Fine analysis of the chromatin structure of the yeast *SUC2* gene and of its changes upon derepression. Comparison between the chromosomal and plasmid-inserted genes

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ABSTRACT

Micrococcal nuclease digestion has been used to investigate some fine details of the chromatin structure of the yeast *SUC2* gene for invertase. Precisely positioned nucleosomes have been found on a 2 kb sequence from the 3' non-coding region, and four nucleosomes also seem to occupy fixed positions on the 5' flank. Eleven nucleosomes lie on the coding region, although their positioning is not as precise as in the flanks. When the gene is derepressed, these latter nucleosomes adopt a more open conformation and so do two of the nucleosomes positioned on the 5' flank. A dramatic change occurs in the 3' flank, whose involvement in the structural transitions of chromatin upon gene activation is postulated. All the observed features are conserved when the gene is inserted in either a single copy centromeric plasmid or in a multicopy, 2 μ m circle-based plasmid.

INTRODUCTION

The study of individual genes for their chromatin structure has constituted a major advance towards understanding the relationships between structure and function of chromatin. Much of the present knowledge is based on the fact that, upon gene activation, the structure of chromatin changes in a manner that results in an increased susceptibility to nucleases. DNase I and micrococcal nuclease (MNase), which were of the utmost importance in the elucidation of bulk chromatin structure during the last decade, have also proved to be among the most useful tools to investigate the transitions between inactive and active chromatin. The former enzyme was used in the first studies on hypersensitivity (1), while the latter is currently used to investigate nucleosome positioning, after the pioneering work of Nedospasov and Georgiev (2). The possible significance of DNase

I hypersensitivity (3) and the implications of nucleosome positioning in gene activation (4) have been recently reviewed.

In this type of research yeast do possess a great potential utility, as recently discussed by Gross *et al.* (5), and nucleases have been used to study several individual genes from *Saccharomyces*, including both genomic (5-16) and episomal (17-22) genes. We have recently reported (13) that in the 5' flank of the 1900 bp *Saccharomyces SUC2* gene for invertase, there are two DNase I hypersensitive sites surrounding the TATA box and a hypersensitive domain that coincides with an upstream regulatory region. In the 3' non-coding region of the gene, two hypersensitive sites map at positions +1920 and +3560. Between them, DNase I generates a ladder of fragments that suggests the existence of precisely positioned nucleosomes (13). Derepression of the gene has a dramatic influence on the sensitivity of the transcribed region and, although the overall DNase I sensitivity of the non-coding regions remained unaffected at low digestion rates, the pattern of fragments generated in both the 3' and 5' flanks exhibit an abrupt change (13). With the hope of obtaining more information, we have undertaken the study of nucleosome positioning along the gene and its flanks. This research is reported in the present paper.

MATERIALS AND METHODS

Materials

Saccharomyces cerevisiae haploid strains X2180 1A (*MATa*, *SUC2*, *CUP1*, *mal0*) and SEY 2101 (*MATa*, *ura3-52*, *leu2-3,112*, *ade2-101*, *suc2-Δ9*, *gal2*) were used in these experiments. Transformants of strain SEY 2101 with pRB58 (23) have been previously characterized by us (13).

Restriction endonucleases, MNase, T4 ligase and alkaline phosphatase were from Boehringer and DNase I was purchased from Worthington Biochemicals.

Construction of plasmid pYCS1

The yeast centromeric plasmid pJW3 (24), containing the sequences *ars1* and *CEN3* and the *ura3* gene, was cleaved at its single *EcoRI* site and digested with alkaline phosphatase. Yeast DNA containing the *SUC2* gene was isolated as an *EcoRI* fragment of 4.3 kb by electrophoresis on low melting point agarose (25) of a digest of plasmid pRB58 (23). This fragment was ligated to the linearized pJW3 with T4 DNA ligase. The ligation mixture was used to transform *E. coli* FB1009, and the transformants were selected by their ampicillin resistance. Plasmids containing the *SUC2* insert were detected by their mobility on agarose gels. The

