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A functional role for nucleosomes in the repression of a yeast promoter

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Induction of the *PHO5* gene in *S.cerevisiae* was previously shown to be accompanied by the removal of four positioned nucleosomes from the promoter. In order to assess the role of nucleosomes in the cascade of gene activation, DNA corresponding to one of these nucleosomes was excised. In its place two foreign DNA segments of the same length were inserted: a fragment from the African green monkey α-satellite DNA which is known to associate with histones in a highly specific fashion to give a uniquely positioned nucleosome or, alternatively, a fragment derived from pBR322 DNA. The promoter constructs were fused to the *lacZ* gene on centromere plasmids and transformed into yeast cells. The satellite fragment formed a nucleosome which persisted under inducing conditions. At the same time the inducibility of the *PHO5* promoter was virtually abolished. When various subfragments containing between 35 and 100 bp of the satellite segment were tested, they were all found to decrease the inducibility of the promoter, full repression required the full length molecule, however. In contrast, the pBR fragment made the promoter weakly constitutive, and induction proceeded to levels even higher than with a promoter lacking an insert. Analysis of the chromatin structure reveals a nucleosome on the pBR segment at noninducing conditions which is removed upon induction. It is concluded that the quality of the histone–DNA interactions at the promoter makes an intrinsic contribution to the regulation of the gene.

Key words: chromatin/nucleosomes/*PHO5*/regulation/*S.cerevisiae*

Introduction

To a first approximation all eukaryotic DNA is organized in nucleosomes (for reviews, see Eissenberg *et al.*, 1985; Pederson *et al.*, 1986; Yaniv and Cereghini, 1986; Gross and Garrard, 1988; Elgin, 1988). Although the primary purpose of this nucleosomal substructure clearly is to help compact the DNA and provide the basis for a hierarchy of superstructures, there is evidence that histone–DNA interactions also participate in the functioning of the DNA.

By *in vitro* reconstitution experiments Lorch *et al.* (1987) showed that promoters can be inactivated when they are reconstituted into nucleosomes. The same conclusion had been reached by Knezetic and Luse (1986). Similarly, the adenovirus major late promoter assembled into nucleosomes in a *Xenopus* oocyte extract was found to be refractory to transcription initiation *in vitro*. However, exposure of the promoter to transcription factors prior to nucleosome assembly relieved this nucleosome-mediated repression (Workman and Roeder, 1987). There is complementary evidence from *in vivo* studies that functionally important DNA elements reside in nucleosome free so-called hypersensitive regions (for reviews see Eissenberg *et al.*, 1985; Pederson *et al.*, 1986; Yaniv and Cereghini, 1986; Elgin, 1988; Gross and Garrard, 1988). The finding of Kayne *et al.* (1989) that an N-terminal deletion of histone H4 abolished repression of the silent mating type loci in yeast points to a direct role for histones in gene regulation. Recently, it was shown that nucleosome positioning can affect the function of a yeast origin of replication (Simpson, 1990).

We have been interested for some time in the role that chromatin structure plays in gene expression and have chosen yeast as an experimental system to address this question. As a major advantage yeast offers the possibility of replacing a wild type gene by a limitless number of single copy mutated alleles which can be tested in parallel for function and for their chromatin organization. This makes it possible to go beyond mere correlations and study directly the function of chromatin elements.

The gene which we have focused our main attention on is the *PHO5* gene, the structural gene for a strongly regulated acid phosphatase in *S.cerevisiae* (Oshima, 1982). We have been able to show that the chromatin structure at the *PHO5* promoter undergoes a massive transition upon induction of the gene (Almer and Hörz, 1986; Almer *et al.*, 1986). In high phosphate media, i.e. conditions under which the gene is repressed, there is a short hypersensitive region located about 370 bp upstream of the gene. This hypersensitive region which contains a major upstream activation site (UAS) is flanked by specifically positioned nucleosomes. Upon induction of the gene, i.e. by starving the cells for phosphate, two nucleosomes upstream and two nucleosomes downstream of this hypersensitive site are selectively removed.

In order to clarify the role that nucleosomes play in the process of *PHO5* induction we have now exchanged DNA underlying one nucleosome immediately adjacent to the UAS by different foreign DNA segments of the same length and have determined in parallel promoter function and the chromatin structure of our constructs.

Results

Nucleosome swapping at the PHO5 promoter

Figure 1 shows the chromatin structure at the *PHO5* promoter with the UAS element residing in a short hypersensitive region which is nucleosome free under inducing as well as noninducing conditions. The four positioned nucleosomes flanking this hypersensitive region which are

