The HCMV IE2 protein negatively autoregulates its own expression as well as represses the transactivation activity of p53. Using the repression domain of IE2 as bait in the yeast two-hybrid system, Nrf1 and Nrf2, members of the CNC-bZIP family, were found to be IE2-interacting proteins. Residues 331–448 encompassing the DNA-binding and the dimerization domains of Nrf1 are sufficient for the interaction. The interaction was further confirmed in vitro by a glutathione S-transferase pull-down assay and in vivo by co-immunoprecipitation. In transient transfection studies, transcription driven by six copies of an NF-E2 site or by chimeric proteins of the CNC-bZIP family is repressed by IE2. Importantly, the DNA binding activity of the Nrf1/MafK heterodimer is not impeded by IE2. In a parallel study, CNC-bZIP factors attenuate the negative autoregulation of IE2. The attenuation could be explained by the finding that Nrf1 functions alone and synergistically with its heterodimerization partner, MafK, in inhibiting the DNA binding activity of IE2. Taken together, these results demonstrate the existence of antagonism between members of the CNC-bZIP family and IE2.

Human cytomegalovirus (HCMV), a member of the beta sub-group of herpesviruses, has a double-stranded DNA genome of 229,354 base pairs with a potential to encode more than 200 proteins (1). A number of immediate-early (IE) proteins of HCMV are made following entry of the virus into cells (2). Among them the IE2 86-kDa protein (referred hereafter as IE2) is most studied. IE2 appears to be a promiscuous transactivator of viral and cellular gene expression (3–5). IE2 stimulates transcription by interacting with general transcription factors and/or gene-specific factors (6–9). The DNA-binding activity of IE2, we isolated several cellular factors, including a member of the CNC-bZIP family, which is also required for its transcriptional activation, DNA binding, and interaction with CREB, c-Jun, RB, TBP, and TFII B (6–9). The DNA-binding and the repression domains of IE2 seem to be overlapping, because an IE2 mutant devoid of DNA binding activity also loses its repression activity (16).

A subset of bZIP proteins sharing a conserved structural domain, called the CNC domain, were recently cloned (17–20). These CNC-bZIP proteins are involved in the developmental processes as well as participate in the regulation of gene expression in the adult (21–26). Members of the CNC-bZIP family bind specifically to a DNA sequence, the NF-E2 binding site, and the repression domains of IE2 seem to be overlapping. These CNC-bZIP factors are completely different in the activation domain.

To further our knowledge about how host cells modulate the autoregulation and the transcriptional repression activities of IE2, we isolated several cellular factors, including a member of the CNC-bZIP family. In this report, we characterized the functional interaction between members of the CNC-bZIP family and IE2.

