Transcription Factor Binding Sites in the Long Control Region of Genital HPVs

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INTRODUCTION

The long control region (LCR) of papillomaviruses (PVs) is a genomic segment, without any major open reading frames, which contains a large number of cis-responsive elements that govern PV gene expression and replication. Its size ranges in different PVs from about 7 to 11% of the total genome, roughly 850 bp in the case of genital HPVs, and it is positioned between the end of the L1, and the start of the E6 gene. While even closely related PV LCRs do not show extensive nucleotide sequence similarities, there are a number of recognizable short motifs that are highly conserved, and it is apparent that in related PVs, e.g., in different genital human PVs (HPVs) [18], or different EV-HPVs [29], there is a similar gross composition of these motifs. Since it is unlikely that a very low amount of overall sequence conservation, combined with highly conserved motifs, evolved by chance alone, most PV researchers share the hypothesis that these motifs would not exist unless each of them were involved in specific and important functions. Different laboratories disagree, however, as to the exact nature of these functions.

So far, only four sequence elements are sufficiently well recognized to be considered common to all PVs. These are (i) a polyadenylation site for late mRNAs, which is fairly close to the 5′ end of the LCR, (ii) E2 protein binding sites, which vary in relative position and in number from 4 in genital HPVs to 10 in BPV-1, (iii) one E1 protein binding site, and (iv) the TATA box of the E6 gene promoter.

The LCR has been functionally studied in six model systems, namely HPV-11, HPV-16, and HPV-18 among the genital HPVs, HPV-8 among the EV-HPVs, BPV-1 among the ungulate fibropapillomaviruses, and BPV-4 among the cutaneous ungulate PVs. As one might expect, the study of viruses from four remotely related groups of PVs [13a] has led to very diverse observations. In many cases, however, it is not clear whether these findings reflect true biological differences, or differences which may have resulted from the use of various cell culture systems which only represent a poor approximation of the natural cellular environment. Some observations may have also originated from particular experimental approaches adopted for historical reasons rather than by design. Two examples of this include the study of BPV-1 gene expression in mouse fibroblasts, rather than in the cutaneous epithelia of ungulates, and of HPVs in HeLa or HaCat cells, which may have altered transcriptional properties as a result of their transformed state. It may be an idiosyncrasy of cell culture systems that most transcripts in the LCR of HPV-8 start at a promoter for the late genes [74], while such a promoter has, so far, not been detected in genital HPVs.

Transcription and replication functions of PV LCRs have been reviewed in recent years by eight independent groups [10,35,43,46,50,57,78,80]. Here, it has not been our intention to present a comprehensive account of PV regulation. Instead, we have concentrated on a detailed comparison of the highly conserved motifs in the LCRs of a number of HPVs, and discuss their possible functions in the context of prevailing views or hypotheses that are controversial, in the hope that we might stimulate new experimental approaches. Furthermore, we have concentrated on the genital HPV types, and only occasionally refer to other unrelated PVs.

ORGANIZATION OF THE LCR OF GENITAL HPVs

Figure 1 is a schematic representation of the HPV-16 LCR, which can be considered as a model for the LCRs of all genital HPVs. Four E2 binding sites serve as landmarks, and two of them divide the LCR into functionally distinct segments, which we have called the 5′, the central, and the 3′ segment.

The 5′ segment of the LCR of genital HPVs. This segment has a size of about 300 bp and is bracketed by the termination codon of L1 and an E2 binding site (marked #1 in fig. 1). This segment contains transcription termination and polyadenylation sites for late transcripts as well as a negative regulatory element acting at the level of late mRNA stability [36,48]. In HPV-11, this segment may also modulate transcription [4], but the details of

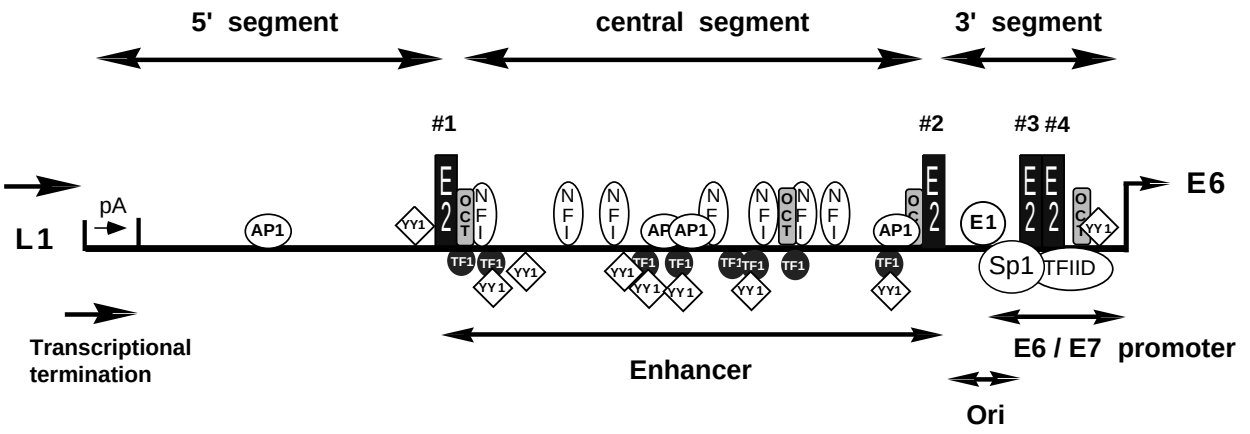


Figure 1. A schematic representation of the HPV-16 LCR, which can be considered as a model for the LCRs of all genital HPVs. Four E2 binding sites serve as landmarks, and two of them divide the LCR into functionally distinct segments, which we have called the 5′ , the central, and the 3′ segment. The 5′ segment contains the transcription termination signal, denoted 'pA', the central segment the epithelial specific enhancer which contains the majority of transcription factor binding sites, and the 3′ segment contains the origin of replication and the E6/E7 promoter. All the transcription factor binding sites are denoted by the abbreviation used in the text with the exception of TEF-1 which is denoted TF1.

this mechanism will require further investigation. A promoter for the late genes is located in this part of the LCR of HPV-8 [74] and BPV-1 [5], but a similar function has not been detected in genital HPVs. The function of the E2 binding site at the 3′ side of this segment is also not known, although it has been reported to influence in vitro transcription from the remote E6/E7 promoter [66].

The central segment of the LCR of genital HPVs. This segment, which is flanked by two E2 binding sites (#1 and #2), has a size of about 400 bp and has been shown to function in HPV-11, HPV-16, and HPV-18 as an epithelial specific transcriptional enhancer [17,20,22,39]. At present it is thought that, rather than there being a tissue specific membrane receptor which could limit viral infection, it is the tissue specific enhancer which may determine the epithelial tropism displayed by HPVs [60,65]. It is, therefore, unfortunate that no single concept has provided a satisfactory explanation as to the basis of this epithelial specificity. Originally, it was thought that perhaps a tissue specific DNA-binding factor(s) might exist. However, DNAse I protection assays have failed to show any obvious differences between epithelially-derived or fibroblast-derived nuclear extracts in their binding to a number of HPV enhancers [22,39]. An alternative hypothesis was proposed which invoked quantitative or qualitative differences in apparently ubiquitous factors [19], and studies along such lines have provided important insights into the question of epithelial specificity. One such study involves different members of the NFI family of transcription factors [2,3], while a second involves different members of the Jun family of factors [77], and both examples will be discussed in more detail in the next section. A third proposal is that tissue specific cofactors may exist which do not bind DNA directly but which may be required for activation through protein-protein interactions. However, no candidate has been found exclusively in epithelial cells to date. In conclusion, it would appear that the epithelial specificity demonstrated by HPV enhancers is defined by a combination of enhancer binding site composition, quantitative and qualitative differences in transcription factors, and perhaps the presence or absence of important regulatory cofactors.

In addition to providing tissue-specificity, the enhancer is also involved in modulating viral gene expression in response to the physiological and differentiation state of the host keratinocyte in the different layers of the stratified squamous epithelium. Such changes are brought about by the differential binding of transcription factors to their cognate sites in the enhancer, and this in turn will be dependent upon the quantitative, and perhaps qualitative, differences in these transcription factors in any given cellular environment.

At least eleven different cellular transcription factors have been shown to bind in vitro to more than twenty different sites of the HPV-16 enhancer. These are AP1, cEBP, glucocorticoid receptor, progesterone receptor, NFI, NF-IL6, Oct-1, PEF-1,TEF-1, TEF-2, and YY1 [14,15,17,18,19,23,37,39,40,47,49,63,73].

At the 3′ side, the central LCR segment is flanked by the #2 E2 binding site, which is involved in replication initiation [16,67,75], and may also modulate transcription of the E6 and E7 genes [66].

