Nucleic Acids Research, 1993, Vol. 21, No. 4 1019–1023

The octamer binding site in the HPV16 regulatory region produces opposite effects on gene expression in cervical and non-cervical cells

Peter J.Morris, Carolyn L.Dent+, Christopher J.Ring and David S.Latchman*
Medical Molecular Biology Unit, Department of Biochemistry, Division of Molecular Pathology, University College London School of Medicine, The Windeyer Building, Cleveland Street, London W1P 6DB, UK

Received September 24, 1992; Revised and Accepted January 18, 1993

ABSTRACT

The upstream regulatory region (URR) of the tumorigenic human papillomaviruses HPV 16 and 18 contains an octamer binding site which is located adjacent to a binding site for the ubiquitous transcription factor NFI. The octamer site binds both the constitutively expressed transcription factor Oct-1 and a novel cervical octamer binding protein. In contrast the URR of the non-tumorigenic viruses HPV6 and HPV11 lacks the octamer binding site although the adjacent NFI site is conserved. Inactivation of the octamer binding site results in a higher level of gene expression in cells which contain only Oct-1 and a lower level in cells containing the cervical octamer binding protein indicating that whilst Oct-1 binding reduces promoter activity, the cervical protein increases it. In agreement with this, over-expression of Oct-1 reduces the level of gene activity directed by this region of the HPV 16/18 URR and inhibits its activation by NFI whilst having no effect on the corresponding region of the HPV 6/11 URR. The significance of these effects is discussed in terms of the cervical-specific activity of the HPV16/18 URR and its role in HPV-mediated transformation.

INTRODUCTION

The human papillomaviruses (HPV) types 16 and 18 which are believed to play a central role in the development of cervical carcinoma exhibit a strict epithelial tropism infecting only the genital mucosa and perigenital epidermis (for reviews see 1, 2). This epithelial tropism is likely to be dependent upon the viral upstream regulatory region (URR) which drives the expression of the genes encoding the E6 and E7 transforming proteins and which is preferentially active in cells of epidermal origin (3, 4). In turn this epithelial specificity of the URR appears to be dependent upon the interplay of several different cellular transcription factors which bind to regulatory sites within the URR (5, 6). Pardoxically however, the great majority of the cellular factors which have been shown to bind to the URR such as NFI (6) API (7) and the glucocorticoid receptor (5) are expressed in all cell types raising the question of how the epithelial-specificity of the URR is produced.

Interestingly, one of the five NFI sites in the URR of HPV 16 and HPV 18 is located adjacent to a sequence (bases 7731 to 7738 in the HPV 16 URR) with a seven out of eight base match to the consensus binding site for cellular octamer binding transcription factors (see Figure 1; for review see 8). The existence of a large family of octamer binding proteins many of which are expressed specifically in different cell types such as B cells (9), embryonal carcinoma cells (10) and the testis (11) suggested the possibility that this site might play a role in the epithelial specificity of the URR by binding an epithelial-specific octamer binding protein. In agreement with this idea several groups have recently shown that this site does indeed represent a functional binding site for octamer binding proteins (12–14) and we have demonstrated that it can bind both the constitutively expressed octamer binding protein Oct-1 and a novel octamer binding protein expressed in cervical cells (15).

Although this octamer binding site is perfectly conserved in the URR of the tumorigenic viruses HPV 16 and HPV 18, inspection of the equivalent region in the URR of HPV 6 and HPV 11, which cause only benign condylomatous warts, revealed a much reduced homology to the octamer motif, although the NFI site remained intact (see Figure 1). We have therefore tested the effect of these changes on the ability of the HPV6/11 sequence to bind Oct-1 and the cervical protein. In addition we have also investigated the ability of the HPV16/18 and HPV6/11 sequences to drive gene expression in both cervical and non-cervical cell types as well as to respond to the over-expression of Oct-1.

