Gene Expression in Yeast. Proceedings of the Alko Yeast Symposium Helsinki 1983, ed. by M. Korhola & E. Väisänen. Foundation for Biotechnical and Industrial Fermentation Research 1 (1983): 19–29.

A RELATIONSHIP BETWEEN CHROMATIN STRUCTURE AND GENETIC ELEMENTS AT THE YEAST HIS3 LOCUS

Kevin Struhl

Department of Biological Chemistry, Harvard Medical School
25 Shattuck St. Boston, Mass. 02115

Genetic analysis of the Saccharomyces cerevisiae (baker's yeast) his3 gene has defined two promoter elements necessary for gene expression and two regulatory elements that are required for inducing higher levels of expression during conditions of amino acid starvation (1-3). Here, I examine the accessibility of his3 DNA sequences in nuclear chromatin by using micrococcal nuclease as a structural probe. The TATA box region is particularly sensitive to micrococcal nuclease cleavage suggesting that in vivo this promoter element is accessible to nuclear proteins. Mutations that delete the upstream promoter element alter the normal chromatin structure in that nuclease cleavage at the TATA box is reduced significantly. On the other hand, the chromatin structure appears unchanged when his3 expression is induced by starving cells for amino acids. Furthermore, regulatory mutations at his3 or unlinked loci do not alter the structure. Thus, the chromatin structure at the his3 locus can be correlated with gene expression (promoter function) but not with gene regulation. The possible significance of this correlation is discussed in light of the properties of the his3 promoter elements.

Results and Discussion

A schematic view of the yeast his3 gene is shown in figure 1. The regulatory sites include sequences 83-96 and 32-41 base pairs upstream from transcribed sequences (3). .The promoter includes sequences 113-126 and 32-52 base pairs before the transcriptional initiation site (1,2, and unpublished results). A striking property of the his3 promoter is that the spacing between the two promoter elements is not critical for gene expression (2). Furthermore, yeast genes are subject to position effects such that their expression can be influenced by DNA sequences located at least 300 base pairs from the mRNA coding region (4). For these reasons, it seemed useful to correlate features of chromatin with the genetic elements necessary for his3 expression and regulation.

Yeast nuclei are prepared by conventional methods and then treated with various amounts of micrococcal nuclease, an enzyme believed to preferentially digest DNA located in spacer regions between nucleosome cores (reviewed in reference 5). The chromatin structure at the his3 locus is examined by standard nucleic acid hybridization procedures, using a small fragment of his3 DNA as a probe. The results of figure 2 show a number of broad hybridization bands, the average lengths being approximately 160 base pairs and multiple integrals thereof. These bands are indistinguishable

