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The Enhancer in the Long Control Region of Human Papillomavirus Type 16 is Up-Regulated by PEF-1 and Down-Regulated by Oct-1

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The minimal enhancer in the long control region of human papillomavirus type 16 regulates cell type and constitutive expression from the promoter P97. This region contains at least four DNase I footprints (fp4e, fp5e, fp6e, and fp7e). We have shown that fp5e is crucial to enhancer function and have described an apparently novel factor (PEF-1) binding fp5e (S. Cuthill, G. J. Sibbet, and M. S. Campo, Mol. Carcinog. 8:96–104, 1993). Further analyses reveal that Oct-1 or an Oct-related factor binds fp5e at a site overlapping that of PEF-1. The binding of Oct-1 to fp5e has been demonstrated by electrophoretic mobility shift assays, by oligonucleotide competition studies, and by using an Oct-1-specific anti-POU serum. The location of the Oct-1 site has been confirmed by a panel of mutants across fp5e. Mutations that block PEF-1 binding to fp5e also block enhancer/promoter activity of the long control region, whereas mutations that block Oct-1 binding significantly increase enhancer/promoter activity. Thus, although both PEF-1 and Oct-1 interact with fp5e, they appear to regulate human papillomavirus expression in opposite ways.

Human papillomaviruses (HPVs) are epitheliotropic tumor viruses with a circular double-stranded DNA genome. Over 80 HPVs have been described, and a subset of these are responsible for initiating anogenital neoplasia (17, 34, 35). HPV type 16 (HPV16) in particular is frequently associated with the initiation of cervical intraepithelial neoplasia and its progression to squamous cell carcinoma. In vivo, the virally encoded oncogenes E6 and E7 are expressed at very low levels in cervical intraepithelial neoplasia but are much more transcriptionally active in squamous cell carcinoma (3, 19, 33). Therefore, increased expression of these transforming genes correlates with tumorigenesis. Indeed, E6 and E7 are together capable of immortalizing human primary keratinocytes in vitro, and their continued expression is required to maintain the transformed phenotype of cervical carcinoma cell lines (4). Thus, HPV16-induced carcinogenesis is critically dependent on the regulatory mechanisms governing papillomavirus E6 and E7 expression.

Transcription of the transforming genes in HPV16 is initiated from the promoter P97 and is regulated by an enhancer in the long control region (LCR). We and other laboratories have previously mapped DNase I footprints of nuclear factors binding within the enhancer (E, F, G, and H [28] or, respectively, fp4e, fp5e, fp6e, and fp7e [13]). These footprints have been shown to bind a variety of transcription factors (6–8, 20) and we have recently described an apparently novel ~110-kDa factor, PEF-1 (papillomavirus enhancer binding factor 1), which binds footprint fp5e (10). We showed that this footprint was important to enhancer function and described a mutation that disrupted both PEF-1 binding and enhancer activity (10). However the mutation in fp5e also down-regulated the enhancer in cells in which PEF-1 was almost absent, indicating that additional factors may bind this site (our unpublished observations). Furthermore, the close proximity of factors binding along the enhancer suggests that they interact synergistically to control the viral enhancer (7). These observations led us to search for other factors that could regulated P97 through footprint fp5e.

We show here that in addition to PEF-1, footprint fp5e binds Oct-1, and that while mutating fp5e in the LCR of HPV16 to block PEF-1 binding causes enhancer/promoter activity to be dramatically reduced, mutations that block Oct-1 binding significantly increase the activity of the enhancer/promoter. Thus, PEF-1 binding and Oct-1 binding to fp5e appear to have opposite effects, PEF-1 up-regulating and Oct-1 down-regulating HPV expression.

MATERIALS AND METHODS

Cell culture. HaCaT cells were grown in Joklik modified minimal essential medium (MEM; Ca^{2+} free; Gibco) supplemented with 10% fetal calf serum (Gibco), 10 mM glutamine, 100 μg of kanamycin per ml, 50 μg of streptomycin per ml, and 37 μg of penicillin per ml (Gibco). HeLa, SiHa, and MRC-5 (human fibroblasts immortalized with simian virus 40) cell lines were grown in Special Liquid Medium (Gibco) supplemented as described above.

