In Vivo Changes of Nucleosome Positioning in the Pretranscriptional State*

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Ernesto Di Mauro‡§, Loredana Verdone§, Barbara Chiappini§, and Micaela Caserta‡†
From the ‡Centro di Studio per gli Acidi Nucleici, CNR, and §Fondazione Istituto Pasteur-Fondazione Cenci Bolognetti, c/o Dipartimento di Genetica e Biologia Molecolare, Università “La Sapienza,” Rome 00185, Italy

The involvement of chromatin structure and organization in transcriptional regulatory pathways has become evident. One unsolved question concerns the molecular mechanisms of chromatin remodeling during in vivo promoter activation. By using a high resolution in vivo analysis we show that when yeast cells are exposed to a regulatory signal the positions of specific nucleosomes change. The system analyzed consists of the basic elements of the Saccharomyces cerevisiae ADH2 promoter, two nucleosomes of which are shown to change the distribution of their positions by few nucleotides in the direction of transcription when the glucose content of the medium is lowered. Such repositioning does not occur in the absence of the ADH2 transcriptional activator Adr1 or in the presence of its DNA-binding domain alone. A construct consisting of the DNA-binding domain plus a 43-amino acid peptide containing the Adr1 activation domain is sufficient to induce the same effect of the full-length protein. Nucleosome repositioning occurs even when the catalytic activity of the RNA polymerase II is impaired, suggesting that the Adr1 activation domain mediates the recruitment of some factor to correctly preset the relevant sequences for the subsequent transcription steps.

Nucleosomes are dynamic particles that must be modified in their structure and/or their location to allow many nuclear processes (1–3). Despite extensive evidence of in vitro nucleosome mobility (4–8) and of the in vitro effects of chromatin remodeling machines (9–20), the actual fate of nucleosomes during in vivo promoter activation (loss, displacement, or structural modification) is still an unresolved point. The two gene systems in which in vivo chromatin remodeling was best characterized (the yeast PHO5 and GAL1-GAL10) (21) were analyzed only at low resolution levels, thus preventing definitive understanding of the underlying mechanism.

We have studied the mechanisms of in vivo chromatin remodeling in Saccharomyces cerevisiae, focusing on the gene coding for the alcohol dehydrogenase II (ADH2) in its natural chromosomal location. The transcriptional activator of the system (22), the key regulatory region (23), and the chromatin organization of the promoter (24) were already defined.

To evaluate what exactly happens in vivo during derepression to the nucleosome particles spanning the ADH2 promoter, we apply here a high resolution method (24–27) consisting of extensive micrococcal nuclease (MN)1 digestion of spheroplasts, isolation, and purification of monomeric nucleosomal DNA, followed by primer extension from selected oligonucleotides allowing precise mapping of the borders of specific nucleosomal particles.

In the ADH2 promoter, we have previously shown that both nucleosomes −1 and +1, spanning, respectively, the TATA box and the RNA initiation sites, consist of a family of rotationally phased translationally alternate particles. A short region encompassed between the two nucleosomes can be alternatively occupied by members of either family (24). Precise mapping of the borders was obtained in a population of cells growing in repressing conditions (3% glucose). The analysis of chromatin remodeling during derepression was so far performed at low resolution by indirect end-labeling (24, 28) showing an increase in MN accessibility inside the particles and a decrease in the frequency of MN cleavage at the borders, thus suggesting a decrease of the protection of DNA by the nucleosomes (24, 28, 29). This is consistent with nucleosomal loss during derepression or with a change of nucleosome location or conformation allowing increased MN access. These possibilities are not distinguishable at low resolution and are not necessarily mutually exclusive.

By using a high resolution in vivo analysis we show that the two promoter nucleosomes change the distribution of their positions by few nucleotides in the direction of transcription and that this repositioning is mediated by the Adr1 activation domain.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—S. cerevisiae strains used in this study are: CY26 (wt) MATα, ura3–52, lys2–801, ade2–101, trp1–Δ1, his3–Δ200, leu2–Δ1, JSY112 (adr1) MATα, same as CY26 except adr1::LEU2; RY260 (ρρ1–1) (28, 30) MATα, ura3–52, ρρ1–1.

