Regulation of Human B19 Parvovirus Promoter Expression by hGABP (E4TF1) Transcription Factor*

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The genetic expression of human B19 parvovirus is only dependent on one promoter in vivo and in vitro. This is the P6 promoter, which is located on the left side of the genome and is a single-stranded DNA molecule. This led us to investigate the regulation of the P6 promoter and the possible resulting variability of the nucleotide sequence. After analysis of the promoter region of 17 B19 strains, only 1.5% variability was found. More exciting was the finding of mutations that were clustered around the TATA box and defined a highly conserved region (nucleotides 113–210) in the proximal part of the P6 promoter. HeLa and UT7/Epo cell extracts were found to protect this region, which contained a core motif for Ets family proteins, with YY1 and Sp1 binding sites on either side. Gel mobility shift assays performed with nuclear proteins from HeLa and UT7/Epo cells identified DNA-binding proteins specific for these sites. By supershift analysis, we demonstrated the binding of the hGABP (also named E4TF1) protein to the Ets binding site and the fixation of Sp1 and YY1 proteins on their respective motifs. In Drosophila SL2 cells, hGABPα and β stimulated P6 promoter activity, and hGABPα/hGABPβ and Sp1 exerted synergistic stimulation of this activity, an effect diminished by YY1.

B19 virus, like other paroviruses, is a nonenveloped icosahedral virus with a single-stranded DNA linear genome composed of 5596 nucleotides that encode one nonstructural protein (NS1), two structural proteins (VP1 and VP2), and several small polypeptides of unknown function (9–11). Both ends of the genome are composed of identical inverted repeat sequences of 383 nucleotides (12). The distal 365 nucleotides are imperfect palindromes that can form a hairpin structure. The transcription map of the B19 parovirus has been determined in infected human bone marrow cells (9, 10). Its only known promoter, named P6 and located in the 5’-terminal region, directs the synthesis of up to nine viral transcripts (13, 14). Although the mRNAs encoding for the capsid proteins and the small polypeptides are spliced, the NS1 mRNA is not (10).

The regulation of the P6 promoter by viral or cellular proteins has not been extensively studied. In erythroid-permissive cells, this regulation might be preponderant. Thus, a recombinant adeno-associated virus, a defective parovirus in which the P5 promoter has been substituted for the B19 P6 promoter, is able to replicate specifically and autonomously in erythroid cells (15). However, isolated in front of a reporter gene, the P6 promoter exhibits strong activity in many cell lines, as demonstrated after transfection (14, 16, 17). Like other paroviruses, the nonstructural protein NS1 can up-regulate the P6 promoter (14, 18–20), but the exact mechanism of this up-regulation is not yet clear. The result of a recent study argues in favor of an indirect effect involving Sp1 and cAMP-response element binding proteins, as already demonstrated for other paroviruses.1 The Sp1 transcription factor has been implicated in the regulation of the P6 promoter (22). Indeed, two GC box motifs located upstream of the TATA box have been implicated in the in vitro up-regulation of promoter transcription. The YY1 transcription factor also binds the P6 promoter to three different motifs (23), which results in a positive P6 promoter regulation.

In this investigation, we first studied the genetic diversity of the B19 P6 promoter. A highly conserved region was characterized after sequencing 17 B19 strains. Within this region, a large sequence protected by erythroid or nonerythroid nuclear proteins was observed using in vitro footprinting analysis. For the first time, as far as we know, we demonstrated the presence of an Ets binding site (EBS)2 in the conserved protected region using electrophoretic mobility shift assays (EMSA). By supershift analysis, we characterized the binding of hGABP proteins, an Ets-related transcription factor so far not found to be in...
olved in regulating a paroviral promoter. In addition to the YY1 transcription factor described above, we demonstrated the fixation of the Sp1 factor to a GC box placed just downstream of the EBS. We then defined a 3-fold sequence composed of the YY1, Ets, and Sp1 binding sites. By transfection analysis of a *Drosophila* cell line, we studied the effect of the B19 P6 promoter regulation by YY1, hGABP, and Sp1 factors. We showed that Sp1 and hGABP activated transcription synergistically throughout this 3-fold sequence. This synergy was abolished by YY1. Of greater interest was the fact that we observed the same results with the P6 native promoter.

**EXPERIMENTAL PROCEDURES**

*Cell Lines and Reagents—*HeLa cells were cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% heat-inactivated fetal calf serum (Eurobio). UT7/Epo cells (24, 25) were maintained in α-modified Eagle’s medium (Life Technologies) containing 10% fetal calf serum and 2 IU/ml erythropoietin (Boehringer Mannheim). *Drosophila* melanogaster SL2 cells (26) were grown in Schneider medium (Life Technologies) supplemented with 10% fetal calf serum.

