Volume 14 Number 24 1986 Nucleic Acids Research

Molecular analysis of the interaction between an enhancer binding factor and its DNA target

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Received 20 October 1986; Accepted 10 November 1986

ABSTRACT

The fine contacts of a mouse nuclear factor, called PEB1, with the B enhancer of polyoma virus were analyzed. It protects against DNaseI attack a region of about 50 base pairs that can be divided in two domains. The first contains a GC-rich palindrome and the homology to the SV40 enhancer. The second is homologous to a sequence in the immunoglobulin (Ig) heavy chain gene enhancer. Methylation interference and protection experiments reveal strong specific contacts only with a purine rich track on the late coding strand of the early proximal part of the palindrome. Deletion analysis show that the minimal sequences necessary for binding include only the first domain. The Ig homology contributes only weakly to the binding. The minimal core is similar to the core of the B enhancer defined in vivo. The interactions we observe here are reminiscent of those of TFIIIA positive transcription factor and the 5SRNA gene of Xenopus.

INTRODUCTION

DNA-protein interactions play an essential role in the regulation of gene expression and DNA replication. Characterization of these interactions can help unravel the mechanisms involved in these processes. Repressor-operator interactions in prokaryotes were the first to be analyzed in great detail : in essence, a repressor dimer interacts with both parts of a palindromic sequence in a symmetric way; a conserved α-helix-turn-α-helix motif fits into the large groove of right-handed B-DNA (1). This model was found to be applicable to activator proteins like the CAP protein (2) or even yeast proteins involved in mating type control (3).

In higher eukaryotes only a few cases of specific DNA-protein interactions have been studied in detail so far. The positive transcription factor TF III A of Xenopus laevis contacts the DNA mainly along the non coding strand (4) and the helical configuration of the DNA may be altered by this binding (5). Knowledge of the primary structure of the protein has led to a model in which small Zn++ binding, fingerlike domains interact with the DNA in a way quite different from bacterial repressors or activators (6). Another

well characterized protein is large T antigen of SV40 or polyoma. Its binding to the origin of replication involves GAGGC repeats (7, 8) ; a 17 bp region in site I of SV40 binds T antigen and contains two of these sites, separated by a 7 bp spacer sequence which may have a structural role (9). The ubiquitous Sp1 transcription factor recognizes a GGGCGG motif, with most contacts along one DNA strand (10, 11). Although the basic motif is repeated 6 times in SV40, one of them is sufficient to provide binding of Sp1 (12). In contrast, the TGGCA binding protein or NF-1 seems to bind a palindromic sequence (13, 14).

Enhancers are cis-acting sequences that activate transcription in an orientation and distance independent manner (15). In view of the unknown mechanism of enhancement, characterization of factors interacting with enhancer sequences is an important goal for the near future. Several reports about proteins interacting with enhancer sequences have been published recently (16, 17, 18, 19, 20, 21). The enhancer of polyoma virus that is studied here is necessary both for transcription from the early promoter and for the replication of viral DNA (22, 23, 24). It could be subdivided into two fragments conserving partial activity : the PvuII-BclI fragment or A enhancer and the PvuII-4 fragment or B enhancer (25). Both fragments could be reduced to a minimal "core" sequence with homologies respectively to the Adenovirus and the SV40 enhancers. These two cores seem to coincide precisely with the α and β elements mapped by Hassel et al. (26) as the minimal sequences required for the activation of polyoma DNA replication. Veldman et al. (27) suggested the existence of two additional subdomains, C and D that act as enhancer auxillary sequences (25). For convenience we propose to designate these domains γ and δ respectively. The location of the different domains along the polyoma enhancer is summarized in figure 1. We revealed recently specific

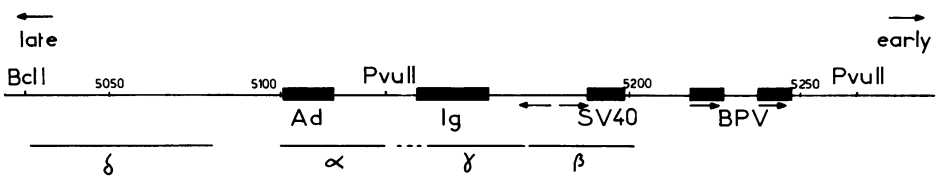

Figure 1 : Functional domains of the polyoma virus enhancer. The 246 bp BclI-PvuII enhancer fragment of polyoma virus is represented. Late and early orientations of the genome are indicated by arrows. The GC-rich palindrome is represented by divergent arrows, the repeats homologous to the BPV1 enhancer by arrows pointing in the same direction. Homologies to respectively the Adenovirus, Immunoglobulin heavy chain gene, SV40 and BPV1 enhancers are represented by black bars. The four functional domains are underlined. See text for further details.

interaction of factor(s) present in mouse 3T6 cells with the PvuII-4 fragment of polyoma containing the α and β domains of the virus enhancer (16). We present here a detailed molecular analysis of this interaction. The DNA-protein contacts we observe are reminiscent of those of TFIIIA transcription factor and the 5S RNA gene of Xenopus pointing to a more general utilization of this type of interaction in gene activation in eucaryotes.

