Neither Reb1p nor Poly(dA-dT) Elements Are Responsible for the Highly Specific Chromatin Organization at the ILV1 Promoter*

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Analysis of the chromatin structure at the yeast ILV1 locus revealed highly positioned nucleosomes covering the entire locus except for a hypersensitive site in the promoter region. All previously identified cis-acting elements required for GCN4-independent ILV1 basal level transcription, including a binding site for the REB1 protein (Reb1p), and a poly(dA-dT) element (26 As out of 32 nucleotides) situated 15 base pairs downstream of the Reb1p-binding site, reside within this hypersensitive site. The existence of a second A/T-rich element (25 As out of 33 nucleotides) present six base pairs upstream of the Reb1p-binding site, suggested that nucleosome exclusion from the hypersensitive site in the ILV1 promoter region might be dictated by synergistic action of the two poly(dA-dT) elements. Replacing one or both of them had, however, no effect on the chromatin structure of the ILV1 promoter, although drastically reduced basal transcription. Similarly, deletion of the Reb1p-binding site, albeit affecting ILV1 expression, had no detectable effect on chromatin at the ILV1 promoter. The absence of a good correlation between effects of these elements on gene activity and on chromatin structure at the ILV1 promoter indicates that the chromatin organization present at the ILV1 promoter is independent of the known regulatory elements and most likely dictated directly by the DNA sequence.

The Saccharomyces cerevisiae anabolic threonine deaminase, encoded by the ILV1 gene, catalyzes the first committed step in the isoleucine biosynthetic pathway. ILV1 basal level expression (defined as the level of expression observed in a Δgcn4 strain grown in minimal medium) is controlled by two cis-acting elements: a binding site for the REB1 protein (Reb1p) and a poly(dA-dT) element (1, 2).

Reb1p (Grf2, Qbp, factor Y or Q) is an essential, abundant DNA-binding protein with numerous binding sites present throughout the genome, many of which are located within regulatory regions for RNA polymerase II transcribed genes. Other Reb1p-binding sites are found in RNA polymerase I regulatory regions and in diverse genetic elements, such as centromeres and telomeres (3–7). Reb1p-binding sites are also found in the recently identified STAR1 (subtelomeric anti-silencing regions) elements (8). These regions function as insulators and can protect neighboring genes from surrounding silencing elements. Interestingly, tandemly repeated binding sites for Reb1p can duplicate this anti-silencing effect and limit telomeric silenced chromatin. Furthermore, Reb1p-binding sites can stimulate or diminish transcription in a context-dependent manner (9–11). Although Reb1p affects transcription of RNA polymerase II transcribed genes, the mechanism by which it does so is not clear. The presence of a Reb1p-binding site in the GAL1-GAL10 promoter correlated with a nucleosome-free region of about 230 bp in vivo (12) suggesting that Reb1p functions to generate a nucleosome-free region allowing auxiliary factors access to adjacent cis-acting elements. Other reports, however, contest this result (13, 14) casting some doubt as to the mechanism of action of Reb1p.

Homopolymeric poly(dA-dT) sequences are present in the promoter region of many yeast genes and have been shown to influence transcription of several genes (2, 15–18). Due to their nucleosome destabilizing properties poly(dA-dT) elements have been proposed to function by virtue of their intrinsic effect on chromatin (15, 18). Interestingly, the yeast poly(dA-dT)-binding protein Dat1p (19) functions as a transcriptional activator of ILV1 expression and this action depends on the presence of the poly(dA-dT) element (2).