Antibodies that recognized the nuclear proteins YY1, Sp1, Fli-1, and hGABP were purchased from Santa Cruz Biotechnologies (Santa Cruz, California). Antibodies against PU-1 and Sp1 were kindly provided by F. Moreau-Gachelin (Institut Curie, Paris, France) against GABP and GABPβ by T. Pfister (Pfizer) and against Ets-1 and Efl-1 by S. T. Smale (UCLA School of Medicine, Los Angeles, CA).

**Oligonucleotides—**Oligonucleotides were synthesized and purified by Genentech (France). Complementary strands were phosphorylated with T4 polynucleotide kinase (New England Biolabs), denatured at 88 °C for 2 min, and annealed at room temperature. A *Hind*III restriction site was introduced at the end of each oligonucleotide to facilitate radioactive labeling. The NFκB probe sequence was 5′-ACGGTACACAGGACTT-TCCGCTA-3′ (27).

**Plasmid Construction**—The pTK plasmid containing the −50 to +55 region of the herpes simplex virus thymidine kinase (TK) promoter linked to the luciferase gene was constructed as follows. The EcoRI/HincII fragment corresponding to the TK promoter was obtained from a pTK-LUC plasmid (30). The orientation and site to obtain the pX-TK-LUC series of plasmids. The orientation and site to obtain the pX-TK-LUC series of plasmids. The orientation and site to obtain the pX-TK-LUC series of plasmids. The orientation and site to obtain the pX-TK-LUC series of plasmids. The orientation and site to obtain the pX-TK-LUC series of plasmids. After digestion with the *Hin*II fragment corresponding to nucleotides 113–360 of the sequence published by Shade et al. (31), we used the primers 5′-AAATGACGTAAT-TGTGCGCTACCT-3′ (nt 113–136) and 5′-AGCGCCGAAAGAAGGAG-3′ (nt 363–384). PCR was run for 30 cycles, each cycle consisting of 30 s at 94 °C, 30 s at 52 °C, and 30 s at 72 °C using Taq polymerase (Promega). The PCR product was fractionated on a 0.8% Tris-acetate-ethylenediaminetetraacetic acid (TAE) gel and stained with ethidium bromide. The same results were obtained with the P6 native promoter. To amplify a 247-bp sequence, two PCR products were obtained by two independent amplifications. Purification of the 247-bp DNA fragment (Wizard DNA clean-up system; Promega), nucleotide sequences were directly through the *Hin*III restriction site (underlined): 5′-GCTCTA-ATGACGATGAATAGAAAGAAGCAGAG-3′ (9 nt 193–1557 was then digested and inserted into the pSm I site to obtain the pX-TK-LUC series of plasmids. The orientation and sequence of the recombinant constructs were verified by DNA sequence analysis (T7 sequencing kit, Amersham).**

**Genetic Analysis—**Seventeen B19-PCR positive sera were collected between 1972 and 1995 in our laboratory. They were obtained from 4 blood donors and 12 patients with erythema, arthralgia, and acute or chronic anemia. One patient was asymptomatic. To amplify a 247-bp fragment corresponding to nucleotides 113–360 of the sequence published by Shade et al. (31), we used the primers 5′-AAATGACGTAAT-TGTGCGCTACCT-3′ (nt 113–136) and 5′-AGCGCCGAAAGAAGGAG-3′ (nt 363–384). PCR was run for 30 cycles, each cycle consisting of 30 s at 94 °C, 30 s at 52 °C, and 30 s at 72 °C using Taq polymerase (Promega). Next, the first 102 bp of the P6 promoter were removed using *Bgl*II and Accl enzymes. After Klenow treatment, the construction was self-ligated to give the pP6-LUC plasmid. The orientation and sequence of the recombinant construct were verified by DNA sequence analysis (T7 sequencing kit, Amersham).

**Preparation of Nuclear Extracts and Proteins**—Nuclear protein extracts were prepared as described previously (32). Briefly, 10² cells were treated with 300 μl of buffer (50 mM Tris-HCl pH 7.9, 10 mM KCl, 0.2% Nonidet P-40, 10% glycerol, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 20 μM/ml NaF, and 1 mM dithiothreitol). After centrifugation, the nuclear proteins were treated with 30 μl of buffer containing 400 mM NaCl, 10 mM KCl, 20% glycerol, 20 μM HEPES, pH 7.9, 1 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 180 μM/ml NaVO₄, and 1 mM dithiothreitol. After another centrifugation, the supernatant was harvested as the nuclear protein extract and stored at −70 °C. Nuclear protein concentration was determined with protein assay reagent (Bio-Rad).**

*In vitro*translated hGABPα, hGABPβ1, and hGABPβ2 proteins were, respectively, purified with 1 µl of supercoiled pET3d-GABPα, pET3d-GABPβ1, and pET3d-GABPβ2 plasmids (30) in the TNT coupled reticulocyte system (Promega) according to the manufacturer’s recommendations.

