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Acidic transcription factors alleviate nucleosome-mediated repression of DNA replication of bovine papillomavirus type 1

RONG LI* AND MICHAEL R. BOTCHAN†

Division of Biochemistry and Molecular Biology, Department of Molecular and Cell Biology, 401 Barker Hall, University of California, Berkeley, CA 94720

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ABSTRACT **The papillomavirus E2 transcription factor is directly involved in viral DNA replication. Previous studies have shown that E2 interacts with both the viral E1 helicase and cellular replication proteins, and thus it may facilitate their targeting to the origin of replication. We demonstrate here that E1-mediated replication of bovine papillomavirus type 1 is repressed by nucleosomal assembly. The E2 protein counteracts this repression, and such activation requires the E2-binding sites adjacent to the origin of replication. These *in vitro* results are consistent with the previous *in vivo* findings that both E2 and specific E2 binding to DNA are absolutely required for replication of bovine papillomavirus. Furthermore, the function of E2 in preventing nucleosome-mediated repression can be achieved as well by several other acidic transcription factors. These data therefore strongly support the idea that a group of enhancer proteins may utilize similar mechanisms to stimulate transcription and replication.**

Recent emerging evidence strongly suggests involvement of both cis and trans transcriptional elements in the regulation of the initiation of DNA replication (for review see refs. 1 and 2). The link between transcription and replication raises an important issue concerning the mechanisms that particular enhancer-binding proteins utilize to activate both processes. It appears that these transcription factors stimulate transcription or replication in at least two ways. Both the basal transcription apparatus and the replication apparatus consist of a distinct family of general factors, each of which is responsible for the assembly of a multicomponent nucleoprotein complex at initiation sites (3, 4). It has been proposed that one function of enhancer proteins is to aid the assembly of such initiation complexes by interacting with one or multiple components of the basal machinery. For example, transcription factors like GAL-VP16 and Sp1 have been shown to target the general transcription factors, in particular components of the TFIID complex or TFIIB (5, 6), thus helping recruit these factors to the promoter region. Analogous to transcriptional activation, some enhancer proteins that stimulate DNA replication have been shown to interact with replication protein A (RPA), an essential replication factor, thus supporting the hypothesis that enhancer proteins may help assemble the DNA replication apparatus (7–9). A second role for enhancer proteins in regulating transcription and replication has been implicated by studies on the impact of chromatin structure on the two processes. Given the fact that nucleosome structure in eukaryotes imposes a formidable impediment to the initiation of both transcription and replication, it follows that factors that overcome the nucleosomal repression will facilitate both processes. Along this line, both genetic and biochemical studies have provided a wealth of evidence for an intricate interplay between transcription activation and the chromatin structure (for review, see ref. 10). In parallel to the work on transcriptional activation, it has also been demonstrated in the studies of simian virus 40 (SV40) replication that enhancer proteins, such as CTF and GAL-VP16, can counteract the nucleosomal repression of *in vitro* replication (11, 12). However, due to the limited number of *in vitro* systems for studying DNA replication, the roles of enhancer proteins in the regulation of DNA replication have not been characterized as extensively as those in transcriptional activation.

Development of an *in vitro* replication system for bovine papillomavirus (BPV) type 1 DNA provides a useful tool for studying the regulation of eukaryotic replication (13). Biochemical studies using this *in vitro* system have led to several important results regarding both cis and trans elements for BPV replication. Besides the host replication machinery, two virus encoded proteins, E1 and E2, are required for efficient replication. E1 has been shown to be a DNA helicase and a site-specific DNA-binding protein that recognizes the replication origin. E2, a typical site-specific DNA-binding transcription factor, can physically interact with E1 and facilitate E1 binding to the replication origin. The characteristics of BPV *in vitro* replication are, in most part, consistent with those revealed by *in vivo* replication assays (14, 15). However, several important differences do exist between the two replication assays; the most prominent one concerns the extent to which E2 and its binding sites are required for BPV replication. While *in vivo* replication absolutely requires both E2 and contact of E2 with specific DNA sequences, a very low but detectable level of *in vitro* replication was observed in the presence of limiting concentrations of E1 alone (13, 16). Moreover, E2 activation of *in vitro* DNA replication at limiting E1 concentrations does not seem to depend on the E2 DNA-binding sites adjacent to the origin of replication.

