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Resolution of a Polyomavirus-Mouse Hybrid Replicon: Viral Function Required for Recombination

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RmI, a circular chimera made of the polyomavirus (Py) genome with an insertion of mouse DNA (Ins), effectively undergoes intramolecular recombination in normal mouse cells, as indicated by the conversion of cloned RmI (RmI_c) into unit-length Py DNA in transfected cultures. To follow the fate of the cellular component of RmI after recombination, the origin of simian virus 40 (SV40) DNA was inserted into the Ins region of RmI_c, generating a new molecular species designated SV-RmI_c. Intramolecular recombination of SV-RmI_c in simian cells synthesizing SV40 large T antigen gave rise to a molecule containing the SV40 origin, as well as the reciprocal product, unit-length Py DNA. However, SV-RmI_c failed to yield unit-length Py DNA in murine cells unless Py large T antigen was provided in *trans*. In murine cells synthesizing SV40 large T antigen, the only detectable product from SV-RmI_c contained only Py sequences, but was heterogeneous in size. These results and others also reported here strongly suggest that Py large T antigen plays a direct role in the resolution of RmI in murine cells.

In the preceding paper (20), we showed that RmI, a polyomavirus (Py)-mouse chimeric DNA whose organization is similar to that of a cointegrate, recombines in normal mouse cells to yield unit-length Py DNA (P155). This process occurs at a high frequency, is inhibited by replication of the precursor DNA, and yields a homogeneous viral DNA product (20). As RmI itself arises via site-specific recombination (29), we were led to envisage a similar mechanism for the production of P155 from RmI (20). However, homologous recombination not having been formally excluded, it appeared pertinent to investigate whether the recombination of RmI yielded only one product, P155. Indeed, it has been proposed recently that unlike site-specific recombination (20), intramolecular homologous recombination is often nonreciprocal, or rather nonconservative (34). For example, homologous recombination within transfected circular dimers of simian virus 40 (SV40) appears to generate not two but one monomeric genome (33). Whether RmI gave rise to P155 alone or to both P155 and the reciprocal product, IR, could not be decided on the basis of the experiments described thus far, because the absence of an origin precluded the amplification of IR (20). To determine the fate of its cellular component after recombination, we decided to modify RmI by introducing an origin in Ins (the inserted mouse DNA sequences), specifically that of SV40 DNA. If reciprocal, recombination in such a construct would be expected to yield P155 and a reciprocal product that would be amplifiable and also detectable with a specific probe. Unexpectedly, the new construct proved to be strongly impaired in its ability to replicate and to yield P155 in normal mouse cells, even though it carried Py sequences identical to those present in RmI. However, the new construct behaved similarly to RmI when transfected into mouse cells synthesizing an active Py large T antigen (LT). These findings and others detailed below demonstrate unambiguously that resolution of RmI involves a function which is lacking in normal mouse cells and which presumably is carried by LT.

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MATERIALS AND METHODS

Cells. We have described elsewhere the origin of the mouse 3T6 cells used in this study and the methods used to extract, purify, and characterize low-molecular-weight DNA (5, 6, 29). SCOP-T1 cells (22) were provided by N. Glaichenhaus, COS-1 cells (11) by J. Weber, NIH-3T3 cells by R. Weinberg, and R1-4 and Ori-1 cells (8) by S. Chen. All of these cells were grown as recommended by the suppliers.

DNA transfections. The assays were performed by the method of Sussman and Milman (28) as already described (20).

Cloning in *Escherichia coli*. The methods and most recombinants used were those described previously (20, 29). To produce a modified RmI carrying the SV40 origin, recombinant plasmid p777, carrying the whole of the SV40 genome inserted at the *Bam*HI site of pBR322 (4), was digested with *Sau*3A. The 1,347-base-pair (bp) fragment of SV40 DNA thus generated, which includes the origin (31), was preparatively isolated and ligated to *Bgl*II-cleaved pB_1-20. Following bacterial transformation and characterization of several recombinant plasmids, one containing the desired insert was used throughout this work ($pSVB_1$-20). Several sister plasmids, representing both orientations of the same insert, and one carrying a dimer of the 1,347-bp SV40 DNA fragment, were used in various biological experiments, with results essentially similar to those obtained with $pSVB_1$-20 (see Results).

