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The Rev Protein of Human Immunodeficiency Virus Type 1 Counteracts the Effect of an AU-Rich Negative Element in the Human Papillomavirus Type 1 Late 3′ Untranslated Region

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We have identified a sequence in the late 3′ untranslated region of human papillomavirus type 1 mRNAs that acts posttranscriptionally to repress gene expression. Deletion analysis localized the inhibitory element to an AU-rich sequence between nucleotides 6958 and 6984 on the human papillomavirus type 1 genome. This sequence inhibits gene expression in an orientation-dependent manner. Upon transfection of eucaryotic cells with plasmids containing this sequence, approximately 4-fold-lower cytoplasmic mRNA levels and 64- to 128-fold-lower protein levels were produced compared with those produced by plasmids lacking the inhibitory sequence. Interestingly, providing the constitutive transport element of simian retrovirus type 1 in sense orientation counteracted inhibition exerted by the human papillomavirus type 1 sequence. Inhibition could also be overcome by the presence of human immunodeficiency virus type 1 Rev protein in *trans* and its target sequence, the Rev-responsive element, in *cis*. Rev is a nuclear protein and acts by promoting nuclear export of human immunodeficiency virus type 1 mRNAs encoding structural proteins. Our results are consistent with a model for human papillomavirus type 1 late-gene expression in which mRNAs containing human papillomavirus type 1 inhibitory sequences enter a nonproductive route in the nucleus, resulting in inefficient mRNA utilization. Rev directs mRNA containing inhibitory sequences to a productive route by interacting with the Rev-responsive element.

Papillomaviruses are epitheliotropic circular double-stranded DNA viruses and are the etiological agents of warts. They are widely distributed in nature and infect the epithelia of both animals and humans. There are more than 70 different genotypes of human papillomaviruses (HPVs), which cause a variety of proliferative epithelial lesions (13). Among the high-risk types, HPV-16 and HPV-18 are the types most frequently found in cervical intraepithelial neoplasias and cervical cancer (56), the second most common cancer of women worldwide. Some types, such as HPV-1, have no evident oncogenic potential. The HPV genome consists of early and late genes as well as a noncoding region (49). The early genes encode proteins with a variety of functions such as control of episomal maintenance of the viral genome and transcriptional activation, while the late genes, L1 and L2, produce viral capsid proteins (49). Expression of HPV early genes occurs throughout the infected epithelium, in contrast to expression of late genes, which is restricted to terminally differentiated keratinocytes (9). This indicates that virus production is linked to the differentiation stage of the epidermal cells. The presence or absence of certain cellular factors in terminally differentiated keratinocytes is likely to determine expression levels of L1 and L2. Investigation of the life cycle of HPVs and their gene expression is hampered by lack of an adequate in vitro cell culture system to efficiently propagate virus. Therefore, it is of interest to study the molecular mechanisms that regulate HPV late-gene expression in human epithelial cells.

Since certain types of HPVs play an etiologic role in human malignancies, development of serological tests and vaccines against HPVs is of great interest (14). However, it has been shown that immunization of cattle with late-gene products of bovine papillomavirus (BPV) produced in bacteria failed to induce neutralizing antibodies (23). Production of HPV proteins in eucaryotic cells may therefore be a prerequisite for vaccine development and serological screening.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS. HIV-1 is a prototypic complex retrovirus characterized by the presence on the genome of several short open reading frames, in addition to *gag*, *pol*, and *env* genes coding for structural proteins. Two proteins encoded by short ORFs, Tat and Rev, regulate gene expression and are essential for replication of the virus. Tat is a transcriptional transactivator, while Rev acts posttranscriptionally (for reviews, see references 10, 25, 29, and 47). The Rev protein interacts with an RNA sequence, termed Rev-responsive element (RRE), which is present on mRNAs coding for the structural proteins Gag, Pol and Env, and promotes the nuclear export of these mRNAs (15, 18, 26, 27, 43, 48). Effects of Rev on mRNA stability and translatability have also been observed (2, 11, 42, 52). In the absence of Rev, HIV-1 mRNAs coding for Gag, Pol, and Env are defective and do not direct protein synthesis. It has been shown that inhibition is caused by the presence on the mRNAs of inhibitory RNA sequences located in the coding sequences of Gag, Pol, and Env (5, 8, 41, 45, 48, 52). Unutilized 5′ and 3′ splice sites have also been implicated in posttranscriptional regulation of HIV-1 expression (6, 28, 40). When functional levels of Rev accumulate in the infected cell, production of the structural proteins Gag, Pol, and Env is induced while production of the early regulatory proteins Tat and Rev is repressed through a feedback mechanism (17). Rev acts as a switch between the early and late phases in the HIV-1 life cycle.

In the work described in this report, we have investigated whether HPV-1 late mRNAs contain *cis*-acting regulatory sequences that inhibit HPV-1 late gene expression in human epithelial cells. We found that the HPV-1 3′ untranslated re-

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gion (UTR) contains posttranscriptionally active inhibitory sequences that strongly repress gene expression. Different portions of the HPV-1 late 3′ UTR were amplified by PCR and cloned downstream of a reporter gene. The effect of the HPV-1 3′ UTR on gene expression was evaluated at both RNA and protein levels upon transfection of plasmids into human epithelial cells. The inhibitory effect was greater at the protein level than at the RNA level. Deletion analysis localized the inhibitory element to a 27-nucleotide AU-rich sequence. This sequence may function as a negative regulator of HPV-1 late-gene expression in undifferentiated cells. Interestingly, the HIV-1 regulatory protein Rev was able to overcome the inhibitory effect of the HPV-1 late 3′ UTR sequence, indicating that expression of late genes of HPVs and human complex retroviruses such as HIV-1 may be regulated by similar mechanisms. Inhibition could also be relieved by providing the constitutive transport element (CTE) of simian retrovirus type 1 (SRV-1) in sense orientation. The CTE element from SRV-1 or Mason-Pfizer monkey virus can functionally replace HIV-1 Rev and RRE (4, 55). Studies on posttranscriptional regulation of HPV-1 may therefore also contribute to the understanding of HIV-1 gene regulation.

MATERIALS AND METHODS

Plasmid constructions. To construct pCHPV1pA, a fragment (nucleotides [nt] 6558 to 7537, where the numbering follows the sequence of the genomic HPV-1 clone [12]) containing the putative HPV-1 poly(A) signals was first PCR amplified from the HPV-1 molecular clone pHPV-1 (12), kindly provided by F. Thierry and M. Yaniv, with oligonucleotides 1S (5′-TCAGCTTTGTGAATTCAAGT TAACTCC-3′) and 1A (5′-CCATAAGGAATTCGCCACAACG-3′). *Eco*RI sites (underlined) were introduced at both ends of the PCR product. The PCR fragment was cloned into a pBluescript plasmid (Stratagene), resulting in pKSHPV1pA, and subsequently transferred as an *Eco*RI fragment into the *Eco*RI site located immediately adjacent to the mutated HIV-1 $p17^{gag}$ gene, p17M1234, in plasmid pCM1234 (50) (see Fig. 1A). The mutated $p17^{gag}$ gene p17M1234 contains multiple point mutations which altered the RNA sequence but maintained the original protein primary sequence (50, 52). This resulted in high $p17^{gag}$ production independent of Rev (50, 52). p17M1234 is used as a reporter gene and is under the control of the human cytomegalovirus immediate-early promoter (nt −671 to +74) (3). These cloning steps resulted in plasmid pCHPV1pA. pCHPV1ΔpA was generated by excising a 515-bp *Xba*I fragment from plasmid pCHPV1pA. pT7-1pA was constructed by introducing a *Sal*I-*Eco*RI fragment, encoding the $p17^{gag}$ gene p17M1234, into pKSHPV1pA. To generate pT7-1ΔpA, plasmid pT7-1pA was digested with *Xba*I and religated.

To construct pCH1pA1M and pCH1pA2M, poly(A) signals pA_1 and pA_2 (nt 7380 to 7385 and nt 7426 to 7431, respectively) in the 3′ UTR of HPV-1 were first mutated by site-directed mutagenesis on uracil-containing single-stranded DNA of pKSHPV1pA with oligonucleotides *Bam*HIpA (5′-AAAGGACAGCTCACT GGATCCGCATATTGCAGGAAA-3′) and *Xho*IpA (5′-TAGGGGTAGGGA TTTCTCGAGGGAGTAGGGAGGGAT-3′), respectively. Restriction sites introduced in the place of the poly(A) signals are underlined. The mutated HPV-1 sequences were transferred as *Eco*RI fragments into *Eco*RI-digested, calf intestinal alkaline phosphatase (CIAP)-treated pCM1234, resulting in plasmids pCH1pA1M and pCH1pA2M. To construct the double mutant pCH1pA12M, pKSHPV1pA was mutated with oligonucleotides *Bam*HIpA and *Xho*IpA simultaneously. The mutated fragment was transferred into pCM1234 as an *Eco*RI fragment as described above. To construct pCH1pAM, the 5′ splice site-like sequence CATGTAAGAT (nt 7032 to 7041) in the 3′ UTR of HPV-1 was first mutated by site-directed mutagenesis on uracil-containing single-stranded DNA of pKSHPV1pA (see above) with oligonucleotide 1D5SS (5′-CTACTTATAC-TAATGTAAGAGCTCTCTATTTAGAACTAAAC-3′), in which a *Sac*I site (underlined) was introduced. The mutated HPV-1 sequence was transferred as an *Eco*RI fragment into the *Eco*RI-digested, CIAP-treated pCM1234, resulting in plasmid pCH1pAM. To construct plasmid pCM1234pA, an *Eco*RI fragment containing simian virus 40 (SV40) early poly(A) signal was directly cloned into *Eco*RI-digested, CIAP-treated pCM1234.

To construct pCHPV1ΔBX and pCH1pA669ΔBX, a *Bss*HII site and an *Xho*I site (underlined) were first introduced at nt 6943 and 7014, respectively, in pKSHPV1pA by site-directed mutagenesis with the oligonucleotides 5′-GT TATATATAATGCGCGCTAAGCCTTACGC-3′ and 5′-TTTAGAACTAAA CTCGAGTAAAAAGTATAAA-3′. To delete sequences between nt 6943 and 7014, the mutated plasmid was digested with *Bss*HII and *Xho*I, sticky ends were filled in with Klenow DNA polymerase, and the plasmid was religated. The mutated HPV-1 DNA was transferred as an *Eco*RI fragment into pCM1234, resulting in pCHPV1ΔBX, or PCR amplified with oligonucleotides 1S-2 and 1A (see below) and blunt-end cloned into the filled-in *Eco*RI site of pCM1234, resulting in pCH1pA669ΔBX.

pCM1R and pCM1RΔpA were constructed by cloning of a 330-nt *Sty*I fragment encompassing the HIV-1 Rev-responsive element (RRE) (53) into *Hpa*I-digested, CIAP-treated pCHPV1pA or pCHPV1ΔpA. pCM1CS and pCM1CA were generated by blunt-end cloning of the SRV-1 CTE (55) into *Hpa*I-digested, CIAP-treated pCHPV1pA. CTE was PCR amplified with the oligonucleotides 5′-GCATCAACGCGTGTCGACGGATCCAGACCACCTCCC CTGCGAG-3′ and 5′-GCATCAGCGGCCGCCTCGAGTCTAGACAAATCCC TCGGAAGCTGCG-3′.

To generate deletions at the 5′ end of the HPV-1 fragment contained in pCHPV1pA, PCR was performed with the following oligonucleotides in combination with oligonucleotide 1A: 1S-1 (5′-CCACCTACGAATTCTCTAGAAG-3′), 1S-2 (5′-GCTACGAATTCCACCACAAAGCGC-3′), 1S-2a (5′-GGCGTAAGA ATTCGTATATATTAT-3′), 1S-2b (5′-GAATTCATATTTATTAGTAGATTAT TTATT-3′), 1S-2c (5′-GAATTCATATATTTTTATATTTTTATAC-3′), 1S-2d (5′-GGTCGACTATTTATTATATATTTTTAT-3′), and 1S-3 (5′-CTTGTTGAATTC TAAATAGACATG-3′). The PCR products were cloned into the filled-in *Eco*RI site in plasmid pCM1234, resulting in plasmids pCH1pA883, pCH1pA669, pCH1pA611, pCH1pA579, pCH1pA555, pCH1pA569, and pCH1pA525, respectively.