Figure 1. Map of plasmids pRB58 (23) and pYCS1 (this work). Thin lines in the plasmids stand for pBR322 DNA; thick, black zones represent yeast chromosomal DNA, and the dashed region represents 2 μ m-circle DNA, including its replication origin. Only the restriction sites relevant to this work have been mapped. The location of relevant genes or other elements is marked by thin-line arcs or arrows. The region containing the *SUC2* gene is shown in greater detail between both plasmids. The gene is marked as an open arrow, and the positions of the TATA box and the upstream regulatory region (38, 39) are shown by thin-line boxes. The probes used in this study are marked as thick lines, and labelled by a two-letter code, according to their restriction end, followed, in some instances, by the probe size in kb. E: *EcoRI*; H: *HindIII*; K: *KpnI*; P: *PstI*; R: *RsaI*. X: *XbaI*.

orientation of inserts was determined by restriction analysis, the plasmid in which the *SUC2* gene has the same orientation as the *ura3* gene being further referred to as pYCS1 (fig. 1). The experimental procedures for alkaline phosphatase digestion, ligation, *E. coli* transformation and restriction were as described by Maniatis *et al.* (26).

Yeast transformation

S. cerevisiae SEY 2101 was transformed with pYCS1 following the procedure of Hinnen *et al.* (27). To select for transformants, uracyl prototrophy was used, and their *SUC*⁺ phenotype was scored by the method of Carlson *et al.* (28) and by invertase assay.

Growth of yeast and isolation of nuclei

The strain X2180 1A was grown in YEPG medium (1% yeast extract, 2% bactopectone, 2% glucose). The transformed strains SEY2101/pRB58 and SEY2101/pYCS1 were grown in minimal medium (0.67% yeast nitrogen base without amino acids (DIFCO), 2% glucose) supplemented with 80 μ g/ml each of leucine and adenine. To derepress the *SUC2* gene, yeast cells were transferred to a medium similar to YEPG, except for low glucose concentration (0.05%), as described by Carlson *et al.* (28). Yeast nuclei were isolated as described elsewhere (29).

Digestion with MNase and location of the cutting sites

Nuclei from strain X2180 1A or lysed protoplasts from transformed yeast cells were digested with MNase as described previously (30). To locate cutting sites in chromatin the indirect end labelling technique (31) was used, combined with MNase digestion (2). The sliding-end-labelling technique (30) was used in some instances to confirm that a given electrophoretic band actually corresponds to a fragment abutting on the restriction site. Electrophoresis, blotting, hybridization, autoradiography and scanning were carried out as described elsewhere (13). To aid the interpretation of results, a map of *SUC2* gene and of the plasmids used is given in figure 1, which also shows the probes employed.

RESULTS

Characteristics of *Saccharomyces cerevisiae* SEY2101 transformed with plasmid pYCS1

We have previously shown that DNA from strain SEY2101 does not hybridize with probe XK, due to the deletion in the *SUC2* gene (13). To calculate the number of copies of plasmid pYCS1 in the transformed cells, we carried out a dot-blot assay in which equal amounts of DNA from transformed cells and from strain X2180 1A, which contains a single copy of the *SUC2* gene, were hybridized with probe XK. The comparison of the intensities of the spots allows a quick estimation of the number of copies of the plasmid pYCS1. Our estimate gave a value of 1-5 copies, in accordance with the expected number of copies for a *CEN*-based plasmid (32).

The *SUC2* gene in pYCS1 can be expressed, as detected by invertase assay, in a glucose-regulated way. The enzymatic activity was about 15-fold increased under low-glucose conditions.

