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A Steroid Hormone Response Unit in the Late Leader of the Noncoding Control Region of the Human Polyomavirus BK Confers Enhanced Host Cell Permissivity

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The effect of steroid hormones on multiplication of the human polyomavirus BK (BKV) was studied. Physiological concentrations of the synthetic glucocorticoid dexamethasone, progesterone R5020, or estrogen 17β-estradiol enhanced the permissivity of the host cell for BKV, resulting in an up to 11-fold (dexamethasone), 5-fold (progesterone), or 3-fold (17β-estradiol) higher virus yield. The increase in virus yield in dexamethasone-stimulated cells correlated with enhanced steady-state levels of viral transcripts. The late leader sequence of the BKV control region contains a hormone response unit composed of a nonconsensus glucocorticoid and/or progesterone response element (GRE/PRE) and a fully consensus estrogen response element (ERE). DNA-protein binding studies showed that the glucocorticoid receptor and the progesterone receptor bound to this BKV GRE/PRE-like sequence, while the estrogen receptor could bind to the BKV ERE motif. By transient transfection assays, we were able to show that these sequences can mediate steroid hormone-induced gene expression. However, no cooperative transactivation effect between the BKV GRE/PRE-like motif and BKV ERE motif was observed. This BKV hormone response unit may play an important role in vivo by enhancing a productive BKV infection, and perhaps also by reactivating a latent infection, during physiological or pathological conditions accompanied by increased steroid hormone levels.

The human polyomavirus BK (BKV) has a worldwide distribution, as shown by seroepidemiological studies (12, 17, 25, 37, 90). Following the initial infection in early childhood, the virus establishes itself in a latent state (14, 26, 36, 90). Immunosuppression, chronic disease, and pregnancy may lead to reactivation of BKV (14, 15, 29, 74), but the stimuli and mechanisms involved are presently unknown.

We chose to investigate the effects of steroid hormones on viral gene expression and multiplication. Gene regulation by steroid hormones is mediated by the interaction of the activated steroid hormone receptor with its cognate DNA sequence, referred to as the hormone response element (HRE [for a review see reference 8]). The sequence elements recognized by different steroid receptors constitute a family of identical or closely related sequences. The consensus glucocorticoid response element (GRE) is the 15-bp imperfect palindromic sequence 5′-GGTACANNNTGTTCT-3′. This sequence cannot be discriminated from the progesterone response element (PRE), the androgen response element, and the mineralocorticoid response element. The minimal functional estrogen response element (ERE) consensus sequence is the 13-bp perfect palindrome 5′-GGTCANNNTGACC-3′ (for a review, see reference 58). Purified glucocorticoid receptor (GR) binds as a dimer to the GRE (34, 83). Initial binding to the TGTTCT half-site of the GRE is followed by cooperative binding of a second GR molecule to the GGTACA half-site (83). Different spacing between half-sites may still allow GR binding, and GR may bind with graded affinity to a broad range of sequences that diverge from the consensus GRE (54). In fact, natural GREs very often deviate from the consensus and seldom correspond to strong, inducible enhancer elements when tested individually. High inducibility is often obtained by imperfect GREs that are clustered or arranged together with other HREs or other regulatory DNA elements, producing a synergistic effect (reviewed in reference 63).

Steroid hormones may not only activate transcription. GRE-like sequence motifs conferring negative regulation have been defined (22, 67, 69; reviewed in reference 2). In addition, various factors exerting positive or negative influence on glucocorticoid-dependent transcription have been described. The spacing between the two half-sites of the GRE (7), overlap with other protein-binding motifs (3, 16, 77), and cross-talk between different signal transduction pathways (20, 42, 47, 52, 72, 75, 89, 92) have been implicated. Cases of interference between the transcription factor AP-1 (consisting of members of the Fos and Jun family) and several nuclear receptors have been well documented. Positive or negative steroid hormone-mediated effects may be determined by the relative amounts of Jun and Fos proteins present (20, 27, 75). Interestingly, both the c-*fos* and the c-*jun* genes are regulated by steroid hormones (40, 51, 87, 88).

Here, we present evidence that the synthetic glucocorticoid dexamethasone, progesterone, or estrogen treatment can enhance BKV infection in vitro. Increased virus yield correlates with increased levels of BKV transcripts, most evidently for the late (structural protein) transcripts. Our results suggest that the increased levels of BKV gene expression by glucocorticoids and/or progesterone and estrogens are mediated by a nonconsensus GRE/PRE-like motif and a consensus ERE, respectively, present in the late leader region of the BKV noncoding

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control region. In vitro DNA-protein binding studies with nuclear extracts of steroid-treated cells, with specific steroid hormone receptor antibodies and with purified receptor, demonstrate the specific binding of the GR, progesterone receptor (PR), and estrogen receptor (ER) to these sequences. Transient expression assays revealed that the BKV hormone response unit can mediate steroid hormone-induced transcription of a reporter gene. Although the BKV hormone response unit is composed of a GRE/PRE-like motif and an ERE, no cooperative transactivation effect was observed. This is probably because simultaneous binding of GR or PR and ER is excluded, since their binding sites partially overlap.

MATERIALS AND METHODS

Plasmids, DNA, and RNA techniques. The plasmids with the reporter gene chloramphenicol acetyltransferase (CAT), pBLCAT2, and pBLCAT3 were kindly provided by B. Luckow (53). The plasmid pGRE15A was a kind gift of G. Klock. This plasmid contains the GRE-II sequence of the tyrosine aminotransferase gene cloned in pBLCAT2 (44). The plasmids pHEO and pSG5 were kindly donated by P. Chambon (48). The plasmids pGRE-L, pERE-L, pERE-M, pEGRE, and pEREvit were constructed by ligating complementary oligonucleotides (see Fig. 3B) into the *Xba*I and *Bam*HI sites of pBLCAT2. Oligonucleotides were synthesized on a Pharmacia-LKB Gene Assembler Plus and purified as recommended by the manufacturer. Annealing of the complementary strands and cloning was done by standard procedures (70). The constructs were verified by DNA sequencing with a Sequenase version 2.0 kit (United States Biochemicals).

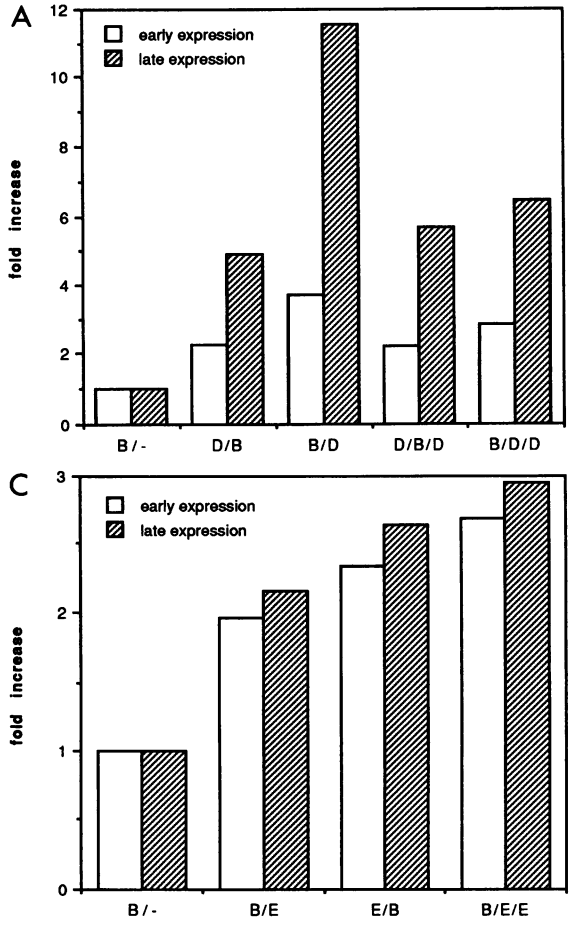
Northern (RNA) blot hybridization with BKV-specific probes was performed as described previously (61). Total RNA from about 10^7 cells was isolated by the guanidinium thiocyanate-CsCl method (55). Densitometric scanning of autoradiographs for quantitation of DNA and RNA was performed with a Hoefer GS 300 scanning densitometer (Hoefer Scientific Instruments) as described by Moens et al. (61).

Cell cultures, virus infections, and hormone treatment. Vero cells (ATCC CCL81) were maintained in Eagle's minimum essential medium supplemented with 2% fetal calf serum, 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 100 mg of streptomycin, and 200 U of penicillin G per ml. HeLa cells (ATCC CCL2) were grown in the same medium except for the addition of 10% fetal calf serum and nonessential amino acids. CV-1 cells (ATCC CCL70) were cultured in Dulbecco's modified essential medium supplemented with 1% HEPES, 2 mM glutamine, 5% fetal calf serum, 200 U of penicillin G, and 100 mg of streptomycin per ml. T47-D cells (ATCC HTB133) were cultured in RPMI 1640 supplemented with 10% charcoal-treated fetal calf serum and insulin (0.6 mg/ml). MCF-7 cells (kindly donated by T. Thorsen) were grown in Dulbecco's modified Eagle's medium without phenol red (D5286; Sigma Chemical Co., St. Louis, Mo.) supplemented with 20 mM HEPES, insulin (0.6 mg/ml), 10% charcoal-treated fetal calf serum, and 100 U of penicillin G and 100 mg of streptomycin per ml. Dextran-coated charcoal treatment was performed to remove endogenous steroids, and phenol red-free medium was employed since trace amounts (10^{-11} M) of estrogen are present (39). All cell cultures were maintained in a humified 5% CO_2 atmosphere at 37°C.

