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Removal of positioned nucleosomes from the yeast PHO5 promoter upon PHO5 induction releases additional upstream activating DNA elements

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The chromatin fine structure in the promoter region of PHO5, the structural gene for a strongly regulated acid phosphatase in yeast, was analyzed. An upstream activating sequence 367 bp away from the start of the coding sequence that is essential for gene induction was found to reside in the center of a hypersensitive region under conditions of PHO5 repression. Under these conditions three related elements at positions −469, −245 and −185 are contained within precisely positioned nucleosomes located on both sides of the hypersensitive region. Upon PHO5 induction the chromatin structure of the promoter undergoes a defined transition, in the course of which two nucleosomes upstream and two nucleosomes downstream of the hypersensitive site are selectively removed. In this way approximately 600 bp upstream of the PHO5 coding sequence become highly accessible and all four elements are free to interact with putative regulatory proteins. These findings suggest a mechanism by which the chromatin structure participates in the functioning of a regulated promoter.

Key words: chromatin structure/hypersensitive sites/nucleosome phasing/gene regulation/acid phosphatase

Introduction

Gene activation in eukaryotes has been shown in many cases to be correlated with changes in the chromatin structure of the gene (for reviews see Mathis *et al.*, 1980; Elgin, 1981, 1984; Igo-Kemenes *et al.*, 1982; Cartwright *et al.*, 1982; Weintraub, 1985). A characteristic feature of many active genes is the presence of so-called hypersensitive sites, narrow regions that are highly susceptible to DNase I and other nucleases.

Although the underlying DNA sequences have been identified in a number of cases as elements important for gene regulation (e.g. Wu, 1980; McGhee *et al.*, 1981; Shermoen and Beckendorf, 1982; Emerson and Felsenfeld, 1984; Lohr, 1984; Fritton *et al.*, 1984; Emerson *et al.*, 1985; Proffitt, 1985), very little is known about the functional role of hypersensitive sites. Hypersensitivity may be a prerequisite for subsequent gene activation, for example by generating torsional stress required for transcription (Weintraub, 1985). Alternatively, the increased sensitivity to nucleases may merely be a consequence of the binding of protein factors that prevent the simultaneous organization of the DNA in nucleosomes. The difficulty in differentiating between such alternatives is due to the fact that in most of the complex systems used for chromatin investigations it is only possible to provide correlations between independently occurring events rather than to establish functional relationships.

In an effort to study cause and effect of chromatin elements we have turned to yeast as an experimental system where it is possible to replace a wild-type gene by a limitless number of mutated alleles and investigate the effects on chromatin structure in parallel to those on gene expression and regulation.

We have begun to characterize the chromatin structure of the tandemly linked structural genes for two acid phosphatases, PHO5 and PHO3 (Almer and Hörz, 1986). The PHO5 gene, which is strongly regulated by phosphate concentration in the medium, is particularly attractive for these chromatin studies since

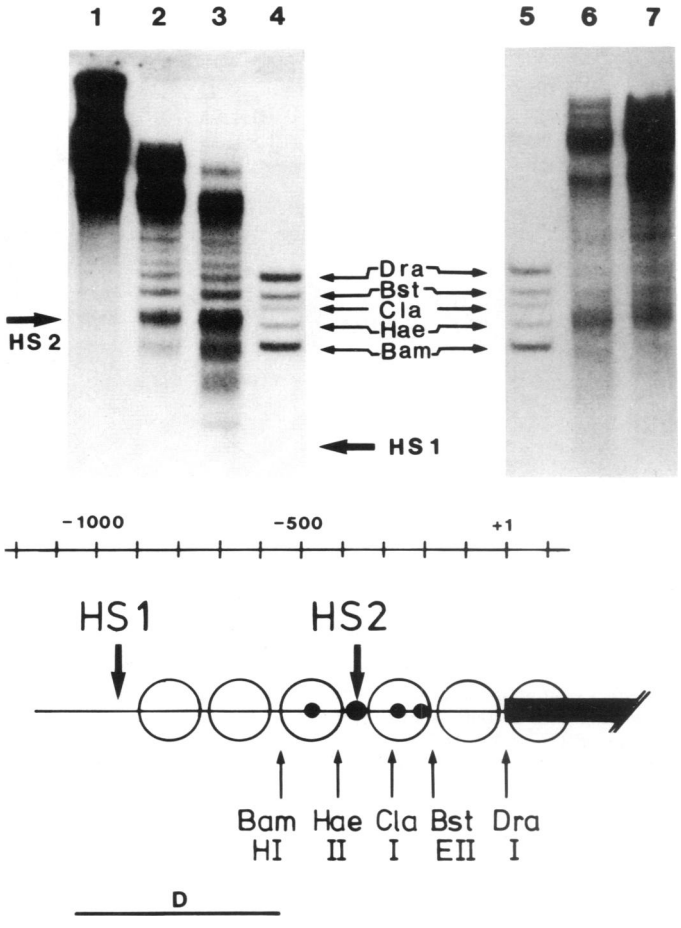

Fig. 1. Chromatin fine structure at the PHO5 promoter. Nuclei from cells grown under conditions of PHO5 repression were digested for 20 min with 0.3, 1, 3 U/ml micrococcal nuclease **(lanes 1–3)** or 1 and 0.3 U/ml DNase I **(lanes 6** and **7)**. DNA was isolated, cut with *Apa*I, separated in a 1.5% agarose gel, blotted and hybridized with probe D. The *Apa*I site used is about 1300 bp upstream of the PHO5 gene. **Lanes 4** and **5** contain a mixture of five different restriction nuclease double digests, each with *Apa*I and in addition a second nuclease as indicated. The chromatin structure of the PHO5 promoter with the hypersensitive sites (HS), positioned nucleosomes and the four 19-bp UAS elements (indicated by solid circles) is shown underneath. The circles are larger than the actual elements. The scale is in base pairs with +1 referring to the initiation codon of the PHO5 coding sequence (part of the gene is shown in solid black underneath).

