GAL4/GAL80-dependent Nucleosome Disruption/Deposition on the Upstream Regions of the Yeast GAL1-10 and GAL80 Genes*

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Metabolic reactivation (incubating spheroplasts with galactose and casamino acids) causes disruption of nucleosomes from the upstream regions of the yeast GAL1, GAL10, and GAL80 genes. The disruption is specific. It depends on the transcription activator Gal4p; it only occurs in galactose-reactivated chromatin from galactose-grown cells; it only affects upstream region, gene-proximal nucleosomes. Due to this specificity and because some of the same regions have shown induction-dependent changes by in vivo analyses, we suggest that the nucleosome-disrupted structure produced by reactivation is the authentic chromatin structure for these regions under conditions of galactose-induced GAL1-10 and GAL80 expression. It is necessary to carry out a spheroplast reactivation treatment in order to observe this disrupted structure in nuclear chromatin because nucleosomes are redeposited onto these regions during the preliminary steps of nuclear isolation (cell harvest/spheroplast preparation) probably in response to the nonphysiological conditions associated with these steps. However, during the same isolation procedures in cells lacking Gal80 protein, there is no nucleosome deposition on these regions, and the in vivo disrupted structure remains present in the nuclear chromatin. Therefore, the nucleosome deposition process that operates in wild-type cells is dependent on Gal80 protein, defining another activity of this negative regulator.

GAL structural gene expression is tightly regulated at the transcriptional level by carbon source (1). GAL1-10, -7, and -2 are induced to very high levels of expression in galactose, via the activator Gal4p, but are completely inactive in other carbon sources. The GAL4 gene and GAL80, the gene that encodes the GAL-specific negative regulator, are expressed in all carbon sources (1) as might be expected of regulatory genes, but their expression levels do vary with carbon source. For example, GAL80 expression occurs at a low basal level in glycerol but is induced 5-10-fold higher, via Gal4p, in galactose (2). This gene family provides excellent opportunities to study the relationship of chromosome structure to gene regulation. Such studies have shown, for example, that under conditions of induced expression (galactose) Gal4p strongly protects the major promoter element, the UASG, on GAL1-10 (3-5) and on GAL80 (6). Gal4p protection of the GAL1-10 UASG is also strong in glycerol (3-5), even though the genes are completely inactive. This reflects the distinctive poised-for-expression status of the structural genes in this carbon source (7). Gal4p protection of the single UASG on GAL80 is weaker in glycerol (6), perhaps to minimize interference with the basal promoter that drives GAL80 expression in glycerol (7).

The UASG elements on GAL1-10 and GAL80 lie within constitutively nonnucleosomal, chromatin hypersensitive regions (4, 6, 8). In the uninduced expression state (glycerol, glucose), positioned nucleosomes are located between these hypersensitive regions and the genes, in the chromosomal copy (6, 8–10) as well as in GAL-containing plasmids (10, 11). The GAL10 TATA, the GAL10 TATA transcription start site, and the GAL80 TATA transcription start site are contained in these nucleosomes. In vivo analysis detected structural changes in two of the GAL1-10 intergenic nucleosomal regions upon galactose-induction of expression (5). However, no such changes were detectable in nuclear chromatin isolated from induced cells (9).

In this paper, we describe experiments that allow us to detect intergenic nucleosome changes in nuclear chromatin, thus reconciling the in vivo and nuclear results. In addition, these experiments yield some unexpected insights on nucleosome disruption/deposition processes taking place on gene control regions.