**MATERIALS AND METHODS**

**Plasmid Constructions**—Plasmids pSG424, pET11d, pIE2, pSV2CAT pBXL1, pLexA-Vp16, and pL6E1bCAT have been described (16, 31–33). pGAL4DB-IE2C was constructed by inserting a DNA fragment encoding residues 290–579 of IE2 between the NcoI and SacI sites of pACT2 (CLONTECH). pGAL4AD-NrF1 was cloned by inserting a DNA fragment encoding residues 1–589 of Nrf2 between the NcoI and SacI sites of pACT2. pGAL4AD-NrF1 was constructed by inserting a DNA fragment encoding residues 1–589 of Nrf2 between the BamHI and the SacI sites of pACT2.
pACT2, pGAL4AD-Nrf1(1–330), pGAL4AD-Nrf1(331–447), pGAL4AD-Nrf1(331–394), pGAL4AD-Nrf1(331–356), and pGAL4AD-Nrf1(395–447) were cloned by inserting DNA fragments encoding the corresponding peptide of Nrf1 residues 1–330, 331–447, 331–394, 331–356, and 395–447, respectively, between the BamHI and the EcoRI sites of pACT2, respectively. pGEM4-NF-E2 and pGEM4-Nrf1 were cloned by inserting, respectively, the NF-E2 p45 and Nrf1 cDNAs between the EcoRI and the XbaI sites of pGEM4 (Promega). pGEM3-Nrf2 was constructed by inserting the Nrf2 cDNA between the BamHI and the SacI sites of pGEM3 (Promega). Plasmids for the expression of histidine-tagged IE2 and MafK were constructed by inserting the corresponding cDNA fragment between the NdeI and the BamHI sites of pET11d. The plasmid for the expression of histidine-tagged Nrf1 was constructed by inserting the Nrf1 cDNA between the NdeI and the XhoI sites of pET11d. pNrf1 was constructed by inserting the Nrf1 cDNA between the HindIII and the XbaI sites of pSG424. pNrf2 was constructed by inserting the Nrf2 cDNA between the HindIII and the SacI sites of pSG424. pSVTATACAT was constructed by replacing the BamHI/XbaI fragment of pE1bCAT with an oligonucleotide consisting of the TATA box (5'-CTAATTTTTTATTTATGCAG-3') of SV40 promoter. pN6CAT was constructed by inserting six copies of an NF-E2 binding site (5'-GCACAGCAATGCTGAGTCATGATGAGTCATGCTG-3') into the BamHI site of pSVTATACAT, pRK5F-LexA-NF-E2, pRK5F-LexA-Nrf1, and pRK5F-LexA-Nrf2 were cloned by inserting a corresponding DNA fragment encoding fusion protein LexA-NF-E2, LexA-Nrf1, and LexA-Nrf2, respectively, between the SmaI and the XbaI

**FIG. 1. Interaction of CNC-bZIP factors with the HCMV IE2.** *Left column*, GAL4 DNA-binding domain (amino acids 1–147) hybrids. *Middle column*, GAL4 activation domain (amino acids 768–881) hybrids. *Right column*, yeast colony color after transformation; the relative color is in parentheses. No colony indicates that no yeast colony was observed after incubation for a standard period of time.

**FIG. 2. The C terminus of Nrf1 is required and sufficient for the interaction with IE2.** *Left column*, the GAL4 DNA-binding domain hybrid, GAL4DB-IE2C. *Middle column*, the GAL4 activation domain hybrid, GAL4AD-Nrf1, and its derivatives. *Right column*, yeast colony color after transformation; the relative color is in parentheses. The Nrf1 frag-

| DNA binding hybrid | Activation hybrid | Colony color |
|--------------------|------------------|--------------|
| 1. GAL4DB IE2C     | GAL4AD NF-E2     | white        |
| 2. GAL4DB IE2C     | GAL4AD Nrf1(1–330) blue (+) |
| 3. GAL4DB IE2C     | GAL4AD Nrf1(331–394) white |
| 4. GAL4DB IE2C     | GAL4AD Nrf1(331–356) white |
| 5. GAL4DB IE2C     | GAL4AD Nrf1(395–447) no colony |
| 6. GAL4DB IE2C     | GAL4AD Nrf2(1–330) no colony |
| 7. GAL4DB IE2C     | GAL4AD Nrf2(331–394) white |
| 8. GAL4DB IE2C     | GAL4AD Nrf2(331–356) white |

The relative positions of CNC (stripes), basic (vertical dashes), and ZIP (diamonds) domains of Nrf1 are also depicted.
Interaction of CNC-bZIP Proteins with HCMV IE2

from beads with buffer D containing 0.1 M glutathione and analyzed by electrophoresis on a 10% SDS-polyacrylamide gel.

