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The papillomaviruses are a large and fascinating group of pathogens infecting skin and internal mucosae where they induce a range of proliferative lesions ranging from the humble wart to cancer. These viruses are not classified serologically but by nucleotide sequence homology and at present about 75 human papillomavirus genotypes (HPVs) have been cloned from clinical biopsies each with a predilection for a cutaneous or mucosal surface. The genotypes infecting the genital tract have received close attention because of the strong association between infection with specific genital HPVs and anogenital cancer, particularly cancer of the uterine cervix (IARC 1995). About 28 HPVs regularly or sporadically infect the genital tract and these can be divided roughly into two groups. There are those HPVs, mainly types 6 and 11, associated with exophytic condyloma acuminata or true genital warts found predominantly in the lower genital tract and those associated with intra-epithelial lesions or flat condyloma particularly of the cervix, HPV types 16, 18, 31, 33, 35 and minor related types. These distinctions are not absolute and HPV 16 and 18 can be detected in 1-2% of genital warts (Coleman et al 1994) and HPV 6 in low grade cervical lesions (IARC 1995). However the association between infection with HPV 16 or 18 and the risk of subsequently developing cervical cancer is strong enough to warrant the description of HPV 16 and 18 as carcinogens (IARC 1995). This raises the possibility that intervention in the natural history of HPV infection in the genital tract either by prevention of infection or treatment of established disease would be an effective anticancer strategy.

Although the oncogenic HPVs receive a great deal of attention disease induced by the low risk genital viruses is not trivial either in terms of morbidity or economic and social costs. Genital warts are the commonest viral sexually transmitted disease in the UK, therapy is generally unsatisfactory with a high frequency of recurrence and a significant proportion of individuals with lesions refractory to treatment. Although immunologically based prophylactic
and therapeutic strategies are attractive options for papillomavirus induced disease there is an increasingly important group of patients with HPV infections who are immunosuppressed either as a consequence of organ transplantation or HIV infection. Not only is infection with both cutaneous and genital types one of the most frequent viral complications in these individuals but the lesions are persistent and refractory to treatment. In addition there is an increased risk of malignant transformation in these lesions both in those in whom oncogenic HPVs are detected but also in cutaneous lesions induced by the non-oncogenic HPVs on sun exposed surfaces. HPV induced disease in immunosuppressed individuals identifies a clear need for chemotherapy in the form of specific anti-HPV drugs for both topical and systemic treatment. This objective is dependent upon an understanding of the biology of the virus and its interaction with the target cell for infection the keratinocyte. The unique replication strategy of pv's in which virus replication and keratinocyte differentiation proceed in parallel with vegetative viral growth confined to terminally differentiating keratinocytes and the difficulties of reproducing this scenario in vitro have contributed significantly to the problems in understanding virus cell interactions and the regulation of viral gene expression during permissive growth.

**Papillomavirus genome organisation**

The application of molecular cloning and sequencing of the genomes of pv's has permitted a systematic analysis of the gens encoded by this group of viruses. The DNA of more than 60 genotypes has now been sequenced and overall show a high degree of conservation of genome organisation (Fig). The HPV genome can be divided into 3 domains, a non-coding upstream regulatory region of approximately 1kb; an early region with open reading frames (ORFs) E6, E7, E1, E2, E5 and E5; a late region encoding two genes L1 (the major capsid protein and L2 (the minor capsid protein). The URR contains the origin (ori) of DNA replication and
binding sites for numerous cellular transcription factors including TBP, Sp1, AP1, YY1 and glucocorticoids.

All viral genes are transcribed from the same strand but despite the apparently simple genetic organisation, the actual protein products are complex because of the use of alternative promoters and multiple RNA splicing events. The E6 and E7 genes of the HPVs encode proteins which, in the context of the viral life cycle deregulate or delay the differentiation programme of the infected keratinocyte permitting viral replication. Inappropriate expression of these genes by the high risk viruses either temporally or spatially is associated with neoplastic progression in vivo and expression of these ORFs is essential for in vitro transformation of primary human keratinocytes by HPV 16 or 18. Both E6 and E7 form complexes with cell proteins which play a role in negatively regulating cell growth and the fact that these interactions are much weaker with the E6 and E7 proteins of the low risk viruses supports the notion that the oncogenic potential of the high risk types is a correlate of the function of these early genes. Several proteins are known to form complexes with E7 and to date the most intensively studies of these is that with the retinoblastoma gene product Rb and the associated pocket proteins p107 and p130. The mechanism underlying these interactions seems to be the disruption of the association between Rb and the transcription factor E2F. E2F responsive promoters are a feature of many genes whose expression is central to the regulation of the cell cycle (cdc2, cyclin A, cyclin B and others) and to the regulation of DNA synthesis (thymidine kinase and DNA polymerase). However in vivo during viral replication high level expression of E7 and E6 is confined to the non-dividing differentiated cells in the superficial layers of the epithelium. Elegant studies using organotypic cultures indicate that the function of E7 in these cells is to induce cellular DNA synthesis thus permitting viral DNA replication (Chiang et al 1995).
The interactions between the E6 proteins of the high risk HPVs and p53 has been intensively investigated (for review see Vousden 1996) and there is general agreement that E6 from high risk HPVs bind to and degrade p53 but that the E6 of low risk HPVs is relatively inefficient in this process. The inhibition of p53 function so engendered, particularly the induction of apoptosis, is probably critical for neoplastic progression. Elegant studies in transgenic mouse models show that E7 expression alone results in apoptosis of differentiated epithelial cells and that this is dependent upon E2F since the effect is lost in E7/E2F-ve mice but expression of E6 together with E7 abrogates this effect. One might speculate in view of this data that therapies for high risk HPVs designed to target E6 alone might be effective since continued expression of E7 could induce apoptosis.

