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Constitutive Expression of Simian Virus 40 Large T Antigen in Monkey Cells Activates Their Capacity To Support Polyomavirus Replication

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Polyomavirus DNA replication is normally restricted to rodent cells, and simian virus 40 (SV40) DNA replication is restricted to primate cells. We demonstrate that DNAs containing the polyomavirus origin can be replicated in monkey cells which constitutively express SV40 large T antigen. Permissivity is most likely caused by SV40 T antigen modification of cellular protein(s) required to replicate the polyomavirus origin. A possible target for the T-antigen-induced modification is DNA polymerase α-DNA primase.

The polyomavirus and simian virus (SV40) core origins of DNA replication are highly conserved; each is composed of a palindrome with several relatively low-affinity viral large T antigen-binding sites bordered on one side by an A+T-rich sequence (10). The core origins are nucleation sites for complexes composed of viral large T antigen and cellular proteins, from which DNA is replicated bidirectionally (10, 22). Despite these very similar features, polyomavirus does not replicate in cells which support efficient SV40 replication, such as monkey CV1 cells (5, 10, 46). Clearly, a high degree of specificity toward origins of DNA replication is exhibited by the proteins involved in replication.

At present we understand only little about what restricts polyomavirus replication in nonpermissive cells. Viral DNA replication depends upon enhancer-activator sequences for function in vivo (12). These elements are reported to confer species specificity upon viral DNA replication, but this has been disputed (5, 11). In vitro, DNA polymerase α-DNA primase from murine cells is required to replicate polyomavirus DNAs; monkey or human cell DNA polymerase α-DNA primase does not support their replication (33, 34). This has been suggested to be the reason why polyomavirus does not replicate in monkey cells. However, here we demonstrate that polyomavirus DNA replication in monkey cells is not unconditionally limited. DNAs containing the polyomavirus origin will replicate efficiently in monkey cells which constitutively express functional SV40 large T antigen. We suggest that the SV40 large T antigen overcomes the cellular restriction upon replication of the polyomavirus origin by modifying the properties of host proteins involved in DNA replication.

We analyzed the replication of test DNAs containing the polyomavirus origin and its regulatory region (M13mp9-DB, M13mp8-BD; 47) or the SV40 origin and its regulatory region (M13mp6-SV04; kindly provided by U. Hansen) in transient DNA replication assays in monkey and mouse cells. Each test DNA (1 μg) was cotransfected by calcium phosphate coprecipitation (35, 48) with helper DNAs (0.2 μg) encoding polyomavirus T antigens (pMKSO11) (44) or SV40 T antigens (pd126) (18) together with 8 μg of salmon sperm DNA. The helper DNAs do not replicate and hence should not efficiently compete with the test DNAs for replication factors. At 48 h posttransfection, low-molecular-weight DNAs were isolated and digested with *Dpn*I and *Eco*RI (for polyomavirus DNAs) or with *Dpn*I and *Hin*dIII (for SV40 DNAs) and analyzed as described previously (36, 43, 44). Results typical of numerous replication assays are shown in Fig. 1. Replication of the SV40 test DNA in monkey CV-1 cells required SV40 T antigens (Fig. 1A, lanes 1 and 2). The polyomavirus test DNA did not replicate in these cells when SV40 T antigens were provided alone (Fig. 1A, lanes 3 and 4), but a very small amount of polyomavirus DNA replication was detected when polyomavirus T antigens were provided, either alone or together with SV40 T antigens (Fig. 1A, lane 5 and 6). This restriction of polyomavirus replication in monkey cells affirms that observed previously by numerous investigators (46).

An alternative means to provide SV40 T antigens in a monkey cell environment is through the use of COS-1 cells (19). The SV40 large T antigen constitutively expressed in COS-1 cells activated the SV40 origin (Fig. 1A, lane 7) but not the polyomavirus origin (Fig. 1A, lane 9). Remarkably, when polyomavirus T antigens were expressed in these cells (by cotransfection of helper DNAs), the polyomavirus origin was activated to about 10% of the level observed for the SV40 origin (Fig. 1A, lane 10). Similar results were observed with three different DNAs containing wild-type polyomavirus origins, and we have observed replication of polyomavirus DNAs in two other types of monkey cells which constitutively express SV40 T antigens (COS-ts-2 cells, see below, and CMT-3 cells, data not shown).