In an attempt to elucidate the mechanism by which the Reb1p-binding site and the downstream poly(dA-dT) element control ILV1 basal expression, we investigated the chromatin structure of the ILV1 locus. We show that the ILV1 promoter and coding regions are assembled into a highly ordered nucleosome array, with a single hypersensitive region encompassing all regulatory cis-acting elements. We also show that deletion of the Reb1p site and/or the adjacent downstream poly(dA-dT) element greatly diminishes ILV1 expression, yet does not cause reconfiguration of the ILV1 chromatin structure. Furthermore, a second A/T-rich element present upstream of the Reb1p-binding site can be deleted, again with no effect on ILV1 chromatin structure. This suggests that neither Reb1p nor the adjacent poly(dA-dT) elements stimulate ILV1 transcription by increasing the accessibility of DNA in chromatin at the promoter, but by another yet unknown mechanism. Sequence insertions and deletions at the hypersensitive region of the ILV1 promoter, albeit affecting expression of the ILV1 gene, do not affect the positioning of the adjacent nucleosomes pointing to a dominant effect of the DNA sequence in organizing the nucleosomal array present at the ILV1 promoter.

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amplificates were cloned into plasmid pGEM-T® (Promega) and the constructs were integrated at the XhoI/HindIII site. Using T-rich tracts in the promoter region. The 5’-most ILV1 A′-rich tract (positions −364 to −28) and a downstream poly(dA:dT) element control ILV1 basal (GCN4-independent) expression (1, 2). The two elements act synergistically, an indication that they might exert their effect through a common activation pathway (2). Sequence inspection revealed an additional A′-rich tract (25 As out of 33 nucleotides) located six bp upstream of the Reb1p-binding site. To determine whether the upstream A′-rich tract also plays a role in ILV1 basal expression we replaced either one or both ILV1 A′-rich tracts by 40% GC content random sequences resulting in strains ILV1(ΔA1), ILV1(ΔA2), and ILV1(ΔA1ΔA2) and an isogenic wild-type strain, TD28 (WT). Total RNA ethidium bromide stainings of the ribosomal bands (28S and 18S) serve as loading control.

The ILV1 Promoter Is Organized in an Ordered Nucleosomal Array—We analyzed the nucleosomal organization of the ILV1 promoter using digestion of yeast nuclei with DNase I, micrococcal nuclease (MNase), or restriction enzymes to map nucleosome cuts by indirect end-labeling (25). Thus, nuclei from strain TD28 (GCN4) were treated with DNase I (see “Materials and Methods”), and the isolated DNA was resolved in an agarose gel after digestion with EcoRI, blotted, and hybridized with a radioactively labeled EcoRI-DraI probe. The obtained DNase I pattern (Fig. 2) shows a characteristic ladder of bands typical of an ordered nucleosomal array, and a strong band corresponding to a hypersensitive site (HS). Mapping of this HS revealed that all previously identified cis-acting elements, namely a Reb1p-binding site, a Gen4p-binding site, and the two poly(dA:dT) elements were located within the hypersensitive region.

To complement the DNase I analysis and confirm our interpretation of the observed banding pattern, we digested nuclei from TD28 cells with various restriction enzymes (Fig. 3, A and B). Hypersensitive sites and linker regions are expected to be susceptible to digestion with endonuclease enzymes, whereas sequences assembled into nucleosomes should be protected. Very strong, nucleosomal sites are resistant to cleavage over a large range in enzyme concentration while non-nucleosomal sites are cut at much lower concentrations. Consequently accessibility reaches plateau levels both for nucleosomal and non-nucleosomal sites. To be certain in our experiments that we have truly reached these plateau values for a given site, we always verify that 3- to 4-fold higher nuclease concentrations still give the same accessibility thus ruling out that the enzyme activity had been limiting. Nuclei from yeast strain TD28 (GCN4) were digested with PvuII, BstUI, DraI, HinfI, or PstI at 500 and 1500 units/ml for 1 h. The results shown in Fig. 3, A and B confirm our conclusions derived from the DNase I assay. Bands were quantified using a phosphorimager equipped with ImageQuant analysis software (Molecular Dynamics, Sunnyvale, CA), and percent accessibility for the various enzymes was calculated from the ratio between the bands corresponding to digested sites and that of undigested DNA. Thus, the two 432 bp sites present in the promoter region showed 50% (position −108) and <5% (position −546) accessibility, consistent with being at the border of the HS and within a nucleosome, respectively. BstUI (position −227) displayed 75% accessibility, correlating well with a position within the hypersensitive region. Conversely, the two DraI sites (positions −364 and −28) were less than 5% accessible, consistent with a location within the nucleosomes flanking the HS. PvuII (position −432) with 30% accessibility locates to an internucleosomal region in the deduced ILV1 chromatin structure. Five HinfI sites were exam-
ined (positions −595, −486, −296, −170, and −132). The sites within the hypersensitive region showed 80% accessibility (positions −170 and −132), whereas the remaining sites displayed less than 5% accessibility, consistent with nucleosomal locations. These results confirm the DNase I analysis and the mapping of nucleosomal positions we derived from that analysis.

