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Rapid Communication

Amino acid substitutions that specifically impair the transcriptional activity of papillomavirus E2 affect binding to the long isoform of Brd4

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Abstract

The E2 protein of papillomaviruses binds to specific sites in the viral genome to regulate its transcription, replication and segregation in mitosis. Amino acid substitutions in the transactivation domain (TAD) of E2, of Arg37 and Ile73, have been shown previously to impair the transcriptional activity of the protein but not its ability to support viral DNA replication. To understand the biochemical basis of this defect, we have used the TADs of a low-risk (HPV11) and a high-risk (HPV31) human papillomavirus (HPV) as affinity ligands to capture proteins from whole cell extracts that can associate with these domains. The major TAD-binding protein was identified by mass spectrometry and western blotting as the long isoform of Brd4. Binding to Brd4 was also demonstrated for the E2 TADs of other papillomaviruses including cutaneous and animal types. For HPV11, HPV31 and CRPV E2, we found that binding to Brd4 is significantly reduced by substitutions of Arg37 and Ile73. Since these amino acids are located near each other in the 3-dimensional structure of the TAD, we suggest that they define a conserved surface involved in binding Brd4 to regulate viral gene transcription.

Keywords: Papillomavirus; E2; Brd4; Bromodomain-containing protein 4; Transcription; Protein–protein interaction; Viral–host interaction

Introduction

Papillomaviruses are a family of small double-stranded DNA viruses that induce benign and malignant hyperproliferative lesions of the differentiating epithelium (reviewed in Zur Hausen and de Villiers, 1994; Lowy and Howley, 2001; Hebner and Laimins, 2005). These viruses infect the basal cell layer where they maintain their double stranded DNA genome as a circular episome in the nucleus of infected cells. Maintenance of the HPV episome in primary keratinocyte cultures requires four viral proteins: the two viral oncogenes, E6 and E7, the E1 replicative helicase and the E2 protein (Howley and Lowy, 2001; Longworth and Laimins, 2004). E2 is a multifunctional protein that binds to specific sites in the regulatory region of the viral genome to promote its replication, regulate its transcription and ensure its proper segregation to daughter cells at mitosis (reviewed in Blachon and Demeret, 2003). E2 is comprised of two functional domains, a N-terminal transactivation domain (TAD) and a C-terminal DNA-binding/dimerization domain separated by a hinge region (Figs. 1A and B). The TAD has been shown to be a protein interaction module that binds to the viral E1 helicase to promote replication of the genome and to cellular transcription factors to regulate viral gene transcription (reviewed in Blachon and Demeret, 2003; Hebner and Laimins, 2005). As a transcription factor, E2 has been shown to act either as an activator or a repressor, depending on the promoter context. Reporter gene assays have shown that E2 can activate transcription from a minimal promoter