Presence of nucleosomes on the *SUC2* gene and its flanks

Figure 2 clearly shows that the chromatin of the genomic *SUC2* gene and its flanks is organized in nucleosomes. A rough estimate of the nucleosomal repeat length gave a value compatible with the 160-165 bp characteristic of bulk yeast chromatin (33, 34). The electrophoretic patterns indicate some differences between the nucleosomal organization of the gene and that of its flanks. The use of a probe from the 3' flank (HH1.3) gives rise to an extremely regular and well resolved ladder of fragments (fig. 2A). Ethidium staining of the original gel (not shown) gave

Figure 2. Presence of nucleosomes in the *SUC2* gene and its flanks. *Saccharomyces cerevisiae* X2180 1A cells were starved under conditions of either repression (R) or derepression (D) of the *SUC2* gene, and their nuclei were digested with MNase to varying extent, as depicted by the horizontal arrows in the bottom of the figure. The DNA was electrophoresed, Southern-blotted and successively hybridized with the following probes: **HH1.3**, corresponding to the 3' flank (panel A); **HH2.1**, covering the transcribing region (panel B), and **EH**, corresponding to the 5' non-coding region. The migration of size markers in bp is given.

a more diffuse pattern; this revealed that the nucleosomal repeat length in the 3' flank of the *SUC2* gene is more regular than in bulk chromatin. Probe **HH2.1**, corresponding almost entirely to the gene itself, also originates a regular series of nucleosomal bands, but those of higher oligomers are broader than in the 3' flank; this fact and the smear between the bands reduce the number of observable bands (fig. 2B). Neither the broadness of the bands nor the smear can be regarded as an electrophoretical artifact, because the same filter was used for successive hybridization with the different probes. The use of a probe from the 5' flank (**EH**) gave a still more diffuse pattern, and this means that the nucleosomal organization in this flank is markedly different from that found in other regions of the gene. On the other hand, the derepression of the *SUC2* gene is accompanied by some changes in the nucleosome patterns, especially in the 5' flank

Figure 3. Detection of nucleosome positioning in the left part of the *SUC2* gene and its upstream flank by indirect end labelling (procedure a). Chromatin from pRB58 was digested with MNase to two levels (lanes b and c), restricted with *Hind*III, electrophoresed, Southern-blotted and hybridized with probe HE. Lane a shows the results of an experiment in which naked DNA was used instead of chromatin. The migration of size markers is given on the left, and on the right margin the MNase cutting sites have been numbered to facilitate their description.

(fig. 2C) that suggest changes in chromatin structure.

Similar experiments were carried out with chromatin from plasmids pRB58 and pYCS1 and they led to comparable results. Whereas the actual relationships between these results and the nucleosomal organization are difficult to envisage, it is obvious that a heterogeneity in nucleosomal size in the different parts of the gene may exist. This, possibly, may result from a fixed nucleosome positioning, and the subsequent experiments were devised to test this hypothesis.

Nucleosome positioning in the repressed gene and its flanks

5' terminal region of the gene and upstream flank. Several procedures have been used to study the nucleosome positioning in the

Figure 4. Detection of nucleosome positioning in the left part of the *SUC2* gene and its upstream flank by indirect end labelling (procedure c), combined with the sliding-end-labelling method. Naked DNA (lane a) and nuclei from strain X2180 1A (repressed *SUC2* gene) were digested with MNase to two different levels (lanes b, c and d), restricted with *Kpn*I (lanes a, b and c) or with *Hpa*I (lane d), electrophoresed, Southern-blotted and hybridized with probe **XX**. All the bands numbered in the figure are 30 bp longer in the *Hpa*I-restricted sample. See the text for further details. The migration of size markers is shown on both sides of the figure.

left part of the gene and its 5' flanking region. The first one (procedure a) included restriction with *Hind*III and probing with **HE**. To study this region in plasmid pYCS1, in which the *Hind*III site at -1100 is not present, DNA was restricted with *Fst*I and

probed with an **PR** probe from the pBR322 DNA adjacent to yeast DNA carrying the *SUC2* gene (see fig. 1). This procedure will be further referred to as procedure **b**.