**In Vitro DNase I Footprinting Analysis**—Nuclear extracts of HeLa and UT7/Epo cells were used for this analysis. The P6 probe was prepared by PCR with a 3²P-labeled primer. A 226-bp fragment corresponding to nucleotides 78–304 of the P6 promoter sequence (31) was amplified from the BP06 plasmid using the primers 5′-ATTTCCT-GTGAACGTACCTTTCGTG-3′ (nt 78–100) and 5′-ACGGTCGACA CCTTATGACGTAAT-TGTGCGCTACCT-3′ (nt 304–283). PCR was run for 35 cycles at 94 °C for 30 s, 56 °C for 30 s, and 68 °C for 2 min/cycle with Klen *Taq* polymerase, according to the manufacturer’s instructions (Advantage-GC cDNA PCR kit; CLONTECH). The labeled fragment was purified by native agarose gel electrophoresis. About 0.5 ng of probe was incubated for 15 min at room temperature with 10 μg of nuclear extract in 25 μl of binding buffer (8 mM HEPES pH 7.9, 5 mM KCl, 0.8% Nonidet P-40, 100 μg/ml NaF, and 0.2% Nonidet P-40, 100 μg/ml NaF, and 0.2% Nonidet P-40, 100 μg/ml NaF). After phenol-chloroform extraction and ethanol precipitation, samples were loaded on an 8% urea, 6% polyacrylamide sequencing gel.

**In Vitro Electrophoretic Mobility Shift Assay**—The EMSA was performed with nuclear extract or *in vitro* translated protein and the DNA probe corresponding to the region spanning nucleotides 126–158 of the P6 promoter sequence (see Fig. 2). This oligonucleotide probe was labeled by filling in with the Klenow fragment of DNA polymerase I in the presence of [α-³²P]dCTP. First, 2.5 μg of cell extract or 1 μl of *in vitro* translated protein were incubated for 10 min at room temperature in
the binding buffer (4% Ficoll, 20 mM Hepes, pH 7.5, 70 mM NaCl, 2 mM dithiothreitol, 100 μg/ml bovine serum albumin, and 0.01% Nonidet P-40) with 1 μg of poly(dl-dc)poly(dl-dc) (Amersham) and 0.5 μg of salmon sperm DNA. Next, either competitor or antibodies were added when indicated and then 1 μl of the 32P-labeled probe (about 20,000 cpm). The preparation was left to stand for 25 min at room temperature and then underwent electrophoresis on 5% polyacrylamide gel in 0.5 × Tris borate/EDTA buffer (45 mM Tris borate, 1 mM EDTA). Lastly, the gel was dried and autoradiographed.

**Transfection and Luciferase Assay**—One day before transfection, SL2 cells were seeded at 2 × 10^6 cells/35-mm well. They were co-transfected with the indicated amount of expression plasmid and 1 μg of reporter plasmid using the calcium phosphate coprecipitation method (33). The total amount of DNA was kept constant at 10 μg by adding nonrecombinant expression plasmid (control plasmid). After the addition of DNA, the plates were left undisturbed until the time of harvest 48 h later. Cells were then washed once with phosphate-buffered saline, and luciferase activity was measured in a luminometer (Lumat LB 9501, Berthold), as described previously (20). To normalize the luciferase assay, the total protein concentration was evaluated for each cell lysate (protein assay reagent, Bio-Rad).

**RESULTS**

**Genetic Diversity Analysis**—As stated above, the 17 B19 PCR-positive sera collected between 1972 and 1995 in our laboratory were obtained from 4 blood donors and 12 patients with erythema, arthralgia, and acute or chronic anemia. One patient was asymptomatic. To analyze the nucleotide composition of the P6 promoter, we amplified a 247-bp fragment including the TATA box. This fragment corresponded to nucleotides 113–360 of the sequence published by Shade et al. (31). For each serum, two PCR products were sequenced independently. Twenty nucleotide modifications (18 substitutions, 1 deletion, and 1 insertion) were observed in comparison with the reference sequence (31) given an average of 1.5% of variation (data not shown). However, these mutations were not equally distributed on the P6 promoter and allowed two regions to be distinguished. All the mutations were situated between nucleotides 210 and 340, and a highly conserved region comprising nucleotides 113–210 was observed on the 5′ side of the amplified promoter. Even though this conserved region corresponded to the palindromic sequence of the hairpin terminus indispensable for parvovirus replication, we could not rule out the possibility that this region has an important role in regulating sable for parvovirus replication, we could not rule out the possibility that this region has an important role in regulating sable for parvovirus replication.

