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Contributions in the domain of cancer research: Review

Human papillomaviruses and their role in cervical cancer

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Abstract. Human papillomaviruses (HPVs) have been linked to a variety of human diseases, most notably cancer of the cervix, a disease responsible for at least 200,000 deaths per year worldwide. Over 100 different types of HPV have been identified and these can be divided into two groups. Low-risk HPV types are the causative agent of benign warts. High-risk HPV types are associated with cancer. This review focuses on the role of high-risk HPV types in cervical tumorigenesis. Recent work has uncovered new cellular partners for many of the HPV early proteins and thrown light on many of the pathways and processes in which these viral proteins intervene. At the same time, structural and biochemical studies are revealing the molecular details of viral protein function. Several of these new avenues of research have the potential to lead to new approaches to the treatment and prevention of cervical cancer.

Key words. Cervical cancer; papillomavirus; gene expression; viral oncogene; early protein; tumour suppressor protein; p53; Rb.

Introduction

Cervical cancer is a major cause of cancer-related death in women. Despite the existence of screening programs, this disease is still responsible for over 10,000 deaths per year in the European Union [1]. In countries with little or no screening, it can even represent the most common cause of cancer-related death in women [2]. This review will first outline the role of high-risk human papillomavirus (HPV) types and some possible viral co-factors in cervical cancer. We will then describe the roles of the viral early proteins (E6, E7, E5, E2 and E1) in HPV infection and tumorigenesis. Finally, we will survey new approaches to the treatment and prevention of this disease.

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Human papillomaviruses

Many of the 100 or so different types of HPV identified to date infect epithelial cells and bring about the formation of benign hyperproliferative lesions more commonly known as warts. For example, HPV types 6 and 11 both infect the genital tract and produce genital warts. In contrast to these so-called low-risk HPV types, some HPVs, for example HPV 16 and 18, are linked to cancer [3]. Genital HPVs infect cells in the basal layer of the cervical epithelium and the differentiation of these cells as they migrate up through the different layers of the epithelium is required for productive viral replication. Until very recently, this requirement for host cell differentiation has been a significant obstacle to research on HPV replication [3, 4].

The HPV genome contains ~8 kb of double-stranded DNA and is usually maintained in cells as a supercoiled closed circular molecule or episome, with the viral open reading frames (ORFs) occupying only one strand of the

DNA (fig. 1). These ORFs encode a variety of proteins that have been classed as early (E) or late (L) depending on their supposed time of expression in infected cells [4] and the properties of these proteins are summarized in Table 1. Several splice variants that encode truncated proteins or fusion proteins containing amino acid sequences from more than one viral protein have also been described. The transcription of all these ORFs is regulated by the viral E2 protein and by a variety of cellular transcription factors. The binding sites for these regulatory proteins are mostly clustered between the start of the E6 ORF and the end of the L1 ORF, a region of the viral genome known as the long control region (LCR) or upstream regulatory region (URR). This region of the viral genome also contains the origin of replication [4].

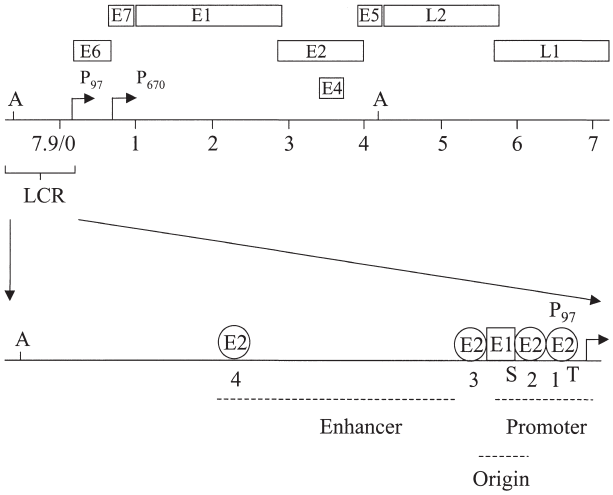

Figure 1. The HPV 16 genome. The top line shows the arrangement of the ORFs within the 7.9-kb HPV 16 genome. The bent arrows represent transcription start sites. Polyadenylation signals are indicated by an A. The bottom line shows an expanded view of the long control region (LCR) with the TATA box and Sp1-binding site indicated by T and S, respectively.

Table 1. The HPV proteins.

Viral protein	Function	Some of their partner proteins
E6	oncoprotein	p53, E6-AP, hDLG, hScrib, Paxillin, Bak, IRF-3
E7	oncoprotein	Rb, p107, p130, TBP, ISGF3, AP1, Mi2β
E5	oncoprotein (?)	EGF receptor, PDGF receptor, vacuolar ATPase.
E2	transcription/ replication factor	E1, Sp1, AMF-1, TBP, TFIIB, p53, CBP, RPA
E1	replication factor	E2, DNA polymerase α-primase, cyclin E-CDK2
E4	facilitate viral escape (?)	
L1	capsid protein	
L2	capsid protein	

HPVs in cervical tumorigenesis

HPV DNA can be detected in virtually all cervical cancers (99.7%) [5]. HPV 16 is the most common viral type found in these tumours (~50%), followed by HPV 18 (~15%), HPV 45 (~8%) and HPV 31 (~5%). The remaining cervical cancers contain any of around a dozen less common high-risk HPV types [5]. These data strongly suggest that HPV infection is a causative agent of cervical cancer. However, it is important to note that HPV infection alone is probably insufficient to bring about cancer, since high-risk HPV infection is common in women with normal cervical cytology and most of these infected women will never go on to develop cervical cancer [6, 7]. In cervical warts, HPV DNA is present as episomal DNA molecules that do not generally integrate into the host genome. In contrast, cervical cancer cells often contain chromosomally integrated HPV DNA or a mixture of both integrated and non-integrated viral DNA (fig. 2) [8–12]. In addition, the episomal HPV DNA present in cervical tumours can be multimeric or contain mutations in the LCR [11–14]. This suggests that integration, or some other event that disturbs the organisation and/or expression of the viral DNA such as multimerisation or mutation, is a critical step in HPV-induced tumorigenesis.

Cells that contain integrated HPV 16 DNA have a selective growth advantage over cells containing non-integrated HPV DNA [15, 16]. This is presumed to be because the integrated HPV genomes retain the E6 and E7 ORFs and are therefore capable of over-producing the E6 and E7 oncoproteins (fig. 2). Deregulated expression of these oncoproteins is thought to lead to cell transformation and eventually to cancer. The sites in the human genome at which HPV DNA integrates do not appear to be a significant factor in tumorigenesis although integration often occurs at common fragile sites [12, 17]. However, relatively few integration sites have been mapped in detail and further studies might reveal locations that are associated with increased cancer risk or variations in tumour aggression [12, 18].

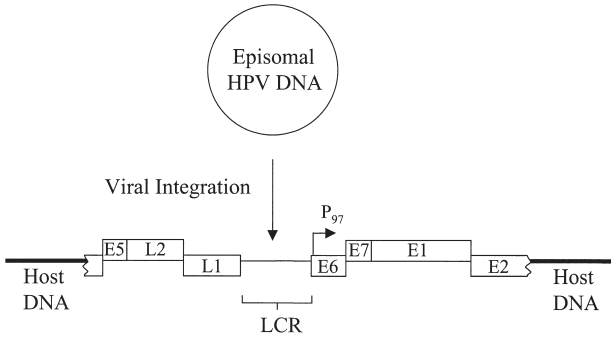

Figure 2. Viral integration in cancer cells. Viral integration often disrupts the E2 ORF. Host chromosomal DNA is represented by the thick line.

