Virology 386 (2009) 353–359

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

Bovine papillomavirus type 1 E2 protein heterodimer is functional in papillomavirus DNA replication in vivo

Reet Kurg [a,*], Piia Uusen [a], Toomas Sepp [c], Mari Sepp [c], Aare Abroi [b], Mart Ustav [a,b]

[a] Institute of Technology, University of Tartu, Nooruse 1, 50411 Tartu, Estonia
[b] Estonian Biocentre, Tartu, Estonia
[c] Institute of Molecular and Cellular Biology, University of Tartu, Estonia

ARTICLE INFO

Article history:
Received 13 October 2008
Returned to author for revision
26 December 2008
Accepted 16 January 2009
Available online 20 February 2009

Keywords:
Papillomavirus
Replication
E2 protein
Heterodimer

ABSTRACT

Papillomaviruses are small DNA viruses that induce epithelial lesions in their host. The viral life cycle is regulated by the family of proteins encoded by the E2 open reading frame. In addition to the full-length E2 protein, the BPV-1 genome encodes two truncated E2 proteins, E2C and E8/E2, which maintain the DNA-binding-dimerization domains, but lack the activation domain. Heterodimers formed between the full-length E2 and truncated E2 proteins serve as activators of E2-dependent transcription and papillomavirus DNA replication. We show that the single activation domain of E2 is sufficient for interaction with viral helicase E1 and for initiation of DNA replication from different papillomavirus origins. Single-chain E2 heterodimer is able to activate papillomavirus DNA replication in the context of entire BPV genome in the absence of other E2 proteins. These data suggest that E2 heterodimers with single activation domain are functional in initiation of papillomavirus replication in vivo.

Introduction

Papillomaviruses are small, double-stranded DNA viruses, which infect a wide variety of vertebrate species and induce proliferative epithelial lesions of skin and mucosa in their host. Studies on BPV1 DNA replication have shown the requirement of two virally encoded proteins, the viral helicase E1 and regulatory protein E2 as well as the origin region containing E1 and E2 binding sites together with A/T-rich region for papillomavirus DNA replication (Ustav and Stenlund, 1991; Ustav et al., 1991). The E1 protein binds with low specificity to the origin of replication, and specific and efficient recognition is achieved by cooperative binding of E1 and E2 to adjacent sites. So, the E2 protein serves as a loading factor and helps to recruit the viral helicase E1 to the origin in the initiation of viral DNA replication within the cells (Sanders and Stenlund, 2001; Sedman and Stenlund, 1995; Sedman et al., 1997).

The E2 open reading frame of BPV1 encodes a family of proteins which form the central regulatory system of the virus, controlling both viral gene expression and replication. The E2 protein is a transcription activator which regulates transcription of viral early promoters through binding to E2 binding sites (Spalholz et al., 1985). E2 participates in initiation of viral DNA replication by loading viral helicase E1 onto the origin of DNA replication (Sedman and Stenlund, 1995; Ustav and Stenlund, 1991), and in virus genome episomal maintenance by tethering viral genomes to mitotic chromosomes during cell division (Ilves et al., 1999; Lehman and Botchan, 1998). E2 is a modular protein consisting of three different structural as well as functional domains; the N-terminal transactivation domain (TAD) (aa 1–200), the C-terminal DNA-binding dimerization domain (DBD) (aa 310–410), and flexible unstructured "hinge region" which functions as a linker between the two functional domains.