MATERIAL AND METHODS

Preparation of nuclear extracts

Nuclear extracts prepared by resuspending nuclei in 0.4 M or 0.55M NaCl as described by Piette et al. (16) were used throughout the experiments.

DNase I footprinting combined with gel retardation experiments

A few nanograms of 3' labelled DNA were incubated at 30°C with 1µg of sonicated salmon sperm DNA and nuclear extract in 10 mM Hepes pH8, 17.5 % glycerol, 0.1 mM EDTA, 100 mM NaCl, 10 mM MgCl2, 2 mM DTT and 100 µg/ml BSA in a total volume of 50 µl. After 10 min incubation, 5 µl of 25 mM CaCl2 and 50 mM MgCl2 solution were added together with 5 µl of a 10 µg/ml DNase I solution. The digestion was slowed down after 1 min by adding 10 µg of sonicated salmon sperm DNA and the mixture was loaded immediately on a 7.5 % polyacrylamide gel. After migration, the gel was exposed for 2 hours at 4°C and the bands corresponding to the DNA-protein complex and free DNA were cut out and eluted (28). The DNase I treated fragments were loaded on a 8% sequencing gel together with the G+A and C>T chemical degradation products obtained with the same labelled fragment to localize the DNase I pattern on the DNA sequence (28).

Methylation interference and protection experiments

In interference experiments, a few nanograms of 3'labelled DNA were treated with dimethylsulfate (28). The methylated DNA was precipitated twice, and rinsed with 80 % ethanol. The modified DNA was then incubated with nuclear extract as described for the DNase I footprinting. After 10 min incubation the mixture was loaded immediately on a 7.5 % polyacrylamide gel. After migration, the gel was exposed for 2 hours at 4°C and the bands corresponding to the DNA-protein complex and free DNA were cut out and eluted (28). Both fragments were further treated with NaOH to cleave the DNA at methylated guanines and adenines (29). They were resuspended in 20 µl of 10 mM sodium phosphate (pH 7.0) and 1 mM EDTA, heated to 90°C for 5 min, followed by the addition of 2µl of 1 M NaOH and further incubation at 90°C for 30 min. The samples were

diluted to 100 ul and precipitated twice with ethanol and rinsed once with 80 % ethanol. The ultimate products were loaded on a 8 % sequencing gel.

In the protection experiments, a few nanogram of 3' labelled DNA were incubated with extract as described for the DNase I footprinting. After 10 mn incubation at 30°C, the mixture was kept on ice for 1 min, then 1µl of dimethyl sulfate was added and the incubation continued for 1 min at 0°C. The reaction was stopped by the addition of mercaptoethanol to a final concentration of 200 mM and immediately loaded on a 7.5 % polyacrylamide gel. Further procedure was like for the DMS interference experiment.

Construction of deletions

The deletions of the polyoma B enhancer were constructed starting from plasmid pPB1 containing the PvuII-4 fragment of polyoma virus cloned between the EcoRI and SalI sites of pML2, a derivative of pBR322 (16). For the Δ E series the plasmid was cut with EcoRI and submitted to a limited digestion by Bal 31 endonuclease. The ends were filled in with Klenow enzyme and a Sal I linker was ligated. The precise end point of the deletions was determined by DNA sequencing. For the ΔS series the plasmid was cut with SalI instead of EcoRI restriction enzyme. Several deletions were cloned into pUC18 to allow the isolation of fragments with addition of a known length of DNA at the deleted end.

Gel retardation assays

The gel retardation assays were performed as described in Piette et al. (16).

RESULTS

A stable complex is formed with the β - γ domains of the polyoma enhancer

The rationale used here to analyze DNA-protein interactions is as follows. We have shown previously that the complex formed between a cellular factor and the PvuII-4 fragment of polyoma is stable and could be studied by the band-shifting method in non denaturing polyacrylamide gels (16). After treatment of the DNA-protein mixture with DNase I, the complexed DNA was separated from free DNA on a polyacrylamide gel and the cleavage pattern of both DNAs compared on a sequencing gel : differences will be due to the binding of the factor to the retarded DNA. In the initial experiments we added EDTA to stop the DNase I reaction. The protection seen on the retarded DNA was only partial under these conditions. We later realized that the addition of EDTA or EGTA partially disrupted the complex in the concentration used (results not shown). To circumvent this problem we slowed down the DNase I