Oligonucleotides. All oligonucleotides were synthesized on an Applied Biosystems model 381A DNA synthesizer. Oligonucleotides for electrophoretic mobility shift assays (EMSAs) were annealed by using standard methods. Double-stranded oligonucleotides were ^{32}P labelled by using T4 polynucleotide kinase (Gibco) as recommended by the manufacturer and were purified by preparative 6% polyacrylamide gel electrophoresis. The HPV16 LCR (−568 to +8 relative to the P97 promoter) was amplified for cloning by PCR with oligonucleotide primers Bam-568 (5′-ctaaagggaatGGATCCCCATTTTGTAGCTTCAACCG-3′) and Hind+8 (5′-ctatagggcAAGCTTTGCAGTTCTCTTTTGGTGCAT-3′) (inserted *Bam*HI and *Hin*dIII restriction sites are underlined, and nonhomologous flanking sequences are in lowercase). The Oct oligonucleotide sequence was from reference 11.

Nuclear extract and plasmids. Nuclear extracts were prepared as previously described (28). The protein concentration of these extracts was typically 3 mg/ml, as determined by the method of Bradford (5). The pGL2-P-based luciferase plasmids and β-galactosidase plasmid were described by Cuthill et al. (10). The pGL2-LCR-based luciferase plasmids were constructed by replacing the simian virus 40 promoter of pGL2-P (Promega) with a PCR-amplified 576-bp fragment corresponding to the enhancer and promoter within the LCR of HPV16 (nucleotides [nt] −568 to +8 relative to the P97 promoter). The upstream and down-

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stream PCR primers contained *Bam*HI and *Hin*dIII restriction sites, respectively, which enabled directional cloning of the PCR product into the *Bgl*II and *Hin*dIII sites of pGL2-P. Mutant LCR constructs were produced by two-stage PCR using the mutant fp5e or fp5eL oligonucleotides as primers along with primers Bam-568 and Hind+8. All wild-type and mutated plasmid constructs were purified by two rounds of CsCl centrifugation and were sequenced directly on both DNA strands.

EMSAs. The conditions used for EMSAs were as follows. Typically 1 μg of nuclear extract was added to 1 to 2 μg poly(dI-dC) and preincubated on ice in a final volume of 50 μl in 20 mM NaCl–8 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.9)–2 mM $MgCl_2$–0.1 mM EDTA–1 mM dithiothreitol–10% glycerol. After 10 min of preincubation ~5 fmol of ^{32}P-labelled oligonucleotide probe was added, and the mixture was incubated for a further 10 min at room temperature before being loaded onto a 6% polyacrylamide gel. Competition gel retardation experiments were carried out under the same conditions except that a 20- to 100-fold molar excess of unlabelled oligonucleotide was included in the incubation. EMSAs with an Oct-1-specific anti-POU (α-POU) serum were performed as described above except that the nuclear extract was preincubated for 30 min on ice with 2 μl of 10-fold-diluted α-POU serum. Autoradiographs of EMSA gels were scanned on a Molecular Dynamics densitometer and displayed with P.D.I. image analysis software.

Transfection protocols. HaCaT cells were transfected with Polybrene and then subjected to a glycerol shock in a procedure similar to that described by Staedel et al. (29). Typically, transfections were performed in triplicate with 2.5×10^5 HaCaT cells plated in 3 ml of medium per 3-cm-diameter dish. Medium on the cells was replaced with 5 μg of pGL2-LCR-based luciferase plasmid per dish in 1.5 ml of Joklik MEM containing 30 μg of Polybrene per ml. After 4 h of transfection, the Polybrene medium was removed, cells were washed with phosphate-buffered saline, and the medium was replaced with 2 ml of serum-free Joklik MEM and 15% glycerol per dish. Following a 4-min glycerol shock, the medium was replaced with 3 ml of standard Joklik MEM. Additional transfections of SiHa and MRC-5 cells were performed in parallel by lipofection with DOTAP (Boehringer, Mannheim, Germany) as recommended by the manufacturer. Typically 10^6 cells were plated in 5 ml per 6-cm-diameter dish. Transfections were performed with 15 μg of pGL2-based luciferase plasmids and 5 μg of β-galactosidase plasmid with 160 μl of DOTAP in 1 ml of Special Liquid Medium per set of four dishes. Cells were harvested and lysed after 40 h and luciferase assays were performed on an LKB1251 luminometer as described by the manufacturer of the assay reagents (Promega).

