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The Replication Activation Potential of Selected RNA Polymerase II Promoter Elements at the Simian Virus 40 Origin

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Binding sites for cellular transcription factors were placed near the simian virus 40 origin of replication, and their effect on replication and TATA-dependent transcription was measured in COS cells. The hierarchy of transcriptional stimulation changed when the plasmids replicated. Only one of seven inserted sequences, a moderately weak transcription element, stimulated replication detectably. However, when two nonstimulatory sites were present in multiple copies they did activate replication. Multiple sites for the chimeric activator GAL4-VP16 did not stimulate replication even though transcription was stimulated strongly. The results indicate that the ability of a binding site to stimulate replication from the simian virus 40 *ori* is not based on its transcriptional activation potential but is instead related to a separate replication activation potential that can be increased by having multiple sites.

The interrelationship between control of cellular replication and transcription has been the subject of extensive experimentation and discussion (10, 11, 19, 38). Because of the uncertainty concerning what constitutes a cellular origin of replication, information has come mostly from viral systems where the origins are defined genetically (6, 10, 21). Among the papova- and adenoviruses, the involvement of transcription regulatory elements in replication control is very well documented (12, 25, 31). Conversely, template replication is typically required for late gene transcription (9, 19). Thus, there is important communication between control of replication and transcription.

The elements jointly controlling DNA and RNA synthesis are generally located near the replication origin in these viruses (5, 12, 22). These are binding sites for different factors in the different viruses, demonstrating that a variety of factors may play dual regulatory roles. These factors can be derived from the host or encoded by the virus. The host proteins are known to function as transcription factors for cellular genes and include, for example, NFI/CTF, used by BK virus and adenovirus, GC box binding factors such as Sp1, used by simian virus 40 (SV40), and a variety of cellular proteins that bind viral enhancer regions (14, 25, 26, 28). Some proteins that influence both processes appear to have separate domains for transcription and replication stimulation (18, 32, 33, 37). Binding sites related to those in the viruses have been found near two putative cellular origins of replication (13). Thus, host factors have been recruited by viruses to act as joint activators of transcription and replication, and these factors have the potential to play similar dual regulatory roles in the host.

Previous studies have suggested that the ability to stimulate replication may be a widespread property of transcription factors when they are bound next to replication origins. Various GAL4 fusion proteins were artificially directed to locations near a polyomavirus origin (1, 2), and their ability to stimulate both replication and transcription was demonstrated. Stimulation of either process required the fused transcription activation domain. There was also a correlation between the strength of transcription activation and that of replication activation (2). This raised the possibility that nearly any transcription factor might have the capacity to stimulate replication when bound near an origin. A potential mechanism for this was that the factor activation domain might interact with a common mediating factor required for both transcription and replication.

A different mechanism was suggested by experiments with the host NFI factor used in BK transcription (7). Those results show that replication is stimulated when the protein is bound near an (SV40) origin of replication. However, in vitro this stimulation only occurs when the template is assembled into chromatin; chromatin assembly represses basal replication activity in vitro. Thus, NFI appears to antagonize the repressive effects of chromatin on replication. This was proposed to occur by a nucleosome exclusion mechanism wherein NFI binding simply prevented the binding of nucleosomes over the adjacent elements required for SV40 replication. In its natural context, the SV40 origin is indeed preferentially cleared of nucleosomes and is adjacent to sites with dual roles in transcription and replication (21, 27). This model also implies that many transcription factors might have the potential to affect replication when bound near origins. Subsequently, a variety of activators have been shown to antagonize the repressive effects of chromatin on transcription (30, 36, 40).