Yeast strains carrying no plasmid were grown in YPD medium (1% yeast extract, 2% Bacto-peptone, 3% glucose). To obtain ADH2 derepression, the cells were collected by centrifugation, washed once with water, and resuspended in the same volume of fresh YP medium containing 0.05% glucose for the appropriate time.

Cells carrying the plasmids described below were grown in YNB medium (0.68% yeast nitrogen base) supplemented with the required amino acids and 3 or 0.05% glucose.

Plasmids—The Adr1 derivatives used are: pADR1Δ172, consisting of the first 172 amino acids of Adr1 inserted in pRS314 (CEN6, ARSH4, TRP1), and pADR1Δ172-AD1 same as above with the addition of a peptide

† To whom correspondence should be addressed: Centro Acidi Nucleici, CNR, c/o Dipartimento di Genetica e Biologia Molecolare, Università “La Sapienza,” P.le Aldo Moro 5, Rome 00185, Italy. Tel.: 39-06-49912659; Fax: 39-06-49912500; E-mail: micaela.caserta@uniroma1.it.

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1 The abbreviations used are: MN, micrococcal nuclease; MOPS, 4-morpholinopropanesulfonic acid; TBE, Tris borate-EDTA; UAS1, upstream activation sequence 1.

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containing amino acids 420–462 of Adr1 (see Ref. 28). These plasmids were used to transform the adr1-disrupted strain JSY112.

**Enzymes**—All nucleases were purchased from Roche, and Zymolyase 100T was purchased from Seikagaku Corp.

**Analysis of Mono-nucleosomal Borders**—The described method (24–27) is slightly modified here. Spheroplasts from cells exponentially growing (ρmax, 0.3–0.5/μl) were treated with nystatin (100 μg/ml) to allow the access of MN to the nucleus. The samples were incubated with MN (120 units/ml, 37 °C, 15 min), and the reaction was stopped with 5 mM EDTA, 1% SDS. After protease K (2 h, 56 °C), phenol-chloroform extraction, and ethanol precipitation, the samples were electrophoresed on preparative 1.5% agarose-TBE gels to isolate the mono-nucleosomal DNA. The monomer-sized DNA was eluted by the “Concert” rapid gel extraction system (Invitrogen), and the amount was determined by the ethidium bromide-based spot test. The samples were primer-extended with Taq polymerase in the presence of labeled oligonucleotides and analyzed by electrophoresis on 6% polyacrylamide-TBE gels. Nucleotide-level mapping was obtained by parallel sequencing lanes.

**Calculation of the Weight Average of the Nucleosomal Borders**—To determine the weight average of each group of borders, we started from the densitometric scansings shown in Figs. 1D and 2C and calculated the area of each border (upstream or downstream) in repressing (R) or derepressing (180°) conditions. These values, expressed as arbitrary units (see Tables I and II), were first corrected by multiplying for a correction factor (calculated as detailed in the legends of Tables I and II), which takes into consideration the intrinsic differences between the upstream and the downstream profiles (namely the differential primer-specific efficiency of annealing and the sequence-dependent Taq polymerase efficiency of elongation) and then transformed in percentage relative to the total (100%). The percentage of each border was then multiplied for the number corresponding to the map position.

By summing up all the values obtained in this way, according to the formula ΣniN1/N2, a number was determined that localizes the center of the distribution of any given group of nucleosomal borders. The correction factors introduced in this calculation were necessary to compare the area values obtained with different oligonucleotides extending on different filaments of the same monomeric DNA template.

**Hybridization**—Nystatin-treated spheroplasts were digested with low amounts of MN (0.5 and 1 unit, 0.25 ml, 37 °C, 15 min). After proteinase K and phenol-chloroform extraction, the purified DNA was primer-extended with Taq polymerase in the presence of labeled oligonucleotides and analyzed by electrophoresis on 6% polyacrylamide-TBE gels.

**RNA Analysis**—For the experiment shown in Fig. 4B, aliquots containing an equal number of cells (0.5 to 1 × 109) were pelleted, and total RNA was prepared as described (31). After spectrophotometric determination of the amount of RNA present in each aliquot, 10 μg of RNA were loaded onto 1.2% agarose-MOPS gels, containing formaldehyde as a denaturing agent and ethidium bromide as an intercalating dye. Northern blot analysis was performed by standard procedures with Hybond N+ nylon paper (Amersham Biosciences, Inc.). For hybridization, a 3'-end-labeled oligonucleotide specific for the ADH2 gene was used. Map positions and sequence are as follows: from +710 to +684, 5′-GGGTGATACCTAAGCAGCTGACATTAAC3′.

