Heterogeneous Nuclear Ribonucleoprotein C Proteins Interact with the Human Papillomavirus Type 16 (HPV16) Early 3′-Untranslated Region and Alleviate Suppression of HPV16 Late L1 mRNA Splicing*

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In order to identify cellular factors that regulate human papillomavirus type 16 (HPV16) gene expression, cervical cancer cells permissible for HPV16 late gene expression were identified and characterized. These cells either contained a novel spliced variant of the L1 mRNAs that bypassed the suppressed HPV16 late, 5′-splice site SD3632; produced elevated levels of RNA-binding proteins SRSF1 (ASF/SF2), SRSF9 (SRp30c), and HuR that are known to regulate HPV16 late gene expression; or were shown by a gene expression array analysis to overexpress the RALYL RNA-binding protein of the heterogeneous nuclear ribonucleoprotein C (hnRNP C) family. Overexpression of RALYL or hnRNP C1 induced HPV16 late gene expression from HPV16 subgenomic plasmids and from episomal forms of the full-length HPV16 genome. This induction was dependent on the HPV16 early untranslated region. Binding of hnRNP C1 to the HPV16 early, untranslated region activated HPV16 late 5′-splice site SD3632 and resulted in production of HPV16 L1 mRNAs. Our results suggested that hnRNP C1 controls HPV16 late gene expression.

Human papillomavirus (HPV)³ is present in 99.7% of all cervical cancers and in many other anogenital cancers as well (1). Of all cancer-associated HPV types, HPV16 is the most common HPV type (2, 3). Although the vast majority of the HPV16 infections are cleared by the immune system, HPV16 may establish persistence and cause cancer (4). These persistent HPV16 infections are characterized by a dysregulated HPV16 gene expression program with continuous expression of the viral oncogenes E6 and E7 and a shutdown of the late genes expressing the highly immunogenic L1 and L2 capsid proteins (5–8). Because aberrant HPV16 gene expression may contribute to cancer progression, it is of interest to determine how HPV16 gene expression is regulated (9). Expression of the HPV16 genes is controlled by at least two promoters named p97 and p670 (10–12), but regulation of alternative splicing and polyadenylation is just as important (9, 13, 14), and HPV16 produces a plethora of alternatively spliced and/or polyadenylated mRNAs (15, 16). The switch from the early to the late gene expression program includes a promoter switch, a poly(A) signal switch, and an activation of late splice signals in a cell differentiation-dependent manner (9).

The early mRNAs produced from the HPV16 genome are polyadenylated at the early poly(A) signal pAE, which is followed by the late region encoding L1 and L2 and the late poly(A) signal pAL (Fig. 1A) (15, 16). Regulation of early polyadenylation is therefore of paramount importance because the activity of the HPV16 pAE controls the levels of both HPV16 early and late gene expression (17–19). The pAE is regulated by cellular factors and by the HPV16 E2 protein, which has been shown to perturb the conformation of the polyadenylation complex, thereby causing a read-through into the late region of the genome (20). Polyadenylation at pAE is dependent on downstream enhancer elements located within the L2 coding region and that interact with CstF-64 and hnRNP H (19, 21). The upstream, early untranslated region also enhances polyadenylation at pAE (17, 18). Efficient splicing to the HPV16 3′-splice SA3358 located upstream of pAE enhances polyadenylation at pAE (22), suggesting that pAE is regulated by the splicing machinery. RNA sequences downstream of SA3358 are essential for utilization of SA3358 and interact with SRSF1 (ASF/SF2), SRSF9 (SRp30c), and SRSF3 (SRp20) (23–27). The early poly(A) signal is competing with HPV16 5′-splice SD3632 that is used exclusively by late mRNAs and is located in between SA3358 and pAE. HPV16 late 5′-splice site SD3632 is used only for production of spliced late L1 mRNAs (15, 16). In undifferen-
entiated cells, SD3632 is suppressed by upstream sequences that encode two AUAGUA motifs and interact specifically with members of the hnRNP D family and hnRNP A2/B1 (28). HPV16 late slice site SD3632 is spliced to late 3′-splice site SA5639 located immediately upstream of the L1 AUG. In mitotic cells, SA5639 is suppressed. Multiple splicing silencers downstream of SA5639 interact with hnRNP A1 (29–31).

Here we have conducted a screen for proteins that are upregulated in cervical cancer cell clones that are permissive for HPV16 late gene expression. We identified one RNA-binding protein named RALYL, which belongs to the hnRNP C family of hnRNPs. We show that both RALYL and hnRNP C1 bind to the HPV16 early UTR and that they can induce HPV16 late L1 mRNA splicing in an HPV16 early 3′-UTR-dependent manner.

Experimental Procedures

Plasmids—The following plasmids have been described previously: pCL086 (32), pBEL (29), pBELDUTr (18), pBELsLuc (28), pBELsLuc (25, 28), pRSVneo (25, 28), pBSpD1MCAT (28), p4xATAGTA (28), p4xMUT (28), and pHPV16AnSL (25, 28). We thank András Nagy (University of Toronto) for providing pCAGGS-nlscre (33).

To generate pBELneo and pTEX4Mneo, a DNA fragment encoding the neo ORF was PCR-amplified from pRSVneo and inserted into pBEL and pTEX4M (24, 25), respectively.

CMV promoter-driven expression plasmids for Myc-tagged RALY (RC210723) and Myc-tagged RALYL (RC206313) were purchased from Origene. To construct RALYL deletion mutant pCRLYL-C, the N terminus was PCR-amplified with primers Sgf1C (5′-GATGACTGACAATATCCAGCTATCTCAATCGGTTATTTGAT-3′) and MluIC (5′-GATGACCGCTTTATCTGTAGAAACAGCTCATGACCCCC-3′) and inserted into Myc-tagged-RALYL plasmid (RC206313, OriGene). pCRLYL-N was constructed by insertion of a restriction site in Myc-tagged-RALYL plasmid (RC206313, OriGene) by site-directed mutagenesis followed by digestion and religation. To generate pChnRNP C1, the hnRNP C1 ORF was PCR-amplified with primers hnrPC1For (5′-GATGACCGCTGACATGGCAGGCAACC-3′) and hnRPc1Rev (5′-GATGACTGACAATATCCAGCTATCTCAATCGGTTATTTGAT-3′) from hps-hnRNP C1 generously provided by Dr. LeStourgeon and inserted into Myc-tagged-RALYL (RC206313) plasmid.

