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YY1 Is the Cellular Factor Shown Previously To Bind to Regulatory Regions of Several Leaky-Late ($\beta\gamma$, γ_1) Genes of Herpes Simplex Virus Type 1

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We have recently reported that a cellular factor, the leaky-late binding factor (LBF), binds to sites in a number of leaky-late ($\beta\gamma$ or γ_1) genes of herpes simplex virus type 1 and that an LBF site is essential for maximum viral transactivation of the major capsid protein (VP5) gene. The results of binding competition, partial proteolysis, and monoclonal antibody inhibition assays presented here establish that LBF is identical to the transcription factor YY1. This, along with our previous observations, suggests that YY1 plays a role in herpes simplex virus type 1 gene regulation.

We recently reported that a nuclear factor from HeLa cells, which we call the leaky-late binding factor (LBF), binds to promoter and internal sites in a number of herpes simplex virus type 1 (HSV-1) genes of the leaky-late ($\beta\gamma$ or γ_1) kinetic class. These include the major capsid protein (VP5), glycoproteins D (gD) and B (gB), and UL37 genes (4). We further demonstrated that the LBF binding site (LBS), having the consensus sequence GGCCATNTT, is required for maximum transactivation of the VP5 promoter by superinfected HSV-1 or cotransfected HSV-1 immediate-early genes ICP0, ICP4, and ICP27 (4). Analyses of binding site sequences suggested that the LBF might be related to the murine transcription factor common factor 1 (CF1) (14), also known as UCRBP (6) or δ (7), the avian factor F-ACT1 (10, 11), and the human counterpart YY1 (16, 17), also known as NF-E1 (13) or MAPF1 (18).

Competitions by oligonucleotides containing CF1 and LBF binding sites. To determine whether LBF is related to CF1, a factor shown previously to bind to several cellular promoters including that of c-*myc* (14), we performed gel shift competition assays as previously described (4) with ^{32}P-labeled promoter fragments from the VP5, gD, and human c-*myc* genes (Table 1). The human c-*myc* promoter, like that of its murine counterpart, contains a CF1 site (at −236 bp relative to its P1 cap site [8]). Double-stranded oligonucleotides containing or lacking LBF or CF1 sites (Table 2) were used as competitors. As we have shown previously (4), the VP5 and gD promoter fragments yielded two major complexes, A and B, (Fig. 1). These complexes footprint over the same site in the VP5 promoter (4), and evidence discussed later suggests that complex B is formed by a proteolytic cleavage product of the protein that forms complex A. The c-*myc*-353 fragment likewise produced two complexes having relative mobilities similar to those observed with the two HSV-1 promoter DNAs (Fig. 1, lane 13). The pattern of complexes formed by the three DNA probes was the same whether uninfected or 8-h HSV-1-infected nuclear extracts were used. Most importantly, excess

TABLE 1. DNA fragments, plasmids, and sources

Gene	DNA fragments (position)[a]	LBS homolog within fragment (position)[a]	Plasmid (source [reference])	Plasmid composition[a]
HSV-1 VP5	*Sal*I-*Hin*dIII (−168 to −4)	GGCCATCTTGAA (−70)	pVP5 (−168) CAT[b] (E. Blair and E. Wagner [2])	HSV-1 VP5 promoter (+4 to −168) linked to CAT
HSV-1 gD	*Pvu*II-*Hin*dIII (−259 to +11)	GGCCATtTTacg (−63)	pP017 (G. Hayward[c])	HSV-1 (MP) 4.9-Kb *Hin*dIII N fragment into pCATB
	*Eag*I-*Hin*dIII (−121 to +11)	GGCCATtTTacg (−63)	pgDCAT (R. Everett [5])	HSV-1 gD promoter (−392 to +11) linked to CAT
Human c-*myc*	*Hin*dIII-*Sma*I (−353 to −101)[d]	TACCATtTTct (−236)[e]	c-*myc*-353 (N. Hay [8])	Human c-*myc* DNA (−353 to +513) linked to CAT

[a] Base pairs are relative to the cap site.
[b] CAT, bacterial chloramphenicol acetyltransferase gene.
[c] Department of Pharmacology and Molecular Sciences, Johns Hopkins School of Medicine, Baltimore, Md.
[d] In this plasmid construct, the *Pvu*II site at position −353 in the c-*myc* promoter has been replaced with *Hin*dIII.
[e] Includes a CF1 binding site (underlined).

