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Transactivation of the human papilloma virus 16 octamer motif by the octamer binding protein Oct-2 requires both the N and C terminal activation domains

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ABSTRACT

The upstream regulatory region (URR) of the human papillomaviruses HPV16 and 18 contains a sequence with a seven out of eight base match to the consensus binding site for octamer binding transcription factors. This motif acts as a target for repression by the Oct-1 transcription factor and therefore inhibits promoter activity in non-cervical cells expressing only Oct-1. In contrast the HPV octamer motif activates promoter activity in cervical cells. Here we show that cervical cells express the activating form of the Oct-2 transcription factor, Oct 2.1 and that this factor can transactivate promoter activity via the HPV16 octamer. This effect is dependent upon both the N and C-terminal activation domains of Oct-2. The expression of specific octamer binding proteins such as Oct-2 in cervical cells thus allows the HPV16 motif to produce opposite effects on gene expression in cervical and non-cervical cells suggesting that it may play a role in the cervical specificity of URR driven gene expression.

INTRODUCTION

The upstream regulatory region (URR) of the human papillomaviruses HPV16 and 18 drives the expression of the genes encoding the E6 and E7 transforming proteins and is preferentially active in cells of epithelial origin (1, 2) paralleling the strict epithelial tropism of these viruses (for reviews see 3, 4). However, the majority of the cellular transcription factors which bind to the URR such as NFI (5) API (6) and the glucocorticoid receptor (7) are ubiquitously expressed and it is therefore unclear how the epithelial specificity of the URR is produced.

Both our laboratory (8) and others (9–11) have recently shown that a site adjacent to one of the NFI sites in the URR of HPV16 and HPV18 (positions 7731 to 7738 in the HPV16 URR) can act as a binding site for cellular octamer binding proteins (for review see 12). In particular we showed (13) that this site can bind the ubiquitous octamer binding protein Oct-1 and that such binding results in an inhibition of promoter activity in non cervical cells which express only Oct-1. However, under certain conditions it appears that this motif can also act as a target for transactivation by specific octamer binding proteins. Thus in cells of cervical origin the presence of this octamer motif enhances promoter activity suggesting that it may play a role in the tissue specific activity of the URR by binding transactivating octamer binding proteins which are present in cervical cells but absent in other cell types. In addition to Oct-1, cervical cells contain both a previously uncharacterized cervical-specific octamer binding protein and the tissue specific octamer binding protein Oct-2 (8) which is also found in B cells (14), neuronal cells (15) and the testis (16) but is absent in most other cell types. Although the cervical-specific octamer binding protein is likely to have an important role in the cervical-specific activity of the URR, the finding that in B cells, Oct-2 acts as a strong activator of octamer containing promoters (17, 18) suggests that it may also play a role in cervical cells. We have therefore further investigated the expression of Oct-2 in cervical cells and its effect on the octamer motif in the HPV16 URR.

MATERIALS AND METHODS

Polymerase chain reaction

Total RNA was prepared from human cervical tissue by the guanidinium isothyocyanate procedure (19) and used as a template for the production of cDNA using random hexanacleotide primers (Pharmacia LKB Biotechnology Ltd.). The cDNA equivalent to 0.01 μg of total RNA was then amplified by polymerase chain reaction according to the method of Kawasaki (20) using twenty cycles of amplification and primers which flank the alternatively spliced region of Oct 2.1, 2.4 and 2.5 (see reference 21 and Figure 1). The identity of the PCR product obtained in each case was verified by Southern blot hybridization with an Oct-2 cDNA probe.

DNA transfection

Transfection of plasmid DNA was carried out according to the method of Gorman (22). Standard transfections were carried out using 10 μg of DNA per 2×10^6 cells on a 90 mm plate. In

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experiments where the effect of Oct-2 on gene expression was being assessed, 10 μg of the reporter plasmid was co-transfected with the indicated amounts of the Oct-2 expression vector with the amount of transfected DNA in each sample being equalized with the parental plasmid. Twenty four hours after transfection cells were harvested for CAT assays. Transfections included a control plasmid in which the MPSV promoter drives expression of the β-galactosidease gene to control for any effects of the experimental plasmids on transfection efficiency or on gene expression driven by an irrelevant promoter.

