
3	-	"
4	stationary/galactose	"
5	log/dextrose	RI-Pvu II in GAL1
6	" /galactose	"
7	-	"
8	stationary/galactose	"

UAS region is a very weak naked DNA site (" o "), while the other (band "3") is also prominent in naked DNA digests.

A subset of the naked DNA sites do coincide with the cleavage sites utilized in repressed chromatin. Because of this coincidence, one cannot distinguish from this data whether the sites cleaved in the chromatin digests are recognized because they lie in nucleosome spacers. However, it is clear that these sites are accessible to the enzyme in nuclear chromatin. In contrast, there are many strong naked DNA cleavage sites which are never cleaved in repressed chromatin ("x", Figure 3, track 3). Thus, on these regions of inactive chromatin there must be something, presumably protein, which protects the naked DNA sites from staphylococcal nuclease cleavage. In general, the size of the region protected cannot be determined from this data. However, where there are many strong naked DNA sites protected between the available sites, such as the 5' end of the coding region of GAL1, the data can suggest a size for the region protected, 170-180 bp in this case.

The chromatin regions corresponding to bands >5, i.e., the GAL1 coding sequences, have a nucleosomal chromatin structure, as shown by other work (13). Thus, the corresponding bands in track 1 must reflect nucleosome cleavage. Digestion profile data shown below suggest that the intergenic region, corresponding to bands 2-5 in Figure 3, has a heterogeneous structure, some of it is nucleosomal while some of it has a structure which appears to differ.

The cleavage site pattern from expressed chromatin is very different from repressed chromatin. All of the naked DNA sites are utilized and the relative intensities of the bands are generally close to those in the naked DNA digests, except that cleavage frequencies in the UAS region are still enhanced compared to naked DNA (cf. band 2, Figure 3 tr. 2). Also, throughout the expressed chromatin pattern there is a quite prominent background of intensity, which is present but not prominent in the naked DNA

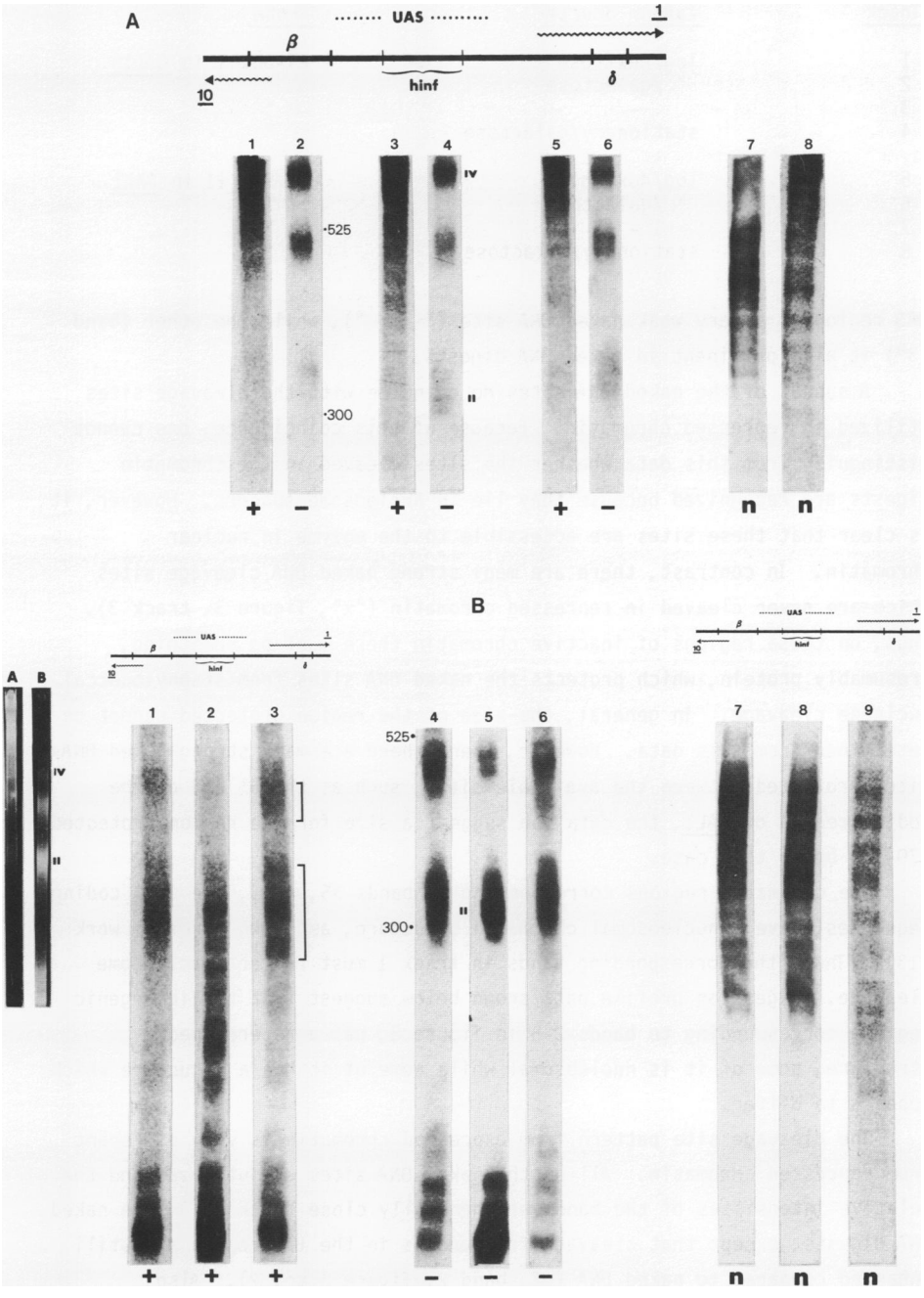
A
UAS
1
β
10
hinf
δ
1 2 3 4 5 6 7 8
·525
·300
IV
II
+ − + − + − n n
B
A B
IV
II
1 2 3 4 5 6 7 8 9
525·
300·
II
+ + + − − − n n n