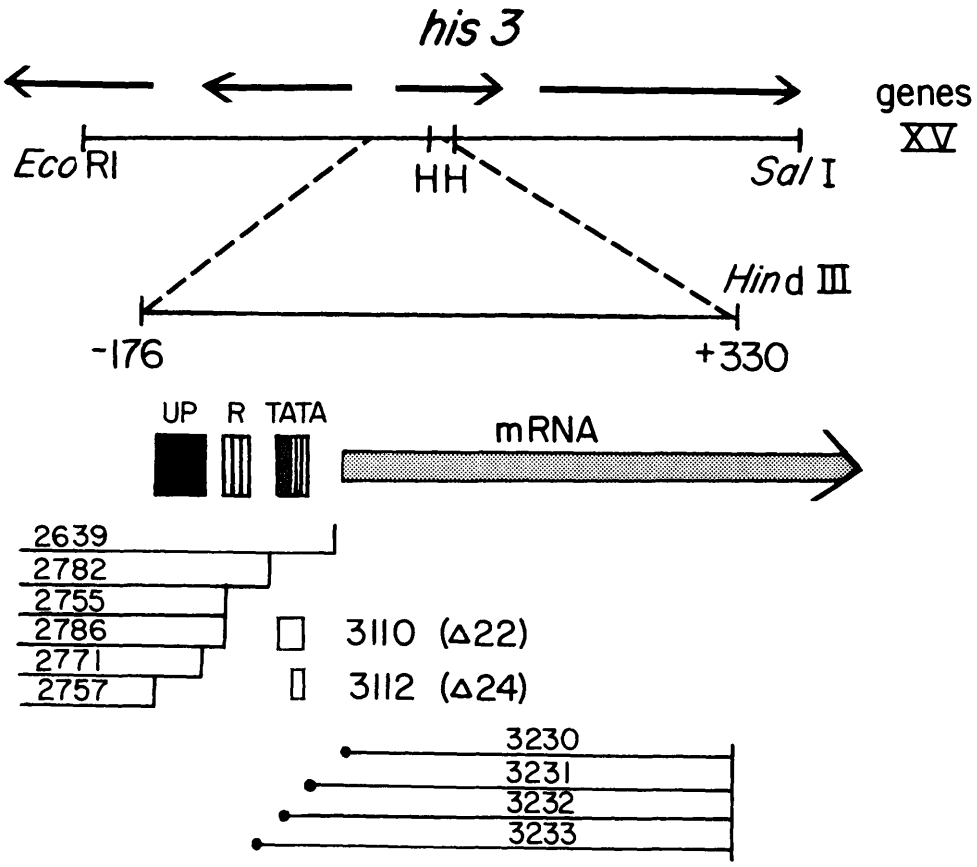

FIG. 1. Structural and functional map of the his3 gene

On the top lines, the locations of his3 and adjacent genes are shown with respect to a 6.1 kb EcoRI-SalI DNA fragment from chromosome XV that contains two internal HindIII (H) sites (7,11). Depicted below in expanded form is a 506 base pair region starting 176 base pairs before the mRNA coding sequences and ending with the HindIII site located 330 base pairs after the mRNA initiation site (7). The locations of the upstream promoter element (UP; see reference 1), regulatory region (R; see reference 3), TATA box promoter/regulatory sequences (TATA; see references 2,3) and mRNA coding sequences are shown with respect to this 506 base pair region. The locations of deleted derivatives (Sc2639, Sc2782, Sc2755, Sc2786, Sc2771, Sc2757, Sc3110, and Sc3112; see references 1,3,6) and of EcoRI-HindIII fragments (Sc3230, Sc3231, Sc3232, and Sc3233) are also shown. These latter fragments were constructed by ligation of an EcoRI octanucleotide linker to various positions of the his3 sequence (2).