RESULTS

Factors other than PEF-1 bind fp5e. Copper-orthophenanthroline footprinting on fp5e had previously shown that the PEF-1 binding site was centered over the CCAAC motif and that mutating this sequence to CCTTC in the fp5e oligonucleotide (fp5e-mt1) completely disrupted PEF-1 binding in EMSA (10). Furthermore, in transfection experiments, the mt1 mutation strongly reduced the activity of the minimal enhancer in primary keratinocytes and SiHa cells (10). However, the mt1 mutation also reduced enhancer activity in cells that contained only very low levels of PEF-1, as assayed by EMSA (data not shown). This finding suggested that additional regulatory transcription factors might bind fp5e but had previously remained undetected by EMSA.

As the length or configuration of the original fp5e oligonucleotide may have selected against the binding of other factors, we performed an EMSA with oligonucleotides that contained the PEF-1 binding site but extended either upstream (fp5eL, nt 7658 to 7687) or downstream (fp5eR, nt 7672 to 7701) of the original fp5e oligonucleotide (nt 7665 to 7691). In addition, mutation mt1, which abrogates PEF-1 binding to fp5e, was also introduced into these oligonucleotides (Fig. 1). The EMSA pattern of complexes formed with oligonucleotide fp5eL was superficially similar to that with fp5e, whereas the ability of oligonucleotide fp5eR to form complexes was dramatically reduced (Fig. 1A). Both oligonucleotide fp5e and oligonucleotide fp5eL formed a main complex, C1. While the C1 complex formed with fp5e contained PEF-1 as previously demonstrated and was sensitive to the mt1 mutation, the C1 complex formed with fp5eL was unaffected by the mt1 mutation, suggesting that fp5eL bound a factor distinct from PEF-1 (Fig. 1A). Oligonucleotide fp5eL also formed a faster-migrating ladder of complexes, which were disrupted by mt1. A very faint band formed

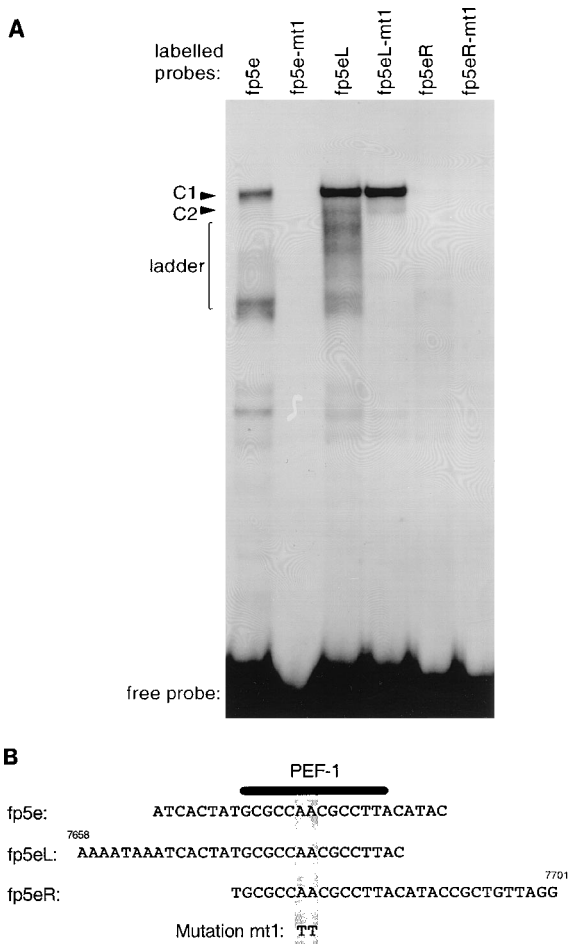

FIG. 1. Comparison of the nuclear factors binding oligonucleotides fp5e, fp5eL, and fp5eR. (A) EMSA with the indicated oligonucleotides containing either the wild-type or mt1 mutated sequence and HeLa nuclear extract. C1, C2, and ladder refer to retarded complexes. (B) Alignment of oligonucleotides fp5e, fp5eL, and fp5eR and their locations in nucleotide map units or relative to the P97 promoter. The A-to-T transversion (mutation mt1) in these oligonucleotides is shown along with the PEF-1 binding site (10).

