DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

159

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS
159

MEELIS KADAJA

Papillomavirus Replication Machinery Induces Genomic Instability in its Host Cell

TARTU UNIVERSITY
PRESS

Institute of Molecular and Cell Biology, University of Tartu, Estonia

The dissertation is accepted for the commencement of the degree of Doctor of Philosophy (in Virology) on 20.01.2009 by the Council of the Institute of Molecular and Cell Biology, University of Tartu.

Supervisor: Prof. Mart Ustav, PhD
Department of Biomedical Technology, Institute of Technology
University of Tartu, Estonia

Opponent: Prof. Michael Botchan, PhD
Department of Molecular & Cell Biology,
University of California, Berkeley, USA

Commencement: April 30, 2009

The publication of this dissertation is granted by the University of Tartu

ISSN 1024–6479
ISBN 978–9949–19–086–7 (trükis)
ISBN 978–9949–19–087–4 (PDF)

Autoriõigus Meelis Kadaja, 2009

Tartu Ülikooli Kirjastus
www.tyk.ee
Tellimus nr. 87

CONTENTS

LIST OF ORIGINAL PUBLICATIONS 6
LIST OF ABBREVIATIONS 7
1. INTRODUCTION 8
2. LITERATURE REVIEW 9
2.1 General introduction to Papillomaviruses 9
2.1.1 Papillomavirus genome organization 9
2.1.2 Papillomavirus life cycle 11
2.2 Initiation of DNA replication in the nucleus of cells infected with papillomaviruses 13
2.2.1 Initiation of genomic DNA replication in mammalian cells ... 14
2.2.2 Initiation of papillomavirus DNA replication 15
2.2.3 Prevention of DNA re-replication and its damage repair by checkpoint pathways 16
2.3 Human papillomaviruses and cervical cancer 17
2.3.1 Physical state of human papillomaviruses in cervical neoplasia 17
2.3.2 Virus-cell interactions leading to the HPV-associated malignant transformation 18
3. RESULTS AND DISCUSSION 20
3.1 Two separate replication modes of the BPV1 origin of replication have different sensitivities to p53 (I) 20
3.2 Genomic instability of the host cell induced by the human papillomavirus replication machinery (II, III) 22
3.3 Mechanisms of Genomic Instability in Cells Infected with High Risk Human Papillomaviruses (III, IV) 24
4. CONCLUSIONS 27
REFERENCES 28
SUMMARY IN ESTONIAN 38
ACKNOWLEDGEMENTS 39
PUBLICATIONS 41

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original papers, which will be referred to by their Roman numerals.

I Ilves I., **Kadaja M.**, Ustav M. (2003) Two separate replication modes of the bovine papillomavirus BPV1 origin of replication that have different sensitivity to p53. *Virus Research,* 96, 75–84.

II **Kadaja M.**, Sumerina A., Verst T., Ojarand M., Ustav E., Ustav M. (2007) Genomic instability of the host cell induced by the human papillomavirus replication machinery. *EMBO Journal,* 26, 2180–91.

III **Kadaja M**., Isok-Paas H., Laos T., Ustav E., Ustav M. (2009) Mechanism of Genomic Instability in Cells Infected with the High Risk Human Papillomaviruses. *PLoS Pathogens* (manuscript in press).

IV **Kadaja M,** Silla T, Ustav E., Ustav M. (2009) Papillomavirus DNA replication – From initiation to genomic instability. *Virology*, 384, 360–8

My contributions to the papers are as follows:

Ref. I I performed the experiments and analyzed the data, except the E1 titration assay;

Ref. II I designed and performed the experiments, except the replication assay of the HPV URR plasmids and the titration of viral replication proteins in HeLa cells. I analyzed the data, and I wrote most of the manuscript;

Ref. III I designed and performed the experiments, except the subcloning of the cells transfected with HPV genomes and their restriction analysis. I analyzed the data and I wrote most of the manuscript;

Ref. IV I wrote most of the manuscript.

LIST OF ABBREVIATIONS

APC	anaphase promoting complex
ATM	ataxia telangiectasia mutated kinase
ATR	ATM and Rad3-related kinase
BPV-1	bovine papillomavirus type 1
BrdU	5-bromo-2-deoxyuridine
cdc	cell division cycle
cdk	cyclin-dependent protein kinase
cdt1	chromatin licensing and DNA replication factor 1
CMV	cytomegalovirus
DSBs	double strand breaks
dsDNA	double stranded DNA
EBV	Epstein – Barr virus
FISH	fluorescent *in situ* hybridization
FS	fragile site
HSIL	High-grade squamous intraepithelial lesion
HPV	human papillomavirus
HR	homologous recombination
LCR	long control region
LSIL	low grade squamous intraepithelial lesion
MCM	minichromosome maintenance
MME	minichromosome maintenance element
mtDNA	mitochondrial DNA
NHEJ	non-homologous end-joining
ORF	open reading frame
ori	origin of DNA replication
PCNA	proliferating cell nuclear antigen
pol	polymerase
pRB	retinoblastoma protein
PV	papillomavirus
Py	polyomavirus
RFC	replication factor C
RPA	replication protein A
SCC	invasive squamous cell carcinoma
ssDNA	single stranded DNA
SV40	Simian virus 40
URR	upstream regulatory region

1. INTRODUCTION

Genetic information in metazoan cells is stored in condensed chromatin, which protects the DNA from damage. However, when this information needs to be accessed, the secured DNA is decondensed as the double helix itself is opened. It is during DNA replication that DNA is the most vulnerable and therefore genomic duplication is highly orchestrated; it is carefully planned during the G1 phase, precisely executed in the S-phase, and all the mistakes are eliminated in the S- and G2 phases before mitosis. The whole process is closely guarded by cellular control factors that intervene immediately at the slightest deviation from the plan.

Papillomaviruses are endogenous parasites that do not require the same kind of fidelity as metazoan cells and can afford some inaccuracy while replicating their small circular double stranded DNA (dsDNA) genome. They have evolved systems either to overrun the cellular defenses and to replicate their genome multiple times per cell cycle or to mimic cellular DNA replication by replicating approximately once per cell cycle. In either case, the genomic integrity of the host cell is not jeopardized during the normal viral life cycle. However, the human papillomavirus (HPV) is occasionally integrated into the host genome. The mechanism of viral integration is currently unknown, but the work presented here will demonstrate that the inability to precisely replicate its DNA once per cell cycle makes the HPV act like a Trojan horse while inside the host genome. If not correctly regulated, it will not only end the viral life cycle, but it can also cause the death of the host by causing chromosomal instability, which may lead to cancer.

2. LITERATURE REVIEW

2.1 General introduction to Papillomaviruses

Papillomaviruses (PV) are a group of nonenveloped, epitheliotropic, species-specific DNA viruses that infect humans and variety of animals. Based on tissue tropism, more than 100 different types of human papillomaviruses (HPV) are divided into subgroups of mucosal or cutaneous papillomaviruses. HPV infection is usually asymptomatic or results in benign epithelial lesions called papillomas, also known as warts on the skin and condylomas on the genitalia. Nevertheless, PVs are also considered to be the strongest known human carcinogen worldwide. The infection with HPV16 or –18 has a stronger association with cervical cancer than tobacco smoking has with lung cancer (Burd, 2003; Ozlu and Bulbul, 2005).

The current literature review concerning papillomaviruses is mainly based on the most studied human papillomaviruses, HPV16 and –18, and on the bovine papillomavirus type 1 (BPV1), which has been a prototype of PV replication studies. From these studies, a general pattern of viral features has been summarized that, with slight modifications, can be applied to all PVs. The specific features of certain papillomaviruses are pointed out where appropriate.

2.1.1 Papillomavirus genome organization

To date, over 100 different human papillomavirus (HPV) types have been identified (de Villiers et al., 2004). These include complete viral genomes, whose L1 open reading frame (ORF) is at least 10% dissimilar when compared to any other HPV type (definition adopted by Papillomavirus Nomenclature Committee in 1995). These different types of HPVs are thought to have arisen through a series of small mutations scattered throughout the genome, rather than by recombination between genomes (Chan et al., 1995).

PV genomes are circular dsDNA about 8 kb in size. Despite the number of PV types, all PV genomes have a highly conserved organization that encodes ORFs from one strand of the viral genome. ORFs that are located in the early region of the genome (E) encode non-structural proteins, while the 2 ORFs in the late region (L) are responsible for the synthesis of structural proteins (Fig. 1).

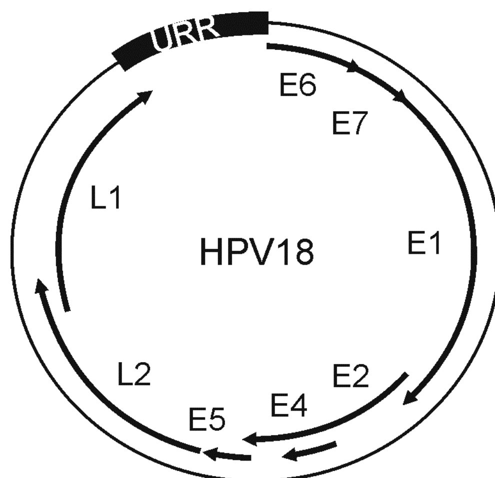

Figure 1. Schematic presentation of the HPV18 genome. Open arrows represent the viral ORFs and the non-coding upstream regulatory region (URR) is shown as an open box.

E1 is a highly conserved PV protein that functions as the origin recognition factor and the ATP-dependent hexameric viral helicase (Sedman and Stenlund, 1998; Stenlund, 2003; Ustav and Stenlund, 1991; Wilson et al., 2002; Yang et al., 1991; Yang et al., 1993).

Full-length E2 protein acts primarily as the viral transcription factor and another origin recognition factor (Bonne-Andrea et al., 1997; Sedman and Stenlund, 1995; Ustav et al., 1993; Ustav and Stenlund, 1991; Yang et al., 1991). While BPV1 E2 is a strong transcription activator, HPV16 and –18 E2 have been shown to be only moderate activators and are known more as transcription repressors of viral oncogenes E6 and E7 at higher concentrations (Demeret et al., 1994; McBride et al., 1991; Romanczuk et al., 1990; Steger and Corbach, 1997; Tan et al., 1994). Also, splice variants of E2 lacking the transactivation domain are expressed that function as repressors of replication and transcription (Lim et al., 1998; Zobel et al., 2003) (Kurg et al., manuscript submitted for publication).

The expression of E4 is linked to differentiation of the epithelial host cell, and its abundant expression in the upper layers of the epithelium is characteristic of late genes (Palefsky et al., 1991). The biological function of the protein is largely unknown, but different HPV E4 proteins have been shown to disrupt cyokeratins and to block the cell cycle in G2/M (Doorbar et al., 1991; Nakahara et al., 2002; Wang et al., 2004). Therefore, E4 is believed to play a role in the release of maturated PV virions.

E5 is the major oncoprotein of BPV1, but in HPVs, its oncogenic ability is not as obvious (DiMaio and Mattoon, 2001). However, it has been demonstrated that HPV16 E5 can induce epithelial tumors in mice through

interaction with the epidermal growth factor receptor (Genther Williams et al., 2005).

E6 is the major oncoprotein of HPVs. Anogenital (mucosal) E6 has a well known ability to prevent cell cycle blockage and apoptosis through the inhibition of p53 (Scheffner et al., 1990). Cutaneous HPV E6 is known to function through the degradation of Bak (Jackson et al., 2000).

E7 is another PV oncoprotein and has been shown to inhibit retinoblastoma protein family members, which are necessary to drive the host cell cycle into S-phase (Boyer et al., 1996).

L1 is the major capsid protein and is one of the most conserved papillomavirus proteins (de Villiers et al., 2004). Together with the minor capsid protein, L2, it is expressed in the upper layers of differentiated epithelium.

Between the late and early regions of the PV genome, there is also an approximately 1 kb non-coding region called the upstream regulatory region (URR)[1] that contains the origin of replication (ori), early promoters, and binding sites for several cellular transcription factors.

2.1.2 Papillomavirus life cycle

Papillomaviruses infect undifferentiated proliferating cells in the basal layer of the epithelium and the progress of their subsequent life cycle within the host depends heavily on the differentiation of the infected cells. Therefore, it has been a challenge to propagate PVs *in vitro*. A significant portion of the knowledge about the early steps in the PV replication cycle is based on BPV1, thanks to its unique ability to infect the monolayer C127 cell-line of mouse fibroblasts. Additionally, *in vitro* models have been developed to recapitulate the epithelial differentiation, such as the "raft" culture system in which the stratified squamous epithelial cells are placed at an air-water interface (Meyers et al., 1997), or placing a suspension of HPV-containing epithelial cells in semisolid medium (Ruesch et al., 1998).

HPV infection is believed to occur through small wounds in the epithelium. It has been suggested that integrin receptors (Oldak et al., 2006) and heparin sulphate are used by PVs for attachment to the cell (Giroglou et al., 2001), leading to the internalization of bound virus through the endocytosis of clathrin coated vesicles (Day et al., 2003; Giroglou et al., 2001). The infection, uncoating, and the entry of HPV DNA into the nucleus of the basal epithelial cells are followed by activation of the early promoter in the upstream regulatory region, resulting in the expression of proteins E1, E2, E6, and E7. E1 and E2 direct the host cell replication factors to the viral origin of replication, which leads to the initial quick rise of the viral genome copy number per cell (Chiang et al., 1992; Ustav and Stenlund, 1991). This process can be simply modeled in cell culture by transient transfection of E1 and E2 expression vectors together

---

[1] also known as long control region (LCR)

with the reporter plasmid carrying the sequences of the viral replication origin (Ustav et al., 1993; Ustav et al., 1991). In epithelial tissue, however, the process is more complicated. When basal epithelial cells divide, some of the daughter cells migrate upward, exit the cell cycle, and undergo terminal differentiation, while the other cells remain in the basal layer as stem cells (Fuchs, 2008). Therefore, at least in differentiating cells, E6 and E7 oncoproteins are also necessary to drive the cell cycle into S-phase and to adjust the intracellular milieu in a way that it is suitable for HPV genome amplification (Garner-Hamrick et al., 2004; Howley, 2001; Thomas et al., 1999). In undifferentiated basal cell layers, it is possible that the pattern of viral gene expression during the latent infection is restricted to E1 and E2, and that the E6 and E7 genes are not required. The expression of E6 and E7 can be kept low due to the tight control by E2 (Romanczuk et al., 1990; Steger and Corbach, 1997).

After the initial amplification, the HPV genome is established as a stable multicopy extrachromosomal plasmid at roughly 50 to 100 copies per cell. Although little is known about the replication mechanisms of PV replicons during viral stable extrachromosomal maintenance, it is generally accepted that the replication initiation process during stable maintenance of PV genomes is guided by a relaxed random-choice control mechanism that results, statistically, once per cell cycle replication of the viral DNA (Piirsoo et al., 1996; Ravnan et al., 1992). It has been also suggested that HPV16 DNA could strictly replicate once per cell cycle (Hoffmann et al., 2006) and that the BPV1 E1 protein is dispensable as soon as stable extrachromosomal maintenance of the viral genome has been established (Kim and Lambert, 2002). However, additional evidence is required to confirm these findings. The strict once per cell cycle replication mode during PV stable maintenance would be expected if the cellular complexes are used for DNA replication initiation, as in the case of Epstein-Barr Virus (EBV) (Chaudhuri et al., 2001; Dhar et al., 2001). Furthermore, 2D analyses of the replication intermediates of BPV1, HPV11, and HPV16 in cells that stably maintain the viral genome have mapped the origin of replication to the same part of the URR that is used in E1-E2-dependent initiation of DNA replication (Auborn et al., 1994; Flores and Lambert, 1997; Schvartzman et al., 1990; Yang and Botchan, 1990).