CAT assays

Assays of CAT activity were carried out as described by Gorman (22) with extracts which were equalised for protein content, as determined by the method of Bradford (23).

RESULTS AND DISCUSSION

Before testing the effect of Oct-2 on the HPV16 octamer motif, it was necessary to define the forms of Oct-2 which are expressed in cervical cells. Thus, although Oct-2 acts as an activator in B cells, it appears to act primarily as a repressor of octamer-containing promoters in neuronal cells (21, 24). This tissue specific difference in activity is controlled by alternative splicing of the Oct-2 RNA with B cells producing predominantly the mRNA encoding Oct 2.1 which contains an intact C-terminal activation domain whereas neuronal cells synthesize predominantly Oct 2.4 and 2.5 which have an altered C-terminal region (Figure 1: 21, 25).

To determine the forms of Oct-2 present in cervical cells, cDNA prepared from human cervical RNA was amplified by the polymerase chain reaction (PCR) using a pair of oligonucleotides which flank the region which is alternatively spliced resulting in the production of the different mRNAs encoding Oct 2.1, 2.4 and 2.5 (Figure 1; for further details see reference 21). The resulting PCR products were electrophoresed on an agarose gel allowing the resolution of the PCR product derived from the Oct 2.1 mRNA from that of the Oct 2.4 mRNA (which lacks 148 base pairs) and that of the Oct 2.5 mRNA (which contains an additional 74 base pairs: 25).

In these experiments (Figure 2) cervical RNA exhibited a high proportion of the Oct 2.1 mRNA with only relative low levels of the Oct 2.4 and 2.5 forms. Hence cervical cells appear to resemble B cells rather than neuronal cells in having a preponderance of the activating form of Oct-2, allthough it should be noted that the proportion of Oct 2.1 in the cervical sample was not as high as we have previously observed in B cells (21).

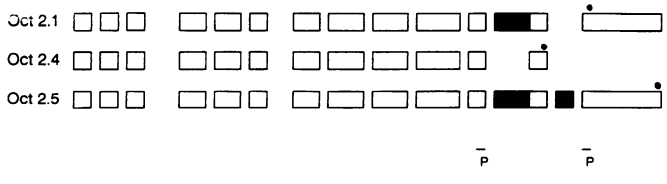


Figure 1. Structure of the different Oct-2 isoforms, Oct 2.1, Oct 2.4 and Oct 2.5. Exons are indicated by boxes with the alternatively spliced exons shaded. Dots indicate the positions of in frame translational stop codons. Arrows labelled P indicate the positions of oligonucleotides used in the polymerase chain reaction to determine the relative proportions of the mRNAs encoding each of the isoforms. Modified from reference 25.

In order to confirm that the Oct 2.1 protein was present in the cervical cells, we carried out a gel shift experiment. In this experiment (Figure 3) a complex of the mobility expected for Oct 2.1 binding to the probe was observed in both the B cell extracts and the cervical extract but was absent in the extract prepared from BHK cells which express only Oct-1. An additional higher mobility complex formed by the cervical-specific octamer binding protein we have previously described (8) was also observed in the cervical extract as expected. The levels of binding

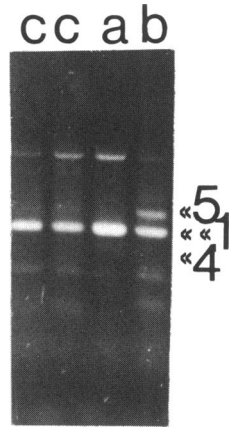


Figure 2. Polymerase chain reaction amplification of cDNA prepared from human cervical RNA (tracks labelled C), A20 lymphocyte RNA (**A**) or brain RNA (**B**) using the primers indicated in Figure 1. The arrows indicate the positions of the predicted PCR products derived from the mRNAs encoding Oct 2.1 (267 base pairs) 2.4 (219 base pairs) and 2.5 (341 base pairs).