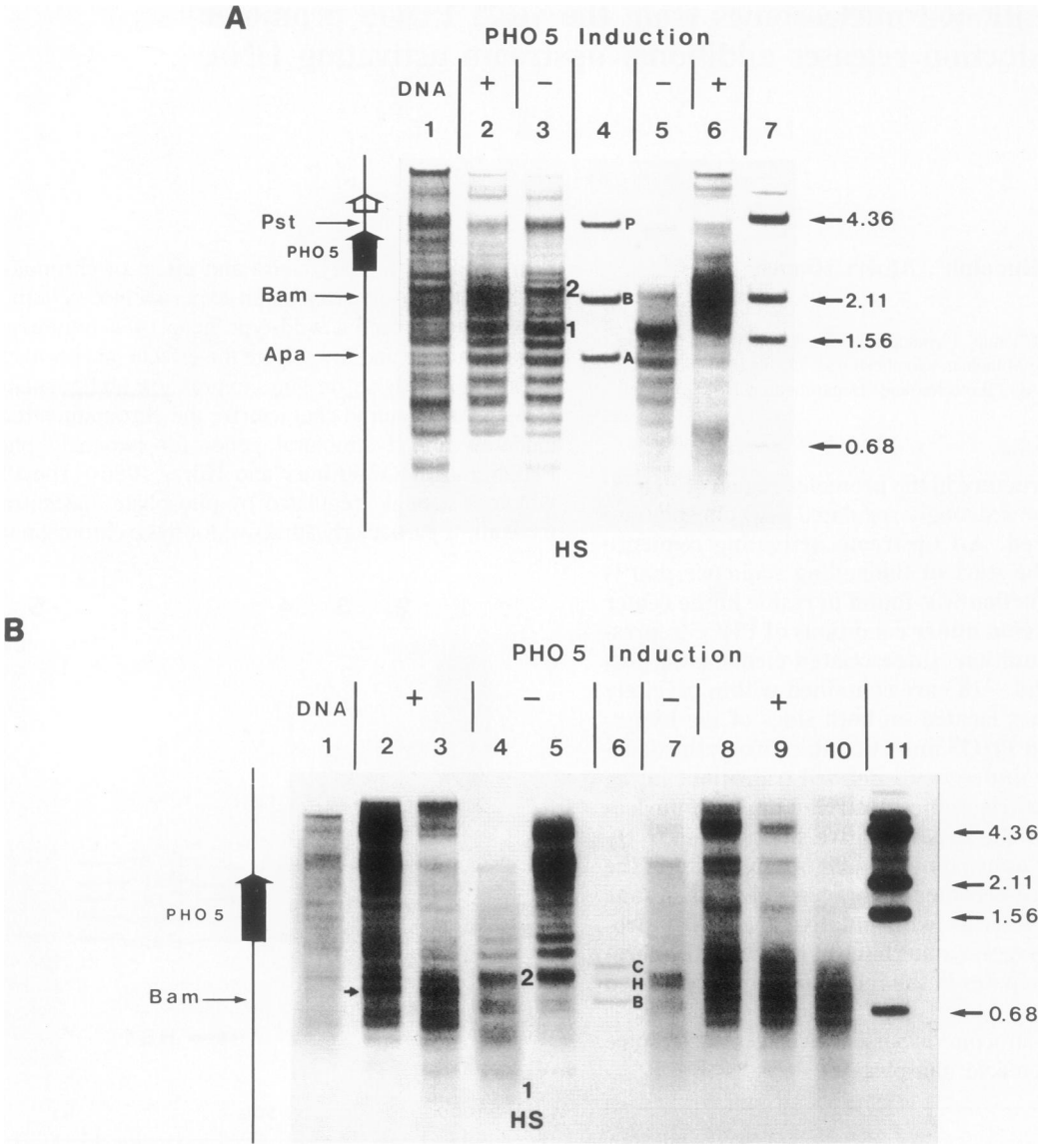

Fig. 2. PHO5 induction leading to a localized change in the chromatin structure confined to the PHO5 promoter. **(a)** Nuclei isolated from cells that had been grown in YPDA (− Induction) or no phosphate medium (+ Induction) were digested with 2 U/ml micrococcal nuclease **(lanes 2** and **3)** or with 1 U/ml DNase I for 20 min **(lanes 5** and **6)**. DNA was isolated, digested with *Eco*RI, blotted and hybridized with an *Eco*RI/*Cla*I subclone (probe **A**). The *Eco*RI site is about 2.6 kb upstream of the PHO5 coding region. A control digest of free DNA with 0.05 U/ml micrococcal nuclease, subsequently cleaved with *Eco*RI, is shown in **lane 1**. **Lane 4** contains three double digests of total yeast DNA, each with *Eco*RI and either *Apa*I (A), *Bam*HI (B) or *Pst*I (P) and **lane 7** contains pBR322 reference fragments with sizes given in kb. **(b)** The same nuclei as in **(a)** were digested with 3, 6, 10 and 3 U/ml micrococcal nuclease **(lanes 2−5)**, or with 1, 1, 2 and 4 U/mol DNase I for 20 min **(lanes 7−10**, respectively). DNA was isolated, digested with *Apa*I and analyzed as in Figure 1. A control digest of free DNA with 0.05 U/ml micrococcal nuclease, subsequently cleaved with *Apa*I, is shown in **lane 1**. **Lane 6** contains three of the five double digests shown in Figure 1 (*Bam*HI, *Hae*II, *Cla*I) and **lane 11** the pBR322 reference fragments. The arrow denotes a prominent fragment characteristic of the active state that is not observed with free DNA (compare **lanes 1** and **2)**.

complementary information at the DNA level has recently become available through a dissection of the promoter that led to the identification of four upstream activating sequence elements (Rudolph and Hinnen, 1986).