MATERIALS AND METHODS

Oligonucleotides and plasmids

Complementary pairs of oligonucleotides with the sequences indicated in Figure 1 were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. In addition to the

* To whom correspondence should be addressed

+ Present address: Wellcome Foundation Ltd., Langley Court, Beckenham, Kent BR3 3BS, UK

sequences shown in the Figure, each oligonucleotide contained the additional sequence GATC at its 5′ end. Following annealing, this sequence remains single stranded allowing the annealed oligonucleotide to be cloned into the Bam HI site in the vector pBL_2 CAT (16). This vector contains the herpes simplex virus thymidine kinase promoter from −105 to +51 driving the chloramphenicol acetyl transferase gene followed by the small T intron and polyadenylation signals from SV40. The effect of a specific sequence on gene expression was therefore assessed by cloning it into the Bam HI site at −105 in this promoter.

DNA mobility shift assays

Oligonucleotides for use in mobility shift assays were labelled following annealing by phosphorylation with gamma 32P ATP and T4 polynucleotide kinase. Nuclear extracts from BHK-21 or HeLa cells were made from about 5×10^7 cells as described by Dignam et al., (17). Binding assays were carried out as previously described (18). Competitions were performed by the addition of a 100-fold excess of unlabelled duplex oligonucleotide to binding reactions.

DNA transfection

Transfection of plasmid DNA was carried out according to the method of Gorman (19). Standard transfections were carried out using 10 μg of DNA per 2×10^6 cells on a 90 mm plate. In experiments where the effect of Oct-1 on gene expression was being assessed, 10 μg of the reporter plasmid was co-transfected with the indicated amounts of the Oct-1 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-galactosidase 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 (19) with extracts which were equalised for protein content, as determined by the method of Bradford (20).

RESULTS AND DISCUSSION

To investigate whether the changes in the octamer motif in HPV6/11 prevented the binding of Oct-1 and the cervical-octamer binding protein we had previously defined (15), nuclear extracts were prepared from a variety of cell types and used in DNA mobility shift assays. The labelled probe used in these assays was a sequence derived from the herpes simplex virus immediate-early 1 gene which we have previously shown to be a high affinity binding site for octamer binding proteins (18).

When extracts from the fibroblast cell line BHK-21 were used in the band shift assay, a single complex formed on this probe representing binding of the constitutively expressed octamer binding protein Oct-1 (Figure 2a). As expected Oct-1 could be specifically removed from the labelled probe by competition with excess unlabelled octamer binding sites including both the octamer binding site from HPV 16/18 as well as the homologous HSV octamer but not by the binding sites for other transcription factors such as Sp1 and NF1. Most interestingly however, the equivalent region of the HPV 6/11 URR was unable to compete for Oct-1 binding in these experiments, even at four hundred fold excess indicating that it does not represent a functional binding site for Oct-1.

When extracts from the HeLa cervical epithelial cell line were used in this assay we observed two complexes as in our previous experiments (Figure 2b). Although both the homologous HSV

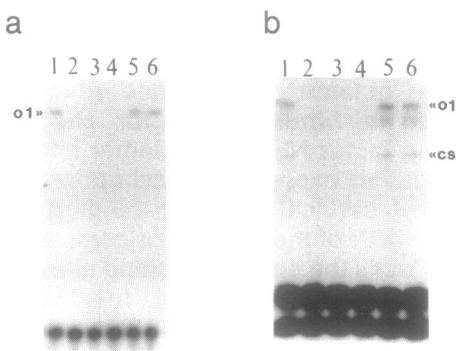

Figure 2. DNA mobility shift assay using a consensus octamer oligonucleotide and extracts from BHK-21 cells (**panel a**) or HeLa cells (**panel b**). The assay was carried out with labelled probe alone (track 1) or in the presence of a one hundred fold excess of unlabelled oligonucleotide containing either the homologous consensus octamer motif (track 2), the HPV 16 octamer-like sequence either alone (track 3) or with the adjacent NF1 site (track 4), the equivalent region of HPV 6 containing the NF1 site and adjacent region (track 5) and a consensus binding site for the unrelated transcription factor Sp1 (track 6). The arrows indicate the positions of Oct-1 (01) and the cervical octamer binding protein (CS).

