The promoter of the galactose-inducible yeast GCY1 gene allows high rates of basal transcription and is kept free of nucleosomes regardless of growth conditions. The general regulatory factor, Reb1p, as well as the nucleotide sequence of a single Gal4p-binding site, structurally cooperate to exclude nucleosomes from about 480 bp of DNA that spans the UAS\textsubscript{GAL}, the Reb1p-binding site, the TATA-box, and the transcriptional initiation sites. Gal4p, which induces transcription of GCY1 about 25-fold in the presence of galactose, is not required for the alteration in chromatin configuration in the promoter upstream region since the hypersensitive site is unchanged when Gal4p is inactive or absent. As soon as either the Reb1p-binding site or the UAS\textsubscript{GAL} or both are mutated, nucleosomes slip into the promoter of Gal4p-induced state to an extent resembling the wild-type promoter, showing that, in principle, activation of most regulated genes requires the removal of one or several nucleosomes before the transcriptional preinitiation complex can be assembled (for example, see Refs. 6–9). Revealingly, in yeast, several promoters can be activated upon experimental nucleosome depletion even in the absence or inactive state of the respective transactivators (10, 11). This means that, in principle, basal transcription can ensue in the absence of specific transactivators as long as the basal transcription machinery has access to the core promoter. On the other hand, promoters of constitutive genes (12, 13) and a number of inducible genes (14–16) have been described that are kept free of nucleosomes permanently, and those nucleosomes flanking the gap, which usually spans the cis-acting sites for regulated transcription activators and/or the basal transcription machinery, occupy quite distinct positions. The molecular or structural bases of permanent nucleosome exclusion are not well understood.

Normal B-helical DNA is compatible with packaging into nucleosomal structures. Persistent nucleosome exclusion may be accomplished by one of at least three different strategies: (i) Either preferred binding of two nucleosomes to two adjacent stretches of prebound DNA positions nucleosomes in such a way that their distance is smaller than required for the accommodation of an additional nucleosome (<145 bp in yeast). Such a nucleosome arrangement, called translational positioning of nucleosomes, has been found to be the basis of accessibility of linker DNA to specific transactivators (8, 13, 17, 18). (ii) As an alternative, a particular nucleotide sequence, which deviates from B conformation, may give rise to a structure that is incompatible with packaging into nucleosomes. Several stretches of poly(dAdT) or poly(dGdC) homologous serquences have been found to be incompatible with packaging into nucleosomes due to their rigid DNA structure (19–23). (iii) As a third possibility, DNA-binding proteins (architectural proteins) may distort the B-helical conformation of the DNA in a way that is incompatible with wrapping around nucleosomes and, thereby, may contribute to the positioning of nucleosomes. The rDNA enhancer-binding protein from yeast (Reb1p), which is among the abundant multifunctional "general regulatory factors," is supposed to play an architectural role and to exclude nucleosomes from the flanks of its binding site on DNA. Reb1p binding to the GAL1–GAL10 intergenic region creates a nucleosome-free gap of about 230 bp (14, 24, 25).

The majority of genes that are transcribed on demand must deal with nucleosomes that cover the respective promoter regions. Therefore, one role of specific transcriptional activator proteins is to induce the disruption of nucleosomal structures in core promoter regions or to recruit accessory proteins that are able to displace nucleosomes from the core promoter. Nucleosome displacement by regulatory proteins was demonstrated, for instance, for the PHO5 promoter, which is transcriptionally activated by the transactivator, Pho4p. Upon induction by phosphate exhaustion, four out of six positioned nucleosomes are removed during the activation process jointly by the regulatory proteins, Pho4p and Pho2p (8, 26). Gal4p, a potent transactivator protein, is able to disrupt nucleosomes in the core promoter regions of galactose-inducible genes, such as the promoters of the GAL1–GAL10 and GAL80 genes (9, 14, 15, 25, 27). Gal4p binds to its target sites in the respective UAS\textsubscript{GAL}, these sites, which are permanently free of nucleo-

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Michaela Angermayr and Wolfhard Bandlow

From the Department Biologie I, Bereich Genetik, Ludwig-Maximilians-Universität München, Maria-Ward-Strasse 1a, D-80638 Munich, Germany

Published, JBC Papers in Press, January 20, 2003, DOI 10.1074/jbc.M210932200

The JOURNAL OF BIOLOGICAL CHEMISTRY Vol. 278, No. 13, Issue of March 28, pp. 11026–11031, 2003

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Printed in U.S.A.
somest, remove repressing nucleosomes from core promoter regions, enabling the basal transcription machinery to assemble and to initiate transcription.

