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E1 protein of human papillomavirus type 1a is sufficient for initiation of viral DNA replication

(origin of replication/replication proteins)

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ABSTRACT **Previous studies on transient replication of papillomaviruses have shown an absolute requirement for the viral E1 and E2 proteins in DNA replication. Here we demonstrate that for human papillomavirus type 1a (HPV-1a) DNA, the E1 protein alone is sufficient for *in vivo* replication of plasmids containing the viral origin of replication. Replication was origin-specific and required the presence of a DNA sequence containing a putative E1 binding site, but the E2 binding sites were dispensable. In the presence of the E1 protein, E2 stimulated replication of plasmids containing the E1 and E2 binding sites, but no stimulation was observed when the origin plasmids lacked E2 binding sites. Conversely, in the presence of E1 alone, the E2 binding sites did not affect replication. Plasmids containing the replication origins of HPV-6b, HPV-18, and bovine papillomavirus type 1 (BPV-1) also replicated efficiently in the presence of the HPV-1a E1 and E2 proteins. However, plasmids containing the origins of HPV-6b and HPV-18 failed to replicate in the presence of HPV-1a E1 alone, whereas a plasmid containing the BPV-1 origin replicated to lower levels than the HPV-1a origin-containing plasmid. These results suggest that replication from papillomaviral origins in the presence of E1 alone is presumably dependent on the strength of E1–origin interactions. Additionally, E1-dependent replication is stimulated by the E2 protein in the presence of E2 binding sites.**

Papillomaviruses are small DNA viruses which infect humans and a wide range of animals. Human papillomaviruses (HPVs) cause mucosal as well as cutaneous epithelial lesions and some HPVs, such as types 16 and 18, are involved in the pathogenesis of cervical cancers (1–3). Papillomaviruses contain a circular, double-stranded genome of ≈8 kb, which is maintained at a relatively fixed copy number (1–4). HPVs provide useful model systems to study regulated DNA replication in eukaryotic cells (5). Numerous studies have been directed toward understanding the role of DNA sequences and viral proteins in the replication of bovine papillomavirus type 1 (BPV-1) and HPVs (4–17). These studies have shown that the origin of replication is located within the viral long control region (LCR). Also, an absolute requirement for the viral E1 and E2 proteins in transient replication of the viral origin DNA has been demonstrated.

The E1 and E2 genes of papillomaviruses are quite homologous and recent studies have shown a lack of specificity of these proteins since they support replication of plasmids containing the replication origins of heterologous papillomaviruses (6–10). The BPV-1 E1 protein is a 68-kDa nuclear phosphoprotein that binds to the origin and has ATPase, DNA helicase, and DNA-unwinding activities (13, 14, 18–23). The BPV-1 E2 protein is both an activator and a repressor of viral transcription (24–32). The BPV-1 E2 open reading frame encodes three proteins that originate from selective promoter usage and alternative mRNA splicing. The 48-kDa transactivator form of BPV-1 E2 protein binds with high affinity to palindromic $ACCGN_4CGGT$ sequences located within the LCR and enhances transcription from viral promoters (24–32). E2 is also known to form a specific complex with E1 and stimulate DNA replication in part by enhancing the binding of E1 to the origin of replication (20, 22, 33, 34). E2 has been shown to bind to replication protein A, and this interaction may also stimulate replication (35).

The origin of replication of BPV-1 includes a binding site for the E1 protein and several E2 binding sites. Previous studies have shown an absolute requirement for the E1 binding site and at least one (or one-half) E2 binding site in BPV-1 DNA replication (16, 17, 19). The BPV-1 E1 protein binds to an 18-bp inverted repeat (IR) element surrounding the *Hpa* I or *Hpa* I-like sequence present within the BPV-1 origin of replication (20, 36). Origins of replication of HPVs also contain an imperfect IR at the corresponding position, and the HPV-11 E1 protein binds weakly to a DNA fragment that includes this IR (22). Ten of 18 nt of this IR are highly conserved among different papillomaviruses and probably correspond to the E1 binding site (7, 20). An *in vitro* system has been developed for the replication of BPV-1 DNA (17–19). The results of these studies are in general agreement with the *in vivo* results. However, at high concentrations the E1 protein alone is capable of supporting replication of BPV-1 *ori* DNA *in vitro*, although under these conditions significant nonspecific replication of the vector DNA is also observed (19).

