Reb1p-dependent DNA Bending Effects Nucleosome Positioning and Constitutive Transcription at the Yeast Profilin Promoter*

Received for publication, February 20, 2003, and in revised form, March 10, 2003
Published, JBC Papers in Press, March 10, 2003, DOI 10.1074/jbc.M301806200

Michaela Angermayr‡, Ulrich Oechsner§, and Wolfhard Bandlow
From the Department Biologie I, Bereich Genetik, Ludwig-Maximilians-Universität München, Maria-Ward-Strasse 1a, D-80638 Munich, Germany

The molecular basis of constitutive gene activation is largely unknown. The yeast profilin gene (PFY1), encoding a housekeeping component of the actin cytoskeleton, is constitutively transcribed at a moderate level. The PFY1 promoter dispenses with classical transcription activators and a consensus TATA box; however, it contains a canonic site for the abundant multifunctional nuclear factor rDNA enhancer-binding protein (Reb1p) combined with a dA\dT element. Reb1p binds specifically in vitro. Mutation of this site reduces PFY1 expression to about 35%. A nucleosome-free gap of about 190 bp is centered at the genomic Reb1p binding site in vivo and spans the presumptive core promoter and transcriptional initiation sites. Nucleosomes at the border of the gap are positioned. Mutation of the Reb1p motif in the genome PFY1 promoter abolishes nucleosome positioning, fills the gap with a non-positioned nucleosome, and reduces transcription by a factor of 3. From permutation studies we conclude that Reb1p induces a strong bend into the DNA. Phasing analyses indicate that it is directed toward the major groove. The data suggest that Reb1p plays an architectural role on DNA and that Reb1p-dependent DNA bending leads to a DNA conformation that is incompatible with packaging into nucleosomes and concomitantly facilitates constitutive transcription. In the absence of other transcription activators, Reb1p excludes nucleosomes and moderately stimulates transcription by distorting DNA.

The actin cytoskeleton is fascinating because of both the complexity of its functions and the dynamics of its structure. In yeast, it has been shown to be involved in the establishment of cell polarity and bud site selection, in intracellular transport, and in signal transduction as well as in cytokinesis. According to the plethora of functions, the organization of the microfilament system and intracellular distribution of actin is highly dynamic and closely linked to the progression of the cell cycle. Despite continuous cell cycle-controlled reorganization of the cytoskeleton, the single actin gene in yeast (ACT1) is expressed constitutively (1). The polymerization state and organization of actin is controlled postranscriptionally by accessibility proteins (2–4), and actin as well as most of the actin-binding proteins are expressed constitutively.

One of the actin-binding proteins is profilin (3, 5–8), which has been thought previously to regulate actin filament formation exclusively by sequestering G-actin monomers and thereby to antagonize actin polymerization (3). However, recent results point to a more complex regulation (9). It involves the binding of phosphoinositides to profilin and their controlled cleavage by phospholipase C, which could provide the basis for the regulation of profilin-actin interaction (10, 11) and, as a consequence, in signaling to actin (9, 12–14). In fact, results of recent experiments on yeast profilin and CAP, a component of the yeast adenyl cyclase complex, functionally link the growth signaling pathway to the control of the cytoskeleton (15). Previously we have isolated and characterized the single structural gene for yeast profilin (PFY1) and shown that its deletion leads to a temperature-conditional phenotype. PFY1 is constitutively transcribed at a moderately high level (8, 16, 17).

Amazingly little is known about the chromatin constellation at constitutive promoters and about transcription factors involved in transcription of housekeeping genes. It is supposed that constitutive transcription reflects a static situation in which the promoter is in a permanently activated state. Accordingly these promoters are presumed to be constitutively kept free of nucleosomes (18, 19), but the principles and mechanisms underlying nucleosome exclusion are far from clear. Moreover it is unknown whether constitutive transcription is basal transcription involving only the basal transcription apparatus or whether specific transactivators are required in addition. Since cis-acting sites for any of the classical transactivators are absent from the 5'-flank of the PFY1 gene, we tested the hypothesis that constitutive transcription of this gene dispenses with classical transcription activators. We have dissected the PFY1 promoter and demonstrate that it harbors a binding site for the rDNA enhancer-binding protein (Reb1p; also known as Grf2p, factor Y, and factor Q).