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TABLE 2. Oligonucleotides[a]

Name	Source of sequence	Sequence
LBS[b]	LBS of HSV-1 VP5 (−70)[c]	CCAGGATCCAGGGCCATCTTGAATGGATCCTGG
CF1[d]	CF1 binding site of murine c-*myc* promoter (−260)	GGGCGCGAGAAGAGAAAATGGTCGGGC
C2[d]	μE3 binding site of murine immunoglobulin H intronic enhancer	CCTTGCCATGACCTGCTTCCT
YY1a[e]	YY1 binding site from AAV[f] P5 promoter (−60)	GATCTCCATGGTTTTGCGACATTTTGCGACA
YY1b[e]	YY1 binding site from AAV P5 initiator (+1)	AGGGTCTCCATTTTGAAGCGGG

[a] All oligonucleotides are double stranded, and all sites homologous to YY1 binding sites are underlined.
[b] Obtained from the Vollum Institute, Oregon Health Sciences University (4).
[c] Base pairs are relative to the cap site.
[d] Gift from K. Riggs and C. Calame, Columbia University (14).
[e] Gift from E. Seto and T. Shenk, Princeton University (17).
[f] AAV, adeno-associated virus.

amounts of unlabeled oligonucleotides containing either the VP5 LBF or the murine CF1 site effectively inhibited formation of complexes A and B by each of the three labeled promoters (Fig. 1). A control oligonucleotide (C2), lacking an LBS or CF1 site, failed to compete significantly (Fig. 1). These results suggest that the two major DNA-protein complexes observed with the VP5, gD, or c-*myc* promoter fragments are formed by the same or similar cellular proteins, and this protein(s) has a binding specificity similar to that of murine CF1.

LBF may be related to transcription factor YY1. Because of binding site similarities and recent reports indicating that CF1 is the murine counterpart of YY1 (13), we tested two oligonucleotides (YY1a and YY1b [Table 2]) containing known YY1 binding sites from adeno-associated virus as competitors