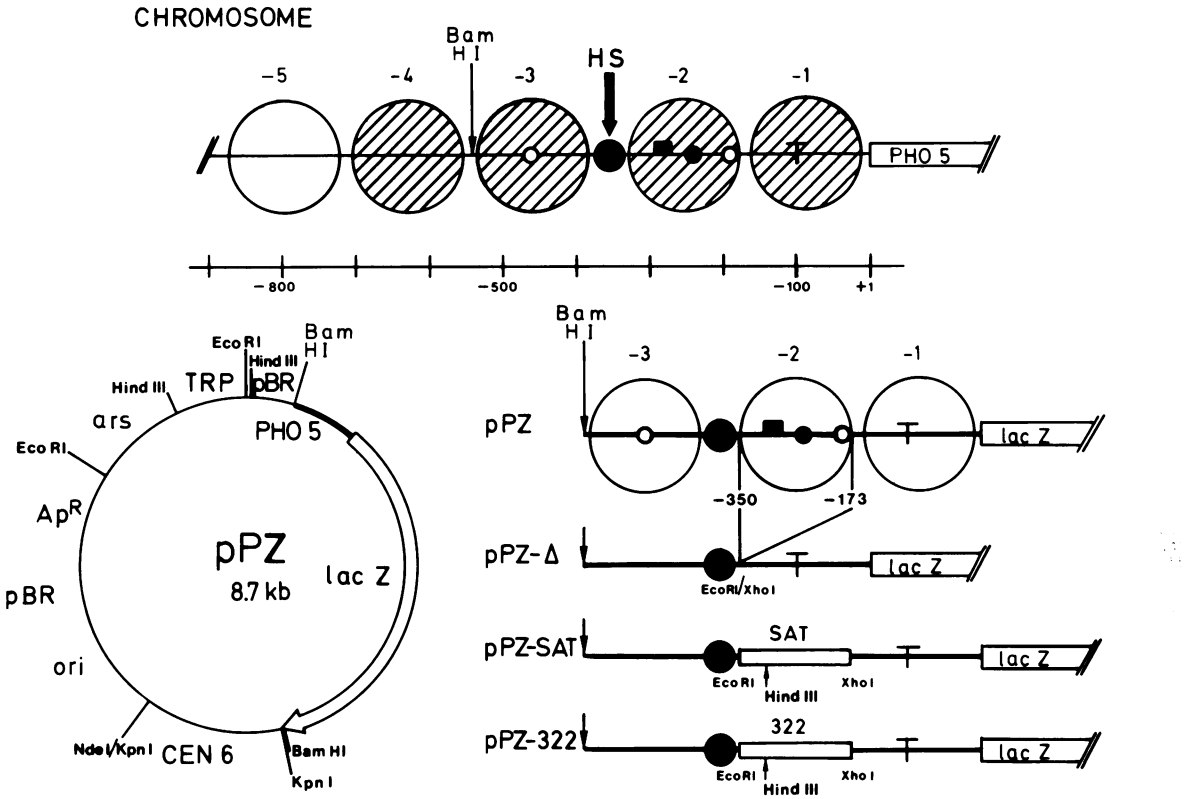

Fig. 1. Design of the *PHO5* promoter/*lacZ* fusion plasmids (pPZ). Schematically shown at the top is the chromatin structure of the inactive chromosomal *PHO5* promoter with positioned nucleosomes and a short hypersensitive region (HS) (Almer *et al.*, 1986). The four shaded nucleosomes are removed upon induction of the promoter. The large solid circle denotes a major UAS at −367, the smaller circles homologous sequences at −489, −245, and −185 (Rudolph and Hinnen, 1987). Circles drawn in solid correspond to binding sites for the positive regulatory protein PHO4 *in vitro* (Vogel *et al.*, 1989). The solid square denotes a site that the positive regulator PHO2 binds to *in vitro* (Vogel *et al.*, 1989). T marks the TATA box.

Shown underneath is the structure of the pPZ plasmids with the *TRP1* marker. pPZ plasmids with the *LEU2* marker had the *LEU2* gene in place of the *Hin*dIII fragment with the *TRP1* gene. The pPZ derivatives are shown on the right (see Materials and methods for details).

removed following induction of the gene are shaded in this diagram.

We decided to replace the DNA underlying the nucleosome immediately adjacent to the major UAS (nucleosome −2 in Figure 1) by foreign DNA segments. By correlating the functional properties of these mutated promoters with their organization in nucleosomes we hoped to be able to better define the contribution of histone−DNA interactions to the regulation of the *PHO5* promoter. These experiments were done with the *PHO5* promoter fused to the *lacZ* (β-galactosidase) gene on centromere containing plasmids. The *PHO5* promoter is fully regulated on such plasmids and undergoes precisely the same chromatin transitions as on the chromosome (Fascher, 1989; K.D.Fascher and W.Hörz, manuscript in preparation). Our strategy has the advantage that any effects related to the mutated *PHO5* promoter can be compared with the intact chromosomal promoter copy.

Figure 1 shows the design of the plasmids used and the strategy employed to replace nucleosome −2 at the *PHO5* promoter. Deletion of the DNA underlying this nucleosome yielded plasmid pPZ-Δ which served as the recipient for foreign DNA segments. The deletion eliminated a PHO2 binding site and in addition a weak UAS element (Rudolph and Hinnen, 1987) which serves as a PHO4 binding site *in vitro* (Vogel *et al.*, 1989). The remaining promoter is driven only by the major UAS at −367 but is still strongly regulated. This simplifies the interpretation of effects by DNA segments to be inserted next to this UAS.

Insertion of a satellite DNA segment into the PHO5 promoter interferes with UAS function

We have shown previously that nucleosomes are located on the African green monkey α-satellite DNA in one major and several additional minor nucleosome positions (Zhang *et al.*, 1983). The prevalence of the major position (frame F) is due to a high affinity of histones for the underlying DNA as shown by nucleosome reconstitution experiments (Neubauer *et al.*, 1986). We therefore decided to introduce frame F DNA into our test plasmid and examine its effect on the regulation of the *PHO5* promoter.

It turned out that insertion of the 147 bp satellite DNA segment into plasmid pPZ-Δ to yield plasmid pPZ-SAT (see Figure 1) left β-galactosidase activity at high phosphate conditions essentially unchanged, but the insertion destroyed the ability of the UAS at −367 to activate transcription of the *lacZ* gene at inducing conditions almost completely (see Table I).