MATERIALS AND METHODS

Strain 21R yeast cells were grown as described previously (9) in glucose/glycerol/ethanol (D); galactose/glycerol/ethanol (G); glycerol/ethanol (g). These media allow growth of any cells in any medium, even 4D cells (disrupted GAL4) in galactose, and thus allow the most controlled comparison of the various strains. Yeast spheroplasts were made as described in Ref. 12. Metabolic reactivation of spheroplasts consists of adding a carbon source to 2%, usually galactose in these studies, and casamino acids (to 0.5%) to the spheroplasts during the last few minutes of the ~25-min incubation with the cell wall lytic enzyme Oxalylase (12). For our strains, the typical time exposed to reactivation conditions is 10–12 min. If reactivation media are added too early in the incubation with Oxalylase, spheroplasting efficiency is affected. If the total incubation with Oxalylase is allowed to proceed too long, spheroplasts begin to lyse. The choice of typical conditions (10 min of reactivation, 25 min of total incubation with Oxalylase) for this set of strains is sufficient for producing striking effects on chromatin (see below) while avoiding significant spheroplast lysis.

Nuclei were isolated by method I (12), and DNase I and MNase digestion were performed on the nuclei as described therein. DNase I and MNase cleavage sites were located by an indirect end label approach (13, 14). The GAL1-10 patterns in Figs. 1, 2, and 5 were obtained by mapping from the essentially coincident Taq I (see Fig. 1) or EcoR I sites, which lie within the coding sequence of GAL10, ~170 bp downstream of the GAL10 transcription start site (cf. Ref. 9). The patterns in Figs. 2 and 5 were obtained by mapping from the EcoR I site within the coding sequence of GAL1, which lies ~1100 bp downstream from the GAL1 transcription start site (cf. Ref. 8).

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1 The abbreviations used are: Gal4p, Gal4 protein; bp, base pair(s); MNase, micrococcal nuclease; UASG, upstream activation sequence; Gal80p, Gal80 protein; HR, hypersensitive region; DBM, diazobenzoyloxymethyl.

2 J. Lopez and D. Lohr, unpublished observations.
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in Fig. 6 were obtained by mapping from the EcoRI site within the coding sequence of GAL80, which lies 400 bp downstream of the GAL80 transcription start site (cf. Ref. 6). DNA from the nuclear digest was purified (cf. Ref. 9). Samples with similar and suitable digestion extents were chosen by analysis of the bulk patterns on mini-gels, cut with the appropriate restriction enzyme, and electrophoresed on denaturing gels (DNase I nuclear footprints) or native gels (MNase profiles) as described previously (9). DNA was electrophoretically transferred to DBM paper using a homemade electrophotograph apparatus. For all hybridizations, small (50–115 bp) probes abluting the mapping restriction sites were used so that probe homology ends below the smallest DNA bands on any of the patterns shown in this work. The probe was radiolabeled by random priming (15) so both strand patterns are detected in all profiles. The DBM paper was washed and exposed to Kodak XAR film as described previously (9).

RESULTS

Why Is the Chromatin Structure of the GAL1–10 Intergenic Region Not Expression-sensitive in Nuclear Chromatin as It Is in Vivo—GAL1–10 intergenic chromatin consists of an ~170-bp nonnucleosomal, UASG-containing hypersensitive region (4, 8), which is surrounded by positioned nucleosomes (8–11). The DNase I cleavage site pattern (Fig. 1) shows generally strong cleavage within the hypersensitive region (→, track 3), except for the UASG elements, which are protected by the nucleosomal regions upstream of GAL10 and between the HR border and the GAL1 TATA, are relatively protected (○, track 3). These chromatin features are not present in naked DNA digests (Fig. 1, tracks 1 and 4) and show little dependence on digestion extent (cf. Fig. 1, tracks 3 and 4). The MNase cleavage site pattern (Fig. 2A) is a high intensity ladder of bands at ~170-bp intervals across the intergenic region (α, β, γ, δ, ε; tracks 2 and 4), with little or no cleavage between these bands. It has been shown that the lack of cleavage in the interband regions α-β, γ-δ, and δ-ε is due to nucleosome protection (8, 9). Regions α-β and γ-δ correspond to the G regions in Fig. 1. Region β-γ, which is the nonnucleosomal hypersensitive region, is simply not cut by MNase in limited digests, even as naked DNA (8, 9).