The 3′ segment of the LCR of genital HPVs. The #2 E2 binding site and the E6 gene mark the boundaries of the 3′ segment of the LCR, which has a size of about 140 bp. This segment contains a single E1 binding site, which identifies the origin of replication. The transcription start site, which is only about 5 bp upstream of the ATG of E6, is located about 90 bp downstream of the E1 binding site. A segment of about 45 bp within these 90 bp contains an SP1 transcription factor binding site and two E2 protein binding sites, as well as a TATA box. These four sites are complex means for modulating E6/E7 promoter activity. The TATA box is required for the binding of TFIID and the establishment of the pre-initiation complex, and the SP1 factor for mediating the long range effect of the enhancer. The E2 sites overlap with the TATA box and with the SP1 site, such that occupancy by E2 proteins displaces TFIID and the SP1 factor [12,26,26a,41,76]. These studies, along with those that have investigated the role of the E2 sites in the initiation of DNA replication, have addressed individual mechanisms of what is probably a series of interconnected regulatory events. If taken in isolation, some of these results may look contradictory. However, taken together they would support a scenario in which the binding of SP1 to its site activates the E6/E7 promoter, but not replication, because SP1 would block E2 binding which could enhance E1-dependent replication initiation. At concentrations of E2 which result in the displacement of SP1, replication is activated and transcription partially repressed [26,76]. The binding of E2 to the promoter proximal site (site #4) would lead to TFIID displacement and further repression. Such a series of events would constitute a switch between replication and transcription initiation which would depend upon the local concentrations of E2.

A computer analysis of transcription factor binding sites in HPV LCRs. Only a small number of HPV LCRs have been studied experimentally to determine whether putative transcription factor binding sites are potentially important for biological activity, but the results of such studies will have been influenced by the cell culture systems used. The motivation behind a computer-based analysis of LCR transcription factor binding sites, is that by comparing the motifs from a larger number of HPV LCRs, including many of those that have never been functionally tested, biologically important sites might become conspicuous due to their highly conserved nature.

It is hoped that two advances might be made from such a study. The first is that those sites that have hitherto been shown in an experimental system to be functionally important might be confirmed, and then extrapolated to include other HPVs if conserved. The second is that previously overlooked motifs that show a high degree of conservation might become an obvious target for future experimental analysis. A good example of the former is the precise spacing of the SP1, TATA, and E2 binding sites at E6/E7 promoters discussed above. As these sites, along with their spacing, are conserved in all genital HPVs, it could be assumed that the same E2-mediated regulation by the displacement of SP1 and TFIID should occur in all of them. As an example, the presence of this conserved region in a number of HPVs such as HPV-2, HPV-3, and HPV-7, which give rise to cutaneous lesions, although belonging phylogenetically to the genital HPVs, could be used to predict the same regulatory mechanism. Another example is the conservation of a composite element at the 3′ end of the central fragment of the LCR in which an octamer site is spaced by exactly 2 bp from an NFI site [63]. Experimental evidence has been provided for the synergistic interaction between Oct-1 and NFI in HPV-16, and its highly conserved nature among those HPVs which infect the genital mucosa would suggest it plays an equally significant role in these viruses.

It is important to note, however, that in spite of the usefulness of such a study, one should keep in mind the following caveats. Firstly, the presence of a potential recognition sequence does not mean that it will always be bound by its cognate transcription factor. For example, while the optimum binding site for a transcription factor has often been determined empirically, this is not the case for all degenerate and low affinity sites. Consequently, certain combinations of degeneracy in a recognition sequence, while being recognized in the computer search, may not be able to bind the cognate transcription factor. Furthermore, many sites that are to be found within the various LCRs, especially within the central segment, are juxtaposed or even overlapping with other sites. Thus, in a given cellular environment, such as columnar epithelia, or the different layers of the squamous epithelium, there may be a particular subset of factors binding giving rise to particular levels of viral gene expression. For example, the recognition sequences for TEF-1, TEF-2 and YY1 are very similar (see figure 2) and consequently a sequence that is capable of binding TEF-1 may also bind YY1 or possibly even TEF-2. Under these circumstances it will be necessary to determine which factor is binding in any given transcriptional environment, and which is responsible for any functional activity.

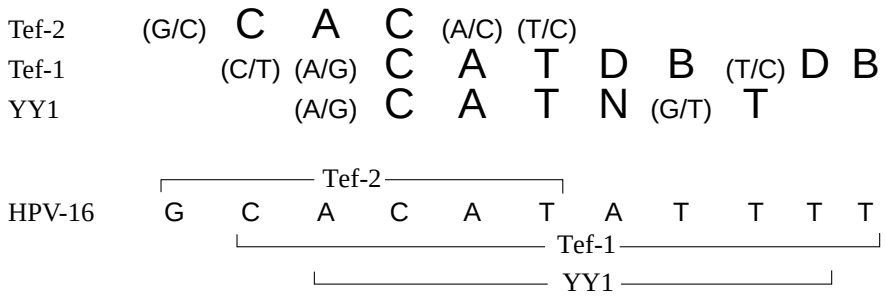


Figure 2. Similarities between the binding sites for TEF-1, TEF-2, and YY1 and their potential overlap in HPV-16. Central to the binding of both TEF-1 and YY1 is the sequence CAT, and because certain degeneracies are accommodated on either side of this core sequence, many sites that can bind TEF-1 can also potentially bind YY1 and vice versa. The 3′ end of the recognition sequence for TEF-2 can also be CAT, thus in certain circumstances an overlap between the recognition sites for all three factors can occur, as exemplified by the HPV-16 regulatory sequence shown above.

The second point to note, is that this study does not provide an exhaustive search of every known transcription factor binding site. The criteria that have been employed in listing binding sites are that (i) previously published information suggested a functional role in HPV expression, and that (ii) at least one binding site existed in most HPV LCRs. Consequently, we have listed the result of searches for AP1, E2, NFI, Oct-1, SP1, TEF-1, and YY1. We have not listed AP2 [18], cEBP [73], glucocorticoid/progesterone receptors [14,64], KRF-1 [54], NF-IL6 [49], PEF-1 [23], or PVF/TEF-2 [18], because either the original publication, or our search suggested that they play a role only in a single, or a limited number of HPVs.

Table 1 provides the search sequences used for each transcription factor. The searches were performed on the LCRs of 20 different HPVs, which were selected as typical representatives of the 11 groups of supergroup A, the genital HPVs. Also included are some representatives of supergroup B (HPV-5) and E (HPV-1 and HPV-41). The results of the search are listed in table form (table 2), and are given as the number of sites present within the LCR region. The exact nucleotide sequence and position of each site is available as a computer file by anonymous ftp from `atlas@lanl.gov`. (Refer to Part V of this compendium for information about how to access HPV information from Los Alamos using ftp or the World Wide Web.) The results are also presented in the form of scale diagrams (figure 3) to denote the positions of the different factor binding sites. Below is a brief description of the transcription factors that have been used in the search and their proposed roles in regulating HPV gene expression.

Table 1 Search patterns used for the identification of putative transcription factor binding sites in HPV LCRs.

Factor	Search pattern[1]	Consensus[2]
NFI	TTGGC	TTGGC
AP1	TKWNTMA	TGANTCA
Oct-1	AANWGYAB	AATTGCAT
Tef-1	YRCATDBYDB	TACATACTTC
YY1	MCATNKT	ACATTTT
E2	$ACC(N)_6GGT$[3]	$ACCG(W)_4CGGT$

[1] The search sequence was obtained by taking into account the known permitted degeneracies for individual nucleotides within a transcription factor recognition site (taken either from published work or empirically determined in our laboratory).

[2] The consensus sequence, usually represents the classical or optimum recognition sequence. Exceptions to this are the octamer motif AATTGCAT which has been shown to bind Oct-1 as well as the classical ATTTGCAT motif, and the TEF-1 consensus, which is made up of the most frequently found individual nucleotides in a number of TEF-1 sites.

[3] The HPV-41 E2 site is represented by the sequence $AAC(N)_6GTT$. In order for a recognition sequence to be included in the results of the search it could not differ by more than 2 nucleotides from the consensus sequence. The exceptions to this are the NFI motifs, in which no degenerate sites were taken, and TEF-1 in which any site which met the original search pattern was taken. The code used for particular sets of nucleotides was standard and includes the following: **K** = G or T; **W** = A or T; **M** = A or C; **R** = G or A; **Y** = T or C; **B** = G, T, C; **D** = G, A, T.

Table 2 Frequency of transcription factor binding sites in HPV LCRs.