The functions of the E5 gene product are imperfectly understood. This ORF encodes a small intensely hydrophobic protein localised predominantly within the membranes of the Golgi and ER. The E5 gene of BPV is a major transforming gene and probably exerts its effect via the interaction with receptors for EGF and PDGF. The E5 proteins of both HPV and BPV complex with the 16kd component of the vacuolar H\(^+\)-ATPase complex inhibiting acidification of the endosome with the consequent retention of non-degraded EGFR and upregulation of receptor density. The interaction with the PDGFR by BPV E5 is different; this is a direct activation by E5 of the PDGFB receptor. There are undoubtedly other functions of E5 and there are preliminary data to suggest that this molecule may influence antigen processing (Cromme personal communication).

A potential target for anti-virals to HPV is inhibition of viral replication. Two viral proteins E1 and E2 have been shown to be essential and sufficient for the initiation of viral replication in cells in monolayer culture in vitro (Ustav 1991). The ability of BPV 1 to infect
mouse C127 cells and generate transformed cells carrying stably replicating copies of BPV DNA (Dvoretzky 1980) permitted the study of BPV replication by mutational analysis and identified the E1 ORF as essential for the maintenance of episomal DNA (Sarver 1984, Lusky and Botchan 1985). The BPV E1 protein has been extensively characterised and shown to be a 68-72 KDa phosphoprotein containing intrinsic ATPase, helicase and specific DNA binding activity (Sun 1990, Santucci 1990, Wilson and LudesMeyers 1991, Yang 1991, Ustav 1991, Laimins 1991, Lambert 1991, Seo 1993). The E1 protein of the papillomaviruses is the only known viral enzyme, it is highly conserved within virus types and is absolutely required for viral replication. Numerous studies have shown that the E2 protein is the major papillomavirus transcriptional regulator (McBride and Howley review 91). Initial observations that established an essential role for BPV E2 in viral DNA replication attributed this requirement to its function as a transcriptional inducer of viral early gene expression including expression of E1 (DiMaio 88 / Rabson 86). However, transient replication assays have shown that the requirement of BPV E2 for origin dependent replication is independent of its role as a transcriptional activator of other genes (Ustav and Stenlund 91). The E2 protein performs its regulatory roles through binding as a dimer to a specific pallindromic sequence repeated several times in the URR and found in all papilloma virus types so far sequenced. The crystal structure of the C-terminal domain of E2 involved in dimerization and DNA binding has been resolved (Hedge and Sigler 92) providing an opportunity for the design of inhibitors of these interactions and the potential for inhibition of viral transcription and replication. In addition to the functions of E1 and E2 described there is evidence that both proteins are involved in recruiting cellular replication factors. Thus it is clear that E1 and E2 have essential roles in viral DNA replication
and are involved in numerous interactions that could be targeted by anti-viral drugs.

Transient replication studies have revealed essential roles for E1 and E2 in viral DNA replication. These studies involve co-transfection of plasmids containing the BPV E1 and E2 ORFs under strong heterogenous promoters into mammalian cell lines, along with a plasmid containing the Papillomavirus URR within which the origin of replication is found. Replication of the URR containing plasmid was shown to be dependent on the expression of E1 and E2. Several recent reports suggest that high concentrations of E1 alone sustain transient DNA replication at a low level. However, the presence of E2 greatly enhances DNA replication and E2 was shown to be indispensable at limiting E1 concentrations (Yang and Botchan 91 / Gopalakrishnan and Khan 94). Similar experiments have indicated a requirement for HPV E1 and E2 gene products in the replication of HPV origin containing plasmids (Ptashne and Brent 85 / Chiang 92/ Delvecchio and Baker 92/ Sverdrup and Khan 94). In addition the E1 and E2 proteins and replication origins from several papillomaviruses can function together and are interchangeable (Delvecchio 92). This observation suggests that an anti-viral compound active against a particular viral type may well be active against other types and may be useful in treatment for a range of HPV induced diseases. A drug screen based on the transient replication assay has recently been set-up (Plumpton and Romanos 95). The screen employs a secreted reporter assay in which the activity of alkaline phosphatase in the cell supernatant gives a rapid measure of DNA replication. This assay has enabled high throughput screening for inhibitors of E1/E2 dependent HPV DNA replication.

The mechanism of E1 and E2 action has still to be completely resolved. It is known that E2 forms a heteromeric complex with E1 in solution (Mohr 90 / Lusky and Fontane 91 / Blitz and Laimins...
It is believed that the E2 protein directs the E1/E2 complex to the origin of replication via the high affinity E2 binding sites which flank the origin whilst stabilizing E1 in a conformation that facilitates origin recognition. Once there E1 binds to a weaker site (an A-T rich inverted repeat) and unwinds the DNA bi-directionally utilizing the hydrolysis of ATP as an energy source (Yang 91 / Lusky 93 / Seo 93). It has been determined that BPV1 E2 associates transiently with E1 during assembly of the initiation complex and is absent in subsequent replication competent complex that contains multimers of DNA associated E1 molecules (Lusky and Seo 94). The intracellular ratios of E1 and E2 may be an important controlling factor in the status of viral DNA replication.