**GCY1** (galactose-inducible crystallin-like yeast protein) was found to encode a glycerol dehydrogenase involved in osmoregulation and osmotolerance of *Saccharomyces cerevisiae* (28, 29). We have investigated transcriptional regulation of the galactose-inducible gene, **GCY1**, which is activated by the specific regulator, Gal4p. Expression of **GCY1** is induced about 25-fold by growth on galactose as carbon source due to Gal4p binding to a single UAS<sub>Gal</sub> in the upstream control region (30). However, **GCY1** is transcribed at a relatively high basal level in the presence of glucose or glycerol as carbon sources. Our previous studies revealed that basal expression of **GCY1** is stimulated 3-fold by the abundant general regulatory factor, Reb1p, which binds to the promoter of **GCY1** about 100 bp upstream the canonical TATA box (31, 32). Reb1p is supposed to exert its binding effect on transcription mainly by excluding nucleosomes from the flanks of its binding motifs and thereby to facilitate binding of other transcription factors to their target sites (14, 24). Surprisingly, the UAS<sub>Gal</sub> contributes to basal transcription of **GCY1** as well even in Gal4p deletion mutants (31). We have analyzed the chromatin structure of the **GCY1** promoter to study the principles of basal expression and constitutive nucleosome exclusion. We have investigated the influence of mutations in the **GCY1** promoter, i.e. deletion of the Reb1p-binding site or mutation of the UAS<sub>Gal</sub>, or the influence of the presence or absence of Gal4p on the nucleosomal array in the upstream control region of **GCY1**. We demonstrate that in wild type, the entire promoter region of **GCY1** is constitutively free of nucleosomes and that the nucleosome-free gap spans an unusually long distance of about 480 bp. Mutation of either the Reb1p-binding site or the UAS<sub>Gal</sub> or both cis-acting elements results in nucleosome packaging of the respective region, indicating that Reb1p binding and the UAS<sub>Gal</sub> are jointly responsible for the permanent exclusion of nucleosomes from the **GCY1** promoter and exert an additive effect.

**EXPERIMENTAL PROCEDURES**

**Plasmids and Strains**—Vectors pBluescript KS (Stratagene, Heidelberg, Germany) or pUC19 were used in ligation reactions. Sure was used as the Escherichia coli host strain (Stratagene). Yeast strain W303-1A (32) served to introduce genomic mutations into the promoter region of **GCY1**, generating the yeast strains YMA1, YMA2, YMA3, or YMA4 (see Fig. 1). YMM707 gal4-A524 (obtained from M. Johnstone, Washington University, Medical Center, Department of Genetics, St. Louis, MO) served for analyzing the chromatin structure of the promoter region of **GCY1** in a gal4-deficient genetic background.

**Introduction of Genomic Mutations into the Promoter of GCY1**—Deletion of the Reb1p-binding site, the point mutation of the UAS<sub>Gal</sub>, as well as the double mutation of both cis-acting elements in the genomic context of the **GCY1** promoter was accomplished by a two-step gene replacement. Construct pKS–GCYAR, in which the natural Reb1p-binding site of the **GCY1** promoter had been replaced by a HindIII restriction site (30), served to insert a 1170-bp HindIII fragment encoding **GCY1** to yield the Ura-prototrophic strain YMA1. After a PvuII restriction site had been introduced into the promoter proximal region of **GCY1** to generate homologous DNA fragment ends, the respective **URA**-containing **GCY1** promoter fragment was excised by PvuII and XhoI and inserted into the genome by homologous recombination using the yeast transformation protocol described by Gietz et al. (34). Recombinants were verified by PCR and restriction digestion. Constructs pKS–GCYAREB1, pKS–GCY-URA<sub>s</sub>, and pKS–GCYAREB1/URA<sub>s</sub> served to replace the URA3 gene from the promoter region of **GCY1** and to introduce the respective mutations of cis-acting elements into the promoter of **GCY1** in the genomic context. DNA fragments carrying the mutation were excised by PvuII and XhoI (see above) and co-transformed with YEP55 (LEU2). Transformants were initially selected by growth on selective media lacking leucine. **URA**3-autotrophic recombinants were identified by replica plating on 5-fluoroorotate-containing media (35) and analyzed by PCR using whole yeast cells. Genomic promoter mutations were verified by restriction digests of the amplify with HinDI (YMA2 = GCYAREB1) or XhoI (YMA3 = GCY-URA<sub>s</sub> or with HindIII and XhoI (YMA4 = GCYAREB1/URA<sub>s</sub>), respectively.