Insertion of a pBR322 segment into the PHO5 promoter enhances UAS function and confers weak constitutivity

It might be argued that any 150 bp DNA insertion into plasmid pPZ-Δ would inactivate the promoter by a mechanism unrelated to the chromatin structure of the promoter. To test the validity of this argument, we inserted a 150 bp control fragment and chose a derivative of a pBR322 DNA segment for that purpose in order to minimize

Table I. β-galactosidase activity of different *PHO5-lacZ* fusion genes.

Plasmid in strain AH220 or YS18	β-galactosidase levels	
	High phosphate	No phosphate
pPZ	4.0	250
pPZ-Δ	3.0	100
pPZ-SAT	2.5	7.3
pPZ-322	32	190

The plasmids were transformed into strains AH220 or YS18. LacZ activities were determined after growing the cells in either high phosphate or no phosphate media as described in Materials and methods. The values are listed in relative units, with the level obtained for plasmid pPZ-Δ in AH220 after induction in no phosphate medium set as 100. Values obtained for the *TRP1* containing plasmid family in AH220 and *LEU2* containing plasmids in YS18 were identical.

the chance of fortuitously selecting another good nucleosome former. When the resulting promoter construct pPZ-322 (see Figure 1) was tested in yeast the promoter was found to be partially derepressed already at high phosphate conditions. At low phosphate conditions β-galactosidase activity was significantly higher than with the parent plasmid pPZ-Δ lacking the insertion and almost reached the level of the wild type promoter as in plasmid pPZ (Table I).

A nucleosome is formed on the satellite DNA insert at the PHO5 promoter and persists under inducing conditions

In order to determine the nucleosomal organization of our constructs, we carried out nuclease digestion experiments and probed the *PHO5* promoter region.

The presence of a nucleosome on the satellite insert of pPZ-SAT could be directly demonstrated by hybridizing extensive micrococcal nuclease digests with a satellite probe. A 150 bp protected fragment was formed, both at high and at low phosphate conditions (Figure 2A, arrow) pointing to a stable nucleosome. At the same time, nucleosome −2 was removed, however, from the wild type *PHO5* promoter which is present on the chromosome. This could be demonstrated by rehybridizing the blot just shown with a probe corresponding to the DNA from nucleosome −2 (Figure 2C). In a further control, it could be shown that the wild type promoter undergoes this same transition from a nucleosomal to a non-nucleosomal state even when present as an extrachromosomal copy on plasmid pPZ (Figure 2D).

The pBR segment at the PHO5 promoter changes from a nucleosomal to a non-nucleosomal state upon induction

The chromatin structure of the pBR insert in the *PHO5* promoter in plasmid pPZ-322 was analyzed by digestion of nuclei with micrococcal nuclease as described above for the satellite sequence. It can be seen that, at high phosphate conditions, this DNA segment shows up as a 150 bp fragment upon extensive digestion with micrococcal nuclease indicative of the association with a histone octamer (Figure 2B, arrow). In contrast, when the cells were starved for phosphate the chromatin structure of the pBR segment changed to a largely non-nucleosomal state (Figure 2B). This transition from a nucleosomal to a non-nucleosomal state correlates well with the strong induction of the *lacZ* gene when driven by the *PHO5* promoter containing the pBR segment (see Table I).

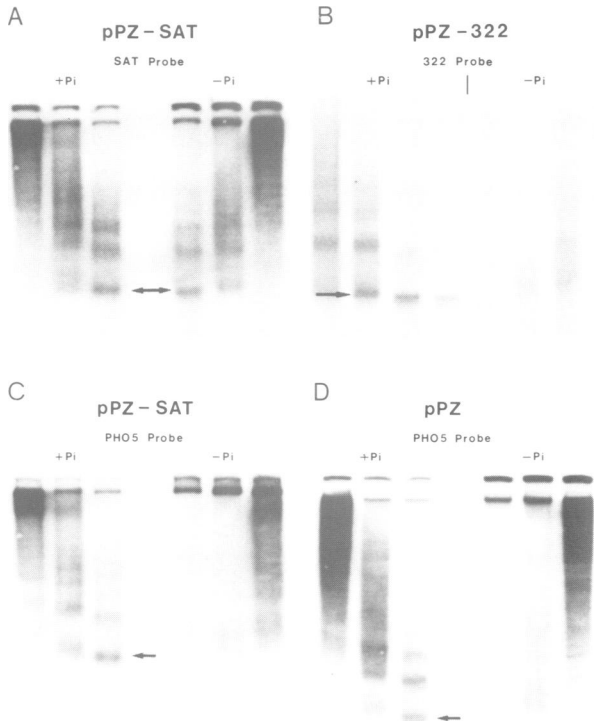

Fig. 2. Fate of nucleosome −2 in different *PHO5* promoter constructs upon promoter induction. Nuclei were isolated from AH220 cells harbouring different plasmids that had been grown either in high phosphate (+Pi) or no phosphate media (−Pi) and were digested for 20 min with 5, 15, and 40 U micrococcal nuclease per ml. DNA was isolated, separated in 2% agarose gels, blotted, and hybridized to the probes indicated. Digests were applied symmetrically with increasing nuclease concentrations from the outside towards the center lane in each gel. The SAT and 322 probes were isolated DNA fragments consisting of the respective promoter inserts. The *PHO5* probe extends from position −349 to −172 which corresponds to nucleosome −2. Note that in C this probe only recognizes the chromosomal *PHO5* promoter, while in D, both the plasmid borne and the chromosomal copy hybridize. The arrows denote the position of the core particle DNA in each case.