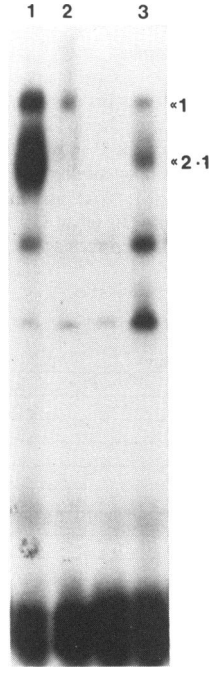


Figure 3. DNA mobility shift assay using a consensus octamer oligonucleotide and extracts prepared from Namalwa B cells (track 1), BHK fibroblasts (track 2) and CaSki cervical cells (track 3). The arrows indicate the complexes formed by Oct-1 and Oct-2.1.

to the probe were similar for Oct-1 and Oct 2.1 suggesting that Oct 2.1 is expressed in cervical cells at physiologically relevant levels to allow it to counteract the inhibitory effect of Oct-1.

To establish whether Oct 2.1 could transactivate the octamer motif in the HPV16 URR we used a construct (13) in which the octamer motif and the adjacent NF1 site (bases 7729–7747 in the HPV16 URR) had been cloned into the Bam HI site in the vector pBL CAT2 which contains the herpes simplex virus thymidine kinase promoter from −105 to +51 driving expression of the chloramphenicol acetyl transferase (CAT) gene (26). This plasmid was co-transfected using the calcium phosphate procedure (22) into BHK-21 cells (27) which contain only Oct-1 (13) together with a plasmid in which the cytomegalovirus (CMV) immediate-early promoter drives the expression of an Oct 2.1 cDNA (25). For comparison the HPV octamer-containing plasmid was also co-transfected with CMV expression vectors driving the expression of Oct 2.4 or Oct-1 as well as the control vector lacking any insert (28).

In these experiments (Figure 4) Oct 2.1 was clearly able to enhance the activity of the construct containing the HPV16 octamer motif compared to the activity observed with plasmid

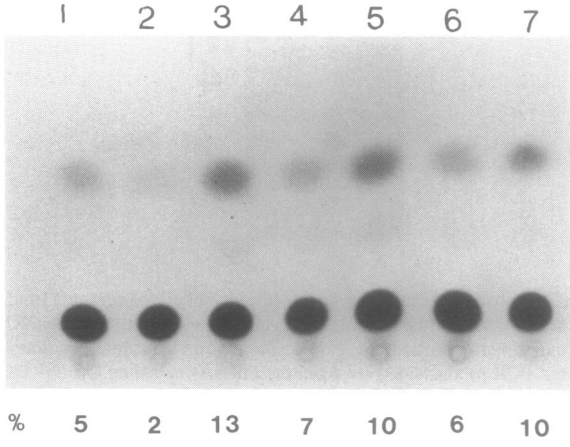


Figure 4. Assay of chloramphenicol acetyl transferase activity following transfection of BHK cells. Tracks 1–4 show the results obtained by co-transfecting a construct containing the HPV16 octamer and adjacent NFI site in the vector pBL CAT2 and either plasmid vector (track 1) an Oct-1 expression clone (track 2), an Oct 2.1 expression clone (track 3) or an Oct 2.4 expression clone (track 4). Tracks 5–7 show the results upon similar co-transfection of the equivalent region from HPV6 in the vector pBL_2 CAT and either plasmid vector (track 5), an Oct 2.1 expression vector (track 6) or an Oct 2.4 expression vector (track 7). The figures indicate the normalized percentage of the available chloramphenicol acetylated in each case.