Octamer consensus	A T T T G C A T	NFI
HPV 16	C T **A A T T G C A T** A T T T G G C A T	
HPV 18	C T **A A T T G C A T** A C T T G G C T T	
HPV 6	T T **A** A A A **G C A T** T T T T G G C T T	
HPV 11	T T **A** A A A **G C A T** T T T T G G C T T	

Figure 1. Relationship of the consensus octamer sequence and the octamer like sequences in the HPV enhancers. The adjacent binding site for nuclear factor 1 (NF1) is indicated. In order to conform to the conventional direction of the papillomavirus genome the octamer sequence has been written as ATTTGCAT rather than the complementary sequence ATGCAAAT which is more often presented.

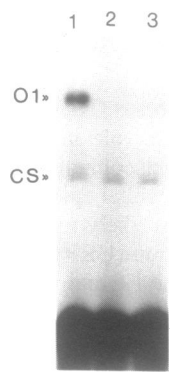

Figure 3. DNA mobility shift assay using the consensus octamer oligonucleotide and extract from HeLa cells. The assay was carried out in the presence of 1 μl of pre-immune rabbit serum (track 1) or 0.5 μl (track 2) or 1 μl (track 3) of polyclonal rabbit antibody to Oct-1. The arrows indicate the positions of Oct-1 (01) and the cervical octamer binding protein (CS).

octamer and that derived from HPV 16/18 readily competed for binding of both the complexes, the sequence from the HPV 6/11 URR was unable to do so. As expected formation of the low mobility complex was inhibited by the addition of antibody to Oct-1 confirming that it contains Oct-1 whereas the antibody had no effect on the formation of the high mobility complex indicating that it is formed by binding of a cervical protein distinct from Oct-1 (Figure 3). Similar results were also obtained in a variety of other cells of cervical origin including cell lines such as SiHa and CaSki as well as cells of limited life span derived from normal cervical epithelium and with no detectable HPV DNA (21).

In order to confirm these results we used the HPV16 sequence as a probe in a DNA mobility shift assay with HeLa cell extract. As in our previous experiments this sequence bound predominantly the cervical protein (Figure 4). However, whilst both the HPV16 sequence and the HSV octamer could readily compete for binding of this protein, the HPV6 sequence could not do so confirming that it does not bind the cervical protein with high affinity (Figure 4).

Hence the two base changes in the octamer-like sequence in HPV 6/11 compared to HPV 16/18 render it non-functional as a binding site both for the constitutively expressed octamer binding protein Oct-1 and the cervical octamer binding protein. In order to investigate the effect of octamer binding proteins upon the level of gene expression driven by the URR we compared the level of gene expression driven by the functional octamer motif with its adjacent NF1 site in the HPV 16/18 URR with that driven by the equivalent region of the HPV 6/11 URR where the octamer motif is non functional. To do this oligonucleotides containing the sequences in this region (as in Figure 1) were cloned upstream of the HSV tK promoter in the BamHI site of the vector pBL_2 CAT (16). The effect of these motifs on promoter activity was then determined by measuring the chloramphenicol acetyl transferase activity obtained following transfection of different cell types.

Following transfection of BHK-21 cells which contain only Oct-1, the HPV 16 motif directed only a relatively weak level of gene activity whereas the HPV 6 motif was considerably more active (Figure 5). This suggests that the ability of the HPV 16 motif to bind Oct-1 which is a relatively weak transactivator (22) inhibits gene expression in non-cervical cells possibly by preventing the binding of the much stronger activator NF1 to its adjacent site. In contrast the HPV 6 motif lacks the octamer binding site and hence directs a higher level of gene expression. A similar effect of Oct-1 in inhibiting binding to an adjacent sph site and thereby preventing gene activation has recently been documented in the SV40 enhancer (22).

In contrast in HeLa cells which contain both Oct-1 and the cervical specific protein, the HPV 16 motif directed a higher level of gene expression than that produced by the HPV 6 motif (Figure 5). This finding is an agreement with that of Chong et