The hormone treatment schemes were designed to investigate whether (i) the number of cells expressing late BKV antigens (i.e., the number of fully permissive host cells [24, 25]) was increased, (ii) the total production of infectious virus particles (i.e., viral yields) was increased, and (iii) the timing and duration of treatment were important for permissivity and viral yields. Expression of viral early and late proteins was monitored by immunoperoxidase staining as described previously (24, 25). This method represents a plaque assay without overlay. Briefly, when staining is performed on day 4 postinfection, only cells infected during the initial adsorption period will express late antigens and produce virus, and hence each stained cell represents one infectious virus particle in the inoculum.

Cells were infected in 24-well culture plates (Costar) with an infectious dose of BKV(Gardner) that resulted in a countable number (50 to 500) of immunoperoxidase-stained cells. The inoculum was allowed to adsorb for 2 h. In the stimulation experiments, the cells were treated with 50 ng of dexamethasone per ml, 10^{-7} M progestin R5020 (Sigma Chemical Co.) or 10^{-8} M 17β-estradiol (Sigma Chemical Co.). For dexamethasone, Vero cells were used, while for progestin and 17β-estradiol we employed MCF-7 cells. For dexamethasone, five different regimens were applied: (i) BKV infection in the absence of glucocorticoids; (ii) treatment for 12 h prior to infection; (iii) treatment for 12 h after infection; (iv) pretreatment plus posttreatment, with no dexamethasone present during virus adsorption; and (v) continuous treatment from the time of infection until harvest 4 days later. The cell cultures were washed twice with medium after the treatment with dexamethasone was ended. In order to enumerate permissive host cells, the monolayers were immunoperoxidase stained for detection of early and late BKV antigen expression 4 days after infection (24, 25). At the same time, the culture media were collected and the volumes were adjusted to equality, whereafter aliquots were used to infect new cultures. Immunoperoxidase staining of, and counting of BKV antigen-expressing cells in, these cultures 4 days later gave a direct measure of virus yields from the dexamethasone-treated and untreated parallels of the key experiment. Similar experiments were performed with progesterone and estrogen, except that regimen ii was not applied. For these experiments, we used MCF-7 cells since these express both the ER and PR (9). Differences in viral yields might theoretically be due to hormone-mediated influence on the relative quantities of intra- and extracellular virus on day 4 postinfection. Therefore, for some hormone-treated and some untreated cultures the cell layers were not immunoperoxidase-stained but harvested, frozen and thawed three times, and sonicated. The extracellular (from media) and intracellular virus yields were thereafter titrated in parallel.

Transient transfection assays. CV-1, MCF-7, and T47-D cells were transfected the day after they had been plated out and had reached 50% confluency, while HeLa cells were transfected at 30% confluency 1 day after plating. Vero cells were transfected at 80 to 90% confluency. Plates with a diameter of 10 cm were used. Transfection was performed by the calcium phosphate-DNA coprecipitation method (31). We routinely used 8 μg of reporter plasmid (62). The day after transfection, fresh medium without serum was added. Dexamethasone was then added at a final concentration of 50 ng/ml, while progestin R5020 and 17β-estradiol were administered to the cells at final concentrations of 10^{-7} M and 10^{-8} M, respectively. Cells were incubated for another 24 h. Assays of CAT activity were performed with whole-cell extracts as described previously (32). Time course experiments were performed to ensure that CAT activity was always measured in the linear range. All transfections were carried out at least three times. Scanning densitometry was used to quantitate the conversion of chloramphenicol to the acetylated forms. All



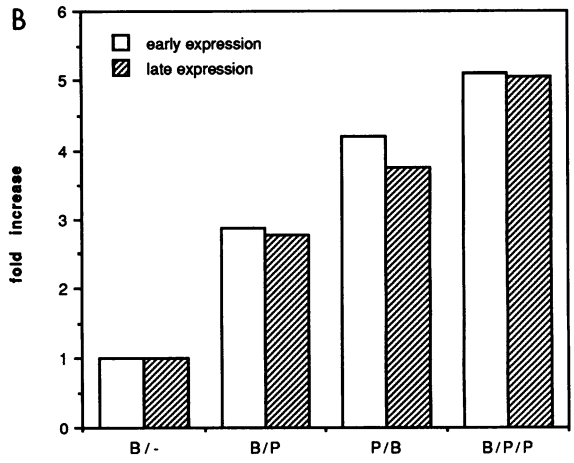


FIG. 1. Influence of steroid hormone treatment on the host cell permissivity for BKV. (A) Vero cell monolayers were infected with BKV(Gardner), and subjected to different regimens of dexamethasone (D) treatment. B/−, BKV-infected cells in the absence of dexamethasone; D/B, cells treated with dexamethasone for 12 h prior to infection, with no dexamethasone added after infection; B/D, cells treated with dexamethasone for 12 h after they had been infected; D/B/D, cells treated with dexamethasone 12 h prior to infection and also for 12 h after infection but not during virus adsorption; B/D/D, cells treated with dexamethasone from the moment they were infected until they were harvested 4 days later. Four days after infection, the culture media were removed and used to infect new Vero cell monolayers, which were immunoperoxidase-stained 4 days later to determine the virus yields from the initial experiments. The results are presented as fold increase in cells expressing early or late antigens. The numbers of untreated infected cells staining for early and late antigens were 1.33×10^4 and 0.32×10^4 cells per ml, respectively. Under the conditions chosen, the number of late antigen-expressing cells is equivalent to the number of infectious virus particles (25). All experiments were performed with six parallels. The inter- and intra-assay variations were less than 20% in all cases. (B) MCF-7 monolayers were infected with BKV(Gardner) and treated with different regimens of progesterone (P). The same regimens as described in A were used, except that stimulation with progesterone prior to and after virus infection was omitted. The numbers of untreated infected cells staining for early and late antigens were 6.5×10^4 cells per ml and 7.0×10^4 cells per ml, respectively. (C) The procedures were as described for B, but cells were treated with 17β-estradiol (E). The numbers of untreated infected cells expressing viral early and late antigens were 7.3 $\times 10^4$ and 6.6×10^4 cells per ml, respectively.

plasmids used in this study were purified by isopycnic centrifugation on CsCl-ethidium bromide gradients (70).

Gel mobility shift assays (GMSA). Nuclear extracts of untreated or dexamethasone-treated Vero cells were prepared as described elsewhere (21). The cells were stimulated with dexamethasone (50 ng/ml) for 12 h before harvesting. Nuclear extracts of CV-1 cells and T47-D cells were prepared as described by Gough (33), while MCF-7 extracts were kindly provided by T. Thorsen. Protein concentrations in the extracts were determined by the method of Bradford (11) with bovine serum albumin as a standard. For binding studies with the GR fusion protein, GRF1, 300 ng of protein was used. This protein contains amino acids 412 to 515 of the human GR (comprising the DNA binding domain) linked to the C terminus of protein A (19). Anti-PR or anti-ER antibodies were preincubated with the nuclear extracts for 20 min at 20°C before labelled probe was added. GMSA were performed essentially as described previously (76). Protein-DNA interaction studies were performed with a total volume of 20 μl in 25 mM HEPES (pH 7.5)–12.5 mM $MgCl_2$–100 mM KCl–1 mM dithiothreitol–10 mM $ZnSO_4$–20% glycerol–0.1% Nonidet P-40. One microgram of nuclear extract and 200 ng of poly(dI-dC) were routinely used. After 15 min of incubation on ice, 250 pg of ^{32}P-labelled probe was added and incubations were continued for an additional 15 min. Probes were end labelled with Klenow polymerase and [α-^{32}P]dCTP (3,000 Ci/mmol). Where competitor DNA was added, this was applied simultaneously with the labelled oligonucleotide. DNA-protein complexes were resolved on 5% polyacrylamide gels in 1 × TBE (89 mM Tris, 89 mM boric acid, 2 mM EDTA) at 7 V/cm at 4°C. Gels were dried and subjected to autoradiography at −70°C with intensifying screens. The oligonucleotides used in the competition studies had the following sequences: GRE, GACCCTA GAGGATCAGAACATGATGTTCTAGATCGAATT CG (kindly provided by J. Å. Gustafsson); TRE, CTAGCGTG ACTCAGCGCGC (5); AP-2, GGGAAGATTTCCCCAGGC AGCTCTTTCAAG (23); CRE, CTAGGAGCCCGTGACGT TTACACTC (60); and NF-κB, AGTTGAGGGGACTTTC CCAGGC (23). GR fusion protein GRF1 was obtained from P. De Vos (19). Anti-PR and anti-ER antibodies were purchased from Affinity BioReagents (Neshanic Station, N.J.).