Most of the replication studies with HPVs to date have utilized viruses that are associated with mucosal lesions and cause benign or malignant disease of the lower genital tract (types 11 and 18). We have studied the *in vivo* replication of HPV-1a, which causes cutaneous lesions such as plantar warts. In this paper, we demonstrate that the E1 protein alone is sufficient for the transient replication of HPV-1a DNA, although the E2 protein stimulates replication. Furthermore, this stimulation by E2 is mediated through its binding sites. Our results show that replication requires a region containing the putative E1 binding site, but the E2 binding sites are dispensable. Although the HPV-1a E1 and E2 proteins supported efficient replication of all the papillomaviral DNAs tested, limited specificity for the cognate HPV-1a origin was observed in the presence of the E1 protein alone.

MATERIALS AND METHODS

Plasmid Constructions. All recombinant DNA procedures were carried out as described (37). The plasmids pUCLCR-1a (HPV-1a nt 6781–7815/1–226), pUCLCR-6b (HPV-6b nt 6499–7902/1–236), pUCLCR-18 (HPV-18 nt 6929–7857/1–

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Abbreviations: BPV, bovine papillomavirus; HPV, human papillomavirus; IR, inverted repeat; LCR, long control region.

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119), and pUCLCR-BPV (BPV-1 nt 6958–7945/1–475) were generated by inserting the appropriate restriction fragments into the multiple cloning sites region of pUC19. To localize the HPV-1a origin within the LCR, the following plasmids were generated by cloning restriction fragments (after filling in the ends with the Klenow fragment of DNA polymerase I and/or chewing back with T4 DNA polymerase, where necessary) into the *Hin*cII site of pUC19 unless otherwise indicated: pori348 (348-bp *Sca* I–*Mae* III fragment, HPV-1a nt 7499–7815/1–31); pori312 (312-bp *Hin*P1I–*Hin*fI fragment, nt 7593–7815/1–89); pori171 (171-bp *Taq* I–*Hpa* II fragment, nt 7767–7815/1–118 inserted into the *Acc* I site of pUC19); pori80 (80-bp *Taq* I–*Mae* III fragment, nt 7767–7815/1–31); pori68 (68-bp *Eco*RI–*Mae* III fragment, nt 7779–7815/1–31); and pori60 (60-bp *Bsi*EI–*Mae* III fragment, nt 7787–7815/1–31).

The vector pSG5, containing the simian virus 40 early promoter, was used for the expression of the HPV-1a E1 and E2 proteins in various cell lines (38). Plasmid pSGE1, which contains the HPV-1a open reading frame was generated by inserting a 2166-bp *Pvu* II fragment (nt 745–2910) into the filled-in *Bam*HI site of pSG5. pSGE2 contains the HPV-1a E2 open reading frame and was obtained by inserting a 1378-bp fragment (nt 2532–3909), modified to contain *Eco*RI and *Bgl* II ends, into the corresponding sites of pSG5.

Transient-Replication Assays. The human cell lines C-33A and 293 were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum. C-33A and 293 are human cervical carcinoma and adenovirus type 5-transformed kidney cell lines, respectively, of epithelial origin. Transfection of cell lines with plasmid DNA was carried out by calcium phosphate coprecipitation (39). Transfection efficiencies were 25–35% for C-33A and 50–60% for 293 cells. Transient-replication assays were done with 3–3.5 $\times 10^5$ cells in 60-mm plates and various amounts of plasmid DNA. Three to 4 days after transfection, low molecular weight DNA was isolated by Hirt extraction (40). The samples were first treated with an appropriate restriction enzyme to either linearize or cleave the plasmids into two fragments, one of which was complementary to the probe. To distinguish between replicated and unreplicated DNA, half of each sample was then treated with an excess of *Dpn* I to remove the unreplicated, methylated input DNA (41). *Dpn* I resistance has previously been used to demonstrate DNA replication in studies with mammalian cells, including studies with other papillomaviruses (7, 8, 16). The DNA samples were analyzed by electrophoresis (in 0.7% agarose gels with Tris/borate/EDTA buffer) followed by Southern blot hybridization (37). The DNA was transferred to GeneScreen membranes (DuPont) and the membranes were hybridized to ^{32}P-labeled pUC19 probe (2–4 $\times 10^7$ cpm) generated by a random-primer labeling kit (Amersham). The specific activity of the probes typically ranged from 4×10^8 to 1×10^9 cpm/μg of DNA. Blots were subjected to autoradiography at −70°C with an intensifying screen. The various bands on the blots were quantitated with an AMBIS Systems 100 radioanalytic detector. To account for small differences in transfection efficiencies of the various samples, the radioactivity in the *Dpn* I-resistant (replicated) plasmid bands was normalized to the radioactivity in the corresponding pSGE1 band not treated with *Dpn* I.