These experiments used test DNAs containing viral origins cloned in phage M13 vectors. To test whether the phage DNA sequences might confer unusual properties upon the viral origins, we measured the extent to which these DNAs are replicated in mouse cells which normally support polyomavirus replication but not SV40 replication. We transfected M13mp6-SV04 or M13mp9-DB DNAs into mouse 3T6 cells and into mouse WOP cells (which constitutively express polyomavirus large T antigens) (9). Regardless of whether the SV40 large T antigen or the polyomavirus large T antigen or a combination of both large T antigens was provided, the M13mp6-SV04 DNA was not replicated (Fig. 1B, lanes 1 through 4, 7, and 8). DNAs with the polyomavi-

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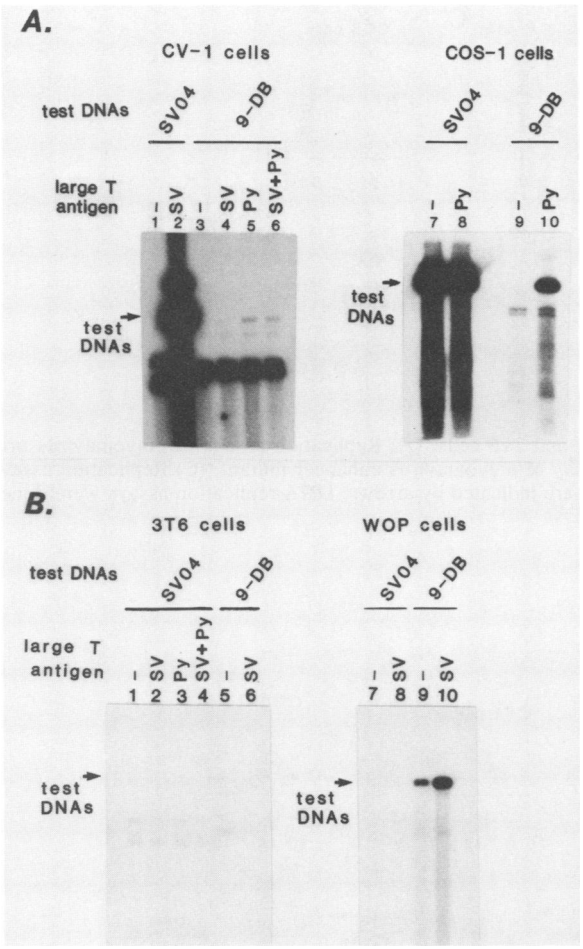

FIG. 1. Replication of DNA containing the polyomavirus origin or the SV40 origin in monkey cells (A) and mouse cells (B). Replicated (test) DNAs are indicated by arrows. Double-stranded M13 DNAs with a wild-type polyomavirus origin (M13mp9-DB) or with a SV40 origin (M13SV04) and helper DNAs which express either polyomavirus T antigens (pMK-SO11) or SV40 T antigen (pd126) were introduced into monkey cells (CV-1 and COS-1 cells) or mouse cells (3T6 and WOP cells) by calcium phosphate coprecipitation. Low-molecular-weight DNAs were isolated and digested with restriction enzymes, fractionated through a 0.8% agarose gel, transferred to nitrocellulose membranes, and hybridized with radiolabeled M13-Py and M13-SV40 DNAs.

rus origin were replicated efficiently in these cells so long as polyomavirus large T antigen was provided (Fig. 1B, lanes 9 and 10). SV40 T antigen alone did not activate the polyomavirus origin (Fig. 1B, lanes 5 and 6). This is to be expected because the arrangement of large T-antigen binding sites differs between the polyomavirus and SV40 core origins and it is unlikely that SV40 large T antigen forms a functional initiation complex at the polyomavirus core origin.

At least one reason that the SV40 origin is not replicated in mouse cells is that the mouse p53 protein forms inhibitory complexes with SV40 large T antigens (3, 17, 50). In addition, the mouse DNA polymerase α-DNA primase fails to function with the SV40 origin and T antigen in cell-free replication assays (34).