Since BPV DNA exists as a minichromosome in the living cell and nucleosome structure has been shown to repress SV40 DNA replication (11), we speculated that BPV replication *in vivo* may require E2 and its binding sites more stringently in the cell than *in vitro*, to efficiently compete with the nucleosomes for occupancy of the replication origin. To test this hypothesis and resolve the differences between the *in vivo* and *in vitro* results, we reconstituted chromatin templates with purified histone octamers and examined the replication efficiency in the *in vitro* replication assays. We observed that the chromatin assembly greatly inhibited BPV *in vitro* replication, and the E1 protein alone was not sufficient to overcome the repression. This repression, however, was substantially relieved by inclusion of E2 and its recognition sites close to the origin of replication. Furthermore, E2, with respect to its ability to alleviate the nucleosomal effect, can be functionally replaced by other acidic transcription factors when these activators are targeted to the replication origin. These results support the idea that nucleosomal

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Abbreviations: BPV, bovine papillomavirus; RPA, replication protein A; SV40, simian virus 40.

*Present address: P.O. Box 100, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.
†To whom reprint requests should be addressed.

antirepression is an important component of the mechanism by which enhancer proteins stimulate DNA replication.

MATERIALS AND METHODS

***In Vitro* Assembly of Chromatin Templates.** Reconstitution of chromatin was performed according to the protocol given by Laybourn and Kadonaga (17). Briefly, core histone octamers were purified from calf thymus and subsequently mixed with high molecular weight poly(glutamic acid). For preparative purposes, supercoiled plasmid DNA was incubated with the histone/poly(glutamic acid) complex at various mass ratios of histone to DNA as described in the text. After incubation, an aliquot of the reaction mixture equivalent to 50 ng of DNA in about 1 μl was added to an *in vitro* replication reaction mixture. For the analytical experiment shown in Fig. 1*A*, the supercoiled DNA was treated with eukaryotic topoisomerase 1 prior to incubation with histone/poly(glutamic acid), topoisomerase 1 was present throughout the assembly reaction. After treatment with proteinase K, the DNA products were extracted with phenol/chloroform, precipitated with ethanol, and analyzed by electrophoresis on a 0.8% agarose/TBE gel (TBE = 90 mM Tris borate, pH 8.3/2 mM EDTA) in the presence or absence of 3 mM chloroquine.

Nuclease Digestion. Nucleosome templates assembled from 2 μg of supercoiled DNA were partially digested with micrococcal nuclease (10 units; Boehringer Mannheim) at 37°C for the period of time indicated in the figure. The DNA products were analyzed by electrophoresis on a 1.5% agarose gel. Native calf thymus chromatin was digested with nuclease in a similar manner and included (17).

Plasmid Construction and Protein Purification. pKSOT, pKSO, and OT-GAL were described previously (7, 13). D112 contains point mutations at the two E2-binding sites: wild-type BS11 ACCGAAACCGGT; mutant BS11 GTCGAAACCGGT; wild-type BS12 ACCATCACCGTT; mutant BS12 TGCATCACCGTT. Standard site-directed mutagenesis methods were employed to introduce these mutations into the pKSO construct.

Expression and purification of various GAL4 derivatives were described previously (7). E1 and E2 proteins were purified according to methods of Yang *et al.* (13).

***In Vitro* Replication.** BPV *in vitro* replication assays were performed as previously described (13, 16). Each reaction mixture (25 μl) contained 30 mM Hepes (pH 7.5), 7 mM $MgCl_2$, 45 mM potassium glutamate, 4 mM ATP, 200 ng of DNA, 40 mM phosphocreatinine, creatinine kinase at 100 μg/ml, 10 μl of cell extract, and E1 and E2 protein. Chromatin templates as prepared above were added into a replication mixture that contained E1, E2, GAL4 derivatives, FM3A extract (13), and other reagents. The reactions proceeded at 37°C for 2 hr. Replication products were subjected to electrophoresis on a 0.8% agarose gel. The dried gel was exposed to x-ray film for 4–16 hr.