DNA transfer and hybridization. The procedures used for transfer and hybridization have been described previously (29). The SV40 DNA used in some gels and for probe synthesis was purchased from Bethesda Research Laboratories. The DNA designated H-B used as the probe for Ins was an *Hin*cII-*Bam*HI fragment from RmII, part of which is also present in RmI (29). This fragment was isolated from recombinant pRH-II-1 (29).

Synthesis of virus-specific RNA. At 24 h after transfection,

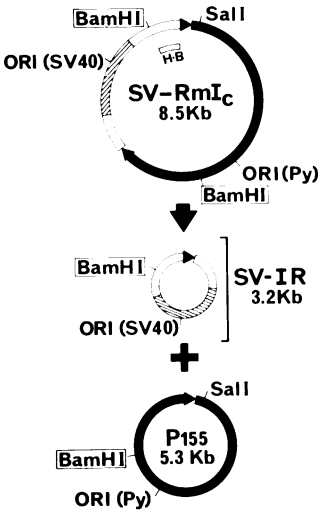

FIG. 1. Maps of SV-RmI$_c$ and its presumptive products. SV-RmI$_c$ is organized like RmI$_c$ except that it carries a fragment of SV40 DNA (hatched area) inserted at the *Bgl*II site of Ins (see Fig. 3 in reference 20). Py sequences are shown by the black areas, and mouse sequences are shown by the white areas. Intramolecular recombination occurring in SV-RmI$_c$ at the Py DNA-mouse DNA junctions is expected to produce SV-IR or P155. Whereas digestion with *Bam*HI simply linearizes P155 (5.3 kb) or SV-IR (3.2 kb), it generates from SV-RmI$_c$ two fragments of 4.6 and 3.8 kb containing, respectively, the Py origin and the SV40 origin. Thus, *Bam*HI will convert a mixture of SV-RmI$_c$, P155, and SV-IR into four readily separable linear species detectable by specific probes (see Fig. 2). The portion of Ins complementary to a cellular DNA probe designated H-B is indicated.

total cell RNA was extracted as described by Murphy et al. (17). Viral RNA was detected by dot blotting (30).

RESULTS

Our initial aim was to determine whether intramolecular recombination occurring in RmI leads to the formation of a product which is the reciprocal of P155. To render such a product readily amplifiable, we inserted the SV40 origin at the unique *Bgl*II site of pB$_1$-20 (20), producing pSVB$_1$-20 (see Materials and Methods). Treatment of pSVB$_1$-20 with *Sal*I and ligase generated a circular chimera designated SV-RmI$_c$ (Fig. 1).

SV-RmI$_c$ replicates and recombines in COS cells. A recombination product inclusive of the SV40 origin was expected to be most readily detectable in a simian cell synthesizing SV40 LT, in which SV-RmI$_c$ itself should also replicate. Therefore, SV-RmI$_c$ was transfected into COS cells (11), and the cells were incubated at 33°C prior to DNA extraction by the Hirt method (13). Two species of circular DNA, both detectable with an SV40 DNA probe as covalently closed and open circular forms, were found to accumulate following transfection (Fig. 2A). One of these two species migrated like SV-RmI$_c$ itself, whereas the second species migrated as expected for the presumptive recombination product of SV-RmI$_c$, designated SV-IR (3.2 kilobases [kb]; Fig. 1). This interpretation was confirmed when the DNA was treated with *Bam*HI prior to electrophoresis and hybridized with various probes (Fig. 2B, C, and D). An SV40 DNA probe revealed two fragments of about 3.8 and 3.2 kb (Fig. 2B), which probably represent the portion of SV-RmI$_c$ inclusive of the SV40 origin and linearized SV-IR, respectively (Fig. 1). Furthermore, the 3.2-kb fragment also annealed with a cellular DNA probe (H-B probe) specific for the portion of Ins included in the 4.6-kb fragment of SV-RmI$_c$ (Fig. 1), whereas the 4.6-kb fragment itself was detectable with the same probe (Fig. 2C). Finally, the 4.6-kb fragment and the 3.8-kb fragment annealed with a Py DNA probe (Fig. 2D), as expected if they both originated from SV-RmI$_c$ (Fig. 1). The presence of one element of the 182-bp repeat in SV-IR made the 3.2-kb fragment detectable with the Py DNA probe, but only after very long exposure times (not shown). Even then, a 5.3-kb fragment that would have represented linearized P155 could not be detected. Since simian cells are nonpermissive for the replication of P155, this experiment could not have been expected to demonstrate the simultaneous production of SV-IR and P155 from SV-RmI$_c$. How-