Figure 4. Staphylococcal Nuclease Digestion Profiles Across the Intergenic Region

DNA from staphylococcal nuclease digestions of yeast nuclei or deproteinized DNA was isolated and electrophoresed on a 3.6% polyacrylamide/0.4% agarose/7 M urea denaturing gel, transferred to DBM paper and hybridized with various intergenic region probes: "β", an ~ 170 bp Dde I fragment containing the transcription initiation region and DNA upstream of GAL10; "hinf", two inseparable ~ 80 bp Hinf1 fragments from the center of the stretch of DNA defined to be the UAS element; "δ", an ~ 50 bp fragment from the 5' end of the coding sequence of GAL1. δ was used because there are no suitable restriction sites which could be used to obtain, in sufficient amounts, a small fragment closer to the UAS. The entire stretch of DNA shown on the map is ~ 900 bp. The initiation sites and directions of transcription of GAL1 and GAL10 transcripts are shown ("⟶"). In the chromatin digestion profiles, the tracks are identified as to state of gene activity: "+", expressed (galactose); "-", inactive (glycerol, stationary in galactose). Repressed chromatin gives a nucleosome-like profile similar to the other inactive states (not shown). Naked DNA profiles are identified by "n". The positions of di- (~ 300 bp) and tetranucleosomal DNA are shown for track 4 and two of the DNA restriction fragments run in the gel are shown to the right of track 2. All profiles are correctly aligned as to DNA size. Electrophoresis is from top to bottom. Other symbols are identified in the text. Figure 4A shows brief digests while 4B shows more extensive digests. Representative chromatin profiles from the ethidium bromide stained gel are shown as an inset to Figure 4B, in order to illustrate the overall extents of digestion in 4A versus 4B. The profiles shown are from gene active chromatin, corresponding to tracks 1,3,5 Figure 4A ("A") or to tracks 1-3, Figure 4B ("B"). The inactive chromatin and the naked DNA samples are digested to comparable extents as the active chromatin samples, as judged by the DNA size distribution through the ethidium bromide profiles.

	Track	Carbon Source	Probe
A:	1	log/galactose	β
	2	" /ethanol	"
	3	" /galactose	hinf
	4	" /ethanol	"
	5	" /galactose	δ
	6	" /ethanol	"
	7	-	hinf
	8	-	δ
B:	1	log/galactose	β
	2	" / "	hinf
	3	" / "	δ
	4	stationary/galactose	β
	5	" / "	hinf
	6	" / "	δ
	7	-	β
	8	-	hinf
	9	-	δ

pattern. This suggests a general nuclease sensitivity enhancement in expressed chromatin.