with fp5eR corresponding to the C1 complex, seen on longer exposures of the EMSA autoradiographs, was disrupted by mt1, suggesting that there was a very weak interaction between fp5eR and PEF-1 (Fig. 1A). The sequences, location of the mt1 mutation, and overlap between oligonucleotides fp5e, fp5eL, and fp5eR are shown in Fig. 1B.

Fp5eL binds regulatory factors other than PEF-1. To confirm that the factor forming C1 with fp5eL was distinct from PEF-1, we performed EMSAs with a set of unlabelled competitor oligonucleotides at 20-fold molar excess. Figure 2A shows the control EMSA without competitors. Figures 2B to D show results of EMSAs in which competitor oligonucleotides fp5e, fp5eL, and fp5eL-mt1, respectively in (Fig. 1B), were used.

Competition with unlabelled fp5e blocked the formation of the PEF-1/fp5e C1 complex but not the formation by fp5eL of either the C1 complex or the faster-migrating ladder (Fig. 2B). Conversely, competition with fp5eL did not affect the PEF-1/fp5e C1 complex but abolished all other complexes formed with either fp5e or fp5eL (Fig. 2C). The fp5eL-mt1 competitor inhibited the formation of C1 and C2 by fp5eL but not the complexes formed by fp5e (Fig. 2D). Therefore, both direct

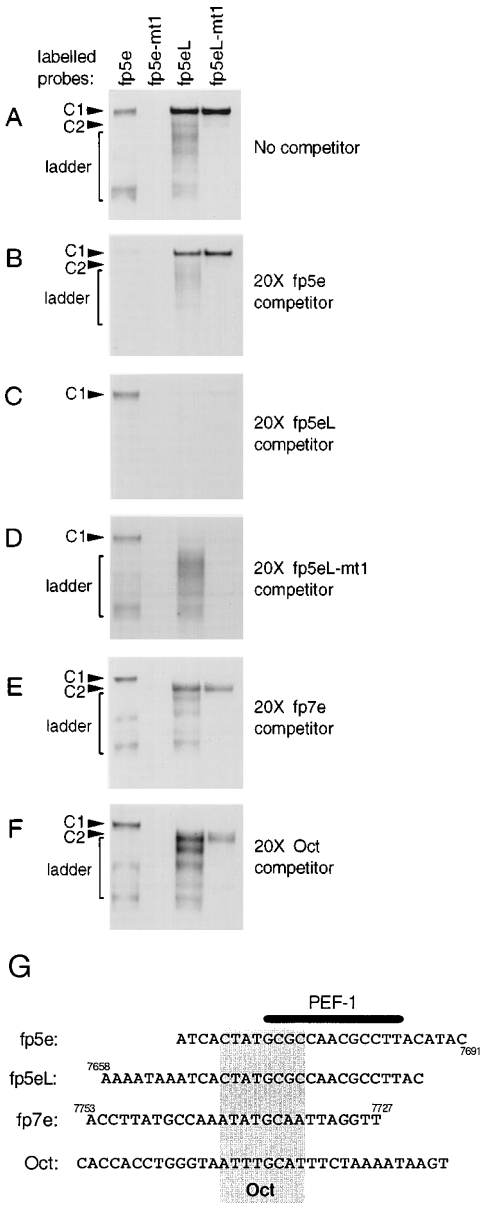

FIG. 2. Competition EMSA of complexes formed with oligonucleotides fp5e and fp5eL and the mt1 mutated oligonucleotides. (A to F) EMSA performed as for Fig. 1 except that nuclear extracts were preincubated with 20-fold-excess unlabelled competitor oligonucleotides. Only the retarded complexes are presented. C1, C2, and ladder are as in Fig. 1. (G) Sequences of the oligonucleotides aligned according to the putative Oct binding site and its location relative to the PEF-1 site in fp5e. The locations of the sequences with the HPV16 genome are shown in map units. The sequence of the Oct-1 binding site in the murine IgH enhancer is from reference 11.