**Protection Analysis of the Highly Conserved P6 Region**—The ability of cellular proteins to bind the highly conserved P6 region was explored using in vitro DNase I footprinting assays. The double-stranded P6 probe covered nucleotides 78–304 of the sequence previously described (31). Only one extremity of the double strand probe was labeled alternatively and incubated with nuclear extracts from epithelial HeLa and erythroid UT7/Epo cells. A large protected region was observed within the conserved region with the nuclear extract from UT7/Epo cells (Fig. 1). By comparison with a DNA ladder, the protected region covered the nucleotides 129–146 (220/203) on the sense strand and nucleotides 129–150 (220/199) on the nonsense one (Fig. 1). The same footprint was observed with the extract from HeLa cells (data not shown). In the protected region, a YY1 binding site was described previously between nucleotides –220 and –212 (23) and an Sp1 binding site between nucleotides –200 and –195 (16, 22). Between these two protected regions, we noticed for the first time as far as we know, an Ets family consensus binding site CCGGAAGT located between nucleotides –208 and –201 (Fig. 2). These results suggested that nuclear proteins from HeLa or UT7/Epo cells were able to protect the nucleotide sequence spanning nucleotides 129–150 (220/199) in the highly conserved region of the P6 promoter.

**Sp1, YY1, and GABP Bind to the Conserved Part of the P6 Region**—To confirm the binding of eukaryotic proteins to nucleotides 129–150 (220/199) of the conserved region, we performed EMSA with the radiolabeled D probe (Fig. 2) corresponding to the binding sites of YY1 (220/212), EBS (208/201), and Sp1 (200/195). When the D probe was incubated with nuclear extracts from HeLa and UT7/Epo cells, it generated seven retarded complexes (Fig. 3A). The migration of six of these complexes was the same in each cell type, whereas the migration of the complex V was more delayed with HeLa proteins than with UT7/Epo proteins (compare lanes 2 and 8). All these seven complexes proved to be specific, since competition with a 30-fold excess of an unrelated sequence (the NFκB region of the HIV-1 long terminal repeat) did not affect their formation (Fig. 3A, lanes 4 and 10). This was confirmed by a competition assay with the cold D probe, which inhibited nucleoprotein complex formation (lanes 3 and 9). Experiments involving competition between the D probe and the cold A, B, C probes were used to link binding sites of the YY1, EBS, or Sp1
transcription factors with the cell protein complexes. The formation of complexes I and II was specifically inhibited by the presence of the cold C probe corresponding to the Sp1 binding site (lanes 7 and 13). The intensity of complex I was not the same with the two cell protein extracts, as the HeLa extract produced a stronger signal than the UT7/Epo, suggesting that different concentrations of the proteins were involved in this complex. Complexes III, IV, and V disappeared when the B probe corresponding to the EBS was used (lanes 6 and 12). Several complexes are usually observed for the EBS since different Ets proteins recognize the same DNA sequence (34). Nevertheless, complex V did not have the same mobility pattern with the two nuclear extracts, suggesting the binding of two different members of the Ets family. Finally, the A probe corresponding to the YY1 binding site inhibited the formation of complexes III and IV (lanes 5 and 11). Complex I formation was also inhibited by competition with the C probe (lanes 7 and 13), suggesting that its formation involved the binding of the two proteins YY1 and Sp1 to the D probe. The two other complexes, VI and VII, probably corresponded, respectively, to the binding of the complete and the truncated forms of the YY1 protein, as previously suggested (35, 36).

To identify the components of the seven complexes, we performed a gel shift assay with the D probe in the presence of specific antisera. Complex I was supershifted by the two polyclonal antisera raised against Sp1 and YY1 (Fig. 3, B and C, lanes 3 and 4). Complexes VI and VII, ascribed to the YY1 binding site, were shifted by the polyclonal antibody that recognized the YY1 factor (lane 3). Complex II was supershifted by the polyclonal antibody raised against Sp1 (lane 4). Among the eight antisera raised against the proteins of the Ets family, only anti-GABPα and GABPβ inhibited the formation of complexes III and IV (lanes 7 and 8). None of these antisera modified the migration of either of the two V complex. All these results were obtained with nuclear extracts from either nonerythroid HeLa cells (Fig. 3B) or erythroid UT7/Epo cells (Fig. 3C). These results provided evidence that Sp1, YY1, and GABPα and -β transcription factors all bind the D probe cor-