RESULTS

Steroid hormones enhance the host cell permissivity for BKV. All the dexamethasone treatment schemes increased the number of cells expressing late (structural) proteins five- to

sixfold. For progesterone and estrogen, the corresponding figures were two- to threefold (data not shown). As shown in Fig. 1A, the different dexamethasone regimens all increased the viral yields considerably. The posttreatment scheme was about twice as efficient as the others, increasing the virus yield compared with those of untreated controls approximately 11-fold. The corresponding results for progesterone and 17β-estradiol are illustrated in Fig. 1B and C, respectively. For the former, 3- to 5-fold and for the latter 2.2- to 3-fold increases in virus yield were measured. Continuous treatment resulted in the highest increases for both hormones. Preliminary experiments with progesterone and 17β-estradiol in T47-D cells also showed increased virus yields in treated cultures (77a).

No differences in the relative amounts of extra- and intracellular virus between cultures treated with any of the hormones and untreated BKV-infected cultures were found. Extracellular virus constituted about 50% of the total yields in all cases, in accordance with earlier published results (24).

Enhanced permissivity following dexamethasone treatment correlates with increased levels of BKV late transcripts. Total RNA extracted 2 days postinfection from untreated BKV-infected Vero cultures, and also from cultures treated 12 h before or 12 h after infection, was subjected to Northern blot analyses. As shown in Fig. 2, increased amounts of viral transcripts were found in infected cells that had received the glucocorticoid both prior to and postinfection. The increase for late transcripts was higher than that for early transcripts (2.6-fold for 16S, 4- to 5-fold for 19S late transcripts, and 1.5-fold for early transcripts). Rehybridization with a probe against 28S rRNA revealed that the differences in hybridization signals were not the result of differences in RNA loading or blotting (Fig. 2C). Thus, these results correlated perfectly with the results obtained by immunoperoxidase detection of viral proteins. Stimulation by dexamethasone seems to work at the transcript level.

In addition to the expected 2.4-kb early transcripts, high-molecular-weight mRNAs were also found to hybridize to the early gene probe. These species may represent fusion transcripts with cellular sequences (71), since we found BKV DNA integrated in the host cell genome of infected cells (61a). Alternatively, these giant transcripts could consist of tandem repeats of the nucleotide sequence of the entire genome as was reported for polyomavirus RNA (1).

In order to determine whether dexamethasone could act by stimulating viral DNA replication, we used the in situ filter hybridization method (59) to examine levels of viral DNA 2 and 4 days postinfection. An expected augmentation after 4 days compared with the level at 2 days was found, but no quantitative differences between dexamethasone-treated and untreated cultures were detected (Fig. 2D). Thus, the increased BKV permissivity seems not to depend on elevated viral DNA replication but rather is the result of larger amounts of viral capsid proteins as a consequence of increased transcript levels.

The late leader of the BKV control region contains a hormone response unit composed of a functional consensus ERE motif and a functional nonconsensus GRE/PRE motif. Since steroid hormones are known to mediate increased transcription via HRE motifs, we used computer analysis to look for such motifs in the BKV genome and particularly in the noncoding control region. In the late leader sequence we identified a putative consensus ERE motif partially overlapping with a nonconsensus GRE/PRE motif. The latter overlaps with the start codon for the putative agnogene sequence (78) (Fig. 3A).

In order to determine whether specific proteins could bind

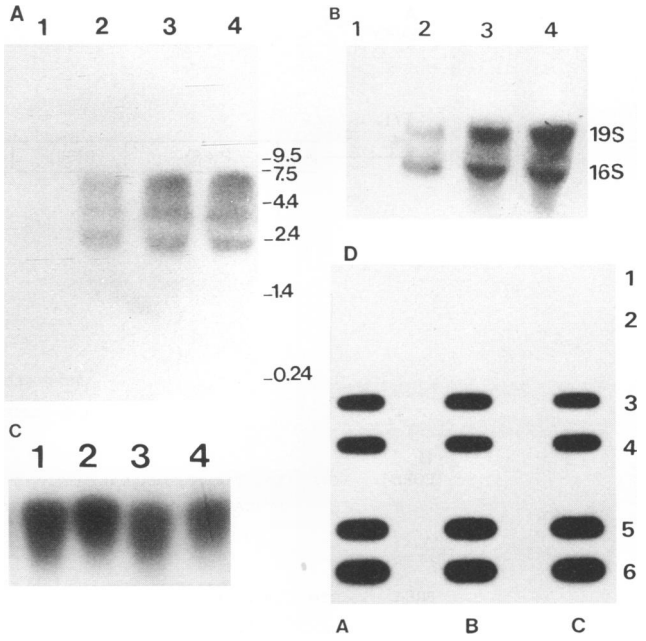


FIG. 2. Northern blot analysis of the steady-state levels of BKV transcripts and in situ filter hybridization of BKV DNA in unstimulated and dexamethasone-stimulated Vero cells. (A) Hybridization with a probe containing part of the early coding region; (B) hybridization with a probe spanning part of the late genes; (C) hybridization to 28S rRNA (61). Lanes 1, uninfected cells; lanes 2, BKV-infected cells; lanes 3, BKV-infected cells treated with dexamethasone (50 ng/ml) for 12 h prior to infection; lanes 4, BKV-infected cells stimulated for 12 h with dexamethasone after infection. Total RNA was extracted 4 days after infection and separated on a 1% agarose gel containing 2.2 M formaldehyde. Molecular marker size is shown in kilobases. (D) Cells were lysed in NaOH and applied to slot blots as described by McIntyre and Stark (59). BKV DNA sequences were detected with a BKV probe containing early region sequences. Samples were applied in three parallels. Row 1, untreated, uninfected cells; row 2, dexamethasone-treated uninfected cells; row 3, untreated BKV-infected cells 2 days after infection; row 4, BKV-infected cells treated with dexamethasone at 2 days after infection; row 5, untreated, BKV-infected cells 4 days after infection; row 6, BKV-infected dexamethasone-treated cells 4 days after infection. Cells were treated with dexamethasone from the moment they were infected until they were harvested.

to the putative GRE/PRE and ERE motifs, GMSA were performed. Nuclear extracts from untreated and dexamethasone-treated Vero cells were incubated with the double-stranded oligonucleotides GRE and GRE-L (Fig. 3B). GRE contains a perfect palindromic GRE consensus, while GRE-L represents the GRE-like motif present in the BKV genome. The results of these experiments are shown in Fig. 4A. No bands of retarded mobility were observed with nuclear extracts from untreated cells (lanes 2 and 5). Two strongly retarded bands were found with extracts from stimulated cells. A weakly retarded band, probably representing nonspecific DNA binding (27, 52), was also seen (lanes 3 and 6). The double bands might represent receptor dimers and monomers (18), the receptor and a proteolytic product (68), or receptor isoforms (38). When nuclear extracts of the dexamethasone-exposed cells and labelled probe were coincubated with a 100-fold excess of unlabelled competitor DNA (with the perfect palindromic GRE), the two major retarded bands were almost completely eliminated by competition (Fig. 4B). Competition studies with a 100-fold molar excess of oligonucleotides con-

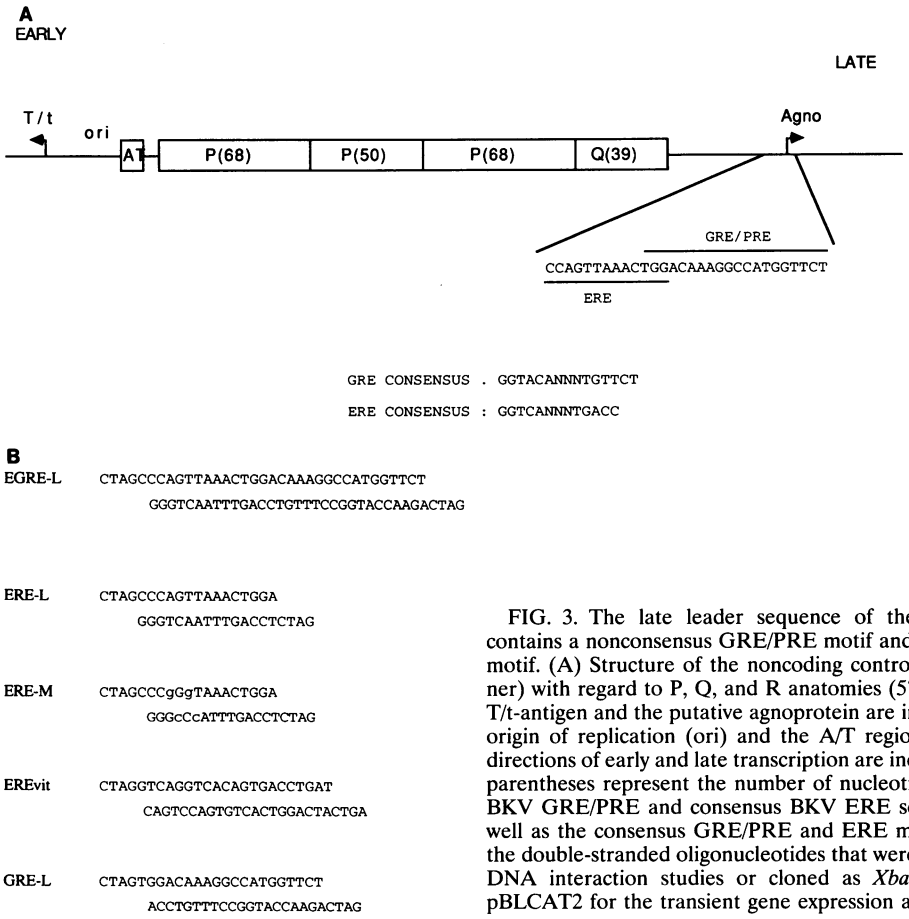


FIG. 3. The late leader sequence of the BKV control region contains a nonconsensus GRE/PRE motif and a fully consensus ERE motif. (A) Structure of the noncoding control region of BKV(Gardner) with regard to P, Q, and R anatomies (57). The start codons for T/t-antigen and the putative agnoprotein are indicated by arrows. The origin of replication (ori) and the A/T region (AT) are given. The directions of early and late transcription are indicated. The numbers in parentheses represent the number of nucleotides. The nonconsensus BKV GRE/PRE and consensus BKV ERE sequences are shown, as well as the consensus GRE/PRE and ERE motifs. (B) Sequences of the double-stranded oligonucleotides that were either used in protein-DNA interaction studies or cloned as *Xba*I-*Bam*HI fragments in pBLCAT2 for the transient gene expression assays are shown.