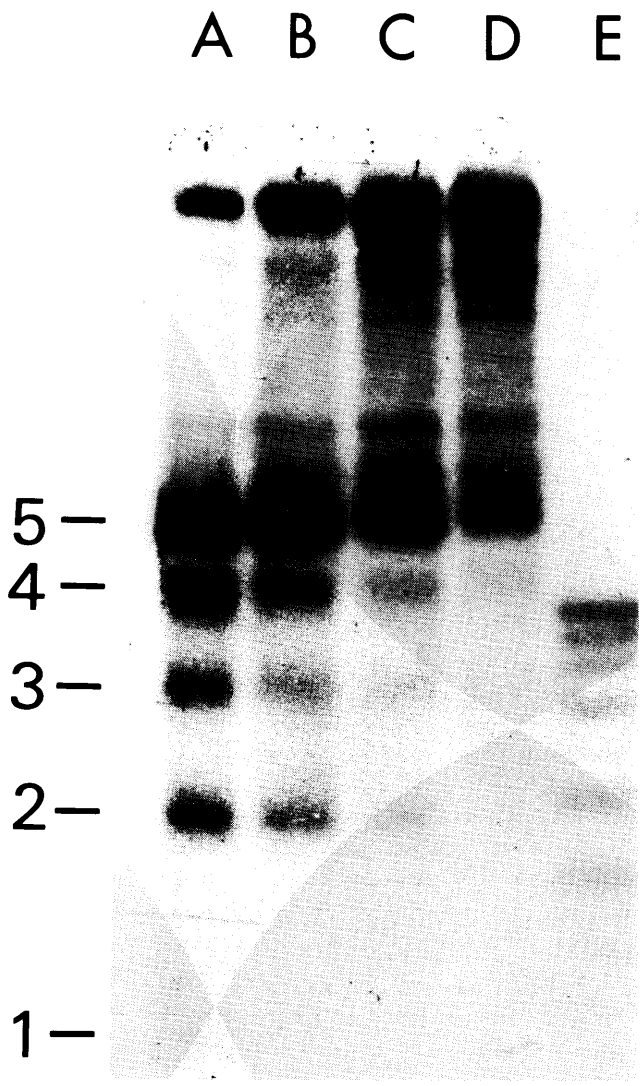

FIG. 2. Nucleosomes at the his3 locus

Spheroplasts from an exponentially growing culture of yeast strain KY29 (trp1-289 ura3-52) were prepared (5) and then lysed by osmotic shock. Nuclei were isolated by differential centrifugation (12), washed twice and then resuspended in nuclease digestion buffer (10mM Tris pH 7.5, 1mM $MgCl_2$. 0.4 ml of nuclei (equivalent to 40 ml of original culture) were treated with various concentrations of micrococcal nuclease (added in 0.1 ml of digestion buffer containing 2.5 mM $CaCl_2$). After 10 minutes at 37 C, DNA was extracted as described in reference 6. Sample DNAs were electrophoretically separated in 2% agarose, transferred to nitrocellulose, and challenged for hybridization with ^{32}P labelled Sc3231 DNA (see figure 1). Lanes A-D represent 1, 0.5, 0.25, and 0.12 micrograms of enzyme; lane E is a size standard prepared by cleaving pBR322 DNA with HaeIII (13). Numbers on the left side of figure indicate positions corresponding monomers, dimers, etc.

in size and shape from those of bulk chromatin (data not shown), thereby suggesting that the spacing of nucleosomes at the his3 locus is typical of yeast chromatin.

Following nuclease treatment, the chromatin structure is disrupted and specific sites of cleavage mapped with respect to a HindIII site located within the structural gene, 330 base pairs from the start of transcription (6; see figure 1). A specific micrococcal nuclease cleavage site is identified by a band of hybridization whose appearance depends upon HindIII treatment. The results are shown in figure 3. At higher micrococcal nuclease concentrations, broad hybridization bands with lengths 160n base pairs are seen. These bands probably correspond to those produced in the absence of HindIII treatment (figure 2); thus they should represent his3 DNA fragments with both ends produced by micrococcal nuclease. Even with the short hybridization probe used in these experiments, such bands are expected for random or non-random nucleosome positioning.

The evidence suggesting that nucleosomes might occupy favored positions comes from the hybridization bands 210, 370, 700, 1900 (and usually 520-550) base pairs in length (Figure 3). Because these bands are not observed in the absence of HindIII treatment (figure 2), their lengths indicate the distance from the HindIII marker to the specific micrococcal nuclease cleavage site. The 370, 700, and 1900 base pair bands are observed even at extremely low concentrations of micrococcal nuclease (average cleavage being about once per 10 kb). Cleavage at the site indicated by the band at 210 base pairs seems less efficient as evidenced by the requirement of higher enzyme concentrations for detection and by the relative weakness of the signal at all concentrations.

Figure 3 also shows the location of these nuclease cleavage sites with respect to the transcriptional map at and around the his3 locus. The sites located 700 and 1900 base pairs from the HindIII marker map near sequences encoding the 5' ends of mRNAs adjacent to the his3 transcript (7). Those located 210 and 370 base pairs from the marker correspond respectively to the his3 mRNA sequences and to the his3 TATA box region.

