Functional divergence between the half-sites of the DNA-binding sequence for the yeast transcriptional regulator Rap1p

Fátima-Zahra IDRISSE and Benjamin PIÑA¹

Centre d'Investigació i Desenvolupament, Consejo Superior de Investigaciones Científicas, Jordi Girona 18, 08034 Barcelona, Spain

The yeast transcriptional regulator Rap1p binds to the DNA consensus sequence ACACCCAYACAYYY. We have previously shown that DNA-binding sites in which all four Y (Y = T or C) positions were Ts (UASrpg sequences) synergized more efficiently to activate transcription than sequences in which all Ys were Cs (telomere sequences) [F.-Z. Idrissi, J. Fernández-Larrea and B. Piña (1998) *J. Mol. Biol.* **284**, 925–935]. Here we provide evidence that the DNA consensus sequence for Rap1p behaves as a combination of two ACAYYY half-sites with different functionality, the presence of Ts in the second half-site being the determinant for the transcriptional behaviour of the UASrpg sequences. DNA structure in the different complexes with Rap1p varied from being relatively uniform to appear rather distorted, this also being dependent on the presence of Ts in the second

half-site. These distortions did not cause sharp bends or kinks in the DNA molecule. Computer analysis suggests that high-affinity binding of Rap1p to UASrpg sequences requires a rearrangement of the C-terminal Myb domain of the protein. We propose that the structural alterations in Rap1p–DNA complexes, both in the DNA and in the protein, affect the transcription potential of the complex in an allosteric manner. We also propose that the dimeric nature of the Rap1 DNA-binding domain is a key structural feature that explains the disparate functions of its DNA-binding sites *in vivo*.

Key words: bandshift, DNA bending, DNA–protein complexes, potassium permanganate, transcription regulation.

INTRODUCTION

The yeast transcriptional regulator Rap1p both activates and represses transcription, depending on the context in which its DNA-recognition sequence is placed [1]. The mechanism for transcriptional silencing by Rap1p depends on its interaction with a set of silencing proteins, Sir2p, Sir3p and Sir4p. It is limited to specific regions of the chromosomes and apparently it only occurs in certain compartments within the yeast nucleus [2,3]. In contrast, transcriptional activation by Rap1p is much more widespread, involving many genes that encode, *inter alia*, glycolytic enzymes and ribosomal proteins [1,4–6]. The knowledge of how Rap1p regulates this large number of genes, many of them with different expression patterns, is central to our understanding the transcriptional control in yeast.

The DNA recognition sequence for Rap1p consists of two internal direct repeats, ACACCC, each of which is covered by one of the two Myb-like domains present in the DNA-binding domain (DBD) of Rap1p [7,8]. Whereas the first of these repeats is very well conserved, the second repeat and the spacer between the two (typically AY, where Y = T or C) show frequent substitutions of Ts for Cs [4,6]. We have recently shown that Rap1p is able to build up structural and functionally different complexes with DNA fragments encompassing two versions of the Rap1p DNA-binding sequence: 5'-ACACCCATACATTT-3' [the upstream activator sequence (UAS)rpg] and 5'-ACACCC-ACACCC-3' (the telomere consensus sequence) [9]. These two sequences differ in their activation potential. When assayed as direct repeats, the UASrpg showed a strong synergistic effect, which was orientation-dependent. In contrast, the telomeric sequence showed a much lower synergism, with no dependence on orientation. Hereafter we refer to this phenomenon as 'the RPG effect'. Studies *in vitro* showed that Rap1p formed complexes with these two sequences with essentially the same affinity;

however, the two complexes were structurally distinguishable by chemical probes [9].