Loss of a nucleosome from the pBR but not from the satellite fragment upon induction is confirmed by restriction nuclease digestion of nuclei

Digestion of nuclei with restriction nucleases constitutes an alternative approach that can be used to map nucleosome free regions in chromatin. The satellite fragment contains a *Hin*dIII restriction site, and the pBR fragment was engineered in such a way as to also contain a *Hin*dIII site at approximately the same location. The accessibility of the *Hin*dIII site in the two inserts was measured by the strategy shown schematically in Figure 3. Nuclei were digested with *Hin*dIII, DNA was isolated and cut with *Eco*RV and *Cla*I. After gel electrophoresis the digests were probed with a fragment from the *lacZ* region. Figure 3 shows the result of the experiment. There was only a small increase in the susceptibility of the *Hin*dIII site of the satellite fragment in pPZ-SAT upon shifting the cells from high phosphate to low phosphate conditions. In contrast, the *Hin*dIII site of the pBR segment of pPZ-322 became fully accessible at low phosphate conditions. The *Xho*I site which flanks the inserted DNA segments on the downstream side (see Figure 1) behaved in exactly the same way. Only in pPZ-322 did this

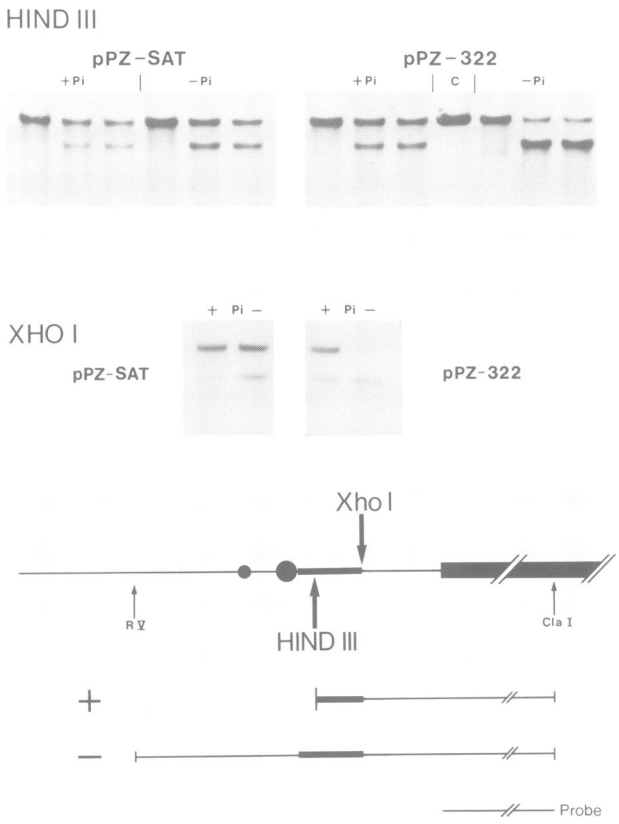

Fig. 3. Accessibility of restriction sites in the chromatin of different promoter constructs. Nuclei were isolated from AH220 or YS18 harbouring the plasmids indicated. Prior growth of the cells in either high phosphate or no phosphate media is denoted by +Pi and −Pi, respectively. Nuclei containing approximately 40 μg of DNA were digested for 60 min in 100 μl with 0, 60, and 120 U *Hin*dIII (from left to right, C refers to DNA from control incubated nuclei). After digestion, DNA was isolated, cleaved with *Eco*RV and *Cla*I, separated in a 1.5% agarose gel and analyzed as in Figure 2. A *Bam*HI−*Cla*I fragment from the *lacZ* region was used as a probe. The principle of the method is shown below with the fragment expected if the *Hin*dIII site had been accessible marked '+' and if inaccessible marked '−'. Accessibility of the *Xho*I site was assayed by the same method with 100 U *Xho*I.

site become accessible upon activation of the *PHO5* promoter while in pPZ-SAT this was not the case (Figure 3).

The pBR but not the satellite fragment in the PHO5 promoter turns hypersensitive to DNase I upon induction of the promoter

DNase I is the most widely employed nuclease to probe the structure of chromatin. Its use has permitted the demonstration of hypersensitive regions which are nucleosome free by a variety of different criteria. We measured therefore accessibility to DNase I by the indirect endlabelling procedure and compared the plasmid containing the satellite segment to our wild type parent plasmid pPZ and the plasmid carrying the pBR segment (Figure 4).

At high phosphate conditions, the promoter region in pPZ-SAT gave a pattern very similar to the one previously demonstrated for the chromosomal *PHO5* copy (Almer *et al.*, 1986) reflecting two positioned nucleosomes at the *PHO5* promoter downstream of the UAS (Figure 4A, lanes 4, 5). That this pattern is truly a property of the chromatin structure is shown by DNA control experiments. Regions highly sensitive in free DNA are protected by the nucleosomes (compare lanes 2, 3 and 4, 5 in Figure 4A). By contrast, the *lacZ* region was fairly accessible in the chromatin digests. After induction there was no change in the digestion patterns (Figure 4A, lanes 6, 7), consistent with nucleosomes persisting on the promoter.