The important role in viral DNA replication played by the interaction between E1 and E2 has led to an extensive analysis of the mechanism of association between the two proteins. The domains of both the E1 and E2 proteins necessary for complex formation have been investigated in attempts to discover the nature of the association. Using an extensive set of mutant E2 proteins two widely separable regions of the E2 protein have been identified which are essential for binding to E1 (Piccini and Banks 95). Investigations of the sites on E1 necessary for interaction with E2 have yielded conflicting results. A number of studies have identified the carboxyl terminus of E1 be necessary and sufficient for binding to E2 (Lusky and Fontane 91 / Sarafi and McBride 95 / Muller and Sapp 96) Whilst other studies suggest that domains sufficient to mediate interaction between E1 and E2 lie in the amino terminal of E1 (Benson and Howley 95 / Thorner and Botchan 93). Information of this kind is vital in the search for potential therapeutic compounds directed towards the inhibition of the E1/E2 association.

**In vitro culture systems**

The absolute dependence upon keratinocyte differentiation for vegetative viral growth is the chief
obstacle in the development of in vitro systems in the assay of anti-virals for HPV. In vitro systems which permit serial passage of keratinocytes without fibroblast overgrowth were described in a seminal series of experiments by Rheinwald and Green (1975). In these submerged culture systems the keratinocyte maintains a proliferative wound phenotype and eventually undergoes replicative senescence. Keratinocyte differentiation markers are not expressed in these systems and they are not permissive for vegetative viral growth. Infection of such monolayer cultures with HPV virions has been disappointing with transient replication only of episomes and rapid loss of HPV DNA from the cells La Porta and Taichmann 1982, Mungal etal 1992. Transfection of primary genital keratinocytes with cloned DNA from the oncogenic HPV's together with a selectable marker has, until recently, resulted in the derivation of immortal lines in which viral DNA is integrated making such lines uninformative for studies on episomal replication. Cell lines in which HPV 16 (Stanley etal 1989) or HPV31b (?Hudson ) are maintained as the episome have been derived as rare events after culture of low grade intra-epithelial lesions.

Since the infectious cycle of the papillomaviruses is absolutely dependent upon the regulated expression of the keratinocyte from stem cell to differentiated squame in vitro systems permissive for HPV must mimic the environment which supports keratinocyte differentiation. The regulation of this programme is extremely complex but depends very substantially on signals from the sub-epithelial dermis or stroma. Culture systems which partially reproduce this connective tissue matrix have been developed and in essence all these systems consist of a collagen matrix seeded with fibroblasts of human or murine origin. Keratinocytes are seeded onto these matrices in submerged culture and when the keratinocyte lawn is confluent the collagen keratinocyte sandwich is raised by placing it on an inert support usually a stainless steel
grid, so that the keratinocyte layer is at the air liquid interface. In such organotypic "raft" cultures the keratinocytes stratify and undergo much of the differentiation programme with the expression of differentiation specific keratins and molecules such as filaggrin and loricrin. Immortal keratinocyte lines transformed by HPV 16 or 18 in which the viral DNA sequences are integrated stratify but incompletely differentiate in raft culture exhibiting a morphology comparable to high grade CIN or VIN. Immortal lines containing HPV as the episome, W12 or CIN 612, stratify and differentiate in raft culture and in situ hybridisation shows that viral DNA amplification occurs in the upper epithelial layers (Bedell 1991, Stanley 1994). Exposure of these cultures to activators of protein kinase C such as the phorbol ester tetradecanoylphorbol-13-acetate (TPA) results in the induction of capsid protein synthesis and the assembly of virions in the superficial cells of the epithelium. Recently Frattini and Laimins (1996) have shown that primary human foreskin keratinocytes lipofected with religated HPV 31b genomes cloned from the CIN 612 line can stably maintain the viral episome at about 50 copies per cell. Furthermore these cells in raft culture are permissive for vegetative viral growth in the absence of TPA treatment. This is a major advance in papillomavirus biology with the opportunity for genetic analysis of papillomavirus gene function during the vegetative life cycle and the downstream consequences of intervening in viral replication. These systems represent powerful tools for the screening of novel anti-viral compounds against the high risk viruses.

This is a significant development but to date only the high risk transforming viruses have been used successfully in these systems and it would seem that the ability of the oncogenic HPVs to over-ride the mechanisms of cellular senescence and immortalise cells is central to the success of these systems. However permissive viral growth of the low risk viruses can be achieved in organotypic culture if explants of condylomas containing HPV 11 are used to initiate the cultures.
(Dollard et al 1992). Stratification and differentiation in these systems is exquisitely dependent upon the source of the fibroblasts embedded in the collagen matrix. Matrices seeded with human dermal fibroblasts do not support viral DNA amplification, late gene expression or virion assembly but matrices seeded with the A31 3T3 strain of mouse fibroblasts support keratinocyte differentiation permissive for viral growth. This dependence upon specific fibroblast populations in the matrix for late gene expression has also been shown for the W12 line (Stanley 1994). Although organotypic culture systems mimic quite faithfully the transit amplifying and differentiated populations of squamous epithelium and submerged cultures the wound keratinocyte phenotype neither sustain stem cells and to date there is no published data in which virions generated in vitro can infect keratinocytes and initiate another round of replication in vitro. In vivo animal models are therefore necessary to assess the efficacy of anti-virals in preventing and treating viral infection particularly for the low risk genital viruses which are refractory on the whole to culture in vitro.

**Animal Models**

There is, understandably, a move toward reducing the number of animals used in experimental science. In the field of papillomavirus research, large amounts of information have been obtained without recourse to animal models, and advances in molecular biology have been critical in the elucidation of viral gene function. However, at present there remains a need for animal models of papillomavirus-associated disease in order to solve problems which are difficult to solve by purely cellular and molecular means. For the testing of anti-viral agents one requires not only a system supporting the complete cycle of viral infection and vegetative growth but also one physiologically and pharmacologically relevant to humans.