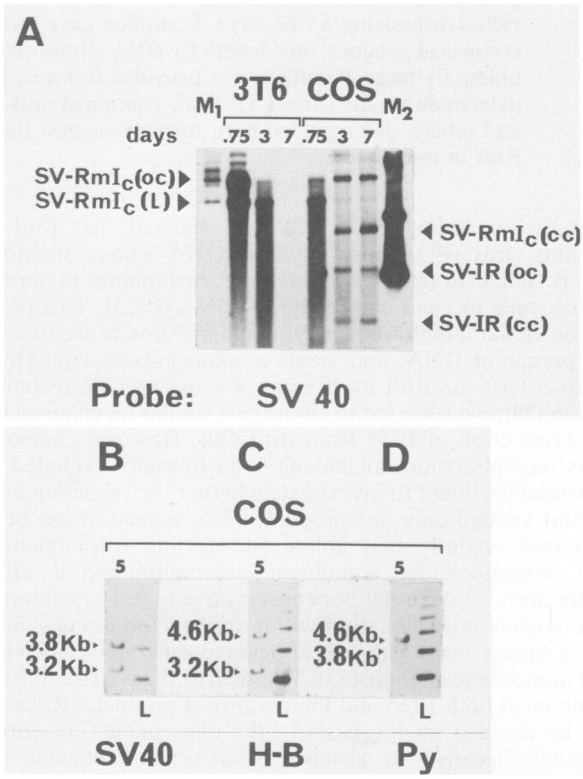

FIG. 2. Fate of SV-RmI$_c$ in mouse 3T6 cells and COS cells. Cells were transfected with SV-RmI$_c$, specifically with pSVB1-20 after it had been treated with *Sal*I and ligase, and incubated at 33°C for 0.75, 3, or 7 days before DNA extraction by the Hirt method (13), electrophoresis, and blotting (26). (A) The DNA, which had not been restricted prior to electrophoresis, was hybridized with an SV40 DNA probe. Except for that found 3 and 7 days after transfection of COS cells, the DNA demonstrated here was completely degradable by *Dpn*I (19) (not shown). Marker DNAs were either that used in the transfection (lane M$_1$) or SV40 DNA from lytically infected monkey cells (lane M$_2$). (B, C, and D) DNA recovered from COS cells 5 days after transfection was digested with *Bam*HI prior to electrophoresis, run in three different tracks, and probed with SV40 DNA (A), H-B DNA (C), and Py DNA (D). H-B DNA is an *Hin*cII-*Bam*HI fragment of mouse DNA isolated from RmII, part of which is also present in RmI. The same radioactive ladder (lane L) was used as a marker in all cases. From bottom to top, the marker fragments contain 3,054, 4,072, 5,090, 6,108, 7,126, and 8,144 bp, respectively. Rehybridization of blot A with a Py DNA probe did not reveal bands undetected by the SV40 DNA probe (not shown). oc, Open circular; cc, covalently closed circular; L, linear.

ever, it has shown that recombination of SV-RmI$_c$ in COS cells results in the formation of at least one product, the reciprocal of P155.

SV-RmI$_c$ neither replicates nor recombines in normal mouse cells. As a control for the experiments reported above, SV-RmI$_c$ was also transfected into mouse 3T6 cells. Unlike RmI$_c$ (20), SV-RmI$_c$ neither accumulated nor yielded detectable P155 in these normal mouse cells (Fig. 2A). The same contrasting results between RmI$_c$ and SV-RmI$_c$ were observed when these molecules were transfected into mouse NIH-3T3 cells (not shown). Together these findings suggested that the SV40 origin interfered with the expression of a function of RmI that was needed for its replication and recombination in normal mouse cells. The validity of this conclusion was tested by two different approaches.

