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Identification of Nuclear and Cytoplasmic Proteins That Interact Specifically with an AU-Rich, *cis*-Acting Inhibitory Sequence in the 3′ Untranslated Region of Human Papillomavirus Type 1 Late mRNAs

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Expression of human papillomavirus late genes encoding L1 and L2 capsid proteins is restricted to terminally differentiated epithelial cells. We have previously identified and characterized an AU-rich, *cis*-acting negative regulatory element in the 3′ untranslated region of human papillomavirus type 1 late mRNAs. This element acts posttranscriptionally to reduce mRNA levels and the translation efficiency of mRNAs. The experiments reported here are a continuation of our previous work. We have used RNA gel shifts and UV cross-linking assays to identify cellular proteins that interact with the inhibitory RNA sequence of human papillomavirus type 1. RNA gel shift assays established that cellular proteins interact with the AU-rich sequence. The binding of nuclear proteins was inhibited by competition with poly(U), whereas the binding of cytoplasmic proteins was inhibited by competition with poly(U) and also by competition with poly(A) and poly(G). Two nuclear proteins and two cytoplasmic proteins that bind specifically to the AU-rich RNA sequence were identified by UV cross-linking. These proteins did not bind to the 3′ untranslated region of human papillomavirus type 1 early mRNAs, which does not show inhibitory activity. The cellular proteins identified in our experiments may therefore be involved in the inhibition of human papillomavirus type 1 late gene expression in nondifferentiated epithelial cells.

Human papillomaviruses (HPVs) are a heterogeneous group of viruses that are associated with benign and malignant cell hyperproliferation (6, 11, 16, 17, 23, 24, 39). HPVs are strictly epitheliotropic but selectively infect certain anatomical sites. HPV type 1 (HPV-1) is a cutaneous HPV type and the etiological agent of warts on the plantar surface of the foot (25). The life cycle of HPV-1 can be divided into an early phase and a late phase. The onset of the late genes coding for capsid proteins L1 and L2 and the production of virions are strictly linked to the differentiation stage of the infected cell, and L1 and L2 proteins are detected primarily in terminally differentiated cells in the upper layers of the epithelium (8, 15, 18). In contrast, HPV-1 genomic DNA and HPV-1 early gene products are detected in all layers of the infected epithelium. The reason for the restriction of L1 and L2 expression to terminally differentiated cells is not clear, and the mechanisms that regulate HPV-1 late gene expression have not been examined in detail.

We have previously identified an AU-rich *cis*-acting inhibitory RNA element located in the HPV-1 late 3′ untranslated region (UTR) (38). This sequence has homology to the sequences of AU-rich elements found in unstable or inefficiently translated cellular mRNAs (33, 38). The presence of this sequence on the mRNA resulted in a decrease in mRNA levels and in inefficient translation of the mRNAs (38). The combined effects resulted in 25- to 50-fold-lower levels of proteins produced. We speculated that the presence of this sequence on HPV-1 late mRNAs may explain the lack of L1 and L2 production in proliferating, nonterminally differentiated keratinocytes. Interestingly, inhibitory sequences have been found also in the 3′ UTR on late mRNAs of mucosal HPV type 16 (HPV-16) (21, 22, 37) and on the late mRNAs of bovine papillomavirus type 1 (9, 10), indicating that inhibitory sequences on papillomavirus late mRNAs are conserved and may have important functions in the virus life cycle.

The inhibitory element we identified in the 3′ UTR of HPV-1 late mRNAs acts in the absence of HPV-1-encoded gene products (38). We reasoned that this RNA element may function by interacting with cellular factors, and we therefore initiated experiments designed to identify such factors. The results revealed that two nuclear proteins and two cytoplasmic proteins from human epithelial cells bind specifically to the inhibitory HPV-1 sequence. These proteins do not bind to the 3′ UTR of the HPV-1 early mRNAs that lacks inhibitory activity, demonstrating that the cellular proteins identified here may be involved in the inhibition of HPV-1 late gene expression in cells other than terminally differentiated keratinocytes.

MATERIALS AND METHODS

Plasmid constructions. To generate plasmids from which HPV-1 RNA probes or competitors could be synthesized, HPV-1 early or late 3′ UTR sequences with various lengths were first PCR amplified with the following sets of oligonucleotides: 1S-2a (5′-GGCGTAAGAATTCGTATATATTAT-3′) and 1A-4 (5′-CAAGCTTATTTAGAACTAAACAAG-3′), 1S-2a and *Xho*I (5′-TTTAGAACTAAACTCGAGTAAAAAGTATAAA-3′), 1S-2a and 1A-5 (5′-TAAGCTTATAATAAATAATCTACT-3′), 1S-2b (5′-GAATTCATATTTATTAGTAGATTATTTATT-3′) and 1A-4, and 1S-2c (5′-GAATTCATATATTTTTATATTTTTATAC-3′) and 1A-4 or H1ES (5′-CGAATTCAATTAATGGAAGTTGATTTTGCTTGG-3′) and H1EA (5′-CGGATCCGAATGTCAGGTGGGCAGGTG-3′). The PCR-amplified fragments were cloned into *Eco*RV-digested, calf intestinal alkaline phosphatase-treated pBluescript (Stratagene), generating plasmids pKSL, pKSF, pKSB, pKSD, pKSC, and pKSH1E.

p17H1ES and p17H1EA were generated by cloning PCR fragments containing the HPV-1 early 3′ UTR sequence (nucleotides [nt] 3798 to 3982) (5) into *Hpa*I-digested, calf intestinal alkaline phosphatase-treated pCM1234pA (38), which contains a mutated human immunodeficiency virus type 1 $p17^{gag}$ gene as a reporter gene (34). To construct p17H16ES and p17H16EA, the HPV-16 early

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3′ UTR sequence (nt 4005 to 4212) (35) was PCR amplified with oligonucleotides 16ES (5′-CGAATTCCAGCCTCTGCGTTTAGGTG-3′) and 16EA (5′-CGGATCCAAAAAACAAACAAACAAACAAAAAAAA-3′) and cloned into *Hpa*I-digested, calf intestinal alkaline phosphatase-treated pCM1234pA (38).

To construct pCCKH1, the chloramphenicol acetyltransferase (CAT) gene was PCR amplified with oligonucleotides CATS and CATA (37) and subsequently cloned blunt end into pCH1pA669 (38), digested with *Sal*I and *Hpa*I, and treated with calf intestinal alkaline phosphatase and T4 DNA polymerase. This procedure replaced the $p17^{gag}$ gene with the CAT reporter gene. pΔKXb was generated by excising a 316-bp *Kpn*I-*Xba*I fragment from pCCKH1 and then performing T4 DNA polymerase treatment and religation. To construct pΔBA and pΔAX, pCCKH1 was first subjected to PCR mutagenesis with oligonucleotides W1-32 (5′-AGTTAACGGTACCGCTACTAGTTCCACCACAAAGCG C-3′), 1A (38), AVRII-S (5′-GATTATTTATCCATGGTTTTTATATTTTTAT AC-3′), and AVRII-A (5′-GTATAAAAATATAAAAACCATGGATAAATAA TC-3′), resulting in the introduction of a *Bss*HII site at nt 6943, an *Avr*II site at nt 6981, and an *Xho*I site at nt 7010 in the HPV-1 late 3′ UTR sequence, generating pCCKH1BAX. To construct pΔBA, pCCKH1BAX was digested with *Bss*HII and *Avr*II, and this digestion followed by T4 DNA polymerase treatment and religation. pΔAX was generated by excising an *Avr*II-*Xho*I fragment from pCCKH1BAX and then performing T4 DNA polymerase treatment and religation.

p17H1LS and p17H1LA have been described previously as pCH1-507 and pCH1-507A (38). Human immunodeficiency virus type 1 $p17^{gag}$ expression plasmid pCM1234pA has been described before (34, 38).

Preparation of nuclear and cytoplasmic extracts of HeLa cells. Nuclear and cytoplasmic extracts used in all the experiments were prepared from subconfluent HeLa cells according to the procedure described by Dignam et al. (7). Protein concentrations of nuclear and cytoplasmic extracts were determined by the Bio-Rad protein assay.