In addition to the full-length E2 protein, the BPV-1 genome encodes two truncated E2 proteins. The full-length E2 is coded by the entire E2 ORF (bp 2608–3840 of BPV-1 genome); the E2C is translated from transcript from promoter P_{3080} with initiator codon corresponding to Met162 in E2; and E8/E2 is translated from alternatively spliced mRNA-s with splice donor at 1235 and splice acceptor at 3225, thus fusing 11 aa from E8 ORF to aa 205 of E2 ORF. The two truncated E2 proteins maintain the DNA-binding-dimerization domains of E2, but lack the activation domain, and therefore act as repressors of E2 dependent transcription. The E2 repressors antagonize the full-length E2 function by competing for E2 DNA binding sites (Barsoum et al., 1992; Lambert et al., 1987; Lim et al., 1998). The promoters for full-length E2 as well as for repressors are themselves controlled by E2 (Baker and Howley, 1987; Li et al., 1989). Despite the E2 repressors shorter half-life (Hubbert et al., 1988) they predominate in the steady state (Kurg et al., 2006). However, the ratio of the repressors to activators changes through the cell cycle (Yang et al., 1991) suggesting that the balance of different E2 proteins is a key event in the regulation of virus life cycle. The truncated E2 proteins are able to form heterodimers with full-length E2 through the common C-terminal DBD (McBride et al., 1989), and the E2 heterodimers with single

* Corresponding author. Fax: +372 7374900.
E-mail address: reet.kurg@ut.ee (R. Kurg).

0042-6822/$ – see front matter
doi:10.1016/j.virol.2009.01.025

activation domain are the most abundant form of E2 containing the full-length E2 protein in virus transformed cells (Kurg et al., 2006). Using the E2 single-chain heterodimer as a model, we have shown that BPV1 E2 heterodimers can function as activators of transcription and replication (Kurg et al., 2006).

Most of our knowledge about the role of E2 in papillomavirus DNA replication is based on studies with the full-length E2 homodimer. In this study, we have investigated in detail the localization and replication function of E2 heterodimers with single activation domain. Our data show that the single activation domain of E2 is sufficient to localize the E2 protein into cellular chromatin, for interaction with viral helicase E1 and for initiation of DNA replication from different papillomavirus origins. E2 heterodimer in the context of entire BPV1 genome is able to initiate the papillomavirus DNA replication, but not to maintain it for a long time.

Results

Localization of E2 heterodimers is determined by the activation domain

Our previous study has shown that E2 heterodimers with single activation domain are the preferential form for BPV1 E2 protein in virus-transformed cells as their amount is four times higher than that of the full-length E2 homodimer (Kurg et al., 2006). E2 heterodimer consists of full-length and truncated E2 proteins which cellular compartmentalization is different: the full-length E2 protein is mostly associated with cellular chromatin while truncated E2C protein is in soluble nucleoplasm fraction (Kurg et al., 2005). In order to study the subnuclear localization of E2 heterodimers with single activation domain, we have used biochemical fractionation approach as depicted in (Fig. 1B, (Kurg et al., 2005)). First we carried out fractionation of BPV1-transformed C127 cells, which maintain and replicate the BPV1 genome as an episome and express all three E2 proteins at low, physiological levels (Hubbert et al., 1988; Kurg et al., 2005). To examine the localization of E2 dimers in virus transformed cells, the UV-treated C127-BPV1 cells were biochemically fractionated as shown in Fig. 1B, and E2 proteins were immunoprecipitated with E2-specific 3F12 antibody (Kurg et al., 1999). UV treatment of cells covalently crosslinks E2 dimers through amino acid Trp360 (Prakash et al., 1992) allowing us to follow the fractionation profile of E2 hetero- and homodimers in virus transformed cells. The efficiency of UV-treatment is approximately 50% explaining why we can see both, E2 monomers and E2 heterodimers on the Western blot. As shown on Fig. 1C, E2 homo- and heterodimers with single activation domain fractionated similar to the full-length E2 protein: the E2 homodimer as well as the E2:E2C and E2:E8/E2 heterodimers all fractionated into 0.4 M salt-sensitive chromatin fraction (lanes 4, 5) while E2C homodimer was found in soluble fraction (lane 2). These data show that E2 heterodimers composed of full-length E2 protein and E2 repressor localize into the salt-sensitive nuclear chromatin fraction similar to full-length E2 homodimer.

