DISSERTATIONES
TECHNOLOGIAE
UNIVERSITATIS
TARTUENSIS
44

EVE SANKOVSKI

Studies on papillomavirus transcription and regulatory protein E2

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS
44

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS
44

EVE SANKOVSKI

Studies on papillomavirus transcription and regulatory protein E2

UNIVERSITY OF TARTU
Press

Institute of Technology, Faculty of Science and Technology, University of Tartu, Estonia

Dissertation was accepted for the commencement of the degree of Doctor of Philosophy in biomedical technology on February 5th, 2018 by the Council of the Institute of Technology, Faculty of Science and Technology, University of Tartu, Estonia.

Supervisor: Mart Ustav, PhD, Professor of Applied Virology, Institute of Technology, University of Tartu, Estonia

Reviewer: Ivar Ilves, PhD, Senior Scientist, Institute of Technology, University of Tartu, Estonia

Opponent: Frank Walter Stubenrauch, Dr. rer. nat., Dipl.-Biol., Professor, Division of Experimental Virology, Institute for Medical Virology and Epidemiology of Viral Diseases, University Hospital Tuebingen, Germany

Commencement: Auditorium 121, Nooruse 1, Tartu, Estonia, at 10.15 on March 8th, 2018.

ISSN 2228-0855
ISBN 978-9949-77-690-0 (print)
ISBN 978-9949-77-691-7 (pdf)

University of Tartu Press
www.tyk.ee

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

(I) Sankovski E, Männik A, Geimanen J, Ustav E, Ustav M. 2014. Mapping of betapapillomavirus human papillomavirus 5 transcription and characterization of viral-genome replication function. Journal of Virology 88: 961–973.

(II) Sankovski E, Karro K, Sepp M, Kurg R, Ustav M, Abroi A. 2015. Characterization of the nuclear matrix targeting sequence (NMTS) of the BPV1 E8/E2 protein – the shortest known NMTS. Nucleus 6: 289–300.

(III) Sankovski E, Abroi A, Ustav M Jr, Ustav M. 2017. Nuclear myosin 1 associates with papillomavirus E2 regulatory protein and influences viral replication.

My contributions to these publications are as follows:

(I) I performed all experiments, except the subcloning of HPV5, 8, 18 U2OS lines. I analysed the data and wrote the manuscript.
(II) I performed the protein biochemical fractionation and localization experiments; participated in data analysis and in writing of the manuscript.
(III) I performed all experiments except transcription assays. I analysed the data and wrote the manuscript.

LIST OF ABBREVIATIONS

bp	–	base pair
BPV	–	bovine papillomavirus
Brd4	–	bromodomain-containing protein 4
BS	–	binding site
C1QBP	–	complement component 1 Q subcomponent-binding protein
CFP	–	cyan fluorescent protein
CIP	–	calf intestine alkaline phosphatase
C-terminal	–	carboxyl-terminal
CTD	–	C-terminal domain
DBD	–	DNA binding domain
dsDNA	–	double-stranded DNA
EBNA1	–	Epstein Barr virus nuclear antigen 1
EFTUD2	–	elongation factor Tu GTP-binding domain-containing protein 2
EIF4A3	–	eukaryotic initiation factor 4A-III
FERM	–	4.1 protein, ezrin, radixin, moesin
FRET	–	fluorescence resonance energy transfer
GAP domains	–	GTPase activating proteins domain
HAT PCAF	–	histone acetyltransferase P300/CBP-associated factor
HMT	–	histone methyltransferase
HPV	–	human papillomavirus
HR-HPV	–	high-risk human papillomavirus
IP	–	immunoprecipitation
KC	–	keratinocyte carcinomas
KDM1B	–	lysine (K)-specific demethylase 1B
LCR	–	long control region
MHC	–	major histocompatibility complex
mRNA	–	messenger RNA
NCOR/SMRT	–	nuclear receptor co-repressor/ silencing mediator for retinoid or thyroid-hormone receptors
NLS	–	nuclear localization signal
NM1	–	nuclear myosin 1
N-terminal	–	amino-terminal
ORF	–	open reading frame
PAAG	–	polyacrylamid gel
PCNA	–	proliferating cell nuclear antigen
PH	–	pleckstrin homology
PML	–	promyelocytic leukemia protein
PTM	–	post-translational modification
RACE	–	rapid amplification of cDNA ends
RFC	–	replication factor C complex
RLM-RACE	–	RNA ligase mediated rapid amplification of cDNA ends

SH3 domains	–	SRC homology 3 domain
siRNA	–	small interfering RNA
Skp2	–	S-phase kinase-associated protein 2
SMC5	–	structural maintenance of chromosomes protein 5
Sp1	–	specificity protein 1
SRPK1/SRPK2	–	serine/arginine-rich proteinspecific kinase
SWI/SNF	–	SWItch/sucrose non-fermentable
TAD	–	transactivation domain
TAP	–	tobacco acid pyrophosphatase
Tax1BP1	–	Tax1-binding protein 1
TBP	–	TATA binding protein
TopBP1	–	topoisomerase 2-binding protein 1
TSS	–	transcription start site
URR	–	upstream regulatory region
WSTF	–	Williams syndrome transcription factor
YFP	–	yellow fluorescent protein

1. INTRODUCTION

Viruses are widespread intracellular parasites that are well-known and often encountered life-forms, which, at the same time, remain mysterious and abstruse infectious agents. Viruses infect all types of organisms, including animals, plants and microorganisms. Papillomaviruses represent a group of non-enveloped DNA viruses that infect basal epithelial cells of the host, where the viral genome is maintained as circular, double-stranded DNA within the cell nucleus. Over the years, hundreds of types of papillomaviruses have been discovered, which infect animals and humans and cause a wide spectrum of diseases. Some of these diseases have no clinically evident symptoms; some cause benign lesions such as genital warts and laryngeal papillomas; and the most dangerous types can cause malignant carcinomas. The most well-studied human cancers associated with papillomaviruses are cervical cancer and head and neck cancers. These malignancies are caused by mucosal human papillomaviruses (HPVs), and as these cancers cause many deaths among the human population. Molecular studies have also been focused mainly on this group of viruses, with less dangerous viruses receiving less attention. However, over the last few decades, there has been increasing interest in studying the other groups of PVs, including cutaneous papillomaviruses, which are now receiving a lot of attention. Cutaneous papillomaviruses infect the human cutaneous epithelium, and in most cases, these infections occur without symptoms. However, in some instances, when the patient is immunocompromised, these viruses can cause lesions and induce squamous cell carcinomas. The most prevalent virus types detected in skin cancer are HPV5 and HPV8. Through this study, we have tried to provide insight into the properties of these viruses, and we focused our attention on HPV5 and on the first part of the life cycle of this virus in cells. Papillomaviruses are known to produce approximately nine proteins, seven of which are expressed early, and two are expressed in late stages of infection. One of the most widely studied proteins over the years of papillomavirus research is the early protein E2, which is a key regulator of many viral life events. It is known that E2 has two truncated or spliced forms, namely, E2C and E8/E2, which are generated by alternative splicing.

The first part of the thesis provides an overview of the papillomavirus life cycle and E2 protein structure and function and gives a general introduction to the NM1 protein. The research part gives deeper insight to papillomavirus transcription and E2 protein, where in our first paper, we present the HPV5 transcription map with the newly identified E8 transcript. In the second paper, we describe a new nuclear matrix localization signal, which targets the BPV1 E8/E2 protein to the nucleus and which appears to be the shortest nuclear matrix targeting signal known to date. We showed that the E8 peptide is essential and sufficient for the localization of proteins to the nuclear matrix. Due to advancements in proteomics, over 200 cellular proteins have been identified as interaction partners of E2. These proteins belong to different protein groups such as

histone modifiers, splicing factors, replication and transcription factors, and chromatin remodelers. In the third paper, we present a new interaction partner of E2, NM1, which is the first myosin family member found to bind to a viral regulatory protein. We characterized this interaction and the role of NM1 in influencing viral replication.

2. LITERATURE REVIEW

2.1. Papillomaviruses – an overview

Papillomaviruses are a group of non-enveloped DNA tumour viruses that infect various animals from birds to mammals, including humans. Papillomaviruses usually cause benign tumours but can sometimes also cause malignancies. In 1982, the first complete papillomavirus genome sequences were published, i.e., the genome sequences of bovine papillomavirus type 1 (BPV1) and human papillomavirus type 1a (1, 2). Since then, BPV1 has served as a useful genetic model for studying papillomaviruses (3) because C127 cells allow BPV1 genome replication upon transfection. The taxonomic classification of papillomavirus types is based on the nucleotide sequence of the L1 gene of the virus. Papillomavirus types belonging to different genera have less than 60% similarity within the L1 gene, and different species within the genus share 60–70% similarity. A novel type must have less than 90% similarity to any other type (4, 5).

Papillomaviruses belong to the taxonomic family Papillomaviridae, which contains 49 genera, 116 species and several hundred types (International HPV Reference Center, http://www.hpvcenter.se/html/refclones.html). Human papillomaviruses (HPV) comprise large and diverse genera of viruses, and new HPV types are frequently discovered. Over 200 types of human papillomaviruses, which are aetiological agents of a wide spectrum of diseases that range from mild asymptomatic infections to malignant carcinomas, have been identified to date (6).

There are five major known HPV genera, namely, *Alpha*-, *Beta*-, *Gamma*-, *Mu*- and *Nupapillomavirus* (4), with the most widely studied genera being *Alpha*- and *Betapapillomavirus*. Alpha-papillomaviruses (alpha-PVs) infect mucosal epithelial cells, which can lead to the induction of benign tumours. These viruses are divided into high-risk (HPV16, HPV18) and low-risk (HPV6, HPV11) categories depending on the ability of the viruses to cause cancerogenic growth. High-risk HPVs are known to be a common cause of cervical cancer, which is fourth most common cause of cancer worldwide. Beta-papillomaviruses infect the cutaneous epithelium and are mostly associated with asymptomatic infections. In some cases, such as individuals with immune disorders, these viruses cause lesions that can become cancerous after long-term infection. Beta-PVs have also been implicated in nonmelanoma skin cancer (7, 8); however, the exact molecular mechanisms of cancer development are still largely unknown.

2.2. Life cycle of papillomaviruses

All papillomaviruses have a similar life cycle that is closely associated with differentiation of the host epithelium. The epithelium consists of a lower basal layer, where cells are mitotically active, and overlaid differentiated layers, which comprise non-dividing cells and are eventually shed from the surface of the epithelium. To initiate infection, papillomaviruses must gain access to the dividing basal cells, for which the viruses require microabrasions or wounds. After entry into the basal layer of the epithelium, viruses need to bind to cellular receptors to ensure viral entry into the cells. The HPV particle is composed primarily of the major capsid protein L1, which co-assembles with the minor capsid protein L2 into the icosahedral capsid around chromatinized viral DNA (9).

At the start of host cell infection, HPV virions first bind to the basement membrane, which is located under the epithelium and consists of fibrous connective tissue. In this extracellular matrix, the virions bind to heparan sulfate proteoglycans (HSPGs), laminin-5 or laminin-322, which are responsible for trapping viral particles (10–13). This initial attachment is dependent only on L1 and is followed by a possible transfer to a secondary receptor on the cell membrane. Binding to HSPGs induces conformational changes in the virions, leading to exposure of the L1 epitope, which is then cleaved by kallikrein-8. This cleavage facilitates the externalization of the N-terminal part of the minor capsid protein L2 from the interior of the capsid by cyclophilins (14). This step is followed by furin-mediated cleavage of the L2 N-terminal proximal peptide to expose the RG-1 epitope (15). It is possible that conformational changes in L2 are necessary to expose a secondary binding site, which enables the virion to bind to secondary receptor complex for entry into the cell. The cell adhesion receptor alpha6-integrin has been suggested to be a secondary receptor that can mediate virion internalization or subsequent events during infection (16–18).

Viral particles enter the cell by either caveolae or clathrin-mediated endocytic pathways (19–21). At this stage, a majority of the L1 capsomers are removed by cyclophilin B (22). Subviral particles are trafficked to the trans-Golgi network (23), which is followed by nuclear import of the viral genome, which occurs during mitosis by association of the viral genome with metaphase chromosomes (24). In the nucleus, the L2 transports the viral DNA to the subnuclear promyelocytic leukemia protein (PML) bodies, where several DNA viruses localize and initiate transcription (25). In the nucleus, the viral genome is amplified from 10 to 100 copies per cell, driving proliferation of the infected basal cell and leading to papilloma formation. Viral genomes replicate in nuclei as extrachromosomal elements. Viral genome amplification, late capsid protein synthesis and virion assembly occur in the upper, terminally differentiated cells of the epithelium. Papillomavirus infections are usually long-lived, and continually dividing basal cells serve as a reservoir of infected cells, which is also the source of malignant tumour growth (26).

2.3. Genome and proteins of papillomaviruses

The genome of papillomaviruses consist of circular double-stranded DNA that is approximately 8000 bp long. The genome is divided into early and late regions depending on the timing of protein expression. The early region contains seven open reading frames, for the E1, E2, E4, E5, E6, E7 and E8 proteins, which are expressed shortly after infection in the lower layers of the epithelium. The late region encodes capsid proteins L1 and L2, which are expressed in the upper, more differentiated layers of the epithelium. Viral RNA is transcribed from one strand and undergoes extensive alternative splicing. Upstream of the coding region lies the upstream regulatory region (URR), which does not encode any proteins but contains important cis-elements such as replication origin, promoters and binding sites for viral and cellular transcription regulators (26) (Fig. 1).

E1 is the primary replication initiator protein of papillomaviruses. An E2 dimer helps load the E1 dimer onto the origin, after which the E2 dissociates from the complex. E1 assembles into double-hexameric ring helicase to unwind the DNA and to allow access to replication factors (27–29). Specific DNA binding is mediated by the DNA-binding domain, while nonspecific binding involves the E1 helicase domain. Initially, the interaction of E2 with the helicase domain of E1 blocks the nonspecific DNA-binding function, but after E2 dissociates, the nonspecific DNA-binding region interacts with the DNA sequences flanking the E1 binding site (30, 31). E1 interacts with many cellular replication factors, such as replication protein A, topoisomerase I, polymerase alpha primase and proliferating cell nuclear antigen (32–35). Overexpression of E1 can cause double-stranded DNA breaks and can activate DNA damage response, leading to cell cycle arrest in the S and G2 phases (36, 37).

The E2 protein is the main regulator of the intracellular life cycle of the papillomavirus genome and is required for replication initiation, transcription regulation and viral genome maintenance during cell division. E2 acts in concert with E1 to bind to the origin and initiate viral replication (27, 38, 39). The E2 protein is more thoroughly discussed in the next chapter.

The E4 protein is usually expressed as an E1^E4 splicing product and is synthesized in the upper layers of the papillomavirus infection site. The E4 ORF is located within the E2 gene, and the spliced product includes the E1 initiation codon (40, 41). The E4 protein is detected during the late stages of infection when vegetative replication begins. This protein interferes with the cell cycle and is important for genome amplification and viral release (42). It has been shown that the cellular cysteine protease calpain cleaves the E1^E4 protein, and the resulting C-terminal fragments can form amyloid-like fibres that disrupt keratin networks, which may help the virus release its particles and exit the cell (43, 41).

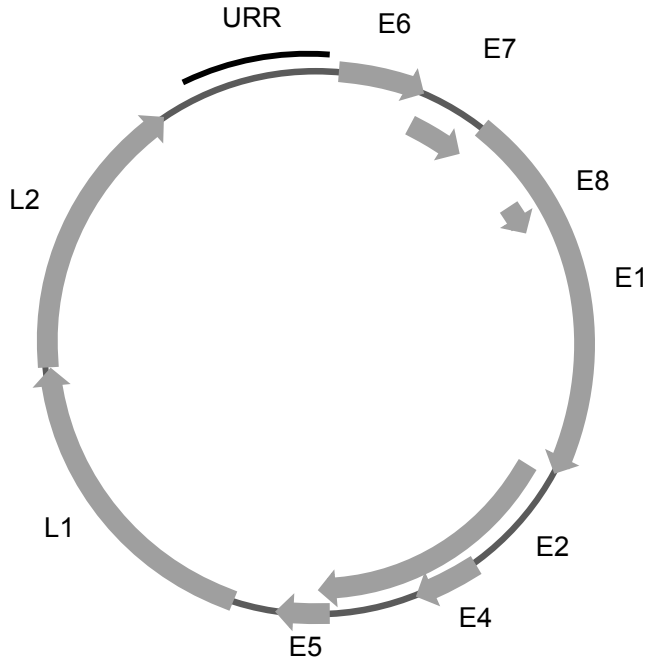


Figure 1. Schematic representation of the papillomavirus genome. The early and late open reading frames are indicated as grey arrows and marked as E1-E8 and L1-L2, respectively. The upstream regulatory region (URR) is shown as a black line.

E5 is a small transmembrane hydrophobic protein that promotes proliferation of infected basal cells by inducing and activating growth factor receptors (44). BPV1 E5 is localized largely to the membranes of the endoplasmic reticulum and Golgi apparatus and functions as a dimer (45, 46). BPV1 E5 does not have intrinsic enzymatic activity but rather induces transformation of the cells by activating the platelet-derived growth factor beta-receptor tyrosine kinase in a ligand-independent fashion (47–49). HPV16 and HPV6 E5 also localize to intracellular membranes, including membranes of the Golgi apparatus, endoplasmic reticulum and nucleus (50, 51), and bind to the epidermal growth factor (52, 53).

The E5 protein protects host cells from apoptosis via two main pathways: inhibition of death-receptor-mediated apoptosis and ER-stress-induced apoptosis (54). The first pathway involves downregulation of the Fas receptor and inhibition of TRAIL-mediated apoptosis by hindering the formation of the death-inducing signalling complex activated by TRAIL (55). The second method involves suppression of three main proteins in the ER stress pathway, namely, COX-2, XBP-1 and IRE1a, which is demonstrated by HPV16 E5 and is beneficial to viral replication and persistence (56). E5 also inhibits acidification of the Golgi apparatus, influencing growth receptor activation and trafficking (57–59), and downregulates surface expression of major histocompatibility

complex class I molecules, helping the virus evade the host immune system (60, 61).

The E6 and E7 proteins also induce cellular proliferation and delay differentiation of the host cells, which prolongs the S-phase and enables viral genome replication. These proteins are powerful oncoproteins (62). The E6 protein, aided by E6AP, binds to the p53 protein and prevents p53 from inducing apoptosis by targeting p53 for degradation via the ubiquitin-proteasome pathway. This prevents growth arrest and apoptosis of abnormal cells, which leads to genetic instability and tumourigenesis (63). In addition, E6 is capable of inhibiting p53-independent apoptosis by binding to different apoptotic signalling pathway proteins (64–67). The E7 protein is a major HPV oncoprotein, and E7 expression in high-risk HPVs is necessary for viral pathogenesis and cellular transformation. The main target of E7 is the antiapoptotic retinoblastoma protein pRB and pRB-associated proteins, p107 and p130 (68). The HPV-16 E7 oncoprotein induces p53-dependent and p53-independent apoptosis, but this oncoprotein may also inhibit apoptosis and cytokine-mediated cell death, depending on the cell and viral types (69, 70). Similar to E5, the oncoproteins E6 and E7 are also important for immune evasion and act by downregulating interferon responsive genes and surface expression of MHC (major histocompatibility complex) Class I proteins (71, 72). Transcription of E6/E7 is regulated by E2, which serves as either an activator or a repressor of transcription, depending on the context of the E2 binding sites within the promoter region (73, 74).