Fig. 3. Binding of eukaryotic nuclear proteins to the 3-fold sequence comprising the YY1, EBS, and Sp1 binding sites. EMSA were performed with the 32P-labeled wild-type 3-fold probe (oligonucleotide D). A, binding of erythroid and nonerythroid proteins. Binding reactions were obtained with 2.5 µg of nuclear extracts from UT7/Epo cells (lanes 2–7) or HeLa cells (lanes 8–13). Experiments were performed either in the absence of competitor oligonucleotide (lanes 1, 2, and 8) or in the presence of a 30-fold molar excess of competitor oligonucleotide (lanes 3–7 and 9–13). Competitors used: oligonucleotide D (lanes 3–9), oligonucleotide NF-κB (lanes 4–10), oligonucleotide A (lanes 5–11), oligonucleotide B (lanes 6–12), and oligonucleotide C (lanes 7–13). The seven retarded complexes are indicated as roman numerals (I–VII) on the left of the figure. B and C, identification of the factors involved in the complexes by supershift analysis. Binding was obtained in the presence of 2.5 µg of nuclear extracts from HeLa cells (B) or UT7/Epo cells (C). Polyclonal antibodies raised against the YY1 (lanes 8, 10, 12), Sp1 (lane 4), Ets transcription factors Fli-1 (lane 5), PU-1 (lane 10), Elf-1 (lane 9), and Spi-B (lane 12) were added. In the control, normal rabbit serum (NRS) was used (lane 11). D, binding of hGABPα and hGABPβ1–2 to the 3-fold sequence. One microliter of in vitro translated hGABPα and/or hGABPβ1–2 were assayed by EMSA (lanes 2–10) as indicated at the top of the figure. A 30-fold excess of oligonucleotide B was added as competitor in lanes 8–10. Nuclear extracts (Nuc Ext.) from HeLa cells (lane 11) or UT7/Epo cells (lane 12) were used. The complexes formed by hGABPα, hGABPβ and hGABPβ1, and hGABPα and hGABPβ2 are indicated by c, a, and b, respectively.
responding to the 3-fold sequence YY1-EBS-Sp1 of the B19 P6 promoter.

To confirm these results, we tested in vitro translated hGABPα, hGABPβ1, and hGABPβ2 proteins for binding to the EBS motif of the 3-fold sequence (Fig. 3D). hGABPα produced a complex c (lane 3), whereas neither hGABPβ1 nor hGABPβ2 alone bound to the D probe (lanes 4 and 5). In contrast, when a mixture of hGABPα and hGABPβ1 or of hGABPα and hGABPβ2 was tested, an intense band a or b of lower mobility was produced along with the abolition of complex c (lanes 6 and 7). The mobility of complexes a and b was very similar to that of bands III and IV produced by HeLa or UT7/Epo nuclear proteins (lanes 12 and 13). Furthermore, these complexes produced by hGABP proteins were specific, because competition with a 30-fold excess of probe A, corresponding to the EBS, inhibited their formation (lanes 9–11).

These data clearly demonstrate that bands III and IV formed by the nuclear extracts corresponded to the heterodimers of hGABPα/hGABPβ1 and hGABPα/hGABPβ2, respectively. In addition, the YY1 protein formed complexes VI and VII, and the Sp1 protein formed complex II. A band comprising Sp1 and YY1 was also detected (1), but no complex was composed of three proteins. Lastly, we were unable to identify the Ets factor involved in complex V.

Effect of Overexpression of YY1, hGABP, and Sp1 on Transcription—The transcriptional effect of the YY1, hGABP, and Sp1 factors on the 3-fold sequence was evaluated by transfection. We isolated the wild-type 3-fold sequence (nt −223/−192, corresponding to oligonucleotide D in Fig. 2) or the sequences mutated on the three different sites (oligonucleotides E, F, G in Fig. 2) upstream of the minimal promoter of the herpes simplex virus TK and the gene encoding the firefly luciferase. The resulting constructs, pD-TK-LUC, pE-TK-LUC, pF-TK-LUC, and pG-TK-LUC were co-transfected with expression vectors for Sp1, YY1, hGABPα, and -β (pPac-Sp1, pPac-YY1, pPac-GABPα, and pPac-GABPβ, respectively) into D. melanogaster SL2 cells, which are devoid of endogenous Sp1 and YY1 (29). After 48 h of incubation, luciferase activity was then estimated in cell extracts. Luciferase activity levels were calculated in relation to the activity in cells in which only the reporter plasmid was transfected. The results illustrated in Fig. 4A show that Sp1 protein activated LUC expression in a dose-dependent manner from the pD-TK-LUC plasmid harboring the wild-type 3-fold sequence, and that this activation was impaired by a mutation that suppressed the binding of Sp1 (Fig. 4A, compare lanes 1–3 with lane 4). On the other hand, YY1 protein had no significant effect on pD-TK-LUC transcription (Fig. 4A, lanes 5–7). When, the effect of hGABP proteins on the activity of the 3-fold sequence was similarly investigated, no activation was observed when each subunit was present alone (Fig. 4B, lanes 1–3). In addition, transcription was activated when hGABPα was coexpressed with hGABPβ1 or β2 (lanes 4–9). Nevertheless, the formation of an increased amount of β subunits reduced transcription activation. Such an effect was not observed with another viral promoter, the adenovirus E4 promoter (30). All these results indicate that Sp1 and hGABP complexes are able to transactivate the 3-fold sequence in SL2 cells. The YY1 transcription factor had no effect on transcription whatever the concentration of expression plasmid used.