As described in the accompanying paper (Almer and Hörz, 1986), the PHO5 gene is organized in a distinct chromatin structure under conditions of gene repression, with hypersensitive sites in the promoter region that are flanked by precisely positioned nucleosomes. In this report, the upstream activating sequence elements are correlated with features of the chromatin structure under repressed conditions, and the chromatin configuration is shown to change at precisely these elements upon gene activation. The chromatin transition observed upon gene induction suggests a general mechanism by which chromatin elements might contribute to gene activation.

Results

A hypersensitive site in the PHO5 promoter corresponds to an upstream activating sequence element

The promoter region of the PHO5 gene has been screened for functionally important DNA sequences by the construction of a comprehensive series of deletion mutants (Rudolph and Hinnen, 1986). Short deletions in two separate regions severely reduced

gene expression. One region contains the well-known TATA box, the other is located further upstream, approximately 370 bp from the PHO5 initiation codon. A DNA element within the latter region at position −367 could be identified that was shown to play a vital role in gene activation (Rudolph and Hinnen, 1986), most likely through interactions with a regulatory protein(s). In its properties it resembles so-called 'upstream activating sequences' (UAS) demonstrated for a number of other yeast genes (Guarente, 1984). Additional elements related to the element at −367 were found at positions −469, −245 and −185. Deletions in at least the latter two were found to reduce gene expression, but not as dramatically as a deletion eliminating the −367 element (Rudolph and Hinnen, 1986).

According to the mapping experiments described in the accompanying paper (Almer and Hörz, 1986), one of the two hypersensitive sites upstream of PHO5 (HS 2) roughly corresponds to the location of the −367 upstream element identified by deletion mutagenesis. In order to determine the precise relation between this element and HS 2, nuclei were digested with micrococcal nuclease and DNase I and indirect end-labeling experiments from a nearby restriction site were carried out. Figure 1 shows the results of these mapping experiments. It was found that the sensitive region starts around a *Hae*II site (position −406), extends for 60−70 bp and ends around position −340, i.e. well before a *Cla*I site (−275). Thus the −367 UAS element is positioned precisely within the hypersensitive region HS 2 and is thereby nucleosome free under conditions of PHO5 repression. The other three elements, on the other hand, are located within positioned nucleosomes under these conditions (see Figure 1).

Changes in the chromatin structure of the PHO5 promoter upon gene activation

Hypersensitivity in the PHO5 promoter region clearly exists under high phosphate conditions, i.e. growth conditions under which PHO5 is repressed. Since hypersensitive sites are usually the hallmark of active genes it was important to determine if PHO5 activation would be accompanied by any changes in the chromatin structure of the PHO5 promoter.

Nuclei from cells grown in conditions with either little or no phosphate (see Materials and methods) were digested with micrococcal nuclease and DNase I. The DNA was digested with *Eco*RI or *Apa*I, separated by gel electrophoresis, blotted and hybridized with the appropriate probe. The view from the far upstream *Eco*RI site is shown in Figure 2a and the close-up from the nearby *Apa*I site in Figure 2b. If the micrococcal nuclease pattern is compared with the control pattern from inactive nuclei the following points emerge. The overall chromatin organization of the PHO5/PHO3 locus remains essentially the same. There are, however, characteristic changes confined to the PHO5 promoter region around HS 2 (compare lanes 2 and 3 of Figure 2a). The changes are seen more clearly by using secondary digestion with *Apa*I (Figure 2b). The regular pattern around HS 2, which is due to positioned nucleosomes that are characteristic of the inactive state, is no longer detectable. Instead the entire region around HS 2 appears to be fairly accessible to micrococcal nuclease, and the pattern is almost indistinguishable from the free DNA control (lane 1 in Figure 2b). The only additional site that is cut by micrococcal nuclease, with some preference in the active state as compared with free DNA, gives rise to a distinct band in the autoradiogram around position −470 (arrow in Figure 2b, lane 2), i.e. about 100 bp upstream of HS 2.