To generate deletions at the 3′ end of the HPV-1 fragment contained in pCHPV1pA, various portions of the HPV-1 3′ UTR were PCR amplified and cloned into the *Hpa*I site upstream of the SV40 early poly(A) signal in plasmid pCM1234pA. The following oligonucleotides were used in combination with oligonucleotide 1S-2: 1A-1 (5′-TAAGCTTGCAGGAAAATAACAC-3′), 1A-2 (5′-TAAGCTTGCCTAACAGGAGGAAAATAG-3′), 1A-3 (5′-TAAGCTTTC ACTATTGTAAATGAT-3′), 1A-4 (5′-CAAGCTTATTTAGAACTAAACAA G-3′), and 1A-5 (5′-TAAGCTTATAATAAATAATCTACT-3′). These cloning steps resulted in the following expression plasmids with the amplified HPV-1 sequence in the sense orientation: pCH1-507, pCH1-350, pCH1-262, pCH1-167, and pCH1-116, respectively; pCH1-507A, with the HPV-1 sequence in the antisense orientation, was also produced. To generate plasmid pCH1-206, a 206-bp fragment was PCR amplified from pCHPV1pA with oligonucleotides 1S-3 and 1A-2 and cloned directly into *Hpa*I-digested, CIAP-treated pCM1234pA.

To generate plasmid pCH1L1UTR, the HPV-1 L1 coding sequence was first PCR amplified from pHPV-1 (12) with oligonucleotides H1L1start 5′-AGCGTCGACAAAGAGCTTATGT-3′ and 1A (see above) and blunt-end cloned into *Eco*RV-digested pBluescript. The L1 coding sequence was transferred as a *Sal*I-*Eco*RI fragment into pCM1234 digested with *Sal*I and *Eco*RI, resulting in pCH1L1UTR. To generate plasmid pCH1L1ΔpA, sequences between nt 6939 and 7184 were deleted from plasmid pCH1L1UTR.

The HIV-1 Rev expression plasmid pNL14A7, the human T-cell leukemia virus type I (HTLV-I) Rex expression plasmid pL3rex, the HIV-1 $p55^{gag}$ expression plasmid pNLvgagSty330, and the SRV-1 CTE-containing plasmid pS12(*Bam*HI-*Xho*I) were kindly provided by G. N. Pavlakis and B. K. Felber and have been described elsewhere (18, 51, 53, 55). Plasmids with the HIV-1 5′ long terminal repeat as promoter were cotransfected with plasmid pCMVtat, which produces the HIV-1 Tat protein.

Cells and transfections. HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% heat-inactivated fetal calf serum. At 24 h prior to transfection, 3×10^5 cells were seeded per 60-mm culture dish or 1×10^6 cells were seeded per 100-mm dish. Transfections were carried out by the calcium phosphate coprecipitation technique (24). The various amounts of plasmids used for transfection were adjusted with pBluescript DNA to 15 μg in 0.5 ml of precipitate for 60-mm dishes or 30 μg in 1 ml of precipitate for 100-mm dishes. Transfected cells were washed twice with phosphate-buffered saline (PBS) 6 h posttransfection, refed with fresh Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, and cultured overnight. The cells were harvested 24 h posttransfection. To evaluate $p17^{gag}$ production from plasmids pT7-1pA and pT7-1ΔpA, HeLa cells were infected for 2 h with recombinant vaccinia virus vTF7-3 (19, 20), producing bacteriophage T7 RNA polymerase, and then plasmid DNA was transfected by the calcium phosphate coprecipitation technique. All plasmid DNAs used for transfection were purified on Qiagen columns (Diagen). pCMVCAT (1 μg) was included as an internal control in each transfection to evaluate transfection efficiency. Chloramphenicol acetyl transferase (CAT) protein was quantitated by a CAT antigen capture enzyme-linked immunosorbent assay (ELISA; Boehringer Mannheim GmbH).

Western immunoblotting. At 24 h posttransfection, cells were harvested in 500 μl of lysis buffer (0.5% Triton X-100, 100 mM Tris-HCl [pH 7.5]) and freeze-thawed three times. Cell lysates were cleared of insoluble material by centrifugation at 12,000 × *g* for 15 min. Proteins were electrophoretically separated on 12% polyacrylamide–sodium dodecyl sulfate (SDS) gels and transferred onto nitrocellulose membranes by electroblotting in transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol) at constant current (250 mA) for 12 to 16 h. The membranes were blocked for 1 h at 37°C with 10% nonfat milk in PBS containing 0.3% Tween 20 (PBS-T). After being washed three times in PBS-T, the blots were incubated for 1 h at 37°C with the HIV-1-positive patient serum (1:4,000 dilution), washed in PBS-T, and incubated with horseradish peroxidase-conjugated donkey anti-human immunoglobulin G (Amersham) diluted 1:10,000 in PBS-T. Specific proteins were visualized with the enhanced chemiluminescence (ECL) detection system (Amersham).

RNA extraction and Northern (RNA) blotting. Total cellular RNA was extracted from HeLa cells by the heparin-DNase procedure 24 h posttransfection (37). Cytoplasmic RNA was isolated by a 10-min in situ lysis of cells on ice in 100-mm dishes containing 1.2 ml of lysis buffer (10 mM Tris-HCl [pH 7.9], 0.15 M NaCl, 1.5 mM $MgCl_2$, 0.65% Nonidet P-40). The cell extracts were centrifuged at 12,000 × *g* for 2 min, and the supernatants were transferred to new tubes containing 300 μl of RPS buffer (0.5 M Tris-HCl [pH 9.0], 50 mM EDTA, 2.5% SDS). Samples were extracted three times with phenol-chloroform, and RNA was precipitated with isopropanol. The RNA pellet was resuspended in DNase I buffer (50 mM Tris-HCl [pH 7.8], 10 mM $MgCl_2$, 1 mM EDTA, 1 mM dithiothreitol) containing 40 U of DNase I (Boehringer Mannheim GmbH), incubated for 1 h at 37°C, and subjected to phenol-chloroform extraction and ethanol precipitation.

Nuclear and cytoplasmic poly(A)$^+$ mRNA was isolated with Dynabeads Oligo $(dT)_{25}$ (Dynal A.S.). Briefly, 24 h posttransfection, cells from 100-mm dishes were washed twice with ice-cold PBS and lysed in 500 μl of lysis buffer D (10 mM Tris-HCl [pH 7.5], 0.14 M NaCl, 5 mM KCl, 1% Nonidet P-40). Low-speed centrifugation at 8,000 × *g* for 2 min was used to separate nuclear and cytoplasmic fractions. To extract cytoplasmic poly(A)$^+$ mRNA, supernatants were mixed with an equal volume of 2× binding buffer (20 mM Tris-HCl [pH 7.5], 1.0 M LiCl, 2 mM EDTA, 0.5% SDS) containing 400 μg of Dynabeads Oligo $(dT)_{25}$. Hybridization was carried out at room temperature for 3 to 5 min, and the Dynabeads Oligo $(dT)_{25}$ were washed three times with 500 μl of washing buffer (10 mM Tris-HCl [pH 7.5], 0.15 M LiCl, 2 mM EDTA). The poly(A)$^+$ mRNAs were eluted with 150 μl of elution buffer (2 mM EDTA [pH 7.5]) at 65°C for 5 min and stored at −70°C until use. Nuclear fractions were washed once with lysis buffer D, and the pellets were resuspended in lysis buffer D without Nonidet P-40. An equal volume of lysis buffer D containing 1% SDS was added, and the samples were incubated on ice for 10 min, and frozen on dry ice. The samples were thawed at room temperature and centrifuged for 2 min at 8,000 × *g*. Supernatants were used for extraction of nuclear poly(A)$^+$ mRNA with Dynabeads Oligo $(dT)_{25}$ as described above.

For Northern blot analysis, 10 μg of total or cytoplasmic RNA was separated on 1% agarose–formaldehyde gels and transferred onto nitrocellulose membranes by standard procedures, and the membranes were baked at 80°C for 2 h. The membranes were probed with [α-^{32}P]CTP-labeled riboprobes generated with T3-RNA polymerase (Stratagene) from pBluescript plasmids containing the HIV-1 p17^{gag} coding sequence. Hybridization was carried out for 16 h at 55°C followed by high-stringency washing in 0.1× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% SDS at 65°C. Filters were then subjected to autoradiography.

RT-PCR. Poly(A)$^+$ mRNA (20 μl) purified by Dynabeads Oligo $(dT)_{25}$ was reverse transcribed at 42°C for 1 h in a total volume of 30 μl containing 4 U of avian myeloblastosis virus reverse transcriptase (RT; Promega); 19 U of RNA guard (Pharmacia); 0.8 mM each dATP, dCTP, dGTP, and dTTP; 4 mM $MgCl_2$; 10 mM Tris-HCl (pH 8.3); 50 mM KCl; and 50 μg of random hexamer oligonucleotides (Pharmacia) per ml. A reaction without RT was performed in parallel to serve as a control for the absence of plasmid DNA contamination. Then 5 μl of the cDNA product was PCR amplified in a 100-μl reaction volume with oligonucleotides 4704 (5′-GCCTGTTAGAAACATCAGAAGGC-3′), and 5912 (5′-CTAGTAATTTTGGCTGACCTG-3′), detecting cDNA of p17^{gag} mRNA, oligonucleotides H1L1start and 1HA (5′-GATGTCCGACCAGCAGTAGA CG-3′), detecting cDNA of L1 mRNA, ACTINS (5′-TGAGCTGCGTGTG GCTCC-3′) and ACTINA (5′-GGCATGGGGGAGGGCATACC-3′), specifically amplifying cDNA of spliced actin mRNA, or ACTINS-1 (5′-CCAGTG GCTTCCCCAGTG-3′) and ACTINA, detecting cDNA of unspliced actin mRNA. To detect cDNA of CAT mRNA produced from the CAT expression plasmid used as internal control for transfection efficiency, oligonucleotides CATS-2 (5′-CGTCTCAGCCAATCCCTGGGTG-3′) and CATA (5′-CTATT AGGCCCCGCCCTGCCACTC-3′) were used.

A 250-bp p17^{gag} fragment was obtained with oligonucleotides 4704 and 5912, a 178-bp product was obtained with H1L1start and 1HA, a 247-bp product was obtained with oligonucleotides ACTINS and ACTINA, a 203-bp product was obtained with oligonucleotides ACTINS-1 and ACTINA, and a 229-bp fragment was obtained with CATS-2 and CATA. Oligonucleotides 5912, ACTINA, and CATA were end labeled with [γ-^{32}P]ATP prior to use. PCR was performed in a total volume of 100 μl with 2 U of *Taq* DNA polymerase (Perkin-Elmer Cetus) for 20 to 25 cycles at 94°C for 1 min, 55°C for 2 min, and 72°C for 3 min with a final extension (after the cycles) at 72°C for 10 min. A 10-μl sample from each RT PCR was analyzed by electrophoresis on 6% polyacrylamide–urea gels. The gels were dried and exposed to X-ray film at −70°C.

RESULTS

The 3′ UTR of the HPV-1 late mRNAs contains sequences that inhibit gene expression. To investigate whether sequences within the 3′ UTR of HPV-1 late mRNAs play a regulatory role in HPV-1 gene expression, we cloned a fragment containing the HPV-1 late 3′ UTR and putative late poly(A) signals downstream of a mutated HIV-1 p17^{gag} gene named p17M1234, used here as a reporter gene (see Materials and Methods). This cloning step resulted in pCHPV1pA (Fig. 1A). Two control plasmids, pCM1234 and pCM1234pA, were also constructed (Fig. 1A). pCM1234 served as a negative control, since poly(A) signal is not present in this plasmid, while pCM1234pA was used as a positive control in which the SV40 early poly(A) signal was utilized. To evaluate p17^{gag} production from pCM1234, pCM1234pA, and pCHPV1pA, HeLa cells were transfected with each plasmid separately and p17^{gag} protein levels were determined by Western immunoblotting. A CAT expression plasmid was included in each transfection experiment to control for transfection efficiency.