**Requirements of an animal model**
In general, an effective animal model should resemble the biology of the comparable human disease as closely as possible, although other more specific criteria have been proposed by which the suitability of an animal system may be judged. Any model should allow rapid, inexpensive induction of the lesion with minimal experimental manipulation. The animal should be widely available, bear multiple offspring, suit modern animal housing facilities, be easily handled, and be free of specific import and export restrictions, such as those affecting endangered species. The animal should be large enough to allow sufficient tissue biopsies to be taken for multiple techniques such as cell culture and histological studies [Leader and Padgett, 1980; Sundberg, 1987]. A short latency period before appearance of the primary lesion would be advantageous, as would the development of neoplastic progression in a reasonable proportion of the lesions (although this is less relevant in investigations of anogenital warts than it is with cancer) [Brandsma, 1994]. The animal should develop lesions on anatomical sites comparable with those in human diseases, and these lesions should be readily accessible for monitoring. Ideally the system should yield large amounts of infectious virus particles for use in both in vivo and in vitro studies. However, the ability to use recombinant DNA as a source of infection may circumvent this requirement to some extent, depending on which part of the viral life cycle is being studied. There is a wide documented range of animals infected by papillomaviruses. These include the domestic cat [Egberink et al., 1992], lion [Sundberg et al., 1996], Elk [Eriksson et al., 1988], Chaffinch [Moreno-Lopez et al., 1984], and coyote [Sundberg et al., 1991] although only a few have been employed as models for human papillomavirus-associated diseases. The main candidates for papillomavirus research are outlined below.

**Rodent models**

Clearly, for reasons of economy and convenience, it would be useful to have a mouse model for
papillomavirus infection. A papillomavirus (MnPv) has been isolated from the multimammate mouse *Mastomys natalensis* [Muller and Gissmann, 1978]. This animal has been shown to carry endogenous papillomavirus genomes which can be reactivated by mechanical irritation [Siegsmund et al., 1991], resulting in papilloma formation and virion production. Extrachromosomal papillomavirus genomes can be found in a variety of tissues, with an increase in copy number with age, leading to tumours at around one year [Amtmann et al., 1984]. The genome has been sequenced, and has some similarity to that of CRPV and some of the cutaneous HPV’s [Tan et al., 1994]. A papillomavirus (MmPV) from the European harvest mouse *Micromys minutus* has been cloned and characterised [O’Banion et al., 1988]. This virus has been associated with a variety of tumours including papillomas and sebaceous carcinomas [Sundberg et al., 1988], and has some homology with rabbit oral papillomavirus (ROPV), MnPV and HPV1a [O’Banion et al., 1988]. Horizontal transmission was successful, although it was not possible to transmit the virus to inbred strains of laboratory mice [Sundberg et al., 1987].

A further mouse model for papillomavirus infection utilises the papillomas induced by ultraviolet irradiation of the hairless mouse strain *Mus musculus* HRA/Skh. Papillomaviral DNA sequences have been detected in papillomas, carcinomas *in situ*, and squamous cell carcinomas, as well as in tumours induced by topical agents [Tilbrook et al., 1989]. No viral particles were seen in the lesions, and papillomavirus group-specific antigens were not detected. Restriction enzyme digests indicated that this papillomavirus was different from MnPV. Tumour extracts containing papillomavirus DNA increased the susceptibility of inoculated hairless mice to UV-induced tumorigenesis [Reeve et al., 1989]. This system has been proposed as a useful model with which to study the association between ultraviolet light, papillomaviruses, and tumour formation.
Various rodent-based models have been used which do not rely on rodent papillomaviruses. Artificial papillomavirus "infections" have been created by using transgenic mice carrying the BPV-1 genome [Lacey et al., 1986]. Since then a variety of mice transgenic for human papillomavirus genes have been developed, enabling studies on tissue specificity [Cid et al., 1993], epidermal neoplasia [Greenhalgh et al., 1994; Lambert et al., 1993], dysplasia and hyperplasia of genital epithelia [Sasagawa et al., 1994], immunological responses to viral oncoproteins [Frazer et al., 1995], and hormonal cofactors in carcinogenesis [Arbeit et al., 1996]. Xenograft systems which reproduce the environment for epithelial differentiation have provided seminal data on viral gene expression during the infectious cycle and are valuable for the assay of anti-virals. The prototype for these is the nude mouse xenograft model developed by Kreider and colleagues (Kreider et al 1985). In this system small chips of human skin or mucosa are incubated with the Hershey strain of HPV 11 for 1-2 hours in vitro and are then implanted under the renal capsule of the nude mouse where after 6-8 weeks condylomatous transformation of the grafts occurs with epithelial cysts containing large numbers of virions which can be purified and serially passaged (Howett, Kreiderr and Cockley 1990). Experimental infection and production of HPV 1 has also been achieved with the renal capsule xenograft model(Kreider et al 1990) and engraftment of skin fragments from patients with epidermodysplasia verruciformis resulted in the expression of EV associated HPVs 5, 8, 9, 12 and 36 within the cysts (Majewski et al 1994). The renal capsule transplantation model requires formidable technical expertise and a more tractable model was described by Sterling et al 1990 in which W12 cells were transplanted into a skin pocket on the flank of the nude mouse using a transplantation technique which permits epithelial reformation (Hammond et al 1987). This results in the expression by W12 cells of the complete keratinocyte differentiation programme and the assembly of HPV 16 virions in the superficial
layers of the epithelium so formed (Sterling et al 1990, 1991).