Polyomavirus DNA replication in mouse cells requires multiple *cis*-acting elements, including cellular protein-binding sites in the enhancer and large T antigen-binding sites at the origin palindrome (8, 10, 31, 47). We examined whether the same DNA sequences are required for polyomavirus DNA replication in monkey COS-1 cells. Polyomavirus DNAs 8-142 and 9-40 contain altered large T antigen-binding sites within the origin palindrome and replicate very poorly in mouse 3T6 cells (47). We observed that these DNAs replicated very poorly in COS-1 cell (Fig. 2A, lanes 1 through 4). Similarly, we measured the replication in COS-1 cells of a polyomavirus DNA (pAd175E$^-$) (14) lacking enhancer sequences between the *BclI* site and the core origin, whose replication in mouse 3T6 cells is greatly reduced (Fig. 2B, lanes 5 and 6). Replication of pAd175E$^-$ in COS-1 cells was similarly restricted (Fig. 2B, lanes 1 through 4). Thus, both the core origin palindrome and the enhancer are required for polyomavirus origin function in COS-1 cells. To assess the importance of the large T- antigen-binding sites adjacent to the core origin palindrome (i.e., large T-antigen site A) (8), we constructed (24) a mutant DNA (M13mp9-pyA*) which contains G→A transitions in the center of each of the large T-antigen recognition motifs. This DNA replicates poorly in mouse 3T6 cells when a limiting amount of large T antigen is provided (W.-J. Tang, and W. R. Folk, manuscript in preparation). In COS-1 cells, mutant pyA* DNA replicated less well than DNA with a wild-type polyomavirus origin (M13mp9-DB) (Fig. 2C, lanes 1 through 4), indicating that site A plays an auxiliary role to the polyomavirus origin in COS-1 cells, just as it does in mouse cells.

Polyomavirus large T antigen binds at the core origin palindrome to activate DNA replication (8, 44). Since the spacing of large T-antigen-binding sites differs between the polyomavirus and SV40 core origins, it is unlikely that SV40 large T antigen forms a functional complex at the polyomavirus origin. This explains the requirement for polyomavirus large T antigen to replicate the polyomavirus origin in COS cells. To ascertain why SV40 large T antigen is required for polyomavirus DNA replication in COS-1 cells, we used several monkey cell lines which express mutant SV40 large T antigens (C6, C11, and COS-ts-2 cells). Monkey cell lines C6 and C11 constitutively express mutant SV40 large T antigens which do not support SV40 DNA replication (1, 30, 41; Fig. 3A, compare lanes 5, 6, 9, and 10 with lanes 13 and 14). Mutant C6 large T antigen interacts weakly with the SV40 origin sequences; mutant C11 large T antigen lacks ATPase and helicase activities (1, 30, 41). DNAs containing the polyomavirus origin replicated very poorly in these cells (less than 1% the level observed in COS-1 cells; Fig. 3A, compare lanes 7, 8, 11, and 12 with lanes 15 and 16). These results suggest that a functional SV40 large T antigen is required to promote polyomavirus DNA replication in monkey cells. However, an alternative explanation for the failure to observe replication is that these defective large T antigens may interfere with the formation of functional replication complexes containing the polyomavirus large T antigens (41). To further evaluate the requirement for functional SV40 large T antigen, we used COS-ts-2 cells.

COS-ts-2 cells constitutively express a thermolabile SV40 large T antigen which activates replication of the SV40 origin at 33 but not at 39.5°C (37) (Fig. 3B, lanes 1, 2, 5, and 6). We observed that DNA with the polyomavirus origin could replicate in COS-ts-2 cells at 33°C (Fig. 3B, lanes 3 and 4) and, surprisingly, also in COS-ts-2 cells shifted to 39.5°C (Fig. 3B, lanes 7 and 8). That polyomavirus DNA replication occurs in COS-ts-2 cells shifted from 33 to 39.5°C, where there is insufficient large T antigen to support SV40 replication, indicates that a functional SV40 large T antigen is not