Figure 1B shows that pCM1234 did not produce detectable levels of p17^{gag} protein as expected, since it lacks poly(A) signals, whereas high levels of p17^{gag} were produced from pCM1234pA. Production of p17^{gag} from pCHPV1pA was undetectable, indicating that either HPV-1 putative poly(A) signals were not efficiently used in HeLa cells or HPV-1 sequences upstream of the putative poly(A) signals inhibited p17^{gag} expression. To test these two possibilities, we deleted 515 bp of the HPV-1 sequence in plasmid pCHPV1pA, generating plasmid pCHPV1ΔpA (Fig. 1A). Western blot analysis showed that p17^{gag} production from pCHPV1ΔpA was dramatically increased. The levels of p17^{gag} produced from pCHPV1ΔpA were comparable to those produced from pCM1234pA. These results demonstrated that HPV-1 sequences between nt 6669 and 7184 strongly inhibited gene expression and that efficiently recognized poly(A) signals were located in the HPV-1 sequence present in pCHPV1ΔpA. The levels of CAT produced from the internal control plasmid pCMVCAT did not vary substantially between transfections. Similar results were obtained when simian cells (Vero cells) or hamster cells (BHK-21 cells) were transfected with pCHPV1pA or pCHPV1ΔpA, respectively (data not shown), indicating that evolutionary conserved processes determine expression levels.

Since there are two putative poly(A) signals present in the HPV-1 late 3′ UTR at nt 7380 (pA_1) and nt 7426 (pA_2), we wished to test if these poly(A) signals were utilized. Therefore, another three plasmids, pCH1pA1M, pCH1pA2M, and pCH1pA12M, were constructed (Fig. 1A). In pCH1pA1M, putative poly(A) signal pA_1 was mutated to a *Bam*HI restriction site; in pCH1pA2M, putative poly(A) signal pA_2 was changed to an *Xho*I site; and in pCH1pA12M, both pA_1 and pA_2 were mutated. Figure 1B shows that both pCH1pA1M and pCH1pA2M produced high levels of p17^{gag}, while p17^{gag} production from pCH1pA12M was substantially reduced. The results show that pA_1 and pA_2 could be used with similar efficiencies, that pA_1 and pA_2 were the major poly(A) signals in this region of HPV-1, and that both of them could be efficiently used in HeLa cells.

The inhibitory HPV-1 sequences reduce cytoplasmic poly(A)$^+$ mRNA levels. To investigate if the low p17^{gag} production from pCHPV1pA was a result of low mRNA levels, cytoplasmic poly(A)$^+$ mRNA was extracted from cells transfected with pCHPV1pA and pCHPV1ΔpA, respectively. The mRNAs were subjected to reverse transcription followed by PCR amplification of a region in the p17^{gag} gene with radioactively labeled oligonucleotides. After determining the linear range of the assay, analysis of amplified products revealed that cytoplasmic poly(A)$^+$ mRNA levels generated from pCHPV1pA were lower than those produced from pCHPV1ΔpA (Fig. 2A). PCR amplification with oligonucleotides specific for CAT mRNA generated from the internal control plasmid pCMVCAT or with oligonucleotides specific for spliced actin mRNA resulted in bands of equal intensity in

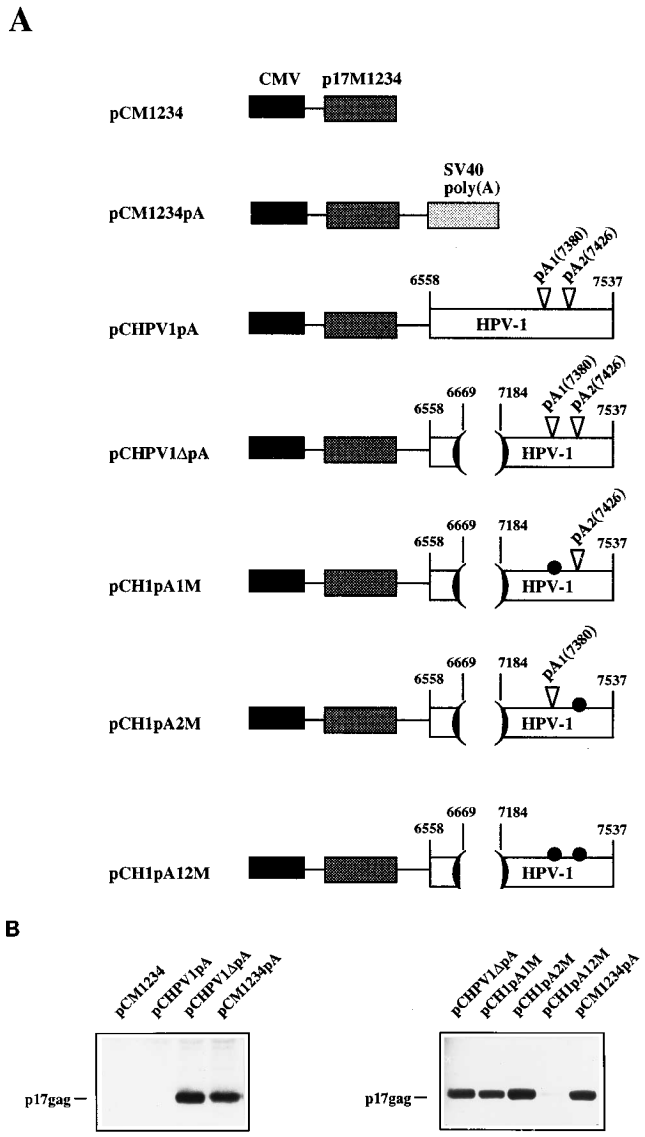


FIG. 1. Sequences within the HPV-1 3′ UTR inhibit HIV-1 p17^{gag} expression. (A) Structures of the p17^{gag} expression plasmids. Black boxes indicate the human cytomegalovirus (CMV) immediate-early promoter, stippled boxes indicate the mutated p17^{gag} gene named p17M1234 (50), the lightly stippled box indicates the SV40 early poly(A) signal-containing sequence, and open boxes indicate the HPV-1 late 3′ UTR-containing sequences. Parentheses mark the limits of a deletion between nt 6669 and 7184. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). Putative polyadenylation signals of the HPV-1 late mRNAs are indicated by triangles. Mutated polyadenylations signals are indicated by black circles. Names of the plasmids are shown on the left. (B) Western blot analysis of lysates from HeLa cells transfected with pCM1234, pCHPV1pA, pCHPV1ΔpA, and pCM1234pA (left panel) and with pCHPV1ΔpA, pCH1pA1M, pCH1pA2M, pCH1pA12M, and pCM1234pA (right panel). Proteins were resolved on 12% polyacrylamide–SDS gels and electrophoretically transferred onto nitrocellulose membranes. The p17^{gag} protein was detected with a serum sample from an HIV-1-positive patient followed by incubation with horseradish peroxidase-conjugated anti-human immunoglobulin G antiserum. The p17^{gag} was visualized with ECL detection reagents. The position of p17^{gag} is indicated on the left.

both samples (Fig. 2A). This demonstrated that the transfection efficiency and the amount of mRNA used for RT-PCR were similar in both samples.

To investigate whether the low cytoplasmic mRNA levels produced from pCHPV1pA was caused by retention of the HPV-1 3′ UTR-containing mRNAs in the nucleus, we analyzed total cellular RNA or total cytoplasmic RNA isolated from cells transfected with pCHPV1pA or pCHPV1ΔpA, respectively. Northern blot analysis of cytoplasmic p17^{gag} mRNA revealed that lower mRNA levels were produced from pCHPV1pA than from pCHPV1ΔpA (Fig. 2B), which is in agreement with the RT-PCR data (Fig. 2A). Similar results were obtained when preparations of total cellular RNA were analyzed (Fig. 2B), demonstrating that the low cytoplasmic mRNA levels produced from pCHPV1pA was not a result of

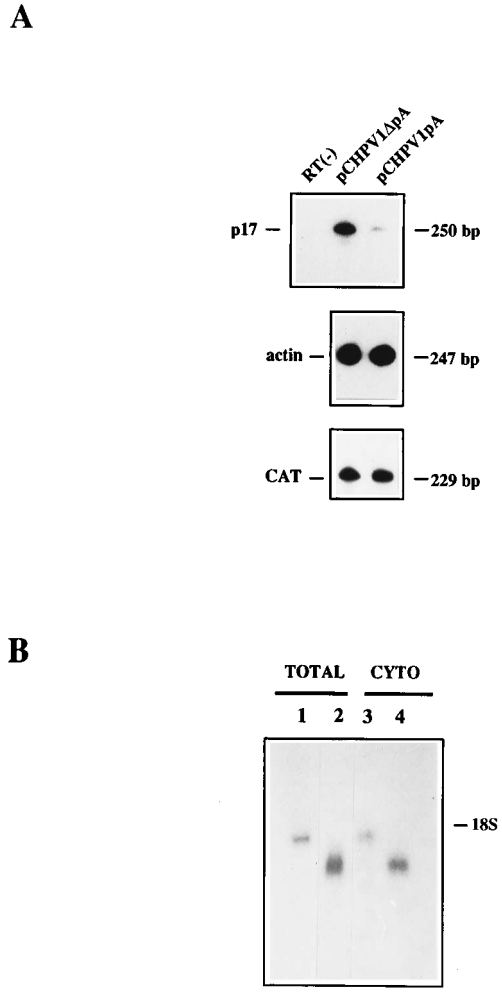


FIG. 2. Effect of the HPV-1 3′ UTR on mRNA levels and subcellular mRNA distribution. (A) RT PCR amplification of cDNA from cytoplasmic poly(A)$^+$ mRNA extracted from cells transfected with pCHPV1ΔpA and pCHPV1pA. Cytoplasmic poly(A)$^+$ mRNA was isolated with Dynabeads Oligo $(dT)_{25}$ and subsequently reverse transcribed. cDNAs were PCR amplified with oligonucleotides specific for p17^{gag} mRNA or spliced actin and CAT mRNA to control for mRNA levels and transfection efficiency. p17^{gag} oligonucleotides generated a 250-bp fragment, actin oligonucleotides generated a 247-bp fragment, and CAT oligonucleotides generated a 229-bp fragment. Oligonucleotides were end labeled with [γ-^{32}P]ATP prior to PCR. The amplified products were analyzed on 6% polyacrylamide–urea gels. Positions of the PCR products are indicated on the left, and their sizes are indicated on the right. RT(−) indicates that RT PCR was performed in the absence of RT in the cDNA synthesis reaction. (B) Northern blot analysis of total and cytoplasmic (CYTO) RNA isolated from cells transfected with pCHPV1pA (lanes 1 and 3) or pCHPV1ΔpA (lanes 2 and 4). A 10-μg portion of each RNA sample was run on a denaturing 1% agarose gel, transferred onto a nitrocellulose membrane, and hybridized with a [α-^{32}P]CTP-labeled riboprobe specifically detecting a portion of the HIV-1 p17^{gag} coding sequence. The position of the 18S rRNA is indicated on the right.

