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HPV-16 RNA processing

Stefan Schwartz

Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, Uppsala, Sweden

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1. ABSTRACT

To understand human papillomavirus type 16 (HPV-16) gene regulation, it is necessary to understand HPV-16 RNA processing. HPV-16 encodes multiple 5'- and 3'-splice sites and two polyadenylation signals pAE and pAL (Figure 1). The major 3'-splice site on the HPV-16 genome (SA3358) is used for generation of E6, E7, E4, L1 and L2 mRNAs. It encodes a suboptimal splice signal but is under control of a strong enhancer that renders SA3358 one of the most efficiently used splice sites on the HPV-16 genome. Thereby SA3358 indirectly blocks HPV-16 late gene expression. The early polyA signal is also under control of the early UTR sequence and multiple RNA elements in the L2 coding region that interact with hnRNP H. The two splice sites SD3632 and SA5639 are used exclusively by late mRNAs and are under control of multiple splicing silencer elements. The silencers at SA5639 are located in the L1 coding region and interact with hnRNP A1. So far, only polypyrimidine tract binding protein (PTB) has been shown to induce late gene expression.

2. INTRODUCTION

2.1. HPV-infection, HPV genome and viral life cycle

Human papillomaviruses (HPV) are strictly epitheliotropic viruses with tropism for mucosal or cutaneous epithelium that belong to the *Papillomaviridae* family (1-3). HPVs infect the basal cell layer of the squamous epithelium through microwounds that expose the lower cell layers to incoming virus. These infections are normally cleared within 12-18 months but a subset of HPVs termed "high-risk"-types may in rare cases persist for decades in the infected host. Such persistent infections may cause the appearance of premalignant lesions named cervical intraepithelial neoplasia (CIN) that can be graded based on the fraction of the epithelium that is covered by the lesions (2). While CIN1 lesions are mild and are likely to regress, the high-grade lesions termed CIN2 and CIN3 are true risk factors for the progression to cervical cancer (2). Although these are rare events, the number of cervical cancers in the world is high due to the high prevalence of HPVs in the human population (4). HPV-16 is one of the most common sexually transmitted mucosal HPV types. It

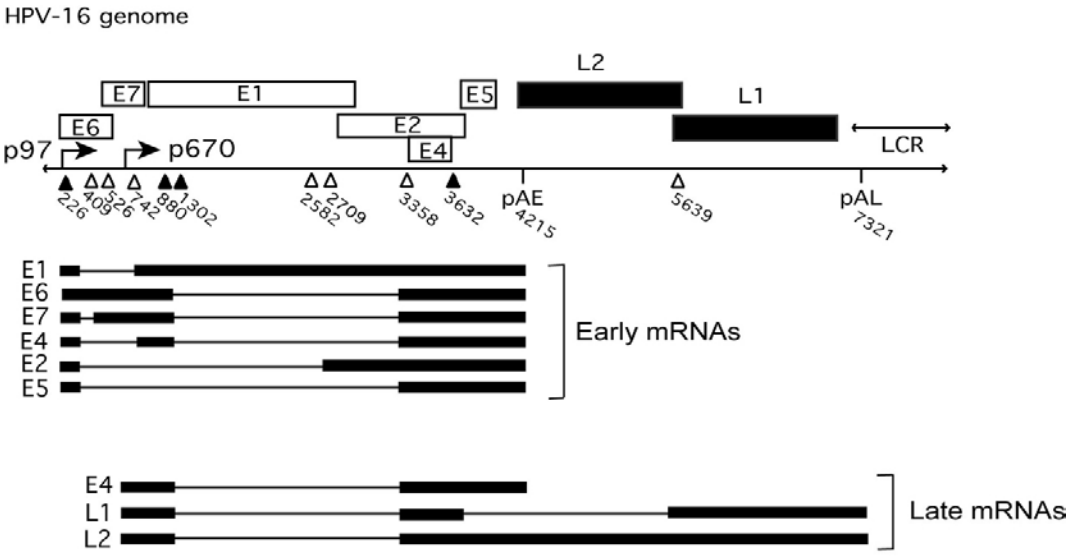

Figure 1. The HPV-16 genome. Early promoter p97 and late promoter p670 are indicated. pAE, early polyA signal and pAL, late polyA signal. Open triangles indicate splice acceptors (3'-splice sites) and filled triangles indicate splice donors (5'-splice sites). Numbers refer to the HPV-16R genome (9). Major mRNA species are shown. Their translation products are indicated to the left.

is also the HPV-type most frequently detected in cervical cancer and it is a prototypic "high-risk" HPV type (5). Persistent HPV-16 infection is a strong risk factor for development of cervical cancer (2).

The HPV-16 virion is simple and consists of a histone-covered, approximately 8-kb double stranded circular genome, with all genes located on the same strand, enclosed in an icosahedral capsid made of viral L1 and L2 protein (1). The genome can be divided into an early region encoding E1, E2, E4-E7 and a late region encoding the two capsid proteins L1 and L2 (Figure 1). One early and one late promoter, and one early and one late polyadenylation signal are also found on the HPV genome. The viral life cycle can be divided into an early stage in which transcription from the early promoter generates mRNAs encoding all early genes that are subsequently polyadenylated at the early polyA signal (pAE) (6, 7). Initially E6 and E7 proteins drive cell proliferation by binding to p53 and pRb (and other factors) and interfering with their functions (8). E1 and E2 interact with each other and replicate the viral DNA genome by binding to viral DNA through E2, and cellular DNA polymerase through E1 (6). High levels of E2 apparently shut down the early promoter concomitantly with activation of the late promoter brought about by terminal cell differentiation as the infected cell reaches the higher layers of the epithelium. The late promoter drives expression of mRNAs encoding E1, E2 and E4 that are polyadenylated at pAE, and L1 and L2 mRNAs that are polyadenylated at pAL (9). This results in the production of progeny virus at the top of the epithelium.

2.2. Regulation of HPV-16 gene expression

The HPV-16 genome contains an early promoter named p97 and a more elusive late promoter termed p670 (10) (Figure 1). Although a switch in use of viral promoters occurs during the viral life-cycle, strict regulation of viral RNA processing is absolutely necessary in order to achieve a temporal expression of the viral genes (11-13). For example, at an early stage of the viral life cycle in which only the early promoter is used, it is of paramount importance that only the early polyA signal and early splice sites are used to avoid expression of the late genes from the early promoter, suggesting that the late polyA (pAL) and the late splice sites (SD3632 and SA5639) are suppressed in dividing cells, whereas early splice sites and polyA signal are highly active. In contrast, when the infected cell differentiates and transcription from the late promoter is induced, splice sites and polyA signal used by late mRNAs must be activated. It is equally important that early splice sites and the early polyA signal allow the late processing signals to compete for splicing factors. HPV-16 RNA processing must be highly flexible and adopt to changes in the differentiating epithelial cell that mark the transit from one stage to another in the cellular differentiation process. Extensive studies of splicing and polyadenylation in bovine papillomavirus (BPV) have shown that splicing occurs in a cell differentiation-dependent manner and multiple splicing regulatory elements have been described (11, 12).

Although the vast majority of all HPV-16 infections are cleared within two years after infection, HPV-16 may persist for decades (14) and give rise to lesions consisting of cells that are driven to proliferate by the continuous expression of HPV-16 E6 and E7 (7). In these cells, the HPV-16 life-cycle is locked at an early stage of the viral replication cycle characterized by continued expression of early genes, while late genes encoding L1 and L2 are suppressed. Cervical cancer cells never express L1 or L2 proteins. It is reasonable to speculate that inhibition of HPV-16 late gene expression is a prerequisite for progression to cancer and that it prevents detection of HPV-16 infected cells by the immune system