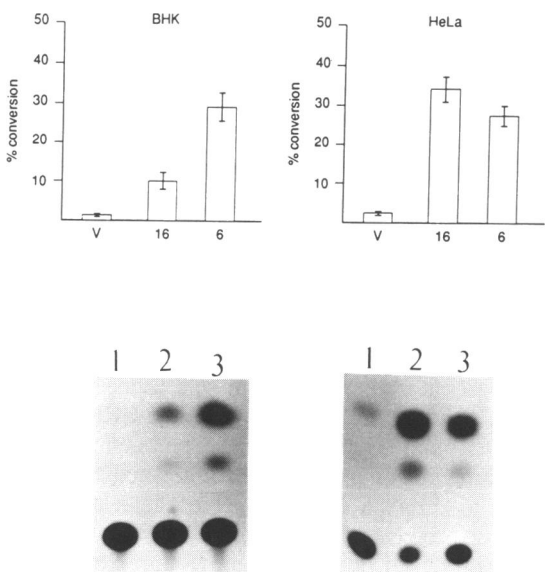

Figure 5. Upper panel: Percentage of chloramphenicol acetylated following transfection of BHK-21 cells or HeLa cells with pBL_2 CAT vector (V) or the same vector with a single copy of the HPV 16 octamer and adjacent NF1 site (16) or the equivalent region of HPV6 (6). Figures are the average of three experiments whose range is shown by the bars. The lower panel shows the result of one of these experiments using either pBL_2 CAT vector alone (track 1) or vector with a single copy of the HPV 16 octamer/NFI sequence (track 2) or with the equivalent region of HPV 6 track 3).

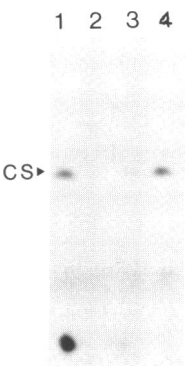

Figure 4. DNA mobility shift assay using the HPV 16 octamer and HeLa cell extract. The assay was carried out with labelled probe alone (track 1) or in the presence of a one hundred fold excess of unlabelled oligonucleotide containing either the homologous HPV 16 sequence (track 2), the consensus octamer motif (track 3) or the equivalent HPV 6 sequence (track 4). The arrow indicates the position of the cervical octamer binding protein (CS).

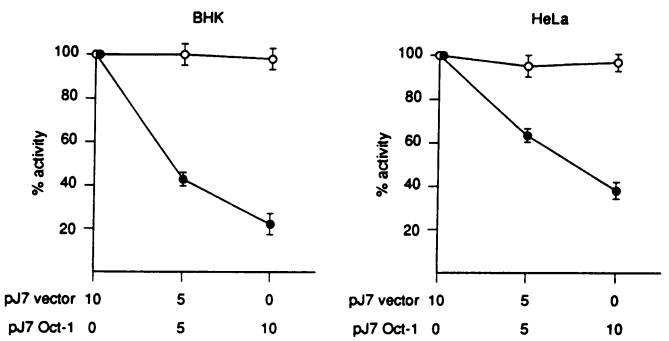

Figure 6. Effect of co-transfecting the pBL_2 CAT constructs containing the HPV 16 octamer/NF1 motif (closed circles) or the corresponding HPV 6 motif (open circles) with the indicated amounts (in micro-grams) of pJ7 plasmid vector or the pJ7 vector expressing Oct-1. Values indicate the CAT activity obtained as a percentage of control and are the average of three experiments whose range is shown by the bars.

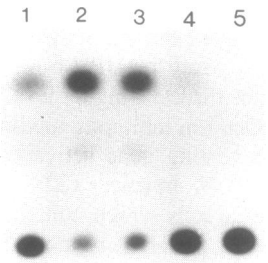

Figure 7. CAT assay of extracts prepared by the transfection of BHK-21 cells with pBL_2 CAT contianing the HPV 16 octamer/NFI motif in the presence of 10 μg of plasmid vector alone (track 1), 10 μg of the RSV-NF4 expression vector (track 2), 10 μg of the RSV-NF21 expression vector (track 3), 5 μg of the RSV-NF4 expression vector with 5 μg of the Oct-1 expression vector (track 4) and 5 μg of the RSV-NF21 expression vector with 5 μg of the Oct-1 expression vector (track 5).

al., (12) who observed a decreased level of gene expression in HeLa cells when the octamer binding site within the HPV-16 URR was deleted. Hence in cells such as HeLa cells which contain the cervical protein, the octamer motif acts as a positive element both in the context of the HPV-16 URR and in a heterologous promoter, resulting in a higher level of gene expression when it is functional. In these cells the cervical protein which has a higher affinity than Oct-1 for the URR (15) will bind to the octamer motif. High level gene expression may then result from this protein being a stronger trans-activator than NF1 allowing it to direct a higher level of gene activation even following displacement of NF1. Alternatively the higher mobility of the shift produced by this protein (Figure 2b) suggests that it may be smaller in size than Oct-1 allowing it to bind adjacent to bound NF1 and activate gene expression synergistically.