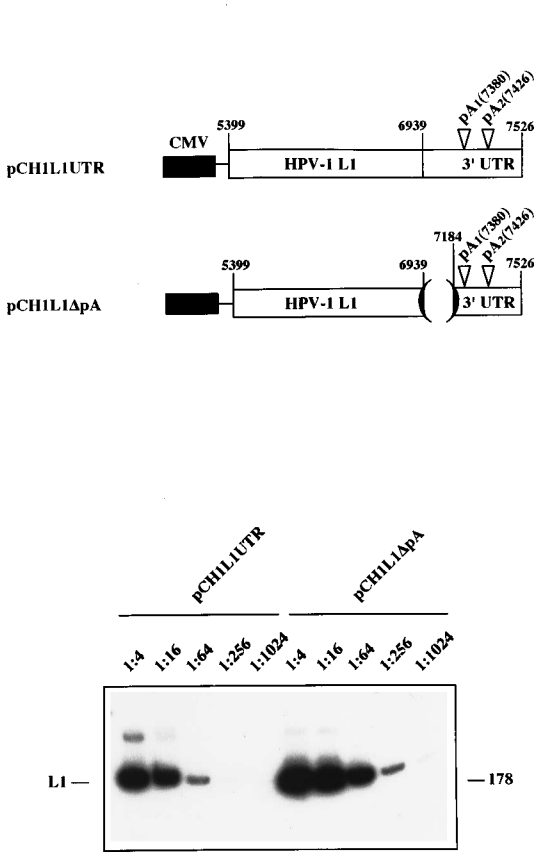


FIG. 3. (A) Schematic structures of HPV-1 L1 expression plasmids. Black boxes indicate the human cytomegalovirus (CMV) immediate-early promoter, and open boxes indicate HPV-1 sequences. The HPV-1 L1 coding sequence is located between nt 5399 and 6939. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). Polyadenylation signals of the HPV-1 late mRNAs are indicated by triangles. Names of the plasmids are shown on the left. (B) RT-PCR analysis of dilutions of cytoplasmic poly(A)$^+$ mRNA. Serial fourfold dilutions of mRNA samples from cells transfected with pCH1L1ΔpA or pCH1L1UTR were performed, and cDNAs were generated from each dilution. PCR amplifications from the cDNA samples with oligonucleotides specific for the L1 sequence present in pCH1L1ΔpA or pCH1L1UTR are shown. Oligonucleotides were end labeled with [γ-^{32}P]ATP prior to PCR. The PCR products were analyzed on a 6% polyacrylamide–urea gel and subjected to autoradiography. The size of the amplified DNA fragments is indicated on the right.

nuclear retention of the HPV-1 late 3′ UTR-containing mRNAs. The possibility exists that mRNAs containing the HPV-1 late 3′ UTR are unstable in the nucleus. In this case, HPV mRNAs would not accumulate in the nucleus even if nuclear export was inefficient. We concluded that the inhibitory HPV-1 sequences acted, at least in part, by reducing cytoplasmic poly(A)$^+$ p17^{gag} mRNA levels.

To exclude the possibility that the inhibitory effect of the HPV-1 late 3′ UTR was related to the expression system used here, we compared mRNA levels produced from pCH1L1UTR (Fig. 3A), containing the HPV-1 L1 coding sequence and late 3′ UTR with poly(A) signals, with mRNA levels produced from pCH1L1ΔpA (Fig. 3A), in which HPV-1 sequences between nt 6939 and 7184 had been deleted. Cytoplasmic poly(A)$^+$ mRNA was extracted and subjected to serial fourfold dilutions followed by RT-PCR with oligonucleotides specific for HPV-1 L1 mRNA. The results shown in Fig. 3B and those from additional independent experiments (data not shown) revealed that mRNA levels generated from pCH1L1ΔpA were fourfold higher than those produced from pCH1L1UTR. CAT protein produced from the internal control plasmid was quantitated by CAT ELISA on the cytoplasmic extract used for isolation of mRNA. CAT levels varied less than 20% (data not shown). We concluded that the presence of the HPV-1 late 3′ UTR resulted in a reduction of HPV-1 L1 mRNA levels, demonstrating that sequences in this region are important determinants that regulate HPV-1 L1 mRNA expression levels.

mRNAs containing the HPV-1 3′ UTR are not efficiently translated. Since mRNAs containing the HPV-1 3′ UTR were present in the cytoplasm, we next investigated whether these mRNAs were efficiently utilized. We first compared the levels of p17^{gag} protein produced from plasmids pCHPV1pA and pCHPV1ΔpA. The levels of p17^{gag} in serial twofold dilutions of cell lysates from cells transfected with pCHPV1ΔpA were compared with the levels of p17^{gag} produced from pCHPV1pA. Western blot analysis revealed that deletion of the 515-nt HPV-1 sequence from plasmid pCHPV1pA increased p17^{gag} production 64- to 128-fold (Fig. 4A). To investigate if the 64- to 128-fold difference in p17^{gag} production could be accounted for by a similar difference at the mRNA level, we quantitatively compared the amounts of cytoplasmic poly(A)$^+$ mRNA produced from pCHPV1pA and pCHPV1ΔpA, respectively. RT PCR was performed on serial fourfold dilutions of each cytoplasmic poly(A)$^+$ mRNA sample. Three independent experiments revealed that cytoplasmic poly(A)$^+$ mRNA levels produced from pCHPV1ΔpA were approximately fourfold higher than those produced from pCHPV1pA (Fig. 4B), while CAT mRNA levels produced from the internal control plasmid pCMVCAT were similar in the two transfections (Fig. 4B). Therefore, the difference in expression between pCHPV1pA and pCHPV1ΔpA was greater at the protein level (64- to 128-fold) than at the mRNA level (4-fold). We propose that the inhibitory HPV-1 sequences act via a bimodal mechanism to inhibit gene expression: while acting partly by reducing mRNA levels, the inhibitory HPV-1 sequences also affect translation of the mRNA.

Inhibition is relieved when transcription occurs in the cytoplasm. As the results described above showed that the mRNAs containing the HPV-1 late 3′ UTR were present in the cytoplasm but were not efficiently translated, we wished to determine if inhibition required nuclear localization of the mRNA. We therefore used the vaccinia virus T7 RNA polymerase-based expression system (19, 20). HeLa cells were first infected with recombinant vaccinia virus vTF7-3 (19, 20), producing bacteriophage T7 RNA polymerase, which localizes to the cytoplasm since it lacks a nuclear localization signal, and subsequently transfected with pT7-1pA or pT7-1ΔpA (Fig. 5A). Plasmids pT7-1pA and pT7-1ΔpA contain the p17^{gag} gene and the HPV-1 sequences present in pCHPV1pA and pCHPV1ΔpA, respectively, under control of the bacteriophage T7 promoter. Analysis of p17^{gag} levels produced after transfection of HeLa cells infected with recombinant vaccinia virus TF7-3 revealed that similar levels of p17^{gag} were produced from pT7-1pA and pT7-1ΔpA (Fig. 5B). These results demonstrated that inhibition exerted by the HPV-1 sequences was overcome when transcription took place in the cytoplasm, indicating that inhibition was dependent on cellular factors in the nucleus. However, it should be noted that RNAs produced with the vaccinia virus T7 system may not be correctly processed and therefore may not be subjected to the control mechanisms regulating expression of mRNAs synthesized in the nucleus.

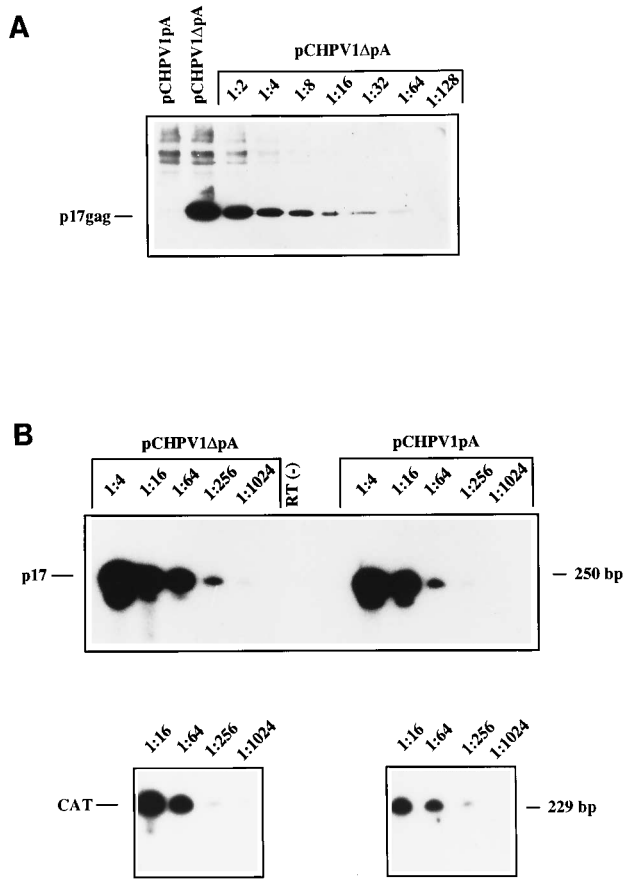


FIG. 4. Comparison of p17^{gag} protein levels and cytoplasmic poly(A)$^+$ mRNA levels from cells transfected with pCHPV1pA and pCHPV1ΔpA. (A) Western blot analysis of p17^{gag} protein from cells transfected with pCHPV1pA or pCHPV1ΔpA. Cell lysates from pCHPV1ΔpA-transfected cells were serially twofold diluted and analyzed by Western immunoblotting as described in Materials and Methods. The position of p17^{gag} is indicated on the left. (B) RT-PCR analysis of dilutions of cytoplasmic poly(A)$^+$ mRNA. Serial fourfold dilutions of mRNA samples from cells transfected with pCHPV1pA or pCHPV1ΔpA were performed, and cDNAs were generated from each dilution. The upper panel shows PCR amplification of the cDNA samples with oligonucleotides specific for the p17^{gag} sequence present in pCHPV1pA and pCHPV1ΔpA. The lower panel shows PCR amplification of the same cDNA samples with oligonucleotides specific for the CAT sequence present in the CAT expression plasmid used here to control for transfection efficiency. Oligonucleotides were end labeled with [γ-^{32}P]ATP prior to PCR. The PCR products were analyzed on a 6% polyacrylamide–urea gel and subjected to autoradiography. The sizes of the amplified DNA fragments are indicated on the right. RT(−) indicates that RT PCR was performed in the absence of RT in the cDNA synthesis reaction.

The effect of the inhibitory HPV-1 sequences can be overcome by interaction of the HIV-1 Rev protein with the RRE. Since the HPV-1 3′ UTR acted posttranscriptionally to inhibit gene expression, we asked whether the HIV-1 posttranscriptional transactivator Rev or the HTLV-I Rex protein could overcome the inhibition. The HIV-1 Rev protein acts by binding to an RNA sequence, termed the RRE, to overcome the effect of inhibitory sequences present on the unspliced and singly spliced HIV-1 mRNAs (5, 6, 8, 15, 27, 41, 45, 48, 50, 52). These sequences act by retaining viral mRNAs in the nucleus, decreasing their stability, and inhibiting translation. Rev and Rex proteins have been shown to be interchangeable, and Rex acts on the HIV-1 RRE (46, 53).

To assess the effects of HIV-1 Rev and HTLV-I Rex on the inhibitory HPV-1 sequence, we inserted the HIV-1 RRE into plasmid pCHPV1pA, resulting in pCM1R (Fig. 6A). As shown in Fig. 6B, cells transfected with either pCHPV1pA or pCM1R produced only low levels of p17^{gag}, while cotransfection of pCM1R with the HIV-1 Rev expression plasmid pNL14A7 gave rise to high levels of p17^{gag}, which were comparable to those produced from pCM1234pA. The results of two parallel transfections are shown. However, the HTLV-I Rex protein failed to induce high p17^{gag} production. This is unexpected, since the Rex protein has been shown to act on the HIV-1 RRE (46, 53). To confirm that functional levels of Rex protein were produced, the Rex expression plasmid was cotransfected with the HIV-1 p55^{gag} expression plasmid pNLvgagSty330. This plasmid contains the same RRE fragment as pCM1R and has previously been shown to respond to Rex (53). As shown in Fig. 6C (right panel), high levels of p55^{gag} were produced when HIV-1 Rev or HTLV-I Rex proteins were provided in *trans*. This demonstrated that functional levels of Rex protein were produced in the transfections. Our data suggest that either the Rev and Rex proteins act by different mechanisms or the Rex protein functions less efficiently on the HIV-1 RRE than does the Rev protein and therefore could not overcome inhibition exerted by the inhibitory HPV-1 sequences.