**Analysis of Chromatin Structure by Digestion with DNAseI or Micrococcal Nuclease**—Yeast cells were grown in rich medium containing 3% glucose, 3% galactose, or 3% glycerol, 2% ethanol as carbon sources. YM707 was cultured on 3% galactose, 3% glycerol, 2% ethanol to simulate galactose-induced physiological conditions. Crude nuclei were prepared and digested with increasing concentrations of DNAseI (Roche Molecular Biochemicals) as described by Almer and Horz (36). Micrococcal nuclease (MBI Fermentas, St. Leon-Rot, Germany) digests were done as described by Thoma (37). Reactions were terminated by the addition of 0.5% SDS, 4 mM EDTA, 50 mM Tris-HCl, pH 8.0, and 200 μg of proteinase K (Merck EuroLab) and incubated at 37 °C for 30 min. DNA was extracted twice with phenol/chloroform and precipitated by ethanol. After the pellet had been resuspended in 10 mM Tris-HCl, pH 7.5, 1 mM EDTA buffer, RNA was digested by RNaseA (Roche Molecular Biochemicals) at 37 °C for 60 min. After extraction once with phenol/chloroform and once with chloroform, DNA was ethanol-precipitated. Purified DNA was digested with EcoRV. Gel electrophoresis was at 100 V in 1.0 or 1.5% agarose gels. After Southern transfer onto nylon membranes (Biodyne A transfer membrane, Pall, Dreieich, Germany), DNA was detected by a randomly primed, radiolabeled 780-bp AsnI/HindIII EcoRV DNA probe that hybridized to the N-terminal portion of the neighboring divergently transcribed **RIO1** gene. DNA marker fragments were excised from a plasmid that contained the entire intergenic region of the **GCY1**/**RIO1** gene pair and coding regions of the genes **GCY1** and **RIO1**. Digestions were performed with EcoRV to generate the distal DNA fragment end and with restriction endonucleases that cleave within the respective regions of interest (EcoRV/AsnI, 780 bp; EcoRV/XhoI, 1060 bp; EcoRV/HindIII, 1280 bp; EcoRV/BamHI, 1480 bp; EcoRV/BglII, 1600 bp; EcoRV/EcoRV, 2130 bp).

**Miscellaneous Procedures**—Molecular operations such as RNA isolation and Northern blotting were performed according to standard protocols (38) or as recommended by the manufacturer. Northern blots were quantified using the ImageQuant analysis software (Amersham Biosciences).

**RESULTS**

**Effect of Genomic Promoter Mutations on Transcription of GCY1**—Previous molecular analyses of the galactose-inducible **GCY1** promoter (30–32) have revealed the presence of three cis-acting elements: an upstream binding motif for Gal4p (positions −369 to −353 with the adenine of the translational start triplet as +1), a canonical Reb1p site (ATTATCAGCG, positions −224 to −215), and a consensus TATAAA core promoter sequence (positions −111 to −116) (Fig. 1A). A poly (dA+dT) block, frequently found adjacent to Reb1p-binding
sites, is absent. To study the bearing of Reb1p or the UAS_{GAL} on the transcriptional activity of the GCY1 promoter, the respective cis-acting elements were mutated in the genomic context. The mutations of the Reb1p site, \( \Delta REB1 \), or the Gal4p site, UAS_{mut}, or the double mutant, \( \Delta REB1/UAS_{mut} \), were brought into the correct genomic context by a two-step gene replacement yielding the respective mutant strains, YMA2 \( \Delta REB1 \) or YMA3 UAS_{mut} (\( U_m \)) or YMA4 \( \Delta REB/UAS_{mut} \) (\( U_m \)), respectively. Lower panel, AKY2 mRNA, loading control. B, quantification of the Northern blot data from panel A normalized to the loading control.

