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Control of viral replication and transcription by the papillomavirus E8Ê2 protein

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ABSTRACT

Human papillomaviruses have adjusted their replication levels to the differentiation state of the infected keratinocyte. PV genomes replicate in undifferentiated cells at low levels and to high levels in differentiated cells. Genome replication requires the viral E1 helicase and the viral E2 transcription/replication activator. The limited replication in undifferentiated cells is predominantly due to the expression of the highly conserved E8Ê2 viral repressor protein, which is a fusion between E8 and the C-terminal half of the E2 protein. E8Ê2 is a sequence-specific DNA binding protein that inhibits viral gene expression and viral genome replication. The E8 domain is required for repression activities, which are mainly due to the interaction with cellular NCoR/SMRT corepressor complexes. In the case of HPV16, the most carcinogenic HPV type, E8Ê2 not only limits genome replication in undifferentiated cells but also productive replication in differentiated epithelium. E8Ê2 is expressed from a separate promoter that is controlled by unknown cellular factors and the viral transcription and replication regulators E1, E2 and E8Ê2. In summary, E8Ê2 is an important negative regulator whose levels may be critical for the outcome of HPV infections.

Contents

1. Introduction

Papillomaviruses are non-enveloped, double-stranded DNA viruses with currently more than 200 different sequenced human genotypes. Infections with human papillomavirus (HPV) may cause different kinds of warts or intraepithelial neoplasias on cutaneous or mucosal epithelia. Importantly, infections with high-risk HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58 and 59 are the major risk factor for the development of cancer of the cervix uteri and anus and contribute to a fraction of cancers of the oro-pharynx, penis, vagina and vulva (Parkin and Bray, 2006).

HPV infect keratinocytes in the basal layer of cutaneous or mucosal skin where limited replication of the viral genomes occurs

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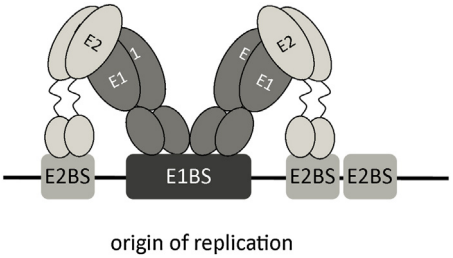

Fig. 1. Binding of E1/E2-complexes to the viral origin of replication. The papillomavirus origin of replication consists of binding sites for viral E1 (E1BS) and E2 (E2BS) proteins. E1 and E2 form a complex in which E2 acts to load E1 onto the origin.

and only early transcripts are expressed. Virus capsid protein expression and amplification of viral genomes to high levels occurs only in the upper layers of the epithelium and is preceded by a re-entry of the infected cells into the cell cycle (Moody and Laimins, 2010). Viral gene expression and replication are tightly controlled in the different phases of the viral replication cycle by both host cell and viral proteins.

2. The E2 Protein

The E2 protein is a key regulator protein that is highly conserved among papillomaviruses. E2 consists of a ~200aa N-terminal domain which is connected by a flexible hinge region of varying length to a C-terminal domain of ~100aa. The C-terminal domain is responsible for the specific recognition of DNA sequences (ACCN6GGT; E2 binding sites (E2BS)) and also for the dimerization of E2 proteins (see for a review: (McBride, 2013). E2 is essential for the replication of PV genomes (Sankovski et al., 2014; Stubenrauch et al., 1998b; Ustav and Stenlund, 1991). This is mainly due to the interaction of E2 with the viral E1 helicase (Bergvall et al., 2013; McBride, 2013). The papillomavirus origin of replication is composed of overlapping E1 binding sites flanked by E2BS (Fig. 1). The E1-E2 protein complex recognizes E2BS with high affinity and thus E2 acts as a helicase loader. E1 unwinds the viral DNA and then recruits cellular replication proteins which replicate the viral genomes (see for a review: (Bergvall et al., 2013)). E2 can also activate transcription and this activity is crucial for bovine papillomavirus 1 (BPV1) gene expression (McBride, 2013). In contrast, activation of transcription by HPV31 E2 is not required for viral genome replication in undifferentiated human keratinocytes but may contribute to the differentiation-dependent replication (Klymenko et al., 2016; Sakakibara et al., 2013; Stubenrauch et al., 1998a). E2 can also act as a repressor of transcription from promoter-proximal E2BS which has been suggested to be important to limit expression of the viral E6 and E7 oncoproteins in carcinogenic HPV. Consistent with this, in the context of hybrid HPV16 genomes that contain the Epstein-Barr virus oriP sequence and an EBNA1 expression cassette, mutations in the N-terminal domain or in the DNA-binding domain (DBD) of E2 increased early viral gene expression (Soeda et al., 2006). However, in the context of full-length HPV16 genomes only mutations in the DBD but not in the N-terminal domain of E2 increased viral early gene expression which supports the idea that E2 repressor proteins that only share the C-terminal but not the N-terminal domain with E2 are mainly responsible for the repression of viral transcription (Lace et al., 2008). Thus, the physiological relevance of E2′s repression activity for the replication of high-risk HPV is still controversial. In addition, E2 has been implicated in the nuclear retention of viral genomes after cell division by attaching the viral genomes to mitotic chromosomes (McBride et al., 2012).