**Antibody Preparation and Immunoprecipitation**—Polyclonal antibodies against Nrf1, MaFk, and IE2 were produced in rabbits according to standard protocols, using purified histidine-tagged Nrf1, MaFk and IE2 proteins as antigens, respectively. Protein A-Sepharose beads (CL-4B, Sigma) were pre-equilibrated overnight in IP buffer (10% glycerol, 50 mM HEPES-KOH, pH 7.3, 100 mM potassium glutamate, 0.5 mM DTT, 6 mM magnesium acetate, 1 mM EGTA, 0.1% Nonidet P-40, and 0.5 mg/ml bovine serum albumin). Bead slurry (40 µl) was spun in a microcentrifuge, and the beads were resuspended in 40 µl of fresh IP buffer. Cell lysate (700 µl) and 2 µl of anti-Nrf1 serum were added to the beads, mixed and rotated for 4 h at 4 ºC. The reactions were then spun in a microcentrifuge, and the supernatant was removed. Beads were washed with 1 ml of IP buffer three times. The beads were then resuspended in 40 µl of loading dye. The presence of IE2 in the immunoprecipitates was detected by Western blotting using antibodies against IE2 after being resolved on a 10% SDS-polyacrylamide gel.

**RESULTS**

Isolation and Identification of Members of the CNC-bZIP Family as IE2-interacting Proteins—In a search for proteins that specifically interact with the autoregulation and repression domain of IE2, we screened a human lymphocyte MATCH-MAKER activation domain library (CLONTECH) using a chimeric protein composed of the C terminus (residues 290–579) of IE2 fused to the GAL4 DNA binding site (16) and fused protein GAL4DB-IE2C(H446L) containing an NF-E2 binding site (16) or a 32P end-labeled double-stranded oligonucleotide (5'-GGACACGAATGCTGAATGATGAGTCATGCTG-3') (33) was used as the probe. When indicated, 0.1 µg of individual recombinant histidine-tagged proteins and approximately 10 µg of serum proteins were used.

**Isolation and Identification of Members of the CNC-bZIP Family as IE2-interacting Proteins—In a search for proteins that specifically interact with the autoregulation and repression domain of IE2, we screened a human lymphocyte MATCH-MAKER activation domain library (CLONTECH) using a chimeric protein composed of the C terminus (residues 290–579) of IE2 fused to the GAL4 DNA binding site (16) and fused protein GAL4DB-IE2C(H446L) containing an NF-E2 binding site (16) or a 32P end-labeled double-stranded oligonucleotide (5'-GGACACGAATGCTGAATGATGAGTCATGCTG-3') (33) was used as the probe. When indicated, 0.1 µg of individual recombinant histidine-tagged proteins and approximately 10 µg of serum proteins were used.
IE2 (row 1). Control experiments confirmed the specificity of the Nrf1-IE2 and Nrf2-IE2 interactions: the introduction of plasmid pGAL4DB-IE2C alone into yeast produced no colony (row 6). Similarly, no colony was observed upon co-transformation of pGAL4DB with either pGAL4AD-Nrf1 (row 7) or pGAL4AD-Nrf2 (row 8). Although co-transformation of pGAL4DB-IE2C(H446L) with pGAL4AD-Nrf1 (row 4) or pGAL4AD-Nrf2 (row 5) did generate some yeast colonies, no LacZ expression was detected. Thus, two conclusions could be drawn from the above experiments. First, not all the members of the CNC-bZIP family interacted with IE2. Second, the bZIP domain of Nrf1 was involved in the binding to IE2. Further supports for the conclusions were provided below (Figs. 2, 3A, and 4B).

Mapping the IE2 Interaction Domain of Nrf1—Because Nrf1 exhibited relatively high affinity toward IE2, we set out to map
Fig. 5. The DNA binding activity of Nrf1/MafK is not impeded by IE2. A 32P end-labeled double-stranded oligonucleotide containing an NF-E2 site was used as a probe in the bandshift assay. The presence (+) or absence (−) of individual protein is indicated above each track of the autoradiogram. Ab, aNrf1, aMafK, and aIE2 are abbreviations for antibody, anti-Nrf1 antibody, anti-MafK antibody, and anti-IE2 antibody, respectively. DNA-protein complexes were separated from the probe by electrophoresis on a 5% nondenaturing polyacrylamide gel. Positions of the DNA-protein complexes are indicated as following: asterisk for the Nrf1/MafK-DNA complex; arrowhead for the Nrf1/MafK-IE2-DNA complex; and square for the aIE2-Nrf1/MafK-IE2-DNA complex. In consistence with previous reports (27, 45), the MafK homodimer failed to bind this particular NF-E2 site (lane 3).