For purposes of this paper, the nuclease site at the TATA box is of primary interest. To refine its location, the size of the relevant fragment is compared to a series of analogous his3 fragments; these are produced by cleavage of appropriate his3 mutant DNAs with HindIII and EcoRI (see figure 1). The EcoRI sites are located between 340 and 400 base pairs from the HindIII site and define his3 nucleotides -8, -32, -52, and -72. As shown in figure 4 (lanes D,E,F), the micrococcal nuclease site maps at nucleotide -42 + 5; this is entirely within the his3 TATA box region (nucleotides -37 to -51).

It is critical to demonstrate that the observed micrococcal nuclease cleavage sites are a reflection of chromatin structure and not simply a consequence of the enzyme's known specificity for particular DNA sequences (8). Two separate lines of evidence strongly suggest this to be the case.

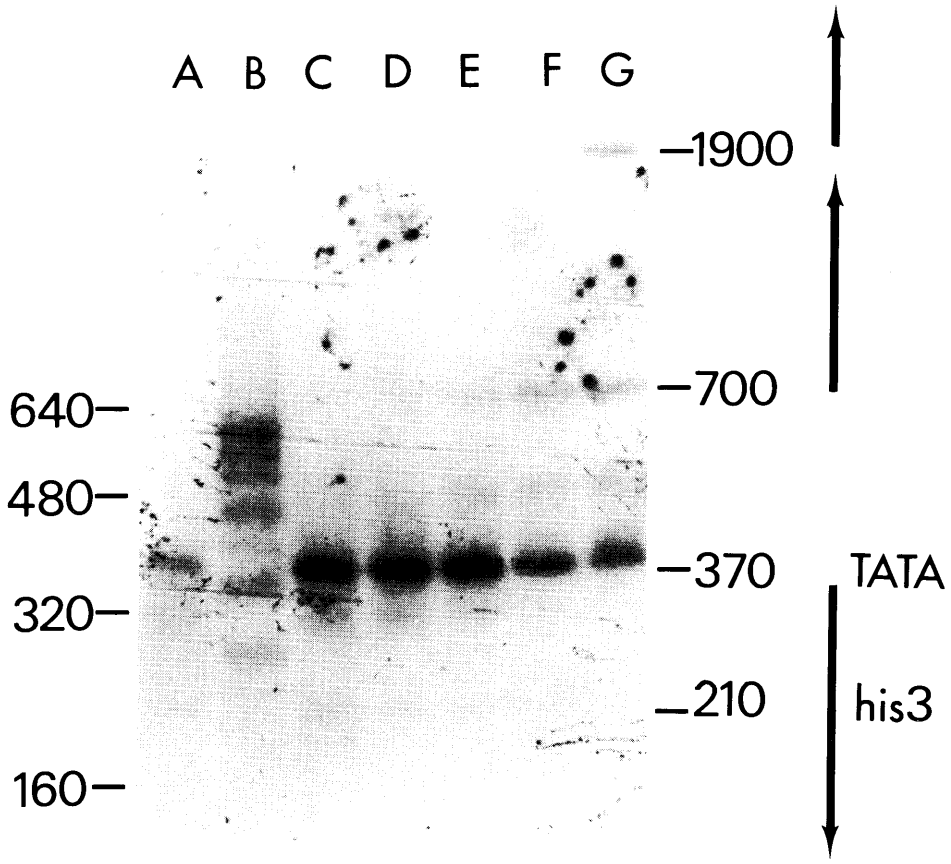

FIG. 3. Micrococcal nuclease hypersensitive sites

The experiment shown in figure 2 was repeated with one exception. After the DNA was purifed, but before electrophoresis, 0.1 micrograms of SV40 DNA were added to each sample; the resulting mixture was digested to completion with HindIII endonuclease (this was monitored by the ethidium bromide staining pattern of the SV40 DNA). Lane A represents treatment with 0.5 micrograms of micrococcal nuclease; lanes C-G represent successive two-fold dilutions of enzyme. The sizes of fragments were determined using HaeIII cleaved pBR322 DNA (lane B) and HindIII cleaved SV40 DNA. Fragment sizes on the left side corrrespond to integral nucleosome units (see figure 2); fragment sizes on the right side refer to specific micrococcal nuclease cleavage sites. The locations of the his3 mRNA, TATA box, and adjacent transcripts are indicated on the right side of the figure.

