Simian virus 40 late gene expression is regulated by members of the steroid/thyroid hormone receptor superfamily

(antiviral drugs/DNA tumor virus/human estrogen-related receptor 1/testis receptor 2/chicken ovalbumin upstream promoter transcription factor)

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ABSTRACT   Transcription of the late genes of simian virus 40 (SV40) is repressed during the early phase of the lytic cycle of infection of binding of cellular factors, called IBP-s, to the SV40 late promoter; repression is relieved after the onset of viral DNA replication by titration of these repressors. Preliminary data indicated that one of the major components of IBP-s was human estrogen-related receptor 1 (hERR1). We show here that several members of the steroid/thyroid hormone receptor superfamily, including testis receptor 2, thyroid receptor $\alpha 1$ in combination with retinoid X receptor α, chicken ovalbumin upstream promoter transcription factors 1 and 2 (COUP-TF1 and COUP-TF2), as well as hERR1, possess the properties of IBP-s. These receptors bind specifically to hormone receptor binding sites present in the SV40 major late promoter. Recombinant COUP-TF1 specifically represses transcription from the SV40 major late promoter in a cell-free transcription system. Expression of COUP-TF1, COUP-TF2, or hERR1 in monkey cells results in repression of the SV40 late promoter, but not the early promoter, in the absence of the virally encoded large tumor antigen. Overexpression of COUP-TF1 leads to a delay in the early-to-late switch in SV40 gene expression during the lytic cycle of infection. Thus, members of this superfamily can play major direct roles in regulating expression of SV40. Possibly, natural or synthetic ligands to these receptors can serve as antiviral drugs. Our findings also provide the basis for the development of assays to screen for the ligands to testis receptor 2 and hERR1.

Expression of the genome of the small primate DNA tumor virus simian virus 40 (SV40) is highly regulated. The late genes, which encode the virion proteins, are expressed efficiently only after the onset of viral DNA replication (1). The early gene product, large tumor (T) antigen, accounts, in part, for activation of the late genes (2). The cis- and trans-acting factors required for efficient transcription from the SV40 major late promoter (MLP) are (i) an upstream enhancer region (3) that binds multiple factors (4) and (ii) three proximal sequence elements (5) that interact with TFIID (6), DAP (7), and, possibly, RNA polymerase II.

Wiley et al. (8) demonstrated that the presence in cells of sequence-specific transcriptional repressors was responsible for the replication dependence of SV40 late gene expression. They showed that (i) the repressor activity, designated IBP-s for initiator binding proteins of SV40, bound with high affinity to sequences located both directly at and 55 bp downstream of the major initiation site in the SV40 late promoter and (ii) these IBP-s binding sites were composed of direct repeats (DRs) similar to 5'-AGGTCA-3' in sequence and separated by 4 and 2 bp, respectively. Interestingly, the sequence 5'-AGGTCA-3' is the consensus half-site sequence for binding by members of the steroid/thyroid hormone receptor superfamily (9). Partial

amino acid sequence analysis revealed that the major 55-kDa component of IBP-s as purified from HeLa cells was identical to human estrogen-related receptor 1 (hERR1) (8). Having been cloned solely on the basis of sequence similarity to estrogen receptor (10), hERR1's binding site specificity, ligand, and function were unknown.

The chicken ovalbumin upstream promoter transcription factors 1 and 2 (COUP-TF1 and COUP-TF2; also named ear-3 and ARP-1, respectively) and human testis receptor 2 (TR2) are other so-called "orphan" members of this superfamily (11–15). Whereas the COUP-TFs have been shown to activate or repress transcription of a variety of genes (16), the binding-site specificity and function, as well as the ligand, of TR2 remain unknown.

We show here that COUP-TF1, COUP-TF2, and hERR1 possess all of the previously identified in vivo and in vitro functional activities of IBP-s, with COUP-TF1 being the second major component of IBP-s. We also show that (i) overexpression of COUP-TF1 delays the SV40 early-to-late switch in gene expression and (ii) thyroid receptor $\alpha 1$ (TR$\alpha 1$) in combination with human retinoid X receptor α (hRXRα) and TR2 bind the +1 and +55 IBP-s binding sites of the SV40 MLP, respectively. Thus, numerous members of the steroid/thyroid hormone receptor superfamily can play major, direct roles in regulating expression of this primate DNA tumor virus.