**Interaction of CNC-bZIP Proteins with HCMV IE2**

Interaction of CNC-bZIP Proteins with IE2 in vitro—To test whether CNC-bZIP transcription factors interact with IE2 in vitro, an affinity matrix consisting of the GST-IE2 fusion protein as a ligand was prepared. Full-length, radiola-

...beled NF-E2, Nrf1, and Nrf2 were incubated with the matrix, and bound CNC-bZIP proteins were analyzed by SDS-PAGE. Fig. 3A shows that, consistent with data obtained with the yeast two-hybrid system (Fig. 1), both Nrf1 (lane 6) and Nrf2 (lane 9) bound to GST-IE2, whereas NF-E2 (lane 3) failed to be retained under the same experimental conditions. Moreover, Nrf1 had higher affinity toward IE2 than Nrf2 did (compare lane 6 with lane 9), also in agreement with the results obtained from the yeast two-hybrid experiments (Fig. 1).

To determine whether CNC-bZIP factors associate with IE2 in human cells, a plasmid expressing IE2 was transfected into H1299 cells that had been selected as a system to study the repression activity of IE2 (15, 16, 33) for co-immunoprecipitation. Further, the transfection efficiency was about 30–40% for H1299 cells at our hands (data not shown). Thus, H1299 cells seemed to provide a desirable system for the experiments. Cell extracts were prepared from the transfected cells and then immunoprecipitated with an antibody against Nrf1. IE2 in immunoprecipitates was detected by Western blot analysis using an antibody against IE2. Although expressed to similar levels (Fig. 3B, panel II, lanes 2 and 4), IE2 was only co-

**Interaction of CNC-bZIP Proteins with IE2 in Vivo—**Because CNC-bZIP factors interacted with IE2 in vivo (Fig. 3), it was important to ask whether the transcriptional activity of CNC-bZIP factors is modulated by IE2. To address this question, two complementary experiments were performed. First, the effect of IE2 on transcription driven by six copies of an NF-E2 site was measured. As shown in Fig. 4A, transcription of the reporter, pN6CAT, was inhibited by IE2 both in WI38 (compare lane 4 with lane 3) and in H1299 cells (compare lane 10 with lane 9). Removal of the NF-E2 sites from the promoter (compare lanes 2 and 8 with lanes 1 and 7) or a substitution of the NF-E2 sites with the SV40 promoter/enhancer (compare lanes 6 and 12 with lanes 5 and 11) rendered the resultant reporters unresponsive to IE2. In other words, the IE2-mediated repression was dependent on the presence of the NF-E2 sites. Second, because multiple CNC-bZIP factors can interact with an NF-E2 site, the identity of CNC-bZIP factors involved in the repression was determined by measuring the effect of IE2 on transcription activated by LexA-CNC-bZIP fusion proteins. As shown in Fig. 4B, the transactivation activity of LexA-Nrf1 and LexA-Nrf2 was largely reduced in the presence of IE2 in both WI38 and H1299 cells (compare lanes 5, 7, 15, and 17). The reduction was specific, because IE2 had little effect toward LexA-VP16 (compare lanes 10 and 20 with lanes 9 and 19). Moreover, although LexA-NF-E2 exhibited a relatively low transactivation activity (lanes 3, 4, 13, and 14), an autoradiogram of longer exposure revealed that IE2 had little effect on LexA-NF-E2 (data not shown). A control experiment demonstrated that IE2 had little effect on the expression of LexA-CNC-bZIP fusion proteins (Fig. 4C, compare lanes 2, 4, and 6 with lanes 3, 5, and 7), eliminating the possibility that a difference in the concentration of fusion proteins might account for the observed IE2-mediated repression (Fig. 4B). As expected, IE2(H446L) did not inhibit transcription driven either by the NF-E2 sites or by the LexA-CNC-bZIP fusion proteins (data not shown). Thus, on the basis of data obtained from the aforementioned experiments, it was concluded that a direct interaction between members of the CNC-bZIP family and IE2 was required for the observed repression.