The results of the analysis of the repressed *SUC2* gene in pRB58 following procedure **a** are given in figure 3. Most, if not all, of the bands are present in the controls of naked DNA, but the cutting patterns of chromatin and deproteinized DNA are different. Restriction of MNase-digested samples with *KpnI* and hybridizing with probe **XX** (procedure **c**) allowed us to examine the chromatin structure of the left part of the gene, and served to confirm some of the data obtained by procedures **a** or **b**. Figure 4 gives, as an example, the results of an experiment carried out with genomic chromatin. Parallel experiments were done with plasmids pYCS1 (not shown) and pRB58 (see fig. 7b). Some of the bands, namely bands 2 and 8-11, were already detected by procedure **a**, but bands 12-18 were only detectable with procedure **c**, as they come from the beginning of the coding region. In order to test whether any of these bands is artifactual, we have applied the sliding-end-labelling method (30). Figure 4d depicts the electrophoretic pattern of a sample similar to that of figure 4c, but restricted with *HpaI* instead of *KpnI*. The *HpaI* site lies 30 bp downstream of the *KpnI* site, so that all the bands that correspond to fragments extending from a single MNase cutting site to the restriction site are 30 bp larger than in lane c of figure 4, and so, they seem to be slid upwards. This is the case of all the bands appearing in figure 4. On the contrary, should the size of a band remained unchanged, it would have corresponded to a fragment generated by two MNase cuts, one of them within the probe, and the size of the band would have led to the wrong mapping of an MNase cutting site.

The absence or faint presence of a particular band in naked DNA controls, or its early appearing during MNase digestion are taken as criteria to decide whether the band may be considered as characteristic of chromatin (see ref. 17). The positions of the most relevant chromatin bands from figures 3 and 4 and from parallel experiments are given in table I. The figures are rounded due to the error in determining band sizes (from ± 10 bp for shorter fragments to ± 40 bp for fragments of ≈ 1500 bp). The dif-

TABLE I. Location of the MNase cutting sites in the *SUC2* locus

Site No.	Position ¹ in		Spacing ²	Site No.	Position ¹ in		Spacing ²
	Genome	pRB58			Genome	pYCS1	
8	-520 ^{a,3}	-520 ^{a,c}	n.d. ⁴	27	+1880 ^d	+1870 ^{d,e}	190
10	-230 ^{a,c}	-260 ^c	n.d.	28	+1980 ^{d,e}	+1960 ^{d,e}	100
11	-40 ^c	-60 ^c	n.d.	29	+2180 ^{d,e}	+2160 ^{d,e}	195
12	+160 ^c	+160 ^c	+140 ^c	30	+2370 ^{d,e}	+2350 ^{d,e}	190
13	+350 ^c	+330 ^c	+310 ^c	31	+2510 ^e	+2510 ^e	150
14	+510 ^c	+510 ^c	+480 ^c	32	+2700 ^e	+2680 ^e	180
15	+650 ^c	+660 ^c	+630 ^c	33	+2860 ^e	+2830 ^e	155
17	+830 ^c	+840 ^c	+800 ^c	34	+3050 ^e	+3070 ^e	215
18	n.d.	n.d.	n.d.	35	n.d.	+3200 ^e	140
19	n.d.	+1150 ^d	n.d.	36	+3410 ^e	+3390 ^e	200
21	+1350 ^d	+1340 ^{d,e}	n.d.	37	+3540 ^e	+3540 ^e	140
23	+1520 ^d	+1490 ^d	+1530 ^d	38	+3730 ^e	+3730 ^e	190
25	+1690 ^d	+1700 ^{d,e}	+1670 ^d	39	+3970 ^e	+3900 ^e	205

¹Relative to the AUG codon (A = +1).²Difference in bp between the averaged positions of every two consecutive chromatin-characteristic bands.³The letters following the figures stand for the procedure used to locate the MNase cutting sites: a, restriction with *Hind*III and probing with HE; b, restriction with *Pst*I and probing with PR; c, restriction with *Kpn*I and probing with XK; d, restriction with *Xba*I and probing with XK (electrophoresis on 1.8% agarose); e, as d, but the electrophoresis was carried out on 1.0% agarose gels. See the text for further details.⁴n.d., position not determined.