It is believed that during stable extrachromosomal maintenance, the replicated PV episomes are distributed evenly into daughter cells. It is not yet clear how this is achieved by HPVs, but in the case of BPV1, it is accomplished through the E2 protein and its binding sites within the URR region known as minichromosome maintenance element (MME) (Piirsoo et al., 1996). MME is responsible for the E2-mediated attachment of full-length BPV1 genomes, as well as URR reporter plasmids, to host cell chromosomes. This process is thought to ensure the partitioning of viral extrachromosomal DNA molecules during host cell mitosis (Ilves et al., 1999; Lehman and Botchan, 1998; Skiadopoulos and McBride, 1998; You et al., 2004).

Current models suggest that when infected cells reach the suprabasal level of differentiated epithelium, the transcription of PV early genes is switched

from the E2-regulated early promoter to the E2-independent late promoter located within the E7 ORF. This switch results in high levels of E1 and E2 (and probably E4 and E5), leading to the increase in viral copy number of up to thousands per cell (Grassmann et al., 1996; Klumpp and Laimins, 1999). The molecular mechanisms of the activation of the late differentiation-dependent promoter are not well understood. It is possible that the promoter is constitutively active and PV protein expression is regulated by alternative splicing patterns in epithelial cells at different stages of differentiation. Alternative splicing could also explain the upregulation of HPV18 protein expression in differentiated keratinocytes, since there is no late promoter found inside the HPV18 E7 ORF. There are two papers suggesting that BPV1, HPV16, and HPV31 might switch from bi-directional DNA replication to the rolling circle mode of replication at this stage of their life cycle (Dasgupta et al., 1992; Flores and Lambert, 1997). First, Flores and Lambert based their conclusions on their ability to detect only Y-shaped DNA replication intermediates of the HPV16 and HPV31 genomes in differentiated cells. However, the study remains inconclusive, since in case of the rolling circles, very characteristic patterns should appear in two dimensional gels as demonstrated in the T4 *in vitro* replication system (Belanger et al., 1996). One of these patterns, the ds-eyebrow, has never been detected when PV replicons have been analyzed. In another study, Dasgupta et al. performed an electron-microscopic analysis of the low molecular weight DNA from the mouse cells harboring high levels of the BPV-1 genome. While the structures of the observed molecules were consistent with rolling circles, the BPV1 specificity was not proven, nor were negative control samples without BPV1 genome presented. Therefore, it may have been possible that the observed DNA molecules represented the replicating mtDNA. Further studies are needed to reveal the viral replication mode in this phase of the PV replication cycle.

Regardless of the exact mechanism of viral replication, the final vegetative PV amplification is accompanied by the expression of L1 and L2 structural proteins and virion assembly in the upper layers of infected tissue. Since papillomaviruses are non-lytic, they are not released until the infected cells are shed from the epithelial surface. The HPV replication cycle takes at least 3 weeks, as this is the time required for the keratinocyte to undergo complete differentiation.

2.2 Initiation of DNA replication in the nucleus of cells infected with papillomaviruses

In general, the initiation of HPV DNA replication resembles the initiation of cellular bi-directional semi-conservative DNA replication (Fig. 2). However, there are some important differences outlined below that allow the PV DNA to replicate more than once per cell cycle.

2.2.1 Initiation of genomic DNA replication in mammalian cells

Genomic DNA is replicated exactly once per cell cycle in eukaryotic cells. The prevention of DNA re-replication is guaranteed by the temporal separation of two critical events: the assembly of the pre-replication complex (licensing of replication) and the actual initiation step of DNA synthesis (Blow and Dutta, 2005; Machida et al., 2005). Pre-replication complex (pre-RC) formation is restricted to G1 phase, while DNA synthesis is activated in S-phase (Fig. 2A).

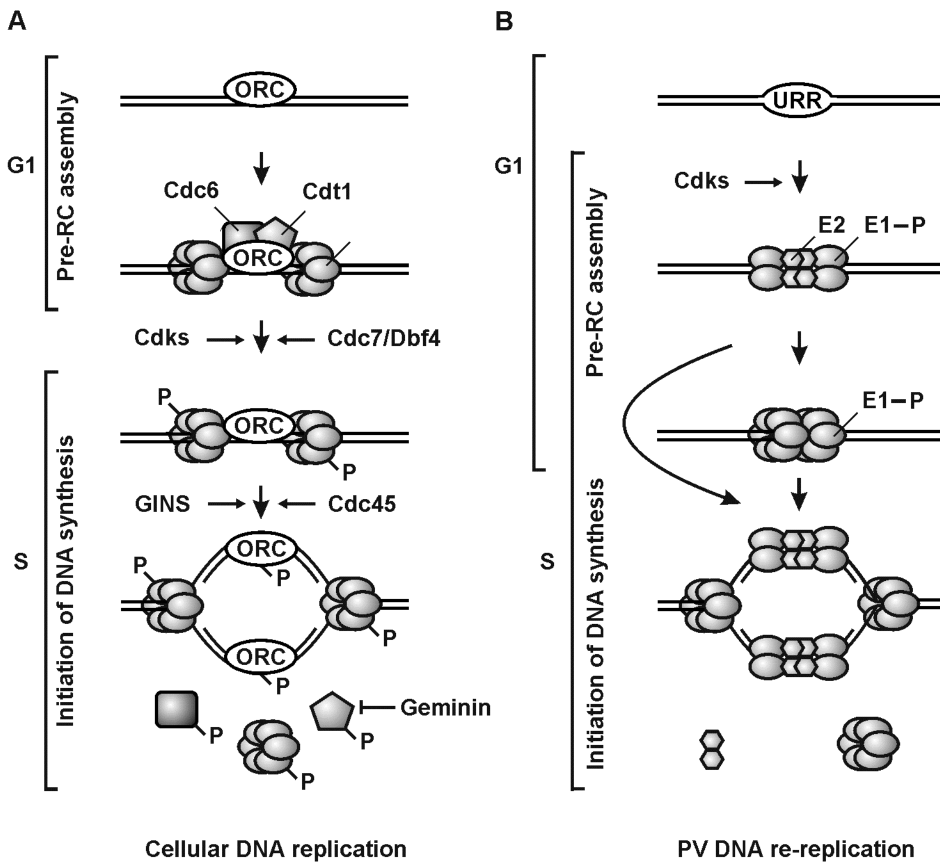

Figure 2. Initiation of cellular DNA replication (A) and viral DNA re-replication (B) in the nucleus of a cell infected with a papillomavirus (IV, Fig. 1). Only the components of the pre-replication complex (pre-RC) are presented. Please see text for details.

The replication of an eukaryotic chromosome starts from multiple origins that are determined during late S- to early G1-phase of the cell cycle by the six-protein origin recognition complex (ORC) (DePamphilis, 2005). In late G1-phase, some of the ORCs are chosen as platforms for pre-replication complex

(pre-RC) formation, which involves the loading of an inactive form of the MCM2-7 helicase complex (MCM2-7) by cdc6 and Cdt1 (Cook et al., 2004; Mailand and Diffley, 2005; Rialland et al., 2002; Yan et al., 1998). In S-phase, DNA replication is activated by the coordinated action of at least 22 additional proteins, including Cdks/cyclins and Cdc7/Dbf4 kinase (Blow and Dutta, 2005; Mendez and Stillman, 2003). Phosphorylation by Cdc7/Dbf4 and Cdks activates the MCM2-7 complex and allows it to recruit several other proteins such as Cdc45 and the GINS complex, which are necessary for loading DNA polymerases and for the start of DNA elongation (Jiang et al., 1999a; Labib and Gambus, 2007; Masai et al., 2006). At the same time, the pre-RCs themselves are disassembled and the reassociation of MCM proteins with origins is not permitted as cdc6 and Cdt1 are inactivated. Cdc6 is phosphorylated by Cdks, which results in its nuclear export (Jiang et al., 1999b; Petersen et al., 1999). Cdt1 is either phosphorylated and degradated or stabilized by geminin in an inactive form until the next G1-phase, when geminin is degradated in an APC-dependent manner (Fujita, 2006; McGarry and Kirschner, 1998; Melixetian and Helin, 2004). Second-round pre-RC formation in humans is also inhibited by the phosphorylation and subsequent degradation of Orc1 during S-phase by ubiquitin-dependent proteolysis (Mendez et al., 2002; Tatsumi et al., 2003). However, the geminin-Cdt1 interaction seems to be a primary factor in preventing DNA re-replication in humans (Blow and Dutta, 2005; Hook et al., 2007).

2.2.2 Initiation of papillomavirus DNA replication

Most of the data regarding the initiation of PV DNA replication were obtained from studies of BPV1. However, this knowledge can be safely extrapolated to the majority of PVs, as the E1-E2-ori interactions are highly conserved in all PVs (Berg and Stenlund, 1997; Chiang et al., 1992).

The viral origin of replication is located within the URR of the genome and contains the binding sites for the E1 and E2 proteins, as well as an AT-rich area (Remm et al., 1992; Ustav et al., 1991). Replication of the PV genome is initiated via the viral pre-RC that involves the cooperative binding of E1 and E2 to the viral origin (Fig. 2B) (Chiang et al., 1992; Russell and Botchan, 1995; Stenlund, 2003; Sverdrup and Khan, 1994; Ustav et al., 1993; Ustav and Stenlund, 1991). The E1 protein acts as a DNA-dependent ATPase and a DNA helicase responsible for the initial melting of the ori, as well as subsequent unwinding of the double helix during replication fork progression (Sedman and Stenlund, 1998; Yang et al., 1993). Phosphorylation by the cyclinE/Cdk2 and cyclinA/Cdk2 complexes has been shown to be important for E1 nuclear localization (Deng et al., 2004; Hsu et al., 2007). E2 has an auxiliary role during the initiation of DNA replication, acting as a specificity factor for E1. It has been demonstrated *in vitro* that E1 can initiate DNA replication from a non-specific DNA sequence in the absence of E2 protein (Bonne-Andrea et al.,

1997; Sedman and Stenlund, 1995). The binding of E1 to E2 is followed by the hexamerization of E1 and the release of E2, as well as the recruitment of host cell replication proteins at the origin (Melendy et al., 1995). It has been demonstrated that various combinations of E1 and E2 proteins from different PVs can initiate DNA replication from the several different PV origins (Chiang et al., 1992).

Unlike the replication of cellular DNA mediated by the ORC and MCM2-7 complexes, the E1 and E2-dependent DNA replication does not follow the once-per-cell cycle initiation mode (Piirsoo et al., 1996; Ravnan et al., 1992). This is mainly because the unscheduled formation of viral pre-RC, orchestrated by viral E1 and E2 proteins, can take place simultaneously in S-phase with the initiation of viral DNA replication (Fig. 2B).

2.2.3 Prevention of DNA re-replication and its damage repair by checkpoint pathways

In the event that genomic DNA re-replication is initiated (Karakaidos et al., 2004; Tatsumi et al., 2006), the ataxia telangiectasia mutated (ATM) kinase as well as the ATM and Rad3-related (ATR) kinase checkpoint pathways are activated to prevent the induction of genomic rearrangements (Lavin, 2007; Lin and Dutta, 2007; Liu et al., 2007; Paulsen and Cimprich, 2007).

In mammalian cells, the ATR-mediated S-phase checkpoint is activated first, at the onset of DNA re-replication after the accumulation of ssDNA and before the appearance of double strand breaks (DSBs). This inhibits re-replication so that significant re-replication is prevented (Liu et al., 2007; Vaziri et al., 2003). The ATR could inhibit DNA re-replication directly by phosphorylation of the MCM complex (Cortez et al., 2004) or indirectly through the p53 and pRB pathways (Liu et al., 2007; Vaziri et al., 2003). It has been demonstrated that the ATR checkpoint is activated in response to the structural problems that occur during DNA re-replication (such as ssDNA) rather than recognizing the re-licensing itself. It is believed that the reloaded MCM proteins are not properly coordinated with other replication proteins, which leads to the functional uncoupling of MCM helicase and DNA polymerases. This results in the accumulation of RPA-coated ssDNA.

If the primarily mission of the ATR pathway is to prevent DNA re-replication, then the ATM pathway is activated when this prevention fails and DSBs are produced. DSBs can be generated in several ways during DNA re-replication including head-to-tail fork collision, the collapse of stalled re-replication forks, or if new re-replication forks encounter Okazaki fragments at the existing forks. DSBs in eukaryotic cells are repaired by homologous recombination (HR) and non-homologous end-joining (NHEJ). The balance between NHEJ and HR shifts during the cell cycle, in which HR is upregulated during the S- and G2 phases of the cell cycle when sister chromatids are available. In addition, it has been demonstrated that DSBs caused by replication

blockage are repaired by HR, whereas "accidental" DSBs in packed chromosomes are frequently repaired by NHEJ (Shrivastav et al., 2008; Sonoda et al., 2006; Weterings and Chen, 2008).

Papillomaviruses can induce the re-replication of their genome in infected cells despite cellular control mechanisms that have evolved to stop cellular DNA re-replication. Most likely, the virus can escape cellular control mechanisms because of the differences in the assembly of viral and cellular pre-RCs and because of the different features of E1 compared to MCM2-7.

2.3 Human papillomaviruses and cervical cancer

Anogenital (mucosal) HPVs are extremely common. They are considered to be the most common sexually transmitted disease worldwide, with 70% of sexually active adults becoming infected with anogenital HPV at some point during their lifetime. It has been estimated that around 291 million women with normal cervical cytology worldwide are carriers of HPV DNA (de Sanjose et al., 2007).

According to the propensity for malignant progression of the HPV infected cells, anogenital HPVs are designated as "high-risk" or "low-risk" viruses (de Villiers et al., 2004; Munger et al., 2004; zur Hausen, 2002). "Low-risk" HPVs (LR-HPV), like HPV6 and HPV11, are considered to be rather normal microflora of the human epithelia since they are generally limited to low-grade lesions or genital warts that rarely progress to malignancy. "High risk" HPV (HR-HPV) infections, however, are associated with more than 99% of cervical neoplasias and invasive cervical carcinomas worldwide (Walboomers et al., 1999). HR-HPVs, such as HPV16 and HPV18, have been shown to be the predominant cause of these diseases, as well as head and neck squamous cell carcinomas (Gillison et al., 2000; Paz et al., 1997). Although the progression to malignancy is relatively rare, anogenital HPVs are one of the most important infectious agents in cancer causation, responsible for 5% of the world cancer burden. Of these, 87% are cervical cancer, 10% are other anogenital cancers, and 3% are oropharyngeal cancers (Parkin, 2006).

Subsequently, the association of "high risk" HPVs with cervical cancer is reviewed.

2.3.1 Physical state of human papillomaviruses in cervical neoplasia

Tumor progression in HR-HPV-associated neoplasia is classified as a low-grade squamous intraepithelial lesion (LSIL), a high-grade squamous intraepithelial lesion (HSIL), or an invasive squamous cell carcinoma (SCC). The physical state of HPV in these different states of neoplastic tissues has been evaluated in several studies over the past few decades. However, only the recent development of quantitative real-time PCR protocols has provided us with the reliable sensitivity needed to understand the physical state of HPV in these

tissues (Andersson et al., 2005; Arias-Pulido et al., 2006; Kulmala et al., 2006; Peitsaro et al., 2002b; Yoshida et al., 2008). In general, it has been found that, although the viral load in tissue samples decreases from LSIL to SCC, the frequency of HPV integration increases according to the degree of transformation. This indicates that HPV integration has an impact on the neoplastic progression of the infected cell. These studies have also demonstrated that HPV integration often results in a situation where the mixed (episomal/integrated) pattern is the most prevalent physical state of HPVs already in PAP smears with normal morphology and in LSIL. In addition, other studies have observed the presence of episomal and integrated HPV within the same cells in clinical samples using *in situ* hybridization (Cooper et al., 1991; Kristiansen et al., 1994). Experiments using the HPV16 positive W12 cell line demonstrated the co-existence of episomal and integrated HPV16 DNA in the same cells in early passages, while the later passages revealed the loss of the HPV plasmids (Pett et al., 2006). A similar result was obtained with a cell line carrying HPV33 (Peitsaro et al., 2002a).