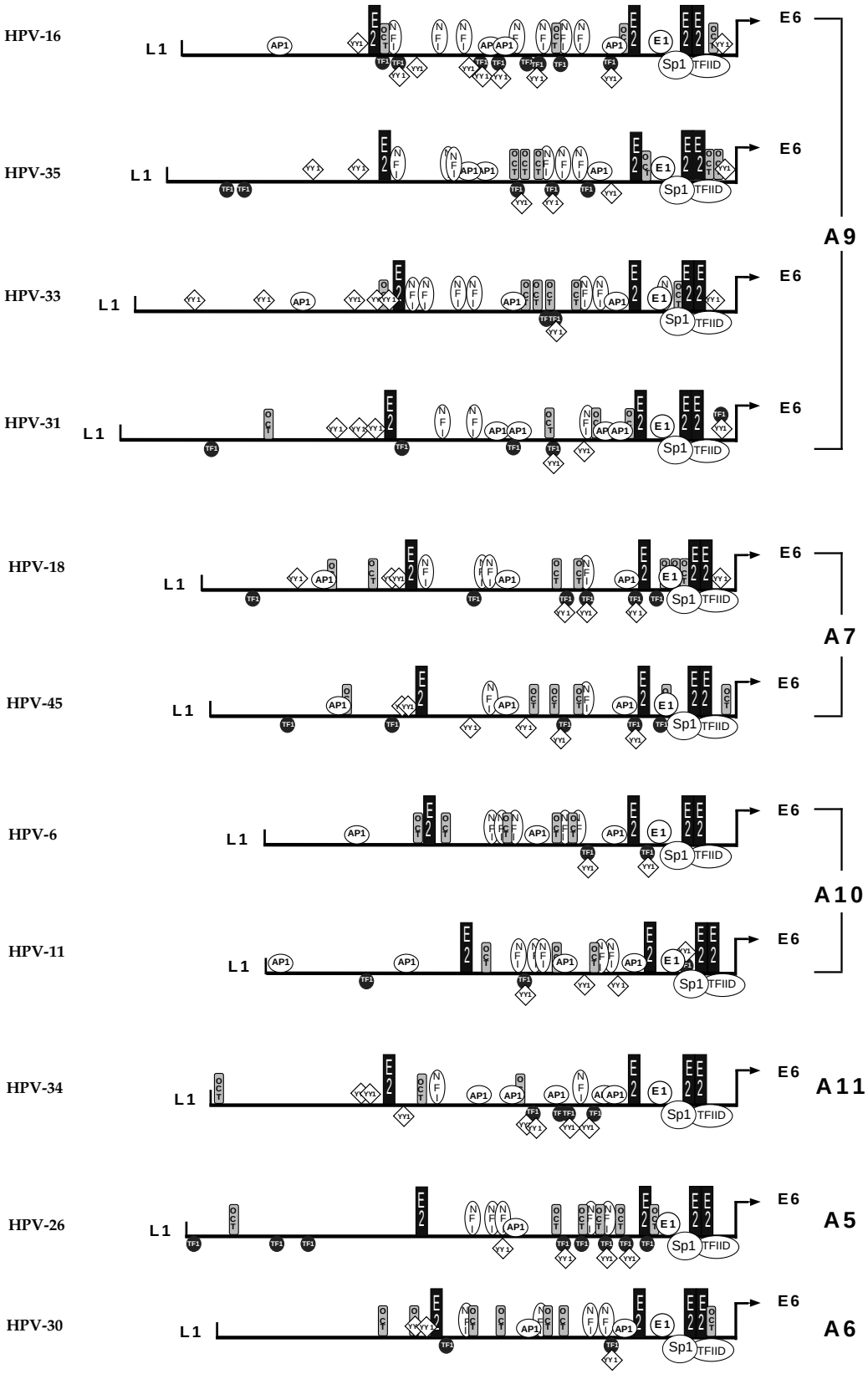
Group	HPV type	E2	NFI	YY1	TEF-1	AP-1	Oct-1
A9	16	4	7	10	8	4	4
A9	35	4	7	5	5	3	7*
A9	33	4	7	7	2	3	6*
A9	31	4	3	8	5	4	4
A7	18	4	4	7	6	3	7*
A7	45	4	2	6	6	3	6*
A10	6	4	5	2	2	3	5*
A10	11	4	5	5	3	4	3*
A11	34	4	2	6	4	5	3
A5	26	4	5	4	8	1	6*
A6	30	4	4	3	2	2	7
A4	2	4	4	4	3	2	4
A4	27	4	3	1	2	2	3*?
A4	57	4	4	1	6	3	3
A2	3	4	3	3	4	4	2
A2	10	4	3	4	4	3	2
A8	7	4	4	6	5	3	3*?
B	5	5	5	1	0	1	1
E	1	4	4	3	2	1	4
E	41	7	3	6	0	2	1

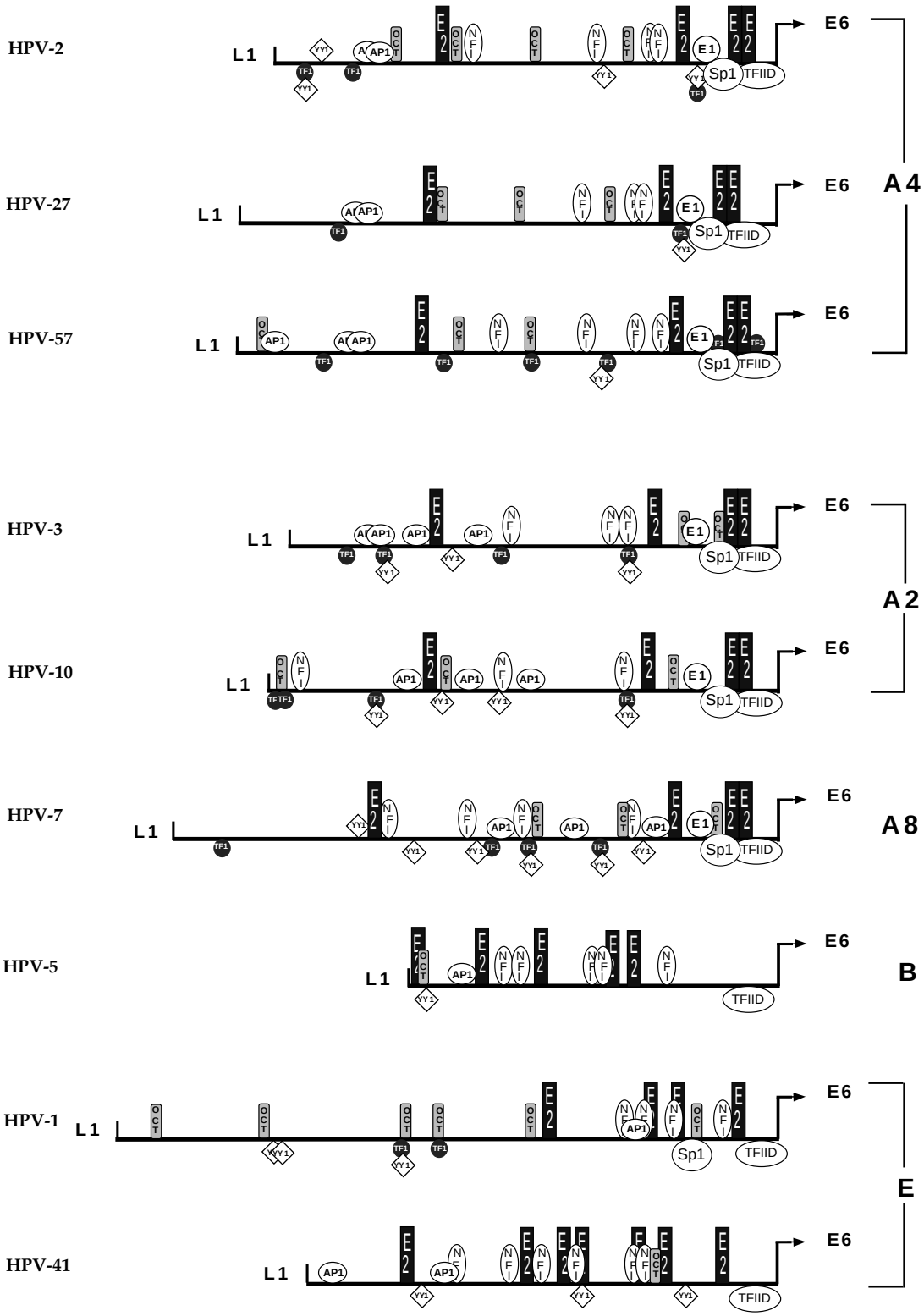
Table 2. Frequency of transcription factor binding sites in HPV LCRs. The numbers represent the number of times a particular site was found within the LCR of any given HPV. An asterisk (*) indicates that one of the octamer motifs was part of the oct/NFI composite element, while *? represents a probable composite element.

Figure 3. (See next two pages.) Distribution of potential transcription factor binding sites within the LCRs of 20 HPVs. The figures are drawn to scale based upon the positions determined by the computer search. Representatives are shown for nearly all of the groups which make up the human genital papillomavirus supergroup A (see reference 13a), as well as representatives for group B and E [13a]. Group numbers are indicated at the right edge of the figure. The striking conservation of E2 sites #1 to #4 can be seen for all of the supergroup A viruses, but is not conserved in HPV-5, HPV-1, and HPV-41.