We conclude that the ILV1 promoter is organized in an ordered nucleosomal array with one strong hypersensitive site encompassing the region where the UAS elements are located. A schematic drawing of the ILV1 locus with restriction sites, cis-acting elements, and inferred chromatin structure is shown in Fig. 3C.

Chromatin Structure of the ILV1 promoter. Nuclei from TD28 cells were digested for 1 h with 500 or 1500 units/ml of various restriction endonucleases. DNA was subsequently isolated from the treated nuclei, and the purified samples were digested with HaeIII and BglIII, electrophoretically resolved on a 4% NuSieve 3:1 agarose gel, blotted, and hybridized with either probe A, a RsaI-BglII labeled fragment (A), or probe B, a radiolabeled HaeIII-HindIII fragment (B). Numbering corresponding to the restriction site position in the promoter is given for the various bands. C, a restriction map of the ILV1 promoter and a schematic presentation of the probes locations and of the inferred nucleosome positioning is depicted. Nucleosomes are presented as filled ellipses, the Reb1p-binding site as a filled triangle, poly(dA·dT) elements as filled circles, and the Gcn4p-binding site as a filled square.

Chromatin Organization of the ILV1 Gene Derepressed by the General Control of Amino Acid Biosynthesis—The S. cerevisiae ILV1 gene is under regulation of the general control of amino acid biosynthesis. A Gcn4p-binding site present at position −127 was found to bind Gcn4p in vitro (26). Indeed, upon amino acid starvation ILV1 expression is increased 2-fold by the Gcn4p activator protein (27). We tried to determine whether derepression by Gcn4p had any effect on ILV1 chromatin structure. For derepression by the general control of amino acid biosynthesis, the tryptophan analog 5-methyl-DL-tryptophan (MeTrp) was added at a final concentration of 0.5 mM to the growth medium (27, 28). Nuclei were prepared from TD28 (GCN4) cells grown to a density of 3 × 10⁶ cells/ml and subjected to nuclease digestion with micrococcal nuclease (MNase) and DNase I, enabling us to complement our structural analysis. RNA was isolated from non-digested nuclei, and Northern analysis performed to confirm that ILV1 expression increased 2-fold upon derepression by the general control of amino acid biosynthesis (data not shown). The pattern obtained with either MNase (Fig. 4) or DNase I (data not shown) was identical to the one previously observed for the basal transcriptional state of the ILV1 gene with a strong hypersensitive site comprising the UAS elements and an ordered nucleosomal array covering the promoter and coding region. Hence, derepression of the ILV1 gene does not modify its chro-
matin structure in a way detectable in our analyses.

Gen4p and Dat1p Are Not Necessary for Nucleosome Positioning in the ILV1 Promoter—We investigated the role of two trans-acting factors, Dat1p and Gen4p, both of which have been shown to bind their cognate sites at the ILV1 promoter in vitro (2, 26). Nuclei isolated from strains TD28 (GCN4), 9994–6C (Δgen4), EWY1002c-1 (DAT1), and BRY1004–3 (Δdat1) grown in minimal medium were digested with DNase I, DNA was isolated, and end-labeling analysis performed as described above. The patterns obtained for all four strains were identical (data not shown), demonstrating that neither Gen4p nor Dat1p are involved in generating the observed ILV1 chromatin structure.