HPV integration studies in cervical tumors have revealed that HPV integrates randomly throughout the genome. However, there is a high correlation between the fragile sites (FS) and HPV integration sites and, up to 60% of the time, fragile sites are the location of HPV integration (Ferber et al., 2003; Thorland et al., 2003; Wentzensen et al., 2002; Wentzensen et al., 2004; Yu et al., 2005). FSs are predetermined chromosomal breakage regions that are prone to recombination. These sites have been seen in metaphase chromosomes mostly after replicative stresses, such as partial inhibition of DNA replication by aphidicolin. Therefore, it might be possible that DSBs generated during the replication of host chromosomes are required for HPV integration to take place. There are at least 104 FSs, of which 24 are rare and 80 are common. Among the common FSs, FRA8C within the c-MYC locus at 8q24 is the most frequent location for HPV18 integration. This integration is often accompanied by amplification of the viral sequences and the flanking cellular sequences, which results in the overexpression of MYC (Ferber et al., 2003; Herrick et al., 2005; Lazo et al., 1989; Macville et al., 1999; Peter et al., 2006a).

2.3.2 Virus-cell interactions leading to the HPV-associated malignant transformation

The integration of HR-HPV DNA into the host cell chromosome is believed to be a key event in the malignant progression of the host cell. Usually only a part of the HPV genome is integrated in cervical carcinomas without any preferred break points inside the integrated HPV sequence. However, the E1 and E2 ORFs are consistently disrupted, while the E6 and E7 ORFs, as well as the URR, are present (Baker et al., 1987; Dall et al., 2008; Kalantari et al., 2001; Luft et al., 2001; Peter et al., 2006a; Ziegert et al., 2003; Wagatsuma et al., 1990). Therefore, it has been suggested that when HPV DNA integration

occurs, the repression function of E2 is switched off, allowing for an increase in the expression of viral oncoproteins E6 and E7 (Corden et al., 1999; Kalantari et al., 2001; Peter et al., 2006b). In turn, E6 and E7 give cells a selective growth advantage primarily due to the disruption of the function of p53 and the retinoblastoma tumor suppressor protein family members (Boyer et al., 1996; Dyson et al., 1989; Jeon et al., 1995; Romanczuk and Howley, 1992; Scheffner et al., 1990). The high-risk E6 proteins are also capable of activating the catalytic hTERT subunit of the telomerase, leading to an increase in telomeric length in infected cells (Klingelhutz et al., 1996; Veldman et al., 2003). HR-HPV E7 can further hinder cell cycle inhibition by increasing the levels of cyclins A and E, as well as by modulating the functions of cyclin-dependent kinase inhibitor $p27^{kip1}$, cyclin A/cdk2 complex, transcription factor E2F1, and cyclin E/cdk2 (Hwang et al., 2002; McIntyre et al., 1996; Ruesch and Laimins, 1998; Zerfass-Thome et al., 1996; Tommasino et al., 1993). The upregulation of HR-HPV oncogene expression is also associated with genomic instability in the HPV infected cells. HPV-16 E7 can produce abnormal centrosome numbers (Duensing and Munger, 2002; Duensing and Munger, 2004; Pett et al., 2004) and HPV-16 E6 can induce abrogation of the G2/M checkpoint (Thomas and Laimins, 1998; Thompson et al., 1997), as well as reduce the efficiency of single-stranded DNA break repair (Iftner et al., 2002).

LR-HPV E6 and E7 proteins have been shown to have lower oncogenic potential (Crook et al., 1991; Heck et al., 1992). Their role in HPV11 episomal maintenance has been shown (Oh et al., 2004) to result in an extended life span, but not the immortalization, of normal keratinocytes in monolayer cultures (Thomas et al., 2001). The inability of integrated “low risk” E6 and E7 to provide a growth advantage in infected cells probably explains why cells harboring integrated LR-HPV are not found *in vivo*, although human foreskin keratinocytes transfected with wild-type HPV-11 genome have been found to contain integrated forms of the viral genome (Oh et al., 2004).

The mechanisms of carcinogenesis of cutaneous HPV types are largely unknown and are probably different from anogenital types. The integration of cutaneous PV DNA is rarely observed, although E6 still seems to be the dominant viral oncogene, and its main anti-apoptotic function is the degradation of Bak (Jackson et al., 2000). This probably leads to delays in the repair of UV-induced DNA damages, resulting in the continuous proliferation of keratinocytes and the onset of epithelial skin cancer.

3. RESULTS AND DISCUSSION

Papillomaviruses do not follow the strict once per cell cycle replication mode at any stage in their life cycle (Piirsoo et al., 1996; Ravnan et al., 1992). This means that PVs must have evolved mechanisms to avoid cellular control pathways that would otherwise hinder the cellular DNA re-replication (Karakaidos et al., 2004; Liu et al., 2007; Tatsumi et al., 2006; Vaziri et al., 2003). This characteristic might become important if "high-risk" HPV DNA carrying the origin of viral DNA replication randomly integrates into the host cell genome. Although ORFs of the two viral replication proteins are disrupted during the integration, there is a short period of time, after the initial HPV integration and before the loss of the HPV episomes, when functional E1 and E2 proteins are still present in these cells (Peitsaro et al., 2002a; Pett et al., 2006). Therefore, it is plausible that E1 and E2 proteins expressed from the HPV plasmid can induce multiple unscheduled initiation events at the integrated HPV origin and thereby endanger the genomic stability of the host and drive malignant transformation.

To clarify the possibilities mentioned above, the work herein was divided into the following aims:

- To use p53 and BPV1 to demonstrate that, in general, the DNA replication mechanisms utilized by papillomaviruses are able to escape from the cellular control mechanisms that have been shown to prevent DNA re-replication;
- To explore the functionality of the HR-HPV replication origin when it is covalently linked to human chromosomes;
- To study the consequences of DNA re-replication initiated from the integrated HPV origin;
- To elucidate the mechanisms by which the DNA re-replication of integrated HPV can cause the genomic instability.

3.1 Two separate replication modes of the BPV1 origin of replication have different sensitivities to p53 (I)

It has been shown that the replication of transiently transfected reporter plasmids carrying the papillomavirus origin of replication are efficiently suppressed by p53 in several cell lines (Lepik et al., 1998). Despite this observation and the fact that BPV1 E6 is unable to induce the degradation of p53 protein, BPV1 can establish itself in p53-expressing cells. Therefore, it is possible that BPV1 might escape the inhibitory effect of p53 at some stage during the establishment of its stable maintenance.

To address this question we decided to use the CHOBgl40 cell line, which has previously been used as a model system to study BPV1 DNA replication during its stable maintenance period (Piirsoo et al., 1996). This cell line

supports constitutive BPV1 E1 and E2 protein expression and stably maintains the BPV1 URR reporter plasmid pNeoBgl40. By transfecting this cell line with another BPV1 URR plasmid, pNeoBgl40HIII, we were able to detect its replication in cells that already maintained a similar plasmid at constant copy number.

By co-transfecting the CHOBgl40 cell line with pNeoBgl40HIII and different p53 expression vectors, we first demonstrated that p53 functions as a suppressor of transient BPV1 amplification while having no effect on the growth of transfected cells (I, Fig. 1). Neither the cell cycle profile, nor the BrdU labeling profile, revealed any significant changes in the growth or viability of the cells that could interfere with the replication analysis of the BPV1 URR reporter plasmids in our experiments (I, Fig. 2). Using the same experimental conditions as in the control experiments, we demonstrated that p53 deletion mutants, which worked as repressors of BPV1 amplifying replication, had no inhibitory effect on the copy number of the reporter plasmid that was stably maintained in the CHOBgl40 cell line (I, Fig. 3). Although E1 expression was slightly decreased in response to p53 over-expression, we showed, by parallel analysis, that it was not the reason for the p53 related sensitivity of BPV1 transient amplification (I, Fig. 4). These data also demonstrated that both the transient amplification and the stable maintenance replication of the BPV1 URR reporter plasmid can take place simultaneously in the same cells. Transient amplification of the BPV1 reporter did not interfere with the simultaneous stable replication of a similar plasmid, as the copy number of the stably replicating reporter remained unchanged.

Therefore, we can conclude that the initial transient amplification and the subsequent stable replication of the BPV1 URR reporter plasmid are mechanistically different processes, well reflected by their different sensitivities to p53. This temporal, spatial, or mechanistic difference is related to the more tightly regulated replication initiation mechanism. Unfortunately, we can only speculate as to what are the exact differences. The most plausible explanation is that the different sensitivity to p53 could appear as an indirect consequence of the altered sub-nuclear localization. It has been demonstrated that long-term stable maintenance of the BPV1 replicon is dependent on its non-covalent attachment to host chromatin, and this process has been linked to efficient partitioning and nuclear retention of the viral genomes during mitosis (Ilves et al., 1999; Lehman and Botchan, 1998; Skiadopoulos and McBride, 1998). We can speculate that the association with chromatin could allow for the access of replication control mechanisms to the BPV1 DNA replication, which are commonly operating during host cell genome replication. It might cause changes in the replication mechanism of the viral DNA that are differentially recognized and affected by p53.

The initial viral amplification is required for successful extrachromosomal establishment of the BPV1 genome after infection. However, we can assume that the uncontrolled over-replication is not favorable for the virus in the long run, as it could induce the cellular responses leading to cell-cycle block or

apoptosis. Therefore, the virus-cell interactions could have evolved to avoid the negative interference of cellular control factors. In this case, p53 might be used to suppress replication of the viral genome molecules that, for some reason, re-initiate uncontrolled amplification during stable extrachromosomal maintenance stage.

3.2 Genomic instability of the host cell induced by the human papillomavirus replication machinery (II, III)

In latently infected cells, HPV genomes have to persist as episomal multicopy circular nuclear plasmids in order to support the viral life cycle. In neoplastic cervical tissues, however, integration of HR-HPV DNA has been reported. The trigger for HPV integration is unknown, but it has been demonstrated that it leads to the rapid loss of HPV plasmids through an intermediate step, in which the episomal and integrated HPV coexist in the same cells (Alazawi et al., 2002; Andersson et al., 2005; Arias-Pulido et al., 2006; Cooper et al., 1991; Kristiansen et al., 1994; Kulmala et al., 2006; Peitsaro et al., 2002a; Peitsaro et al., 2002b; Pett and Coleman, 2007; Pett et al., 2006). This raises the intriguing possibility that the E1 and E2 proteins expressed from the HPV plasmids could induce unscheduled replication from the integrated viral origin within the host chromosomal DNA.

By using HeLa and SiHa cells as model systems, we demonstrated that DNA replication could indeed be initiated from the integrated HPV origin when HPV E1 and E2 proteins are expressed (II, Fig. 1–5). We showed that high concentrations of HR-HPV E1 can produce a heterogeneous mixture of DNA fragments, which is referred to as the “onion skin”-type of replication mode. At the same time, the elevated E1 levels did not cause unspecific initiation of DNA replication, and the origin-specific replication initiation occurred at any E1 concentration (II, Fig. 2). Our data demonstrated that the DNA replication initiated from the HPV origin could extend into the flanking cellular sequences on either side to a distance of at least 12.6 kb from the HPV origin, under the experimental conditions used (II, Fig. 4). Since the transfection efficiency was approximately 40%, the level of amplification in the cell, as well as the replicon size is likely to be greater than the estimated values. Interestingly, we observed that even if the amplification of the integrated HPV16 origin was highly dependent on E1 concentration, the dependence of the replication of distal sequences on E1 concentration decreased as the distance from the replication origin increased. Therefore, the elongation of replication forks at considerable distances from the initiation site is determined presumably more by proper configuration of the template and the ability of the replication complex to function on it, and less by the level of E1. Subsequently, we evaluated the ability of HPV18 plasmid to prepare the integrated HPV18 origin for

unscheduled replication in HeLa cells at native E1 and E2 proteins levels. By extracting the transfected cells from the total population, we reproducibly succeeded in detecting replication of episomal HPV18 plasmid at a level of 8 copies per cell, together with simultaneous two fold amplification of the integrated HPV18 sequences (II, Fig. 5).

Next, we explored the possibility that the E1-driven virus-host DNA replication could become responsible for the genomic instability in HPV-infected cells. SiHa cells were co-transfected with HPV16 E1 and E2 expression plasmids and the stability of the integrated HPV16 locus was determined by restriction analyses of the subclones of transfected cells. The results demonstrated that the co-expression of E1 and E2 in SiHa cells resulted in a novel HPV16-specific restriction pattern in some of the transfected cells (II, Fig. 6), representing either an internal rearrangement or reintegration at a novel site. Subcloning of SiHa cells transfected with the HPV18 genome confirmed these results under physiological conditions (III, Fig. 8). More precise analysis suggested that *in situ* duplication of the HPV16 genome together with 3' cellular DNA had taken place in one of the subclones, in which DNA replication from integrated HPV had been initiated (II, Fig. 6). FISH analysis of metaphase cells revealed *de novo* cross-chromosomal translocation of the integrated HPV16 with the entire q-arm of chromosome 13 in another subclone (III, Fig. 7). As a result, there was a third 13q arm in the genome of the subclone. In control cells, which only expressed HPV16 E1, no changes were detected within the integrated HPV16 locus (II, Fig. 6).

Current study suggests that during the intermediate step of HPV integration, when both episomal and integrated HPV DNA are present in the same cells, the expression of E1 and E2 from the HPV plasmid could induce the amplification of integrated HR-HPV and flanking cellular DNA sequences. These amplified loci could serve as targets for repair/recombination machinery, resulting in genomic rearrangements, which were also observed in this study. The exact outcome of the rearrangements is difficult to predict, but it is reasonable to assume that modifications of the genome could affect any number of oncogenes, leading to the transformation of cells into cancer cells. The genomic changes may contribute to the transformation directly or indirectly by increasing the expression of viral oncogenes E6 and E7, as well as affecting the regulation of host genes nearby. Recently, it has been published that cellular regions at the MYC locus, encompassing HPV sequences, are amplified in several cell lines derived from tumors (Herrick et al., 2005). It was concluded that the amplification had already taken place in the primary tumors and the integration of viral DNA must have occurred prior to MYC gene amplification. Mapping the integration sites showed that the integrated HPV DNA sequences were located at the center of the amplicon (Peter et al., 2006a). In addition, cytogenetic characterization revealed the dispersion and co-amplification of c-MYC and HPV18 DNA in HeLa cells (Macville et al., 1999). These data suggest that MYC amplification occurred after (and was most likely triggered by) the viral insertion at a single integration site.

There is evidence from previous studies demonstrating the presence of different papillomavirus subtypes in the same tissue in clinical samples, suggesting that the frequency of co-infection may be quite high (Kulmala et al., 2006). Therefore, we can speculate that cells containing integrated HPV could also be targets for *de novo* infection of papillomaviruses, resulting in an intracellular mixture of episomal and integrated HPV. This could then lead to the amplification of integrated HPV DNA together with flanking cellular sequences. We can call this a "hit-and-run" mechanism since "low-risk" or "high-risk" HPV plasmids themselves could be lost quickly after the infection, but the damage done through chromosome-associated HR-HPV amplification remains. This is especially remarkable since LR-HPVs are considered to be harmless due to the lower oncogenic potential of their E6 and E7 proteins (Crook et al., 1991; Heck et al., 1992; Oh et al., 2004). However, we have demonstrated their ability to initiate DNA replication from the integrated HR-HPV origin (II, Fig 3).

In addition to the amplification of regulatory sequences or genes driving the cell cycle, there is also the possibility of deletion of genomic sequences. The SiHa cell line itself can be seen as an example of this, since the 5' and 3' cellular-viral junction sites of HPV16 integration (el Awady et al., 1987) are ~300 kb apart within the intact chromosome 13 according to the 36th assembly of the human genome (NCBI, released in November 2005). This deletion could have been due to the integration of HPV16.