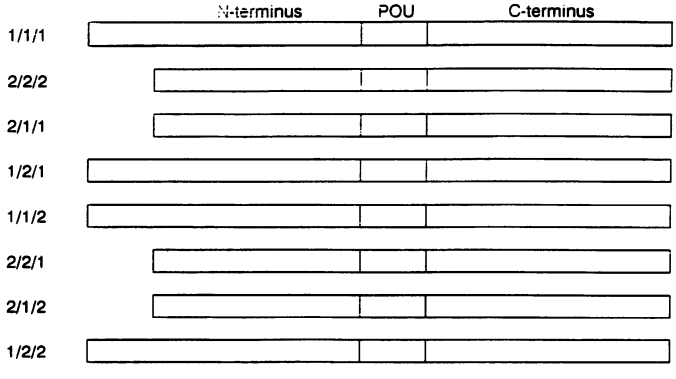
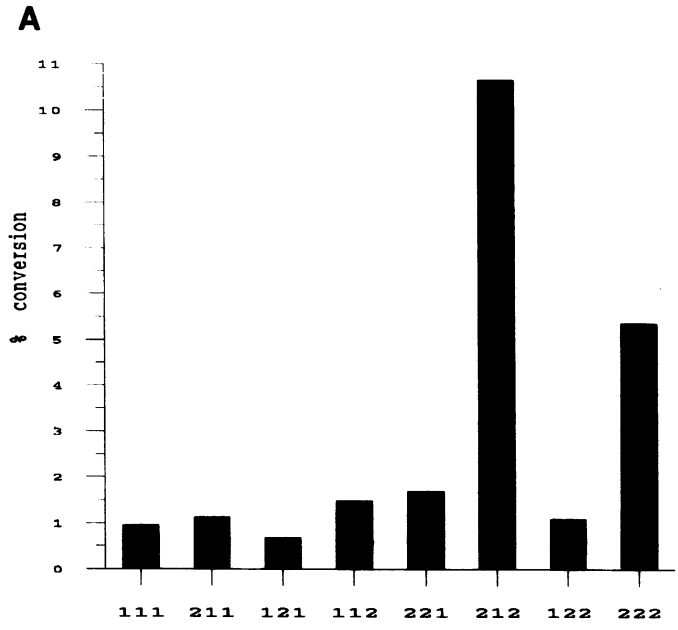


Figure 5. Structure of the Oct 1/Oct 2 chimaeric plasmids used in this study. The regions derived from Oct-1 are shaded.



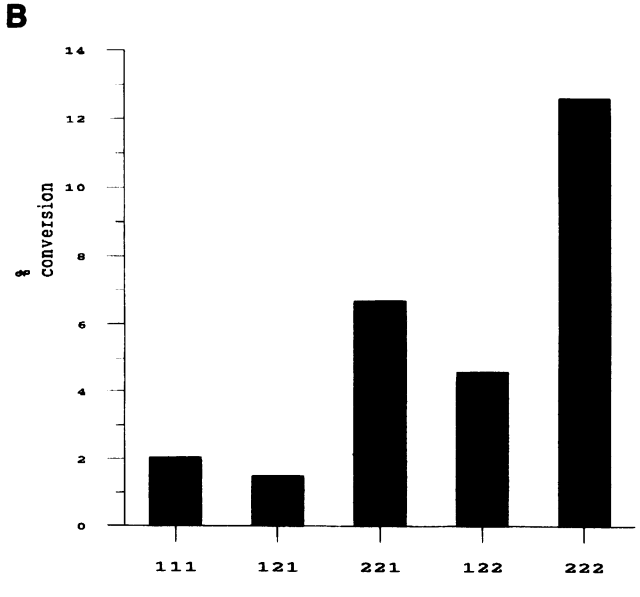


Figure 6. Assay of chloramphenicol acetyl transferase activity upon co-transfection of plasmids containing either the HPV16 octamer and adjacent NFI site (panel **a**) or a consensus octamer motif (panel **b**) cloned in the vector pBL CAT2 with the plasmids expressing the indicated chimaeras of Oct 1/Oct 2 illustrated in Figure 5.

