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Recognition of specific DNA sequences by the c-*myb* protooncogene product: Role of three repeat units in the DNA-binding domain

(DNA-binding protein/oncoprotein/sequence recognition)

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ABSTRACT **The DNA-binding domain of c-Myb consists of three homologous tandem repeats of 52 amino acids. The structure of the third (C-terminal) repeat obtained by NMR analysis has a conformation related to the helix–turn–helix motif. To identify the role of each repeat in the sequence recognition of DNA, we analyzed specific interactions between c-Myb and DNA by measuring binding affinities for systematic mutants of Myb-binding DNA sites and various truncated c-Myb mutants. We found that specific interactions are localized unevenly in the AACTGAC region in the consensus binding site of c-Myb: The first adenine, third cytosine, and fifth guanine are involved in very specific interactions, in which any base substitutions reduce the binding affinity by >500-fold. On the other hand, the interaction at the second adenine is less specific, with the affinity reduction in the range of 6- to 15-fold. The seventh cytosine involves a rather peculiar interaction, in which only guanine substitution abolishes the specific binding. The binding analyses, together with the chemical protection analyses, showed that the c-Myb fragment containing the second and third repeats covers the AACTGAC region from the major groove of DNA in such an orientation that the third repeat covers the core AAC sequence. These results suggest that the third repeat recognizes the core AAC sequence very specifically, whereas the second repeat recognizes the GAC sequence in a more redundant manner. The first (N-terminal) repeat, which covers the major groove of DNA only partially, is not significant in the sequence recognition, but it contributes to increase the stability of the Myb–DNA complex. The presence of an N-terminal acidic region upstream of the first repeat, which is important for the activation of c-*myb* protooncogene, was found to reduce the binding affinity by interfering with the first repeat in binding to DNA.**

The protooncogene c-*myb* codes for the nuclear protein (c-Myb) that binds to DNA in a sequence-specific manner (1, 2). The c-Myb protein is supposed to function as an activator or repressor of transcription (3–6). The DNA-binding domain of c-Myb consists of three homologous tandem repeats of 52 amino acids [repeat 1 (R1), repeat 2 (R2), and repeat 3 (R3) from the N terminus] (7–9). Each repeat has three conserved tryptophans spaced 18–19 aa apart (10). R1 can be deleted without significant loss of DNA-binding activity (9, 11). Thus, R1 is thought to be a minor player in sequence recognition. The solution structure of R3 has been obtained by NMR analysis (12). The analysis showed that the conserved tryptophans form a hydrophobic core, as predicted from sequence and mutagenesis analyses (13, 14), and that the α-helices fold into a conformation related to the helix–turn–helix (HTH) motif. The model of Myb–DNA complex suggests that one of the helices directly interacts with base pairs and recognizes the core AAC sequence in the consensus Myb-binding sites. The role of R2 has been suggested to be that of increasing stability by electrostatic interaction with DNA. In contrast, Gabrielsen *et al.* (15) have suggested that R2 may take a HTH-related structure and, together with R3, contribute to sequence recognition, on the basis of the experimental observation in which mutations of corresponding amino acids in R2 and R3 affected DNA-binding activity similarly. However, it is unclear how R2 and R3 recognize the target sequences. The N-terminal region next to R1, which is characterized by abundant acidic amino acids, appears to be involved in the regulation of DNA binding by c-Myb as well. This region contains two serine residues, the phosphorylation of which by casein kinase II seems to inhibit the binding of Myb to DNA (16). Both v-Myb proteins encoded by the chicken leukemia viruses avian myeloblastosis virus and E26 are N- and C-terminally truncated versions of c-Myb (17). Furthermore, integration of chronically transforming retroviruses into the c-*myb* locus can also result in truncation of the N or C terminus of c-Myb, inducing myelogenous diseases in mice and B-cell lymphomas in chickens (18, 19). In most of the c-Myb versions activated by N-terminal truncation, the N-terminal region, including the phosphorylation site and a part of R1, is deleted (20). However, the mechanism by which N-terminal truncation alters the c-Myb activity is not yet known.