Previous work (13) had suggested an explanation for the smearing of the staphylococcal nuclease nucleosomal ladder noted on active chromatin (13,24,25); there is a conformational transition in the core particles of active chromatin, resulting in significant staphylococcal nuclease accessibility increases in the core domain, relative to spacer cleavage. The cleavage site mapping results in Figure 3 are quite consistent with this explanation. For example, the naked DNA cleavage sites masked in the repressed pattern become accessible but there is still significant cleavage at the same nucleosome intervals seen on inactive chromatin.

Other inactive chromatin states, for example from cells grown to stationary phase in galactose (Figure 3, tr. 4) or from cells grown in glycerol (not shown), where the genes are inducible but not expressed, show the same results as repressed chromatin, a nucleosome-like cleavage site map and an enhanced accessibility of the UAS. These results suggest that the transition from inactive to active chromatin must not involve large scale rebuilding since these genes can be induced to express from the unexpressed state in a few minutes. The similarity of stationary galactose to repressed and unexpressed patterns suggests that the transition is also quite reversible.

Mapping cleavage sites from the RI site in GAL1 shows the same results (Figure 3, trs. 5-8). From inactive chromatin, there is a pattern of bands at about nucleosome intervals, which is not reproduced by the naked DNA cleavage pattern, although there is again some coincidence of sites cleaved. The strongest naked DNA cleavage sites again lie near the 5' end of GAL1 (and GAL10). Although the intensities of the bands from the UAS region in the inactive chromatin patterns may not be quite as large as in tracks 1 and 4, the intergenic region is still strongly cleaved, confirming the enhanced cleavage of the UAS region suggested in tracks 1 and 4. The relative weakness of band 2 in track 5 is probably an artifact, since this band is strong in other repressed chromatin tracks, especially in very brief digests (not shown) and in other inactive chromatin patterns such as stationary phase (Figure 3, tr. 8) and unexpressed chromatin (not shown). The active chromatin patterns (Figure 2, track 6) again differ markedly from inactive chromatin and resemble the naked DNA pattern.

Cleavage Profiles Across the Intergenic Region

Data like that shown above is very useful in locating nuclease cleavage sites and interesting regions of chromatin. A complementary approach, which

emphasizes structural information, is the analysis of the fragment profiles produced by digests using only the "nonspecific" nuclease. For example, if the partial coincidence of inactive chromatin and naked DNA staphylococcal nuclease cleavage sites (Figure 3) is the explanation for the nucleosome like pattern in the chromatin tracks, then the digestion profiles for the two should also look similar.

To analyze the chromatin structure within the intergenic region, three small probes were used (cf. Figure 4). By using small probes and concentrating on the smaller DNA sizes in the profiles, it is possible to obtain structural information which is largely localized to the chromatin region homologous to the probe used. For example, for DNA of mono- and dinucleosomal size (~ 300 bp), most of the signal obtained from the hinf1 probe arises from the 365 bp UAS region, since these probes cover ~ 160 bp from the center of the UAS, roughly spanning the region containing the hypersensitive sites (Figure 2), with approximately 100 bp of UAS on either side.

In agreement with the cleavage site mapping results, there is a distinctive nucleosome-like profile on the UAS region and surrounding intergenic region chromatin when the genes are inactive (Figure 4A, trs. 2,4,6; 4B, trs. 4-6). This nucleosome profile is seen at all times of digestion (cf. Figure 4A, tr. 4; Figure 4B, tr. 5). The profile is just as clear as the very distinctive nucleosome profile found throughout the coding sequences of inactive GAL1 chromatin (13). One can resolve up to 7-8 nucleosome peaks in these profiles but since the larger DNA sizes necessarily carry information from a large region, only the first few nucleosome peaks are shown.

As in the cleavage site maps, there is smearing of the nucleosome profile when the genes are expressed, in all three regions (Figure 4A, trs. 1 vs. 2, 3 vs. 4, 5 vs. 6), and at all extents of digestion (Figures 4A, trs. 1,3,5; 4B trs. 1-3). The extent of profile disruption in the β and δ profiles resembles that seen on other parts of coding sequence GAL1 chromatin (13), for there remain broadened, residual peaks at approximately nucleosome periodicities in both profiles (Figure 4B, tr. 3 "]"). The smearing is more uniform in hinf1 profiles (cf. Figure 4B, tr. 2). These differences are not digestion extent dependent, for β and δ profiles digested to the same extent as hinf1 still show the same peak/interpeak intensities. These differences can also be seen, although less clearly, in brief digests (cf. Figure 4A, trs. 1,5).