As reported previously (9), the intergenic region nuclear chromatin profiles from induced cells, which actively express the GAL1–10 genes, are basically the same as profiles from cells in which GAL1–10 are inactive, whether the inactivity is due to growth in a noninducing carbon source (Fig. 1, compare tracks 2 and 3) or to the absence of the transcription activator Gal4p (Fig. 2A, compare tracks 4 and 2). The single difference around the GAL1 5’ end in MNase profiles will be discussed below. This basic similarity would suggest that the chromatin structure of the intergenic region is insensitive to gene activity (9). However, in vivo analysis (5) detected induction-dependent structural changes within two nucleosomal portions of the intergenic region (X to the right of Fig. 1, track 5, and Fig. 2A, track 3). Since nucleosome structure on upstream regions may be an aspect of gene control (16, 17), we wanted to determine why these nucleosomes do not seem to be sensitive to gene activity in our nuclear chromatin. This issue was particularly perplexing because intergenic nucleosome changes were observed in a previous nuclear chromatin analysis (8), which used a yeast strain of uncharacterized GAL genotype. The strains used in Ref. 9 (and below) comprise an isogenic series with a well characterized GAL pedigree and are widely used in GAL analysis. Note that in vivo and nuclear chromatin approaches only differ for the nucleosomal regions; the two approaches give similar results concerning UASG protection and the absence of induction-dependent change in the nonnucleosomal hypersensitive region.

Galactose and Gal4p-dependent Intergenic Nucleosome Changes Are Observed in Nuclear Chromatin When Nuclei Are Isolated from Metabolically Reactivated Spheroplasts—Isolating yeast nuclei involves a number of cell washing, harvesting, and spheroplasting steps that require 1–2 h at 0–4 °C in nonphysiological solutions (cf. Ref. 12). This corresponds to starvation conditions for yeast cells. It was shown some time ago that incubation of spheroplasts in nutrients (a carbon source plus casamino acids) just prior to their lysis enhances the transcription level in nuclei isolated from the treated spheroplasts (18). This treatment restores the spheroplasted cells to a metabolically active state, allowing them to recover from the starvation during the preliminary steps. To test whether this spheroplast reactivation treatment, which is not ordinarily used, might affect GAL1–10 chromatin structure, we have carried out comparative experiments in which cells harvested from a single culture were divided just prior to the final step in making spheroplasts, treatment with lytic enzyme. One aliquot was reactivated (see “Materials and Methods”) during the lytic treatment; the other was not. Nuclei were isolated from both sets of spheroplasts and digested with nucleases in parallel. The two sets of chromatin digests only differ in the presence of nutrients for a few minutes during spheroplast formation, thus...
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Fig. 2. MNase digestion patterns from the GAL1–10 intergenic region. MNase cleavage sites were mapped from the EcoRI site within GAL10 by indirect end label analysis on 2.8% polyacrylamide, 0.6% agarose nondenaturing gels, as described under "Materials and Methods." In A, the tracks show from left to right: track 1, a naked DNA digest; tracks 2 and 3, digests from wild-type galactose-grown cells minus (track 2) or plus (track 3) spheroplast reactivation with galactose (G⁺); track 4, a digest from 4⁰/80⁰ (disrupted GAL4/disrupted GAL80) cells grown in galactose and reactivated with galactose. In B, the tracks show from left to right: track 1, a naked DNA digest; tracks 2–4, digests from wild-type, galactose-grown cells without (track 2) or with spheroplast reactivation with galactose for 3 min (track 3) or 9 min (track 4); track 5, a digest from galactose-grown, galactose-reactivated 4⁰ cells; tracks 6 and 7, digests from wild-type glycerol/ethanol-grown (track 8) or galactose-grown (track 9) cells, both reactivated with galactose. DNA sizes were determined by comparison to the mobilities of φX174/HaeIII marker fragments run on the gel (some of which are shown to the right of track 4; b, 1078 bp; c, 872 bp; d, 603 bp; e, 310 bp; h, 234 bp). The UAS₁, the major TATA boxes (T), and the GAL1 and 10 transcription start sites (wavy lines) are shown to the left of track 1. The location of the hypersensitive region (HR) is shown to the right of track 3 in A. Other symbols are described in the text. Note that the intensity response in these blots is size dependent (19). This causes α-β region intensity to be generally diminished relative to the other interband regions so that longer exposures are required to visualize α-β interband intensities in the reactivated profiles.

excluding chromatin differences due to variations in cell growth or in day to day nuclear isolation/nuclease digestion conditions.