To understand the mechanism of DNA sequence recognition by c-Myb and clarify the role of the three repeat units and the N-terminal region in the sequence recognition, the following experiments were done. First, we analyzed the sequence specificity by measuring the binding affinities between the c-Myb DNA-binding domain and oligonucleotides containing a Myb-binding site and by examining the effects of systematic base-pair substitutions on binding affinity. As already demonstrated for the repressor binding (21, 22), such analyses provide valuable information about the location of specific interactions, their energetic contribution, and details of interactions between amino acids and base pairs. We then analyzed the binding of various truncated mutants of c-Myb to DNA to identify the part of the c-Myb DNA-binding domain important for the sequence-specific binding. We also analyzed methylation interference to obtain geometrical information on Myb–DNA interactions. These experiments were combined to decipher the role of the specific repeat structure of the c-Myb DNA-binding domain in the recognition of its target sequences.

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Abbreviations: HTH, helix–turn–helix; R1, repeat 1; R2, repeat 2; R3, repeat 3; R123, R1 + R2 + R3; R23, R2 + R3; NR123, R123 fragment with N-terminal acidic region; MBS-I, Myb-binding site in simian virus 40 enhancer.

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MATERIALS AND METHODS

Construction of Expression Vectors of c-Myb Deletion Mutants. All plasmids to express various c-Myb proteins were constructed by using a modified T7 expression vector, pAR2156*Nco*I, in which an *Nco* I site (CCATGG) was introduced by site-specific mutagenesis (23) into a site overlapping the ATG initiation codon. The *Nco* I fragment encoding various portions of the c-Myb DNA-binding domains was prepared by combinations of the previously described c-Myb mutant cDNAs (9) or by using PCR and cloned into the *Nco* I site of pAR2156*Nco*I. NR123 contains N-terminal 193 aa of c-Myb. R123 has all three repeats (R1 + R2 + R3; aa 38–193), and R23 has the C-terminal two repeats (R2 + R3; aa 90–193) of the c-Myb DNA-binding domain. Δ110, Δ122, and Δ127 contain aa 111–193, 123–193, and 128–193, respectively.

Expression and Purification of c-Myb Mutants. A fresh overnight culture of *Escherichia coli* BL21(DE3) (24) harboring one of the above-mentioned c-Myb expression plasmids was diluted in superbroth (25) containing ampicillin (100 μg/ml) and grown at 37°C. After this reached an absorption at 550 nm of ≈1.0, isopropyl β-D-thiogalactopyranoside was added to a final concentration of 1 mM, and the bacterial growth was continued for ≈6 hr. Cells of a 12-liter culture were harvested, washed with phosphate-buffered saline, and suspended in 200 ml of buffer A (50 mM Tris·HCl, pH 8.0/0.5 mM EDTA/0.3 M NaCl) containing 0.5% Nonidet P-40, 0.1 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride). The bacteria were then lysed on ice by sonication and centrifuged at 15,000 rpm for 20 min.

Procedure for purification of the bacterially expressed c-Myb will be described elsewhere. Briefly, various c-Myb proteins were purified by phosphocellulose, gel filtration, and CM-cellulose column chromatography. The purity of each c-Myb mutant protein was checked to be ≈95% by SDS/PAGE. The purified R123 was suspended in 100 mM potassium phosphate buffer (pH 7.5) containing 20 mM KCl, 0.1 mM EDTA, bovine serum albumin at 500 μg/ml, and 10 mM dithiothreitol. The synthetic R2 and R3 fragments are from S. Aimoto, Osaka University (26).

Preparation of Oligonucleotides. The 22-mer oligonucleotide CACCCTAACTGACACACATTCT, containing Myb-binding site in simian virus 40 enhancer (MBS-I) (2), and its mutants and truncated versions were synthesized and purified by HPLC with a C_{18} reverse-phase column (WAKO-SIL DNA). The purified DNA was suspended in 1× STE (0.1 M NaCl/10 mM Tris·HCl/1 mM EDTA, pH 8.0), and complementary strands were annealed and end-labeled with [γ-^{32}P]ATP (NEN) using T4 polynucleotide kinase (Toyobo, Osaka). The labeled DNAs were purified by passing them through a Sephadex G-50 column equilibrated with the potassium phosphate buffer.