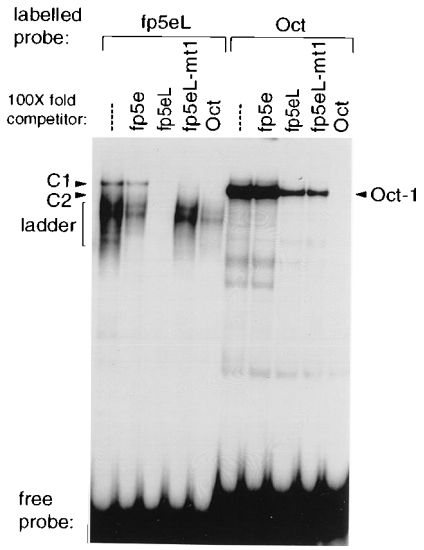

FIG. 3. Direct comparison of the nuclear factors binding oligonucleotide fp5eL and Oct-1 binding the murine IgH enhancer oligonucleotide Oct. The retarded complexes formed with both labelled oligonucleotides fp5eL and Oct were competed for with 100-fold-excess oligonucleotides. C1, C2, and ladder are as in Fig. 1.

binding EMSA and competition EMSA confirm that the C1 complex formed with fp5eL is insensitive to the mt1 mutation and thus does not contain PEF-1.

Oligonucleotide fp7e was also chosen as a competitor oligonucleotide since sequence analysis of fp5e or fp5eL reveals a weak homology to fp7e (Fig. 2G). Chong et al. had previously shown that fp7e binds Oct-1 (7), which suggested to us that fp5eL might likewise bind Oct-1. Competition with oligonucleotide fp7e abolished only the C1 complex formed by fp5eL and had no effect on C2 or the other, faster-migrating complexes (Fig. 2E). Furthermore, an oligonucleotide (Oct) containing a high-affinity Oct-1 binding site from the murine immunoglobulin heavy-chain (IgH) enhancer also specifically abolished the fp5eL C1 complex but not the faster-migrating ladder of bands (Fig. 2F). These results indicate that the C1 complex of fp5eL contains an Oct factor rather than PEF-1.

Fp5eL binds an Oct factor. To directly compare the C1 complex formed by fp5eL with the Oct-1 complex formed by the murine IgH enhancer Oct oligonucleotide, we labelled these oligonucleotides and performed competition EMSAs with 100-fold-molar-excess unlabelled oligonucleotides (Fig. 3). These experiments showed that while the fp5eL C1 complex and the Oct oligonucleotide complex comigrated, the Oct oligonucleotide competed for the fp5eL C1 complex with a much higher efficiency than oligonucleotide fp5eL competed for the murine IgH oligonucleotide Oct-1 complex. Thus, an Oct factor binds to fp5eL but with significantly lower affinity than to the Oct-1 binding site of the murine IgH enhancer. Furthermore, the mt1 mutation had no effect on the affinity of the Oct factor for fp5eL, since the fp5eL and fp5eL-mt1 oligonucleotides competed for Oct-1 binding to the murine IgH enhancer oligonucleotide to equivalent extents, and both completely abolished formation of C1 with fp5eL (Fig. 3).

The Oct factor binding site on fp5eL overlaps the PEF-1 site on fp5e. The location of the Oct factor binding site in fp5eL, partly overlapping the PEF-1 binding site, is suggested by sequence comparison with oligonucleotide fp7e (Fig. 2G). The putative site has good homology to the consensus Oct motif in its 5′ half but is GC rich and has poor homology in the 3′ half overlapping the PEF-1 site. To confirm the location by direct EMSAs, a series of mutations was introduced into both fp5e and fp5eL oligonucleotides within and flanking the putative Oct site (Fig. 4C). Mutation mt1 was described above. Mutation mt2 is a C-to-G transversion within the GC-rich region of the site and would be expected to have little effect on Oct binding. In contrast, mutation mt3, which is within the region of best homology, would be expected to abolish Oct factor binding. Mutation mt4 maintained the similarity of the puta-

