Regulation of splicing-associated SR proteins by HPV-16

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Abstract

HPV-16 (human papillomavirus type 16) is a small dsDNA (double-stranded DNA) virus which infects mucosal epithelial tissue of the cervix. Epithelial tissue is composed of a basal layer of cells, capable of division, and a number of suprabasal layers, wherein the cells become more differentiated the closer to the surface of the epithelium they become. Expression of viral proteins is dependent upon epithelial differentiation status, and, within the HPV-16 genome, several elements have been found which control expression both transcriptionally and post-transcriptionally. Expression of the highly immunogenic capsid proteins, L1 and L2, is restricted to only the most differentiated cells, where immune surveillance is limited. However, L1 and L2 transcripts can be detected in less differentiated cells, suggesting post-transcriptional mechanisms exist to prevent their expression in these cells. Indeed, a number of *cis*-acting RNA elements have been observed within the HPV-16 late region which may be involved in control of capsid gene expression. Mechanisms controlling HPV-16 capsid gene expression and the cellular RNA-processing factors involved will be the focus of this article.

We have demonstrated that, during differentiation of uninfected epithelial cells and tissue, cellular levels of RNA-processing proteins are down-regulated as cellular function begins to shut down. General down-regulation of these proteins is observed during differentiation of HaCaT cells, a spontaneously immortalized epithelial cell line, which is virus-uninfected [1]. However, late gene expression from HPV-16 (human papillomavirus type 16) requires extensive RNA processing to ensure efficient production of late viral proteins (S.G. Milligan and S.V. Graham, unpublished work). Therefore the virus must regulate at least a subset of the factors controlling RNA processing to ensure completion of its life cycle. One particular family of splicing-related proteins, known as SR proteins (serine- and arginine-rich proteins), are regulated during HPV-16 infection. These are essential for both constitutive and alternative splicing, and bring together protein complexes which form upon 5′ and 3′ splice sites [2]. Furthermore, SR proteins interact with ESEs (exonic splicing enhancers) to direct splicing to proximal splice sites [3,4]. In contrast, another family of RNA-binding protein, hnRNPs (heterogeneous nuclear ribonucleoproteins), are known to associate with a second type of RNA element, known as ESSs (exonic splicing silencers) [4]. This interaction directs splicing to more distal splice sites, allowing for regulation of splice patterns through the use of competing ESEs and ESSs and varying levels of these antagonistic factors [3].

Key words: *cis*-acting RNA element, epithelial differentiation, human papillomavirus type 16 (HPV-16), RNA processing, serine- and arginine-rich protein (SR protein).

Abbreviations used: CIN I, cervical intraepithelial neoplasia, grade I; ESE, exonic splicing enhancer; ESS, exonic splicing silencer; hnRNP, heterogeneous nuclear ribonucleoprotein; HPV, human papillomavirus; LRE, late regulatory element; ORF, open reading frame; pAE, early polyadenylation signal; SF2/ASF, splicing factor 2/alternative splicing factor; SR protein, serine- and arginine-rich protein.

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The key SR protein, SF2/ASF (splicing factor 2/alternative splicing factor), and hnRNP A1 are up-regulated in HPV-16-infected differentiated epithelial cells

W12 is a cell line derived from an HPV-16-infected CIN I (cervical intraepithelial neoplasia, grade I; benign cervical lesions that are the precursors to carcinomas), in which the virus genome is episomal and maintains the natural pattern of HPV infection [5]. These cells also exhibit typical epithelial cell morphology consistent with CIN I lesions and can be differentiated *in vitro*, allowing for the comparison of gene expression, and the effects thereof, between undifferentiated and differentiated cells. Although most RNA-processing proteins are not affected by HPV-16 infection, levels of both SR protein, SF2/ASF and hnRNP A1 are elevated in response to differentiation. Quantification of SF2/ASF and hnRNP A1 in these cells shows ~4–8-fold up-regulation of each protein in differentiated W12 cells. Furthermore, HPV-16 encodes a transcription factor, E2, which may regulate levels of these proteins, and SF2/ASF is up-regulated in cells stably expressing E2 [1].