Nude mice although T cell depleted are not NK or macrophage depleted, the latter have considerable cytotoxic capacity and graft take in nude mice is relatively low. Severe combined immunodeficiency (SCID) mice are lymphoid depleted and HPV 11 infected foreskin grafts under the renal capsule in SCID mice show enhanced graft take and size (Bonnez et al 1993) and furthermore such grafts will grow in the subcutis and peritoneum. HPV 6 and 11 infected biopsies from genital and laryngeal warts grafted onto SCID mice using the transplantation chamber technique resulted in growth of the grafts and expression of viral capsid proteins although these grafts could not be serially passaged (Sexton et al 1995) A promising system was described by Brandsma and colleagues 1995, in which human foreskin inoculated with HPV 16 DNA genomes by gene gun delivery was subsequently engrafted in a sub-cutaneous pocket onto the flank of SCID mice. A papillomatous lesion in which viral capsid proteins were expressed developed on the engrafted skin. This system generates easily accessible macroscopic papillomas, and provides a useful model of developing lesions in which antiviral therapies can be tested.

Some of these models are limited by the difficulties of monitoring lesion progression, such as with grafts located under the renal capsule, within the peritoneum, or in the subcutis. Other limitations include low levels of virion production, or a dependence on cell lines rather than clinical isolates as an initial source, although the ability to use naked recombinant viral DNA rather than intact virus makes the latter point less restrictive.

**Rabbit papillomaviruses**

Cottontail rabbit papillomavirus (CRPV) is a naturally occurring infection causing cutaneous
papillomas, some of which progress to squamous cell carcinomas in the natural host *Sylvilagus floridanus* [Rous and Beard, 1935]. CRPV has been studied extensively since its description by Shope [Shope, 1933]. The natural host, the cotton tail rabbit is not easily available and does not do well in captivity but the ability to transmit CRPV to domestic rabbits (*Oryctolagus cuniculus*) has increased the utility of this system. In the domestic rabbit, malignant conversion occurs at high frequency [Syverton, 1952], although virion production is insignificant [Wettstein and Stevens, 1982]. CRPV infection in domestic rabbits has been used as a model system for the testing of possible therapeutic agents. Since the lesions develop on the dorsal skin, they are easily visualised and can be directly counted and measured to allow evaluation of the efficacy of therapeutic agents. Ostrow and others [Ostrow et al., 1992] used domestic rabbits infected with CRPV, and tested the effects of ribavirin on the development of the resulting cutaneous papillomas. Daily intradermal injection of ribavirin resulted in a dose-dependant reduction in the number of warts, reduction in wart mass, and prolongation of the time until first wart appearance.

Domestic rabbits infected with CRPV were used to evaluate the efficacy of topical podofilox therapy on papillomas [Kreider et al., 1992]. Podofilox treatments were found to strongly inhibit papilloma growth, with CRPV DNA being undetectable by PCR or Southern blots of tissue from cured sites. The same system was used to investigate immune cell infiltration in the treated papillomas [Okabayashi et al., 1993]. Another putative topical therapeutic agent, the cobalt-containing complex CTC-96, was found to cause earlier appearance of tumours, at a greater number of sites, than with the control group [Ostrow et al., 1994]. Photodynamic therapy has been tested in the CRPV-domestic rabbit system. Early trials with a haematoporphyrin derivative demonstrated marked regression of tumours [Shikowitz et al., 1986] without recurrence and with
disappearance of CRPV DNA from regressed lesions [Shikowitz et al., 1988]. More recent work using m-tetra (hydroxyphenyl) chlorin [Lofgren et al., 1994], achieved a 75% cure rate with papillomas under 100mm² in area. A similar model was used to evaluate protoporphyrin IX (PPIX), produced by metabolism of delta-aminolaevulinic acid (ALA) [Lofgren et al., 1995], achieving a 3 month cure rate of 82% with papillomas below a certain size. This type of therapy has the potential to damage adjacent tissues, and the larynx of normal rabbits has been used to investigate the distribution of photoactive agents, and the resulting tissue damage and healing after photodynamic therapy [Kleemann et al., 1996].

These experiments demonstrate clearly the ability of the different CRPV-based systems to generate lesions with easily quantified progression and regression. Additionally the lesions are large enough to provide sufficient tissues for histological and molecular studies, and useful amounts of blood can be withdrawn without harming the animal. The flexibility of the CRPV system is evident from work which demonstrated its ability to model latent papillomavirus infections. Low concentrations of a viral inoculum resulted in delayed and reduced efficiency of wart production. Low levels of viral DNA could be detected in sites where papillomas did not form, and the latent virus could be reactivated by mechanical irritation of the skin [Amella et al., 1994]. This system could be used to study mechanisms of viral activation and provides a means for evaluating therapies aimed at preventing viral activation or eliminating latent virus.

An in vitro system, based on infection of a cottontail rabbit epidermal cell line with CRPV, was able to yield CRPV-specific transcripts, providing a possible model for analysis of the early events of viral infection [Angell et al., 1992], although productive infection was not achieved. A further development of the rabbit system has been the production of a neoplastic cell line arising from a CRPV-induced lesion. The papillomavirus-induced squamous cell carcinoma VX-2 cell
line has been used in rabbits to investigate perilesional interleukin-2 as a possible treatment for squamous cell carcinoma [Carroll et al., 1995]. Tumour growth was inhibited in animals receiving IL-2, although lower doses of IL-2 tended to enhance tumour growth. These findings suggested that high dose recombinant IL-2 has a potential role in tumour therapy. The VX-2 carcinoma has also been used to investigate the effect of oxygen radicals produced locally by arterial administration of xanthine oxidase and venous administration of hypoxanthine [Yoshikawa et al., 1995]. Significant suppression of tumour growth was reported, indicating the potential utility of oxygen-radical based therapies.