Here we assayed DNA sequences showing mixed features of both UASrpg and telomeric sequences both *in vivo* and *in vitro*. We defined the bases that determine the presence of the distinctive structural cues for each type of sequence as well as the sequence requirements for the RPG effect. The bases responsible for both structural and synergistic differences between the two sequences lie in the second half of the site, which is covered by the C-terminal Myb domain of Rap1p [7]. Computer simulation of a Rap1p DBD molecule bound to the UASrpg sequence suggests that this complex would not be stable should the protein adopt the conformation observed when it binds to the telomeric sequence [7]. We propose that the structural differences between complexes of Rap1p with both types of sequences involve not only the DNA molecule, but also this C-terminal Myb domain of Rap1p, which possibly accommodates to the different sequences it binds. As suggested for other transactivators, this may result in an allosteric effect able to modulate transcriptional activation by determining the interaction of Rap1p with different subsets of co-activators [10].

METHODS

Plasmids and strains

The yeast strain YPH499 was obtained from the Yeast Stock Center, Berkeley, CA, U.S.A. The different reporter constructs were based on plasmid pSLFA-178K, a derivative of pLGA-312 [11] constructed by S. L. Forsburg [9]. Appropriate oligonucleotides were cloned into the unique *Xho*I site, at position –178 of the *CYC1* promoter. The oligonucleotide sequences were (upper sequences only): RPG1: 5'-TCGACACCCATACATT-3'; RPGC1: 5'-TCGACACCCACACATT-3'; TEL1: 5'-TCG-ACACCCACACACCC-3'; TELT1: 5'-TCGACACCCATAC-

Abbreviations used: DBD, DNA-binding domain; RPG, ribosomal-protein genes; UAS, upstream activation sequence.

¹ To whom correspondence should be addressed (e-mail bpbcmbc@cid.csic.es).

ACCC-3'; RPG2: 5'-TCGACACCCATACATTTACACACACCCATACATT-3'; RPGC2: 5'-TCGACACCCACACATTTACACACACCCACACATT-3'; TEL2: 5'-TCGACACCCACACACCCACACACCCACACACCC-3'; TELT2: 5'-TCGACACCCATACACCCATATACACCCATACACCC-3'.

Nucleotide variations from the UASrpg consensus sequence are shown in **bold**; positions outside consensus are underlined. For convenience we refer to as 'direct' the orientation in which these oligonucleotides are in the upper strand. The final sequences of the different constructs were determined by the T7 polymerase method (Pharmacia). Oligonucleotides RPG2 and TEL2 were also cloned into the *SaII* site of pBEND2 [12,13] for circular permutation analysis.

β -Galactosidase assays

YPH499 cells were transformed by the lithium acetate method described in [14]. Transformed cells were grown in selective media until medium-late exponential phase. β -Galactosidase assays were performed as described in [15] after permeabilization of whole cells with chloroform and SDS.

Preparation of recombinant Rap1p-DBD

Plasmids expressing Rap1p-DBD in bacteria [16,17] were generously given by Professor D. Rhodes (MRC Laboratory of Molecular Biology, Cambridge, U.K.). Bacterial extracts were obtained as described in [9].

KMnO₄ 'footprinting'

5'-End (C-strand) or 3'-end (G-strand)-labelled DNA fragments were incubated with different amounts of bacterial extracts containing Rap1p-DBD in 20 mM Tris/HCl (pH 8.0)/60 mM KCl/0.1 mM EDTA/20% glycerol/0.25 mg/ml BSA, containing 0.2 mg/ml of poly(dA-dT)·poly(dA-dT) (Sigma) and 1.5 ng/ μ l of pSLFA-178 as competitor DNA. Binding was allowed to proceed for 60 min at room temperature. Formation of the complexes was checked by electrophoresis in TBE (Tris/borate/EDTA)/5% (v/v) polyacrylamide gels; more than 90% of the input DNA molecules were found forming complexes with two molecules of Rap1p DBD (see [9]). Binding-reaction mixtures (30 μ l) were incubated with 100 mM KMnO₄ as described [16] for 2 min at 0 °C. The reaction was terminated with 140 mM 2-mercaptoethanol, and DNA was extracted with phenol/chloroform. Modified DNA was cleaved with 1 M piperidine for 30 min at 90 °C. The resulting DNA fragments were analysed on 10%-(w/v)-polyacrylamide sequencing gels.