Figure 5. Comparative detection of nucleosome positioning in the 3' terminal region and downstream flank of the repressed *SUC2* gene in genomic or episomal chromatin. The indirect end labelling technique has been used following procedure **d**, i. e., restriction with *Xba*I and probing with **XX** (electrophoresis on 1.8% agarose gels). The origin of chromatin is indicated at the top of the figure. Two controls of naked DNA have also been included (lanes a and b). Two levels of MNase digestion are shown for every sample. The two bands marked with an asterisk do not correspond to single MNase cutting sites (see the text for further details). Other symbols as in the legend of figure 3.

ference in bp between every two consecutive chromatin characteristic bands is compatible in most cases with nucleosomal repeat length. The separation of bands 8 and 10 is, however, very large and considering that DNase I hypersensitivity exists between -430 and -540 and at -290 (13), it can be concluded that if a nucleo-

some is positioned between sites 8 and 10, it will be placed between -430 and -290.

3' region of the gene and non-coding flank. To study the nucleosome positioning in the 3' terminal region of the repressed gene and its downstream flank, DNA resulting from MNase digestion was restricted with *Xba*I and probed with **XX**. The electrophoresis was carried out either on 1.8% (procedure **d**) or 1.0% (procedure **e**) agarose gels. The results of experiments with genomic *SUC2* gene, plasmid pRB58 and plasmid pYCS1, all following procedure **d**, are shown in figure 5. The poor resolution of the pYCS1 gels does not allow an accurate determination of the band sizes, except for a few cases recorded in table I, but, from a qualitative point of view, it is clear that the structure of pYCS1 chromatin is similar to that of pRB58 or genomic chromatin.

A pair of strong bands that map around +1900 are easily detectable in the genomic and episomal chromatin (bands 27 and 28). The resolution was improved by using procedure **e**, as it will be shown in figure 8. Both upstream and downstream of these sites highly regular ladders of fragments can be seen, those in the 3' flank being especially clear in figure 8f. The sizes of these bands, measured by either procedure **b**, **e** or both, allowed us to map the MNase cutting sites, which are also given in table I. The two bands marked with an asterisk in figure 5 have not been included in the table: by applying the sliding-end-labelling method, we have previously shown that they are generated by MNase double cutting (30).

The DNA between MNase cutting sites in the 3' non-coding region is extremely protected (figs. 5 and 8). While MNase digestion of deproteinized DNA gave a large number of bands and a remarkable smear, bands 28-40 in chromatin from repressed cells are quite definite and the smear between them is negligible. These results could be interpreted in terms of a precise nucleosome positioning starting at about +1990 and extending for over 2 kb. Distinct arrays of positioned nucleosomes have also been found in the 3' and 5' distal regions of the yeast *HSP82* gene (35).

Changes in the chromatin structure upon derepression

Figures 6-8 show that some precise and definite changes in chromatin structure take place upon activation of the *SUC2* gene.

Figure 6. Effects of derepressing the *SUC2* gene on the nucleosome positioning in the 5' flank. The figure shows the results of an experiment similar to that of figure 3, except that genomic instead of pRB58 chromatin was used, and that a sample from the repressed gene (R, lane b) is compared to a sample from the derepressed (D, lane c) state. Control of naked DNA is included in lane a.

Changes occurring in the 5' flank imply an enhanced exposure of DNA between sites 7-8 (fig. 6) and at site 9 (figs. 6 and 7), while the DNA between sites 8 and 9 seems to be more protected in active chromatin (figs. 6 and 7).

Several interesting changes are seen in the 3' non-coding region. Band 35 disappears on activation and a prolonged smear appears between bands 32 and 34. In experiments with pRB58, the two extra bands marked in figure 8i, which map at +2790 and +2930, are clearly visible in active chromatin. These bands are present in naked DNA, which means that the region between sites 32 and 34 becomes accessible to MNase in active chromatin, while the region between sites 34 and 36 is now protected.

Figure 7. Effects of derepressing the *SUC2* gene on the nucleosome positioning in the left part of the coding region and proximal zones of the 5' flank. The figure shows the results of an experiment similar to that of figure 4, lanes a-c, except that a repressed (R) sample and a derepressed (D) one from pRB58 chromatin are compared. All the symbols are as in figure 4.