Chromosomal integration of the HPV genome commonly results in the disruption of the viral E2 ORF and, as a consequence, loss of the E2 protein [19, 20]. In addition, disruption of the E2 ORF has been shown to increase the ability of HPV 16 to immortalise cells [21]. These observations suggest that the loss or inactivation of the E2 protein might be important in cervical tumorigenesis [22]. Since the E2 protein regulates transcription of E6 and E7, loss of the E2 protein must bring about the deregulated expression of these oncoproteins. However, recent work from a number of laboratories indicates that this might not be the entire story and that the E2 protein (or its absence) might also play a direct role in tumorigenesis [23–26].

Co-factors in cervical tumorigenesis

A number of possible co-factors for HPV infection in cervical tumorigenesis have been identified, the most plausible of which are immunosuppression, smoking and the steroid hormones progesterone and oestrogen. Immunosuppressed individuals, such as renal transplant patients and persons infected with human immunodeficiency virus (HIV), show increased levels of HPV infection and cervical cancer [27–31]. Smoking also appears to increase the risk of cervical cancer [32]. Cigarette smoke by-products are present in the cervical mucus of active and passive smokers and these chemicals could increase cancer risk via their mutagenic effects on cervical cells [33]. Smoking is also thought to decrease the number of antigen-presenting Langerhans cells in the cervix, which might lessen the local immunity to HPV infection [34, 35].

The possibility that progesterone and/or oestrogen from oral contraceptives might play a role in HPV-induced cervical cancer has been investigated intensively. The available evidence suggests that any increase in risk associated with oral contraceptive use is very small [36]. However, both progesterone and oestrogen can influence HPV 16 and HPV 18 gene expression [37–42]. In addition, most cases of cervical cancer arise in a region of the cervix known as the transformation zone, which is believed to be particularly oestrogen sensitive [43, 44]. This region of the cervix is not called the transformation zone because it is the area in which HPV-transformed cells arise. Rather, the term refers to the area in which columnar epithelium, typical of the endocervix (inside surface), has been replaced by squamous epithelium, typical of the ectocervix (outside surface). The oestrogen metabolite 16α-hydroxyestrone is known to cause DNA damage [45] and one intriguing possibility is that this damage might result in the accumulation of mutations that act synergistically with HPV to induce tumorigenesis [46, 47].

The E6 protein

The HPV 16 E6 and E7 proteins can act in concert to immortalise human foreskin keratinocytes [48], and although E6 alone is unable to immortalise these cells in vitro, this protein can produce tumours in transgenic mice [49]. The E6 protein is relatively small and has no intrinsic enzymatic activity. Instead, E6 seems to exert its transforming functions by interacting with cellular proteins, most notably the tumour suppressor gene p53, which it targets for ubiquitin-mediated degradation [50–52]. The HPV 16 E6 protein contains 151 amino acids and is approximately 18 kDa in size. Gel filtration and analytical ultracentrifugation have shown that E6 is probably monomeric in solution but can form dimers at high protein concentrations [53]. A truncated form of E6, known as E6*, has also been identified in some HPV-transformed cell lines [54]. The E6 and E7 ORFs show clear homology and may have been formed by duplication of a 33-amino-acid unit containing a cysteine doublet (C-x-x-C) [55]. Such cysteine doublets often co-ordinate a zinc ion and can mediate nucleic acid binding and/or protein-protein interactions. E6 contains four C-x-x-C motifs, suggesting that the protein has two zinc fingers. Consistent with this view, the E6 protein has two zinc-binding domains [56, 57]. However, remarkably little is known about the structure of E6. Efforts to determine the structure of this protein have been hampered by the difficulty to produce it in large quantities and also because it appears to be relatively unstable. These difficulties are due, in part at least, to the unusually high proportion of cysteine residues in this protein (around 10% in HPV 16 E6) and their effect on protein solubility [58]. Two recent studies have overcome these problems, one by mutating non-zinc finger cysteines to serine, and one by removing the C-terminal nine residues of the protein [53, 58]. Like the wild-type protein, these E6 derivatives bind two zinc ions per molecule [53, 58].

Over-expression of the E6 protein can influence the activity of cellular promoters [59–62] and although this could be an indirect effect of E6 mediated by cellular proteins, the presence of potential zinc fingers suggests that E6 itself might be able to bind DNA and function as a transcription factor [57]. In fact, the C-terminal zinc-binding domain of E6 shows significant sequence similarity to part of endonuclease VII from the T4 bacteriophage [63]. T4 endonuclease VII binds and cleaves four-way DNA junctions formed as intermediates in DNA recombination. Recent work has shown that the HPV 16 E6 protein can also bind specifically to four-way DNA junctions [58]. These observations suggest a direct role for E6 in gene regulation. However, in the case of bovine papillomavirus 1 (BPV 1) E6 protein, mutants that fail to transform cells retain the ability to activate transcription [61]. As a consequence of this and the work outlined in

the following section, the interactions of E6 with cellular proteins have received most attention in recent years.

E6 and cellular proteins

E6 exerts at least part of its biological activity by binding to the cellular tumour suppressor protein p53 [50–52]. The central importance of p53 in tumour suppression is illustrated by the fact that it is inactivated by mutation in around half of all human tumours [64]. In response to a number of stimuli, including ionising radiation, cell stress or viral infection, the p53 protein mediates either cell cycle arrest or apoptosis, a form of programmed cell death. Thus, for example, the activation of p53 in response to ionising radiation gives cells time to repair any damaged DNA or, if the insult is too great to be countered effectively, induces cell suicide (fig. 3). p53 is a DNA-binding protein that can regulate transcription from promoters that contain p53-binding sites [65]. Some of the cellular responses to p53 activation stem from the effects of p53 on promoter activity. However, some responses to p53 activation are independent of its effects on gene expression [66–68].

The E6 proteins from high-risk HPV types bind to p53 in conjunction with a ubiquitin-ligase known as E6-AP or ubiquitin protein ligase 3A (fig. 3) [69, 70]. The ubiquitination of p53 that occurs as a result of complex formation targets this protein for proteasome-mediated degradation, and significantly reduces the half-life of p53 [50–52]. The decrease in p53 levels brought about by E6 is thought to be important for viral replication, since over-expression of p53 can inhibit this process [71, 72]. Furthermore, the decrease in p53 levels is probably important for the tumorigenic effects of high-risk HPV types [53, 73]. In effect, the presence of the E6 protein substitutes for the mutations that frequently inactivate p53 in non-HPV-induced tumours. A common polymorphism results in either arginine or proline at amino acid 72 of p53, and in cells, p53-R72 appears to be more susceptible to E6-induced degradation than p53-P72 [74]. An analysis of a small number of cervical tumours and normal controls suggested that the presence of the p53-R72 allele could be a risk factor in HPV-induced cancer [74]. However, studies with larger sample sizes have generally failed to show a correlation between the presence of this allele and tumorigenesis [75–78]. The picture is complicated by the fact that within particular HPV types, there are E6 sequence variants which may interact differently with different forms of p53 [79, 80].

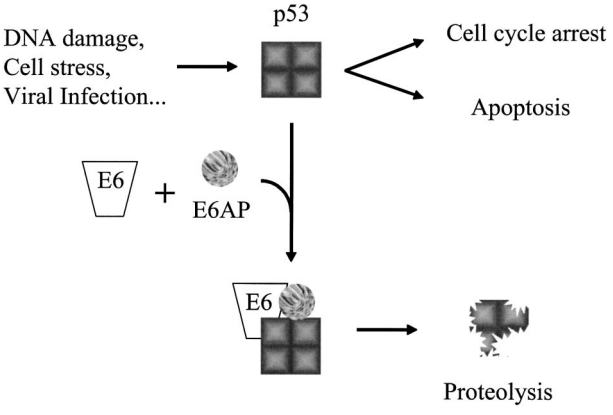

Figure 3. E6 targets p53. Activated p53 induces either cell cycle arrest or apoptosis. E6 binds to p53 in conjunction with E6-AP and targets p53 for proteolysis.

