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Interactions of the cellular CCAAT displacement protein and human papillomavirus E2 protein with the viral origin of replication can regulate DNA replication

Janaki Narahari [a,b], John C. Fisk [c], Thomas Melendy [c], Ann Roman [a,b,*]

[a] Department of Microbiology and Immunology, Indiana University School of Medicine and Walther Cancer Institute, Indianapolis, IN 46202, USA
[b] Walther Oncology Center, Indiana University School of Medicine and Walther Cancer Institute, Indianapolis, IN 46202, USA
[c] Departments of Microbiology and Immunology and Biochemistry, University at Buffalo School of Medicine and Biomedical Sciences, Buffalo, NY 14214, USA

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Abstract

Previously, we and others have shown that CCAAT displacement protein (CDP) negatively regulates the papillomavirus promoters. Overexpression of CDP has been shown to inhibit high-risk human papillomavirus virus (HPV) and bovine papillomavirus DNA replication in vivo presumably through reduction in expression of viral replication proteins, E1 and E2. Sequence analysis of the HPV origin indicates several potential CDP-binding sites with one site overlapping the E1-binding site. Therefore, CDP could also negatively regulate papillomavirus replication directly by preventing the loading of the initiation complex. We show here that purified CDP inhibits in vitro HPV DNA replication. Footprint analysis demonstrated that CDP binds the E1-binding site and the TATA box, and that the binding of purified CDP to the E1-binding site is decreased by the addition of purified E2 protein. Consistent with this, E2-independent in vitro HPV replication is inhibited by CDP to a greater extent than E2-dependent replication. These results suggest that binding of E2 at the E2-binding site may play an important role in overcoming the inhibition of E1 initiation complex formation caused by the binding of negative regulators like CDP to the origin of replication.

Keywords: Human papillomaviruses; Regulation of HPV replication; CCAAT displacement protein

Introduction

Human papillomaviruses (HPVs) are double-stranded DNA viruses that infect mucosal or cutaneous epithelia causing hyperproliferative lesions or warts. HPVs are considered etiologic agents for the development of cervical cancer (zur Hausen, 2002). The papillomaviruses are termed 'high risk' if they are associated with development of cancer (e.g., HPV 16, 18, and 31) or 'low risk' when seen in warts but not typically associated with cancer [e.g., HPV 6 and 11 (zur Hausen, 2002)].

All papillomaviruses have a similar genomic organization divided into three regions: the long control region (LCR) which contains the major cis regulatory sequences and the origin of replication, the early region that encodes six to eight proteins, and the late region that codes for 2 late structural proteins (Laimins, 1993; Turek and Smith, 1996). In the case of low-risk HPV, three promoters have been identified, the E6, E7, and E1 promoters (Karlen et al., 1996; Rapp et al., 1997; Smotkin et al., 1989). High-risk HPV appears to have only E6 and E1 promoters (Smotkin et al., 1989), although a putative E7 promoter has been recently identified in HPV 16 (Glahder et al., 2003). All the promoters give rise to polycistronic messages. In infected epithelia, early genes are expressed in the dividing cells of the basal cell layer, and as the cells divide, viral DNA is maintained at approximately 50–100 episomal copies per cell by limited replication. The productive amplification takes place as the cells differentiate (Stubenrauch and Laimins, 1999). Very

* Corresponding author. Department of Microbiology and Immunology, Indiana University School of Medicine, Indianapolis, IN 46202, USA. Fax: +1 317 278 3331.
E-mail addresses: jnarahar@iupui.edu (J. Narahari), johnfisk@buffalo.edu (J.C. Fisk), TMelendy@buffalo.edu (T. Melendy), aroman@iupui.edu (A. Roman).

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little is known about the changes within the cells that promote this substantial increase in viral replication.

The E1 and E2 proteins and the papillomavirus origin are essential for viral DNA replication in vivo (Chiang et al., 1992; Kuo et al., 1994; Remm et al., 1992; Ustav and Stenlund, 1991; Yang et al., 1991). The remaining machinery necessary for DNA replication is derived from the host cell. While both E1 and E2 proteins participate in formation of the pre-initiation complex, E1 alone is sufficient for initiation and elongation of viral DNA replication in vitro (Kuo et al., 1994; Seo et al., 1993; Stenlund, 2003; Yang et al., 1993). The binding sites for E1 are highly conserved among papillomaviruses (Wilson et al., 2002). The E1 DNA-binding site consists of an A + T-rich palindromic sequence. Using various techniques, e.g., DNAse I footprinting, mutational analysis of the binding sites, and sequence analysis, the binding site for E1 was shown to be an 18 bp imperfect palindrome (Frattini and Laimins, 1994a, 1994b; Sun et al., 1996; for a review, see Wilson et al., 2002). The E1-binding site in HPV 11 was found to be ACTTAATAACAATCTTAG (Sun et al., 1996). Using the DNA-binding domain of BPV E1, variants of an AACAAT sequence were identified as the basic E1 DNA recognition site. There are 6 copies of these sequences arranged in overlapping arrays in E1-binding sites (Chen and Stenlund, 2001). E1 proteins expressed and purified from bacteria or insect cells have been shown to have DNA binding, ATPase and helicase activities (Bream et al., 1993; Hughes and Romanos, 1993; White et al., 2001). The papillomavirus E2 protein has sequence specific DNA-binding activity. The E2-binding site was mapped to a 12-bp palindromic sequence ACCN6GGT (Androphy et al., 1987; Frattini and Laimins, 1994a; Hawley-Nelson et al., 1988; Hirochika et al., 1988; Li et al., 1989; McBride et al., 1989) which is present in multiple copies in papillomavirus genomes. There is a high degree of similarity between various papillomavirus origins of replication as well as among the replication proteins. E1 and E2 proteins from HPV11 and BPV1 can efficiently replicate a plasmid containing the LCR of HPV 6b and also the origins of many other human and animal papillomaviruses to various degrees [data not shown and (Conger et al., 1999; Zou et al., 1998)].