Therefore, it is important to learn how wide a variety of cellular proteins can jointly stimulate replication and transcription and to learn how this occurs. We have attempted to address these issues by placing a variety of sites for binding of cellular factors (39) near the SV40 origin and measuring effects on both replication and transcription. The sites are studied on replicating plasmids in COS7 cells and include those known to bind seven different transcription factors. The results show that joint activation from single binding sites is rare but is somewhat more common when sites are present in multiple copies. The potential ability to stimulate

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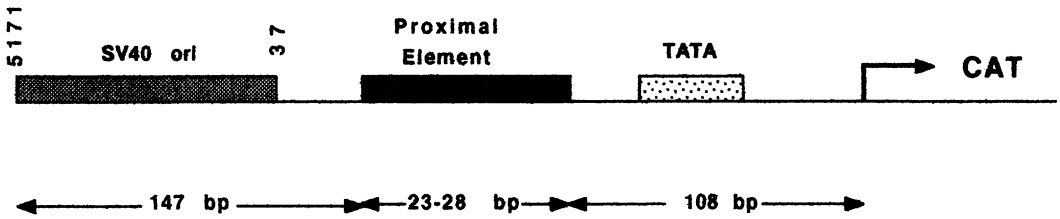

FIG. 1. Schematic diagram showing the arrangement of the SV40 origin of replication and promoter elements. The origin is placed so that its late side is approximately 23 bp upstream from the nearest proximal element and 43 to 48 bp from an adenoviral major late promoter TATA box (15). The *ori* sequence corresponds to SV40 nucleotides 5171 to 37, which includes the core *ori* and large-T-antigen binding site I.

replication is not correlated with the ability to stimulate transcription and instead appears to be a separate and varying property of each site and its cognate factor.

MATERIALS AND METHODS

Plasmid constructs. Starting with the unit vector pAC ATX_{10} (39), the SV40 origin of replication from SV40 nucleotide 5171 to nucleotide 37, which includes the aux-1 sequence (large-T-antigen binding site I) (10), was cloned directionally between the *Bgl*II and *Xba*I sites. In this construct, $pACATX_{10}$ + *ori*, the endogenous SV40 early gene promoter is directed away from the chloramphenicol transcriptional unit start site. The proximal elements shown in Fig. 1 were inserted with 23 bp separating the *Nco*I boundary of the *ori* and the *Bcl*I site boundary of the element. When necessary, the TATA box was removed by *Sph*I digestion and recloning.

The exchange of the BK-NFI binding site obtained from pUC-BK.NFI (7) into the mouse mammary tumor virus (MMTV)-NFI binding site of $pMACATX_{10}$ was done via the compatible *Alw*NI site of the two vectors to create pBKA $CATX_{10}$ + *ori*; the other incompatible (*Eco*RI and *Xho*I) ends were made blunt with Klenow polymerase before ligation. An additional construct, pBK + *ori*, was also made whereby the CAT reporter gene and TATA box element were removed through blunt-ended *Hin*dIII and *Sac*I sites of $pBKACATX_{10}$ + *ori*.

Multiples of MMTV-NFI and ATF sites were constructed by sequentially adding sites in tandem. This was made possible via flanking upstream *Pst*I and downstream *Xho*I sites of the parental vector containing either an MMTV-NFI or an ATF site.

The construct containing five tandem 17-bp GAL4 binding sites was a kind gift from M. Carey. These sites were then cloned into $pACATX_{10}$ + *ori* at the *Bcl*I position. This is the identical position through which other proximal elements used in this study are cloned (39). The original GAL4-VP16 transactivator construct, also a gift from M. Carey, was reconstructed to replace the SV40 early promoter sequence with the equally strong adenovirus E3 promoter (15). The SV40 early promoter includes the *ori* sequence which, when cotransfected in increasing amounts with the reporter constructs, inhibited replication of the reporter plasmid (24a). All constructs made were sequenced, and their identities were confirmed. Also, all constructs used in subsequent replication and transcription studies were CsCl banded twice in at least three separate preparations.