**RESULTS AND DISCUSSION**

**Change of Positioning of the –1 Nucleosomal Family**—The nucleotide level mapping of the –1 nucleosomal family, protecting the ADH2 TATA box, was previously performed in a population of cells growing in repressing conditions (3% glucose) (24). We therefore asked what would happen to the same groups of nucleosome particles when analyzed during transcriptional activation by studying the distribution of their borders after extensive MN digestion of the spheroplasts. We reasoned that, depending on the type of mechanisms underlying the ADH2 chromatin remodeling, the high resolution pattern of MN-induced cuts would be different, as follows.

(i) If nucleosomal loss is the major mechanistic route, taking place upon derepression, with no partial relaxation occurring, the intensity of the entire borders profile should decrease homogeneously. In this instance, MN may have access to the DNA sites that have lost their nucleosomal organization and degrade them. The corresponding nucleosomal borders would be lost.

(ii) If a conformational change occurs upon derepression in part or in all the members of a nucleosomal family, the MN accessibility of the modified members of the family would be different from that of the non-modified ones. This would cause a partial or total modification of the distribution of the border intensities, respectively.

(iii) If the chromatin modification occurring upon derepression consists of a directional repositioning of nucleosomal particles, the intensities of the borders should vary in a predictable way. The variation of the border profile should consist of the decrease of the borders on one side, matched by the increase of the corresponding borders on the opposite side. If the repositioning occurs over several helical repeats, the appearance of new bands on one side of the initial border distribution should be observed (accompanied by a decrease or disappearance in the opposite side).

Fig. 1 shows the results obtained for the nucleosome –1, in a wild type and its isogenic adr1 strain. Two divergent oligonucleotides (Fig. 1C) were used with the same amount of material: oligonucleotide 1 anneals in the center of the population and extends rightward, away from the UAS1, thus defining all the downstream borders of the nucleosome family –1 (Fig. 1A); oligonucleotide 2 anneals in the center and extends leftward, toward the UAS1, thus defining all the upstream borders of the family (Fig. 1B).

The nucleotide level mapping of the borders and the identification of the single particles is reported in Fig. 1C. Fig. 1A (downstream distribution) shows that the intensity of some borders (–1, 1.1, 1.2, 1.3, 1.4) in the wild type but not in the adr1 strain does indeed decrease with a different rate during derepression whereas the intensity of the others (–1.5, –1.6) increases. The decreased frequency is expected from, and explained by, the accumulation of MN cleavages inside certain monomeric particles, thus causing their absence as substrates for primer extension. The increased frequency of certain borders is explained considering that some members of the family –1 “disappear” from one place to “reappear” in a different place, consistent with a shift of the position of the particles in the direction of transcription. In addition, some new bands appear above the –1.6 border (see arrowheads in Fig. 1A, left panel, and in the bottom panel showing a longer autoradiographic track of the specific area). Fig. 1B (upstream distribution) shows a stronger decrease in the intensities of some borders (–1.1, –1.2, –1.3, –1.4), a less pronounced decrease in the intensities of the others (–1.5, –1.6), and the appearance of new bands below the –1.6 border (see arrowheads in Fig. 1B, left panel). The new bands, present on one side only of the initial distribution, i.e. beyond the –1.6 particle (more clearly visible for the same nucleosome in Fig. 4 and Fig. 5A, arrowheads, and for the nucleosome +1 in Fig. 2A, arrowheads), are not consistent with a nucleosome loss (i) or a nucleosome conformational change mechanism (ii) but rather with a repositioning mechanism (iii), in which the most upstream particles of the –1 family move in the direction of transcription. Fig. 1D shows a densitometric evaluation of part of these data: for both the upstream and the downstream profiles only the two extreme situations, r (repressing conditions, 3% glucose) and 180° (derepressing conditions, 0.05% glucose), are shown. To describe more quantitatively this phenomenon, we calculated the weight average for each group of borders and defined the variation of the nucleosome positioning. The results are shown in Table I. After 180 min of activation, the weight average of the upstream borders of the nucleosome covering the TATA box shifts by 6 base pairs to the right (from position 953 in repressing conditions to 959 after promoter induction). The same numerical change (6 base pairs) is reported for the weight average of the downstream borders. This number represents only the minimal estimate of the real shift because the intensities of the
new bands appearing beyond the initial distribution have not been included in this calculation.