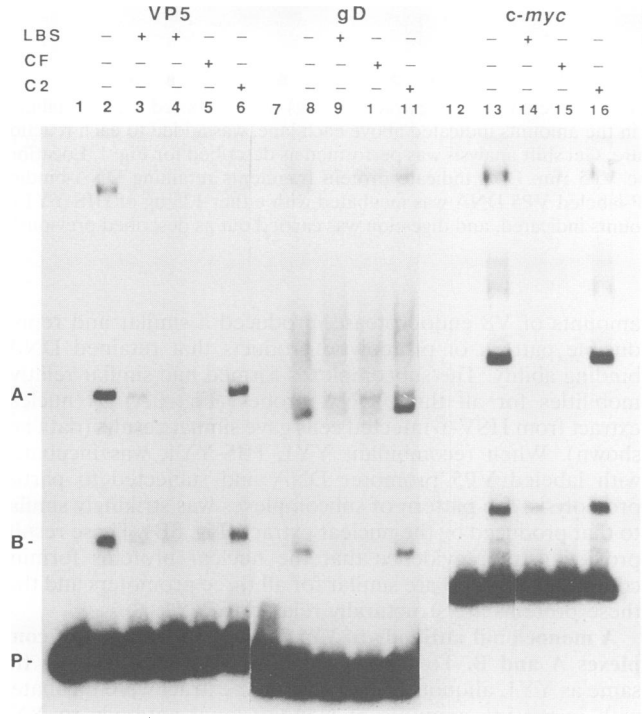

FIG. 1. Inhibition of VP5, gD, and c-*myc* promoter DNA complexes by LBS or CF1 oligonucleotides. Uninfected nuclear extract (1.5 μg of protein) was incubated with ^{32}P-labeled promoter fragments from HSV-1 VP5 (−4 to −168), gD (−259 to +11), or human c-*myc*-353 (−353 to −101) genes (base pairs relative to cap site). Competing unlabeled double-stranded oligonucleotides were included in the reactions as indicated. VP5 LBS was added at 150-fold (lane 3) or 300-fold (lanes 4, 9, and 14) molar excess of probe. Both CF1 (lanes 5, 10, and 15) and C2 (lanes 6, 11, and 16) were at 300-fold excess of probe. Complexes (A and B) and free probe (P) are indicated. Polyacrylamide gels (4%) were run at 250 V for 2.25 h in 1× nonrecirculating buffer (DBB-1 [3]).

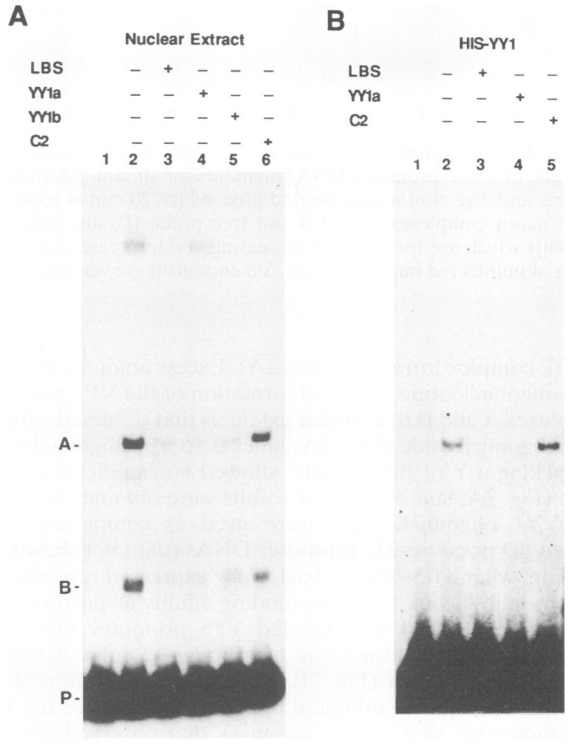

FIG. 2. Inhibition of VP5 promoter complex formation by oligonucleotides containing YY1 binding sites. Electrophoretic gel shift and competition assays were performed as described in the legend to Fig. 1 with ^{32}P-labeled VP5 promoter DNA. (A) Uninfected nuclear extract was incubated at 1.5 μg per reaction mixture with ^{32}P-labeled VP5 DNA (lanes 2 to 6). The following unlabeled oligonucleotides (described in Table 2) were added at 300-fold excess of labeled probe: LBS (lane 3) and YY1a (lane 4) and YY1b (lane 5), containing YY1 binding sites from the adeno-associated virus P5 promoter at −60 and +1, respectively, and C2 (lane 6), a control oligonucleotide lacking YY1 binding sites. (B) HIS-YY1 (125 ng) was incubated with labeled VP5 DNA. Unlabeled oligonucleotides, added at 300-fold excess, were LBS (lane 3), YY1a (lane 4), and C2 (lane 5).