The naked DNA profiles from these regions do not look exactly like either chromatin profile (Figures 4A, trs. 7,8; 4B, trs. 7-9). Clearly, they bear no resemblance to nucleosome profiles and thus cannot explain the nucleosome ladders from inactive chromatin. While both expressed chromatin and naked DNA digests show DNA throughout the profile, the naked DNA profiles contain a large number of discrete bands, superimposed on a smear, while the expressed chromatin profiles are mainly smeared (Figure 4A, trs. 3 vs 7, 5 vs. 8; 4B, trs. 1 vs. 7, 2 vs. 8, 3 vs. 9). The appearance of the naked DNA profiles is predicted from the naked DNA cleavage site map. The strongly recognized naked DNA sites are all frequently cleaved and their many combinations give rise to the large number of discrete bands, while the smear arises from the low level of cleavage at all the sites throughout the region. In the expressed chromatin profiles, the sharp bands are obscured by the higher level of smear, undoubtedly resulting from the more pronounced general level of cleavage noted previously in the expressed chromatin cleavage site maps.

In both inactive and active chromatin digests, the UAS region chromatin seems to be digested somewhat faster than the surrounding chromatin, as judged by comparison of the relative yields of mono- versus higher molecular weight DNA in the hinf1 compared to β or δ profiles (Figure 4B, trs. 2 vs. 1,3; 5 vs. 4,6). This is consistent with the cleavage site data shown in Figure 3. This faster digestion must reflect chromatin features, for in the naked DNA profiles the δ region is cleaved faster (Figure 4B, trs. 8,9). This is in agreement with the cleavage site map data for naked DNA (Figure 3, trs. 3,7), which shows that the strongest cleavage sites lie around the 5' end of the GAL1 coding sequence, the location of the δ probe. For each of the comparisons of the three chromatin regions, the same DBM strip was used, so there can be no artifactual explanations for these interregion differences and even small differences are meaningful.

DNase I digests provide another way of assessing chromatin structure, particularly the nature of the DNA/protein interaction. Nucleosomal chromatin yields a distinctive profile of 10.5 nucleotide spaced bands on denaturing gels. This feature has been shown to remain present even on actively expressed GAL1 coding sequence chromatin (13). Analysis of the DNase I digestion profiles across the intergenic region yields a very surprising result; there is not a DNase I ladder profile on hinf1 chromatin (Figure 5, trs. 2,3). The smeared profile is produced in all stages of gene activity, although the smearing does appear to be somewhat less pronounced

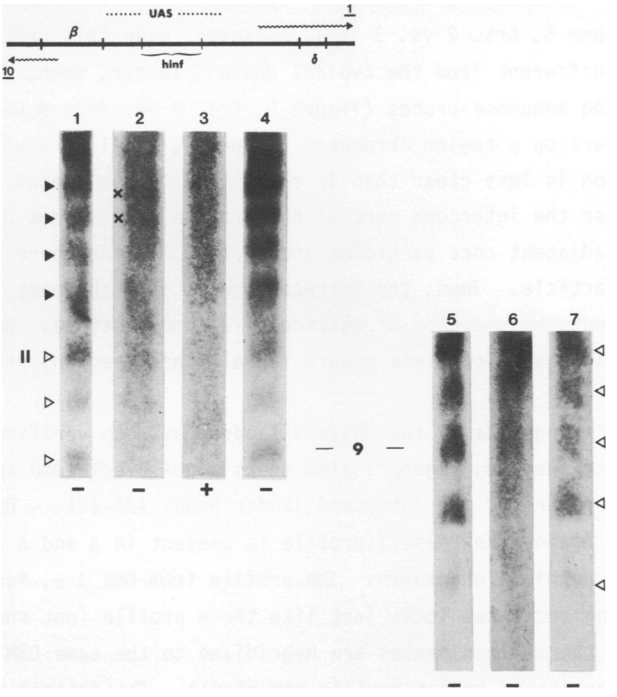

Figure 5. DNase I Digestion Profiles Across the Intergenic Region

DNA from DNase I digestions of yeast nuclei was electrophoresed on 5.4% polyacrylamide/0.5% agarose/7 M urea denaturing gels, transferred and hybridized as in Figure 4. The maps shown describe the same features as in Figure 4. The tracks are identified as to state of gene activity: "+", expressed (galactose); "-", inactive (dextrose, stationary in galactose). Bands from the intracore (#6-11, ~ 63-115 nucleotides) DNase I profile are identified by "▷", while bands from the intercore profile (> ~ 120 nucleotides) are identified by "▶". The first intercore band visualized is #12.5. Intracore bands #9 and #11 are located in the profiles. Probably because δ is so small, it was not possible from a normally loaded gel to get a profile in which the signal was strong enough or the background low enough to show up properly in a photograph, so for track 4 an ~ 200 bp probe from the region immediately downstream of δ was used instead (δ*).