One further feature emerges when comparing the profiles of the wild type and of the adr1 strains, as in Fig. 1A. Additional bands are present between the borders of nucleosomes −1.4 and −1.5 in the adr1 strain. The same feature is evident also in the adr1 strain transformed with a reduced version of Adr1 (see Fig. 4A) or in another mutant, the rpb1−1 strain (see Fig. 5A), although the behavior of these bands depends on the genetic context. The accessibility to MN in this specific region may be altered in these mutants because the absence of a full-length activator or the presence of a defective RNA polymerase II (rpb1−1 strain) could favor the assembly of alternative protein complexes.

Change of Positioning of the −1 Nucleosomal Family—Because of the close proximity of the nucleosome family −1, which
covers the TATA box region, with the family +1, which covers the RNA initiation sites, we argued that the repositioning of the group of particles −1 could influence the translational positioning of the adjacent group of +1 particles. We therefore extended the high resolution analysis to the family of nucleosomes +1; the results are shown in Fig. 2. A second couple of

FIG. 2. Change of the distribution of the members of the +1 nucleosomal family. A, the upstream and downstream nucleosomal borders are identified by numbers (+1.1 to +1.6). Matching positions from the two divergent oligonucleotides, whose distance approaches the monomer size (146 bp), have the same number. R, repressing conditions, 3% glucose. Derepression: increasing times after shifting the cells to low glucose (0.05%). Arrowheads indicate the additional bands appearing during derepression outside the initial border distribution of the repressing conditions. Lanes N, M, A, C, and T as in Fig. 1B; map of the relevant ADH2 promoter sequences. The positions of the +1 nucleosomal borders are identified by vertical bars. RIS, RNA initiation sites; arrowheads indicate the two major and the two minor ADH2 transcription start sites. Oligonucleotide 3, 5'-TTAATGATATCATTGCAAATTGCTTT-3'; oligonucleotide 4, 5'-TGGAATAGACATTGTGTATTACGATATAT-3'. C, densitometric scanning analysis of selected lanes from Fig. A. Ordinate, arbitrary units. The two extreme situations are compared: lane R (repressing conditions) and lane 180' (the longest time after the shift to derepressing conditions).
differently overlapping oligonucleotides (map position in Fig. 2B) was used: oligonucleotide 3 points toward the downstream borders, and oligonucleotide 4 toward the upstream ones. As in the case of the nucleosome –1, by inspection of the downstream nucleosomal borders (right part of the figure) some members of the family (+1.2, +1.3) show a decrease in occupancy during derepression, some remain constant (+1.1, +1.4), whereas others (+1.5, +1.6) show an increase, and even new positions (see arrowheads above the +1.6 band) become evident. Again, the effect is observed only in the wild type and not in the adr1 strain (data not shown). Fig. 2C shows a densitometric evaluation of part of these data: for both the upstream and the downstream profiles only the two extreme situations, r (repressing conditions, 3% glucose) and 180° (derepressing conditions, 0.05% glucose), are shown. From these scannings we calculated the weight average for each group of nucleosome borders and defined the change of nucleosome positioning. The results are shown in Table II; after 180 min of activation, the weight average of the nucleosome +1 upstream borders shift by 6 base pairs to the right (from position 1126 in repressing conditions to 1132 after promoter induction). The same numerical change (6 base pairs) is reported for the weight average of the downstream borders.