vector alone. In contrast Oct 2.4 had virtually no effect on promoter activity and Oct-1 inhibited promoter activity compared to that observed with plasmid vector alone in agreement with our previous results (13). Similar transactivation by Oct 2.1 was observed on a construct containing the HPV16 octamer motif without the adjacent NF1 site (data not shown). Transactivation by Oct 2.1 was dependent upon the presence of the HPV16 octamer motif since neither Oct 2.1 or Oct 2.4 transactivated a pBL CAT2 plasmid containing the equivalent region from HPV6 (Figure 4) which contains an NFI site but lacks a functional octamer motif capable of binding proteins and is therefore not inhibited by Oct-1 (13). Indeed Oct 2.1 actually reduced the level of gene expession directed by the HPV6 motif probably via a non-specific squelching effect. Hence as well as acting as a target for inhibition by Oct-1, the HPV16 octamer motif can also activate promoter activity in the presence of other octamer binding proteins such as Oct-2. In view of the expression of Oct 2.1 in cervical cells but not in fibroblasts, the divergent effects of Oct-1 and Oct 2.1 on the HPV16 octamer motif are likely to play a critical role in the opposite effects of this motif on gene expression in cervical and non cervical cells (13).

To determine the basis for the different effects of Oct-1 and Oct 2.1 we used a series of constructs which following transfection can direct the expression of similar levels of molecules in which either the N or C termini or the central POU domain of Oct-1 had been exchanged for the equivalent region of Oct 2.1 (Figure 5; 29). These constructs were transfected into BHK cells with the HPV16 octamer construct and CAT activity measured as before. In these experiments (Figure 6a), the construct containing intact Oct-2 and the construct with the N and C terminal domains of Oct-2 and the POU domain of Oct-1 (designated 212, Figure 5) both strongly transactivated the HPV16 octamer construct with the 212 construct actually producing a higher level of promoter activity than intact Oct-2. Thus any differences in the POU domains of Oct-2 and Oct-1 are not responsible for the difference in their effect on gene activity. In contrast replacement of either the N or C terminal regions of Oct-2 with the equivalent region of Oct-1 (as in the 211, 221, 112 and 122 constructs) resulted in much lower levels of promoter activity whilst replacement of both these regions (in the 121 construct) resulted in a level of promoter activity lower than that observed with intact Oct-1.

These results indicate therefore that differences in both the N and C terminal domains of Oct-1 and Oct-2 are responsible for the differences in their effects on the HPV16 octamer motif with both these domains of Oct-2 being required for trans-activation via this motif. Thus Oct 2.4 which has a truncated C-terminal region fails to activate the HPV16 construct whilst the larger Oct-1 protein represses its activity since it cannot transactivate and prevents the binding of the NFI activator to its adjacent site (13).

Previous studies have indicated that Oct-2 contains both N and C-terminal activation domains and that functional co-operation between these domains is required for maximal transactivation of a promoter containing a consensus octamer motif (29, 30). A similar requirement for both the N and C-terminal activation domains is also observed therefore in the case of the HPV16 octamer. However the requirements for trans-activation via a consensus octamer motif appear to be less stringent than for the HPV16 octamer (which differs from the consensus by one base). Thus a consensus octamer motif can be activated by a construct containing the C-terminus derived from Oct-2 and the N terminus derived from Oct-1 (29) whereas in the case of the HPV16 motif the 122 construct produced only weak promoter activity. In order to directly confirm this difference between the HPV 16 motif and the consensus motif, we cloned a consensus octamer motif into the pBL CAT2 vector and co-transfected it with the chimaeric Oct 1/2 constructs. In this experiment (Figure 6b), the consensus octamer was transactivated by constructs containing either the N or the C terminal region of Oct-2 alone although maximal transactivation required as expected that both these regions were derived from Oct 2. Hence either the N or C terminal activation domains of Oct-2 can transactivate a consensus octamer motif when combined with the appropriate region of Oct-1 whereas this is not the case for the HPV16 octamer.

In summary therefore it is clear that the HPV16 octamer motif can act as a target for transactivation by octamer binding proteins but is only transactivated by those octamer binding proteins such as Oct-2 with strong N and C-terminal activation domains. In turn such stringent requirements for transactivation allow this motif to exert a tissue specific effect on promoter activity, resulting in repression of activity in non-cervical cells expressing Oct-1 and activation in cervical cells expressing other octamer binding proteins such as Oct-2 and the cervical-specific octamer binding protein we have previously characterised (8).

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