Synergistic Activation by hGABP and Sp1—To observe the possible functional interplay between the Sp1, YY1, and hGABP factors, we performed co-transfection experiments in SL2 cells using the pD-TK-LUC plasmid, the reporter plasmid construction pB6-LUC, which included the entire promoter region (nt 102–480) and the expression plasmids for the factors. When the reporter plasmid pD-TK-LUC was used, as depicted
interaction. Nevertheless, this hypothesis is actually under investigation in our laboratory.

DISCUSSION

Contrary to other parvoviruses whose genetic expression is controlled by two functional promoters located on the left and the middle of the genome, only one promoter has been described for the human parvovirus B19. We therefore attempted to analyze its variability by PCR amplification and sequencing of the promoter region of 17 B19 virus strains. Our data show that YY1 had any effect on the 3-fold sequence or the P6 promoter, which is relieved by EIA proteins (21). For B19 parvovirus, the positive effect of YY1 on transcription was described by Momoeda et al. in HeLa cells but was very weak, i.e. 1.3–1.9-fold above basal transcription (23). We did not find that YY1 had any effect on the 3-fold sequence or the P6 promoter in SL2 cells. This difference may be due to the type of cells transfected.

The present study demonstrates, for the first time as far as we know, that the specific DNA-binding proteins for the CCGGAAGT motif of the human B19 parvovirus promoter is very likely to be hGABP, as indicated by the following results. (i) The DNA protein complex detected in the gel shift assay was abolished by the competition assay, (ii) antibodies against GABPs and β-subunits supershifted this complex, and (iii) the combination of in vitro translated hGABPs and β-proteins produced a complex with essentially the same mobility as that of the minute virus of mice and was also located immediately upstream from the GC box that binds the Sp1 transcription factor (37). Using EMSA with synthetic oligonucleotidic probes and competition assays with the corresponding probes, we confirmed the binding of YY1 previously observed by Momoeda et al. (23). Binding was also detected at this site for Sp1, contrarily to the findings of Liu et al. (16). The results were similar whether the extract used was from HeLa or UT7/Epo cells. Supershift analysis allowed us to establish that the Ets motif at nucleotides −208/−201 in the conserved B19 promoter region is recognized by hGABPα, a ubiquitously expressed Ets protein. In addition, antibodies confirmed that HeLa and UT7/Epo binding complexes contain proteins that are immunologically related to both hGABPα and hGABPβ. The complexes produced by the hGABP proteins were specific, as evaluated by probe competition. hGABP (also named E4TF1) is indeed composed of three distinct polypeptides: hGABPα (60 kDa), hGABPβ1 (53 kDa), and hGABPβ2 (47 kDa), all of which are required for high affinity DNA binding (α subunit) and transcriptional activation (α and β subunits) (38, 39). hGABP binds to a purine-rich cis-regulatory element required for the VP16-mediated activation of herpes simplex virus immediate early gene and regulates adenovirus E4 gene transcription (39, 40). We therefore investigated the possible involvement of hGABP in the regulation of the B19 promoter. hGABP was shown to activate a 3-fold sequence comprising YY1-GABP-Sp1 binding sites with the TK minimal promoter in Drosophila SL2 cells; this activation was also found with the P6 promoter. It is noteworthy that our GABP binding site was immediately adjacent to the Sp1 site. Ets-related transcription factors such as hGABP are often found in large complexes with other transcription factors (41–47). For example, Ets-1 and Sp1 interact to activate synergistically the human T-cell lymphophotic virus long terminal repeat (29). In addition, Sp1 activity is known to be modulated by factors that recognize the DNA elements flanking or overlapping a GC box (48, 49). In the present work, Sp1 transactivated the 3-fold sequence and the P6 promoter and displayed synergistic activation with hGABP. Similarly, by co-transfection experiments using also Drosophila SL2 cells, the P4 promoter of minute virus of mice was found to be transactivated synergistically by Ets-1, the prototype member of the Ets family of transcription factors, and the Sp1 factor that binds to a GC box flanking the EBS motif (37). In our study, the mutations of the GABP and Sp1 sites suggest that the combined synergistic effect of the corresponding transcription factors seems to incriminate DNA binding but also protein interactions. Whatever the precise mechanism under investigation, this cooperation was partially inhibited by YY1 protein. In adeno-associated virus, YY1 was found to act as a repressor of transcription from the adeno-associated virus P5 promoter, which is relieved by EIA proteins (21). For B19 parvovirus, the positive effect of YY1 on transcription was described by Momoeda et al. in HeLa cells but was very weak, i.e. 1.3–1.9-fold above basal transcription (23). We did not find that YY1 had any effect on the 3-fold sequence or the P6 promoter in SL2 cells. This difference may be due to the type of cells transfected.