taining CRE-, NF-κB-, or AP-2-binding sites did not interfere with protein-DNA complex formation (data not shown). To confirm that the GR could bind to the BKV GRE-like motif, GMSA were performed with the GR fusion protein, GRF1. This protein contains amino acids 412 to 515 of the human GR (comprising the DNA binding domain) linked to the C terminus of protein A (19). The results shown in Fig. 4C clearly demonstrate that the BKV GRE binds GRF1 (lane 4), while no binding was observed with an oligonucleotide containing a CRE motif. Densitometric scanning revealed that consensus GRE bound 2.3-fold more GRF1 than GRE-L.

Similar GMSA were performed with complementary oligonucleotides containing the BKV GRE/PRE sequences or the ERE sequence and nuclear extracts of MCF-7 and T47-D cells. These cells both express the PR and ER (39). As a negative control, extracts from CV-1 cells were used. These cells do not express the PR or the ER (9). The oligonucleotides used are shown in Fig. 3B. EREvit contains the well-characterized ERE of the *Xenopus laevis* vitellogenin A2 gene (86), while ERE-L represents the ERE motif found in the BKV late leader region. We also used a mutated BKV ERE motif (EREL-M) which contained two point mutations in the 5′ half of the motif (Fig. 3B). The results of these GMSA are shown in Fig. 5A. While no binding was observed for any of the oligonucleotides tested with extracts from CV-1 cells, clear binding was found for the EREvit probe with both MCF-7 and T47-D extracts. For the ERE-L probe, similar retarded bands were obtained, although less protein was bound. The mutated BKV ERE motif bound some protein very weakly. Competitor experiments with a 100-fold molar excess of EREvit sequences completely abolished the formation of protein-DNA complexes (data not shown), while 100-fold molar excess with a nonspecific competitor with an AP-2 consensus motif did not affect the protein-DNA complexes (Fig. 5B). On the other hand, a 100-fold molar excess of ERE-1 sequences strongly reduced protein formation with the EREvit probe, while a 10-fold molar excess completely abolished the protein-ERE-L complex (data not shown). To further characterize the protein complexes, GMSA were performed in the presence of a monoclonal anti-ER antibody. The antibody used was directed against the DNA binding domain (82). Increasing amounts of antibodies inhibited the formation of the protein-DNA complex (Fig. 5B). Studies with nonspecific competitor DNA or with monoclonal antibodies against the PR revealed that the protein in MCF-7 extracts bound the BKV GRE/PRE sequence specifically and was indeed the PR (Fig. 5C).

HREs of BKV can mediate hormone-induced expression of a CAT reporter gene. To investigate whether the BKV GRE/PRE and ERE motifs could activate transcription, we cloned the noncoding control region of BKV and the double-stranded oligonucleotides shown in Fig. 3B into pBLCAT3 and pBLCAT2, respectively. pBLCAT3 is a promoterless plasmid, while pBLCAT2 contains the basal promoter from the herpes simplex virus thymidine kinase gene upstream of the CAT gene. This promoter does not contain any HRE (44). Transient gene expression studies were performed in different cell lines.

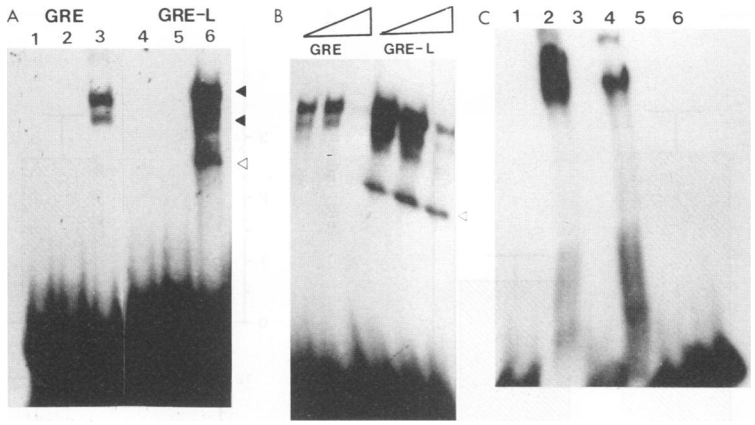


FIG. 4. Protein-DNA interaction studies of the BKV GRE-like motif as analyzed by GMSA. (A) Nuclear extracts of Vero cells and dexamethasone-exposed Vero cells were incubated with ^{32}P-labelled complementary oligonucleotides GRE and GRE-L as described in the legend to Fig. 3B. Lanes 1 to 3, palindromic GRE; lanes 4 to 6, GRE-L. In lanes 1 and 4, no nuclear extract was added, while in lanes 2 and 5 and lanes 3 and 6 extract of Vero cells and dexamethasone-treated Vero cells, respectively, were incubated with the labelled DNA. Specific complexes are indicated by closed triangles, while the open triangle probably represents nonspecific binding. (B) GMSA of nuclear proteins of dexamethasone-exposed cells with labelled palindromic GRE or BKV GRE-L in the presence of increasing amounts of perfectly palindromic GRE competitor DNA (0-, 10-, and 100-fold molar excesses, respectively). Increasing amounts of competitor are indicated by triangles at the top of the figure. The open triangle represents nonspecific complexes. (C) Binding of GR fusion protein to BKV GRE-L. Lanes 1 and 2, palindromic GRE; lanes 3 and 4, GRE-L; lanes 5 and 6, CRE. Lanes 1, 3, and 5, no protein added; lanes 2, 4, and 6, 300 ng of GR fusion protein added.

The plasmid pG_L, containing the noncoding control region of BKV, could mediate dexamethasone-induced expression in Vero cells (fold increase in CAT activity, 2 ± 0.3; $n = 4$ [Fig. 6A]) and in HeLa cells (data not shown). The noncoding region of BKV(AS), a natural mutant with a deletion in its late leader that removes the GRE-like sequence (81), could not mediate dexamethasone-induced transcription of the CAT reporter gene. To further identify the sequences responsible for induced transcription, transient transfections with the plasmid pGRE-L, which contains the BKV GRE-like motif, were performed in HeLa cells. These cells express the GR and have been extensively used for this kind of study (reference 9 and references therein). The BKV GRE-like motif increased CAT activity about threefold more than pBLCAT2 after dexamethasone stimulation. This is slightly higher than found for the consensus GRE represented by pGRE15A. These results, summarized in Fig. 6B, correlate well with the increased late gene expression demonstrated by both immunoperoxidase staining and Northern blot analyses. As expected, none of these plasmids were able to mediate induction of CAT activity by dexamethasone in CV-1 cells (data not shown), which do not express the GR (9).

Transient transfection studies with plasmids pERE-L, pEREM-L, and pEREvit (Fig. 3B) were performed with both HeLa cells and T47-D cells. Since the former do not express