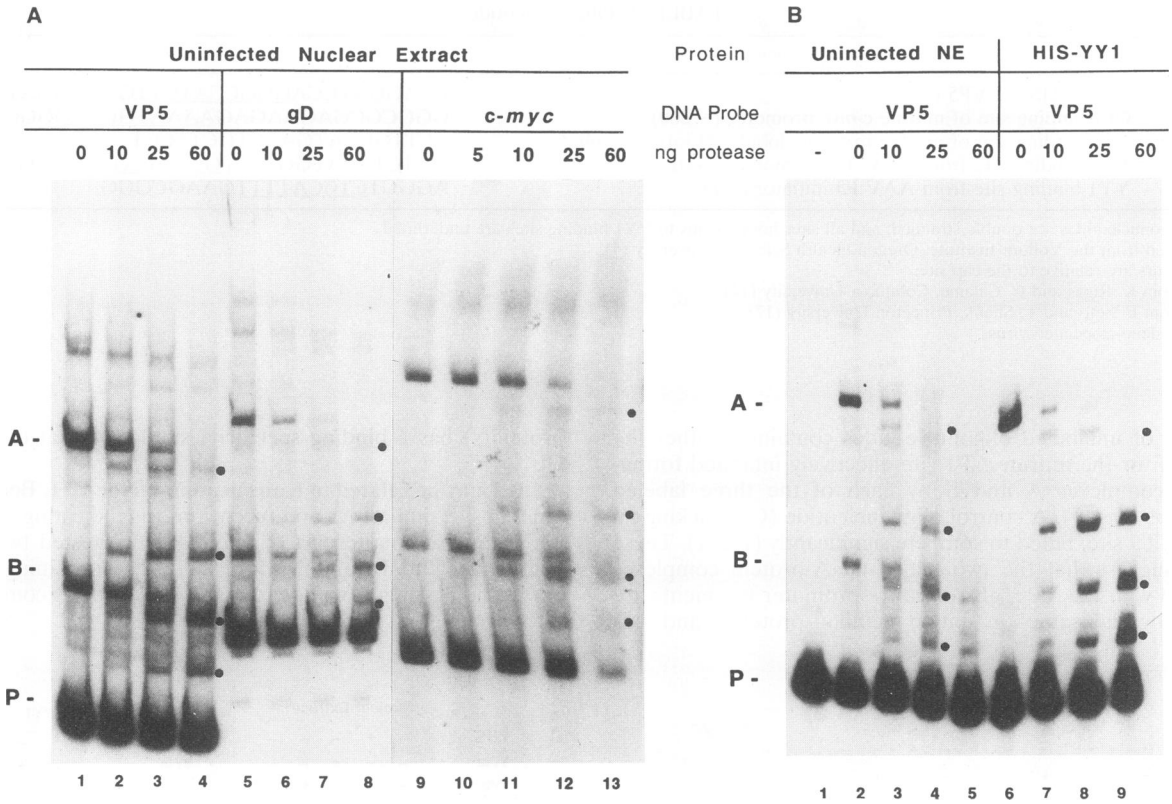

FIG. 3. Partial proteolytic band-clipping analysis by V8 endoprotease. (A) Uninfected nuclear extract (2.4 μg) was incubated with ^{32}P-labeled VP5, gD, or c-*myc* promoter DNA fragments for 10 min. V8 endoprotease, in the amounts indicated above each lane, was added to each reaction mixture, and digestion was allowed to proceed for 20 min at room temperature. Gel shift analysis was performed as described for Fig. 1. Locations of the major complexes A and B and free probe (P) are indicated for the VP5 run. Dots indicate protein fragments retaining DNA-binding capability which are formed with increasing endoprotease digestion. (B) ^{32}P-labeled VP5 DNA was incubated with either 125 ng of HIS-YY1 or 2.4 μg of uninfected nuclear extract. V8 endoprotease was added in the amounts indicated, and digestion was carried out as described previously.

of LBF complex formation (Fig. 2A). Excess amounts of either YY1 oligonucleotide inhibited formation of the VP5 promoter complexes A and B to a similar extent as that observed with the LBS oligonucleotide (Fig. 2A, lanes 3 to 5). Oligonucleotide C2, lacking a YY1 binding site, showed no significant competition (Fig. 2A, lane 6). Similar results were obtained when the two YY1 oligonucleotides were used as competitors with labeled gD or c-*myc*-353 promoter DNAs (data not shown). In addition, when HIS-YY1, a bacterially expressed recombinant YY1 protein having the same binding affinity as purified YY1 (17), was incubated with labeled VP5 promoter DNA, the complex formed had the same mobility as complex A formed by the nuclear extract (Fig. 2B, 3B, and 4). Formation of this complex was likewise inhibited by excess amounts of the LBS oligonucleotide to a similar extent as that observed with the YY1 oligonucleotide YY1a (Fig. 2B, lanes 3 and 4). Excess amounts of C2 failed to inhibit complex formation (Fig. 2B, lane 5). These results strongly suggest that LBF is the same as, or is closely related to, YY1.