Including a spheroplast reactivation step in the isolation of nuclei from galactose-induced cells results in exposure of the protected chromatin regions to DNase I cleavage (Fig. 1, tracks 6 and 7), and of the α-β, γ-δ, and δ-ε interband regions to MNase cleavage (Fig. 2A, track 3). The exposure of DNA that is nucleosome-protected in unreactivated chromatin indicates that reactivation causes a significant nucleosome structural alteration, either unfolding or nucleosome loss. Reactivation also causes a loss of the high intensity MNase ladder pattern, due to reduced cleavage at sites β, γ, and δ (Fig. 2B, tracks 2–4 and 7). All of the reactivation-induced changes depend on functional Gal4p (Fig. 2B, tracks 4 and 5) and can only be triggered when cells are grown and spheroplasts are reactivated in galactose. Even spheroplasts from glycerol-grown cells show no evidence of nucleosome alterations (Fig. 2B, track 6), even if reactivated with galactose for up to 25 min (not shown), a period of time that is longer than the time required for galactose to induce full GAL1–10 expression in cells growing in glycerol. Reactivating spheroplasts from induced cells with nongalactose carbon sources also fails to produce any GAL1–10 chromatin changes (not shown). Thus, when isolated from reactivated spheroplasts, nuclear chromatin demonstrates galactose- and GAL4-dependent change in the GAL1–10 nucleosome regions.

We can exclude a number of artifactual explanations for the DNA exposure produced by reactivation. Endogenous nuclease activity is negligible in both types of nuclei (not shown) and thus cannot explain the results. Spheroplast preparation can cause a heat shock-like response, which can be prevented by azide treatment of cells prior to harvest; reactivation effects are the same with or without this treatment (not shown). Treatment with cycloheximide just prior to harvest does not alter the reactivation effect (not shown), so protein synthesis is apparently not required. Most importantly, histone profiles of nuclei from reactivated or unreactivated spheroplasts are very similar (Fig. 3, tracks 1 and 2). Thus, the reactivation-induced DNA exposure does not reflect a proteolysis artifact.

Reactivation Affects all Three GAL1–10 Intergenic Nucleosomes, Probably Equally; Reactivation-induced Changes Are Restricted to These Intergenic Nucleosomes—The response of the three intergenic nucleosome regions to reactivation appears to vary in extent. However, we suggest that reactivation causes a similar and significant change on all three regions because reactivation causes each of the three to become similar

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3 W. Garrard, personal communication.
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to its corresponding naked DNA profile in both relative intensity level and cleavage pattern. For example, in reactivated chromatin profiles interband region γ-δ has the highest intensity, as it does in naked DNA profiles, and as in the naked profiles, this high intensity is limited to the GAL1-proximal half of the region (Fig. 2A, tracks 1 and 3). On the other hand, δ-ε region intensity is lower but spread through the whole region. In the α-β region, both DNase I (Fig. 1, tracks 6 and 7 versus 8), and MNase (not shown) reactivated chromatin cleavage patterns resemble naked patterns. Note particularly the region around the GAL10 TATA (Fig. 1). The similarities of reactivated and naked DNA profiles and the fact that changes are always observed in each of the three regions suggest that the same change occurs on all three nucleosomes and that the change involves major or complete nucleosome loss, but the possibility that any or all of these nucleosome changes are only partial or reflect only nucleosome unfolding cannot be excluded. We will refer to these changes as nucleosome disruption. The γ-δ nucleosome was suggested by in vivo approaches to be disrupted (20).