Transfection of HeLa, C33A, C33A2, and 293T Cells—HeLa, C33A, C33A2, and 293T cells were cultured in Dulbecco’s modified Eagle’s medium with 10% heat-inactivated fetal bovine calf serum and penicillin/streptomycin. Transfections were carried out using TurboFect according to the manufacturer’s instructions (Fermentas). Briefly, the mixture of 2 µl of Turbofect and 100 µl of Dulbecco’s modified Eagle’s medium without serum was added to 1 µg of plasmid DNA and incubated at room temperature for 15 min prior to dropwise addition to 60-mm plates with subconfluent HeLa cells. Plasmid pCMVSEAP or pCMV5sLuc was included in the transfection experiments to control for transfection efficiency. Cells were harvested at 24 h posttransfection. Each plasmid was transfected in triplicate in a minimum of two independent experiments. For stable transfections of C33A cells with pBELneo, the transfected cells were split at 24 h posttransfection and seeded into five 100-mm plates. The day after transfer to 100-mm plates, the medium was replaced by neomycin-containing medium (1.2 mg/ml neomycin), and culturing was continued with frequent medium changes until single-cell-derived, neomycin colonies appeared. The colonies were expanded and analyzed as described below.

Propagation and Transfection of Human Primary Keratinocytes—Propagation of neonatal human epidermal keratinocytes has been described previously (28). Briefly, neonatal human epidermal keratinocyte cells were purchased from Gibco and were propagated in EpiLife medium supplemented with human keratinocyte growth supplement (Gibco). For transfection, 150,000 cells were seeded per 60-mm plate and transfected with 1.5 µg of plasmid in Fugene 6. For analysis of episomal HPV16, plasmid pHPV16AnSL was cotransfected with plasmid pCAGGS-nlscre (generously provided by Dr. András Nagy, University of Toronto), which expresses the cre recombinase that releases the HPV16 genome from the plasmid at two flanking lox sites. Each plasmid was transfected in triplicates, in a minimum of two independent experiments. Medium was harvested for analysis of secreted luciferase at different time points after transfection.

Secreted Luciferase Assay—The Metridia longa secreted luciferase activity in the medium of the transfected cells was monitored with the help of the Ready To Glow secreted luciferase reporter assay according to the instructions of the manufacturer (Clontech). Briefly, 50 µl of cell culture medium were added to 5 µl of 0.5× secreted luciferase substrate/reaction buffer in a 96-well plate, and luminescence was monitored in a Tristar LB941 luminometer.

RNA Extraction and RT-PCR—Nuclear and cytoplasmic RNA was extracted 24 h posttransfection, as described previously (28). Total RNA was extracted using an RNasy minikit (Qiagen, Valencia, CA) according to the manufacturer’s protocol. 200 ng of RNA were reverse transcribed in a 25-µl reaction at 42 °C by using Superscript II and random hexamers according to the protocol of the manufacturer (Invitrogen). Two microliters of cDNA were subjected for PCR amplification of cDNA.

E4 mRNAs spliced from SD880 to SA3358 were amplified with primers 757S and 757A (Fig. 1A), and L1 and L1i mRNAs were amplified with primers 757S and L1A (Fig. 1A) (25). For analysis of stably transfected, neomycin-resistant cell clones, L2 mRNAs were amplified with primers L2S (5′-CTTTAGTATCAGGCTCTGATTACCC-3′) and L2R (5′-TACAGATGCTTTAAGATGATC-3′) from pHS-hnRNP C1 generously provided by Dr. LeStourgeon and inserted into Myc-tagged-RALYL (RC206313) plasmid.

Transfection of HeLa, C33A, C33A2, and 293T Cells—HeLa, C33A, C33A2, and 293T cells were cultured in Dulbecco’s modified Eagle’s medium with 10% heat-inactivated fetal bovine calf serum and penicillin/streptomycin. Transfections were carried out using TurboFect according to the manufacturer’s instructions (Fermentas). Briefly, the mixture of 2 µl of Turbofect and 100 µl of Dulbecco’s modified Eagle’s medium without serum was added to 1 µg of plasmid DNA and incubated at room temperature for 15 min prior to dropwise addition to 60-mm plates with subconfluent HeLa cells. Plasmid pCMVSEAP or pCMV5sLuc was included in the transfection experiments to control for transfection efficiency. Cells were harvested at 24 h posttransfection. Each plasmid was transfected in triplicate in a minimum of two independent experiments. For stable transfections of C33A cells with pBELneo, the transfected cells were split at 24 h posttransfection and seeded into five 100-mm
MiniOpticon (Bio-Rad) using the Sso Advanced SYBR Green Supermix (Bio-Rad) according to the manufacturer’s instructions. Primers were those described above, and all cDNA quantitations were normalized to GAPDH mRNA levels. GAPDH cDNA was amplified with primers F-GAPDH (5’-ACCCAGAAGACTGTGGATGG-3’) and R-GAPDH (5’-TTCTAGACAUGCAGGTCAAGT-3’).

**Microarray Analysis**—Total cellular RNA was extracted at two occasions from pBELneo- or pRSVneo-stably transfected cells using the RNeasy minikit (Qiagen). The RNA quality was determined using a Bioanalyzer (Agilent, Santa Clara, CA). RevertAidTM (Fermentas Inc., Glen Burnie, MD) was used for synthesis of first strand cDNA from 1 μg of RNA using random hexamer primers. Each sample was analyzed in triplicates on Human Gene 1.0 ST Arrays covering 36,079 transcripts. Microarray analysis was performed using the Affymetrix expression system at SCIBLU Genomics (Lund University).

Basic Affymetrix chip and experimental quality analysis were performed using the Expression Console Software version 1.12 (Affymetrix, Santa Clara, CA). Robust multiarray analysis was performed for data normalization (34). Statistical analysis was performed using the TMEV version 4.0 software (35). Array results have been submitted to the Gene Expression Omnibus database repository and have received the following ID: GSE65166.