Reb1p is among the abundant so-called "general regulatory factors." It is multifunctional, as it is involved in transcriptional termination (20, 21), binds to telomeres and centromeres (22), and plays a role in transcriptional regulation of a plethora of functionally unrelated genes transcribed by either polymerase I or II (18, 22–26). However, the direct transcriptional activation potential of Reb1p is marginal compared with specific activator proteins, but combinations of Reb1p binding sites with cognate motifs for weak transcription activators or dA\dT elements cause considerable synergistic effects (22, 27–29). Interactions between Reb1p and the basal transcription machinery are discussed as well (1, 22, 26, 30, 31). Reb1p is encoded by an essential gene (32); however, the reason for its indispensability has not yet been established. The 125-kDa protein binds as a monomer to its site on DNA with the consensus YNNYYACCCG, and its DNA-binding domain, which...
bears some similarity to the vertebrate proto-oncogene myb, is extraordinarily large (about 400 amino acids) (32, 33). The analysis of the chromatin structure at the GAL1-GAL10 promoter, which contains a Reb1p site overlapping with a motif for binding of Gal4p, has revealed a nucleosome-free gap of 230 bp. Previous studies indicated that Reb1p binding is responsible for nucleosome exclusion from the GAL1-GAL10 promoter (34). However, more recent results have demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter dispenses with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter disposes with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter disposes with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter disposes with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter disposes with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter disposes with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.

We show that constitutive transcription at the PFY1 promoter disposes with classical transactivators. We demonstrate that Reb1p has an architectural role, and its DNA binding is necessary and sufficient to keep nucleosomes off the DNA region spanning the core promoter and the transcription initiating is necessary and sufficient to keep nucleosomes off the DNA. We demonstrated that the chromatin structure in this intergenic region is not influenced by Reb1p binding (35). Thus, the importance of Reb1p binding for the arrangement of nucleosomes and the efficiency of transcriptional initiation is still obscure. Whether nucleosome exclusion is a general feature of Reb1p remains to be elucidated and is controversially discussed (22, 29, 31). More importantly, if nucleosome exclusion is a general feature, it is yet unknown which property of Reb1p prevents assembly of nucleosomes in the flanks of its binding site extending over distances as long as about 100 bp to either side.
digested with HindIII. The randomly primed, radiolabeled 384-bp BfI/HindIII fragment was used as the 3' hybridization probe. The following isolated natural or PCR-amplified and cloned DNA fragments served as standards for length calibration and evaluation as described previously (46) (see Fig. 4): BfI/HindIII (360 bp), BglII/HindIII (590 bp), NdeI/HindIII (950 bp), NdeImutant NsiI site (1270 bp), SupI (1520 bp), and BamHI/HindIII (1830 bp).

Miscellaneous Procedures—β-Galactosidase activities of promoter-lacZ fusion proteins were determined as described previously (28, 47). Values give the mean expression activity of at least four independent clones. Generally the values varied in the order of 5–15%. Protein concentrations were determined according to the method described by Bradford (48). Yeast were transformed using the procedure described by Gietz et al. (49). Other molecular operations were performed according to standard procedures (43) or as recommended by the manufacturer.

RESULTS

Promoter Truncations and Reporter-based Expression Studies—The PFY1 gene is encoded on chromosome XV in tandem with LEO1, a non-essential gene coding for an acidic, highly polar protein (50), the function of which has not yet been established. The intergenic distance comprises 287 bp, and the non-transcribed region was truncated stepwise from the 5'-end, and β-galactosidase activities were measured in homogenates of the respective yeast transformants (Fig. 1B).