In order to confirm the inhibitory effect of Oct-1 on the HPV 16 motif, we constructed an Oct-1 expression vector by cloning a human Oct-1 cDNA clone (23) under the control of the strong cytomegalovirus immediate-early promoter in the vector pJ7 (24). The HPV 16 and HPV 6 motifs cloned into pBL_2 CAT were then co-transfected into BHK cells with this construct or with pJ7 vector alone. Over-expression of Oct-1 decreased the level of expression driven by the HPV 16 motif whilst having no effect on the much higher level of expression driven by the HPV 6 motif and the extent of repression observed was dependent upon the degree of over-expression of Oct-1 (Figure 6). A similar effect of Oct-1 on the HPV 16 motif was also observed in HeLa cell transfections although to a lesser extent indicating that at higher levels Oct-1 can compete with the cervical protein for binding to the octamer motif (Figure 6).

These data indicate therefore that the octamer motif in HPV 16/18 can act as a target for repression by Oct-1. In order to elucidate the manner in which Oct-1 acts, we co-transfected the HPV 16 octamer and adjacent NF1 site cloned in pBL_2 CAT into BHK cells with expression vectors containing different forms of NFI either alone or in combination with the Oct-1 expression vector. As shown in Figure 7, the expression vectors NF4 and NF21 (which contain cDNAs for two distinct NFI proteins under the control of the RSV promoter:—a kind gift of Dr A.Nicosia) were both able to activate the HPV 16 construct. However, such activation was dramatically reduced in the presence of the Oct-1 expression vector. Hence Oct-1 is likely to act by inhibiting the stimulatory effect of endogenous NFI on the HPV 16 motif, presumably by inhibiting NFI binding to its overlapping site.

The octamer motif in HP 16/18 is therefore able to mediate the inhibition of gene expression by Oct-1 and can confer a tissue specific pattern of activity on a heterologous promoter with neither of these effects being observed with the corresponding motif in HPV 6/11. When taken together with the data of Chong et al (12) who observed a decrease in the activity of the HPV-16 URR in HeLa cells when this motif was deleted, this indicates that this motif may play a role in the level of gene expression driven by the URR and in its epithelial specificity. Thus this motif acts to enhance gene activity in the presence of the cervical protein and to minimize gene activation by NF1, in the presence of Oct-1 alone. In contrast the non tumourigenic viruses HPV 6/11 would not be subject to such cell type-specific modulation in activity via this region since they do not contain this functional octamer binding site.

Interestingly the effect of Oct-1 on the octamer motif at 7731 to 7738 in HPV 16/18 which we have documented here may be only one of multiple effects of Oct-1 on the HPV URR. Thus Mack and Laimins (25) have shown that another site distinct from the one characterized here and located at nucleotides 7641–7675 in the HPV 18 URR also binds both Oct-1 and a keratinocyte-specific protein although this site is not conserved in the HPV 16 URR. Moreover this site is contained within a short region of the HPV 18 URR whose activity has been shown to be inhibited by Oct-1 in co-transfection assays and which does not contain the Oct-1 target site we have characterized here (13). Hence Oct-1 represents a prime candidate for the intra-cellular surveillance factor proposed by zur Hausen (26, 27) which inhibits HPV gene expression in non transformed cells. Interestingly however, the data presented here suggest that activation of HPV gene expression may not be dependent on decreased expression of Oct-1, which in any case is known to be expressed at enhanced levels in rapidly proliferating transformed cells (28, 29). Rather it is likely to depend on the expression by cervical cells of a novel octamer binding protein which displaces Oct-1 from its binding site in the URR and activates gene expression.

ACKNOWLEDGEMENTS

We thank Winship Herr for the Oct-1 cDNA and antibody to Oct-1, Hartmut Land for the pJ7 vector, Nick Lakin for constructing the Oct-1 expression plasmid and Alfredo Nicosia for the RSV-NFI expression plasmids. This work was supported by the Cancer Research Campaign and Birthright, The Royal College of Obstetricians and Gynaecologists.