Reactivation produces a strong MNase band at the GAL1 5′ end. This band has similar mobility to a band in the naked DNA profile and is a doublt like the naked band (Fig. 2B, tracks 1, 3, and 7). It probably reflects exposure of the DNA at the GAL1 5′ end due to the reactivation-induced loss of the δ-ε nucleosome that covered this site. The GAL1 5′ end region is extensively protected in unreactivated profiles from induced cells, over an even larger region than the nucleosome protection seen in the inactive state (Fig. 2A, tracks 2 and 4). This extensive protection is the one feature that differs between MNase digests of inactive and induced (unreactivated) chromatin. It may reflect the presence of stalled transcription-associated complexes near the GAL1 5′ end due to the nonphysiological conditions during harvest/spheroplast pretreatment (below).

Reactivation produces no detectable change in the UASg footprint or within the hypersensitive region (Fig. 1, tracks 6 and 7) and, except for the extreme 5′ end, has no effect on the GAL1 intragenic chromatin pattern. As shown previously (8, 10), galactose induction of expression abolishes the very regular intragenic nucleosome pattern of inactive GAL1 (Fig. 4, track 4), resulting in more heterogeneous nuclease cutting and new bands (Fig. 4, track 5). However, reactivated and unreactivated patterns from galactose-induced cells are quite similar (Fig. 4, tracks 5 and 6). In these same profiles, the striking reactivation-dependent changes on the GAL1–10 intergenic region can be seen clearly (Fig. 4, tracks 2 and 3). Thus, reactivation only affects the intergenic nucleosomes.

The GAL1–10 Intergenic Nucleosomes Are Disrupted without Reactivation in Cells That Lack Gal80p. Providing Evidence That Gal80p Plays a Role in Nucleosome Occupancy on This Region—Nuclear chromatin isolated from galactose-induced cells that lack functional Gal80p (80D cells) shows the same features that are produced in wild-type chromatin by reactivation: nucleosome accessibility of the DNA within the Ω-protected regions (Fig. 1, track 5) and the α-β, γ-δ, δ-ε interband regions (Fig. 5, track 5) and weak cleavage at sites β, γ, and δ. However, these features are present in unreactivated chromatin from cells that lack Gal80p. Moreover, reactivation of these spheroplasts does not produce any further chromatin change (Fig. 5, track 4). Thus, the nucleosome-disrupted structure produced by reactivation in wild-type is present without reactivation in nuclear chromatin isolated from cells lacking Gal80p.

The chromatin regions affected by reactivation in wild-type spheroplasts include most of the sites affected in vivo by the induction of GAL1–10 expression (Fig. 1 and 2). Because of
both wild-type and 80D and they are isogenic strains, the ab-
Gal80p is absent. Since the same isolation protocol is used for
occur on the intergenic regions during the isolation steps when
when cells lack Gal80p. Thus, nucleosome deposition does not
disrupted structure is maintained through these same steps
starvation) conditions associated with these steps. Reactivation
the region during the 1–2 h of cell harvest/spheroplast pre-
mination start sites of
GAL1–10
region to cleavage. That only limited changes are observed prob-
this replacement because the disruption site used to make 80D
region during cell harvest/pretreatment by a
invivo
disrupted under induced conditions
reactivation-induced changes are
UAS region (Fig. 6,
GAL10
and
GAL1
tracks
GAL4
D

GAL80
expression
GAL10
and
GAL80
profiles were obtained
profiles in Figs. 2 and 4.