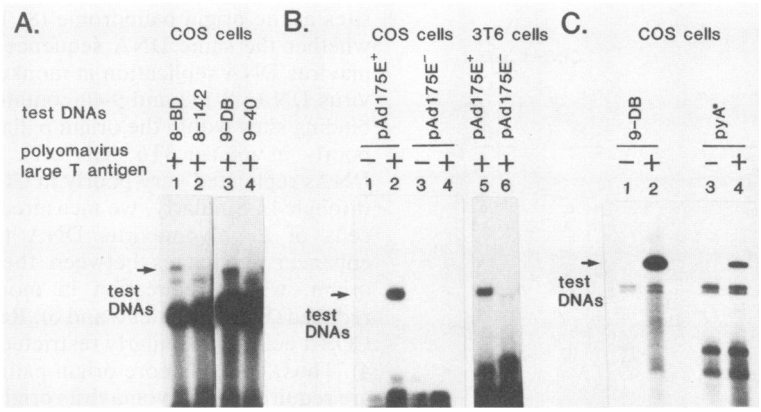

FIG. 2. Elements required for polyomavirus DNA replication in COS-1 and 3T6 cells. (A) Replication assays of polyomavirus origin sequences with point mutations at the core palindrome. (B) Replication assay of polyomavirus enhancer mutant. (C) Replication assay of DNA with wild-type origin and with site A mutant pyA*. Replicated DNAs are indicated by arrows. DNA replication assays were done as described in the legend to Fig. 1.