**Protein Extraction and Western Blotting**—Western blotting was performed as described previously (20). Briefly, cells were lysed in RIPA buffer (50 mM Tris, pH 7.8, 300 mM NaCl, 1% sodium deoxycholate, 0.1% SDS, 1% Triton X-100, and protease inhibitors) and subjected to electrophoresis on 12% SDS-polyacrylamide gels. Proteins were transferred onto nitrocellulose membranes, blocked with 5% nonfat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween 20, and stained with the following antibody: anti-hnRNP A1 antibody (sc-10032), anti-hnRNP I antibody (sc-56709), anti-hnRNP C1 antibody (sc-32308), anti-HuR antibody (sc-56709), anti-Srp30c antibody (sc-134036), anti-RALY antibody (ab105866), anti-RALYL antibody (ab66263), anti-SF2 antibody AK96 (Millipore), anti-hnRNP D antibody (Abcam 61193), anti-Myc tag antibody (sc-789), anti-CPSF30 antibody (ab-103142), or anti-actin antibody (sc-134036, 23), and we therefore speculated that rare cells exist within a population that produces high levels of factors that stimulate HPV16 late gene expression. Identification and characterization of such rare cells could identify cellular factors that regulate HPV16 late gene expression at the level of RNA processing. To do so, we used a subgenomic expression plasmid named pBEL that consists of early and late HPV16 genes (Fig. 1A) (29) but expresses only the early genes, primarily the spliced E4 mRNA. Our experimental approach was to stably transfect this plasmid into HPV-negative C33A cells and select cell clones that were permissive for HPV16 late gene expression. Characterization of these clones might lead to the identification of cellular factors that control HPV16 late gene expression. We have shown previously that the late genes from this plasmid can be induced by overexpression of HPV16 E2 (20) but also by cellular proteins PTB (37) and SRSp9/SRSp30c (23), and we therefore speculated that rare cells exist within a population that produces high levels of factors that stimulate HPV16 late gene expression. Identification and characterization of such rare cells could identify cellular factors that control HPV16 late gene expression.

**Results**

**Identification of Cervical Cancer Cells That Are Permissive for HPV16 Late Gene Expression**—Our aim was to identify cellular factors that regulate HPV16 late gene expression at the level of RNA processing. To do so, we used a subgenomic expression plasmid named pBEL that consists of early and late HPV16 genes (Fig. 1A) (29) but expresses only the early genes, primarily the spliced E4 mRNA. Our experimental approach was to stably transfect this plasmid into HPV-negative C33A cells and select cell clones that were permissive for HPV16 late gene expression. Identification and characterization of such rare cells might lead to the identification of cellular factors that control HPV16 late gene expression. We have shown previously that the late genes from this plasmid can be induced by overexpression of HPV16 E2 (20) but also by cellular proteins PTB (37) and SRSp9/SRSp30c (23), and we therefore speculated that rare cells exist within a population that produces high levels of factors that stimulate HPV16 late gene expression. Identification and characterization of such rare cells could identify cellular factors that control HPV16 late gene expression.

**To identify cells that are permissive for HPV16 late gene expression, we first modified the previously described pBEL plasmid to fit our purpose (Fig. 1A) (29, 38). A portion of the HPV16 L1 gene was replaced with the poxvirus internal ribosome entry site (IRES) followed by the G418 resistance gene.
hnRNP C Family Interacts with HPV16 Early UTR

To demonstrate that pTEX4Mneo efficiently expresses HPV16 late genes and that pBELneo does not, these plasmids were transfected pairwise into C33A or HaCaT cells with three different transfection reagents (T, F, and L), followed by selection in G418 and monitoring of neomycin-resistant, single-cell-derived colonies. The results revealed that pTEX4Mneo gave rise to 14–39 times more colonies than pBELneo (Fig. 1B). Note that transfection of HaCaT with transfection reagent T did not yield any colonies with pBELneo (Fig. 1B). The results validated the use of pBELneo for the identification of cell clones that are permissive for HPV16 late gene expression.

Identification of a Novel, Alternatively Spliced L1 mRNA—To identify individual cell clones that were permissive for HPV16 late gene expression, pBELneo was transfected into the HPV-negative, cervical cancer cell line C33A, followed by selection in G418. As a control, C33A cells were also transfected with RSV-neo plasmid and selected in parallel. A number of clones were obtained, expanded, and subjected to RNA extraction and analysis by RT-PCR to monitor levels of early HPV16 E4 mRNAs spliced from SD880 to SA3358 (primers 880S and E4a) and late L1 mRNAs spliced between SD880-SA3358-SD3632-SA5639 or L1i mRNAs spliced from SD880 to SA5639 (primers 880S and L1A) (Fig. 2A). In addition, late L2 mRNAs were amplified with primers L2S and L1A (Fig. 2A). RT-PCR of the HPV16 E4 mRNAs identified HPV16-positive neomycin-resistant clones. Of those, one clone produced all three late mRNAs (L2, L1, and L1i) (clone 1f (Fig. 2B)), three produced L2 and L1i mRNAs (clones 1.2a, 1.2c, and 1.2f) (Fig. 2C) produced L1 mRNAs that differed in size from the L1 mRNA produced by clone 1f. The size of the L1 mRNA produced by clones 1.2a, 1.2c, and 1.2f appeared to be smaller than the L1 mRNA produced by clone 1f, which produced both L1 and L1i mRNAs of correct sizes. We therefore cloned and sequenced the L1 cDNA products of 1.2a, 1.2c, and 1.2f. The results revealed that the smaller “L1” mRNAs produced by clones 1.2a, 1.2c, and 1.2f were alternatively spliced and did not use the suppressed late splice site SD3632.

FIGURE 1. A, schematic representation of the HPV16 genome and subgenomic HPV16 expression plasmids. The early and late viral promoters p97 and p670 and the long control region (LCR) are indicated. Numbers indicate nucleotide positions of 5’ (filled circles) and 3’-splice sites (open circles) or the early and late poly(A) sites pAE and pAL, respectively. The major late mRNAs are indicated (15). A schematic representation of the pBELneo and pTEX4Mneo plasmids is shown. The human CMV, the poliovirus IRES, and the neomycin resistance gene (neo) are indicated. Locations of mutations that inactivate the splicing enhancer downstream of SA3358 and the splicing silencers downstream of SA5639 are shown (24, 29). The structures of the E4 mRNAs produced by pBELneo and the E4 and L1i mRNAs produced by pTEX4Mneo are displayed below the plasmids. B, graphs display the number of neomycin-resistant colonies observed at 14 days posttransfection of pBELneo or pTEX4Mneo into C33A or HaCaT cells following selection in 1 mg/ml G418. Three different transfection reagents were used: Turbofect (T), Fugene 6 (F), and Lipofectamine (L).
This mRNA was called XL1 mRNA and is displayed in Fig. 3A. It was spliced from SD880 to SA3358, as expected, and thereafter a novel 5' splice site SD3519 was used to bypass the strongly suppressed late splice site SD3632 (Fig. 3A), followed by splicing to a novel 3' splice site in the L2 region (SA4980) and further from a novel 5' splice site in L2 (SD5011) to L1 splice site SA5639 (Fig. 3A). The small exon in the L2 region was AU-rich (78% AU) and encompassed 32 nucleotides (Fig. 3A).