L1 and L2 are papillomavirus capsid proteins and form an icosahedral structure with a 55–60-nm diameter. L1 is the major capsid protein and can assemble into virus-like particles (75, 76). L2, the minor capsid protein, is important for packaging the viral genome into the capsid during the late phase of infection (77, 78). At early stages of infection, L2 helps transport the viral DNA to the nucleus to establish permissive infection (25).

2.4. Papillomavirus E2 protein

All papillomaviruses encode the E2 protein, which is the master regulator of the viral life cycle. E2 is a multifunctional protein that is involved in many aspects of the viral life cycle, such as as replication, transcription and maintenance of the viral genome during cell division. E2 is expressed at the early and intermediate stages of viral infection.

2.4.1. E2 structure

The full-length E2 protein consists of a conserved N-terminal domain named the transactivation domain (TAD), which is approximately 200 amino acids long, and a C-terminal domain named the DNA-binding and dimerization domain

(DBD), which is approximately 100 amino acids long. The two domains are connected by a flexible sequence called a hinge (Fig. 2). The crystal structure of the TA domain has been solved for BPV1, HPV11, HPV16 and HPV18, and this domain has been shown to form a cashew-shaped structure. The N-terminal region of this domain is composed of three alpha helices in a bundle, and the C-terminal region is composed of anti-parallel beta sheets (79). Between the two regions there is a two-turn helix called a fulcrum (80). The TA domain is involved in transcriptional activation and repression and in replication. Residues R37 and I73 are important in transcription regulation and are located on the convex side of the domain, and the E39 residue, which is involved in replication and E1 interaction, is located on the opposite, concave side of the domain. The TA domain has also been implicated in self-interaction (BPV1, HPV16) and is important in the looping of DNA through the widely spaced E2 binding sites (81–83).

E2 binds DNA via the DNA-binding domain. The DNA-binding domain forms a dimeric beta-barrel structure that positions two DNA-recognition alpha helices in contact with the consensus binding site (84). The first DNA-binding domain structure was solved for BPV1 E2 in 1992, and since then, the structures of several homologous DNA-binding domains have been solved, such as those of HPV6, HPV16, HPV18 and HPV31 (85–89).

The E2 consensus binding sites are located mainly in the URR, and the sites with the highest affinity have the sequence $ACCG(N)_4CGGT$ (90, 91). E2 also uses non-consensus binding sites with lower affinity (92), such as $AAC(N)_6GGT$ or $AAC(N)_6GTT$ in BPV1, and binds to these various binding sites with affinities that differ over 300-fold (91). The number and sequence of E2-binding sites vary between genera, for example, BPV1 contains 17 E2-binding sites, but HPV18 contains four conserved E2-binding sites. E2 forms a highly stable dimer via the DBD domain; the dimer contains a hydrophobic core with numerous packed side chains (93, 84).

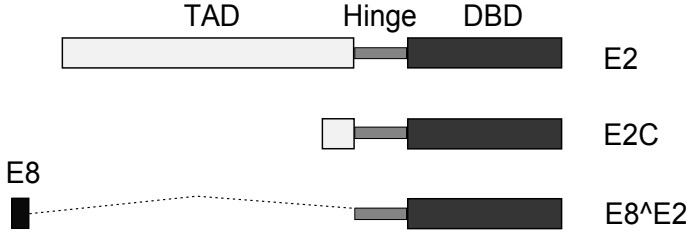


Figure 2. Schematic representation of E2 and truncated forms of E2. The trans-activation domain (TAD) is represented as light grey boxes; the DNA-binding and dimerization domain (DBD) is represented as dark grey boxes; and the two are connected by the hinge region. E8 is shown as a black box.

The E2 hinge region lies between N- and C-terminal domains and varies among the papillomavirus genera by length and sequence; the hinge sequence is not well conserved (94, 95). The E2 hinge is unstructured and forms a flexible link between the TA and DBD domains (96). The hinge region contains auxiliary elements that are conserved only among related viruses within genera. For example, in the BPV1 E2 hinge, a short sequence is phosphorylated by CK2, which induces proteasomal degradation of the E2 (97, 98). In alpha-PVs, a highly conserved KRXR motif promotes nuclear localization and association of E2 with the nuclear matrix (99, 100). In beta-PVs, a highly conserved RXXS motif in the hinge is phosphorylated by PKA, which promotes binding to mitotic chromosomes and stabilizes E2 (101–103).

2.4.2. Truncated forms of E2

In addition to full-length E2 protein, all papillomaviruses have the potential to encode shorter forms of E2 (Fig. 2). One E2 isoform called E8^E2 consists of an 11–13 amino acids long peptide encoded by an alternative reading frame of the E1 region fused to the DBD domain of E2. The E8 promoter is tightly regulated by cellular proteins that bind to conserved elements. In addition, this promoter is positively regulated by viral E1 and E2 proteins and negatively regulated by the E8^E2 protein, which implies that E8^E2 transcription is autoregulated in an E8-domain-dependent manner (104).

E8^E2 is encoded by a spliced RNA transcript, and the start codon and splice donor and acceptor sites are highly conserved among HPVs. The E8^E2 protein is a strong negative regulator of viral genome replication and downregulates the expression of HPV transcripts (105–108). These transcripts have been described for cottontail rabbit PV and HPV1, 5, 11, 16, 18, 31 and 33 (109–114, 105, 115). Recently, it has been found that the repressive function of E8^E2 is mediated by an E8-dependent interaction with NCOR/SMRT complexes, which, in addition to inhibiting transcription, are also involved in controlling the replication of HPV origins (116). The E8 peptide domain is rich in basic and hydrophobic residues and has been shown to associate with cellular proteins such as histone deacetylases, histone methyl transferase, nuclear coreceptor 1, chromodomain-helicase DNA-binding protein, and NCOR/SMRT repressor complexes (117–119).

Another short E2 isoform is found in BPV1 and is called E2C or E2-TR. This isoform is transcribed from the internal promoter P3080 in the BPV1 genome, which lies in E2 ORF TA domain (120, 109). The truncated E2 repressor proteins can regulate genome copy number and limit runaway replication. Mutations that eliminate E2C expression in BPV1 can boost replication (121, 122), which is explained by the fact that the E2C protein contains the DNA-binding and dimerization domain of E2 and can inhibit the activity of full-length E2 protein by competing for E2-binding sites or by forming heterodimers (123, 120, 124).

In BPV1-infected cells, the three different E2 isoforms, E2, E2C and E8^E2, are expressed in a ratio of 1:10:3 (125), implying that most full-length E2 proteins are present in the cells as heterodimers, which support transcription and replication but not viral genome partitioning (124, 126).

2.4.3. E2 stability

The half-life of full-length E2 is 40 min, and the shorter forms of E2, E2-TR and E8^E2, have half-lives of 10 min and 15 min, respectively (125). The main target for proteasomal degradation is the N-terminal region of E2, and association of the N-terminus with cellular or viral proteins stabilizes E2 and prolongs the life of this protein (127). The main cellular interaction partners of the N-terminal region of E2, Brd4 and Tax1BP1, stabilize E2 by preventing association of the E3 ubiquitin ligase cullin-3 with E2 (128, 129). The E2 TA domain interacts with Skp2, which is part of the Skp1/Cullin1/F-box Skp2 ubiquitin ligase complex, in a Brd4-independent manner, which degrades E2 at the end of G1 phase (130), thereby enabling the expression of the viral oncogenes E6/E7 and cell transformation by HPV. Association of E2 with viral E1 and E1/E4 also increases E2 stability (131, 132).

BPV1 E2 has two major phosphorylation sites, serines 298 and 301, and a minor phosphorylation site, serine 235 (133). In the cellular environment, the phosphorylation is carried out by casein kinase II and results in conformational switching and rapid turnover of E2 by the ubiquitin-proteasome pathway (133). HPV8 E2 is phosphorylated on residue 253 by PKA during the S phase, and this phosphorylation increases protein stability and promotes the binding of E2 to host chromatin (103).

2.4.4. E2 functions

2.4.4.1 Transcription

The E2 protein is the main regulator of papillomavirus transcription (120, 109). The E2 protein regulates transcription by binding to cognate binding sites and recruiting cellular factors to the viral genomes, which function as transcription activators or repressors. E2 binding can sterically hinder the binding of cellular transcription regulators such as Sp1 and TBP (134–136). E2 can activate or repress transcription in a dose-dependent manner (137, 138). The shorter forms of E2 act as repressors by competing with E2 for binding sites, recruiting repressor complexes and forming heterodimers (120, 117). In HPV-associated cancers, E2 is often disrupted, which causes reduction in E2-mediated repression and increased expression of the E6 and E7 oncogenes (139, 140). Restoration of E2 function in HPV-positive cervical cancer cell lines leads to cellular senescence (141). High levels of E2 cause read-through at the early polyadenylation signal into the late region of the HPV genome, inducing the expression of L1 and L2

mRNAs (142). It is possible, that E2 disruption acts in a similar manner to over-expression of E2 as both of these processes turn off the expression of oncogenes E6 and E7 and induce the expression of late genes. Expression of E2 inhibits cell growth in both HPV-positive and HPV-negative cells. In HPV-positive cells, E2 represses the early viral promoter and downregulates E6 and E7. Most HPV positive cancer cells are dependent on viral oncogenes for sustained growth and depletion of E6 and E7 inhibits cell propagation (143, 141). In HPV-negative cells, E2 induces apoptosis via p53-dependent and independent pathways and by interacting with caspase 8 (144, 145), which may be relevant the promotion of cell differentiation. As has been shown, keratinocytes that survive E2 expression develop phenotypes that are typical of terminally differentiated cells, suggesting that E2 may use this feature to promote viral function late in the life cycle (146).

2.4.4.2. Initiation of viral DNA replication

E2 participates in the initiation of viral DNA replication by loading the E1 helicase onto the replication origin, which contains at least an E1 binding site, an A/T-rich region and one E2 binding site (38, 147). After loading, E1 is converted to a double-hexameric replicative helicase upon oligomerization to unwind the dsDNA, and E2 is displaced from the origin (29). The E1 loads the cellular replication complex at the replication forks to initiate replication, and E1-binding specificity at HPV origins is determined by the E2 protein (148).

Viral DNA replication occurs in transient replication centres, and the formation of these centres is dependent on E2 (36, 37). These replication centres contain DNA damage response factors, which are characteristic to replication stress and might be important in the recruitment of cellular repair proteins to help synthesize new viral DNA (149, 37).

E2 is also required for vegetative viral DNA replication. In undifferentiated cells, most E2-encoding mRNAs are transcribed from the early promoter of the viral genome, but as cells differentiate in the mid-layers of the epithelium, the late promoter of the viral genome is used for transcription (150, 151). During viral DNA amplification, the transactivating function of E2 is not necessary, but the replication function is required (152).

2.4.4.3. Genome maintenance

To maintain the viral genome as an independent, episomal element, the genome needs be preserved and divided uniformly among daughter cells during mitosis. The E2 protein anchors the viral genome to the host mitotic chromosomes to ensure that the genome is not lost during partitioning by binding via the DBD region to E2-binding sites on the viral genome and via the TA domain to chromosomal protein(s) at the other end (153, 154). In addition to one E2-binding site, which was enough for minimal origin replication, genome maintenance requires

additional E2 binding sites, for example, BPV1 requires at least eight E2 binding sites in cis to the origin (155). Heterodimers of E2, which are formed by combining long and short forms of E2, can support replication but are defective in genome partitioning (124, 126).

The E2 proteins from different PV genera bind to different chromosomal regions of host cells. Alpha-PV E2 is not tightly bound to chromosomes during the cell cycle and is mostly found at pericentromeric regions of mitotic chromosomes (156). The proposed targets of alpha-PV E2 on chromosomes are Brd4, mitotic spindle, ChlR1 and TopBP1 (157, 158, 156, 159, 160). Beta-PV E2s also bind primarily to pericentromeric regions that overlap with the loci of the ribosomal rRNA genes (161, 101). The beta-PV E2 proteins require only the E2 DBD domain and a short phosphorylated peptide from the hinge region to bind to chromosomes, and the possible interaction partner of these proteins is Brd4 (102, 103). E2 proteins from delta-PVs bind as punctate foci all over the arms of mitotic chromosomes (153, 161). BPV1 and HPV1 E2 proteins bind with high affinity to Brd4 via the TA domain, and several other cellular proteins, such as ChlR1 and Mklp2, can be important E2-tethering partners (162–164).

2.4.4.4. Apoptosis

Several HPV proteins are associated with apoptotic cell death. The E5 protein protects cells from apoptosis by inhibition of death-receptor-mediated apoptosis and ER-stress-induced apoptosis (54). The oncoprotein E6 inhibits apoptosis by binding to and accelerating the degradation of several proteins that are important in apoptotic signalling, including caspase-8 and p53 (165), which in turn prevents growth arrest and apoptosis of abnormal cells, leading to genetic instability and tumourigenesis (166). Oncoprotein E7 is involved in both apoptosis activation and inhibition (70). The E2 protein is able to induce apoptosis by downregulating the transcription of E6/E7 mRNA and by binding with p53. The E2 protein from HPV16 can induce apoptosis in HPV-transformed and in non-HPV-transformed cells (167, 168), while the E2 proteins from low-risk HPVs fail to do so (167). HPV16 E2 appears to use at least two pathways to induce apoptosis. One pathway involves the binding of E2 to p53, which can occur in both HPV-transformed and non-HPV-transformed cells. The second pathway requires the binding of E2 to the viral genome, and this can only occur in HPV-transformed cells (167). It has been shown that HPV16 E2 interacts with c-FLIP, a key regulator of apoptotic cell death, and activates the apoptotic signalling factors caspase-8 and caspase-3. It has been suggested that HPV16 E2 abrogates the apoptosis-inhibitory function of c-FLIP, leaving cells hypersensitive to apoptotic signals (168). HPV18 E2 has been shown to bind to procaspase-8, which also leads to caspase-8 activation and subsequent cell death (169). Low-risk HPV E2 proteins remain nuclear, but high-risk HPV E2 proteins are present in both nucleus and cytoplasm. Data indicate that induction of apoptosis is caused by accumulation of E2 in the cellular cytoplasm following caspase-8

activation. It is speculated that disruption of the E2 gene during viral genome integration in cervical carcinoma makes it possible for cells to avoid E2-induced apoptosis, thus allowing the initiation of carcinogenesis (170).

2.4.5. Interaction partners of E2

E2 is the master regulator of the viral life cycle and functions by binding to the viral genome and recruiting a large number of cellular proteins to carry out its different functions. E2 interacts with a wide range of cellular proteins, including transcription regulators, replication factors, RNA-processing proteins, chromatin modifiers, cell cycle regulators and apoptosis-promoting proteins. There have been two major proteomic studies about the interaction partners of E2. One study was by Caroline Demeret's group in France, who mapped the virus-host protein interactions of the E2 proteins from 12 different HPV genotypes using yeast two-hybrid screenings and a cell-based interaction assay for validation of identified partners (171). The other work was by Alison McBride's group in the USA, who used tandem affinity purification followed by mass spectrometric analyses to identify the major host proteins interacting with 11 HPV types (172). Using different approaches, more than 200 cellular proteins have been identified as nuclear interaction partners of PV E2 proteins (171, 172) (Table 1).

The most widely studied partner of E2 from many PV genera is the chromatin adaptor protein Brd4, which is the major binding factor for delta-, lambda-, kappa-, mu- and tau-PV E2 proteins. Interestingly, alpha-PV E2 was shown to bind to Brd4 with low affinity and did do not stabilize the interaction of Brd4 with chromatin (173, 156). Beta-PV HPV8 E2 binds to Brd4 in vitro, but binds to the rDNA loci on acrocentric chromosomes in a Brd4-independent manner (156, 101). Interaction between E2 and Brd4 is important in several E2-dependent processes, such as transcription activation (174) and transcription repression (175), and in replication (163, 176). In addition to Brd4, other proteins involved in transcriptional regulation were identified, for example, TBP, MGA and TP53 (171). E2 also interacts with histone modifiers such as the acetyl transferases INO80 and p400; deacetyltransferases NCOR1/HDAC; demethylase KDM1B; and methylases WDR5 and WHSC (172). An additional chromosomal protein group that interacts with E2 is the chromosomal maintenance proteins SMC5 and SMC6, which have been implicated in chromosome replication, segregation and repair, roles that are associated with the functions of E2 in the viral life cycle.

Beta-PV HPV8 has been shown to colocalize with splicing speckles (99, 102), and consistent with this finding, HPV8 E2 has been shown to co-precipitate with mRNA-processing proteins such as SRPK1/SRPK2 (serine/arginine-rich protein-specific kinase), EFTUD2 (elongation factor Tu GTP-binding-domain-containing protein 2) and EIF4A3 (eukaryotic initiation factor 4A-III) (172). Several E2 proteins were found to interact with PCBP1, a protein that is involved in the inhibition of translation of the late mRNA encoding the L2 capsid protein (171). E2 binds to the protein C1QBP, which interacts with splicing

factors and binds to many viral proteins, such as EBNA1, adenovirus core protein V, and herpesvirus IE63. C1QBP has structural and functional similarities to the histone chaperones NAP-1 and nucleophosmin, which are both interaction partners of E2 (177, 171).

Many E2 targets are involved in ubiquitination. Given that E2 are ubiquitinated and degraded by the proteasome, some of these interactions probably mediate E2 degradation. However, the binding of E2 to ubiquitin ligases could have a functional impact by altering the degradation of the natural targets of the ligases (171, 127). These ligases include ubiquitin ligases of the HECT domain family (HUWE1, WWP2) as well as substrate adaptors of cullin-based ubiquitin ligase complexes (BTBD1, SPOP, CDC20, CDH1) (171).

Table 1. Cellular protein groups interacting with PV E2 proteins (171, 172)

Category	Examples of E2 interaction partners
Nuclear matrix	MATR3, HNRNPU, HNRNPA1
PTM	CSNK2A1, SRPK1/2, BAZ1B
DNA replication/repair	RFC2-5, ATAD5, WICH (BAZ1B, SMARCA5)
Transcription regulation	BNC2, YY1, TBP, MGA, p53
RNA processing	Spliceosome factors, C1QBP, PCBP1
Apoptosis	p53, CASP8, TAX1BP1
Ubiquitination	HUWE1, WWP2, BTBD1
Histone acetylase	INO80, P400, TRRAP
Histone deacetylase	NCOR1, HDAC, SAP18
Histone demethylase	KDM1B
Histone methyltransferase	WDR5, WHSC, PRMT5
Intracellular trafficking	VPS52, clathrin, RAB3IP
Chromatin binding	BRD4, Polycomb
Chromatin remodelling	SWI/SNF, NuRD, WICH (BAZ1B, SMARCA5), INO80, EP400, TRRAP

Many E2 interaction partners are Brd4-binding factors, and Brd4 likely mediates the association of these factors with E2. For example, replication factor C (RFC) proteins consist of five subunits, and Brd4 is shown to directly bind with the largest subunit, ATAD5, of this complex (178–180).

Apoptosis regulation is a common function of viral proteins because manipulation of cell-death-associated pathways is often a key process in viral infection. Apoptosis is generally prevented in early phases of viral infection to allow viral replication, and induction of apoptosis occurs when viral particles are produced. E2 proteins target both positive and negative regulators of apoptosis such as p53, CASP8 and TAX1BP1 (171).