Also conserved in the super group A PVs, is the precise distancing between the #3 and #4 E2 sites and the SP1 and TATA motifs. All genital HPVs possess a number of non-palindromic TTGGC NFI motifs within the central segmant of the LCR. All viruses also possess putative recognition sequences for AP1, Oct-1, and YY1, while all but HPV-5 and HPV-41 also possess putative TEF-1 sites. The position of the E1 binding site and likely origin of replication is also indicated and is highly conserved (see figure 4 for E1 sequences). Again HPV-5, HPV-1, and HPV-41 differ from the other viruses shown here in that they have less easily identifiable E1 sites and consequently have not been shown here. Overall these results show a number of extremely well conserved features thought to be important for genital HPV biology and these are discussed further in the text.

Figure 3A


HPV-16
HPV-35
HPV-33
HPV-31
HPV-18
HPV-45
HPV-6
HPV-11
HPV-34
HPV-26
HPV-30
L1
E6
A9
A7
A10
A11
A5
A6
AP1
E1
E2
Sp1
TFIID


HPV-2
HPV-27
HPV-57
HPV-3
HPV-10
HPV-7
HPV-5
HPV-1
HPV-41
L1
E6
E1
E2
Sp1
TFIID
AP1
OCT
NFI
YY1
TF1
A4
A2
A8
B
E

DESCRIPTION OF TRANSCRIPTION FACTORS

NFI This factor was originally identified through its role in the replication of the adenovirus genome, where it binds to the sequence TTGGC(N)$_6$ CCAA [27,61]. In HPVs all NFI sites appear to be made up of the lower affinity non-palindromic or half-site TTGGC, however the NFI protein still binds as a dimer, which forms in solution [58]. As NFI is a family of transcription factors, there are multiple genes which give rise to proteins with the same DNA binding and dimerization domains, but which differ in their activation domains and in their biological properties. Furthermore, as each gene produces a number of differentially spliced products there is an array of possible dimers which may bind to the TTGGC motif. In epithelial cells, where the HPV enhancer is active, the major forms of NFI present consist of dimers made up of the three splice products from the NFI-C gene, CTF-1, CTF-2 and CTF-3. In contrast, in fibroblast cells, which don't support HPV transcription, spliced isoforms of the NFI-X gene seem to predominate [2]. Furthermore, the overexpression of NFI-X in epithelial cells leads to the down regulation of the HPV-16 enhancer [3], suggesting an important role for NFI sites in determining the epithelial-specificity of HPV enhancers.

Statistically, a TTGGC motif could have an *a priori* frequency of 1/1024. However, the search revealed that every single HPV LCR contained NFI sites and on average more than 4 sites were present, with some LCRs having as many as 7 sites. Even more striking is the fact that in genital HPVs, all of these NFI sites occur within the central segment of the LCR, which is only 300–400 bp long. The probability that this would occur by chance is negligible. It is also worth noting that for NFI sites we have not looked for degenerate sites, such as TTGGA, which have also been reported to bind NFI, and this could mean that the numbers of potential NFI sites presented here is an underestimate.

It is not yet clear why there should only be non-palindromic NFI sites present in HPV enhancers. However, due to the clustering of these sites within the central segment of the LCR, it is tempting to speculate that NFI could be playing an architectural role within the HPV enhancer, as has been proposed for certain factors in determining the stereospecificity of other enhancers [79]. Finally, while NFI sites were shown to be important for HPV-18 enhancer function, similar experiments using much larger fragments encompassing the whole LCR gave conflicting results [13]. At present, there is no obvious explanation for these different observations.

AP1 This factor, which binds as heterodimers made up of proteins derived from the jun, fos and ATF gene family, recognizes DNA motifs related to the sequence TGANTCA and serves as a downstream target of intracellular signalling mechanisms [82]. The AP1 sites present in HPV enhancers may therefore modulate viral gene expression in response to growth factors such as EGF, KRF and tumor growth factors via the protein kinase C pathway. Evidence for this comes from the use of phorbolesters which mimic the signalling pathways and strongly activate E6 and E7 expression [15]. It has also been proposed that AP1 may contribute towards epithelial-specific activation since the genes that give rise to its subunits are differentially expressed [77].

The search results show that all genital HPV LCRs contain at least one AP1 site, with most possessing 3 or 4 sites. Of these, usually all, or all but one, fall within the central segment of the LCR in the epithelial-specific enhancer. Furthermore, it would appear that they show some conservation with respect to their positioning, with all genital mucosal viruses possessing one or two AP1 sites in the central portion of the enhancer, and one or two sites at the very 3′ end of the enhancer. The functional importance of this observation however, if any, is not known. The only HPVs which do not at first sight appear to possess any AP1 sites within their enhancers (HPV-2, 27, and 57) do, on closer inspection, possess one or more degenerate sites with the sequence TGTTTAA, TTGCTCA, or TGCATTA, which may bind AP1.

Although it still remains to be established, AP1 sites could translate increased mitogenic signals resulting from E5 action [51], into increased levels of viral gene transcription. Another potential role of the AP1 sites might also be to mediate the repressive effect of retinoids on HPV gene expression, which has previously been observed [6], as AP1 and retinoids are molecular antagonists [30].

Oct-1 This factor has been shown to regulate a large number of viral and cellular genes [68], and is a member of the POU factor family [66a]. All POU factors possess a highly homologous, bi-partite DNA binding domain. Within the POU domain, an N-terminal POUS subdomain recognizes the 5′ end of the recognition sequence, ATGCAAAT for Oct-1 [81a], and this is separated by a linker, which varies in length and sequence in different POU factors, from the C-terminal POUH subdomain which recognizes the 3′ end of the recognition sequence [81a]. Even quite degenerate versions of the octamer motif can be recognized by Oct-1 [9, 63]. However, for degenerate sites, the flanking three base pairs play a role in determining binding affinity [9].

Initial observations in which the overexpression of Oct-1 resulted in a down-regulation of HPV enhancer activity, did not require the binding of the Oct-1 factor to the HPV enhancer [46]. This suggests that repression could have occurred as a result of the "squelching of cofactor(s) necessary for promoter stimulation [38,55] which might have resulted from the high, non-physiological levels of Oct-1 generated during these experiments. More recent studies have shown that, at the physiological levels present in epithelial cells, Oct-1 activates the HPV enhancer by binding to a conserved composite regulatory element present at the 3′ end of the enhancer [63]. This composite element consists of an octamer motif separated by exactly 2 bp from a TTGGC motif, and Oct-1 does not appear to activate the enhancer directly, but rather does so by stabilizing the binding of NFI to the composite element, which in turn results in higher levels of enhancer activity.

The results of the computer search indicate that the octamer/NFI composite element is highly conserved amongst the genital mucosal HPVs, being found in 8 out of 11 of the enhancers tested here, as well as in HPV-51, and even RhPV-1. In addition to the octamer motif present in the composite element, there are always at least 2 other octamer motifs elsewhere in the central region of the LCR, with up to a maximum of 7 sites, and further sites can also be found outside the central portion of the LCR in all but two of the genital HPVs (HPV-11 and HPV-27).

Studies of the histone H2B gene have indicated that Oct-1 may play a role in cell cycle-dependent transcriptional regulation [32], a hypothesis that is supported by the fact that Oct-1 is differentially phosphorylated during the cell cycle [11]. It is feasible therefore that Oct-1 could modulate HPV expression in a cell-cycle dependent manner. One important point to note, however, is that in the different layers of the squamous epithelium, it may not be Oct-1, but other POU factors which may bind the octamer motifs present in the HPV enhancer and subsequently regulate transcriptional activity. Obvious candidates would include POU factors that were epithelial specific and, while no human homolog has been found for the rat POU factors Skn1a and Skn1i [1], quite recently an unrelated human epithelial specific POU factor, Oct-6, was isolated, and shown to be expressed at high levels in all layers of the stratified squamous epithelium [31]. The presence of numerous octamer motifs, as well as the octamer/NFI composite element could, therefore, be important in regulating HPV expression in the different layers of the stratified epithelium.

TEF-1 and TEF-2 (PVF). Transcriptional enhancer factor (TEF)-1 and TEF-2 were both originally identified as factors which bound to the enhancer of SV40 [24,34], and TEF-2 was also identified as the factor which bound to the CAC box of the human β-globin promoter [25]. TEF-1 has been shown to contribute towards HPV-16 enhancer activity [47] and the purified protein can bind, in vitro, to several sites within the HPV-16 enhancer. However, it is not yet clear which sites are bound by TEF-1 in the context of nuclear extracts where other transcription factors may compete for binding sites. This is an important point to note due to the similarity between TEF-1, TEF-2, and YY1 recognition sequences (see figure 2). A potentially new transcription factor, originally termed PVF [18], was shown in later studies to co-migrate with TEF-2 when comparing the HPV-16 PVF motif with the human β-globin CAC and the SV40 GT-IC motifs. Overlapping with the TEF-2 site is a potential YY1 site, and a TEF-1 site, as shown by DNAse I protection analysis with purified TEF-1 protein [47]. Thus, in this particular case any one of these factors might be contributing to enhancer function. As we have no clear indication that TEF-2 is important for HPV-16 enhancer function at this time, we have not included the computer search for TEF-2 sites. However, there are a number of potential TEF-2 sites in the majority of genital HPVs. Their positions can be identified in the electronic data file.