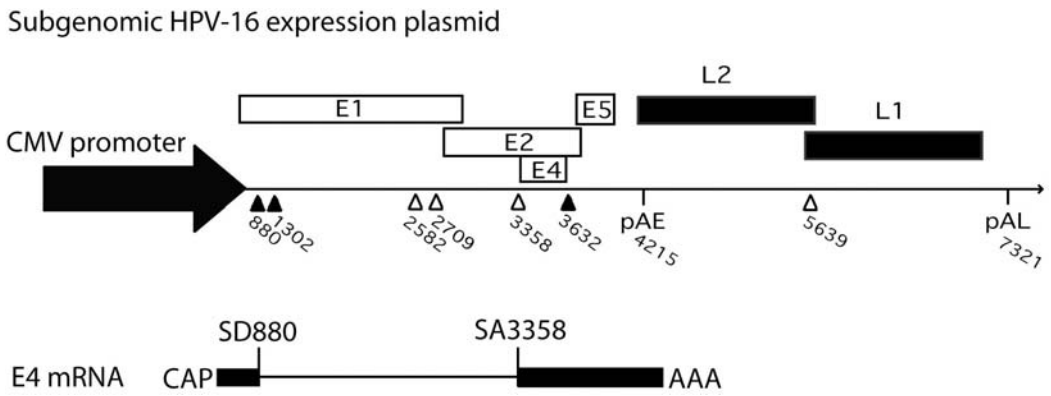

Figure 2. Replacing the HPV-16 late promoter with a strong CMV promoter does not activate HPV-16 late gene expression. The strong human cytomegalovirus immediate early promoter (CMV) has been inserted in place of the late promoter p670 to generate a subgenomic HPV-16 expression plasmid named pBEL (22). pAE, early polyA signal and pAL, late polyA signal. Open triangles indicate splice acceptors (3'-splice sites) and filled triangles indicate splice donors (5'-splice sites). Numbers refer to the HPV-16R genome (9). The major mRNA species produced by plasmid pBEL upon transfection into human cells is the E4 mRNA indicated below plasmid pBEL (22).

of the host. In stark contrast, E6 and E7 expression is a prerequisite for cell proliferation (8), whereas E1 and E2 expression is required (6), at least at low levels, as long as the HPV-16 genome remains as an episome, regardless of grade of lesion or cancer. In some cases the HPV-16 genome integrates in cervical cancer cells, presumably as a result of genetic instability of the cancer cell (15, 16). To summarize, it is clear that the HPV gene expression profile has bearing on cancer progression.

2.3. Early and late splice sites and polyA signals

A number of splice sites have been mapped to the early region of the HPV-16 genome including the 5'-splice sites SD226, SD880 and SD3632 and the 3' splice sites SA426, SA509, SA742, SA2709 and SA3358, whereas only one splice site SA5639 is located in the late region (Figure 1) (17-19). Splicing has also been extensively studied in HPV-31 (20). However, the physical location of each splice site does not necessarily reveal whether they function early or late in the viral life cycle (9). Based on function, the viral splice sites can be divided into those used exclusively by the early mRNAs, such as SD226, SA426, SA509 and SA724, those used by both early and late mRNAs including SD880, SA2709 and SA3358, and those used exclusively by the late mRNAs (SDS3632 and SA5639) (Figure 1). Similarly, the early polyA signal pAE must be used at both early and late stages in the viral life cycle, whereas the late polyA signal pAL, is used exclusively by late mRNAs, during the late productive stage of the infection (Figure 1).

2.4. The utilization efficiency of each splice site determines the levels of the individual viral mRNAs in the infected cells

Although the HPV-16 genome encodes early and late promoters (p97 and p670) that are subject to regulation (10), these promoters do not affect the abundance of individual transcripts, they turn on or off groups of viral mRNAs (Figure 1). To determine exactly how much of a certain mRNA that should be synthesized at a given time in the viral life cycle, the HPV splice sites are strictly regulated. Many splice sites are used in a mutually exclusive manner. The utilization frequency of one splice site can therefore indirectly affect the levels of an alternatively spliced mRNA (12, 13). This was initially shown for BPV and later for HPV-16 (11, 12). Even relatively minor splice sites may play important roles during the viral replication cycle (21). For these reasons, it is of major importance to understand how HPV RNA processing is regulated and how usage of each splice site is controlled.

A simple experiment that high-lights the importance of RNA processing in the HPV-16 gene expression program has been to replace differentiation-inducible late viral promoter p670 with a constitutively active promoter (Figure 2) (22). Replacing the late promoter p670 with the strong, constitutively active human cytomegalovirus (CMV) immediate early promoter did not activate HPV-16 late gene expression (Figure 2) (22). Although additional promoters have been identified in the E4 and E5 regions of HPV-16 and HPV-31 that could potentially express the late genes (19, 20), insertion of the strong CMV promoter in the same region is not sufficient to activate late gene expression either (23, 24). We concluded that posttranscriptional events block HPV-16 late gene expression in mitotic cells and that alleviation of this block is needed for induction of late gene expression in differentiated cells. This strongly supports a model in which late gene expression is regulated at the level of RNA processing (13).

3. REGULATION OF HPV-16 RNA PROCESSING.

3.1. HPV-16 mRNA splicing

3.1.1. Regulation of early splice sites SD226, SA409 and SA526

SD226 is used exclusively by the early mRNAs (Figure 1). One role of SD226 is to splice to SA409 or SA526, also located in the E6 ORF (Figure 1). Removal of

the E6 intron is necessary for generation of mRNAs that can be efficiently translated into E7 (25, 26). The E6 AUG is weak and further weakened by its close proximity to the 5'-end of the mRNA (Figure 1). However, mRNAs on which the E6 ORF is intact are poor producers of E7. Too efficient splicing of the E6 intron would result in insufficient quantities of E6. Partial splicing is therefore a prerequisite for production of correct levels of E6 and E7 proteins. One explanation for the inefficient splicing of the E6 intron may be the suboptimal branch point (27). As continued expression of both E6 and E7 is needed for progression to cancer, identification of the splicing regulatory elements in E6 is of interest.

While some investigators concluded that E7 was translated as a result of leaky scanning at the E6 AUG (28), others have suggested that translation initiation at the E7 AUG occurs by a reinitiation mechanism (29). Remm *et al* showed that ribosomes initiating translation at the E1 AUG, downstream of the E6 and E7 AUGs, belonged to a different pool of ribosomes than those translating the upstream E7 open reading frame, indicating that the E1 AUG was recognized by scanning ribosomes or by a shunting mechanism (30). Translation of downstream ORFs in the HPV genome appears to be affected by splicing in the E6 region. Experiments on HPV-31 demonstrated that the corresponding splice sites in HPV-31, and the E6 intron, were important for production of the E1 protein (31), perhaps suggesting that the HPV-31 splice donor in the E6 ORF, corresponding SD226 in HPV-16, is required for production of E1 mRNAs.

In addition to splicing in E6 being important for expression of downstream ORFs, it appears as if the various splice variants of E6 give rise to E6 proteins with distinct functions. While the full-length E6 protein binds and targets p53 and procaspase 8, for degradation, the short version of E6 produced from the spliced mRNA appears to counteract these two functions of E6 (32, 33). Splicing of the E6 intron may therefore also affect the function of the E6 protein.

3.1.2. Regulation of splice sites SA3358 and SA2709

By far the most efficiently used splice site in the HPV-16 genome is SA3358 (Figure 1 and 2). The E4 mRNAs is one of the most abundant HPV-16 mRNAs produced during an HPV-16 infection (Figure 2). In cells experimentally infected with HPV-31, mRNAs spliced to the corresponding 3'-splice site in HPV-31 (SA3295) are among the most abundant mRNAs and is detected as early as 8 hrs post infection (34). The HPV-16 SA3358 is used by both early and late mRNAs to generate E4 mRNAs (Figure 1). mRNAs using SD226, the newly discovered SA742, SD880 and SA3358 is probably the major E4 mRNA produced in the infected cell (Figure 1) (19, 29, 35, 36). Surprisingly, SA3358 does not conform well to the consensus 3'-splice site (9) and is therefore predicted to be inefficiently recognized by the splicing machinery. A deletion analysis of the E4 region of the HPV-16 genome identified an 85-nucleotide AC-rich splicing enhancer located downstream of SA3358 (Figure 3) (23). Transfection experiments revealed that recognition of SA3358 is totally dependent on this downstream enhancer (23). Interestingly, not only SA3358 relied on the enhancer for function, the early polyA signal pAE was no longer used when SA3358 was bypassed. Alternative 3'-splice sites that could potentially be activated when SA3358 was inactivated are those of E2 (SA2709) and L1 (SA5639) (Figure 1). Of those, SA2709 appears to be an excellent 3'-splice site, but usage of SA2709 does not increase significantly when SA3358 is knocked down (23), suggesting that the SA2709 is under the influence of strong splicing suppressors. A mutation designed to inactivate a 3'-splice site (SA3295) in the HPV-31 genome (37) that corresponds to HPV-16 SA3358, did not affect levels of E4 mRNAs, since the mutation activated a cryptic 3'-splice site, 3-nucleotides downstream of HPV-31 SA3295. This strongly suggests that the E4 splice acceptor in HPV-31 is under control of a strong splicing enhancer, similarly to HPV-16 SA3358. In CONCLUSION, the AC-rich splicing enhancer at SA3358 in HPV-16 not only promotes usage of SA3358, an important 3'-splice site in E6, E7, E4 and L1 mRNAs, it also promotes polyadenylation at pAE, thereby indirectly inhibiting HPV-16 late gene expression (23). At an early stage in the viral life cycle, competition between SA3358 and late splice sites also contributes to inhibition of late gene expression in mitotic cells (23). This was experimentally verified by the demonstration that simultaneous mutational inactivation of the E4 enhancer and the L1 splicing silencers (see below) in a subgenomic HPV-16 clone, resulted in a complete switch from E4 mRNA production only, to L1 mRNA production only (13, 23).