The presence of HIV-1 Rev and RRE increases utilization of cytoplasmic mRNAs containing inhibitory HPV-1 sequences. In light of previous studies which showed that Rev promotes nuclear export of RRE-containing mRNAs sequestered in the nucleus, we were interested in investigating the subcellular distribution of mRNAs produced from pCM1R in the absence

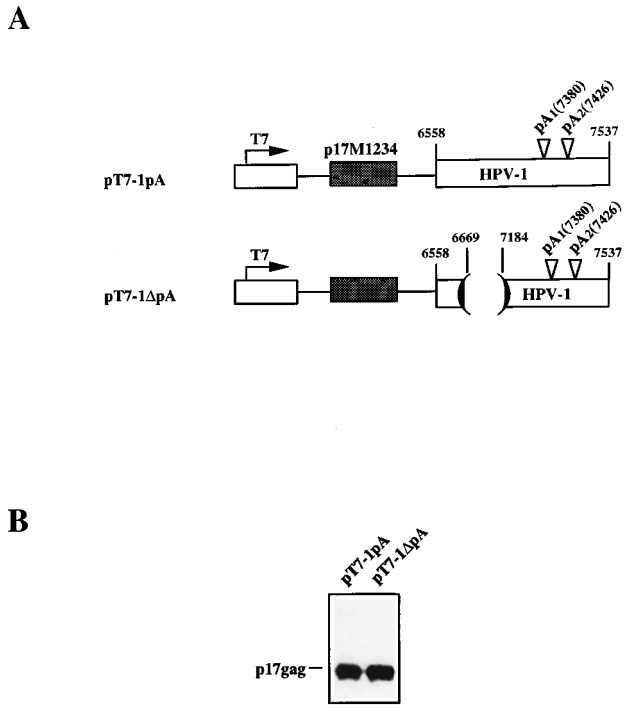
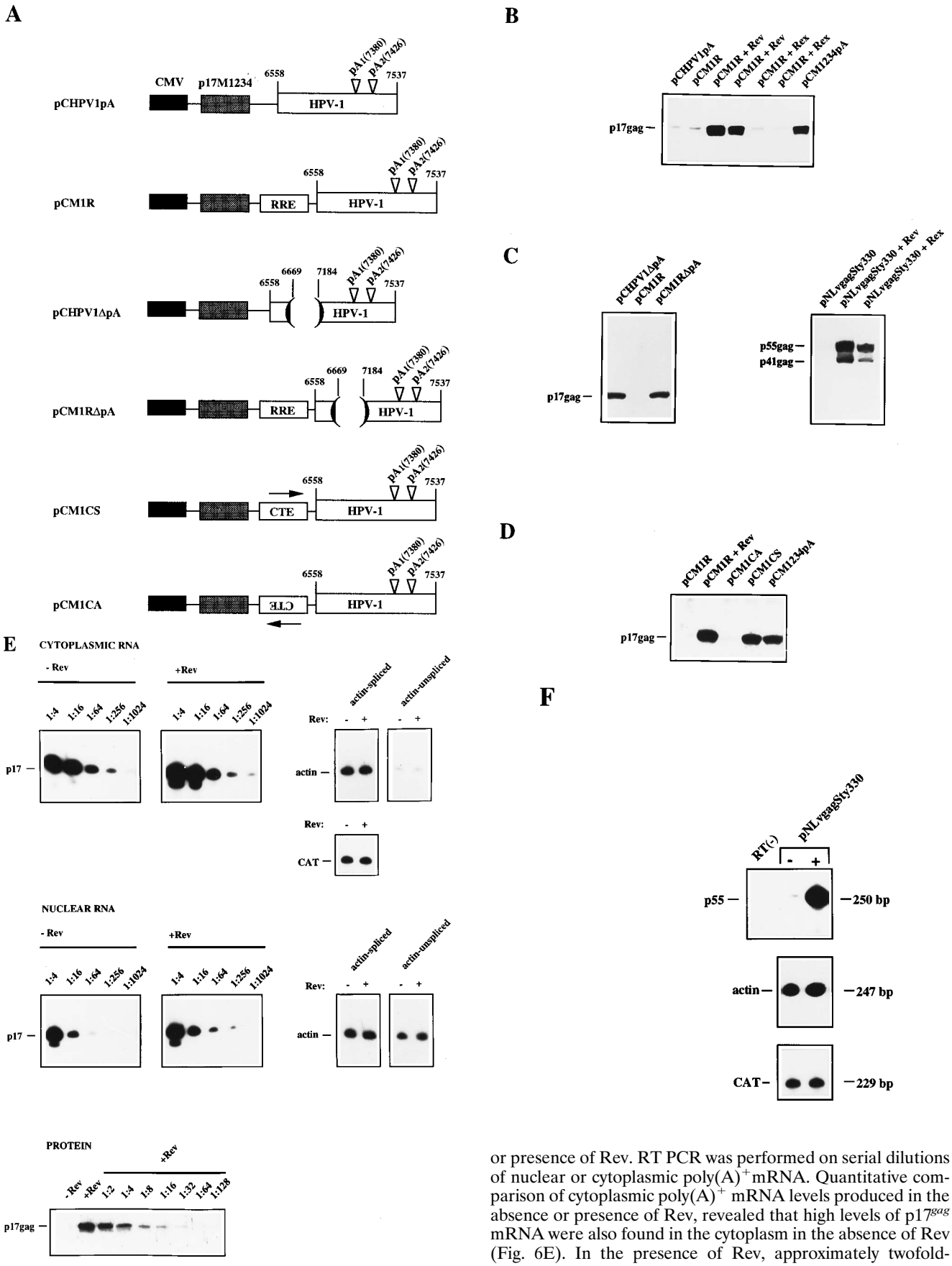


FIG. 5. Transcription in the cytoplasm relieves inhibition. (A) Structures of the p17^{gag} expression plasmids. Open boxes indicate the bacteriophage T7 promoter, and stippled boxes indicate the mutated p17^{gag} gene named p17M1234 (50). The HPV-1 late 3′ UTR-containing sequences are shown. Parentheses mark the limits of a deletion between nt 6669 and 7184. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). Polyadenylation signals of the HPV-1 late mRNAs are indicated by triangles. Names of the plasmids are indicated on the left. (B) HeLa cells were infected with recombinant vaccinia virus vTF7-3 (19, 20) and then subjected to transfection with pT7-1pA or pT7-1ΔpA. Production of p17^{gag} was analyzed by Western immunoblotting as described in the legend to Fig. 1B. The position of p17^{gag} is indicated on the left.



or presence of Rev. RT PCR was performed on serial dilutions of nuclear or cytoplasmic poly(A)$^+$mRNA. Quantitative comparison of cytoplasmic poly(A)$^+$ mRNA levels produced in the absence or presence of Rev, revealed that high levels of p17^{gag} mRNA were also found in the cytoplasm in the absence of Rev (Fig. 6E). In the presence of Rev, approximately twofold-

FIG. 6. The inhibitory effect of the HPV-1 3′ UTR can be overcome by HIV-1 Rev in *trans* and the RRE in *cis* or by the SRV-1 CTE in *cis*. (A) Structures of the HIV-1 $p17^{gag}$ expression plasmids with or without the HIV-1 RRE sequence or SRV-1 CTE. A 173-bp PCR fragment encompassing the SRV-1 CTE (55) or a 330-nt *Sty*I fragment containing the HIV-1 RRE (53) was inserted between the $p17^{gag}$ gene and the HPV-1 3′ UTR-containing sequence. Arrows indicate the orientation of the CTE. Parentheses indicate a deletion between nt 6669 and 7184. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). Triangles represent HPV-1 poly(A) signals. Names of the plasmids are indicated on the left. CMV, human cytomegalovirus immediate-early promoter. (B) Western blot analysis of extracts from HeLa cells transfected with pCHPV1pA or pCM1R in the absence or presence of HIV-1 Rev or HTLV-I Rex. Western immunoblotting was performed as described in Materials and Methods. The position of $p17^{gag}$ is indicated on the left. (C) Western blot analysis of extracts from HeLa cells transfected with pCHPV1ΔpA, pCM1R, or pCM1RΔpA (left panel) or pNLvgagSty330 in the absence or presence of HIV-1 Rev or HTLV-I Rex (right panel). The $p17^{gag}$ protein produced by pCHPV1ΔpA, pCM1R, and pCM1RΔpA and the $p55^{gag}$ and $p41^{gag}$ proteins generated from pNLvgagSty330 are indicated on the left of each immunoblot. (D) Western blot analysis of extracts from HeLa cells transfected with pCM1R in the absence or presence of Rev, pCM1CA, pCM1CS, or pCM1234pA. The position of $p17^{gag}$ is indicated on the left. (E) The top left panel shows PCR amplification of cDNA synthesized from serially fourfold-diluted cytoplasmic poly(A)$^+$ mRNA isolated from cells transfected with pCM1R in the absence or presence of HIV-1 Rev. The top right panel shows PCR amplification of cDNA from spliced or unspliced actin mRNA to control for the cell fractionation technique and from CAT mRNA to control for transfection efficiency. The number of cycles used for PCR amplification of cDNA from actin mRNA or CAT mRNA was adjusted so as to detect cDNA in the linear range of the assay. The middle left panel shows PCR amplification of cDNA synthesized from serially diluted nuclear polyadenylated mRNA isolated from cells transfected with pCM1R in the absence or presence of HIV-1 Rev. The middle right panel shows PCR amplification of cDNA from spliced and unspliced nuclear actin mRNA. The positions of the amplified DNA fragments are indicated on the left. Oligonucleotides were end labeled with [γ-^{32}P]ATP prior to PCR. The PCR products were analyzed on a 6% polyacrylamide–urea gel and then subjected to autoradiography. The bottom panel shows Western immunoblotting of extracts from HeLa cells transfected with pCM1R in the absence or presence of Rev. Extract obtained from cells transfected with pCM1R in the presence of Rev was serially twofold diluted, as indicated, and subjected to immunoblotting as described in the legend to Fig. 1B. The position of $p17^{gag}$ is indicated on the left. (F) PCR amplification of cDNA synthesized from cytoplasmic polyadenylated mRNA extracted from HeLa cells transfected with pNLvgagSty330 in the absence or presence of Rev. PCR was performed with oligonucleotides specific for the $p55^{gag}$ mRNA produced by pNLvgagSty330 (top panel), spliced actin mRNA to control for mRNA levels (middle panel), or CAT mRNA produced from the internal control plasmid producing CAT mRNA (bottom panel). The number of PCR cycles required for linear range of amplification was first determined for all primer pairs. Positions of PCR products are indicated on the left, and their sizes are indicated on the right.

higher levels of cytoplasmic $p17^{gag}$ mRNA were observed, while CAT mRNA levels produced from the internal control plasmid were unaffected (Fig. 6E). Analysis of $p17^{gag}$ mRNA levels in the nucleus, in the absence or presence of Rev, revealed that these levels also increased approximately twofold in the presence of Rev (Fig. 6E).

To exclude the possibility that the high levels of $p17^{gag}$ mRNA found in the cytoplasm in the absence of Rev was caused by nuclear leakage during cell fractionation, we performed RT-PCR with oligonucleotides specific for unspliced and spliced actin mRNA. As expected, unspliced actin mRNA was found primarily in the nuclear fraction (Fig. 6E), while spliced actin mRNA was detected in both nuclear and cytoplasmic fractions (Fig. 6E). To further control for the cell fractionation technique, we analyzed cytoplasmic poly(A)$^+$ mRNA levels produced from pNLvgagSty330 in the absence or presence of Rev. Previous work has established that $p55^{gag}$ mRNA produced from this plasmid is trapped in the nucleus in the absence of Rev and can be detected in the cytoplasm only in the presence of Rev (18). Figure 6F shows that high levels of cytoplasmic $p55^{gag}$ mRNA are detected only in the presence of Rev, confirming that leakage of mRNA from the nucleus does not occur during cell fractionation. Levels of CAT mRNA produced from the control plasmid pCMVCAT and levels of spliced actin mRNA were similar in the absence and presence of Rev (Fig. 6F), demonstrating that mRNA levels and transfection efficiency were similar in the absence or presence of Rev.

We next quantitated the levels of $p17^{gag}$ protein produced from pCM1R in the absence or presence of Rev. This analysis showed a difference of greater than 32-fold at the protein level (Fig. 6E). In conclusion, inhibition of $p17^{gag}$ expression from mRNAs containing HPV-1 sequences could not be explained by sequestration of these mRNAs in the nucleus. Furthermore, rescue of $p17^{gag}$ production from pCM1R by HIV-1 Rev and RRE was not associated with large alterations in subcellular mRNA distribution. Therefore, Rev acted by increasing the utilization of cytoplasmic $p17^{gag}$ mRNAs containing inhibitory HPV-1 sequences.