This novel HPV16 L1 mRNA termed XL1 has not been detected in HPV16-infected cells to our knowledge, but a late mRNA with highly similar structure has been identified in HPV31-infected cells (Fig. 3A) (15, 16, 39). The alternative splicing pattern of the XL1 mRNA is complex and appears to serve the purpose of bypassing the suppressed late splice site by activating an upstream splice site that splices into another splice site in the L2 coding region: SA4980. In this respect, it is interesting to note that seven nucleotides upstream of SD3519 are identical to the seven nucleotides encompassing the 3' splice site in the L2 coding region (Fig. 3B), suggesting that sequence homology might contribute to pairing of the splice

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**FIGURE 2.** A, schematic representation of the HPV16 subgenomic expression plasmid pBELneo. Numbers indicate the nucleotide positions of 5' (filled circles) and 3' (open circles) or the early and late poly(A) sites pAE and pAL, respectively, and the borders of deletions. B, RT-PCR with primers 880S and L1A (L1 and L1i mRNAs), L2S and L1A (L2 mRNAs), and 880S and E4a (E4 mRNAs) on cDNA of cytoplasmic RNA extracted from the various cell clones indicated at the top of the gels, as well as from the C33A control cells transfected with pRSVneo. C, RT-PCR with primers 880S and L1A (L1 and L1i mRNAs) on cDNA of cytoplasmic RNA extracted from clones indicated at the top of the gel. The alternatively spliced XL1 mRNA is indicated (for structure of the XL1 mRNA, see Fig. 3A). D, ratio of L1 mRNAs over E4 mRNA levels determined by RT-qPCR with primers 880S and L1A or E4a, respectively. RNA from the three cell clones 1c, 1d, and 1.4a was analyzed. E, Western blot analysis of cell extracts from the pBELneo-transfected and G418-selected cell clones indicated at the top of the gels, as well as from the C33A control cells transfected with pRSVneo and selected in G418, with antibodies to SRSF1, SRSF9, hnRNP A1, hnRNP I, or HuR. Error bars, S.D.
A

HPV-16 genome

p97

p670

E1

E2

E4

E5

E6

E7

pAE

pAL

LCR

7372

L2

L1

XL1

ACUUAUUACAUAGAUAAUCUUGCAUAGAAG

SD3519: ACCCCUGCCACACCACUAAGU
SA4980: UUGUAACCACUCCCACUAA
SD3322: CCUUUGCUGGGGAUUGU
SA4369: CCGGCUCUGGUACU

B

HPV16:
SD3519: ACCCCUGCCACACCACUAAGU
SA4980: UUGUAACCACUCCCACUAA

HPV31:
SD3322: CCUUUGCUGGGGAUUGU
SA4369: CCGGCUCUGGUACU

C

anti-HPV16 L1 ab

FIGURE 3. A, schematic representation of the HPV16 genome. The early and late viral promoters p97 and p670 are indicated. Numbers indicate the nucleotide positions of 5′- (filled circles) and 3′-splice sites (open circles) or the early and late poly(A) sites pAE and pAL, respectively. LCR, long control region. HPV16 L2 and L1 mRNAs are indicated, as is the novel, alternatively spliced XL1 mRNA. Note that the E1 AUG followed by part of the E4 ORF is fused in frame with a short sequence from the L2 region (but not in the L2 reading frame) and the entire L1 ORF, creating an mRNA with the potential to produce an E4-L1 fusion protein. An mRNA with similar structure identified in HPV31-infected cells is indicated (mRNA E1^E4*) (15, 16, 39). The E4 and L1 ORFs are not in frame on the HPV31 mRNA. B, sequences immediately upstream of the HPV16 cryptic 5′-splice site SD3519 are shown and aligned with sequences encompassing the downstream cryptic 3′-splice site SA4980. The splice sites are underlined (the first or last dinucleotides of the intron). The seven nucleotides immediately upstream of SD3519 are 100% homologues to seven nucleotides encompassing SA4980. C, Western blot analysis of extracts from cells transfected with cDNA plasmids expressing either the L1 ORF or the XL1 ORF.
hnRNP C Family Interacts with HPV16 Early UTR

TABLE 1
Top 20 up-regulated genes in neomycin-resistant, pBEL-neo transfected C33A cell clone 1.4a

| Gene     | Gene accession | -Fold change | Gene description                              |
|----------|----------------|--------------|-----------------------------------------------|
| 1. DSCR8 | NR_026839      | 9.7          | Down syndrome critical region 8               |
| 2. LGALS3BP| NM_005667      | 5.2          | Lectin, galactose-binding, soluble, 3 binding protein |
| 3. SNSN3 | NM_104666      | 3.7          | Member of the srin family of stress-induced proteins Poly(ADP-ribose) polymerase (PARP) |
| 4. PARP8 | NM_001178055   | 5.0          | Transmembrane glycoprotein of the immunoglobulin superfamily |
| 5. EM9   | NM_198449      | 4.5          | Interferon-induced protein 35                 |
| 6. IDS5  | NM_005533      | 4.4          | Cell adhesion molecule 1                      |
| 7. CADM1 | NM_014333      | 4.1          |                                             |
| 8. C4orf3| NM_173487      | 3.7          |                                             |
| 9. RALYL | NM_173848      | 3.6          | RALY RNA-binding protein-like (RALYL)         |
| 10. HIT1  | NM_001548      | 3.5          | Interferon-induced protein with tetratricopeptide repeats 1 |
| 11. CSorf3| NM_004772      | 3.3          | Neuronal regeneration-related protein (NREP) |
| 12. BEX1 | NM_018476      | 3.1          | Brain-expressed, X-linked 1                   |
| 13. CA8  | NM_004056      | 3.0          | Carbonic anhydrase VIII, lacking activity     |
| 14. TINAG| NM_014464      | 2.9          | Tubulointerstitial nephritis antigen          |
| 15. TLR3 | NM_009265      | 2.8          | Toll-like receptor 3                          |
| 16. PTGIS | NM_000961      | 2.8          | Prostaglandin I2 (prostacyclin) synthase (PTGIS) or CYPA1 |
| 17. LRRC16A| NM_017640      | 2.7          | Binds CAPZA2 decreases CAPZA2 affinity for actin barbed ends. |
| 18. ISL1 | NM_002201      | 2.7          | Member of the LIIM/homeodomain family of transcription factors |
| 19. F2R  | NM_001992      | 2.7          | High affinity receptor for activated thrombin coupled to G proteins |
| 20. LPCAT3| NM_005768      | 2.6          | Atyltransferase                               |