MATERIALS AND METHODS

Oligonucleotides and Plasmids. The double-stranded oligonucleotides used (Fig. 1) were synthesized, annealed, and gel purified by standard protocols (17). Plasmids pCOUP-TF1, pCOUP-TF2, and pRSh-COUP-TF1 (18) were gifts from M.-J. Tsai. Plasmids pRSV-hERR1 (10), pCDMα1(hTRα1) (19), pSGhRXRα (20), and pSG5-TR2 (21) were kindly provided by R. Evans, L. DeGroot, P. Chambon, and C. Chang, respectively. Plasmid pSV1773, containing an SV40 genome with a frameshift mutation in its VP1-coding region (22), was used as "wild-type" (WT) SV40 in transfection experiments. Mutant pm322C×LS26 is a variant of pSV1773 in which the +1 and +55 IBP-s binding sites have been inactivated (8). In cell-free transcription reactions, the SV40 genomes lacked the 72-bp enhancer region as well (8). Plasmid pSVE-luc, containing the luciferase reporter driven by the SV40 early promoter, was constructed by subcloning SV40 nt 5243–446 into the HindIII site of pGL-Basic (Promega). Plasmid pSVL-luc(WT) (23) is a derivative of pSV1773 containing (i) a substitution of the VP1-coding region by luciferase-encoding sequences, (ii)

Abbreviations: COUP-TF, chicken ovalbumin upstream promoter transcription factor; DR, direct repeat; hERR1, human estrogen-related receptor 1; RARα, retinoic acid receptor α; hRXRα, human retinoid X receptor α; TRα1, human thyroid receptor α1; TR2, human testis receptor 2; IBP-s, initiator binding proteins of SV40; MLP, major late promoter; SV40, simian virus 40; T antigen, large tumor antigen; WT, wild type.
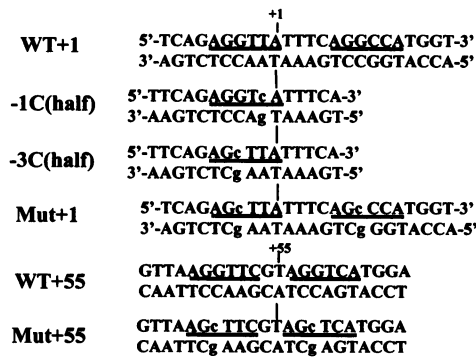*To whom reprint requests should be addressed.

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WT+1
5'-TCAG<u>AGGTTA</u>TTTC<u>AGGCCA</u>TGGT-3'
3'-AGTCTCCAATAAAGTCCGGTACCA-5'

-1C(half)
5'-TTCAG<u>AGGTc</u>ATTTCA-3'
3'-AAGTCTCCA**g**TAAAGT-5'

-3C(half)
5'-TTCAG<u>AGc TTA</u>TTTCA-3'
3'-AAGTCTC**g**AATAAAGT-5'

Mut+1
5'-TCAG<u>AGc TTA</u>TTTC<u>AGc CCA</u>TGGT-3'
3'-AGTCTC**g**AATAAAGTC**g**GGTACCA-5'

WT+55
GTTA<u>AGGTTC</u>GT<u>AGGTCA</u>TGGA
CAATTCCAAGCATCCAGTACCT

Mut+55
GTTA<u>AGc TTC</u>GT<u>AGc TCA</u>TGGA
CAATTC**g** AAGCATC**g** AGTACCT

FIG. 1. Sequences of double-stranded oligonucleotides used for gel mobility-shift and cell-free transcription assays. Nucleotide numbers are relative to the initiation site of the SV40 MLP. Lowercase letters indicate alterations from the wild-type (WT) SV40 sequence. The underlines indicate the locations of putative half-site sequences for binding of IBP-s.

an inactivated viral origin of DNA replication, and (*iii*) a frameshift mutation in the sequence encoding the amino-terminal region of T antigen (24). Plasmid pSVL-luc(-3C) differs from pSV-luc(WT) solely by a G → C change at SV40 nt 322.

Gel Mobility-Shift Assays. Recombinant receptor proteins were synthesized in a coupled transcription–translation rabbit reticulocyte lysate system (Promega). Gel mobility-shift and supershift assays were performed as described (18) with minor modifications (23). The COUP-TF-specific antiserum and the preimmune serum used in the supershift experiments were gifts from M.-J. Tsai (11). Quantitative analyses were performed with a PhosphorImager (Molecular Dynamics).

Cell-Free Transcription Assays. Cell-free transcription reactions were performed essentially as described (8). Final reaction volumes (50 μl) contained ≈150 μg of protein from HeLa cell nuclear extract and 200 ng of SV40 DNA. The DNA templates were preincubated, where indicated, with the specified amounts of *in vitro* synthesized recombinant COUP-TF1 prior to the addition of the nuclear extract. The resulting RNAs were analyzed by primer extension (22). Synthetic oligonucleotides corresponding to SV40 nt 446–422 and 5178–5201 were used as primers for detecting late- and early-strand transcripts, respectively.