It has been suggested that HIV-1 RRE has a negative effect on gene expression (5, 45). It may be argued that a negative effect of RRE on gene expression could interfere with the interpretations of the results presented here. To exclude this, RRE was inserted into plasmid pCHPV1ΔpA, which does not contain inhibitory HPV-1 sequences and produces high levels of $p17^{gag}$. This resulted in pCM1RΔpA (Fig. 6A). Figure 6C (left panel) shows that similar levels of $p17^{gag}$ were produced from pCHPV1ΔpA and pCM1RΔpA, demonstrating that insertion of RRE does not affect $p17^{gag}$ production.

The effect of the inhibitory HPV-1 sequences is overcome by the presence in *cis* of the SRV-1 constitutive transport element. The 3′ UTR of Mason-Pfizer monkey virus and SRV-1 contains a sequence named CTE, which can substitute for HIV-1 Rev and RRE when inserted into Rev$^-$ mutant proviruses (4, 55). We wished to test if the SRV-1 CTE could overcome inhibition exerted by the inhibitory HPV-1 sequences. A 241-nt fragment spanning the SRV-1 CTE was cloned into plasmid pCHPV1pA in sense and antisense orientations, resulting in plasmids pCM1CS and pCM1CA, respectively (Fig. 6A). Figure 6D shows that high levels of $p17^{gag}$ were produced from pCM1CS, while pCM1CA produced only low levels, demonstrating that SRV-1 CTE acted in an orientation-dependent manner to revert inhibition exerted by the HPV-1 sequence. The $p17^{gag}$ levels were similar to those produced from pCM1R in the presence of Rev (Fig. 6D), indicating that CTE acted with similar efficiency to Rev and RRE in overcoming inhibition caused by the HPV-1 sequence.

Mutating the 5′ splice site-like sequence CATGTAAGAT within the HPV-1 3′ UTR does not relieve inhibition. Since the HIV-1 Rev protein could reverse the inhibition caused by the HPV-1 3′ UTR, we asked whether the HPV-1 3′ UTR contained sequences similar to inhibitory RNA elements found on HIV-1 mRNAs. The presence on HIV-1 mRNAs of unutilized splice sites has been implicated in negative regulation of expression of HIV-1 structural proteins in the absence of Rev (6, 28, 40). Since deletion of a 515-bp fragment in the HPV-1 3′ UTR-containing sequence resulted in a large increase in $p17^{gag}$ production, this sequence was analyzed for the presence of 5′ splice site-like sequences. One sequence (CATGTAAGA) with substantial homology to the consensus 5′ splice site (CAGGTAAGT) was found between nt 7032 and 7040 in the 3′ UTR (Fig. 7A). To test if this sequence was responsible for the strong inhibitory effect exerted by the HPV-1 3′ UTR, the 5′ splice site-like sequence was mutated to GAGCTC by site-directed mutagenesis, resulting in plasmid pCH1pAM (Fig. 7A). However, cells transfected with pCH1pAM did not pro-

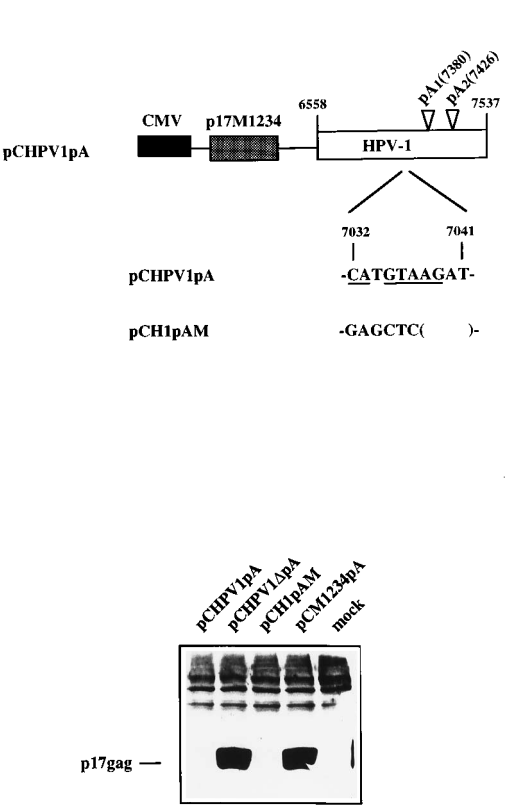


FIG. 7. The 5′ splice site-like sequence in the HPV-1 3′ UTR does not contribute to inhibition of p17^{gag} production. (A) Structure of p17^{gag} expression plasmids containing unmutated or mutated HPV-1 5′ splice site-like sequence. The DNA sequence of the 5′ splice site-like sequence between nt 7032 and 7041 in the HPV-1 3′ UTR is shown. Nucleotides that comply with the consensus 5′ splice site (CAGGTAAGT) are underlined. The 5′ splice site-like sequence in plasmid pCHPV1pA was mutated by site-directed mutagenesis, generating plasmid pCH1pAM. A *Sac*I site, GAGCTC, was introduced, and 4 nt were deleted. Triangles represent HPV-1 poly(A) signals. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). CMV, human cytomegalovirus immediate-early promoter. (B) Western blot analysis of p17^{gag} production from plasmid pCH1pAM. HeLa cells transfected with pCHPV1pA, pCHPV1ΔpA, pCH1pAM, or pCM1234pA were harvested 24 h posttransfection. The production of p17^{gag} was analyzed by Western immunoblotting as described in the legend to Figure 1B. The position of p17^{gag} is indicated on the left.

duce elevated levels of p17^{gag} (Fig. 7B). In contrast, high levels of p17^{gag} were produced from both pCHPV1ΔpA and pCM1234pA (Fig. 7B). Our data demonstrate that the 5′ splice site-like sequence within the HPV-1 3′ UTR is not required for inhibitory activity, indicating that sequences distinct from 5′ splice site-like motifs regulate gene expression.

A negative element located between nt 6926 and 7035 in the HPV-1 genome inhibits gene expression in an orientation-dependent manner. To identify the minimal inhibitory element within the HPV-1 3′ UTR, the HPV-1 sequence was subjected to deletion analysis. Different regions of the HPV-1 poly(A)-containing sequences were PCR amplified and cloned immediately downstream of p17^{gag}, as described in Materials and Methods. These cloning steps generated plasmids pCH1pA883, pCH1pA669, pCH1pA611, and pCH1pA525 (Fig. 8A). As shown in Fig. 8B, production of p17^{gag} was strongly inhibited in cells transfected with either pCHPV1pA or pCH1pA883, while slightly increased levels of p17^{gag} were produced form pCH1pA669 and pCH1pA611. In contrast, pCH1pA525 gave rise to high levels of p17^{gag}, similar to those produced from the control plasmid, pCM1234pA (Fig. 8B). These results localized the 5′ boundary of the inhibitory HPV-1 element between nt 6926 and 7012.

To determine the 3′ boundary of the inhibitory element, we used oligonucleotides and PCR amplification to create a series of 3′ deletions. Since these deletions excluded the HPV-1 poly(A) sites, the fragments were cloned upstream of the SV40 early poly(A) signal in pCM1234pA. This resulted in five expression plasmids: pCH1-507, pCH1-350, pCH1-262, pCH1-167, and pCH1-206 (Fig. 9A). Figure 9B shows that the levels of p17^{gag} produced from pCH1-206 were similar to those produced from pCM1234pA, indicating that the HPV-1 sequence contained in this plasmid did not inhibit gene expression. Interestingly, all the other plasmids (pCH1-507, pCH1-350, pCH1-262, and pCH1-167) produced low levels of p17^{gag}, demonstrating that the inhibitory HPV-1 element was retained in these plasmids. This demonstrated that the 3′ boundary of the inhibitory HPV-1 element is located upstream of nt 7035.

To investigate if the inhibitory element acted in an orientation-dependent manner, the fragment contained in plasmid pCH1-507 was also cloned in the antisense orientation, resulting in plasmid pCH1-507A. The results shown in Fig. 9C revealed that inhibition caused by the HPV-1 element is orientation dependent, since transfection of plasmid pCH1-507A gave rise to high levels of p17^{gag}. The levels were similar to

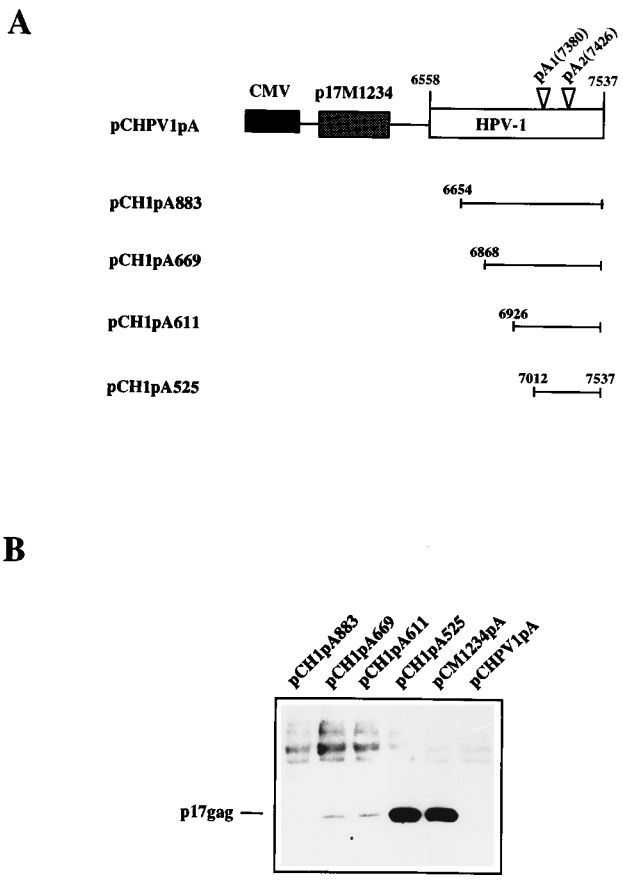


FIG. 8. Identification of the 5′ boundary of the inhibitory HPV-1 element. (A) Structures of the p17^{gag} expression plasmids with progressive deletions at the 5′ end of the HPV-1 sequence. Names of plasmids are indicated on the left. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). CMV, human cytomegalovirus immediate-early promoter. (B) Western blot analysis of p17^{gag} production from various p17^{gag} expression plasmids as indicated. Western immunoblotting was performed as described in the legend to Figure 1B. The position of p17^{gag} is indicated on the left.

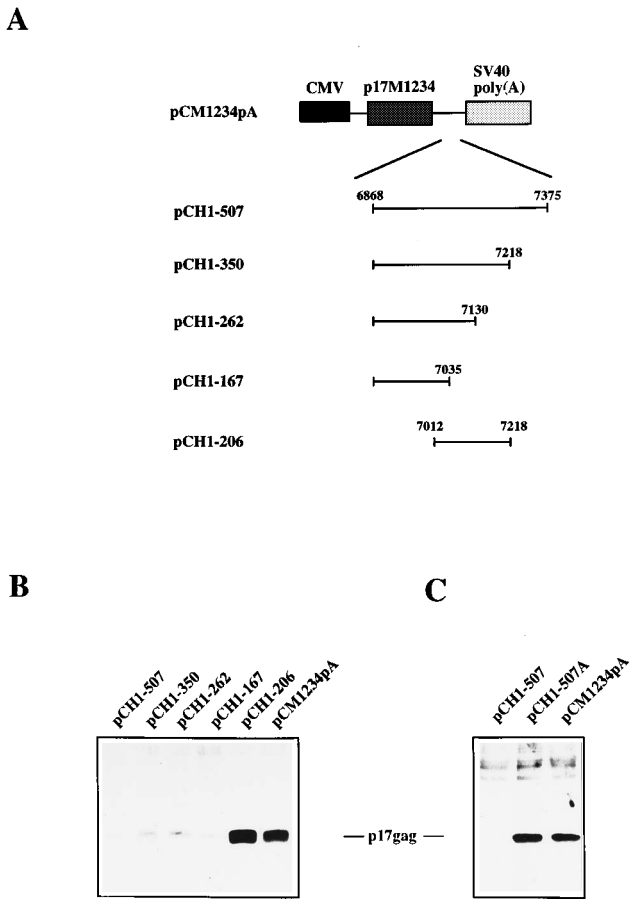


FIG. 9. Identification of the 3′ boundary of the inhibitory HPV-1 element. (A) Structures of the p17^{gag} expression plasmids with progressive deletions at the 3′ end of the HPV-1 3′ UTR-containing sequence. The SV40 early poly(A) signal is used in these constructs. Names of plasmids are indicated on the left. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). CMV, human cytomegalovirus immediate-early promoter. (B) Western blot analysis of p17^{gag} production from various p17^{gag} expression plasmids as indicated. Western immunoblotting was performed as described in the legend to Fig. 1B. The position of p17^{gag} is indicated on the right. (C) Inhibition exerted by the HPV-1 sequence is orientation dependent. Production of p17^{gag} from plasmids with the HPV-1 sequence in sense or antisense orientation was analyzed by Western immunoblotting as described in the legend to Fig. 1B. The position of p17^{gag} is indicated on the left.

those produced from pCM1234pA (Fig. 9C), indicating that inhibitory function was abolished when the sequence was placed in antisense orientation. We concluded that the inhibitory HPV-1 element is located between nt 6926 and 7035 and acts in an orientation-dependent manner to inhibit gene expression.