Circular permutation assays

DNA fragments from pBEND-RPG2 and pBEND-TEL2 cut with appropriate restriction enzymes were 5'-end-labelled and incubated with different amounts of bacterial extracts containing Rap1p-DBD as described above. Complexes were analysed in 30-cm-long 0.5 $\times$ TBE/6% polyacrylamide gels run at 260 V for 12 h at 4 °C. DNA bending angles for the complexes were calculated as described in [12].

Computer analysis

Atomic co-ordinates were obtained from the European Bioinformatics Institute (EBI), Cambridge, U.K. Unreleased material was also generously provided by Professor D. Rhodes and Dr. L. Chapman (the latter also at the MRC Laboratory of Molecular Biology, Cambridge, U.K.). Base-pairs at positions 12, 13

and 14 were substituted by A-T base-pairs using the TURBO program in a Silicon Graphics Station.

RESULTS

Transcriptional activity of hybrid Rap1p-binding DNA sequences

The UASrpg sequence occurs frequently in the promoters of ribosomal protein genes [6,18] and glycolytic-enzyme genes [4]. Its main characteristic is the presence of Ts in positions 8, 12, 13 and 14 of the Rap1p-binding DNA consensus sequence ([9], see Figure 4 for base numbering). We assayed two Rap1p-binding DNA sequences in which some of these positions were occupied by Cs. The sequence RPGC has a C at position 8 and Ts at positions 12, 13 and 14. In contrast, sequence TELT has a T at position 8, but Cs at positions 12, 13 and 14. As references, we assayed a canonical UASrpg (sequence RPG) and a telomeric consensus sequence (sequence TEL).

When assayed as single copies in a *cyc1-lacZ* reporter construct, all these sequences showed an identical activation capacity, regardless of their orientation relative to the promoter (Figure 1). Albeit that they are low, the transcription levels obtained with these constructs were significantly higher (more than 10-fold) than the values obtained with the parental plasmid without UAS (results not shown; [9]). When we tested the capacity of these constructs to synergize by assaying them as two copies, we observed clear differences between them. As previously reported, UASrpg sequences (pRPG2) showed a much stronger synergistic effect and an equally stronger orientation-dependence than their telomeric counterparts (pTEL2; Figure 1; [9]). Interestingly, this RPG effect was also observed for the hybrid construct pRPGC2, but not for the pTELT2 construct (Figure 1). Evidently the main

Figure 1 Transcriptional activity of hybrid Rap1p-binding DNA sequences

The values are β -galactosidase activity (means $\pm$ S.D., in Miller units [15]) for at least three independent yeast transformants. Names of the different plasmids correspond to the sequences described in the Methods section. For each sequence, '1' and '2' indicate the number of Rap1p sites in each construct. Lower-case 'd' or 'r' indicates the direct or reverse orientation of these sites, as defined in the text. Bar fillings indicate the different constructs, from RPG sequences (black) to TEL sequences (empty bars). Values from the parental plasmid pSLFA-178 are also included.

Figure 2 C-strand KMnO_4 -hypersensitive sites

Tracks from left to right correspond to complexes of DBD with TEL2, RPGC2, TELT2, and RPG2 sequences respectively. The hypersensitive sites are indicated by ★. A + G lanes correspond to Maxam and Gilbert's purine reactions. Lanes labelled with a '+' correspond to reaction including appropriate amounts of recombinant DBD; lanes labelled with a '-' correspond to reactions where BSA was added instead. Relevant sequences are depicted on the right side of each A + G lane.

determinant for the transcriptional behaviour of these constructs was the presence of the three Ts at positions 12, 13 and 14. These data also reinforces our former observation [9] that the intervening sequence between Rap1p sites does not influence either transcription or the RPG effect. Note that pTEL2 and pTEL22 differ on this intervening sequence (ACAC versus ATAT); nevertheless, they were indistinguishable in terms of their transcriptional potential *in vivo*. We have also assayed a construct presenting two copies of the RPG1 oligonucleotide in tandem. Although this construct showed an only two-base spacing between Rap1p-binding sites (CG), it also displayed the RPG effect (result not shown). Rap1p binds *in vitro* with identical affinity to all these DNA sequences in a non-co-operative manner (results not shown; [9]).