That the wild type *PHO5* promoter turns hypersensitive to DNase I also when present on the constructs used in this study is shown in Figure 4B. The same results as previously obtained for the chromosomal *PHO5* copy (Almer *et al.*, 1986) were obtained for plasmid pPZ: a cutting pattern reflecting positioned nucleosomes at high phosphate conditions and uniform sensitivity of the promoter to DNaseI after induction (Figure 4B).

A similar situation as found with plasmid pPZ was also found for plasmid pPZ-322. After induction, the promoter (including the pBR segment) became hypersensitive, while the presence of positioned nucleosomes was clearly visible before induction (Figure 4B).

Subfragments from the satellite segment confer partial repression of the PHO5 promoter

Our results with the satellite fragment do not rule out the possibility that the fortuitous presence of a negative regulatory element on this DNA serving as a target for a regulatory factor was the main reason for repression of the *PHO5* promoter. To address this possibility we divided the satellite fragment into different subfragments and tested each one separately. Such subfragments are not related in sequence since the 172 bp α-satellite repeat unit is not internally repetitious (Rosenberg *et al.*, 1978).

A subfragment containing a putative negative transcriptional element should have a much stronger repressing effect than a neighbouring fragment lacking such an element. We would therefore expect a subfragment to either have a very pronounced negative effect or have virtually no effect at all since elements of that kind are usually fairly short. If, on the other hand, generation of a stable nucleosome is responsible for the repression we would instead expect to find low level repression that would be similar for all subfragments. The reason for expecting some repression also with subfragments is that even satellite half molecules retain sufficient structural information to organize a positioned nucleosome by maintaining the original histone−DNA contacts and making new contacts in the rest of the core particle (Neubauer *et al.*, 1986). This shows that formation of a stable nucleosome on the satellite segment is the result of multiple additive histone−DNA interactions distributed along the entire segment.

The first two fragments tested bore out the expectation inherent in the nucleosome model quite well (see Figure 5). Inducibility of the promoter dropped from 100% to 55% and 46% upon inserting the subfragments SAT_a and SAT_{ab}, respectively, as opposed to 8% for the entire fragment. Surprisingly, however, fragment SAT_b by itself repressed more strongly than SAT_{ab} (even though it is fully contained in SAT_{ab}) and decreased inducibility to 16%. Also unexpected was the finding that the SAT_c fragment gave a value of 29%. It should be noted, however, that any nucleosome forming over the latter two subfragments in such a way as to preserve the original favorable histone−DNA contacts, would incorporate the UAS element itself into the core particle. If this were the reason for the unexpectedly