REFERENCES

1. Peto, R. and zur Hausen, H. (1986). Viral etiology of cervical cancer. Banbury Report 21. Cold Spring Harbor Laboratory, New York.
2. Syranen, K.J., Gissmann, L. and Koss, L.G. (1987). Papilloma viruses and human disease. Springer Verlag, Berlin.
3. Cripe, T.C., Haugen, T.H., Turk, J.P., Tabatabai, F., Schmid, P.G., Duerst, M., Gissmann, L., Roman, A. and Turek, L. (1987). EMBO Journal 6, 3745–3753.
4. Gloss, B., Bernard, H.U., Seedorf, K. and Klock, G. (1987). EMBO Journal 6, 3735–3743.
5. Chan, W-K., Klock, G. and Bernard, H-U. (1989). Journal of Virology 63, 3261–3269.
6. Gloss, B., Yeo-Gloss, M., Meisterenst, M., Rogge, L., Wimocker, E.L. and Bernard, H-U. (1989). Nucleic Acids Research 17, 3517–3533.

7. Chan, W-K., Chong, T., Bernard, U. and Klock, G. (1990). Nucleic Acids Research 18, 763–769.
8. Falkner, F.G., Moickat, R. and Zachau, H.G. (1986). Nucleic Acids Research 13, 7847–7863.
9. Scheidereit, C., Heguy, A. and Roeder, R.G. (1987). Cell 51, 783–793.
10. Lenardo, M.J., Staudt, L., Robbins, P., Kuang, A., Milligan, R.C. and Baltimore, D. (1989). Science 243, 544–546.
11. Goldsborough, A., Ashworth, A. and Willison, K. (1990). Nucleic Acids Research 18, 1634.
12. Chong, T., Apt, D., Gloss, B., Isa, M. and Bernard, H-U. (1991). Journal of Virology 65, 5933–5943.
13. Hoppe-Seyler, F., Butz, K. and zur Hausen, H. (1991). Journal of Virology 65, 5613–5618.
14. Royer, H-D., Freyaldenhover, M.P., Napierski, I., Spitkousky, D.D., Bauknecht, T. and Dathan, N. (1991). Nucleic Acids Research 19, 2363–2371.
15. Dent, C.L., McIndoe, G.A.J. and Latchman, D.S. (1991). Nucleic Acids Research 19, 4531–4535.
16. Luckow, B. and Schutz, G. (1987). Nucleic Acids Research 15, 5490.
17. Dignam, J.D., Lebovitz, R.M. and Roeder, R.G. (1983). Nucleic Acids Research 11, 1475–1489.
18. Dent, C.L. and Latchman, D.S. (1991). Biochemical Journal 277, 541–545.
19. Gorman, C.M. (1985). *In*: DNA cloning a practical approach. Ed. Glover, D.M., IRL Press, vol. 2. pp 143–190.
20. Bradford, M. (1976). Anal. Biochem. 72, 248–254.
21. Bal, V., McIndoe, A., Denton, G., Hudson, D., Lombard, G., Lamb, J. and Lechler, R. (1990). Eur. J. Immunol. 20, 1893–1897.
22. Tanaka, M. and Herr, W. (1990). Cell 60, 375–386.
23. Sturm, R.A., Das, G. and Herr, W. (1988). Genes and Dev. 2, 1582–1599.
24. Morgenstern, J.P. and Land, H., (1990). Nucleic Acids Research 18, 1068.
25. Mack, D.H. and Laimins, L.A. (1991). Proc. Natl. Acad. Aci., USA., 88, 9102–9106.
26. zur Hausen, H. (1982). Lancet 2, 1370–1372.
27. zur Hausen, H. (1986). Lancet 2, 489–491.
28. Dent, C.L., Lillycrop, K.A., Bybee, A., Latchman, D.S. and Thomas, N.S.B. (1991). Journal of Biological Chemistry 266, 20888–20892.
29. Ito, M., Sharma, A., Lee, A.S. and Maxson, R. (1989). Mol. Cell. Biol. 9, 869–873.