Fig. 2. Transcriptional analysis of wild-type and mutant GCY1 promoter. A, upper panel. Northern analysis of GCY1 mRNA from wild-type grown on glucose (Glc), glycerol/ethanol (Gro/EtOH), or galactose (Gal) or mutants YMA2 \( \Delta REB1 \) or YMA3 UAS_{mut} (\( U_m \)) or YMA4 \( \Delta REB/UAS_{mut} \) (\( U_m \)), respectively. Lower panel, AKY2 mRNA, loading control. B, quantification of the Northern blot data from panel A normalized to the loading control.

The \( \Delta REB1/UAS_{mut} \) double mutation reduces GCY1 transcription to about 10\%, showing that the contributions of both elements to transcriptional activation of the GCY1 promoter are about additive (Fig. 2B).

In Vivo Footprinting of the GCY1 Wild-type Promoter—To study chromatin structure and the effect of transcriptional induction by galactose on the nucleosomal array at the GCY1 promoter, nuclear footprints were performed in vivo using DNasel or micrococcal nuclease digestion of native chromatin. Since expression of GCY1 is induced by galactose and weakly repressed by glucose, and to analyze the influence of the carbon source on the arrangement of nucleosomes, analyses of the GCY1 promoter were performed after growth of yeast cells on galactose, glucose, or glycerol/ethanol (Fig. 3A and B). DNasel- or micrococcal nuclease-treated chromatin from yeast cells after growth on all three carbon sources (glucose, galactose, or glycerol/ethanol) displayed no obvious differences in the nucleosomal organization of the GCY1 promoter and revealed that under any condition, the upstream control region of GCY1 was free of nucleosomes. The nucleosome-free gap extends over a region of about 480 bp that contains the transcriptional initiation sites, the TATA element, the Reb1p-binding site, and the UAS_{GAL}. The protected regions flanking the gap on the 3'-GCY1-coding side as well as over the two distal poly(dA-dT) blocks at the 5'-side are narrow (about 140 bp), indicating that these nucleosomes are positioned.

Evidently, in the GCY1 wild-type promoter, the Gal4p protein does not contribute to the nucleosome-free state of the GCY1 upstream promoter region since the nucleosomal array is identical after growth on glucose (Fig. 3A), where the expression level of Gal4p is extremely low, or on glycerol/ethanol, where Gal4p is essentially inactive and complexed with Gal80p, or on galactose (Fig. 3B). To confirm this observation and test directly whether Gal4p has any influence on the array of nucleosomes, we analyzed the chromatin structure of the GCY1 promoter in a gal4 background (Fig. 3B). Indeed, the large nucleosome-free gap was detected likewise in wild type and a strain that lacked functional Gal4p, corroborating that nucleosome exclusion from the regulatory GCY1 upstream region is not caused by binding of Gal4p to DNA. As an alternative possibility, we examined whether destruction of the binding sites for Gal4p or Reb1p influence the array of nucleosomes.

Mutations of Reb1p-binding Site or UAS_{GAL} Result in Packaging of the GCY1 Promoter into Nucleosomes—Our previous studies on expression of Gcy1/\( \beta \)-galactosidase reporter proteins demonstrated that basal expression of GCY1 is mainly influenced by binding of the general regulatory factor Reb1p to the upstream control region on the one hand and on the other hand by the single UAS_{GAL}. Basal expression of the Gcy1/\( \beta \)-galactosidase reporter (absence of galactose) is diminished to one-third after deletion of the Reb1p-binding site and reduced to about the same degree by point mutations in the UAS_{GAL} in line with the Northern blots shown in Fig. 2. In the double mutant, which was simultaneously deleted for the Reb1p-binding site and for the 5'-flank of the UAS_{GAL}, basal transcription of GCY1 was reduced to \( \frac{1}{10} \) of wild type (30), suggesting that the two sites operate independently of one another and that their effects are about additive. We also showed that the stimulating effect of the UAS_{GAL} was not due to Gal4p activity in the absence of galactose as carbon source as it was also observed in a gal4 genetic background. To investigate whether Reb1p binding or/and the UAS_{GAL} activate transcription of GCY1 via an alteration of the chromatin configuration to yield a nucleosome-free gap, the respective promoter mutations were introduced into the original genomic context by a two-step gene replacement (see above and also see Figs. 1, B and C), and the arrange-
ment of nucleosomes was analyzed. The introduction of these mutations into the genomic situation by homologous recombination was necessary to exclude artifacts that could result from plasmid constructs or ectopic insertion of the respective promoter into the genome by means of integrative plasmids.