Given all this emphasis on the E6-induced degradation of p53, it is important to point out that mutations in HPV 16 E6 that block the ability of this protein to induce p53 degradation do not block immortalisation [81, 82]. This suggests that other aspects of E6 function are important in tumorigenesis. Interestingly, another mechanism whereby E6 can modulate the activity of p53 has recently been uncovered. p53 can recruit the transcriptional co-activator protein CBP/p300 to p53-responsive promoters and this plays a role in transcription activation [83–85]. The binding of E6 to p53 blocks the interaction of p53 with CBP/p300 and this can down-regulate p53-dependent transcription [86, 87]. Although E6 induces increased p53 degradation, HPV-transformed cells that express the E6 protein still contain some functional p53 protein and can be stimulated to undergo p53-mediated growth arrest and apoptosis [88]. The effects of E6 on p53-dependent transcription may thus represent a *bona fide* mechanism whereby E6 can modulate p53 activity.

E6 interacts with a wide variety of other cellular proteins, some of which may contribute to tumorigenicity. As mentioned previously, E6 binds to E6-AP [70]. The binding of HPV 16 E6 to E6-AP triggers the ubiquitination and degradation of E6-AP, which could be important in E6-induced transformation [82]. The E6 proteins from HPV 16 and 18 also bind to the tumour suppressor human discs large (hDlg) and target this protein for ubiquitin-mediated degradation [89]. The decrease in hDlg levels probably plays a role in E6-mediated deregulation of the cell cycle and loss of cell differentiation [89–91]. Scribble is a *Drosophila* protein that co-operates with Dlg in a pathway controlling epithelial cell growth [92]. The E6 proteins from HPV 16 and 18 bind to hScrib, the human homologue of scribble, and target the protein for degradation [92]. The targeting of both these cellular proteins for degradation suggests that this is an important component of the biological activity of E6 proteins from high-risk HPV types.

Further E6-interacting proteins include Paxillin and Bak; although others have been identified, for the sake of brevity they will not be discussed here. Paxillin is a focal adhesion protein implicated in integrin signalling and cell transformation [93]. The E6 proteins from high- but not low-risk HPV types bind to Paxillin, suggesting that this

interaction might be important in tumorigenesis [94, 95]. Furthermore, BPV 1 E6 mutants that fail to interact with Paxillin also fail to transform cells [96]. The E6 proteins from both high- and low-risk HPV types bind to the pro-apoptotic protein Bak (a member of the Bcl-2 family) and target this protein for degradation [97]. The effect of HPV 11 E6 is weaker than that of the HPV 16 and HPV 18 E6 proteins, suggesting that the ability of E6 to circumvent this apoptotic trigger may be important in transformation [97].

The regulation of E6 activity

E6 and E7 are under the control of the same promoter and are translated from a bicistronic mRNA [98]. The E6/E7 mRNA is present at low levels in the basal layer of organotypic raft cultures and its expression seems to precede immortalisation [99]. Higher levels of the E6/E7 mRNA are found in tumours and this has often been referred to as derepression [100, 101]. Little is known about how E6 protein levels or activity are regulated. The E6 protein is a short-lived phosphoprotein and is difficult to detect in HPV-transformed cells [57]. At least two kinases phosphorylate E6. Phosphorylation of E6 by protein kinase A (PKA) blocks the E6-hDlg interaction and inhibits E6-dependent degradation of this protein [102]. However, phosphorylation by PKA does not seem to inhibit the E6-dependent degradation of p53, suggesting that phosphorylation can determine the targets of E6 in vivo [102]. Protein kinase N (PKN) binds tightly to the E6 proteins from HPV 16 and 18 but poorly to the E6 proteins from HPV 6 and 11 [103]. PKN phosphorylates HPV 16 E6 in vivo and mutants of E6 that fail to bind PKN are unable to immortalise normal mammary epithelial cells [103]. This would seem to indicate that the binding of PKN to E6 and its subsequent phosphorylation are probably important in tumorigenesis.

The E7 protein

E7 can co-operate with an activated ras oncogene to transform primary cells [104]. In HPV-infected cells, E7 perturbs cell differentiation and induces DNA synthesis, creating a favourable environment for viral replication [105]. In large part at least, E7 brings about these effects by binding to the retinoblastoma tumour suppressor protein (Rb) and the Rb-related proteins p107 and p130 [106–108]. The binding of E7 to these and other cellular proteins is also thought to be responsible for the transforming activity of this protein.

The E7 protein is around 100 amino acids in length but has an apparent molecular weight of around 21 kDa. The protein appears to be predominantly nuclear and is phosphorylated in vivo [109–113]. The N-terminal half of E7 has sequence and functional similarities to the adenovirus E1a protein and the SV40 large T antigen. A 17-amino-acid sequence within this N-terminal region contains a conserved LXCXE motif and is essential for binding to Rb [114, 115]. This region also contains two serines that are substrates for phosphorylation by casein kinase II (CKII) [112]. Mutations that disrupt the LXCXE motif or prevent phosphorylation by CKII reduce the transforming ability of E7 [112, 116–118]. The C-terminal half of the E7 protein contains two C-x-x-C motifs that form an unconventional zinc finger and bind a zinc ion [55, 56, 119, 120]. E7 is predominantly a dimer and the zinc-finger region is thought to be important for dimerisation [121, 122]. The role of the zinc-binding region in transformation is less clear, with opinion divided as to whether or not the region is necessary for immortalisation [121–123].

E7 and the pocket proteins

E7 can force quiescent cells to enter the cell cycle and, in some circumstances, it can also induce apoptosis. Some of the effects of E7 on the cell cycle are mediated by the so-called 'pocket proteins', a family of closely related proteins that regulate cell proliferation [124]. There are three members of this family: Rb, p107 and p130. These proteins contain a conserved domain, or pocket, that binds members of the E2F family of transcription factors and a variety of other proteins. Each pocket protein binds to a particular set of partners. For example, whilst Rb binds to E2F-1, -2, -3 and -4, p107 and p130 bind to E2F-4 and -5 [125]. Although E7 and the E2F family members bind to separate sites within the pocket, the binding of E7 brings about the release of these E2F proteins [126]. The binding of E7 to each of the pocket proteins may be important during the HPV life cycle. Interestingly, whereas the Rb gene is frequently mutated in non-HPV-induced cancers, the genes encoding p107 and p130 are usually wild-type [127].

The interaction of each pocket protein with E2F family members is regulated through phosphorylation by cyclin-dependent kinases (CDKs) [128]. Hypophosphorylated Rb binds to E2F and brings about the repression of E2F-dependent genes. As cells move towards S phase, Rb is hyperphosphorylated by cyclin E-CDK2 and cyclin D-CDK4 or cyclin D–CDK6. Hyperphosphorylation of Rb brings about the release of E2F and the activation of E2F-dependent genes. The activity of these cyclin-CDK complexes is regulated by kinase inhibitors. For example, the kinase inhibitors $p16^{INK4}$ and p21 inhibit cyclin D- and cyclin A-, and cyclin E-associated kinases, respectively [128]. However, E7-expressing cells continue to cycle in the presence of high levels of both $p16^{INK4}$ and p21 [129, 130]. The binding of E7 to Rb thus overrides these kinase inhibitors, and the consequent release of E2F brings

about the continuous activation of E2F-dependent genes and unchecked cell proliferation (fig. 4). p53 induces p21 in response to DNA damage and this usually brings about cell cycle arrest. E7 overcomes p21-mediated inhibition of CDK2 activity and neatly side-steps p53-induced cell cycle arrest [131]. The inappropriate activation of E2F-dependent genes appears to be responsible for many of the other effects of E7. For example, E7 induces abnormal centrosome duplication that may lead to genome instability, but this is blocked after inhibition of E2F [91, 132]. Similarly, E7 can induce apoptosis in some cells, and again this is due, at least in part, to increased E2F activity [133, 134].