The Hypersensitive Region in the ILV1 Promoter Is Not Caused by the Reb1p-binding Site or the Downstream poly(dA:dT) Element—A Reb1p-binding site present at position −180 is required for GCN4-independent ILV1 basal level transcription. Deletion of the Reb1p site reduces ILV1 basal level expression 10- to 15-fold (1). Additionally, the poly(dA:dT) element located between positions −164 and −135, which is also necessary for wild-type ILV1 basal level expression (see above), is situated 15 bp downstream of the Reb1p site, and the two elements cooperatively stimulate ILV1 transcription (2). This synergistic activation could be the result of a common mechanism of action.

A Reb1p-binding site in the GAL1-GAL10 promoter was correlated with the presence of a nucleosome-free region of about 230 bp (12). Furthermore, poly(dA:dT) sequences stimulate Gen4p-activated transcription and were suggested to function by changing chromatin structure and increasing accessibility of adjacent Gen4p sites (18). Thus, the cooperative activation by the ILV1 Reb1p site and the downstream poly(dA:dT) element could be due to interactions between these two elements leading to increased accessibility of the adjacent Gen4p-binding site. To test this possibility, we analyzed previously characterized promoter constructs showing decreased ILV1 basal expression (2). In these constructs the ILV1 coding region has been replaced by the E. coli lacZ gene encoding β-galactosidase (1).

To determine whether the replacement of the ILV1 coding region with lacZ affected the structure of the ILV1 promoter, we compared MNase digests of strain TD28 with those of strain TG561, an isogenic TD28 derivative containing a ILV1 wild-type promoter, but with the ILV1 coding sequence replaced by lacZ. The digestion pattern for strain TD28 (Fig. 5, ILV1) is the expected one, a strong HS located in the promoter region, and an ordered nucleosomal array covers the coding region and promoter. Strain TG561 where the ILV1 coding sequence was substituted with lacZ shows a different pattern (Fig. 5, lacZ). The ILV1 promoter conserved its structure with one exception: the hypersensitive site was slightly decreased in size. This appears to be due to a shifted position of the nucleosome positioned at the downstream boundary of the hypersensitive region. Moreover, the lacZ sequence appears strongly nuclease-sensitive suggesting this gene to be less prone to undergo an ordered nucleosome organization. To confirm these conclusions and to determine that no accessibility changes had occurred in the hybrid promoter as compared with the wild-type promoter, we performed restriction enzyme analysis on the two strains. The results (Fig. 6) show that BarI accessibility is almost identical (75% in ILV1 and 70% in lacZ) but that the PstI site at position −108 changed from 50% accessibility in the wild-type situation (Fig. 6, ILV1) to <5% accessibility in the hybrid construct (Fig. 6, lacZ), consistent with going from a location at the border of the hypersensitive site to being covered by the adjacent nucleosome. The original position of this nucleosome had incorporated about 20 bp of coding sequence. Apparently, in the lacZ derivative, this positioned nucleosome is shifted upstream by about 20 bp thus excluding the new coding sequence altogether. At any rate, we conclude that the structure of the hybrid promoter construct reflects the wild-type situation in a sufficiently adequate manner for our study.