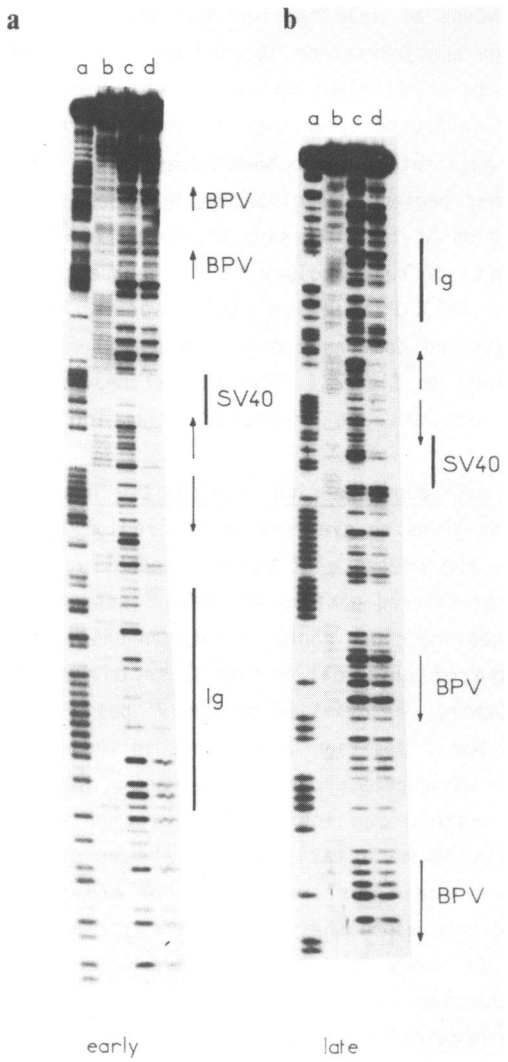

Figure 2 : DNase I footprinting of the PEB1-DNA complex. The PvuII-4 fragment was either 3' labelled at the late proximal PvuII site to give the footprint of the early coding strand (a) or 3' labelled at the early proximal PvuII site to give the footprint of the late coding strand (b). Incubation with 10 ug of a 0.55 M nuclear extract of 3T6 cells, DNase I digestion and further procedure was as described in material and methods. G + A and C>T specific degradation products are loaded in lanes (a) and (b), control DNA in lanes (c) and DNA that was complexed with the nuclear factor in lane (d). Typical features of the sequence are indicated : homologies with the SV40 and Ig-heavy chain genes enhancer by black bars, the GC-rich palindrome by divergent arrows and the repeats homologous to the BPV1 enhancer by two arrows pointing in the same direction.

reaction with an excess of cold carrier DNA before loading the preparative gel. Addition of high concentrations of cold DNA at the end of the incubation had also the advantage of eliminating all remaining non specific protein-DNA complexes. As shown in figure 2, an almost complete protection of the GC-rich palindrome and the adjacent sequence homologous to the Weiher-Gruss consensus (30) is evident : this region is enclosed by hypersensitive sites on the late strand. A second region at the late side of the palindrome is also protected : this region is part of the homology to a sequence present in the Ig-heavy chain gene enhancer (31). Protection can be seen on both DNA strands. The position of the protected domains and of the hypersensitive sites along the DNA sequence are shown on figure 6. For several reasons discussed below, we believe that both segments are protected by a single protein or protein complex.

Specific contacts with a purine-rich tract on the late strand of the domain

A DNase I footprint gives an overview of the region involved in DNA-protein interactions : both the presence of protein and structural modifications in the DNA can induce an altered pattern of DNase I sensitivity (32, 33). A more refined analysis revealing close contacts between bases and the protein can be obtained by the use of chemical probes like dimethyl sulfate (34). In interference experiments, methylation of the 7' position of guanines in the major groove or of the 3' position of adenines in the minor groove of the DNA helix, can prevent binding of a protein. Conversely, in protection experiments the presence of a protein can inhibit methylation at the sites of close contact. An increase in methylation is sometimes observed in the domain interacting with a protein, presumably by the creation of a hydrophobic pocket. We have used both approaches to obtain a picture of specific contacts with the DNA helix. As shown in figure 3, and schematized in figure 6, there is a striking interaction with the purine-rich track constituting the late strand of the early proximal side of the GC-rich palindrome : these residues are protected against methylation in the presence of the protein and their methylation in turn prevents complex formation. Surprisingly, the symmetrical counter part of the palindrome on the early strand shows no interference or protection. In contrast, two guanines on the latter strand show an enhanced methylation on the early proximal part of the palindrome. The two 5' proximal guanines of the Weiher and Gruss consensus sequence (GTGTGGTTT) show clear interference and protection (note that the first one is also a member of the palindrome). A weak protection of the fourth guanine is also observed. Methylation of G residues in the domain homologous to the Ig enhancer did