In vitro transcription reactions. In vitro transcription reactions were performed essentially as described previously (32), with some minor modifications. With 40 μCi of [^{32}P]UTP (3,000 Ci/mmol) (Amersham) being used, linearized plasmids pKSL, pKSF, pKSB, pKSD, pKSC, and pKSH1E were transcribed in vitro with bacteriophage T3 or T7 RNA polymerase (0.4 U/μl) in 25 μl of transcription buffer (20 mM Tris-HCl [pH 7.5], 3 mM $MgCl_2$, 0.1 mM spermidine, 10 mM dithiothreitol) in the presence of 0.4 mM ATP, CTP, and GTP (each) and 4 μM UTP at 37°C for 1 h. The samples were treated with RNase-free DNase (1 U/μl) (Boehringer GmbH, Mannheim, Germany) at 37°C for 15 min, extracted twice with phenol-chloroform (1:1 [vol/vol]), and ethanol precipitated. The radiolabeled RNAs were pelleted, dried, and dissolved in 250 μl of RNase-free distilled water and stored at −70°C until use. Unlabeled RNA competitors were transcribed under similar conditions, except that the radiolabeled nucleotide was substituted with 0.4 mM UTP. The unlabeled transcripts were dissolved in RNase-free distilled water to a concentration of about 2 μg/μl. The quality of the probes or RNA competitors was examined by electrophoresis on 5% polyacrylamide gels (acrylamide-bisacrylamide, 60:1). RNA was visualized by ethidium bromide staining or autoradiography.

RNA mobility shift assays. RNA-protein binding reactions were performed by incubating radiolabeled RNA probe L (50,000 cpm) with 20 to 40 μg of cytoplasmic extract or with 10 to 20 μg of nuclear extract, unless otherwise stated, at room temperature in 20 μl of binding buffer (60 mM KCl, 10 mM HEPES [*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid] [pH 7.6], 3 mM $MgCl_2$, 1 mM dithiothreitol, and 5% [vol/vol] glycerol). After 15 min, 100 μg of heparin (Sigma) was added to each reaction mixture, and incubation was continued for 15 min at room temperature. For competition experiments, unlabeled specific or nonspecific competitor RNA was preincubated with nuclear or cytoplasmic extracts at room temperature for 15 min prior to the addition of radiolabeled probe L. The molar excess or amount of each competitor used is indicated in the figure legends. For the RNase T_1 protection experiments, reaction mixtures containing RNA-protein complexes or unbound RNAs were digested at 37°C for 15 min with different amounts of RNase T_1 (Boehringer GmbH). To digest proteins in cellular extracts, nuclear or cytoplasmic extracts were preincubated with various amounts of proteinase K at 37°C for 15 min prior to the addition of radiolabeled probe L. The poly(A), poly(U), poly(G), and poly(C) used in the competition experiments were purchased from Pharmacia; tRNA (baker's yeast) and *Escherichia coli* 16S and 23S rRNA were from Boehringer GmbH. The RNA-protein complexes were resolved on nondenaturing 5% polyacrylamide gels (acrylamide-bisacrylamide, 60:1). The gels were dried, and the RNA-protein complexes were visualized by autoradiography.

UV cross-linking. Cross-linking of ^{32}P-labeled RNA to protein was carried out by UV irradiation of RNA-protein complexes formed under the same conditions as those described for RNA mobility shift assays, except that 50 to 100 μg of cytoplasmic extract was used for UV cross-linking. The reaction mixtures were transferred into 96-well plates, placed on ice, and irradiated for 15 min at a 5-cm distance with a Philips G15T8 UV lamp. After the reaction mixtures were treated with RNase A (2 U/μl) (Boehringer GmbH) at 37°C for 15 min, the samples were heated and loaded onto 10% polyacrylamide–sodium dodecyl sulfate (SDS) gels (acrylamide-bisacrylamide, 29:1) under reducing conditions and electrophoresed at 180 V. The gels were dried and autoradiographed at −70°C. For competition experiments, nuclear or cytoplasmic extracts from HeLa cells were preincubated with the indicated nonradioactive competitors at room temperature for 15 min prior to the addition of radiolabeled probe L.

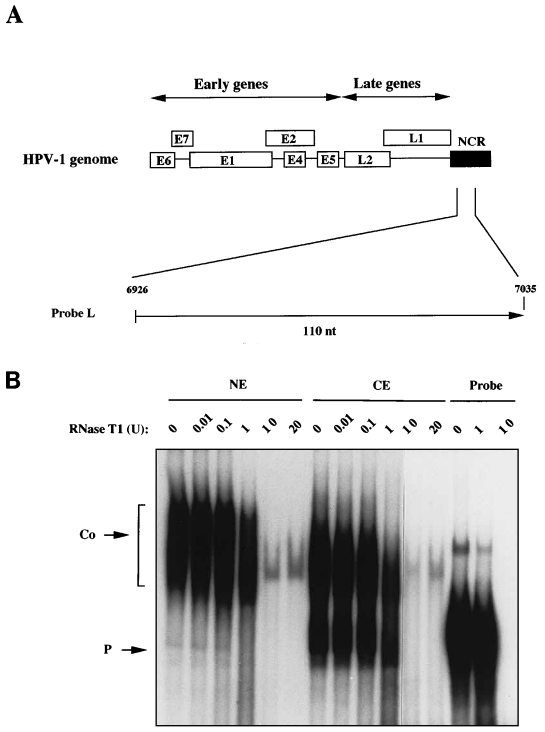


FIG. 1. (A) Schematic representation of the HPV-1 genome. Open boxes represent open reading frames encoding early and late proteins, and the solid box indicates the noncoding region (NCR). Riboprobe L contains the HPV-1 inhibitory RNA sequences (38). Numbers refer to nucleotide positions in HPV-1 genomic clone pHPV-1 (5). (B) The RNA-protein complexes are resistant to RNase T_1 digestion. Nuclear extracts (NE) or cytoplasmic extracts (CE) were incubated with radiolabeled riboprobe RNA L as described in Materials and Methods. The binding mixtures or free probe alone was treated with various amounts of RNase T_1 at 37°C for 15 min and subsequently was analyzed by electrophoresis on nondenaturing 5% polyacrylamide gels. U, RNase T_1 units; Co, RNA-protein complexes; P, radiolabeled free probe.

Cells, transfections, CAT ELISA, and Western blot (immunoblot) analysis. HeLa cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum plus 100 U of penicillin per ml and 100 μg of streptomycin per ml. Transfections were performed by the calcium phosphate coprecipitation technique (14) as previously described (38). Production of CAT protein was quantitated by a CAT antigen capture enzyme-linked immunosorbent assay (ELISA; Boehringer GmbH) according to the manufacturer's instructions. Western blot analysis of the human immunodeficiency virus type 1 $p17^{gag}$ protein was performed as previously described (38).

RESULTS

Specific binding of nuclear and cytoplasmic proteins to an inhibitory RNA sequence in the HPV-1 late 3′ UTR. The 3′ UTR of HPV-1 late mRNA contains sequences that inhibit gene expression in human epithelial cells (38). To investigate whether cellular factors interact specifically with these sequences, RNA-protein interactions were studied by gel retardation analysis as described in Materials and Methods. Since previous deletion analysis localized the major inhibitory element to an AU-rich region between nt 6943 and 7014 (the numbers refer to genomic HPV-1 molecular clone pHPV-1 [5]) on the HPV-1 genome (38), we first cloned an HPV-1 fragment (nt 6926 to 7035) (Fig. 1A) into a pBluescript (Stratagene) vector that was used for in vitro synthesis of riboprobe L (Fig. 1A). To perform RNA-protein binding reactions, radiolabeled RNA probe L was incubated with either nuclear or cytoplasmic extracts from HeLa cells. Retarded bands were observed on the gels after electrophoresis (Fig. 1B), suggesting

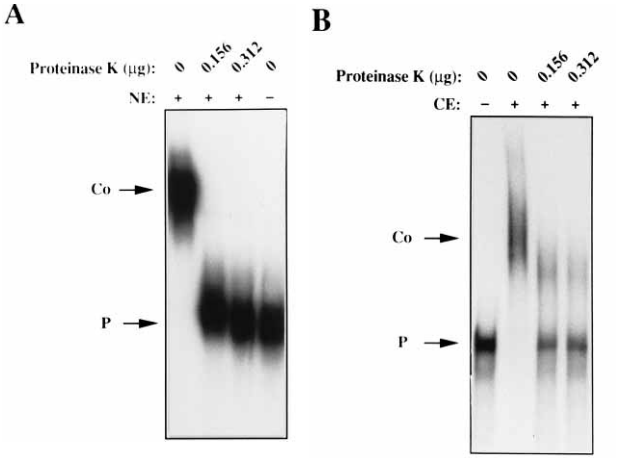


FIG. 2. Proteinase K treatment of nuclear or cytoplasmic extract from HeLa cells prior to the binding reactions prevents the formation of band-shifted complexes. Nuclear (A) or cytoplasmic (B) extracts from HeLa cells were incubated at 37°C for 15 min in the presence of various amounts of proteinase K, and this incubation was followed by the addition of radiolabeled riboprobe L and continued incubation as described in Materials and Methods. The reaction mixtures were analyzed by electrophoresis on nondenaturing 5% polyacrylamide gels. NE, nuclear extract; CE, cytoplasmic extract; Co, RNA-protein complexes; P, radiolabeled free probe.