DISCUSSION

Equivalence of the chromatin structure of the genomic and episomal *SUC2* gene

It had been shown that several features of the structure of the genomic *PHO5* gene of *S. cerevisiae* (7) are preserved when the gene is inserted in several multicopy plasmids (20, 21) and that the nucleosome positioning around the chromosomal centromeric sequences *CEN3* and *CEN11* is indistinguishable from that present in yeast plasmids carrying these sequences (36). Our present results and those reported before (13) extend the comparison at a fine level to a DNA region of 5 kb containing the

Figure 8. Effects of derepressing the *SUC2* gene on the chromatin structure of the 3' terminal region of the gene and downstream flank. Naked DNA, genomic or pRB58 chromatin from either repressed (R) or derepressed (D) cells, as shown in the figure, were digested with either DNase I or MNase, as indicated in the upper part of the figure. When appropriate, *e. g.*, lanes d and e, or f and g, two different levels of nuclease digestion are included. Lanes h and j contain size markers of 3584, 1693, 1545, 1125, 779, 622 and 527 bp.

SUC2 gene and support the idea that the features of chromatin structure and their changes upon activation are maintained when a chromosomal gene is inserted into a plasmid. This offers an important practical applicability. Many questions on the structure of active chromatin could be investigated if a sufficient amount

Figure 9. Model of the chromatin structure of the *Saccharomyces cerevisiae* *SUC2* gene and the adjacent non-coding regions. In the upper part of the figure, under the map scale (+1 stand for the first letter of the initiation codon), the proposed model of chromatin structure in the repressed gene is depicted. Selected regions from both the 5' and the 3' flanks are depicted below at a greater magnification, to show the proposed changes upon derepression. Arabic numerals (from 4 to 39) stand for the MNase cutting sites characterized in this study, and Roman numerals designate the positioned nucleosomes and other protected regions of the chromatin. Arrowheads point to DNase I hypersensitive sites (see ref. 13) and the thick horizontal arrow represent the mRNA. The circles represent positioned nucleosomes. A dotted circle mean that there are not sufficient data to decide whether the nucleosome is present or not in active chromatin. The structures depicted as triangles and rectangles may represent altered nucleosomes or some other structural motif that results in a protection of DNA towards MNase digestion. The shaded areas are regions especially protected in the active state. The positions of the TATA box and the upstream regulatory region (URR) are also given.

of chromatin from a single gene would be available. The incorporation of such a gene in a multicopy plasmid and the preparative isolation of native episomal chromatin, whose possibility has been demonstrated (22), open a promising way to these studies.

Our results pose another interesting question. The fine details of chromatin structure and their changes upon gene activation are, probably, determined by sequence-specific DNA-protein interactions, and it is currently thought that the number of copies of these proteins is small. However, the plasmid pRB58 is present in 30-40 copies (13) and yet all the structural features are well preserved. This means that, at least in this instance,

the number of copies of regulatory proteins must be in the range of several dozen.

Relationships between DNase I and MNase hypersensitivity

Although DNase I and MNase usually recognize different motifs in chromatin structure, there are some common target sites for their hydrolytic action. Hypersensitive sites are often heavily attacked by both nucleases. Renaud and Ruiz-Carrillo have recently conducted an elegant comparison of these common sites in the histone H5 gene chromatin of chicken erythroid cells (37) and a coincidence between DNase I and MNase hypersensitivity has been also found in the yeast *HSP82* gene (35). This is also the case in the yeast *SUC2* locus. Bands 27 and 28 (fig. 5) and 41 (fig. 8) are indicative of high MNase sensitivity, at sites corresponding to the previously described (13) DNase I hypersensitive regions at +1920 and +4390 respectively. The role of these hypersensitive domains remains to be determined. In the 5' flank, bands 8 and 10 (fig. 3) are clearly indicative of hypersensitivity. The first one maps at about -520, that is, within the upstream regulatory region of the gene (38, 39), in which three closely-spaced DNase I hypersensitive sites have been described. The second one, band 10, maps in the middle of the DNase I hypersensitive region between -220 and -290 (13).