Filter Binding Assay. The equilibrium dissociation constants K_d for the Myb–DNA binding were measured by the filter-binding assay. Labeled DNA and various amounts of Myb were incubated in 100 μl of binding buffer (100 mM potassium phosphate buffer, pH 7.5/20 mM KCl/0.1 mM EDTA/bovine serum albumin at 500 μg/ml/5% (vol/vol) glycerol/10 mM dithiothreitol) on ice for 10–15 min, and the samples were filtered through a nitrocellulose filter (Schleicher & Schuell, BA-85, 0.45 μm) under suction. Filters were dried and counted by a liquid scintillation counter. K_d was obtained from the binding titration curve. The binding free energy change associated with the base-pair substitution was calculated by $\Delta\Delta G = -RT\ln K_d(\text{mutant})/K_d(\text{wild type})$.

Methylation Interference. Two hundred nanograms of the sense strand of MBS-I oligonucleotide was end-labeled by T4 polynucleotide kinase and annealed with the complementary oligonucleotide. The annealed DNA was ethanol-precipitated, suspended in 200 μl of 50 mM sodium cacodylate (pH 8.0), and incubated for 5 min at 20°C after addition of 1 μl of dimethyl sulfate. The reaction was stopped by the addition of 50 μl of dimethyl sulfate stop buffer (1.5 M sodium acetate/1 M 2-mercaptoethanol/0.5 M Tris·HCl, pH 7.5/5 mM $MgCl_2$), and the DNA was isolated by ethanol precipitation. For binding of c-Myb to the probe DNA, ≈0.3 μg of end-labeled DNA and 20–30 μg of c-Myb mutant proteins were incubated for 20 min at 25°C in the reaction containing 25 mM Hepes (pH 7.9), 0.5 mM EDTA, 50 mM KCl, and 0.5 mM dithiothreitol. The reaction mixture was then put onto a 8% polyacrylamide gel in 0.25× TBE (1× TBE is 90 mM Tris base/90 mM boric acid/2 mM EDTA), electrophoresed, and bound and free DNAs were isolated by electroelution. DNA was suspended in 100 μl of 1 M piperdine and heated at 90°C for 30 min. After precipitation, DNA cleavage products were fractionated on denaturing 20% polyacrylamide gels.

RESULTS

Size of Target DNA Required for Myb Binding. We have synthesized various lengths of oligonucleotides containing the Myb-binding site MBS-I, which are shown in Fig. 1, and measured the binding affinity with the R123 fragment containing the three repeats of c-Myb DNA-binding domain by a filter-binding assay. The K_d for the 22-mer MBS-I is 4.0×10^{-10} M. R123 bound to the 22-mer, 19-mer, and 16-mer fragments in a sequence-specific manner with similar affinities, but for the 13-mer and 10-mer fragments, binding affinity was greatly reduced ($K_d > 10^{-7}$ M). Thus, the 16-mer MBS-I is sufficient for the specific binding with R123. In the following mutational analyses, however, we use the 22-mer to avoid possible end effects.

Mapping of Specific Contacts in the DNA Complexed with R123. We have introduced systematic base-pair substitutions at each position from 6 to 16 of the MBS-I 22-mer fragment, replacing the base pairs with the other 3 bp. The binding-affinity change represented by binding free energy change due to base-pair substitutions is shown in Fig. 2. All measurements in this analysis were repeated at least twice. Typical experimental errors for the binding free energy change are <0.2 kcal/mol. A positive free energy change means that the binding is weakened by the substitutions, and a negative one corresponds to an increased binding affinity. The reduction of the binding constant by 10-fold will increase the binding free energy change by ≈1.3 kcal/mol. Fig. 2 shows that most substitutions reduced the binding affinity, indicating that the wild-type MBS-I is, indeed, a highly specific binding site. Any substitutions from the original base pairs at positions 7, 9, and 11 greatly reduced the binding affinity (reduction of binding constant by >500-fold). Because the K_d for the nonspecific DNA is in the order of 10 μM, the nonspecific binding does not interfere with the mutational effects. The very large effects by these single mutations indicate that very specific interactions must be involved between R123 and the original base pairs at these