The ability of E7 to bind Rb and release E2F is, however, not sufficient for efficient cell transformation [117]. This means that in addition to activating E2F-responsive genes, E7 must deregulate other important cellular pathways. Expression of the E7 protein in several cell types leads to a reduction in Rb protein levels [108, 135]. E7 brings about this change by binding to Rb and targeting this protein for ubiquitin-mediated degradation by the proteasome [108]. This E7-induced degradation of Rb appears to be necessary to efficiently overcome $p16^{INK4}$-imposed cell cycle arrest [136]. Interestingly, the E7 protein from HPV 1, a low-risk type, binds to Rb but fails to bring about its degradation [136]. These observations suggest that Rb degradation and E2F up-regulation, rather than simply the up-regulation of E2F activity, is the primary oncogenic activity of the E7 protein from high-risk HPV types.

E7 and other cellular proteins

Although over-expression of E7 can affect cellular promoter activity [104, 137], there is no evidence to suggest that this protein binds DNA. However, E7 does interact with transcription factors and can thereby indirectly modulate gene expression. For example, E7 binds to the C-terminal region of the TATA box-binding protein (TBP) [138, 139]. The phosphorylation of E7 by CKII significantly enhances binding to TBP, suggesting that the interaction might be regulated in vivo [138, 139]. However, the functional significance of this interaction is not yet understood. E7 also binds to interferon-stimulated gene factor 3 (ISGF3) and blocks the induction of interferon (IFN)-α-responsive genes [140]. IFN-α affects cell growth and differentiation but its main function appears to be antiviral. E7 can thus compromise the antiviral responses normally induced by IFN-α [140, 141]. Cervical tumours that respond to IFN-α treatment have lower levels of E7 than those which fail to respond [142]. However, the E7 proteins from low-risk HPV types also inhibit IFN-α signalling, suggesting that this viral strategy to escape the immune system does not correlate with tumorigenesis [140, 141]. In contrast, E7 binds to members of the AP1 family of transcription factors, including c-Jun, JunB, JunD and c-Fos, and this does appear to be important in tumorigenesis [143, 144]. A mutant of c-Jun that binds to E7, but which lacks the ability to regulate transcription, is a transdominant negative regulator of E7-mediated transformation [143]. Presumably, the defective c-Jun protein binds to E7 and blocks its interaction with functional Jun family members. This implies that the interaction with Jun family members is important for tumorigenesis. E7 also binds to the Mi2β component of the NURD (nuclear remodelling and histone deacetylase) complex [145]. Although E7 might influence chromatin remodelling via this route, the importance of the E7-Mi2β interaction in tumorigenesis is unclear.

E7 binds to a variety of other cellular proteins including insulin-like growth factor-binding protein 3 (IGFBP-3) and pyruvate kinase [146, 147]. IGFBP-3 can suppress cell proliferation and induce apoptosis and the protein is present at high levels in senescent cells. E7 binds to IGFBP-3 and targets this protein for proteasome-mediated degradation [146]. The binding of HPV 16 E7 protein to pyruvate kinase is thought to stimulate glycolysis [147]. The HPV 11 E7 protein fails to bind this protein, suggesting that the effects of E7 on glycolytic flux are important in transformation [147, 148].

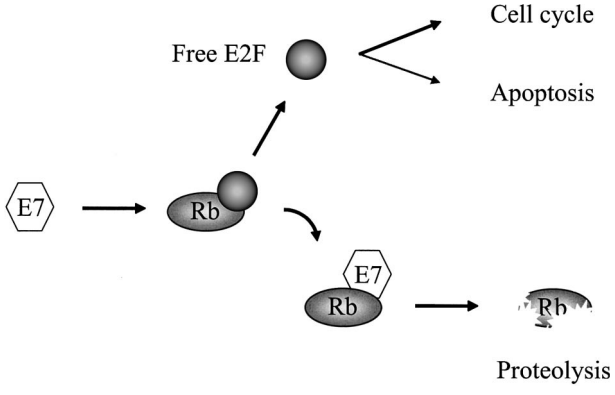

Figure 4. E7 targets Rb. E7 binds to Rb and brings about the release of E2F. Free E2F proteins promote cell cycle progression and can also induce apoptosis. The binding of E7 to Rb targets Rb for proteolysis.

The regulation of E7 activity

As mentioned earlier, E7 and E6 are under the control of the same promoter and are translated from a bicistronic mRNA [98]. The expression of E7 from this mRNA appears to depend on ribosomes scanning through the E6 coding sequence and initiating translation at the E7 start codon [149]. However, E7 is also expressed from a variety of spliced mRNAs that do not encode the full-length E6 protein [4, 98]. The E7 protein is short-lived, with a half-life of around 30 min, and is degraded by the ubiquitin-proteasome pathway [150, 151]. E7 is phosphorylated in vivo and the pattern and level of phosphorylation

change during the cell cycle [152]. Since phosphorylation of E7 is important in transformation, these changes are probably important in tumorigenesis [110, 112, 118].

The E5 protein

The E5 protein is a dimeric membrane protein of around 80 amino acids in length [153, 154]. Membrane proteins are difficult subjects for X-ray diffraction and nuclear magnetic resonance (NMR) methods and the structure of E5 has yet to be determined. However, polarised-infared spectroscopy has shown that the E5 protein from BPV 1 is predominantly α-helical and that it traverses the membrane [155]. The protein appears to be expressed throughout the viral life cycle and is found in the lower layers of the epithelium [156]. E5 is capable of inducing anchorage-independent growth and enhancing tumorigenesis in nude mice [157, 158]. These effects of E5 are thought to result from the ability of this protein to enhance signalling by growth factor receptors [158, 159]. E5 binds to the 16-kDa subunit of the vacuolar ATPase, and this is thought to inhibit the acidification of the endosome [160], thereby inhibiting the degradation of internalized growth factor receptors and enhancing signalling [161]. In support of this hypothesis, some mutants of the vacuolar ATPase can themselves transform cells [162]. The E5 proteins from some HPV types associate with the epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) receptors [163]. However, in the case of the BPV E5 protein at least, binding to the PDGF receptor is not required for transformation [164]. BPV E5 induces increases in the pH of the Golgi complex and this may or may not be important in transformation [165]. Viral integration often abolishes the expression of E5, suggesting that this protein is not involved in tumorigenesis. However, E5 is expressed in some cancers that contain episomal HPV DNA [166] and this protein may have a role in the establishment of some or all cervical tumors.