The longest PFY1 promoter-lacZ fusion construct, H4R2, started in the coding region of the 5'-adjacent gene LEO1 and, thus, contained the termination region of LEO1 as well as the complete non-transcribed upstream region of PFY1. With progressing promoter truncation, expression remained at a relatively constant level until it dropped to about 12% of the original value in the interval between positions −150 and −108 (Fig. 1B, compare activities with constructs H0R2 and H6R2). This segment contains the presumptive Reb1p binding site and the dAdT element. This indicated that sequences 5'−150 altogether are dispensable for PFY1 transcription and that the element(s) deleted in the interval between −150 and −108 is important for the expression of PFY1. To examine more directly the role of the presumptive Reb1p element in transcription of PFY1, the sequence was mutated by site-directed in vitro mutagenesis.

Two-base pair exchanges in the most conserved part of the recognition sequence of Reb1p in mutant H2GmR2 (see “Materials and Methods”), which abolished Reb1p binding (Fig. 2C, below), reduced PFY1 expression to about one-third of the wild type. The comparison of the activities obtained with H2GmR2 and H6R2 reveals that some additional element 3′ of the Reb1p site is important for expression of the reporter. We tested the oligo(dT) element (pos. −124 to −105) that is immediately 3'-adjacent to the Reb1p binding motif. Indeed deletion of this element (construct H2Δ1) reduced reporter activity to about 30% of the wild type promoter (Fig. 1C), i.e. this element has
Reb1p-induced Chromatin Modeling

Reb1p binding to the upstream promoter region of PFY1. A. homologous competition experiments after incubation with 20 μg of yeast nuclear extract. B, gel shift assay after incubation with 200 ng of recombinant Reb1p from E. coli. Specific binding of Reb1p was confirmed by homologous competition with a 25-, 50-, and 100-fold molar excess of unlabeled DNA fragment (REB1-Oligo) or by heterologous competition with a 100-, 200-, and 400-fold molar excess of unlabeled DNA fragment containing the functional Reb1p binding site of the GCY1 promoter (REB1-GCY). C, DNA binding of recombinant (E) or yeast (Y) Reb1p to a set of DNA fragments differing in length on their 5’ side. In fragment H2GmR1, the binding site of Reb1p has been mutated. Retention signals due to binding of Reb1p are indicated.

about the same importance as the Reb1p binding site. Simultaneous deletion of both sites and further extension in the 3’ direction in construct H2Δ3 (pos. −136 to −75) reduced expression to near background, although the transcriptional initiation sites were left untouched. Since in yeast the core promoter usually is 40–120 bp upstream of the first transcriptional initiation site (in PFY1 at pos. −41 relative to the start triplet), these data imply that the core promoter has been deleted in this construct in addition to the Reb1p site and the oligo(dt) tract (interval between pos. −104 and −75). The presence of the core promoter in this segment is in line with the lowered activity obtained with construct H2Δ2 in which the Reb1p site and the oligo(dt) element have been left intact.

Reb1p binds specifically to the PFY1 promoter—To verify binding of Reb1p to the presumptive site in the PFY1 promoter, a 77-bp double-stranded oligonucleotide containing this motif (see “Materials and Methods”) was incubated with yeast nuclear extracts (Fig. 2A) or recombinant Reb1p from E. coli (Fig. 2B) and used in electrophoretic mobility shift assays. Specificity of Reb1p binding was demonstrated by homologous competition with a 25-, 50-, and 100-fold molar excess of the respective unlabeled DNA fragment (Fig. 2, A and B) or by heterologous competition with a 100- or 400-fold molar excess of an unlabeled DNA fragment containing the functional Reb1p binding site of the GCY1 promoter (2B). Both recombinant Reb1p from E. coli or from yeast nuclear extracts specifically binds to the oligonucleotide, and the Reb1p site of the PFY1 promoter is a more efficient competitor than that of the GCY1 promoter.