Transient-Transfection Assays. CV-1PD and HeLa cells were grown and transfections were performed as described (22). Viral sequences were excised from their cloning vector and ligated to form monomer circles before transfection. The relative amounts of early and late SV40 RNA present at the indicated times after transfection were determined by quantitative S1 nuclease mapping (3). Viral DNA isolation and Southern blot analyses were performed as described (24). Luciferase activities were determined with an assay kit (Promega) 54 hr after transfection and normalized to total protein present in the lysate as determined by Bradford assays (Bio-Rad). Essentially identical results were obtained by cotransfection with pRSV-CAT and normalization to chloramphenicol acetyltransferase (CAT) activity (data not shown).

RESULTS

COUP-TFs Comprise Most of IBP-s Activity. Preliminary Western blot analysis indicated that highly purified preparations of IBP-s contained an ≈45-kDa component that cross-reacted with an antiserum specific for COUP-TFs (23). Gel mobility-shift assays performed with the radiolabeled +55 and +1 region probes indicated that both of these proteins specifically bound the putative DR2 (+55 site) and DR4 (+1 site) elements present in the SV40 MLP (ref. 23; see also Fig. 3). To
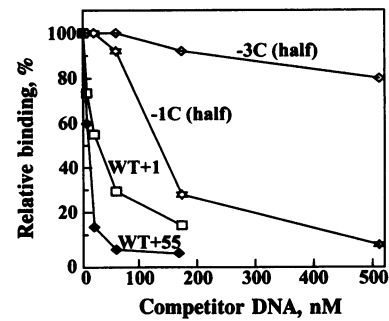
FIG. 2. Relative affinities of COUP-TF1 for a half-site sequence and the +1 and +55 region sites in the SV40 MLP. Quantitative gel mobility-shift assays were performed with 55 fmol of 5' end-labeled WT+55 DNA as probe, a constant amount of *in vitro* synthesized COUP-TF1, and the indicated amounts of unlabeled competitor DNA (see Fig. 1 for sequences).

determine the relative affinities of COUP-TF1 for these IBP-s binding sites, we performed quantitative competition gel mobility-shift assays. These data (Fig. 2) indicated that the affinity of COUP-TF1 for the WT+55 sequence was 4- and 10-fold higher than it was for the WT+1 and optimal half-site [−1C (half)] sequence, respectively. Thus, COUP-TF1 can probably bind a half-site in this sequence context. On the other hand, its affinity for a mutated single half-site sequence [−3C (half)] is extremely low. Similar relative binding affinities were also observed with recombinant COUP-TF2 (23). The relative affinities of these proteins for these binding sites are remarkably similar to those observed previously with IBP-s (8, 25).