The results of the computer search for TEF-1 sites show that while potential TEF-1 motifs are not present in all HPVs (being absent in HPV-5 and HPV-41), the majority do possess them in numbers ranging from 2 to 8. Many of these sites, however, are also capable of binding YY1, as indicated in figure 3, and consequently the number of sites actually binding TEF-1 may be lower.

While TEF-1 appears to contribute to HPV enhancer function, it is unlikely that it is by itself able to generate epithelial specificity. This is because TEF-1 and its cofactor have only been shown to be absent from lymphoid cells. Moreover, TEF-1 sites are not present in the epithelial specific enhancers of all HPVs searched, which would be expected from a factor with a major role in determining epithelial specificity.

YY1 This zinc finger protein was originally termed "yin and yang 1 due to its ability to either repress or activate the adeno-associated virus (AAV) P5 promoter [71]. The binding of YY1 upstream of the P5 promoter led to downregulation, however, this repression was translated into activation upon the interaction of E1A protein with bound YY1 [52,71]. While the exact mechanism behind the ability of YY1 to repress P5 gene expression is not known, there have been numerous documentations of the ability of YY1 to interact directly with transcriptional regulators [52,70,72], and in some instances YY1 has been shown to displace transcriptional activators or bend DNA [42,50a,62]. Presumably, it is these characteristics that will play a role in the repression of promoter activity. On the other hand, co-transfected YY1

has been shown to activate the c-myc promoter [83] and there is good evidence that YY1 can act as a transcription initiation factor [70,81]. Thus, the role of YY1 binding sites within regulatory regions will depend upon enhancer / promoter context and upon the cellular environment.

YY1 was first implicated in the regulation of HPV gene expression when a silencing element in HPV-18 demonstrated YY1 binding ability, and mutations which prevented YY1 binding relieved repression [7]. More recent studies have suggested that YY1 binding to this silencer element can actually activate the HPV-18 promoter in the context of the whole LCR due to the presence of an upstream "switch region [8]. In HPV-16, evidence has been presented which suggests that the binding of YY1 to certain sites within the enhancer can repress HPV gene expression. Naturally occurring deletions or point mutations of these sites in the episomal HPV genomes obtained from cervical carcinomas, give rise to elevated levels of enhancer activity which may contribute towards tumorigenesis [28,56].

The computer search reveals the existence of YY1 recognition sequences in all of the HPVs tested, with up to a maximum of 9 sites present in HPV-16. Many of the sites present in the HPV enhancers overlap with potential TEF-1 sites as mentioned above. Most HPVs also possess one or more YY1 sites in the 5′ or 3′ segment of the LCR. In HPV-16 there are also two quite degenerate sites that have been shown to bind YY1 in vitro [56] which were not picked up by the search. This suggests that there could be even more potential sites for YY1 which will not have been detected by this search.

YY1 may play a role in the down-regulation of gene expression in a number of viruses associated with persistant infection including AAV, adenovirus [71] and EBV [59]. It is possible, therefore, that YY1 may serve to help maintain lower levels of HPV transcription which might facilitate long term infections, while changes in the transcriptional environment, or mutation of YY1 sites [28,56] could result in elevated levels of HPV gene expression.

E2 The functions of the E2 sites have been described in the earlier section. What is most striking from the computer analysis is the degree of conservation of spacing between E2 sites #2, #3, and #4. As well as this, another interesting observation is that the #2 E2 site is usually the most degenerate site, and therefore, presumably, has the lowest affinity for E2 protein. This invites hypotheses about variable occupancy of E2 sites giving rise to differential regulation. Lastly, the strict conservation of E2 site #1 implies a more important function than the minor modulation of enhancer function reported for this site.

E1 The initiation of replication of papillomaviruses is modulated by the binding of the E1 protein to a single specific site in the LCR. The recognition of the origin of replication by E1 may be enhanced by complexing with E2 protein, which binds to either of the two flanking E2 binding sites [16,33,53,75]. The E1 protein has been thought to possess low sequence specificity, the binding site even being occasionally referred to as just an A/T rich segment. In Figure 4 we have extended published E1 binding sites and presumed homologies [44] and provide an E1 consensus site based on the sequences from 17 HPVs and the homologous sequences from BPV-1 and CRPV. The conserved region spans 20 base pairs with a highly conserved 3′ end and a modal consensus which is composed entirely of A/T sequences except for a single nucleotide, which shows no specific requirements. In figure 4 we have also included the consensus sequence found in over 90% of the HPVs tested, as well as the distances of the putative E1 site from the #3 E2 site and the TATA box. The conservation of many nucleotide sequences, as well as the conserved distance to the TATA box of the E6 promoter and one of the E2 sites influencing both the E6 promoter and replication initiation, argues for the existence of an E1 site in the same position in each HPV LCR.

HPV type	Start	Sequence	Distance (bp) from E2	Distance (bp) from TATA
HPV-16	7892	ATA**TAATAATACTAAACTACAAT**AATTC	21	51
HPV-35	7854	TTT**TTATTATAGTTAgTAACAAT**TATCC	21	51
HPV-33	7899	TTT**aTATAATAGTAAACTATAAT**GCCAA	21	51
HPV-31	7900	TTC**TTTTTATACTTAATAATAAT**AATCT	22	51
HPV-18	7853	TTT**TcATTAaTACTTTTAACAAT**TGTAG	18	49
HPV-45	7851	CTT**TTCTTAaTACTTTTAACAAT**TATAC	18	49
HPV-6	7892	TCC**TTCTTATAGTTAATAACAAT**CTTGG	17	46
HPV-11	7921	TCC**TTCTTATACTTAATAACAAT**CTTAG	17	46
HPV-34	7709	TTA**TAATTATAAAAAACTATAAT**CCTAC	18	49
HPV-26	7840	TCT**TTATAATAACTTTTAACAAT**TATAT	17	48
HPV-30	7843	TTA**TAATTATTGAAAgTTACAAg**CATAG	17	48
HPV-2	7856	GGT**TTATAATgTATAACTATAAT**CCTTT	18	49
HPV-27	6	GGT**TTATAATATATAACTATAAT**CCTTT	18	47
HPV-57	7856	GGT**TTATAATATATAACTATAAT**CCTTC	19	48
HPV-3	3	TAA**cTATAATTATAAATAACAAT**GCACA	19	49
HPV-10	7911	TAA**cTATAATTATAAACTATAAT**CTAGA	19	49
HPV-7	8016	TTC**TAGTTATTATgTTTAATAAT**TGTAC	17	47
BPV-1	7934	GAA**TAATTgTTGTTAACAATAAT**CACAC	none	43
CRPV	7858	TGA**TggTTgTTGCTAACAATAAT**TAAGA	21	115
Consensus:				
Modal		**TTATTATANTTAATAATAAT**		
90%		**TWNTWATWNHWWWYWAYAAT**		

Figure 4. Conservation of the E1 binding site in PVs. Putative E1 binding sites from 17 HPVs and the homologous sequences from BPV-1 and CRPV have been aligned and show a high degree of conservation with respect to both sequence and position within the LCR. The conserved region spans 20 base pairs with a highly conserved 3′ end and a modal consensus which is composed entirely of A/T sequences except for a single nucleotide, which shows no specific requirements. Also shown is the consensus sequence which is found in over 90% of the HPVs above, as well as the distances of the putative E1 site from the #3 E2 site and the TATA box. The conservation of many nucleotide sequences, as well as the conserved distance to the TATA box of the E6 promoter and one of the E2 sites influencing both the E6 promoter and replication initiation, argues for the existence of an E1 site in the same position in each HPV LCR.