In vivo, DNase I or micrococcal nuclease digests were performed with each of the two single GCY1 promoter mutants (YMA2 ΔREB1 and YMA3 UASmut) as well as with the double mutant (YMA4 ΔREB1/UASmut). In each of the single mutants as well as in the double mutant, the chromatin structure was strikingly different from the wild-type situation (Fig. 4). When only one of the cis-acting elements was eliminated, we observed that the hypersensitivity decreased, and two bands of slightly sensitive linker DNA signals emerged instead. With micrococcal nuclease, we observed similar patterns as with DNase I with both single mutants. In the presence of galactose, the chromatin structure of the ΔREB1 single mutant was identical to that of the wild-type promoter. This demonstrates that, once nucleosomes cover the promoter as in the ΔREB1 mutant, Gal4p is able to bind to nucleosomal DNA and to disrupt repressing nucleosomes at the core promoter in vivo on its own, i.e. independently of Reb1p. This property is not required in the wild-type situation of the Reb1p site due to the architectural activity of Reb1p.

In the double mutant, the patterns of nucleosomes are similar to each of the single mutants with the exception that the three protected regions are short (about 150 bp) and slightly more distinct as compared with the single mutants, and nucleosomes are separated by narrow linkers. This implies that three positioned nucleosomes occupy the GCY1 upstream region in the double mutant.

DISCUSSION

The molecular mechanisms allowing basal transcription are poorly understood. Our previous analyses demonstrated that GCY1 is expressed at a relatively high basal level in the ab-

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**FIG. 3. Chromatin structure at the GCY1 Promoter.** A, chromatin structure at the GCY1 promoter in cells grown on glucose. Chromatin and “naked” DNA were digested with increasing concentrations of DNase I (symbolized by wedges) as described under “Experimental Procedures.” Hypersensitive regions of DNA and arrangement of nucleosomes are schematized on the right. Reb1p-binding site, UASGAL, distal oligo(dA:dT) blocks, core promoter (T), and transcriptional initiation sites (i) are indicated. B, wild-type cells were grown on the carbon sources indicated (Glc, glucose; Gal, galactose; Gro/EtOH, glycerol/ethanol), the gal4 strain was grown on galactose/glycerol/ethanol, and chromatin or naked DNA was digested by increasing concentrations of DNase I (left) or micrococcal nuclease (right). Positions of marker fragments are indicated on the margins. The nucleosomal arrangement is schematized in the center as described in the legend for Fig. 2.
sence of galactose as a carbon source. Basal expression in the absence of active Gal4p was found to be in part dependent on the general regulatory factor, Reb1p, which binds to its target site about 100 bp 5′ of the TATA box and about 140 bp 3′ of the UAS<sub>GAL</sub> (31). The UAS<sub>GAL</sub> contributes to basal transcription of <i>GCY1</i> as well, an effect that is independent of Gal4p binding. In the present work, we analyzed the chromatin structure of the Gal4p-inducible gene, <i>GCY1</i>. Surprisingly, the complete promoter region of <i>GCY1</i>, 480 bp in length, is permanently free of nucleosomes independent of growth conditions. DNaseI as well as micrococcal nuclease digestions of native chromatin revealed nucleosomes independent of growth conditions. DNaseI as well as micrococcal nuclease digestions of native chromatin revealed no obvious differences in the chromatin organization after growth of yeast cells on glucose, galactose, or glycerol/ethanol. The nucleosome-free gap of about 480 bp comprises the UAS<sub>GAL</sub>–the Reb1p recognition site, the TATA box, and the transcriptional initiation sites. Although <i>GCY1</i> is transcriptionally regulated by Gal4p in the presence of galactose as carbon source, no alterations of the chromatin structure can be detected upon Gal4p activation. These observations have been corroborated by analyses of the chromatin structure of the <i>GCY1</i> promoter region in a Gal4p-deficient yeast background. In the absence of the specific transactivator Gal4p, the nucleosomal array is identical to that of the <i>GAL4</i> wild-type yeast strain, suggesting that the Gal4 protein on its own has no bearing on the chromatin organization of the <i>GCY1</i> upstream region. Investigations on the <i>GAL1–GAL10</i> intergenic region and the <i>GAL80</i> promoter demonstrated that the UAS<sub>GAL</sub> sites are constitutively free of nucleosomes as well (15, 25, 27). However, Gal4p levels are very low, and binding to its target site is not detectable when yeast cells are grown on glucose (15), whereas the factor binds to its recognition site on glycerol/ethanol, but with its activation domain blocked by Gal80p, and is active only when bound under conditions of galactose induction (reviewed by Johnston (40)). Therefore, nucleosome exclusion cannot be attributed to Gal4p action but must be due either to structural properties of the DNA at the UAS<sub>GAL</sub> per se or to another DNA-binding protein that binds to the same or an overlapping motif and inhibits the assembly of nucleosomes. We have excluded the possibility that the UAS<sub>GAL</sub> region is strongly bent or kinked, and as a consequence, incompatible with packaging into nucleosomes by using permutation analyses (data not shown). However, we cannot decide whether this cis-acting element displays a quite rigid DNA structure that cannot be assembled into chromatin or whether an additional protein binds to an overlapping sequence within the UAS<sub>GAL</sub> apart from Gal4p. No indication in favor of the latter possibility could be detected by electrophoretic mobility shift assays with nuclear extracts from glucose-grown cells.