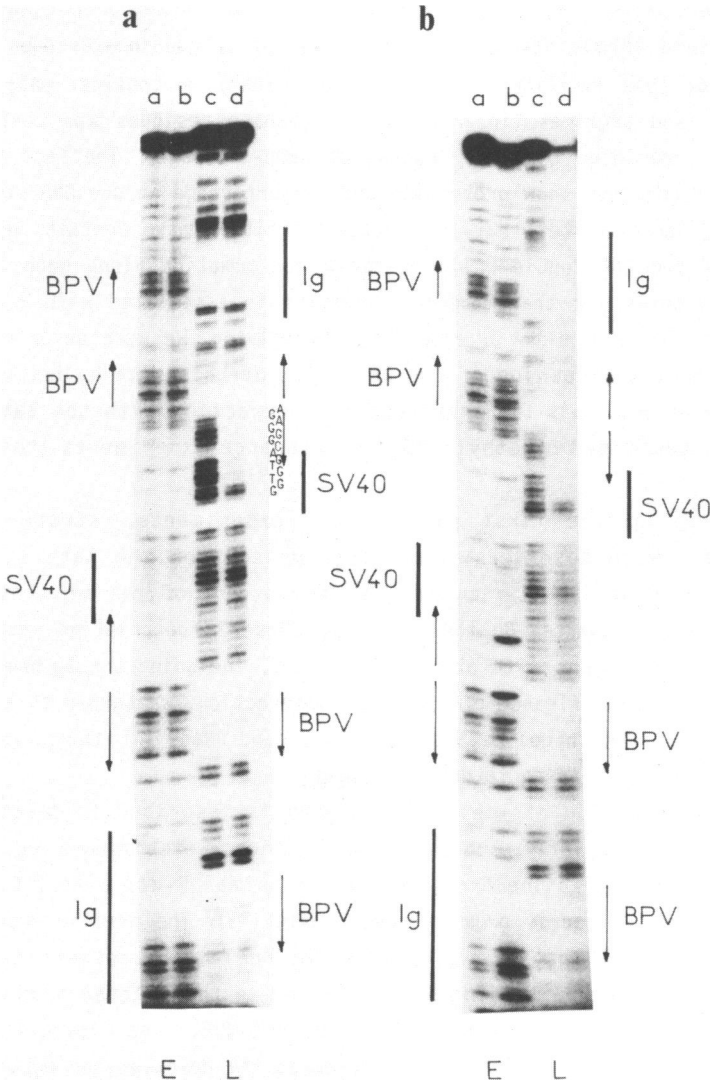

Figure 3 : Methylation interference and protection experiments. Interference experiments are shown in (a), protection experiments in (b). The PvuII-4 fragment was either 3' labelled at the late proximal PvuII site (lanes a and b) or 3' labelled at the early proximal PvuII site (lanes c and d). The experiments were performed as described in material and methods. Incubation was with 10 µg of a 0.55 M nuclear extract of 3T6 cells. Control DNA is loaded in lanes (a) and (c), DNA that was complexed by the nuclear factor in lanes (b) and (d). Symbols are as in figure 2. In the protection experiment given in lane d, the yield of longer DNA fragments was strongly reduced probably due to non specific cross linking of proteins to DNA. The residual protection pattern is still identical to that of the interference pattern.

not interfere with complex formation. It must be noted that cleavage also occurs at some thymidines or cytosines adjacent to guanine-residues ; as the mechanism of this auxiliary reaction is not clear, we consider only cleavage at guanines and adenines (interference at guanine residues was confirmed by performing piperidine cleavage instead of NaOH-cleavage). The fact that both guanines and adenines show protection and interference on one DNA strand over at least 11 bp indicates that the protein(s) either make contacts with about one turn of the DNA double helix in the B conformation along both the minor and major grooves, or that complex formation is associated with a change in the conformation of the DNA or even with its melting. We were nevertheless not able to detect any opening of the DNA helix at this site by the method of Kirkegaard et al. (35). The preferential interaction with the late coding strand was confirmed by ethylation interference experiments (results not shown).

To sum up the first part of our experiments, strong specific interactions were observed with a core present on the late strand and containing half of the GC-rich palindrome and part of the adjacent Weiher-Gruss consensus sequence. On the contrary, DNase I footprinting experiments pointed to interactions with other regions too, including the Ig homology. To investigate the significance of this last interaction, we turned to a deletion analysis combined with gel retardation assays and DNase I footprinting.

A single factor is binding to domains γ and β

The construction of deletion mutants is described in detail in the experimental part. Briefly, two series of deletions were constructed by Bal 31 digestion : the first one from the late proximal PvuII site (ΔE) and the second one from the early proximal PvuII site (ΔS). The precise endpoints of the relevant deletions were determined by nucleotide sequencing and are indicated in figure 6. Binding of the factor to the deleted fragments was analyzed by band-shifting in polyacrylamide gels (36, see figure 4). We have to mention that the fragments are bordered at the deleted side by a eigth bp SalI linker GGTCGACC, which could partially replace deleted nucleotides. Starting from the late side of the PvuII-4 fragment, removal of residues up to nt 5139 (ΔE3) does not affect binding of the cellular factor. Further deletion, however, up to nt 5165 (ΔE1) greatly decreases complex formation, complex which is no more detectable when the GC-rich palindrome is reached (ΔE17 and ΔE15). A smear in the gel may be due to less stable interactions with the remaining DNA. Thus, both sequences located between nt 5139 and 5165, and between 5165 and 5174 may be important for the formation of the complex,