Table 3 Transcription Factor Binding Sites in HPV LCRs

Group	HPV type	E2	NFI	YY1	Tef-1	AP-1	Octamer factor-1
A9	16	7454, 7861, 7942, 7957	7476, 7557, 7590, 7678, 7714, 7745, 7770	7438, 7483, 7517, 7600, 7619, 7647, 7706, 7822, 7983	7189, 7466, 7617, 7617, 7688, 7705, 7738, 7821	7306, 7634, 7651, 7814	7469, 7735, 7839 7975
A9	35	7442, 7823, 7904, 7919	7459, 7542, 7554, 7563, 7697, 7722, 7745	7135, 7405, 7658, 7705, 7794, 7973	7197, 7227, 7657, 7704, 7759	7578, 7595, 7778	7646, 7665, 7687, 7844, 7945, 7955
A9	33	7503, 7867 7949, 7964	7518, 7540, 7597, 7619, 7786, 7810, 7923	7188, 7293, 7429, 7468, 7487, 7748, 7791	7733, 7747	7352, 7677, 7832	7475, 7696, 7715, 7736, 7776, 7935
A9	31	7478, 7869, 7951, 7965	7568, 7608, 7799	7232, 7394, 7443, 7462, 7680, 7737, 7786, 7990	7214, 7490, 7678, 7736, 7989	7646, 7684, 7807, 7836	7291, 7725, 7813, 7850
A7	18	7459, 7823, 7900, 7916	7476, 7570, 7583, 7732	7284, 7424, 7444, 7555, 7693, 7809, 7936	7210, 7554, 7692, 7725, 7808, 7842	7326, 7609, 7793	7334, 7404, 7681, 7722, 7850, 7863, 7874
A7	45	7479, 7821, 7898, 7914	7581, 7730	7445, 7464, 7553, 7637, 7691, 7807	7272, 7428, 7552, 7690, 7806, 7845	7352, 7607, 7791	7361, 7651, 7679, 7720, 7861, 7947
A10	6	7549, 7862, 7938, 7953	7646, 7658, 7677, 7757, 7777	7792, 7886	7791, 7885	7437, 7714, 7835	7532, 7571, 7661, 7767, 7739
A10	11	7593, 7891, 7967, 7982	7670, 7690, 7702, 7786, 7806	7568, 7678, 7768, 7821, 7915	7431, 7677, 7914	7284, 7494, 7737, 7834	7730, 7614, 7796
A11	34	7298, 7678, 7756, 7772	7372, 7593	7252, 7268, 7321, 7509, 7525, 7574, 7601	7524, 7563, 7572, 7612	7435, 7483, 7558, 7637, 7651	7020, 7341, 7495
A5	26	7470, 7809, 7886, 7902	7543, 7573, 7586, 7724, 7749	7581, 7683, 7753, 7782	7104, 7244, 7291, 7682, 7717, 7751, 7766, 7810	7612	7168, 7671, 7714, 7735, 7769, 7826

Table 3 TranscriptionTranscription Factor Binding Sites in HPV LCRs (cont)

Group	HPV type	E2	NFI	YY1	Tef-1	AP-1	Octamer factor-1
A6	30	7491, 7812, 7889, 7906	7543, 7657, 7734, 7757	7459, 7481, 7763	7511, 7761	7638, 7796	7415, 7466, 7546, 7594, 7664, 7686, 7924
A4	2	7502, 7826, 7903, 7918	7708, 7540, 7778, 7792	7313, 7334, 7713, 7848	7312, 7379, 7846	7401, 7411	7435, 7517, 7624, 7749
A4	27	7478, 7801, 7876, 7891	7683, 7753, 7767	7823	7355, 7821	7377, 7387	7493, 7601, 7724?
A4	57	7494, 7828, 7904, 7919	7710, 7592, 7780, 7794	7850	7350, 7362, 7369, 7446, 7848, 7946	7288, 7390, 7400	7627, 7509, 7638
A2	3	7469, 7762, 7871, 7886	7568, 7703, 7728	7397, 7485, 7731	7343, 7396, 7559, 7729	7378, 7391, 7439, 7520	7800, 7857
A2	10	7554, 7850, 7959, 7974	7379, 7652, 7813	7337, 7644, 7668, 7819	7337, 7360, 7481, 7817	7524, 7603, 7692	7351, 7888
A8	7	7578, 7986, 8062, 8077	7595, 7706, 7774, 7926	7554, 7631, 7717, 7790, 7888, 7941	7371, 7741, 7788, 7887	7745, 7846, 7963	7795, 7917?, 8037
B	5	7492, 7582, 7647, 7759, 7789	7595, 7617, 7713, 7726, 7813	7504	none	7535	7485
E	1	3987, 4122, 4161, 4235	4085, 4118, 4154, 4216	3611, 3623, 3788	3789, 3831	4104	3460, 3597, 3788, 3836, 3957
E	41	7346, 7390, 7436, 7462, 7539, 7575, 7653	7292, 7364, 7409, 7455, 7543, 7554	7241, 7463, 7604	none	7130, 7281	7557

CONCLUSIONS

The analysis of potential transcription factor binding sites within the LCRs of genital HPVs was carried out in the hope that biologically important sites might become conspicuous due to their highly conserved nature. Probably the most obvious feature of the LCRs of all genital HPVs searched is the positioning of their E2 sites, with the highly conserved spacing of sites #2, #3 and #4, reflecting what is almost certainly a common theme for the regulation of E6/E7 promoter activity, and possibly replication as well. In nearly all cases the positioning of the #1 E2 site appears to delineate the 5′ end of the enhancer in which the vast majority of transcription factor binding sites can be found. Other obviously conserved features include the clustering of the NFI sites within the central portion of the LCR, the conservation of the octamer/NFI composite element at the 3′ end of the enhancer, and the positioning of the AP1 sites. Further sites that show conservation with respect to positioning may also represent functionally important aspects of HPV biology, either throughout the genital HPVs or among closely related viruses that may occupy a similar biological niche. In the end, only experimental analysis will determine if their conserved positioning is truly important.

Another outstanding feature of HPV LCRs is that degenerate binding sites seem to be the rule rather than the exception. One possible explanation for this observation is that degenerate sites with lower affinity for their cognate factors could result in lower levels of HPV gene expression. This might be an important part of the virus' strategy for long term persistence, unlike fulminant infections typical of many other viruses which may require high levels of gene expression. A second possible advantage to HPVs possessing degenerate sites is that they would have an inherent sensitivity to changes in the transcriptional environment, which would not be the case for those with enhancers made up of only high affinity sites. Degenerate sites could, for example, allow for a more subtle level of regulation as different degeneracies may have different affinities, thus allowing for differential occupancy of sites at a given local concentration of transcription factor. Degeneracy of sites would also play an important role in determining which particular sequences were bound by which factor in those situations in which more than one site overlap.

Finally, while the issue of epithelial specificity is still not resolved, it is unlikely that a single epithelial specific DNA binding factor, responsible for the specificity of all HPVs, would have been missed by researchers. This would suggest that either epithelial specific activity originates from several of the factors described above acting collectively, or alternatively, that an epithelial specific cofactor(s) may be involved. At present there have been epithelial specific functional aspects described for both NFI and AP1, however, no candidate epithelial specific cofactor has been found.

REFERENCES

[1] Andersen B, Schonemann M, Flynn S, Pearse R, Singh H, and Rosenfeld M: Skn-1a and Skn1i: Two functionally distinct Oct-2 related factors expressed in epidermis. *Science* 1993; **260**:78–82.

[2] Apt D, Chong T, Liu Y and Bernard HU: Nuclear factor I and epithelial cell-specific transcription of human papillomavirus type 16. *J. Virol*. 1993; **67**: 4455–4463.

[3] Apt D, Liu Y and Bernard HU: Cloning and functional analysis of spliced isoforms of human nuclear factor I-X: interference with transcriptional activation by NFI/CTF in a cell-type specific manner. *Nucleic Acids Res*. 1994; **22**:3825–3833.

[4] Auborn KJ and Steinberg BM: A key DNA-protein interaction determines the function of the 5′ URR enhancer of human papillomavirus type 11. *Virology* 1991;**181**:132–138.

[5] Baker CC and Howley PM: Differential promoter utilization by the bovine papillomavirus in transformed cells and productively infected wart tissues. *EMBO J*. 1987; **61**:1027–1035.

[6] Bartsch D, Boye B, Baust C, zur Hausen H, and Schwarz E: Retinoic acid-mediated repression of human papillomavirus 18 transcription and different ligand regulation of the retinoic acid receptor beta gene in non-tumorigenic and tumorigenic HeLa hybrid cells. *EMBO J*. 1992;**11**:2283–2291.

[7] Bauknecht T, Angel P, Royer H-D, and zur Hausen H: Identification of a negative regulatory domain in the human papillomavirus type 18 promoter: interaction with the transcriptional repressor YY1. *EMBO J*. 1992; **11**:4607–4617.

[8] Bauknecht T, Jundt F, Herr I, Oehler T, Delius H, Shi Y, Angel P, and zur Hausen H: A switch region determines the cell type-positive or negative action of YY1 on the activity of the human papillomavirus type 18 promoter. *J. Virol*. 1995; **69**:1–12.

[9] Baumruker T, Sturm R, and Herr W: OBP100 binds remarkably degenerate octamer motifs through specific interactions with flanking sequences. *Genes Dev*. 1988; **2**:1400–1413.

[10] Bernard HU and Apt D: Transcriptional control and cell type specificity of HPV gene expression. *Arch. Dermatol*. 1994;**130**:210–215.

[11] Boseman Roberts S, Segil N, and Heintz N: Differential phosphorylation of the transcription factor Oct-1 during the cell cycle. *Science* 1991; **253**:1022–1026.