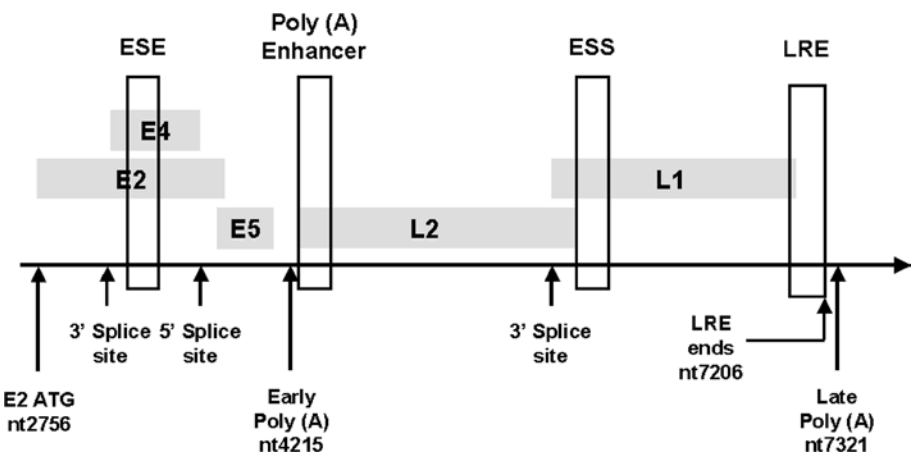
Cis-acting RNA element within the HPV-16 late region

Within the late region of the HPV-16 genome, and particularly within the long exonic sequence encoding the L1

Table 1 | **Cellular RNA-processing factors interacting with HPV-16 late *cis*-acting RNA elements**
snRNP, small nuclear ribonucleoprotein.

Element	Associated cellular proteins	Reference(s)
E4 ESE	Not known	
L2 polyadenylation enhancer	hnRNP H; CstF-64	[10]
L1 ESS	hnRNP A1	[6]
LRE	U1 snRNP-like complex; U2AF-65; SF2/ASF; CstF-64; HuR; hnRNP A1	[1,12,16,17]; T. Veerapraditsin and S.V. Graham, unpublished work

Figure 1 | ***Cis*-aciting RNA elements with the HPV-16 late region**
A schematic diagram of the 3′ portion of the HPV-16 genome, showing the late region (not to scale). ORFs, grey rectangles; splice sites and polyadenylation signals, vertical arrows; *cis*-acting RNA elements, open rectangles.



capsid protein, a number of predicted ESEs are observed (S.V. Graham, unpublished work). Many of these are likely to be involved in directing appropriate splicing of late transcripts depending on the differentiation status of the cell. Zhao et al. [6] have revealed an ESS within the 5′ region of L1 transcripts, which inhibits the use of a 3′ splice site immediately upstream in undifferentiated cells (Figure 1). Inhibition of splicing to this splice site is necessary, as its use could allow production of L1-containing transcripts in undifferentiated cells, owing to deletion of the early polyadenylation signal (pAE). Consistent with its action as an ESS, hnRNP A1 has been shown to associate with this element, and this interaction inhibits splicing *in vitro* (Table 1). Furthermore, an ESE within the E4 ORF (open reading frame) enhances splicing from a weak 3′ splice site within the E2 ORF (Figure 1) [7]. In addition, deletion of this element leads to loss of polyadenylation at pAE, resulting in production of late transcripts encoding L1 and L2. However, as yet, cellular proteins interacting with the E4 ESE have not been identified. Furthermore, both the L1 ESS and E4 ESE may also act as RNA-instability elements as their deletion results in elevated levels of late viral mRNAs [6,7].

Further regulatory elements have been observed within the L2 ORF. These are generally less well defined, but have been shown to inhibit translation and result in instability of L2-containing mRNAs [8]. Proteins interacting with the L2 RNA elements include hnRNP K and PCBP-1 and -2 [poly(rC)-binding protein 1 and 2], causing inhibition of translation [9]. Furthermore, an element within the 5′ region of L2 enhances polyadenylation from pAE (Figure 1) [10]. This region encodes multiple GGG motifs and can interact with polyadenylation factor, CstF-64 and, more strongly, with hnRNP H (Table 1). Consistent with a role for hnRNP H in enhancing polyadenylation from pAE, levels of the protein are seen to diminish upon differentiation of apparently normal cervical epithelium. However, as mentioned, levels of certain RNA-processing proteins are regulated during the HPV-16 life cycle, indicating that hnRNP H abundance could also respond to infection.