The CRPV genome has been cloned and sequenced [Giri et al., 1985] which, combined with an efficient means for inoculating rabbits with DNA [Brandsma et al., 1991], has allowed the effects of specific mutations to be studied by infecting rabbits with molecular clones [Brandsma et al., 1991; Wu et al., 1994]. Frameshift mutations were made in the E1 and E2 regulatory genes, and the resulting mutant CRPV genomes failed to induce papillomas in domestic rabbits. This system has demonstrated the requirement for E1, E2, E6, E7 and L1 genes for papilloma formation, although the L1 gene is dispensable for transformation [Meyers et al., 1992; Nasseri et al., 1989]. An interesting demonstration of the potential for ribozyme-based therapies has been examined using a CRPV system [Wisotzkey et al., 1993]. A plasmid was designed which contained a gene for a hammerhead ribozyme which specifically cleaved CRPV E7 transcripts in vitro suggesting that these genes may have potential as targets for therapeutic intervention.

To overcome some of the difficulties involved in infecting domestic rabbits with CRPV, transgenic rabbits have been created which carry either CRPV DNA alone, or both CRPV DNA and EJ-ras [Peng et al., 1993]. The rabbits carrying CRPV DNA alone developed extensive skin papillomas at an early age, with transcripts of CRPV DNA found only in skin or papillomas. The
rabbit transgenic for both CRPV DNA and EJ-ras suffered extensive cutaneous squamous cell carcinomas, also at an early age. It was proposed that extensive methylation of the CRPV genome, especially in the upstream regulatory region, may be a factor controlling tissue-specific expression of the genome [Peng et al., 1995]. These models may provide clues as to the factors affecting the tissue-specificity of papillomaviruses. A better understanding of the mechanisms controlling genome expression may give clues for the rational design of therapies.

The main limitations of the CRPV system include the limited availability of the natural host, the non-productive infections in the domestic rabbit and the fact that it affects haired skin rather than mucosal surfaces. A further virus, the rabbit oral papillomavirus (ROPV) affects domestic rabbits, causing oral papillomas (not transmissible to the genital tract), with abundant virion production [Sundberg et al., 1985]. The genome has been cloned and partially sequenced [O'Banion et al., 1988], showing similarities with CRPV, HPV-1a, HPV-16 and BPV-5. Rabbit tongue tissues have been incubated with ROPV and placed subrenally into athymic mice, producing stocks of infectious ROPV particles [Christensen et al., 1996]. As a mucosal animal papillomavirus, ROPV clearly has potential as an experimental system for modelling human mucosal papillomavirus infections.

**Canine papillomavirus**

The existence of transmissible warts in the dog was noted almost a hundred years ago [Penberthy, 1898]. Since then, experimental transmission by cell-free extracts [Chambers and Evans, 1959], the histologic and electron microscopic appearances [Watrach et al., 1969], and the sequencing of the viral genome [Delius et al., 1994] have confirmed the involvement of a papillomavirus. Papillomas have been identified in the oral cavity (including buccal mucosa, tongue, and soft
palate), skin, conjunctiva, penis and vulva [Belkin, 1979; Bonney et al., 1980; Hare and Howard, 1977; Sansom et al., 1996; Sundberg et al., 1984; Tokita and Konishi, 1975]. The cutaneous lesions include both squamous papillomas [Campbell et al., 1988; Watrach, 1969], cutaneous inverted papillomas [Campbell et al., 1988; Shimada et al., 1993], and squamous cell carcinomas [Watrach et al., 1970]. Squamous cell carcinomas have been associated with the use of a live COPV vaccine [Bregman et al., 1987; Ghim et al., 1995]. The canine "pigmented epidermal nevus" is associated with a papillomavirus, and some parallels have been drawn with human epidermodysplasia verruciformis [Nagata, 1995]. It is thought that there are several types of canine papillomavirus [Campbell et al., 1988; Delius et al., 1994], each having a different tissue tropism which may be expanded by natural or iatrogenic immunosuppression.

Experimental studies of COPV infection reported incubation periods of between 3 and 8 weeks, with 69% developing within 4-5 weeks. The longest wart duration was 21 weeks, with the modal duration being 4 - 8 weeks [Chambers and Evans, 1959]. The time of regression appeared to be related to concentration of viral inoculate, with delayed regression in dogs inoculated with greater dilutions.

The dog has been used to model the side effects on the larynx of photodynamic therapy [Lofgren et al., 1994]. Recent work has concentrated on using the canine papillomavirus model to evaluate both formalin-inactivated virus and recombinant viral capsid (L1) proteins for use as vaccines [Bell et al., 1994; Ghim et al., 1995; Suzich et al., 1995]. There are disadvantages associated with the use of dogs, including their relatively high purchase and maintenance costs, although not when compared with other systems such as the bovine
model. However, the canine model has several advantages over other animal models. The dog is a standard laboratory animal, and much is known about its physiology. A broad range of canine immunological reagents are available with which to characterise cellular and humoral events. Additionally, COPV is a mucosal papillomavirus, with obvious similarities to the important mucosal papillomavirus infections of humans. The lesions are easily induced and monitored, yield high titres of infectious virions [Chambers and Evans, 1959; Konishi et al., 1972], and the animals suffer no significant adverse effects, recovering completely with immunity to further infection.