Partial proteolysis with *Staphylococcus aureus* V8 endoprotease. To further establish whether the protein(s) forming complexes A and B with the VP5, gD, and c-*myc* promoters was related to YY1, we carried out a proteolytic clipping bandshift assay (9, 14, 15) of the complexes with V8 endoprotease. The resulting subcomplexes were separated and identified by gel shift analysis (Fig. 3). Each of the three probes, when incubated with uninfected nuclear extract and increasing amounts of V8 endoprotease, produced a similar and reproducible pattern of proteolytic products that retained DNA-binding ability. The subcomplexes formed had similar relative mobilities for all three DNA probes (Fig. 3A). A nuclear extract from HSV-1-infected cells gave similar results (data not shown). When recombinant YY1, HIS-YY1, was incubated with labeled VP5 promoter DNA and subjected to partial proteolysis, the pattern of subcomplexes was strikingly similar to that produced by the nuclear extract (Fig. 3B). These results provide further evidence that the nuclear proteins forming complexes A and B are similar for all three promoters and that these proteins are structurally related to YY1.

A monoclonal antibody to YY1 inhibited formation of complexes A and B. To further establish whether LBF was the same as YY1, aliquots of HeLa nuclear extract were incubated with increasing amounts of a monoclonal antibody to YY1 (anti-YY1) for 5 to 10 min at room temperature prior to complex formation with labeled VP5 promoter DNA (16). (Anti-YY1, produced by Y. Shi in the laboratory of T. Shenk, Princeton University, was previously shown to inhibit the formation of YY1-DNA complexes.) The resulting complexes were analyzed by gel mobility shift assay (Fig. 4). Increasing amounts of anti-YY1 inhibited the formation of complex A and, to a lesser but significant extent, complex B (Fig. 4, lanes 3 and 4). The same amount of a control monoclonal antibody (anti-HSV ICP4, lanes 5 and 6), or two other nonrelated antibodies (anti-gB or anti-HSV ICP0 [data not shown]), had