In contrast to the <i>GCY1</i> promoter, the hypersensitive regions within the UAS<sub>GAL</sub> of the <i>GAL1–GAL10</i> and <i>GAL80</i> promoters span only about 150–230 bp and do not include the TATA elements or the transcriptional start sites (15, 25). Gal4p action induces alterations of the chromatin structure in these promoters, i.e. repressing nucleosomes are disrupted in the core promoters (9, 15, 25). Since at the <i>GCY1</i> promoter, the TATA box and the initiation sites are not concealed in chromatin, there is no need for Gal4p to remove nucleosomes from the basal promoter of <i>GCY1</i>. Therefore, the permanent exclusion of nucleosomes from the core promoter of <i>GCY1</i> provides a plausible explanation for the relatively high basal transcription rate.

In addition to Gal4p, the general regulatory factor, Reb1p, has been shown to bind to the upstream control region of <i>GCY1</i>. This abundant DNA-binding protein stimulates basal transcription of <i>GCY1</i> about 3-fold (31). Reb1p is supposed to act mainly by nucleosome exclusion from the flanks of its binding site over a region of about 230 bp (13). One way of its action may be linked to the recent observation with Reb1p action on the promoter of the profilin gene, where it was found that the binding of Reb1p strongly bends DNA. Therefore, it has been concluded that the DNA deviates from the normal B conformation and assumes a structure that is incompatible with packaging into nucleosomes. Whether in addition Reb1p assists recruitment of chromatin remodeling machines or histone deacetylases to the promoter site, however, remains to be elucidated. Nevertheless, the effect of Reb1p on expression of <i>GCY1</i> is small and restricted to basal transcription of the gene (31).

On the other hand, the nucleosome-free gap in the <i>GCY1</i> promoter comprises about 480 bp and thus cannot be attributed to Reb1p binding alone. Our investigations imply that Reb1p binding to the upstream region of the <i>GCY1</i> gene and the UAS<sub>GAL</sub> together contribute to the permanent exclusion of nucleosomes.