Structural features of Rap1p complexes with different DNA binding sites

Binding of Rap1p (or its DBD) produces an ill-defined DNA structural alteration that results in the appearance of KMnO_4 -hypersensitive sites [9,16]. The UASrpg sequence shows a very intense site at base T^8 in the C-rich strand, as well as two further sites in the G-rich strand, one between bases T^7 and A^8 and the other between bases G^5 and G^6 . The telomeric sequence shows a rather weak site at base C^8 in the C-strand and no hypersensitive sites in the G-strand [9].

Analysis of the KMnO_4 -hypersensitive sites in complexes between DBD and sequences RPG2, RPGC2, TEL2 and TELT2 showed several interesting features. First, analysis of the C-rich strand in complexes RPG2 and TEL2 (Figure 2) showed a pattern identical with that observed in their single-copy counterparts (results not shown; see [9]). Complex RPGC2 showed a very similar pattern to complex TEL2, whereas the TELT2 sequence showed the same pattern as the RPG2 sequence. These data suggest that the only determinant for the stronger attack of KMnO_4 at base T^8 of the UASrpg sequence was the chemical nature of the base itself.

Analysis of the G-strand showed a somewhat different picture. First, hypersensitive sites observed between bases T^7 and A^8 , and between bases G^5 and G^6 were only observed in RPG and in TELT sequences; they were probably linked to the strong attack on T^8 on the opposite strand. However, these two constructs differed in the intensity of these sites. The RPG sequence showed a 3–5-fold more intense cleavage than the TELT sequence, both as single copies (★ in Figure 3A) and as two tandem repeats (★ in Figure 3B). In addition, we observed two new hypersensitive sites (▶ in Figure 3B) between the two UASrpgs in complexes RPG2 and RPGC2, but not in TEL2 or TELT2, or in any of the complexes made on single copies (Figures 3A and 3B). The position of the hypersensitive sites for all sequences tested in both strands is depicted in Figure 4.

Bending of DNA binding sites by Rap1p–DBD

Rap1p bends DNA in two ways. The whole molecule produces a sharp bending from 90 to 100° in position 3' of its binding site, both in UASrpg-like and in telomeric sequences [16,19]. In addition, the DBD produces a smaller bending (between 20 and 30°; [16]) in the centre of its DNA recognition sequence. The centre of bending by DBD coincides approximately with the region where KMnO_4 -hypersensitive sites occur. We therefore undertook the study of whether the differing intensities of these sites and the appearance of new sites between the two UASrpgs could result in the bending of the UASrpg-like sequences being greater than that of telomeric sequences.

Figure 5 shows a circular-permutation experiment using the plasmid pBEND2; either sequence pTEL2 (left) or pRPG2 (right) was cloned in the *SaII* site. In both cases the DBD complexes on DNA fragments that contained the Rap1p–DNA recognition sequence in their centre (*StuI*, *PvuII* and *XhoI*) showed a slightly larger electrophoretic retardation than the complexes formed on DNA fragments where these sequences were at their ends (*BamHI* and *MluI*). The effect was more conspicuous for the complexes C2, which carried two DBD molecules. For the TEL2 sequence the bending corresponded to a curvature of DNA of $21.3 \pm 5.5^\circ$ for complex C1 and of $27.8 \pm 5.6^\circ$ for complex C2. For the RPG2 sequence, the corresponding values were $22.7 \pm 7.0^\circ$ for the C1 complex and $32.0 \pm 7.3^\circ$ for complex C2. Means and S.D. values correspond to five independent determinations, using the relative mobility of *PvuII* (centre) and *MluI* (end) DNA fragments, as described in

Figure 3 G-strand KMnO_4 -hypersensitive sites

Tracks from left to right correspond to complexes of DBD with TEL, RPGC, TELT and RPG sequences respectively, either as single copies (A) or as tandem repeats (B). A conspicuous hypersensitive site appearing in all constructs is marked with a '*'; it can be used as a reference. Hypersensitive sites occurring only in RPGC2 and RPG2 complexes are marked ►; other relevant sites are labelled ►. Labels are as in Figure 2.