The DNase I digests similarly reflect the increased accessibility

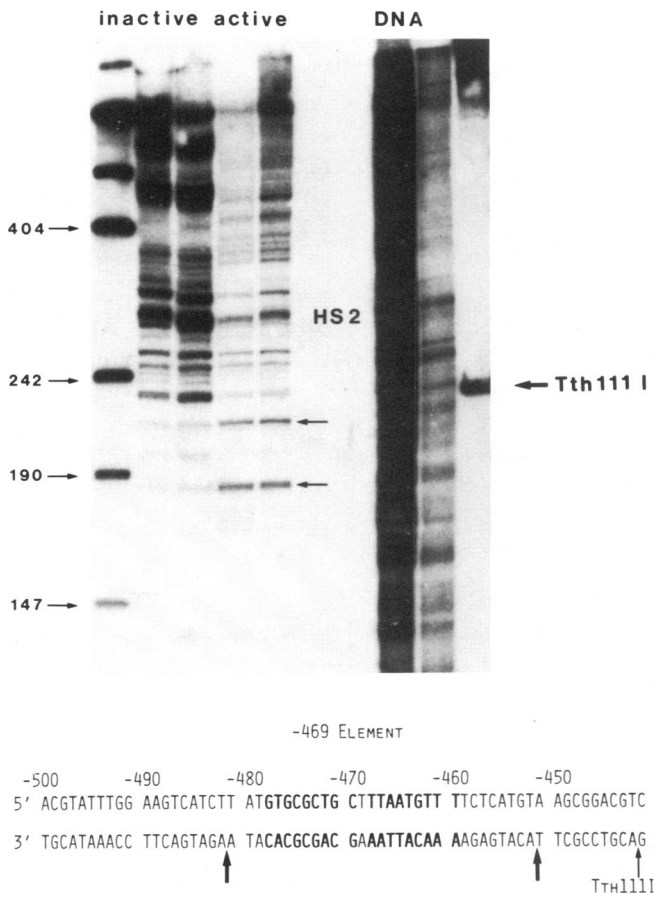

Fig. 3. High-resolution analysis of micrococcal nuclease cuts upstream of HS 2. Inactive nuclei, active nuclei and DNA were digested for 20 min with 3, 10, 10, 6, 0.1 and 0.05 U/ml micrococcal nuclease (from left to right). DNA from the chromatin digests were cut with *Nde*I, applied to an 8% sequencing gel, blotted, and hybridized as described in Materials and methods. The *Nde*I site used is around position −760. A 120-bp *Nde*I/*Bam*HI probe denoted F was labeled so that it only recognizes the lower strand in the sequence shown below (equivalent to the PHO5 coding strand). Arrows denote enhanced cutting by the nuclease in the active state. A *Tth*111I/*Nde*I double digest is shown on the right and a *Hpa*II digested pBR322 clone on the left with fragment sizes listed in nucleotides. The positions of enhanced cutting by micrococcal nuclease in the active state are shown underneath in the DNA sequence (Bajwa *et al.*, 1984). Nucleotides belonging to the −469 element are in heavy print. The positions of the two cuts were independently confirmed by co-electrophoresis with *Nde*I/*Hha*I and *Nde*I/*Bbv*I double digests.

of a region much larger than HS 2 (Figure 2a, lane 6 and Figure 2b, lanes 8−10). The boundaries of the DNase I sensitive region map around position −50 and −650. Thus altogether about 600 bp are preferentially cleaved by the nuclease under conditions of PHO5 activation.

Interestingly, this transition in the chromatin structure is not always observed as dramatically as shown in Figure 2. Different strains respond differently, and the extent of PHO5 induction seems to play a role. For example, even at conditions of maximal PHO5 induction strain S288c yields patterns that resemble the inactive patterns more closely than the active ones shown in Figure 2. In addition, even with strain IH2 that was used throughout this work, it was found that nuclei from cells that had been induced to 30−40% of the maximal value gave patterns virtually indistinguishable from those of inactive nuclei and clearly different from patterns obtained when three to four parts fully active nuclei were mixed with six to seven parts fully inactive nuclei

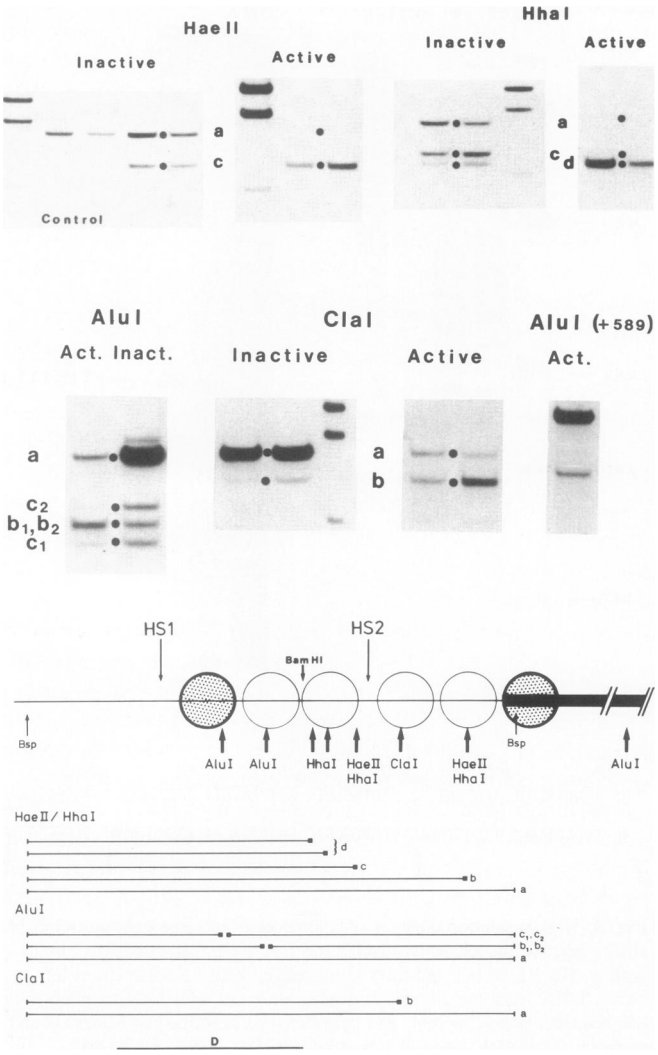

Fig. 4. Analysis of the PHO5 promoter from active and inactive nuclei by digestion with restriction nucleases. Nuclei containing approximately 50 μg of DNA were digested for 60 min in 100 μl with the restriction nuclease as indicated, DNA was isolated, cleaved with *Bsp*RI (an isoschizomer of *Hae*III), separated in a 1.5% agarose gel and hybridized to probe D after Southern transfer. Restriction nucleases were usually used at two concentrations to check how close the digests were to limit digests. The second lane corresponds to the higher concentration in each case. The controls shown in the *Hae*II panel are DNA before and after incubation of nuclei without enzyme. The individual fragments are identified underneath. The nucleosomal structure in the inactive state is indicated. Non-stippled nucleosomes denote those removed upon activation. In order to determine the extent of cleavage at a particular restriction site in chromatin, the relative amount of each fragment was evaluated by measuring the radioactivity directly in a scintillation counter. The *Alu*I site at position +589 within the coding sequence (last panel) was monitored by treating the same digest that was used to analyze the two upstream *Alu*I sites with *Bam*HI and *Pst*I and hybridizing it to probe G. The fragment at the top of the gel is the *Bam*HI/*Pst*I fragment, while the lower one is a *Bam*HI/*Alu*I fragment which signals cleavage by *Alu*I at that site in chromatin. Nuclease units (U) per sample were as follows: *Hae*II, 50, 150; *Hha*I, 50, 150; *Alu*I, 50; *Cla*I, 30, 100.

prior to digestion. It might be, therefore, that this chromatin transition is a characteristic of the fully induced state (see Discussion).