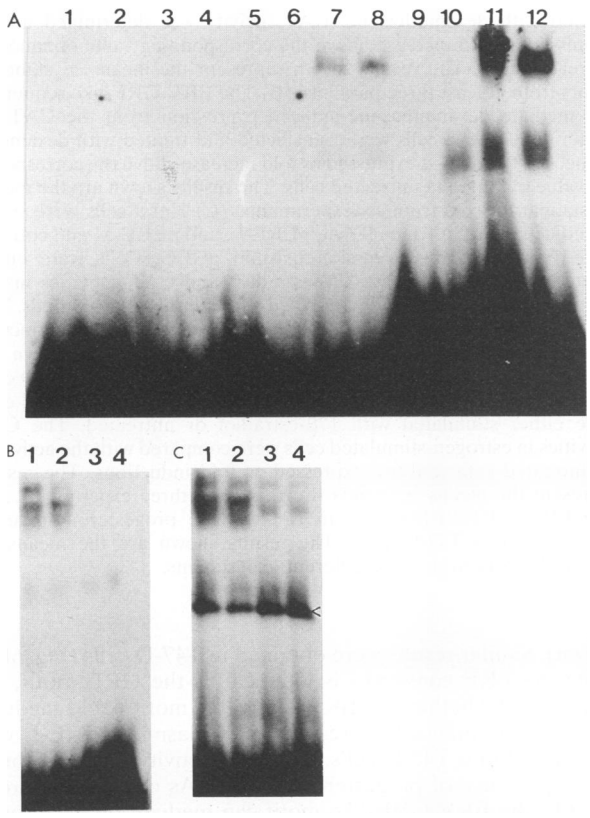


FIG. 5. Protein-DNA interaction studies with the BKV ERE consensus sequence and BKV GRE/PRE sequences. (A) Nuclear extracts of CV-1, MCF-7, and T47-D cells were incubated with labelled complementary oligonucleotides ERE-M, ERE-L, and EREvit (see Fig. 3B), and complexes were analyzed by GMSA. Lanes 1, 5, and 9, no extracts added; lanes 2, 6, and 10, CV-1 extracts added; lanes 3, 7, and 11, MCF-7 extracts added; lanes 4, 8, and 12, T47-D extracts added. Lanes 1 to 4, ERE-M; lanes 5 to 8, ERE-L; lanes 9 to 12, EREvit. (B) GMSA in the presence of nonspecific competitor DNA and anti-ER antibodies. ERE-L was incubated with a 100-fold molar excess of nonspecific AP-2 competitor DNA (lane 1) or with increasing amounts (1, 2, and 3 μg) of anti-ER antibodies (lanes 2, 3, and 4, respectively). (C) Binding of the PR to the BKV GRE/PRE motif. Labelled complementary oligonucleotide GRE-L was incubated with MCF-7 extracts in the presence of nonspecific AP-2 competitor DNA or anti-PR antibodies. Lane 1, 100-fold molar excess of AP-2 competitor DNA; lanes 2 to 4, 1, 2, and 3 μg of anti-PR antibodies, respectively. The open triangle probably represents a nonspecific protein-DNA complex.

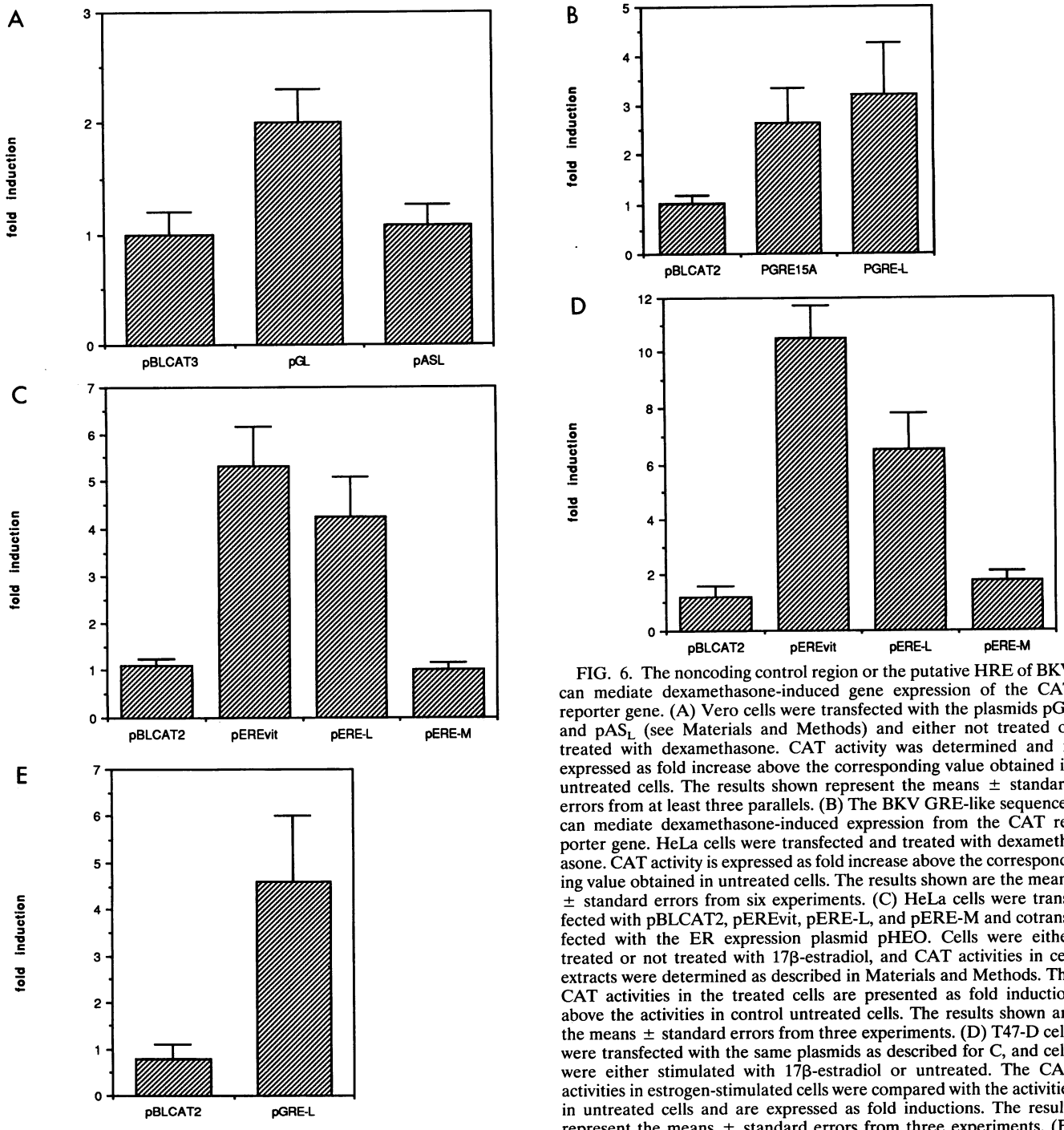


FIG. 6. The noncoding control region or the putative HRE of BKV can mediate dexamethasone-induced gene expression of the CAT reporter gene. (A) Vero cells were transfected with the plasmids pG_L and pAS_L (see Materials and Methods) and either not treated or treated with dexamethasone. CAT activity was determined and is expressed as fold increase above the corresponding value obtained in untreated cells. The results shown represent the means ± standard errors from at least three parallels. (B) The BKV GRE-like sequences can mediate dexamethasone-induced expression from the CAT reporter gene. HeLa cells were transfected and treated with dexamethasone. CAT activity is expressed as fold increase above the corresponding value obtained in untreated cells. The results shown are the means ± standard errors from six experiments. (C) HeLa cells were transfected with pBLCAT2, pEREvit, pERE-L, and pERE-M and cotransfected with the ER expression plasmid pHEO. Cells were either treated or not treated with 17β-estradiol, and CAT activities in cell extracts were determined as described in Materials and Methods. The CAT activities in the treated cells are presented as fold induction above the activities in control untreated cells. The results shown are the means ± standard errors from three experiments. (D) T47-D cells were transfected with the same plasmids as described for C, and cells were either stimulated with 17β-estradiol or untreated. The CAT activities in estrogen-stimulated cells were compared with the activities in untreated cells and are expressed as fold inductions. The results represent the means ± standard errors from three experiments. (E) The BKV GRE/PRE-like motif can mediate progesterone-induced transcription in T47-D cells. The results shown are the means ± standard errors from three different experiments.

the ER, cotransfection with the ER expression plasmid pHEO was done. This plasmid contains the coding sequence of the human ER cloned in the eukaryotic expression vector pSG5 (48). The results are shown in Fig. 6C. A slight induction of CAT activity for pBLCAT2 was measured for 17β-estradiol. This was also found in the studies done by Klock et al. (44). A markedly increased CAT activity was measured for the plasmids carrying the BKV ERE or the vitellogenin ERE motif. The mutated BKV ERE motif was unable to mediate estrogen-induced transcription. No induction in CAT expression was seen when cotransfection was done with pSG5 (data not shown). Similar results were obtained in T47-D cells (Fig. 6D). Since the PRE consensus is identical to the GRE motif, we investigated whether the BKV GRE-like motif could mediate progesterone-induced expression. The plasmid pGRE-L was transfected into T47-D cells, and CAT activity was monitored in the presence of progesterone R5020. As can be seen from Fig. 6E, the BKV GRE-like motif can mediate progesterone-

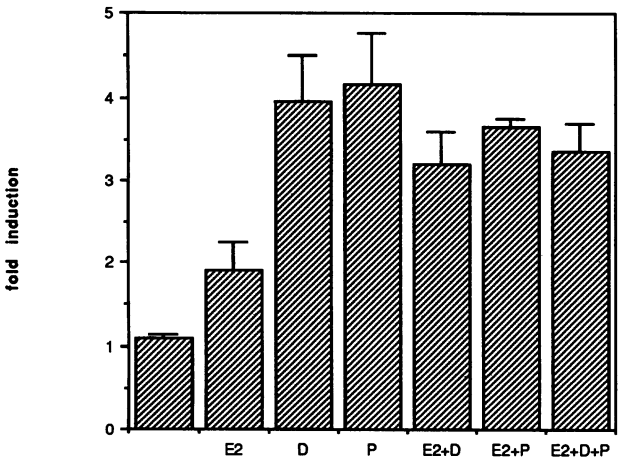


FIG. 7. No cooperative effect in transactivation is observed between the BKV ERE and GRE/PRE-like sequences. T47-D cells were transfected with the plasmid pEGRE. This plasmid contains both the BKV ERE and GRE/PRE sequences. Transfected cells were either not treated (−) or treated with 17β-estradiol (E2), dexamethasone (D), progesterone (P), or a combination of these steroid hormones. CAT activities in stimulated cells were compared with those in unstimulated cells and are shown as fold inductions. The results are the means ± standard errors from three parallels.

induced expression. A 4.5-fold increase in CAT activity in the presence of progesterone R5020 was measured.