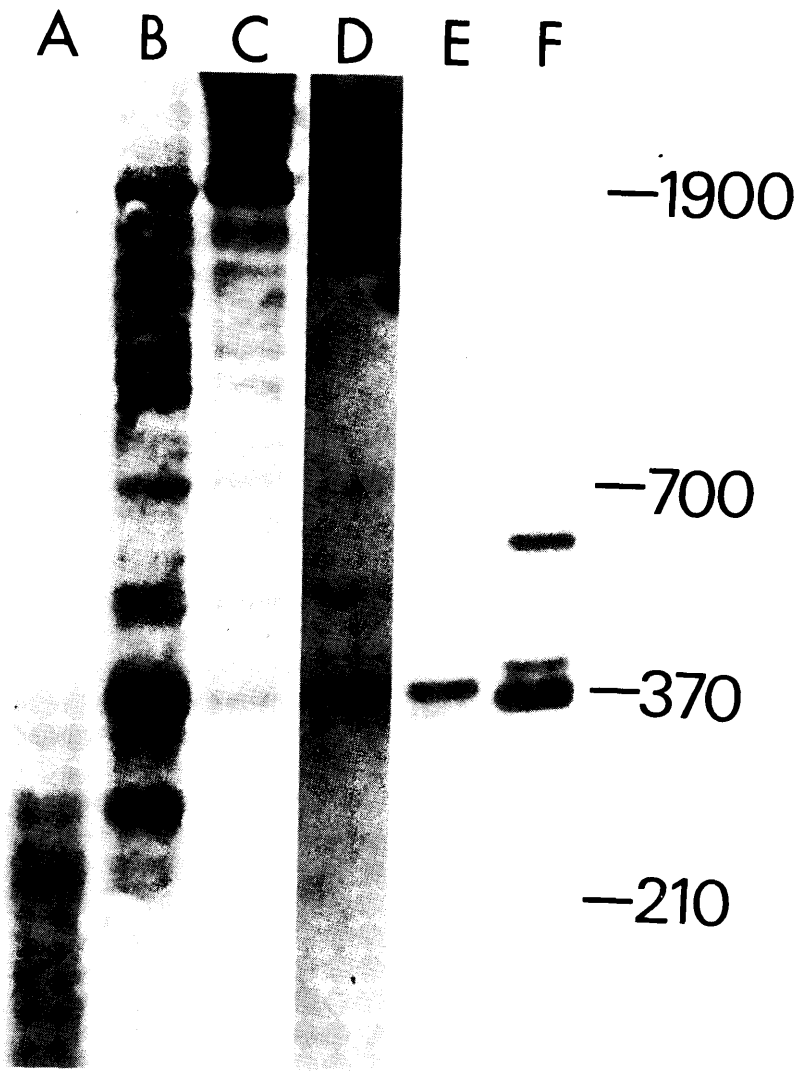

FIG. 4. Fine structure mapping
In lanes A-C, the procedure of figure 3 was followed except that 0.1 micrograms of a λhis3 (λgt4-Sc2601((11) was used instead of yeast nuclei. Each lane represents a 5-fold successive reduction in the amount of micrococcal nuclease. Lane D is the identical sample as shown in figure 3, lane D. Lanes E,F show the results of HindIII-EcoRI cleavage of Sc3230, Sc3232 (E) and Sc3231, Sc3233 (F). The upper doublets in these lanes result from partial cleavage with HindIII. All the samples were examined on the identical gel; however different exposure times were used for various lanes.