RESULTS

***In Vitro* Reconstitution of Chromatin Templates.** There are several methods available to assemble chromatin complexes for *in vitro* studies. We adopted the protocol described previously by Laybourn and Kadonaga (17), as this technique employs purified components and therefore does not introduce unknown factors to the replication system. Purified histone octamers were deposited upon circular DNA by mixing with poly(glutamic acid), and well-defined nucleosomal templates at various histone-to-DNA ratios were subsequently added to the BPV *in vitro* replication system.

The efficiency of the chromatin reconstitution was analyzed by gel electrophoresis and partial digestion with micrococcal nuclease (Fig. 1). The starting materials used in Fig. 1*A* were relaxed circular DNA (lane 2), and nucleosomes were assembled with various amounts of histones in the continuous presence of topoisomerase 1. The products were deproteinized, and the different topoisomers were resolved by gel electrophoresis in the absence or presence of chloroquine. Nucleosome assembly introduces negative supercoils into relaxed circular DNA, and the superhelical density of the DNA increases with increasing numbers of nucleosomes formed on the template. As shown in Fig. 1*A*, the majority of the DNA migrates at the position for supercoiled DNA (form I) at mass ratios of 0.8–1.2 of core histone to DNA. This indicates quantitative formation of densely packed nucleosomes. To further characterize the reconstituted chromatin templates, we treated the histone–DNA complexes with micrococcal nuclease for limited periods of time. As shown in Fig. 1*B*, digestion of the naked plasmid DNA generated only small oligonucleotides of heterogeneous size (lanes