Reactivation Causes Disruption of the Gene-proximal Up-
stream Nucleosome on the GAL Gene—As is the case on
GAL10 and GAL1, the UASG on GAL80 lies within a constitut-
ive, nonnucleosomal HR (Fig. 6, track 1) that is immediately
flanked on the gene-proximal side by a positioned nucleosome

(Ref.6; Fig. 6, c). This nucleosomal region is flanked on the gene-proximal side by a positioned nucleosome

region. Why
then are nucleosomes found on this region in unreactivated
nuclear chromatin from wild-type induced cells? The most
likely explanation is that these nucleosomes were deposited on
the region during the 1–2 h of cell harvest/spheroplast pre-
treatment, probably in response to the nonphysiological (0 C/
starvation) conditions associated with these steps. Reactivation
then causes disruption of these deposited nucleosomes, restor-
ing the in vivo structure. On the other hand, the nucleosome-
disrupted structure is maintained through these same steps
when cells lack Gal80p. Thus, nucleosome deposition does not
occur on the intergenic regions during the isolation steps when
Gal80p is absent. Since the same isolation protocol is used for
both wild-type and 80D and they are isogenic strains, the ab-
scence of nucleosome repositioning in 80D cells indicates that the
nucleosome deposition process that occurs in wild-type during
isolation depends on Gal80p, directly or indirectly. This ex-
plains why GAL80-dependent structural features were ob-
verved in nuclear chromatin (9) but not in vivo (5).

Reactivation Causes Disruption of the Gene-proximal Up-
stream Nucleosome on the GAL80 Gene—As is the case on
GAL10 and GAL1, the UAS on GAL80 lies within a constitut-
ive, nonnucleosomal HR (Fig. 6, track 1) that is immediately
flanked on the gene-proximal side by a positioned nucleosome

fig. 5. MNase digestion patterns in chromatin from 80° cells.
MNase cleavage sites were mapped from the EcoRI site within GAL10
by indirect end label analysis on 2.8% polyacrylamide, 0.6% agarose
nondenaturing gels, as described under “Materials and Methods.” The
tracks show from left to right: track 1, a naked DNA digest; track 2 and
3, digests from wild-type galactose-grown cells minus (track 2) or plus
(track 3) spheroplast reactivation with galactose; tracks 4 and 5, digests
from 80D (disrupted for GAL80) galactose-grown cells plus (track 4) or
minus (track 5), spheroplast reactivation with galactose. DNA sizes were
determined by comparison to the mobilities of φX174/HaeIII marker
fragments run on the gel (some of which are shown to the right of track
5: b, 1078 bp; c, 872 bp; d, 603 bp; e, 310 bp; h, 234 bp). The UAS, the
major TATA boxes (T), and the GAL1 and 10 transcription start sites
(wavy lines) are shown to the left of track 1. Other symbols are described
in the text.

fig. 6. MNase digestion patterns on the GAL80 upstream region.
MNase cleavage sites were mapped from the EcoRI site within
GAL80 by indirect end label analysis, as discussed under “Materials
and Methods.” The tracks show from left to right: 0, a naked DNA
digest; tracks 1 and 3, digests from wild-type galactose-grown cells,
unreactivated; tracks 2, 5, and 8, digests from wild-type galactose-
grown cells, reactivated with galactose (G); track 4, a digest from
wild-type glucose-grown cells; track 6, a digest from wild-type glucose/ethanol-grown cells, reactivated with galactose; track 7, a digest from
4 D (disrupted for GAL4) galactose-grown cells, reactivated with galac-
tose. DNA sizes were determined by comparison to the mobilities of
φX174/HaeIII marker fragments run on the gel (some of which are
shown to the right of track 8: c, 872 bp; d, 603 bp; e, 310 bp; f, 281 bp).
The UAS region (U), the TATA box (T), and the GAL80 transcription
start site (wavy line) are shown to the left of track 1. The location of
the hypersensitive region (HR) is shown to the right of track 1. Other
symbols are described in the text.
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**DISCUSSION**

**Nucleosome Disruption**— Metabolic reactivation with galactose results in exposure of GAL1, GAL10, and GAL80 DNA sequences that are nucleosome-protected in unreactivated, or inactive, chromatin (Fig. 7). Thus, reactivation causes a disruption of the structure of these nucleosomes. The nucleosomes affected lie between the transcription units and the upstream regions, and the GAL1, GAL10, and GAL80 TATA and GAL1 and GAL80 transcription start sites. One of the GAL1-10 nucleosomes, B (Fig. 7), was shown by photofootprinting in vivo to undergo disruption when GAL1 expression is induced by galactose (20). For this reason and because the reactivation-induced changes depend on Gal4p and are specific to galactose-grown/galactose-reactivated chromatin, it is likely that the disrupted structure produced by reactivation is the authentic chromatin structure for these upstream sequences under conditions of galactose-induced expression and that the nucleosome-disruption process triggered by galactose reactivation in spheroplasts is a process that normally acts in cells during induction of GAL1-10 and GAL80.