Figure 4 Location of the different structural variations

Topmost and bottommost marks correspond to Rap1p complexes with UASrpg; central marks correspond to telomeric sequences. ● Indicate guanines protected from DMS ([20,25]; results not shown). ▲ and △ represent KMnO_4 -hypersensitive sites; △ show those sites only observed when the sequences were assayed as tandem repeats. Boxes include DNA bases contacting with the N-terminal Myb domain (dark grey), the C-terminal one (white), or the C-terminal tail of Rap1p DBD (light grey) [7]. Broken lines indicate differences, on hydroxyl-radical footprinting, between the two sequences [9].

[12]. Albeit small, we found a consistently greater retardation for the RPG2 complexes with the *PvuII* DNA fragment than for the corresponding TEL2 complexes, especially for complex C2 (see Figure 5). These differences vanished for DBD complexes on *MluI*-cut DNA fragments, as expected.

Computer simulation of a DBD–UASrpg complex

The structure of the Rap1p DBD with a telomeric sequence has been solved at atomic level by X-ray crystallography [7]. We have taken advantage of the availability of these atomic co-ordinates to simulate a putative complex of the Rap1p DBD with the RPG sequence, where the protein retained the same position relative to the DNA molecule as observed in the original structure. Analysis of such a hypothetical complex suggested that it might have energetically less favourable interactions. We found that replacement of bases G^{12} and G^{13} by As would destroy the key hydrogen bonds between amino groups from residues Arg^{546} and Arg^{542} and the respective O^6 groups of the bases, which would be replaced by amino groups. In addition, the substitution of base C^{12} by a T would result on a steric clash between the 5-methyl group of the base and the residue Asn^{539} . Figure 6 shows the interaction of residues Arg^{546} and Asn^{539} with the base-pair at position 12 of an RPG (simulated, left) or a TEL (from [7], right) sequence. The picture would be essentially identical for the base-pair in position 13, with the difference that there would be no steric impediment to accommodate the 5-methyl group of C^{13} . The same analysis indicated that the nature of the base-pair at position 14 seemed irrelevant in terms of interaction with the DBD. The interactions of Arg^{546} and Arg^{542} with G^{12} and G^{13}

Figure 5 Circular permutation analysis of DBD complexes

TEL2 (left) and RPG2 (right) sequences were cloned into pBEND2. The corresponding restriction sites are indicated at the top of the gel. The label 'DNA' indicates the band corresponding to free DNA fragments. Bands labelled 'C1' and 'C2' correspond to complexes containing one or two molecules of DBD respectively.

Figure 6 Predicted interactions of base-pair 12 with DBD

The Figure shows the two DBD relevant residues Arg⁵⁴⁶ and Asn⁵³⁹. The left-hand panel shows the crystal structure of a DBD complex with a telomeric sequence (this part of the Figure is our interpretation of the atomic co-ordinates from [7]). The right-hand panel shows a computer simulation of a putative DBD complex with a UASrpg sequence, where the protein maintains the same co-ordinates as the previous one. Phosphorus, nitrogen and oxygen atoms are represented by open circles encircling a 'P', by grey circles and by black circles respectively. Broken lines joining two atoms indicate hydrogen bonds; specially relevant are those occurring between the two amino groups of residue Arg⁵⁴⁶ and groups N⁷ and O⁶ of base G¹² in the telomeric sequence. By analogy, a similar hydrogen bond is postulated between one of the amino groups of residue Arg⁵⁴⁶ and the N⁷ group of base A¹² in the UASrpg sequence. Predicted unfavourable interactions between protein residues and bases in the UASrpg sequence are indicated by sectors of circles of the corresponding van der Waals radii.

appear to be essential for Rap1p binding to the telomeric sequence, since methylation of either G¹² or G¹³ results on the loss of Rap1p binding to the telomeric sequence ([20]; F.-Z. Idrissi and B. Piña, unpublished work; Figure 4). Nevertheless, Rap1p showed a very similar affinity for telomeric and UASrpg sequences, although, in the latter, G¹² and G¹³ are replaced by As [9].