The present study demonstrated, for the first time as far as we know, that the specific DNA-binding proteins for the CCGGAAGT motif of the human B19 parvovirus promoter is very likely to be hGABP, as indicated by the following results. (i) The DNA protein complex detected in the gel shift assay was abolished by the competition assay, (ii) antibodies against GABPs and β-subunits supershifted this complex, and (iii) the combination of in vitro translated hGABPs and β-proteins produced a complex with essentially the same mobility as that
produced by the HeLa or UT7/Epo cell extract. Lastly, our results clearly demonstrated that in nonerythroid cells, hGABP proteins, ubiquitously expressed Ets protein, stimulate the expression of the human B19 parvovirus promoter. The precise mechanism of the synergy exerted by hGABP and Sp1, which is diminished by YY1, is currently under investigation.

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REFERENCES

1. Berns, K. I., Bergoin, M., Lederman, M., Muzykant, N., Siegl, G., Tal, J., and Tattersall, P. (1994) Arch. Virol. Suppl. 10, 166–178
2. Brown, K. E., Young, N. S., and Liu, J. M. (1994) Crit. Rev. Oncol. Hematol. 16, 1–31
3. Mortimer, P. P., Humphries, R. K., Moore, J. G., Purcell, R. H., and Young, N. S. (1983) Nature 302, 426–429
4. Takahashi, T., Ozawa, K., Takahashi, K., Asano, S., and Takaku, F. (1990) Blood 75, 603–610
5. Ozawa, K., Kurtzman, G., and Young, N. (1986) Science 233, 885–886
6. Pallier, C., Green, A., Le Junter, J., Saib, A., Vassias, I., and Morinet, F. (1997) J. Virol. 71, 9412–9429
7. Srivastava, A., Bruno, E., Bridgell, B., Cooper, R., Srivastava, C., van Besien, K., and Hoffman, R. (1990) Blood 76, 1997–2004
8. Leruez, M., Pallier, C., Vassias, I., Elsouf, J. F., Romeo, P., and Morinet, F. (1994) J. Virol. 75, 1475–1478
9. Luo, W., and Astell, C. R. (1993) Virology 195, 448–455
10. Ozawa, K., Ayub, J., Hao, Y. S., Kurtzman, G., Shimada, T., and Young, N. (1987) J. Virol. 61, 2385–2406
11. Cotmore, S. F., McKie, V. C., Anderson, L. J., Astell, C. R., and Tattersall, P. (1986) J. Virol. 60, 548–558
12. Deiss, V., Tratschin, J. D., Weitz, M., and Siegl, G. (1990) Virology 175, 247–254
13. Blundell, M. C., Beard, C., and Astell, C. R. (1987) Virology 157, 534–538
14. Doering, C., Hirt, B., Antonietti, J. P., and Beard, P. (1990) J. Virol. 64, 387–396
15. Wang, X. S., Yoder, M. C., Zhou, S. Z., and Srivastava, A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 12416–12420
16. Liu, J. M., Green, S. W., Hao, Y. S., McDonagh, K. T., Young, N. S., and Shimada, T. (1991) Virology 182, 361–364
17. Liu, J. M., Fujii, H., Green, S. W., Komatsu, N., Young, N. S., and Shimada, T. (1991) Virology 182, 361–364
18. Leruezville, M., Vassias, I., Pallier, C., Cecille, A., Hazan, U., and Morinet, F. (1990) J. Virol. 74, 215–219
19. Moffatt, S., Tanaka, N., Tada, K., Nese, M., Nakamura, M., Muraoka, O., Hirano, T., and Sugamura, K. (1996) J. Virol. 70, 8485–8491
20. Sol, N., Morinet, F., Alizon, M., and Hazan, U. (1996) J. Virol. 74, 2011–2014