To analyze the importance of the Reb1p recognition site and

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* M. Angermayr and W. Bandlow, unpublished.
the UAS\textsubscript{GAL} for the organization of the chromatin in the GCY1 upstream control region, we introduced mutations of the respective \textit{cis}-acting elements into the original genomic context. Mutation of either the Reb1p target sequence or the UAS\textsubscript{GAL} resulted in a loss of the nucleosome-free gap of 480 bp. Three unpositioned nucleosomes occupy this region in the respective promoter single mutants. The nucleosomal arrays are essentially identical in both single mutants. For the promoter double mutant, in which the Reb1p-binding site and the UAS\textsubscript{GAL} have been destroyed simultaneously, the pattern is quite similar to the one in the single mutants. However, some hypersensitivity is still detectable in the single mutants, whereas in the double mutant, the protected regions are more pronounced, and the linkers between the nucleosomes are more distinct, indicating that the additional nucleosomes that cover the double mutant promoter are positioned. We propose that mutations of either the Reb1p recognition motif or the UAS\textsubscript{GAL} result in packaging into chromatin of the upstream control region but that the nucleosomes are not tightly fixed to a definite position but rather allow to some extent the basal transcription machinery to assemble at the TATA box. In contrast, in the promoter double mutant, the transcriptional initiation complex is largely excluded from its target since the nucleosomes seem to be positioned. Both the Reb1p target site as well as the UAS\textsubscript{GAL} are essential for nucleosome exclusion over a stretch of 480 bp in the GCY1 promoter. Neither of the two elements is sufficient to perform this task on its own. As soon as one of the \textit{cis}-acting elements is eliminated, basal transcription drops to about 1/3 of the wild-type level. Gal4p does not play a role in nucleosome exclusion from the GCY1 promoter since the chromatin structures are identical in the wild type and in a Gal4p-deficient background. This indicates that Gal4p acts mainly by stimulating recruitment of the basal transcription machinery to the GCY1 promoter. However, the Reb1p-site mutation of the GCY1 promoter is loosely covered with nucleosomes in the absence of galactose but displays the same nucleosome-free gap as the wild-type promoter after growth of yeast cells on galactose. This demonstrates that, once the chromatin is closed as in the mutant at the Reb1p site, active Gal4p is required to open the chromatin and to make the promoter accessible, whereas in the wild type, this task is constitutively fulfilled by the architectural activity of Reb1p. This simultaneously explains why Reb1p stimulates basal transcription of GCY1 but does not affect induction of GCY1 by Gal4p action since Gal4p, in its active state, does not depend on the nucleosome excluding function of Reb1p because it is able to disrupt nucleosomes on its own.

In summary, and when the transcription data are compared with the chromatin analyses, the following conclusions can be drawn: (i) In the wild type, the chromatin is constitutively open (independent of growth conditions and independent of the presence of Gal4p). The level of GCY1 mRNA synthesis exclusively reflects the cellular concentration and activation status of Gal4p. (ii) Analysis of the mutant promoters reveals a particular DNA conformation that is due to the binding of Reb1p and a sequence element comprising the UAS\textsubscript{GAL}. Both \textit{cis}-acting elements cooperate to exclude nucleosomes from the core promoter and promote assembly of the basal transcription machinery and, thus, allow high rates of basal transcription.

Acknowledgment—We thank M. Johnston, St. Louis, MO, for yeast strain YMT07 gal4-542.