The change in positioning occurring to the nucleosomal family +1 appears therefore to be very similar to that occurring to the neighboring group of –1 particles. If one influences the other or if the two events are unrelated remains to be established. Moreover, the constant intensity of the downstream boundary of nucleosomes +1.1 and +1.4 could potentially represent the consequence of the simultaneous influence of the two adjacent nucleosomes (–1 and +2). To confirm the Adr1-dependent change of translational positioning observed upon derepression in the nucleosomal families –1 and +1, as obtained through the analysis of populations of isolated monomeric particles, we performed a high resolution analysis using chromatin samples obtained from mildly MN-digested spheroplasts. This approach allows the study of an entire array of nucleosomes providing information (Fig. 3) on the occupancy of the whole area encompassed between the two adjacent families of nucleosomes –1 and +1 and on its variations upon derepression. Oligonucleotide 1 (as in Fig. 1A) was used for the primer extension. The bands indicated as –1.1 to –1.6 and +1.1 to +1.6 were unambiguously attributed to one or the other nucleosomal family, based on the nucleotide level mapping previously obtained with the purified monomeric populations (see Figs. 1C and 2B). In the samples dubbed “60°” (derepressing conditions), the intensity of some of the –1 and +1 intermingled borders decreases (–1.1, –1.2, –1.3, –1.4, +1.1, +1.2, +1.3), whereas the intensity of few others remain constant (–1.5, +1.4) and the intensity of the remaining ones increases (–1.6, +1.5, +1.6) relative to the samples dubbed “R” (repressing conditions). Again, as in Figs. 1 and 2, the effect is directional, with a clear increase in site occupancy in the direction of transcription. In addition, to locate and to allow the analysis of nucleosome boundaries, MN is able to recognize and cut the DNA that has lost or is in the process of losing its contacts with the histone octamer, as we have observed by low resolution analysis (24, 28, 29) and can as be clearly seen by inspection of Fig. 3. An increase in accessibility inside the +1 nucleosomal family after derepression is observed when comparing the lanes R with the lanes 60°. Moreover, when comparing the lanes 60° with the in vitro-treated samples (lanes N), it appears that some bands coincide, suggesting a local loss of contact with histones, whereas other bands at the samples’ N are not visible in the samples 60°, indicating that these latter DNA sites are still protected by one or more members of the +1 nucleosomal family.

One interesting possibility would be that the concerted repositioning of the –1 and the +1 families of nucleosomes allows the RNA initiation sites (indicated by arrowheads in Fig. 3) to become accessible to the transcriptional apparatus.

Nucleosome Repositioning Is Mediated by the Adr1 Activation Domain—The Adr1 dependence of the change of positioning of the promoter nucleosomes can be explained by two different alternatives: (i) when the glucose in the medium becomes limiting, the activator protein occupies the UAS1 and because of its large size (1323 amino acids) pushes the more upstream borders of the –1 nucleosome toward the nearby +1 nucleosome; (ii) in derepressing conditions, Adr1 binds the UAS1 and recruits additional factors which in turn cause the nucleosomes to slide.

To distinguish between these two possibilities, we have an-

| Map position | AU | CF | Σ,ρiN/Σ,ρi | AU | CF | Σ,ρiN/Σ,ρi |
|--------------|----|----|------------|----|----|------------|
| –1.1         | 934| 4.25| 0.82       | 1.3| 1.42|           |
| –1.2         | 944| 6.7 | 2.7        | 1.35| 3.22|           |
| –1.3         | 949| 22.8| 1.87       | 5.55| 1.58|           |
| –1.4         | 957| 48.65| 0.86      | 11.9| 1.21| 959       |
| –1.5         | 968| 55.7| 0.13       | 14.1| 0.69|           |
| –1.6         | 978| 30.25| 0.19       | 10.35| 0.87|           |

\[ \text{Table I} \]

Determination of the weight average for each group of the –1 nucleosome family

\( R \) and 180 min, repressed (3% glucose) and derepressed (180 min in low glucose) conditions, respectively. AU, arbitrary units for the evaluation of the areas. The values are based on the densitometric scanings shown in Fig. 1D. \( CF \) and \( CF_{180} \) correction factors consisting of the ratios between the downstream and the upstream area values for each particle in repressed and derepressed (180 min) conditions, respectively. \( n_i \), percentage of the area of each border relative to the total (100%). \( N \), map position of each border (see left columns and Fig. 1C). For a more detailed description of the calculation procedure see “Experimental Procedures.”
**Table II**