Based on the data presented, it can be concluded that papillomavirus replication machinery could be active in changing cell genomic make-up, and it sets the stage for the induction of genomic instability that could contribute considerably to the oncogenic transformation of HPV-infected cells.

3.3 Mechanisms of Genomic Instability in Cells Infected with High Risk Human Papillomaviruses (III, IV)

Subsequently, we clarified the mechanisms by which DNA re-replication of integrated HPV caused internal rearrangements and inter-chromosomal translocations at the HPV integration locus. First, we demonstrated that linear fragments, branched and open circular molecules, as well as covalently closed circular plasmids of heterogeneous size were produced as a result of the "onion skin"-type of replication mode of integrated HPV (III, Fig. 1). Since the HPV origin-containing plasmids, which arise from integrated HPV, are the potential templates for E1 and E2-driven replication, we can speculate that this might be a mechanism for gene amplification. Viral oncogenes E6 and E7 could be primarily amplified because they are located next to the viral origin and their expression is regulated by promoters located in the URR.

We continued by developing a single cell-based immunofluorescence assay to analyze the cellular response to integrated HPV replication (III, Fig. 2 and Fig. 3). We demonstrated that the DSBs are generated by re-replication of the

HPV locus and recognized by the initiating factors of NHEJ and HR (III, Fig. 4). Our data suggest that, although HR is the primary DNA repair mechanism in the S-phase, the slow HR pathway could become saturated by the generation of abundant DSBs during the replication of integrated HPV, and the rest of the DSBs are repaired by NHEJ. The NHEJ machinery plays a significant role in maintaining genome stability and suppressing tumorigenesis (Ferguson et al., 2000; Karanjawala et al., 1999; Sharpless et al., 2001), but it is also responsible for the vast majority of tumorigenic chromosomal translocations. Even the "correct" re-joining of broken ends by NHEJ often results in mutations at junction sites (Zhang and Rowley, 2006). Therefore, NHEJ might contribute to the development of genetic instability found in HPV-associated cancer. NHEJ might even be responsible for generating the tandem repeats of the integrated HPV and neighboring cellular sequences commonly found in HPV-associated cancers. The HPV E6 and E7 oncoproteins can decrease the fidelity of DSB repair further by interacting with and disrupting the functions of the p53 and pRB pathways (Boyer et al., 1996; Scheffner et al., 1990; Shin et al., 2006).

The re-replication of the cellular DNA is prevented in cells containing the intact ATR pathway. Despite this, HPV E1- and E2-driven pre-RC assembly, DNA unwinding by E1, and the expression of E6 and E7 oncogenes gives HPV the ability to replicate its 8 kb DNA genome multiple times in a single cell cycle during normal viral infection. Current study demonstrated that although ATR-interacting protein (ATRIP) was located at the sites of integrated HPV replication (III, Fig. 5), HPV re-replication was not prevented even when the HPV origin was covalently linked with the host cell chromosome. As a result, we observed the clear localization of ATM and its main downstream effector kinase, Chk2, into the replication sites of the integrated HPV (III, Fig. 5). In addition, clear phosphorylation of Chk2 kinase was detected in the cell population where the replication of integrated HPV took place (III, Fig. 6). The ATM pathway is thought to be involved when the prevention of DNA re-replication fails and DSBs are produced. There are several possible explanations as to why the ATR pathway does not work properly if DNA re-replication is initiated from the integrated HPV origin. First, it is possible that the weak localization of ATRIP and Chk1 (S317) into the sites of integrated HPV DNA was not caused by the uncoupling of the viral helicase and cellular polymerases at the properly extending replication fork; rather, it was caused by the availability of the RPA-coated ssDNA at the sites of fork collision and dissociation (III, Fig. 9). However, even if the ATR and ATRIP proteins recognize the sites of integrated HPV replication, the potential to inhibit HPV replication might still be limited as there are only a few targets available for ATR, compared with the complex initiation mechanisms of cellular DNA replication. Phosphorylation of E1 has been extensively studied and the ability of the ATR to phosphorylate HPV E1 protein has not been determined (Lentz et al., 2006). In addition, the ATR indirect signaling pathways through p53 and pRB might be cut off by HPV E6 and E7 oncoproteins in cells harboring the "high risk" HPV.

Supported by the observation that Cdt1 is overexpressed in human cancer cells lacking the biological functions of p53 or pRB proteins, it has been suggested that DNA re-replication can lead to chromosomal instability and malignant transformation (Karakaidos et al., 2004; Liu et al., 2007; Tatsumi et al., 2006; Vaziri et al., 2003). However, except for the formation of short linear dsDNA molecules (Davidson et al., 2006), there is no evidence from previous studies of cellular DNA replication to support this hypothesis. Although, chromosomal excision and the formation of heterogeneous pools of circular molecules, as well as reintegration have been detected when DNA re-replication occurs in cells with integrated polyomavirus in the presence of large T antigen (Botchan et al., 1979; Bullock et al., 1984; Hanahan et al., 1980; Syu and Fluck, 1997). The current study demonstrates for the first time by metaphase FISH analysis that DNA re-replication can lead to chromosomal instability (III, Fig. 7). Although we mostly used high levels of E1 expression in our experiments, we have also demonstrated that the presence of the HPV episomal genome could induce rearrangements in the genomic content of SiHa cells (III, Fig. 8). Furthermore, in W12 cells, Dall et al. recently demonstrated that local rearrangements occur frequently and shortly after natural HPV16 integration, during the phase when episomal and integrated viral genomes are present in the same cell (Dall et al., 2008). Similar translocations of the viral-host DNA have also been detected in several cell lines derived from invasive genital carcinomas with native expression levels of the viral proteins (Brink et al., 2002; Couturier et al., 1991; Peter et al., 2006a). These observations indicate that regardless of the expression level, E1 and E2-driven DNA replication lacks the mechanism to restrict it to once per cell cycle and, therefore, results in re-replication and chromosomal instability (IV, Fig. 2).

4. CONCLUSIONS

1. The transient amplifying replication of a BPV1 URR reporter plasmid can take place in cells that already maintain stable extrachromosomal copies of similar reporter plasmids. At the same time, the copy number of the stably maintained plasmid is not affected. The stable maintenance replication of the BPV1 URR reporter plasmid, unlike its transient amplifying replication, is not sensitive to p53 expression in the same cells. These data suggest that the initial amplifying and subsequent long-term stable maintenance replication of BPV1 may use two separable mechanisms. It also indicates that PV DNA replication has the potential to avoid the negative effect of cellular control mechanisms, which might be important for the successful establishment of the stable extrachromosomal maintenance.
2. The integrated HR-HPV origin is effectively mobilized for replication by the HPV E1 and E2 proteins produced from the respective expression vectors, and more importantly, from episomal HPV genomes transiently replicating in the same cells. Current study demonstrates that E1 and E2-dependent initiation of DNA replication from the integrated HPV origin follows the "onion skin"-type replication mode, leading to the generation of a heterogeneous population of replication intermediates, including supercoiled plasmids containing the integrated HPV and flanking cellular sequences. These data provide experimental proof for the hypothesis that similar amplification of the integrated HR-HPV sequences could occur in HPV-infected cells in LSIL and HSIL if episomal HPV genomes producing viral replication proteins are present.
3. We show by immunofluorescence analysis that the replication of integrated HPV takes place in the DNA repair/recombination centers, which incorporate viral and cellular replication proteins, the MRE complex, Ku70/80, ATM, ATRIP, and Chk2. Activation of the ATM-Chk2 pathway was also confirmed by the IP-Western blot analyses. These data show the active processing of replication intermediates by cellular DNA repair/recombination machinery, by which the most of the irregularities are fixed. But in some cases, DNA rearrangements within the HPV integration site are generated. Clonal analysis of the cells in which replication from the integrated HPV origin was induced demonstrated the duplication of the HPV-containing and flanking cellular sequences, as well as *de novo* cross-chromosomal translocation. These data suggest that the papillomavirus replication machinery and the cellular DNA repair complexes might be responsible for inducing the genomic changes that contribute to the formation of the HPV-associated cancer cells.

REFERENCES

Alazawi, W., Pett, M., Arch, B., Scott, L., Freeman, T., Stanley, M.A. and Coleman, N. (2002) Changes in cervical keratinocyte gene expression associated with integration of human papillomavirus 16. *Cancer Res*, **62**, 6959–6965.

Andersson, S., Safari, H., Mints, M., Lewensohn-Fuchs, I., Gyllensten, U. and Johansson, B. (2005) Type distribution, viral load and integration status of high-risk human papillomaviruses in pre-stages of cervical cancer (CIN). *Br J Cancer*, **92**, 2195–2200.

Arias-Pulido, H., Peyton, C.L., Joste, N.E., Vargas, H. and Wheeler, C.M. (2006) Human papillomavirus type 16 integration in cervical carcinoma in situ and in invasive cervical cancer. *J Clin Microbiol*, **44**, 1755–1762.

Auborn, K.J., Little, R.D., Platt, T.H., Vaccariello, M.A. and Schildkraut, C.L. (1994) Replicative intermediates of human papillomavirus type 11 in laryngeal papillomas: site of replication initiation and direction of replication. *Proc Natl Acad Sci U S A*, **91**, 7340–7344.

Baker, C.C., Phelps, W.C., Lindgren, V., Braun, M.J., Gonda, M.A. and Howley, P.M. (1987) Structural and transcriptional analysis of human papillomavirus type 16 sequences in cervical carcinoma cell lines. *J Virol*, **61**, 962–971.

Belanger, K.G., Mirzayan, C., Kreuzer, H.E., Alberts, B.M. and Kreuzer, K.N. (1996) Two-dimensional gel analysis of rolling circle replication in the presence and absence of bacteriophage T4 primase. *Nucleic Acids Res*, **24**, 2166–2175.

Berg, M. and Stenlund, A. (1997) Functional interactions between papillomavirus E1 and E2 proteins. *J Virol*, **71**, 3853–3863.

Blow, J.J. and Dutta, A. (2005) Preventing re-replication of chromosomal DNA. *Nat Rev Mol Cell Biol*, **6**, 476–486.

Bonne-Andrea, C., Tillier, F., McShan, G.D., Wilson, V.G. and Clertant, P. (1997) Bovine papillomavirus type 1 DNA replication: the transcriptional activator E2 acts in vitro as a specificity factor. *J Virol*, **71**, 6805–6815.

Botchan, M., Topp, W. and Sambrook, J. (1979) Studies on simian virus 40 excision from cellular chromosomes. *Cold Spring Harb Symp Quant Biol*, **43 Pt 2**, 709–719.

Boyer, S.N., Wazer, D.E. and Band, V. (1996) E7 protein of human papilloma virus–16 induces degradation of retinoblastoma protein through the ubiquitin-proteasome pathway. *Cancer Res*, **56**, 4620–4624.

Brink, A.A., Wiegant, J.C., Szuhai, K., Tanke, H.J., Kenter, G.G., Fleuren, G.J., Schuuring, E. and Raap, A.K. (2002) Simultaneous mapping of human papillomavirus integration sites and molecular karyotyping in short-term cultures of cervical carcinomas by using 49-color combined binary ratio labeling fluorescence in situ hybridization. *Cancer Genet Cytogenet*, **134**, 145–150.

Bullock, P., Forrester, W. and Botchan, M. (1984) DNA sequence studies of simian virus 40 chromosomal excision and integration in rat cells. *J Mol Biol*, **174**, 55–84.

Burd, E.M. (2003) Human papillomavirus and cervical cancer. *Clin Microbiol Rev*, **16**, 1–17.

Chan, S.Y., Delius, H., Halpern, A.L. and Bernard, H.U. (1995) Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *J Virol*, **69**, 3074–3083.

Chaudhuri, B., Xu, H., Todorov, I., Dutta, A. and Yates, J.L. (2001) Human DNA replication initiation factors, ORC and MCM, associate with oriP of Epstein-Barr virus. *Proc Natl Acad Sci U S A*, **98**, 10085–10089.

Chiang, C.M., Ustav, M., Stenlund, A., Ho, T.F., Broker, T.R. and Chow, L.T. (1992) Viral E1 and E2 proteins support replication of homologous and heterologous papillomaviral origins. *Proc Natl Acad Sci U S A*, **89**, 5799–5803.

Cook, J.G., Chasse, D.A. and Nevins, J.R. (2004) The regulated association of Cdt1 with minichromosome maintenance proteins and Cdc6 in mammalian cells. *J Biol Chem*, **279**, 9625–9633.

Cooper, K., Herrington, C.S., Stickland, J.E., Evans, M.F. and McGee, J.O. (1991) Episomal and integrated human papillomavirus in cervical neoplasia shown by non-isotopic in situ hybridisation. *J Clin Pathol*, **44**, 990–996.

Corden, S.A., Sant-Cassia, L.J., Easton, A.J. and Morris, A.G. (1999) The integration of HPV–18 DNA in cervical carcinoma. *Mol Pathol*, **52**, 275–282.

Cortez, D., Glick, G. and Elledge, S.J. (2004) Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases. *Proc Natl Acad Sci U S A*, **101**, 10078–10083.

Couturier, J., Sastre-Garau, X., Schneider-Maunoury, S., Labib, A. and Orth, G. (1991) Integration of papillomavirus DNA near myc genes in genital carcinomas and its consequences for proto-oncogene expression. *J Virol*, **65**, 4534–4538.

Crook, T., Tidy, J.A. and Vousden, K.H. (1991) Degradation of p53 can be targeted by HPV E6 sequences distinct from those required for p53 binding and trans-activation. *Cell*, **67**, 547–556.

Dall, K.L., Scarpini, C.G., Roberts, I., Winder, D.M., Stanley, M.A., Muralidhar, B., Herdman, M.T., Pett, M.R. and Coleman, N. (2008) Characterization of naturally occurring HPV16 integration sites isolated from cervical keratinocytes under noncompetitive conditions. *Cancer Res*, **68**, 8249–8259.

Dasgupta, S., Zabielski, J., Simonsson, M. and Burnett, S. (1992) Rolling-circle replication of a high-copy BPV–1 plasmid. *J Mol Biol*, **228**, 1–6.

Davidson, I.F., Li, A. and Blow, J.J. (2006) Deregulated replication licensing causes DNA fragmentation consistent with head-to-tail fork collision. *Mol Cell*, **24**, 433–443.

Day, P.M., Lowy, D.R. and Schiller, J.T. (2003) Papillomaviruses infect cells via a clathrin-dependent pathway. *Virology*, **307**, 1–11.

de Sanjose, S., Diaz, M., Castellsague, X., Clifford, G., Bruni, L., Munoz, N. and Bosch, F.X. (2007) Worldwide prevalence and genotype distribution of cervical human papillomavirus DNA in women with normal cytology: a meta-analysis. *Lancet Infect Dis*, **7**, 453–459.

de Villiers, E.M., Fauquet, C., Broker, T.R., Bernard, H.U. and zur Hausen, H. (2004) Classification of papillomaviruses. *Virology*, **324**, 17–27.

Demeret, C., Yaniv, M. and Thierry, F. (1994) The E2 transcriptional repressor can compensate for Sp1 activation of the human papillomavirus type 18 early promoter. *J Virol*, **68**, 7075–7082.

Deng, W., Lin, B.Y., Jin, G., Wheeler, C.G., Ma, T., Harper, J.W., Broker, T.R. and Chow, L.T. (2004) Cyclin/CDK regulates the nucleocytoplasmic localization of the human papillomavirus E1 DNA helicase. *J Virol*, **78**, 13954–13965.

DePamphilis, M.L. (2005) Cell cycle dependent regulation of the origin recognition complex. *Cell Cycle*, **4**, 70–79.

Dhar, S.K., Yoshida, K., Machida, Y., Khaira, P., Chaudhuri, B., Wohlschlegel, J.A., Leffak, M., Yates, J. and Dutta, A. (2001) Replication from oriP of Epstein-Barr virus requires human ORC and is inhibited by geminin. *Cell*, **106**, 287–296.