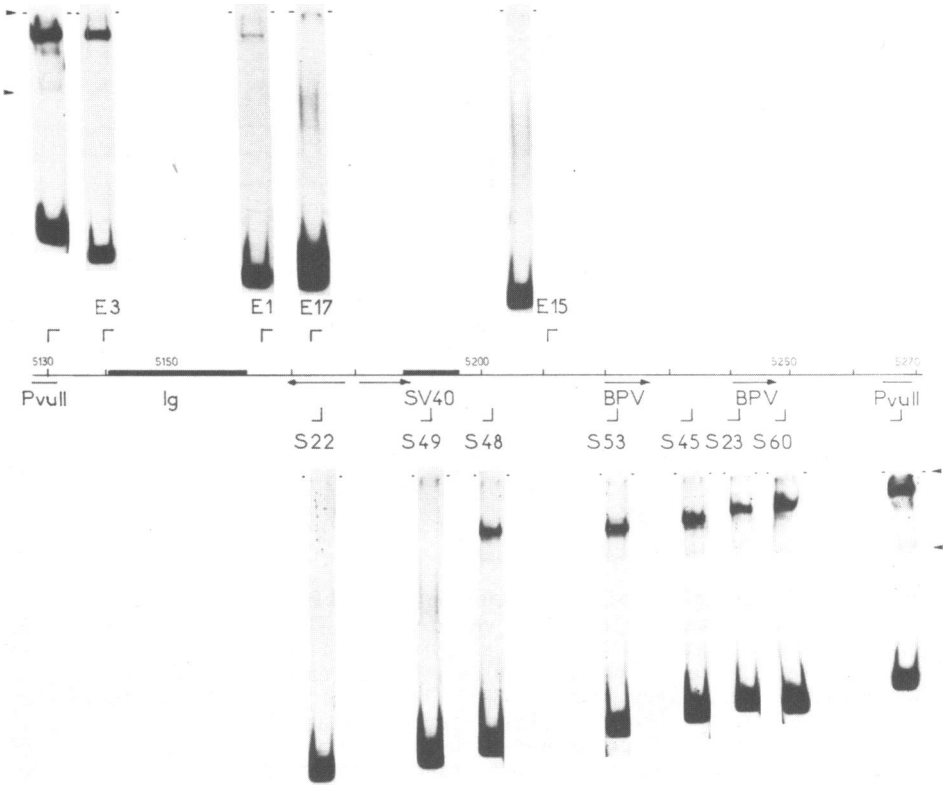

Figure 4 : Analysis of complex formation on deleted PvuII-4 fragments. Gel retardation assays were performed as described in experimental procedures with 10 µg of a 0.55 M nuclear extract of 3T6 cells. The PvuII-4 fragment is represented with typical features as symbolized in figure 1. The endpoint of the fragments used is indicated for each deletion by "⌐" above the figure for the ΔE series and below for the Δ S series. The pattern of gel migration is inserted next to the cognate deletion. The ΔE (except ΔE17) and ΔS series were incubated in the same conditions and run on the same 7.5 % polyacrylamide gel. The slots are indicated by an arrow as also the lowermost band of the doublet series.

in concordance with the DNaseI footprinting results. To test if the drop in binding efficiency we observe is due to the loss of specific contacts with the deleted DNA or just to the removal of any DNA sequence, we replaced the polyoma DNA with pBR322 DNA : almost complete recovery of the initial yield of complex is obtained with Δ E1', while weaker recovery is observed with ΔE17' (figure 5). Footprinting of Δ E1' complexes indicated that no interactions detectable by DNaseI protection occur with the plasmid DNA replacing the Ig