To determine directly whether the COUP-TFs are major components of IBP-s, we examined whether IBP-s–DNA complexes could be specifically retarded in electrophoretic mobility by incubation with a COUP-TF-specific polyclonal antiserum (Fig. 3*A*). Addition of the antiserum resulted in ≈80% of the IBP-s–DNA complexes being shifted to a slower mobility (lane 2). Thus, most, but not all, of IBP-s activity contains COUP-TF1, COUP-TF2, or other related proteins that crossreact with this serum.
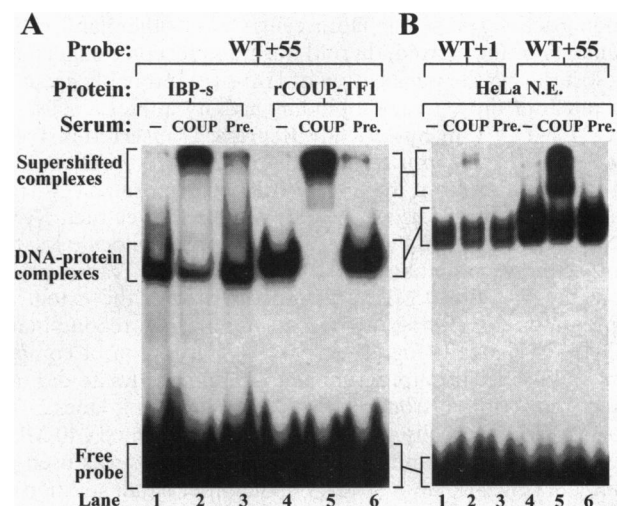
FIG. 3. Most of the IBP-s activity crossreacts with a COUP-TFs-specific antiserum. (*A*) Mobility-shift assays performed with radiolabeled WT+55 DNA as probe, a preimmune (lanes 3 and 6) or COUP-TFs-specific (lanes 2 and 5) serum, and highly purified IBP-s (lanes 1–3) or recombinant (r) COUP-TF1 synthesized in a reticulocyte lysate (lanes 4–6). (*B*) Mobility-shift assays performed with HeLa cell nuclear extract (N.E.) and radiolabeled WT+1 (lanes 1–3) or WT+55 (lanes 4–6) DNA as probe in the presence of COUP-TF-specific (lanes 2 and 5) or preimmune (lanes 3 and 6) serum.
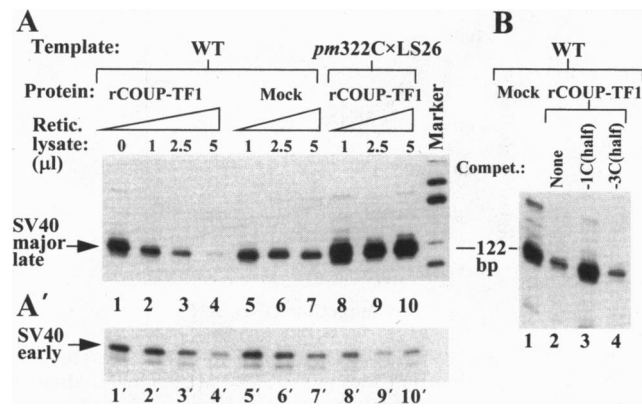
FIG. 4. COUP-TF1 represses transcription from the SV40 MLP *in vitro* in a sequence-specific, titratable manner. (*A* and *A'*) Autoradiograms showing the effect of addition of COUP-TF1 on SV40 late (*A*) versus early (*A'*) RNA synthesis in a cell-free transcription system. Cell-free transcription assays were performed by incubation of the indicated amounts of *in vitro* synthesized recombinant (r) COUP-TF1 at 4°C for 10 min, followed by 15 min at 26°C with 200 ng of supercoiled plasmid containing WT SV40 DNA (lanes 1–4) or the double IBP-s binding-site mutant *pm*322C×LS26 (lanes 8–10). As a control, reactions were also performed in parallel with similar amounts of reticulocyte lysate to which pCOUP-TF1 had not been added (Mock, lanes 5–7). (*B*) Autoradiogram showing the effect of addition of specific competitor oligonucleotides on repression by rCOUP-TF1. Reactions were performed as in *A*, lane 2, except that a 100-fold molar excess of the indicated double-stranded oligonucleotide competitor was included as well (see Fig. 1 for sequences).

To confirm that a majority of the IBP-s binding activity present in HeLa cells does, indeed, contain COUP-TF cross-reacting material, a HeLa cell nuclear extract was incubated with radiolabeled +1 or +55 region probe in the absence or presence of the COUP-TF-specific antiserum (Fig. 3B). The mobilities of two-thirds of the protein–DNA complexes formed on the +55 region probe were retarded by the COUP-TF-specific antiserum (Fig. 3B, lane 5). In contrast, few of the protein–DNA complexes formed with the +1 region probe were retarded (Fig. 3B, lane 2). Thus, COUP-TFs (or COUP-TF-like proteins) account for most of the +55 region IBP-s binding activity present in HeLa cells. On the other hand, other members of the steroid/thyroid hormone receptor superfamily, lost during the purification of IBP-s (8), probably account for much of the +1 region binding activity in HeLa cells.

COUP-TF1 Can Specifically Repress Transcription from the SV40 MLP *in Vitro*. To determine the effect of COUP-TF1 on transcription from the SV40 MLP, we performed cell-free transcription assays using a HeLa cell nuclear extract, SV40 DNA as template, and various amounts of reticulocyte lysate-synthesized recombinant COUP-TF1 (Fig. 4A). Transcription from the WT SV40 MLP was repressed, with the extent of repression correlating with the amount of recombinant COUP-TF1 added (Fig. 4A, lanes 1–4). Addition of comparable quantities of unprogrammed reticulocyte lysate did not affect transcription from the SV40 MLP (Fig. 4A, lanes 5–7). No repression was observed when a variant of the SV40 MLP mutated in the +1 and +55 IBP-s binding sites was used as template (Fig. 4A, lanes 8–10). On the other hand, addition of reticulocyte lysate, whether or not it was programmed to synthesize COUP-TF1, led to only slight, nonspecific repression of transcription from the SV40 early promoter present on the same plasmid DNA in the same reactions (Fig. 4A', lanes 1'–10'). Thus, COUP-TF1 can specifically repress transcription from the SV40 MLP and does so in a manner dependent upon the IBP-s binding sites.