that factors in the nuclear and cytoplasmic extracts of HeLa cells bound to probe L. The retarded bands obtained from nuclear extracts migrated more slowly than the complexes obtained with cytoplasmic extracts (Fig. 1B), suggesting that factors binding to probe L were more abundant in the nucleus than in the cytoplasm or that complexes formed in the nuclear extracts had compositions different from those formed in the cytoplasmic extracts. Treatment of the complexes with RNase T_1 prior to electrophoresis revealed that factors in the nuclear and cytoplasmic extracts protected a portion of RNA probe L from digestion (Fig. 1B) but that the free probe was completely digested after being treated with the same amounts of RNase T_1 (Fig. 1B). These results indicated that HPV-1 RNA probe L bound tightly to the factors in the HeLa cell extracts. Pretreatment of nuclear or cytoplasmic extracts with proteinase K prior to the binding reaction prevented complex formation (Fig. 2A and B), demonstrating that cellular proteins were present in the complexes.

To test the specificity of the RNA-protein interactions, we performed competition experiments with up to 400-fold molar excesses of unlabeled competitor RNA preincubated with either nuclear or cytoplasmic extracts before the addition of radiolabeled probe L. We found that the formation of RNA-protein complexes could be inhibited by competition with excess unlabeled RNA transcript L (Fig. 3A and B) but that tRNA failed to inhibit complex formation by competition (Fig. 3A and B). These results demonstrated that the binding of nuclear and cytoplasmic proteins to the inhibitory RNA sequence in the HPV-1 late 3′ UTR was specific.

Efficient competition of binding with RNA spanning the U-rich 3′ half of the inhibitory sequence or poly(U) being used as competitor. We next wished to determine if the cellular proteins interacted with the AU-rich 5′ half of probe L or with the U-rich 3′ end of probe L (Fig. 4A). We therefore performed competition experiments with five unlabeled RNA competitors named L, F, B, D, and C (Fig. 4B). The sizes (in nucleotides) and percentages of A and U nucleotides in each RNA competitor are shown in Fig. 4C. Up to 400-fold molar excesses of each competitor were used. With cytoplasmic extracts being used, our results showed that RNA competitor B, which spans the 5′ 59 nt of the probe L sequence, did not compete efficiently for proteins that interact with probe L (Fig. 4D) and that competitors C and F had a higher ability to compete for binding to radiolabeled probe L (Fig. 4D). The ability of RNA competitor D to compete was relatively high but was lower than that of competitor L (Fig. 4D). There was no substantial competition when the antisense RNA strand of D, named RNA DA, was used (Fig. 4D). Since RNA competitor L was the most efficient competitor, we concluded that multiple proteins bind to probe L. Alternatively, maintenance of the capacity of RNA competitor L to bind to cellular proteins may require the integrity of a specific secondary structure retained in full-length RNA competitor L. Similar results were obtained with nuclear extracts: tRNA, RNA B, and RNA DA failed to compete, while RNA L, C, F, and D competed for binding (Fig. 4E). Since RNA C competed for binding while RNA B failed to efficiently compete, most cellular proteins in the complexes apparently interacted with the U-rich 3′ half of RNA probe L.

We next wished to test whether the proteins binding to radiolabeled probe L had affinities for certain nucleotides. We therefore performed competition experiments with poly(A), poly(U), poly(C), and poly(G). These competitors were first preincubated with nuclear extract, and this preincubation was followed by the addition of the radiolabeled probe. The results

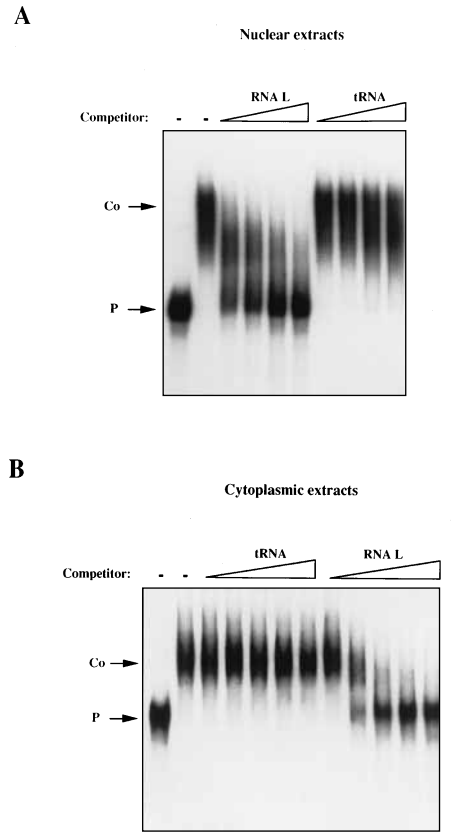
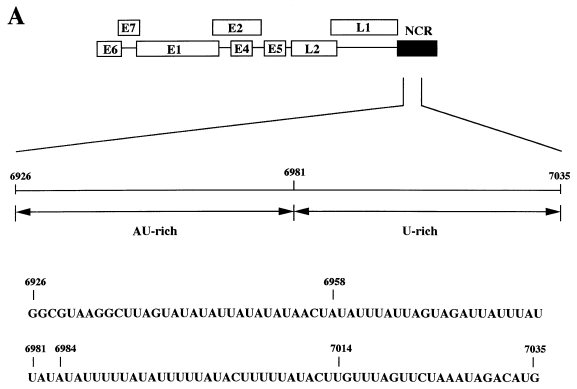
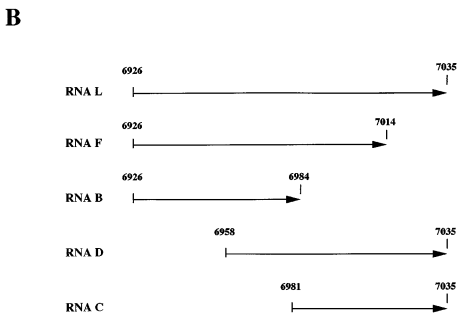


FIG. 3. RNA band shift assays with nuclear (A) or cytoplasmic (B) extract from HeLa cells in the absence (−) or presence of competitor RNAs. Competitions were performed by preincubating nuclear or cytoplasmic extracts with 50-, 100-, 200-, 400-, or 800-fold molar excesses of specific competitor RNA L or nonspecific competitor tRNA prior to the addition of radiolabeled probe L, as described in Materials and Methods. The positions of RNA-protein complexes (Co) and radiolabeled free probe (P) are indicated on the left.