High resolution mapping reveals enhanced cutting by micrococcal nuclease on both sides of an upstream activating element

In addition to the sequence element at position −367 that was shown to be essential for PHO5 activation, three other elements

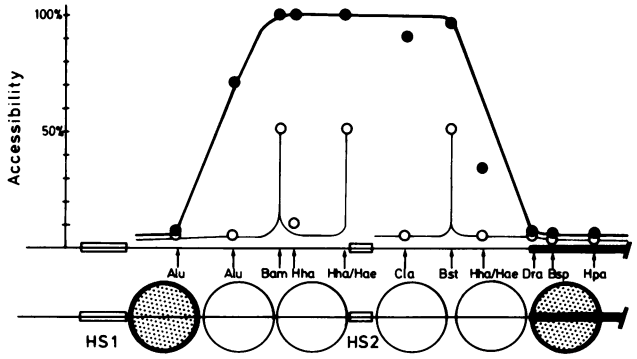

Fig. 5. The PHO5 promoter is rendered accessible to restriction nucleases upon PHO5 activation. Accessibility measurements for various restriction sites in chromatin, as shown in Figure 4, are summarized. Solid circles refer to active nuclei, open circles to inactive nuclei. The non-stippled nucleosomes denote those that are absent in active nuclei.

were detected on the basis of their sequence homology in the vicinity, at positions −469, −245 and −185 (Rudolph and Hinnen, 1986). The location of the first element roughly corresponds to the position of enhanced cutting by micrococcal nuclease in the active state (see arrow in lane 2 of Figure 2b). In order to determine the precise location of these cuts, sequencing gels were used for indirect end-labeling experiments with strand-specific DNA probes by the method of genomic sequencing (Church and Gilbert, 1984) (Figure 3).

Firstly, it is again apparent that there is a change in the micrococcal nuclease pattern upon PHO5 activation. The strong bands due to digestion in nucleosome linker regions downstream of HS 2 (top part of the gel) that are characteristic of the inactive state are not observed in the digestion of active chromatin. Side-by-side comparison of inactive and active material reveals that the enhanced cutting upstream of HS 2 that was noticed before in the analysis of the active state (Figure 2b) occurs at positions −481 and −451 (arrows in Figure 3). This is precisely at the border of element I (see Figure 3) which extends from position −478 to −460. The sequence element itself, on the other hand, is resistant to micrococcal nuclease despite the fact that it contains a preferred target sequence for micrococcal nuclease (5′ CATTAAAG 3′) (Hörz and Altenburger, 1981) and is readily cleaved at that position in free DNA (Figure 3).

Nucleosomes are absent from the active PHO5 promoter: evidence from restriction nuclease experiments

The results of the indirect end-labeling experiments presented above might indicate a redistribution of nucleosomes from a positioned array characteristic of the inactive state to random arrangements with less densely packed nucleosomes. Alternatively, nucleosomes might be absent altogether from that region upon gene induction. The answer to this question is obviously relevant for our understanding of PHO5 activation. In order to discriminate between the two possibilities, two types of experiments were performed. First, the accessibility of restriction sites to the nucleases in the active chromatin were assayed in comparison with inactive nuclei. Secondly, micrococcal nuclease digests were hybridized directly with very short DNA probes from the regions in question in order to assay for the absence or presence of nucleosomal DNA bands.

The effect of PHO5 activation on the accessibility of different restriction sites was monitored in a region starting 800 bp upstream of the PHO5 gene and extending 1000 bp into the coding sequence. This is shown for some of the restriction nucleases