Tissue culture and transfection. COS7 cells are maintained at subconfluent concentration in high-glucose Dulbecco modified Eagle medium supplemented with 10% fetal bovine serum and antibiotics. Twenty hours prior to transfection, the cells were seeded at approximately 10^5 cells per ml into plates (100 by 20 mm) at 10 ml per plate. $CaPO_4$-mediated transfection was as described previously (39). For replication studies, 1 μg of each plasmid was used. For transcription studies, 10 μg was used except as indicated in the text. For both transcription and replication studies using the GAL4 system, 0.5 μg of the reporter constructs bearing five GAL4 binding sites was cotransfected with 2 μg of the GAL4-VP16 transactivator.

Replication and transcription assays. To determine the total amount of DNA replicated from the transfected constructs at 44 to 48 h posttransfection, total cell lysate was made by the method of Hirt (24). This time was chosen since Guo et al. (21) showed that it was representative of replication activity for SV40 constructs containing four different auxiliary elements. After the removal of cellular DNA by using high salt and selective centrifugation, the lysate was treated with RNase A and proteinase K for 1 h each. Following successive phenol-chloroform extractions and ethanol precipitation, the DNA was suspended in a volume of 50 μl. Prior to Southern blot analysis, approximately 10 μg of each sample was digested with *Dpn*I and *Stu*I. The sample was then electrophoresed through a 1% agarose gel, blotted onto a Nytran filter, and probed with either one of two ^{32}P-end-labeled 17-bp primers. Primer W2 is located 35 bp from the initiation site of the CAT gene, whereas primer 3 binds within the T-antigen binding site I of core *ori*. A third random-primer (Prime-It random primer kit; Stratagene, La Jolla, Calif.) -synthesized and labeled probe of the CAT gene fragment was also used, and results were compared with those obtained from either oligo probe.

Detection of transcriptional activity was done by CAT assay as described previously (17) at 44 to 48 h posttransfection. Results obtained from either replication or transcription assays were repeated at least three times.

RESULTS

The organization of the family of plasmids used for studying replication and transcription is shown in Fig. 1. Each plasmid contains an origin of SV40 replication oriented so that its late side sequences face a marker gene for chloramphenicol acetyltransferase (CAT). Various synthetic sites, studied previously in a nonreplicating context and modeled after elements with known involvement in transcription (39), are placed between the *ori* and CAT. The distance between *ori* and CAT is approximately 160 bp and includes transcription sites placed 23 bp from the edge of *ori*. This close distance was chosen since it is well within the domain covered by a nucleosome, which would be required in the nucleosome exclusion model for replication activation, and because initial experiments confirmed that it allows stimulation by the BK-NFI site (reference 7 and see below). The sites studied (39) include a CACCC element from the rat

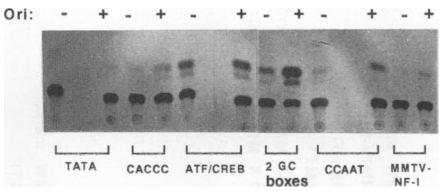

FIG. 2. CAT assay of promoters with (+) and without (−) the SV40 origin of replication. The promoter elements examined are indicated below each lane (39). All promoters are in the context of the adenovirus major late promoter TATA box.

tryptophan oxygenase promoter, an ATF site from the adenovirus E3 promoter, two tandem GC boxes that bind Sp1 with high affinity, a CCAAT box from the α-globin promoter (35), an NFI site from the long terminal repeats of the MMTV, a BK-NFI site (from reference 7), and a binding site to which GAL4-VP16 can be directed. In all of the constructs shown, a TATA box is placed between the synthetic element and CAT. The transcription start site has been mapped to approximately 30 bp downstream of TATA (39). All plasmids have multiple AP1 sites in a far downstream position to enhance the transcription signal.