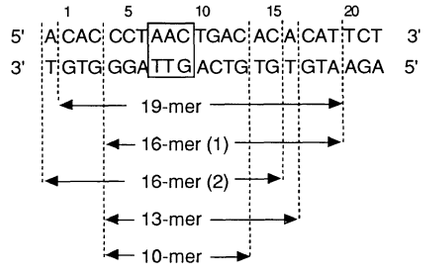

FIG. 1. Deletion mutants of MBS-I sequence. The core AAC sequence is indicated by a box.

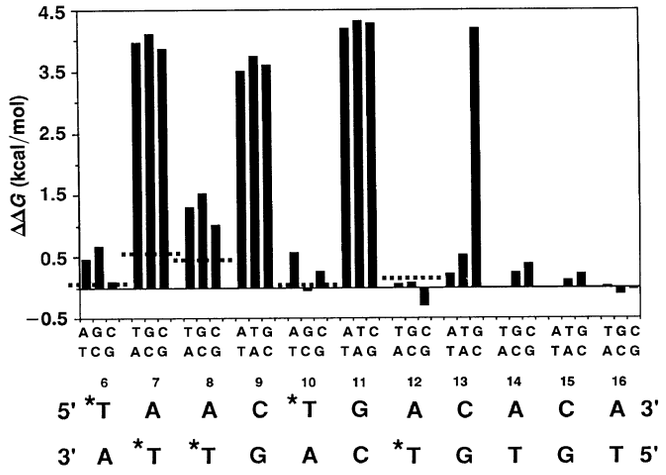

FIG. 2. Relative binding free energy changes ($\Delta\Delta G$) in binding of Myb R123 to MBS-I upon base substitutions. Sequence shown at bottom is MBS-I. Each solid bar represents $\Delta\Delta G$ from the indicated single-base substitution, and each dashed line represents $\Delta\Delta G$ from the T → U substitution (asterisks). The K_d values for wild-type MBS-I and all single-substitution mutants were measured by the filter-binding assay, and $\Delta\Delta G$ values were calculated from the K_d values, as described in text. K_d for wild-type MBS-I is 0.4 nM under our assay conditions.

positions. Positions 7 and 9 are within the core AAC sequence. The substitutions at the middle of the sequence (position 8) reduced the affinity by 6 to ≈15-fold, indicating that less-specific interactions are involved at this position than the adjacent base pairs. At position 13, a transversion from cytosine to guanine severely reduced the binding, but the other substitutions did not affect the binding significantly. The other positions in the binding region were not much affected by base-pair substitutions.

We also replaced the thymines indicated in Fig. 2 with uracils to examine the effects of methyl group on the binding. The substitutions at positions 7 and 8 reduced the binding affinity to some extent, and no significant effects were observed at other positions. Although the affinity changes are rather small, the amount of change at position 8 accounts for a significant portion of the affinity change due to base-pair substitutions, indicating that the methyl group of this thymine plays some role in the specific interaction with Myb.

Deletion Analysis of Myb DNA-Binding Domain. To identify which part of the Myb DNA-binding domain confers the specificity described above, we examined the binding affinities for various truncated fragments of the Myb DNA-binding domain shown in Fig. 3. We also examined synthetic fragments of R2 and R3, and a fragment with the N-terminal acidic region (NR123). The dissociation constant for the R23