**Bovine papillomaviruses**

Six types of bovine papillomaviruses have been characterised. Bovine papillomavirus (BPV) types 1, 2 and 5 [Campo et al., 1981; Chen et al., 1982; Lancaster, 1979] cause fibropapillomas, in which there is a marked dermal proliferative component. Fibropapillomas are found on the skin, rumen, omasum, and anogenital squamous epithelium. Each viral type is associated with certain anatomical sites. These fibropapillomas are distinct from the important papillomavirus-associated human lesions, and do not undergo malignant progression. Despite their dissimilarities with the human papillomavirus-related disease, these bovine viruses have proved to be important model systems.

Because of their large viral content, bovine fibropapillomas have been used to quantify some of the health risks associated with carbon dioxide laser therapy, used to vapourise warts. Vapour collected from bovine fibropapillomas after laser treatment was found to contain intact BPV DNA, with intact viral DNA being liberated also from laser-treated human plantar warts, creating potentially infectious aerosols [Garden et al., 1988]. This work was extended to investigate the risks associated with electrocoagulation and laser-therapy, using both human plantar warts and
bovine fibropapillomas to recover viral DNA in aerosols [Sawchuk et al., 1989]. Bioassay revealed that both treatment modalities liberated infectious BPV DNA, most of which could be filtered by a surgical mask.

The high viral content of bovine fibropapillomas has allowed large amounts of virus to be obtained for use in vitro, and BPV has the ability to transform cultured cells [DiMaio, 1991; Nakabayashi et al., 1984], and can induce tumours in hamsters [Cheville, 1966; Robl and Olson, 1968]. Cloning and characterization of the viral genomes [Campo and Coggins, 1982; Chen et al., 1982; Coggins et al., 1985] has enabled gene functions to be investigated [Androphy et al., 1985; Jareborg et al., 1992], and in vitro mutagenesis studies have revealed much about the molecular biology of BPV-1 [DiMaio, 1991; Lentz et al., 1993]. Additionally, there is good evidence that BPV-1 and BPV-2 can remain as latent infections, being reactivated by immunosuppression or physical trauma to cause cutaneous papillomas or urinary bladder cancer [Campo et al., 1994]. This finding obviously raises the possibility that this system could act as a more general model of papillomavirus latency.

BPV types 3, 4 and 6 [Jarrett et al., 1984; Patel et al., 1987; Pfister et al., 1979] cause true epithelial papillomas on the skin, upper alimentary tract, and udder, respectively. The papillomas caused by BPV-4 are of interest since they affect mucosal epithelium and can progress to squamous cell carcinoma, with obvious parallels with the human mucosal papillomaviruses. BPV-4 therefore provides an important model of mucosal papillomavirus infections, with the ability to create and monitor lesions in the oral cavity. Unfortunately it is difficult to monitor the progression of these tumours in the upper alimentary tract, and there are mechanistic differences in the oncogenic process itself, since BPV-4 genes do not need to be present continually for malignant progression to occur [Campo, 1987]. Cattle are obviously expensive and inconvenient
as a model system for papillomavirus studies, but have nevertheless provided important data in studies on vaccination and potential therapies [Jarrett et al., 1990]. Virus-like particles composed of either the L1 and L2 capsid proteins, or L1 protein alone, of BPV-4 have been used as both prophylactic and therapeutic vaccines in cattle [Kirnbauer et al., 1996]. Prophylaxis was effective with either preparation, although therapeutic vaccination was not particularly effective in animals with established papillomas, despite some apparent increase in the rate of regression compared with the unvaccinated controls.

Despite the large size and inconvenient husbandry requirements of cattle, they have been used in various therapeutic trials. Local injection of IL-2 as a therapy for papillomas and carcinomas has been demonstrated in a bovine model [Hill et al., 1994]. An effect on tumour load was observed in over 80% of treated animals, with complete regression in some. Regression was not restricted to the injected tumours in most cases. The same model was used to test the efficacy of intrallesional injection of live Bacillus Calmette-Guerin (BCG), causing total regression of all of six carcinomas and limited regression of advanced papillomas [Hill et al., 1994].

Most therapeutic studies using BPV have utilised in vitro systems rather than a whole animal approach. The E2 gene product has been suggested as a target for antivirals, and the potential therapeutic action of antisense oligonucleotides complementary to E2 mRNA has been assessed [Cowsert et al., 1993]. E2-dependant transactivation and viral focus formation was reduced in a sequence-specific and concentration-dependant manner by antisense oligonucleotides complementary to the mRNA cap region and the translation initiation region for the full-length E2 transactivator region. An antisense oligonucleotide to the translation initiation region of HPV-6 and HPV-11 E2 mRNA was able to inhibit E2-dependant transactivation, suggesting that antisense oligonucleotides may have a role in the treatment of genital warts.
It is clear that BPV models have made very important contributions in the field of papillomavirus research. For whole animal studies of potential therapeutic strategies however, the bovine remains a less than ideal system due to the expense and inconvenience of the husbandry requirements.