Transcription. Most of the replicating plasmids were transfected into COS7 cells, and 44 h later CAT activity was determined by assaying extracts. The results from one set of assays are shown in Fig. 2 (lanes labeled +). The data show that the least active construct is the one containing a TATA box without any additional element. The conversion to acetylated chloramphenicol is barely detectable in this case. Slightly higher activities are observed from constructs bearing additional CACCC or MMTV-NFI elements. The most active construct of this set contained tandem GC boxes, and very significant activity was obtained by using the α-globin CCAAT element or the adenovirus ATF element. The average of at least three separate experiments of this type is shown in Table 1. All of the added elements cause increased activity when combined with TATA. The increases range from a marginal 2-fold for two elements to a significant 4-, 9-, and 21-fold for three others.

Another series of constructs, identical to this set except that they do not contain a replication origin, were studied in parallel. Figure 2 shows that the CAT activities were generally much lower for this set (see lanes marked −). All the plasmids are more active when attached to a replication origin. We confirm below that all origin-containing plasmids are in fact replicated. However, not all the constructs have the same dependence of transcription on DNA replication. This is most evident by comparing the results with the ATF and GC box constructs. In the absence of an origin, the ATF construct is the stronger of the two (lanes marked −). But in the presence of an origin, the promoter strength is reversed, with the GC boxes directing stronger transcription than the ATF element (lanes marked +). This reversal is due to a combination of strong stimulation of the GC boxes by replication and barely detectable stimulation of the ATF promoter. Repeated experiments show that the other promoters are much more stimulated by replication than ATF and somewhat less stimulated than the GC boxes.

TABLE 1. Stimulation of transcription among promoter elements in the presence of *ori*

Element	Activity[a]
TATA	1
CACCC	2
ATF	9
2 GC boxes	21
CCAAT	4
MMTV-NFI	2
BK-NFI	6
5× GAL4-VP16[b]	170

[a] Each datum point was normalized to the activity of TATA alone and is the average of results from three or more assays.
[b] For 5× GAL4-VP16, one-fifth the amount of DNA was used.

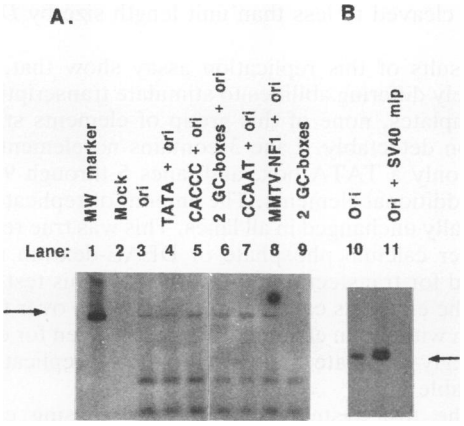

FIG. 3. Southern blot showing the effects of various promoter elements on replication. In blot A (lanes 1 to 9), the arrow denotes *Stu*I linearized and *Dpn*I-resistant replicated DNA. In separate experiments, the element ATF/CREB directed equivalent replication (data not shown). Blot B (lanes 10 and 11) is a positive control showing the relative replication efficiency of pOR2 (*ori* alone) and pOR4 (*ori* plus six GC boxes and one copy of the 72-bp enhancer repeats).

Collectively, these results describe a set of promoters that are active to various extents on replicating templates. Each promoter element is present in the same location, approximately 23 bp from the replication origin. Other transcription elements have been shown to influence replication from such a nearby position (1, 7), and in one case there was a general correlation between the strength of stimulation of transcription and replication (2). The ability of the promoters in this set to stimulate replication will now be tested.