Retention of the DNA Binding Activity of Nrf1/MafK in the Presence of IE2—It is known that small Maf proteins lack a

| Protein | Nrf1 | MafK | IE2 |
|---------|------|------|-----|
| Presence (+) or absence (−) of individual protein is indicated above each track of the autoradiogram. Ab, aNrf1, aMafK, and aIE2 are abbreviations for antibody, anti-Nrf1 antibody, anti-MafK antibody, and anti-IE2 antibody, respectively. DNA-protein complexes were separated from the probe by electrophoresis on a 5% nondenaturing polyacrylamide gel. Positions of the DNA-protein complexes are indicated as following: asterisk for the Nrf1/MafK-DNA complex; arrowhead for the Nrf1/MafK-IE2-DNA complex; and square for the aIE2-Nrf1/MafK-IE2-DNA complex. In consistence with previous reports (27, 45), the MafK homodimer failed to bind this particular NF-E2 site (lane 3).
transactivation domain, and homodimers of small Maf proteins have been shown to act as transcriptional repressors by binding to certain NF-E2 sites (27). Conceivably, IE2 inhibits the transactivation activity of CNC-bZIP factors by blocking the DNA binding activity of CNC-bZIP/Maf heterodimers, therefore favoring the occupation of the NF-E2 sites by homodimers of small Maf proteins. This hypothesis gains support from the observation that the DNA-binding domain of Nrf1 was involved in the interaction with IE2 (Fig. 2). Alternatively, IE2 does not interfere with the DNA binding activity of CNC-bZIP/Maf heterodimers. Rather, it inhibits CNC-bZIP factors, as in the case of p53 (16), by tethering a repression domain to them. The observations that transcription driven by LexA-CNC-bZIP proteins was nonetheless repressed by IE2 (Fig. 4B) is consistent with this idea.

To distinguish between these possibilities, an electrophoretic gel mobility shift assay was performed to investigate whether the DNA binding activity of CNC-bZIP factors is blocked in the presence of IE2. Because Nrf1 (17) and MafK (28, 29) were ubiquitously expressed, these two factors are likely to be partners in vivo. We therefore examined whether binding of the Nrf1/MafK heterodimer to the NF-E2 site was affected by IE2. When Nrf1, MafK, or IE2 alone was incubated with the NF-E2 probe, no specific DNA-protein complex was observed (Fig. 5, lanes 2–4). A complex was formed by co-incubation of the probe with Nrf1 and MafK (lane 5), which was efficiently competed out with the addition of the unlabeled double-stranded NF-E2 DNA oligonucleotide but not with a nonspecific one (data not shown). Importantly, inclusion of IE2 in the reaction did not block the formation of DNA-protein complex. Instead, the mobility of the complex was further retarded (compare lanes 5 and 6), suggesting that IE2 participated in the formation of the new complex. The identity of individual components in the new complex was examined by the addition of specific antibodies to the incubation: an antibody against Nrf1 eliminated the complex (lane 7), whereas those against MafK and IE2 further reduced the mobility (lanes 8 and 9). In contrast, a preimmune serum had little effect on the mobility of the IE2-Nrf1/MafK ternary complex (lane 10). Taken together, these data demonstrate that IE2 did not interfere with the DNA binding activity of the Nrf1/MafK heterodimer. Instead, the resultant ternary complex consisting of Nrf1, MafK, and IE2 was still able to bind the NF-E2 site, favoring the model that IE2 represses the transactivation activity of CNC-bZIP factors by tethering a repression domain to them.