[12] Bouvard V, Storey A, Pim D, and Banks L: Characterization of the human papillomavirus E2 protein: evidence of trans-activation and trans-repression in cervical keratinocytes. *EMBO J*. 1994;**13**:5451–5459.

[13] Butz K, and Hoppe-Seyler, F: Transcriptional control of human papillomavirus (HPV) oncogene expression: Composition of the HPV type 18 upstream regulatory region. *J. Virol*. 1993; **67**:6476–6486.

[13a] Chan SY, Delius H, Halpern AL, and Bernard HU: Analysis of genomic sequences of 95 papillomavirus types: Uniting typing, phylogeny, and taxonomy. *J. Virol*. 1995, **69**:3074–83.

[14] Chan WK, Klock G, and Bernard HU: Progesterone and glucocorticoid response elements occur in the long control regions of several human papillomaviruses involved in anogenital neoplasia. *J. Virol*. 1989; **63**:3261–3269.

[15] Chan WK, Chong T, Bernard HU, and Klock G: Two AP1 sites in the long control region of human papillomavirus type 16 lead to phorbolester stimulation of the viral E6/E7 promoter. *Nucleic Acids Res*. 1990;**18**:763–769.

[16] Chiang CM, Dong G, Broker TR and Chow LT: Control of human papillomavirus type 11 origin of replication by the E2 family of transcription regulatory proteins. *J. Virol*. 1992; **66**:5224–5231.

[17] Chin MT, Broker TR, and Chow LT: Identification of a novel constitutive enhancer element and an associated binding protein: implications for human papillomavirus type 11 enhancer regulation. *J. Virol*. 1989;**63**: 2967–2977.

[18] Chong T, Chan WK, and Bernard HU: Transcriptional activation of human papillomavirus 16 by nuclear factor I, AP1, steroid receptors and a possibly novel transcription factor, PVF: a model for the composition of genital papillomavirus enhancers. *Nucleic Acids Res*. 1990; **18**:465–470.

[19] Chong T, Apt D, Gloss B, Isa M, and Bernard HU: The enhancer of human papillomavirus type 16: Binding sites for the ubiquitous transcription factors oct-1, NFA, TEF-2, NFI, and AP-1 participate in epithelial cell-specific transcription. *J. Virol*. 1991;**65**:5933–5943.

[20] Cid A, Auewarakul P, Garcia-Carranca A., Ovseiovich R, Gaissert H, and Gissmann L: Cell-type-specific activity of the human papillomavirus type 18 upstream regulatory region in transgenic mice and its modulation by tetradecanoyl phorbol acetate and glucocorticoids. *J. Virol*. 1993;**67**:6742–6752.

[21] Cripe TP, Alderborn A., Anderson RD, Parkkinen S, Bergman P, Haugen TH, Petterson U, and Turek LP: Transcriptional activation of the human papillomavirus-16 P97 promoter by an 88-nucleotide enhancer containing distinct cell-dependent and AP-1 responsive modules. *New Biol*. 1990;**2**:450–463.

[22] Cripe TP, Haugen TH, Turk JP, Tabatabai F, Schmid PG, Dürst M, Gissmann L., Roman A, and Turek L: Transcriptional regulation of the human papillomavirus-16 E6-E7 promoter by a keratinocyte dependent enhancer, and by viral E2 transactivator and repressor gene products: implications for carcinogenesis. *EMBO J*. 1987;**6**:3745–3753.

[23] Cuthill S, Sibbet GJ and Campo MS: Characterization of a nuclear factor, papillomavirus enhancer binding factor-1, that binds the long control region of human papillomavirus type 16 and contributes to enhancer activity. *Mol. Carcinog*. 1993;**8**:96–104.

[24] Davidson I, Xiao JH, Rosales R, Staub A, and Chambon P: The HeLa cell protein TEF-1 binds specifically and cooperatively to two SV40 enhancer motifs of unrelated sequence. *Cell* 1988; **54**:931–942.

[25] deBoer E, Antoniou M, Mignotte V, and Grosveld F: The human β-globin promoter: nuclear protein factors and erythroid specific induction of transcription. *EMBO J*. 1988; **13**:4203–4212.

[26] Demeret C, Yaniv M and Thierry F: The E2 transcriptional repressor can compensate for SP1 activation of the human papillomavirus type 18 early promoter. *J. Virol*.1994;**68**:7075–7082.

[26a] Dostatni N, Lambert PF, Sousa R, Ham J, Howley PM, and Yaniv M. The functinal BPV-1 E2 transactivating protein can act as a repressor by preventing formation of the initiation complex. *Genes Dev*. 1991;**5**:1657–1671.

[27] deVries E, van Driel W, Tromp M, van Boom J, and van der Vliet PC: Adenovirus DNA replication in vitro: site-directed mutagenesis of the nuclear factor I binding site of the Ad2 origin. *Nucleic Acids Res*. 1985;**13**:4935–4952.

[28] Dong XP, Stubenrauch F, Beyer-Finkler E, and Pfister H: Prevalence of deletions of YY1-binding sites in episomal HPV-16 DNA from cervical cancers. *Int. J. Cancer* 1994;**58**:803–808.

[29] Ensser A. and Pfister H: Epidermodysplasia verruciformis associated human papillomaviruses present a subgenus-specific organization of the regulatory genome region. *Nucleic Acids Res*. 1990;**18**:3919–3922.

[30] Fanjul A, Dawson MI, Hobbs PD, Jong L, Cameron JF, Harlev E, Graupner G, Lu XP, and Pfahl M: A new class of retinoids with selective inhibition of AP-1 inhibits proliferation. *Nature* (London) 1994;**372**:107–111.

[31] Faus I, Huey-Juang H, and Fuchs E: Oct-6: a regulator of keratinocyte gene expression in stratified squamopus epithelia. *Mol. Cell Biol*. 1994; **14**:3263–3275

[32] Fletcher C, Heintz N, and Roeder, R: Purification and characterization of OTF-1, a transcription factor regulating cell cycle expression of a human histone H2B gene. *Cell* 1987; **51**:773–781.

[33] Frattini MG and Laimins LA: Binding of the human papillomavirus E1 origin recognition protein is regulated through complex formation with the E2 enhancer-binding protein. *Proc. Natl. Acad. Sci. USA* 1994;**91**:12398–12402.

[34] Fromental C, Kanno M, Nomiyama H, and Chambon P: Cooperativity and hierarchical levels of functional organization in the SV40 enhancer. *Cell* 1988; **54**:943–953.

[35] Fuchs, PG and Pfister H: Transcription of papillomavirus genomes. *Intervirology* 1994;**37**:159–167.

[36] Furth PA and Baker CC: An element in the bovine papillomavirus late 3′ untranslated region reduces polyadenylaed cytoplasmic RNA levels. *J. Virol*. 1991;**65**:5806–5812.

[37] Garcia-Carranca A, Thierry F, and Yaniv M: Interplay of viral and cellular proteins along the long control region of human papillomavirus type 18. *J. Virol*. 1988;**62**: 4321–4330.

[38] Gill G, and Ptashne M: Negative effect of the transcriptional activator GAL4. *Nature* 1988; **334**:721–724.

[39] Gloss B, Bernard HU, Seedorf K and Klock G: The upstream regulatory region of the human papillomavirus-16 contains an E2 protein independent enhancer which is specific for cervical carcinoma cells and regulated by glucocorticoid hormones. *EMBO J*. 1987; **6**: 3735–3743.

[40] Gloss B, Chong T, and Bernard HU: Numerous nuclear proteins bind the long control region of human papillomavirus type 16: A subset of 6 of 23 DNAseI-protected segments coincides with the location of the cell-type-specific enhancer. *J. Virol*. 1989; **63**:1142–1152.

[41] Gloss B and Bernard HU: The E6/E7 promoter of human papillomavirus type 16 is activated in the absence of E2 proteins by a sequence-aberrant Sp1 distal element. *J. Virol*. 1990;**64**:5577–5584.

[42] Gualberto A, Lepage D, Pons G, Mader S, Park K, Atchinson M, Walsh K: Functional antagonism between YY1 and serum responsive factor. *Mol. Cell Biol*. 1992; **12**:4209–4214.

[43] Ham J, Dostatni N, Gauthier JM, and Yaniv M: The papillomavirus E2 protein: a factor with many talents. *Trends Biochem. Sci*. 1991;**16**:440–444.

[44] Holt SE, Schuller G, and Wilson VG: DNA binding specificity of the bovine papillomavirus E1 protein is determined by sequences contained within an 18 bp inverted repeat element at the origin of replication. *J. Virol*. 1994;**68**:1094–1102.

[45] Hoppe-Seyler F and Butz K, and zur Hausen, H: Repression of the human papillomavirus type 18 enhancer by the cellular transcription factor Oct-1. *J. Virol*. 1991; **65**:5613–5618.