Since the intrinsic potential of Reb1p to activate transcription directly is negligible as with any of the so-called general regulatory factors, we tested whether we could detect any additional non-histone protein binding to this region as a candidate for a transcriptional activator (Fig. 2C). We used a set of promoter fragments with variable extensions into the 5’-flank of the PFY1 gene and harboring a functional Reb1p site (H0R1, H1R1, H2R1, and H3R1 using reverse primer R1) or lacking it (H2GmR1 containing a mutated Reb1p site) (see Fig. 1 for the constructs). DNA fragments were incubated with either recombinant Reb1p isolated from E. coli or with nuclear extracts from yeast, and DNA-protein complexes were separated by native polyacrylamide gel electrophoresis. With all wild type DNA fragments very similar retention signals were found using either recombinant Reb1p or yeast nuclear extracts (similar to the oligonucleotide in Fig. 2, A and B). Since constitutive transcription reflects a static situation of permanent promoter activation, one would expect to detect by this assay the binding of any additional transactivator protein even if its cellular concentration is low. Together with the deletion data, this result strengthens the conclusion that no transcription factor binds to the 5’-upstream region of PFY1 in addition to Reb1p.

Effect of the Mutation of the Reb1p Site on PFY1 Expression in Vivo—The above data show that a 2-base pair point mutation in the Reb1p motif diminishes β-galactosidase expression from plasmid-borne PFY1 promoter-reporter fusion constructs (Fig. 1C). To corroborate that this element is also important in vivo, we have replaced the Reb1p motif in the genuine genomic context of the PFY1 promoter with the same 2-base pair point mutation as above. Although deletion of the PFY1 gene has been reported to be conditionally lethal (16), this promoter mutation is correlated with no temperature-sensitive phenotype, although cells are slightly enlarged resembling 3pfy1 cells in this respect. This suggests that the mutant promoter is partly functional despite the fact that Reb1p does not bind, which is in line with the in vitro data. We have analyzed the cellular concentration of PFY1 mRNA in wild type and mutant by Northern blot analysis (Fig. 3A) and of Pfy1p in a Western blot using anti-profilin antibodies (Fig. 3B).

The concentration of PFY1 mRNA was standardized relative to that of the ACT1 messenger (both probes of comparable length were labeled to about the same specific radioactivity and then mixed for hybridization and detection). In wild type and two different mutant clones the ACT1 mRNA was constant, whereas the concentration of the PFY1 mRNA differed between wild type and mutants (Fig. 3A). The densitometric evaluation revealed that in the wild type PFY1-specific mRNA amounted to ~35–40% of ACT1 mRNA. In the mutant clones this value dropped to about one-third of the wild type (about 14% of ACT1 mRNA). This value is in good agreement with the data obtained from the Western blot (Fig. 3B) and from reporter expression studies with PFY1 promoter-lacZ fusion constructs (compare with Fig. 1). Taken together, the destruction of the presumptive motif for Reb1p binding both on a plasmid and in the genomic context reduces transcription of PFY1 by a factor of about 3.

Chromatin array at the PFY1 promoter—We studied the nucleosome arrangement at the PFY1 promoter in the genomic
context to test whether Reb1p excludes nucleosomes from this region. The chromatin of wild type cells was digested either with DNase I (Fig. 4A, left panel) or with micrococcal nuclease (Fig. 4A, right panel) and visualized using a probe hybridizing 756–1120 bp downstream of the 5′-end of the PFY1-coding region. The results obtained with the two nucleases were very similar and, due to the slightly different preferences of DNA cleavage, mutually complementary. In wild type chromatin, the array of nucleosomes displayed a pronounced gap of hypersensitive DNA spanning about 190 bp. Standardization and sequence alignment revealed that, with the exception of about 90 bp in the center of the gap, the PFY1 promoter region was unprotected in the chromatin context. In chromatin, the center of the gap was resistant to both nucleases to a similar extent, but it displayed normal sensitivity to either nuclease with protein-free naked DNA. Thus, the protection to degradation in wild type chromatin was not attributable to the cleavage specificity of one of the respective nucleases but rather was a protein-dependent property of the chromatin at the PFY1 promoter. Most likely it was related to the binding of a protein or protein complex. The protected region within the gap was centered at the Reb1p binding site.