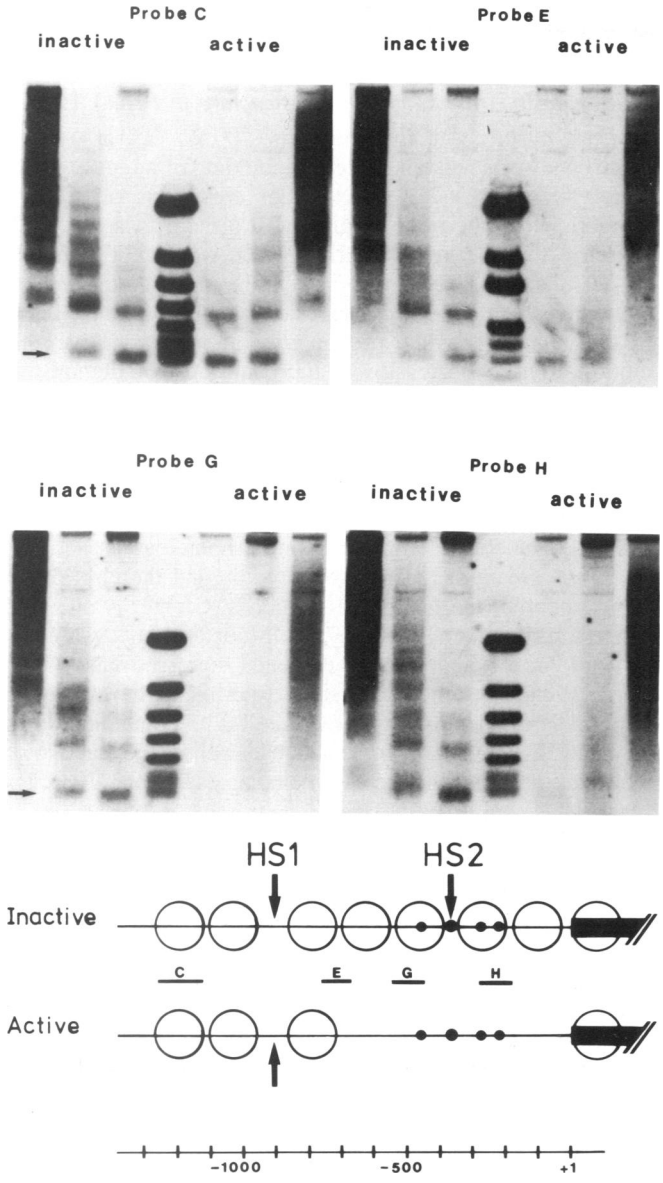

Fig. 6. Absence of nucleosomes in the PHO5 promoter region following gene activation. Active and inactive nuclei were digested for 20 min with 6, 20 and 50 U/ml micrococcal nuclease. DNA was isolated and hybridized after Southern transfer to the probes indicated. The same digests were used in all four experiments. Identical results were obtained upon rehybridizing a filter with a second probe after washing off the first one. *Hpa*II-digested pBR322 DNA was used as a mol. wt reference. The position of the mononucleosomal DNA fragments of about 150 bp is indicated by an arrow on the gel.

in Figure 4, and all results are schematically summarized in Figure 5. The most striking effect is a massive opening up of the chromatin structure between position −650/700 and the start point of the actual gene, i.e. the region where precisely positioned nucleosomes are located on both sides of HS 2 in the inactive state. For example, a *Hha*I site at position −514 becomes susceptible upon activation, whereas it had been largely inaccessible before (Figure 4). The *Hha*I site at position −405, which is at the very border of a positioned nucleosome in the inactive state, is 50% susceptible before activation and 100% afterwards (Figure 4). Of two *Alu*I sites, about 640 and 760 bp upstream of the gene, both cleaved in only a few per cent of the nuclei in the inactive state; the −640 site becomes accessible upon induction while the site at −760 remains resistant to the nuclease. An *Alu*I site at position +589 within the coding sequence similarly stays largely inaccessible after gene activation (Figure 4). An analogous picture for the actual coding region emerges when other restriction nucleases are employed: the accessibility for five different nucleases (*Bsp*RI, *Hpa*II, *Sal*I, *Bst*EII, *Alu*I) was in the 5−15% range regardless of the state of PHO5 activation.

It is important to realize that the ability to cut DNA in chromatin differs for different restriction nucleases and does not only depend on the actual accessibility of a particular cleavage site. The chromatin environment may affect some enzymes more adversely than others, and the actual size of the enzyme may play a role. Consequently, susceptibility measurements can only be taken as relative values. For example, the resistance of a site to a restriction nuclease in inactive chromatin is only meaningful if it can be shown in the same digest that another site for that nuclease located elsewhere is indeed cleaved or, even better, if the same site is susceptible when active nuclei are used. All sites in Figures 4 and 5 fulfil either one of these criteria.

Nucleosomes are absent from the active PHO5 promoter: evidence from micrococcal nuclease experiments

Figure 6 shows the results obtained by digesting nuclei from cells grown in either low or high phosphate conditions and hybridizing the DNA, without any secondary digestion, to different short DNA probes (100−120 bp) from the PHO5 promoter region. Appearance of a 145 bp DNA band is taken to reflect the presence of a nucleosome at the region recognized by the probe.

Probe C from a DNA region upstream of HS 1, i.e. a region not implicated in PHO5 regulation, was used as a control and did indeed confirm that both active and inactive nuclei yield a nucleosomal ladder upon digestion. Probe E still recognizes core-size DNA fragments with active nuclei, but dinucleosomal DNA, abundant in inactive nuclei, is barely detectable. With probes G and H there is essentially no evidence of a nucleosome at the corresponding locations in the active nuclei, while a nucleosomal ladder is clearly present in the inactive state. It is important to realize that the same two DNA digests were probed in all four experiments.

The outcome of these experiments therefore fully agrees with the indirect end-labeling experiments and the restriction nuclease analyses. Of the three nucleosomes upstream of HS 2, only the most distal one persists after gene activation — the other two are absent. The nucleosome flanking HS 2 at the downstream boundary that is found under conditions of gene repression similarly disappears upon gene induction.

The fate of the nucleosome positioned immediately upstream of the actual coding sequence that contains the TATA box in its very center could not be determined with the same certainty as the others. In the indirect end-labeling experiments its position coincides with the boundary of the accessible domain found after PHO5 activation. Direct hybridization of micrococcal digests with a probe derived from that region is not possible since the probe cross-hybridizes with a homologous region in the PHO3 and possibly also the PHO11 promoter. Finally, the third approach which measured the accessibility of restriction sites, was applicable only to a *Hae*II/*Hha*I site located next to the TATA box. The susceptibility of this site to the two nucleases increased from about 5% to 35% upon gene activation. In the same digests, the *Hae*II/*Hha*I sites located further upstream became 100% susceptible (Figure 4). These results are interpreted to mean that the positioned nucleosome on the TATA box also disappears upon gene induction but that the transcriptional apparatus confers some

protection against restriction nuclease attack. We cannot exclude the possibility, however, that heterogeneously positioned nucleosomes persist around the TATA box in the active state as opposed to a precisely positioned nucleosome.