The E2 protein

The HPV E2 ORF encodes a DNA-binding protein of around 360 amino acids in length that regulates viral gene expression. The E2 protein also binds to the E1 protein and this interaction is required for efficient viral replication. E2 is composed of two domains that are thought to be separated by a flexible hinge (fig. 5A) [167]. The C-terminal domain mediates sequence-specific DNA binding. The N-terminal domain mediates transcription activation and the enhancement of viral replication. The structure of the iso-

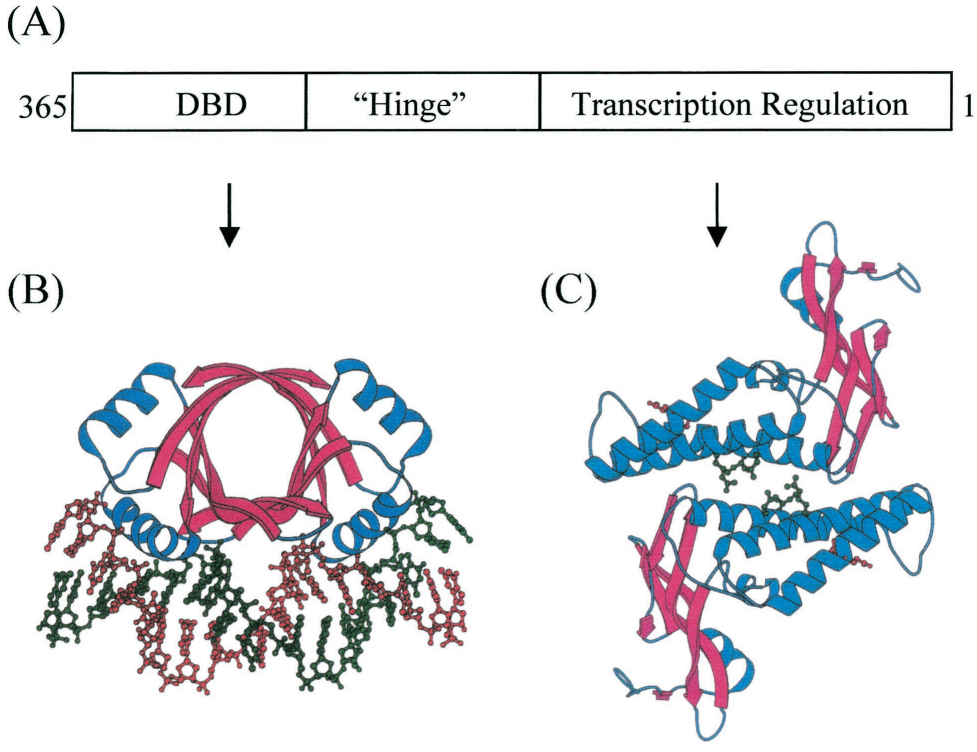

Figure 5. The E2 protein (pictures kindly provided by Richard Sessions). (*A*) The domain structure of the HPV 16 E2 protein. DBD indicates the DNA-binding domain. (*B*) A model of the HPV 16 E2 DBD bound to DNA based on the crystal structure of the BPV E2-DNA complex. (*C*) The HPV 16 N-terminal domain. Amino acids R37, Q76, and I73 (side chains in green) are important for dimerisation. Amino acids Q12 and Q39 (side chains in red) are important for binding to the E1 protein.

lated C-terminal domain has been determined by X-ray crystallography and NMR [168, 169]. The structure of the isolated N-terminal domain has been determined using X-ray crystallography [170, 171]. However, the structure of the full-length E2 protein remains elusive.

The E2 C-terminal DNA-binding domain (DBD) is a dimer that recognises inverted repeats conforming to the consensus sequence 5′AACCGN$_4$CGGTT 3′, where N represents any base pair [172 and references therein]. The two subunits of the E2 dimer form a β barrel that carries a pair of surface-exposed α helices which make specific contacts with the base pairs in two successive major grooves of the DNA [173, 174] (fig. 5B). Although the E2 protein does not contact the central four base pairs of its binding site, the sequence of these base pairs does have an effect on the binding of the HPV 16 and HPV 18 E2 proteins. In general, these E2 proteins bind more tightly to sites that contain an A:T-rich central sequence. This selectivity is a result of the changes in DNA and protein conformation that occur on complex formation [172, 173, 175–178].

The N-terminal domain is made up of three α helices that form a twisted plane separated by a short helical region and two loops from a region that is predominantly antiparallel β sheet [170] (fig. 5C). Conserved amino acids in two of the three α helices in the twisted plane appear to mediate dimerisation. The monomer-monomer interactions are predominantly via hydrogen bonds and include few hydrophobic interactions [170]. Two amino acids important for interaction with the E1 protein (Q12 and Q39) lie on the outer surface of the three-helix plane, suggesting that E1 contacts this region (side chains indicated in red in fig. 5C). In contrast, several amino acids important for transcription activation lie at the dimer interface (indicated in green in fig. 5C), suggesting that mutations at these positions disrupt dimerisation. Interestingly, these mutations do not seem to affect replication. This suggests that dimerisation of the N-terminal domain might be required for the activation of transcription by E2 but is of little (or lesser) importance for the stimulation of replication [170]. These observations highlight the value of structural information in mutagenic studies.

The full-length BPV 1 E2 protein binds co-operatively to DNA fragments with multiple E2 recognition sites and can form DNA loops [179–181]. In contrast, the isolated C-terminal DBD does not bind co-operatively to multiple specific sites or form DNA loops [181]. This suggests that the N-terminal domains of DNA-bound E2 dimers mediate these interactions. Analytical ultracentrifugation has shown that dimerisation of the N-terminal domain only occurs at relatively high protein concentrations (K_d ~10 µM) [170], whereas dimerisation of the C-terminal domain occurs at very low protein concentrations (K_d < nM). However, once two C-terminal domains have dimerised, the local concentration of N-terminal domains will become very high leading to their dimerisation. Thus in cells, E2 proteins probably form dimers in which both the C- and N-terminal domains form paired structures separated by their two hinge regions (fig. 6A). The formation of a bidentate link between the N- and C-terminal dimers would constrain the relative movement of the two domains implying that the hinge region might have less freedom of movement than previously thought. Two E2 dimers have been proposed to associate via their N-terminal domains to form tetramers (fig. 6B) [170]. This would neatly explain how DNA-bound E2 proteins bring about DNA looping. This model does not rule out the possibility that E2 might also form higher-order structures such as hexamers or octamers (fig. 6C). Electron microscopy has revealed hexameric complexes on DNA fragments carrying three E2-binding sites [181] and one is tempted to speculate that the four E2-binding sites present in the HPV LCR might be occupied by an octameric E2 complex composed of four E2 dimers.

E2 and the regulation of HPV gene expression

The HPV LCR generally contains four binding sites for the E2 protein as well as numerous binding sites for a variety of cellular transcription factors (fig. 1). The locations of the E2-binding sites are conserved between all

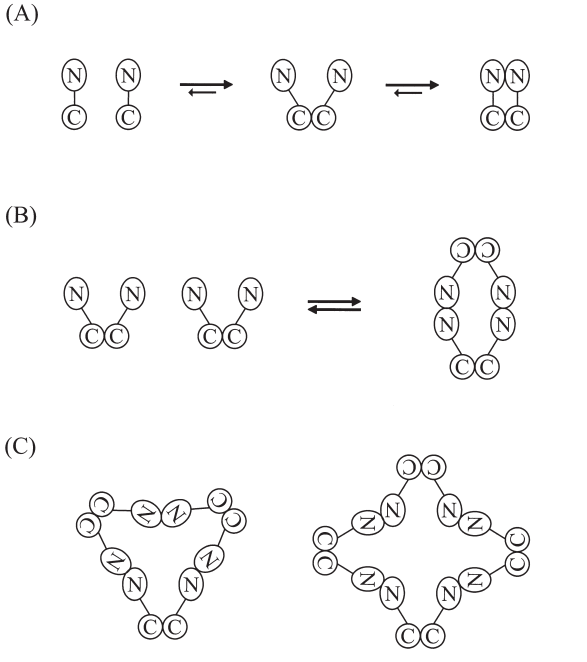

Figure 6. E2 dimers and other possibilities. (*A*) Each E2 monomer is made up of a C-terminal DBD (*C*) linked via a flexible hinge (|) to an N-terminal transcription activation domain (N). E2 monomers form stable dimers via their C-terminal DBDs. Once the C-terminal domains have found partners, the local concentration of N-terminal domains becomes very high and they also dimerise. (*B*) E2 dimers might form tetramers. (*C*) Hexamers and octamers may also form. Note that in all situations, the interactions between the N-terminal domains are identical.