The inhibitory element coincides with an AU-rich sequence in the HPV-1 3′ UTR. To fine map the inhibitory element located between nt 6926 and 7035, further deletions were introduced in this region. Expression plasmids pCH1pA579, pCH1pA569, and pCH1pA555 (Fig. 10A) were generated as described in Materials and Methods. These plasmids were transfected into HeLa cells, and expression levels of p17^{gag} were compared with those from pCH1pA611 and pCH1pA525 (Fig. 8A and 10A). Western blot analysis showed that cells transfected with pCH1pA611 or pCH1pA579 produced low levels of p17^{gag} (Fig. 10B), while cells transfected with pCH1pA569, pCH1pA555, and pCH1pA525 produced high levels of p17^{gag} (Fig. 10B). The HPV-1 fragment present in pCH1pA579 lacks a sequence with the potential to form a stem-loop structure (Fig. 10A) but retains two AUUUA motifs as well as three repeats of the U-rich sequence, UUUUUAUA (Fig. 10A). Apparently, the stem-loop structure is dispensable for inhibition, since pCH1pA579 still produced low levels of p17^{gag}. Therefore, the 5′ boundary of the HPV-1 inhibitory element was mapped to nt 6958. When deletions were introduced in the AU-rich sequence between nt 6958 and 7012, p17^{gag} production increased substantially (Fig. 10B). Since similar high levels of p17^{gag} were produced from pCH1pA569, pCH1pA555, and pCH1pA525, we concluded that the three UUUUUAUA motifs alone did not have a strong inhibitory effect on p17^{gag} production. Therefore, the AU-rich sequence between nt 6958 and 6982 appeared to be necessary for inhibition of gene expression.

The AU-rich HPV-1 sequence located between nt 6868 and 6984 is necessary and sufficient for inhibition. To determine whether the AUUUA-containing sequence between nt 6958 and 6982 acts in concert with the downstream UUUUUAUA repeats, a fragment (nt 6868 to 6984) containing the potential

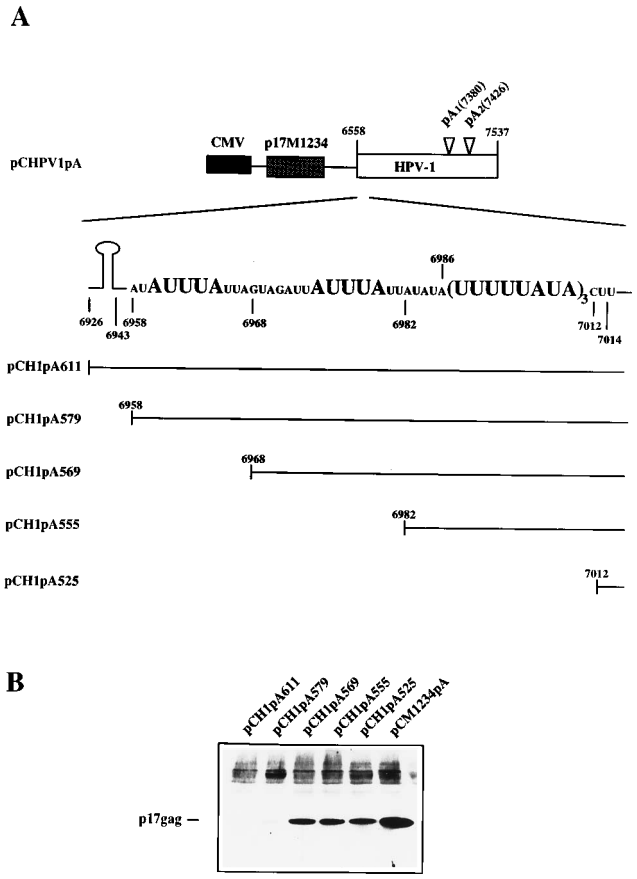


FIG. 10. Fine mapping of the HPV-1 inhibitory element. (A) Structures of the p17^{gag} expression plasmids containing different portions of the HPV-1 3′ UTR. Names of plasmids are indicated on the left. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). The mRNA sequence from nt 6958 to 7014 of the HPV-1 3′ UTR is shown. A potential stem-loop structure at the 5′ end of mRNA is also displayed. $(UUUUUAUA)_3$ indicates that the UUUUUAUA sequence motif is repeated three times. For simplicity, a cytidine present between the second and third UUUUUAUA motifs is not shown. CMV, human cytomegalovirus immediate-early promoter. Triangles represent poly(A) signals of the HPV-1 late mRNAs. (B) Western blot analysis of p17^{gag} production from different expression plasmids. Western immunoblotting was performed as described in the legend to Fig. 1B. The position of p17^{gag} is indicated on the left.

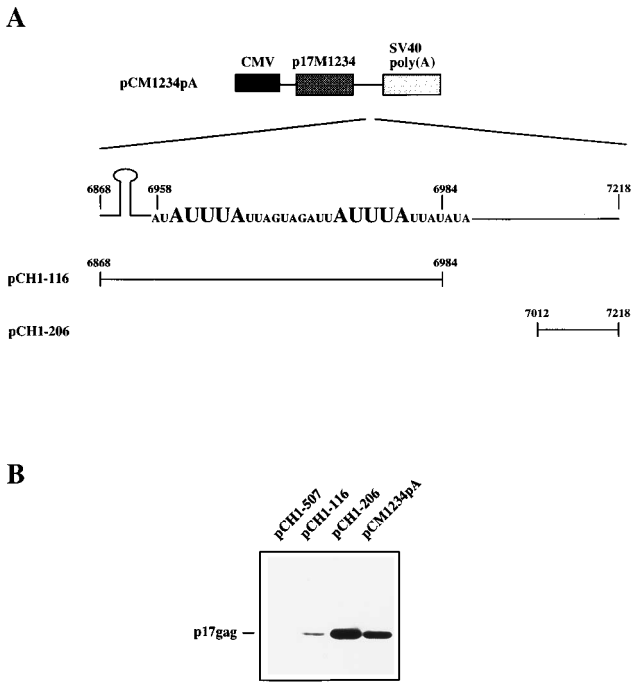


FIG. 11. The sequence between nt 6868 and 6984 in the HPV-1 late 3′ UTR is sufficient for inhibition. (A) Structures of p17^{gag} expression plasmids. The AU-rich sequence between nt 6958 and 6984 is displayed. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). A potential stem-loop structure upstream of the AU-rich sequence is indicated. Names of the plasmids are indicated on the left. CMV, human cytomegalovirus immediate-early promoter. (B) Western blot analysis of p17^{gag} production. Western immunoblotting was performed as described in the legend to Fig. 1B. The position of p17^{gag} is indicated on the left.

stem-loop structure and the AUUUA-containing sequence between nt 6958 and 6982 but lacking the UUUUUAUA repeats was cloned upstream of the SV40 early poly(A) signal in plasmid pCM1234pA. This resulted in pCH1-116 (Fig. 11A). Western blot analysis revealed that this plasmid produced significantly less p17^{gag} than did plasmid pCM1234pA and plasmid pCH1-206 (Fig. 11B), which contains an HPV-1 fragment without inhibitory activity. These results demonstrated that the sequence information present downstream of nt 6984 is not required for strong inhibition. However, the p17^{gag} levels produced from pCH1-116 were elevated compared with those produced from pCH1-507 (Fig. 11B). Therefore, the downstream U-rich sequences may act to enhance the effect of the AUUUA-containing sequence located between nt 6958 and 6982.

To test whether deletion of the AU-rich sequence in the HPV-1 3′ UTR would abolish inhibition, a 70-bp sequence (nt 6943 to 7014) containing two AUUUA motifs and three UUUUUAUA repeats was deleted from plasmids pCHPV1pA and pCH1pA669, generating plasmids pCHPV1ΔBX and pCH1pA669ΔBX, respectively (Fig. 12A). Western blot analysis showed that high levels of p17^{gag} were produced from pCHPV1ΔBX and pCH1pA669ΔBX compared with their parental plasmids (Fig. 12B). These results established that the AU-rich sequence between nt 6943 and 7014 is the major inhibitory element in the HPV-1 fragment analyzed here.

DISCUSSION

In this paper, we report the identification of a negative regulatory RNA element in the HPV-1 3′ UTR. The inhibitory effect mediated by this element is greater at the protein level (64- to 128-fold) than at the mRNA level (4-fold), demonstrating that mRNAs produced from pCHPV1pA are translated less efficiently than those produced from pCHPV1ΔpA. These results indicate a bimodal mechanism of inhibition in which both mRNA levels and mRNA translatability are affected. To investigate the reason for low cytoplasmic mRNA levels, the stability of cytoplasmic mRNAs was determined. Actinomycin D treatment of cells transfected with pCHPV1pA or pCHPV1ΔpA did not reveal a major difference in half-lives of the mRNA transcripts (data not shown). This is in agreement with results of a previous study on the BPV-1 3′ UTR (21). In that study, Furth and Baker identified inhibitory sequences in the BPV-1 3′ UTR but did not detect an effect of these sequences on mRNA stability. Interestingly, we found that inhibition exerted by the HPV-1 3′ UTR could be overcome by providing the HIV-1 Rev protein in *trans* and the RRE in *cis*. Rev is a regulatory protein that functions primarily in the nucleus and acts by facilitating nuclear export of viral mRNAs containing the RRE (15, 18, 27, 43). In the presence of Rev, we observed a 2- to 4-fold increase at the nuclear and cytoplasmic mRNA levels, while the amounts of protein produced in-

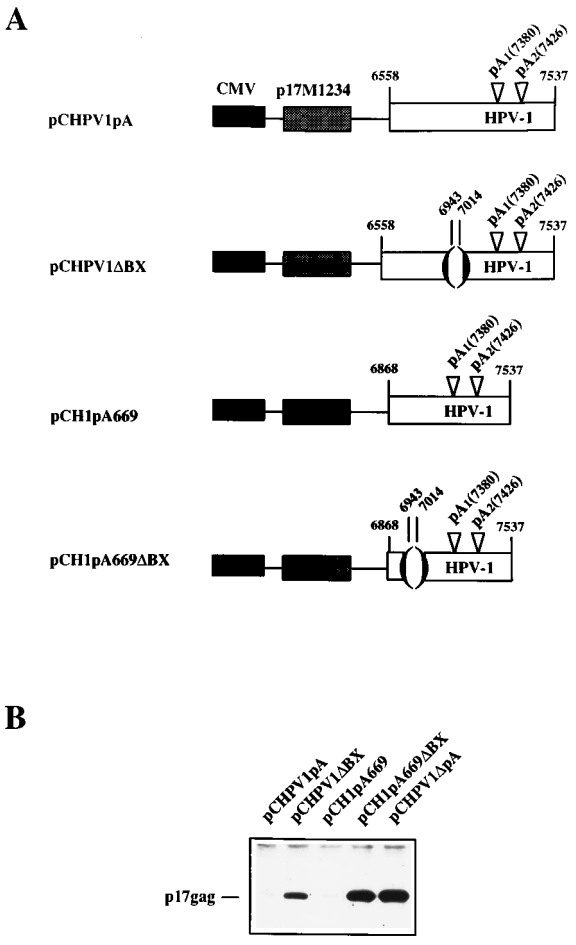


FIG. 12. Internal deletions in the HPV-1 3′ UTR. (A) Structures of p17^{gag} expression plasmids. Parentheses indicate deletion between nt 6943 and 7014. Numbers indicate nucleotide positions on the genomic HPV-1 clone (12). Triangles represent polyadenylation signals. CMV, human cytomegalovirus immediate-early promoter. (B) Western blot analysis of p17^{gag} production from the various expression plasmids. Western immunoblotting was performed as described in the legend to Fig. 1B. The position of p17^{gag} is indicated on the left.