Muller and colleagues have shown that E2 interacts with proteins involved in intracellular trafficking. VPS52 is involved in trafficking between the Golgi

apparatus and endosomes; clathrin light chain A (CLTA) participates in HPV entry pathways as do the Rab-family proteins (RAB3IP), molecular motors (KIF20A), and endosomal/lysosomal trafficking factors (VPS39) (171).

Recently, many studies have shown that E2 interacts with proteins that are part of chromatin remodelling complexes, which contain multiple proteins and modify chromatin via ATP-dependent catalytic subunits. These complexes activate or repress gene expression; regulate RNA polymerase I-, II-, and III-mediated transcription; maintain chromatin structure; and regulate DNA replication and repair (181). It has been shown that viral DNA is also packed into nucleosomes like host DNA, and interactions between viral and chromatin remodelling proteins enable the modulation of viral transcription and replication (182, 183). According to many researchers, HPV E2 interacts with the SWI/SNF chromatin remodelling complex proteins. For example, HPV8 from the beta-papillomavirus group binds to several chromatin remodelling complex proteins, including SMARCA4 (BRG1), SMARCB1 (SNF5), WSTF and INO80D (172). BPV1 E2 was also shown to interact with several chromatin remodelling proteins (172). Alpha-PV HPV18 E2 interacts with the SMARCB1 (SNF5) protein (184), and it has been demonstrated that SMARCB1 and SMARCA4 enhance HPV18 E2-dependent transcription activation and replication (182). Alpha-PV HPV16 E2 interacts with the SMARCA2 (Brm) protein from the SWI/SNF complex via its N-terminal domain (185), and HPV16 E7 binds to another protein from this complex, named Brg1, to control the cell cycle (186). Another major viral replication protein, HPV E1, also associates with SMARCB1 (184), making it plausible that viral E1 engages these enzymes during the S phase to help drive viral replication. E2 also binds to papillomavirus proteins such as helicase E1, minor capsid protein L2 (25, 187), E7 (188), E6 (189) and E1^E4 (131).

2.5. Papillomavirus replication and transcription

Papillomavirus transcription and replication is closely associated with the three phases of the viral life cycle, where the first and second phase occur in basal cells in the bottom layer of the epithelium, and the third phase occurs in the differentiated cells in the upper layer of the epithelium. The first replication phase is called initial amplificational replication, where the viral genome copy number increases to establish infection in dividing cells. The major viral proteins in this phase are the early proteins E1 and E2, which are sufficient for initiation and elongation of replication (27, 190). The minimal replication origin in the long control region (LCR) contains an E1-binding site and an E2-binding site (27, 147). Dimerized E1 and E2 bind cooperatively to their binding sites, forming a pre-replication complex (191). After binding, E2 dissociates from the complex, and E1 forms a double-hexameric ring helicase, which is an ATP-dependent process (192). This initial binding requires site-specific binding of E1, which is provided by the DBD domain. However, after E2 dissociates, the

non-specific binding enables the helicase domain to freely bind to the A/T-rich region flanking E1 BS (30). Single-stranded DNA is threaded through the E1 hexamer as the hexamer rotates down the DNA (193). The E1 helicase interacts with many cellular replication factors that are needed for viral DNA synthesis. The major cellular replication factors are replication protein A, which stabilizes single-stranded DNA; topoisomerase I, which relieves torsional stress; and cellular DNA polymerase alpha-primase, which primes replication (32, 34). During initial replication, papillomaviruses use both bi-directional theta replication and recombinational replication (194).

The second replication phase of PVs is stable maintenance replication, during which viral genomes are maintained as extrachromosomal elements in infected basal cells at a constant copy number to sustain infection. The genome copy number in this phase is up to 200 copies per cell. In stable replication, E2 exhibits its distinct function in genome maintenance; E2 partitions genomes between daughter cells by binding to the viral genome via its DBD domain and to the chromosome via its TAD domain (153). For this function, the genome needs multiple E2-binding sites in cis to the origin, and the minimal origin region is not sufficient (155). Generally, it is believed that both initiation and maintenance of replication require viral proteins E1 and E2, as has been demonstrated in BPV1 (195). However, some studies state that E1 is not required for maintenance of replication and is substituted by cellular proteins (196, 197). However, the need for E1 may be dependent on cell type, differentiation state and initial load of viral DNA.

Amplificational replication occurs in differentiated keratinocytes when these cells move upwards in the epidermal layer. At this stage, viral genomes are amplified up to five times followed by packing of the DNA into virions and release from the cells. In the cells, along with the amplification of genomes, elevated levels of E1 and E2 are detected (97). During differentiation, the cellular environment changes, and virus begins to express its late genes. At this stage, rolling circle replication, which enables rapid synthesis of viral DNA, might be prevalent (198, 194).

HPV replication is also associated with genomic instability. When HPV is integrated into the host cell chromosome, the papillomavirus episomes that express physiological levels of viral replication proteins can induce genomic instability in the host cell DNA (199). E1- and E2-dependent DNA replication from the integrated HPV origin follows the “onion skin”-type replication mode and generates a heterogeneous population of replication intermediates, which in turn integrate into host cell DNA and are processed by the activated cellular DNA repair/recombination machinery. This process results in cross-chromosomal translocation and can lead to the development of HPV-associated cancer (199). It has been shown that high-risk, but not low-risk, HPV E2 proteins promote a mitotic block, which is often followed by metaphase-specific apoptosis, independent of the viral oncogenes E6 and E7. High-risk HPV E2-expressing cells also exhibit polyploidy, chromosomal mis-segregation and centrosome amplification, leading to genomic instability (200).

2.6. Nuclear myosin 1

Myosins are crucial and specific cellular proteins that have the unique ability to transform free chemical energy from ATP into mechanical movement. By phylogenetic analysis, myosins have been grouped into 15 distinct classes (201). The best known myosins are those from class II, also called conventional myosins because this was the only class of myosins known for over 60 years (202). Myosin II has a two-headed structure; the dimerized tail of this myosin forms filaments; and this myosin is found mostly in muscles but also in the cytoplasm of cells. Unconventional myosins are less well known and are classified into several groups. One of these myosins are myosins from class I, which were discovered next and became known as monomeric, non-processive and slow-rate molecules (203). Myosin 1c was the first single-headed myosin isolated from mammals (203, 204). The human MYOIC gene encodes three isoforms: (I) myosin 1c isoform C is the classical 1063 amino acid myosin and was first detected only in the cytoplasm but was later also detected in the nucleus (204, 205); (II) myosin 1c isoform B, also known as nuclear myosin 1 (NM1), includes 16 additional amino acids at its N-terminus (206, 207); and (III) the newest myosin 1c isoform is isoform A, which includes 35 additional amino acids at its N-terminus and is found in the cell nucleus (208). Nuclear myosin 1 was discovered fortuitously when testing affinity-purified polyclonal antibodies against adrenal myosin 1. This antibody was also seen to stain a 120-kDa nuclear protein that was shown to have ATPase activity and could bind ATP, actin and calmodulin, which all indicated that this protein belonged to the class of unconventional myosins (206). A mass spectrometric analysis performed to identify the new immunopurified protein showed that this protein was highly homologous to the previously identified cytoplasmic Myo1c protein. Since it was the only nuclear myosin known at the time, it was named nuclear myosin 1 (207).

2.6.1. Structure of nuclear myosin 1

Myosins constitute a large superfamily of proteins that share a common domain structure and have been shown to interact with actin, hydrolyse ATP and produce movement in all cases examined to date. Similar to all myosins, nuclear myosin 1 is composed of three functional subdomains:

(1) The motor domain, which interacts with actin, binds ATP and has a unique 16-residue amino-terminal extension. The structures of the motor domains of the myosin family are relatively conserved with the exception of several surface loops and the amino terminus (206, 202) (Fig. 3).

(2) The neck domain, located between the head and tail, contains three IQ motifs that bind calmodulin (206). The IQ motif is a helical structure that has a consensus sequence of IQXXXRGXXXR, and this motif is located in the neck region of all myosins that bind light chains and calmodulin (209). The number of IQ motifs present in the neck domains of different myosins

can vary between zero and six (202). The nuclear localization signal (NLS) of NM1 lies in the second IQ motif, and the nuclear import mechanism involves importins (210).

(3) The tail domain, which is too short to self-aggregate into filaments. However, the NM1 tail domain does contain regions such as a positively charged domain and pleckstrin homology domain (PH) that bind various molecules such as DNA (211) and acidic phospholipids. The latter allows NM1 to bind actin with its head domain, and acidic phospholipids bind to the PH domain in the tail region, thus fulfilling the class I myosin functions, i.e., linking membranes and membrane-coated vesicles to actin-rich structures such as cytoskeletons (212, 213).

The tail domains are the most diverse domains in myosin proteins and vary widely in length and sequence. The tail contains often functional motifs, such as SH3 domains, GAP domains, FERM domains, and pleckstrin homology (PH) domains. In addition, the tails of many myosins contain coiled-coil-forming sequences, which allow the molecules to dimerize and produce two-headed molecules (202).

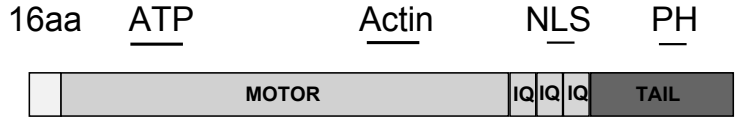


Figure 3. Schematic representation of the nuclear myosin 1 structure. Shown are the motor and tail domains connected with IQ motifs. ATP- and actin-binding sites are marked with lines, as are the nuclear localization signal (NLS) and pleckstrin homology (PH) domain.

2.6.2. Nuclear myosin 1 functions

Myosins are actin-based motors that are known or hypothesized to play fundamental roles in many forms of eukaryotic motility, such as cell crawling, cytokinesis, phagocytosis, growth cone extension, maintenance of cell shape, and organelle/particle trafficking (214).

NM1 is an unconventional myosin that works with actin and other nuclear components to regulate different steps in the gene expression pathway (215–217) and affects chromosome repositioning (218). Using specific antibodies generated against its N-terminal epitope, NM1 can be detected predominantly in the nuclei, nucleoli and at the plasma membranes of interphase cells (206, 219, 220). NM1 plays important roles in DNA transcription, RNA maturation, chromatin remodelling and cell motility.

During transcription, NM1 associates with chromatin and localizes to both nuclear and nucleolar transcription sites in an RNA-dependent manner (219–

221). At the rRNA gene promoter, the interaction between the chromatin-bound NM1 and RNA polymerase I (Pol I)-associated actin is required for transcription activation (221). The interaction of NM1 with Pol I is indirect and is mediated by TIF-1A, a transcription factor phosphorylated by growth-associated kinases (222). The NM1-TIF-1A interaction suggests that NM1 plays a role in the growth-dependent regulation of rDNA transcription (223). NM1 is also necessary for forming the first phosphodiester bond during transcription initiation, and purified NM1 stimulates transcription by RNA polymerase II (224).

During RNA maturation, NM1 associates with rRNA in the nucleolus (219), is loaded onto nascent preribosomal subunits and accompanies export-competent ribosomal subunits to the nuclear pore basket (225).

NM1 plays an important role in chromatin remodelling, where NM1 is part of a multiprotein assembly, B-WICH, that contains the WICH chromatin remodelling complex with the subunit WSTF and the ATPase SNF2h (226–228). On the rDNA, WSTF bookmarks the position of the chromatin remodelling complex, while NM1 interacts with SNF2h to stabilize B-WICH, leading to recruitment of the HAT PCAF for H3K9 acetylation (221). NM1 has therefore been proposed to link Pol I with the rDNA via direct interactions with the Pol I-associated actin and chromatin, respectively. Since this mechanism depends on myosin ATPase activity, and the catalytic activity of NM1 is required for Pol I transcription, NM1 is likely to function as an actin-based motor that activates transcription by providing a permissive chromatin state for rapid Pol I transcription activation (223, 221, 229). It has been demonstrated that B-WICH along with NM1 activates RNA pol III transcription (230).

Recently, it has been shown by Almuzzaini and colleagues that NM1, SNF2h and WSTF are enriched at active class II promoters, where NM1 specifically maintains hypophosphorylated Pol II levels and modulates B-WICH assembly, including SNF2h recruitment for local chromatin remodelling. They demonstrated that this process leads to the recruitment of the HAT PCAF and the HMT Set1/Ash2 for H3K9 acetylation and H3K4 trimethylation, respectively (231).

The motor function of nuclear myosin is crucial for cell motility and for functional compartmentalization of the nucleus. NM1 links the cell plasma membrane to the adjacent cytoskeleton and regulates the plasma membrane tension, which determines cell shape and influences processes such as cell motility, spreading, endocytosis and exocytosis (232). The nuclear centre of the cell includes early replicating chromosomes, whereas the periphery contains late-replicating heterochromatic chromosomal regions (233). It has been shown that the movement of an engineered chromosome locus is an active process powered by actin and NM1, and overexpression of mutated NM1 that lacks motor activity inhibits this effect (234). Several studies have demonstrated that chromosome repositioning requires an NM1-containing motor complex (235, 236, 218).

3. OBJECTIVES OF THE STUDY

Beta-papillomaviruses are a group of viruses that have been studied much less thoroughly than alpha-papillomaviruses, which have received most of the scientific spotlight. The main reasons why beta-PVs have remained overshadowed by alpha-PVs are the absence of a suitable model system for studying replication of beta-PV genomes, the transient nature of beta-PV infections in humans; and their unclear association with carcinogenesis in humans. Beta-PV research has been mostly limited to medical studies about viral loads and types in humans, but molecular mechanisms are understudied due to the challenges in this area.

The general objective of this study was to provide a more thorough molecular biological characterization of beta-papillomavirus HPV5 and to expand the knowledge about main regulatory protein of papillomaviruses, E2, which plays key roles in all viral life events. The specific objectives of this study were as follows:

1) To identify and characterize the HPV5 transcription start sites and polyadenylation cleavage sites in the early region of the viral genome, to identify the HPV5 E8 transcript, and to demonstrate the function of this protein in genome replication (I).
2) To identify the BPV1 E8/E2 nuclear targeting signal and demonstrate its function in protein localization inside the cell (II).
3) To characterize a new interaction partner of papillomavirus E2 protein, identified as NM1, and demonstrate the role of NM1 in viral replication and transcription (III).

4. MATERIALS AND METHODS

For characterization of the HPV5 transcription map, we used U2OS cells to generate HPV5 mRNA and analysed this mRNA by the rapid amplification of cDNA ends (RACE) method. According to Geimanen and colleagues (237), U2OS has proved to be a useful cell line for studying different papillomaviruses, including the beta-papillomaviruses HPV5 and HPV8. U2OS cells, derived from human bone osteosarcoma, is highly chromosomally altered, moderately differentiated and expresses wild-type pRb and p53 proteins. U2OS cells can be transfected with high efficiency and enable the study of viral genome replication in transient and stable phases but also support viral genome amplification, which is characteristic to the late phase of the viral life cycle. The transcription map of HPV18 is very similar in U2OS cells and in infected raft cultures (238, 151), which proves that the U2OS cell line is highly compatible for studying different phases of the papillomavirus life cycle.

For identification of the early promoter regions and polyadenylation cleavage sites of HPV5, we used the RNA-ligase-mediated rapid amplification of cDNA ends method (RLM-RACE), which allows the amplification of cDNA only from full-length, capped mRNA, omitting incomplete messages that lack 5' ends (Ambion FirstChoice RLM-RACE Kit, Thermo Fisher Scientific, Waltham, MA). A major limitation of traditional 5' RACE is that there is no selection for the amplification of fragments corresponding to the actual 5' ends of mRNA because all cDNAs are acceptable templates in the reaction. Additionally, the PCR step selects the most efficient amplicons, which can be the smallest, favouring the amplification of products with sizes lower than the full-length product, producing a heterogeneous population of amplified products. Briefly, the RLM-RACE procedure is as follows: RNA is first treated with calf intestinal alkaline phosphatase (CIP) to remove free 5' phosphates from molecules such as ribosomal RNA, fragmented mRNA, tRNA, and contaminating DNA, all of which can interfere with the determination of the real 5' ends of intact mRNA, which have a cap structure that protects the mRNA from CIP treatment. The RNA is then treated with tobacco acid pyrophosphatase (TAP) to remove the cap structure from the full-length mRNA, leaving a 5' monophosphate. RNA adapter oligonucleotide is ligated to the RNA population using T4 RNA ligase. The adapter cannot be ligated to dephosphorylated RNA because these molecules lack the 5' phosphate necessary for ligation, which ensures that only true 5' ends of transcripts are amplified. During ligation, a majority of the full-length, decapped mRNA acquires the adapter sequence. A randomly primed reverse transcription reaction and nested PCR then amplifies the 5' end of a specific transcript.

In the characterization of HPV5 transcripts (I) and in the identification of nuclear myosin as an E2-binding partner (III), we used HPV minicircle genomes for transfection of U2OS cells (239). Traditionally, viral genomes are inserted into bacterial plasmids, and to produce a complete viral genome the genome has

to be cut out from the plasmid and religated before transfection into the cells. Instead of using this time-consuming procedure to generate the circular HPV genome, we used an arabinose-inducible bacterial recombination system, where the ΦC31 integrase produces minicircle genomes, and the SceI endonuclease destroys the plasmid backbone, ensuring that only minicircle DNA is produced, without plasmid contamination.

A complete overview of methods used in this study are described in the appropriate sections of each publication.

5. RESULTS AND DISCUSSION

5.1. Characterization of the HPV 5 transcription map in U2OS cells (Ref. I)

By 1960, it was already thought that HPV infections can cause cutaneous warts, although the multiplicity of the HPV types was not yet fully understood. Harald zur Hausen was the first to propose a role for HPV in the development of cervical cancer, for which he received a Nobel Prize in 2008. Due to the work of many researchers, the critical role of HPV in the development of different types of cancer was established, and it has now been found that approximately 4,8% of new cancers develop due to HPV infections. These estimates are based on the carcinogenic potentials of the high-risk HPV types from the genus *Alphapapillomavirus*, which infect only mucosal epithelia and do not cause keratinocyte carcinomas (KCs). Keratinocyte carcinomas (basal and squamous cell carcinomas) are the most common cancers among Caucasian populations. The main factors causing KCs are solar UV radiation, chemicals, Rontgen radiation, smoking and immunosuppressive medication; however, there is growing evidence that HPVs the genus *Betapapillomavirus* act as co-factors in the development of UV-induced KCs, especially in immunosuppressed patients. Therefore, all research that helps reveal the molecular biology of beta-PVs is necessary for understanding the mechanisms of viral infection that lead to carcinogenesis.