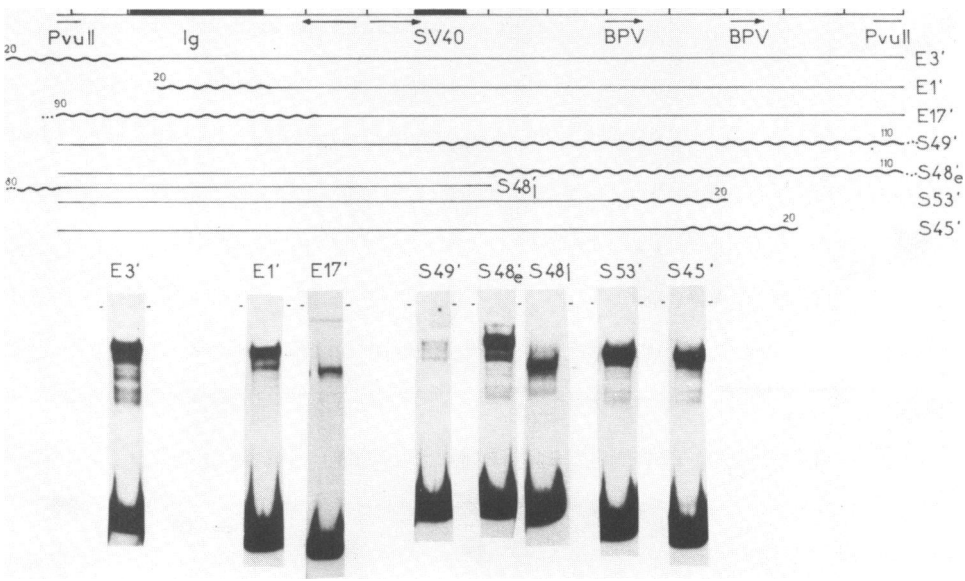

Figure 5 : Analysis of complex formation on deleted PvuII-4 fragments in which the deleted DNA was replaced by vector DNA. The PvuII-4 fragment is represented with typical features as symbolized in figure 1. Below, the fragments used in the gel retardation assays are represented by a solid line (=polyoma DNA) and a wavy line (= pBR322 DNA). The number of nucleotides of pBR322 DNA added is indicated. Gel retardation assays were performed as described in experimental procedures with 10 µg of a 0.55 M nuclear extract of 3T6 cells. Gel migration patterns are inserted at the bottom of the figure. Incubations and migration on 6 % polyacrylamide gels were the same for all fragments shown except for ΔE17'.

homology : only the GC-rich palindrome and the SV40 homology are protected (result not shown). These results suggest that the specific interactions with the Ig homology evidenced by DNase I footprinting but not methylation experiments contribute only weakly to the overall stability of the complex. Although it forms a separate binding domain on the PvuII-4 fragment, the binding to this sequence is dependent on the initial association of the factor with the β domain. The fact that no increase in the mobility of the complex occurs when this region is removed (compare complexes ΔE1' and ΔE3' in figure 5) suggests that the same protein that binds to the β domain is also binding to the γ domain. However we cannot exclude that we deal with a tight complex of two proteins, one interacting with the β domain, the other with the γ domain. We define this factor as PEB1 for polyoma enhancer binding factor 1. The assumption that a single factor composed of one or more polypeptide chains is binding to both domains is further supported by following

observations : the binding activities cosedimented on glycerol gradients and eluted as a single peak from a heparine-agarose column (results not shown).

Binding of the PEB1 factor may alter DNA conformation

Progressive deletions from the early side of the PvuII-4 fragment show that DNA can be removed up to nt 5199 (ΔS48) without decrease in binding efficiency (figure 4). However, removal of seven more nucleotides (ΔS49), dramatically affects complex formation : only a weak, less retarded doublet is seen, which disappears when part of the GC-rich palindrome is removed (ΔS22). Only a very faint band is observed at the original position in the gel after replacement of the deleted DNA of ΔS49 by plasmid DNA (figure 5, ΔS49'). Thus, both nucleotides of the SV40 homology and the GC-rich palindrome play a crucial role in the formation of the complex. Striking with this set of deletions is the progressive increase in mobility of the retarded bands with decreasing fragment size : only the mobility of the lowermost minor band (indicated by an arrow in figure 4) is not affected by the increasing size of the deletions. To test if this effect is due either to the reduction in size of the fragments or rather to the removal of nucleotides from the early side of the PvuII-4 fragment we analyzed the behaviour in the band-shifting test of two ΔS48 fragments to which pBR322 sequences were added either at the early side (ΔS48'e) or at the late side (ΔS48'l) to obtain fragments of similar size : the complex formed with ΔS48'l migrates clearly faster than that formed with ΔS48'e excluding the former possibility (see figure 5). To verify now if there is a requirement for specific sequences at the early side of the complex we added different lengths of pBR322 sequences at this side and compared the migration of the complexes formed on those fragments. As can clearly be seen in figure 5, addition of pBR322 sequences greatly decreases the mobility of the retarded bands and this proportionally to the length of DNA added (compare ΔS48e' and ΔS53'). Footprinting of a deleted fragment (ΔS53) or of a fragment in which the deleted sequence was replaced by plasmid DNA (ΔS48e') gave the same protection (results not shown). We conclude that the nature of the sequences at the early side of nt 5199 is of no importance for complex formation and that only the length of the DNA at this side determines the speed of migration of the complex in polyacrylamide gels. At this stage of our work, we cannot completely exclude the possibility that small proteins not detected by DNaseI footprinting, pile up along the DNA at the early side of the core. However we favor the alternative explanation that an important structural alteration, perhaps bending of the DNA occurs at the early side of the core complex. The position of a bend with respect to the ends of a DNA