Splicing to SA3358 to generate the E4 mRNA is dependent on SD880 as the E4 ORF obtains start codon and initial codons from the E1 ORF, upstream of SD880 (9). In contrast, inhibition of SD880 is required for production of mRNAs encoding the E1 protein. The importance of the corresponding 5'-splice site in HPV-11 (SD847) was investigated by mutational inactivation of HPV-11 SD847. This resulted in aberrant E1 to E2 protein ratio with effects on viral replication as a consequence (38). The importance of producing correct relative levels of E1 and E2 have been documented (38, 39). This predicts that alterations in the corresponding HPV-16 splice sites may impair HPV-16 replication as well.

Late mRNAs expressed from the late promoter p670 are presumably spliced from SD880 to SA3358 to generate the L1 mRNA (Figure 1). An additional splicing event from SD3632 to SA5639 is required (9). A potential problem with this mRNA is that the L1 mRNA is located downstream of the E4 ORF that contains the E1 AUG. This AUG is strong according to Kozak rules (40) and would not allow translation initiation at downstream AUGs. Indeed, analysis of translation of the corresponding mRNA in HPV-6b, revealed that mutation inactivation of the upstream E1 AUG resulted in a 30-fold increase in translation initiation at the L1 AUG (41). Alternative L1 mRNAs which are initiated from putative promoters located in the E4-E5 region and that do not contain the E1 AUG have been identified in HPV-16 and HPV-31 (19, 20). Such late mRNAs would only use splice sites that are

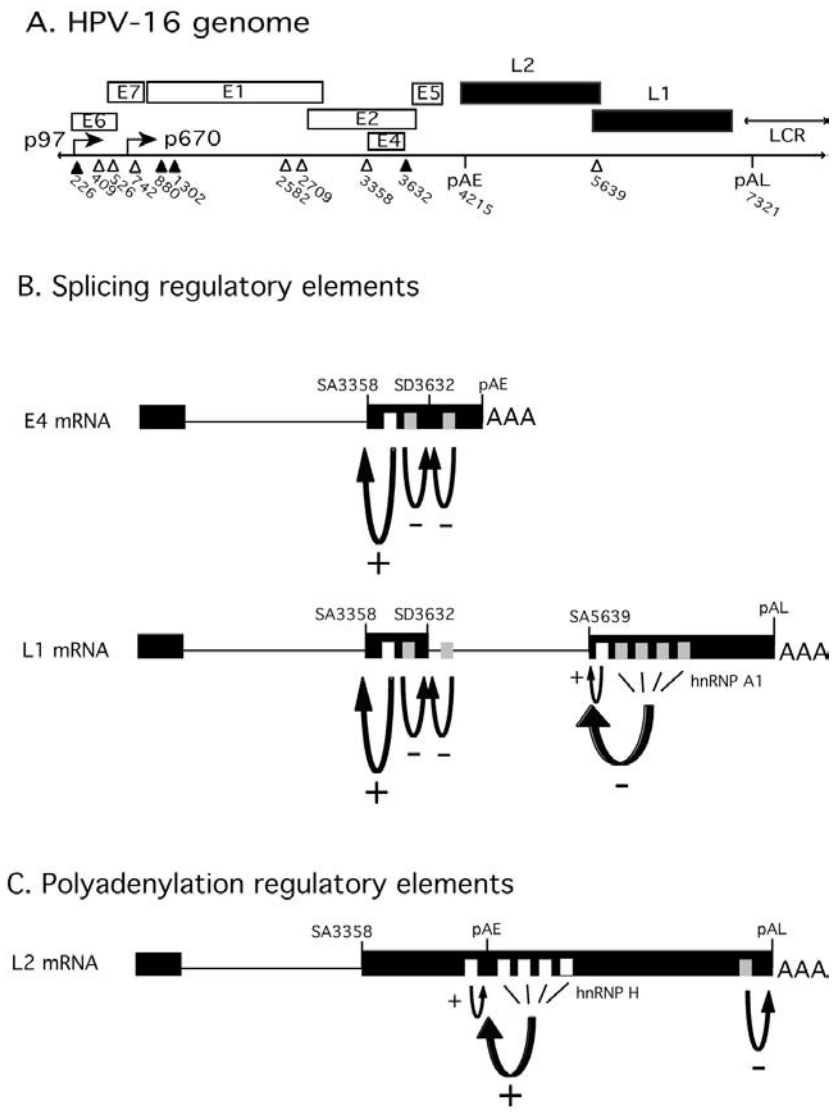

Figure 3. Regulatory elements on HPV-16 mRNAs. A. HPV-16 genome with early and late promoters p97 and p670, respectively, and early and late polyA signals, pAE and pAL, respectively. Open triangles indicate splice acceptors (3'-splice sites) and filled triangles indicate splice donors (5'-splice sites). Numbers refer to the HPV-16R genome (9). B. HPV-16 splicing regulatory elements are displayed as white boxes (splicing enhancers) located at SA3358 (23) and at SA5639 (42) or grey boxes (splicing suppressors) located at SD3632 (23, 24) and SA5639 (22). The splicing silencers in HPV-16 L1 coding region interact with hnRNP A1 (22, 42, 44). C. HPV-16 polyadenylation regulatory elements are displayed as white boxes (polyadenylation stimulatory elements) located in the early UTR (Zhao 2005) and in the L2 coding region (52) or grey box (polyadenylation inhibitor) located in the late UTR (55-57). The polyadenylation elements in HPV-16 L2 coding region interact with hnRNP H (52). Similar elements in HPV-31 have been shown to interact with CstF-64 (50). The HPV-16 late 3'-UTR element interacts with U1snRNP (55-57).

specific for the late mRNAs, i.e SD3632 and SA5639. It would be interesting to determine how efficiently the various spliced late mRNAs are translated into L1 and L2 protein.

3.1.3. Regulation of late splice site SD3632

SD3632 is used exclusively by late mRNAs and is suppressed in mitotic cells. It is located right between the efficiently used SA3358 and the early polyA signal pAE (Figure 1). We have shown that deletions immediately upstream or downstream, of SD3632 activate this splice site, indicating that it is suppressed by adjacent splicing silencers (Figure 3) (23). Similarly, optimizing the SD3632 by increasing complementarity to U1 snRNA enhances splicing (23). However, the enhancing effect of these mutations and deletions are primarily seen when suppression of the downstream splice acceptor SA5639 (see below) is relieved by mutations that destroy splicing silencers located in the L1 coding region (22, 23).

Recent experiments have shown that overexpression of polypyrimidine tract binding protein (PTB) activates HPV-16 late gene expression by interfering with sequences that suppress SD3632 (24). These results suggest that PTB activates HPV-16 late gene expression directly or interferes with factors that regulate SD3632 through splicing silencers. To understand the mechanism behind this effect fully, it is necessary to characterize the splicing elements further and to identify the factors that interact with the splicing suppressor sequences at SD3632.

3.1.4. Regulation of late splice site SA5639

Late 3'-splice site SA5639 is used exclusively by late mRNAs and is tightly regulated by cis-acting elements located in the L1 coding region (Figure 3) (22). The first 17 nucleotides located immediately downstream of SA5639 harbor a splicing enhancer (Figure 3) (42). This sequence is necessary for splicing into SA5639. Although the enhancer is active in dividing cells, it is overridden by multiple splicing silencers located downstream of the enhancer, within the coding region of L1 (42). The multiple splicing silencers were discovered by the introduction of point mutations in L1 that altered the L1 RNA sequence but not the protein sequence (Figure 3) (43). These mutations destroyed the splicing silencers and induced splicing to SA5639 (22), thereby demonstrating that HPV-16 had evolved to contain splicing silencers in the L1 coding region. The splicing silencer sequences were active *in vitro* as well as in transfected cells (22). Multiple splicing silencers were subsequently discovered in L1 and it was shown that hnRNP A1 interacted specifically with these splicing silencers (Figure 3) (42).