Nucleosome positioning

Figure 9 gives a hypothetical model of nucleosome positioning in the *SUC2* locus, consistent with the results reported in this paper. The most precisely positioned nucleosomes lie on the 3' flank, as shown, especially, by the absence of background between the linker bands in figure 8. It is clear (lanes b/c and f) that DNase I preferentially attacks linker DNA in this region, as anticipated by us (13). A similar preference of DNase I for linker DNA has been shown to occur in the highly positioned nucleosomes of yeast centromeres (36) and in the yeast *HSP82* gene (35). This preference has also been found at the early stages of *Xenopus* chromatin assembly, when histone H1 has not yet bound the linkers (40). Whether the absence of H1 is actually required for DNase to preferentially attack the linker DNA, escapes our present knowledge, but, if it were, the absence of an H1-like histone in yeast would explain our results and those mentioned above

(35, 36). Between sites 33 and 37 four protected regions exist in chromatin (fig. 8f), which show a pattern of alternate long and short lengths (table I). It is not clear whether these protected regions actually correspond to nucleosomes, but if so, they must exhibit some unique properties, in view of their behaviour on activation (see below).

Eleven nucleosomes (V-XV) may be placed on the coding sequence although, probably, their positioning is not as precise as in the 3' flank, as the broadness of the bands from the linker seems to indicate (figs. 4 and 5). Finally, present studies suggest that 4 nucleosomes may exist within the 1000 bp analysed in the 5' non-coding region. They are tentatively located in figure 9 with the aid of the data on DNase I hypersensitivity (13).

Changes in nucleosome positioning upon gene derepression

The changes that occur in the 5' flank of the gene upon activation are better interpreted if nucleosome III is removed from chromatin in the active state. This would explain the increased accessibility of DNA at site 9 and between this site and site 10 (figs. 6 and 7). On the other hand, the already mentioned enhanced protection of DNA between sites 8 and 9 in the active state may be explained by a change in the protein factors bound to that zone. It has been suggested that the proteins coded by the *SSN6* or *SSN20* genes may act as repressors of the *SUC2* gene (41-43). Upon derepression, several factors may be substituted or modified, changing the overall chromatin structure of the region. Nucleosome II may be somewhat opened in the active state, and this would justify the relative enhancement of MNase accessibility to DNA sequences between 7 and 8 (fig. 6).

Lorch *et al.* (44), by using *in vitro* reconstituted templates from promoter DNA and histone octamers, have recently described that RNA polymerases cannot initiate transcription in a nucleosome, but we have not found any clear derepression-dependent change between sites 10 and 11, where the TATA box lies. It should be kept in mind that the *SUC2* gene can be constitutively transcribed to an 1.8 kb mRNA, although the transcription rate is much lower than that of the repressible 1.9 kb mRNA (23). The transition from a repressed to a derepressed state is not, therefore, an absolute "off-on" change, and this might explain our

discrepancy with the results of Lorch *et al.* (44). If these results were not extrapolable to *in vivo* systems, nucleosome IV would not need to be removed to initiate transcription if the useful information contained in the TATA box remains accessible on the surface of the positioned nucleosome IV (see ref. 4).

The changes observed within the coding region are better explained if the nucleosomes adopt a relaxed conformation in the actively transcribing gene. The existence of "half nucleosomes" in an actively transcribing yeast gene has been proposed (35), and this may justify that intracore DNA becomes accessible to MNase in the active *SUC2* gene.

Finally, the changes that occur in the 3' non-coding region deserve some attention. It has been already mentioned that the spacing between sites 34 through 37 is irregular. We do not know whether the structures depicted as rectangles and triangles in figure 9 (XXI-XXIV) actually are nucleosomes, but we speculate that this region is involved in the binding of a regulatory protein in active chromatin, which leads to a profound alteration of chromatin structure, as depicted in the model of figure 9. There is an obvious relationship between the loss of nucleosomes and the change in the DNase I cutting pattern (fig. 8). An attractive, though highly conjectural, possibility is that the proposed 3' regulatory protein simultaneously binds some of the 5' regulatory sequences, bringing the gene to an exposed conformation. Preliminary evidence from this laboratory indicates that removal of the *Hind*III fragment from the 3' flank results in a change of the chromatin structure of the promoter, suggesting that a long-distance structural relationship may exist between the 3' and 5' flanks. This topic is now under further study.

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