In our study, we focused on the characterization of the transcription map of the beta-papillomavirus HPV5. Only one study has been published about HPV5 differentiation-dependent transcription; Haller et al. (240) identified multiple HPV5 transcripts from EV patients via in situ hybridization. The main obstacle in beta-papillomavirus research has been the lack of suitable model systems that can enable the study of viral gene transcription and viral genome replication. We found that the human osteosarcoma cell line U2OS is suitable for studying both alpha- and beta-papillomaviruses, supporting viral genome replication and transcription in transient and stable replication modes. For this study, we used a bacterial recombination system to generate covalently closed circular HPV5 genome minicircles, cloning the whole viral genome into the appropriate vector, pMC.BESPX. This vector facilitates the production of the viral genome as a supercoiled miniplasmid, containing only a minimal portion (92 bp) of non-viral sequences located in the HPV5 L2 gene. Although we successfully generated the HPV5 and HPV8 positive subclones in the U2OS cell line and confirmed the episomal status of the viral genomes, we did not see amplification of beta-papillomavirus genomes after cultivation in subconfluent conditions; viral DNA amplification under these conditions in alpha-papillomaviruses HPV16 and HPV18 has been observed. The lack of beta-PV genome amplification in confluent U2OS cells might reflect the absence of certain critical factors required for the amplification of the HPV5 and HPV8 genomes in U2OS cells. It is also

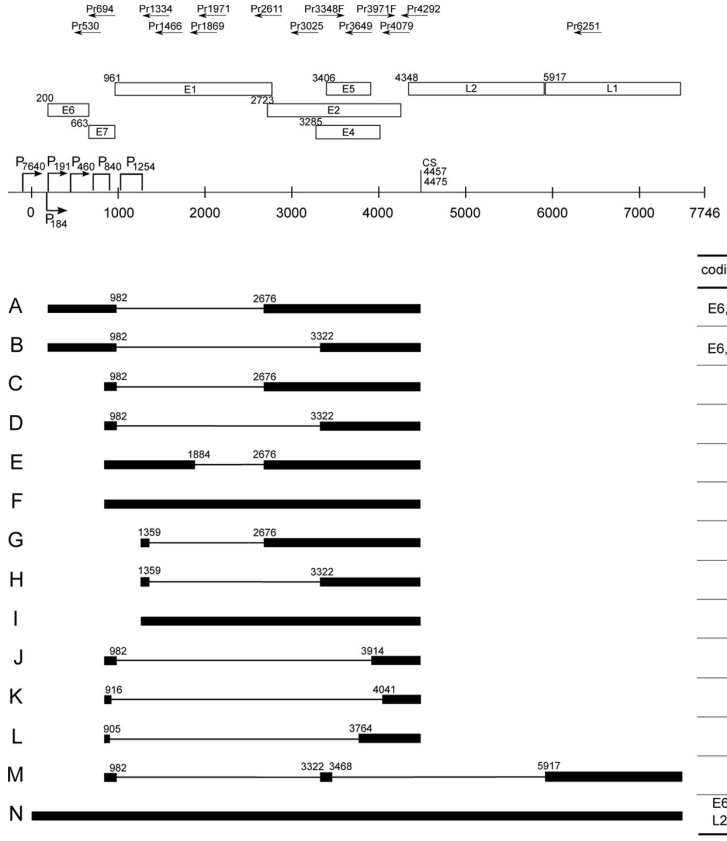
possible that beta-PV does not enter a strong amplification phase, which is plausible given the asymptomatic nature and ubiquity of beta-PV infections.

First, we established that HPV5 genome replication is dependent on E1 and E2 expression, which was confirmed by transient replication analysis of the HPV5 wild-type (wt) genome and E1- or E2-defective genomes in U2OS cells. To precisely demonstrate that the HPV5 genome cannot replicate without the E1 and E2 proteins, we generated two mutant genomes encoding truncated E1 and truncated E2 by inserting stop codons into the corresponding ORFs, resulting in frameshifts in the coding sequences of the replication proteins. Episomal DNA analysis of the replication of mutated viral genomes showed that the E1- and E2-defective genomes failed to replicate in U2OS cells, while the wt HPV5 genome replicated well, showing an increase in replication signal over the 5-day period (Ref. I, Fig. 1A). This result was also confirmed by a complementation assay, where co-transfection of both mutated genomes resulted in restoration of the replication signal. The overall result of this assay was expected because E1 and E2 are the main initial replication factors across PV genera, as demonstrated in 1991 by Ustav and colleagues in a BPV1 model system (27).

After successfully establishing U2OS subclones carrying an episomal HPV5 genome, our next aim was to characterize the HPV5 mRNA products. Many HPV types, such as HPV1, 11, 16, 18, 31 and 33, express negative regulators of viral genome replication, that is, splicing products of the E8 and E2 genes. Using 5´RACE analysis of transiently expressed HPV5 transcripts, we identified HPV5 E8 ORF at nt 1328 to 1359 in the E1 gene. After mutation of the E8 start codon, we observed an increase in the replication signal, which was approximately 100 times higher than that of the wt genome (Ref. I, Fig. 2A), which proves that E8^E2C is an important repressor of HPV5 gene expression. Sequencing of E8-containing transcripts revealed two E8-ORF-containing transcripts, E8^E1C and E8^E2C. Replication analysis indicated that E8^E1C did not affect HPV5 E8 replication, and only E8^E2C had a strong effect on viral genome replication, where co-transfection with E8^E2C successfully reduced HPV5 E8 replication.

Our main aim was to map the HPV5 transcription start sites. For this purpose, we used the 5´RACE technique, using total RNA extracted from U2OS cells transfected with the HPV5 genome or HPV5 subclone no. 15. We successfully identified four HPV5 early promoter regions with transcription start sites (TSSs) at nucleotides 184/191, 460, 840, and 1254 and the HPV late promoter with a TSS at nt 7640. The early TSS at 184/191, upstream of the E6 ORF, is analogous to other PV promoter regions such as HPV8 P175 and HPV16 P97. We noticed that transcripts starting at this TSS all begin from the same nucleotide, which is unlike alpha-PVs, where broad heterogeneity of TSSs is common. We believe that the reason behind this observation may be the more complex and variable environment of the mucosal epithelia, where alpha-PVs propagate.

A second early promoter within the E7 ORF represents a larger region, in which the TSSs range from nt 811 to 888. The heterogenous promoter region in the E7 ORF is found also in alpha-PVs. We found a new promoter in the E1 ORF, P1254, which is the start site for transcripts of repressor protein and truncated E1. A similar promoter region, P1193, is located in the HPV18 genome (151). The HPV5 late promoter was mapped to nt 7640, which is similar to the HPV8 late promoter P7535.



coding potential	trans replic	sub-confl line	confl line	EV skin
E6,E7,E2	+	+	+	+
E6,E7,E1^E4	+	+	+	+
E2	+	+	+	+
E1^E4	+	+	+	+
E2		+		
E1,E2	+	+	+	
E8^E1C	+	+	+	
E8^E2C	+	+	+	
E1*,E2	+	+	+	
E2C		+	+	
E2C	+			
E2C			+	
L1		+	+	+
E6,E7,E1,E2, L2,L1	+	+	+	

Figure 4. Schematic representation of HPV5 transcripts mapped in U2OS cells. The HPV5 genome is shown on top with ORFs (open boxes), start codons (numbers above), promoters (arrows for single sites and brackets for regions) and PA cleavage sites (CSs). For details, see (Ref. I).

We also wanted to map early polyadenylation cleavage sites (PA CSs) for early mRNAs, which are important in the maturation of pre-mRNAs. Upon analysis of 39 clones obtained by the 3'RACE technique, we identified early mRNA cleavage sites at nts 4457 and 4475, which are located at the beginning of the L2 ORF. In HPV11, an analogous CS is located before the L2 ORF (241), but in HPV18, an early PA CS is also located at the beginning of the L2 ORF (151).

Analysis of the HPV5 transcript splice sites revealed the main splice acceptor sites to be at nts 2676 and 3322. These sites were identical to those identified from the data obtained from EV patients with HPV5-positive skin lesions. Altogether, we identified 14 different HPV5 mRNA species from U2OS cells harbouring the HPV5 genome (Ref. I, Fig. 4), which is much more than has been identified in previous work (240).

A complete transcription map of the viral genome is a basic tool that provides the means to perform a variety of studies on the current virus. The online papillomavirus episteme database (http://pave.niaid.nih.gov) currently contains transcription maps for eleven papillomaviruses. Considering that more than 150 HPV types have been identified to date, eleven transcription maps does not seem like much; however, the most important viruses associated with carcinogenesis, such as HPV11, 16, and 18, and HPV5 and 8 are included in this database. Given the advancements in current methodologies, there are now detailed protocols available for the construction of papillomavirus transcription maps based on recently published studies, which have used methods such as 5' and 3'RACE to map transcription start sites and polyadenylation cleavage sites, primer walking RT-PCR to study the splicing junction of RACE products, and RNase protection assay and primer extension methods.

5.2. Identification of the nuclear matrix targeting sequence (NMTS) of the BPV1 E8/E2 protein (Ref. II)

Modern proteomic tools have provided much new information about proteins and protein isoforms. Protein isoforms can have different localization and functions in the cell compared to the full-length protein. Understanding the importance of alternative splicing and the complexity of protein localization signals helps us understand the biological functions of these proteins in the complex cellular network.

The main regulatory protein of papillomaviruses, E2, has two isoforms, E2C and E8/E2, that are generated by alternative splicing. We used biochemical fractionation to study three E2 isoform localizations in the cell (Ref. II, Fig. 2). Biochemical fractionation included the treatment of transfected cells with different buffers containing increasing concentrations of NaCl and supplemented with nucleases such as DNase or RNase. Each time new buffer was added, the proteins that were solubilized under the conditions used and the proteins that stayed in the insoluble cellular fraction with the cellular matrix and other insoluble compartments were collected separately and analysed by SDS-PAGE and by immunoblotting with specific antibodies. This method enables the separation of cytoplasmic proteins from nuclear proteins using a mild buffer with a low concentration of the non-ionic detergent Triton-X100 and physiological concentrations of NaCl, which permeabilizes the cellular and nuclear membranes and releases soluble proteins, which are not strongly bound to the matrix structures. Upon treatment with the next buffer, containing a higher NaCl concent-

ration, we could solubilize the proteins that had higher affinity for the matrix. The nuclease DNase destroys DNA and releases proteins bound to chromatin, such as histones, while RNase degrades RNA, and all proteins bound to RNA are released into the soluble fraction. By this method, we established that all three forms of E2 have different subnuclear localizations. The truncated E2 isoform E2C was extracted from nuclear structures with mild buffer conditions; and E2 was extracted with 0,4 M NaCl buffer; but E8/E2 remained bound to nuclear structures after treatment with 0,4 M NaCl, RNase, DNase and 2 M NaCl. We refer to the insoluble fraction of the isolated cell nuclei, which is resistant to salt and nuclease treatment, as the nuclear matrix. Further experiments with the proteins where the E8 peptide was added as a linker or was deleted showed that the E8 peptide is essential and sufficient for localization of the proteins to the nuclear matrix (Fig. 5). Mutational analysis of the E8 peptide helped identify the critical residue for nuclear matrix localization, i.e., the conserved lysine residue at position 2. The mutated E8 K2A was no longer retained in the nuclear matrix but was easily extracted from cells by mild treatment with CSK buffer.

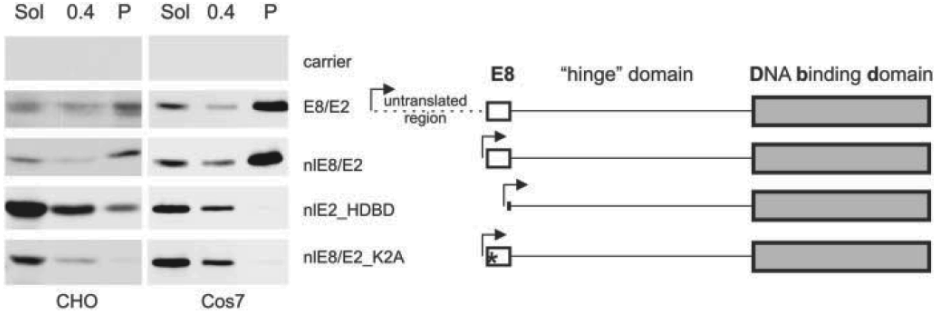


Figure 5. The E8 part of the E8/E2 protein is required for targeting the protein to the nuclear matrix. Western blot of biochemical fractions from CHO and Cos7 cells are shown. Sol, CSK soluble fraction; 0.4, CSK soluble fraction with 0,4 M NaCl; P, CSK insoluble fraction containing nuclear matrix proteins. For details, see (Ref. II).

We wanted to further establish the nature of E8 localization in the nuclear matrix. Data obtained by biochemical fractionation are indirect and can imply that E8 and the E8 mutant K2A localize into different subnuclear compartments or that the mutant K2A has weaker affinity for the same nuclear compartment to which wild-type E8 is attached. We used a FRET assay to determine whether E8 and E8K2A localize close to each other or far apart. The FRET assay enables the measurement of the proximity of a donor-acceptor pair of fluorescence proteins in living cells. Notably, fixation of the cells is not necessary as fixation may alter the structure of the cell compartments and the data may not be reflective of the actual situation. Our experiments showed that expression of E8 together with E8 K2A resulted in a much weaker FRET signal than expression

of E8-E8 proteins, which demonstrates that E8 and its mutant have different spatial localizations in live cells (Ref. II, Fig. 6).

Overall, we successfully characterized the localization of the papillomavirus E8/E2 repressor protein in the cell and identified the determinants of localization. According to nuclear matrix database (www.rostlab.org/db/NMPdb), E8 is the shortest nuclear matrix targeting signal identified to date. Similar localization signals have been described for NF-90 and other proteins, which indicates that we have just started to understand the complex world of protein trafficking in subcellular compartments. We do not yet know the biological significance of the strong attachment of E8 to nuclear matrix compartments. This phenomenon could be associated with the important repressor functions of E8; therefore, when the cell divides, E8 is still present in each cell to control viral genome protein expression. We have shown that in HPV5 and HPV18, E8 has a strong influence on the expression levels of some mRNAs and subsequently controls viral genome replication. It would also be interesting to identify the interaction partners of E8 in the nuclear matrix and to determine whether this localization increases E8 stability. Resent studies in the field of virology have provided insight into the life of viruses in cellular systems and indicated how these two systems are very closely entangled, forming a unified balanced network where everything is connected.

5.3. Identification of the new interaction partner, NM1, of the papillomavirus E2 protein and of the role of NM1 in viral replication (Ref. III)

E2 is the master regulator of the life cycle of papillomaviruses, affecting all the main viral life events. Proteins that form strong or weak complexes with E2 to modify, help or restrict the various functions of E2 have always received a lot of interest. There are many published studies about the identification of cellular targets of the papillomavirus E2 protein, including chromatin binders, modifiers and remodelers; RNA-processing proteins; and replication and transcription factors. In this study, we identified a new interaction partner of the papillomavirus E2 protein, namely, nuclear myosin 1c (NM1), which is a known chromatin remodeler and is part of the B-WICH complex. NM1 is the first myosin that has been identified in the cell nucleus (206). Mass-spectrometric analysis of this protein showed that it was highly homologous to the myosin Myo1c, which was the first single-headed myosin isolated from mammals. It was later discovered that the ratios between Myo1c and NM1 in the nucleus and cytoplasm are roughly equal, and both isoforms are mutually redundant during transcription (205). NM1 associates with RNA polymerases I, II and III and is required for transcription, nuclear transport and chromatin relocation (207, 222, 230).

By performing an immunoprecipitation assay, we showed that the BPV-1 E2 protein co-precipitated with three proteins, which were later identified by mass-

spectrometry as myosin 9, nuclear myosin 1c and beta-actin (Ref. III, Fig. 1). By washing the immunoprecipitated material with washing buffers containing increasing concentrations of NaCl, we demonstrated that the E2 protein affinity towards myosin 9 was weaker than that towards NM1. In the immunoprecipitation assays, the beta-actin signal was generally weak, and we hypothesized that beta-actin was a myosin partner but was not directly bound to E2. We wanted to characterize the interaction between E2 and NM1 more closely and generated truncated E2 and NM1 protein mutants in which only the N-terminal or C-terminal regions were expressed. Using an immunoprecipitation assay, we established that both E2 and NM1 interact through their N-terminal regions. Myosins use ATP to generate a motive force, which changes the myosin conformation. We found that addition of ATP to immunoprecipitated complexes causes NM1 to loose its interaction with E2. We concluded that ATP binding and hydrolysis cause conformational changes in full-length NM1 that lead to lower affinity for E2, as non-hydrolysable γATP and ADP released considerably less NM1 into the supernatant from the NM1-E2 complex. BPV1 E2 has been characterized thoroughly by creating alanine-scanning mutants, and this has revealed critical residues for replication, transcription and genome maintenance function. Previous studies have demonstrated that the E2 double mutant R37A/I73A supports E1- and E2-dependent BPV replication initiation, but the transcription activation ability of this mutant is impaired, and the mutant is unable to bind mitotic chromosomes or the Brd4 protein. We found that the binding of E2 R37A/I73A to NM1 was weaker that of wtE2 or the E2 mutants E39A and R68A, which could be due to conformational changes or because the structural determinants of E2 are also involved in the binding to Brd4 and NM1 (Ref. III, Fig. 2).

The chromatin adapter protein Brd4 is the most well-studied interaction partner of E2, and it has been shown that the interaction between Brd4 and E2 is important in E2-dependent transcriptional activation and repression (156, 242). Brd4 might be involved in E2-dependent replication and is been shown to mediate BPV1 genome segregation to mitotic chromosomes (164). Brd4 interacts with E2 through its C-terminal domain (CTD), and this interaction increases E2 stability in cells by preventing the association of E3 ubiquitin ligase with E2 (129). In our experiments, when we transcribed proteins in vitro and subsequently used these proteins in immunoprecipitation assays, we observed that Brd4CTD co-immunoprecipitates with the E2-NM1 complex, but Brd4CTD and NM1 do not directly interact with one another (Ref. III, Fig. 3). The results of these experiments also confirmed that beta-actin and Brd4 CTD are not essential for the interaction of E2 with NM1. This finding was similarly illustrated by washing IP complexes with buffers of different ionic strengths; E2 appeared to be bind to NM1 with greater affinity than to Brd4CTD or beta-actin.

Papillomavirus E2 proteins are not well conserved across genera; however, we wanted to test whether the interaction between E2 and NM1 is a unique feature of BPV1 or whether it also occurs with E2 proteins from other genera. For this experiment, we selected HPV5 E2 and HPV18 E2, which have 26%

and 25% identity to BPV1 E2 at the amino acid level. Our immunoprecipitation experiments showed that HPV5 and HPV18 bind effectively to full-length NM1 and to Myo1C, which is another isoform. NM1 is part of the multiprotein complex B-WICH, which includes the transcription factor WSTF and the ATPase SNF2h. We demonstrated that BPV1 E2, HPV5 E2 and HPV18 E2 are present in the complex with NM1, WSTF and SNF2h, allowing us to conclude that the interaction of E2 with the chromatin remodelling complex B-WICH may be required to promote the transcription of viral genes by facilitating the generation of permissive DNA structures.

The main function of E2 is the initiation of viral replication by binding to viral helicase E1 and to E2-binding sites in the viral promoter region to facilitate the unwinding of the viral DNA. We wanted to test whether NM1 can influence E2-dependent replication. For this purpose, we used NM1-directed siRNA to downregulate NM1 expression in the cells, which caused replication growth in BPV1 and HPV5 but not in HPV18 (Fig. 6).