Furthermore, L1 cDNAs are infamous for the low levels of mRNA and protein produced when transfected into human cells. Experiments with the mutant L1 cDNAs in which the splicing silencers had been inactivated by multiple point mutations, revealed that they expressed high levels of HPV-16 L1 mRNA and protein in transfected cells (43-45). Immunisation of mice with these genes induced a vigorous immune response towards L1 protein, including humoral and cell-mediated responses (46). In contrast, wild type L1 genes did not induce detectable immune responses against L1 (46). These results demonstrate that splicing silencers binding to hnRNP A1 can inhibit expression from cDNAs, in the absence of splicing (44). Any cDNA that is poorly expressed may contain splicing regulatory sequences in the coding region that interact with splicing factors to regulate splicing when the mRNAs is expressed directly from the gene (47, 48). However, when the same protein-coding region is expressed from a cDNA that is unable to splice, the splicing regulatory factors that bind to the mRNA may be retained on the mRNAs as it is never spliced. Under these circumstances, splicing regulatory elements inhibit gene expression (47, 48). This is presumably a result of nuclear entrapment or premature degradation of "mRNAs" that are regarded as defective since they fail to splice although they encode splicing regulatory elements. In case of the HPV-16 L1 cDNA, we have shown that binding of hnRNP A1 to the multiple splicing silencers in L1 is responsible for inhibiting expression of L1 from L1 cDNAs (44).

3.2. HPV-16 POLYADENYLATION

3.2.1. Regulation of the early polyA site pAE

We have recently shown that mutations that inactivate the early pAE of HPV-16 resulted in read-through into that late region of the HPV-16 genome, confirming the role of HPV-16 pAE as an important regulator of late gene expression (35). However, induction of late gene expression was small and a detailed analysis of the early mRNAs produced from the pAE mutant revealed that a cryptic polyA site at position 3820 was activated by the mutational inactivation of pAE (35). While pAE contains a canonical AAUAAA sequence, the cryptic polyA site did not (35). AU-rich sequences were located within the 50 upstream nucleotides. In spite of this, the cryptic polyA site was used very efficiently, suggesting that strong enhancer elements were located in the vicinity of pAE. These regulatory elements presumably activate the cryptic polyA signal in the absence of pAE (35). Mutational inactivation of the early polyadenylation signal in bovine papillomavirus also resulted in the activation of upstream, cryptic polyadenylation signals (49). Similar results were obtained with HPV-31 (50, 51). In conclusion, the pAE functions as a barrier to late gene expression.

Further analysis of pAE revealed that the early UTR stimulated polyadenylation at pAE, but this effect was relatively weak (Figure 3) (35). The early UTR was shown to interact with the polyadenylation factor Fip-1, which interacts directly with CPSF in the polyadenylation complex, and CstF-64 and PTB (35). The role of these factors in HPV-16 early polyadenylation remains to be determined.

The first 400 nucleotides of the HPV-16 L2 sequence were shown to enhance polyadenylation at pAE substantially (Figure 3) (52). Insertion of a mutant L2 sequence in which the RNA sequence had been mutated without affecting the protein-coding region (45), resulted in high read-through into the late region of the HPV-16 genome (52). This experiment showed that the HPV-16 genome had evolved to contain polyadenylation enhancers that spanned the L2 coding region (Figure 3). Multiple triple-G motifs were present in this region and selected mutations of the triple-G sites increased read-through into the late region. The triple-G motifs interacted specifically with hnRNP H (52). If hnRNP H stimulates polyadenylation at pAE, one would expect hnRNP H levels to decrease in response to cell differentiation. This has been shown to be the case in cervical epithelium (52). However, the polyadenylation element in L2 extends far beyond the hnRNP H binding sites (52), suggesting that secondary structure of the L2 RNA was important for function, or that other regulatory sequences are located in the region. Previous studies of HPV-31 had identified sequences in the L2 coding region that stimulated polyadenylation at HPV-31 pAE (50, 51). These elements also extended several hundred nucleotides into L2, and were shown to interact with CstF-64. Knocking down CstF-64 enhanced polyadenylation at HPV-31 pAE (50), suggesting that CstF-64 may act also on HPV-16. In bovine papillomavirus,

regions of transcriptional termination were identified in the L2 region, indicating that transcripts that extend past pAE in BPV are terminated before they reach the late polyA signal (53).

3.2.2. Regulation of the late polyA site pAL

A late 3'-UTR element in BPV that reduces the levels of late BPV mRNAs in the cytoplasm (54), contains a sequence perfectly matching U1snRNA (55). This sequence was required for the inhibitory effect of the BPV late UTR (55). It was shown to interact with U1snRNA in transfected cells. Similar sequence motifs were found in the HPV-16 late UTR (Figure 3) (55). Subsequent experiments showed that U1-70K protein in the U1snRNP particle binding to the BPV late UTR element interacted with polyA polymerase and inhibited polyadenylation at the late BPV polyA signal (Figure 3) (56). U1snRNPs binding to the U1snRNA binding sites in HPV-16 did not contain detectable levels of U1-70K and may act by a different mechanism (57). A recent publication shows that the HPV-16 late UTR element interacts with CUG binding protein 1 (CUGBP1) and that this interaction mediates the inhibitory effect of the HPV-16 late UTR element in transfected cells (58). It would be interesting to see the expression profile of CUGBP1 in cervical epithelium.

3.3. HPV-16 mRNA stability

3.3.1. RNA instability elements in the HPV-16 L1 and L2 coding regions

We have previously reported that insertion of the HPV-16 L1 and L2 coding regions after a CAT reporter gene reduced mRNA levels produced from these plasmids in transiently transfected cells (43, 59). In contrast, insertion of the L1 or L2 coding sequence in the antisense orientation affect mRNA levels to a much lower extent (43, 59). Furthermore, HPV-16 L1 and L2 sequences reduced mRNA levels to a higher extent than L1 and L2 sequences from the highly productive, low risk HPV type 1 (HPV-1) (59). The existence of inhibitory RNA instability elements in the HPV-16 L1 coding region was confirmed by other investigators using hybrids between HPV-16 L1 and firefly luciferase (60). Generation of HPV-16 L1 and L2 genes in which we had introduced multiple point mutations that altered the L1 or L2 RNA sequence without affecting the protein coding sequence had a dramatic effect on mRNA levels and protein production (43, 59, 61). Interestingly, similar elements were found in canine oral papillomavirus L1 (62), indicating a conserved and important role of these RNA elements in the papillomavirus life cycle. It is not clear if the intragenic L1 and L2 elements regulate RNA stability or if the effect on mRNA half-life is caused by binding of cellular polyadenylation- or splicing- factors to these mRNAs (see above) (22, 44, 52).

3.3.2. RNA instability elements in the HPV-16 early UTR

The early UTR of HPV-16 has an exceptionally high U-content, a hallmark of many unstable mRNAs (63). However, AUUUA-motifs found in many AU-rich RNA instability elements are not present in the early UTR. Nevertheless, Jeon *et al* have reported that the HPV-16 early UTR reduces mRNA stability when inserted in the stable globin mRNA (64, 65). This mRNA was driven by the inducible c-fos promoter, contained a heterologous polyA signal and was expressed in murine cells. In contrast, HPV-1 and HPV-16 early UTRs did not reduce the stability of reporter mRNAs or HPV-1 or HPV-16 early mRNAs expressed from a CMV promoter in HeLa cells (66). Other experiments describe an inhibitory effect on mRNA levels and mRNA translatability when the HPV-16 early region from position 3358 to 4214 was inserted downstream of the GUS reporter gene (67). In this case also splicing regulatory elements were included in the inserted fragment. It remains to be seen if the HPV-16 early UTR has a regulatory role in HPV-16 early gene expression.