DiMaio, D. and Mattoon, D. (2001) Mechanisms of cell transformation by papillomavirus E5 proteins. *Oncogene*, **20**, 7866–7873.

Doorbar, J., Ely, S., Sterling, J., McLean, C. and Crawford, L. (1991) Specific interaction between HPV–16 E1-E4 and cytokeratins results in collapse of the epithelial cell intermediate filament network. *Nature*, **352**, 824–827.

Duensing, S. and Munger, K. (2002) The human papillomavirus type 16 E6 and E7 oncoproteins independently induce numerical and structural chromosome instability. *Cancer Res*, **62**, 7075–7082.

Duensing, S. and Munger, K. (2004) Mechanisms of genomic instability in human cancer: insights from studies with human papillomavirus oncoproteins. *Int J Cancer*, **109**, 157–162.

Dyson, N., Howley, P.M., Munger, K. and Harlow, E. (1989) The human papilloma virus–16 E7 oncoprotein is able to bind to the retinoblastoma gene product. *Science*, **243**, 934–937.

el Awady, M.K., Kaplan, J.B., O'Brien, S.J. and Burk, R.D. (1987) Molecular analysis of integrated human papillomavirus 16 sequences in the cervical cancer cell line SiHa. *Virology*, **159**, 389–398.

Ferber, M.J., Thorland, E.C., Brink, A.A., Rapp, A.K., Phillips, L.A., McGovern, R., Gostout, B.S., Cheung, T.H., Chung, T.K., Fu, W.Y. and Smith, D.I. (2003) Preferential integration of human papillomavirus type 18 near the c-myc locus in cervical carcinoma. *Oncogene*, **22**, 7233–7242.

Ferguson, D.O., Sekiguchi, J.M., Chang, S., Frank, K.M., Gao, Y., DePinho, R.A. and Alt, F.W. (2000) The nonhomologous end-joining pathway of DNA repair is required for genomic stability and the suppression of translocations. *Proc Natl Acad Sci U S A*, **97**, 6630–6633.

Flores, E.R. and Lambert, P.F. (1997) Evidence for a switch in the mode of human papillomavirus type 16 DNA replication during the viral life cycle. *J Virol*, **71**, 7167–7179.

Fuchs, E. (2008) Skin stem cells: rising to the surface. *J Cell Biol*, **180**, 273–284.

Fujita, M. (2006) Cdt1 revisited: complex and tight regulation during the cell cycle and consequences of deregulation in mammalian cells. *Cell Div*, **1**, 22.

Garner-Hamrick, P.A., Fostel, J.M., Chien, W.M., Banerjee, N.S., Chow, L.T., Broker, T.R. and Fisher, C. (2004) Global effects of human papillomavirus type 18 E6/E7 in an organotypic keratinocyte culture system. *J Virol*, **78**, 9041–9050.

Genther Williams, S.M., Disbrow, G.L., Schlegel, R., Lee, D., Threadgill, D.W. and Lambert, P.F. (2005) Requirement of epidermal growth factor receptor for hyperplasia induced by E5, a high-risk human papillomavirus oncogene. *Cancer Res*, **65**, 6534–6542.

Gillison, M.L., Koch, W.M., Capone, R.B., Spafford, M., Westra, W.H., Wu, L., Zahurak, M.L., Daniel, R.W., Viglione, M., Symer, D.E., Shah, K.V. and Sidransky, D. (2000) Evidence for a causal association between human papillomavirus and a subset of head and neck cancers. *J Natl Cancer Inst*, **92**, 709–720.

Giroglou, T., Florin, L., Schafer, F., Streeck, R.E. and Sapp, M. (2001) Human papillomavirus infection requires cell surface heparan sulfate. *J Virol*, **75**, 1565–1570.

Grassmann, K., Rapp, B., Maschek, H., Petry, K.U. and Iftner, T. (1996) Identification of a differentiation-inducible promoter in the E7 open reading frame of human papillomavirus type 16 (HPV–16) in raft cultures of a new cell line containing high copy numbers of episomal HPV–16 DNA. *J Virol*, **70**, 2339–2349.

Hanahan, D., Lane, D., Lipsich, L., Wigler, M. and Botchan, M. (1980) Characteristics of an SV40-plasmid recombinant and its movement into and out of the genome of a murine cell. *Cell*, **21**, 127–139.

Heck, D.V., Yee, C.L., Howley, P.M. and Munger, K. (1992) Efficiency of binding the retinoblastoma protein correlates with the transforming capacity of the E7 oncoproteins of the human papillomaviruses. *Proc Natl Acad Sci U S A*, **89**, 4442–4446.

Herrick, J., Conti, C., Teissier, S., Thierry, F., Couturier, J., Sastre-Garau, X., Favre, M., Orth, G. and Bensimon, A. (2005) Genomic organization of amplified MYC genes suggests distinct mechanisms of amplification in tumorigenesis. *Cancer Res*, **65**, 1174–1179.

Hoffmann, R., Hirt, B., Bechtold, V., Beard, P. and Raj, K. (2006) Different modes of human papillomavirus DNA replication during maintenance. *J Virol*, **80**, 4431–4439.

Hook, S.S., Lin, J.J. and Dutta, A. (2007) Mechanisms to control rereplication and implications for cancer. *Curr Opin Cell Biol*, **19**, 663–671.

Howley, P.M., Lowy, D.R. (2001) *Papillomaviruses and Their Replication.* Lippincott Williams & Wilkins, Philadelphia.

Hsu, C.Y., Mechali, F. and Bonne-Andrea, C. (2007) Nucleocytoplasmic shuttling of bovine papillomavirus E1 helicase downregulates viral DNA replication in S phase. *J Virol*, **81**, 384–394.

Hwang, S.G., Lee, D., Kim, J., Seo, T. and Choe, J. (2002) Human papillomavirus type 16 E7 binds to E2F1 and activates E2F1-driven transcription in a retinoblastoma protein-independent manner. *J Biol Chem*, **277**, 2923–2930.

Iftner, T., Elbel, M., Schopp, B., Hiller, T., Loizou, J.I., Caldecott, K.W. and Stubenrauch, F. (2002) Interference of papillomavirus E6 protein with single-strand break repair by interaction with XRCC1. *Embo J*, **21**, 4741–4748.

Ilves, I., Kivi, S. and Ustav, M. (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.

Jackson, S., Harwood, C., Thomas, M., Banks, L. and Storey, A. (2000) Role of Bak in UV-induced apoptosis in skin cancer and abrogation by HPV E6 proteins. *Genes Dev*, **14**, 3065–3073.

Jeon, S., Allen-Hoffmann, B.L. and Lambert, P.F. (1995) Integration of human papillomavirus type 16 into the human genome correlates with a selective growth advantage of cells. *J Virol*, **69**, 2989–2997.

Jiang, W., McDonald, D., Hope, T.J. and Hunter, T. (1999a) Mammalian Cdc7-Dbf4 protein kinase complex is essential for initiation of DNA replication. *Embo J*, **18**, 5703–5713.

Jiang, W., Wells, N.J. and Hunter, T. (1999b) Multistep regulation of DNA replication by Cdk phosphorylation of HsCdc6. *Proc Natl Acad Sci U S A*, **96**, 6193–6198.

Kalantari, M., Blennow, E., Hagmar, B. and Johansson, B. (2001) Physical state of HPV16 and chromosomal mapping of the integrated form in cervical carcinomas. *Diagn Mol Pathol*, **10**, 46–54.

Karakaidos, P., Taraviras, S., Vassiliou, L.V., Zacharatos, P., Kastrinakis, N.G., Kougiou, D., Kouloukoussa, M., Nishitani, H., Papavassiliou, A.G., Lygerou, Z. and Gorgoulis, V.G. (2004) Overexpression of the replication licensing regulators hCdt1 and hCdc6 characterizes a subset of non-small-cell lung carcinomas: synergistic effect with mutant p53 on tumor growth and chromosomal instability--

evidence of E2F–1 transcriptional control over hCdt1. *Am J Pathol*, **165**, 1351–1365.
Karanjawala, Z.E., Grawunder, U., Hsieh, C.L. and Lieber, M.R. (1999) The nonhomologous DNA end joining pathway is important for chromosome stability in primary fibroblasts. *Curr Biol*, **9**, 1501–1504.
Kim, K. and Lambert, P.F. (2002) E1 protein of bovine papillomavirus 1 is not required for the maintenance of viral plasmid DNA replication. *Virology*, **293**, 10–14.
Klingelhutz, A.J., Foster, S.A. and McDougall, J.K. (1996) Telomerase activation by the E6 gene product of human papillomavirus type 16. *Nature*, **380**, 79–82.
Klumpp, D.J. and Laimins, L.A. (1999) Differentiation-induced changes in promoter usage for transcripts encoding the human papillomavirus type 31 replication protein E1. *Virology*, **257**, 239–246.
Kristiansen, E., Jenkins, A. and Holm, R. (1994) Coexistence of episomal and integrated HPV16 DNA in squamous cell carcinoma of the cervix. *J Clin Pathol*, **47**, 253–256.
Kulmala, S.M., Syrjanen, S.M., Gyllensten, U.B., Shabalova, I.P., Petrovichev, N., Tosi, P., Syrjanen, K.J. and Johansson, B.C. (2006) Early integration of high copy HPV16 detectable in women with normal and low grade cervical cytology and histology. *J Clin Pathol*, **59**, 513–517.
Labib, K. and Gambus, A. (2007) A key role for the GINS complex at DNA replication forks. *Trends Cell Biol*, **17**, 271–278.
Lazo, P.A., DiPaolo, J.A. and Popescu, N.C. (1989) Amplification of the integrated viral transforming genes of human papillomavirus 18 and its 5'-flanking cellular sequence located near the myc protooncogene in HeLa cells. *Cancer Res*, **49**, 4305–4310.
Lavin, M.F. (2007) ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. *Oncogene*, **26**, 7749–7758.
Lehman, C.W. and Botchan, M.R. (1998) Segregation of viral plasmids depends on tethering to chromosomes and is regulated by phosphorylation. *Proc Natl Acad Sci U S A*, **95**, 4338–4343.
Lentz, M.R., Stevens, S.M., Jr., Raynes, J. and Elkhoury, N. (2006) A phosphorylation map of the bovine papillomavirus E1 helicase. *Virol J*, **3**, 13.
Lepik, D., Ilves, I., Kristjuhan, A., Maimets, T. and Ustav, M. (1998) p53 protein is a suppressor of papillomavirus DNA amplificational replication. *J Virol*, **72**, 6822–6831.
Lim, D.A., Gossen, M., Lehman, C.W. and Botchan, M.R. (1998) Competition for DNA binding sites between the short and long forms of E2 dimers underlies repression in bovine papillomavirus type 1 DNA replication control. *J Virol*, **72**, 1931–1940.
Lin, J.J. and Dutta, A. (2007) ATR pathway is the primary pathway for activating G2/M checkpoint induction after re-replication. *J Biol Chem*, **282**, 30357–30362.
Liu, E., Lee, A.Y., Chiba, T., Olson, E., Sun, P. and Wu, X. (2007) The ATR-mediated S phase checkpoint prevents rereplication in mammalian cells when licensing control is disrupted. *J Cell Biol*, **179**, 643–657.
Luft, F., Klaes, R., Nees, M., Durst, M., Heilmann, V., Melsheimer, P. and von Knebel Doeberitz, M. (2001) Detection of integrated papillomavirus sequences by ligation-mediated PCR (DIPS-PCR) and molecular characterization in cervical cancer cells. *Int J Cancer*, **92**, 9–17.
Machida, Y.J., Hamlin, J.L. and Dutta, A. (2005) Right place, right time, and only once: replication initiation in metazoans. *Cell*, **123**, 13–24.

Macville, M., Schrock, E., Padilla-Nash, H., Keck, C., Ghadimi, B.M., Zimonjic, D., Popescu, N. and Ried, T. (1999) Comprehensive and definitive molecular cytogenetic characterization of HeLa cells by spectral karyotyping. *Cancer Res*, **59**, 141–150.

Mailand, N. and Diffley, J.F. (2005) CDKs promote DNA replication origin licensing in human cells by protecting Cdc6 from APC/C-dependent proteolysis. *Cell*, **122**, 915–926.

Masai, H., Taniyama, C., Ogino, K., Matsui, E., Kakusho, N., Matsumoto, S., Kim, J.M., Ishii, A., Tanaka, T., Kobayashi, T., Tamai, K., Ohtani, K. and Arai, K. (2006) Phosphorylation of MCM4 by Cdc7 kinase facilitates its interaction with Cdc45 on the chromatin. *J Biol Chem*, **281**, 39249–39261.

McBride, A.A., Romanczuk, H. and Howley, P.M. (1991) The papillomavirus E2 regulatory proteins. *J Biol Chem*, **266**, 18411–18414.

McGarry, T.J. and Kirschner, M.W. (1998) Geminin, an inhibitor of DNA replication, is degraded during mitosis. *Cell*, **93**, 1043–1053.

McIntyre, M.C., Ruesch, M.N. and Laimins, L.A. (1996) Human papillomavirus E7 oncoproteins bind a single form of cyclin E in a complex with cdk2 and p107. *Virology*, **215**, 73–82.

Melendy, T., Sedman, J. and Stenlund, A. (1995) Cellular factors required for papillomavirus DNA replication. *J Virol*, **69**, 7857–7867.

Melixetian, M. and Helin, K. (2004) Geminin: a major DNA replication safeguard in higher eukaryotes. *Cell Cycle*, **3**, 1002–1004.

Mendez, J. and Stillman, B. (2003) Perpetuating the double helix: molecular machines at eukaryotic DNA replication origins. *Bioessays*, **25**, 1158–1167.

Mendez, J., Zou-Yang, X.H., Kim, S.Y., Hidaka, M., Tansey, W.P. and Stillman, B. (2002) Human origin recognition complex large subunit is degraded by ubiquitin-mediated proteolysis after initiation of DNA replication. *Mol Cell*, **9**, 481–491.

Meyers, C., Mayer, T.J. and Ozbun, M.A. (1997) Synthesis of infectious human papillomavirus type 18 in differentiating epithelium transfected with viral DNA. *J Virol*, **71**, 7381–7386.

Munger, K., Baldwin, A., Edwards, K.M., Hayakawa, H., Nguyen, C.L., Owens, M., Grace, M. and Huh, K. (2004) Mechanisms of human papillomavirus-induced oncogenesis. *J Virol*, **78**, 11451–11460.

Nakahara, T., Nishimura, A., Tanaka, M., Ueno, T., Ishimoto, A. and Sakai, H. (2002) Modulation of the cell division cycle by human papillomavirus type 18 E4. *J Virol*, **76**, 10914–10920.

Oh, S.T., Longworth, M.S. and Laimins, L.A. (2004) Roles of the E6 and E7 proteins in the life cycle of low-risk human papillomavirus type 11. *J Virol*, **78**, 2620–2626.

Oldak, M., Smola-Hess, S. and Maksym, R. (2006) Integrin beta4, keratinocytes and papillomavirus infection. *Int J Mol Med*, **17**, 195–202.

Ozlu, T. and Bulbul, Y. (2005) Smoking and lung cancer. *Tuberk Toraks*, **53**, 200–209.

Palefsky, J.M., Winkler, B., Rabanus, J.P., Clark, C., Chan, S., Nizet, V. and Schoolnik, G.K. (1991) Characterization of in vivo expression of the human papillomavirus type 16 E4 protein in cervical biopsy tissues. *J Clin Invest*, **87**, 2132–2141.

Parkin, D.M. (2006) The global health burden of infection-associated cancers in the year 2002. *Int J Cancer*, **118**, 3030–3044.

Paz, I.B., Cook, N., Odom-Maryon, T., Xie, Y. and Wilczynski, S.P. (1997) Human papillomavirus (HPV) in head and neck cancer. An association of HPV 16 with squamous cell carcinoma of Waldeyer's tonsillar ring. *Cancer*, **79**, 595–604.