RESULTS

Transient Replication of Plasmids Containing the HPV-1a LCR. We transfected 0.5 μg of an LCR-containing plasmid (pUCLCR-1a) into C-33A cells along with various amounts of the pSGE1 and pSGE2 plasmids. The LCR plasmid replicated efficiently, as evidenced by *Dpn* I resistance of this DNA (Fig. 1). This conclusion is also supported by the lack of *Dpn* I-resistant bands corresponding to pSGE1 and

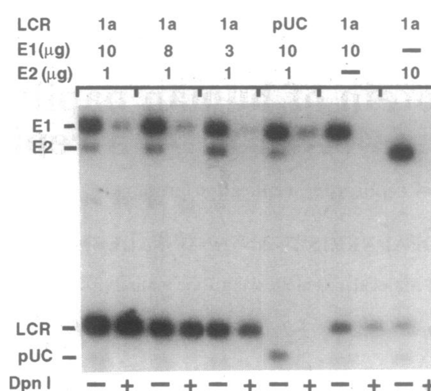

FIG. 1. Replication of the pUCLCR-1a plasmid in C-33A. C-33A cells were transfected with 0.5 μg of pUCLCR-1a and indicated amounts of the pSGE1 and pSGE2 plasmids. Cells were harvested 4 days after transfection, and low molecular weight DNA was isolated and digested with *Eco*RI. This treatment linearized pSGE2 and generated two fragments each for the pSGE1 and pUCLCR-1a plasmids, one of which contains vector sequences complementary to the probe. Half of each sample was then treated with *Dpn* I. The samples were subjected to agarose gel electrophoresis followed by Southern blot hybridization as described in *Materials and Methods*. The positions of the pUCLCR-1a (LCR), pUC19 (pUC), pSGE1 (E1), and pSGE2 (E2) plasmids are indicated. The autoradiogram was exposed for 1 hr.

pSGE2, which were transfected in much higher amounts than pUCLCR-1a. Replication of pUCLCR-1a increased with increasing amounts of the cotransfected pSGE1 plasmid, although this increase was not linear. The highest level of replication was obtained in the presence of 10 μg of pSGE1 and 1 μg of pSGE2 (Fig. 1). Low levels of pSGE2 plasmid (1 μg) stimulated replication, but no further increase in replication was seen at higher levels of this plasmid (data not shown). Surprisingly, pUCLCR-1a replicated to detectable levels upon cotransfection with pSGE1 alone (Fig. 1). This is in contrast to previous results with BPV-1 and some HPVs where an absolute requirement for both E1 and E2 was demonstrated. No replication of pUCLCR-1a plasmid was obtained in the presence of pSGE2 alone. Finally, the vector pUC19 did not replicate under any condition, demonstrating that the replication of the HPV-1a LCR plasmid was specific. In some samples, weak *Dpn* I-resistant signals were observed with pSGE1 (Fig. 1). However, this was not reproducible and may have been due to the higher levels of this plasmid used as compared with pUCLCR-1a. Based on the comparison of the signal in lanes untreated and treated with *Dpn* I, >90% of the DNA present in the transfected cells represented replicated DNA under optimal conditions. On the other hand, only 40% of the pUCLCR-1a DNA present in the sample with E1 alone was *Dpn* I-resistant, suggesting limited replication.