Discussion

Chromatin organization of DNA elements implicated in PHO5 regulation

A comprehensive analysis of *in vitro* engineered deletion mutants in the PHO5 promoter region has revealed the existence of an upstream activating sequence (UAS) element around position −360 to −380 that is essential for PHO5 activation (Rudolph and Hinnen, 1986). A 20-bp sequence within that region (at position −367) is thought to be responsible for the activation effect by interacting with a regulatory protein(s). Our experiments show that this sequence element is in the center of a 60−70 bp hypersensitive region. Interestingly, hypersensitivity at this position is found equally at low as well as high phosphate conditions, i.e. independently of gene activation.

In addition to the element at −367, three other DNA elements with striking homology had been found close by, at positions −469, −245 and −185. From the analysis of the deletion mutants, the −245 and the −185 elements appear to contribute to PHO5 activation, albeit at a lower level than the −367 element, while a large deletion that destroys the −469 element had no effect (Rudolph and Hinnen, 1986).

At high phosphate conditions, the hypersensitive site containing the −367 element is surrounded by precisely positioned nucleosomes to the effect that the −469 and −245 elements are in the very center of nucleosome core particles, while the −185 element is at the boundary of a nucleosome core (Figure 1). This situation changes dramatically upon activation of PHO5: nucleosomes seem to be absent altogether from that region of the PHO5 promoter. Our conclusion is based on three types of experiments. (i) Indirect end-labeling experiments show that micrococcal nuclease generated bands that reflect linker regions disappear upon gene activation, and a much larger region becomes susceptible to DNase I. (ii) Hybridizing extensive micrococcal nuclease digests to short probes from the region in question fails to uncover a 145-bp core particle DNA band at low phosphate conditions that is otherwise present. (iii) The accessibility of the entire chromatin region to restriction nucleases increases dramatically to almost 100% after PHO5 activation.

The observed changes are confined to a very short region. Of the three nucleosomes upstream of HS 2, only the two proximal ones are affected, and one to two nucleosomes in the downstream direction, i.e. towards the gene, are removed (Figures 5 and 6). The chromatin structure of the transcribed region itself does not undergo any major changes and remains largely protected against nuclease cleavage even after gene activation.

Previous analyses of the PHO5 promoter region have led to the conclusion that hypersensitivity is only established following gene activation (Bergman and Kramer, 1983). It might be, however, that in these experiments only the extensive open domain formed upon gene activation was detected by nuclease digestion, and that hypersensitivity in the inactive state confined to a shorter region was originally not recognized and only later taken to be a 'long nucleosome linker region' (Bergman *et al.*, 1986). In the same paper it was reported that the superhelical density of a PHO5 containing plasmid in yeast decreases by about three turns upon PHO5 induction (Bergman *et al.*, 1986). They do not ascribe this effect to the loss of nucleosomes but instead to other topological changes. However, our data for the chromosomal PHO5 gene clearly show the loss of three to four nucleosomes upon PHO5 activation, and it might be that the plasmid undergoes a similar transition.

A possible role for the chromatin structure in PHO5 activation

Nuclease hypersensitivity at position −400 to −350 at high phosphate conditions most likely reflects binding of a regulatory protein(s) to the −367 element under conditions of phosphate repression (see below). Binding of a protein to a short DNA stretch within the hypersensitive region would not be detected by our digestion experiments. A similar situation has been encountered at the *Drosophila* heat shock locus, where the binding of a heat shock activator protein within a hypersensitive region did not affect hypersensitivity and could only be demonstrated by additional digestion of the chromatin with exonuclease III (Wu, 1984).

For the *GAL1/GAL10* genes that seem to be regulated in a fashion closely resembling that of the PHO5 gene, it was found by *in vivo* methylation experiments that the *GAL4* product, a positive regulator protein, binds to four homologous sites within a 120-bp stretch at the promoter region and thereby activates transcription of the *GAL1/GAL10* genes (Giniger *et al.*, 1985). Interestingly, binding of *GAL4* to all four elements was equally shown to occur in the *GAL80* mediated repressed state (Giniger *et al.*, 1985). Chromatin digestion experiments have revealed a large hypersensitive region of about 200 bp containing all four elements, again present in the active as well as the inactive state which is consistent with the *GAL4* binding data (Lohr, 1984; Proffitt, 1985).

Although there is a close similarity at the DNA level between the PHO5 promoter and the *GAL1/GAL10* promoter, their organization in chromatin is different in one important aspect. In the GAL system a 200 bp region containing all four activating sequence elements is constitutively accessible, regardless of the state of activation (Lohr, 1984; Proffitt, 1985). For PHO5, on the other hand, only the −367 element is clearly available at conditions of PHO5 repression. The other three elements are contained within positioned nucleosomes. Even though DNA wrapped around the histone octamer might still be able to interact with the regulatory proteins, the change in the chromatin structure seen upon PHO5 activation suggests a different sequence of events. At limiting phosphate concentrations an activating protein(s) might modify the interaction of the regulatory protein with its target in such a way as to locally unfold the chromatin, for example by enhancing an intrinsic tendency of the underlying DNA to adopt an alternative DNA conformation (counteracted otherwise by the regulatory protein). That a regulatory protein can indeed induce an alternative DNA structure has been shown for the *Escherichia coli lac* promoter which assumes a bent conformation upon interaction with the CAP protein (Wu and Crothers, 1984).