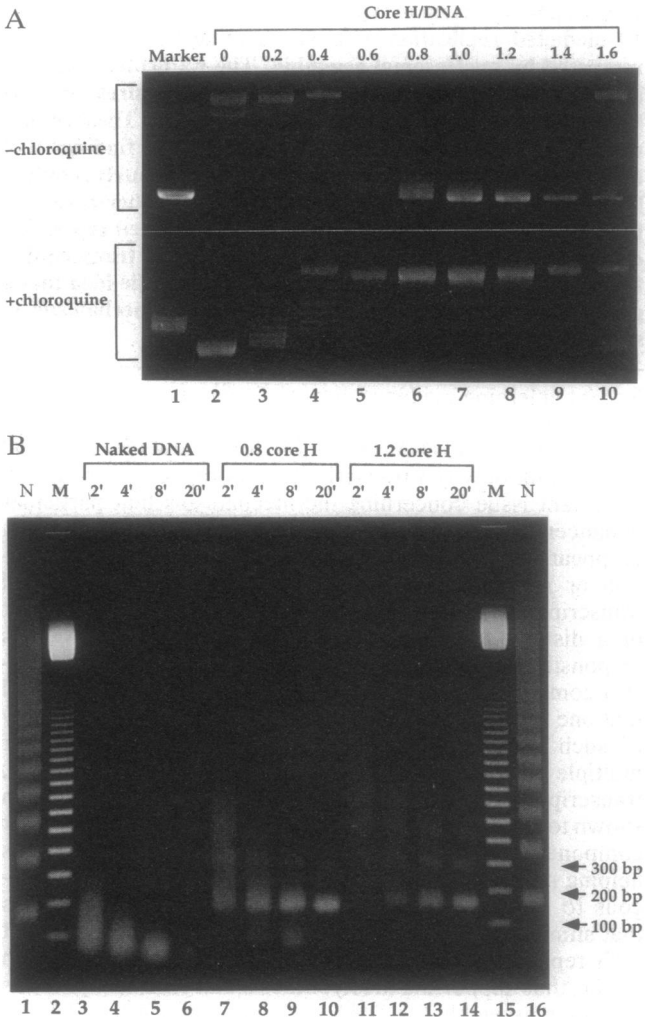

FIG. 1. Characterization of the reconstituted chromatin templates. (*A*) Supercoiling measurement of the reconstitution efficiency. Core histone octamers were deposited on the plasmid pKSO in the presence of topoisomerase 1. The DNA samples were subsequently analyzed by agarose gel electrophoresis in the absence or presence of chloroquine. The various mass ratios of core histones (core H) to DNA are indicated on the top of the gel. The marker represents the starting supercoiled DNA. (*B*) Limited digestion of the chromatin DNA by micrococcal nuclease. After reconstitution with different amounts of core histones, the reaction products were treated with the nuclease for various periods of time, as indicated in minutes at the top of the gel. The resulting fragments were resolved on a 1.5% agarose gel. As a comparison, native chromatin DNA from calf thymus (N) was also digested with the nuclease (lanes 1 and 16). M represents the 100-bp-unit ladder (Pharmacia), and the positions for 100, 200, and 300 bp are indicated on the right.

3–6). In contrast, digestion of the assembled chromatin yielded a DNA ladder with repeat units roughly 150 bp in length (lanes 7–14), strongly indicating that the reconstituted chromatin templates contained nucleosomes. We have noticed, however, that the repeat size of the digested reconstituted nucleosomes was smaller than that of the native nucleosomes isolated from calf thymus (≈180 bp; lanes 1 and 16); this is most likely due to the absence of histone H1 in the reconstituted material. In addition, when the *in vitro* assembled minichromosomes were centrifuged through a sucrose gradient, the sedimentation coefficient of the materials was found to increase with increasing concentration of input histones (data not shown), presumably due to a more compact structure and increased mass as more nucleosomes formed on the DNA.

E2 Antagonizes the Nucleosomal Repression of BPV DNA Replication. To test the effect of nucleosomal formation on BPV DNA replication *in vitro*, we initially focused on two replication templates, pKSOT and pKSO. [As illustrated in Fig. 3*A*, one prominent difference between the two plasmids is that pKSOT does not contain the two E2-binding sites (BS11 and BS12) and therefore does not replicate *in vivo* (R. Mendoza and M.R.B., unpublished observations; ref. 15).] Fig. 2 shows the *in vitro* replication of both templates under various conditions. As expected, neither template replicated in the absence of both E1 and E2 (lanes 1–6) (13). When E1 was added to the replication reaction mixture alone, both naked DNA templates generated a low replication signal (lanes 7 and 10) (13), which was greatly repressed by the chromatin assembly (lanes 8, 9, 11, and 12). Titrating E1 over a 3-fold concentration range showed that E1 by itself was not sufficient for antirepression (data not shown). This implies that the E1 protein alone is not sufficient to support efficient replication on chromatin templates, consistent with the previous report on BPV *in vivo* replication. Upon addition of E2 to the reaction mixture, replication of the two naked templates was stimulated to a similar level (compare lanes 13 and 16 with lanes 7 and 10) (13), however, replication of the reconstituted chromatin templates behaved very differently. While replication of pKSOT was severely reduced by nucleosome assembly (lanes 14 and 15), the template pKSO replicated at an only slightly lower level with increasing amounts of core histones (lanes 17 and 18). These results are shown in a quantitative manner in Fig. 2*B*. Taken together, the data indicate that the E2 protein substantially prevents the nucleosomal repression of BPV replication and that such antagonism relies on certain cis elements, presumably the E2 binding sites, around the origin of replication.

To assess more directly the importance of the two E2 binding sites present in the pKSO chromatin template, point mutations were introduced into the two sites (BS11 and BS12) that abolish E2 binding (data not shown). The resulting mutant template, D112, was assembled into a minichromosome and subsequently tested for its ability to replicate *in vitro*. The reconstitution efficiencies of the wild-type and the mutant templates were essentially the same, as examined by gel electrophoresis (data not shown). As shown in Fig. 3*B*, while a robust replication signal of pKSO was detected at all ratios of histone to DNA (lanes 4–6), replication of D112 was dramatically compromised by the incorporation of the histone octamers, as it was in the case of pKSOT (lanes 1–3 and 7–9). The repression by the histone octamers is quantitated in Fig. 3*C*. It is clear that the nucleosomal repression on the templates lacking the E2-binding sites was much more significant than that on the wild-type template. We thus infer that specific contact with the DNA sites is crucial for E2 to help in effectively competing with the nucleosome repression. It is also interesting that, although the E2 sites are not absolutely required for *in vitro* replication of naked templates, their presence does moderately enhance the replication signal (compare lane 4 with lanes 1 and 8). A more extensive comparison of the pKSO and pKSOT DNAs as