**Determination of the weight average for each group of borders of the +1 nucleosome family**

$R$ and 180 min, repressed (3% glucose) and derepressed (180 min in low glucose) conditions, respectively. AU, arbitrary units for the evaluation of the areas. The values are based on the densitometric scanings shown in Fig. 2C. $C_{R}$ and $C_{180\text{ min}}$ correction factors consisting of the ratios between the upstream and the downstream area values for each particle in repressed and derepressed (180 min) conditions, respectively. $n_{i}$, percentage of the area of each border relative to the total (100%). $N_{i}$, map position of each border (see left columns and Fig. 2B). For a more detailed description of the calculation procedure see “Experimental Procedures.”

| Map position | $R$ | 180 min |
|-------------|-----|---------|
|             | AU  | $\Sigma n_{i}/\Sigma n_{i}$ | AU  | $\Sigma n_{i}/\Sigma n_{i}$ |
| Upstream borders |
| +1.1 | 1112 | 73.1 | 13.5 |
| +1.2 | 1121 | 30.2 | 6.2 |
| +1.3 | 1125 | 57.4 | 7.5 |
| +1.4 | 1131 | 55.45 | 13.3 |
| +1.5 | 1138 | 58.9 | 16.3 |
| +1.6 | 1147 | 14.6 | 23.55 |

| Downstream borders |
|-------------------|-----|---------|
|                    | AU  | $\Sigma n_{i}/\Sigma n_{i}$ | AU  | $\Sigma n_{i}/\Sigma n_{i}$ |
| +1.1 | 1284 | 63.6 | 1.15 |
| +1.2 | 1271 | 15 | 2.01 |
| +1.3 | 1276 | 20.45 | 2.81 |
| +1.4 | 1282 | 39.55 | 1.4 |
| +1.5 | 1288 | 9.3 | 6.33 |
| +1.6 | 1299 | 2.65 | 5.51 |

**Fig. 3. Footprint of the -1 and +1 nucleosome borders.** Chromatin samples, obtained by mild digestion of nystatin-treated spheroplasts with MN (0.5, and 1 units/0.25 ml), were digested with HphI. $R$, repressing conditions (3% glucose) 60', time after the shift to derepressing conditions (0.05% glucose). $N$, naked chromosomal DNA treated in vitro with MN (0 and 0.15 units/0.1 ml) and digested with HphI (map position 1538). All the samples were primer-extended with oligonucleotide 1. Arrowheads indicate the two major and the two minor ADH2 transcription start sites.

**TABLE II**

A couple of isogenic *adr1* strains transformed with two different *adr1* derivatives: pADR1$_{172}$-AD, consisting of the first 172 amino acids (of 1323) containing the Adr1 DNA-binding domain, and pADR1$_{172-AD}$, consisting of the Adr1 DNA-binding domain plus a peptide (amino acids 420–462) containing the Adr1 activation domain (see Ref. 28). The results are shown in Fig. 4. When using oligonucleotide 1 to elongate a monomeric population prepared from *adr1* cells containing pADR1$_{172}$ we did not observe any change in the distribution of nucleosome borders (Fig. 4A, left panel), whereas in the presence of the construct pADR1$_{172-AD}$, a change of the intensity of the borders is visible (Fig. 4A, right panel), just as observed in the presence of the full-length protein (Fig. 1A, *wt*). When using oligonucleotide 2 to elongate the same samples, again a change in the distribution of nucleosomes borders is visible only in *adr1* cells containing pADR1$_{172-AD}$ (compare Fig. 4B, right panel, with Fig. 4B, left panel). The lack of sliding in cells containing the DNA-binding domain alone is not due to instability of the Adr1 derivative because the same molecule is capable of binding UAS1 and of inducing a conformational change in the adjacent −1 nucleosome in the absence of transcription (28).

The results obtained with these two constructs indicate that the nucleosome repositioning is not simply induced as a consequence of Adr1 binding but is specifically mediated by the Adr1 activation domain.