Localization of the HPV-1a Origin of Replication. The requirement for the E1 and E2 binding sites present within the HPV-1a LCR was investigated by testing the ability of plasmids containing various subregions of the viral LCR (Fig. 2*A*) to replicate in C-33A cells when cotransfected with pSGE1 and pSGE2. All plasmids containing the putative E1 binding site replicated to detectable levels (Fig. 2*B*). The plasmid pori60, containing the putative E1 binding site but lacking the E2 binding sites (Fig. 2*A*), replicated to detectable levels, demonstrating that the E2 binding sites are not absolutely required for HPV-1a replication. A plasmid containing two E2 binding sites but lacking the putative E1 binding site (pori81) did not replicate (data not shown). The plasmids pori80 and pori171, containing one and two E2 binding sites, respectively, replicated ≈5- and ≈15-fold better than pori60 (Table 1). These results suggest that the E2 binding sites stimulate replication in a cooperative manner. Interestingly, maximal replication

FIG. 2. Localization of the origin of replication of HPV-1a. (*A*) Organization of the HPV-1a LCR. Locations of the putative E1 and E2 binding sites are indicated. The sequence of the 60-bp region containing the minimal origin of HPV-1a is shown. The 16-bp IR surrounding the *Hpa* I sequence is underlined and compared with the corresponding sequences of BPV-1, HPV-6b, and HPV-18. Numbers correspond to the published sequence of HPV-1a (42). P, perfect E2 binding site; I, imperfect E2 binding site. (*B*) Transient-replication analysis of HPV-1a *ori* plasmids. C-33A cells were cotransfected with 0.5 μg of pUCLCR-1a or equimolar amounts of the indicated *ori* plasmids along with 10 μg of pSGE1 and 1 μg of pSGE2. In competition experiments, 0.5 μg of pUCLCR-1a along with equimolar amounts of the various *ori* plasmids were cotransfected. Four days after transfection, low molecular DNA was treated with *Eco*RI for noncompetition experiments. This treatment linearized pSGE2 and generated two fragments of pSGE1 and various *ori* plasmids. For competition experiments, the Hirt fractions were treated with *Hin*dIII, which linearized pSGE1 and the *ori* plasmids and generated two fragments of pSGE2. The positions of pUCLCR-1a and other *ori* bands are indicated. The autoradiogram was exposed for 1 hr.

was obtained with pori171 which replicated ≈3-fold more efficiently than pUCLCR-1a and pori312 (Table 1). This was surprising, since pori171 contains one high-affinity (E2BS-3) and one imperfect (E2BS-4) E2 binding site which, along with one or two additional imperfect E2 binding sites, are also contained within pori312 and pUCLCR-1a, respectively (Fig. 2*A*). To further investigate the replication efficiency of the various *ori* plasmids, competition experiments were carried out in which 0.5 μg of pUCLCR-1a and equimolar amounts of various test *ori* plasmids were cotransfected. The level of replication of the various *ori* plasmids compared with pUCLCR-1a indicates their relative efficiency of replication.

Table 1. Replication efficiencies of the various *ori* plasmids in the presence of the E1 and E2 expression plasmids

Plasmids	Relative replication efficiency	
	No competition	Competition
pUCLCR-1a	1	1
pori348	0.55	0.16
pori312	0.92	0.9
pori171	2.9	7.6
pori80	1.53	1.9
pori68	0.31	0.28
pori60	0.23	0.1

The relative replication efficiency of the *ori* plasmids was determined by using an AMBIS 100 systems radioanalytic detector to compare the radioactivity present in the *ori* bands in Southern blots. The numbers obtained with the pUCLCR-1a plasmid were arbitrarily assigned a value of 1 and the relative efficiency of replication of other *ori* plasmids is given with reference to it. Most values represent an average of three independent experiments.

In general, the replication pattern obtained in competition experiments was similar to that obtained in the absence of competition (Fig. 2*B* and Table 1). For example, pori171 replicated to ≈8-fold higher levels than pUCLCR-1a and pori312. These results indicate that the region between HPV-1a nt 7593 and 7767 (boundaries of pori312 and pori171) may contain sequences that are inhibitory for DNA replication. Replication of pori348 was about 6-fold lower than replication of pUCLCR-1a. This may be due to the fact that while pori348 contains two imperfect and one perfect E2 binding site (E2BS-1, -2, and -3), it lacks E2BS-4, which is located adjacent to the putative E1 binding site (Fig. 2*A*).