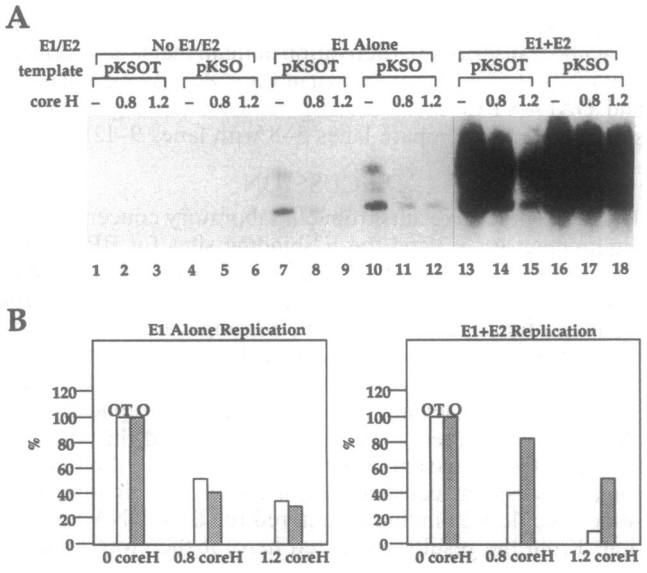

FIG. 2. E2 antirepresses the nucleosome-mediated repression of BPV *in vitro* replication. (*A*) After incubation of the plasmids pKSOT or pKSO with various amounts of core histones, the DNA templates were added to the *in vitro* replication reaction mixtures, with no viral proteins (lanes 1–6), with 90 ng of E1 (lanes 7–12), or with both 90 ng of E1 and 6 ng of E2 (lanes 13–18). The same amounts of E1 and E2 were used in experiments described later in this study. (*B*) Quantitation of the replication results in *A*. The replication efficiencies of the chromatin templates were compared to the efficiency of the naked DNA, which was designated as 100%. The replication of pKSOT (OT) and pKSO (O) are represented by blank and shaded bars, respectively.

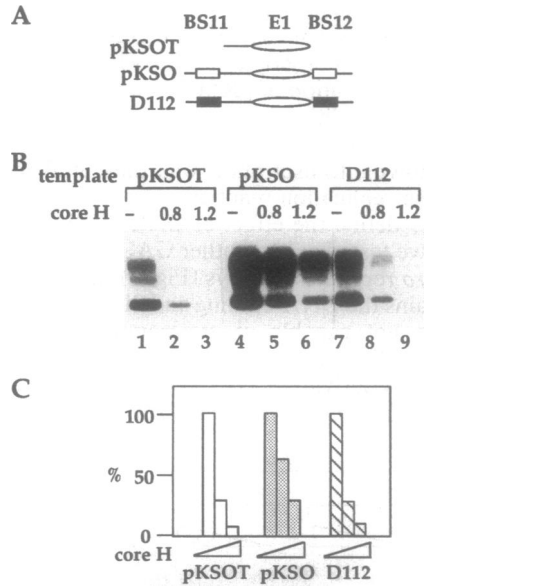

FIG. 3. The antirepression of E2 is dependent upon the E2-binding sites. (*A*) Diagram of the DNA templates pKSOT, pKSO, and D112. The oval represents the E1-binding site; the two boxes indicate the two E2-binding sites, BS11 and BS12. The plasmid D112 contains point mutations in the two E2-binding sites, as indicated by the black boxes. (*B*) Autoradiogram showing the replication products of the naked (−) or chromatin templates at two different mass ratios of core histone to DNA (0.8 and 1.2). (*C*) Quantitation of the *in vitro* replication shown in *B*. The replication signal of each naked DNA template is designated 100%.

templates for DNA replication will be provided elsewhere (ref. 18, and P. Park and M.R.B., unpublished results). This result argues that, even in the absence of nucleosomal structure, the specific interaction between E2 and its DNA site facilitates the initiation of replication.