Tracks	Carbon Source	Probe
1	log/dextrose	β
2	" "	hinf
3	" /galactose	"
4	" /dextrose	δ*
5	stationary/galactose	β
6	" "	hinf
7	" "	δ

in the inactive chromatin profiles, for bands are sometimes visible through the smear (Figure 5, trs. 2 vs. 3 "x"). However, even this profile is significantly different from the typical DNase I ladder, seen, for example, with GAL1 coding sequence probes (Figure 5, trs. 2 vs. 4). A DNase I ladder profile reappears on β region chromatin (Figure 5, tr. 1). However, the intercore region is less clear than in the typical DNase I profiles, probably because the intercore part of the profile arises from digestion involving two adjacent core particles and on the UAS side there is not a typical core particle. Thus, the intracore bands give the best local information about the presence or absence of a core particle. Naked DNA DNase I digestions show complete smears for all three regions probed (not shown).

This surprising loss of the DNase I ladder has been verified on several DBM papers. For example, Figure 5 also shows a gel overloaded to allow visualization of more of the intracore ladder bands (#6-#11, ~ 65-115 nucleotides). Again, the DNase I profile is present in β and δ profiles, but is smeared on hinf1 chromatin. The profile from Dde I α, further into the GAL10 coding sequences looks just like the β profile (not shown). Again, because the various probes are hybridized to the same DBM strip, digest or gel artifacts cannot explain the result. The absence of a DNase I ladder from the hinf1 region is remarkable, for it is the first piece of chromatin I have noted from which a DNase I ladder has not been obtainable, including ~ 9 kb of the 35S gene in yeast and the coding sequences of GAL1 in either the active or inactive states. It is also surprising that this region shows no DNase I ladder in the inactive state since staphylococcal nuclease yields a nucleosome-like profile. This difference could arise from the inherently greater precision in the DNase I analysis or because these nucleases probe somewhat different features of chromatin.

DISCUSSION

The UAS control element has a novel chromatin structure. It is the locus of a pattern of symmetrical DNase I hypersensitive sites, it shows a disrupted DNase I ladder profile in more extensive digests and has an enhanced staphylococcal nuclease sensitivity. These features are present within ~ 170-180 bp near the center of the UAS element defined by Guarente et al. (15). Since the minimum stretch of DNA needed for function is not known, this subsection may contain all the necessary sequences.

The novel structure is present whether the gene is active or

inactive. Such permanent structural distinction of a control region may be necessary because there are regulatory proteins that induce the formation of the active state and proteins like RNA polymerase that function after the gene is activated.

The chromatin structure of the regions surrounding the UAS looks different. On the stretch of chromatin corresponding to β, there is no DNase I hypersensitivity and a DNase I ladder reappears. Expressed chromatin from these surrounding regions shows some digestion pattern and digestion profile differences from the UAS. These surrounding regions also contain strong naked DNA cleavage sites ("x"), which are protected in inactive chromatin. Since protection of cleavage sites requires the presence of protein, then, at least in inactive chromatin, the regions surrounding the UAS element must be nucleoprotein.

This nucleoprotein region includes RNA polymerase control sequences near the gene. For example, the 170 bp β probe contains the first 100 bp of upstream sequence and thus includes the TATA box region. Several of the naked DNA sites which are covered in inactive chromatin lie just 5' upstream from the GAL10 gene, including one exactly coincident with the (presumed) TATA box. There are also chromatin protected, naked DNA sites in the region upstream of GAL1, although the (presumed) TATA box itself lies between an exposed and a protected site. This information is summarized in Figure 6. When the gene is expressed, cleavage of both TATA box regions and all the sequences around them is strongly increased.

This upstream nucleoprotein structure may be a nucleohistone structure and in fact looks similar to that characterized previously on the GAL1 coding sequences (13). For example, the β region shows a DNase I ladder. It is not quite a typical ladder but this is not surprising since there is not a neighboring prototypical nucleosome on the UAS side to contribute to the intercore pattern. The β region yields nucleosome-like digestion profiles which resemble the profiles from the GAL1 coding sequences (13). The changes in chromatin structure upon gene expression on this region also resemble, in all details, the changes on the GAL1 coding sequences. The same structure is probably present on the region upstream of GAL1, based on the cleavage site map and digestion profile similarities.