21. Shi, Y., Seto, E., Chang, L. S., and Shenk, T. (1991) Cell 67, 377–388
22. Blundell, M. C., and Astell, C. R. (1989) J. Virol. 63, 4814–4823
23. Momoea, M., Kawase, M., Jane, S. M., Miyamura, K., Young, N. S., and Kajigaya, S. (1994) J. Virol. 68, 7159–7168
24. Shimomura, S., Komatsu, N., Frichkofen, N., Anderson, S., Kajigaya, S., and Young, N. S. (1992) Blood 79, 18–24
25. Komatsu, N., Nakauchi, H., Miwa, A., Ishihara, T., Eguchi, M., Morii, M., Okada, M., Sato, Y., Wada, H., and Yawata, Y. (1991) Cancer Res. 51, 341–348
26. Schneider, I. (1972) J. Embryol. Exp. Morphol. 27, 355–365
27. Hazan, U., Thomas, D., Alcamis, J., Bachelier, F., Israel, N., Yssel, H., Virelizier, J. L., and Arenzana-Seisdedos, F. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 7861–7865
28. Ham, J., Dostatni, N., Arslan, F., and Yaniv, M. (1991) EMBO J. 10, 2893–2900
29. Geonoge, A., Bossutet, R., Bailly, R. A., and Ghysdael, J. (1993) EMBO J. 12, 1169–1176
30. Sawa, C., Goto, M., Suzuki, F., Watanabe, H., Sawada, J., and Handa, H. (1996) Nucleic Acids Res. 24, 4954–4961
31. Shade, R. O., Blundell, M. C., Cenmore, S. F., Tattersall, P., and Astell, C. R. (1986) J. Virol. 58, 921–936
32. Andrews, N. C., and Faller, D. V. (1991) Nucleic Acids Res. 19, 2499
33. Chen, C., and Okayama, H. (1987) Mol. Cell. Biol. 7, 2745–2752
34. Wasylyk, C., Kerkdael, J. P., and Wasylyk, B. (1992) Gene 96, 965–974
35. Klug, J., and Beato, M. (1996) Mol. Cell. Biol. 16, 6398–6407
36. Sekenik, J. L., Chen, D., Bradley, M. E., Chen, S. J., and Lee, T. C. (1997) Nucleic Acids Res. 25, 843–849
37. Fukas, F., Deleu, L., Dinsart, C., Rommeelaere, J., and Faisst, S. (1996) J. Virol. 70, 1331–1339
38. Sawa, C., Goto, M., Sawa, C., Watanabe, H., and Handa, H. (1994) EMBO J. 13, 1396–1402
39. Watanabe, H., Sawa, J., Yano, K., Yamaguchi, K., Goto, M., and Handa, H. (1993) Mol. Cell. Biol. 13, 3385–3391
40. Thompson, C. C., Brown, T. A., and McNight, S. L. (1991) Science 253, 762–768
41. Wotton, D., Ghysdael, J., Wang, S., Speek, N. A., and Owen, M. J. (1994) Mol. Cell. Biol. 14, 840–850
42. Pongubala, J. M., Nagalapally, S., Klun, M. J., McKeerher, S. R., Maki, R. A., and Atchison, M. L. (1992) Mol. Cell. Biol. 12, 368–378
43. Wasylyk, B., Hahn, S. L., and Giovane, A. (1993) Eur. J. Biochem. 211, 7–18
44. Rossman, A. G., Caprio, D. G., Kirsch, D. G., Handa, H., and Simkevich, C. P. (1995) J. Biol. Chem. 270, 23627–23633
45. Sadavasian, E., Cedeno, M. M., and Rothenberg, S. P. (1994) J. Biol. Chem. 269, 4725–4735
46. Fitzsimmons, D., Hodosi, W., Wheat, W., Maira, S., Wasylyk, B., and Hayman, J. (1996) Genes Dev. 10, 2195–2211
47. Dittmer, J., Pese-Maison, C. A., Clemens, K. E., Choi, K.-S., and Brady, J. N. (1997) J. Biol. Chem. 272, 4953–4958
48. Fischer, K. D., Haase, A., and Nowock, J. (1993) J. Biol. Chem. 268, 23915–23923
49. Perkins, N. D., Edwards, N. L., Dukett, C. S., Agranoff, A. B., Schmid, R. M., and Nabil, G. J. (1993) EMBO J. 12, 3551–3558
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