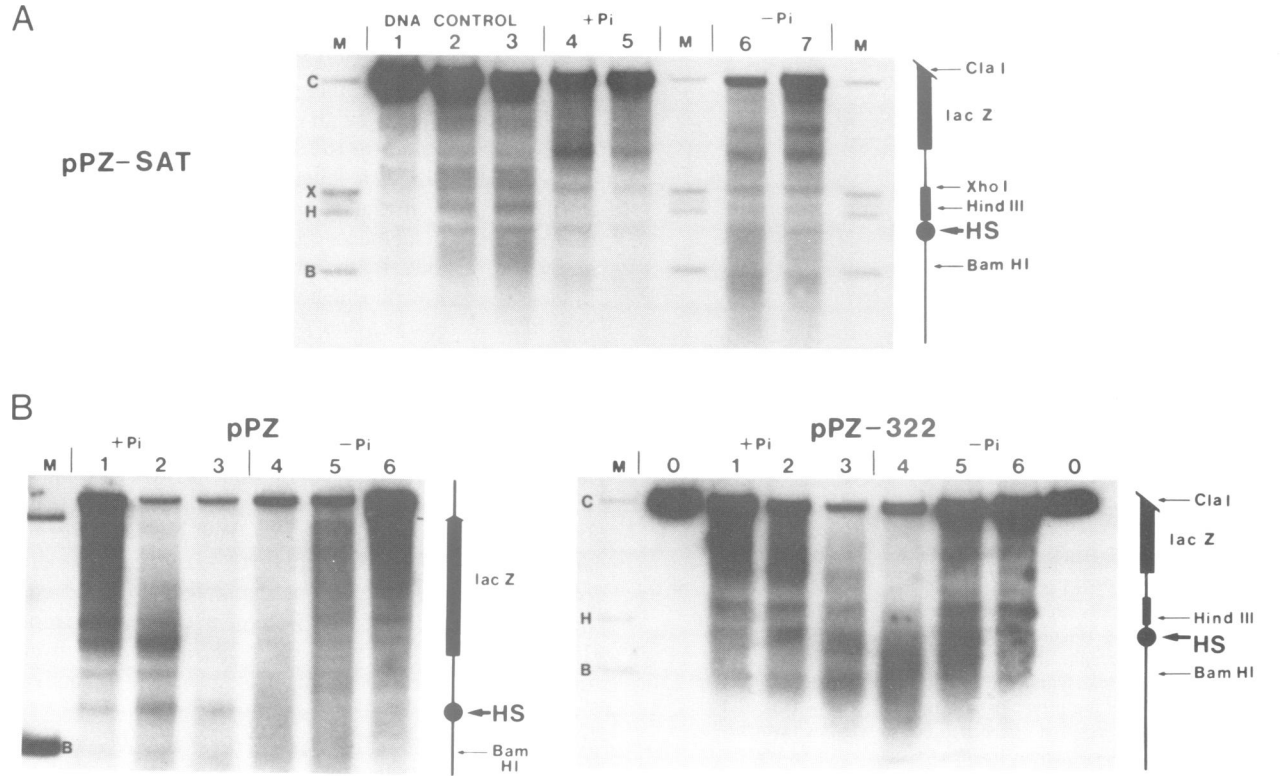

Fig. 4. Measuring DNase I hypersensitivity at the *PHO5* promoter. Nuclei were isolated from AH220 or YS18 harbouring the plasmids indicated. Prior growth of the cells in either high phosphate or no phosphate media is denoted by +Pi and −Pi, respectively. The design of the plasmids analysed including the position of the major UAS (solid circle) in the hypersensitive site (HS) and the location of the pertinent restriction sites is shown in maps drawn to scale with gels. **A**. Nuclei were digested for 20 min with 3 and 1 U of DNase I per ml (lanes 4, 5 and 6, 7 respectively). As a control, deproteinized DNA was digested also for 20 min with 0.2, 0.4, and 0.8 U of DNase I per ml (lanes 1−3, respectively). The positions of the DNase I cuts were analysed by the indirect endlabelling protocol. To that end DNA was isolated, cleaved with *Cla*I separated in a 1.5% agarose gel, blotted, and hybridized to a 350 bp *Hin*dIII−*Bam*HI fragment from pBR322 DNA (see Figure 1). M refers to marker fragments generated by digestion of DNA with either *Cla*I alone (C), *Cla*I plus *Xho*I (X), *Cla*I plus *Hin*dIII (H), or *Cla*I plus *Bam*HI (B). **B**. Digestion of nuclei was performed as in A with 1, 3, and 9 U of DNaseI per ml (lanes 1−3 and 6−4, respectively). 0 refers to DNA from nuclei that had not been incubated, and M to marker fragments generated for pPZ by digestion of DNA with *Hin*dIII and *Bam*HI (B), and for pPZ-322 with *Cla*I alone (C), *Cla*I plus *Hin*dIII (H), and *Cla*I plus *Bam*HI (B).

strong effect of SAT_b and SAT_c then reversing the polarity of SAT_c should alleviate the problem since a nucleosome would now extend in the downstream direction as in the case of SAT_a and SAT_{ab}. This is exactly what was found. Fragment $SAT_{c(rev)}$ has a much weaker repressing effect than SAT_c, with inducibility reaching a level of 65%, similar to what was found for the other subfragments conforming to this orientation.

The results obtained with the four subfragments would be difficult to reconcile with a negative regulatory element as the cause of repression by the satellite fragment, and they fully support our conclusion that it is histone−DNA interactions that are responsible.

Discussion

Repression of the PHO5 promoter by insertion of a satellite fragment

Our analyses have shown that DNA inserted into a promoter between a UAS and a TATA-box can have profound effects on the function of that promoter. A fragment from the African green monkey α-satellite abolished inducibility of the promoter almost completely. In our construct, this fragment is organized in a positioned nucleosome in much the same way as in its native environment (Zhang *et al.*, 1983). Furthermore, the same specific positioning as found in African green monkey cells had also been found by *in vitro* reconstitution experiments using DNA and only core histones (Neubauer *et al.*, 1986) demonstrating that histone−DNA interactions are responsible for nucleosome positioning on this DNA. It comes as no surprise, therefore, that a positioned nucleosome is also formed on the satellite DNA fragment after insertion into the *PHO5* promoter in yeast where it substitutes for another specifically positioned nucleosome.

The nucleosome on the satellite DNA segment cannot be removed upon shifting the cells to inducing conditions, and the promoter is rendered permanently inactive. That proteins bound to DNA between a UAS element and the TATA-box can repress a promoter has a precedent. A *GAL1* promoter carrying a *lexA* operator between the upstream activator sequence and the TATA region was repressed 10-fold by LexA protein (Brent and Ptashne, 1984). Similarly the *CYC1* transcriptional terminator can diminish *GAL1* transcription when it is inserted into the *GAL1* promoter (Brent and Ptashne, 1984). In those cases, however, it was specific nonhistone proteins which were responsible for the repression, and yeast cells do not usually have to cope with a LexA repressor protein.

The novel aspect of our findings is that formation of a

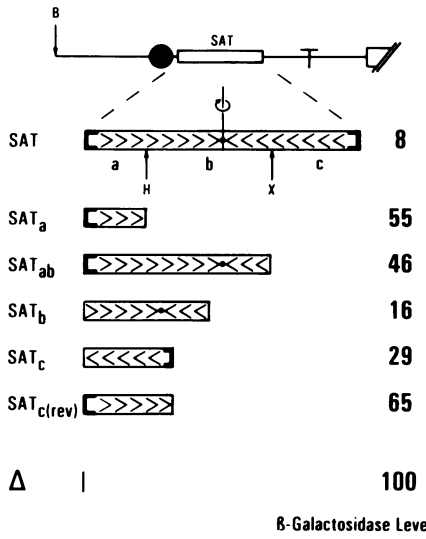

Fig. 5. Effect of subfragments derived from the satellite segment on inducibility of the *PHO5* promoter. The design of the plasmids tested is shown schematically on the left, with SAT referring to pPZ-SAT and Δ to pPZ-Δ (see Figure 1). The dyad axis for a core particle forming over the satellite fragment is indicated with arrow heads denoting the rotational symmetry. a, b, and c refers to the satellite segments present in the promoter inserts shown below (see Materials and methods). The position of the left and right DNA boundary as present in the original satellite fragment is marked in heavy set brackets. H and X denote the *Hin*dIII and *Xmn*I site in the satellite segment. LacZ activities were determined after growing the cells with the different plasmids in no phosphate media. The values are listed in relative units (c.f. Table I), with the level obtained for pPZ-Δ set as 100.