The nuclease digestion pattern of chromatin on the 5′-side of the gap revealed a positioned nucleosome as the protected region was short (about 145 bp) and the signals of the linker sequences were distinct and narrow. The farther upstream adjacent nucleosomes were more mobile since the linkers were wider and the boundaries of the nucleosomes were more fuzzy. The 3′-end of the LEO1 gene lies in a moderately sensitive region, presumably comprising the transcriptional terminator region of this gene.

The first nucleosome on the 3′-side of the gap has a fixed position as well according to the same criteria. Farther PFY1-proximal, i.e. 3′-adjacent to the first nucleosome in the PFY1-coding region, a wide nucleosome-sensitive linker coincided with the 3′-end of the intron. The downstream adjacent non-occupied two nucleosomes were bounded by two consecutive hypersensitive sites that lay in the bidirectional overlapping termination region of the two convergently transcribed genes PFY1 and GCY1 (51, 52), the 3′-mRNA ends of which overlap in this region by 37 nucleotides (40).

To analyze the contribution of Reb1p to the creation of the observed nucleosome-free gap in the PFY1 promoter and, in addition, to examine whether the protected region in the center of the nucleosome-free promoter region was attributable to Reb1p binding, the genomic PFY1 promoter mutant, which had been used in the expression studies described in Fig. 2, was analyzed. It carried a 2-base pair change of the Reb1p binding motif in the promoter region of the genomic PFY1 gene so that Reb1p binding was abolished (see “Materials and Methods”). The 2-base pair change did not influence the cleavage pattern of naked DNA by either DNase I (not shown) or micrococcal nuclease (Fig. 4B).

In the mutant, the pattern of digested chromatin resembled that of naked DNA in the region of the PFY1 core promoter, and the 190 bp gap is absent. This reveals that, in the absence of a binding site for Reb1p, the nucleosomes are randomly arranged and not positioned (compare with Ref. 53). This interpretation is in agreement with the expression data that showed that destruction of the Reb1p binding site did not abolish PFY1 expression completely but reduced it to about 35%. In addition, the 90-bp stretch of uncleaved DNA found in the wild type in the center of this region is not detected in mutant chromatin. This shows that Reb1p binding is responsible simultaneously for exclusion of nucleosomes from 190 bp of promoter DNA and for protection (or distortion to a non-cleaveable conformation) of a stretch of DNA comprising its own binding site in the wild type.

**Analyses of Reb1p-induced DNA Bending**—To elucidate the mechanism by which Reb1p effects nucleosome exclusion, we investigated by permutation analyses (54) whether Reb1p induces a DNA bend upon binding to its recognition site that might lead to a DNA conformation that is incompatible with wrapping around nucleosomes. The PFY1 promoter fragment containing the high affinity Reb1p target site was ligated into E. coli and separated in non-denaturing polyacrylamide gels. As a control, the mobilities of the free DNA fragments were determined. DNA fragments were detected by Southern hybridization. The relative mobilities of the permuted DNA-protein complexes differed (Fig. 5B). When the Reb1p recognition sequence was located in the center of the DNA fragment, the DNA-protein complexes were maximally retarded. The closer the Reb1p target sequence was to one of the ends of the DNA fragment the less the mobilities of the respective DNA-protein complexes were affected. However, the mobilities of the corresponding free DNA fragments varied slightly as well. These results indicate that the PFY1 promoter region contains a slight intrinsic bend on its own that likely is attributable to the oligo(dAdT) stretch, which is slightly phased (e.g. Refs. 55–57). A bending angle of ~25° for the respective protein-free PFY1 promoter DNA was calculated (58). After the relative mobilities of the DNA-protein complexes had been corrected for the differences in the mobilities of the respective free DNA fragments, the bending angle caused by Reb1p binding to its target site was calculated (58) to be ~120°. By correlating the relative mobilities of the distinct DNA-protein complexes with respect to the position of the Reb1p target site on the DNA fragment the bending center was determined to be in the immediate 5′-flank of the canonic Reb1p binding sequence (Fig. 5C).