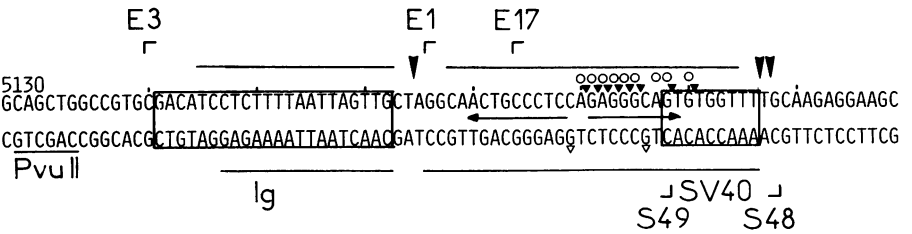

Figure 6 : Summary of the protection and interference experiments and position of the deletion endpoints on the DNA sequence. Part of the sequence of the enhancer region is represented with homologies to the immunoglobulin heavy chain gene enhancer and SV40 enhancer boxed. The palindromes are indicated by divergent arrows. Endpoints of deletions are indicated by "⌐", protection against DNase I by lines, enhanced cleavage sites by vertical arrows. DMS-interference and DMS-protection = o , DMS-enhancement : ▽ , ENU-interference = ▼. Only the strong interferences and protections are indicated.

fragment critically influences the migration of this fragment (37, 38). The different subbands we see in the retardation gels would then represent different conformations of the same complex : no bending for the lowermost band to maximal bending for the uppermost band. Indeed, the lowermost bands display the same DNase I footprint as the major one (results not shown) ; in addition the fact that their migration is less affected by removal of sequences at the early side suggests a different conformation of the DNA.

DISCUSSION

Gel retardation assays reveal in mouse cells a nuclear factor that interacts specifically with the polyoma B enhancer sequences. In the present study, we have undertaken a detailed analysis of the interaction of this cellular factor that we defined as polyoma enhancer binding protein 1 (PEB1), with the PvuII-4 enhancer fragment of polyoma virus. From enzymatic and chemical protection and interference experiments on one hand and deletion analysis on the other, we concluded that the minimal sequences necessary to ensure binding of this nuclear factor are contained in a 25 bp stretch : remarkably this includes mainly the early proximal part of a GC-rich palindrome, and the sequence homologous to the Weiher-Gruss SV40 core enhancer consensus sequence. All the contacts mapped by methylation and ethylation protection or interference experiments are located in this 25 bp core and more particularly on the late strand, only weaker ethylation interference and strong methylation enhancement are observed on the complementary strand in the same region. DNase I footprinting experiments

showed additional protection further to the late side in the PvuII-4 fragment, including the late proximal part of the palindrome and a sequence showing homology to the Ig-heavy chain gene enhancer. Deletion of this Ig homology greatly reduced the yield of complex formation and further removal of part of the GC-rich palindrome completely abolished any detectable binding. Replacement of the deleted sequences by plasmid DNA restored partially the ability to form a stable complex indicating that the proximity of the end of the DNA fragment to the binding site could be detrimental to complex formation. However, no protection against DNaseI digestion of the plasmid DNA replacing the Ig homology was anymore observed, suggesting that the interactions detected at this site are partially sequence specific. We can thus distinguish at least two binding domains on the DNA : one is essential and includes the GC-rich palindrome (at least the early part) and the SV40 homology, the other, the Ig homology, is dispensable but contributes to the stability of the complex. The sequences essential for complex formation are part of the core of the B enhancer defined by Herbomel et al. (25). They correspond almost precisely to the core of the domain 2 of the polyoma enhancer or the minimal element that activates replication as defined by Hassel et al. (26). This coincidence strongly suggests that the interaction disclosed here is functionnally important. The Ig homology unable by itself to bind the PEB1 factor, does not function as an enhancer in single or multiple copies (27).

The binding of the PEB1 factor to its recognition sequence induces alterations in the conformation of the DNA as revealed by enhanced cleavage at certain positions and by variations in the electrophoretic mobility of the complex. We detected strong contacts with both the major and minor grooves and with the phosphates of the DNA molecule along one strand of the helix over at least 11 bp. To maximize the DNA-protein interaction bending of the DNA may be induced as suggested for the cro-repressor (39). Alternatively, factor binding could induce a non-B-DNA conformation ; transition points between alternative DNA structures and B-DNA are preferred sites for DNA bending as suggested by model building (40). Such transition points could be correlated with the presence of DNase I hypersensitive sites in vitro and in vivo (41). The behavior of the multiple bands in the gel retardation assays is also worth mentionning : the migration of the lower bands is independent of the position of the binding site relative to the ends of the DNA fragment. On the contrary that of the major band, the uppermost one strongly depends on the amount of extra DNA present on the early proximal side of the SV40

homology. Since both bands gave identical footprints, they may represent different conformations of the same complex. Bends or even wrapping of the DNA around a protein core can be stabilized by additional contacts with PEB-1 or by other small protein molecules interacting with PEB-1. However, if present, these additional contacts do not seem to block accessibility to DNaseI.