E1 Protein Alone Is Sufficient to Support Replication of *ori* Plasmids. We further investigated the ability of the various HPV-1a *ori* plasmids to replicate in the presence of pSGE1 alone. All plasmids containing the E1 binding site—including pori60, which lacks the E2 binding sites—replicated to significant levels (Fig. 3). The vector pUC19 did not give a detectable signal. These results clearly show that a 60-bp sequence (HPV-1a nt 7787–7815/1–31) containing a putative E1 binding site is sufficient for E1-dependent replication. Approximately 2- to 3-fold higher levels of replication were obtained with pori312, pori171, and pori68 than with pUCLCR-1a. The significance of this observation remains to be evaluated. In another experiment, the vector pUC19 DNA was cotransfected along with pori171 and various amounts of the E1-expressing plasmid. While pori171 replicated to significant levels even in the presence of low amounts of the E1-expressing plasmid, no replication of pUC19 was observed even at higher levels of the pSGE1 plasmid (Fig. 4). Thus, replication of pori171 in the presence of E1 was specific.

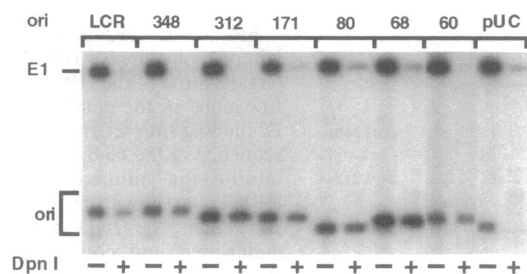

FIG. 3. Replication of the HPV-1a *ori* plasmids in the presence of HPV-1a E1 alone. One-half microgram of pUCLCR-1a or equimolar amounts of the other plasmids were transfected into C-33A cells along with 10 μg of pSGE1. Four days after transfection, low molecular DNA was isolated, treated with *Eco*RI, and analyzed by Southern blot hybridization.

Replication of Other Papillomavirus DNAs Supported by the HPV-1a E1 and E2 Proteins. We tested the ability of the HPV-1a E1 and E2 proteins to support replication of plasmids containing the LCRs of HPV-6b and -18 and BPV-1. All plasmids replicated efficiently, as indicated by the presence of *Dpn* I-resistant bands corresponding to the LCR plasmids (Fig. 5*A*). While pUCLCR-1a, pUCLCR-18, and pUCLCR-BPV replicated to similar levels, pUCLCR-6b replicated 4- to 5-fold more efficiently than the cognate *ori* plasmid. This was also apparent in competition experiments where pUCLCR-6b replicated to significantly higher levels than the HPV-1a origin-containing plasmid (Fig. 5*A*). Transient replication assays with plasmids containing the LCRs from various papillomaviruses in the presence of pSGE1 alone revealed that pUCLCR-1a replicated to significant levels, as observed earlier. On the other hand, pUCLCR-BPV replicated to much lower levels, and pUCLCR-6b and pUCLCR-18 failed to replicate (Fig. 5*B*). This is intriguing, since pUCLCR-6b replicated more efficiently than pUCLCR-1a in the presence of both E1 and E2. These results suggest that E1 may be the main determinant of papillomavirus-type replication specificity and that E2 stimulates replication by strengthening the interaction of E1 and possibly cellular proteins with the viral origins.

DISCUSSION

Our results demonstrate that plasmids containing the LCR of HPV-1a can replicate efficiently in human C-33A and 293 cells when cotransfected with plasmids expressing the viral E1 and E2 proteins. The minimal origin of replication of HPV-1a is contained within a 60-bp sequence (HPV-1a nt 7787–7815/1–31) (Fig. 2*A*). Interestingly, this region does not contain an E2 binding site but includes a 16-bp imperfect IR that presumably corresponds to the binding site for the HPV-1a E1 protein (refs. 20 and 36; Fig. 2*A*). These results show that limited replication can occur in the absence of the E2 binding sites. This is in contrast to the BPV-1, HPV-11, and HPV-18 systems, where at least one E2 binding site is essential for replication (6, 10, 12, 16, 17, 22). Although an E2 binding site is not absolutely required for HPV-1a replication, it stimulates replication to significant levels. Plasmid pori60, which contains the putative E1 binding site but lacks the E2 binding sites, replicated to similar levels in the presence of E1 alone and both E1 and E2 (Figs. 2*B* and 3; data not shown). These results show that stimulation of replication by E2 requires its binding sites. It is likely that the E2 binding sites stimulate replication of *ori* plasmids by significantly increasing the recruitment of the E1 protein to the origin. It is also possible that the E2 protein promotes a better recruitment of the chromosomal replication proteins. The plasmid pori171 replicated to significantly higher levels than pUCLCR-1a and pori312, suggesting that the region between HPV-1a nt 7593 and 7767 (boundaries of pori312 and pori171) may be inhibitory for replication, at least in transient assays.