3.3.3. RNA instability elements in the HPV-16 late UTR

The HPV-16 late UTR contains a GU-rich negative regulatory element (NRE) that acts as an RNA instability element, at least *in vitro* (68, 69). It interacts with a range of cellular factors including ASF/SF2, CstF-64, U2AF65, hnRNP A1 and HuR (70-73). Inhibitory effects on late mRNA polyadenylation by U1snRNP-binding sites within the negative regulatory element in the late UTR are clear (56). In addition, we have shown that a 55-kDa protein binds specifically to GUUUG-elements in the HPV-16 negative regulatory element (74). This is the same 55-kDa factor that binds specifically to the AUUUA-motifs in the HPV-1 AU-rich RNA instability element in the HPV-1 late UTR (74). This factor remains to be identified.

The late UTR of the cutaneous and benign HPV-1 contains a 57-nucleotide AU-rich RNA instability element named h1ARE (75, 76). This element contains two AUUUA- and three AUUUUUA-motifs which all contribute to premature mRNA degradation (75, 77). The h1ARE element interacts specifically with the same factors as the c-fos AU-rich RNA instability element (66, 78, 79), including HuR and hnRNP C (78, 80). These factors do not bind to mutant h1ARE sequences that do not mediate premature mRNA degradation, suggesting a functional role for these proteins in HPV-1 late gene expression. The role of this element in the viral life cycle has not been determined but one may speculate that it either inhibits late gene expression in mitotic cells or serves as positive regulatory element needed for efficient export of stable L1 and L2 mRNAs at a late stage in the viral life cycle.

Many HPV types contain RNA elements in the late UTR that act by reducing mRNA levels, but not all HPV late UTRs contain AUUUA-motifs (74). However, all HPV late UTRs contain one or more variations of the $PuU_{3-5}Pu$ sequence (74). This suggests that the presence of regulatory elements in the late UTR is conserved property of HPVs although these RNA elements may act by different mechanisms. It would be interesting to determine the role of the negative elements in the late HPV UTRs in the context of the viral genome and in the viral replication cycle.

3.4. HPV-16 mRNA EXPORT

Many HPV-16 mRNAs are incompletely spliced and as such contain unutilized splice sites or splicing

regulatory elements that may retain these mRNAs in the nucleus where they are prematurely degraded. This property is shared by many different viruses that make use of the host cell splicing machinery such as polyomaviruses and retroviruses. The HIV-1 Rev protein binds to the Rev-response element (RRE) RNA element on partially spliced HIV-1 mRNAs and promotes nuclear export of these mRNAs by interacting with CRM1 (81, 82). We have shown that HIV-1 Rev can induce nuclear export of HPV-1 and HPV-16 late mRNAs if the Rev-response element was inserted in these mRNAs. HIV-1 Rev could overcome the inhibitory effect of RNA sequences in the HPV-16 late UTR as well as of RNA elements in HPV-16 L1 coding sequences (45, 61, 76). In addition, the constitutive transport element (CTE) from simian retrovirus type 1 (SRV-1) that interacts directly with the TAP protein to induce nuclear export of partially spliced retroviral mRNAs also stabilizes and induces nuclear export of HPV-1 and HPV-16 late mRNAs (45, 61, 76). It has been observed that HPV-16 L1 mRNAs are retained in the nucleus in undifferentiated epithelial W12 cells (71). Collectively, these results strongly suggest that late HPV-1 and HPV-16 mRNAs are inefficiently exported from the nucleus, at least in mitotic cells, and that nuclear export of the late HPV mRNAs may be induced by a yet unidentified factor. The shuttling HuR protein that binds both HPV-1 and HPV-16 late UTRs (71, 78, 83), and the shuttling HPV-RNA binding proteins hnRNP A1 and ASF/SF2 are candidate export factors (22, 42, 70, 73).

3.5. HPV-16 mRNA TRANSLATION

3.5.1. Inhibition of translation by the HPV-16 late UTR

We initially observed that inhibition of gene expression caused by the late UTR of HPV-1 and HPV-16 displayed a stronger inhibitory effect at the protein level than at the RNA level (61, 75, 76), suggesting that translation was inhibited by the late UTR element. This was later proven by direct transfection into the HeLa cell cytoplasm of capped and polyadenylated, *in vitro* synthesized mRNAs containing the HPV-1 late UTR element (77). While HPV mRNAs with a mutant late UTR were efficiently translated, mRNAs with the wild type late UTR produced very little protein as a result of poor translation (77). The AU-rich RNA element affected translation only of mRNAs with polyA tail and the AU-rich element was shown to bind polyA-binding protein (PABP), suggesting that the AU-rich element inhibited translation by interfering with PABP (77). In general, inhibition of gene expression by the late UTR of multiple HPV types was shown to be higher at the level of protein than at the level of RNA (74), suggesting that the late UTR of other HPV types may inhibit translation as well.

3.5.2. Inhibition of translation by the HPV-16 early region

One publication has presented data indicating that insertion of an HPV-16 early sequence (from 3358 to 4214) downstream of the GUS reporter gene had an inhibitory effect on translation (67). The RNA element that inhibits translation remains to be identified.

3.5.3. Inhibition of translation by the HPV-16 L1 and L2 coding regions

The HPV-16 L1 and L2 genes were first shown to contain inhibitory sequences when these genes were inserted downstream of the CAT reporter gene and were shown to reduce CAT RNA and protein levels upon transfection into HeLa cells. The effect was greater at the protein level than at the RNA level suggesting an inhibitory effect on mRNA translation (59, 61), in addition to the documented effect on RNA stability (43, 59). This was also seen when hybrids between the efficiently expressed equine infectious anemia virus and HPV-16 L1 or L2 genes were constructed and expressed in human cells (43, 45). Cellular factors binding specifically to RNA derived from the 3'-end of the HPV-16 L2 coding region were identified as polyC binding proteins 1 and 2 (PCBP-1 and -2) (also known as hnRNP E1 and E2) and hnRNP K (84). Binding of these factors to the HPV-16 L2 coding region inhibited translation of HPV-16 L2 mRNA *in vitro* (84). Although it has been shown that PCBPs and hnRNP K bind to the 3'-UTR of 15-lipoxygenase mRNAs and inhibit translation of these mRNAs (85), it remains to be investigated if PCBPs and hnRNP K inhibit translation of L2 mRNAs in HPV-16 infected cells.

Codon modification of the BPV or HPV-6b L1 and L2 coding regions resulted in a dramatic increase in production of L1 and L2 protein while an effect on mRNA levels was undetectable (86). These investigators concluded that replacing rare codons used in the BPV or HPV-6b genes with codons that are more commonly used in human genes resulted in late papillomavirus mRNAs that were more efficiently translated in mitotic cells (86). Overexpression of a rare tRNA ($tRNA^{ser\ (CGA)}$) in mammalian cells transfected with wild type bovine papillomavirus L1 expression plasmid resulted in induction of L1 protein production without significantly affecting steady state L1 mRNA levels (86). These investigators also speculated that the relative levels of tRNAs change with differentiation and that papillomavirus codon usage of the late genes reflects the conditions in terminally differentiated cells. However, also early papillomavirus mRNAs contain rare codons and replacing rare codons in HPV-16 E5 (87), E6 (88) and E7 (89, 90) with more frequently used codons enhanced expression also of the early genes in transfected cells as well as in DNA immunized animals. The presence of rare codons may be a general property of all genes on HPV genomes. It would be interesting to investigate if there are differences in composition in RNA/protein complexes formed on wild type and "codon optimized" BPV and HPV-6b L1 or L2 sequences. RNA binding proteins specifically interacting with the wild type L1 or L2 mRNAs may inhibit translation of these mRNAs in transfected cells (44).

4. PERSPECTIVE

In order to understand how expression of the HPV-16 genes is regulated, it is necessary to understand how processing of the viral mRNAs is regulated. As the expression levels of the various HPV-16 genes change during the viral life cycle and in response to a

differentiating environment, each HPV-16 RNA splice site and polyadenylation signal is subject to regulation. They are regulated by cis-acting RNA elements and trans-acting cellular factors. In addition, stability and efficiency of nuclear export of the viral mRNAs will affect the final levels of each mRNA species. The utilization efficiency of each RNA processing signal will affect the final concentrations of the viral proteins made in the infected cell. A detailed knowledge of the various HPV-16 RNA processing signals will increase our understanding of HPV-16 infection and the viral life-cycle, and may uncover novel targets for antiviral therapy.

The most frequently used 3'-splice site of the HPV-16 genome, SA3358, is totally dependent on a downstream AC-rich enhancer (23) (Figure 3). It is used by E4, E6, E7, L1 and L2 mRNAs. This enhancer is therefore one of the major regulatory elements on the HPV-16 genome, but the factors interacting with the RNA elements remain to be identified. In addition, identification of the splicing regulatory elements that determine the ratio of the E6 and E7 mRNAs in infected cells, in particular in cancer cells, may lay the ground for therapy against cervical cancer.