**Nucleosome Repositioning in the Absence of Transcription**—Because of the requirement for the Adr1 activation domain, one could think that the change of the translational positioning of the two promoter nucleosomes during derepression is due to the Adr1-induced wave of transcription. To better investigate this possibility, we analyzed (Fig. 5) the populations of isolated monomeric particles in the temperature-sensitive strain *ADH2 rpb1−1* (30), in which the major catalytic subunit of the RNA polymerase II can be heat-inactivated. Cells growing at the permissive temperature (25 °C) in *ADH2* repressing conditions (3% glucose) were shifted to the restrictive temperature (37 °C) in *ADH2* derepressing conditions (0.05% glucose) and analyzed after 60, 120, and 180 min (Fig. 5A). Even in the complete absence of transcription (RNA analysis in Fig. 5B) the positions of the nucleosomes change, suggesting that the nucleosome repositioning actually precedes transcription and that the activation domain is required to recruit some factor(s) to correctly preset the relevant sequences for the subsequent transcription steps. Chromatin remodeling upon *ADH2* gene activation is there-
fore due at least in part to the translational repositioning of nucleosomes following activator binding but preceding the actual start of mRNA accumulation. This nucleosome fluidity is likely to be facilitated by ATP-dependent chromatin remodeling complexes and/or by changing the acetylation level of the histone tails, as proposed (2), or it could be caused by TATA-bending protein-induced DNA binding as recently hypothesized (32).

Nevertheless, this change of nucleosome distribution cannot by itself represent the only process occurring upon activation: additional features of chromatin remodeling are likely to involve conformational changes of the nucleosome particles that alter their accessibility, as indicated by the low resolution analysis of the involvement of the various Adr1 domains (28) and as shown in the footprint presented in Fig. 3.

**Fig. 4.** Nucleosome repositioning is mediated by the Adr1 activation domain. A and B, comparative analysis of the change of the distribution of the −1 nucleosome borders in *adr1* cells transformed with two different Adr1 derivatives: pADR1<sub>1-172</sub>, consisting of the first 172 amino acids containing the Adr1 DNA-binding domain (left panel), and pADR1<sub>1-172-AD</sub>, consisting of the Adr1 DNA-binding domain plus a peptide (amino acids 420–462) containing the Adr1 activation domain (right panel, see Ref. 28). The downstream (A) and upstream (B) nucleosomal borders are identified by numbers (−1.1 to −1.6). Matching positions determined by the two divergent oligonucleotides are referred to by the same number. R, repressing conditions, 3% glucose. Derepression: increasing times after shifting the cells to low glucose (0.05%). Arrowheads indicate the additional bands appearing during derepression outside the initial border distribution of the repressing conditions.

**Fig. 5.** Nucleosome repositioning in the absence of transcription. A, *rpbl-1* cells (strain RY260) (29, 30) were grown at the permissive temperature (25 °C) in *ADH2* repressing conditions and analyzed (lane R, 3% glucose). After centrifugation, they were resuspended in derepressing medium (0.05% glucose) at the restrictive temperature (37 °C) and analyzed at different times after the shift (60', 120', and 180'). The eluted monomer-sized DNA was then primer-extended with oligonucleotides 1 or 2. The nucleosome borders are identified by numbers (−1.1 to −1.6). Arrowheads indicate the additional bands appearing during derepression outside the initial border distribution of the repressing conditions. N, naked chromosomal DNA digested with HphI (map positions 773 and 1358). M, molecular size marker, pBR322 MspI-digested. A, C, and T, sequencing reactions obtained by primer extending, with the same oligonucleotide, the plasmid DNA pFA (28). B, a small aliquot of *rpbl-1* cells from the treatment described in Fig. 4A was used to isolate total RNA and analyzed by Northern. Lane dubbed C (hybridization control) contains the same amount of total RNA from a culture of wild type cells grown in derepressing conditions (0.05% glucose) for 180 min.

The *in vivo* existence of multiple nucleosome positionings in different eukaryotic systems (24–27, 33–35), even in the repressed state, suggests the intrinsic mobility of the nucleosomal particles. Multipositioning implies that each individual cell may locate the regulatory nucleosome in one of different
positions, inside a gaussian distribution of possible alternatives. Thus, individual cells (most of them genetically identical) are confronted with different localization choices. Being independent on the genetic background, these alternative localizations are epigenetic in their nature. The present observations show that yeast cells actually use this potentiality and distribute nucleosomes along a series of alternatives. The fact that nucleosome positions vary in a controlled manner upon a physiological event adds genetic and regulatory interest to their multiplicity.