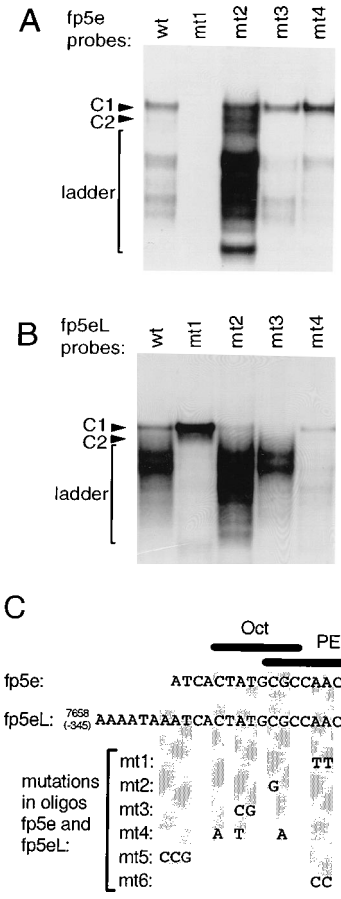

FIG. 4. Mapping of the nuclear factor binding sites along oligonucleotides fp5e and fp5eL by mutations mt1 to mt6. (A) Direct EMSA comparison of the wild-type (wt) fp5e oligonucleotide with mutants mt1, mt2, mt3, and mt4. (B) As in panel A, but with fp5eL-based oligonucleotides. C1, C2, and ladder are as in Fig. 1. (C) Mutations mt1 to mt6 are shown relative to oligonucleotides fp5e and fp5eL. The locations of the PEF-1 site and the Oct-1 binding homology site are also shown.

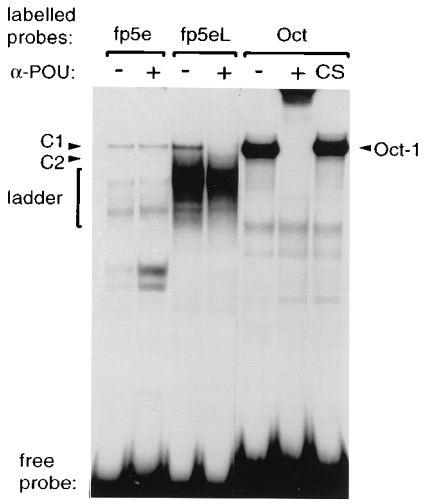

FIG. 5. Identification of the nuclear factor binding fp5eL to form complex C1. Lanes +, preincubation of HeLa nuclear extract with Oct-1-specific α-POU serum prior to EMSA with labelled oligonucleotides fp5e, fp5eL, and Oct. CS, control serum. C1, C2, and ladder are as in Fig. 1.

tive site to the consensus and introduced a C-to-A transversion into the GC-rich region of the site. An additional pair of mutations, mt5 and mt6, were also used to examine the role of flanking sequences on Oct factor binding (Fig. 4C).

As expected for oligonucleotide fp5e, mutations mt2, mt3, and mt4 had little effect on the C1 complex containing PEF-1, although mutation mt2 strongly enhanced the ladder of faster-migrating complexes (Fig. 4A). The identical mutations in oligonucleotide fp5eL did, however, modulate the Oct factor C1 complex. Mutation mt3 completely abolished the fp5eL C1 complex but had no effect on the faster-migrating ladder of bands. Conversely, mutations mt2 and mt4 in fp5eL both maintained C1 formation, or Oct factor binding, but with reduced affinity (Fig. 4B). However, these mutations had opposite effects on the ladder of faster-migrating complexes, since mt2 in fp5eL strongly enhanced their formation, as it did with oligonucleotide fp5e, whereas mt4 specifically disrupted the ladder of complexes.

Additional EMSA competition studies showed that Oct-factor binding to fp5eL was specifically blocked by mutation mt5 but unaffected by mutation mt6. PEF-1 binding to fp5e was reduced by mutation mt6 but not completely blocked (data not shown). Thus, by EMSA, mutations mt3 and mt5 behaved alike, as did mutations mt1 and mt6, and the locations of the mutations modulating the fp5eL C1 complex are consistent with the presence of an Oct factor binding site adjacent or overlapping that of PEF-1.