In the first series of experiments, the effect of the SV40 origin on the expression of some of the functions encoded by RmI was investigated. Monolayer cultures of mouse 3T6 cells were transfected with 1 μg of DNA, which was either viral DNA selectively extracted from mouse cells lytically infected with *ts*P155 or recombinant plasmid DNA originating from *E. coli* (26). The recombinant DNA had either undergone no enzymatic treatment (pI-1) or else been treated with *Sal*I and ligase to excise and recircularize the cloned insert, RmI$_c$ or SV-RmI$_c$ (20). At 24-h after transfection, total RNA was extracted, digested with DNase, and dot blotted onto a nitrocellulose sheet (see Materials and Methods). After hybridization with a Py DNA probe, the resulting autoradiogram was scanned, and the area under the peaks was estimated by weighing the pieces of paper with a microbalance. The values for *ts*P155, pI-1, RmI$_c$, and SV-RmI$_c$ were 0.0488, 0.0055, 0.0059, and 0.0008 g (grams of paper), respectively. Note that cloned RmI produces 5 to 10 times less viral RNA than viral DNA originating from tissue culture cells, and SV-RmI$_c$ 5 to 10 times less RNA than RmI$_c$ or pI-1. SV-RmI$_c$ was five to 10 times less effective than RmI$_c$ in initiating the synthesis of Py-specific RNA in mouse 3T6 cells. A difference of a similar magnitude was observed when the same cells were examined for the production of either early or late viral proteins detectable by immunofluorescence (A. Piché and E. Herring-Gillam, unpublished observations).

In a second series of experiments, SV-RmI$_c$ was transfected into Py-transformed cells known to complement early temperature-sensitive mutants of Py for growth at the restrictive temperature (SCOP cells) [22]). Accumulation of both SV-RmI$_c$ and P155 was readily detectable in these cells

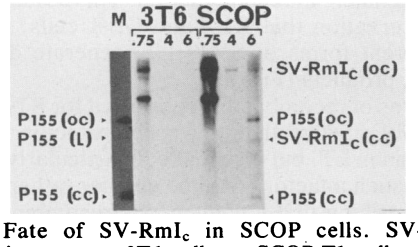

FIG. 3. Fate of SV-RmI$_c$ in SCOP cells. SV-RmI$_c$ was transfected into mouse 3T6 cells or SCOP-T1 cells, which were incubated at 33°C for 0.75, 4, or 6 days before DNA extraction. DNA was subjected to electrophoresis and annealed with a Py DNA probe. The marker DNA (lane M) was extracted from mouse 3T6 cells lytically infected with *ts*P155 virus. Even after longer exposures, bands similar to those detected for the SCOP cells 4 and 6 days after transfection remained undetectable in the case of the 3T6 cells. See Fig. 2 legend for abbreviations.

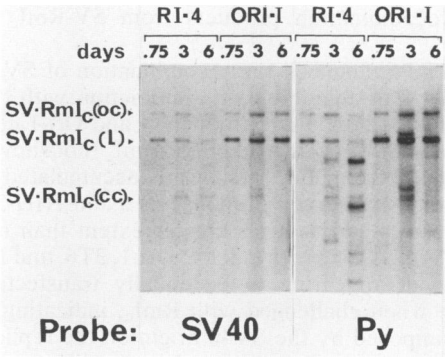

FIG. 4. Fate of SV-RmI$_c$ in R1-4 cells and Ori-1 cells. SV-RmI$_c$ was transfected into cell cultures, which were then incubated at 33°C for 0.75, 3, or 6 days before DNA extraction. Untreated DNA was subjected to electrophoresis and annealed with an SV40 DNA probe (left). After autoradiography, a second hybridization was performed with a Py DNA probe (right). See Fig. 2 legend for abbrevations.

(Fig. 3). By day 6 after transfection, P155 had become as abundant as SV-RmI$_c$, indicating again that extensive accumulation of the precursor was nonessential to the formation of a readily detectable recombination product. As the unit-length viral DNA encoded all the functions needed for its own replication in permissive cells, this result suggested that the release of P155 from SV-RmI$_c$ required a function expressed in SCOP cells but not in mouse 3T6 cells.