the high-risk HPV types, suggesting that their precise arrangement is important for normal viral function. Two adjacent E2-binding sites lie between an Sp1-binding site and the viral TATA box (sites 1 and 2 in fig. 1). The binding of E2 to these sites blocks the binding of Sp1 and TBP, respectively, resulting in transcriptional repression [182– 184]. The remaining two E2-binding sites are located further upstream of the promoter (E2 site 3 and E2 site 4), and binding of E2 to these sites is thought to mediate transcriptional activation [185, 186]. Whether E2 represses or activates HPV promoter activity probably depends on the relative affinity of E2 for each of its four binding sites and the amount of E2 within the cell [187, 188]. In vitro binding experiments have shown that in HPV 16, the E2 protein binds tightly to E2 site 4, less tightly to E2 sites 1 and 2, and relatively weakly to E2 site 3 [172, 189]. During viral replication, E2 levels are thought to be tightly regulated. E2 is present at low levels in HPV-infected basal cells and this may result in transcription activation. As these cells begin to differentiate E2 levels rise, possibly resulting in transcriptional repression, and the viral DNA is replicated [172, 189]. However, E2 binds equally well to all four of its binding sites in the LCR of HPV 11 [190]. This suggests that in different virus types, E2 utilises different mechanisms to regulate viral gene expression and/or that transcriptional regulation by E2 is more complex than has been imagined previously.

The N-terminal transcription domain of E2 is required for transcription activation and interacts with the basal transcription factors TBP and TFIIB [191–194], and the transcriptional co-activator AMF-1 [195]. Two truncated forms of the E2 protein, both of which lack this N-terminal domain, are expressed in BPV and are known as E2-TR and E8^E2C. These truncated E2 proteins can repress viral transcription and can also form transcriptionally inactive heterodimers with the full-length E2 protein [196]. Transcripts encoding E8^E2C proteins have been identified in some of the high-risk HPV types [197, 198]. In HPV 31, the E8^E2C protein can repress viral transcription in both the absence and presence of the full-length E2 protein [198, 199]. An understanding of the relative roles of E2 and E8^E2C and of the interplay between these proteins will require detailed investigation of the expression of these proteins throughout the viral life cycle.

E2 and cell proliferation

There are at least two mechanisms whereby the E2 protein can have effects on cell proliferation (fig. 7). First, E2 can influence cell proliferation indirectly via its effects on the expression of E6 and E7 [200, 201]. Second, E2 can have a direct effect on cell proliferation via as yet poorly defined pathways [26]. In HPV-infected cells, changes in the level or activity of the E2 protein would be expected to result in either up- or down-regulation of E6 and E7. Although this indirect effect of E2 on cell proliferation has not been demonstrated in HPV-infected cells, it is seen in HPV-transformed cells [200, 201]. Expression of the BPV 1 E2 protein induces growth arrest in cervical carcinoma cell lines that contain integrated HPV genomes with intact E6 and E7 ORFs [23, 24, 202, 203]. In these cells, BPV 1 E2 represses transcription of E6 and E7 and this blocks the transition from G1 to S phase [200, 201]. In addition, the E2 proteins from HPV 16, 18, and 33 have been shown to induce apoptotic cell death in cervical carcinoma cell lines [25, 26, 133, 134]. As in the case of E2-induced growth arrest, the induction of apoptosis by these E2 proteins is thought to result at least in part from changes in the levels of E6 and/or E7 [134, 201].

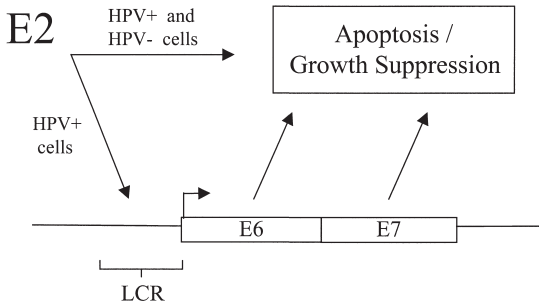

Figure 7. E2 induces apoptosis by two pathways. In HPV-transformed cells (HPV+), the E2 proteins from HPV 16 and HPV 18 can modulate transcription of E6 and E7 by binding to sites within the integrated HPV LCR. Changes in the levels of E6 or E7 can induce apoptosis. In non-HPV-transformed cells (HPV–) and HPV-transformed cells (HPV+), these E2 proteins can also induce apoptosis independently of E6 and E7.

Direct effects of E2 on cell proliferation have also been observed and these E6/E7-independent consequences of E2 expression might also play a role in the viral life cycle and HPV-induced tumorigenesis. The HPV 16 E2 protein can induce apoptosis in several HPV-negative cell lines [26]. Similarly, the HPV 31 E2 protein can induce apoptosis in HPV-negative normal human foreskin keratinocytes [133]. Furthermore, the HPV 16 E2 protein can also influence the proliferation of HPV-transformed cells independently of its ability to modulate E6 and E7 expression. Mutations in the HPV 16 E2 DBD that eliminate DNA-binding activity, and thereby block the effects of E2 on the expression of E6 and E7, do not prevent E2-induced apoptosis in HeLa cells [26]. This suggests that these direct effects of E2 on cell proliferation probably arise as a consequence of the interaction of E2 with cellular proteins involved in apoptosis. The HPV 16 E2 protein has been shown to interact with p53 [204], and the HPV 18 E2 protein has been shown to interact with CBP [205], a co-activator protein important in some forms of p53-dependent apoptosis [83]. HPV 16 E2-induced apop-

tosis can be blocked by over-expression of the HPV 16 E6 protein, suggesting that it is p53 dependent [26; J. Parish, and K. Gaston, unpublished data]. However, E6 also has p53-independent anti-apoptotic activity [206]. Furthermore, HPV 18 E2-induced apoptosis precedes the accumulation of p53 and is not accompanied by an increase in the transcription of Bax, a p53-responsive gene that can induce cell death [207]. Although these data suggest that in this case E2-induced apoptosis is p53 independent, several studies have suggested that neither p53 accumulation nor transcription activation by p53 are required for some forms of p53-dependent apoptosis [66–68, 208].

The HPV 16 E7 protein increases cell proliferation and, like the HPV 16 E2 protein, E7 can also induce apoptosis [26, 105, 207, 209]. However, over-expression of the HPV 16 E6 protein blocks both E2- and E7-induced apoptosis, presumably by down-regulating p53 levels [26]. This suggests that during viral infection, pro-apoptotic signals generated by E7 and E2 may be counterbalanced by the anti-apoptotic effect of E6. Perhaps random integration events that disrupt the E2 ORF upset this balance between pro- and anti-apoptotic signals. Alternatively, or in addition, these random integration events may simply block the repression of E6 and E7 expression normally mediated by E2, resulting in increased levels of E6 and/or E7. Tumour supressor proteins such as Rb and p53 generally act as negative regulators of cell proliferation. When these proteins are inactivated, either by mutation or by the action of viral oncoproteins, cell proliferation is deregulated, leading to increased cancer risk. In many ways, the HPV E2 proteins from high-risk HPV types seem to act like tumour suppressor proteins. Disruption of the E2 ORF and/or the loss of E2 protein expression produces cells that are more likely to proliferate and, as a consequence, more likely to produce cervical cancer.