Fp5eL binds Oct-1. To further distinguish between and identify the factors binding fp5e and fp5eL, the wild-type and mutated oligonucleotides were subjected to EMSA in the presence of an Oct-1-specific α-POU serum under conditions that would specifically disrupt Oct-1 binding. As predicted, the α-POU serum had no effect on any of the complexes formed with fp5e but specifically abrogated formation of the C1 complex with the fp5eL oligonucleotide (Fig. 5). None of the mutants in oligonucleotide fp5e formed complexes that were disrupted by the α-POU serum, whereas mutants mt1, mt2, and mt4 of oligonucleotide fp5eL all formed C1 complexes susceptible to disruption by the α-POU serum (data not shown). This finding confirmed that Oct-1 binds fp5eL and forms the C1 complex.

Oct-1 binding negatively regulates the fp5e PEF-1 site. To correlate the binding of PEF-1 and Oct-1 to the functional role of fp5e in the HPV16 enhancer, the mutations were introduced into the LCR of HPV16. The wild-type and mutated LCRs were inserted into the pGL2-P luciferase vector. The plasmids were transfected into the keratinocyte cell line HaCaT, and luciferase activity was assayed, comparing two independent clones of wild-type LCR with the mutant LCRs. HaCaT cells were chosen since they contain both PEF-1 and Oct-1, are free of papillomaviral DNA, and retain the capacity to differentiate, therefore providing a cellular environment more like normal keratinocytes.

Mutation mt1, which abrogates PEF-1 binding without affecting Oct-1, dramatically reduced enhancer/promoter function when introduced into the LCR. In contrast, mutation mt3, which specifically blocks Oct-1 binding, significantly enhanced LCR enhancer/promoter expression (Fig. 6). Likewise, mutation mt6, which reduced PEF-1 binding, also reduced enhancer/promoter activity, while mutation mt5, which blocked Oct-1 binding, markedly increased activity (Fig. 6). Mutations mt2 and mt4, which only marginally affected PEF-1 or Oct-1 binding (C1 complexes), also had only a limited effect on enhancer/promoter function of the LCR (Fig. 6), despite the dramatically altered levels of the faster-migrating ladder complexes

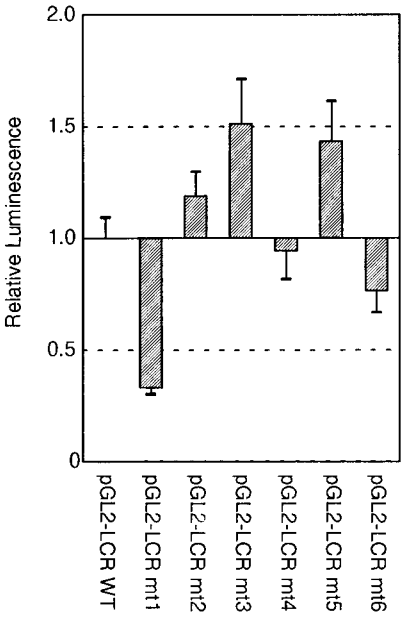

FIG. 6. Functional assays of the pGL2-LCR-based luciferase reporter plasmids transfected into HaCaT keratinocytes. Mutations mt1 to mt6 (shown in Fig. 4C) were introduced into the LCR (nt −568 to +8 relative to the P97 promoter) of pGL2-LCR. The activities of the mutated LCR luciferase reporter plasmids are shown relative to the activities of two independent wild-type (pGL2-LCR WT) clones. The means and standard errors of six duplicate transient transfections are shown.

(Fig. 4A and B). Thus, the overall contribution to enhancer function of the factors forming the faster-migrating ladder is small in comparison with that of PEF-1 and Oct-1. These functional assays in conjunction with the EMSA data are therefore consistent with PEF-1 acting as an up-regulatory factor and Oct-1 acting as a down-regulatory factor.

DISCUSSION

The HPV16 enhancer is nucleosome free and densely bound by a wide variety of transcription factors (2, 7, 9, 13, 14, 16, 20, 25, 26, 28, 31). Our previous studies of the factors binding the enhancer of HPV16 have revealed that footprint fp5e is critical to enhancer activity and is bound by an apparently novel factor, PEF-1 (10, 28).