To determine whether one activation domain is sufficient to localize the E2 protein into cellular chromatin, we analyzed the fractionation profile of "single-chain heterodimer" of E2 (scE2) constructed by fusing the coding region of C-terminal DBD in frame to the full-length E2 (Fig. 1A). scE2 heterodimer could bind to E2 binding sites similar to the full-length E2 suggesting that this molecule is structurally and functionally intact and can be used as a model for E2 heterodimer with single activation domain. Using "single-chain E2 heterodimer" we have seen only formation of

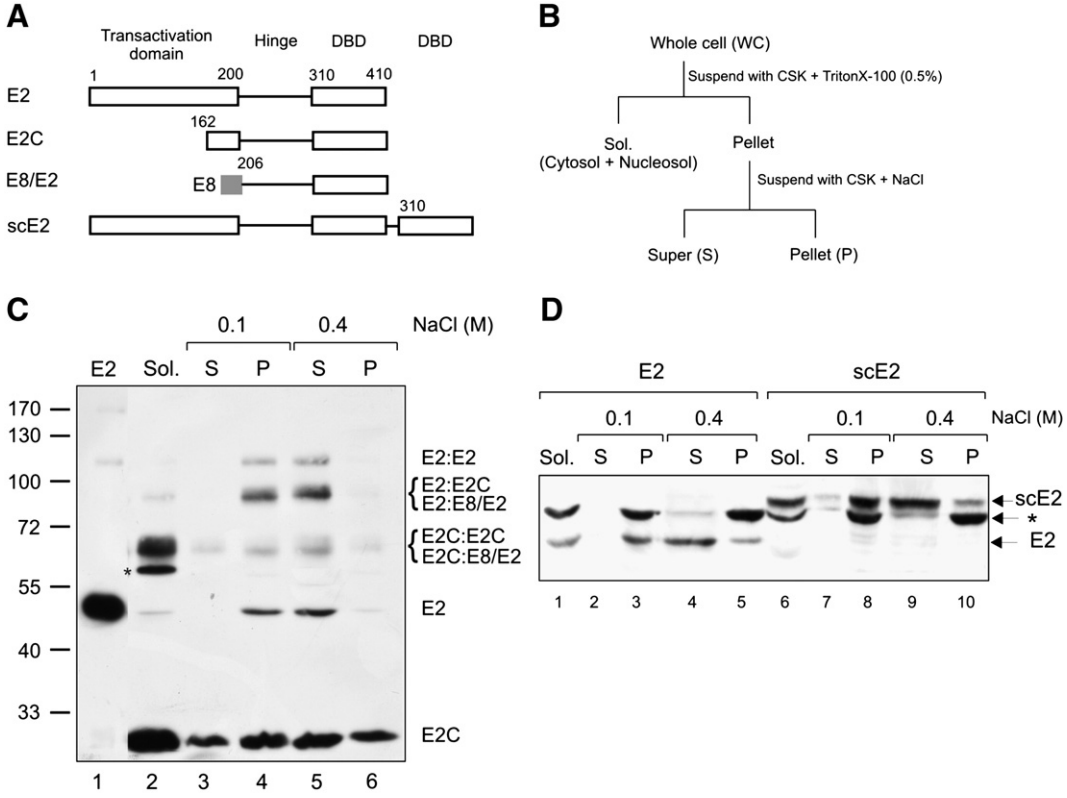

Fig. 1. Subnuclear localization of E2 proteins. (A) Schematic representation of E2 proteins used in this study. (B) Protocol for biochemical fractionation. (C) Subnuclear localization of E2 proteins expressed in BPV1 transformed C127 cells. The UV-treated C127-BPV cells were biochemically fractionated, and E2 proteins were immunoprecipitated from all fractions and analyzed by immunoblotting with E2 specific antibody. Localization of E2 homo- and heterodimers is indicated on the right. Non-specific signal with cellular protein is shown by asterisk. Lane 1, 15 ng of bacterially purified E2 protein. (D) U2OS cells, transfected with 100 ng of expression plasmids for E2 or scE2, were fractionated 24 h after electroporation and subjected to biochemical fractionation. Equal volume of each fraction was subjected to SDS-PAGE and immunoblotted with HRP-conjugated E2-specific antibody 5E11. Non-specific signal with cellular protein is shown by asterisk.