CNC-bZIP Factors Attenuate the Autoregulation Activity of IE2 by Preventing It from Binding to CRS—Reciprocally, because CNC-bZIP factors interacted with the autoregulation domain of IE2, their influence on the IE2 activity was investigated. As shown in Fig. 6, in the absence of IE2, both Nrf1 and Nrf2 had little effect toward the expression of a reporter driven by the HCMV MIEP (compare lanes 2 and 3 with lane 1) or by a control promoter (compare lanes 8 and 9 with lane 7). IE2 negatively autoregulated MIEP (compare lane 4 with lane 1). However, the IE2-mediated repression of MIEP was largely relieved by co-transfection of a plasmid expressing either Nrf1 or Nrf2 (compare lanes 5 and 6 with lane 4). The antagonistic effect of CNC-bZIP factors was specific for the IE2-mediated autoregulation, because it had little effect toward an IE2-unresponsive reporter driven by the SV40 promoter/enhancer (compare lanes 11 and 12 with lane 10).

It is well known that IE2-mediated autoregulation is caused by binding of IE2 to a specific DNA sequence, called CRS, located immediately downstream of the MIEP TATA box. Thus, the observation that CNC-bZIP factors reduced the autoregulation activity of IE2 strongly suggests that association of IE2 with a CNC-bZIP factor may result in the disruption of its CRS binding activity. To test this idea, an electrophoretic gel mobility shift assay was performed. As shown in Fig. 7, the CRS probe interacted only with IE2 to form a complex (compare lane 2 with lanes 3–5). Nrf1 alone or in association with MafK blocked the binding of IE2 to the CRS probe (compare lanes 6 and 8 with lane 2). Interestingly, although MafK by itself did not inhibit binding of IE2 to the CRS probe (lane 7), it appeared to augment the ability of Nrf1 in reducing the CRS binding activity of IE2 (compare lane 8 with lane 6) by an unknown mechanism.

DISCUSSION

Based on the results presented here and previous studies on the repression of p53 by IE2 (15, 16), we propose a model for the antagonism between Nrf1 and IE2. IE2 loses its DNA binding activity when complexed with Nrf1/MafK (Fig. 7), which probably underlies the mechanism of how members of the CNC-bZIP family attenuate the autoregulation activity of IE2 (Fig. 6). In contrast, binding of IE2 to Nrf1/MafK has little effect on the DNA binding activity of the heterodimer (Fig. 5). Rather, the resultant IE2-Nrf1/MafK ternary complex can still bind to the NF-E2 site and inhibits the autoregulation of Nrf1/MafK (Fig. 4) possibly by, as in the case of IE2-mediated inhibition of p53 activity (16), tethering a repression domain to Nrf1/MafK. Moreover, the IE2-mediated inhibition may not be restricted to the Nrf1/MafK heterodimer on the basis of the following evidence. First, IE2 also interacts with Nrf2 (Fig. 1), and LexA-Nrf2 is repressed by IE2 (Fig. 4B). Second, CNC-bZIP proteins appear to form obligatory heterodimers with one or another of small Maf proteins (21, 27–29).

IE2 is an important regulator of HCMV, and thus its activities need to be strictly controlled. Negative autoregulation is one of the most studied functions of IE2. Nonetheless, little is known about how this IE2 activity is regulated. Recent work implicates that phosphorylation plays an important role in controlling the DNA binding activity of IE2 (14). The current studies demonstrate the antagonism between CNC-bZIP factors and IE2 and thus uncover another potential mechanism for host cells to modulate the autoregulation activity of IE2.
IE2  -  +  -  -  -  +  +  +  
Nrf1  -  -  +  +  -  +  +  +  
MafK  -  -  +  +  +  +  +  +  

FIG. 7. Nrf1/MafK inhibits the DNA binding activity of IE2. A $^{32}$P end-labeled double-stranded CRS oligonucleotide was used as a probe in the bandshift assay. The presence (+) or absence (−) of IE2, Nrf1 and MafK is indicated above each track of the autoradiogram. The DNA-protein complex was separated from the probe by electrophoresis on a 5% nondenaturing polyacrylamide gel.