Acidic Transcription Factors Can Provide for E2 in Nucleosomal Antirepression. As they do in their activation of transcription, enhancer proteins often stimulate replication synergistically, and in some cases rather promiscuously. For an example, it has been shown that CTF and GAL4-VP16 have similar effects on SV40 replication by relieving the nucleosomal repression (11, 12). Therefore we were interested in knowing whether the role of E2 in overcoming the nucleosomal repression could be functionally played by other transcription factors. Toward this end, we investigated the effect of GAL4-VP16 on the replication of a plasmid called OT-GAL, in which five GAL4-binding sites are fused adjacent to the replication origin in pKSOT (7). As shown in Fig. 4, due to the lack of the E2 binding sites on either template, replication of both pKSOT and OT-GAL templates in the presence of E1 and E2 were strongly inhibited by the incorporation of the core histones (lanes 1–4 and 9–12). However, when the GAL4-VP16 protein was included in the replication reactions, several intriguing observations were made. First, GAL4-VP16 stimulated replication of the naked DNA template of OT-GAL (compare lanes 1 and 5), consistent with our previous results. The stimulation was most likely due to the physical interaction between the acidic activation domain of VP16, a viral enhancer protein, and RPA, an essential component in the eukaryotic replication machinery (7). Second, the magnitude of GAL4-VP16 stimulation on the OT-GAL replication persisted with increasing amount of the core histones (lanes 6–8). In fact, the replication signal did not appreciably change even at the highest ratio of core histones to DNA used in the experiment (lane 8). This result indicates that, similar to the E2 protein, GAL4-VP16 can antagonize the nucleosomal repression of BPV replication. Finally, the results in Fig. 4 also demonstrate that the stimulation of GAL4-VP16 requires its specific binding to the GAL4 sites near the replication origin, as GAL4-VP16 did not have any effect on the replication of pKSOT (compare lanes 5–8 with lanes 13–16). In addition, since GAL4-VP16 enhancement of replication was insensitive to the inhibitor α-amanitin (ref. 7; data not shown), transcription was thus unlikely to be involved in the replication reaction.

To further define the effect of GAL4-VP16 on chromatin templates, we tested several other GAL4 protein derivatives in the *in vitro* replication assays (Fig. 5). GAL4-(1–147) (19), which contains the DNA-binding and dimerization domain of GAL4, was not capable of overcoming the nucleosomal repression of BPV DNA replication (Fig. 5*A*, lanes 13–16). Another GAL4 derivative, GAL-VP16-(413–454), contains a truncated activation domain of VP16 and possesses only

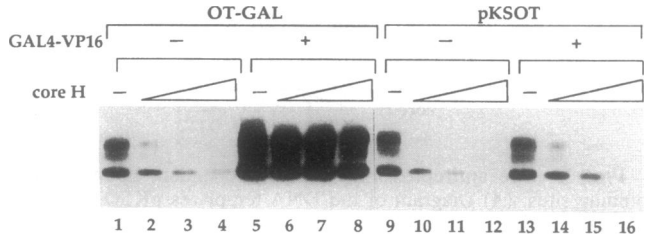

FIG. 4. GAL4-VP16 can functionally replace the antirepressive activity of E2. The chromatin templates of OT-GAL and pKSOT were reconstituted at histone to DNA mass ratios 0.8, 1.0 and 1.2 and subsequently tested in a replication reaction mixture that included the FM3A extract, E1, and E2. In lanes 5–8 and 13–16, 36 ng of GAL4-VP16 was added to the reaction mixture.