In mouse cells transformed by SV40, recombination occurs but generates different products. The transfections performed thus far indicated that SV-RmI$_c$ contains a Py origin active in Py-transformed mouse cells and an SV40 origin active in SV40-transformed monkey cells. However, the SV40 origin was rendering SV-RmI$_c$ inactive in an environment permissive for Py. Could such inhibition be reversed if the SV40 LT was provided in *trans*?

Chen et al. (8) derived from mouse NIH-3T3 cells two transformed cell lines, designated R1-4 and Ori-1, that synthesize different forms of SV40 LT: whereas Ori-1 cells synthesize only regular lytic SV40 LT, R1-4 cells synthesize in addition a larger form designated super-T. When transfected into these cells, SV-RmI$_c$ underwent limited replication, as could be demonstrated by annealing the DNA recovered from the cells with an SV40 DNA probe (Fig. 4, left). SV-RmI$_c$ also generated DNA of lower molecular weight, as evidenced by rehybridizing the same blot with a Py DNA probe (Fig. 4, right). However, this probe demonstrated not one but several molecular forms smaller than SV-RmI$_c$. These forms were likely to have arisen from SV-RmI$_c$ via recombination. Indeed, they lacked detectable SV40 sequences, as if they had specifically lost the SV40 origin that was inhibitory to replication.

The accumulation of DNA illustrated in Fig. 4 was verified to involve on one hand the supply of the thermolabile Py LT encoded by SV-RmI$_c$ and its products, since no such accumulation occurred in cells incubated at 39 instead of 33°C (not shown). This accumulation involved, on the other hand, the participation of SV40 LT or SV40 super-T, as indicated by two findings. First, we had observed previously that no replication or recombination of SV-RmI$_c$ was taking place in NIH-3T3 cells, from which R1-4 cells and Ori-1 cells had been derived (see above). Second, R1-4 and Ori-1 cells, which had been shown to synthesize different forms of SV40 LT (8), were reproducibly found to generate somewhat

different recombination products from SV-RmI$_c$ (Fig. 4, right).

If SV40 LT influenced the recombination of SV-RmI$_c$ in mouse cells, was this due to its interaction with the SV40 origin? To answer this question, R1-4 and Ori-1 cells were transfected with RmI$_c$ instead of SV-RmI$_c$ (not shown). Two observations were made. First, RmI$_c$ accumulated in these cells as already observed in mouse 3T6 and NIH-3T3 cells (20; this report) and to a far greater extent than noted for SV-RmI$_c$ (Fig. 4). Therefore, R1-4, Ori-1, 3T6, and NIH-3T3 cells were all appearing to be equally transfectable and permissive when challenged with RmI$_c$, indicating that the inhibition imposed by the SV40 origin on the replication of SV-RmI$_c$ was only partly relieved in the SV40 transformants. The second observation was that recombination of SV-RmI$_c$ yielded a product detectable with a Py DNA probe which, although difficult to discern in the presence of large amounts of RmI, appeared to be clearly more heterogeneous than that produced from RmI$_c$ in mouse 3T6 and NIH-3T3 cells (20; this report). Therefore, the pattern of recombination noted for R1-4 and Ori-1 cells was linked not to the presence of the SV40 origin in SV-RmI$_c$, but to a function acquired by these cells during transformation by SV40.

DISCUSSION

Replication of SV-RmI$_c$. The results described above demonstrate clearly that both viral origins of SV-RmI$_c$ are functional. The chimeric DNA replicates readily in either monkey cells supplying SV40 LT (COS cells) or mouse cells providing Py LT (SCOP cells), as does the recombination product with the "homologous" origin, SV-IR in COS cells and P155 in SCOP cells. In normal mouse cells, however, SV-RmI$_c$ is largely unable to replicate, presumably because the SV40 origin exerts a *cis* effect preventing the synthesis of adequate amounts of Py LT. Whether the converse inhibition would also take place could not be tested readily, as SV-RmI$_c$ does not include a complete SV40 early coding region. In SV40-transformed mouse cells such as R1-4 and Ori-1, synthesis of Py LT took place, allowing for the replication of SV-RmI$_c$ and of recombination products that included a Py origin. Such relief of the inhibition imposed by the SV40 origin probably reflected the availability of proteins which bind specifically to this origin, such as SV40 LT.