REFERENCES

1. Widom, J. (1998) Annu. Rev. Biophys. Biomol. Struct. 27, 285–327
2. Kingston, R. E., and Narlikar, G. J. (1999) Genes & Dev. 13, 2339–2352
3. Wolffe, A. P., and Guschin, D. (2000) J. Mol. Biol. 279, 102–122
4. Beard, P. (1978) Cell 15, 955–967
5. Pennings, S., Meersseman, G., and Bradbury, E. M. (1994) J. Mol. Biol. 220, 101–110
6. Van Holde, K. E., Lohr, D. E., and Robert, C. (1992) J. Biol. Chem. 267, 2837–2840
7. O’Donohue, M. F., Duband-Goulet, I., Hamiche, A., and Prunell, A. (1994) Nucleic Acids Res. 22, 937–945
8. Studitsky, V. M., Kassavetis, G. A., Geiduschek, E. P., and Felsenfeld, G. (1997) Science 278, 1960–1963
9. Varga-Weisz, P. D., Duband-Goulet, I., Hamiche, A., and Prunell, A. (1994) Nucleic Acids Res. 22, 937–945
10. Pazin, M. J., Bhargava, P., Geiduschek, E. P., and Kadonaga, J. T. (1997) Science 276, 609–612
11. Schnitzler, G., Sif, S., and Kingston, R. E. (1998) Cell 94, 17–27
12. Lorch, Y., Cairns, B. R., Zhang, M., and Kornberg, R. D. (1998) Cell 94, 29–34
13. Lorch, Y., Zhang, M., and Kornberg, R. D. (1999) Cell 96, 389–392
14. Travers, A. A. (1999) Cell 96, 311–314
15. Bazett-Jones, D. P., Côté, J., Landel, C. C., Peterson, C. L., and Workman, J. L. (1999) Mol. Cell. Biol. 19, 1470–1478
16. Hamiche, A., Sandaltzopoulos, R., Gdula, D. A., and Wu, C. (1999) Cell 97, 833–842
17. Lengast, G., Bonte, E. J., Corona, D. F. V., and Becker, P. B. (1999) Cell 97, 843–852
18. Whitehouse, I., Flaus, A., Cairns, B. R., White, M. F., Workman, J. L., and Owen-Hughes, T. (1999) Nature 400, 784–787
19. Guschin, D., Wade, P. A., Kikyo, N., and Wolffe, A. P. (2000) Biochemistry 39, 5238–5245
20. Jaskelioff, M., Gavino, I. M., Peterson, C. L., and Logie, C. (2000) Mol. Cell. Biol. 20, 3658–3668
21. Gregory, P. D., and Horz, W. (1998) Eur. J. Biochem. 251, 9–18
22. Denis, C. L., and Young, E. T. (1983) Mol. Cell. Biol. 3, 360–370
23. Beier, D. R., and Young, E. T. (1982) Nature 300, 724–728
24. Verdine, L., Camilloni, G., Di Mauro, E., and Caserta, M. (1996) Mol. Cell. Biol. 16, 1978–1988
25. Buttinelli, M., Di Mauro, E., and Negri, R. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 9315–9319
26. Venditti, P., Costanzo, G., Negri, R., and Camilloni G. (1994) Biochim. Biophys. Acta 1219, 677–689
27. Costanzo, G., Di Mauro, E., Negri, R., Pereira, G., and Hollenberg, C. (1995) J. Biol. Chem. 270, 11091–11097
28. Di Mauro, E., Kendrick, S. G., and Caserta, M. (2000) J. Biol. Chem. 275, 7612–7618
29. Verdine, L., Cesari, F., Denis, C. L., Di Mauro, E., and Caserta, M. (1997) J. Biol. Chem. 272, 30828–30834
30. Nonet, M., Scafe, C., Sexton, J., and Young, R. (1987) Mol. Cell. Biol. 7, 1602–1611
31. Schmitt, M. E., Brown, T. A., and Trumpower, B. L. (1990) Nucleic Acids Res. 18, 3091
32. Lomvardas, S., and Thanos, D. (2001) Cell 106, 685–696
33. Xian-Yang, Z., and Horz, W. (1984) J. Mol. Biol. 176, 105–129
34. Fragoso, G., John, S., Roberts, M. S., and Hager, G. L. (1995) Genes Dev. 9, 1933–1947
35. Tanaka, S., Livingstone-Zatchej, M., and Thom, F. (1996) J. Mol. Biol. 257, 919–934
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Ernesto Di Mauro, Loredana Verdone, Barbara Chiappini and Micaela Caserta

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