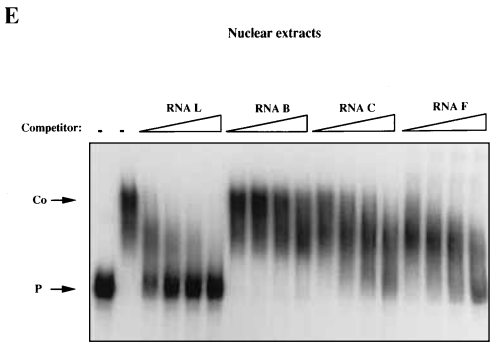


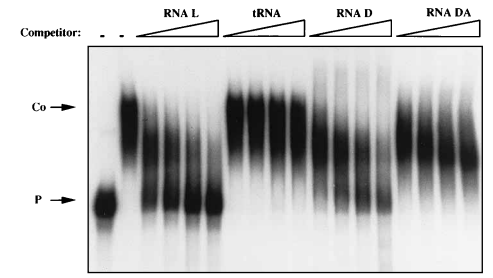


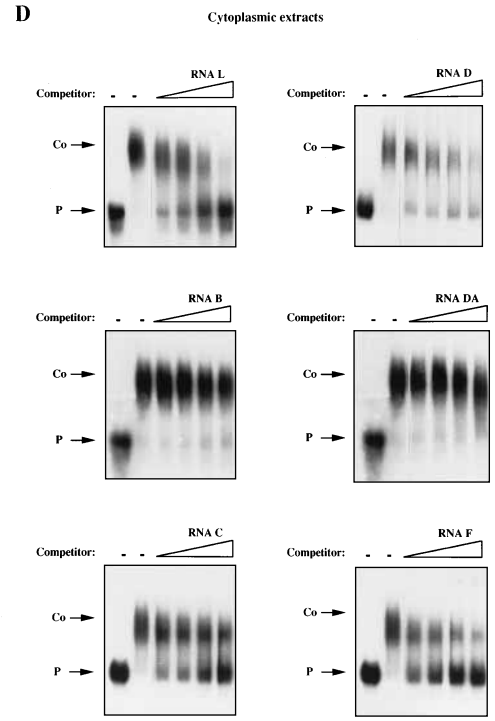
C

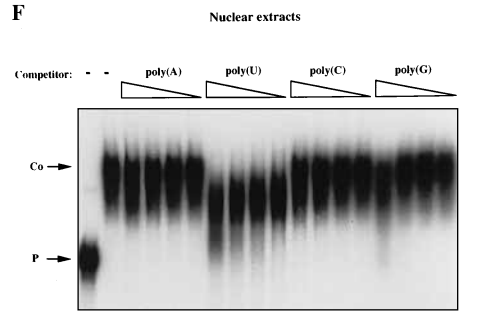
Features of competitor RNAs

RNA	L	F	B	D	C
Size (nt)	110	89	59	78	55
A%	32	31	36	31	29
U%	51	54	46	55	55









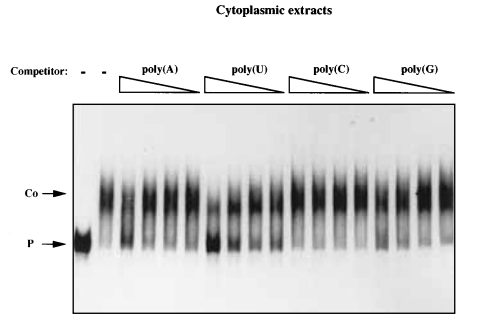


FIG. 4. RNA band shift assays with nuclear or cytoplasmic extracts in the presence of RNA competitors with various lengths. (A) Schematic diagram of the HPV-1 genome, the AU-rich and U-rich regions, and the complete nucleotide sequence of probe L. Numbers refer to nucleotide positions in HPV-1 genomic clone pHPV-1 (5). NCR, noncoding region. (B) Schematic representation of various RNA competitors. (C) Features of different RNA competitors. Sizes (in nucleotides) indicate the lengths of the various RNA competitors. (D) RNA band shift assays with cytoplasmic extracts from HeLa cells and radiolabeled riboprobe L were performed in the absence (−) or presence of 50-, 100-, 200-, or 400-fold molar excesses of various RNA competitors, as indicated. Co, RNA-protein complexes; P, radiolabeled free probe; RNA DA, the antisense transcript of RNA D. (E) RNA band shift assays with nuclear extracts from HeLa cells and radiolabeled riboprobe L were performed in the absence (−) or presence of 50-, 100-, 200-, or 400-fold molar excesses of different RNA competitors, as indicated. Co, RNA-protein complexes; P, radiolabeled free probe; RNA DA, the antisense transcript of RNA D. (F) RNA band shift assays with nuclear or cytoplasmic extracts from HeLa cells and radiolabeled riboprobe L in the absence (−) or presence of decreasing amounts (4, 1, 0.25, and 0.0625 μg) of poly(A), poly(U), poly(C), or poly(G). Co, RNA-protein complexes; P, radiolabeled free probe.

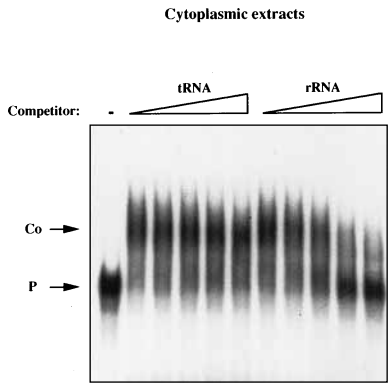


FIG. 5. rRNA competes with HPV-1 RNA for cellular proteins. RNA band shift assays were performed with cytoplasmic extracts from HeLa cells and radiolabeled probe L. Competitions were carried out with increasing amounts (0, 0.1, 0.8, 4, or 8 μg) of each competitor (tRNA or rRNA). Co, RNA-protein complexes; P, radiolabeled free probe.

showed that the complexes formed in the presence of nuclear extracts could be substantially affected by the addition of increasing amounts of poly(U) (Fig. 4F). However, the competition of poly(U) is incomplete, indicating that some but not all proteins in the complex bind to U-rich sequences. Using the same competitors before the incubation of radiolabeled RNA probe L with cytoplasmic extracts (Fig. 4F), we found that the complexes could be efficiently inhibited by the addition of increasing amounts of poly(U). Poly(A) and poly(G) also inhibited the complexes (Fig. 4F), whereas poly(C) did not (Fig. 4F). Taken together, these results implied that nuclear proteins that bind to probe L have a strong binding affinity for U-rich sequences but that the interaction of cytoplasmic proteins with probe L may involve U-rich as well as purine-rich sequences.

We have previously shown that the HPV-1 late 3′ UTR on the mRNA acts in *cis* to reduce utilization of the mRNA (38). It is therefore possible that the HPV-1 RNA sequence may bind to proteins that interact with rRNAs. For this reason, we tested if rRNA could compete for binding factors. The results revealed that the formation of complexes between riboprobe L and cytoplasmic extracts could be partially inhibited by preincubation of the cytoplasmic extract with high amounts of rRNA (Fig. 5) and that competition was not observed with tRNA (Fig. 5). Therefore, at least some of the cytoplasmic proteins that bind to probe L may be associated with ribosomes.

The 3′ UTR of HPV-1 early mRNAs does not contain inhibitory sequences. In order to identify the proteins that bind to the inhibitory 3′ UTR sequence, we wished to use UV crosslinking. Since this sequence is part of the HPV-1 late 3′ UTR, proteins that are involved in 3′ end processing of mRNAs but do not have inhibitory activity may also bind specifically to probe L. For this reason, we wished to compare the UV crosslinking results obtained with the RNA probe L with the results obtained with a 3′ UTR mRNA sequence that lacks inhibitory activity. The best control RNA would be the 3′ UTR of the HPV-1 early mRNAs. To confirm that inhibitory activity is restricted to the HPV-1 late 3′ UTR and is not encoded by the early 3′ UTR, we cloned the entire HPV-1 early 3′ UTR (nt 3798 to 3982) lacking poly(A) signals (5) downstream of the human immunodeficiency virus type 1 p17M1234 reporter gene (Fig. 6A) (34, 38), previously used for identification and characterization of the inhibitory sequences in the HPV-1 late 3′ UTR (38). The HPV-1 early 3′ UTR sequence was cloned downstream of the p17M1234 gene in a sense or an antisense orientation (Fig. 6A), generating plasmids p17H1ES and p17H1EA, respectively. These plasmids were transiently transfected into HeLa cells. Western blot analysis revealed that plasmids p17H1ES and p17H1EA produced similar high levels of $p17^{gag}$ protein (Fig. 6B). The levels were similar to those produced from plasmid pCM1234pA (Fig. 6B), which does not contain HPV-1 sequences. In contrast, plasmid p17H1LS, which contains HPV-1 late 3′ UTR sequences in a sense orientation, produced only low levels of $p17^{gag}$ (Fig. 6B), while p17H1LA, which contains the late 3′ UTR sequence in an antisense orientation, produced high levels of $p17^{gag}$, as was expected (Fig. 6B). The absence of inhibitory sequences in the early 3′ UTR appears to be a conserved property among HPVs, since plasmids p17H16ES and p17H16EA, which contain the HPV-16 early 3′ UTR in a sense and an antisense orientation, respectively, produced similar high levels of $p17^{gag}$ (Fig. 6B). Taken together, these results demonstrated that inhibitory sequences are present in the HPV-1 late 3′ UTR but not in the HPV-1 early 3′ UTR. Therefore, proteins binding to the HPV-1 late 3′ UTR but not to the early 3′ UTR could potentially be mediators of inhibitory activity.