No cooperativity observed between the BKV GRE/PRE and the BKV ERE. Cooperative transactivation of steroid receptors has been well documented (reviewed in reference 63). We therefore investigated whether such cooperativity existed between the BKV GRE/PRE and ERE. The plasmid pEGRE, which contained both the BKV GRE/PRE and ERE motifs, was transfected into T47-D cells. The cells were then treated with different steroids or with combinations of steroids, and CAT activities in the cellular extracts of these cells were determined. The results are shown in Fig. 7. All hormones stimulated the CAT activity, but the most profound effect was seen with R5020. No cooperative transactivation was seen when a combination of steroid hormones was used.

DISCUSSION

In the present work, we show that the synthetic glucocorticoid dexamethasone, progesterone, and 17β-estradiol enhance the permissivity of host cells for BKV infection by increasing the fraction of cells expressing viral capsid proteins, resulting in an up to 11-fold (dexamethasone), 5-fold (progesterone), or 3-fold (estrogen) higher virus yield. Our results suggest that the mechanism responsible for this enhancement is an increased transcription rate, particularly of the late genes expressing structural proteins. We attribute the increased transcription rate to the presence of a functional nonconsensus GRE/PRE sequence and a consensus ERE sequence located in the late leader (overlapping the putative agnogene start codon) of the BKV control region. This is supported by both DNA-binding studies and transient transfection assays. In addition to increasing transcriptional initiation, steroid hormones may stabilize some mRNAs (reference 66 and references therein). We tried to address this through experiments with the transcription inhibitor actinomycin D. However, because of the extended period before late transcription can be detected following BKV infection (24; this study), we were unable to find conditions where actinomycin D did not abolish all late transcription. Although these experiments remain inconclusive with regard to a possible effect of steroid hormones on BKV mRNA stability, we feel that our data are more compatible with a scenario where the HREs increase late gene transcription (see Results). This is further supported by our findings that the noncoding region of BKV(AS) could not mediate dexamethasone-induced transcription of the CAT reporter gene. BKV(AS) has a deletion in its late late leader that removes the GRE-like sequence found in all other BKV strains sequenced so far (81). Furthermore, we were unable to detect quantitative differences between the viral DNAs of dexamethasone-treated and untreated cultures. In line with this, O'Connor and Subramani (65) demonstrated that replication of a plasmid containing the simian virus 40 origin of replication and enhancer linked to a GRE was not influenced by dexamethasone treatment and studies with polyomavirus showed that estrogen did not effect viral replication in polyomavirus-infected mice (35).

GMSA showed that nuclear extracts of dexamethasone-, progesterone-, and 17β-estradiol-treated cells formed complexes with the BKV GRE, PRE, and ERE, respectively. Competition studies with nonspecific and specific competitor DNAs confirmed the specificity of the protein-DNA complexes, while studies with either purified receptor (GR) or monoclonal antibodies against the receptors (PR and ER) clearly identified the complexes as being the respective steroid receptor proteins. Recent studies by Klungland and coworkers have shown that the ER can bind to two ERE-like motifs in the gonadotropin-releasing hormone gene promoter region of Atlantic salmon. Each ERE-like motif is composed of two palindromic ERE half-sites interspaced by 8 and 9 nucleotides, respectively (45). Our results here and studies by others (54) show that different spacing between half-sites of a GRE may still allow binding of the GR.

Cooperative transactivation between steroid hormone receptors or other transcription factors is a well known phenomenon (reviewed in reference 63). Although the BKV genome contains functional GRE, PRE, and ERE motifs, we were unable to detect any cooperative transactivation between these receptors. One plausible explanation is that since the binding sites for these receptors overlap, simultaneous binding of two receptors is sterically impossible. We found that pEGRE responded remarkably better to progesterone and dexamethasone than to estradiol (Fig. 7). This suggests a stronger affinity of PR or GR for their GRE/PRE than ER for its cognate ERE sequence. This is rather unexpected, since the BKV ERE motif is a full consensus sequence while the BKV GRE/PRE motif is a nonconsensus motif. Alternatively, activated PR could be a stronger transactivator than activated ER. This is supported by the transient transfection and further confirmed by the in vitro infection studies which showed higher virus yield after dexamethasone (up to 11-fold) or progesterone (up to 5-fold) stimulation than in the presence of estrogens (up to 3-fold). Another explanation may be that the variation in inducibility by steroid hormones may be linked to the endogenous concentration of each steroid receptor present in the cell. Finally, it is possible that interaction of a cellular factor(s) with the steroid receptor is required for full transcriptional transactivation. Recently, several such factors have been described (49, reference 91 and references therein). One possible candidate is nuclear factor-1 (NF-1). Studies by us (61a) and others (13) demonstrated a functional NF-1 binding site that overlaps with the BKV GRE/PRE sequence.

Previous studies with other human viruses have illustrated a stimulating or inhibiting role of steroid hormones on viral

replication (herpes simplex virus type 2 [4], vaccinia virus [4], herpes simplex virus type 1 [64], Epstein-Barr virus [6], human immunodeficiency virus type 1 [56], human cytomegalovirus [46, 79, 80], and adenovirus [41]). In other human viruses, a GRE has been identified. Best studied are the hepatitis B virus (84, 85), Epstein-Barr virus (50, 73), the human papillomavirus type 16 (30), and the human immunodeficiency virus (28, 43). Although the GREs contained in these viral genomes can mediate glucocorticoid-induced expression, an increase in the expression of viral proteins due to glucocorticoid treatment has been demonstrated only for hepatitis B virus, yet no direct proof for increased production of infectious virus was presented (85). As far as we know, our results are the first showing a striking increase in the production of a human polyomavirus upon treatment of host cells with different steroid hormones.

In conclusion, our results show that steroid hormones can increase the levels of BKV transcripts and virus production in in vitro infected cells. Transient gene expression studies suggest that the activity is exerted at the level of transcription, although we cannot fully exclude some contribution by the stabilization of the RNA. Since we were able to boost the yield of infectious particles through steroid hormone treatment, we think that the degree of host cell permissivity is closely linked to late gene expression, although regulation of early gene expression or viral DNA replication may also contribute. We think that the BKV hormone response unit may play an important role in vivo by enhancing a productive BKV infection, and perhaps also by reactivating a latent infection, during physiological or pathological conditions accompanied by increased steroid hormone levels.

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REFERENCES

1. **Acheson, N. H.** 1978. Polyomavirus giant RNAs contain tandem repeats of the nucleotide sequence of the entire viral genome. Proc. Natl. Acad. Sci. USA **75**:4754–4758.
2. **Akerblom, I. W., and P. L. Mellon.** 1991. Repression of gene expression by steroid and thyroid hormones, p. 175–196. *In* M. G. Parker (ed.), Nuclear hormone receptors. Molecular mechanisms, cellular functions, clinical abnormalities. Academic Press Ltd., London.
3. **Akerblom, I. W., E. P. Slater, M. Beato, J. D. Baxter, and P. L. Mellon.** 1988. Negative regulation by glucocorticoids through interference with a cAMP response enhancer. Science **241**:350–353.
4. **Amstey, M. S.** 1977. Effect of pregnancy hormones on herpesvirus and other deoxyribonucleic acid viruses. Am. J. Obstet. Gynecol. **15**:159–163.
5. **Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and M. Karin.** 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell **49**:729–739.
6. **Bauer, G.** 1983. Induction of Epstein-Barr virus early antigens by corticosteroids: inhibition by TPA and retinoic acid. Int. J. Cancer **31**:291–295.
7. **Beato, M.** 1987. Induction of transcription by steroid hormones. Biochim. Biophys. Acta **910**:95–102.
8. **Beato, M.** 1989. Gene regulation by steroid hormones. Cell **56**:335–344.
9. **Bocquel, M. T., V. Kumar, C. Stricker, P. Chambon, and H. Gronemeyer.** 1989. The contribution of the N- and C-terminal regions of steroid receptors to activation of transcription is both receptor and cell-specific. Nucleic Acids Res. **17**:2581–2595.
10. **Borgatti, M., F. Costanzo, M. Portolani, C. Vullo, L. Osti, M. Masi, and G. Barbanti-Brodano.** 1979. Evidence for reactivation of persistent infection during pregnancy and lack of congenital transmission of BK virus, a human papovavirus. Microbiologica **2**:173–179.
11. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
12. **Brown, P., T. Tsai, and G. D. Gajdusek.** 1975. Seroepidemiology of human papovaviruses. Discovery of virgin populations and some unusual patterns of antibody prevalence among remote people of the world. Am. J. Epidemiol. **102**:331–340.
13. **Chakraborty, T., and G. C. Das.** 1991. Proteins of the nuclear factor-1 family act as an activator of the late promoter in human polyomavirus BK *in vitro*. J. Gen. Virol. **72**:1935–1942.
14. **Chester, P. M., J. Heritage, and D. J. McCane.** 1983. Persistence of DNA sequences of BK virus and JC virus in normal tissues and in diseased tissues. J. Infect. Dis. **147**:676–684.
15. **Coleman, D. V., R. A. Daniel, S. D. Gardner, A. M. Field, and P. E. Gibson.** 1977. Polyomavirus in urine during pregnancy. Lancet **ii**:709–710.
16. **Cordingley, M. G., A. T. Rieget, and G. L. Hager.** 1987. Steroid-dependent interaction of transcription factors with the inducible promoter of mouse mammary tumor virus *in vivo*. Cell **48**:261–270.
17. **Dei, R., F. Marmo, D. Corte, M. G. Sampietro, E. Franceschini, and P. Urbano.** 1982. Age-related changes in the prevalence of precipitating antibodies to BK virus in infants and children. J. Med. Microbiol. **15**:285–291.
18. **DeMarzo, A. M., C. A. Beck, S. A. Onata, and D. P. Edwards.** 1991. Dimerization of mammalian progesterone receptors occurs in the absence of DNA and is related to the release of the 90-kDa heat shock protein. Proc. Natl. Acad. Sci. USA **88**:72–76.
19. **De Vos, P., F. Claessens, B. Peeters, W. Rombauts, W. Heyns, and G. Verhoeven.** 1993. Interaction of androgen and glucocorticoid receptor DNA-binding domains with their response elements. Mol. Cell. Endocrinol. **90**:R11–R16.
20. **Diamond, M. I., J. N. Minner, S. Yoshinaga, and K. R. Yamamoto.** 1990. Transcription factor interactions: selectors of positive or negative regulation from a single DNA element. Science **249**: 1266–1272.
21. **Dignam, J. D., R. M. Lebowitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11**:1475–1489.
22. **Drouin, J., M. A. Trifiro, R. K. Plante, M. Nemer, P. Eriksson, and Ö. Wrange.** 1989. Glucocorticoid receptor binding to a specific DNA sequence is required for hormone-dependent repression of pro-opiomelanocortin gene transcription. Mol. Cell. Biol. **9**:5305–5314.
23. **Faisst, S., and S. Meyer.** 1992. Compilation of vertebrate-encoded transcription factors. Nucleic Acids Res. **20**:3–26.
24. **Flægstad, T., and T. Traavik.** 1987. BK virus in cell culture: infectivity quantitation and sequential expression of antigens detected by immunoperoxidase staining. J. Virol. Methods **16**:139–146.
25. **Flægstad, T., T. Traavik, K. E. Christie, and J. Jørgensen.** 1986. Neutralization test for BK virus: plaque reduction detected by immunoperoxidase. J. Med. Virol. **9**:287–296.
26. **Gardner, S. D., E. F. D. McKenzie, C. Smith, and A. A. Porter.** 1984. Prospective study of the human polyomaviruses BK and JC and cytomegalovirus in renal transplant recipients. J. Clin. Pathol. **37**:578–586.
27. **Gaub, M.-P., M. Bellard, I. Scheuer, P. Chambon, and P. Sassone-**