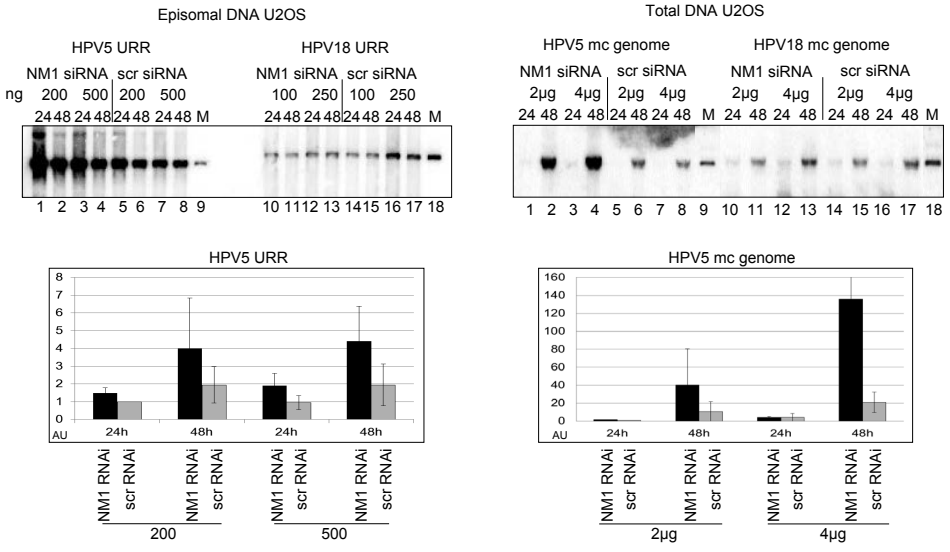


Figure 6. NM1 downregulation by siRNA enhances HPV5 replication but does not influence HPV18 replication. Southern blot analysis of U2OS cells transfected with siRNA and the HPV URR-containing plasmid together with the E1- and E2-constructs are shown. For details, see (Ref. III).

We propose that the difference between HPV5 and HPV18 replication is dependent on the upstream regulatory regions of these viruses, which are type-specific and contain different binding sites for cellular replication factors. The NM1 downregulation effect in BPV1 and HPV5 replication enhancement is probably caused by the availability of free E2, which increases when the NM1

level decreases, enhancing E2 functions. Transcription studies showed that NM1 downregulation has no significant effect on E2 transcription regulation.

Currently, there are more than 200 known cellular interaction partners of E2, which were identified by proteomic analysis and the functions of most of which are unknown. We consider the interaction of NM1 with E2 to be very interesting as the functions of NM1 in chromatin remodelling and replication initiation are very intriguing. We have managed to shed a little light on the cooperation between the key viral regulator E2 and NM1. However, more interesting discoveries remain to be made in the future. In this study, we did not examine the interaction between myosin 9 and E2, which could also be very interesting and warrants further research.

6. CONCLUSIONS

The cutaneous beta-papillomavirus HPV5 is much less well-studied than the strongly oncogenic mucosal papillomaviruses HPV16 and HPV18. We wanted to characterize HPV5 more thoroughly at the RNA level. At the same time, in our research group, the main regulatory protein of papillomaviruses, E2, has been the primary subject of research as it influences all the main viral life events. In this study we have achieved the following:

1) We successfully characterized the HPV transcription map, including the early start sites and polyadenylation cleavage sites. We also identified the HPV5 E8 transcript and demonstrated the role of this protein in genome replication.
2) We identified the BPV1 E8/E2 nuclear targeting signal, and using different protein constructs, we showed that this signal localizes the proteins to the nucleus.
3) We discovered a new E2 interaction partner, NM1, which influences BPV1 and HPV5 replication but not HPV18 replication. However, the E2 protein of all the mentioned viruses interacts with NM1, which is part of the WICH chromatin remodelling complex. The exact role of NM1 in the viral life cycle remains to be determined in future studies.

SUMMARY IN ESTONIAN

Papilloomiviiruse transkriptsiooni ja regulaatorvalgu E2 uurimine

Papilloomiviirused on viiruste perekond, mis nakatab nii inimeste kui loomade epiteelrakke ja põhjustab papilloome ehk näsakasvajaid, mis võivad teatud tingimustes areneda halvaloomulisteks kasvajateks. Organismi nakatumiseks peab viirus sisenema aktiivselt paljunevatesse keratinotsüütide tüvirakkudesse. Edasine viiruse elutsükkel sõltub rakkude diferentseerumisest ja seetõttu saavad uued viiruse osakesed tekkida ainult naha ülemise kihi mittepaljunevates rakkudes, kust uued virionid vabanevad rakkude irdumisel.

Inimese papilloomiviiruseid on teada üle 200 tüübi ja nad jagatakse kaheks grupiks nende koespetsiifilisuse alusel: limaskesti nakatavad papilloomiviirused ja naharakke nakatavad papilloomiviirused. Limaskesti nakatavad viirused jaotatakse omakorda kaheks grupiks nende vähitekke riski alusel, nimelt madala- ja kõrge riski papilloomiviirusteks. Tänaseks päevaks on selgunud, et enamik inimese emakakaelavähi juhtumitest on põhjustatud kõrge riski papilloomiviiruste poolt. Lisaks emakakaelavähile põhjustavad need viirused ka teisi anogenitaalseid ja suulimaskesta kasvajaid. Naha papilloomiviirused on siiani pälvinud vähem tähelepanu, sest nende võimalik seos nahakasvajatega on tulnud ilmsiks alles viimasel kümnendil. Meie keskendusime oma töös naha papilloomiviirusele HPV5, mida on viimasel ajal hakatud seostama nahakasvajate tekkega, aga mida on siiani ikkagi suhteliselt vähe uuritud. Meie töö tulemusena valmis HPV5 transkriptsiooni kaart, mille iseloomustamiseks me kasutasime inimese rakuliini U2OS. Varasemalt takerdusid paljud uurimistööd sobiva mudelsüsteemi puudumise taha, kuna rakkude diferentseerimine laboritingimustes on keeruline, kuni meie laboris avastati HPV genoomi paljunemise jaoks sobiv rakuliin U2OS. U2OS on inimese sääreluu kasvajast pärinev rakuliin, mis sobib erinevate HPV genoomide replikatsiooni uurimiseks. Selle rakuliini abil õnnestus meil kirjeldada 14 erinevat viiruse mRNAd. Me kirjeldasime ka HPV5 uut viirusvalgu E8 transkripti ja näitame selle represseerivat mõju viiruse DNA paljunemisele.

Me keskendusime oma töös viirusvalgu E2 uurimisele, mis on põhiline viiruse elutsükli regulaator. E2 valgul on lisaks täispikale valgule veel kaks isovormi, valgu C-terminaalset osa sisaldav E2C ja alternatiivse splaissinguga saadud E8/E2. Me uurisime E2 paiknemist rakus ja leidsime, et lühike E8 valgu järjestus on piisav selleks, et valk suunata raku tuuma. Meile teadaolevalt on see järjestus lühim tuuma suunav järjestus, mis on siiani teada. E2 valgu edasisel uurimisel keskendusime tema rakuliste partnervalkude otsimisele. Tänu proteoomika arengule on siiani leitud üle 200 E2 partnervalgu, millest enamuse funktsioon viiruse elutsüklis on siiani teadmata. Meie leidsime uue E2-ga seonduva valgu, milleks on tuumamüosiin 1 (NM1), mis on esimene müosiini perekonda kuuluv E2 partnervalk. Müosiinide roll rakus on väga oluline, hõlmates kõike seda, mis on seotud raku liikumisega, fagotsütoosiga, raku kujuga ning organellide ja partiklite transpordiga. NM1 valk osaleb rakus DNA transkript-

sioonis, RNA küpsemises ja kromatiini ümberkorraldamises. Lisaks on tal ka väga oluline roll raku motoorikas. Me näitasime, et E2 ja NM1 seostuvad omavahel mõlema valgu esimeste osade kaudu ja see interaktsioon sõltub ATPst. E2-NM1 valgukompleks kuulub ka rakulisse WICH kompleksi, mis osaleb kromatiini ümberkorraldustes. Me leidsime, et NM1 mõjutab BPV1 ja HPV5 viiruse DNA replikatsiooni, aga see mõju puudus HPV18 puhul.

Kokkuvõtvalt võib öelda, et antud töö käigus kirjeldati põhjalikumalt naha papilloomiviiruse HPV5 elutsükli varasemat osa ja iseloomustati viiruse olulise regulaatorvalgu E2-e uusi aspekte.

REFERENCES

1. **Chen, E. Y., P. M. Howley, A. D. Levinson, and P. H. Seeburg.** 1982. The primary structure and genetic organization of the bovine papillomavirus type 1 genome. Nature **299:**529–534.
2. **Danos, O., M. Katinka, and M. Yaniv.** 1982. Human papillomavirus 1a complete DNA sequence: a novel type of genome organization among papovaviridae. The EMBO journal **1:**231–236.
3. **Zheng, Z. M., and C. C. Baker.** 2006. Papillomavirus genome structure, expression, and post-transcriptional regulation. Front Biosci **11:**2286–2302.
4. **Bernard, H. U., R. D. Burk, Z. Chen, K. van Doorslaer, H. Hausen, and E. M. de Villiers.** 2010. Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. Virology **401:**70–79.
5. **de Villiers, E. M., C. Fauquet, T. R. Broker, H. U. Bernard, and H. zur Hausen.** 2004. Classification of papillomaviruses. Virology **324:**17–27.
6. **Bzhalava, D., C. Eklund, and J. Dillner.** 2015. International standardization and classification of human papillomavirus types. Virology **476:**341–344.
7. **Feltkamp, M. C., M. N. de Koning, J. N. Bavinck, and J. Ter Schegget.** 2008. Betapapillomaviruses: innocent bystanders or causes of skin cancer. J Clin Virol **43:**353–360.
8. **Arroyo Muhr, L. S., M. Hortlund, Z. Bzhalava, S. Nordqvist Kleppe, D. Bzhalava, E. Hultin, and J. Dillner.** 2017. Viruses in case series of tumors: Consistent presence in different cancers in the same subject. PloS one **12:**e0172308.
9. **Holmgren, S. C., N. A. Patterson, M. A. Ozbun, and P. F. Lambert.** 2005. The minor capsid protein L2 contributes to two steps in the human papillomavirus type 31 life cycle. Journal of virology **79:**3938–3948.
10. **Giroglou, T., L. Florin, F. Schafer, R. E. Streeck, and M. Sapp.** 2001. Human papillomavirus infection requires cell surface heparan sulfate. Journal of virology **75:**1565–1570.
11. **Culp, T. D., L. R. Budgeon, M. P. Marinkovich, G. Meneguzzi, and N. D. Christensen.** 2006. Keratinocyte-secreted laminin 5 can function as a transient receptor for human papillomaviruses by binding virions and transferring them to adjacent cells. Journal of virology **80:**8940–8950.
12. **Selinka, H. C., L. Florin, H. D. Patel, K. Freitag, M. Schmidtke, V. A. Makarov, and M. Sapp.** 2007. Inhibition of transfer to secondary receptors by heparan sulfate-binding drug or antibody induces noninfectious uptake of human papillomavirus. Journal of virology **81:**10970–10980.
13. **Broutian, T. R., S. A. Brendle, and N. D. Christensen.** 2010. Differential binding patterns to host cells associated with particles of several human alphapapillomavirus types. The Journal of general virology **91:**531–540.
14. **Cerqueira, C., P. Samperio Ventayol, C. Vogeley, and M. Schelhaas.** 2015. Kallikrein-8 Proteolytically Processes Human Papillomaviruses in the Extracellular Space To Facilitate Entry into Host Cells. Journal of virology **89:**7038–7052.
15. **Richards, R. M., D. R. Lowy, J. T. Schiller, and P. M. Day.** 2006. Cleavage of the papillomavirus minor capsid protein, L2, at a furin consensus site is necessary for infection. Proceedings of the National Academy of Sciences of the United States of America **103:**1522–1527.

16. **Evander, M., I. H. Frazer, E. Payne, Y. M. Qi, K. Hengst, and N. A. McMillan.** 1997. Identification of the alpha6 integrin as a candidate receptor for papillomaviruses. Journal of virology **71:**2449–2456.
17. **McMillan, N. A., E. Payne, I. H. Frazer, and M. Evander.** 1999. Expression of the alpha6 integrin confers papillomavirus binding upon receptor-negative B-cells. Virology **261:**271–279.
18. **Yoon, C. S., K. D. Kim, S. N. Park, and S. W. Cheong.** 2001. alpha(6) Integrin is the main receptor of human papillomavirus type 16 VLP. Biochemical and biophysical research communications **283:**668–673.
19. **Bousarghin, L., A. Touze, P. Y. Sizaret, and P. Coursaget.** 2003. Human papillomavirus types 16, 31, and 58 use different endocytosis pathways to enter cells. Journal of virology **77:**3846–3850.
20. **Day, P. M., D. R. Lowy, and J. T. Schiller.** 2003. Papillomaviruses infect cells via a clathrin-dependent pathway. Virology **307:**1–11.
21. **Hindmarsh, P. L., and L. A. Laimins.** 2007. Mechanisms regulating expression of the HPV 31 L1 and L2 capsid proteins and pseudovirion entry. Virology journal **4:**19.
22. **Bienkowska-Haba, M., C. Williams, S. M. Kim, R. L. Garcea, and M. Sapp.** 2012. Cyclophilins facilitate dissociation of the human papillomavirus type 16 capsid protein L1 from the L2/DNA complex following virus entry. Journal of virology **86:**9875–9887.
23. **Lipovsky, A., A. Popa, G. Pimienta, M. Wyler, A. Bhan, L. Kuruvilla, M. A. Guie, A. C. Poffenberger, C. D. Nelson, W. J. Atwood, and D. DiMaio.** 2013. Genome-wide siRNA screen identifies the retromer as a cellular entry factor for human papillomavirus. Proceedings of the National Academy of Sciences of the United States of America **110:**7452–7457.
24. **Aydin, I., S. Weber, B. Snijder, P. Samperio Ventayol, A. Kuhbacher, M. Becker, P. M. Day, J. T. Schiller, M. Kann, L. Pelkmans, A. Helenius, and M. Schelhaas.** 2014. Large scale RNAi reveals the requirement of nuclear envelope breakdown for nuclear import of human papillomaviruses. PLoS pathogens **10:**e1004162.
25. **Day, P. M., C. C. Baker, D. R. Lowy, and J. T. Schiller.** 2004. Establishment of papillomavirus infection is enhanced by promyelocytic leukemia protein (PML) expression. Proceedings of the National Academy of Sciences of the United States of America **101:**14252–14257.
26. **McBride, A. A.** 2008. Replication and partitioning of papillomavirus genomes. Adv Virus Res **72:**155–205.
27. **Ustav, M., and A. Stenlund.** 1991. Transient replication of BPV-1 requires two viral polypeptides encoded by the E1 and E2 open reading frames. The EMBO journal **10:**449–457.
28. **Yang, L., I. Mohr, E. Fouts, D. A. Lim, M. Nohaile, and M. Botchan.** 1993. The E1 protein of bovine papilloma virus 1 is an ATP-dependent DNA helicase. Proceedings of the National Academy of Sciences of the United States of America **90:**5086–5090.
29. **Sanders, C. M., and A. Stenlund.** 1998. Recruitment and loading of the E1 initiator protein: an ATP-dependent process catalysed by a transcription factor. The EMBO journal **17:**7044–7055.
30. **Stenlund, A.** 2003. E1 initiator DNA binding specificity is unmasked by selective inhibition of non-specific DNA binding. The EMBO journal **22:**954–963.

31. **Schuck, S., and A. Stenlund.** 2006. Surface mutagenesis of the bovine papillomavirus E1 DNA binding domain reveals residues required for multiple functions related to DNA replication. Journal of virology **80:**7491–7499.
32. **Kuo, S. R., J. S. Liu, T. R. Broker, and L. T. Chow.** 1994. Cell-free replication of the human papillomavirus DNA with homologous viral E1 and E2 proteins and human cell extracts. The Journal of biological chemistry **269:**24058–24065.
33. **Conger, K. L., J. S. Liu, S. R. Kuo, L. T. Chow, and T. S. Wang.** 1999. Human papillomavirus DNA replication. Interactions between the viral E1 protein and two subunits of human dna polymerase alpha/primase. The Journal of biological chemistry **274:**2696–2705.
34. **Loo, Y. M., and T. Melendy.** 2004. Recruitment of replication protein A by the papillomavirus E1 protein and modulation by single-stranded DNA. Journal of virology **78:**1605–1615.
35. **Clower, R. V., J. C. Fisk, and T. Melendy.** 2006. Papillomavirus E1 protein binds to and stimulates human topoisomerase I. Journal of virology **80:**1584–1587.
36. **Sakakibara, N., R. Mitra, and A. A. McBride.** 2011. The papillomavirus E1 helicase activates a cellular DNA damage response in viral replication foci. Journal of virology **85:**8981–8995.
37. **Reinson, T., M. Toots, M. Kadaja, R. Pipitch, M. Allik, E. Ustav, and M. Ustav.** 2013. Engagement of the ATR-Dependent DNA Damage Response at the Human Papillomavirus 18 Replication Centers during the Initial Amplification. J Virol **87:**951–964.
38. **Ustav, M., E. Ustav, P. Szymanski, and A. Stenlund.** 1991. Identification of the origin of replication of bovine papillomavirus and characterization of the viral origin recognition factor E1. The EMBO journal **10:**4321–4329.
39. **Winokur, P. L., and A. A. McBride.** 1996. The transactivation and DNA binding domains of the BPV-1 E2 protein have different roles in cooperative origin binding with the E1 protein. Virology **221:**44–53.
40. **Doorbar, J., S. Ely, J. Sterling, C. McLean, and L. Crawford.** 1991. Specific interaction between HPV-16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. Nature **352:**824–827.
41. **Doorbar, J.** 2013. The E4 protein; structure, function and patterns of expression. Virology **445:**80–98.
42. **Davy, C. E., D. J. Jackson, Q. Wang, K. Raj, P. J. Masterson, N. F. Fenner, S. Southern, S. Cuthill, J. B. Millar, and J. Doorbar.** 2002. Identification of a G(2) arrest domain in the E1 wedge E4 protein of human papillomavirus type 16. Journal of virology **76:**9806–9818.
43. **Khan, J., C. E. Davy, P. B. McIntosh, D. J. Jackson, S. Hinz, Q. Wang, and J. Doorbar.** 2011. Role of calpain in the formation of human papillomavirus type 16 E1^E4 amyloid fibers and reorganization of the keratin network. Journal of virology **85:**9984–9997.
44. **DiMaio, D., and D. Mattoon.** 2001. Mechanisms of cell transformation by papillomavirus E5 proteins. Oncogene **20:**7866–7873.
45. **Horwitz, B. H., A. L. Burkhardt, R. Schlegel, and D. DiMaio.** 1988. 44-amino-acid E5 transforming protein of bovine papillomavirus requires a hydrophobic core and specific carboxyl-terminal amino acids. Molecular and cellular biology **8:**4071–4078.