To corroborate these findings we repeated the permutation analysis using a truncated version of recombinant Reb1p that exclusively harbored the 364 amino acids comprising the DNA-binding domain (33). The bending angle caused by the DNA-binding domain of Reb1p was calculated to be in the range of 100° and thus comparable to that of the full-length protein (Fig. 6). As with full-length Reb1p, the bending center caused by the
DNA-binding domain of Reb1p was determined to be in the 5' vicinity of the canonic consensus sequence (Fig. 6B).

To determine the relative direction of the apparent bend induced by Reb1p binding, a DNA fragment containing the Reb1p binding site was fused to a set of fragments of phased oligo(dA) tracts of known magnitude and direction of bending in which the spacing between the two sites was varied in increments of 2 bp over a full helical turn (59–62). The phasing analyses with both wild type and Reb1p truncation-derived protein-DNA complexes demonstrated that Reb1p binding bends the DNA toward the major groove (data not shown).

**DISCUSSION**

**Reb1p Influences Transcription and Nucleosome Arrangement at the PFY1 Promoter**—Yeast general regulatory factors constitute an interesting class of DNA-binding proteins. On the one hand, they abound and have many target sequences throughout the genome, explicitly in a great number of promoters. On the other hand, their potential to activate transcription directly is negligible, and deletion of their binding elements from a particular promoter in most instances leads to only marginal reduction of transcription capacity of the respective gene (e.g. Refs. 25 and 31). Nevertheless, apart from CBF1, the genes encoding general regulatory factors are essential, but the molecular basis of the mode of action of Cbf1p, Rap1p, or Abf1p is still far from clear, and, in the case of Reb1p, it is even an enigma.

As an approach to understand the role of Reb1p, we have analyzed the constitutive PFY1 promoter. We show that the relatively short activation region of the PFY1 gene dispenses with binding sites for classical transactivators. Since the constitutive PFY1 promoter reflects a static chromatin array, the...
presence of additional activators would have been detected. Instead the PFY1 promoter contains only a single canonic motif for DNA binding of Reb1p to which Reb1p binds specifically so that the impact of this factor on chromatin structure and transcription activation can be studied free of the influence of other factors. PFY1 is moderately strongly transcribed, and we have shown that Reb1p modestly enhances PFY1 mRNA transcription.

We also show that the core promoter of PFY1 is nucleosome-free and that binding of Reb1p to its cognate site is responsible for the exclusion of nucleosomes. Mutation of the recognition motif of Reb1p leads to encroachment of the promoter by randomly arranged nucleosomes. So Reb1p has two effects on the PFY1 promoter: (i) exclusion of nucleosomes and (ii) subtle stimulation of transcription.

Contradictory Results at Other Promoters Suggest Context-dependent Action of Reb1p—Reb1p binding sites have been found both in regulated and constitutive promoters. Much work has been devoted to the analysis of Reb1p in regulated promoters in which its importance is mainly restricted to a moderate stimulation of basal, but not of induced, transcription (31, 52, 63).