In contrast to the HPV31 mRNA, the HPV16 mRNA splicing event fuses the E4 ORF in frame with the L1 ORF, thereby creating an mRNA with the potential to produce an E4-L1 fusion protein (Fig. 3A), at the same time presumably alleviating the inhibitory effect on L1 translation exerted by the strong E1 ATG. To confirm that the XL1 cDNA can express L1 protein, the XL1 cDNA was recreated and inserted after a CMV promoter. Because the L1 coding region consists acting negative RNA elements, a previously described codon-modified sequence of L1 was used. The results revealed that the XL1 cDNA produced L1 protein levels that were similar to those produced by a codon-modified L1 gene described previously (Fig. 3C). We concluded that the XL1 mRNA was fully capable of expressing the novel L1 protein.

Elevated Expression of the RNA-binding Protein RALYL in Cells Permissive for HPV16 Late Gene Expression—Next we focused our attention on the other neomycin-resistant and HPV16-positive cell clones 1c, 1d, and 1.4a, which produced high levels of the L1i mRNA (Fig. 2B). Because the RT-PCR shown on the gel was saturated, we first quantified E4 and L1 mRNA levels and calculated the L1/E4 ratio. The results revealed that clone 1.4a displayed the highest L1/E4 ratio, suggesting that alleviation of late gene suppression was highest in clone 1.4a (Fig. 3C). We also sought to explain the high levels of L1i mRNA production in these three clones by monitoring levels of proteins that have been shown previously to affect HPV16 late gene expression, including SRSF1 (ASF/SF2), SRSF9, and HuR, whereas levels of hnRNP A1, hnRNP I/PTB, and HuR were not significantly altered in cell clone 1.4a compared with the parental C33A cells, and analysis of SRSF1 and SRSF9 revealed only a minor increase of these two proteins in clone 1.4a compared with C33A cells (Fig. 2E). Clone 1.4a was therefore investigated further.

Duplicate samples of RNA from C33A clone 1.4a were subjected to Human Gene 1.0 ST Array analysis in triplicates and compared with the C33A control cells, which were stably transfected with an RSV-neo plasmid. A list of the 20 most up-regulated genes in clone 1.4a compared with C33A identified one RNA-binding protein named RALY-like protein L (RALYL) (Table 1). RALYL is a member of the hnRNP C family of hnRNP s, but little is known about its function. We therefore investigated the role of RALYL in HPV16 gene expression on RALYL. We confirmed that the RALYL protein was overexpressed in cell clone 1.4a compared with C33A control cells (Fig. 4A). RT-PCR of RALYL mRNA revealed that RALYL mRNA is expressed in C33A cells, as expected, but also in human foreskin keratinocytes (HFKs) and in a previously described, in vitro HPV16-immortalized HFK cell line, C97HFK (Fig. 4B) (25). Immunofluorescence on the C33A cells showed a strong nuclear/nucleolar staining of RALYL (Fig. 4C). These results demonstrated that RALYL is expressed in normal human keratinocytes, in HPV16-immortalized keratinocytes, and in at least one cervical cancer cell line (C33A).

RALYL Induces HPV16 Late Gene Expression—To investigate whether overexpression of RALYL could induce HPV16 late gene expression, a CMV-driven RALYL cDNA was co-transfected with subgenomic HPV16 expression plasmid pBEISLuc (Fig. 5A) (28). The pBEISLuc plasmid encodes the secreted luciferase (sLuc) gene as a marker for HPV16 late genes, and induction of late genes results in production of sLuc in the cell culture medium. Cotransfection of pBEISLuc with serially diluted pCRALYL revealed a RALYL protein dose-dependent induction of sLuc in 239T-, C33A-, HeLa- and HFK cells (Fig. 5, B and C). RALYL also induced late gene expression from a mutant version of pBEISLuc named pBEISMsLuc, in which the splicing silencers downstream of late splice site SA5639 had been mutationally inactivated (Fig. 5, A and D) (29). These results demonstrated that RALYL did not induce HPV16 late gene expression by interfering with the splicing silencer elements in the HPV16 L1 coding region (Fig. 5D). The induction of HPV16 late gene expression required the full RALYL protein, which suggested that RALYL binds to HPV16 mRNAs (Fig. 5E). However, we do not know whether RALYL
binds RNA directly. RNA analysis of C33A cells cotransfected with pBELsLuc and pCRALYL revealed that pBELsLuc produced ~7-fold higher L1 mRNA levels and 3-fold higher L2 mRNA levels in the presence of RALYL (Fig. 5, F and G). We concluded that RALYL induced HPV16 late gene expression predominantly by activating production of spliced L1 mRNA.

**hnRNP C1 Induces HPV16 Late Gene Expression**—To determine whether RALY could induce HPV16 late gene expression also from a full-length HPV16 genome, plasmid pHPV16ANsL (Fig. 6A) (25, 28) was cotransfected with pCRALYL. To create episomal structures of the HPV16 genomes, the HPV16 plasmids were cotransfected with a cre-expressing plasmid that efficiently excises the HPV16 genome at two lox sites flanking the HPV16 genome, as described previously. The results revealed that RALYL induced HPV16 late gene expression from the episomal form of the HPV16 genome cre-loxed from the same plasmid (Fig. 6B). Similar results were obtained in primary HFKs (Fig. 6B). In conclusion, RALYL induced HPV16 late gene expression from both full-length HPV16 genomes and from subgenomic HPV16 expression plasmids.