Once histone−DNA interactions are labilized to an extent that a significant proportion of the DNA becomes transiently free, the DNA might then interact with the regulatory protein by virtue of the other three elements. Through cooperative binding of more copies of the regulatory protein at the additional sites, the conformational transition initiated at the hypersensitive site might spread to the nearby DNA. This way a conformational change in a protein would serve as the primary signal that would be amplified into a large open chromatin domain spanning 600 bp immediately upstream of the gene.

The PHO5 promoter is an unusually strong and tightly regulated yeast promoter. In this context it is significant that the extensive chromatin changes are found only at maximal induction of PHO5, while at lower levels of PHO5 expression a nearly

'inactive' chromatin configuration can be found. Under the latter conditions gene activation might proceed solely via the −367 element, which is always nucleosome free. This might be analogous to the activation of PHO11, another regulated acid phosphatase which cannot, however, be induced to levels as high as PHO5 (Oshima, 1982). At the DNA level this might be explained by the presence of only one UAS element that is almost identical to the −367 PHO5 element (Rudolph and Hinnen, 1986). We are currently investigating the possibility that the chromatin structure of PHO11 does not show any chromatin changes at low phosphate conditions despite its activation, which would lend credibility to the hypothesis that maximal induction of PHO5 is a two-stage phenomenon.

Mechanism of nucleosome removal

The PHO5 promoter is the first promoter that has been directly shown to undergo a transition from a nucleosomal to a non-nucleosomal state upon activation. The fact that nucleosomes are also removed from DNA that is located upstream of the presumed recognition sites for the regulatory protein and that is not required for PHO5 regulation (Rudolph and Hinnen, 1986) makes it unlikely that the absence of the nucleosomes in the active state is simply the result of competition between the histone octamer and the regulatory protein for the same DNA sequence. A change in the DNA conformation, on the other hand, would be expected to extend beyond the primary site of nucleation to nearby sequences and affect histone binding properties of a larger DNA domain. Enhanced micrococcal nuclease cutting near the −469 element (see Figure 3) might be due to binding of the regulatory protein at that element or it might also reflect the induction of an alternative DNA conformation at that sequence. High resolution mapping shows that the two positions preferentially cleaved by micrococcal nuclease in the active state mark the boundaries of a 12-bp and a 6-bp run of alternating purine/pyrimidine residues, the first one overlapping the −469 element.

Chromatin structure and gene regulation

There have been several reports that the chromatin structure in yeast can be correlated with promoter function but not with gene regulation. This is based on the finding that hypersensitivity in putative control regions of a particular gene similarly exists under conditions of gene activation as well as repression (e.g. Sledziewski and Young, 1982; Struhl, 1982; Lohr, 1984; Proffitt, 1985). Our results on the PHO5 gene add a new perspective to this picture. On the one hand, we have confirmed the view that, in yeast, hypersensitivity is not only established following gene activation but predates it. On the other hand, the chromatin structure does change dramatically upon activation.

In promoter fusion experiments a PHO5 activating element has been shown to actually repress an otherwise constitutively expressed gene when the cells were grown in high phosphate medium (Rudolph and Hinnen, 1986). This is consistent with the concept that a protein(s) binds to that element under conditions of PHO5 repression. A similar conclusion was reached in the *GAL1/GAL10* system by directly assaying for protein−DNA interactions (Giniger *et al.*, 1985). Since hypersensitivity most likely reflects the binding of regulatory proteins, it would not, therefore, be expected to depend on gene activation.

Although hypersensitivity in the PHO5 promoter exists prior to gene induction, the chromatin structure does respond to conditions of gene activation. The PHO5 gene is therefore the first metabolically controlled gene in yeast that has been shown to undergo a localized transition in its chromatin structure at the promoter region upon activation. Detailed analysis of the role that regulatory proteins play in this chromatin transition might provide an insight into the functioning of a promoter and into the mechanism by which upstream activating sequences induce high rates of transcription by RNA polymerase.

Materials and methods

Yeast strains and media

The haploid yeast strain IH2 (*a, his-3-11, his3-15, leu2-112, ura3-251, ura3-328, ura3-373, ade2*) (Rudolph *et al.*, 1985) was used in all experiments shown here. The strain was either grown in YPDA medium [2% Bacto Peptone (Difco), 1% Bacto Yeast Extract (Difco), 2% glucose and 100 mg/l adenine], i.e. conditions of PHO5 repression, or in low phosphate medium (Meyhack *et al.*, 1982) to induce PHO5 activity. An alternative protocol for PHO5 induction was to grow the cells in YPDA to an OD_{600} of 5, harvest them, wash them twice in water, and resuspend them in a phosphate-free medium at an OD_{600} of 0.5. As described by Field and Schekman (1980), acid phosphatase was fully induced after growing the cells for 8−10 h. They were harvested and nuclei isolated as described (Almer and Hörz, 1986). High phosphate medium (Meyhack *et al.*, 1982) gave identical results to YPDA.

Isolation of yeast nuclei, nuclease digestion, gel electrophoresis, hybridization, DNA probes

The methods used are described in the accompanying paper (Almer and Hörz, 1986). High resolution mapping of nuclease cuts was essentially by the method of genomic sequencing described by Church and Gilbert (1984). DNA was analyzed in 0.5 mm thick 8% sequencing gels, the DNA was transferred electrophoretically onto nylon membranes (GeneScreen Plus, New England Nuclear). Single-stranded specific probes were prepared from pBR322 clones containing 100−120 bp inserts. The circular DNA was opened with a restriction nuclease at one end of the insert, and the linear DNA treated with exonuclease III to remove about 300 nucleotides from the 3′ ends. The single-stranded regions were filled in with DNA polymerase I (large fragment) in the presence of two [α-^{32}P]deoxynucleosidetriphosphates (800 Ci/mmol). The linear molecule was cut with a second restriction nuclease that cleaves at the other end of the insert, and the DNA was then used for hybridization.

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