Paulsen, R.D. and Cimprich, K.A. (2007) The ATR pathway: fine-tuning the fork. *DNA Repair (Amst)*, **6**, 953–966.

Peitsaro, P., Hietanen, S., Johansson, B., Lakkala, T. and Syrjanen, S. (2002a) Single copy heterozygote integration of HPV 33 in chromosomal band 5p14 is found in an epithelial cell clone with selective growth advantage. *Carcinogenesis*, **23**, 1057–1064.

Peitsaro, P., Johansson, B. and Syrjanen, S. (2002b) Integrated human papillomavirus type 16 is frequently found in cervical cancer precursors as demonstrated by a novel quantitative real-time PCR technique. *J Clin Microbiol*, **40**, 886–891.

Peter, M., Rosty, C., Couturier, J., Radvanyi, F., Teshima, H. and Sastre-Garau, X. (2006a) MYC activation associated with the integration of HPV DNA at the MYC locus in genital tumors. *Oncogene*, **25**, 5985–5993.

Petersen, B.O., Lukas, J., Sorensen, C.S., Bartek, J. and Helin, K. (1999) Phosphorylation of mammalian CDC6 by cyclin A/CDK2 regulates its subcellular localization. *Embo J*, **18**, 396–410.

Pett, M. and Coleman, N. (2007) Integration of high-risk human papillomavirus: a key event in cervical carcinogenesis? *J Pathol*, **212**, 356–367.

Pett, M.R., Alazawi, W.O., Roberts, I., Dowen, S., Smith, D.I., Stanley, M.A. and Coleman, N. (2004) Acquisition of high-level chromosomal instability is associated with integration of human papillomavirus type 16 in cervical keratinocytes. *Cancer Res*, **64**, 1359–1368.

Pett, M.R., Herdman, M.T., Palmer, R.D., Yeo, G.S., Shivji, M.K., Stanley, M.A. and Coleman, N. (2006) Selection of cervical keratinocytes containing integrated HPV16 associates with episome loss and an endogenous antiviral response. *Proc Natl Acad Sci U S A*, **103**, 3822–3827.

Piirsoo, M., Ustav, E., Mandel, T., Stenlund, A. and Ustav, M. (1996) Cis and trans requirements for stable episomal maintenance of the BPV–1 replicator. *Embo J*, **15**, 1–11.

Ravnan, J.B., Gilbert, D.M., Ten Hagen, K.G. and Cohen, S.N. (1992) Random-choice replication of extrachromosomal bovine papillomavirus (BPV) molecules in heterogeneous, clonally derived BPV-infected cell lines. *J Virol*, **66**, 6946–6952.

Remm, M., Brain, R. and Jenkins, J.R. (1992) The E2 binding sites determine the efficiency of replication for the origin of human papillomavirus type 18. *Nucleic Acids Res*, **20**, 6015–6021.

Rialland, M., Sola, F. and Santocanale, C. (2002) Essential role of human CDT1 in DNA replication and chromatin licensing. *J Cell Sci*, **115**, 1435–1440.

Romanczuk, H. and Howley, P.M. (1992) Disruption of either the E1 or the E2 regulatory gene of human papillomavirus type 16 increases viral immortalization capacity. *Proc Natl Acad Sci U S A*, **89**, 3159–3163.

Romanczuk, H., Thierry, F. and Howley, P.M. (1990) Mutational analysis of cis elements involved in E2 modulation of human papillomavirus type 16 P97 and type 18 P105 promoters. *J Virol*, **64**, 2849–2859.

Ruesch, M.N. and Laimins, L.A. (1998) Human papillomavirus oncoproteins alter differentiation-dependent cell cycle exit on suspension in semisolid medium. *Virology*, **250**, 19–29.

Ruesch, M.N., Stubenrauch, F. and Laimins, L.A. (1998) Activation of papillomavirus late gene transcription and genome amplification upon differentiation in semisolid medium is coincident with expression of involucrin and transglutaminase but not keratin–10. *J Virol*, **72**, 5016–5024.

Russell, J. and Botchan, M.R. (1995) cis-Acting components of human papillomavirus (HPV) DNA replication: linker substitution analysis of the HPV type 11 origin. *J Virol*, **69**, 651–660.

Scheffner, M., Werness, B.A., Huibregtse, J.M., Levine, A.J. and Howley, P.M. (1990) The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. *Cell*, **63**, 1129–1136.

Schvartzman, J.B., Adolph, S., Martin-Parras, L. and Schildkraut, C.L. (1990) Evidence that replication initiates at only some of the potential origins in each oligomeric form of bovine papillomavirus type 1 DNA. *Mol Cell Biol*, **10**, 3078–3086.

Sedman, J. and Stenlund, A. (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. *Embo J*, **14**, 6218–6228.

Sedman, J. and Stenlund, A. (1998) The papillomavirus E1 protein forms a DNA-dependent hexameric complex with ATPase and DNA helicase activities. *J Virol*, **72**, 6893–6897.

Sharpless, N.E., Ferguson, D.O., O'Hagan, R.C., Castrillon, D.H., Lee, C., Farazi, P.A., Alson, S., Fleming, J., Morton, C.C., Frank, K., Chin, L., Alt, F.W. and DePinho, R.A. (2001) Impaired nonhomologous end-joining provokes soft tissue sarcomas harboring chromosomal translocations, amplifications, and deletions. *Mol Cell*, **8**, 1187–1196.

Shin, K.H., Ahn, J.H., Kang, M.K., Lim, P.K., Yip, F.K., Baluda, M.A. and Park, N.H. (2006) HPV–16 E6 oncoprotein impairs the fidelity of DNA end-joining via p53-dependent and -independent pathways. *Int J Oncol*, **28**, 209–215.

Shrivastav, M., De Haro, L.P. and Nickoloff, J.A. (2008) Regulation of DNA double-strand break repair pathway choice. *Cell Res*, **18**, 134–147.

Skiadopoulos, M.H. and McBride, A.A. (1998) Bovine papillomavirus type 1 genomes and the E2 transactivator protein are closely associated with mitotic chromatin. *J Virol*, **72**, 2079–2088.

Sonoda, E., Hochegger, H., Saberi, A., Taniguchi, Y. and Takeda, S. (2006) Differential usage of non-homologous end-joining and homologous recombination in double strand break repair. *DNA Repair (Amst)*, **5**, 1021–1029.

Steger, G. and Corbach, S. (1997) Dose-dependent regulation of the early promoter of human papillomavirus type 18 by the viral E2 protein. *J Virol*, **71**, 50–58.

Stenlund, A. (2003) Initiation of DNA replication: lessons from viral initiator proteins. *Nat Rev Mol Cell Biol*, **4**, 777–785.

Sverdrup, F. and Khan, S.A. (1994) Replication of human papillomavirus (HPV) DNAs supported by the HPV type 18 E1 and E2 proteins. *J Virol*, **68**, 505–509.

Syu, L.J. and Fluck, M.M. (1997) Site-specific in situ amplification of the integrated polyomavirus genome: a case for a context-specific over-replication model of gene amplification. *J Mol Biol*, **271**, 76–99.

Zerfass-Thome, K., Zwerschke, W., Mannhardt, B., Tindle, R., Botz, J.W. and Jansen-Durr, P. (1996) Inactivation of the cdk inhibitor p27KIP1 by the human papillomavirus type 16 E7 oncoprotein. *Oncogene*, **13**, 2323–2330.

Zhang, Y. and Rowley, J.D. (2006) Chromatin structural elements and chromosomal translocations in leukemia. *DNA Repair (Amst)*, **5**, 1282–1297.

Ziegert, C., Wentzensen, N., Vinokurova, S., Kisseljov, F., Einenkel, J., Hoeckel, M. and von Knebel Doeberitz, M. (2003) A comprehensive analysis of HPV integration loci in anogenital lesions combining transcript and genome-based amplification techniques. *Oncogene*, **22**, 3977–3984.

Zobel, T., Iftner, T. and Stubenrauch, F. (2003) The papillomavirus E8-E2C protein represses DNA replication from extrachromosomal origins. *Mol Cell Biol*, **23**, 8352–8362.

zur Hausen, H. (2002) Papillomaviruses and cancer: from basic studies to clinical application. *Nat Rev Cancer*, **2**, 342–350.

Tan, S.H., Leong, L.E., Walker, P.A. and Bernard, H.U. (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.

Tatsumi, Y., Ohta, S., Kimura, H., Tsurimoto, T. and Obuse, C. (2003) The ORC1 cycle in human cells: I. cell cycle-regulated oscillation of human ORC1. *J Biol Chem*, **278**, 41528–41534.

Tatsumi, Y., Sugimoto, N., Yugawa, T., Narisawa-Saito, M., Kiyono, T. and Fujita, M. (2006) Deregulation of Cdt1 induces chromosomal damage without rereplication and leads to chromosomal instability. *J Cell Sci*, **119**, 3128–3140.

Thomas, J.T., Hubert, W.G., Ruesch, M.N. and Laimins, L.A. (1999) Human papillomavirus type 31 oncoproteins E6 and E7 are required for the maintenance of episomes during the viral life cycle in normal human keratinocytes. *Proc Natl Acad Sci U S A*, **96**, 8449–8454.

Thomas, J.T. and Laimins, L.A. (1998) Human papillomavirus oncoproteins E6 and E7 independently abrogate the mitotic spindle checkpoint. *J Virol*, **72**, 1131–1137.

Thomas, J.T., Oh, S.T., Terhune, S.S. and Laimins, L.A. (2001) Cellular changes induced by low-risk human papillomavirus type 11 in keratinocytes that stably maintain viral episomes. *J Virol*, **75**, 7564–7571.

Thompson, D.A., Belinsky, G., Chang, T.H., Jones, D.L., Schlegel, R. and Munger, K. (1997) The human papillomavirus–16 E6 oncoprotein decreases the vigilance of mitotic checkpoints. *Oncogene*, **15**, 3025–3035.

Thorland, E.C., Myers, S.L., Gostout, B.S. and Smith, D.I. (2003) Common fragile sites are preferential targets for HPV16 integrations in cervical tumors. *Oncogene*, **22**, 1225–1237.

Tommasino, M., Adamczewski, J.P., Carlotti, F., Barth, C.F., Manetti, R., Contorni, M., Cavalieri, F., Hunt, T. and Crawford, L. (1993) HPV16 E7 protein associates with the protein kinase p33CDK2 and cyclin A. *Oncogene*, **8**, 195–202.

Ustav, E., Ustav, M., Szymanski, P. and Stenlund, A. (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.

Ustav, M. and Stenlund, A. (1991) Transient replication of BPV–1 requires two viral polypeptides encoded by the E1 and E2 open reading frames. *Embo J*, **10**, 449–457.

Ustav, M., Ustav, E., Szymanski, P. and Stenlund, A. (1991) Identification of the origin of replication of bovine papillomavirus and characterization of the viral origin recognition factor E1. *Embo J*, **10**, 4321–4329.

Wagatsuma, M., Hashimoto, K. and Matsukura, T. (1990) Analysis of integrated human papillomavirus type 16 DNA in cervical cancers: amplification of viral sequences together with cellular flanking sequences. *J Virol*, **64**, 813–821.

Walboomers, J.M., Jacobs, M.V., Manos, M.M., Bosch, F.X., Kummer, J.A., Shah, K.V., Snijders, P.J., Peto, J., Meijer, C.J. and Munoz, N. (1999) Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. *J Pathol*, **189**, 12–19.

Wang, Q., Griffin, H., Southern, S., Jackson, D., Martin, A., McIntosh, P., Davy, C., Masterson, P.J., Walker, P.A., Laskey, P., Omary, M.B. and Doorbar, J. (2004)

Functional analysis of the human papillomavirus type 16 E1=E4 protein provides a mechanism for in vivo and in vitro keratin filament reorganization. *J Virol*, **78**, 821–833.

Vaziri, C., Saxena, S., Jeon, Y., Lee, C., Murata, K., Machida, Y., Wagle, N., Hwang, D.S. and Dutta, A. (2003) A p53-dependent checkpoint pathway prevents rereplication. *Mol Cell*, **11**, 997–1008.

Veldman, T., Liu, X., Yuan, H. and Schlegel, R. (2003) Human papillomavirus E6 and Myc proteins associate in vivo and bind to and cooperatively activate the telomerase reverse transcriptase promoter. *Proc Natl Acad Sci U S A*, **100**, 8211–8216.

Wentzensen, N., Ridder, R., Klaes, R., Vinokurova, S., Schaefer, U. and Doeberitz, M.K. (2002) Characterization of viral-cellular fusion transcripts in a large series of HPV16 and 18 positive anogenital lesions. *Oncogene*, **21**, 419–426.

Wentzensen, N., Vinokurova, S. and von Knebel Doeberitz, M. (2004) Systematic review of genomic integration sites of human papillomavirus genomes in epithelial dysplasia and invasive cancer of the female lower genital tract. *Cancer Res*, **64**, 3878–3884.

Weterings, E. and Chen, D.J. (2008) The endless tale of non-homologous end-joining. *Cell Res*, **18**, 114–124.

Wilson, V.G., West, M., Woytek, K. and Rangasamy, D. (2002) Papillomavirus E1 proteins: form, function, and features. *Virus Genes*, **24**, 275–290.

Yan, Z., DeGregori, J., Shohet, R., Leone, G., Stillman, B., Nevins, J.R. and Williams, R.S. (1998) Cdc6 is regulated by E2F and is essential for DNA replication in mammalian cells. *Proc Natl Acad Sci U S A*, **95**, 3603–3608.

Yang, L. and Botchan, M. (1990) Replication of bovine papillomavirus type 1 DNA initiates within an E2-responsive enhancer element. *J Virol*, **64**, 5903–5911.

Yang, L., Li, R., Mohr, I.J., Clark, R. and Botchan, M.R. (1991) Activation of BPV–1 replication in vitro by the transcription factor E2. *Nature*, **353**, 628–632.

Yang, L., Mohr, I., Fouts, E., Lim, D.A., Nohaile, M. and Botchan, M. (1993) The E1 protein of bovine papilloma virus 1 is an ATP-dependent DNA helicase. *Proc Natl Acad Sci U S A*, **90**, 5086–5090.

Yoshida, T., Sano, T., Kanuma, T., Owada, N., Sakurai, S., Fukuda, T. and Nakajima, T. (2008) Quantitative real-time polymerase chain reaction analysis of the type distribution, viral load, and physical status of human papillomavirus in liquid-based cytology samples from cervical lesions. *Int J Gynecol Cancer*, **18**, 121–127.

You, J., Croyle, J.L., Nishimura, A., Ozato, K. and Howley, P.M. (2004) Interaction of the bovine papillomavirus E2 protein with Brd4 tethers the viral DNA to host mitotic chromosomes. *Cell*, **117**, 349–360.

Yu, T., Ferber, M.J., Cheung, T.H., Chung, T.K., Wong, Y.F. and Smith, D.I. (2005) The role of viral integration in the development of cervical cancer. *Cancer Genet Cytogenet*, **158**, 27–34.

SUMMARY IN ESTONIAN

Papilloomiviiruse DNA replikatsioon põhjustab peremeesrakus geneetilist ebastabiilsust

Normaalselt kulgeva viiruse elutsükli korral püsib inimese papilloomiviiruse (HPV) genoom latentselt nakatunud rakkudes episomaalse multikoopialise plasmiidina. Samas on aga avastatud, et enamuses emakakaelavähi rakkudes on osa viiruse DNAst, mis sisaldab viiruse replikatsiooni alguspunkti ning E6 ja E7 avatud lugemisraame, integreerunud peremeesraku genoomi. Lisaks on tõestatud, et HPV integratsiooni käigus läbitakse etapp, kus plasmiidne ja integreerunud HPV DNA eksisteerivad ühtedes rakkudes. Seega on võimalik, et vähitekkeni viinud geneetilised ümberkorraldused on vähemalt osaliselt saanud alguse integreeritud HPV DNA re-replikatsioonist, mis algatati plasmiidselt HPV-lt ekspresseeritud viiruse replikatsioonivalkude vahendusel.