Regarding late gene expression, we have learnt that late splice sites SD3632 and SA5639 are under control of strong splicing silencers that suppress these sites in mitotic cells (23) (Figure 3). Although the silencers at SA5639 bind to hnRNP A1 (23), a better understanding of the splicing suppressors at SD3632 (23) and identification of the factors they interact with is desirable. The PTB protein counteracts these elements and induces late gene expression, but the identity of the inhibitory factors is unknown (23). The mysterious L2 mRNA deserves special attention in that it should be inefficiently exported, since it is partially spliced, and it should be inefficiently translated as the L2 ORF is never first on any of the cloned and sequenced L2-encoding mRNAs. Alternative ways of RNA export and translation may operate on the L2 mRNA and studies on L2 expression may be informative.

Little is known of how the partially spliced E1 mRNA is actually escaping the nuclear control that targets incompletely spliced mRNAs for premature degradation. Especially the control of nuclear export of E1 mRNA would be of interest. The ratio between E1 and E2 is of major importance since alterations in this ratio immediately has a bearing on viral DNA replication (38, 39). Efficient use of 5'-splice site SD880 prevents production of E1 and favors E4 production. The 3'-splice site upstream of E2 has a long polypyrimidine tract and should be recognized quite well by the splicing machinery, but is not. E2 regulates viral transcription and replication, induces apoptosis and may act as the master switch that promotes entry into the late phase of the viral life cycle. E2 expression must therefore be well controlled and the E2 3'-splice site is likely to be under control of strong splicing silencers.

Studies of the role of the regulatory RNA elements in the viral life-cycle would be very interesting. These experiments would determine how important each splicing- or polyadenylation-element or trans-factor is for completion of the replication cycle of the papillomavirus.

Finally, an area of research that will expand in the future is that on the role of microRNAs in HPV gene regulation, as well as in viral pathogenesis, as microRNAs will undoubtedly be involved in many aspects of the viral life cycle (91, 92).

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6. REFERENCES

1. Howley, P. M. & D. R. Lowy: Papillomaviridae and their replication. In: *Virology*. Eds: B. N. Fields, D. M. 2.Knipe&P. M. Howley. Lippincott/The Williams & Wilkins Co. Philadelphia, Pa, (2001)
2. zur Hausen, H.: Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer*, 2, 342-350 (2002)
3. de Villiers, E. M., C. Fauquet, T. R. Broker, H. U. Bernard & H. zur Hausen: Classification of papillomaviruses. *Virology*, 324, 17-27 (2004)
4. de Sanjosé, S., M. Diaz, X. Castellsagué, G. Clifford, L. Bruni, N. Muñoz & F. X. Bosch: Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect. Dis.,* 7, 453-459 (2007)
5.Munoz, N., F. X. Bosch, S. de Sanjose, R. Herrero, X. Castellsague, K. V. Shah, P. J. Snijders & C. J. Meijer: Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N Engl J Med*, 348, 518-27 (2003)
6. Hebner, C. M. & L. A. Laimins: Human papillomaviruses: basic mechanisms of pathogenesis and oncogenicity. *Rev Med Virol,* 16, 83-97 (2006)
7. Doorbar, J.: The papillomavirus life cycle. *J Clin Virol*, 32 Suppl 1, S7-15 (2005)
8. Münger, K. & P. Howley: Human papillomavirus immortalization and transformation functions. *Virus Res*, 89, 213-228 (2002)
9. Baker, C. & C. Calef: Maps of papillomavirus mRNA transcripts. In: *Human papillomaviruses: A compilation and analysis of nucleic acid and amino acid sequences*. Eds: S. R. Billakanti, C. E. Calef, A. D. Farmer, A. L. Halpern&G. L. Myers. Theoretical biology and biophysics, Los Alamos National Laboratory, Los Almos (1997)
10. Norrild, B., C. H. Hansen & J. A. Glahder: Identification and activity of promoters in the HPV genome. In: Human papillomavirus gene regulation and transformation. Ed: B. Norrild. *Transworld Research Network*, (2007)
11. Baker, C. C.: Posttranscriptional regulation of papillomavirus gene expression. In: *Human papillomaviruses: a compilation and analysis of nucleic acid and amino acid sequences.* Eds: S. R. Billakanti, C. E. Calef, A. D. Farmer, A. L. Halpern&G. L. Myers.

Theoretical biology and biophysics, Los Alamos national laboratory, Los Alamos (1997)
12. Zheng, Z. M. & C. C. Baker: Papillomavirus genome structure, expression, and posttranscriptional regulation. *Front. Biosci*., 11, 2286-2302 (2006)
13. Schwartz, S., M. Somberg, X. Zhao, M. Rush, D. Öberg, H. Cassidy, J. Fay & H. Lambkin: Regulation of HPV gene splicing and expression. In: Human papillomavirus gene regulation and transformation. Ed: B. Norrild. *Transworld Research Network*, (2007)
14. Schiffman, M. & S. K. Kjaer: Chapter 2: Natural history of anogenital human papillomavirus infection and neoplasia. *J Natl Cancer Inst Monogr*14-9 (2003)
15. Pett, M. & N. Coleman: Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? *J. Pathol.,* 212, 356-367 (2007)
16. Duensing, S. & K. Münger: Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. *Int. J. Cancer*, 109, 317-326 (2004)
17. Doorbar, J., A. Parton, K. Hartley, L. Banks, T. Crook, M. Stanley & L. Crawford: Detection of novel splicing patterns in a HPV16-containing keratinocyte cell line. *Virology*, 178, 254-262 (1990)
18. Grassmann, K., B. Rapp, H. Maschek, K. U. Petry & T. Iftner: Identification of a differentiation-inducible promoter in the E7 open reading frame of human papillomavirus type 16 (HPV-16) in raft cultures of a new cell line containing high copy numbers of episomal HPV-16 DNA. *J Virol*, 70, 2339-2349. (1996)
19. Milligan, S. G., T. Veerapraditsin, B. Ahamet, S. Mole & S. V. Graham: Analysis of novel human papillomavirus type 16 late mRNAs in differentiated W12 cervical epithelial cell. *Virology*, 360, 172-181 (2007)
20. Ozbun, M. A. & C. Meyers: Characterization of late gene transcripts expressed during vegetative replication of human papillomavirus type 31b. *J Virol*, 71, 5161-5172. (1997)
21. Poppelreuther, S., T. Iftner & F. Stubenrauch: A novel splice donor at nt 1534 is required for long-term maintenance of HPV-31 genomes. *Virology*, 370,93-101, (2007)
22. Zhao, X., M. Rush & S. Schwartz: Identification of an hnRNP A1 dependent splicing silencer in the HPV-16 L1 coding region that prevents premature expression of the late L1 gene. *J. Virol*., 78, 10888-10905 (2004)
23. Rush, M., X. Zhao & S. Schwartz: A splicing enhancer in the E4 coding region of human papillomavirus type 16 is required for early mRNA splicing and polyadenylation as well as inhibition of premature late gene expression. *J. Virol*., 79, 12002-12015 (2005)
24. Somberg, M., X. Zhao, M. Fröhlich, M. Evander & S. Schwartz: PTB induces HPV-16 late gene expression by interfering with splicing inhibitory elements at the major late 5'-splice site SD3632. *J. Virol*., 81, in press (2008)
25. Belaguli, N. S., M. M. Pater & A. Pater: Splice sites of human papillomavirus type 16 E6 gene or heterologous gene required for transformation by E7 and accumulation of E7 RNA. *J Med Virol*, 47, 445-53 (1995)
26. Zheng, Z. M., M. Tao, K. Yamanegi, S. Bodaghi & W. Xiao: Splicing of a cap-proximal human papillomavirus 16 E6E7 intron promotes E7 expression, but can be restrained by distance of the intron from its RNA 5' cap. *J Mol Biol*., 337, 1091-1108 (2004)
27. De la Rosa-Rios, M. A., M. Martínez-Salazar, M. Martínez-Garzia, C. Gonzaléz-Bonilla & N. Villegas-Sepúlveda: The intron of HPV-16 has a suboptimal branch point at a guanosine. *Virus Res*, 118, 46-54 (2006)
28. Stacey, S. N., D. Jordan, P. J. F. Snijders, M. Mackett, J. M. M. Walboomers & J. R. Arrand: Translation of the human papillomavirus type 16 E7 oncoprotein from bicistronic mRNA is independent of splicing events within the E6 open reading frame. *J. Virol*., 69, 7023-7031 (1995)
29. Tang, S., M. Tao, J. P. McCoy & Z.-M. Zheng: The E7 oncoprotein is translated from spliced E6*I transcripts in high-risk human papillomavirus type 16- or 18-positive cervical cancer cell lines via translation reinitiation. J. Virol., 80, 4249-4263 (2006)
30. Remm, M., A. Remm & M. Ustav: Human papillomavirus type 18 E1 protein is translated from polycistronic mRNA by a discontinuous scanning mechanism. *J.Virol*., 73, 3062-3070 (1999)
31. Hubert, W. G. & L. A. Laimins: Human papillomavirus type 31 replication modes during the early phases of the viral life cycle depend on transcriptional and posttranscriptional regulation of E1 and E2 expression. *J. Virol*., 76, 2263-2273 (2002)
32. Filippova, M., M. M. Johnson, M. Bautista, V. Filippov, N. Fodor, S. S. Tungteakkhun, K. Williams & P. J. Duerksen-Hughes: The large and small isoforms of human papillomavirus type 16 E6 bind to and differentially affect procaspase 8 stability and activity. *J. Virol*., 81, 4116-4129 (2007)
33. Pim, D., P. Massimi & L. Banks: Alternatively spliced HPV-18 E6* protein inhibits E6 mediated degradation of p53 and suppresses transformed cell growth. *Oncogene*, 15, 257-64 (1997)
34. Ozbun, M. A.: Human papillomavirus type 31b infection of human keratinocytes and the onset of early transcription. *J. Virol*., 76, 11291-11300 (2002)
35. Zhao, X., D. Öberg, M. Rush, J. Fay, H. Lambkin & S. Schwartz: A 57 nucleotide upstream early polyadenylation element in human papillomavirus type 16 interacts with hFIP1, CstF-64, hnRNP C1/C2 and PTB. *J. Virol*., 79, 4270-4288 (2005)
36. Ordonez, R. M., A. M. Espinosa, D. J. Sanchez-Gonzalez, J. Armendariz-Borunda & J. Berumen: Enhanced oncogenicity of Asian-American human papillomavirus 16 is associated with impaired E2 repression of E6/E7 oncogene transcription. *J. Gen. Virol*., 85, 1433-1444 (2004)
37. Klumpp, D., F. Stubenrauch & L. A. Laimins: Differential effects of the splice acceptor at nucleotide 3295 of human papillomavirus 31 on stable and transient viral replication. *J. Virol*., 71, 8186-8194 (1997)
38. Deng, W., G. Jin, B. Y. Lin, B. A. Van Tine, T. R. Broker & L. T. Chow: mRNA splicing regulates human papillomavirus type 11 E1 protein production and DNA replication. *J Virol*, 77, 10213-26 (2003)
39. Mannik, A., K. Runkorg, N. Jaanson, M. Ustav & E. Ustav: Induction of the bovine papillomavirus origin "onion skin"-type DNA replication at high E1 protein concentrations *in vivo*. *J. Virol*., 76, 5835-5845 (2002)