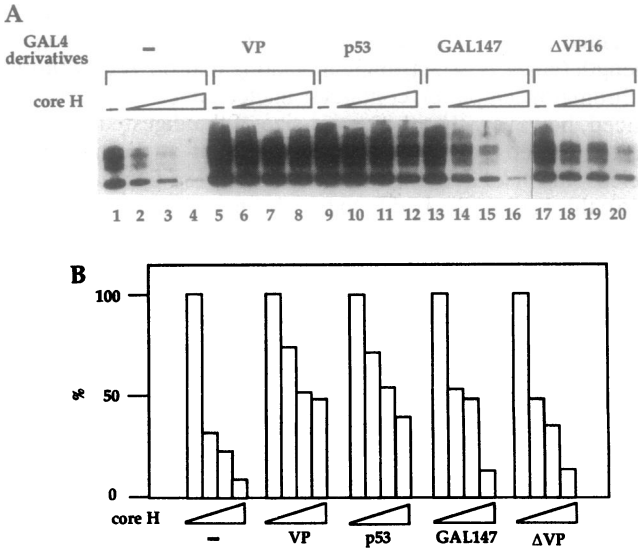

FIG. 5. Comparison of the antirepressive ability of various GAL4 derivatives for BPV replication *in vitro*. (*A*) The plasmid OT-GAL was incubated with core histones at mass ratios of histone to DNA of 0.8, 1.0, and 1.2. The resulting chromatin templates were examined in the replication assay in the presence of GAL4-VP16 (VP; lanes 5–8), GAL4-p53-(1–73) (p53; lanes 9–12), GAL4-(1–147) (GAL147; lanes 13–16), or GAL4-VP16-(413–454) (ΔVP16; lanes 17–20). See the text for detailed description of each fusion protein. Each GAL4 derivative used in the experiment was normalized by the DNA-binding activity measured by a DNase 1 footprint assay. (*B*) Quantitation of the replication data shown in *A*.

partial transcriptional activity (20). When added to the replication reactions, this fusion protein slightly enhanced the replication efficiency of the chromatin templates (lanes 17–20). Finally, we tested GAL4-p53-(1–73), which contains the N-terminal 73 amino acids of the tumor suppressor p53 (for review, see ref. 21). This region of the p53 protein contains an acidic transcriptional activation domain that is as potent as that of VP16 (22, 23). As shown in Fig. 5*A*, GAL4-p53-(1–73) and GAL4-VP16 overcame the nucleosomal repression to similar extents (compare lanes 5–8 with lanes 9–12).

DISCUSSION

The initial *in vitro* results from our laboratory concerning the requirement for E2 and the E2-binding sites for BPV DNA replication seemed to differ from the *in vivo* observations made by Ustav *et al.* (14, 15). This study, using chromatin templates for *in vitro* replication, has implicated the nucleosome structure as the likely reason for the differences between *in vitro* and *in vivo*. First, we have shown that E1 alone is insufficient to alleviate repression of *in vitro* replication by chromatin structures. Second, we have found that the E2-binding sites are essential for E2 to antagonize the nucleosomal repression, consistent with the *in vivo* findings that some specific E2 binding is required for BPV DNA replication. From the results presented here, it therefore appears that additional protein–protein interaction (E1–E2) and protein–DNA interaction (E2–E2 sites) are necessary to strengthen the replication preinitiation complex in a competition with the nucleosome structure for the origin of DNA replication. It is worthwhile to point out that the poly(glutamic acid) method used in this study is in fact an artificial way of creating nucleosomal templates. Although this method apparently does efficiently deposit the core histones on the DNA templates, the assembled nucleosomal DNA may differ from native chromatin in certain respects, such as linker space and distribution. In addition, the chromatin templates used in this study lack histone H1 and nonhistone