We carried out a DNase I analysis on four constructs, which had previously been used to identify the Reb1p site and the downstream poly(dA:dT) element as important basal regulatory elements in the ILV1 promoter (1, 2). The promoter derivatives are either deleted for the Reb1p-binding site and/or the poly(dA:dT) element. Again, the pattern observed is identical in all three derivatives and indistinguishable from the wild-type pattern (Fig. 7, A and B and data not shown) suggesting that neither the Reb1p-binding site nor the poly(dA:dT) are responsible for generating of the HS present at the ILV1 promoter. To rule out the possibility that deletion of the Reb1p-binding site or/and the neighboring poly(dA:dT) element has more subtle effects on chromatin not detected by DNase I digestion, we performed a restriction enzyme analysis of three of the constructs as well (Fig. 7C). BarI (position −227) accessibility is similar in the tested constructs (75–70%), and the PstI site at position −108, as shown before, displayed <5% accessibility (Fig. 7C, AREB1 Δ(dA:dT)) in the lacZ constructs. These results show that neither the ILV1 Reb1p-binding site nor the downstream poly(dA:dT) element are responsible for generating the hypersensitive region we observe in the ILV1 promoter.
The Hypersensitive Region at the ILV1 Locus Is Persistent—We have shown that ILV1 basal level expression depends on the distance separating the Reb1p-binding site and the downstream poly(dA·dT) element, since insertion of spacing DNA into a XhoI site created between the Reb1p-binding site and the poly(dA·dT) element in the 15X construct reduces promoter activity (2). We reasoned that if the hypersensitive site in the ILV1 promoter were caused by any cis-acting element present within the nuclease accessible region, the insertion of 41 bp or 74 bp of DNA corresponding to a size increase of 22% and 41%, respectively, might cause some rearrangement of the borders of the hypersensitive region thus affecting promoter activity.

DNase I analysis of the constructs containing insertions of DNA between the Reb1p-binding site and the downstream poly(dA·dT) element is shown in Fig. 8. The basic structure and relative positioning of nucleosomes is preserved in the constructs containing insertions relative to the wild-type situation. The main difference is the progressive increase in the size of the nuclease-sensitive region. The resulting pattern clearly shows that the entire promoter region structure is similar between all the constructs and that insertion of increasingly larger spacing DNA is compensated for by correspondingly larger hypersensitive regions.

We considered the possibility that the presence of two A-T-rich tracts placed symmetrically within the HS and located at roughly the same distance from the borders of the HS triggers nuclease exclusion from this region. Such a mechanism would also explain the lengthening of the hypersensitive site observed as a consequence of introducing random sequences in between the two A-T rich tracts. We therefore replaced either one or both ILV1 A-T-rich tracts by 40% GC content random sequences resulting in strains ILV1(AA1), ILV1(AA2), and ILV1(AA1AA2), respectively. MNase analysis of these strains is shown in Fig. 9. The obtained pattern is identical to the one previously detected in the ILV1 promoter. Neither the typical nucleosomal ladder nor the hypersensitive region shows any significant difference to the wild-type situation. We conclude that the strong nucleosome positioning which we observe at the ILV1 locus is not dependent on the presence of the two A-T-rich tracts present in the promoter region.

**DISCUSSION**

**Effect of Reb1p in the Chromatin Structure of ILV1**—We have reported that ILV1 basal level expression is controlled in a synergistic manner by a Reb1p-binding site and a downstream poly(dA·dT) element (2). Reb1p has been proposed to antagonize nucleosomal repression by creating an accessible chromatin structure, thereby allowing other factors to gain access to their cognate sites (12). Other reports, however, dispute this conclusion and interpret the data differently (13, 14). Reb1p has also been implicated as a regulatory factor both in Pol II and Pol I transcribed genes (1, 6, 9).

Synergistic interactions between Reb1p-binding sites and poly(dA·dT) elements have been described by several authors (2, 3, 29, 30), raising the possibility that the two elements cooperate to increase accessibility of adjacent elements since also homopolymeric poly(dA·dT) elements have been proposed to stimulate transcription through an effect on chromatin structure (15, 18). Given the presence of a Reb1p-binding site and two poly(dA·dT) elements in the ILV1 promoter and the fact that at least one poly(dA·dT) element synergizes with Reb1p to sustain a relatively high basal level expression of the ILV1 gene, it is possible that the two elements act to increase chromatin accessibility, perhaps by keeping the promoter free from nucleosomes.