DISCUSSION

Rap1p displays a wide range of functions in yeast cells. It acts as a structural protein required for transcriptional silencing in the

telomeres, as well as a pure transcriptional activator in the MAT α gene promoter [1,21]. In most promoters in which Rap1p acts as a transactivator, it does so in combination with other UASs, most frequently either binding sites for Gcr1p or T-rich elements ([1]; see below). In these cases, the recognition sequences for Rap1p are predominantly UASrpg-like rather than telomeric-like [9]. As Rap1p binds to both types of sequences with similar affinity, the prevalence of the former may be attributable to the mechanisms of transcriptional activation.

Our previous observation that UASrpg sequences were synergistically more efficient than telomeric sequences [9] prompted us to identify the features of the sequence that determine this characteristic, which we dubbed the RPG effect. Our data indicate that the RPG effect depends upon the presence of Ts at positions 12, 13 and 14 of the DNA recognition site for Rap1p. In contrast, the existence of a C or a T at position 8 did not appear to influence the ability of Rap1p to activate our constructs. However, the presence of a T at position 8 may be functionally important in different contexts, given its conservation in the different UASrpgs. Comparative analysis shows that position 8 is occupied by a T in 70–75% of UASrpgs found in ribosomal protein gene promoters [6,18], as well as in 60% of UASrpgs from glycolytic-enzyme genes [4]. Despite this high conservation rate, this base position seems irrelevant for the binding affinity of Rap1p for the sequence [9,22]. As for position 14, both our data and all the references cited above indicate that it has very little influence both in the binding affinity and in the biological effect of Rap1p DNA-binding sites.

Our results also demonstrate that the RPG effect was not related to the intervening sequences between Rap1p binding sites, for several changes of these sequences produced no effect on transcription (see also [9]). Although we have not explored it systematically, we believe that the spacing between the two binding sites is also irrelevant as regards the RPG effect. Apart from the obvious fact that all the constructs shown here have the same spacing, and therefore spacing alone could not account for the differences between them, a construct where two RPG1 sequences were cloned in tandem also showed the RPG effect, despite its short spacing of only two bases. Spacing between 2 and 6 bp between adjacent UASrpg sites is very common in promoters of ribosomal protein genes [6,18]. The spacing we used here should allow binding of two molecules of Rap1p without major clashes between them (D. Rhodes, personal communication).

Analysis of the structure of the different Rap1p DNA-binding sites showed a rather gradual picture. The presence of a strong KMnO₄-hypersensitive site at position 8 of the C-strand seemed strongly dependent on the chemical nature of the base occupying it. Our data are consistent with the higher sensitivity of Ts to KMnO₄ [23]. KMnO₄-hypersensitive sites between positions 7 and 8, and between positions 5 and 6 on the G-strand also appeared to be related to the presence of a T at position 8 on the complementary strand. However, the intensity of these sites depended apparently on the sequence context. The RPG sequence showed a stronger attack on these positions than the sequence TELT, although position T⁸ on the C-strand was equally attacked in the two constructs. This suggests that the DNA molecule has a somewhat different structure on each of these two complexes, and that this difference is not merely due to the presence of a T or a C at position 8 on the C-strand.

The appearance of two new KMnO₄-hypersensitive sites on the G-strand between the two UASrpgs on the RPG2 and the RPGC2 complexes reinforces the hypothesis that UASrpgs adopt a peculiar structure when bound to Rap1p [9]. These sites were not present in TEL2 or TELT2 constructs; neither did they

occur in complexes of Rap1 with single copies of any of the DNA sequences studied. The most prominent of these sites occurred between A¹³ and A¹², bases that are not particularly sensitive to KMnO₄ [23]. The theoretically much more sensitive complementary bases T¹² and T¹³ on the C-strand were not attacked. This suggests a very specific distortion of the DNA molecule, rather than a local unpairing of the affected bases.