The E1 protein

Although HPV DNA is copied by the host cell machinery, replication cannot proceed efficiently in the absence of the viral E2 and E1 proteins [210, 211]. The E1 protein is an ATP-dependent DNA helicase, an enzyme that couples the hydrolysis of nucleoside triphosphates to the unwinding of double-stranded DNA [for a review see ref. 212]. Interestingly, E1 binds poorly to the viral origin of replication in the absence of the E2 protein [213]. Once E2 has enabled E1 to bind to the origin, E1-mediated unwinding results in the separation of the DNA strands that is required for the initiation of DNA replication. The E1 proteins form doughnut-shaped hexameric assemblies that unwind DNA in the 3′ to 5′ direction [214]. One strand of the DNA is thought to pass through the hole in the doughnut whilst the second strand is passed around the outside [212]. However, there is evidence to suggest that in the absence of DNA, E1 can also exist as monomers and that the monomer-hexamer transition might be an important step in origin recognition [215].

Each E1 monomer is around 600 amino acids in length. Helicase activity requires all except the first 100 or so amino acids at the N terminus and the final 50 or so amino acids at the C terminus [216, 217]. The helicase region appears to be divided into an N-terminal DBD and a C-terminal ATP-binding domain [216, 217]. The structure of the E1 DBD from BPV has been determined by X-ray crystallography [218]. The BPV E1 DBD is a monomer in solution but appears to bind to DNA as a dimer. The E1 DBD consists of an anti-parallel β sheet with two α helices lying on one side of the sheet and three α helices lying on the other (fig. 8) [218]. Several mutations that prevent DNA binding are localised to a positively charged surface formed by a loop and the N-terminal end of one of the α helices (indicated in red in fig. 8), suggesting that this surface binds DNA [218]. Dimerisation of the E1 DBD appears to be mediated by hydrophobic interactions between the non-DNA-binding α helix located on the same side of the β sheet as the putative DNA-binding helix. Two positions at which mutations impair dimerisation are indicated in green in figure 8 [218]. Dimerisation along this interface would allow a pair of DNA-binding surfaces to contact two successive major grooves in the E1-binding site.

In addition to its role in HPV replication, the E1 protein may also be involved in cell transformation and viral gene regulation. For example, disruption of the HPV 16 E1 ORF enhances the ability of this virus to immortalise primary keratinocytes [21]. Why the loss of E1 expression should augment immortalisation is not clear. However, as mentioned previously, cervical carcinomas often contain integrated viral genomes in which expression of the E1 ORF is lost. This suggests that the loss of E1, or the production of a truncated E1 protein, might be important in

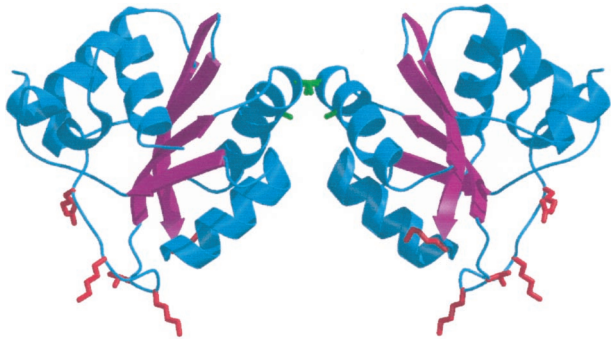

Figure 8. The E1 DNA-binding domain. The crystal structure of the BPV E1 DBD. Amino acids V202 and A206 (side chains in green) are important for dimerisation. Amino acids R180, K183, K186, T187, and K241 (side chains in red) are important for DNA binding. Picture kindly provided by Leemor Joshua-Tor and Arne Stenlund.

tumorigenesis. One possibility is that E1, like E2, might negatively regulate the expression or activities of E6 and E7. Since E1 and E2 interact, it is hardly surprising that over-expression of E1 can influence transcriptional regulation by E2, and several studies have shown that the BPV E1 protein can modulate the transcription activity of the BPV E2 protein. However, over-expression of BPV E1 has been reported to either down- or up-regulate transcription activation by BPV E2 depending on the experimental system [219–222]. A clearer understanding of the role of the HPV E1 protein in transcriptional regulation of viral gene expression will probably require mutants of E1 that are wild type for some functions but defective in others.

E1 and E2 in HPV replication

The HPV origin of replication contains binding sites for E1 and E2. However, the E1-binding site is not essential for HPV replication and two adjacent E2 sites can function as a minimal replication origin in transient replication assays [223]. E1 and E2 can interact in the absence of DNA and the interacting regions have been mapped. Although reports from different groups conflict somewhat, the C-terminal 300 or so amino acids of the E1 protein that encompass the E1 ATP-binding domain seem to bind to the N-terminal transcription activation domain of E2 [216]. The BPV E1 protein also interacts with the DNA-binding domain of the BPV E2 protein [224]. Obviously, the interaction of E1 with either or both of these domains could explain how E1 modulates the effects of E2 on transcription.

Elegant footprinting studies have examined the binding of E1 and E2 to the BPV origin in detail [225]. These studies have shown that E1 alone can bind to the origin and that this results in a distortion of the DNA that probably corresponds to strand separation. ATP is not required for E1 binding or origin distortion but does seem to promote these events [225]. The presence of the E2 protein stimulates binding of E1, lowering the E1 concentration needed to bring about unwinding. E2 thus seems to act as a loading factor for E1 [226, 227]. In the presence of both E1 and E2 proteins, two distinct complexes are formed at the replication origin (fig. 9). One complex contains a dimer of E2 and what appears to be a monomer of E1 and this is thought to be the initial complex formed at the origin [228]. The second complex contains only multimers of E1 and appears to be the final initiation complex [228]. Strand separation or conformational changes that accompany the transition from E1 monomers to hexamers could displace E2. Alternatively, E1 could join the complex as a hexamer and the conformational changes that accompany ATP binding, or the opening and closing of the E1 ring to encircle the DNA, could displace E2. However, experiments with the HPV 11 E1 and E2 proteins suggest that a final complex containing hexameric E1 and a single dimer of E2 can form in solution [229]. Once hexameric E1 complexes have been assembled at the origin, ATP-dependent DNA unwinding proper can begin. The details of this step are also far from being completely understood. Two hexameric E1 complexes would be required to unwind the viral DNA bidirectionally and how the second hexameric ring binds is not known. Also not known is whether the two hexameric rings track around the circular HPV genome, or whether single-stranded DNA is extruded from a complex formed from two hexamers.

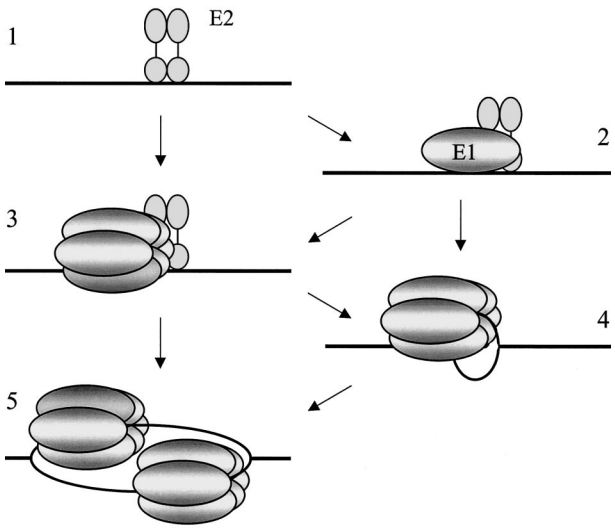

Figure 9. A model to illustrate the assembly of an HPV replication complex. The binding of E2 to a specific site (1) is followed by the recruitment of either monomeric E1 (2) or hexameric E1 (3). The E2-hexameric E1 complex (3) may displace E2 (4) and then be converted to a double hexamer E1 complex (5) or proceed directly to this complex (5). The E2-monomeric E1 complex (2) may proceed either via the recruitment of more E1 monomers to form the E2-hexameric E1 complex (3) or via displacement of E2 (4).