A chromatin response to spheroplast treatments is not limited to GAL genes nor to gene activation. Incubation of spheroplasts with phosphate results in specific nucleosome deposition on the PHOS promoter region, assayed, as here, in nucleosomes isolated from treated spheroplasts (21). The presence of these nucleosomes is associated with the inactive state of PHO5, and phosphate is the normal inactivation signal. Thus, on PHO5 as on the GAL genes, a metabolic signal can exert its gene-specific chromatin effect in spheroplasts. Such responsiveness should not be unexpected; spheroplasts are intact cells and on solid media can regenerate a cell wall and resume normal growth. Spheroplast treatment should thus be included in all studies of gene-specific yeast chromatin structure.

**Nucleosome Redeposition**— We suggest that it is necessary to carry out a spheroplast reactivation treatment in order to observe the authentic (nucleosome-disrupted) GAL1-10/GAL80 upstream structure in nuclear chromatin from induced cells because nucleosomes are deposited back onto these regions during the steps of cell harvest/spheroplast preparation, probably in response to the nonphysiological conditions (0°C/starvation) encountered during these steps. However, under the same conditions, such nucleosome deposition does not occur in cells that lack Gal80p, and the in vivo nucleosome-disrupted structure remains present through the isolation. Thus, the nucleosome deposition that occurs in wild-type cells during these steps depends on Gal80p. The structure produced by this "isolation-induced" nucleosome deposition is quite similar to the inactive chromatin structure produced by GAL-specific regulatory processes, as seen for example in chromatin from glyceral- or glucose-grown cells. Thus, the same Gal80p-dependent process may also function in vivo to place nucleosomes on these upstream regions in response to GAL-specific inactivation.

In this regard, it is noteworthy that the GAL1-10 intergenic region is never as completely nucleosome-protected in cells that lack Gal80p function, even in glucose, as it is in wild-type conditions.
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The Role of the GAL-specific Factors—The observations in this work describe a link between upstream region nucleosome occupancy and GAL gene regulation, implemented via the GAL-specific regulatory factors. Upstream nucleosome removal is associated with gene activity and depends on the Gal4p activator; upstream nucleosome replacement, which is associated with gene inactivity, depends on the negative regulator Gal80p. Gal4p and Gal80p are in contact in all carbon sources (7), including galactose (24). Moreover, the contact site of Gal80p with Gal4p lies in the Gal4p transcription activation region (1, 7), the region whose activity is associated with the nucleosome removal process. The structural organization of these two factors is thus consistent with the suggestion that they function in reciprocal nucleosome disruption/deposition processes. The limitation of these reciprocal processes to the upstream5′ region nucleosomes is also consistent with this suggestion because Gal4p-Gal80p are probably restricted to these regions. The occurrence of these processes on both GAL1-10 and GAL80 suggests they may be a general GAL feature. Gene expression also affects the nucleosomes within genes (cf. Ref. 25), including GAL1 (8, 10). However, GAL1 intragenic patterns are not sensitive to reactivation, and therefore, the intragenic nucleosomes must not be subject to a GAL80-dependent reorganization process like that acting on the intergenic nucleosomes. Intragenic nucleosome changes may be due to polymerase passage (25) and thus reflect a different process than the upstream nucleosome changes described in this work. Cavalli and Thoma (10) have suggested that the loss of the GAL1 intragenic pattern in galactose reflects mainly nucleosome rearrangements, with at most partial nucleosome loss.