stable nucleosome can similarly repress transcription. The results obtained with subfragments derived from the satellite segment cannot be explained by the presence of a negative regulatory element present on the satellite fragment. For one, we would expect full repression for the one subfragment containing the putative repressor sequence while the other fragment should have little or no effect. We found, however, that all subfragments repress induction to an intermediate extent. This is consistent with the additive nature of histone–DNA interactions in nucleosome formation (Neubauer *et al.*, 1986). Secondly, the concept of a repressor protein binding to a target sequence cannot explain why increasing the length of a given fragment without changing its distance to the proximal promoter elements (SAT$_{ab}$ versus SAT$_b$ in Figure 5) can decrease the degree of repression rather than leaving it unaffected or if anything increasing repression. Thirdly, one hallmark of upstream regulatory sequences is that they function in either orientation. We have found, however, that reversing the polarity of one of the subfragments strongly affects repression. These effects are, on the other hand, what one would expect if repression by the subfragments were due to nucleosome formation. This is because a nucleosome positioned in such a way as to recruit the adjacent UAS element into the actual core particle would be even more detrimental to inducibility than the same nucleosome interspersed between the UAS and the downstream promoter elements.

Promoter regulation by nucleosomes

A pBR322 DNA segment inserted into the *PHO5* promoter has effects opposite to those observed for the satellite fragment. Not only does it confer weak constitutively to the promoter under high phosphate conditions, but it raises the inducibility to a level which is 2-fold higher than in the absence of any insert rather than lowering it 13-fold as the satellite does. The strength of the promoter thus generated is almost equivalent to the *PHO5* wild type promoter.

We interpret the low level constitutivity of our construct to be due to an instability of the nucleosome formed over the prokaryotic segment, which, however, is hard to pick up by our methods. By the same token the exceptionally high inducibility at low phosphate is consistent with the complete loss of a nucleosome and the generation of a long hypersensitive domain which is very similar to what we find for the wild type promoter.

An independent line of evidence demonstrating that nucleosomes can indeed affect promoter function comes from the work of Han *et al.* (1988), who showed that repression of histone H4 synthesis in appropriately constructed yeast strains leads to nucleosome loss from the *PHO5* promoter and strong activation of the *PHO5* gene even under high phosphate conditions. This activation is also observed after deletion of the *PHO5* UAS element (Han and Grunstein, 1988), which means that removal of histones from the downstream promoter element is sufficient for activation.

Mechanism of nucleosome removal and biological role

One important conclusion regarding the mechanism of nucleosome removal can be drawn from the analysis of the chromatin structure of pPZ-322. The change from a nucleosomal to a nonnucleosomal state is instigated by the nearby UAS and depends on *PHO4* (Fascher *et al.*, 1990). A participation of the acidic domain of the PHO4 protein (Legrain *et al.*, 1986) in this process could provide a mechanism for the chromatin transition. Even though this might not be the main function of the acidic domain (Guarente, 1988), turning the argument around, it is difficult to envision how the acidic region once it is liberated, presumably by release of the *PHO80* product (Oshima, 1982) could keep from labilizing histone–DNA interactions in the vicinity.

It should be noted that as we pointed out before (Pavlovic and Hörz, 1988), we cannot distinguish between total absence of nucleosomes upon *PHO5* induction and a persistence of nucleosomes with drastically altered properties, such as no longer being able to protect DNA against restriction nucleases and micrococcal nuclease. If nucleosomes can indeed be removed or structurally altered on preexisting chromatin templates in the absence of DNA replication this would greatly expand the flexibility of gene regulation by nucleosomes.

In a reevaluation of previous crosslinking experiments, Solomon *et al.* (1988) presented evidence that there are still histone–DNA contacts in the hypersensitive region of the induced *Drosophila* hsp70 promoter although fewer than before heat shocking. That there are stages intermediate between presence of canonical nucleosomes and their complete absence is also indicated by recent findings of Nacheva *et al.* (1989). Applying a newly devised crosslinking protocol they raised the possibility that, in actively transcribed chromatin, nucleosomes unfold but that histones remain associated with DNA via their flexible N- or C-terminal tails.

These new concepts might provide a framework for a

participation of nucleosomes during the rapid regulation of promoters also in higher eukaryotes. An intriguing picture has emerged from an analysis of the glucocorticoid mediated induction of the MMTV promoter (Richard-Foy and Hager, 1987). In the absence of hormone, a regular array of nucleosomes was mapped across the promoter region. In the presence of hormone, a region of about 200 bp which contains the hormone receptor binding site becomes hypersensitive within few minutes presumably by the displacement of a specific nucleosome as part of the activation process, and binding sites for additional transcription factors become available (Cordingley *et al.*, 1987). From *in vitro* reconstitution experiments there is evidence that the hormone–receptor complex can bind to a specifically positioned nucleosome on the MMTV promoter (Perlman and Wrange, 1988; Pina *et al.*, 1990). Further studies may reveal subsequent steps by which the nucleosome is displaced.

The recently discovered functionality of activator proteins from mammalian cells in yeast (Guarente, 1988) offers the possibility of testing these newly emerging concepts by the powerful techniques available in yeast and of elucidating the contribution of histone–DNA interactions to transcriptional repression and activation.