Recombination of SV-RmI$_c$. In normal mouse cells, SV-RmI$_c$ was obviously far less effective than RmI$_c$ in yielding P155. This could not be the consequence of the reduced ability of SV-RmI$_c$ to replicate, since we had observed earlier that P155 was more readily generated from cloned RmI when replication of the latter was impeded (20). In this work also, recombination products eventually became as abundant as SV-RmI$_c$ even though SV-RmI$_c$ was partially handicapped for replication (Fig. 2, 3, and 4). Thus, the relative inability of SV-RmI$_c$ to yield P155 indicates that the underlying recombination mechanism is dependent on a function that is not expressed in normal mouse cells. That this function is a viral function encoded by RmI or evoked by a virally encoded function is suggested by two types of observations. First, the Py genes of SV-RmI$_c$ appear to be poorly expressed in normal mouse cells (see Results). Second, SV-RmI$_c$ recombines readily in cells in which early viral functions are expressed, such as SCOP cells and COS cells.

Mechanism of recombination. We have already proposed that RmI may recombine in normal mouse cells via a site-specific mechanism (20). This proposal was almost entirely based on the observation of an effective conversion of RmI into P155, an exclusively viral recombination product of high apparent homogeneity. The fact that SV-RmI$_c$ will also yield recombination products of high apparent homogeneity, such as P155 and its reciprocal, SV-IR, in SCOP cells and COS cells, respectively, is in keeping with our proposal. What appears to be particularly significant, however, is the fact that this homogeneity is not a mere reflection of the internal homology of 182 bp in RmI. Indeed, in cells such as R1-4 and Ori-1, the recombination product arising from SV-RmI$_c$ is not P155. This is contrary to what would be expected if all that were needed for RmI to recombine in mouse cells were the internal homology and the enzymes available in this permissive environment.

Function required for recombination. Py LT is most likely to carry the specific function involved in the resolution of RmI. First, Py LT is encoded by RmI, as expected if it were involved in the site-specific resolution of this chimera (20). Second, recent evidence suggests that LT may often be supplied as a limiting factor. For instance, it has been shown that SV40 DNA replication depresses early viral transcription and thus the synthesis of LT (14, 15). High amounts of LT, such as may be needed for site-specific recombination, may thus be available only when viral DNA replication is depressed. This would explain why P155 is produced late after transfection with RmI$_c$ and early after transfection with pI-1 (20). Third, that Py LT is required for recombination of RmI would also explain why P155 is readily produced in SCOP cells transfected with SV-RmI$_c$. The release of SV-IR from SV-RmI$_c$ in COS cells would then indicate that SV40 LT could play a role similar to that of Py LT in the recombination of SV-RmI$_c$. While not unreasonable in view of the respective requirements of SV40 LT and Py LT for DNA binding (24), the participation of SV40 LT in recombination is suggested by another observation. Transfection of SV-RmI$_c$ into R1-4 cells and Ori-1 cells leads to the production of molecules comprising exclusively of Py sequences that are distinct from P155. Since SV-RmI$_c$ does not appear to become altered as it replicates (Fig. 4, left), this finding indicates that the recombination event under study has lost here some or all of its original specificity. It is possible that oligomerization of LT is essential to its activity in recombination, just as it is essential to its function in the initiation of replication, a function which requires binding of this protein to the viral DNA (25). It is also possible that in R1-4 and Ori-1 cells, heterologous oligomers are formed between the SV40 LT encoded by the resident viral genome and the Py LT specified by the transfected DNA, and that such heterologous oligomers function differently from homologous oligomers in recombination. This assumption suits well the observation that R1-4 and Ori-1 cells, which synthesize different forms of SV40 LT, generate different recombination products (Fig. 4).

The patterns of recombination observed for R1-4 and Ori-1 cells not only suggest that the specific factor needed for recombination is LT, but also make it particularly difficult to assume that such a factor could be cellular rather than viral. It is indeed striking that homogeneous recombination products were formed in 3T6, NIH-3T3, SCOP, and COS cells and heterogeneous products in R1-4 and Ori-1 cells. However, it appears unlikely that while either Py or SV40 would evoke a cellular factor yielding a homogeneous product, a heterogeneous product would be formed when such a factor(s) would be evoked simultaneously by the two viruses.