First, mapping nuclease cleavage sites in purified DNA reveals approximately 15 sites located 200-2000 base pairs from the HindIII marker (figure 4, lanes A-C): Of these, only a subset of them (370, 700, 1900, and possibly 530) are cleaved in nuclear chromatin. Thus, although DNA sequences are recognized preferentially by micrococcal nuclease per se, they are selectively cleaved in the chromatin structure. Furthermore, the chromatin cleavage site located in the structural gene (indicated by the band at 210 base pairs) is barely, if at all, observed in purifed DNA, and the purified DNA site at 280 base pairs is not seen in chromatin.

Second, cleavage at the his3 TATA box was examined in nuclei prepared from yeast strains in which the normal his3 gene on chromosome XV is replaced by one copy of various his3 DNAs. All five strains tested have the entire TATA box region, but delete to various extents sequences further upstream (see figure 1). From the results shown in figure 5, micrococcal nuclease cleavage at the his3 TATA box is reduced significantly in three out of the five cases. Since the TATA box region is identical in DNA sequence in all these strains, the differences should reflect differences in chromatin structure.

The micrococcal nuclease cleavage site at the TATA box is of particular interest because DNA sequences in this region are important for his3 expression per se (promoter function) and for regulation of this expression in response to conditions of amino acid starvation. The evidence from figure 5 suggests that the chromatin structure at the his3 TATA box can be correlated with promoter function, specifically to the presence of the upstream element. Deletions that remove this element (Sc2771, Sc2755, and Sc2782) severely affect the level of expression and reduce nuclease action at the TATA box. Furthermore, an analogous deletion mutant that retains the upstream element (Sc2757) is indistinguishable both with respect to gene expression and chromatin structure. On the other hand, there is no evidence for a correlation between chromatin structure and gene regulation. Nuclease cleavage at the TATA box is apparently normal when nuclei are prepared from wild type cells starved for amino acids, conditions that result in a 10-fold increase in his3 mRNA levels (Figure 6). Moreover, chromatin structure is unchanged when nuclei are prepared during normal or starvation conditions from strains harboring any of the following mutations: aas2-5039, an unlinked mutation causing basal levels of his3 expression under all growth conditions (9); tra3-1, an unlinked mutation which expresses his3 at the induced rate under all growth conditions (9); and his3-Δ21 or Δ24, mutations at the his3 locus that prevent regulation but express the gene at the normal basal level.

Thus, the results described here constitute evidence for a relationship between his3 genetic elements and chromatin structure. What might this relationship signify in mechanistic terms? Micrococcal nuclease is generally assumed to cleave chromatin in spacer regions between nucleosome cores (5). By this interpretation, the his3 TATA box would lie preferentially in spacer DNA. If correct,