Experiments described here demonstrate that the HPV-1a E1 protein alone is both necessary and sufficient for the transient replication of its cognate *ori* DNA (Figs. 3 and 4). This is in contrast to previous *in vivo* studies with other papillomaviruses, where an absolute requirement for the E2

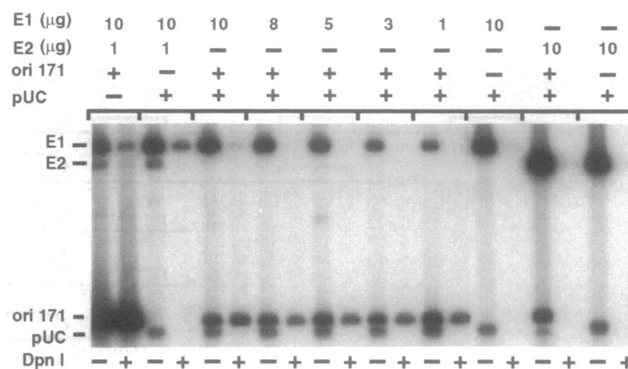

FIG. 4. The HPV-1a E1 protein alone supports the specific replication of the pori171 plasmid. C-33A cells were transfected with 0.5 μg of pori171 and equimolar amounts of the pUC19 DNA along with the indicated amounts of pSGE1. The Hirt fractions were treated with *Eco*RI. The autoradiogram was exposed for 6 hr.

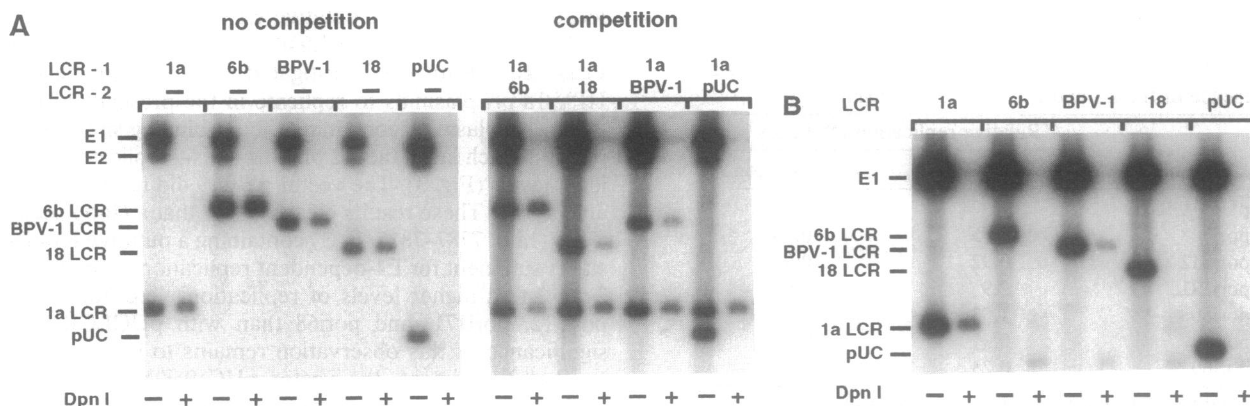

FIG. 5. Replication of plasmids containing the LCR of various papillomaviruses. One-half microgram of pUCLCR-1a or equimolar amounts of the other LCR-containing plasmids were transfected into human 293 cells along with pSGE1 (10 μg) and pSGE2 (1 μg) (*A*) or with 10 μg of pSGE1 alone (*B*). In competition experiments, 0.5 μg of pUCLCR-1a and equimolar amounts of other LCR plasmids were used. Transient-replication assays were carried out as described in *Materials and Methods*. The positions of various LCR plasmids and pSGE1 and pSGE2 are indicated.