Corsi. 1990. Activation of the ovalbumin gene by the estrogen receptor involves the Fos-Jun complex. Cell **63**:1267–1276.
28. **Ghosh, D.** 1992. Glucocorticoid receptor-binding site in the human immunodeficiency virus long terminal repeat. J. Virol. **66**:586–590.
29. **Gibson, P. E., A. M. Field, S. D. Gardner, and D. V. Coleman.** 1981. Occurrence of IgM antibodies against BK and JC polyomaviruses during pregnancy. J. Clin. Pathol. **34**:674–679.
30. **Gloss, B., H. U. Bernard, K. Seedorf, and G. Klock.** 1987. The upstream regulatory region of the human papillomavirus-16 contains an E2 protein-independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. EMBO J. **6**:3735–3743.
31. **Gorman, C. M.** 1985. High efficiency gene transfer into mammalian cells, p. 143–190. *In* D. M. Glover (ed.), DNA cloning, vol. II. IRL Press, Oxford.
32. **Gorman, C. M., L. F. Moffat, and B. H. Howard.** 1982. Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. Mol. Cell. Biol. **2**:1044–1051.
33. **Gough, N.** 1988. Cytoplasmic RNA extraction protocol. Anal. Biochem. **173**:93–95.
34. **Härd, T., K. Dahlman, J. Carlstedt-Duke, J. Å. Gustafsson, and R. Rigler.** 1990. Cooperativity and specificity in the interaction between DNA and the glucocorticoid receptor DNA-binding domain. Biochemistry **29**:5358–5364.
35. **Heindel, R. M., S. Z. Haslam, and M. M. Fluck.** 1992. Effect of estrogen in polyomavirus replication, gene expression and mammary tumor genesis. Proc. Am. Assoc. Cancer Res. **33**:391.
36. **Heritage, J., P. M. Chester, and D. J. McCane.** 1981. The persistence of papovavirus BK DNA sequences in normal renal tissue. J. Med. Virol. **8**:143–150.
37. **Hogan, T. F., E. C. Borden, J. A. McBain, B. L. Padgett, and D. L. Walker.** 1980. Human polyomavirus infection with JC virus and BK virus in renal transplant patients. Ann. Intern. Med. **92**:373–378.
38. **Hollenberg, S. M., C. Weinerberger, E. S. Ong, G. Cerelli, A. Oro, R. Lebo, E. G. Thompson, M. G. Rosenfeld, and R. M. Evans.** 1985. Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature (London) **318**:635–641.
39. **Horwitz, K. B., and W. L. McGuire.** 1978. Estrogen control of progesterone receptor in human breast cancer: correlation with nuclear processing of estrogen receptor. J. Biol. Chem. **253**:2223–2228.
40. **Hyder, S. M., G. M. Stancel, Z. Nawaz, D. P. McDonnell, and D. S. Loose-Mitchell.** 1992. Identification of an estrogen response element in the 3′-flanking region of the murine c-*fos* protooncogene. J. Biol. Chem. **267**:18047–18054.
41. **James, C. B. L., E. A. Vanderpool, and P. Roane.** 1992. Acceleration of adenovirus replication and increased virion production by treatment with the steroid hormone 17β-estradiol. Microbiol. Immunol. **36**:99–103.
42. **Jonat, C., H. J. Rahmsdorf, K.-P. Park, A. C. B. Cato, S. Gebel, H. Ponta, and P. Herrlich.** 1990. Antitumor promotion and antiinflammation: down-modulation of AP-1 (Fos/Jun) activity by glucocorticoid hormone. Cell **62**:1189–1204.
43. **Katsanakis, C. D., C. E. Sekeris, and D. A. Spandisos.** 1991. The human immunodeficiency virus long terminal repeat contains sequences showing partial homology to glucocorticoid responsive element. Anticancer Res. **11**:381–383.
44. **Klock, G., U. Strähle, and G. Schütz.** 1987. Oestrogen and glucocorticoid response elements are closely related but distinct. Nature (London) **329**:734–736.
45. **Klungland, H., Ø. Andersen, G. Kisen, P. Aleström, and L. Tora.** 1993. Estrogen receptor binds to the salmon GnRH gene in a region with long palindromic sequences. Mol. Cell. Endocrinol. **95**:147–154.
46. **Koment, R. W.** 1985. Lytic cytomegalovirus replication and the hormones of human pregnancy. J. Med. Virol. **15**:149–156.
47. **Köning, H., H. Ponta, H. J. Rahmsdorf, and P. Herrlich.** 1992. Interference between pathway-specific transcription factors: glucocorticoids antagonize phorbol ester-induced AP-1 activity without altering AP-1 site occupation *in vivo*. EMBO J. **11**:2241–2246.
48. **Kumar, V., S. Green, G. Stack, M. Berry, J. R. Jin, and P. Chambon.** 1987. Functional domains of the human estrogen receptor. Cell **51**:941–951.
49. **Kupfer, S. R., K. B. Marschke, E. M. Wilson, and F. S. French.** 1993. Receptor accessory factor enhances specific DNA binding of androgen and glucocorticoid receptors. J. Biol. Chem. **268**:17519–17527.
50. **Kupfer, S. R., and W. C. Summer.** 1990. Identification of a glucocorticoid response element in Epstein-Barr virus. J. Virol. **64**:1984–1990.
51. **Lee, H., Y.-T. Shaw, S.-T. Chiou, W.-C. Chang, and M. D. Lai.** 1991. The effects of glucocorticoid hormone on the expression of c-*jun*. FEBS Lett. **280**:134–136.
52. **Lucibello, F., E. P. Slater, K. U. Joos, M. Beato, and R. Müller.** 1990. Mutual transrepression of Fos and the glucocorticoid receptor: involvement of a functional domain in Fos which is absent in FosB. EMBO J. **9**:2827–2834.
53. **Luckow, B., and G. Schütz.** 1987. CAT constructions with unique restriction sites for the functional analysis of eukaryotic promoters and regulatory sequences. Nucleic Acids Res. **15**:5490.
54. **Luisi, B. F., W. Xu, Z. Otwinowski, L. P. Freedman, K. R. Yamamoto, and P. B. Sigler.** 1991. Crystallographic analysis of the interaction of the glucocorticoid receptor with DNA. Nature (London) **352**:497–505.
55. **MacDonald, R. J., G. H. Swift, A. E. Przybyla, and J. M. Chrigwin.** 1987. Isolation of RNA using guanidinium salts. Methods Enzymol. **152**:219–227.
56. **Markham, P. D., S. Z. Salahuddin, K. Veren, S. Orndorff, and R. C. Gallo.** 1986. Hydrocortisone and some other hormones enhance the expression of HTLV-III. Int. J. Cancer **37**:67–72.
57. **Markowitz, R.-B., and W. S. Dynan.** 1988. Binding of cellular proteins to the regulatory region of BK virus DNA. J. Virol. **62**:3388–3398.
58. **Martinez, E., and W. Wahli.** 1991. Characterization of hormone response elements, p. 125–153. *In* M. G. Parker (ed.), Nuclear hormone receptors. Molecular mechanisms, cellular functions, clinical abnormalities. Academic Press Ltd., London.
59. **McIntyre, P., and G. R. Stark.** 1988. A quantitative method for analyzing specific DNA sequences directly from whole cells. Anal. Biochem. **174**:209–214.
60. **Moens, U., S. Nanthakumar, B. Johansen, and J. Aarbakke.** 1993. The c-*fos* cAMP-response element: regulation of gene expression by a β2-adrenergic agonist, serum and DNA methylation. Biochim. Biophys. Acta **1173**:63–70.
61. **Moens, U., A. Sundsfjord, T. Flægstad, and T. Traavik.** 1990. BK virus early RNA transcripts in stable transformed cells: enhanced levels induced by dibutyryl cyclic AMP, Forskolin and 12-O-tetradecanoylphorbol 13-acetate treatment. J. Gen. Virol. **71**: 1461–1471.
61a. **Moens, U., and T. Traavik.** Unpublished data.
62. **Moens, U., P. Villand, B. Johansen, and T. Traavik.** 1992. A simple and convenient method to standardize transient chloramphenicol acetyltransferase activity. Methods Mol. Cell. Biol. **2**:169–175.
63. **Muller, M., C. Baniahmad, C. Kaltschmidt, R. Schüle, and R. Renkawitz.** 1991. Co-operative transactivation of steroid receptors, p. 155–174. *In* M. G. Parker (ed.), Nuclear hormone receptors. Molecular mechanisms, cellular functions, clinical abnormalities. Academic Press Ltd., London.
64. **Notter, M. F. D., and J. J. Docherty.** 1978. Steroid hormone alteration of Herpes simplex virus type 1 replication. J. Med. Virol. **2**:247–252.
65. **O'Connor, D., and S. Subramani.** 1988. Do transcriptional enhancers also augment DNA replication? Nucleic Acids Res. **16**:11207–11222.
66. **Parker, M. G., and O. Bakker.** 1991. Nuclear hormone receptors: concluding remarks, p. 377–395. *In* M. G. Parker (ed.), Nuclear hormone receptors. Molecular mechanisms, cellular functions, clinical abnormalities. Academic Press Ltd., London.
67. **Ray, A., K. S. LaForge, and P. B. Seghal.** 1990. On the mechanisms for efficient repression of the interleukin-6 promoter by glucocorticoids: enhancer, TATA box, and RNA start site (Inr motif) occlusion. Mol. Cell. Biol. **10**:5736–5746.
68. **Sabbah, M., F. Gouillieux, B. Sola, G. Redeuilh, and E.-E. Baulieu.** 1991. Structural differences between the hormone and antihor-