To ascertain if Reb1p or the poly(dA·dT) elements have an effect on chromatin at ILV1, we examined the structure of the ILV1 locus and mapped the nucleosomal of this gene (Fig. 3C). The promoter and coding region are covered by an ordered nucleosomal array that is interrupted by a single strong nucleosome-sensitive site comprising the region where the cis-acting elements required for normal expression of the ILV1 gene are located. The structure of the locus is unaltered by Gcn4p-mediated derepression (Fig. 4). This is not an unexpected result since ILV1 basal level expression is relatively high and ILV1 is up-regulated only 2-fold by amino acid starvation (26, 27). Additionally, neither eliminations of Dat1p nor of Gcn4p, two trans-acting factors required for normal levels of expression,
abolish it (\( /H9252 \)).

### Chromatin Organization of the ILV1 Gene

The Two ILV1 A-T-rich Tracts Do Not Affect ILV1 Chromatin Structure—Poly(dA-dT) elements stimulate transcription of several yeast genes (15–18). Circular dichroism studies, x-ray fiber diffraction and an analysis of the helical repeat of homopolymeric poly(dA-dT) tracts clearly showed that the homopolymer is structurally different from canonical B-DNA (31–33). Failed attempts to assemble homopolymers into nucleosomes in vitro (34, 35) and the observation that cloned oligoadenosine regions were excluded from nucleosome formation (36) provided the basis for the view that poly(dA-dT) tracts are refractory to nucleosome assembly. More recent reports, however, present a different view on homopolymeric sequences function and structure. Herrera and Chaires (37) showed that poly(dA-dT) tracts can be assembled into nucleosomes, in some cases even more favorably than heterogeneous-sequence DNA (38–41). We have shown that a poly(dA-dT) element present in the ILV1 promoter is required for efficient basal level expression and that the activity of the element is partially dependent on Dat1p, a poly(dA-dT) DNA-binding protein (2). Analysis of ILV1 chromatin in the absence of Dat1p and in an ILV1-lacZ fusion construct containing a deletion of the downstream poly(dA-dT) element, showed no difference to a wild-type situation (data not shown), suggesting that the ILV1 downstream poly(dA-dT) element was not responsible for the nucleosome exclusion observed in the promoter region.

If the nuclease accessible site is caused by a strong setting preference of the nucleosomes positioned at the HS borders, we would expect that insertion of 74 bp of DNA into a previously 180-bp long stretch might allow the assembly of an additional nucleosome, thereby eliminating or splitting the nucleosome-free region. On the other hand, if a single cis-acting element present within the promoter region were responsible for the nucleosome-free region, then insertions would cause an asymmetric shift in the position of the HS. Insertion of spacing DNA in the center of the hypersensitive region (Fig. 8), clearly shows that the entire promoter structure remains unaltered, accommodating the inserted sequences within the hypersensitive site with a concomitant enlargement of the nucleosome-free region.
The observed size increase suggested to us that two elements might be required to generate the hypersensitive region, such as the two A–T-rich tracts flanking the ILV1 Reb1p-binding site. However, simultaneous deletion of both A–T-rich tracts present in the ILV1 promoter had no effect on the chromatin structure of the locus (Fig. 9), excluding the possibility that these elements are responsible for the nucleosomal exclusion.

In summary, we have established the chromatin structure of the ILV1 locus, with a positioned array of nucleosomes covering the entire gene and promoter, and a hypersensitive region comprising all known cis-acting sequences. We have shown that Reb1p does not visibly affect chromatin structure of the ILV1 promoter even though the Reb1p-binding site is required for normal expression. This shows that Reb1p functions at the ILV1 promoter to the loss of individual factor binding sites and to localized DNA insertions and deletions. Shen and Clark (42) recently reported that also at the yeast CUP1 gene DNA sequence is likely to play a very important role in positioning nucleosomes, which together with our data suggests a major role for DNA sequence in dictating nucleosome positioning and exclusion in vivo.

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REFERENCES
1. Remacle, J. E., and Holmberg, S. (1992) Mol. Cell. Biol. 12, 5516–5526
2. Moreira, J. M. A., Remacle, J. E., Kielland-Brandt, M. C., and Holmberg, S. (1998) Mol. Gen. Genet. 258, 95–103
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