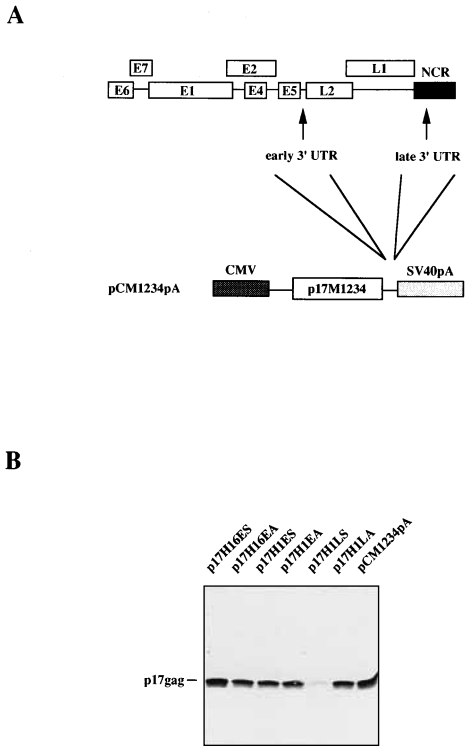


FIG. 6. Effects of HPV early and late 3′ UTR sequences on human immunodeficiency virus type 1 $p17^{gag}$ reporter gene p17M1234 (34) expression. (A) Schematic representation of the HPV genome. Open boxes represent open reading frames encoding early and late proteins, and the solid box indicates the noncoding region (NCR). HPV-1 early or late or HPV-16 early 3′ UTR sequences were cloned in a sense or an antisense orientation downstream of human immunodeficiency virus type 1 $p17^{gag}$ reporter gene p17M1234 (34) in plasmid pCM1234pA (38), as indicated. CMV, cytomegalovirus promoter. (B) Western blot analysis of $p17^{gag}$ production from HeLa cells transfected with the indicated plasmids. 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 a human immunodeficiency virus type 1-positive patient, and this detection was followed by incubation with horseradish peroxidase-conjugated anti-human immunoglobulin G antiserum. $p17^{gag}$ was visualized with the enhanced chemiluminescence detection reagent (Amersham). The position of the $p17^{gag}$ protein is indicated on the left.

Identification of cellular proteins that bind specifically to the inhibitory HPV-1 late 3′ UTR sequence. We next used UV cross-linking to identify cellular proteins that bind specifically to the HPV-1 late 3′ UTR sequence. In vitro-synthesized radiolabeled probe L, which contains the inhibitory RNA sequence in the HPV-1 late 3′ UTR, was incubated with nuclear or cytoplasmic extract. The complexes were irradiated with UV light to mediate covalent cross-linking between RNA and the proteins. After digestion with RNase A, the samples were subjected to 10% polyacrylamide–SDS gel electrophoresis. As can be seen from Fig. 7A, multiple proteins were radiolabeled in both nuclear and cytoplasmic extracts. To investigate the specificity of the binding, we performed UV cross-linking with labeled probe L in the presence of a 200-fold molar excess of three different unlabeled RNA competitors. The first competitor, designated RNA L, contained the same HPV-1 late 3′ UTR sequence as probe L (nt 6926 to 7035) (5). Competitor RNA E contained the entire HPV-1 early 3′ UTR sequence (nt 3798 to 3982) (5). tRNA was used as the third competitor RNA. The experiments revealed that the protein bands formed by the interaction of probe L with nuclear or cytoplasmic extracts could be eliminated by competitor RNA L (Fig. 7A) but not by the same amount of competitor RNA E or tRNA (Fig. 7A), demonstrating specific interaction between the HPV-1 late 3′ UTR RNA and cellular proteins.

Identification of nuclear and cytoplasmic proteins that bind to the inhibitory HPV-1 late 3′ UTR sequence but not to the HPV-1 early 3′ UTR. To identify proteins that bind to the HPV-1 late 3′ UTR sequence in probe L but not to the early 3′ UTR (probe E), we performed UV cross-linking of probe E or L by using nuclear or cytoplasmic extracts. The cross-linked proteins were analyzed in parallel on polyacrylamide-SDS gels. Our results showed that two proteins in the nuclear extract (molecular masses of approximately 52 and 38 kDa) cross-linked to probe L but not to probe E (Fig. 7B). These proteins were undetectable in the cytoplasmic extract (Fig. 7B). Two proteins with molecular masses of approximately 74 and 50 kDa that cross-linked to probe L but not to probe E were detected in the cytoplasmic extracts (Fig. 7B). These proteins were not detected in the nuclear extracts (Fig. 7B). A nuclear protein with an estimated molecular mass of 46 kDa and a cytoplasmic protein with a size of approximately 45 kDa appeared to be present at low levels or cross-linked inefficiently to probe L, but they were consistently cross-linked to probe L but not to probe E.

Although the amounts of cytoplasmic extracts used in the UV cross-linking experiment were fivefold higher than the amounts of nuclear extracts used, the intensities of the signals obtained with cytoplasmic extracts were weaker than the signals obtained with nuclear extracts (Fig. 7A and B). This result suggested that the levels of the RNA-binding proteins were higher in the nuclear extracts than in the cytoplasmic extracts, which is consistent with results obtained with the RNA band shift assay (Fig. 1B and data not shown).

Mapping of the binding sites of the cellular proteins within the inhibitory RNA sequence. To map the protein binding sites within probe L, the five RNA fragments, L, F, B, D, and C (Fig. 4B), were radiolabeled and used as probes in UV cross-linking experiments. The results obtained with nuclear extracts revealed that the 38-kDa protein could be detected with probe L, C, or D and that probe F bound weakly (Fig. 7C). In contrast, probe B did not bind to the 38-kDa protein (Fig. 7C), indicating that the 38-kDa protein binds to the U-rich 3′ half of probe L. The 38-kDa protein could also be detected in the cytoplasmic fraction in the experiment shown here (Fig. 7C). This result is probably due to leakage into the cytoplasm. The 52-

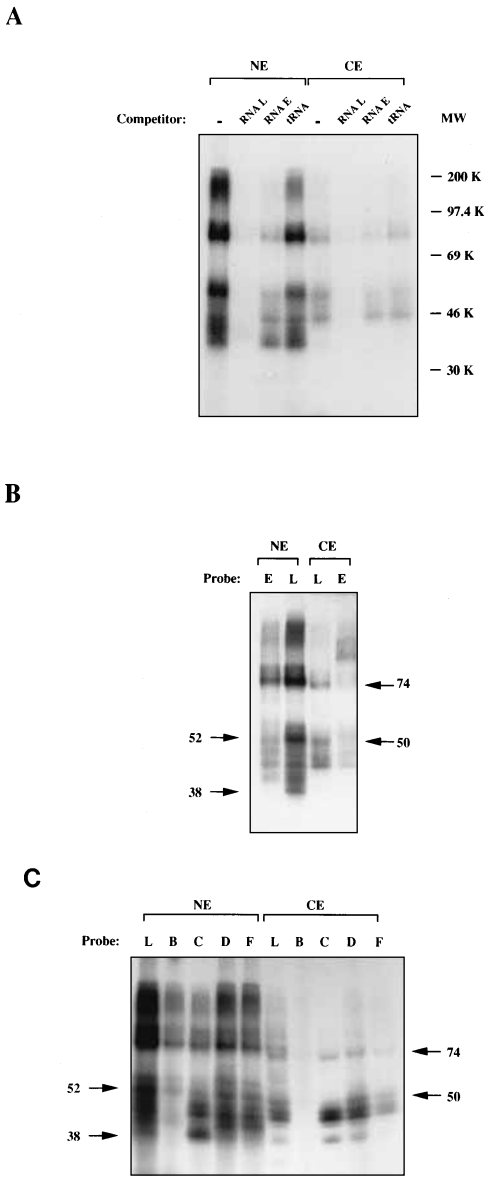


FIG. 7. UV cross-linking of nuclear and cytoplasmic proteins from HeLa cells to radiolabeled RNA L containing the HPV-1 late 3′ UTR inhibitory sequence or to riboprobe RNA E containing the HPV-1 early 3′ UTR sequence. (A) Nuclear (NE) or cytoplasmic (CE) extracts from HeLa cells were preincubated with a 200-fold molar excess of each nonradiolabeled RNA competitor (RNA L, RNA E, or tRNA) or no competitor (−) at room temperature for 15 min prior to the addition of radiolabeled riboprobe L, and this preincubation was followed by UV cross-linking. The reaction mixtures were treated with RNase A and were analyzed on 10% polyacrylamide–SDS gels under reducing conditions. Molecular weight markers (MW) are indicated on the right. (B) Identification of nuclear and cytoplasmic proteins in HeLa cells that bind to RNA L but not to RNA E. The UV cross-linked products were analyzed on 10% polyacrylamide–SDS gels under reducing conditions. NE, nuclear extracts; CE, cytoplasmic extracts; E, radiolabeled RNA E; L, radiolabeled RNA L. The positions of proteins that cross-link to probe L but not to probe E are indicated on the left and right (the numbers indicate sizes in kilodaltons). (C) Mapping of the protein-binding sites on RNAs containing HPV-1 late 3′ UTR sequences. Proteins that were UV cross-linked to the various radiolabeled RNAs were analyzed on 10% polyacrylamide–SDS gels under reducing conditions. Proteins that were UV cross-linked to RNA probe L but not to RNA probe E are indicated. NE, nuclear extract; CE, cytoplasmic extract. L, B, C, D, and F refer to probes RNA L, RNA B, RNA C, RNA D, and RNA F, respectively (Fig. 4B). The positions and sizes (in kilodaltons) of the proteins are indicated at the sides of the gel.