The cellular hnRNP proteins can be divided into various families, and RALYL belongs to the hnRNP C family, which consists of RALY, RALYL, hnRNP C, and hnRNP CL1 (40). We therefore investigated whether RALY and hnRNP C1 could also activate HPV16 late gene expression. Although RALY could not induce HPV16 late gene expression from pBELsLuc (Fig. 7B), it activated late gene expression from pBELsLuc in a dose-dependent manner (Fig. 7, A and C), suggesting that RALY could overcome suppression of late gene expression only if the inhibitory sequences at late splice site SA5639 were inactivated. These results further supported the idea that the hnRNP C family of proteins induced HPV16 late gene expression independently of the splicing silencers in the L1 coding region. In contrast to RALY, hnRNP C1 induced HPV16 late gene expression from both pBELsLuc and pBELsLuc and to a higher extent than both RALY and RALYL (Fig. 7, B and C). We also transfected pCRALYL and pChnRNPC1 into a previously described reporter cell line, C33A2 (28), which contains pBELsLuc stably integrated into its genome. The results confirmed that both RALY and hnRNP C1 could induce HPV16 late gene expression from pBELsLuc and that hnRNP C1 did so more efficiently than RALY and RALYL (Fig. 7D). RT-PCR revealed that both proteins induced L1 mRNA production (Fig. 7E). Quantitation of the HPV16 mRNA levels by RT-qPCR showed that RALY and hnRNP C1 induced 3.2- and 9.1-fold higher levels of L1 mRNAs, respectively, whereas L2 mRNAs were induced 1.8- and 2.3-fold, respectively (Fig. 7F). In contrast, the early E4 mRNAs were largely unaffected (Fig. 7F). We concluded that RALY and hnRNP C1 induced HPV16 late gene expression primarily by activating HPV16 L1 mRNA splicing.

**Induction of HPV16 Late Gene Expression by RALYL and hnRNP C1 Is Dependent on the HPV16 Early Untranslated Region**—We have previously reported that hnRNP C1 can bind to U-rich regions in HPV1 and HPV16 UTR sequences (18, 41, 42). We therefore investigated whether induction of HPV16 late gene expression was dependent on the HPV16 early UTR. pCRALYL, pCRALYL, and pChnRNPC1 were individually cotransfected into C33A cells with pBEL, which contains the entire early UTR, or with pBELDUTR (18), in which the early UTR had been deleted (Fig. 8A). RT-PCR revealed that E4 mRNA production from both pBEL and pBELDUTR was relatively unaffected by RALYL and hnRNP C1 (Fig. 8B). In contrast, both RALYL and hnRNP C1 induced production of L1 mRNAs from pBEL but had no or very little effect on pBELDUTR (Fig. 8B), indicating that the HPV16 early UTR was required for the induction of HPV16 L1 mRNA production by RALYL and hnRNP C1. The results were confirmed in a transfection experiment with 293T cells (Fig. 8C).

Quantitation of the HPV16 L1 mRNAs by RT-qPCR revealed that L1 mRNA levels were 12 and 14.5 times higher in the presence of RALYL or hnRNP C1, respectively (Fig. 8D). This effect was totally dependent on the HPV16 early UTR, because L1 mRNA levels produced from pBELDUTR were unaffected by RALYL and hnRNP C1 (Fig. 8D). Furthermore, the L2 mRNA levels were also increased in an HPV16 early UTR-dependent manner by the presence of hnRNP C1, but only around 4-fold (Fig. 8D). The levels of L2 mRNAs did not change significantly in the presence of RALYL (Fig. 8D). HPV16 E4 mRNAs were unaffected by hnRNP C1, as expected (Fig. 8D), whereas RALYL appeared to cause a 3-fold increase in E4 mRNAs from both pBEL and pBELDUTR, suggesting a more general effect on mRNA synthesis or stability that was independent of the HPV16 early UTR (Fig. 8D). We concluded that hnRNP C1 and RALYL induced high levels of HPV16 late, spliced L1 mRNAs.

It has recently been suggested that hnRNP C1 promotes nuclear export of cellular mRNAs (43). We therefore investigated whether overexpression of hnRNP C1 altered the nucleocytoplasmic ratio of the HPV16 L1 mRNAs. As can be seen...
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A HPV-16 genome

B pCRALYL (ng)

C pBELsLuc

D pBELMsLuc

E RALYL

F M PCL086 pCRALYL

G L1/L1i mRNA (fold induction)
from the RT-PCR, hnRNP C1 induced high levels of L1 mRNAs both in the nucleus and in the cytoplasm, whereas E4 mRNA levels were unaffected (Fig. 8E). Analysis of unspliced and spliced actin mRNAs confirmed proper cell fractionation (Fig. 8E). Furthermore, the nuclear levels of L1 mRNAs produced from pBELDUUTR were unaffected by hnRNP C1 (Fig. 8F), confirming that the effect of hnRNP C1 on HPV16 late gene expression was dependent on the HPV16 early UTR and extending these observations to include nuclear events. These results demonstrated that hnRNP C1 acted on the HPV16 mRNAs in the nucleus, prior to nuclear export, consistent with an enhancing effect of hnRNP C1 on HPV16 late L1 mRNA splicing and an inhibitory effect on HPV16 early polyadenylation. We speculated that hnRNP C1 induced L1 mRNA splicing by alleviating inhibition of HPV16 late 5′-splice site SD3632, thereby enhancing HPV16 L1 mRNA splicing.

**hnRNP C1 Enhances Splicing from HPV16 Late 5′-Splice Site SD3632**—To test the idea that hnRNP C1 enhances splicing from HPV16 late 5′-splice site SD3632, we transfected hRNP C1 with HPV16 reporter plasmid pBSpD1MCAT (Fig. 9A) (28), which contains only two HPV16 splice sites: late splice sites SD3632 and SA5639. In pBSpD1MCAT, splicing silencers in the L1 coding region at SA5639 were mutationally inactivated (L1M), and late gene expression is suppressed primarily by splicing silencers at SD3632, as reported previously (29).