Käesolevas töös näidati esmalt veise papilloomiviiruse tüüp 1 (BPV1) ja p53 valgu näitel, et papilloomiviirustel on potentsiaali varjata oma genoomi re-replikatsiooni toimumist rakuliste kontrollmehhanismide eest. Järgnevalt demonstreeriti, et HPV valgud E1 ja E2 on võimelised algatama DNA replikatsiooni ka integreeritud HPV-lt ning, et see viib madalmolekulaarsete DNA molekulide, muulhulgas ekstrakromosomaalsete plasmiidide, tekkeni. Lisaks selgus, et integreerunud HPV-lt alguse saanud DNA replikatsioon ulatub ka ümbritsevate rakuliste järjestusteni ning, et see viib teatud ümberkorraldusteni raku genoomis. Antud töös tuvastati nii HPV integratsioonilookuse duplikatsioon kui ka kromosoomide vaheline translokatsioon. Immunofluorestsentsanalüüsi ja immunosadestamise tulemused näitasid, et integreeritud HPV replikatsiooni poolt tekitatud DNA katkete likvideerimiseks aktiveeritakse rakulised DNA reparatsiooni faktorid.

Kokkuvõttes võib järeldada, et integreeritud papilloomiviiruse DNA replikatsioon ning sellele vastuseks peremeesraku poolt käivitatud DNA reparatsioon võivad viia geneetilise ebastabiilsuseni ja vähkkasvaja tekkeni.

ACKNOWLEDGEMENTS

First of all, I would like to thank my wife Heli for her constant support and belief in me. Studying for so long would not have been possible without the support of my mother, father, sister, and brother.

I am very grateful to my mentor Mart Ustav for the excellent guidance and his positive attitude. I am thankful to Ene Ustav for sharing her vast experience in lab and her kindness.

I am grateful to the coauthors of the papers: Helen Isok-Paas, Triin Laos, Mari Ojarand, Alina Sumerina, and Tatjana Verst.

I thank Ivar Ilves for the first supervision in lab and Aare Abroi, Andres Merits, Andrei Nikonov, Reet Kurg, and Toomas Silla for the fruitful discussions.

I am indebted to Arne Stenlund for the opportunity to participate in the undergraduate research program at CSHL. I thank the former URPs, Lakshmi and Timothy Sonbuchner, for their very useful advice while I was looking for the postdoc position.

Special thanks go to Saul Kivimäe and Anne Kalling for injecting self-confidence in me during the dry time.

I highly appreciate the help of all the colleagues whose phone numbers are in my cell phone, especially the former and current members of Ustav's group: Mihkel Allik, Helena Faust, Jelizaveta Geimanen, Nele Jaanson, Niilo Kaldalu, Kaja Kiiver, Anri Kivil, Ann Kilk, Andres Männik, Kadri Peil, Merike Petuhov, Regina Pipitš, Tormi Reinson, Eve Sankovski, Aksel Soosaar, Eva Žusinaite, Ingrid Tagen, Urve Toots, Mart Ustav Jr, and Agne Velthut. I am also very grateful to Daima and Tõnis Örd.

I would like to use this opportunity to acknowledge the teachers in Kivilinna Gymnasium, especially Tiia Krass, Anne Limak, Helgi Muoni, and Rita Zobel, as well as all the lecturers at the University of Tartu, especially Agu Laisk and Rein Sikut, whose dedication built the foundation of my education.

I am grateful to the organizations that provided scholarships for my studies: Archimedes Foundation, Celecure Ltd, Cold Spring Harbor Laboratory, Estonian Biocentre, Estonian Genome Foundation, Estonian Students Foundation, Estonian World Council, Federation of European Biochemical Society, and Tartu University Foundation.

PUBLICATIONS

CURRICULUM VITAE

Meelis Kadaja

Date and place of birth:	January 20th, 1979 in Tartu, Estonia
Address:	Tartu University Institute of Technology, Nooruse 1, 50411 Tartu
Phone:	+372 737 4880
Fax:	+372 737 4900
E-mail:	meelis.kadaja@ut.ee

Education and professional employment

2008–	scientist in Estonian Biocentre
2006–2007	extraordinary researcher in Institute of Molecular and Cell Biology, University of Tartu
2003–2005	scientist in Estonian Biocentre
2002–	Ph.D student in Institute of Molecular and Cell Biology, University of Tartu
2001–2002	M.Sc student in Institute of Molecular and Cell Biology, University of Tartu, *magister scientiarum* diploma in virology
2001 summer	Undergraduate Research Program in Cold Spring Harbor Laboratory, New York
1997–2001	undergraduate studies in Faculty of Biology and Geography, University of Tartu, graduated with *cum laude* diploma in molecular biology and genetics
1986–1997	Tartu Kivilinna Gymnasium

Scientific work

I work in the lab of Prof. Mart Ustav, where I study the genomic instability of the host cell induced by the DNA replication of human papillomaviruses.

List of publications

1. Kadaja M., Isok-Paas H., Laos T., Ustav E., Ustav M. (2009) Mechanism of Genomic Instability in Cells Infected with the High Risk Human Papillomaviruses. *PLoS Pathogens* (manuscript in press).
2. Kadaja M, Silla T, Ustav E., Ustav M. (2009) Papillomavirus DNA replication – From initiation to genomic instability. *Virology, 384, 360–8.*

3. Kadaja M., Sumerina A., Verst T., Ojarand M., Ustav E., Ustav M. (2007) Genomic instability of the host cell induced by the human papillomavirus replication machinery. *EMBO Journal,* 26, 2180–91.
4. Ilves I., Kadaja M., Ustav M. (2003) Two separate replication modes of the bovine papillomavirus BPV1 origin of replication that have different sensitivity to p53. *Virus Research,* 96, 75–84.

CURRICULUM VITAE

Meelis Kadaja

Sünniaeg ja -koht:	20. jaanuar 1979, Tartu
Aadress:	Tartu Ülikooli Tehnoloogiainstituut, Nooruse 1, 50411 Tartu
Telefon:	+372 737 4880
Faks:	+372 737 4900
E-post:	meelis.kadaja@ut.ee

Haridus ja erialane teenistuskäik

Alates 2008	Eesti Biokeskuse teadur
2006–2007	Tartu Ülikooli Molekulaar- ja Rakubioloogia Instituudi erakorraline teadur
2003–2005	Eesti Biokeskuse teadur
Alates 2002	doktoriõpe Tartu Ülikooli Molekulaar- ja Rakubioloogia Instituudis
2001–2002	magistriõpe Tartu Ülikooli Molekulaar- ja Rakubioloogia Instituudis, *magister scientiarum* kraad viroloogia erialal
2001 suvi	praktika Cold Spring Harbor Laboratooriumis, New York
1997–2001	bakalaureuseõpe Tartu Ülikooli bioloogia-geograafia-teaduskonnas, *cum laude* diplom molekulaarbioloogia ja geneetika erialal
1986–1997	Tartu Kivilinna Gümnaasium

Teadustegevus

Uurin Mart Ustavi juhitavas teaduslaboris inimese papilloomiviiruse DNA replikatsiooni mehhanisme ning selle poolt põhjustatud geneetilisi muutusi peremeesraku genoomis.

Publikatsioonid

1. Kadaja M., Isok-Paas H., Laos T., Ustav E., Ustav M. (2009) Mechanism of Genomic Instability in Cells Infected with the High Risk Human Papillomaviruses. *PLoS Pathogens* (käsikiri on trükis).
2. Kadaja M, Silla T, Ustav E., Ustav M. (2009) Papillomavirus DNA replication — From initiation to genomic instability. *Virology, 384, 360–8.*

3. Kadaja M., Sumerina A., Verst T., Ojarand M., Ustav E., Ustav M. (2007) Genomic instability of the host cell induced by the human papillomavirus replication machinery. *EMBO Journal,* 26, 2180–91.
4. Ilves I., Kadaja M., Ustav M. (2003) Two separate replication modes of the bovine papillomavirus BPV1 origin of replication that have different sensitivity to p53. *Virus Research,* 96, 75–84.

DISSERTATIONES BIOLOGICAE UNIVERSITATIS TARTUENSIS

1. **Toivo Maimets**. Studies of human oncoprotein p53. Tartu, 1991, 96 p.
2. **Enn K. Seppet**. Thyroid state control over energy metabolism, ion transport and contractile functions in rat heart. Tartu, 1991, 135 p.
3. **Kristjan Zobel**. Epifüütsete makrosamblike väärtus õhu saastuse indikaatoritena Hamar-Dobani boreaalsetes mägimetsades. Tartu, 1992, 131 lk.
4. **Andres Mäe**. Conjugal mobilization of catabolic plasmids by transposable elements in helper plasmids. Tartu, 1992, 91 p.
5. **Maia Kivisaar**. Studies on phenol degradation genes of *Pseudomonas* sp. strain EST 1001. Tartu, 1992, 61 p.
6. **Allan Nurk**. Nucleotide sequences of phenol degradative genes from *Pseudomonas sp.* strain EST 1001 and their transcriptional activation in *Pseudomonas putida.* Tartu, 1992, 72 p.
7. **Ülo Tamm**. The genus *Populus* L. in Estonia: variation of the species biology and introduction. Tartu, 1993, 91 p.
8. **Jaanus Remme**. Studies on the peptidyltransferase centre of the *E.coli* ribosome. Tartu, 1993, 68 p.
9. **Ülo Langel**. Galanin and galanin antagonists. Tartu, 1993, 97 p.
10. **Arvo Käärd**. The development of an automatic online dynamic fluorescense-based pH-dependent fiber optic penicillin flowthrought biosensor for the control of the benzylpenicillin hydrolysis. Tartu, 1993, 117 p.
11. **Lilian Järvekülg**. Antigenic analysis and development of sensitive immunoassay for potato viruses. Tartu, 1993, 147 p.
12. **Jaak Palumets**. Analysis of phytomass partition in Norway spruce. Tartu, 1993, 47 p.
13. **Arne Sellin**. Variation in hydraulic architecture of *Picea abies* (L.) Karst. trees grown under different enviromental conditions. Tartu, 1994, 119 p.

13. **Mati Reeben**. Regulation of light neurofilament gene expression. Tartu, 1994, 108 p.
14. **Urmas Tartes**. Respiration rhytms in insects. Tartu, 1995, 109 p.
15. **Ülo Puurand**. The complete nucleotide sequence and infections *in vitro* transcripts from cloned cDNA of a potato A potyvirus. Tartu, 1995, 96 p.
16. **Peeter Hõrak**. Pathways of selection in avian reproduction: a functional framework and its application in the population study of the great tit (*Parus major*). Tartu, 1995, 118 p.
17. **Erkki Truve**. Studies on specific and broad spectrum virus resistance in transgenic plants. Tartu, 1996, 158 p.
18. **Illar Pata**. Cloning and characterization of human and mouse ribosomal protein S6-encoding genes. Tartu, 1996, 60 p.
19. **Ülo Niinemets**. Importance of structural features of leaves and canopy in determining species shade-tolerance in temperature deciduous woody taxa. Tartu, 1996, 150 p.

20. **Ants Kurg**. Bovine leukemia virus: molecular studies on the packaging region and DNA diagnostics in cattle. Tartu, 1996, 104 p.
21. **Ene Ustav**. E2 as the modulator of the BPV1 DNA replication. Tartu, 1996, 100 p.
22. **Aksel Soosaar**. Role of helix-loop-helix and nuclear hormone receptor transcription factors in neurogenesis. Tartu, 1996, 109 p.
23. **Maido Remm**. Human papillomavirus type 18: replication, transformation and gene expression. Tartu, 1997, 117 p.
24. **Tiiu Kull**. Population dynamics in *Cypripedium calceolus* L. Tartu, 1997, 124 p.
25. **Kalle Olli**. Evolutionary life-strategies of autotrophic planktonic microorganisms in the Baltic Sea. Tartu, 1997, 180 p.
26. **Meelis Pärtel**. Species diversity and community dynamics in calcareous grassland communities in Western Estonia. Tartu, 1997, 124 p.
27. **Malle Leht**. The Genus *Potentilla* L. in Estonia, Latvia and Lithuania: distribution, morphology and taxonomy. Tartu, 1997, 186 p.
28. **Tanel Tenson**. Ribosomes, peptides and antibiotic resistance. Tartu, 1997, 80 p.
29. **Arvo Tuvikene**. Assessment of inland water pollution using biomarker responses in fish *in vivo* and *in vitro.* Tartu, 1997, 160 p.
30. **Urmas Saarma**. Tuning ribosomal elongation cycle by mutagenesis of 23S rRNA. Tartu, 1997, 134 p.
31. **Henn Ojaveer**. Composition and dynamics of fish stocks in the gulf of Riga ecosystem. Tartu, 1997, 138 p.
32. **Lembi Lõugas**. Post-glacial development of vertebrate fauna in Estonian water bodies. Tartu, 1997, 138 p.
33. **Margus Pooga**. Cell penetrating peptide, transportan, and its predecessors, galanin-based chimeric peptides. Tartu, 1998, 110 p.
34. **Andres Saag**. Evolutionary relationships in some cetrarioid genera (Lichenized Ascomycota). Tartu, 1998, 196 p.
35. **Aivar Liiv**. Ribosomal large subunit assembly *in vivo*. Tartu, 1998, 158 p.
36. **Tatjana Oja**. Isoenzyme diversity and phylogenetic affinities among the eurasian annual bromes (*Bromus* L., Poaceae). Tartu, 1998, 92 p.
37. **Mari Moora**. The influence of arbuscular mycorrhizal (AM) symbiosis on the competition and coexistence of calcareous crassland plant species. Tartu, 1998, 78 p.
38. **Olavi Kurina**. Fungus gnats in Estonia (*Diptera: Bolitophilidae, Keroplatidae, Macroceridae, Ditomyiidae, Diadocidiidae, Mycetophilidae*). Tartu, 1998, 200 p.
39. **Andrus Tasa**. Biological leaching of shales: black shale and oil shale. Tartu, 1998, 98 p.
40. **Arnold Kristjuhan.** Studies on transcriptional activator properties of tumor suppressor protein p53. Tartu, 1998, 86 p.

41. **Sulev Ingerpuu.** Characterization of some human myeloid cell surface and nuclear differentiation antigens. Tartu, 1998, 163 p.
42. **Veljo Kisand.** Responses of planktonic bacteria to the abiotic and biotic factors in the shallow lake Võrtsjärv. Tartu, 1998, 118 p.
43. **Kadri Põldmaa.** Studies in the systematics of hypomyces and allied genera (Hypocreales, Ascomycota). Tartu, 1998, 178 p.
44. **Markus Vetemaa.** Reproduction parameters of fish as indicators in environmental monitoring. Tartu, 1998, 117 p.
45. **Heli Talvik.** Prepatent periods and species composition of different *Oesophagostomum* spp. populations in Estonia and Denmark. Tartu, 1998, 104 p.
46. **Katrin Heinsoo.** Cuticular and stomatal antechamber conductance to water vapour diffusion in *Picea abies* (L.) karst. Tartu, 1999, 133 p.
47. **Tarmo Annilo.** Studies on mammalian ribosomal protein S7. Tartu, 1998, 77 p.
48. **Indrek Ots.** Health state indicies of reproducing great tits (*Parus major*): sources of variation and connections with life-history traits. Tartu, 1999, 117 p.
49. **Juan Jose Cantero.** Plant community diversity and habitat relationships in central Argentina grasslands. Tartu, 1999, 161 p.
50. **Rein Kalamees.** Seed bank, seed rain and community regeneration in Estonian calcareous grasslands. Tartu, 1999, 107 p.
51. **Sulev Kõks.** Cholecystokinin (CCK) — induced anxiety in rats: influence of environmental stimuli and involvement of endopioid mechanisms and erotonin. Tartu, 1999, 123 p.
52. **Ebe Sild.** Impact of increasing concentrations of O_3 and CO_2 on wheat, clover and pasture. Tartu, 1999, 123 p.
53. **Ljudmilla Timofejeva.** Electron microscopical analysis of the synaptonemal complex formation in cereals. Tartu, 1999, 99 p.
54. **Andres Valkna.** Interactions of galanin receptor with ligands and G-proteins: studies with synthetic peptides. Tartu, 1999, 103 p.
55. **Taavi Virro.** Life cycles of planktonic rotifers in lake Peipsi. Tartu, 1999, 101 p.
56. **Ana Rebane.** Mammalian ribosomal protein S3a genes and intron-encoded small nucleolar RNAs U73 and U82. Tartu, 1999, 85 p.
57. **Tiina Tamm.** Cocksfoot mottle virus: the genome organisation and translational strategies. Tartu, 2000, 101 p.
58. **Reet Kurg.** Structure-function relationship of the bovine papilloma virus E2 protein. Tartu, 2000, 89 p.
59. **Toomas Kivisild.** The origins of Southern and Western Eurasian populations: an mtDNA study. Tartu, 2000, 121 p.
60. **Niilo Kaldalu.** Studies of the TOL plasmid transcription factor XylS. Tartu 2000. 88 p.