Another major element regulating HPV-16 late gene expression, the LRE (late regulatory element), is found within the late 3′-UTR (untranslated region) (Figure 1) [11]. This region is known to confer negative regulatory activity upon reporter gene expression in undifferentiated cells [12]. It is thought that the LRE is multifunctional, and a number of proteins involved in splicing, RNA stability and polyadenylation are known to interact with it, including SF2/ASF and hnRNP A1 (Table 1) [1]. SF2/ASF not only has roles during splicing, but also is able to shuttle between the nucleus and the cytoplasm [13], can regulate nucleocytoplasmic shuttling [14] and also regulates translation [15]. It is therefore hypothesized that association of SF2/ASF with late

transcripts, via the LRE, may regulate late gene expression at several stages post-transcriptionally.

Gene expression from HPV-16 is highly complex and is controlled at both transcriptional and post-transcriptional levels. There are many *cis*-acting RNA elements within late transcripts which restrict expression of capsid proteins to the most differentiated cells of epithelial tissue. We hypothesize that these elements respond to changes in levels of certain RNA-processing factors, some of which are controlled during HPV-16 infection.

References

1 McPhillips, M.G., Veerapraditsin, T., Cumming, S.A., Karali, D., Milligan, S.G., Boner, W., Morgan, I.M. and Graham, S.V. (2004) J. Virol. **78**, 10598–10605
2 Sanford, J.R., Ellis, J. and Cáceres, J.F. (2005) Biochem. Soc. Trans. **33**, 443–446
3 Cáceres, J.F., Stamm, S., Helfman, D.M. and Krainer, A.R. (1994) Science **265**, 1706–1709
4 Wang, Z., Xiao, X., Van Nostrand, E. and Burge, C.B. (2006) Mol. Cell **23**, 61–70
5 Stanley, M.A., Browne, H.M., Appleby, M. and Minson, A.C. (1989) Int. J. Cancer **43**, 672–676
6 Zhao, X., Rush, M. and Schwartz, S. (2004) J. Virol. **78**, 10888–10905
7 Rush, M., Zhao, X. and Schwartz, S. (2005) J. Virol. **79**, 12002–12015
8 Oberg, D., Collier, B., Zhao, X. and Schwartz, S. (2003) J. Virol. **77**, 11674–11684
9 Collier, B., Goobar-Larsson, L., Sokolowski, M. and Schwartz, S. (1998) J. Biol. Chem. **273**, 22648–22656
10 Oberg, D., Fay, J., Lambkin, H. and Schwartz, S. (2005) J. Virol. **79**, 9254–9269
11 Kennedy, I.M., Haddow, J.K. and Clements, J.B. (1991) J. Virol. **65**, 2093–2097
12 Cumming, S.A., McPhillips, M.G., Veerapraditsin, T., Milligan, S.G. and Graham, S.V. (2003) J. Virol. **77**, 5167–5177
13 Cáceres, J.F., Screaton, G.R. and Krainer, A.R. (1998) Genes Dev. **12**, 55–66
14 Cazalla, D., Zhu, J., Manche, L., Huber, E., Krainer, A.R. and Cáceres, J.F. (2002) Mol. Cell. Biol. **22**, 6871–6882
15 Sanford, J.R., Gray, N.K., Beckmann, K. and Cáceres, J.F. (2004) Genes Dev. **18**, 755–768
16 Dietrich-Goetz, W., Kennedy, I.M., Levins, B., Stanley, M.A. and Clements, J.B. (1997) Proc. Natl. Acad. Sci. U.S.A. **94**, 163–168
17 Koffa, M.D., Graham, S.V., Takagaki, Y., Manley, J.L. and Clements, J.B. (2000) Proc. Natl. Acad. Sci. U.S.A. **97**, 4677–4682

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