In the case of the GAL1-GAL10 intergenic region, Reb1p binding was supposed to be responsible for nucleosome exclusion (34). However, more recent analyses demonstrate that Reb1p binding has no bearing on the chromatin structure or on
Reb1p-induced Chromatin Modeling

the transcription of GAL1 or GAL10 (35). Similarly it has been found that Reb1p binding does not influence chromatin organization at the LEV1 promoter but positively influences Gcn4p-independent transcription in combination with two AT-rich elements (29). At the HSC82 promoter, Reb1p has only a mild effect on nucleosome positioning but rather facilitates and stabilizes binding of the heat shock transcription factor and the TATA-binding protein TBP (31). However, at the Gal4p-regulated GCY1 promoter, containing a Reb1p site remote from a Gal4p binding site, it has recently been demonstrated that Reb1p and the nucleotide sequence, i.e. presumably a particular DNA structure comprising the dG-dC-rich Gal4p binding site, contribute simultaneously to activation of basal transcription and nucleosome exclusion at the GCY1 promoter in an additive fashion (25, 52).

In constitutive promoters, the role of Reb1p is not well established. The ACT1 gene (1) encoding a cytoskeletal protein like profilin (PFY1) also is expressed constitutively. Strikingly both promoters lack binding sites for classical transcription activators and instead contain sites for the binding of the general regulatory factor Reb1p in combination with poly(dA:dT) elements (1). However, it has not been investigated yet whether Reb1p influences the organization of the chromatin structure of the ACT1 promoter. Another example for a constitutively transcribed strong promoter is that of the triosephosphate isomerase (TPII) gene (18). Reb1p moderately stimulates basal transcription of TPII and is supposed to exclude nucleosomes, thereby allowing binding of additional activators such as Gcr1p or Rap1p. However, an immediate involvement of Reb1p in nucleosome exclusion has not been demonstrated.

Taken together, a general feature of Reb1p action is stimulation of basal or constitutive transcription (18, 25, 31, 52, 63). However, the mechanisms by which Reb1p exerts these positive effects seem to differ at the respective promoters. At some promoters, e.g. PFY1 and GCY1, Reb1p has the capacity to prevent nucleosome formation in the vicinity of its binding site and to position nucleosomes at a distance (52), whereas in other Reb1p-containing promoters, this function is evidently not required since other mechanisms, i.e. specific transactivators or the intrinsic DNA sequence (see below), fulfill this task (31, 64, 65).

Reb1p Bends DNA upon Binding—Based on permutation studies, we have shown that Reb1p induces a bend into its target sequence with the bending center in the immediate vicinity (5') of the canonical binding sequence. Because the extent of curvature is important to exclude nucleosomes from DNA, the Reb1p site cannot be replaced by the soft curvature of phased oligo(dA:dT) sequences. The induced bend angle, although its magnitude may have been overestimated by the procedure used, is relatively large but comparable to other general regulatory factors from yeast such as Abf1p, Cpf1p, and Rap1p (66–69). The general regulatory factors from yeast may differ from many other DNA-binding proteins in that they may differ from many other DNA-binding proteins in that they have an extraordinarily large DNA interaction domain and, in contrast to certain mammalian basic helix-loop-helix transcription factors like the API-binding Fos-Jun heterodimer or yeast Gcn4p that were found to cause only a small DNA deformation (62, 70), induce large bend angles.

Nucleosome exclusion from core promoters facilitates transcriptional initiation. In line with the above conclusions, it has been observed that at least some promoters can be maximally activated even in the absence of transactivators by experimental deprivation of nucleosomes (71, 72). Moreover constitutive promoters have been found that dispense with any classical gene activators or other DNA-binding proteins and yet display nucleosome-free core promoters. These apparently rely on structural peculiarities of the DNA that render it incompatible with packaging into nucleosomes, e.g. the promoter of the major adenylate kinase gene Akr2 (19) and the RIO1 promoter. These results together demonstrate that loosening (73, 74), removal (75, 76), or absence (19, 52) of nucleosomes from the core promoters is necessary and, at least at some promoters, sufficient to allow transcription even in the absence of trans-activators. In addition, this points out that, apart from translational positioning of nucleosomes as found in many promoters (55, 73–77), several alternative mechanisms exist to keep nucleosomes off a certain cis-site. Exclusion of nucleosomes in the vicinity of a Reb1p binding site due to protein-dependent distortion of the DNA may add to this list. Thus, Reb1p plays its role mainly by distorting normal DNA structure.