proteins that are normally associated with the native chromatin DNA. H1 has been shown to impose repression on transcription in addition to the core histone octamers, and other structural proteins may also play a role in the interplay between transcription factors and the chromatin structure. Thus, it is reasonable to suggest that the addition of other repressive proteins such as H1 to preassembled chromatin structures will only enhance the requirements for E2 and its DNA-binding sites. Nevertheless, it will be informative in future studies to compare the results from assays using various reconstitution methods and incorporating other structural components on the chromosomal DNA. In fact, Guo and DePamphilis (24) found that GAL-VP16 could not stimulate the SV40 core provided with proximal GAL-4 DNA sites for replication in COS cells, although in other cell types utilizing a similar construct of the polyomavirus, the core origin could be activated by the fusion protein. These results emphasize that *in vitro* conditions do not necessarily mimic those found *in vivo*, as Cheng *et al.* (12) found that in a cell-free system, GAL-VP16 could activate SV40 chromatin replication. In particular, it is possible that strong enhancer proteins such as GAL-VP16 may, *in vivo* in a given cell type, activate transcription at the expense of replication for certain constructs where strong core promoter elements overlap with replication determinants.

Considerable evidence suggests an important role for transcription factors in the regulation of DNA replication. The data described here, together with the previous findings, establish that these enhancer binding proteins may have multiple functions in stimulating DNA replication. The BPV E2 protein has been shown to physically interact with the origin-binding protein E1 and thus bind cooperatively to a DNA site (13, 25–27). Moreover, the recent observation that E2 also interacts with the cellular replication protein RPA suggests that E2 may play a role in recruiting components of the cellular replication machinery during assembly of the preinitiation replication complex (7). The results presented here show that E2 effectively relieves the nucleosome-mediated repression of BPV replication, thus suggesting that E2 has several functions in DNA replication. At present, it is not clear whether the nucleosomal antirepression defines an independent activity of E2, since this property of E2 may simply manifest as a result of a tight and specific contact with DNA and the rest of the replication apparatus. We wish to emphasize that, both *in vivo* and *in vitro*, GAL-VP16 cannot replace E2 for replication activation (7, 14). Thus, the ability of E2 to cooperatively bind with E1 to the origin site and to help initiate assembly of a preinitiation complex is critical and highly specific. We suggest that antirepression is a consequence, rather than a cause, of this assembly. Similarly, it seems likely that the ability of GAL-VP16 to interact with RPA (through the activation domain) gives some of the interaction energy that allows for preinitiation assembly, and this leads consequently to antirepression. This latter point is indicated by the requirements for an activation domain associated with the DNA-binding domain of the GAL-4 derivatives to effectively antirepress (see Fig. 5). On naked DNA or chromatin templates, GAL-VP16 does not stimulate the replication signal without E2 (ref. 7 and data not shown). Indeed, further work may show that GAL-VP16 cannot effectively compete with nucleosome occupancy over the origin site on templates such as pKSO without E1 and E2 and a variety of cellular factors.

The data presented here extend to another system the observations that were made by Kelly and his colleagues (11, 12) for SV40 chromatin replication. In both systems, the transcription factors were found to alleviate repression of nucleosomal structures, and intact activation domains of the factors are so required. The issue remains as to how such activation on chromatin templates comes about. One suggestion relies simply upon a thermodynamic argument for competition on the origin site by two different nucleoprotein complexes—the histone octamer and associated proteins, or the preinitiation complex. Given the fact that GAL4-(1–147) binds to certain chromatin templates with the same efficiency as does GAL4-VP16 (12, 28), it appears that mere occupancy of the GAL4 sites is not sufficient and that the activation domain of VP16 is required for the antirepression of the nucleosomal effect. We and others have recently demonstrated that several acidic transcription factors bind to the cellular RPA factor, and we have suggested that this targeting helps stimulate DNA replication. Clearly, this interaction could favor the formation of a replication complex at the expense of a nucleosome. In fact, we have argued elsewhere (7) that binding interactions between E1, E2, and RPA and respective DNA–protein interactions could all effectively be required to efficiently compete with the nucleosome. However, this thermodynamic model may not be sufficient to describe the mechanism of activation. Other cellular cofactors may be required to effectively assemble a preinitiation complex on chromatin. For example, such factors as SNF/SWI (29) may be chaperones which assist in this assembly. It will be interesting to ask if depletion of such activities from the cellular extracts interferes with E2 activation.

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