REFERENCES

1. Kernberg, R. D., and Lorch, Y. (1999) \textit{Cell} \textbf{98}, 285–294
2. Struhl, K. (1999) \textit{Cell} \textbf{98}, 1–4
3. Workman, J. L., and Kingston, R. E. (1998) \textit{Annu. Rev. Biochem.} \textbf{67}, 545–579
4. Gregory, P. D., and Horz, W. (1998) \textit{Curr. Opin. Cell Biol.} \textbf{10}, 339–345
5. Keer, P., and Horz, W. (2002) \textit{Annu. Rev. Biochem.} \textbf{71}, 247–273
6. Piña, B., Barettoni, D., Truss, M., and Beato, M. (1990) \textit{J. Mol. Biol.} \textbf{216}, 975–990
7. Archer, T. K., Cordingly, M. G., Wolford, R. G., and Hager, G. L. (1991) \textit{Mol. Cell. Biol.} \textbf{11}, 688–698
8. Fascher, K. D., Schmitz, J., and Horz, W. (1990) \textit{EMBO J.} \textbf{9}, 2523–2528
9. Axelrod, J. D., Reagan, M. S., and Majors, J. (1993) \textit{Genes Dev.} \textbf{7}, 857–869
10. Han, M. H., and Grunstein, M. (1988) \textit{Cell} \textbf{55}, 1137–1145
11. Durrin, L. K., Mann, R. K., and Grunstein, M. (1992) \textit{Mol. Cell. Biol.} \textbf{12}, 1621–1629
12. McLean, M., Hubberstey, A. V., Bouman, D. J., Pece, N., Mastrangelo, P., and Wildeman, A. G. (1995) \textit{Mol. Microbiol.} \textbf{18}, 605–614
13. Angermayr, M., Oechsner, U., Gregor, K., Schroth, G. P., and Bandlow, W. (2002) \textit{Nucleic Acids Res.} \textbf{30}, 4199–4207
14. Fedor, M. J., Lue, N. F., and Kornberg, R. D. (1988) \textit{J. Mol. Biol.} \textbf{204}, 109–127
15. Lehr, D. (1993) \textit{Proc. Natl. Acad. Sci. U.S.A.} \textbf{90}, 10628–10632
16. Moreira, J. M. A., Horz, W., and Holmgren, S. (2002) \textit{J. Biol. Chem.} \textbf{277}, 3292–3299
17. Travers, A. A. (1990) \textit{Cell} \textbf{60}, 177–180
18. Straka, C., and Horz, W. (1991) \textit{EMBO J.} \textbf{10}, 361–368
19. Brandl, C. J., and Struhl, K. (1990) \textit{Cell} \textbf{60}, 4256–4265
20. Tanaka, S., Zatzeck, M., and Thoma, F. (1999) \textit{EMBO J.} \textbf{18}, 1187–1193
21. Tanaka, S., Livingstone-Zatzeck, M., and Thoma, F. (1999) \textit{J. Mol. Biol.} \textbf{257}, 919–934
22. Iyer, V., and Struhl, K. (1995) \textit{EMBO J.} \textbf{14}, 2570–2579
23. Chasman, D. I., Lo, N. F., Buchman, A. R., LaPinte, J., Lorch, Y., and Kornberg, R. D. (1990) \textit{Genes Dev.} \textbf{4}, 1604–1614
24. Lehr, D., and Hopper, J. E. (1985) \textit{Nucleic Acids Res.} \textbf{13}, 8409–8423
25. Schmid, A., Fascher, K. D., and Horz, W. (1992) \textit{Cell} \textbf{71}, 853–864
26. Cavalli, G., and Thoma, F. (1985) \textit{EMBO J.} \textbf{12}, 4603–4613
27. Nakao, T., and Thoma, F. (1999) \textit{FEBS Lett.} \textbf{436}, 195–200
28. Nakao, T., and Thoma, F. (1999) \textit{EMBO J.} \textbf{18}, 187–193
29. Norbeck, J., and Blomberg, A. (2000) \textit{Yeast} \textbf{16}, 121–137
30. Angermayr, M., and Bandlow, W. (1997) \textit{Mol. Gen. Genet.} \textbf{256}, 682–689
31. Angermayr, M., and Bandlow, W. (1997) \textit{J. Biol. Chem.} \textbf{272}, 31630–31635
32. Angermayr, M., and Bandlow, W. (1997) \textit{Mol. Gen. Genet.} \textbf{256}, 682–689
33. Angermayr, M., and Bandlow, W. (1997) \textit{J. Biol. Chem.} \textbf{272}, 31630–31635
34. Crivellone, M. D., Wu, M., and Tzagoloff, A. (1988) \textit{J. Biol. Chem.} \textbf{263}, 14325–14333
35. Gotte, R. D., Schiestl, R. H., Willems, A. R., and Woods, R. A. (1995) \textit{Yeast} \textbf{11}, 355–369
36. Almer, A., and Horz, W. (1986) \textit{EMBO J.} \textbf{5}, 2681–2687
37. Thoma, F. (1996) \textit{Methods Enzymol.} \textbf{274}, 197–214
38. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) \textit{Molecular Cloning: A Laboratory Manual}, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
39. Oechsner, U., Magnolen, V., Zoglowek, C., Hacker, U., and Bandlow, W. (1988) \textit{FEBS Lett.} \textbf{231}, 187–190
40. Johnston, M. (1987) \textit{Microbiol. Rev.} \textbf{51}, 458–476