Role of LT in the resolution of RmI. If LT indeed participates in the resolution of RmI, what would be its role in this

process? LT is known for its affinity for the origin of viral DNA, and our results could be interpreted to indicate that binding of LT to this site modifies the conformation of RmI into a more "recombinogenic" one. However, we have no reason to exclude the possibility that LT would bind specifically to the origin and also to other portions of RmI, as for instance the 182-bp repeats, which are of the size of some sites involved in site-specific recombination events (1, 23).

LT and recombination. A role of LT in integration, rearrangement, and excision of viral DNA has been well established, but these roles were always assumed to reflect the ability of this protein to trigger viral DNA replication (2, 3). However, a number of mutants have been described for both SV40 and Py which appear to code for an LT defective in viral DNA replication but not in the establishment of transformation (10, 12, 16, 21). Also, rearrangement of integrated SV40 DNA was found to depend on the availability of LT, but on neither the presence of a functional origin of replication (G. Blanck, R. Pollack and S. Chen, personal communication) nor the ability of LT to bind to this origin (32). Finally, we have observed ourselves that supplying a lytic LT that is fully active in the initiation of viral DNA replication does not promote the excision of an otherwise inducible viral genome, such as that integrated in Cyp cells (9). Together these observations point to an involvement of LT in phenomena such as integration, excision, and rearrangement of DNA which could be unrelated to its role in the initiation of viral DNA replication. If this were the case, we may be able to understand why SV40 LT is essential to SV40 DNA excision while seeming to have no effect on the frequency of either homologous or nonhomologous recombination in SV40 DNA (18, 27).