The precise structure within the UAS element remains unclear. The smeared DNase I digestion profiles from the UAS region do not necessarily require that proteins be absent. A nucleoprotein structure which does not periodically restrict the access of DNase I to the DNA would be consistent

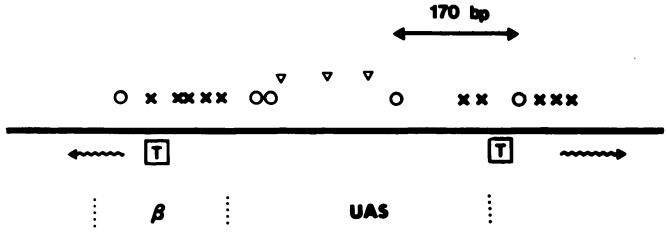

Figure 6. Superposition of Nuclease Cleavages and Functional Control Sequences on the GAL1-GAL10 Intergenic Region

Staphylococcal nuclease naked DNA cleavage sites ("O" and "x") and DNase I (chromatin) hypersensitive sites ("▽") are shown above the line. Naked DNA sites which are available to staphylococcal nuclease in inactive chromatin are shown as "O" while naked DNA sites which are not cleaved are shown as "x". Below the line are shown the transcription initiation sites of GAL1 and GAL10, the TATA like sequences near the two genes and the UAS region. The β region is also shown. Four naked DNA sites on the β region can be seen in Figure 2, track 3. The fifth site, near the UAS border can be seen on the autoradiogram and thus has been included here. The GAL10 gene is on the left side of the diagram and GAL1 is on the right.

with these profiles. Such a DNA/protein interaction differs from DNA/histone (26) or DNA/gyrase (27) interactions, but may have an analog in prokaryotic regulatory protein/DNA interactions (cf. 28). Unfortunately, the UAS contains no strong staphylococcal nuclease naked DNA sites whose accessibility can be assessed. There is a weak background of naked DNA cleavage in the region between bands 2 and 3 (Figure 3), which is not seen in the inactive chromatin digests. Staphylococcal nuclease digestion profiles of inactive chromatin also show a band of core particle length DNA (147 bp) from this region while the strong naked DNA cleavage sites in the UAS are 170 bp apart, at the minimum. However, none of these data are compelling and other analyses of this small region are in progress. Using other approaches, it has been suggested that the Drosophila hsp70 upstream region (29) and ~ 1/3 of the molecules in the population from a β-globin hypersensitive upstream region (3) are protein free DNA.

A region like the UAS may be present around other eukaryotic genes. For example, a number of genes have nuclease hypersensitive 5' flanking regions in locations analogous to the UAS (1,3,4). In β-globin chromatin, there is a hypersensitive region, from -70 to -260 bp, flanked by a less sensitive, perhaps nucleosomal structure toward the coding sequences (3). This resembles the UAS organization, a small sequence with a very anomalous structure, surrounded by more typical chromatin. The UAS region is thought

to be the binding site for a regulatory protein. Analogously, Emerson and Felsenfeld have recently identified a protein factor which reconstitutes the hypersensitivity in the β-globin upstream region (30).

This work also suggests one possible explanation for DNase I hypersensitive regions. Since DNase I ladders reflect a DNA/protein interaction in which the nuclease has periodically restricted access (~ every 10 nucleotides) to the DNA (26,27), the absence of a ladder suggests the absence of such periodic restriction, i.e., DNase I may have greater access to UAS DNA than to nucleosomal DNA. Such increased access could allow both DNA strands at any place on the DNA to be simultaneously accessible to the nuclease. This is a reasonable suggestion because restriction endonucleases can cleave the analogous stretches of DNA in other genes (3,4). Since double stranded DNA is the strongly preferred substrate for DNase I (31), even though the enzyme makes single strand nicks (31,32), the increased and simultaneous accessibility of both strands in the UAS region could strongly enhance its digestion, compared to the adjacent, nucleoprotein bound DNA, in which the access of DNase I to DNA is more limited. The variations in digestion rate of naked DNA due to this factor can be orders of magnitude (31). This explanation is not exclusive of other explanations of hypersensitive sites, such as alternative secondary structures of DNA (33), but may merely be one of several contributions. Since many DNase I hypersensitive sites are found in locations analogous to the UAS, i.e., upstream of the CAAAT and TATA box motifs (1), this explanation may have some general applicability.

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