To confirm the sequence specificity of this repression, cell-free transcription assays were also performed in the
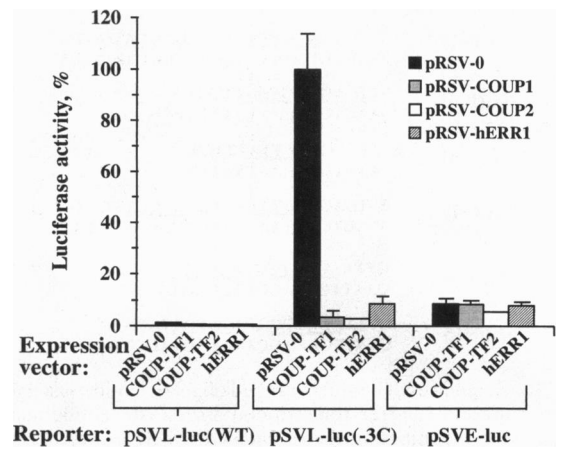

FIG. 5. COUP-TF1, COUP-TF2, and hERR1 specifically repress SV40 late RNA synthesis in the absence of T antigen and viral DNA replication in transient-transfection assays. CV-1PD cells in 6-cm dishes were cotransfected in parallel with 0.25 μg of the expression vectors and 0.4 μg of the reporter plasmids indicated. Luciferase activities are presented relative to the activity obtained with SVL-luc(−3C) plus pRSV-0. These data are means of the results obtained from two experiments performed with triplicate sets of dishes; error bars indicate SEM.

presence of competitor oligonucleotides (Fig. 4B). As expected, the presence in trans of double-stranded oligonucleotide containing the optimal half-site sequence 5'-AG-GTCA-3' resulted in a 10-fold relief of repression by exogenous COUP-TF1 (Fig. 4B, lane 3 vs. lane 2). However, addition of a similar oligonucleotide lacking this binding site sequence had no effect on transcription (Fig. 4B, lane 4 vs. lane 2). Therefore, as with IBP-s (8), repression of transcription from the SV40 MLP by recombinant COUP-TF1 is both sequence-specific and titratable in trans.

COUP-TFs and hERR1 Can Specifically Repress Transcription from the SV40 Late Promoter *in Vivo* in the Absence of T Antigen and Viral DNA Replication. During the early phase of the lytic cycle of infection, the virally encoded protein large T antigen has not yet accumulated and the viral DNA has not yet begun to replicate. To investigate the effect of COUP-TF1 on transcription from the SV40 late promoter under *in vivo* conditions similar to this situation, we constructed a variant of SV40, SVL-luc(WT), in which (*i*) the origin of DNA replication and the T antigen-coding region were mutationally inactivated and (*ii*) the SV40 VP1-coding region (a late gene) was replaced with sequences encoding the reporter luciferase. This SV40 variant was cotransfected into CV-1PD cells along with plasmids encoding recombinant COUP-TF1, COUP-TF2, or hERR1 (Fig. 5). As expected, transcription from the WT SV40 late promoter on this plasmid was not affected by the presence of these receptor-encoding DNAs, since the amount of endogenous IBP-s activity was sufficient in these cells to repress SV40 late transcription when viral template copy number was low [Fig. 5, pSVL-luc(WT); ref. 8]. To reduce repression by endogenous IBP-s activity, cotransfections were also performed with pSVL-luc(-3C), a plasmid which differs from pSVL-luc(WT) solely by a G → C alteration within the +1 site (Fig. 1). This single-base-pair change resulted in a 50-fold increase in the synthesis of luciferase in cotransfections with the parental expression vector pRSV-0 [Fig. 5, pSVL-luc(-3C)], presumably reflecting a partial derepression of the late promoter due to the reduced affinity of endogenous IBP-s for the mutated +1 site. However, cotransfection with pRSV-COUP-TF1, pRSV-COUP-TF2, or pRSV-hERR1 reduced the synthesis of luciferase almost back down to the level observed with the WT SV40 late promoter [Fig. 5, pSVL-luc(-3C)]. Thus, partial derepression can be overcome by the
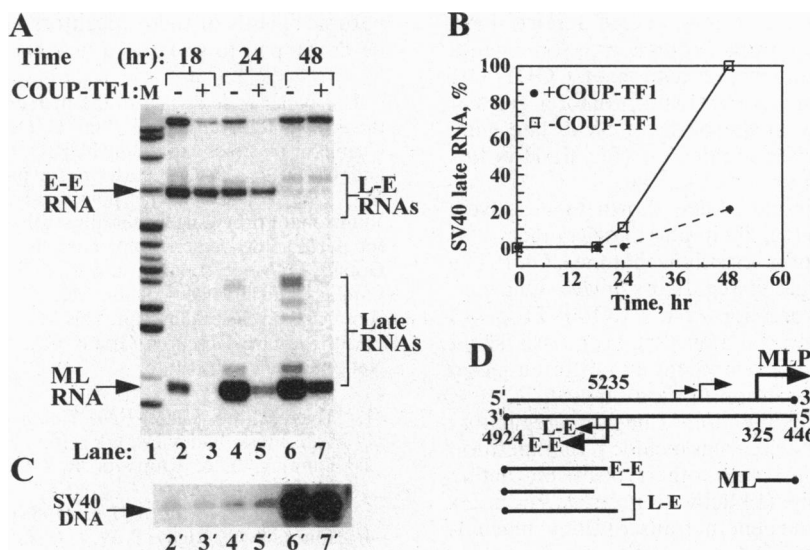
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FIG. 6. Effect of COUP-TF1 overexpression on the early-to-late switch in SV40 expression during the lytic cycle of infection. (*A*) Autoradiogram of a quantitative S1 nuclease mapping experiment using the probe indicated in *D*, to determine the relative amounts of whole-cell SV40 early- and late-strand RNA accumulated by 18, 24, or 48 hr after transfection. WT SV40 DNA (1.2 μg) was cotransfected into CV-1PD cells along with 0.5 μg of pRSV-COUP-TF1 (+) or pRSV-0 (−). Each reaction mixture contained 10% (lanes 2 and 3), 2.5% (lanes 4 and 5), or 0.25% (lanes 6 and 7) of the total RNA sample. Markers (M; lane 1) were *Msp* I-cut fragments of pBR322 DNA. Arrows indicate the DNAs protected by each of the viral RNAs shown in *D*. E-E, early-early; L-E, late-early; ML, major late. (*B*) Accumulation of SV40 late RNA in the presence (●) or absence (□) of exogenous COUP-TF1 as determined by quantitative analysis of the experiment in *A*. (*C*) Southern blot analysis of the replicated SV40 DNA in the samples analyzed in *A* for viral RNA. Each lane contained 3% (lanes 2′–5′) or 1% (lane 6′ and 7′) of the total DNA present in the sample. (*D*) Probe used in the S1 nuclease mapping experiment shown in *A* and fragments protected by hybridization with each of the major viral RNAs.