61. **Dina Lepik.** Modulation of viral DNA replication by tumor suppressor protein p53. Tartu 2000. 106 p.
62. **Kai Vellak.** Influence of different factors on the diversity of the bryophyte vegetation in forest and wooded meadow communities. Tartu 2000. 122 p.
63. **Jonne Kotta.** Impact of eutrophication and biological invasionas on the structure and functions of benthic macrofauna. Tartu 2000. 160 p.
64. **Georg Martin.** Phytobenthic communities of the Gulf of Riga and the inner sea the West-Estonian archipelago. Tartu, 2000. 139 p.
65. **Silvia Sepp.** Morphological and genetical variation of *Alchemilla L.* in Estonia. Tartu, 2000. 124 p.
66. **Jaan Liira.** On the determinants of structure and diversity in herbaceous plant communities. Tartu, 2000. 96 p.
67. **Priit Zingel.** The role of planktonic ciliates in lake ecosystems. Tartu 2001. 111 p.
68. **Tiit Teder.** Direct and indirect effects in Host-parasitoid interactions: ecological and evolutionary consequences. Tartu 2001. 122 p.
69. **Hannes Kollist.** Leaf apoplastic ascorbate as ozone scavenger and its transport across the plasma membrane. Tartu 2001. 80 p.
70. **Reet Marits.** Role of two-component regulator system PehR-PehS and extracellular protease PrtW in virulence of *Erwinia Carotovora* subsp. *Carotovora*. Tartu 2001. 112 p.
71. **Vallo Tilgar.** Effect of calcium supplementation on reproductive performance of the pied flycatcher *Ficedula hypoleuca* and the great tit *Parus major,* breeding in Nothern temperate forests. Tartu, 2002. 126 p.
72. **Rita Hõrak.** Regulation of transposition of transposon Tn*4652* in *Pseudomonas putida*. Tartu, 2002. 108 p.
73. **Liina Eek-Piirsoo.** The effect of fertilization, mowing and additional illumination on the structure of a species-rich grassland community. Tartu, 2002. 74 p.
74. **Krõõt Aasamaa.** Shoot hydraulic conductance and stomatal conductance of six temperate deciduous tree species. Tartu, 2002. 110 p.
75. **Nele Ingerpuu.** Bryophyte diversity and vascular plants. Tartu, 2002. 112 p.
76. **Neeme Tõnisson.** Mutation detection by primer extension on oligonucleotide microarrays. Tartu, 2002. 124 p.
77. **Margus Pensa.** Variation in needle retention of Scots pine in relation to leaf morphology, nitrogen conservation and tree age. Tartu, 2003. 110 p.
78. **Asko Lõhmus.** Habitat preferences and quality for birds of prey: from principles to applications. Tartu, 2003. 168 p.
79. **Viljar Jaks.** p53 — a switch in cellular circuit. Tartu, 2003. 160 p.
80. **Jaana Männik.** Characterization and genetic studies of four ATP-binding cassette (ABC) transporters. Tartu, 2003. 140 p.
81. **Marek Sammul.** Competition and coexistence of clonal plants in relation to productivity. Tartu, 2003. 159 p

82. **Ivar Ilves.** Virus-cell interactions in the replication cycle of bovine papillomavirus type 1. Tartu, 2003. 89 p.
83. **Andres Männik.** Design and characterization of a novel vector system based on the stable replicator of bovine papillomavirus type 1. Tartu, 2003. 109 p.
84. **Ivika Ostonen.** Fine root structure, dynamics and proportion in net primary production of Norway spruce forest ecosystem in relation to site conditions. Tartu, 2003. 158 p.
85. **Gudrun Veldre.** Somatic status of 12–15-year-old Tartu schoolchildren. Tartu, 2003. 199 p.
86. **Ülo Väli.** The greater spotted eagle *Aquila clanga* and the lesser spotted eagle *A. pomarina*: taxonomy, phylogeography and ecology. Tartu, 2004. 159 p.
87. **Aare Abroi.** The determinants for the native activities of the bovine papillomavirus type 1 E2 protein are separable. Tartu, 2004. 135 p.
88. **Tiina Kahre.** Cystic fibrosis in Estonia. Tartu, 2004. 116 p.
89. **Helen Orav-Kotta.** Habitat choice and feeding activity of benthic suspension feeders and mesograzers in the northern Baltic Sea. Tartu, 2004. 117 p.
90. **Maarja Öpik.** Diversity of arbuscular mycorrhizal fungi in the roots of perennial plants and their effect on plant performance. Tartu, 2004. 175 p.
91. **Kadri Tali.** Species structure of *Neotinea ustulata*. Tartu, 2004. 109 p.
92. **Kristiina Tambets.** Towards the understanding of post-glacial spread of human mitochondrial DNA haplogroups in Europe and beyond: a phylogeographic approach. Tartu, 2004. 163 p.
93. **Arvi Jõers.** Regulation of p53-dependent transcription. Tartu, 2004. 103 p.
94. **Lilian Kadaja.** Studies on modulation of the activity of tumor suppressor protein p53. Tartu, 2004. 103 p.
95. **Jaak Truu.** Oil shale industry wastewater: impact on river microbial community and possibilities for bioremediation. Tartu, 2004. 128 p.
96. **Maire Peters.** Natural horizontal transfer of the *pheBA* operon. Tartu, 2004. 105 p.
97. **Ülo Maiväli.** Studies on the structure-function relationship of the bacterial ribosome. Tartu, 2004. 130 p.
98. **Merit Otsus.** Plant community regeneration and species diversity in dry calcareous grasslands. Tartu, 2004. 103 p.
99. **Mikk Heidemaa.** Systematic studies on sawflies of the genera *Dolerus, Empria,* and *Caliroa* (Hymenoptera: Tenthredinidae). Tartu, 2004. 167 p.
100. **Ilmar Tõnno.** The impact of nitrogen and phosphorus concentration and N/P ratio on cyanobacterial dominance and N_2 fixation in some Estonian lakes. Tartu, 2004. 111 p.
101. **Lauri Saks.** Immune function, parasites, and carotenoid-based ornaments in greenfinches. Tartu, 2004. 144 p.

102. **Siiri Rootsi.** Human Y-chromosomal variation in European populations. Tartu, 2004. 142 p.
103. **Eve Vedler.** Structure of the 2,4-dichloro-phenoxyacetic acid-degradative plasmid pEST4011. Tartu, 2005. 106 p.
104. **Andres Tover.** Regulation of transcription of the phenol degradation *pheBA* operon in *Pseudomonas putida*. Tartu, 2005. 126 p.
105. **Helen Udras.** Hexose kinases and glucose transport in the yeast *Hansenula polymorpha*. Tartu, 2005. 100 p.
106. **Ave Suija.** Lichens and lichenicolous fungi in Estonia: diversity, distribution patterns, taxonomy. Tartu, 2005. 162 p.
107. **Piret Lõhmus.** Forest lichens and their substrata in Estonia. Tartu, 2005. 162 p.
108. **Inga Lips.** Abiotic factors controlling the cyanobacterial bloom occurrence in the Gulf of Finland. Tartu, 2005. 156 p.
109. **Kaasik, Krista.** Circadian clock genes in mammalian clockwork, metabolism and behaviour. Tartu, 2005. 121 p.
110. **Juhan Javoiš.** The effects of experience on host acceptance in ovipositing moths. Tartu, 2005. 112 p.
111. **Tiina Sedman.** Characterization of the yeast *Saccharomyces cerevisiae* mitochondrial DNA helicase Hmi1. Tartu, 2005. 103 p.
112. **Ruth Aguraiuja.** Hawaiian endemic fern lineage *Diellia* (Aspleniaceae): distribution, population structure and ecology. Tartu, 2005. 112 p.
113. **Riho Teras.** Regulation of transcription from the fusion promoters generated by transposition of Tn*4652* into the upstream region of *pheBA* operon in *Pseudomonas putida*. Tartu, 2005. 106 p.
114. **Mait Metspalu.** Through the course of prehistory in india: tracing the mtDNA trail. Tartu, 2005. 138 p.
115. **Elin Lõhmussaar.** The comparative patterns of linkage disequilibrium in European populations and its implication for genetic association studies. Tartu, 2006. 124 p.
116. **Priit Kupper.** Hydraulic and environmental limitations to leaf water relations in trees with respect to canopy position. Tartu, 2006. 126 p.
117. **Heili Ilves.** Stress-induced transposition of Tn*4652* in *Pseudomonas Putida.* Tartu, 2006. 120 p.
118. **Silja Kuusk.** Biochemical properties of Hmi1p, a DNA helicase from *Saccharomyces cerevisiae* mitochondria. Tartu, 2006. 126 p.
119. **Kersti Püssa.** Forest edges on medium resolution landsat thematic mapper satellite images. Tartu, 2006. 90 p.
120. **Lea Tummeleht.** Physiological condition and immune function in great tits (*Parus major* l.): Sources of variation and trade-offs in relation to growth. Tartu, 2006. 94 p.
121. **Toomas Esperk.** Larval instar as a key element of insect growth schedules. Tartu, 2006. 186 p.

122. **Harri Valdmann.** Lynx (*Lynx lynx*) and wolf (*Canis lupus*) in the Baltic region: Diets, helminth parasites and genetic variation. Tartu, 2006. 102 p.
123. **Priit Jõers.** Studies of the mitochondrial helicase Hmi1p in *Candida albicans* and *Saccharomyces cerevisia*. Tartu, 2006. 113 p.
124. **Kersti Lilleväli.** Gata3 and Gata2 in inner ear development. Tartu, 2007. 123 p.
125. **Kai Rünk.** Comparative ecology of three fern species: *Dryopteris carthusiana* (Vill.) H.P. Fuchs, *D. expansa* (C. Presl) Fraser-Jenkins & Jermy and *D. dilatata* (Hoffm.) A. Gray (Dryopteridaceae). Tartu, 2007. 143 p.
126. **Aveliina Helm.** Formation and persistence of dry grassland diversity: role of human history and landscape structure. Tartu, 2007. 89 p.
127. **Leho Tedersoo.** Ectomycorrhizal fungi: diversity and community structure in Estonia, Seychelles and Australia. Tartu, 2007. 233 p.
128. **Marko Mägi.** The habitat-related variation of reproductive performance of great tits in a deciduous-coniferous forest mosaic: looking for causes and consequences. Tartu, 2007. 135 p.
129. **Valeria Lulla.** Replication strategies and applications of Semliki Forest virus. Tartu, 2007. 109 p.
130. **Ülle Reier**. Estonian threatened vascular plant species: causes of rarity and conservation. Tartu, 2007. 79 p.
131. **Inga Jüriado**. Diversity of lichen species in Estonia: influence of regional and local factors. Tartu, 2007. 171 p.
132. **Tatjana Krama.** Mobbing behaviour in birds: costs and reciprocity based cooperation. Tartu, 2007.
133. **Signe Saumaa.** The role of DNA mismatch repair and oxidative DNA damage defense systems in avoidance of stationary phase mutations in *Pseudomonas putida.* Tartu, 2007. 172 p.
134. **Reedik Mägi**. The linkage disequilibrium and the selection of genetic markers for association studies in european populations. Tartu, 2007. 96 p.
135. **Priit Kilgas.** Blood parameters as indicators of physiological condition and skeletal development in great tits (*Parus major*): natural variation and application in the reproductive ecology of birds. Tartu, 2007. 129 p.
136. **Anu Albert**. The role of water salinity in structuring eastern Baltic coastal fish communities. Tartu, 2007. 95 p.
137. **Kärt Padari.** Protein transduction mechanisms of transportans. Tartu, 2008. 128 p.
138. **Siiri-Lii Sandre.** Selective forces on larval colouration in a moth. Tartu, 2008. 125 p.
139. **Ülle Jõgar.** Conservation and restoration of semi-natural floodplain meadows and their rare plant species. Tartu, 2008. 99 p.
140. **Lauri Laanisto.** Macroecological approach in vegetation science: generality of ecological relationships at the global scale. Tartu, 2008. 133 p.
141. **Reidar Andreson**. Methods and software for predicting PCR failure rate in large genomes. Tartu, 2008. 105 p.

142. **Birgot Paavel.** Bio-optical properties of turbid lakes. Tartu, 2008. 175 p.
143. **Kaire Torn.** Distribution and ecology of charophytes in the Baltic Sea. Tartu, 2008, 98 p.
144. **Vladimir Vimberg.** Peptide mediated macrolide resistance. Tartu, 2008, 190 p.
145. **Daima Örd.** Studies on the stress-inducible pseudokinase TRB3, a novel inhibitor of transcription factor ATF4. Tartu, 2008, 108 p.
146. **Lauri Saag.** Taxonomic and ecologic problems in the genus *Lepraria* (*Stereocaulaceae*, lichenised *Ascomycota*). Tartu, 2008, 175 p.
147. **Ulvi Karu.** Antioxidant protection, carotenoids and coccidians in greenfinches – assessment of the costs of immune activation and mechanisms of parasite resistance in a passerine with carotenoid-based ornaments. Tartu, 2008, 124 p.
148. **Jaanus Remm.** Tree-cavities in forests: density, characteristics and occupancy by animals. Tartu, 2008, 128 p.
149. **Epp Moks.** Tapeworm parasites *Echinococcus multilocularis* and *E. granulosus* in Estonia: phylogenetic relationships and occurrence in wild carnivores and ungulates. Tartu, 2008, 82 p.
150. **Eve Eensalu.** Acclimation of stomatal structure and function in tree canopy: effect of light and CO_2 concentration. Tartu, 2008, 108 p.
151. **Janne Pullat**. Design, functionlization and application of an *in situ* synthesized oligonucleotide microarray. Tartu, 2008, 108 p.
152. **Marta Putrinš.** Responses of *Pseudomonas putida* to phenol-induced metabolic and stress signals. Tartu, 2008, 142 p.
153. **Marina Semtšenko.** Plant root behaviour: responses to neighbours and physical obstructions. Tartu, 2008, 106 p.
154. **Marge Starast.** Influence of cultivation techniques on productivity and fruit quality of some *Vaccinium* and *Rubus* taxa. Tartu, 2008, 154 p.
155. **Age Tats.** Sequence motifs influencing the efficiency of translation. Tartu, 2009, 104 p.
156. **Radi Tegova.** The role of specialized DNA polymerases in mutagenesis in *Pseudomonas putida.* Tartu, 2009, 124 p.
157. **Tsipe Aavik.** Plant species richness, composition and functional trait pattern in agricultural landscapes – the role of land use intensity and landscape structure. Tartu, 2008, 112 p.
158. **Kaja Kiiver.** Semliki forest virus based vectors and cell lines for studying the replication and interactions of alphaviruses and hepaciviruses. Tartu, 2009, 104 p.