Comparison of replication and transcription. All replication assays in this study used the standard procedure of measuring *Dpn*I-resistant DNA (34). The input bacterial plasmid DNA contains sites susceptible to *Dpn*I cleavage which are converted to *Dpn*I-resistant sites when DNA replication-dependent methylation changes occur in the COS cells. Thus, only when the DNA is replicated in the mammalian COS cells does it become resistant to cleavage by this *Dpn*I. The DNA samples isolated from transfected COS cells are first treated with *Dpn*I to digest the unreplicated plasmids. Next, the samples are cleaved with the unique site enzyme *Stu*I, which cuts plasmid DNA into a unit-length linear species. The position corresponding to *Stu*I unit size-replicated DNA on a Southern blot is shown by using the purified plasmid DNA sample in lane 1 of Fig. 3A (upper arrow). Lane 9 is a control showing that no *Dpn*I-resistant DNA is seen on a Southern blot of DNA isolated from cells transfected with the plasmid containing no SV40 origin, as expected. The lower bands in lane 9 and the other lanes represent input transfected DNA that did not replicate and

thus was cleaved to less than unit length size by *Dpn*I and *Stu*I.

The results of this replication assay show that, despite their widely differing abilities to stimulate transcription from these templates, none of this group of elements stimulates replication detectably. Lane 3 contains no element, lane 4 contains only a TATA box, and lanes 5 through 9 contain various additional elements. The amount of replicated DNA is essentially unchanged in all lanes. This was true regardless of whether calcium phosphate or DEAE-dextran methods were used for transfection (not shown). In this test system, none of the elements can enhance replication over the basal level seen without an element. This is true even for elements that strongly stimulate transcription on the replicating templates (Table 1).

That the test system is capable of sensing enhanced replication is shown in lanes 10 and 11 of Fig. 3B. Figure 3B compares the plasmids pOR2 and pOR4, which are known to replicate to differing extents (10). Both contain the same SV40 *ori* used above, but pOR4 contains an adjacent approximately 120-base-pair region known to stimulate replication. By using the same assay just applied, the results show that the pOR4 plasmid (lane 11) replicated better than the pOR2 plasmid (lane 10). Thus the additional region in pOR4 can stimulate replication of a test plasmid in the same context that the other elements could not. Several additional positive controls of elements that can stimulate replication will be shown below.

One possible complication in deducing the role of these elements in this context is that they may be required to act simultaneously in replication and transcription. That is, when the sites are bound by factors it is possible that the presence of a nearby TATA box causes the factor to assemble into a transcription complex to the detriment of a potential replication complex. Therefore, we removed the TATA box from several of these constructs and reassayed replication. We showed previously that removal of the TATA box eliminates specific transcription of these plasmids in HeLa cells (39). No increase in replication was seen when the TATA box was removed (not shown), suggesting that this complication was not relevant in this case.

Replication can be activated when multiple sites are present. It is possible that these sites contain weak replication stimulatory activities that are not revealed by using the conditions of this assay. To test this possibility, we initially chose two of these sites and constructed plasmids that contained multiple copies of each. These were then retested in the same replication assays to compare with the lack of replication activation by single sites.

Figure 4 shows that multiple copies of the MMTV-NFI site now stimulate replication detectably. Lanes 4, 5, and 6 of Fig. 4A differ in that the plasmids contain one, three, and five tandem copies of the MMTV-NFI site. The result shows that more DNA is replicated when the number of sites is increased from one to three or one to five. It is not clear whether there is a small increase in replication as the sites increase from three to five. Figure 4B also shows the result of increasing the number of ATF sites from one to three or one to six. There is slightly more replication with six sites (lane 11) than with a single site (lane 9); densitometric scanning indicates a 30% increase. Thus, multiple sites can stimulate replication, whereas no stimulation is detected with a single site.