mone estrogen receptor complexes bound to the hormone responsive element. Proc. Natl. Acad. Sci. USA **88**:390–394.

69. **Sakai, D. D., S. Helms, J. Carlstedt-Duke, J. Å. Gustafsson, F. M. Rottman, and K. R. Yamamoto.** 1988. Hormone-mediated repression of transcription: a negative glucocorticoid responsive element from the bovine prolactin gene. Genes Dev. **2**:1144–1154.
70. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
71. **Schneider-Gädicke, A., and E. Schwarz.** 1986. Different human cervical carcinoma cell lines show similar transcription patterns of human papillomavirus type 18 early genes. EMBO J. **9**:2285–2292.
72. **Schühle, R., P. Rangarajan, S. Kliewer, L. J. Ransone, J. Bolado, N. Yang, I. M. Verma, and R. M. Evans.** 1990. Functional antagonism between oncoprotein c-Jun and the glucocorticoid receptor. Cell **62**:1217–1266.
73. **Schuster, C., S. Chasserot-Golaz, G. Urier, G. Beck, and A. Sergeant.** 1991. Evidence for a functional glucocorticoid responsive element in the Epstein-Barr virus genome. Mol. Endocrinol. **5**:267–272.
74. **Shah, K. V., R. W. Daniel, D. Madden, and S. Stagno.** 1980. Serological investigation of BK papovavirus infection in pregnant women and their offspring. Infect. Immun. **30**:29–35.
75. **Shemshedini, L., R. Knauthe, P. Sassone-Corsi, A. Pornon, and H. Gronemeyer.** 1991. Cell-specific inhibitory and stimulatory effects of Fos and Jun on transcription activation by nuclear receptors. EMBO J. **10**:3839–3849.
76. **Shingh, H., R. Sen, D. Baltimore, and P. Sharp.** 1986. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. Nature (London) **319**:154–158.
77. **Strömstedt, P.-E., L. Poellinger, J. Å. Gustafsson, and J. Carlstedt-Duke.** 1991. The glucocorticoid receptor binds to a sequence overlapping the TATA box of the human osteocalcin promoter: a potential mechanism for negative regulation. Mol. Cell. Biol. **11**:3379–3383.

77a. **Subramaniam, N., U. Moens, and T. Traavik.** Unpublished data.

78. **Sundsfjord, A., T. Johansen, T. Flægstad, U. Moens, P. Villand, S. Subramani, and T. Traavik.** 1990. At least two types of control regions can be found among naturally occurring BK variants in vivo and in vitro. J. Virol. **64**:3864–3871.
79. **Tanaka, J., T. Ogura, S. Kamiya, H. Sato, T. Yoshie, H. Ogura, and M. Hatano.** 1984. Enhanced replication of human cytomegalovirus in human fibroblasts treated with dexamethasone. J. Gen. Virol. **65**:1759–1767.
80. **Tanaka, J., T. Ogura, S. Kamiya, T. Yoshie, Y. Yabuki, and M. Hatano.** 1984. Dexamethasone enhances human cytomegalovirus replication in human epithelial cell cultures. Virology **136**:448–452.
81. **Tavis, J. E., D. L. Walker, S. D. Gardner, and R. J. Frisque.** 1989. Nucleotide sequence of the human polyomavirus AS virus, an antigenic variant of BK virus. J. Virol. **63**:901–911.
82. **Traish, A., R. Ettinger, N. Kim, A. Marshak-Rothstein, and H. Wotiz.** 1990. Development and characterization of monoclonal antibodies to a specific domain of human estrogen receptor. Steroids **55**:196–208.
83. **Tsai, S. Y., J. Carlstedt-Duke, M. L. Weigel, K. Dahlman, J. Å. Gustafsson, M.-J. Tsai, and B. O'Malley.** 1988. Molecular interactions of steroid hormone receptor with its enhancer element: evidence for receptor dimer formation. Cell **55**:361–369.
84. **Tur-Kaspa, R., R. D. Burk, Y. Shaul, and D. A. Shafritz.** 1986. Hepatitis B virus DNA contains a glucocorticoid responsive element. Proc. Natl. Acad. Sci. USA **83**:1627–1631.
85. **Tur-Kaspa, T., and O. Laub.** 1990. Corticosteroids stimulate hepatitis B virus DNA, mRNA and protein production in a stable expression system. J. Hepatol. **11**:34–36.
86. **Wahli, W., E. Martinez, B. Corthésy, and J.-R. Cardinaux.** 1989. *Cis* and *trans*-acting elements of the estrogen-regulated vitellogenin gene B1 of *Xenopus laevis*. J. Steroid Biochem. **34**:17–32.
87. **Weisz, A., L. Cicatiello, E. Persico, M. Scalona, and F. Bresciani.** 1990. Estrogen stimulates transcription of c-*jun* protooncogene. Mol. Endocrinol. **4**:1041–1050.
88. **Weisz, A., and R. Rosales.** 1990. Identification of an estrogen response element upstream of the human c-*fos* gene that binds the estrogen receptor and the AP-1 transcription factor. Nucleic Acids Res. **18**:5097–5106.
89. **Yang-Yen, H.-F., J.-C. Chambard, Y.-L. Sun, T. Smeal, T. J. Schmidt, J. Drouin, and M. Karin.** 1990. Transcriptional interference between c-Jun and the glucocorticoid receptor: mutual inhibition of DNA binding due to direct protein-protein interaction. Cell **62**:1205–1215.
90. **Yoshiike, K., and K. K. Takemoto.** 1986. Studies with BK virus and monkey lymphotropic papovavirus, p. 295–326. *In* N. P. Salzman (ed.), The Papovaviridae, vol. 1. The polyomaviruses. Plenum Publishing Corp., New York.
91. **Yoshinaga, S. K., C. I. Peterson, I. Herskowitz, and K. R. Yamamoto.** 1992. Roles of SWI1, SWI2 and SWI3 proteins for transcriptional enhancement by steroid receptors. Science **258**: 1598–1604.
92. **Zhang, X.-K., J.-M. Dong, and J.-F. Chiu.** 1991. Regulation of the α-fetoprotein gene expression by antagonism between AP-1 and the glucocorticoid receptor at their overlapping binding site. J. Biol. Chem. **266**:8248–8254.