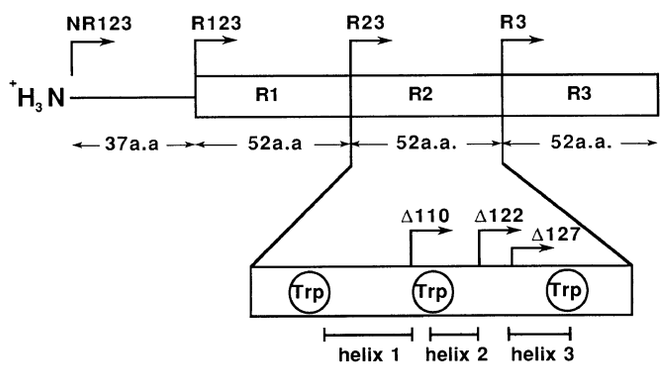

FIG. 3. Schematic representation of c-Myb proteins used. Three mutants, Δ110, Δ122, and Δ127, lack either one or two of the putative helices in R2.

fragment against the 22-mer MBS-I fragment is 2.2 nM. Thus, the R23 fragment has a lower affinity than R123 by about one-sixth, but it still retains the sequence specificity (i.e., base mutations affect the binding affinity). On the other hand, all fragments truncated inside R2 (Δ110, Δ122, and Δ127) weakly bind to the DNA (K_d = 1 μM to ≈10^{-5} M), and the binding is no longer sequence specific (i.e., base mutations do not affect the binding). This result may indicate that R2 forms a compact conformation necessary for the DNA binding. Also, the synthetic fragments R2 and R3 by themselves form only weak complexes with the DNA ($K_d \gtrsim 10^{-5}$ M) without sequence specificity. Thus, these results suggest that R23 is the minimal unit that binds to its target DNA in a sequence-specific manner. Interestingly, NR123 binds to the DNA less strongly than R123, with the dissociation constant of 1.2 nM.

Because the MBS-I sequence does not have 2-fold symmetry, the orientation of R123 with respect to the DNA sequence needs to be identified. For this end, we did the following experiment: as described above, the 16-mer fragment of MBS-I retains the specific binding with Myb. We shifted this frame, as shown in Fig. 1, and measured the binding affinity of these fragments against R123 and R23. Table 1 shows that the binding affinity of R123 to the shifted 16-mer was reduced by one-seventh, compared with the original 16-mer. On the other hand, R23 bound to the two fragments with similar affinity. The reduction of binding affinity due to the frame shift is nearly equal to that caused by the deletion of R1 from R123. Therefore, this result suggests that DNA segment between positions 16 and 19 interacts with the R1 portion of R123.

Methylated Bases that Interfere with Myb Binding. To establish which guanine bases within MBS-I are in close contact with each c-Myb deletion mutant, methylation-interference experiments were done with NR123, R123, and R23. Fig. 4 shows that the two consecutive guanine bases, 9 and 13, on the bottom strand interfered with binding of R23 when methylated. In addition to these two guanine residues, the guanine residue at 15 also interfered with binding of R123 when methylated. However, this residue did not interfere with binding of NR123. From these results, we conclude that R23 portion of R123 covers the consensus AAC region and its adjacent sequences toward its 3′ direction from the major groove of DNA, that R1 portion of R123 covers the guanine residue at position 15 but not at position 17 from the major groove, and that the N-terminal region upstream of R1 blocks the interaction between the guanine residue at position 15 and R1.

DISCUSSION

The DNA-binding domain of c-Myb has a distinctive structure in that it consists of three homologous tandem repeats of 52 amino acids. We have confirmed that both R2 and R3 are required for the sequence-specific binding, and these repeats bind to the target DNA sequences from the major groove of DNA. The present mutational analyses showed that specific interactions are unevenly localized in the AACTGAC region of the Myb-binding site. The first adenine (position 7 of Fig. 2) and the third cytosine (position 9) and the fifth guanine (position 11) are involved in very specific interactions with Myb, whereas the interactions at the second adenine (posi-

Table 1. Dissociation constants for the 16-mer MBS-I fragments with R123 and R23

Myb fragment	K_d, nM	
	MBS-I 16-mer (1)	MBS-I 16-mer (2)
R123	.55	3.8
R23	3.8	5.2