overexpression of these orphan receptors. On the other hand, overexpression of the COUP-TFs or hERR1 had no effect on transcription from the SV40 early promoter (Fig. 5, SVE-luc). Therefore, their effects are promoter-specific, rather than indirect or a consequence of "squelching." Results similar to these were also obtained with HeLa cells (23). These findings show that the COUP-TFs and hERR1 can specifically repress transcription from the SV40 late promoter under physiological conditions in living cells.

Overexpression of COUP-TF1 Delays the Early-to-Late Switch. To test directly whether members of the steroid/thyroid hormone receptor superfamily can affect regulation of the early-to-late switch in transcription of SV40 during its lytic cycle of infection, WT SV40 DNA was cotransfected into CV-1PD cells along with pRSV-COUP-TF1 (Fig. 6). As a control, cells were cotransfected in parallel with pRSV-0. Whereas the accumulation of the early-strand RNAs was unaffected by overexpression of COUP-TF1, accumulation of the late-strand RNAs was severely delayed (Fig. 6*A*; summarized in Fig. 6*B*). Interestingly, the switch in the use of

Table 1. Relative affinities of various receptors for the SV40 IBP-s binding sites

Recombinant protein(s)	Binding	
	WT+1	WT+55
COUP-TF1	++	+++
COUP-TF2	++	+++
hRARα	−	−
hRXRα	−	−
hRARα/hRXRα	−	−
hTRα2	−	−
hTRα1	+	−
hTRα1/hRXRα	+++	−
hTR2	−	++

Binding assays were performed as described in the legend to Fig. 2, using the oligonucleotides shown in Fig. 1 as probes and receptors synthesized in a reticulocyte lysate. −, No binding detected; +++, ++, and +, binding with high, moderate, and low affinity, respectively.

early-early (E-E) to late-early (L-E) initiation sites in the SV40 early promoter was not affected. Thus, these switches in transcription occur via different mechanisms. Southern blot analysis showed that the time course of accumulation of viral DNA was not affected by overexpression of COUP-TF1 (Fig. 6*C*). Considering our earlier findings (8), we conclude that the early-to-late switch in expression of the SV40 genome can be regulated by the ratio of IBP-s-like activities to SV40 DNA, with efficient transcription from the late promoter being delayed until viral template copy number becomes high enough to titrate out IBP-s-like activities present in the cells.