Once the origin has been recognized, cellular proteins must be added to the complex for replication to begin. E1 interacts with DNA polymerase α-primase and can thereby engage the replication machinery [220, 230]. DNA polymerase α-primase seems to compete with E2 for binding to E1. This suggests that E1 may only be free to recruit the primase to the origin once E2 has been displaced [231, 232]. E2 interacts with replication protein A and this may also be important in complex assembly [233]. Replication is bidirectional and produces two daughter molecules by semi-conservative replication; however, there is evidence to suggest that this might switch to a rolling-circle mode as infected cells differentiate and production of HPV particles begins [234].

The BPV E2 protein is required to maintain the viral genome as an episome [235]. E2 binds to mitotic chromosomes and this may be required to ensure equal segregation during cell division and the long-term episomal maintenance of viral genomes within infected cells [235,

236]. Somewhat surprisingly, the site-specific DNA-binding activity of E2 is not thought to be required for the interaction of this protein with mitotic chromosomes [236]. The considerable non-specific DNA-binding activity of E2 [189, 237] might anchor E2 on mitotic chromosomes. Dimerisation between E2 molecules bound to host chromosomes and E2 molecules bound to the viral genome might then facilitate equal partitioning of viral DNA between daughter cells.

Targets for therapeutic intervention

Over the last 50 years or so, the UK has seen a 50% reduction in the number of deaths per year caused by cervical cancer [1]. This has been brought about by the introduction of screening programs and by advances in the treatment of pre-cancerous lesions. However, this disease is still responsible for over 1000 deaths per year and at best only around 70% of the women with this condition will survive for 5 years after diagnosis [238]. The current treatment for cervical cancer is surgery or radiotherapy or their combination, and occasionally radiotherapy and chemotherapy [239, 240]. Pre-cancerous lesions can be treated using a variety of approaches including cryotherapy, laser therapy, electrosurgery, and surgical excision. Several cytotoxic agents are also available, including 5-fluorouracil, trichloroacetic acid, podophyllin, and podofilox [241]. None of these chemical treatments specifically attack HPV-infected cells and they all require multiple applications. Unfortunately, they all have failure rates of around 30% and even after successful treatment, recurrence is commonly as high as 70% [241]. New approaches to the treatment and prevention of both cervical cancer and pre-cancerous lesions would obviously be of great potential benefit.

Vaccination seems to offer the possibility of preventing both pre-cancerous lesions and cervical cancer [242, 243]. Several vaccination trials are in progress or about to start. These vaccines employ recombinant capsid proteins often in the form of virus-like particles either alone or as fusions to amino acid sequences from the E6 and/or E7 proteins. The results of phase I and phase II clinical trials suggest that these vaccines might be useful in both the treatment and prevention of genital warts [244]. However, multiple HPV infections seem to be quite common in nature [6, 7], suggesting that a vaccine that protects against one HPV type will be unlikely to protect against others. One way around this problem would be a vaccine containing proteins from several HPV types. However, we know very little about the mechanism whereby these vaccines might disrupt the HPV life cycle. A disturbing possibility is that a vaccine might block HPV replication but not block initial viral infection or the subsequent viral integration events that lead to cancer. If this were to be the case, a vaccine might be counterproductive in that it could mask pre-cancerous lesions that are currently picked up by screening, but it might not block the eventual formation of cervical cancers.

The E1 helicase is the only HPV protein that has enzymatic activity and has therefore been the subject of intense investigation. Most screens for drugs that can inhibit E1 have been based on selecting compounds that block ATP-dependent DNA strand displacement. Unfortunately, this approach is likely to select drugs that bind to the ATPase active site and inhibit its activity. Compounds that act in this way may not be sufficiently specific to be useful clinically. The interaction of E1 and E2 provides a second target for drug discovery. However, drugs that block a specific protein-protein interaction are difficult to select or design. Short peptides based on the amino acid sequence of the N-terminus of HPV 16 E2 block the interaction of E1 and E2 and the replication of HPV 16 and HPV 11 in vitro [245]. If drugs could be produced that mimic the activity of these peptides, they would be useful in the treatment of pre-cancerous HPV-induced lesions. Since functional E1 protein is not required by HPV-induced cervical tumour cells, these drugs would probably not be useful in the treatment of cervical cancer.

E6 and E7 are required for the growth of HPV-transformed cells, so these proteins, and their cellular partners, are obvious targets for drug discovery. Any compounds that block the activity of these proteins would be expected to block HPV replication and would thus be useful in the treatment of viral infection as well as cancer. The E6* protein inhibits E6-directed degradation of p53 and induces p53-dependent apoptosis [246]. E6* could thus form the basis of a gene therapy approach to cervical cancer [246]. Peptides that bind specifically to the HPV 16 E6 protein can also induce apoptosis and increase the levels of p53 in HPV-transformed cells [247]. Similarly, the compound 4,4′dithiodimorpholine removes the zinc from E6 and induces apoptosis and increases p53 levels in HPV-transformed cells [248]. E7 is also a zinc-binding protein and this approach might also be taken with this protein. However, a cell contains many zinc-binding proteins and although E6 and E7 probably represent highly individual if not unique zinc-binding pockets, achieving specificity will be difficult. Another possible approach that might knock out E6 and/or E7 function is antisense regulation [249]. Antisense E7 has been shown to reduce the proliferation of HPV-transformed cells in vitro and their tumorigenicity in nude mice [250]. The main problem with this approach will be ensuring that the oncogenes are permanently turned off rather than temporarily down-regulated. The cellular partners of E7 and E6 make poor targets for therapeutic intervention since any drugs that block their activities are likely to have dramatic effects on both uninfected and untransformed cells. An exception might be drugs that stabilize or activate p53. The

treatment of cervical carcinoma cell lines with the nuclear export inhibitor leptomycin B in conjunction with actinomycin D leads to p53 accumulation and apoptosis [251].

At first thought, the E2 protein might seem an unlikely target for drug research since, like E1, it is not required for the growth of cervical cancer cells. However, any drugs that block E2 function would prevent HPV replication and might thereby block HPV-induced tumorigenesis. Compounds that bind to the HPV 31 E2 protein and block its DNA-binding activity have been identified and these could lead to the development of specific inhibitors of HPV replication [252]. In addition, the BPV E2 protein can suppress the growth of HPV-transformed cells and the HPV 16 and HPV 18 E2 proteins can induce apoptosis. This suggests that the E2 protein itself might be useful in the treatment of cervical cancer. The use of E2 would have two main advantages. First, E2 could kill HPV-transformed cells by apoptosis [25, 26]. Growth suppression is not very useful since without stable transformation, E2 protein expression would eventually be lost and growth could continue. The second advantage of using E2 is that it might evoke an immune response. E2 immunity is thought to play a role in the control of HPV infection [253], and induced immunity to E2 could be protective against further infection. It is even possible that local long-lived mucosal E2 immunity in combination with a capsid-based vaccine might be more useful than a capsid-based vaccine alone. To be an effective treatment, either the action of the E2 protein must be specific to cancer cells, or the protein must only be delivered to cancer cells in vivo. Viral delivery of BPV E2 has been tested in an animal model and seems to suppress tumour growth effectively [254, 255]. Real therapies based on E2 might emerge over the next few years.

Conclusion

Knowing the cause of a particular type of cancer does not always mean that the incidence of the disease can be reduced. For example, the link between cigarette smoking and lung cancer is very firmly established, yet young people still take up smoking and lung cancer is set to remain the most common cause of cancer-related death. The link between HPV and cervical cancer is equally well established. However, in this case, the combination of screening programs, HPV vaccines, and new drug therapies that specifically target the virus offer the possibility that deaths from this disease might be totally eliminated.

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