kDa protein bound to probe L, D, or F much more strongly than it did to probes C and B (Fig. 7C), suggesting that the binding site for the 52-kDa protein was located in the middle of probe L. Alternatively, there could be two binding sites for the 52-kDa protein, one in RNA B and the other in RNA C. As probes B and C contained part of the middle sequence of probe L, the decrease in their abilities to bind to the 52-kDa protein may be due to conformational changes in the secondary structure of these RNAs.

The results obtained with cytoplasmic extracts revealed that the 74-kDa protein bound to probes L, C, and D but not to probe B (Fig. 7C). Probe F, which lacks the 3′ end of probe L, bound weakly to the 74-kDa protein. Taken together, these results indicated that the binding site for the 74-kDa protein was at the immediate 3′ end of probe L. The 50-kDa protein bound closer to the middle portion of probe L since it was cross-linked to RNA L, D, and F but not to RNA B or C.

The binding sites of the cellular proteins are required for inhibitory activity. Since the 50- and the 52-kDa proteins bind to the middle portion of RNA probe L while the 38- and 74-kDa proteins bind to the U-rich sequences at the 3′ end of the L RNA, we wished to investigate if both the AU-rich 5′ half and the U-rich 3′ half were required for inhibitory activity. We constructed plasmid pΔBA (Fig. 8A), which lacks the AU-rich sequence present in probe B, and plasmid pΔAX (Fig. 8A), which lacks the U-rich sequence present in probe C. Plasmid pΔKXb (Fig. 8A) lacks both the AU-rich and the U-rich regions and served as a positive control, while plasmid pCCKH1 (Fig. 8A) contains the complete inhibitory sequence. The four plasmids were separately transfected into HeLa cells, and CAT protein levels were determined by CAT capture ELISA. The results revealed that plasmid pΔKXb produced CAT protein levels that were approximately 25 times higher than those produced by pCCKH1 (Fig. 8B), which is a result in agreement with our previous results (38). Interestingly, deletion of either the AU-rich region or the U-rich region resulted in a substantial loss of inhibitory activity (Fig. 8B), demonstrating that both regions were important for inhibition. As a result, the four proteins (38- and 52-kDa nuclear proteins and 50- and 74-kDa cytoplasmic proteins) that bind either to the middle portion of probe L or to its 3′ end may be involved in the inhibition of HPV-1 late gene expression.

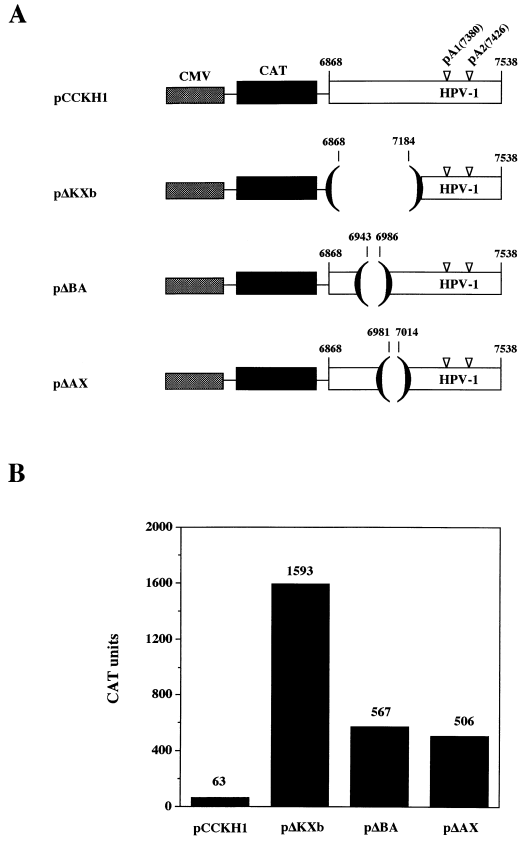


FIG. 8. AU-rich and U-rich sequences in the HPV-1 late 3′ UTR are required for inhibitory activity. (A) Structures of the CAT expression plasmids. Stippled boxes indicate the human cytomegalovirus (CMV) immediate-early promoter, solid boxes indicate the CAT gene, and open boxes indicate HPV-1 late 3′ UTR-containing sequences. The large parentheses mark the limits of the deletions in the HPV-1 late 3′ UTR sequences. Numbers indicate nucleotide positions on the genomic HPV-1 clone (5). Poly(A) signals on the HPV-1 late mRNAs are indicated by triangles (38). The names of the plasmids are shown on the left. (B) CAT protein levels in cell lysates of HeLa cells transfected with the indicated plasmids were monitored with a CAT capture ELISA kit as described in Materials and Methods. The CAT protein levels are measured in CAT units.

DISCUSSION

In a previous study, we reported that the HPV-1 late 3′ UTR contains a negative *cis*-acting regulatory RNA element that acts posttranscriptionally to inhibit gene expression. Here, we present evidence that cellular proteins in HeLa cell extracts interact specifically with the inhibitory HPV-1 RNA sequence. Binding proteins were identified in both the nuclear and the cytoplasmic fractions.

The HPV-1 RNA inhibitory sequence in the late 3′ UTR is rich in A and U nucleotides. Riboprobe L, which we used in the band shift assays or UV cross-linking experiments, contains 110 nt and has an A+U content of 83%. For convenience, probe L can be divided into AU-rich and U-rich regions. The former is present in the 5′ half of riboprobe L, whereas the latter is in the 3′ half. We found at least four cellular proteins (with sizes of 38, 50, 52, and 74 kDa) that bind to riboprobe L, which contains the HPV-1 inhibitory sequence, but not to the early 3′ UTR sequence, which lacks inhibitory activity. The 38- and 52-kDa proteins were present primarily in the nucleus, whereas the 50- and 74-kDa proteins were found in the cytoplasm. Our UV cross-linking experiments showed that the 38-kDa nuclear protein binds to the U-rich region at the 3′ half of probe L, whereas the 52-kDa nuclear protein binds to the middle part of probe L. As for the cytoplasmic proteins, the 74-kDa protein appears to bind to the U-rich sequence at the immediate 3′ end of probe L, whereas the binding site for the 50-kDa protein seems to be close to the middle part of probe L. Our data also revealed that deletion of inhibitory sequences in the HPV-1 late 3′ UTR resulted in approximately 25-fold-higher levels of CAT. Deletion of either the AU-rich region or the U-rich region resulted in a substantial loss of inhibitory activity, indicating that both parts may be important for inhibitory activity. This result suggests that more than one binding factor may be involved in the inhibition of HPV-1 late gene expression in nondifferentiated cells.