TRα1/RXRα Heterodimers and TR2 Also Bind to the SV40 MLP. We used gel mobility-shift assays to identity other members of the steroid/thyroid hormone receptor superfamily that might bind the SV40 MLP. As summarized in Table 1, an isoform of the thyroid receptor, TRα1, when heterodimerized with RXRα, was found to bind the +1 site, a DR4, with high affinity. By contrast, the orphan testis receptor TR2 bound the +55 site, a DR2, instead. Other receptors, including retinoic acid receptor α (RARα), TRα2, and RXRα, bound neither site. Thus, several members of this superfamily can bind one, the other, or both of the two major IBP-s binding sites present in the SV40 MLP.

DISCUSSION

We have shown (Figs. 2 and 3) that the orphan receptors COUP-TF1 and COUP-TF2, as well as hERR1 (8), are major components of IBP-s in HeLa cells. This result is consistent with the previous report that hERR1 copurifies from HeLa cells with the COUP-TFs (12). We have demonstrated both *in vitro* (Fig. 4) and *in vivo* (Figs. 5 and 6) that recombinant COUP-TF1 represses transcription from the SV40 late promoter in a sequence-specific and titratable manner. Thus, COUP-TF1 has the functional activities attributed to IBP-s (8).

The two isoforms of the COUP-TFs are expressed in many tissues, albeit at different levels. They are especially abundant in kidney cells (13, 14), the primary tissue in which SV40 replicates (1). Although the COUP-TFs were originally iden-

tified as positive transcription factors, recent reports have indicated that they can also repress expression of some genes (16). Our data indicate that overexpression of COUP-TF1 represses transcription from the SV40 late promoter (Figs. 4 and 5), resulting in a delay in the onset of SV40 late gene expression during the lytic cycle of infection (Fig. 6). How this delay affects virion production is not known.

Although the COUP-TFs have been shown to negatively regulate numerous genes (16), their physiologic function(s) remain unclear. Our present findings show that the COUP-TFs may, indeed, function as negative regulators *in vivo.* Qiu *et al.* (26) have reported that expression of the COUP-TF genes exhibits spatial and temporal regulation. One hypothesis is that the COUP-TFs play roles in development and differentiation by turning off specific genes at appropriate times.

How might the COUP-TFs function as negative regulators of transcription? Plausible mechanisms include (*i*) competition for binding to the promoter with other, positively acting members of this superfamily, (*ii*) active repression via interaction with components of the general transcriptional machinery (e.g., TFIIB; ref. 27), and (*iii*) competition for formation of heterodimers with other members of this superfamily.

The SV40 MLP provides an example of a promoter in which COUP-TF-responsive elements are located at and downstream of the transcription initiation site, rather than upstream of it. Thus, as an alternative, but not mutually exclusive, fourth plausible mechanism of repression, we hypothesize that binding of COUP-TF1 or COUP-like factors to the SV40 MLP could prevent formation of preinitiation complexes by directly competing with RNA polymerase II and general transcription factors for binding to the promoter. Data from order-of-addition experiments are consistent with this latter hypothesis (23).

hERR1 and the human TR2 were originally isolated on the basis of cross-hybridization with probes derived from the estrogen and androgen receptors, respectively (10, 15). Thus, their ligands, sequence specificities, target genes, and functions remain unknown. Data presented here identify binding sites for these receptors on a functional promoter (Fig. 5; Table 1). This information should be useful for both (*i*) determination of the sequence specificities of these receptors and (*ii*) development of simple assays to screen for their ligands and cellular target genes.

What are the effects of natural ligands and their synthetic analogs on expression of the SV40 late genes? Unfortunately, this question cannot yet be answered for the orphan receptors COUP-TFs, hERR1, and TR2. On the other hand, our finding that the heterodimer $TR\alpha1/RXR\alpha$ binds to the SV40 +1 site with high affinity makes some experiments with ligands possible. Preliminary data indicate that addition of the thyroid hormone 3,3',5-triiodothyronine to CV-1PD cells cotransfected with SV40 DNA and $pTR\alpha1$ specifically relieves repression of the SV40 MLP by this receptor (23). Thus, ligands to some steroid/thyroid hormone receptor superfamily members may be directly involved in regulating infection of animals by SV40 and related polyomavirus family members. Given our finding that numerous different receptors with distinct tissue distributions (10, 15, 16, 19) can bind the SV40 MLP (Table 1), we speculate that different members of the steroid/thyroid hormone receptor superfamily may play roles in the regulation of SV40 late gene expression in different tissues.

Binding sites for members of the steroid/thyroid hormone receptor superfamily have been found in the promoters of numerous viruses, including the clinically relevant human immunodeficiency virus type 1 (28, 29), human hepatitis B virus (ref. 30; X.-M. Yu and J.E.M., unpublished data), and herpes simplex virus 1 (31). Thus, members of this receptor superfamily and their ligands may play direct, significant roles in modulating gene expression of many viruses. Possibly, the

natural ligands of these receptors and analogs of them could be developed to serve as a new class of antiviral drugs.