Materials and methods

Plasmids

PHO5-lacZ fusion plasmids were constructed by blunt end ligating the *Bam*HI–*Dra*I *PHO5* promoter fragment from pBR322(*HIS3*)*PHO5*/*PHO3* which includes the first two *PHO5* codons (Meyhack *et al.*, 1982) to the *lacZ Sma*I–*Sal*I fragment from plasmid pMC1871 (Pharmacia LKB Biotechnology, Freiburg, FRG). The *Sal*I site was converted to an *Nde*I site and the resulting *Bam*HI–*Nde*I fragment cloned into the *Bam*HI–*Nde*I sites of YRp7 (Tschumper and Carbon, 1980). The *Nde*I site was then converted to a *Kpn*I site and a 1.15 kb *Kpn*I–*Hin*dIII fragment from CEN6 (Panzeri and Philippsen, 1982) inserted after conversion of the *Hin*dIII site to a *Kpn*I site. The resulting plasmid was called pPZ.

Deletion of nucleosome −2 DNA to give pPZ-Δ was from position −350 to −173. Position are given relative to the translational start of the +1 reading frame and denote the first and last nucleotide remaining after the deletion. An *Eco*RI linker (GGAATTCC) was attached to position −350, cleaved, sticky ends were filled in, and an *Xho*I linker (CCGCTCGAGCGG) was added. At position −173 the same *Xho*I linker was attached. The two *Xho*I sites were cleaved and ligated together, to generate in effect an intact *Eco*RI and *Xho*I site next to each other.

Plasmid pPZ-SAT was generated from pPZ-Δ by insertion of a 147 bp segment extending from position 143 to position 117 of the cloned African green monkey satellite α-sat.1 fragment (see Figure 1 of Neubauer *et al.*, 1986) after attaching an *Eco*RI linker (CCGGAATTCCGG) to position 143 and the *Xho*I linker used before to position 117.

The following plasmids with subfragments derived from the 147 bp satellite segment were used (the position of the first and last nucleotide relative to the 147 bp satellite segment is listed). pPZ-SAT_a, 1–35; pPZ-SAT_{ab}, 1–100; pPZ-SAT_b, 31–100; pPZ-SAT_c 101–147; pPZ-$SAT_{c(rev)}$ 101–147 (see Figure 5). The first two plasmids were constructed from pPZ-SAT by attaching an *Xho*I linker to the filled in *Hin*dIII site (SAT_a) or the *Xmn*I site (SAT_{ab}) of the satellite insert and connecting the new *Xho*I site to the *Xho*I site present in pPZ-Δ. pPZ-SAT_b was generated from pPZ-SAT_{ab} by attaching a *Sal*I linker to the filled in *Hin*dIII site, excising the desired satellite fragment as a *Sal*I–*Xho*I fragment and ligating it into the *Xho*I site of pPZ-Δ. pPZ-SAT_c and pPZ-$SAT_{c(rev)}$ were generated by adding a *Sal*I linker to the *Xmn*I site in pPZ-SAT and inserting the satellite fragment as a *Sal*I–*Xho*I fragment into pPX-Δ as described for pPZ-SAT_b. The polarities were determined from the location of the regenerated *Xho*I site.

The insert in plasmid pPZ-322 was constructed from a 136 bp *Xmn*I–*Mnl*I fragment (positions 2034–1898 of the pBR322 sequence, respectively (Sutcliffe, 1978). A 12 bp *Hin*dIII linker (CGCAAGCTTGCG) was inserted into an *Alu*I site (position 1998 of the pBR322 sequence), so the resulting fragment is 148 bp long. After attachment of the 12 bp *Eco*RI linker to the *Xmn*I site and the *Xho*I linker used before to the *Mnl*I site, the resulting fragment was inserted into the *PHO5* promoter. The sequence of the constructs was confirmed by direct sequence analysis.

Derivatives of all plasmids in which the *TRP1* gene was replaced by the *LEU2* gene were constructed by adding *Hin*dIII linkers to a 2.2 kb *Xho*I–*Sal*I fragment containing the *LEU2* gene (Andreadis *et al.*, 1984) and substituting the resulting fragment for a *Hin*dIII fragment with the *TRP1* gene (see Figure 1).

Yeast strains

Two strains obtained from A.Hinnen, Basel (AH220 and YS18) that were wild type with regard to regulation of the *PHO5* promoter were used in this study. They gave identical results in *lacZ* expression measurements and in the chromatin analyses. AH220 (*a, trp1, his3, leu2, pho5, pho3*) were derived from AH216 (Meyhack *et al.*, 1982). YS18 (*a, his3, leu2, ura3*) is described by Sengstag and Hinnen (1987).

β-galactosidase assays

Cells were grown either in YNB medium without amino acids (Difco Laboratories) supplemented with the appropriate nutrients, i.e. conditions of *PHO5* repression, or pregrown in the same medium and transferred into a synthetic phosphate-free medium as described (Almer *et al.*, 1986) and harvested after 10–15 hours. Toluene-permeabilized cells were assayed for LacZ activity as described by Guarente (1983) using the A_{600} value of the culture for normalization.

Chromatin analyses

Nuclei were isolated by a modification of the procedure of Wintersberger *et al.* (1973) as described (Almer *et al.*, 1986). Nuclease digestion, gel electrophoresis and hybridization were performed as described before (Almer and Hörz, 1986). Nylon membranes (GeneScreen Plus, New England Nuclear) were used for Southern transfer. The DNA probes used were DNA fragments which were gel purified from pBR322 subclones and radioactively labelled by the random primer method (Feinberg and Vogelstein, 1983).

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