However, PEF-1 is not the only factor to bind fp5e. For example, Gloss et al. had previously described the very weak binding of NF-I/CTF to this footprint (14). More recently, List et al. discovered a novel methylation-sensitive transcription factor, MSPF, that binds fp5e (21). We have not looked at the methylation sensitivity of PEF-1 binding to fp5e, but comparative EMSAs with the unrelated oligonucleotide tat-GRU-D (glucocorticoid response unit of the *tat* gene) which also binds MSPF (21), should enable a direct analysis of both factors. While the relationship, if any, between PEF-1 and MSPF remains to be determined, we have found that the transcription factor Oct-1 also binds to fp5e, at a site adjacent to or overlapping that of PEF-1. The Oct-1 binding activity was characterized by cross-competition EMSA, use of an α-POU serum, and analysis of a panel of mutations (mt1 to mt6) across fp5e.

Binding of Oct factor to the site is revealed only with oligonucleotide fp5eL, which extends 5′ of the original fp5e and presumably provides a larger target sequence. Given the apparent overlap between the two sites, we might expect the binding of PEF-1 and Oct-1 to be mutually exclusive. We have not observed any complexes containing both factors, but until this is tested directly by using a larger oligonucleotide encompassing both fp5e and fp5eL, we cannot formally exclude the possibility that PEF-1 and Oct-1 bind fp5e together. Nevertheless, in functional studies, the two factors have opposite effects on LCR activity, which is consistent with PEF-1 and Oct-1 competing for fp5e.

It has to be stressed that the effect of PEF-1 and Oct-1 binding to their respective sites has been monitored in the setting of a 576-bp LCR fragment containing the enhancer and the P97 promoter of HPV16, and therefore in the context of all other cellular transcription factors known to regulate enhancer function and with physiological levels of PEF-1 and Oct-1. Furthermore, the analysis has been conducted in HaCaT cells, which do not contain HPV DNA, and thus in the absence of overlaying effects of virally encoded transcription factors. The binding of PEF-1 or Oct-1 can be selectively abolished by appropriate mutations. The abrogation of PEF-1 binding results in down-regulation of LCR activity (mutations mt1 and mt6), whereas abolition of Oct-1 binding leads to an increase in activity (mutations mt3 and mt5). PEF-1 and Oct-1 are therefore antagonists, and the balance between the two may determine the overall level of LCR activity in epithelial cells.

The interplay between PEF-1 and Oct-1 resembles the situation reported for simian virus 40 enhancer repeats, in which case the activating *Sph*I motifs are sterically hindered by Oct-1 binding (30). Similarly, KRF-1, thought to be a novel tissue-specific activating factor which regulates the HPV18 enhancer, appears to be blocked by Oct-1 binding at an overlapping site (22). Overexpression of Oct-1 has also been shown to down-regulate transcription through a region of the HPV18 enhancer which includes the overlapping Oct site and KRF-1 site, although this may be due to squelching rather than steric hindrance (18).

A role for Oct factors in cell type and cell cycle regulation of papillomaviral and cytokeratin gene expression has recently emerged (1, 12, 15, 23). The human cell line HeLa has been shown to contain an Oct factor which is absent in BHK cells. This Oct factor causes up-regulated expression of a reporter plasmid through fp7e of HPV16, while Oct-1 binding causes down-regulation (23). This role for Oct-1 binding fp7e is, however disputed, since it has been recently demonstrated that mutating the site to reduce Oct-1 binding dramatically reduces enhancer function (24). Another Oct-1-related factor, p92, has been found in HeLa cells (27). Nuclear factor p92 binds the enhancer of HPV18 at sites including the KRF-1/Oct-1 site and is cell cycle regulated by an inhibitor I-92 (32). It would be interesting to know whether p92 binds fp5eL and confers cell cycle regulation upon HPV16 expression. Other novel cell-type-specific Oct factors such as Skn-1a (Oct-11) and Oct-6 have been shown to regulate cytokeratin gene expression, and thus they may play a similar role in regulating HPV expression (1, 12).

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