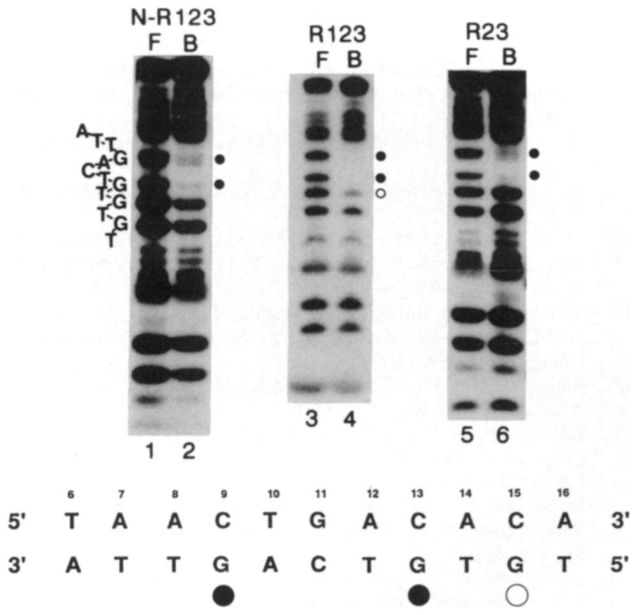

FIG. 4. Methylated bases that interfere with binding of c-Myb mutant proteins. MBS-I oligonucleotides, which was ^{32}P-labeled at the 5′-end of the bottom strand, were treated with dimethyl sulfate, and the methylated DNA was incubated with the c-Myb mutant proteins shown above. Free DNA (F) and Myb–DNA complexes (B) were separated by electrophoresis in a nondenaturing polyacrylamide gel. DNA was eluted from the gel and cleaved at modified guanine bases. Cleavage products were fractionated by electrophoresis in denaturing 20% polyacrylamide gels. Positions of completely and partially interfering bases are indicated by closed and open circles, respectively. DNA sequence of the Myb-binding site is shown below. Note that to judge the degree of interference, the differences in the densities of bands in both free and bound lanes were normalized by measuring the density of each band by Bioimage analyzer (Fuji).

tion 8) and the seventh cytosine (position 13) are less specific. This result is qualitatively consistent with the sequence bias compiled from various Myb-binding sites (27, 28), although the latter cannot reveal the details of specificity.

Our results suggest that R3 covers the core AAC region of the Myb consensus binding sequence. The structure of R3 obtained by NMR analysis has a conformation related to the HTH motif (12). Thus, the third α-helix may be used as the recognition helix to interact with base pairs of DNA, from the analogy with other HTH proteins. In the NMR structure, Asn-179, Lys-182, Asn-183, Asn-186, and Ser-187 are exposed in the DNA-binding surface of the third helix (12). Considering the previous observations in Cro and λ repressor (21, 22), the first adenine is likely to interact with one of the asparagines, which has the same H-bond donor-acceptor pattern as glutamine, and the third guanine of the opposite strand interacts with Lys-182. The second adenine is involved in less-specific interactions than the first adenine. One possibility is that this base forms H-bonds with another asparagine, such that the geometry deviates from the optimal H-bond geometry. A similar situation was found in the λ repressor, where the specific interaction between glutamine and adenine in the nonconsensus side is weaker than that in the consensus side (22). Although we assume that the affinity changes caused by the base mutations are from the direct interaction between bases and amino acids, it is also possible that some of the changes may be contributed by the indirect effects, such as the conformation changes of DNA.