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LITERATURE CITED

1. **Abremski, K., R. Hoess, and N. Sternberg.** 1983. Studies on the properties of P1 site-specific recombination: evidence for topologically unlinked products following recombination. Cell **32**:1301–1311.
2. **Botchan, M., J. Stringer, T. Mitchison, and J. Sambrook.** 1980. Integration and excision of SV40 DNA from the chromosome of a transformed cell. Cell **20**:143–152.
3. **Botchan, M., W. Topp, and J. Sambrook.** 1978. Studies on simian virus 40 excision from cellular chromosomes. Cold Spring Harbor Symp. Quant. Biol. **43**:709–719.
4. **Bouchard, L., C. Gélinas, C. Asselin, and M. Bastin.** 1984. Tumorigenic activity of polyoma virus and SV40 DNAs in newborn rodents. Virology **135**:53–64.
5. **Bourgaux, P., L. Delbecchi, K. K. Y. Yu, E. Herring, and D. Bourgaux-Ramoisy.** 1978. A mouse embryo cell line carrying an inducible, temperature-sensitive, polyoma virus genome. Virology **88**:348–360.
6. **Bourgaux, P., B. S. Sylla, and P. Chartrand.** 1982. Excision of polyoma virus DNA from that of a transformed mouse cell: identification of a hybrid molecule with direct and inverted repeat sequences at the viral-cellular joints. Virology **122**:84–97.
7. **Bushman, W., S. Yin, L. L. Thio, and A. Landy.** 1984. Determinants of directionality in lambda site-specific recombination. Cell **39**:699–706.
8. **Chen, S., D. S. Grass, G. Blanck, N. Hoganson, J. L. Manley, and R. E. Pollack.** 1983. A functional simian virus 40 origin of replication is required for the generation of a super T antigen with a molecular weight of 100,000 in transformed mouse cells. J. Virol. **48**:492–502.
9. **Delbecchi, L., D. Gendron, and P. Bourgaux.** 1981. Inducible permissive cells transformed by a temperature-sensitive polyoma virus: superinfection does not allow excision of the resident viral genome. J. Virol. **39**:196–206.
10. **Galup, C., E. Herring-Gillam, B. S. Sylla, and P. Bourgaux.** 1984. The temperature-sensitive defect in polyoma virus P155 mutant. Virus Res. **1**:469–475.
11. **Gluzman, Y.** 1981. SV40-transformed simian cells support the replication of early SV40 mutants. Cell **23**:175–182.
12. **Gluzman, Y., and B. Ahrens.** 1982. SV40 early mutants that are defective for viral DNA synthesis but competent for transformation of culture rat and simian cells. Virology **123**:78–92.
13. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. **26**:365–369.
14. **Lebkowski, J. S., S. Clancy, and M. P. Calos.** 1985. Simian virus 40 replication in adenovirus-transformed human cells antagonizes gene expression. Nature (London) **317**:169–171.
15. **Lewis, E. D., and J. L. Manley.** 1985. Repression of simian virus 40 early transcription by viral DNA replication in human 293 cells. Nature (London) **317**:172–175.
16. **Manos, M. M., and Y. Gluzman.** 1984. Simian virus 40 large T-antigen point mutants that are defective in viral DNA replication but competent in oncogenic transformation. Mol. Cell. Biol. **4**:1125–1133.
17. **Miller, J., Bullock, P., and M. Botchan.** 1984. Simian virus 40 T antigen is required for viral excision from chromosomes. Proc. Natl. Acad. Sci. USA **81**:7534–7538.
18. **Murphy, D., P. M. Brickell, D. S. Latchman, K. Willison, and P. W. J. Rigby.** 1983. Transcripts regulated during normal embryonic development and oncogenic transformation share a repetitive element. Cell **35**:865–871.
19. **Peden, K. W. C., J. M. Pipas, S. Pearson-White, and D. Nathans.** 1980. Isolation of mutants of an animal virus in bacteria. Science **209**:1392–1396.
20. **Piché, A., and P. Bourgaux.** 1987. Resolution of a polyomavirus-mouse hybrid replicon: release of genomic viral DNA. J. Virol **61**:840–844.
21. **Pintel, D., N. Bouck, and G. di Mayorca.** 1981. Separation of lytic and transforming functions of the simian virus 40 a region: two mutants which are temperature sensitive for lytic functions have opposite effects on transformation. J. Virol. **38**:518–528.
22. **Rautmann, E., N. Glaichenhaus, Z. Nahgashfar, R. Breathnach, and M. Rassoulzadegan.** 1982. Complementation of a tsa mutant and replication of a recombinant DNA carrying the viral ori region in mouse cells transformed by polyoma virus. Virology **122**:306–317.
23. **Reed, R. R.** 1981. Resolution of cointegrates between transposons γδ and Tn3 defines the recombination site. Proc. Natl. Acad. Sci. USA **78**:3428–3432.
24. **Scheller, A., and C. Prives.** 1985. Simian virus 40 and polyomavirus large tumor antigens have different requirements for high-affinity sequence-specific DNA binding. J. Virol. **54**:532–545.
25. **Schürmann, C., M. Montenarh, M. Kohler, and R. Henning.** 1985. Oligomerization of simian virus 40 tumor antigen may be involved in viral DNA replication. Virology **146**:1–11.
26. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
27. **Subramani, S., and P. Berg.** 1983. Homologous and nonhomologous recombination in monkey cells. Mol. Cell. Biol. **3**:1040–1052.
28. **Sussman, D. J., and G. Milman.** 1984. Short-term, high-efficiency expression of transfected DNA. Mol. Cell. Biol. **4**:1641–1643.
29. **Sylla, B. S., D. Huberdeau, D. Bourgaux-Ramoisy, and P. Bourgaux.** 1984. Site-specific excision of integrated polyoma DNA. Cell **37**:661–667.

30. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
31. **Tooze, J. (ed.).** 1980. Molecular biology of tumor viruses, vol. 2. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Verderame, M. F., and R. Pollack.** 1986. Expression of 100,000-M_r simian virus 40 (SV40) tumor antigen in mouse fibroblasts transfected with replication-defective SV40 genomes. J. Virol. **57**:857–863.
33. **Wake, C. T., T. Gudewicz, T. Porter, A. White, and J. H. Wilson.** 1984. How damaged is the biologically active subpopulation of transfected DNA? Mol. Cell. Biol. **4**:387–398.
34. **Wake, C. T., F. Vernaleone, and J. H. Wilson.** 1985. Topological requirements for homologous recombination among DNA molecules transfected into mammalian cells. Mol. Cell. Biol. **5**:2080–2089.