Nonetheless, it is interesting to note that the efficiency of autoregulation by IE2 seems to be cell-dependent (4, 10, 13). In this regard, IE2-mediated autoregulation was prominent in H1299 cells, and the expression of exogenous CNC-bZIP factors was required to attenuate the repression (Fig. 6). Perhaps, the relative expression levels of IE2 and CNC-bZIP factors and/or a difference in the phosphorylation status of IE2 may account for the cell-dependent autoregulation. Because of the resolution limit of the SDS-PAGE gel, it was difficult to tell which IE2 species interacted with CNC-bZIP factors (Fig. 3B). Future work is required to solve this issue. Furthermore, several cellular genes have a CRS-like element strategically positioned around the TATA box.2 It would be important to determine whether the expression of these genes is subjected to the modulation by the IE2-CNC-bZIP antagonistic interaction.

Previous studies demonstrate that IE2 negatively autoregulates its own expression by a passive mechanism (12). The current work, in conjunction with previous studies (15, 16), provides strong evidence to support the notion that IE2 can actively repress gene transcription as well by tethering a repression domain to DNA-binding proteins, for example, p53, Nrf1, and Nrf2. This type of IE2 repression activity is observed with many different cells, including primary human corneal smooth muscle cells (15), the osteosarcoma cell line Saos-2 (16), as well as the lung cancer cell line H1299 and an HCMV permissive cell line W138 (this work). Because IE2 contains a trans-repression domain (16), it is presumed that IE2 represses transcription by interacting with a general transcription factor(s) in a nonproductive manner (38) and/or recruiting a putative co-repressor(s) (39, 40) to the promoter.

Many cellular genes, including proinflammatory cytokines tumor necrosis factor and interleukin 4, contain NF-E2-like sites in their promoters (41–43). By interacting with CNC-bZIP factors, IE2 may shift the balance between activation and repression of these cellular genes, resulting in the modulation of immune responses to HCMV infection. However, it must be pointed out that IE2 is both a positive (3–5) and a negative (10, 11, 15, 16, 33) regulator of gene expression. The presence of an NF-E2 site(s) in the promoter of a gene does not necessarily imply that the gene is under negative control by IE2. In fact, the promoter of NQO2 (quinone oxidoreductase 2) (44) is activated by IE2; however, elimination of the NF-E2 site from its promoter renders the mutant promoter more responsive to the IE2-mediated activation.2 In light of the dichotomy of IE2, the SV40 TATA box, which is unresponsive to IE2 (Fig. 4), was chosen in purpose to investigate the relatively weak repression of CNC-bZIP factors by IE2.

As an additional level of complexity, CNC-bZIP factors were reportedly able to interact with members of Jun/Fos and ATF families (42) as well as with nuclear hormone receptors (21). Besides, IE2 has activities other than autoregulation and trans-repression, such as transcriptional activation and interaction with CREB, c-Jun, RB, TBP, and TFIIB (6–9). However, the influence of the IE2-CNC-bZIP interaction on the aforementioned activities and the relative IE2-binding affinity among those factors are currently unknown. Further investigation into the antagonism between IE2 and CNC-bZIP factors should shed light on the role of IE2 in modulating viral and cellular gene expression and thus contribute to our knowledge of the biology and pathology of HCMV infection.

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Chien-Fu Huang, Yeau-Ching Wang, Der-An Tsao, Shiu-Feng Tung, Young-Sun Lin and Cheng-Wen Wu

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