Enhancement of HPV16 late L1 mRNA splicing from pBSpD1MCAT therefore monitors the activity of HPV16 late 5′-splice site SD3632. Cotransfection of pBSpD1MCAT with hnRNP C1 resulted in a small but significant effect on HPV16 late L1 mRNA production (Fig. 9B), confirming that hnRNP C1 can activate SD3632. We have previously reported that HPV16 late 5′-splice site SD3632 is suppressed by an adjacent splicing silencer located upstream of SD3632 and consisting of two ATAGTA motifs (28). Replacing this sequence upstream of SD3632 in pBSpD1MCAT with four copies of ATAGTA, as in p4xATAGTA, or four copies of ACACGTG, a mutant-inactive sequence, as in p4xMUT (Fig. 9A) (28), revealed a 23-fold difference in L1 mRNA production between the two plasmids (Fig. 9C). Overexpression of hnRNP C1 induced HPV16 L1 mRNA production from p4xATAGTA but not from p4xMUT (Fig. 9D), suggesting either that hnRNP C1 acted on the splicing silencer directly or that suppression of HPV16 SD3632 is required for induction of HPV16 late mRNA splicing by hnRNP C1. RALYL also induced HPV16 L1 mRNA production from p4xATAGTA but not from p4xMUT (Fig. 9E). The effect of RALYL was lower than the effect of hnRNP C1. We concluded that hnRNP C1 and RALYL can induce HPV16 late L1 mRNA production by derepressing the HPV16 late 5′-splice site SD3632.
RALYL and hnRNP C1 Interact with the HPV16 Early Untranslated Region—Because the results suggested that RALYL and hnRNP C1 interacted directly with the HPV16 early UTR to activate mRNA splicing, we performed an RNA CLIP analysis on either pBEL- or pBELDUTR-transfected cells in the absence or presence of RALYL or hnRNP C1. We monitored levels of immunoprecipitated HPV16 E4 mRNAs because they contain the HPV16 early UTR. As can be seen from Fig. 10A, HPV16 E4 mRNA could be immunoprecipitated with antibodies to the Myc tag on the RALYL protein (Fig. 10A), demonstrating that RALY binds to these mRNAs in the living cells. The levels of E4 mRNAs were >100-fold higher than those pulled down by the IgG negative control (Fig. 10B). Furthermore, the anti-Myc tag antibodies failed to immunoprecipitate HPV16 E4 mRNAs from cells transfected with the HPV16 early UTR-less plasmid pBELDUTR (Fig. 10C), strongly
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suggesting that RALYL binds directly to the HPV16 early UTR in the living cells.

Similar experiments were performed with hnRNP C1, with the exception that an antibody to the native hnRNP C1 protein was used that also detected endogenous hnRNP C1. Therefore, the hnRNP C1 antibody pulled down HPV16 E4 mRNAs both in the absence and in the presence of cotransfected hnRNP C1 plasmid, but with different efficiency (Fig. 10D), whereas plain IgG did not (Fig. 10D). However, the HPV16 E4 mRNAs could not be efficiently immunoprecipitated with anti-hnRNP C1 antibody from cells transfected with pChnRNP C1 and pBELDUTR, which lacks the HPV16 early UTR (Fig. 10E). The difference in immunoprecipitation of E4 mRNA was seen only when hnRNP C1 was overexpressed (Fig. 10F), which is in line with the increase in HPV16 late gene expression from pBEL, and not from pBELDUTR, in the presence of exogenous hnRNP C1 (Fig. 8, B–D and F). Quantitation of immunoprecipitated E4 mRNA levels from pBEL and pBELDUTR was determined in the absence of exogenous hnRNP C1. The results revealed that levels of immunoprecipitated E4 mRNAs produced from pBEL plasmid increased by 2.5-fold as a result of hnRNP C1 overexpression, whereas the levels of E4 mRNAs immunoprecipitated...
from pBELDUTR-transfected cells were unaffected (Fig. 10G). These results suggested that the HPV16 early UTR is a major hnRNP C1 binding site on HPV16 mRNAs and further strengthened the conclusion that hnRNP C1 can bind to the HPV16 early UTR and control HPV16 late gene expression.

**hnRNP C1 Binds to the HPV16 Early Untranslated Region in Vitro and Is Present in the Protein Complex on the Splicing Silencer Upstream of SD3632—**

Our results suggested that hnRNP C1 and RALYL bind to the HPV16 early UTR in living cells. To investigate whether RALYL and hnRNP C1 bind to the HPV16 early UTR in vitro, we performed pull-down experiments with biotinylated oligonucleotides representing wild type and mutant HPV16 early UTR sequences (Fig. 11A). The results revealed that WT RNA BRUWT2 could pull down hnRNP C1 and RALYL from a nuclear extract, whereas the mutant RNA (BRUMUT2) was less efficient and pulled down very low levels of hnRNP C1 and RALYL (Fig. 11B). We concluded that hnRNP C1 and RALYL bind to the HPV16 early UTR with sequence specificity. If there is cross-talk between hnRNP C1 and/or RALYL in the HPV16 early UTR protein complex and the splicing silencer complex containing primarily hnRNP D proteins, hnRNP D might be pulled down by the BRUWT2 RNA too. Indeed, low levels of hnRNP D were pulled down by the BRUWT2 RNA but not by the BRUMUT2 RNA (Fig. 11B). Based on these results, one should be able to find hnRNP C1 and RALYL in the protein complex that forms on
the splicing silencer sequence upstream of SD3632. Proteins pulled down by the HPV16 wild type splicing silencer RNA (BRAUA) were also subjected to Western blotting with hnRNP C1 and RALYL antibody (Fig. 11C). Both hnRNP C1 and RALYL were pulled down by the splicing silencer RNA BRAUA (Fig. 11C) and less efficiently by the mutant splicing silencer RNA BRCUC (Fig. 11C). As expected, hnRNP D bound to the WT splicing silencer and not to the mutant (Fig. 11C). Finally, we wished to investigate whether hnRNP C1 binds directly to the HPV16 early UTR RNA. We performed an RNA-protein UV cross-linking experiment with in vitro synthesized, radiolabeled RNA representing the entire early UTR and recombinant His-tagged hnRNP C1. The results demonstrated that hnRNP C1 binds directly to the HPV16 UTR probe (Fig. 11D) and demonstrated that this interaction was specific for the U-rich region of the HPV16 early UTR because preincubation with a cold RNA competitor encompassing the entire UTR competed well with the probe for hnRNP C1, whereas a shorter HPV16 UTR RNA lacking the U-rich 3’-end did not (Fig. 11D). The full UTR competed to the same extent as an artificial RNA containing five copies of and hnRNP C1 binding site AUUUUUA (C1BP) (Fig. 11D). Taken together, these results strongly suggested that hnRNP C derepresses HPV16 late 5’-splice site SD3632 by interfering with the splicing silencer complex upstream of SD362.