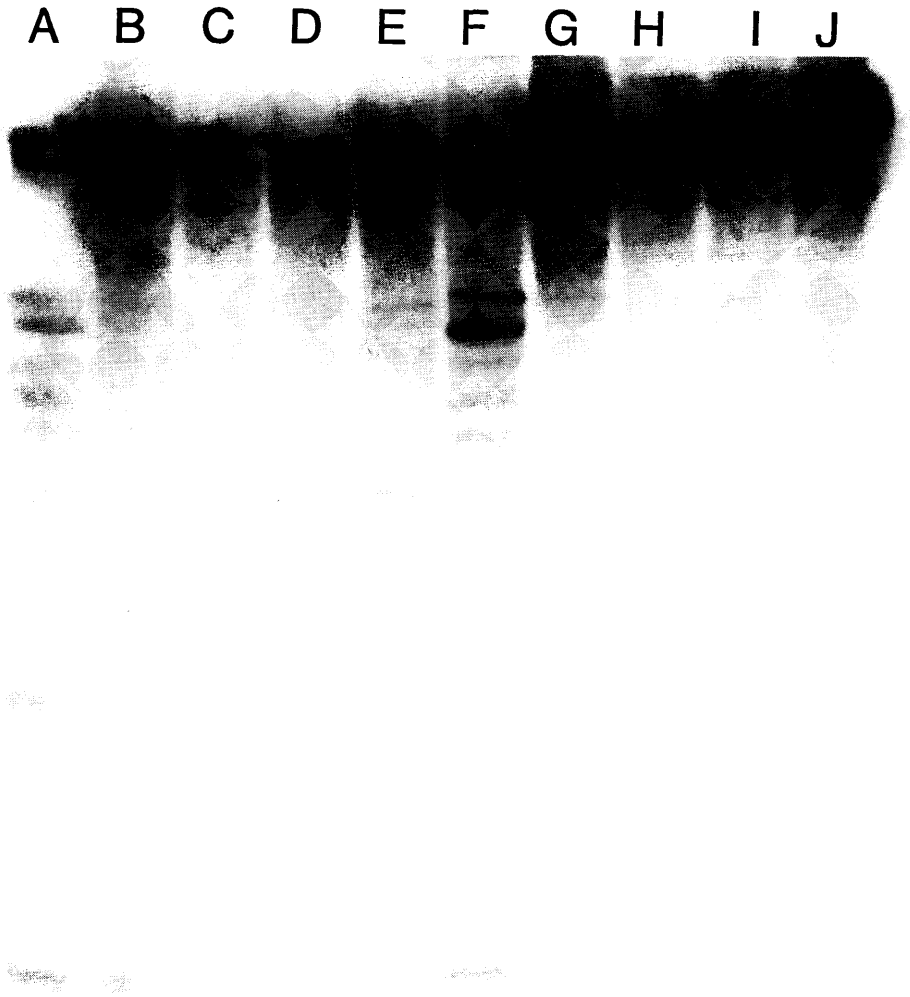

FIG 5. Chromatin structure of his3 mutants

His3 derivatives cloned into the yeast vector YRp21 (1) were introduced into strain KY160 (ura3-52 trp1-289 his3-Δ200). His3-Δ200 was constructed by Michael Fasullo; it deletes the entire his3 locus and sequences as far upstream as -200. Stable variants of Trp^+ transformants were isolated (1,14). The standard procedure was carried out; lanes A-E have four times the nuclease concentration as lanes F-J. The strains used are KY29 (wild type, lanes A and F), Sc2757 (B,G), Sc2771 (C,H), Sc2755 (D,I), and Sc2782 (lanes E,J). The position corresponding to the TATA box is indicated by an arrow. The absence of bands with lengths greater than 370 base pairs in the mutant DNAs indicates that DNA is not sensitive to nuclease in native chromatin.