In nearly all systems tested to date, several transcription factors play important roles in DNA replication. Transcription factors can activate replication through various mechanisms, including inducing conformational changes in DNA structure making it more accessible for binding of initiation proteins, by remodeling chromatin, and/or by recruiting replication proteins to initiation complexes (Heintz, 1992 and references therein). In papillomaviruses, the E2 protein functions as both a replication and a transcription factor. It binds to and increases the specificity and affinity of origin binding of papillomavirus E1 protein for the formation of the replication initiation complex (Chiang et al., 1992; Frattini and Laimins, 1994a; Kuo et al., 1994; Muller et al., 1994; Sedman and Stenlund, 1995; Stenlund, 2003; Yang et al., 1991; Zou et al., 1998). Also, papillomavirus replication is inhibited by transcription factors such as Yin-yang 1 (YY1), CCAAT displacement protein (CDP), and the TATA-binding protein (TBP). TBP prevents formation of the HPV 11 E1E2 complex at the origin of replication in vitro (Hartley and Alexander, 2002), while YY1 has been demonstrated to inhibit replication of high-risk and low-risk viruses in vivo by sequestering the E2 protein (Lee et al., 1998). Using transient co-transfection of an HPV 16 origin containing plasmid and a CDP expression vector into cells stably expressing E1 and E2 proteins, overexpression of CDP was shown to repress replication of HPV 16 in vivo (O'Connor et al., 2000). A similar effect of CDP on the replication of HPV 31 and BPV1 using cell lines containing episomal copies of the respective viral DNA was also reported (O'Connor et al., 2000). The mechanism underlying the repression was not established, although potential CDP-binding sites were identified by sequence analysis in the E1 DNA-binding region. CDP and YY1 both regulate gene expression from the promoters of papillomaviruses, thereby controlling the amounts of E1 and E2 proteins being expressed. While CDP inhibits transcription from all promoters of low- and high-risk papillomaviruses (Ai et al., 1999, 2000; O'Connor et al., 2000), YY1 regulates the E1 promoter of the low-risk papillomavirus (Ai et al., 2000) and the E6 promoter of the high-risk viruses (Bauknecht et al., 1992; Kanaya et al., 1997; May et al., 1994). YY1 does not bind the E1 promoter of high-risk viruses (Ai et al., 2000). Therefore, the control of replication exerted by CDP and YY1 could be multifaceted.

CDP is expressed in undifferentiated but not in differentiated cells (Ai et al., 1999; Pattison et al., 1997 and reviewed in Nepveu, 2001). Full-length CDP has four highly conserved DNA-binding domains, of which three are Cut repeats (CR1, CR2, and CR3), and the fourth is a homeodomain (HD) (Andres et al., 1994; Aufiero et al., 1994; Harada et al., 1995; Neufeld et al., 1992). CDP binds to a wide range of DNA-binding sequences with relaxed sequence specificity (Narahari and Roman, 2002; Nepveu, 2001). The reported sequences include CCAAT, ATCGAT, TCGATAA, ATCGATTA, GGGGCGGTTGTATATCAGGGCC, Sp1 sites, and AT-rich matrix attachment regions (Chattopadhyay et al., 1998; Coqueret et al., 1998; Liu et al., 1997; Luo and Skalnik, 1996; Wang et al., 1999).

This report shows that CDP specifically inhibits in vitro replication of a plasmid containing the HPV 11 origin of replication. The DNA-binding sites for CDP on the HPV 6 and 11 origin of replication were mapped to three sites using the nuclease protection assay. CDP inhibits E2-independent in vitro replication of HPV 11 to a greater extent than E2-dependent replication. Data are also presented to show that E2 decreases the binding of CDP at the E1-binding site. Based on our present and previous results, we conclude that CDP inhibits papillomavirus replication during the initial infection by (1) binding the HPV promoters and inhibiting transcription of the replication proteins in undifferentiated cells and (2) competing with the replication proteins for DNA binding. We therefore propose that E2, in addition to enhancing the binding of E1, plays an important role in replication initiation by outcompeting host cell negative regulators, such as CDP, for the viral origin of replication.