40. Kozak, M.: Regulation of translation initiation in eucaryotic systems. *Annu. Rev. Cell Biol*., 8, 197-225 (1992)
41. Tomita, Y. & B. Simizu: Translational properties of the human papillomavirus type-6 L1-coding mRNA. *Gene*, 133, 223-225 (1993)
42. Zhao, X., J. Fay, H. Lambkin & S. Schwartz: Identification of a 17-nucleotide splicing enhancer in HPV-16 L1 that counteracts the effect of multiple hnRNP A1-binding splicing silencers. *Virology*, 369, 351-363 (2007)
43. Collier, B., D. Öberg, X. Zhao & S. Schwartz: Specific inactivation of inhibitory sequences in the 5' end of the human papillomavirus type 16 L1 open reading frame results in production of high levels of L1 protein in human epithelial cells. *J Virol*, 76, 2739-2752 (2002)
44. Zhao, X. & S. Schwartz: Inhibition of HPV-16 L1 expression from L1 cDNAs correlates with the presence of hnRNP A1 binding sites in the L1 coding region. *Virus Genes*, 36,45-53, (2007)
45. Öberg, D., B. Collier, X. Zhao & S. Schwartz: Mutational inactivation of two distinct negative RNA elements in the human papillomavirus type 16 L2 coding region induces production of high levels of L2 in human cells. *J. Virol*., 77, 11674-11684 (2003)
46. Rollman, E., L. Arnheim, B. Collier, D. Öberg, H. Hall, J. Klingström, J. Dillner, D. V. Pastrana, C. B. Buck, J. Hinkula, B. Wahren & S. Schwartz: HPV-16 L1 genes with inactivated negative RNA elements induce potent immune responses. *Virology*, 322, 182-189 (2004)
47. Zhou, W., R. F. Cook, S. J. Cook, S. A. Hammond, K. Rushlow, N. N. Ghabrial, S. L. Beger, R. C. Montelaro & C. J. Issel: Multiple RNA splicing and the presence of cryptic RNA splice donor and acceptor sites may contribute to low expression levels and poor immunogenicity of potential DNA vaccines containing the env gene of equine infectious anemia virus (EIAV). *Veterinary Micorbiol*, 88, 127-151 (2002)
48. Taniguchi, I., K. Masuyama & M. Ohno: Role of purine-rich exonic splicing enhancers in nuclear retention of pre-mRNAs. *Proc Natl Acad Sci U S A*, 104, 13684-9 (2007)
49. Andrew, E. M. & D. DiMaio: Hierarchy of polyadenylation site usage by bovine papillomavirus in transformed cells. *J. Virol*., 67, 7705-7710 (1993)
50. Terhune, S. S., W. G. Hubert, J. T. Thomas & L. A. Laimins: Early polyadenylation signals of human papillomavirus type 31 negatively regulate capsid gene expression. *J. Virol*., 75, 8147-8157 (2001)
51. Terhune, S. S., C. Milcarek & L. A. Laimins: Regulation of human papillomavirus 31 polyadenylation during the differentiation-dependent life cycle. *J. Virol*., 73, 7185-7192 (1999)
52. Öberg, D., B. Collier, X. Zhao & S. Schwartz: A downstream polyadenylation element in human papillomavirus type 16 encodes multiple GGG-motifs and interacts with hnRNP H. *J Virol*, 79, 9254-9269 (2005)
53. Baker, C. C. & J. S. Noe: Transcriptional termination between bovine papillomavirus type 1 (BPV-1) early and late polyadenylation sites blocks late transcription in BPV-1-transformed cells. *J. Virol*., 63, 3529-3534 (1989)
54. Furth, P. A. & C. C. Baker: An element in the bovine papillomavirus late 3' untranslated region reduces polyadenylated cytoplasmic RNA levels. *J Virol*, 65, 5806-5812. (1991)
55. Furth, P. A., W. T. Choe, J. H. Rex, J. C. Byrne & C. C. Baker: Sequences homologous to 5' splice sites are required for the inhibitory activity of papillomavirus late 3' untranslated regions. *Mol Cell Biol*, 14, 5278-5289. (1994)
56. Gunderson, S. I., M. Polycarpou-Schwarz & I. W. Mattaj: U1 snRNP inhibits pre-mRNA polyadenylation through a direct interaction between U1 70K and poly (A) polymerase. *Molecular Cell*, 1, 255-264 (1998)
57. Cumming, S. A., M. G. McPhillips, T. Veerapraditsin, S. G. Milligan & S. V. Graham: Activity of the human papillomavirus type 16 late negative regulatory element is partly due to four weak consensus 5' splice sites that bind a U1 snRNP-like complex. *J. Virol*., 77, 5167-5177 (2003)
58. Goraczniak, R. & S. I. Gunderson: The regulatory element in the 3' untranslated region of human papillomavirus 16 inhibits expression by binding CUG binding protein 1. *J. Biol. Chem*, 283: 2286-96 (2007)
59. Sokolowski, M., W. Tan, M. Jellne & S. Schwartz: mRNA instability elements in the human papillomavirus type 16 L2 coding region. *J Virol*, 72, 1504-1515. (1998)
60. Mori, S., S. Ozaki, T. Yasugi, H. Yoshikawa, Y. Taketani & T. Kanda: Inhibitory cis-element-mediated decay of human papillomavirus type 16 L1-transcript in undifferentiated cells. *Mol. Cell. Biochem*., 288, 47-57 (2006)
61. Tan, W., B. K. Felber, A. S. Zolotukhin, G. N. Pavlakis & S. Schwartz: 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. (1995)
62. Berg, M., J. DiFatta, E. Hoiczyk, R. Schlegel & G. Ketner: Viable adenovirus vaccine prototypes: High-level production of a papillomavirus capsid antigen from the major late transcription unit. *Proc Natl Acad Sci U S A*, 102, 4590-4595 (2005)
63. Barreau, C., L. Paillard & H. B. Osborne: AU-rich elements and associated factors: are there unifying principles? *Nucleic Acids. Res*., 33, 7138-7150 (2006)
64. Jeon, S., B. L. Allen-Hoffmann & P. F. Lambert: Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. *J.Virol*, 69, 2989-2997 (1995)
65. Jeon, S. & P. F. Lambert: 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 U S A*, 92, 1654-1658 (1995)
66. Zhao, C., W. Tan, M. Sokolowski & S. Schwartz: Identification of nuclear and cytoplasmic factors that interact specifically with an AU-rich, cis-acting inhibitory sequence in the 3' untranslated region of human papillomavirus type 1 late mRNAs. *J Virol*, 70, 3659-3667 (1996)
67. Vinther, J., M. W. Rosenstierne, K. Kristiansen & B. Norrild: The 3' region of human papillomavirus type 16 early mRNAs decrease expression. *BMC Infect. Dis*., 5, 83 (2005)