Are there any other factors interacting with the polyoma PvuII-4 enhancer fragment ? A factor that interacts with the small palindrome at nt 5158 to 5172 was recently described by Fujimura (43). We could detect this factor only after fractionation of a nuclear extract on a heparine agarose column (unpublished observations). It constitutes a minor species in our extracts and its presence is most probably obscurred by the binding of the dominant PEB1 factor. In fact, the binding of both factors could be mutually exclusive because their binding sites are overlapping. The balance between both factors could thus ensure a fine tuning of enhancer activity in different tissues or during the viral cycle. One of the activities recently described by Böhnlein and Gruss (44) is most probably the same as PEB1, while the other is less clear but may be similar to the protein described by Fujimura (43).

There is strong evidence that the factor we have described here is involved at least in the enhancer dependent activation of the replication of polyoma virus. (i) As already mentioned, the minimal sequence of the PvuII-4

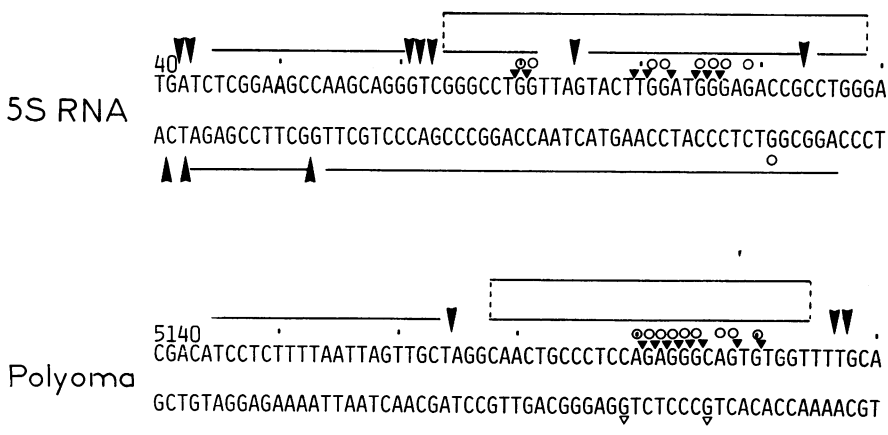

Figure 7 : Comparison of the DNA protein interaction of the PEB1-factor and the polyoma PvuII-4 fragment with that of TFIIIA positive factor and the 5SRNA gene of Xenopus. Data of the TFIIIA interaction are from Sakonju and Brown (4) and Smith et al. (45). Symbols are like in figure 6. Also shown is the domain of the 5S RNA gene interacting with the 20 kd protease product of TFIIIA and the domain of the ΔE1' deletion interacting with PEB1 factor.

fragment able to complement in cis the origin core for replication (= β core) coincides almost precisely with the sequence defined here as essential for binding the PEB1 factor (26 and J. Hassel, personal communication). (ii) Both DMS and DNase I footprinting studies performed in vivo on the polyoma minichromosome indicated similar contacts with the β-γ domains as disclosed here in vitro (M.H. Kryszke, unpublished results). (iii) A strong affinity site for PEB1, lacking any homology to Fujimura's palindrome, is able to complement the origin core for replication of the viral DNA (unpublished results).

Could the observations reported here offer some clues on the mechanism of enhancer function ? Strong interaction with only one of the two DNA strands at the binding site are reminiscent of the interaction of the TFIIIA positive transcription factor of Xenopus laevis with the 5S RNA gene (4, 45). The two binding sites with their salient features are displayed in figure 7. Similarities include the extent of DNase I protection, the strong interaction with the non coding strand in the case of 5S rDNA and the late strand in the case of polyoma and the existence of two DNA domains, one of them being sufficient to provide binding specificity. In spite of this structural similarity, only very limited sequence homology is noticed. We were unable to detect any interaction of TFIIIA with the polyoma enhancer, or of the PEB1-factor with the 5S RNA gene of Xenopus (unpublished observations). Nevertheless, the similarity in DNA-protein interaction may indicate a common structural organization and even similarity in the mode of action of the two factors. It is worth noting in this respect that several other eukaryotic regulatory proteins were found to have fingerlike domains (6), in species as distant as yeast (46) and Drosophila (47). PEB1 factor may thus be the first enhancer factor belonging to this larger family of DNA binding proteins. This type of DNA-protein interaction may turn out to be involved quite frequently in gene activation in eukaryotes.

ACKNOWLEDGEMENTS

We thank P. Herbomel for critical reading of the manuscript, J. A. Hassel and M.H. Kryszke for communicating unpublished results, H. Denis for the gift of TFIIIA protein and J. Ars for help in preparation of the manuscript. This work was supported by grants from the CNRS (UA041149 et ATP biologie moléculaire du gène et oncogènes), from INSERM (CRL 85/698), from the ARC and from the Fondation pour la Recherche Médicale Française. J. Piette was supported by a longterm fellowship from EMBO and by a fellowship from the ARC.

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