[46] Hoppe-Seyler F and Butz K: Cellular control of human papillomavirus oncogene transcription. *Mol. Carcinog*. 1994;**10**:134–141.

[47] Ishiji T, Lace MJ, Parkinen S, Anderson RD, Haugen RH, Cripe TP, Xiao JH, Davidson I, Chambon P, and Turek LP: Transcriptional enhancer factor (TEF)-1 and its cell-specific coactivator activate human papillomavirus-16 E6 and E7 oncogene transcription in keratinocytes and cervical carcinoma cells. *EMBO J*. 1992;**11**: 2271–2281.

[48] Kennedy IM, Haddow JK, and Clements JB: A negative regulatory element in the human papillomavirus type 16 genome acts at the level of late mRNA stability. *J. Virol*. 1991;**65**:2093–2097.

[49] Kyo S, Inoue M, Nishio Y, Nakanishe K, Akira S, Inoue H, Yutsudo M, Tanizawa O, and Hakura A: NF-IL6 represses early gene expression of human papillomavirus type16 through binding to the noncoding region. *J. Virol*. 1993; **67**:1058–1066.

[50] Lambert PF: Papillomavirus DNA replication. *J. Virol*. 1991;**65**:3417–3420.

[50a] Lee T-C, Shi Y, and Schwartz R: Displacement of BrdUrd-induced YY1 by serum responsive factor activates skeletal a-actin transcription in embryonic myoblasts. *Proc. Natl. Acad. Sci. USA* 1992; **89**:9814–9818.

[51] Leechanachai P, Banks, L, Moreau, F, Matlashewski G: The E5 gene from human papillomavirus type 16 is an oncogene which enhances growth factor-mediated signal transduction to the nucleus. *Oncogene* 1992;**7**:19–25.

[52] Lewis B, Tullis G, Seto E, Horikoshi N, Weinmann R, and Shenk T: Adenovirus E1A proteins interact with the cellular YY1 transcription factor. *J. Virol*. 1995; **69**:1628–1636.

[53] Lu JZJ, Sun YN, Rose RC, Bonnez W, and McCance DJ: Two E2 binding sites (E2BS) alone or one E2BS plus an A/T-rich region are minimal requirements for the replication of the human papillomavirus type 11 origin. *J. Virol*. 1993;**67**:7131–7139.

[54] Mack DH and Laimins LA: A keratinocyte specific transcription factor, KRF-1, interacts with AP-1 to activate expression of human papillomavirus type 18 in squamous epithelial cells. *Proc. Natl. Acad. Sci. USA* 1991;**88**:9102–9106.

[55] Martin K, Lillie J, and Green, M: Evidence for interaction of different eukaryotic transcriptional activators with distinct cellular targets. *Nature* 1990; **346**:147–152.

[56] May M, Dong XP, Beyer-Finkler E, Stubenrauch F, Fuchs PG, and Pfister H: The E6/E7 promoter of extrachromosomal HPV-16 DNA in cervical cancers excapes from cellular repression by mutation of target sequences for YY1. *EMBO J*. 1994; **13**:1460–1466.

[57] McBride AA, Romanszuk H, and Howley PM: The papillomavirus E2 regulatory proteins. *J. Biol. Chem*. 1991;**266**:18411–18414.

[58] Mermod N, O'Neill EA, Kelly TJ, and Tjian R: The proline-rich transcriptional activator of CTF/NFI is distinct from the replication and DNA binding domain. *Cell* 1989;**58**:741–753.

[59] Montalvo E, Shi Y, Shenk T, and Levine A: Negative regulation of the BZLF1 promoter of Epstein-Barr virus. *J. Virol*. 1991; **65**:3647–3655.

[60] Müller M, Gissmann L, Cristiano RJ, Sun XY, Frazer IH, Jenson AB, Alonso A, Zentgraf H, and Zhou J: Papillomavirus capsid biding and uptake by cells from different tissues and species. *J. Virol*. 1995;**69**:948–954.

[61] Nagata K, Guggenheimer RA, and Hurwitz J: Specific binding of a cellular DNA replication protein to the origin of replication of adenovirus DNA. *Proc. Natl. Acds. Sci. USA* 1983;**80**:6177–6181.

[62] Natesan S, and Gilman M: DNA bending and orientation-dependent function of YY1 in the c-fos promoter. *Genes Dev*. 1993; **7**:2497–2509.

[63] O'Connor M and Bernard HU: Oct-1 activates the epithelial-specific enhancer of human papillomavirus type 16 via a synergistic interaction with NFI at a conserved composite regulatory element. *Virology* 1995;**207**:77–88.

[64] Pater MM, Hughes GA, Hyslop DE, Nakshatri H and Pater A: Glucocorticoid-dependent oncogenic transformation by type 16 but not by type 11 human papillomavirus DNA. *Nature* (London) 1988; **335**:832–835.

[65] Roden RBS, Kirnbauer R, Jenson AB, Lowy DR, and Schiller JT: Interaction of Papillomaviruses with the cell surface. *J. Virol*. 1994;**68**:7260–7266.

[66] Romanczuk H, Thierry F, and Howley PM: Mutational analysis of cis-elements involved in E2 modulation of human papillomavirus type 16 P97 and type 18 P105 promoters. *J. Virol*. 1990;**64**:2849–2859.

[66a] Rosefeld MG: POU-domain transcription factors: Pou-er-ful developmental regulators. *Genes Dev*. 1991;**5**:897–907.

[67] Russel J and Botchan MR: cis-acting components of human papillomavirus (HPV) DNA replication: linker substitution analysis of the HPV type 11 origin. *J. Virol*. 1995;**69**:651–660.

[68] Ruvkun G, and Finney M: Regulation of transcription and cell identity by POU domain proteins. *Cell* 1991; **64**:475–478.

[69] Seto E, Shi Y, and Shenk T: YY1 is an initiator sequence-binding protein that directs and activates transcription in vitro. *Nature* 1991; **354**:241–245.

[70] Seto E, Lewis B, and Shenk T: Interaction between transcription factors Sp1 and YY1. *Nature* 1993; **365**:462–464.

[71] Shi Y, Seto E, Chang L-S, and Shenk T: Transcriptional repression by YY1, a human GLI-Kruppel-related protein, and relief of repression by adenovirus E1A protein. *Cell* 1991; **67**:377–388.

[72] Shrivastava D, Saleque S, Kalpana G, Artandi S, Goff S, Calame K: Inhibition of transcriptional regulator YY1 by association with c-myc. *Science* 1993; **262**:1889–1892.

[73] Sibbet GJ and Campo MS: Multiple interactions between cellular factors and the non-coding region of human papillomavirus type 16. J. Gen. Virol.1990;**71**:2699–2707.

[74] Stubenrauch F, Malejczyk J, Fuchs PG, and Pfister H: Late promoter of human papillomavirus type 8 and its regulation. *J. Virol*. **1994**:**66**:3485–3493.

[75] Sverdrup F and SA Khan: Two E2 binding sites alone are sufficient to function as the minimal origin of replication of human papillomavirus type 18 DNA. *J. Virol*. 1995;**69**:1319–1323.

[76] Tan SH, Leong LEC, Walker PA, and Bernard HU: The human papillomavirus type 16 transcription factor E2 binds with low cooperativity to two flanking binding sites and represses the E6 promoter by displacement of Sp1 and TFIID. *J. Virol*. 1994; **68**:6411–6420.

[77] Thierry F, Spyrou G, Yaniv M, and Howley PM: Two AP1 sites binding junB are essential for human papillomavirus type 18 transcription in keratinocytes. *J. Virol*. 1992; **66**:3740–3748.

[78] Thierry F: Proteins involved in the control of HPV transcription. *Papillomavirus Rep*. 1993;**4**:27–32.

[79] Tjian R and Maniatis, T: Transcriptional activation: A complex puzzle with few easy pieces. *Cell* 1994; **77**:5–8.

[80] Turek LP: The structure, function and regulation of papillomavirus genes in infection and cervical cancer. *Adv. Virus. Res*. 1994; **44**:305–356.

[81] Usheva A, and Shenk T: TATA-binding protein-independent initiation:YY1, TFIIB, and RNA polymerase II direct basal transcription on supercoiled template DNA. *Cell* 1994; **76**:1115–1121.

[81a] Verrijzer CP, Arnoud JK, and van der Vliet PC: The Oct-1 homeo domain contacts only part of the octamer sequence and full Oct-1 DNA binding activity requires the POU-specific domain. *Genes Dev*. 1990;**4**:1964–1974.

[82] Vogt P, and Bos T: Jun oncogene and transcription factor. *Adv. Cancer Res*. 1990; **55**:1–35.

[83] Wang H-G, Rikatake Y, Carter M, Yaciuk P, Abraham S, Zerler B, and Moran E: Identification of specific adenovirus E1A N-terminal residues critical to the binding of cellular proteins to the control of cell growth. *J. Virol*. 1993; **67**:476–488.