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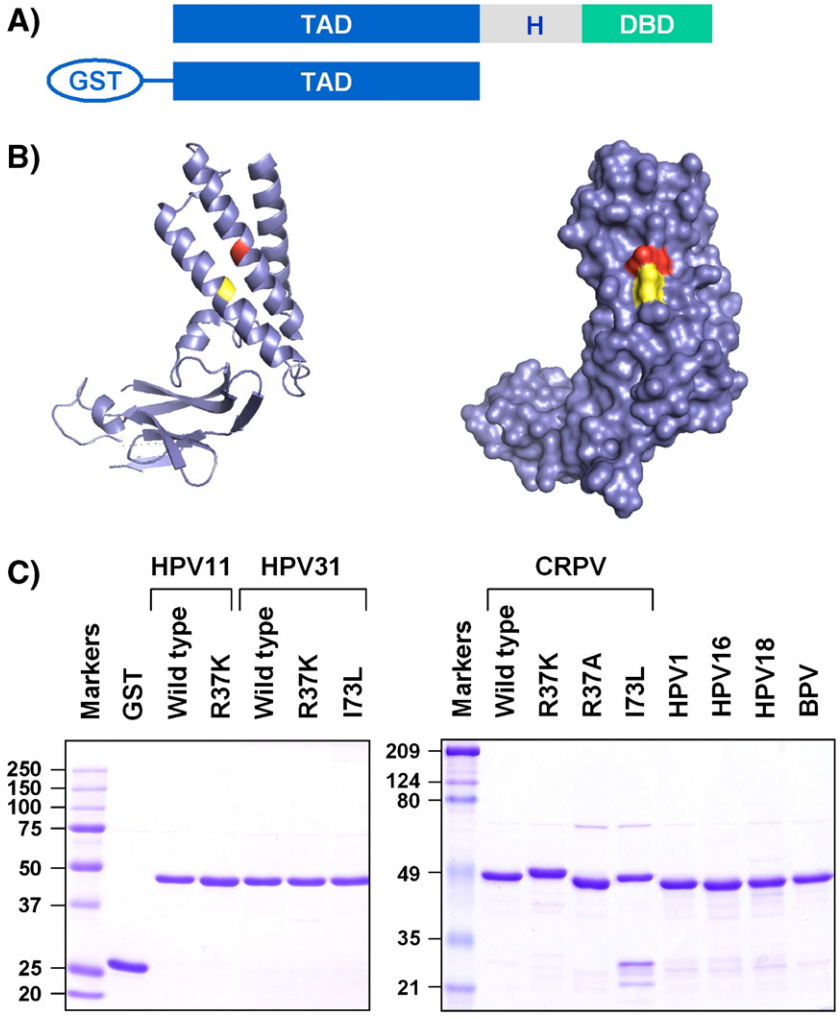

Fig. 1. Purified GST-E2 TAD proteins used in this study. (A) Domain structure of the complete E2 protein. The transactivation domain (TAD) is linked by a hinge (H) to the C-terminal dimerization and DNA binding domain (DBD). (B) Ribbon (left) and surface (right) representations of the HPV11 E2 TAD (Wang et al., 2004). Arg37 and Ile73 have been colored in red and yellow, respectively. (C) Purified GST-E2 TAD proteins used in this study. 3 μg of each protein was separated on a 10% SDS-PAGE and stained with Coomassie blue. The sizes (kDa) of the molecular weight markers are shown on the left.

under the control of multimerized E2 binding sites (Kovelman et al., 1996). In contrast, in the context of the viral genome, E2 is primarily a repressor of viral transcription initiated in the LCR (Thierry and Yaniv, 1987; Bernard et al., 1989; Demeret et al., 1997; Soeda et al., 2006). As a segregation factor, E2 tethers the viral episome to mitotic chromatin (Lehman and Botchan, 1998; Skiadopoulos and McBride, 1998; Bastien and McBride, 2000; Ilves et al., 1999). Studies using the E2 protein from bovine papillomavirus (BPV) have shown that the long isoform of the bromodomain-containing protein 4, Brd4(L), is the receptor to which E2 binds on mitotic chromosomes (You et al., 2004, 2005; McPhillips et al., 2005; reviewed in McBride et al., 2004). However, a recent study by Oliveira et al. (2006) has suggested that there are important differences in the way that alpha-papillomavirus E2, like those of HPV11 and HPV31, bind to mitotic chromosomes. One group also suggested that HPV11 E2 associates primarily with the mitotic spindle and with centrosomes, rather than chromatin in mitosis (Van Tine et al., 2004).

Mutagenesis of the E2 TAD led to the identification of amino acid substitutions that genetically separate the transcription from the replication function of the protein. In particular, substitutions of arginine 37 for alanine and of isoleucine 73 for leucine or alanine have been shown to impair the ability of E2 to activate and repress transcription while having little or no effect on its ability to interact with E1 and support viral DNA replication (Abroi et al., 1996; Breiding et al., 1996; Brokaw et al., 1996; Ferguson and Botchan, 1996; Sakai et al., 1996; Grossel et al., 1996; Cooper et al., 1998; Nishimura et al., 2000). Interestingly the conserved R37 and I73 are located close to each other at the surface of the TAD, in a region opposite to that involved in binding the E1 helicase (Fig. 1B). To understand the biochemical basis of this defect, we have used the TADs of a low-risk (HPV11) and a high-risk (HPV31) HPV type as affinity ligands to capture proteins from whole cell extracts that can associate with wild type but not transactivation-defective versions of these domains.