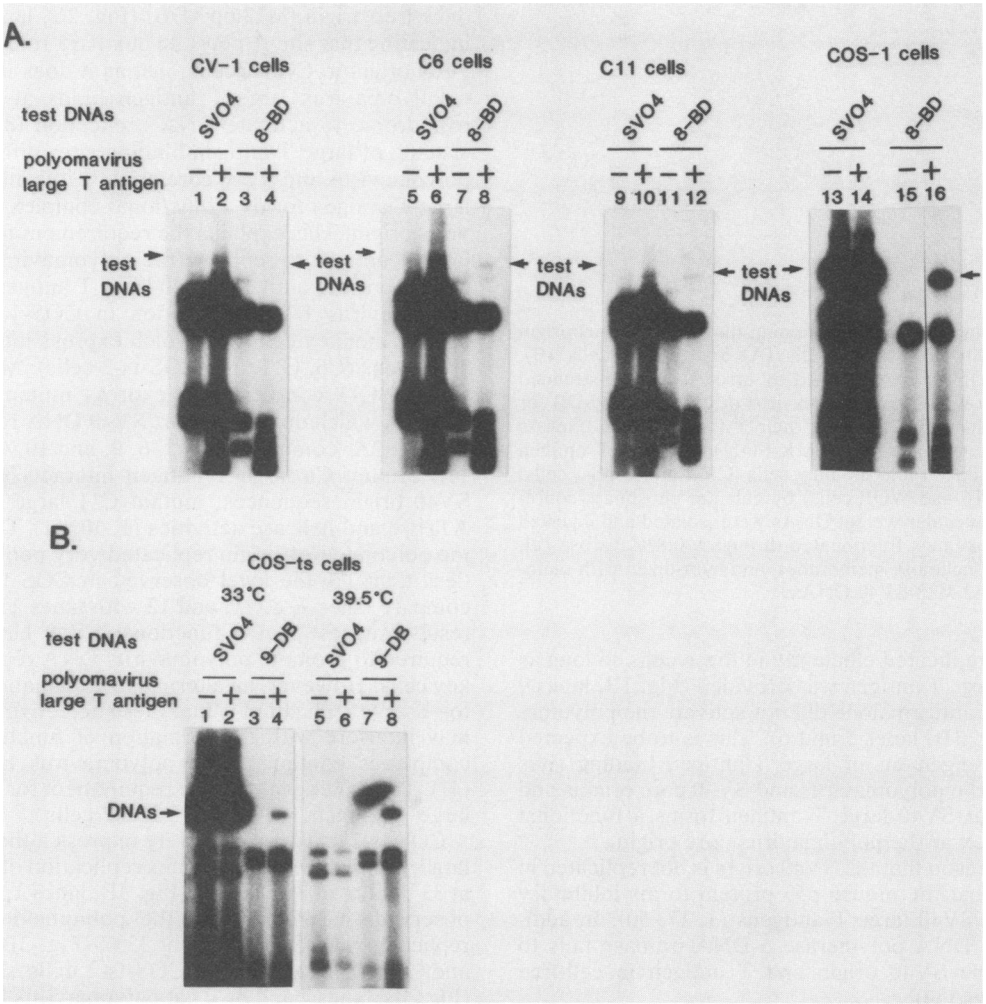

FIG. 3. Replication of DNAs with SV40 polyomavirus origins in CV-1, C6, C11, and COS-1 cells (A) and COS-ts-2 monkey cells (B). COS-ts-2 cells were maintained at 37°C before transfection in panel B, lanes 1 through 8. DNA replication assays were done as described in the legend to Fig. 1.

absolutely required to promote polyomavirus DNA replication.

A likely explanation for these results is that the SV40 large T antigen in COS-1 cells and Cos-ts-2 cells does not act directly upon the polyomavirus origin, rather it is the result of the capacity of this protein to modify the cellular replication apparatus which engenders permissivity. A slow modification process might also explain why the expression of SV40 T antigens in CV-1 cells did not engender permissivity during the transient replication assay with cotransfected polyomavirus DNA (Fig. 1). The failure to observe polyomavirus replication in C6 and C11 cells may be caused by interference between these mutant T antigens and the polyomavirus T-antigen replication complex assembled at the origin (41).

Other reports have demonstrated that cells can be altered in their capacity to support polyomavirus DNA replication. Polyomavirus replication in semipermissive rodent cells is enhanced after exposure to agents which damage DNA (15). A *trans*-acting factor responsible for this activity has been partially purified (38). Human embryonic kidney and human lymphoma cells, which are normally semipermissive toward SV40, will efficiently support SV40 DNA replication if modified by the adenovirus E1A proteins or by c-*myc* gene expression, respectively (7, 26, 27). Our observations provide the first evidence that SV40 T antigen will alter a species-specific restriction for DNA replication.

How might large T antigen modify the cellular replication apparatus to permit polyomavirus origins to be replicated? The large T antigen has pleiotropic activities, including intrinsic ATPase, DNA helicase, and DNA-binding activities (4, 40, 45); it both activates and represses transcription (2, 20, 21), and it interacts with numerous cellular proteins, including protein kinases, the DNA polymerase α-primase, the Rep C protein, enhancer binding proteins, and proteins involved in cell growth control (6, 16, 25, 28, 29, 32, 39, 45, 49, 50). Any or all of these activities might be directly or indirectly responsible for altering the specificity of the DNA replication apparatus (e.g., see reference 42). Understanding what restricts viral replication and how these restrictions are lifted should provide insights into the mechanisms by which cells control their DNA replication. The most fruitful approach to this question will derive from the analysis of in vitro DNA synthesis and the purification and characterization of the requisite proteins.

A likely target for the SV40 T antigen to effect the types of changes we have observed is the cellular DNA polymerase α-DNA primase. If the observations made in vitro regarding incompatibility between the monkey cell DNA polymerase α-DNA primase and the polyomavirus origin also apply in vivo (33, 34), this restriction must be overcome in COS cells. Recent evidence suggests that DNA polymerase α is subjected to phosphorylation (13) and that the phosphorylated forms have altered fidelity and kinetic properties (23). Comparison of the primer sites synthesized on SV40 DNA by purified DNA polymerase α-DNA primase with those observed to be used during SV40 DNA replication in vivo (51) suggests that the specificity of DNA polymerase α-DNA primase is altered during viral replication. Such modifications might alter the ability of DNA polymerase α-DNA primase to interact productively with the polyomavirus origin.

At least two other hypotheses can be put forth to explain these observations. SV40 large T-antigen action may permit DNA polymerase α-DNA primase to be replaced by another DNA polymerase (such as polymerase δ) to help initiate DNA replication. Alternatively, the SV40 large T antigen may be capable of forming a mixed initiation complex with polyomavirus large T antigen at the polyomavirus origin and thereby promote replication of the polyomavirus origin. However, we believe that neither of these latter suggestions is as likely as the first. We are currently testing these possibilities.

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