1. Tooze, J., ed. (1982) *DNA Tumor Viruses* (Cold Spring Harbor Lab. Press, Plainview, NY), Rev. 2nd Ed.
2. Fanning, E. & Knippers, R. (1992) *Annu. Rev. Biochem.* **61,** 55–85.
3. Hertz, G. Z. & Mertz, J. E. (1988) *Virology* **163,** 579–590.
4. Jones, N. C., Rigby, P. W. J. & Ziff, E. B. (1988) *Genes Dev.* **2,** 267–281.
5. Ayer, D. E. & Dynan, W. S. (1988) *Mol. Cell. Biol.* **8,** 2021–2033.
6. Wiley, S. R., Kraus, R. J. & Mertz, J. E. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 5814–5818.
7. Ayer, D. E. & Dynan, W. S. (1990) *Mol. Cell. Biol.* **10,** 3635–3645.
8. Wiley, S. R., Kraus, R. J., Zuo, F.-R., Murray, E. E., Loritz, K. & Mertz, J. E. (1993) *Genes Dev.* **7,** 2206–2219.
9. Tsai, M.-J. & O'Malley, B. W. (1994) *Annu. Rev. Biochem.* **63,** 451–486.
10. Giguère, V., Yang, N., Segui, P. & Evans, R. (1988) *Nature (London)* **331,** 91–94.
11. Wang, L.-H., Tsai, S. Y., Cook, R. G., Beattie, W. G., Tsai, M.-J. & O'Malley, B. W. (1989) *Nature (London)* **340,** 163–166.
12. Wang, L.-H., Ing, N. H., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1991) *Gene Expression* **1,** 207–216.
13. Miyajima, N., Kadowaki, Y., Fukushige, S., Shimizu, S., Semba, K., Yamanashi, Y., Matsubara, K., Toyoshima, K. & Yamamoto, T. (1988) *Nucleic Acids Res.* **16,** 11057–11074.
14. Ladias, J. A. A. & Karathanasis, S. K. (1991) *Science* **251,** 561–565.
15. Chang, C. & Kokontis, J. (1988) *Biochem. Biophys. Res. Commun.* **155,** 971–977.
16. Qiu, Y., Tsai, S. Y. & Tsai, M.-J. (1994) *Trends Endocrinol. Metab.* **5,** 234–239.
17. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
18. Cooney, A. J., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1992) *Mol. Cell. Biol.* **12,** 4153–4163.
19. Naikai, A., Sakurai, A., Bell, G. I. & Degroot, L. J. (1988) *Mol. Endocrinol.* **2,** 1087–1092.
20. Leid, M., Kastner, P., Lyons, R., Nakshatri, H., Saunders, M., Zacharewski, T., Chen, J.-Y., Staub, A., Garnier, J.-M., Mader, S. & Chambon, P. (1992) *Cell* **68,** 377–395.
21. Chang, C., Kokontis, J., Acakpo-Satchivi, L., Liao, S., Takeda, H. & Chang, Y. (1989) *Biochem. Biophys. Res. Commun.* **165,** 735–741.
22. Good, P. J., Welch, R. C., Ryu, W.-S. & Mertz, J. E. (1988) *J. Virol.* **62,** 563–571.
23. Zuo, F. (1995) Ph.D. thesis (Univ. of Wisconsin, Madison).
24. Hertz, G. Z. & Mertz, J. E. (1986) *Mol. Cell. Biol.* **6,** 3513–3522.
25. Wiley, S. R. (1993) Ph.D. thesis (Univ. of Wisconsin, Madison).
26. Qiu, Y., Cooney, A. J., Kuratani, S., DeMayo, F. J., Tsai, S. Y. & Tsai, M.-J. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 4451–4455.
27. Cooney, A. J., Leng, X., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1993) *J. Biol. Chem.* **268,** 4152–4160.
28. Cooney, A. J., Tsai, S. Y., O'Malley, B. W. & Tsai, M.-J. (1991) *J. Virol.* **65,** 2853–2860.
29. Lee, M.-O., Hobbs, P. D., Zhang, X.-K., Dawson, M. I. & Pfahl, M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 5632–5636.
30. Garcia, A. D., Ostapchuk, P. & Hearing, P. (1993) *J. Virol.* **67,** 3940–3950.
31. Park, H.-Y., Davidson, D., Raaka, B. M. & Samuels, H. H. (1993) *Mol. Endocrinol.* **7,** 319–330.