46. **Burkhardt, A., M. Willingham, C. Gay, K. T. Jeang, and R. Schlegel.** 1989. The E5 oncoprotein of bovine papillomavirus is oriented asymmetrically in Golgi and plasma membranes. Virology **170:**334–339.
47. **Petti, L., L. A. Nilson, and D. DiMaio.** 1991. Activation of the platelet-derived growth factor receptor by the bovine papillomavirus E5 transforming protein. The EMBO journal **10:**845–855.
48. **Petti, L. M., and F. A. Ray.** 2000. Transformation of mortal human fibroblasts and activation of a growth inhibitory pathway by the bovine papillomavirus E5 oncoprotein. Cell growth & differentiation : the molecular biology journal of the American Association for Cancer Research **11:**395–408.
49. **Mattoon, D., K. Gupta, J. Doyon, P. J. Loll, and D. DiMaio.** 2001. Identification of the transmembrane dimer interface of the bovine papillomavirus E5 protein. Oncogene **20:**3824–3834.
50. **Conrad, M., V. J. Bubb, and R. Schlegel.** 1993. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. Journal of virology **67:**6170–6178.
51. **Oetke, C., E. Auvinen, M. Pawlita, and A. Alonso.** 2000. Human papillomavirus type 16 E5 protein localizes to the Golgi apparatus but does not grossly affect cellular glycosylation. Archives of virology **145:**2183–2191.
52. **Straight, S. W., P. M. Hinkle, R. J. Jewers, and D. J. McCance.** 1993. The E5 oncoprotein of human papillomavirus type 16 transforms fibroblasts and effects the downregulation of the epidermal growth factor receptor in keratinocytes. Journal of virology **67:**4521–4532.
53. **Auvinen, E., K. Crusius, B. Steuer, and A. Alonso.** 1997. Human papillomavirus type 16 E5 protein (review). International journal of oncology **11:**1297–1304.
54. **Hu, L., T. A. Potapova, S. Li, S. Rankin, G. J. Gorbsky, P. C. Angeletti, and B. P. Ceresa.** 2010. Expression of HPV16 E5 produces enlarged nuclei and polyploidy through endoreplication. Virology **405:**342–351.
55. **Kabsch, K., and A. Alonso.** 2002. The human papillomavirus type 16 E5 protein impairs TRAIL- and FasL-mediated apoptosis in HaCaT cells by different mechanisms. Journal of virology **76:**12162–12172.
56. **Sudarshan, S. R., R. Schlegel, and X. Liu.** 2010. The HPV-16 E5 protein represses expression of stress pathway genes XBP-1 and COX-2 in genital keratinocytes. Biochemical and biophysical research communications **399:**617–622.
57. **Goldstein, D. J., M. E. Finbow, T. Andresson, P. McLean, K. Smith, V. Bubb, and R. Schlegel.** 1991. Bovine papillomavirus E5 oncoprotein binds to the 16K component of vacuolar H(+)-ATPases. Nature **352:**347–349.
58. **Schapiro, F., J. Sparkowski, A. Adduci, F. Suprynowicz, R. Schlegel, and S. Grinstein.** 2000. Golgi alkalinization by the papillomavirus E5 oncoprotein. The Journal of cell biology **148:**305–315.
59. **Suprynowicz, F. A., J. Sparkowski, A. Baege, and R. Schlegel.** 2000. E5 oncoprotein mutants activate phosphoinositide 3-kinase independently of platelet-derived growth factor receptor activation. The Journal of biological chemistry **275:**5111–5119.
60. **Zhang, B., P. Li, E. Wang, Z. Brahmi, K. W. Dunn, J. S. Blum, and A. Roman.** 2003. The E5 protein of human papillomavirus type 16 perturbs MHC class II antigen maturation in human foreskin keratinocytes treated with interferon-gamma. Virology **310:**100–108.

61. **Ashrafi, G. H., D. R. Brown, K. H. Fife, and M. S. Campo.** 2006. Down-regulation of MHC class I is a property common to papillomavirus E5 proteins. Virus research **120:**208–211.
62. **Androphy, E. J., N. L. Hubbert, J. T. Schiller, and D. R. Lowy.** 1987. Identification of the HPV-16 E6 protein from transformed mouse cells and human cervical carcinoma cell lines. The EMBO journal **6:**989–992.
63. **Huibregtse, J. M., M. Scheffner, and P. M. Howley.** 1991. A cellular protein mediates association of p53 with the E6 oncoprotein of human papillomavirus types 16 or 18. The EMBO journal **10:**4129–4135.
64. **Underbrink, M. P., H. L. Howie, K. M. Bedard, J. I. Koop, and D. A. Galloway.** 2008. E6 proteins from multiple human betapapillomavirus types degrade Bak and protect keratinocytes from apoptosis after UVB irradiation. J Virol **82:**10408–10417.
65. **Gewin, L., and D. A. Galloway.** 2001. E box-dependent activation of telomerase by human papillomavirus type 16 E6 does not require induction of c-myc. Journal of virology **75:**7198–7201.
66. **White, E. A., R. E. Kramer, M. J. Tan, S. D. Hayes, J. W. Harper, and P. M. Howley.** 2012. Comprehensive analysis of host cellular interactions with human papillomavirus E6 proteins identifies new E6 binding partners and reflects viral diversity. Journal of virology **86:**13174–13186.
67. **Wise-Draper, T. M., and S. I. Wells.** 2008. Papillomavirus E6 and E7 proteins and their cellular targets. Frontiers in bioscience : a journal and virtual library **13:**1003–1017.
68. **Garnett, T. O., and P. J. Duerksen-Hughes.** 2006. Modulation of apoptosis by human papillomavirus (HPV) oncoproteins. Archives of virology **151:**2321–2335.
69. **Liu, X., A. Clements, K. Zhao, and R. Marmorstein.** 2006. Structure of the human Papillomavirus E7 oncoprotein and its mechanism for inactivation of the retinoblastoma tumor suppressor. The Journal of biological chemistry **281:**578–586.
70. **McLaughlin-Drubin, M. E., and K. Munger.** 2009. The human papillomavirus E7 oncoprotein. Virology **384:**335–344.
71. **Ronco, L. V., A. Y. Karpova, M. Vidal, and P. M. Howley.** 1998. Human papillomavirus 16 E6 oncoprotein binds to interferon regulatory factor-3 and inhibits its transcriptional activity. Genes & development **12:**2061–2072.
72. **Hasan, U. A., E. Bates, F. Takeshita, A. Biliato, R. Accardi, V. Bouvard, M. Mansour, I. Vincent, L. Gissmann, T. Iftner, M. Sideri, F. Stubenrauch, and M. Tommasino.** 2007. TLR9 expression and function is abolished by the cervical cancer-associated human papillomavirus type 16. Journal of immunology **178:**3186–3197.
73. **Rapp, B., A. Pawellek, F. Kraetzer, M. Schaefer, C. May, K. Purdie, K. Grassmann, and T. Iftner.** 1997. Cell-type-specific separate regulation of the E6 and E7 promoters of human papillomavirus type 6a by the viral transcription factor E2. Journal of virology **71:**6956–6966.
74. **Wells, S. I., D. A. Francis, A. Y. Karpova, J. J. Dowhanick, J. D. Benson, and P. M. Howley.** 2000. Papillomavirus E2 induces senescence in HPV-positive cells via pRB- and p21(CIP)-dependent pathways. The EMBO journal **19:**5762–5771.
75. **Kirnbauer, R., F. Booy, N. Cheng, D. R. Lowy, and J. T. Schiller.** 1992. Papillomavirus L1 major capsid protein self-assembles into virus-like particles that are highly immunogenic. Proceedings of the National Academy of Sciences of the United States of America **89:**12180–12184.

76. **Kirnbauer, R., J. Taub, H. Greenstone, R. Roden, M. Durst, L. Gissmann, D. R. Lowy, and J. T. Schiller.** 1993. Efficient self-assembly of human papillomavirus type 16 L1 and L1-L2 into virus-like particles. Journal of virology **67:**6929–6936.
77. **Day, P. M., R. B. Roden, D. R. Lowy, and J. T. Schiller.** 1998. The papillomavirus minor capsid protein, L2, induces localization of the major capsid protein, L1, and the viral transcription/replication protein, E2, to PML oncogenic domains. Journal of virology **72:**142–150.
78. **Zhao, K. N., X. Y. Sun, I. H. Frazer, and J. Zhou.** 1998. DNA packaging by L1 and L2 capsid proteins of bovine papillomavirus type 1. Virology **243:**482–491.
79. **McBride, A. A.** 2013. The papillomavirus E2 proteins. Virology **445:**57–79.
80. **Antson, A. A., J. E. Burns, O. V. Moroz, D. J. Scott, C. M. Sanders, I. B. Bronstein, G. G. Dodson, K. S. Wilson, and N. J. Maitland.** 2000. Structure of the intact transactivation domain of the human papillomavirus E2 protein. Nature **403:**805–809.
81. **Knight, J. D., R. Li, and M. Botchan.** 1991. The activation domain of the bovine papillomavirus E2 protein mediates association of DNA-bound dimers to form DNA loops. Proceedings of the National Academy of Sciences of the United States of America **88:**3204–3208.
82. **Hernandez-Ramon, E. E., J. E. Burns, W. Zhang, H. F. Walker, S. Allen, A. A. Antson, and N. J. Maitland.** 2008. Dimerization of the human papillomavirus type 16 E2 N terminus results in DNA looping within the upstream regulatory region. Journal of virology **82:**4853–4861.
83. **Sim, J., S. Ozgur, B. Y. Lin, J. H. Yu, T. R. Broker, L. T. Chow, and J. Griffith.** 2008. Remodeling of the human papillomavirus type 11 replication origin into discrete nucleoprotein particles and looped structures by the E2 protein. Journal of molecular biology **375:**1165–1177.
84. **Hegde, R. S., S. R. Grossman, L. A. Laimins, and P. B. Sigler.** 1992. Crystal structure at 1.7 A of the bovine papillomavirus-1 E2 DNA-binding domain bound to its DNA target. Nature **359:**505–512.
85. **Bussiere, D. E., X. Kong, D. A. Egan, K. Walter, T. F. Holzman, F. Lindh, T. Robins, and V. L. Giranda.** 1998. Structure of the E2 DNA-binding domain from human papillomavirus serotype 31 at 2.4 A. Acta crystallographica. Section D, Biological crystallography **54:**1367–1376.
86. **Hegde, R. S., and E. J. Androphy.** 1998. Crystal structure of the E2 DNA-binding domain from human papillomavirus type 16: implications for its DNA binding-site selection mechanism. Journal of molecular biology **284:**1479–1489.
87. **Kim, S. S., J. K. Tam, A. F. Wang, and R. S. Hegde.** 2000. The structural basis of DNA target discrimination by papillomavirus E2 proteins. The Journal of biological chemistry **275:**31245–31254.
88. **Nadra, A. D., T. Eliseo, Y. K. Mok, C. L. Almeida, M. Bycroft, M. Paci, G. de Prat-Gay, and D. O. Cicero.** 2004. Solution structure of the HPV-16 E2 DNA binding domain, a transcriptional regulator with a dimeric beta-barrel fold. Journal of biomolecular NMR **30:**211–214.
89. **Hooley, E., V. Fairweather, A. R. Clarke, K. Gaston, and R. L. Brady.** 2006. The recognition of local DNA conformation by the human papillomavirus type 6 E2 protein. Nucleic acids research **34:**3897–3908.
90. **Androphy, E. J., D. R. Lowy, and J. T. Schiller.** 1987. Bovine papillomavirus E2 trans-activating gene product binds to specific sites in papillomavirus DNA. Nature **325:**70–73.

91. **Li, R., J. Knight, G. Bream, A. Stenlund, and M. Botchan.** 1989. Specific recognition nucleotides and their DNA context determine the affinity of E2 protein for 17 binding sites in the BPV-1 genome. Genes & development **3:**510–526.
92. **Newhouse, C. D., and S. J. Silverstein.** 2001. Orientation of a novel DNA binding site affects human papillomavirus-mediated transcription and replication. Journal of virology **75:**1722–1735.
93. **Mok, Y. K., G. de Prat Gay, P. J. Butler, and M. Bycroft.** 1996. Equilibrium dissociation and unfolding of the dimeric human papillomavirus strain-16 E2 DNA-binding domain. Protein science : a publication of the Protein Society **5:**310–319.
94. **Giri, I., and M. Yaniv.** 1988. Structural and mutational analysis of E2 trans-activating proteins of papillomaviruses reveals three distinct functional domains. The EMBO journal **7:**2823–2829.
95. **McBride, A. A., J. C. Byrne, and P. M. Howley.** 1989. E2 polypeptides encoded by bovine papillomavirus type 1 form dimers through the common carboxyl-terminal domain: transactivation is mediated by the conserved amino-terminal domain. Proc Natl Acad Sci U S A **86:**510–514.
96. **Gauthier, J. M., J. Dillner, and M. Yaniv.** 1991. Structural analysis of the human papillomavirus type 16-E2 transactivator with antipeptide antibodies reveals a high mobility region linking the transactivation and the DNA-binding domains. Nucleic acids research **19:**7073–7079.
97. **Penrose, K. J., and A. A. McBride.** 2000. Proteasome-mediated degradation of the papillomavirus E2-TA protein is regulated by phosphorylation and can modulate viral genome copy number. J Virol **74:**6031–6038.
98. **Garcia-Alai, M. M., M. Gallo, M. Salame, D. E. Wetzler, A. A. McBride, M. Paci, D. O. Cicero, and G. de Prat-Gay.** 2006. Molecular basis for phosphorylation-dependent, PEST-mediated protein turnover. Structure **14:**309–319.
99. **Lai, M. C., B. H. Teh, and W. Y. Tarn.** 1999. A human papillomavirus E2 transcriptional activator. The interactions with cellular splicing factors and potential function in pre-mRNA processing. The Journal of biological chemistry **274:**11832–11841.
100. **Zou, N., B. Y. Lin, F. Duan, K. Y. Lee, G. Jin, R. Guan, G. Yao, E. J. Lefkowitz, T. R. Broker, and L. T. Chow.** 2000. The hinge of the human papillomavirus type 11 E2 protein contains major determinants for nuclear localization and nuclear matrix association. Journal of virology **74:**3761–3770.
101. **Poddar, A., S. C. Reed, M. G. McPhillips, J. E. Spindler, and A. A. McBride.** 2009. The human papillomavirus type 8 E2 tethering protein targets the ribosomal DNA loci of host mitotic chromosomes. J Virol **83:**640–650.
102. **Sekhar, V., S. C. Reed, and A. A. McBride.** 2010. Interaction of the betapapillomavirus E2 tethering protein with mitotic chromosomes. J Virol **84:**543–557.
103. **Sekhar, V., and A. A. McBride.** 2012. Phosphorylation regulates binding of the human papillomavirus type 8 E2 protein to host chromosomes. Journal of virology **86:**10047–10058.
104. **Straub, E., J. Fertey, M. Dreer, T. Iftner, and F. Stubenrauch.** 2015. Characterization of the Human Papillomavirus 16 E8 Promoter. Journal of virology **89:**7304–7313.
105. **Jeckel, S., E. Loetzsch, E. Huber, F. Stubenrauch, and T. Iftner.** 2003. Identification of the E9/E2C cDNA and functional characterization of the gene product reveal a new repressor of transcription and replication in cottontail rabbit papillomavirus. Journal of virology **77:**8736–8744.

106. **Zobel, T., T. Iftner, and F. Stubenrauch.** 2003. The papillomavirus E8-E2C protein represses DNA replication from extrachromosomal origins. Molecular and cellular biology **23:**8352–8362.
107. **Stubenrauch, F., E. Straub, J. Fertey, and T. Iftner.** 2007. The E8 repression domain can replace the E2 transactivation domain for growth inhibition of HeLa cells by papillomavirus E2 proteins. International journal of cancer **121:**2284–2292.
108. **Fertey, J., J. Hurst, E. Straub, A. Schenker, T. Iftner, and F. Stubenrauch.** 2011. Growth inhibition of HeLa cells is a conserved feature of high-risk human papillomavirus E8^E2C proteins and can also be achieved by an artificial repressor protein. Journal of virology **85:**2918–2926.
109. **Choe, J., P. Vaillancourt, A. Stenlund, and M. Botchan.** 1989. Bovine papillomavirus type 1 encodes two forms of a transcriptional repressor: structural and functional analysis of new viral cDNAs. J Virol **63:**1743–1755.
110. **Palermo-Dilts, D. A., T. R. Broker, and L. T. Chow.** 1990. Human papillomavirus type 1 produces redundant as well as polycistronic mRNAs in plantar warts. Journal of virology **64:**3144–3149.
111. **Rotenberg, M. O., L. T. Chow, and T. R. Broker.** 1989. Characterization of rare human papillomavirus type 11 mRNAs coding for regulatory and structural proteins, using the polymerase chain reaction. Virology **172:**489–497.
112. **Doorbar, J., A. Parton, K. Hartley, L. Banks, T. Crook, M. Stanley, and L. Crawford.** 1990. Detection of novel splicing patterns in a HPV16-containing keratinocyte cell line. Virology **178:**254–262.
113. **Snijders, P. J., A. J. van den Brule, H. F. Schrijnemakers, P. M. Raaphorst, C. J. Meijer, and J. M. Walboomers.** 1992. Human papillomavirus type 33 in a tonsillar carcinoma generates its putative E7 mRNA via two E6* transcript species which are terminated at different early region poly(A) sites. Journal of virology **66:**3172–3178.
114. **Stubenrauch, F., M. Hummel, T. Iftner, and L. A. Laimins.** 2000. The E8E2C protein, a negative regulator of viral transcription and replication, is required for extrachromosomal maintenance of human papillomavirus type 31 in keratinocytes. Journal of virology **74:**1178–1186.
115. **Kurg, R., P. Uusen, L. Vosa, and M. Ustav.** 2010. Human papillomavirus E2 protein with single activation domain initiates HPV18 genome replication, but is not sufficient for long-term maintenance of virus genome. Virology **408:**159–166.
116. **Dreer, M., S. van de Poel, and F. Stubenrauch.** 2017. Control of viral replication and transcription by the papillomavirus E8^E2 protein. Virus research **231:**96–102.
117. **Ammermann, I., M. Bruckner, F. Matthes, T. Iftner, and F. Stubenrauch.** 2008. Inhibition of transcription and DNA replication by the papillomavirus E8-E2C protein is mediated by interaction with corepressor molecules. Journal of virology **82:**5127–5136.
118. **Powell, M. L., J. A. Smith, M. E. Sowa, J. W. Harper, T. Iftner, F. Stubenrauch, and P. M. Howley.** 2010. NCoR1 mediates papillomavirus E8;E2C transcriptional repression. Journal of virology **84:**4451–4460.
119. **Fertey, J., I. Ammermann, M. Winkler, R. Stoger, T. Iftner, and F. Stubenrauch.** 2010. Interaction of the papillomavirus E8–E2C protein with the cellular CHD6 protein contributes to transcriptional repression. Journal of virology **84:**9505–9515.