TABLE 1. Comparison of AU-rich sequences present in the 3′ UTR of unstable cellular mRNAs and HPV-1 late mRNAs

mRNA[a]	Sequence[b]
GM-CSF	.....UAAUAUUUAUAUAUUUAUA**UUUUU**AAAAUAUUUAUUUAUUUAUUUAUUUAA
c-*fos*	.....G**UUUUU**AAUUUAUUUAUUAAGAUGGAUUCUCAGAUAUUUAUA**UUUUUUAUUU**
c-*myc*	.....UAA**UUUUUUUU**AUUUAAGUACA**UUUUGCUUUUU**AAAGUUGA**UUUUUUUUCU**A
IL-1	.....**UUAUUUUUU**AAUUAUUAUUUUAUAUAUGUAUUUAUAAAUAUAUUUUAAGAUAAU
HPV-1	.....UAUAUAUUAUAUAUAACUAUAUUUAUUAGUAGAUUAUUUAUUAUAUA**UUUUUAUAUUUUU**AUAC**UUUUU**AUA

[a] GM-CSF, granulocyte-monocyte colony-stimulating factor; IL-1, interleukin-1.
[b] AUUUA motifs are underlined, and U-rich sequences are in boldface.

creased 32- to 64-fold. Since the mRNAs containing inhibitory HPV-1 sequences did not accumulate in the nucleus in the absence of Rev, the major effect of Rev was on mRNA utilization. Our data support a model in which mRNAs containing the complete HPV-1 3′ UTR enter a nonproductive pathway in the nucleus, which results in mRNA degradation and inability to associate with the cellular translation machinery within the cytoplasm. Rev may rescue the mRNAs in the nucleus by directing them to a productive nuclear export pathway.

Rev proteins are regulatory proteins essential for retrovirus replication and are produced by several complex ungulate and primate retroviruses. Some of these proteins are interchangeable and appear to act by similar mechanisms to relieve nuclear retention of mRNAs encoding viral structural proteins. Some viruses in the oncoretrovirus group, i.e., HTLV-I, HTLV-II, and bovine leukemia virus, produce Rex proteins similar in function to the lentivirus Rev proteins (16). Interestingly, the HTLV-I Rex protein, shown to be interchangeable with HIV-1 Rev (46), did not increase $p17^{gag}$ production from plasmid pCM1R. The reason for this may be that the Rex protein interacts less efficiently with the HIV-1 RRE than does the HIV-1 Rev protein (53). If Rev or Rex competes for the mRNA with a negative cellular factor binding to the inhibitory sequences, the affinity of the RNA-binding protein to its target sequence may play a decisive role. Indeed, it has been shown that HTLV-I Rex binds with higher affinity to the Rex-responsive element than to HIV-1 RRE (54). Alternatively, the Rex protein may not act by the same mechanism as that used by Rev.

Several different viruses have been shown to produce mRNAs containing *cis*-acting RNA elements that determine the fate of the viral mRNAs (1, 4, 10, 31, 33, 34). Posttranscriptional regulation of viral gene expression often involves interactions of viral mRNAs with cellular splicing machinery. Experiments performed on HIV-1 have suggested that cellular splicing factors interact with unutilized splice sites present on mRNAs encoding structural proteins (6, 28, 40). As a result, the mRNAs were retained in the nucleus in the absence of Rev. Furth et al. have reported that sequences with homology to the 5′ splice site are located in the BPV-1 and HPV-16 late 3′ UTRs and that these sequences repress gene expression (22). The HPV-1 late 3′ UTR contains one sequence motif with significant homology to a consensus 5′ splice site (44). However, removal of the 5′ splice site-like sequence (CATG TAAGAT) in the HPV-1 3′ UTR by site-directed mutagenesis did not increase $p17^{gag}$ production, demonstrating that sequences distinct from 5′ splice site-like motifs are required for inhibitory activity. Furthermore, deleting the AU-rich inhibitory HPV-1 sequences but leaving the 5′ splice site-like sequence intact (for example, pCH1pA525 [Fig. 8] and pCH1-206 [Fig. 9]) resulted in high $p17^{gag}$ production, demonstrating that the 5′ splice site-like sequence in the HPV-1 3′ UTR does not confer inhibition. HIV-1 mRNAs also contain inhibitory sequences distinct from splice sites. Such sequences have been mapped to the coding regions of the HIV-1 *gag*, *pol*, and *env* genes (5, 8, 41, 45, 48, 52). Interestingly, some of these sequences coincide with AU-rich regions but do not generally contain AUUUA motifs found in the 3′ UTRs of many labile cellular mRNAs. In HPV-16, inhibitory sequences have been mapped to the 3′ UTR of late mRNAs (35, 36). Also, in this case the sequences were AU rich but did not contain AUUUA elements. However, sequences which differed from AUUUA motifs in single nucleotide positions were located within the inhibitory HPV-16 element. It remains to be investigated whether AUUUA sequences in the HPV-1 late 3′ UTR and AUUUA-like sequences in the HPV-16 late 3′ UTR play a role in inhibition of HPV late-gene expression. Alternatively, HPV-1 and HPV-16 may use different elements in the 3′ UTR to regulate gene expression. Furth et al. have suggested that 5′ splice site-like sequences in the HPV-16 late 3′ UTR are important for inhibition (22). This may reflect the difference in tropism between these two HPV types. HPV-1 typically causes plantar warts in cutaneous epithelium, while HPV-16 infects genital mucosa. Various types of epithelia may produce different regulatory factors, which may interact specifically only with certain types of HPVs.

Initial deletion analysis mapped the 5′ and 3′ boundaries of the HPV-1 inhibitory element to nt 6926 and 7035. This sequence consists of a potential stem-loop structure (nt 6936 to 6957), tandemly arranged AUUUA motifs, and three consecutive UUUUUAUA repeats, except that the second and the third UUUUUAUA motifs are separated by a cytidine. The expression plasmid pCH1pA579, lacking the stem-loop structure, did not produce elevated levels of $p17^{gag}$ (Fig. 10B). This pointed to the importance of the AU-rich region between nt 6958 and 7035, which contains 87% A+U nucleotides. AU-rich elements constitute a common feature of the 3′ UTRs of many unstable mRNAs encoding cellular regulatory proteins such as c-Myc, c-Fos and granulocyte-monocyte colony-stimulating factor. The AU-rich elements usually contain various numbers of AUUUA motifs and have a high A+U content. Several lines of evidence indicate that AU-rich sequences from the 3′ UTRs of the mRNAs for, e.g., c-Myc, c-Fos, granulocyte-monocyte colony-stimulating factor, and beta interferon reduce mRNA stability. Similarly to the HPV-1 3′ UTR, the AU-rich elements of cellular mRNAs also contain U-rich sequences (Table 1). Detailed studies on the 3′ UTR of the c-*fos* mRNA demonstrated that an AUUUA-containing sequence functioned as an RNA destabilizer itself, while a downstream 20-nucleotide U-rich sequence enhanced the destabilizing effect of the AUUUA motifs (7). The inhibitory HPV-1 sequence contains AUUUA motifs followed by three U-rich UUUUUAUA octanucleotides. Deletion of the sequences between nt 6943 and 7014, as in pCHPV1ΔBX and pCH1pA669ΔBX, resulted in elevated $p17^{gag}$ production, demonstrating that this AU-rich region is the most important determinant for inhibition in the HPV-1 late 3′ UTR. Our data indicate that the portion of this sequence that encodes

AUUUA motifs contains the strongest negative element, while the UUUUUAUA repeats contribute to inhibition to a lower extent. The two closely arrayed AUUUA motifs may play a role in inhibition, as has been proposed for some cellular mRNAs. Alternatively, other sequence motifs may constitute the core of the inhibitory element or act in concert with the AUUUA or UUUUUAUA motifs. Mutational analysis of sequences within this region is in progress and will elucidate the exact role of AUUUA and UUUUUAUA motifs in the regulation of HPV-1 gene expression.

The presence on various mRNAs of AU-rich sequences may result in low mRNA stability. However, we did not detect gross differences in mRNA steady-state levels with or without the HPV-1 late 3′ UTR (data not shown), indicating that the major effect of the HPV-1 3′ UTR is not on mRNA stability. The inhibitory HPV-1 sequences have a greater effect at the protein level than at the mRNA level, suggesting that translation of the HPV-1 3′ UTR-containing mRNAs is affected. Interestingly, it has been reported that AUUUA-containing mRNAs are translated less efficiently than AUUUA-lacking mRNAs after injection into *Xenopus* oocytes (38). Our findings are also consistent with previous studies which demonstrated that AU-rich sequences within the 3′ UTRs of beta interferon and tumor necrosis factor mRNAs have an inhibitory effect on translation (30, 39).

It is well established that papillomavirus gene expression can be divided into an early and a late phase. Early gene products of HPVs are expressed continuously in the infected epithelia, while expression of late genes is restricted to terminally differentiated keratinocytes. Keratinocyte differentiation-specific factors may be involved in regulation of HPV late-gene expression. Since our results demonstrated that the inhibitory effect of the HPV-1 3′ UTR could be overcome by the HIV-1 Rev and RRE, it is tempting to speculate that expression of HPV-1 late mRNAs is regulated posttranscriptionally by a Rev-like cellular protein which is absent during the early stage of HPV life cycle but is expressed in terminally differentiated keratinocytes. Either a Rev-like cellular factor or an HPV protein competes with the inhibitory factor(s), or production of inhibitory factors is down-regulated as the cell differentiates. Further experiments are necessary to elucidate the molecular mechanism behind the tight coupling between cell differentiation and HPV late gene expression.

It was recently shown that sequences in the 3′ UTRs of the retroviruses Mason-Pfizer monkey virus and SRV-1 contain a sequence named CTE which can substitute for HIV-1 Rev and RRE and render HIV-1 structural gene expression and viral replication Rev independent (4, 55). It was postulated that this element might interact with cellular factors to facilitate transport of intron-containing HIV-1 mRNAs from the nucleus to the cytoplasm. The SRV-1 CTE could overcome inhibition caused by the negative element in HPV-1 when the CTE was present in the sense orientation. Similar results were obtained with cell lines of different origin (data not shown). These results suggest that a cellular factor interacting with the CTE is present in the cells examined. This interaction could counteract the inhibitory HPV-1 sequence. The fact that a cellular machinery is active and can overcome the effect of negative HPV-1 elements when appropriate *cis*-acting sequences are present on the mRNA favors a model for regulation of HPV late-gene expression in which a cellular factor acting on the inhibitory HPV sequence to prevent HPV late-gene expression is down-regulated as differentiation of the epithelial cell proceeds. Alternatively, sequences similar in function to the CTE may be present on HPV late mRNAs. Such sequences may be activated through interactions with cellular factors induced in terminally differentiated epithelial cells.

It is interesting that both HPV-1 and HIV-1 utilize AU-rich *cis*-acting repressive sequences to posttranscriptionally regulate expression of viral late-gene products. Despite the fact that HIV-1 and SRV-1 are not related to HPV-1, HIV-1 Rev and RRE, and SRV-1 CTE can overcome inhibition caused by the HPV-1 3′ UTR. Interplay between viral *cis*-acting RNA sequences and viral or cellular *trans*-acting factors may be a general mechanism by which both DNA and RNA viruses regulate expression of late, structural genes. A *cis*-acting sequence that facilitates the transport and utilization of viral mRNA transcripts was also found on hepatitis B virus mRNAs (31, 32). Identification of viral regulatory *cis*-acting sequences that interact with viral or cellular factors will improve our understanding of the mechanisms of viral and cellular gene regulation and may help to establish a tissue culture system for efficient propagation of HPVs.

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