By contrast, a single NFI site from BK virus (lane 3) does stimulate replication, confirming a previous experiment (7). Because there are a few inherent sequence differences

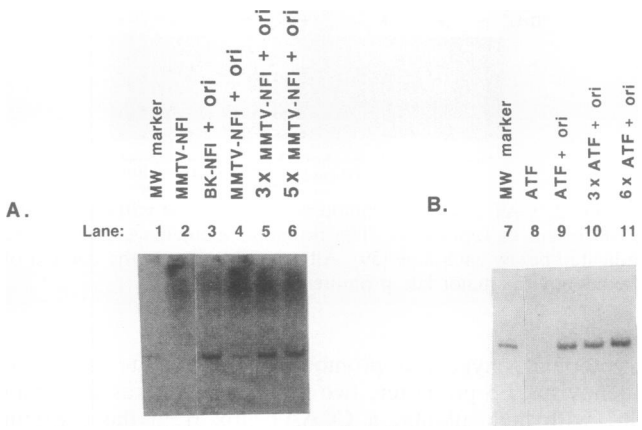

FIG. 4. Multiple MMTV-NFI and ATF sites stimulate replication activity. Blot A compares replication among constructs bearing one, three, and five MMTV-NFI sites or one BK-NFI site. Blot B is a separate experiment of replication derived from plasmids with one, three, and six ATF/CREB sites.

between the BK- and MMTV-NFI sites (see Fig. 5B), we reconstructed plasmids so that each NFI sequence type was included in both its original context and in the original context of the other. The replication assays of Fig. 5A show that in either vector context a single BK site stimulates replication, although weakly. The extent of stimulation is

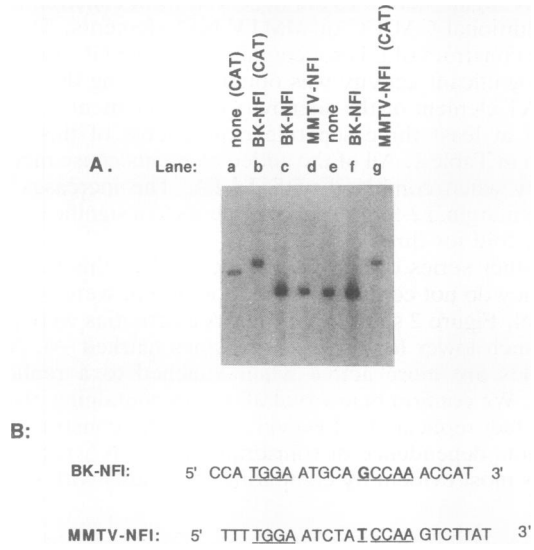

FIG. 5. (A) Southern blot showing differential stimulation of *ori* replication activity by BK-NFI and MMTV-NFI elements. Plasmids used in lanes e and f were from Cheng and Kelly (7). The BK-NFI site from this plasmid was inserted into a CAT-containing vector in lane b and into a non-CAT-containing vector in lane c. The MMTV-NFI site was inserted into the vector used by Cheng and Kelly (7) in lane d. Lane g contains the same construct used in Fig. 3, lane 8. (B) Comparison of BK-NFI and MMTV-NFI binding sequences. The BK-NFI recognition sequence is from the enhancer region of the BK viral regulatory region, whereas the MMTV-NFI recognition sequence is found within the long terminal repeats of the MMTV. Underlined nucleotides denote the core NFI binding sequence. The bold and underlined nucleotides denote the 1-bp difference within the core binding sequence, G versus T, between the two similar NFI binding sites.

much lower than reported previously (7). This lesser stimulation was confirmed in multiple experiments using both calcium phosphate and DEAE-dextran transfection protocols. The result is likely related to differences in COS cell lines since the extent of replication varies with the cell line (7). In any case, the comparison shows that the BK-NFI site is unique with regard to replication stimulation in that it is the only site tested that does not require multiple copies to stimulate. Gel shift experiments using a COS7 cell extract confirmed that the BK-NFI site bound a different factor than did the MMTV-NFI site, as evidenced by altered mobility complexes (data not shown).

Since a previous study (2) showed that there was a general correlation between the strength of stimulation of replication and transcription of various activators, it was possible that the BK-NFI site was a much stronger transcriptional activator than the others. Therefore, we measured CAT activity directed by the replicating BK-NFI plasmid (Table 1). We found that its activity was not outside the range of stimulation defined by the collection of elements. Its activity is roughly comparable to that of the ATF and CCAAT sites, less than that of the two tandem GC boxes, and greater than that of the CACCC and MMTV-NFI elements. Thus the ability to stimulate replication in this series is unrelated to the ability to stimulate transcription.