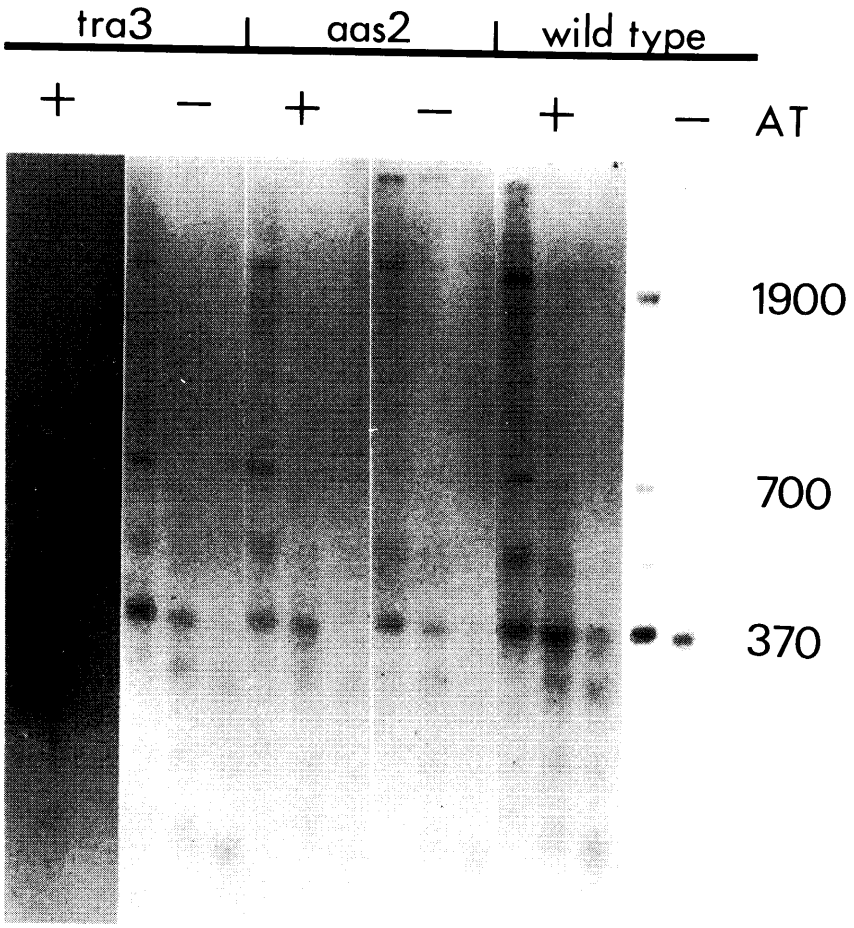

FIG. 6. Chromatin structure in regulatory mutants

The following strains were used: KY29 (wild type), KY107 (aas2-5039), KY108 (tra3-1). Cells were grown in glucose minimal medium in the absence (−) or presence (+) of 10mM aminotriazole. This compound starves yeast cells for histidine by virtue of competitive inhibition of IGP dehydratase, the his3 gene product (15). The figure is a composite of several different experiments. Indistinguishable results were obtained from derivatives Sc3110 (Δ22) and Sc3112 (Δ24).

this predicts micrococcal nuclease cleavage sites at positions displaced from the TATA box by the length of nucleosome units (160 base pairs in yeast). Indeed, the site in the structural gene is located the expected distance from the TATA box. With respect to a site located one nucleosome unit upstream from the TATA box (i.e. 530 base pairs from the HindIII marker), it is difficult to separate a potential desired band (530 base pairs in length) from the observed broad band that corresponds to nucleosome trimers (averaging 480 base pairs).

Taken together, all the data is consistent with Kornberg's view (10) that nucleosome arrangement is generally insensitive to DNA sequence, but that the organization at particular sequences (especially those implicated in gene expression) may be non -random However, even if this specific interpretation is not correct, preferentiaal micrococcal nuclease cleavage of chromatin should be a measure of enzyme access to DNA. Thus in the intact cell, it seems likely that the TATA box region should be exposed to the action of nuclear proteins just like it is to micrococcal nuclease in isolated nuclei. Since this region encodes both a promoter and a regulatory element, such nuclear proteins could include RNA polymerase II and possibly regulatory molecules.

The results here are relevant in terms of the properties of the upstream promoter element. Based on its relatively large distance from the transcriptional initiation site and the striking observation that it can be moved with respect to the TATA box without major effects on gene expression, I suggested that the upstream element might not interact directly with RNA polymerase II (1,2). One proposed role for the upstream element is that it creates an open domain of chromatin which allows polymerase to bind at a favored site (the TATA box?). The data neatly fit this view. However, the correlation between promoter function and chromatin structure does not imply any particular cause and effect relationship. For example, it may not reflect the presence of an upstream promoter element per se, but merely transcription of the gene. In other words, the process of transcription itself could alter the chromatin structure. The simplest relationship with transcription seems unlikely because the ten fold increase in his3 mRNA during amino acid starvation (7) is not accompanied by a detectable change in structure. Furthermore, unlinked suppressor mutations that allow deletion mutants lacking the upstream promoter element to express his3, nevertheless do not regain TATA box sensitivity.

The analysis of chromatin from more his3 mutants should be useful in understanding the nature of the correlation between structure and function.

I thank Michael Fasullo for sending me a strain containing his3-Δ200 and the National Institute of Health for supporting this work (Grant GM30186 from the division of general medical sciences).