**Ovine papillomavirus**

Papillomas occur occasionally on the limbs, ears, and muzzle of sheep, and papillomavirus-like particles been demonstrated by electron microscopy [Gibbs et al., 1975; Vanselow and Spradbury, 1983; Vanselow et al., 1982]. DNA extracted from ovine papillomas has been shown to hybridise to HPV-16 DNA under conditions of low stringency [Hayward et al., 1993]. The demonstration by DNA hybridization of papillomavirus-related sequences in squamous cell carcinomas of the perineum of sheep indicates a possible role for papillomaviruses in the development of these lesions [Tilbrook et al., 1992]. Papillomavirus DNA has also been detected in aural precancerous lesions of sheep [Trenfield et al., 1990], with a restriction digest pattern close to that of BPV-2. It is thought that there may be a progression of papillomas to squamous cell carcinoma, and that exposure to sunlight may play a role [Tilbrook et al., 1992; Vanselow et al., 1982]. Ovine warts can be transmitted experimentally to other sheep, and can also induce fibromas in hamsters [Gibbs et al., 1975]. Ovine papillomavirus has been classified into Supergroup C along with the other ungulate fibropapillomaviruses [Chan et al., 1995], although there may be more than one ovine papillomavirus, since both fibropapillomas and squamous papillomas have been described [Gibbs et al., 1975; Hayward et al., 1993]. When considering their potential as an animal model of papillomavirus-associated disease, the smaller size of sheep gives them an economical and practical advantage over cattle and horses. Against this must be balanced the large amount of information already available for the bovine models, together with
the availability of better characterised alternatives such as the rabbit and dog.

**Equine papillomas**

Papillomas are moderately common in the horse [Sundberg et al., 1977], and can be transmitted experimentally [Hamada et al., 1990]. Experimental transmission caused papillomas to appear within 19-26 days of infection, reaching maximum size by 39-54 days. The papillomas are often found on the penis or vulva. Squamous cell carcinomas are common in the horse, and are found in sites similar to those of papillomas. Although viral antigens were detected in genital papillomas, they were not found in genital squamous cell carcinomas [Junge et al., 1984]. Papillomavirus antigens have been detected in papillomas contiguous with invasive equine penile squamous cell carcinoma [Sundberg, 1987].

An equine cutaneous papillomavirus has been cloned and characterized [O'Banion et al., 1986], and the difference in restriction patterns between the cutaneous isolate and one from a penile papilloma indicates the existence of more than one viral type. Equine sarcoids, which have both a fibrous and epithelial proliferative component, have been found to contain DNA with restriction enzyme profiles characteristic of BPV-1 or BPV-2 [Angelos et al., 1991], indicating that these viruses or close relatives are involved in the pathogenesis of equine sarcoids. The frequency of natural papillomavirus infections in the horse, together with the occurrence of genital forms of infection, make this an interesting model.

Unfortunately, horses require considerable effort in their upkeep, and most laboratory workers are unfamiliar with this species although this has not prevented the bovine models from being utilised effectively.

**Primate Papillomaviruses**
Despite the broad host range of papillomaviruses [Sundberg et al., 1984], there are only a few reports of the disease in primates. Papillomaviruses have been isolated from the pygmy chimpanzee *Pan paniscus* [Van-Ranst et al., 1992], the rhesus monkey *Macaca mulatta* [Ostrow et al., 1991], and two species of colobus monkey (*Colobus polykomus* and *C. guereza*) [Boever and Kern, 1976; Reszka et al., 1991].

Of particular interest is the rhesus papillomavirus (RhPV-1). This virus can be transmitted sexually and has been associated with squamous cell carcinoma of the cervix, and a carcinoma of the penis [Kloster et al., 1988; Ostrow et al., 1990]. RhPV-1 has similarities with the high risk HPV types 16 and 18, being integrated in tumour DNA, and has been detected in rhesus monkeys from several different geographical locations [Ostrow et al., 1995]. The genome has been sequenced, confirming similarities with HPV-16 [Ostrow et al., 1991]. Some *in vitro* work has established cooperation of RhPV-1 with cellular oncogenes during transformation of rat epithelial cells [Schneider et al., 1991], and transforming abilities have been localised to E5, E6, and E7 open reading frames [Ostrow et al., 1993].

The rhesus monkey is not the only non-human primate in which sexually-transmitted papillomavirus infections have been described. Although the Colobus monkey papillomaviruses have been associated with cutaneous papillomas [Boever and Kern, 1976; Rangan et al., 1980], a papilloma from the penis of a colobus monkey was found to contain papillomavirus group-specific antigens, viral particles and papillomavirus DNA, providing a further non-human primate model for venereal papillomavirus-associated diseases [O'Banion et al., 1987]. CgPV-1 isolated from the penile lesion has similarities with HPV types 16 and 18, and can transform cells. The transformed cells were found to have integrated and partially deleted CgPV-1 DNA, indicating parallels with the fate of HPV-16 and 18 in some cervical cancer cell lines [Reszka et
A different isolate from a cutaneous papilloma (CgPV-2) was found to be extrachromosomal [Kloster et al., 1988], and a laryngeal carcinoma from a colobus monkey was found to contain nucleotide sequences very closely related to RhPV-1 [Kloster et al., 1988]. These lesions obviously indicate the potential for non-human primate models of papillomavirus-induced genital and laryngeal tumours.

A further example of a non-human primate papillomavirus infection is that seen in the pygmy chimpanzee Pan paniscus. A papillomavirus was associated with clinical signs similar to those of oral focal epithelial hyperplasia of humans (caused by HPV-13)[Van-Ranst et al., 1991]. The PcPV-1 genome was found to have 85% sequence homology with that of HPV-13, indicating its potential as a model for HPV-13-associated disease, and was classified along with the other HPV types 6, 11, 43, and 44 [Van-Ranst et al., 1992]. Non-human primates are of particular interest as models of disease due to their close taxonomic relationships with humans. However, primate models are expensive, they may present important zoonotic disease risks, and their use is associated with important ethical considerations. In the final analysis, no animal disease can be regarded as a totally accurate model of its human counterpart.

Although the recent advances in the use of transgenic animals, the various xenograft systems, and the potential applications of chimaeric viral genomes [Brandsma, 1994; Jenkins et al., 1990] will provide useful experimental systems, the complexity of the viral-host relationship means that animal models still remain critical elements in papillomaviral research.