The GAL4-dependence of nucleosome disruption and GAL80-dependence of nucleosome deposition could reflect the direct action of these factors or indirect action mediated via other factors. For example, Gal4p might act via Swi/Snf (26), which have been implicated in the disruption of TATA-bound nucleosomes in vitro (27). Concerning the nucleosome target, it has been observed that removal of histone H4 N-terminal tails results in a significant reduction in the in vivo level of GAL1-induced expression (28). This reduction might reflect involvement of the H4 tails in mediating the Gal4p-dependent nucleosome disruption associated with induction. Conversely, removal of histone H3 N-terminal tails results in an increased level of GAL1 induced expression (29), roughly similar to that produced by removal of Gal80p function (22). Perhaps H3 tails mediate Gal80p-dependent nucleosome deposition and loss of these tails (or of Gal80p) hinders this process, thereby affecting expression.

A Model for Upstream Region Nucleosome Occupancy—These upstream nucleosome disruption/deposition processes can apparently take place rapidly. Disruption effects can be detected by 3 min and are well in place by 9 min of reactivation treatment (Fig. 2B), while deposition in our experiments occurs sometime within the 1–2 h of cell harvest/spheroplast preparation. However, nucleosome deposition may also be quite rapid; on PHO5 it occurs within minutes (21). This rapidity and the involvement of opposing regulatory functions in these reciprocal disruption/deposition processes suggest that nucleosome presence on the TATA/start site regions might involve an ongoing competition of Gal4p-dependent nucleosome disruption and Gal80p-dependent nucleosome deposition. Activation conditions (signals) favor disruption; inactivity, or simply a lack of activation signals, favors deposition. Such a competition could facilitate rapid changes in expression state and fits the observation that the precise degree of disruption of the γ-δ nucleosome correlates with the precise strength of the Gal4p activator (20). The increased level of GAL1-induced expression when Gal80p function is removed indicates that Gal80p normally modulates GAL1 expression in the induced state (7). This modulation might involve Gal80p maintenance of at least partial nucleosome occupancy on the GAL1 upstream region in galactose, thus reflecting the operation of this competition even in the active state.

Nucleosome loss on upstream regions in association with gene expression is common on yeast and other eukaryotic genes (16, 17). On many genes, the nucleosomes are lost from genespecific promoter elements. However, on GAL1–10 and GAL80, the specific promoter elements, the UASG, lie in constitutively nonnucleosomal regions (4, 6, 8). The disrupted nucleosomes come from the TATA/ transcription start site regions, and their disruption/deposition occurs without any effect on activator-UASG interactions. Thus on GAL genes, the activator-specific promoter interaction and disruptable nucleosome occupation probably reflect distinct levels of control. This can allow cells to respond to fluctuating carbon sources or growth conditions in stages. In noninducing carbon sources like glycerol, the activator is bound to the UASG, but subsequent stages of expression, which involve upstream nucleosome disruption, are not implemented. If galactose becomes available, the inhibition at these subsequent stages can be quickly released. Transcription-associated DNA melting occurs ∼20 bp downstream of the GAL1 and GAL10 TATA (30), in regions that are exposed by the upstream nucleosome disruption. In addition to making this DNA more accessible, nucleosome disruption might also aid the DNA melting process by liberating the constrained negative supercoiling residing in these upstream nucleosomes. Negative supercoiling is generated behind a transcribing polymerase (31). Negative supercoiling favors nucleosome formation and thus would favor the redeposition of nucleosomes on these TATA/start site sequences after a polymerase initiates transcription. Transcription initiation might therefore involve a cycle of upstream nucleosome disruption/redisposition. Such a cycle is consistent with the structural view presented above, of a balanced competition of Gal4p-dependent nucleosome disruption and Gal80p-dependent nucleosome deposition processes operating on the upstream nucleosomes, and may play a facilitating role in the events that take place at transcription initiation of GAL genes. We also note that the need for metabolic reactivation in the disruption of GAL nucleosomes is consistent with the recent demonstration that nucleosome disruption is energy requiring (32).

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