3. E2 repressor proteins

3.1. Transcripts for PV repressor proteins

Studies with BPV1 indicated that additional proteins are derived from the E2 gene that are called E2TR (or E2C) and E8/E2 (Choe et al., 1989; Hubbert et al., 1988; Lambert et al., 1987). E2TR is derived from a RNA initiating at the P3080 promoter within the E2 gene and gives rise to an N-terminally truncated E2 that starts at residue 162 (Lambert et al., 1987). The E8/E2 mRNA is derived from the P890 promoter within the E1 gene and is spliced from nt. 1235 to nt. 3225 and the corresponding protein consists of residues 1-11 of E8 fused to residues 207-410 of E2 (Choe et al., 1989). The analysis of RNA from HPV1, 5, 11, 16, 18, 31 and 33-positive cells and cottontail rabbit PV (CRPV)-induced papillomas indicated that HPV and CRPV express an RNA homologous to the BPV1 E8/E2 mRNA (Fig. 2) (Doorbar et al., 1990; Fertey et al., 2011; Isok-Paas et al., 2015; Jeckel et al., 2003; Lace et al., 2008; Palermo-Dilts et al., 1990; Rotenberg et al., 1989; Snijders et al., 1992; Stubenrauch et al., 2000; Wang et al., 2011). The respective HPV gene products have been labeled E2C, E8Ê2C or E8Ê2. Experts in the field (A. Abroi, T.H. Haugen, P.M. Howley, A.A. McBride, P.F. Lambert, F. Stubenrauch, Z.M. Zheng) have now agreed on E8Ê2 as the official designation. Comparable to BPV1, the HPV E8Ê2 mRNA is generated from a separate promoter within the E1 gene with transcriptional start sites located ~70-150 nt upstream of the E8 ATG start codon (Fig. 2) (Chen et al., 2014; Isok-Paas et al., 2015; Lace et al., 2008; Milligan et al., 2007; Sankovski et al., 2014; Straub et al., 2015; Toots et al., 2014; Wang et al., 2011). Quantitative transcript analyses also indicated that the major transcript expressed by this promoter is the E8Ê2-encoding transcript (Straub et al., 2015). The HPV16 E8 promoter displays basal activity in HPV-negative keratinocytes. In contrast to the major viral early promoter P97, the basal activity of the E8 Promoter is not modulated by enhancer elements in the URR (Straub et al., 2015). Instead, constitutive activity of the HPV16 E8 promoter requires two conserved elements (CE2 and CE3, Fig. 2) close to the transcription start site which bind to unknown cellular proteins (Straub et al., 2015). In addition, the E8 promoter is regulated by viral proteins. E8Ê2 inhibits its own promoter and E2 weakly activates it (Straub et al., 2015). Interestingly, the combination of E1 and E2 activates the E8 promoter to higher levels than the P97 promoter (Straub et al., 2015). Since the E8 promoter is both positively and negatively regulated by viral replication proteins, it might act as a sensor and modulator of viral copy number.

Similar to BPV1 E8/E2, HPV E8Ê2 consists of 12-16 residues encoded by the E8 exon fused to the hinge/DBD/dimerization domains of E2 (Fig. 2). Bioinformatic analyses suggest that the potential to generate E8Ê2 transcripts and the corresponding fusion proteins is highly conserved as E8 exons can be found in more than 300 mammalian PV genomes, including all HPV types in the alpha, beta, gamma and mu-genera (Fertey et al., 2011; Puustusmaa and Abroi, 2016). Both E2TR and E8Ê2 (E8/E2) share the hinge and dimerization/DNA binding domain with E2 and thus are able to form homo- and heterodimers and interact with E2BS in the viral genome (Kurg et al., 2006; McBride et al., 1989). In BPV1 infected cells E2TR is the predominant species followed by E8/E2, whereas E2 is the least abundant form (Hubbert et al., 1988; Kurg et al., 2006; Lambert et al., 1989).

3.2. Phenotypes of E2 repressor knock-out genomes

The analysis of BPV1 E2TR- genomes revealed that E2TR limits BPV1 replication in C127 cells (Table 1) (Lambert et al., 1987; Riese et al., 1990). In contrast, the knock-out of E8/E2 had no discernible phenotype (Table 1) (Lambert et al., 1990). Surprisingly, BPV1 E8/E2-/E2TR- genomes showed both a decreased replication

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