The crystal structure of DBD over a telomeric sequence shows a rather moderate distortion of the DNA double helix [7], as well as a bending of approx. 20° of the DNA molecule. Our analysis of bending of RPG2 and TEL2 sequences is consistent with this observation, and suggests that the differences in the structure of complexes on the two sequences have a rather small effect on the total bending. This almost rules out the possibility that the KMnO₄-hypersensitive sites between the two UASrpgs in the RPG2 complex were due to a sharp bend or a kink in the DNA molecule. Although unlikely, we can not rule out the possibility that the transcriptional machinery, as suggested for the thyroid-hormone receptor [24], might sense even these small differences in DNA bending.

Rap1p binds DNA through its two Myb domains [7]. However, the N-terminal Myb domain seems to be much more important for DNA binding than the C-terminal domain. DNA sequences isolated as Rap1p binders by the selected-and-amplified-binding technique ('SAAB') showed a high conservation of bases C²–A⁷, but a very poor conservation for the rest of bases, except for C¹⁰ [22]. The non-conserved bases are the same as those involved in direct contact with the second Myb domain [7]. In contrast, a single base change from C⁵ to T⁵, in the middle of the binding sequence for the N-terminal Myb domain, is sufficient to abolish binding of Rap1p [25]. The structural differences between Rap1p complexes with UASrpg and telomeric sequences involved mainly bases contacting either the second Myb domain or the C-terminal tail of Rap1p–DBD [9]; the present paper (see Figure 4). The second Myb domain is likely to have a high degree of flexibility that would allow it to adopt different configurations and thus interact with different DNA sequences (see, for example, [26]). This hypothesis is reinforced by the finding that a portion of the C-terminal Myb domain of Rap1p DBD was not ordered enough to be solved by X-ray diffraction [7]. Our computer simulation of a DBD–UASrpg complex also indicates that this Myb domain cannot maintain in this complex the same configuration that it adopts when bound to the telomeric sequence. We propose that a structural rearrangement would allow new, and still uncharacterized, contacts with the UASrpg sequence that would substitute for the missing ones. A very similar case has been proposed for c-Myb [26]; perhaps this is a common feature for regulatory proteins with duplicated Myb domains.

There is growing evidence that binding of activators to DNA sites of different sequence may involve structural changes of both the DNA molecule and the protein [26–29]. These alterations may have an allosteric effect on the activation function of the protein, with interesting regulatory consequences. Activators might expose different activation (or repression) domains depending upon their particular structure when bound to a given DNA sequence, and thus interact with different sets of co-activators or co-repressors (reviewed in [10]). This model has been invoked to explain the disparate, and in some cases contradictory, effects of binding of nuclear receptors to their cognate sequences [10,24,27,30]. Although telomeric sequences may serve as activators when placed in front of a promoter, natural sequences found in Rap1p-regulated promoters have a

strong bias towards RPG-like sequences placed in tandem with a spacing and orientation similar to our RPG2d construct. This is the disposition that, in our hands, gave the strongest activation [18]. We propose that Rap1p also interacts with different co-activators depending on its conformation when bound to different DNA-binding sites, and that this may explain the different roles of Rap1p-binding sites in the genome.

We thank Dr. J. Portugal (CID-CSIC) for his technical advice and for his critical reading of the manuscript before its submission. We also thank Professor M. Coll (CID-CSIC) for his help in producing Figure 6 and for his advice, and Professor D. Rhodes, Dr. R. Giraldo and Dr. L. Chapman (MRC Laboratory of Molecular Biology, Cambridge, U.K.) for the Rap1p–DBD expression plasmids, for their sending of the unpublished co-ordinates of the DBD–DNA complex and for their advice. In addition we thank Dr. R. F. Lascaris, Professor W. H. Mager and Professor R. J. Planta (Department of Biochemistry and Molecular Biology, IMBW, Biocentrum Amsterdam, Vrije Universiteit, Amsterdam, The Netherlands) for sending us unpublished results. This work has been supported by the grants PB92-0051 and PB95-0433 from the Ministerio de Educación y Cultura (Spain). F.-Z. I. was a recipient of a fellowship from the Ministère d'Éducation (Morocco).

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