The distortion of the DNA structure may facilitate and/or stabilize binding of additional DNA-binding proteins, presumably including TBP. Prebending of the core promoter structure has been shown to facilitate binding of transcription factor IID and to allow spontaneous assembly of the basal transcription machinery in the absence of transcription activators (78–81). Since TBP-TATA box complexes induce a strong bend in the core promoter that also is directed toward the major groove (78, 81), it appears plausible to assume that prebending effected by Reb1p binding causes a synergistic effect. Moreover it could stabilize binding and facilitate interactions between DNA-bound proteins that are separated by long distances as the intervening DNA sequences would loop out. Reb1p, binding to the vertex of the loop, could bring several weak activator proteins into close proximity. Thereby it could promote direct protein-protein contacts between them or facilitate interactions with bridging coactivators or with components of the basal transcription machinery. In line with this conclusion, it has been found that Reb1p binding stabilizes interactions of TBP with core promoter DNA and/or binding of other factors such as heat shock transcription factor or Gcr1p (18, 31). Thus, the architectural influence of Reb1p binding on DNA structure, possibly endorsed by the combination with a dA:dT element (82), simultaneously may lead to nucleosome exclusion and facilitates bending of the basal transcription machinery to the core promoter in the vicinity of a Reb1p binding site. In line with this interpretation, the core promoter of the PFY1 gene, presumably a dC- and dT-rich stretch of DNA, has been found <40 bp downstream of the Reb1p site and about 40 bp upstream of the first (major) transcriptional initiation site (8).

Acknowledgments—The skillful technical assistance of G. Strobel is gratefully acknowledged. E. coli transformant strains either expressing recombinant Reb1p or harboring the cloning vector pET11a were donated to us by J. Warner (Bronx, NY). Anti-profilin and anti-actin antisera were kindly provided by S. Brown (Ann Arbor, MI) and A. Adams (Tucson, AZ), respectively.

REFERENCES

1. MeLean, M., Hubberstey, A. V., Bouman, D. J., Pece, N., Mastrangelo, P., and Wildeman, A. G. (1995) Mol. Microbiol. 18, 605–614.
2. Way, M., and Weeds, A. (1990) Nature 344, 292–294.
3. Stessel, T. P., Chaponnier, C., Ezell, R. M., Hartwig, J. H., Jimney, P. A., Kwiatkowski, D. J., Lind, S. E., Smith, D. B., Southwick, F. S., Yun, H. L., and Zaner, K. S. (1985) Annu. Rev. Cell Biol. 1, 353–402.
4. Weeds, A. (1982) Nature 296, 811–816.
5. Carlson, L., Nystrom, L.-E., Lindberg, U., Kannan, K. K., Cid-Dresdner, H., Lovgren, S., and Jornvall, H. (1976) J. Mol. Biol. 105, 353–366.
6. Pollard, T. D., and Cooper, J. A. (1986) Annu. Rev. Cell Biol. 2, 197–213.
7. Rechsteiner, M. (1977) J. Biol. Chem. 252, 10741–10749.
8. Magnolen, V., Oechsner, U., Müller, G., and Bandlow, W. (1988) Mol. Cell. Biol. 8, 5108–5115.
9. Goldeismordon, P., and Janet, P. A. (1991) Cell 66, 419–421.
10. Lassing, D., and Lindberg, U. (1985) Nature 314, 472–474.
11. Lassing, D., and Lindberg, U. (1988) J. Cell. Biol. 107, 255–267.
12. Hartwig, J. H., Chambers, K. A., Hopea, K. L., and Kwiatkowski, D. J. (1989)