To examine the range of DNA covered by each repeat of Myb, we modeled the interaction on computer graphics. If the third helix of R3 is placed on the AAC region and R2 is assumed to take a compact domain structure similar to R3, the placement of R2 in the major groove in van der Waals contact with R3 causes R2 to cover the GAC region (see Fig. 5), although it is still possible that amino acids from the nonhelical region of R3 interact with the fifth guanine. Gabrielsen *et al.* (15) suggested that Gln-129, Arg-133, and Asn-136 are important for the sequence recognition. Asparagine or glutamine may not uniquely specify guanine according to the H-bond pattern. The computer modeling suggests that Lys-128 can specifically interact with the fifth guanine. The interaction at position 13 is rather peculiar in that only guanine substitution abolished the binding. We have not observed this kind of base recognition in the repressor system. Gabrielsen *et al.* (15) suggested a double HTH model, in which corresponding amino acids in the HTH-related motifs of R2 and R3 recognize sequence. Our results show that R2 recognizes sequence, but the pattern of base recognition by R2 is quite different from that by R3, and the recognition is more redundant. We have also found subtle differences in conformation and stability in the spectroscopic and stability analyses of R2 and R3 (29). These distinct characteristics of R2 suggest that the role of R2 in sequence recognition may differ from that of R3.

We have confirmed that R1 does not contribute to the sequence-specific binding. However, we have found that R1 increases the stability of the Myb–DNA complex. This additional stability seems partially cancelled by the presence of the N-terminal acidic region adjacent to R1. The methylation-protection analysis shows that R1 partly covers the major groove of DNA, but the N-terminal region hinders the protection. Thus, both binding and protection analyses suggest that the effect of the N-terminal acidic region is to block binding of R1 to DNA. This blockade may account for the role of the N-terminal acidic region, the deletion of which activates c-*myb* protooncogene. Although the effects by R1 and the N-terminal region are not very large, they may have significant consequences in c-Myb function. The sequence specificity of c-Myb is rather low, and it binds to multiple sequences; slight changes in the binding affinity may affect the relative occupancy of the multiple binding sites and therefore be used for the differential control of binding at different sites. Furthermore, phosphorylation of the N-terminal region by casein kinase II might enhance the inhibitory effect of the N-terminal region by increasing the negative

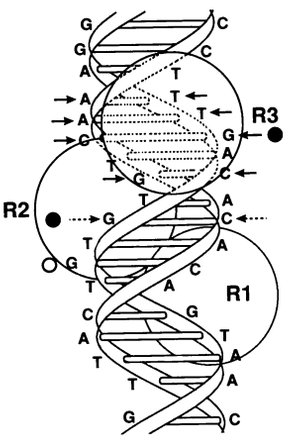

FIG. 5. Schematic model for the Myb–DNA complex. The size of each repeat is drawn so as to reflect the van der Waals volume of the R3 structure (12), which is roughly a sphere of 25-Å diameter. The base pairs at which specific binding was abolished by any base substitutions are indicated by arrows. The C·G bp at which specific binding was abolished by transversion is indicated by dashed arrows. The guanines protected from methylation by dimethyl sulfate are indicated by solid circles, and the partially protected guanine is indicated by an open circle.

charge of this region. Interestingly, the casein kinase II phosphorylation completely inhibits Myb binding to low-affinity sites but is not very efficient at inhibiting Myb binding to high-affinity sites (16). This result appears consistent with our proposition that the small effect by the N-terminal region may affect the binding of Myb only to limited sites. Thus, R1, in cooperation with the adjacent acidic region, might play a much more important role than we expect.

The DNA-binding domain of c-Myb resembles a zinc finger in that both have a tandem-repeat structure. According to the crystal structure of Zif268–DNA complex (30), the zinc fingers bind in the major groove of DNA and wrap part way around the double helix. The relative orientation of the three fingers with respect to the DNA is almost superimposable, and each finger contacts a 3-bp subsite. Our result of methylation interference for the Myb–DNA interaction suggests that R3 and R2 bind in the major groove but R1 covers the major groove only partially (see Fig. 5); it may be anchored to the phosphate backbone or partly extended into the minor groove. Also, R3 and R2 have different specific interactions with DNA, indicating some difference in the relation with DNA structure. Thus, the DNA-binding mode of the c-Myb repeat structure appears quite different from that of zinc fingers.