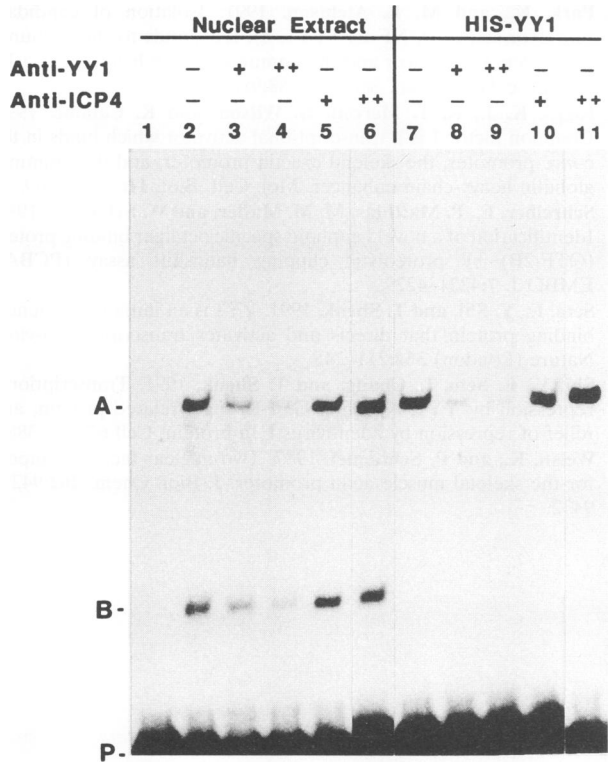

FIG. 4. Inhibition of VP5 complex formation by a YY1 monoclonal antibody. ^{32}P-labeled VP5 promoter DNA was incubated with either uninfected cell nuclear extract (1.5 μg) (lanes 2 through 6) or HIS-YY1 (250 ng) (lanes 7 through 11). Anti-YY1 was added at 1.2 μg (lanes 3 and 8) or 2.4 μg (lanes 4 and 9). A control antibody (anti-ICP4, provided by W. C. McClements) was added at 1.2 μg (lanes 5 and 10) or 2.4 μg (lanes 6 and 11). Gel shift assays were performed as described for Fig. 1.

little effect. Complex formation by the recombinant YY1 protein HIS-YY1 was inhibited to a similar extent by anti-YY1 but not by the control antibodies (Fig. 4, lanes 7 to 11). These results provide further evidence that the protein forming complex A of the LBF is the same as transcription factor YY1. Additionally, these results are consistent with the hypothesis that complex B is formed by a proteolytic product of the protein that generates complex A, factor YY1.

Relationship of complexes A and B to factor YY1. We have consistently observed two major complexes, A and B, in gel shift assays with HeLa nuclear extracts and DNA probes containing LBF or YY1 sites. Our previous DNA footprint analyses (4) and the observation that a 27-bp oligonucleotide containing a single VP5 LBF site formed the two complexes (11a) indicate that these two complexes involve the same binding site. Furthermore, a number of observations strongly indicate that the protein forming the smaller complex (B) is structurally related to the one forming the larger complex (A) and that the protein forming complex A is YY1. (i) Formation of the two LBF complexes on VP5, gD, and c-*myc* promoter fragments was inhibited by oligonucleotides containing binding sites for LBF, CF1, or YY1 (Fig. 1 and 2A). (ii) Formation of an HIS-YY1 complex with the VP5 promoter fragment was inhibited by an oligonucleotide containing YY1 binding sites (Fig. 2B). (iii) Partial proteolysis with V8 endoprotease yielded the same pattern of protein-DNA subcomplexes with either HeLa nuclear extracts or HIS-YY1 (Fig. 3). (iv) A monoclonal antibody specific for human YY1 inhibited LBF complex formation by a HeLa nuclear extract (Fig. 4).

These results further suggest either that two structurally related forms of YY1 exist in the cell or that the smaller complex is the result of proteolysis of YY1 before and/or during nuclear extract preparation. Two observations indicate that the latter possibility is the most likely one. (i) A complex having mobility similar to that of complex B was shown to be formed by proteolysis of F-ACT1 (a chicken homolog of YY1) (9, 11). (ii) Incubation of a HeLa nuclear extract with a crude control antibody preparation containing endogenous protease activity resulted in an increase in complex B and decrease in complex A with the HeLa nuclear extract and the conversion of complex A to complex B when HIS-YY1 was used (11a).

In summary, we have presented several lines of evidence supporting the conclusion that the cellular protein, previously referred to as the LBF (4), is the same as transcription factor YY1 (17), also known as CF1 (14), UCRBP (6), δ (7), NF-E1 (13), F-ACT1 (10, 11), and MAPF1 (18). This factor has been shown to act as either a repressor (1, 6, 10–13, 17) or activator (7, 14, 16, 17) of viral and cellular gene transcription. The results of studies presented here, taken together with our previous analyses of the VP5 promoter in transient assays (4) and preliminary results from analyses of point mutations in the YY1 binding site of the HSV-1 gD promoter (11a), indicate that factor YY1 also plays a role in the transactivation of certain HSV-1 βγ genes by viral factors.

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ADDENDUM IN PROOF

Riggs et al. have recently published results showing that CF1 is identical to YY1 (K. J. Riggs, S. Saleque, K.-K. Wong, K. T. Merrell, J.-S. Lee, Y. Shi, and K. Calame, Mol. Cell. Biol. **13**:7487–7495, 1993).

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