protein in replication has been shown (6, 8, 10, 12, 16, 22). Although we have not measured the levels of the E1 protein, it is possible that replication of HPV-1a DNA in the presence of E1 alone is due to its high levels in transiently transfected cells. Under these conditions the E2 binding sites may be dispensable for replication. Another possibility is that the E1 protein of HPV-1a may differ from those of other papillomaviruses in its DNA replication activity. In previous *in vivo* studies with BPV-1, HPV-11, and HPV-18, no replication was observed even at high levels of the E1-expressing plasmids (refs. 7, 8, 12, and 22; data not shown). The above results also suggest that the HPV-1a E1 protein is capable of interacting *in vivo* with the cellular replication factors that assemble at the origin during the initiation of replication. *In vitro* replication experiments with BPV-1 have shown that the E1 protein alone can support replication of BPV-1 *ori* plasmids to a limited degree at high concentrations (18, 19). However, the vector plasmid also replicated to about one-fifth the levels obtained with the BPV-1 ori plasmids (18, 19). Our *in vivo* results are similar to these *in vitro* results, except that no origin-independent replication was observed even at higher levels of the E1-expressing plasmid (Fig. 4).

Plasmids containing the HPV-6b, HPV-18, and BPV-1 LCR also replicated efficiently in the presence of pSGE1 and pSGE2 plasmids (Fig. 5*A*). Interestingly, pUCLCR-6b replicated better than pUCLCR-1a even in competition experiments. Similar results were obtained in experiments with the E1 and E2 proteins of HPV-18, where the HPV-6b *ori* plasmid replicated more efficiently than the HPV-18 *ori* plasmid (12). It is possible that the E1–E2 complexes of HPV-1a and HPV-18 interact more efficiently with the HPV-6b origin because it contains more high-affinity E2 binding sites (7, 28). While the HPV-6b origin includes three perfect E2 binding sites having the consensus sequence $ACCGN_4CGGT$, that of HPV-18 contains two perfect and one imperfect E2 binding site, $ACCN_6GGT$ (7). The HPV-1a origin contains only one E2 binding site with a perfect match to the consensus sequence. In the presence of the E1-expressing plasmid alone, pUCLCR-1a replicated to significant levels while pUCLCR-BPV showed limited replication (Fig. 5*B*). However, pUCLCR-6b and pUCLCR-18 failed to replicate under these conditions. The lack of replication of pUCLCR-6b is especially striking, since this plasmid replicated more efficiently than the pUCLCR-1a plasmid in the presence of both pSGE1 and pSGE2. These results suggest that replication in the presence of HPV-1a E1 alone may be dependent upon the ability of this protein to bind to the 16-bp IR sequence representing its putative binding site (Fig. 2*A*). The corresponding 18-bp IR present within the BPV-1 origin has been shown to be the binding site of the BPV-1 E1 protein (20, 36). Thirteen out of 16 nt in the 16-bp imperfect IR of HPV-1a are conserved in HPV-6b and BPV-1, while only 10 of these nucleotides are conserved in HPV-18 (Fig. 2*A*). The variable nucleotides in the 16-bp IR of HPV-1a may be critical for DNA-binding and replication activities of the HPV-1a E1 protein. In addition, sequences surrounding the 16-bp IR and present in pori60 (HPV-1a nt 7787–7815/1–31) may also be required for E1-dependent replication. Mutational analyses of the origin of replication of BPV-1 have shown that binding of E1 to the origin is necessary but not sufficient for DNA replication (11, 20). Thus, factors other than the ability of E1 to bind DNA may also be important in DNA replication. The presence of the E2 protein may overcome the inability of the HPV-1a E1 protein to bind to the origins of HPV-6b and HPV-18 and stimulate their replication. Although the viral E1 and E2 proteins support replication of heterologous origins in various cell lines (7, 8, 12), HPVs display strict tissue tropism *in vivo* (1–3). Thus, tissue specificity of these viruses may be determined at the level of expression of the E1 and E2 proteins. The ability of the HPV-1a E1 protein alone to support *in vivo* replication of *ori* DNA provides a good model system to study papillomavirus DNA replication in which the sequence requirements and specificity of replication can be studied in the absence of the stimulatory effects of E2. Also, since E1 is the most conserved and the only viral protein which is absolutely required for the replication of all papillomaviruses studied to date, it may provide an ideal target for antiviral drugs.

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