Other investigators have described cellular proteins which bind to AU-rich sequences of labile cellular mRNAs or to AUUUA multimers. Some of these proteins are similar in size to the 38-kDa protein identified here. You et al. described a U-rich sequence-binding protein with a size of 37 kDa that interacts with the AU-rich element of the 3′ UTR of c-*fos* mRNAs (42). This binding could be inhibited by competition with poly(U) (42). This result is in agreement with the results obtained by our experiments with the HPV-1 late 3′ UTR and the 38-kDa protein. These investigators appropriately named

the binding factors U-rich sequence-binding proteins (42). Vakalopoulou et al. identified a 32-kDa nuclear protein in HeLa cells that interacts with a number of 3′ UTR sequences in labile mRNAs, including the c-*fos* mRNA (40). It could be found also in the cytoplasmic extract, although it accumulated primarily in the nucleus. Binding of this protein to the 3′ UTR sequence of granulocyte-macrophage colony-stimulating factor (GM-CSF) could be inhibited by competition with poly(U). This outcome is in agreement with the preferred binding to the U-rich region in the HPV-1 late 3′ UTR of the 38-kDa nuclear protein identified in our experiments. A 33-kDa protein with similar characteristics was found in nuclear extracts of 293 cells (1). The cDNA of AUH, a 32-kDa protein with properties similar to those of the 32-kDa protein initially described by Vakalopoulou et al. (40), has been cloned and sequenced (29). The recombinant AUH protein bound specifically to the AU-rich elements of interleukin-3, GM-CSF, and c-*myc* and c-*fos* mRNAs. Brewer identified a cytosolic factor named AUF that consisted of a 37- and a 40-kDa protein (4). Binding of this factor to the AU-rich sequence in the c-*myc* 3′ UTR could be inhibited by competition with poly(A) or poly(U). It was suggested that AUF accelerated degradation of c-*myc* mRNA in a cell-free mRNA decay system. AUF-1 is a 37-kDa protein located in the nuclear and cytoplasmic compartments (43). If AUF-1 is required for mRNA degradation, other factors are apparently required as well, since removal of more than 95% of AUF-1 does not result in increased stability for c-*myc* mRNAs in cell-free decay reactions (43). Bojhanen et al. (3) have described a 34-kDa nuclear protein, AU-A, which is constitutively expressed in T cells. AU-A binds to the 3′ UTR of GM-CSF, interleukin-2, tumor necrosis factor alpha, and c-*myc* mRNA (3). Interestingly, the binding could be inhibited by competition with poly(U). Apparently, AU-A shuttles between the nucleus and the cytoplasm. Shuttling between nuclear and cytoplasmic compartments has been described also for the AU-rich RNA binding activities identified by Müller et al. (27).

A factor named AU-B, with a molecular weight similar to that of AU-A described above, was detected in cytoplasmic extracts after stimulation with anti-CD3 antibody (2). AU-B binds specifically to AU-rich sequences on lymphokine mRNAs and displays kinetics that parallels high levels of lymphokine mRNAs (2). It may be involved in mRNA stabilization. Studies on factors that determine the intracellular levels of β-adrenergic receptor mRNAs identified a prominent 35-kDa protein that binds to β-adrenergic receptor mRNAs which undergo agonist-induced down-regulation (30). Binding of this protein to the AU-rich 3′ UTR of the mRNA could be inhibited by competition with poly(U). The levels of β-adrenergic receptor mRNAs inversely correlated to the abundance of the 35-kDa protein. The authors named this protein βARB and speculated that it may be involved in the stabilization of the receptor mRNA. Gillis and Malter reported on a cellular factor named AUBF that is found in the cytoplasm of T cells and that interacts with 3′ UTR sequences from interleukin-3, GM-CSF, gamma interferon, and c-*fos* and c-*myc* mRNAs (13, 26). The RNase T_1-resistant RNA-protein complex migrated as a 44-kDa band in polyacrylamide-SDS gels under nonreducing conditions. AUBF is located on polysomes, and its removal results in mRNA destabilization by in vitro decay systems (31). It was suggested that AUBF may prevent GM-CSF mRNA decay. In line with these observations, it was observed that production of AUBF was induced in activated T cells. An AUBF-like activity was also identified in preadipocytes treated with tetradecanoyl phorbol acetate, tumor necrosis factor alpha, cyclic AMP, or okadaic acid (36). Treatment of cells with these substances resulted in stabilization of glucose transporter (GLUT-1) mRNA to which the inducible AUBF-like activity binds in vitro. The questions of whether the 38-kDa protein identified in our experiments regulates HPV late gene expression and whether it acts to repress or activate expression remain to be investigated. It will be of interest to test the relatedness of the 38-kDa protein identified in our experiments to the factors identified by others.

Using UV cross-linking, Zhang et al. identified a 75-kDa protein that interacts with 3′ UTR sequences of c-*myc* mRNA as well as c-*fos* mRNA (43). Here, we identified a cytoplasmic 74-kDa protein that interacts with the AU-rich HPV-1 late 3′ UTR sequence but not with the HPV-1 early 3′ UTR. This protein binds to the U-rich region in the 3′ end of the negative element, and its binding may be sensitive to competition with poly(U). Poly(A) could partially inhibit the formation of complexes in cytoplasmic extracts (Fig. 4F), whereas complexes formed by nuclear extracts were not affected by preincubation with poly(A) (Fig. 4F). It is reasonable to assume that the 50-kDa cytoplasmic protein that binds to the AU-rich region in the middle of the inhibitory RNA sequence has affinity for A nucleotides and may be inhibited by competition with poly(A). This protein could be one of the factors contributing to the regulation of the stability of polyadenylated HPV-1 late mRNA (20, 41).

It was previously reported that the HPV-16 early 3′ UTR contains sequences that affect mRNA stability (19), whereas our results showed that the HPV-16 early 3′ UTR did not substantially reduce the expression of the $p17^{gag}$ reporter gene used here. The exact explanation for these differences is not yet known. We have transiently transfected human HeLa cells, whereas stably transfected NIH 3T3 cells were analyzed in the previous study (19). The usage of different reporter genes and assay systems may account for the different observations.

The AU-rich sequences found in the 3′ UTR of many labile cellular mRNAs are involved in the destabilization of their respective mRNAs (33). Some investigators have reported an effect of the 3′ UTR sequence on the translation efficiency of the mRNA (33). Our previous experiments indicated that the inhibitory sequence in the HPV-1 late 3′ UTR acted in *cis* to reduce mRNA levels approximately fourfold (38), suggesting that some of the proteins identified here may act by promoting mRNA degradation. However, we reported that the effect of the inhibitory sequence was greater at the protein level (an approximately 50-fold inhibition) than at the RNA level (an approximately 4-fold reduction) (38). Therefore, the mRNAs which contain the inhibitory sequence and enter the cytoplasm are less efficiently translated compared with mRNAs that lack the inhibitory sequence. The proteins identified here may act by different mechanisms to inhibit gene expression. Alternatively, some proteins may play dual roles in mRNA metabolism. It will be interesting to investigate if the intracellular concentrations of the cross-linked proteins identified here are affected when cells differentiate. We are currently analyzing the presence of these proteins in cell lines of different origins and in differentiated cells.

The AU-rich sequences of certain mRNAs have been implicated in tumorigenesis. It has been proposed that AU-rich viral RNA sequences directly affect cell transformation (12, 28). It was proposed that U RNAs produced by herpesvirus saimiri compete directly with AU-rich elements of cellular proto-oncogene mRNAs for factors that mediate degradation of the cellular proto-oncogene mRNAs. This competition may result in the stabilization of the proto-oncogene mRNAs. HPV infection triggers proliferation of the infected cell (23, 39). How this triggering occurs in HPV-1-infected cells remains unclear. The sequestering of cellular factors that mediate degradation of cellular mRNAs encoding proto-oncogenes and/or growth

factors may therefore contribute to the ability of HPV-1 to induce cell proliferation.