120. **Lambert, P. F., B. A. Spalholz, and P. M. Howley.** 1987. A transcriptional repressor encoded by BPV-1 shares a common carboxy-terminal domain with the E2 transactivator. Cell **50:**69–78.
121. **Lambert, P. F., B. C. Monk, and P. M. Howley.** 1990. Phenotypic analysis of bovine papillomavirus type 1 E2 repressor mutants. Journal of virology **64:**950–956.
122. **Riese, D. J., 2nd, J. Settleman, K. Neary, and D. DiMaio.** 1990. Bovine papillomavirus E2 repressor mutant displays a high-copy-number phenotype and enhanced transforming activity. Journal of virology **64:**944–949.
123. **Barsoum, J., S. S. Prakash, P. Han, and E. J. Androphy.** 1992. Mechanism of action of the papillomavirus E2 repressor: repression in the absence of DNA binding. Journal of virology **66:**3941–3945.
124. **Kurg, R., H. Tekkel, A. Abroi, and M. Ustav.** 2006. Characterization of the functional activities of the bovine papillomavirus type 1 E2 protein single-chain heterodimers. J Virol **80:**11218–11225.
125. **Hubbert, N. L., J. T. Schiller, D. R. Lowy, and E. J. Androphy.** 1988. Bovine papilloma virus-transformed cells contain multiple E2 proteins. Proc Natl Acad Sci U S A **85:**5864–5868.
126. **Kurg, R., P. Uusen, T. Sepp, M. Sepp, A. Abroi, and M. Ustav.** 2009. Bovine papillomavirus type 1 E2 protein heterodimer is functional in papillomavirus DNA replication in vivo. Virology **386:**353–359.
127. **Bellanger, S., C. Demeret, S. Goyat, and F. Thierry.** 2001. Stability of the human papillomavirus type 18 E2 protein is regulated by a proteasome degradation pathway through its amino-terminal transactivation domain. Journal of virology **75:**7244–7251.
128. **Gagnon, D., S. Joubert, H. Senechal, A. Fradet-Turcotte, S. Torre, and J. Archambault.** 2009. Proteasomal degradation of the papillomavirus E2 protein is inhibited by overexpression of bromodomain-containing protein 4. J Virol **83:**4127–4139.
129. **Zheng, G., M. R. Schweiger, G. Martinez-Noel, L. Zheng, J. A. Smith, J. W. Harper, and P. M. Howley.** 2009. Brd4 regulation of papillomavirus protein E2 stability. J Virol **83:**8683–8692.
130. **Bellanger, S., C. L. Tan, W. Nei, P. P. He, and F. Thierry.** 2010. The human papillomavirus type 18 E2 protein is a cell cycle-dependent target of the SCFSkp2 ubiquitin ligase. Journal of virology **84:**437–444.
131. **Davy, C., P. McIntosh, D. J. Jackson, R. Sorathia, M. Miell, Q. Wang, J. Khan, Y. Soneji, and J. Doorbar.** 2009. A novel interaction between the human papillomavirus type 16 E2 and E1–E4 proteins leads to stabilization of E2. Virology **394:**266–275.
132. **King, L. E., E. S. Dornan, M. M. Donaldson, and I. M. Morgan.** 2011. Human papillomavirus 16 E2 stability and transcriptional activation is enhanced by E1 via a direct protein-protein interaction. Virology **414:**26–33.
133. **Penrose, K. J., M. Garcia-Alai, G. de Prat-Gay, and A. A. McBride.** 2004. Casein Kinase II phosphorylation-induced conformational switch triggers degradation of the papillomavirus E2 protein. J Biol Chem **279:**22430–22439.
134. **Romanczuk, H., F. Thierry, and P. M. Howley.** 1990. Mutational analysis of cis elements involved in E2 modulation of human papillomavirus type 16 P97 and type 18 P105 promoters. Journal of virology **64:**2849–2859.

135. **Dostatni, N., P. F. Lambert, R. Sousa, J. Ham, P. M. Howley, and M. Yaniv.** 1991. The functional BPV-1 E2 trans-activating protein can act as a repressor by preventing formation of the initiation complex. Genes & development **5:**1657–1671.
136. **Tan, S. H., L. E. Leong, P. A. Walker, and H. U. Bernard.** 1994. The human papillomavirus type 16 E2 transcription factor binds with low cooperativity to two flanking sites and represses the E6 promoter through displacement of Sp1 and TFIID. J Virol **68:**6411–6420.
137. **Bouvard, V., A. Storey, D. Pim, and L. Banks.** 1994. Characterization of the human papillomavirus E2 protein: evidence of trans-activation and trans-repression in cervical keratinocytes. The EMBO journal **13:**5451–5459.
138. **Fujii, T., J. L. Brandsma, X. Peng, S. Srimatkandada, L. Li, A. Canaan, and A. B. Deisseroth.** 2001. High and low levels of cottontail rabbit papillomavirus E2 protein generate opposite effects on gene expression. J Biol Chem **276:**867–874.
139. **Thierry, F., and M. Yaniv.** 1987. The BPV1-E2 trans-acting protein can be either an activator or a repressor of the HPV18 regulatory region. The EMBO journal **6:**3391–3397.
140. **Thierry, F., and P. M. Howley.** 1991. Functional analysis of E2-mediated repression of the HPV18 P105 promoter. The New biologist **3:**90–100.
141. **Goodwin, E. C., and D. DiMaio.** 2000. Repression of human papillomavirus oncogenes in HeLa cervical carcinoma cells causes the orderly reactivation of dormant tumor suppressor pathways. Proceedings of the National Academy of Sciences of the United States of America **97:**12513–12518.
142. **Johansson, C., M. Somberg, X. Li, E. Backstrom Winquist, J. Fay, F. Ryan, D. Pim, L. Banks, and S. Schwartz.** 2012. HPV-16 E2 contributes to induction of HPV-16 late gene expression by inhibiting early polyadenylation. The EMBO journal **31:**3212–3227.
143. **Dowhanick, J. J., A. A. McBride, and P. M. Howley.** 1995. Suppression of cellular proliferation by the papillomavirus E2 protein. J Virol **69:**7791–7799.
144. **Webster, K., J. Parish, M. Pandya, P. L. Stern, A. R. Clarke, and K. Gaston.** 2000. The human papillomavirus (HPV) 16 E2 protein induces apoptosis in the absence of other HPV proteins and via a p53-dependent pathway. The Journal of biological chemistry **275:**87–94.
145. **Thierry, F., and C. Demeret.** 2008. Direct activation of caspase 8 by the pro-apoptotic E2 protein of HPV18 independent of adaptor proteins. Cell death and differentiation **15:**1356–1363.
146. **Burns, J. E., H. F. Walker, C. Schmitz, and N. J. Maitland.** 2010. Phenotypic effects of HPV-16 E2 protein expression in human keratinocytes. Virology **401:**314–321.
147. **Ustav, E., M. Ustav, P. Szymanski, and A. Stenlund.** 1993. The bovine papillomavirus origin of replication requires a binding site for the E2 transcriptional activator. Proc Natl Acad Sci U S A **90:**898–902.
148. **Sedman, J., and A. Stenlund.** 1995. Co-operative interaction between the initiator E1 and the transcriptional activator E2 is required for replicator specific DNA replication of bovine papillomavirus in vivo and in vitro. The EMBO journal **14:**6218–6228.
149. **Fradet-Turcotte, A., F. Bergeron-Labrecque, C. A. Moody, M. Lehoux, L. A. Laimins, and J. Archambault.** 2011. Nuclear accumulation of the papillomavirus E1 helicase blocks S-phase progression and triggers an ATM-dependent DNA damage response. Journal of virology **85:**8996–9012.

150. **Johansson, C., and S. Schwartz.** 2013. Regulation of human papillomavirus gene expression by splicing and polyadenylation. Nature reviews. Microbiology **11:**239–251.
151. **Toots, M., A. Mannik, G. Kivi, M. Ustav, Jr., E. Ustav, and M. Ustav.** 2014. The transcription map of human papillomavirus type 18 during genome replication in U2OS cells. PloS one **9:**e116151.
152. **Alderborn, A., N. Jareborg, and S. Burnett.** 1992. Evidence that the transcriptional trans-activating function of the bovine papillomavirus type 1 E2 gene is not required for viral DNA amplification in division-arrested cells. The Journal of general virology **73 (Pt 10):**2639–2651.
153. **Ilves, I., S. Kivi, and M. Ustav.** 1999. Long-term episomal maintenance of bovine papillomavirus type 1 plasmids is determined by attachment to host chromosomes, which Is mediated by the viral E2 protein and its binding sites. J Virol **73:**4404–4412.
154. **Bastien, N., and A. A. McBride.** 2000. Interaction of the papillomavirus E2 protein with mitotic chromosomes. Virology **270:**124–134.
155. **Piirsoo, M., E. Ustav, T. Mandel, A. Stenlund, and M. Ustav.** 1996. Cis and trans requirements for stable episomal maintenance of the BPV-1 replicator. The EMBO journal **15:**1–11.
156. **McPhillips, M. G., J. G. Oliveira, J. E. Spindler, R. Mitra, and A. A. McBride.** 2006. Brd4 is required for e2-mediated transcriptional activation but not genome partitioning of all papillomaviruses. J Virol **80:**9530–9543.
157. **Dao, L. D., A. Duffy, B. A. Van Tine, S. Y. Wu, C. M. Chiang, T. R. Broker, and L. T. Chow.** 2006. Dynamic localization of the human papillomavirus type 11 origin binding protein E2 through mitosis while in association with the spindle apparatus. Journal of virology **80:**4792–4800.
158. **Gammoh, N., E. Isaacson, V. Tomaic, D. J. Jackson, J. Doorbar, and L. Banks.** 2009. Inhibition of HPV-16 E7 oncogenic activity by HPV-16 E2. Oncogene **28:**2299–2304.
159. **Parish, J. L., A. M. Bean, R. B. Park, and E. J. Androphy.** 2006. ChlR1 is required for loading papillomavirus E2 onto mitotic chromosomes and viral genome maintenance. Molecular cell **24:**867–876.
160. **Donaldson, M. M., W. Boner, and I. M. Morgan.** 2007. TopBP1 regulates human papillomavirus type 16 E2 interaction with chromatin. Journal of virology **81:**4338–4342.
161. **Oliveira, J. G., L. A. Colf, and A. A. McBride.** 2006. Variations in the association of papillomavirus E2 proteins with mitotic chromosomes. Proc Natl Acad Sci U S A **103:**1047–1052.
162. **Baxter, M. K., and A. A. McBride.** 2005. An acidic amphipathic helix in the Bovine Papillomavirus E2 protein is critical for DNA replication and interaction with the E1 protein. Virology **332:**78–88.
163. **Ilves, I., K. Maemets, T. Silla, K. Janikson, and M. Ustav.** 2006. Brd4 is involved in multiple processes of the bovine papillomavirus type 1 life cycle. J Virol **80:**3660–3665.
164. **You, J., J. L. Croyle, A. Nishimura, K. Ozato, and P. M. Howley.** 2004. Interaction of the bovine papillomavirus E2 protein with Brd4 tethers the viral DNA to host mitotic chromosomes. Cell **117:**349–360.

165. **Yuan, C. H., M. Filippova, J. L. Krstenansky, and P. J. Duerksen-Hughes.** 2016. Flavonol and imidazole derivatives block HPV16 E6 activities and reactivate apoptotic pathways in HPV(+) cells. Cell death & disease **7:**2060.
166. **Blanchette, P., and P. E. Branton.** 2009. Manipulation of the ubiquitin-proteasome pathway by small DNA tumor viruses. Virology **384:**317–323.
167. **Parish, J. L., A. Kowalczyk, H. T. Chen, G. E. Roeder, R. Sessions, M. Buckle, and K. Gaston.** 2006. E2 proteins from high- and low-risk human papillomavirus types differ in their ability to bind p53 and induce apoptotic cell death. Journal of virology **80:**4580–4590.
168. **Wang, W., Y. Fang, N. Sima, Y. Li, W. Li, L. Li, L. Han, S. Liao, Z. Han, Q. Gao, K. Li, D. Deng, L. Meng, J. Zhou, S. Wang, and D. Ma.** 2011. Triggering of death receptor apoptotic signaling by human papillomavirus 16 E2 protein in cervical cancer cell lines is mediated by interaction with c-FLIP. Apoptosis : an international journal on programmed cell death **16:**55–66.
169. **Singh, N., S. Senapati, and K. Bose.** 2016. Insights into the mechanism of human papillomavirus E2-induced procaspase-8 activation and cell death. Scientific reports **6:**21408.
170. **Blachon, S., S. Bellanger, C. Demeret, and F. Thierry.** 2005. Nucleo-cytoplasmic shuttling of high risk human Papillomavirus E2 proteins induces apoptosis. The Journal of biological chemistry **280:**36088–36098.
171. **Muller, M., and C. Demeret.** 2012. The HPV E2-Host Protein-Protein Interactions: A Complex Hijacking of the Cellular Network. The open virology journal **6:**173–189.
172. **Jang, M. K., D. E. Anderson, K. van Doorslaer, and A. A. McBride.** 2015. A proteomic approach to discover and compare interacting partners of papillomavirus E2 proteins from diverse phylogenetic groups. Proteomics **15:**2038–2050.
173. **McPhillips, M. G., K. Ozato, and A. A. McBride.** 2005. Interaction of bovine papillomavirus E2 protein with Brd4 stabilizes its association with chromatin. J Virol **79:**8920–8932.
174. **Schweiger, M. R., J. You, and P. M. Howley.** 2006. Bromodomain protein 4 mediates the papillomavirus E2 transcriptional activation function. J Virol **80:**4276–4285.
175. **Schweiger, M. R., M. Ottinger, J. You, and P. M. Howley.** 2007. Brd4-independent transcriptional repression function of the papillomavirus e2 proteins. J Virol **81:**9612–9622.
176. **Wang, X., C. M. Helfer, N. Pancholi, J. E. Bradner, and J. You.** 2013. Recruitment of Brd4 to the human papillomavirus type 16 DNA replication complex is essential for replication of viral DNA. J Virol **87:**3871–3884.
177. **Rehtanz, M., H. M. Schmidt, U. Warthorst, and G. Steger.** 2004. Direct interaction between nucleosome assembly protein 1 and the papillomavirus E2 proteins involved in activation of transcription. Molecular and cellular biology **24:**2153–2168.
178. **Maruyama, T., A. Farina, A. Dey, J. Cheong, V. P. Bermudez, T. Tamura, S. Sciortino, J. Shuman, J. Hurwitz, and K. Ozato.** 2002. A Mammalian bromodomain protein, brd4, interacts with replication factor C and inhibits progression to S phase. Molecular and cellular biology **22:**6509–6520.
179. **Rahman, S., M. E. Sowa, M. Ottinger, J. A. Smith, Y. Shi, J. W. Harper, and P. M. Howley.** 2011. The Brd4 extraterminal domain confers transcription activation

independent of pTEFb by recruiting multiple proteins, including NSD3. Molecular and cellular biology **31:**2641–2652.
180. **Ulrich, H. D.** 2013. New insights into replication clamp unloading. Journal of molecular biology **425:**4727–4732.
181. **Witkowski, L., and W. D. Foulkes.** 2015. In Brief: Picturing the complex world of chromatin remodelling families. The Journal of pathology.
182. **Cha, S., and T. Seo.** 2011. hSNF5 is required for human papillomavirus E2-driven transcriptional activation and DNA replication. Intervirology **54:**66–77.
183. **Euskirchen, G., R. K. Auerbach, and M. Snyder.** 2012. SWI/SNF chromatin-remodeling factors: multiscale analyses and diverse functions. J Biol Chem **287:**30897–30905.
184. **Lee, D., H. Sohn, G. V. Kalpana, and J. Choe.** 1999. Interaction of E1 and hSNF5 proteins stimulates replication of human papillomavirus DNA. Nature **399:**487–491.
185. **Kumar, R. A., S. R. Naidu, X. Wang, A. N. Imbalzano, and E. J. Androphy.** 2007. Interaction of papillomavirus E2 protein with the Brm chromatin remodeling complex leads to enhanced transcriptional activation. J Virol **81:**2213–2220.
186. **Lee, D., C. Lim, T. Seo, H. Kwon, H. Min, and J. Choe.** 2002. The viral oncogene human papillomavirus E7 deregulates transcriptional silencing by Brm-related gene 1 via molecular interactions. J Biol Chem **277:**48842–48848.
187. **Okoye, A., P. Cordano, E. R. Taylor, I. M. Morgan, R. Everett, and M. S. Campo.** 2005. Human papillomavirus 16 L2 inhibits the transcriptional activation function, but not the DNA replication function, of HPV-16 E2. Virus research **108:**1–14.
188. **Gammoh, N., H. S. Grm, P. Massimi, and L. Banks.** 2006. Regulation of human papillomavirus type 16 E7 activity through direct protein interaction with the E2 transcriptional activator. Journal of virology **80:**1787–1797.
189. **Grm, H. S., P. Massimi, N. Gammoh, and L. Banks.** 2005. Crosstalk between the human papillomavirus E2 transcriptional activator and the E6 oncoprotein. Oncogene **24:**5149–5164.
190. **Chiang, C. M., M. Ustav, A. Stenlund, T. F. Ho, T. R. Broker, and L. T. Chow.** 1992. Viral E1 and E2 proteins support replication of homologous and heterologous papillomaviral origins. Proc Natl Acad Sci U S A **89:**5799–5803.
191. **Sedman, T., J. Sedman, and A. Stenlund.** 1997. Binding of the E1 and E2 proteins to the origin of replication of bovine papillomavirus. Journal of virology **71:**2887–2896.
192. **Sedman, J., and A. Stenlund.** 1996. The initiator protein E1 binds to the bovine papillomavirus origin of replication as a trimeric ring-like structure. The EMBO journal **15:**5085–5092.
193. **Enemark, E. J., and L. Joshua-Tor.** 2006. Mechanism of DNA translocation in a replicative hexameric helicase. Nature **442:**270–275.
194. **Orav, M., J. Geimanen, E. M. Sepp, L. Henno, E. Ustav, and M. Ustav.** 2015. Initial amplification of the HPV18 genome proceeds via two distinct replication mechanisms. Scientific reports **5:**15952.
195. **Ilves, I., M. Kadaja, and M. Ustav.** 2003. Two separate replication modes of the bovine papillomavirus BPV1 origin of replication that have different sensitivity to p53. Virus research **96:**75–84.
196. **Kim, K., and P. F. Lambert.** 2002. E1 protein of bovine papillomavirus 1 is not required for the maintenance of viral plasmid DNA replication. Virology **293:**10–14.