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1. Biedenkapp, H., Borgmeyer, U., Sippel, A. E. & Klempnauer, K.-H. (1988) *Nature (London)* **335,** 835–837.
2. Nakagoshi, H., Nagase, T., Kanei-Ishii, C., Ueno, Y. & Ishii, S. (1990) *J. Biol. Chem.* **265,** 3479–3483.
3. Nishina, Y., Nakagoshi, H., Imamoto, F., Gonda, T. J. & Ishii, S. (1989) *Nucleic Acids Res.* **17,** 107–117.
4. Weston, K. & Bishop, J. M. (1989) *Cell* **58,** 85–93.
5. Nakagoshi, H., Nagase, T., Ueno, Y. & Ishii, S. (1989) *Nucleic Acids Res.* **17,** 7315–7324.
6. Ness, S. A., Marknell, A. & Graf, T. (1989) *Cell* **59,** 1115–1125.
7. Gonda, T. J., Gough, N. M., Dunn, A. R. & de Blaquiere, J. (1985) *EMBO J.* **4,** 2003–2008.
8. Klempnauer, K.-H. & Sippel, A. E. (1987) *EMBO J.* **6,** 2719–2725.
9. Sakura, H., Kanei-Ishii, C., Nagase, T., Nakagoshi, H., Gonda, T. J. & Ishii, S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 5758–5762.
10. Anton, I. A. & Frampton, J. (1988) *Nature (London)* **336,** 719 (lett.).
11. Howe, K. M., Reaks, C. F. L. & Watson, R. J. (1990) *EMBO J.* **9,** 161–169.
12. Ogata, K., Hojo, H., Aimoto, S., Nakai, T., Nakamura, H., Sarai, A., Ishii, S. & Nishimura, Y. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 6428–6432.
13. Kanei-Ishii, C., Sarai, A., Sawazaki, T., Nakagoshi, H., He, D.-M., Ogata, K., Nishimura, Y. & Ishii, S. (1990) *J. Biol. Chem.* **265,** 19990–19995.
14. Saikumar, P., Murali, R. & Reddy, E. P. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8452–8456.
15. Gabrielsen, O. S., Sentenac, A. & Fromageot, P. (1991) *Science* **253,** 1140–1143.
16. Lüsher, B., Christenson, E., Litchfield, D. W., Krebs, E. G. & Eisenman, R. N. (1990) *Nature (London)* **344,** 517–522.
17. Klempnauer, K.-H., Gonda, T. J. & Bishop, J. M. (1982) *Cell* **31,** 453–463.
18. Sheng-Ong, G. L., Morse, H. C., III, Potter, M. & Mushinski, J. F. (1986) *Mol. Cell. Biol.* **6,** 380–392.
19. Pizer, E. & Humphries, E. H. (1989) *J. Virol.* **63,** 1630–1640.
20. Sheng-Ong, G. L. (1990) *Biochem. Biophys. Acta* **1032,** 39–52.
21. Takeda, Y., Sarai, A. & Rivera, V. M. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 439–443.
22. Sarai, A. & Takeda, Y. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 6513–6517.
23. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. (1987) *Methods Enzymol.* **154,** 367–382.
24. Rosenberg, A. H., Lade, B. N., Chui, D.-S., Lin, S.-W., Dunn, J. J. & Studier, F. W. (1987) *Gene* **56,** 125–135.
25. Ramsay, R. G., Ishii, S., Nishina, Y., Soe, G. & Gonda, T. J. (1989) *Oncogene Res.* **4,** 259–269.
26. Hojo, H. & Aimoto, S. (1991) *Bull. Chem. Soc. Jpn.* **64,** 111–117.
27. Howe, K. M. & Watson, R. J. (1991) *Nucleic Acids Res.* **19,** 3913–3919.
28. Weston, K. (1992) *Nucleic Acids Res.* **20,** 3043–3049.
29. Sarai, A., Uedaira, H., Morii, H., Yasukawa, T., Ogata, K., Nishimura, Y. & Ishii, S. (1993) *Biochemistry* **32,** 7759–7764.
30. Pavletich, N. P. & Pabo, C. O. (1991) *Science* **252,** 809–817.