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REFERENCES

1. **Bickel, M., Y. Iwai, D. H. Pluznik, and R. B. Cohen.** 1992. Binding of sequence-specific proteins to the adenosine- plus uridine-rich sequences of the murine granulocyte/macrophage colony-stimulating factor mRNA. Proc. Natl. Acad. Sci. USA **89:**10001–10005.
2. **Bohjanen, P. R., B. Petryniak, C. H. June, C. B. Thompson, and T. Lindsten.** 1991. An inducible cytoplasmic factor (AU-B) binds selectively to AUUUA multimers in the 3′ untranslated region of lymphokine mRNA. Mol. Cell. Biol. **11:**3288–3295.
3. **Bohjanen, P. R., B. Petryniak, C. H. June, C. B. Thompson, and T. Lindsten.** 1992. AU RNA-binding factors differ in their binding specificities and affinities. J. Biol. Chem. **267:**6302–6309.
4. **Brewer, G.** 1991. An A+U-rich element RNA-binding factor regulates c-*myc* mRNA stability in vitro. Mol. Cell. Biol. **11:**2460–2466.
5. **Danos, O., M. Katinka, and M. Yaniv.** 1982. Human papillomavirus 1a complete DNA sequence: a novel type of genome organization among papovaviridae. EMBO J. **1:**231–236.
6. **de Villiers, E.** 1994. Human pathogenic papillomavirus types: an update. Curr. Top. Microbiol. Immunol. **186:**1–12.
7. **Dignam, J. D., R. M. Lebovitz, and R. G. Roeder.** 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. Nucleic Acids Res. **11:**1475–1489.
8. **Eng, A. M., Y.-T. Jin, L. Y. Matsuoka, C. V. Grgurich, J. Robinson, and A. Armin.** 1985. Correlative studies of verruca vulgaris by H & E, PAP immunostaining, and electron microscopy. J. Cutaneous Pathol. **12:**46–54.
9. **Furth, P. A., and C. C. Baker.** 1991. An element in the bovine papillomavirus late 3′ untranslated region reduces polyadenylated cytoplasmic RNA levels. J. Virol. **65:**5806–5812.
10. **Furth, P. A., W.-T. Choe, J. H. Rex, J. C. Byrne, and C. C. Baker.** 1995. Sequences homologous to 5′ splice sites are required for the inhibitory activity of papillomavirus late 3′ untranslated regions. Mol. Cell. Biol. **14:** 5278–5289.
11. **Galloway, D. A., and J. K. McDougall.** 1989. Human papillomaviruses and carcinomas. Adv. Virus Res. **37:**125–170.
12. **Geck, P., M. M. Medveczky, C.-S. Chou, A. Brown, J. Cus, and P. G. Medveczky.** 1994. Herpesvirus saimiri small RNA and interleukin-4 mRNA AUUUA repeats compete for sequence-specific factors including a novel 70K protein. J. Gen. Virol. **75:**2293–2301.
13. **Gillis, P., and J. S. Malter.** 1991. The adenosine-uridine binding factor recognizes the AU-rich elements of cytokine, lymphokine, and oncogene mRNAs. J. Biol. Chem. **266:**3172–3177.
14. **Graham, F. J., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52:**456–460.
15. **Grussendorf, E.-I., and H. zur Hausen.** 1979. Localization of viral DNA-replication in sections of human warts by nucleic acid hybridization with complementary RNA of human papilloma virus type 1. Arch. Dermatol. Res. **264:**55–63.
16. **Howley, P. M.** 1991. Papillomaviridae and their replication, p. 743–768. *In* B. N. Fields and D. M. Knipe (ed.), Fundamental virology. Raven Press, New York.
17. **Howley, P. M.** 1991. Role of the human papillomaviruses in human cancer. Cancer Res. **51:**5019–5022.
18. **Jenson, A. B., J. D. Rosenthal, C. Olson, F. Pass, W. D. Lancaster, and K. Shah.** 1980. Immunologic relatedness of papillomaviruses from different species. JNCI **64:**495–500.
19. **Jeon, S., and P. F. Lambert.** 1995. Integration of human papillomavirus type 16 DNA into the human genome leads to increased stability of E6 and E7 mRNAs: implications for cervical carcinogenesis. Proc. Natl. Acad. Sci. USA **92:**1654–1658.
20. **Keller, W.** 1995. No end yet to messenger RNA 3′ end processing! Cell **81:**829–832.
21. **Kennedy, I. M., J. K. Haddow, and J. B. Clements.** 1990. Analysis of human papillomavirus type 16 late mRNA 3′ processing signals in vitro and in vivo. J. Virol. **64:**1825–1829.
22. **Kennedy, I. M., J. K. Haddow, and J. B. Clements.** 1991. A negative regulatory element in the human papillomavirus type 16 genome acts at the level of late mRNA stability. J. Virol. **65:**2093–2097.
23. **Laimins, L. A.** 1993. The biology of human papillomaviruses: from warts to cancer. Infect. Agents Dis. **2:**74–86.
24. **Lambert, P. F.** 1991. Papillomavirus DNA replication. J. Virol. **65:**3417–3420.
25. **Laurent, R., J. L. Kienzler, O. Croissant, and G. Orth.** 1982. Two anatomoclinical types of warts with plantar localization: specific cytopathogenic effects of papillomavirus. Arch. Dermatol. Res. **274:**101–111.
26. **Malter, J. S.** 1989. Identification of an AUUUA-specific messenger RNA binding protein. Science **246:**664–666.
27. **Müller, W. E. G., H. Slor, K. Pfeifer, P. Hühn, A. Bek, S. Orsulic, H. Ushijima, and H. C. Schröder.** 1992. Association of AUUUA-binding protein with A+U-rich mRNA during nucleo-cytoplasmic transport. J. Mol. Biol. **226:**721–733.
28. **Myer, V. E., S. I. Lee, and J. A. Steitz.** 1992. Viral small nuclear ribonucleoproteins bind a protein implicated in messenger RNA destabilization. Proc. Natl. Acad. Sci. USA **89:**1296–1300.
29. **Nakagawa, J., H. Waldner, S. Meyer-Monard, J. Hofsteenge, P. Jenö, and C. Moroni.** 1995. AUH, a gene encoding an AU-specific RNA binding protein with intrinsic enoyl-CoA hydratase activity. Proc. Natl. Acad. Sci. USA **92:**2051–2055.
30. **Port, J. D., L. Y. Huang, and C. C. Malbon.** 1992. β-Adrenergic agonists that down-regulate receptor mRNA up-regulate a Mr 35,000 protein(s) that selectively binds to β-adrenergic receptor mRNAs. J. Biol. Chem. **267:** 24103–24108.
31. **Rajagopalan, L. E., and J. S. Malter.** 1994. Modulation of granulocyte-macrophage colony-stimulating factor mRNA stability in vitro by the adenosine-uridine binding factor. J. Biol. Chem. **269:**23882–23888.
32. **Roehl, H. H., and B. L. Semler.** 1994. *In vitro* biochemical methods for investigating RNA-protein interactions in picornaviruses. Methods Mol. Genet. **4:**169–182.
33. **Ross, J.** 1995. mRNA stability in mammalian cells. Microbiol. Rev. **59:**423–450.
34. **Schwartz, S., M. Campbell, G. Nasioulas, J. Harrison, B. K. Felber, and G. N. Pavlakis.** 1992. Mutational inactivation of an inhibitory sequence in human immunodeficiency virus type 1 results in Rev-independent *gag* expression. J. Virol. **66:**7176–7182.
35. **Seedorf, K., G. Krämer, M. Dürst, S. Suhai, and W. G. Röwekamp.** 1985. Human papillomavirus type 16 DNA sequence. Virology **145:**181–185.
36. **Stephens, J. M., B. Z. Carter, P. H. Pekala, and J. S. Malter.** 1992. Tumor necrosis factor alpha-induced glucose transporter (GLUT-1) mRNA stabilization in 3T3-L1 preadipocytes. J. Biol. Chem. **267:**8336–8341.
37. **Tan, W., B. K. Felber, A. S. Zolotukhin, G. N. Pavlakis, and S. Schwartz.** 1995. Efficient expression of the human papillomavirus type 16 L1 protein in epithelial cells by using Rev and the Rev-responsive element of human immunodeficiency virus or the *cis*-acting transactivation element of simian retrovirus type 1. J. Virol. **69:**5607–5620.
38. **Tan, W., and S. Schwartz.** 1995. 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. J. Virol. **69:** 2932–2945.
39. **Turek, L. P.** 1994. The structure, function and regulation of papillomaviral genes in infection and cervical cancer. Adv. Virus Res. **44:**305–356.
40. **Vakalopoulou, E., J. Schaack, and T. Shenk.** 1991. A 32-kilodalton protein binds to AU-rich domains in the 3′ untranslated regions of rapidly degraded mRNAs. Mol. Cell. Biol. **11:**3355–3364.
41. **Wahle, E.** 1992. The end of the message: 3′-end processing leading to polyadenylated messenger RNA. Bioessays **14:**113–118.
42. **You, Y., C.-Y. A. Chen, and A.-B. Shyu.** 1992. U-rich sequence-binding proteins (URBPs) interacting with a 20-nucleotide U-rich sequence in the 3′ untranslated region of c-*fos* mRNA may be involved in the first step of c-*fos* mRNA degradation. Mol. Cell. Biol. **12:**2931–2940.
43. **Zhang, W., B. J. Wagner, K. Ehrenman, A. W. Schaefer, C. T. DeMaria, D. Crater, K. DeHaven, L. Long, and G. Brewer.** 1993. Purification, characterization, and cDNA cloning of an AU-rich element RNA-binding protein, AUF1. Mol. Cell. Biol. **13:**7652–7665.