197. **Egawa, N., T. Nakahara, S. Ohno, M. Narisawa-Saito, T. Yugawa, M. Fujita, K. Yamato, Y. Natori, and T. Kiyono.** 2012. The E1 protein of human papillomavirus type 16 is dispensable for maintenance replication of the viral genome. Journal of virology **86:**3276–3283.
198. **Orav, M., L. Henno, H. Isok-Paas, J. Geimanen, M. Ustav, and E. Ustav.** 2013. Recombination-Dependent Oligomerization of Human Papillomavirus Genomes upon Transient DNA Replication. J Virol **87:**12051–12068.
199. **Kadaja, M., H. Isok-Paas, T. Laos, E. Ustav, and M. Ustav.** 2009. Mechanism of genomic instability in cells infected with the high-risk human papillomaviruses. PLoS pathogens **5:**e1000397.
200. **Bellanger, S., S. Blachon, F. Mechali, C. Bonne-Andrea, and F. Thierry.** 2005. High-risk but not low-risk HPV E2 proteins bind to the APC activators Cdh1 and Cdc20 and cause genomic instability. Cell cycle **4:**1608–1615.
201. **Sellers, J. R.** 1999. Unphosphorylated crossbridges and latch: smooth muscle regulation revisited. Journal of muscle research and cell motility **20:**347–349.
202. **Sellers, J. R.** 2000. Myosins: a diverse superfamily. Biochimica et biophysica acta **1496:**3–22.
203. **Barylko, B., M. C. Wagner, O. Reizes, and J. P. Albanesi.** 1992. Purification and characterization of a mammalian myosin I. Proceedings of the National Academy of Sciences of the United States of America **89:**490–494.
204. **Wagner, M. C., B. Barylko, and J. P. Albanesi.** 1992. Tissue distribution and subcellular localization of mammalian myosin I. The Journal of cell biology **119:**163–170.
205. **Venit, T., R. Dzijak, A. Kalendova, M. Kahle, J. Rohozkova, V. Schmidt, T. Rulicke, B. Rathkolb, W. Hans, A. Bohla, O. Eickelberg, T. Stoeger, E. Wolf, A. O. Yildirim, V. Gailus-Durner, H. Fuchs, M. H. de Angelis, and P. Hozak.** 2013. Mouse nuclear myosin I knock-out shows interchangeability and redundancy of myosin isoforms in the cell nucleus. PLoS One **8:**e61406.
206. **Nowak, G., L. Pestic-Dragovich, P. Hozak, A. Philimonenko, C. Simerly, G. Schatten, and P. de Lanerolle.** 1997. Evidence for the presence of myosin I in the nucleus. J Biol Chem **272:**17176–17181.
207. **Pestic-Dragovich, L., L. Stojiljkovic, A. A. Philimonenko, G. Nowak, Y. Ke, R. E. Settlage, J. Shabanowitz, D. F. Hunt, P. Hozak, and P. de Lanerolle.** 2000. A myosin I isoform in the nucleus. Science **290:**337–341.
208. **Ihnatovych, I., M. Migocka-Patrzalek, M. Dukh, and W. A. Hofmann.** 2012. Identification and characterization of a novel myosin Ic isoform that localizes to the nucleus. Cytoskeleton **69:**555–565.
209. **Cheney, R. E., and M. S. Mooseker.** 1992. Unconventional myosins. Current opinion in cell biology **4:**27–35.
210. **Dzijak, R., S. Yildirim, M. Kahle, P. Novak, J. Hnilicova, T. Venit, and P. Hozak.** 2012. Specific nuclear localizing sequence directs two myosin isoforms to the cell nucleus in calmodulin-sensitive manner. PLoS One **7:**e30529.
211. **Hofmann, W. A., T. Johnson, M. Klapczynski, J. L. Fan, and P. de Lanerolle.** 2006. From transcription to transport: emerging roles for nuclear myosin I. Biochemistry and cell biology = Biochimie et biologie cellulaire **84:**418–426.
212. **Adams, R. J., and T. D. Pollard.** 1989. Binding of myosin I to membrane lipids. Nature **340:**565–568.
213. **Hokanson, D. E., and E. M. Ostap.** 2006. Myo1c binds tightly and specifically to phosphatidylinositol 4,5-bisphosphate and inositol 1,4,5-trisphosphate. Proceedings

of the National Academy of Sciences of the United States of America **103:**3118–3123.
214. **Berg, J. S., B. C. Powell, and R. E. Cheney.** 2001. A millennial myosin census. Molecular biology of the cell **12:**780–794.
215. **Visa, N., and P. Percipalle.** 2010. Nuclear functions of actin. Cold Spring Harbor perspectives in biology **2:**a000620.
216. **de Lanerolle, P., and L. Serebryannyy.** 2011. Nuclear actin and myosins: life without filaments. Nature cell biology **13:**1282–1288.
217. **Sarshad, A. A., and P. Percipalle.** 2014. New insight into role of myosin motors for activation of RNA polymerases. International review of cell and molecular biology **311:**183–230.
218. **Mehta, I. S., M. Amira, A. J. Harvey, and J. M. Bridger.** 2010. Rapid chromosome territory relocation by nuclear motor activity in response to serum removal in primary human fibroblasts. Genome biology **11:**R5.
219. **Fomproix, N., and P. Percipalle.** 2004. An actin-myosin complex on actively transcribing genes. Experimental cell research **294:**140–148.
220. **Kysela, K., A. A. Philimonenko, V. V. Philimonenko, J. Janacek, M. Kahle, and P. Hozak.** 2005. Nuclear distribution of actin and myosin I depends on transcriptional activity of the cell. Histochemistry and cell biology **124:**347–358.
221. **Sarshad, A., F. Sadeghifar, E. Louvet, R. Mori, S. Bohm, B. Al-Muzzaini, A. Vintermist, N. Fomproix, A. K. Ostlund, and P. Percipalle.** 2013. Nuclear myosin 1c facilitates the chromatin modifications required to activate rRNA gene transcription and cell cycle progression. PLoS genetics **9:**e1003397.
222. **Philimonenko, V. V., J. Zhao, S. Iben, H. Dingova, K. Kysela, M. Kahle, H. Zentgraf, W. A. Hofmann, P. de Lanerolle, P. Hozak, and I. Grummt.** 2004. Nuclear actin and myosin I are required for RNA polymerase I transcription. Nature cell biology **6:**1165–1172.
223. **Ye, J., J. Zhao, U. Hoffmann-Rohrer, and I. Grummt.** 2008. Nuclear myosin I acts in concert with polymeric actin to drive RNA polymerase I transcription. Genes & development **22:**322–330.
224. **Hofmann, W. A., G. M. Vargas, R. Ramchandran, L. Stojiljkovic, J. A. Goodrich, and P. de Lanerolle.** 2006. Nuclear myosin I is necessary for the formation of the first phosphodiester bond during transcription initiation by RNA polymerase II. Journal of cellular biochemistry **99:**1001–1009.
225. **Obrdlik, A., E. Louvet, A. Kukalev, D. Naschekin, E. Kiseleva, B. Fahrenkrog, and P. Percipalle.** 2010. Nuclear myosin 1 is in complex with mature rRNA transcripts and associates with the nuclear pore basket. FASEB journal: official publication of the Federation of American Societies for Experimental Biology **24:**146–157.
226. **Cavellan, E., P. Asp, P. Percipalle, and A. K. Farrants.** 2006. The WSTF-SNF2h chromatin remodeling complex interacts with several nuclear proteins in transcription. J Biol Chem **281:**16264–16271.
227. **Percipalle, P., N. Fomproix, E. Cavellan, R. Voit, G. Reimer, T. Kruger, J. Thyberg, U. Scheer, I. Grummt, and A. K. Farrants.** 2006. The chromatin remodelling complex WSTF-SNF2h interacts with nuclear myosin 1 and has a role in RNA polymerase I transcription. EMBO reports **7:**525–530.
228. **Vintermist, A., S. Bohm, F. Sadeghifar, E. Louvet, A. Mansen, P. Percipalle, and A. K. Ostlund Farrants.** 2011. The chromatin remodelling complex B-WICH

changes the chromatin structure and recruits histone acetyl-transferases to active rRNA genes. PLoS One **6:**e19184.
229. **Sarshad, A. A., M. Corcoran, B. Al-Muzzaini, L. Borgonovo-Brandter, A. Von Euler, D. Lamont, N. Visa, and P. Percipalle.** 2014. Glycogen synthase kinase (GSK) 3beta phosphorylates and protects nuclear myosin 1c from proteasome-mediated degradation to activate rDNA transcription in early G1 cells. PLoS genetics **10:**e1004390.
230. **Sadeghifar, F., S. Bohm, A. Vintermist, and A. K. Ostlund Farrants.** 2015. The B-WICH chromatin-remodelling complex regulates RNA polymerase III transcription by promoting Max-dependent c-Myc binding. Nucleic Acids Res **43:**4477–4490.
231. **Almuzzaini, B., A. A. Sarshad, A. K. Farrants, and P. Percipalle.** 2015. Nuclear myosin 1 contributes to a chromatin landscape compatible with RNA polymerase II transcription activation. BMC biology **13:**35.
232. **Venit, T., A. Kalendova, M. Petr, R. Dzijak, L. Pastorek, J. Rohozkova, J. Malohlava, and P. Hozak.** 2016. Nuclear myosin I regulates cell membrane tension. Scientific reports **6:**30864.
233. **Spector, D. L.** 2003. The dynamics of chromosome organization and gene regulation. Annual review of biochemistry **72:**573–608.
234. **Chuang, C. H., A. E. Carpenter, B. Fuchsova, T. Johnson, P. de Lanerolle, and A. S. Belmont.** 2006. Long-range directional movement of an interphase chromosome site. Current biology : CB **16:**825–831.
235. **Dundr, M., J. K. Ospina, M. H. Sung, S. John, M. Upender, T. Ried, G. L. Hager, and A. G. Matera.** 2007. Actin-dependent intranuclear repositioning of an active gene locus in vivo. The Journal of cell biology **179:**1095–1103.
236. **Hu, Q., Y. S. Kwon, E. Nunez, M. D. Cardamone, K. R. Hutt, K. A. Ohgi, I. Garcia-Bassets, D. W. Rose, C. K. Glass, M. G. Rosenfeld, and X. D. Fu.** 2008. Enhancing nuclear receptor-induced transcription requires nuclear motor and LSD1-dependent gene networking in interchromatin granules. Proceedings of the National Academy of Sciences of the United States of America **105:**19199–19204.
237. **Geimanen, J., H. Isok-Paas, R. Pipitch, K. Salk, T. Laos, M. Orav, T. Reinson, M. Ustav, Jr., M. Ustav, and E. Ustav.** 2011. Development of a cellular assay system to study the genome replication of high- and low-risk mucosal and cutaneous human papillomaviruses. J Virol **85:**3315–3329.
238. **Wang, X., C. Meyers, H. K. Wang, L. T. Chow, and Z. M. Zheng.** 2011. Construction of a full transcription map of human papillomavirus type 18 during productive viral infection. Journal of virology **85:**8080–8092.
239. **Kay, M. A., C. Y. He, and Z. Y. Chen.** 2010. A robust system for production of minicircle DNA vectors. Nature biotechnology **28:**1287–1289.
240. **Haller, K., F. Stubenrauch, and H. Pfister.** 1995. Differentiation-dependent transcription of the epidermodysplasia verruciformis-associated human papillomavirus type 5 in benign lesions. Virology **214:**245–255.
241. **Nasseri, M., R. Hirochika, T. R. Broker, and L. T. Chow.** 1987. A human papilloma virus type 11 transcript encoding an E1–E4 protein. Virology **159:**433–439.
242. **Smith, J. A., E. A. White, M. E. Sowa, M. L. Powell, M. Ottinger, J. W. Harper, and P. M. Howley.** 2010. Genome-wide siRNA screen identifies SMCX, EP400, and Brd4 as E2-dependent regulators of human papillomavirus oncogene expression. Proc Natl Acad Sci U S A **107:**3752–3757.

ACKNOWLEDGMENTS

I would like to thank my supervisor, Mart Ustav, who has shown great patience during my long years as a Ph.D. student. He personally taught me molecular biological techniques, provided countless ideas for experiments (some of them crazy, and some very practical) and encouraged me throughout my studies. Although he complains that his phone battery runs out because of how much he calls me, I hope it has been worth it.

I have warm and funny memories from my years as a Master's student, when we studied together, supported each other and worked side by side: Lele, Eve-Ly, Gabriela – what a dream team!

I am very grateful to all my numerous co-workers from Citrina lab and from the 4th floor at Nooruse street for their continuous support and advice.

I would like to specially thank my course mate Andres Männik from Icosagen for all his invaluable advice and friendly support, especially during the HPV5 project.

I feel greatest gratitude towards my family, who have taught me to value education and have supported me during my years at university.

PUBLICATIONS

CURRICULUM VITAE

Name: Eve Sankovski
Born: January 31th, 1972, Viljandi
Citizenship: Estonia

Education and work experience:

1987–1990	Viljandi Secondary School No. 5
1990–1995	University of Tartu, BSc in Biology
1995–1997	Institute of Zoology and Botany, technician
1997–1999	University of Tartu, MSc in Molecular Biomedicine
1999–2005	University of Tartu, PhD studies in Molecular Biomedicine
2005–2008	University of Tartu, researcher
2008–2015	Estonian Biocentre, specialist
2015–	University of Tartu, specialist

Publications:

Sankovski E, Männik A, Geimanen J, Ustav E, Ustav M. 2014. Mapping of betapapillomavirus human papillomavirus 5 transcription and characterization of viral-genome replication function. Journal of virology 88: 961–973.

Sankovski E, Karro K, Sepp M, Kurg R, Ustav M, Abroi A. 2015. Characterization of the nuclear matrix targeting sequence (NMTS) of the BPV1 E8/E2 protein – the shortest known NMTS. Nucleus 6: 289–300.

Sankovski E, Abroi A, Ustav M Jr, Ustav M. 2017. Nuclear myosin 1 associates with papillomavirus E2 regulatory protein and influences viral replication.

ELULOOKIRJELDUS

Nimi: Eve Sankovski
Sünniaeg: 31. jaanuar 1972, Viljandi
Kodakondsus: Eesti

Haridus ja töökogemus:

1987–1990	Viljandi V keskkool
1990–1995	Tartu ülikool, BSc bioloogias
1995–1997	Zooloogia ja botaanika instituut, vanemlaborant
1997–1999	Tartu ülikool, MSc molekulaarse biomeditsiini erialal
1999–2005	Tartu ülikool, doktoriõpe molekulaarse biomeditsiini erialal
2005–2008	Tartu ülikool, erakorraline teadur
2008–2015	Eesti biokeskus, spetsialist
2015–	Tartu ülikool, spetsialist

Publikatsioonid:

Sankovski E, Männik A, Geimanen J, Ustav E, Ustav M. 2014. Mapping of betapapillomavirus human papillomavirus 5 transcription and characterization of viral-genome replication function. Journal of virology 88: 961–973.

Sankovski E, Karro K, Sepp M, Kurg R, Ustav M, Abroi A. 2015. Characterization of the nuclear matrix targeting sequence (NMTS) of the BPV1 E8/E2 protein – the shortest known NMTS. Nucleus 6: 289–300.

Sankovski E, Abroi A, Ustav M Jr, Ustav M. 2017. Nuclear myosin 1 associates with papillomavirus E2 regulatory protein and influences viral replication.

DISSERTATIONES TECHNOLOGIAE UNIVERSITATIS TARTUENSIS

1. **Imre Mäger**. Characterization of cell-penetrating peptides: Assessment of cellular internalization kinetics, mechanisms and bioactivity. Tartu 2011, 132 p.
2. **Taavi Lehto**. Delivery of nucleic acids by cell-penetrating peptides: application in modulation of gene expression. Tartu 2011, 155 p.
3. **Hannes Luidalepp**. Studies on the antibiotic susceptibility of *Escherichia coli*. Tartu 2012, 111 p.
4. **Vahur Zadin**. Modelling the 3D-microbattery. Tartu 2012, 149 p.
5. **Janno Torop**. Carbide-derived carbon-based electromechanical actuators. Tartu 2012, 113 p.
6. **Julia Suhorutšenko**. Cell-penetrating peptides: cytotoxicity, immunogenicity and application for tumor targeting. Tartu 2012, 139 p.
7. **Viktoryia Shyp**. G nucleotide regulation of translational GTPases and the stringent response factor RelA. Tartu 2012, 105 p.
8. **Mardo Kõivomägi**. Studies on the substrate specificity and multisite phosphorylation mechanisms of cyclin-dependent kinase Cdk1 in *Saccharomyces cerevisiae*. Tartu, 2013, 157 p.
9. **Liis Karo-Astover**. Studies on the Semliki Forest virus replicase protein nsP1. Tartu, 2013, 113 p.
10. **Piret Arukuusk**. NickFects–novel cell-penetrating peptides. Design and uptake mechanism. Tartu, 2013, 124 p.
11. **Piret Villo**. Synthesis of acetogenin analogues. Asymmetric transfer hydrogenation coupled with dynamic kinetic resolution of α-amido-β-keto esters. Tartu, 2013, 151 p.
12. **Villu Kasari**. Bacterial toxin-antitoxin systems: transcriptional cross-activation and characterization of a novel *mqsRA* system. Tartu, 2013, 108 p.
13. **Margus Varjak**. Functional analysis of viral and host components of alphavirus replicase complexes. Tartu, 2013, 151 p.
14. **Liane Viru**. Development and analysis of novel alphavirus-based multifunctional gene therapy and expression systems. Tartu, 2013, 113 p.
15. **Kent Langel**. Cell-penetrating peptide mechanism studies: from peptides to cargo delivery. Tartu, 2014, 115 p.
16. **Rauno Temmer**. Electrochemistry and novel applications of chemically synthesized conductive polymer electrodes. Tartu, 2014, 206 p.
17. **Indrek Must**. Ionic and capacitive electroactive laminates with carbonaceous electrodes as sensors and energy harvesters. Tartu, 2014, 133 p.
18. **Veiko Voolaid**. Aquatic environment: primary reservoir, link, or sink of antibiotic resistance? Tartu, 2014, 79 p.
19. **Kristiina Laanemets**. The role of SLAC1 anion channel and its upstream regulators in stomatal opening and closure of *Arabidopsis thaliana.* Tartu, 2015, 115 p.

20. **Kalle Pärn**. Studies on inducible alphavirus-based antitumour strategy mediated by site-specific delivery with activatable cell-penetrating peptides. Tartu, 2015, 139 p.
21. **Anastasia Selyutina**. When biologist meets chemist: a search for HIV-1 inhibitors. Tartu, 2015, 172 p.
22. **Sirle Saul**. Towards understanding the neurovirulence of Semliki Forest virus. Tartu, 2015, 136 p.
23. **Marit Orav**. Study of the initial amplification of the human papillomavirus genome. Tartu, 2015, 132 p.
24. **Tormi Reinson**. Studies on the Genome Replication of Human Papillomaviruses. Tartu, 2016, 110 p.
25. **Mart Ustav Jr**. Molecular Studies of HPV-18 Genome Segregation and Stable Replication. Tartu, 2016, 152 p.
26. **Margit Mutso**. Different Approaches to Counteracting Hepatitis C Virus and Chikungunya Virus Infections. Tartu, 2016, 184 p.
27. **Jelizaveta Geimanen**. Study of the Papillomavirus Genome Replication and Segregation. Tartu, 2016, 168 p.
28. **Mart Toots**. Novel Means to Target Human Papillomavirus Infection. Tartu, 2016, 173 p.
29. **Kadi-Liis Veiman**. Development of cell-penetrating peptides for gene delivery: from transfection in cell cultures to induction of gene expression *in vivo*. Tartu, 2016, 136 p.
30. **Ly Pärnaste**. How, why, what and where: Mechanisms behind CPP/cargo nanocomplexes. Tartu, 2016, 147 p.
31. **Age Utt**. Role of alphavirus replicase in viral RNA synthesis, virus-induced cytotoxicity and recognition of viral infections in host cells. Tartu, 2016, 183 p.
32. **Veiko Vunder**. Modeling and characterization of back-relaxation of ionic electroactive polymer actuators. Tartu, 2016, 154 p.
33. **Piia Kivipõld**. Studies on the Role of Papillomavirus E2 Proteins in Virus DNA Replication. Tartu, 2016, 118 p.
34. **Liina Jakobson**. The roles of abscisic acid, CO_2, and the cuticle in the regulation of plant transpiration. Tartu, 2017, 162 p.
35. **Helen Isok-Paas**. Viral-host interactions in the life cycle of human papillomaviruses. Tartu, 2017, 158 p.
36. **Hanna Hõrak**. Identification of key regulators of stomatal CO_2 signalling via O_3-sensitivity. Tartu, 2017, 160 p.
37. **Jekaterina Jevtuševskaja**. Application of isothermal amplification methods for detection of *Chlamydia trachomatis* directly from biological samples. Tartu, 2017, 96 p.
38. **Ülar Allas.** Ribosome-targeting antibiotics and mechanisms of antibiotic resistance. Tartu, 2017, 152 p.
39. **Anton Paier.** Ribosome Degradation in Living Bacteria. Tartu, 2017, 108 p.
40. **Vallo Varik.** Stringent Response in Bacterial Growth and Survival. Tartu, 2017, 101 p.

41. **Pavel Kudrin.** In search for the inhibitors of *Escherichia coli* stringent response factor RelA. Tartu, 2017, 138 p.
42. **Liisi Henno.** Study of the human papillomavirus genome replication and oligomer generation. Tartu, 2017, 144 p.
43. **Katrin Krõlov.** Nucleic acid amplification from crude clinical samples exemplified by *Chlamydia trachomatis* detection in urine. Tartu, 2018, 118 p.