One possible complication concerns the availability of various factors in vivo. For example, if a limited amount of factor was bound to the input templates, then there might be little available to distribute to templates during replication. This seems unlikely for those templates that are transcribed during replication. For the tandem GC box element that did not stimulate replication, transcription increased as the amount of transfected template increased, arguing further against this complication (not shown). The amount of DNA used here ranged from the 1 μg used for replication studies to the 10 μg used for transcription studies. In the following studies using GAL4 sites, excess activator protein will be provided by cotransfection.

We note parenthetically that the replication-dependent stimulation of transcription by most of these sites is impressive when one considers the low levels of replicated DNA compared to input DNA. Evidence of this observation is shown in Fig. 3A, where the upper replicated bands are present in very low amounts compared with the lower input DNA. Nevertheless, this small amount of additional DNA, likely less than a 10% increase, raises the level of CAT activity severalfold (Fig. 2). These observations confirm that replicated DNA is an excellent template for transcription (9, 18).

Multiple GAL4-VP16 sites stimulate transcription but not replication. It has been reported that multiple binding sites for GAL4-VP16 can stimulate replication when placed next to the polyomavirus core origin and when the activator is provided by cotransfection (1, 2). There was also a correlation between the extent of activation of transcription and that of replication (2). We initiated similar experiments to see if this also applied to the SV40 origin. The reporter plasmids contained multiple GAL4 binding sites. The GAL4-VP16 expression vector was reconstructed to remove the SV40 *ori* and cotransfected in an amount which previous titration has shown to be below the amount that leads to inhibition by "squelching" (16).

The results of transcription assays show that these plasmids are very strongly activated by GAL4-VP16 (Fig. 6, top). There is an average of 170-fold activation (Table 1) of the five-site construct over that of the control transfections

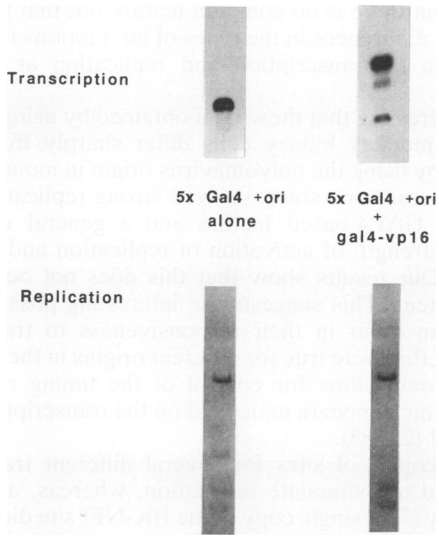

FIG. 6. Transcription and replication activities with five tandem GAL4 binding sites and ori in the presence and absence of the transactivator GAL4-VP16. Transcription activity is shown by CAT assays as in Fig. 2. Replication activity is shown by Southern blot as in Fig. 3.

lacking GAL4-VP16. In strong contrast, repeated replication assays in the presence and absence of GAL4-VP16 revealed no stimulation of replication (Fig. 6, bottom). The experiments shown in Fig. 6 used the same cells, protocols, and placement of sites that showed stimulation by using NFI sites (above). We also deleted 4 bp between the *ori* and the GAL4 sites to alter the phasing, but again no replication stimulation was observed (not shown). Both the cell line assayed and the origin differ, however, from those of the previous studies showing stimulation, in which a polyomavirus core origin was used. We conclude that even a very strong transcriptional activator can still fail to stimulate SV40 origin replication, even when placed very close to the *ori*.