68. Kennedy, I. M., J. K. Haddow & J. B. Clements: Analysis of human papillomavirus type 16 late mRNA 3' processing signals *in vitro* and *in vivo*. *J Virol*, 64, 1825-1829. (1990)
69. Kennedy, I. M., J. K. Haddow & J. B. Clements: A negative regulatory element in the human papillomavirus type 16 genome acts at the level of late mRNA stability. *J Virol*, 65, 2093-2097. (1991)
70. Cheunim, T., J. Zhang, S. G. Milligan, M. G. McPhilips & S. V. Graham: The alternative splicing factor hnRNP A1 is up-regulated during virus-infected epithelial cell differentiation and binds the human papillomavirus type 16 late regulatory element. *Virus Res*, in press, (2007)
71. Koffa, M. D., S. V. Graham, Y. Takagaki, J. L. Manley & J. B. Clements: The human papillomavirus type 16 negative regulatory RNA element interacts with three proteins that act at different posttranscriptional levels. *Proc Natl Acad Sci U S A*, 97, 4677-4682. (2000)
72. Dietrich-Goetz, W., I. M. Kennedy, B. Levins, M. A. Stanley & J. B. Clements: A cellular 65-kDa protein recognizes the negative regulatory element of human papillomavirus late mRNA. *Proc Natl Acad Sci U S A*, 94, 163-168. (1997)
73. McPhillips, M. G., T. Veerapraditsin, S. A. Cumming, D. Karali, S. G. Milligan, W. Boner, I. M. Morgan & S. V. Graham: SF2/ASF binds the human papillomavirus type 16 late RNA control element and is regulated during differentiation of virus-infected epithelial cells. *J Virol*, 78, 10598-10605 (2004)
74. Zhao, X., M. Rush, A. Carlsson & S. Schwartz: The presence of inhibitory RNA elements in the late 3'-untranslated region is a conserved property of human papillomaviruses. *Virus Res*., 125, 135-144 (2007)
75. Sokolowski, M., C. Zhao, W. Tan & S. Schwartz: AU-rich mRNA instability elements on human papillomavirus type 1 late mRNAs and c-fos mRNAs interact with the same cellular factors. *Oncogene*, 15, 2303-2319. (1997)
76. Tan, W. & S. Schwartz: 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. (1995)
77. Wiklund, L., M. Sokolowski, A. Carlsson, M. Rush & S. Schwartz: Inhibition of translation by UAUUUAU and UAUUUUUAU motifs of the AU-rich RNA instability in the HPV-1 late 3' untranslated region. *J Biol Chem*, 277, 40462-40471 (2002)
78. Sokolowski, M., H. Furneaux & S. Schwartz: The inhibitory activity of the AU-rich RNA element in the human papillomavirus type 1 late 3' untranslated region correlates with its affinity for the elav-like HuR protein. *J Virol*, 73, 1080-1091. (1999)
79. Zhao, C., M. Sokolowski, W. Tan & S. Schwartz: Characterisation and partial purification of cellular factors interacting with a negative element on human papillomavirus type 1 late mRNAs. *Virus Res*, 55, 1-13 (1998)
80. Sokolowski, M. & S. Schwartz: Heterogeneous nuclear ribonucleoprotein C binds exclusively to the functionally important UUUUU-motifs in the human papillomavirus type-1 AU-rich inhibitory element. *Virus Res*, 73, 163-175. (2001)
81. Felber, B. K. & G. N. Pavlakis: Molecular biology of HIV-1: positive and negative regulatory elements important for virus expression. *AIDS*, 7, S51-62 (1993)
82. Pollard, V. W. & M. H. Malim: The HIV-1 Rev protein. *Annu Rev Microbiol*, 52, 491-532 (1998)
83. Carlsson, A. & S. Schwartz: Inhibitory activity of the human papillomavirus type 1 AU-rich element correlates inversely with the levels of the elav-like HuR protein in the cell cytoplasm. *Arch Virol*, 145, 491-503 (2000)
84. Collier, B., L. Goobar-Larsson, M. Sokolowski & S. Schwartz: Translational inhibition *in vitro* of human papillomavirus type 16 L2 mRNA mediated through interaction with heterogenous ribonucleoprotein K and poly (rC)-binding proteins 1 and 2. *J Biol Chem*, 273, 22648-56. (1998)
85. Ostareck, D. H., A. Ostareck-Lederer, I. N. Shatsky & M. W. Hentze: Lipoxygenase mRNA silencing in erythroid differentiation: The 3'UTR regulatory complex controls 60S ribosomal subunit joining. *Cell*, 104, 281-290 (2001)
86. Gu, W., M. Li, W. M. Zhao, N. X. Fang, S. Bu, I. H. Frazer & K.-N. Zhao: tRNASer (CGA) differentially regulates expression of wild-type and codon-modified papillomavirus L1 genes. *Nucleic Acids Res*, 32, 4448-4461 (2004)
87. Disbrow, G. L., I. Sunitha, C. C. Baker, J. Hanover & R. Schlegel: Codon optimization of the HPV-16 E5 gene enhances protein expression. *Virology*, 311, 105-114 (2003)
88. Lin, C.-T., Y.-C. Tsai, L. He, R. Calizo, H.-H. Chou, T.-C. Chang, Y.-K. Soong, C.-F. Hung & C.-H. Lai: A DNA vaccine encoding a codon-optimised human papillomavirus type 16 E6 gene enhances CTL response and anti-tumor activity. *J. Biomed. Sci.*, 13, 481-488 (2006)
89. Cid-Arregui, A., V. Juarez & H. zur Hausen: A synthetic E7 gene of human papillomavirus type 16 that yields enhanced expression of the protein in mammalian cells and is useful for DNA immunization studies. *J Virol*, 77, 4928-4937 (2003)
90. Liu, W. J., F. Gao, K. N. Zhao, W. Zhao, G. J. Fernando, R. Thomas & I. H. Frazer: Codon modified human papillomavirus type 16 E7 DNA vaccine enhances cytotoxic T-lymphocyte induction and anti-tumour activity. *Virology*, 301, 43-52 (2002)
91. Cai, X., G. Li, L. A. Laimins & B. R. Cullen: Human papillomavirus genotype 31 does not express detectable microRNA levels during latent or productive virus replication. *J. Virol*., 80, 10890-10893 (2006)
92. Martinez, I., A. S. Gardiner, K. F. Board, F. A. Monzon, R. P. Edwards & S. A. Khan: Human papillomavirus type 16 reduces the expression of microRNA-218 in cervical carcinoma cells. *Oncogene*, in press, (2007)

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Send correspondence to: Stefan Schwartz, Department of Medical Biochemistry and Microbiology, Uppsala University, BMC, Husargatan 3, Box 582, 75123 Uppsala, Sweden, Tel: 46184714239, Fax: 46184714673, E-mail: Stefan.Schwartz@imbim.uu.se

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