Discussion

We have previously reported that the HPV16 late 5’-splice site SD3632 is strongly suppressed in cervical cancer cells (22, 28). These results were further substantiated here. When we selected cervical cancer cells that were permissive for HPV16 late gene expression, many of these cells bypassed SD3632 by activating novel, perhaps cryptic, splice sites to circumvent SD3632. Whether these splicing events are part of the HPV16 gene expression program and take place in vivo remains to be seen. However, utilization of alternative splice sites as well as direct splicing from SD880 to SA5639 to circumvent SD3632 certainly supports the idea that SD3632 is strongly suppressed in cancer cells. The results also suggested that suppression is mediated by factors that are essential for dividing cells or essential to the tumor cell phenotype, because very rarely was SD3632 used in the permissive HPV16 clones. Instead, other splice sites that obviated the need for SD3632 were used. Indeed, the hnRNP D proteins and hnRNP A2/B1 that sup-
press SD3632 are both overexpressed in cervical cancer cells (44), suggesting an important role for these proteins in carcinogenesis.

A number of alternatively spliced HPV16 mRNAs encoding L1 have been found in HPV16-infected cells (15, 16). However, the major L1 mRNA candidate mRNAs expressed from the late promoter p670 are either spliced SD880-SA3358-SD3632-SA5639 (L1 mRNA) or SD880-SA5639 (L1i mRNA), and both contain the E1/E4 ATG upstream of the L1 ATG. Because the E1/E4 ATG matches the Kozak sequence for efficient translation initiation very well (45), it is likely to constitute an obstacle to L1 translation. Indeed, it has been shown to have a negative impact on translation of L1 (46). The novel XL1 mRNA detected here solves that problem by fusing the E1/E4 ATG to the L1 ORF with the E1/E4 ORF, thereby using the strong E1/E4 ATG for L1 production. However, the E1/E4 ORF and the little exon in L2 add 70 amino acids to the N terminus of L1. It remains to be seen whether this protein is produced in vivo and whether it can function as a capsid protein. A similarly spliced mRNA found in HPV31 that does not fuse the E1/E4 ATG to L1 (39), would seem to argue that these splicing events occur to bypass suppressed splice sites rather than translation start codons.

Human cells contain a large number of RNA binding hnRNPs, many of which have been shown previously to interact with HPV mRNAs and/or regulate HPV gene expression, including hnRNP A1 (29–31, 47, 48), hnRNP A2/B1 (28), hnRNP C1/C2 (18, 41, 42), hnRNP D (28), hnRNP E (49), hnRNP H (21), hnRNP I (37), and hnRNP K (49). We have shown that hnRNP C1 binds specifically to the HPV16 early UTR. Because the HPV16 early UTR stimulates polyadenylation at pAE (18) and, as such, prevents HPV16 late gene expression, it was surprising to find that hnRNP C1 and, to a lesser extent, RALYL enhanced HPV16 late gene expression by activating splicing of HPV16 late 5′-splice site SD3632. Binding of hnRNP C1 to the HPV16 early UTR appeared to have less impact on HPV16 early polyadenylation, because the effect of hnRNP C1 on the levels of L2 mRNAs in general was lower than on the spliced L1 mRNAs. A recent publication of individual nucleotide resolution UV cross-linking and immunoprecipitation data on hnRNP C1 revealed that hnRNP C1 can bind to the polypyrimidine tract upstream of 3′-splice sites and to the polyU tract downstream of 5′-splice sites (50). The U-rich HPV16 early UTR may therefore function as a landing pad for hnRNP C1 proteins that control the activity of HPV16 late 5′-splice site SD3632. The role of hnRNP C1 in the regulation of splicing of cellular mRNAs has often been as a repressor of splicing (50) or exon exclusion (51), but hnRNP C1-mediated enhancement of splicing has been suggested as well (52). This is consistent with the activation of HPV16 late 5′-splice site SD3632 reported here. We speculate that this effect of hnRNP C1 RNA binding is dependent on the exact context of the RNA binding site, as recently suggested for hnRNP L (53).

Our results indicate that hnRNP C1 and RALYL can activate splicing from the suppressed HPV16 late 5′-splice site SD3632 by interacting with the HPV16 early UTR. One may therefore speculate that hnRNP C1 and/or RALYL control SD3632 by interacting with the hnRNP D and hnRNP A2/B1 proteins in the splicing silencer complex upstream of SD3632 (28). Indeed, we found that hnRNP C1 was present in this splicing silencer complex, but at low levels. The hnRNP C family of hnRNPs consists of hnRNP C1/C2, hnRNP CL1, RALY, and RALYL (40), and it has been shown that hnRNP A2/B1 and hnRNP D are part of the interactome of RALY (54), suggesting that the hnRNP C1, hnRNP D, and hnRNP A2/B1 proteins are in contact in the cells.

hnRNP C1/C2 is one of the more common RNA-binding proteins in the cell and is known as a strictly nuclear protein (55). Despite its nuclear localization, it is involved in many aspects of RNA processing, ranging from mRNA polyadenylation to splicing, nuclear export, and translation. The subcellular localization of hnRNP C1 can be altered, depending on the conditions of the cell. For example, cells that are stressed or simply mitotic exhibit elevated levels of hnRNP C1 protein in the cytoplasm (56, 57). Thus, hnRNP C1 could also control translation of the cellular amyloid precursor mRNA (58). Effects on translation are also associated with a perturbed nucleocytoplasmic ratio of the hnRNP C1 protein brought about by virus infections. hnRNP C1 has been shown to bind specifically to the 5′- and/or 3′-untranslated regions of various RNA viruses that replicate in the cytoplasm (59–62) and to control viral replication (60). Although the subcellular distribution of hnRNP C1 in uninfected or HPV16-infected human keratinocytes has not been studied, levels of expression of hnRNP C1 have been investigated by immunohistochemistry in low or high grade cervical lesions as well as in cervical cancer biopsies obtained for the purpose of cervical cancer screening (44). The results revealed that hnRNP C1 was overexpressed in the HPV-infected cells and that the levels of hnRNP C1 correlated with the severity of the HPV16-infected lesion (44). These results strongly suggested that the levels of hnRNP C1 increased as a result of the HPV16 infection. In contrast, in low grade HPV16-positive lesions, as in uninfected cervical epithelium, the levels of hnRNP C1 were highest in suprabasal layers and decreased again at the very top of the epithelium (44). Thus, high hnRNP C1 expression correlates with high levels of HPV16 replication, suggesting that hnRNP C1 may contribute to HPV16 late gene expression as viral DNA replication peaks. In conclusion, it appears that many viruses depend on the presence of hnRNP C1 in the infected cell and that many also alter the levels or subcellular distribution of hnRNP C1.

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