DISCUSSION

Previous experiments have shown that a member of the NFI family (7) and a variety of GAL4 hybrid transcription activators (1, 2) can stimulate both replication and transcription when their binding sites are near viral replication origins. The mechanisms by which this occurred appeared to differ in the two studies. BK-NFI was suggested to work by nucleosome exclusion, freeing the nearby SV40 origin for replication. The GAL4 fusion proteins were proposed to use their various transcription activation domains directly to stimulate both replication and transcription, in this case at the polyomavirus core origin (2). This latter proposal was suggested by the strict correlation observed between the ability to stimulate replication and the ability to stimulate transcription in the series of fusion plasmids studied.

In this study, we have shown that among a wide variety of sites there is no correlation between the ability to stimulate the two processes at the SV40 origin. Even a construct which is stimulated by 170-fold in transcription, and in which the GAL4-VP16 transcription factor is provided in optimal amounts, fails to stimulate replication detectably. We infer that the interaction used to stimulate replication is different from that used to stimulate transcription. This does not

suggest that there is no common feature but that there must be critical differences in the types of interactions that lead to stimulation of transcription and replication at the SV40 origin.

It is interesting that these data obtained by using the SV40 origin in monkey kidney cells differ sharply from results obtained by using the polyomavirus origin in mouse cells. In the latter case, one study showed strong replication stimulation by GAL4-based factors and a general correlation between strength of activation of replication and transcription (2). Our results show that this does not occur in the SV40 system. This suggests the interesting possibility that origins can differ in their responsiveness to transcription factors. If this were true for different origins in the same cell, then it would allow for control of the timing of firing of origins, which appears to depend on the transcriptional state of the cell (23, 38).

Single copies of sites for several different transcription factors did not stimulate replication, whereas, as reported previously (7), a single copy of the BK-NFI site did stimulate replication. Some of these single sites stimulated transcription better than the BK-NFI site, indicating that they are bound by active factors. Taken together with the GAL4-VP16 result, this supports the view that simple binding of a transcription factor is not sufficient to cause activation of replication.

We have found that although certain sites do not stimulate replication detectably in single copies, they can stimulate replication when present in multiple copies. A related phenomenon occurs at the natural SV40 origin where decreasing the number of GC box elements decreases replication (see reference 3 for an example). This indicates that each factor may contain a domain for replication stimulation and that, as for transcription (4, 29), multiple domains can cause stimulation whereas a single domain cannot. Our data cannot yet indicate whether the activation of replication is synergistic; more detailed studies in this regard are in progress.

Overall, these results point to a model in which each transcription factor has a unique potential to stimulate replication which can be enhanced by the binding of multiple factors. The domains responsible for this could overlap with the transcription activation domain, as implied by the GAL4 studies at the polyomavirus origin (2), or could overlap with the DNA binding domain, as indirectly implied by the nucleosome exclusion model (7). The structural domains of certain members of the NFI family do contain apparently separable domains for activation of transcription and replication (25, 32). Either domain might have the ability to disturb the local chromatin structure and thereby allow increased accessibility of regulatory factors. In the set of sites studied here, the BK-NFI factor should have a potent replication activation domain since it can stimulate replication even from a single site. An important future challenge will be to learn what makes a domain a potent activator of replication, which, along with the parallel study of transcription activation domains, might reveal how one achieves the important coordination of these two critical processes.

ADDENDUM

After submission of this work, two relevant papers appeared (8, 20). The results of Guo and DePamphilis (20) are in excellent agreement with our results in that there is a lack of correlation between replication and transcription activity of selected elements when tested in the context of an SV40 origin. Cheng et al. (8) find that GAL4-VP16 can stimulate SV40 replication in vitro, presumably by acting as an antirepressor for the inhibitory effect of chromatin. However, both this study and that of Guo et al. (20) fail to observe GAL4-VP16 stimulation in vivo. It appears that the repressive effects of chromatin structure observed in vitro are not dominant in vivo and that more-direct interactions between transcription factors and the replication machinery need to be explored.

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