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Genetics

Another chromosomal assignment for a simian virus 40 integration site in human cells

(transformed cells/cell hybrids/DNA blotting technique/viral integration)

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ABSTRACT **Somatic cell hybrids derived from fusion of GM637, a human cell line transformed by simian virus 40, and mouse B82 cells were examined for simian virus 40 T antigen, V antigen, and viral DNA. All hybrid cell lines that contained viral DNA were T-antigen positive. Cells that did not have viral DNA were T-antigen negative. We determined that there is a single viral insertion in these hybrid cells. Correlation of T-antigen expression and viral DNA with the partial complements of the human genome retained in the hybrids showed that the inserted viral genome is in human chomosome 8. The integrated viral DNA is stable; free viral DNA found in GM637 does not insert at other potential sites in the human genome.**

Simian virus 40 (SV40), a papovavirus, grows in African green monkey cells and transforms human and a number of rodent cells. The viral DNA persists in the transformed cells, and continued viral gene expression is necessary to maintain the transformed state (1–9). At least part of the integrated viral DNA is usually present as an intact genome, because functional virus particles can be obtained by fusion of the transformed cells with permissive cells (10). The features of viral integration sites are of interest because of possible interactions between integrated viral DNA and adjacent host sequences. Furthermore, the structure of viral insertions may reveal something about the mechanisms of somatic recombination. Two different approaches have been traditionally used to study this problem. On the one hand somatic cell genetic techniques have been employed to determine the specificity of SV40 viral insertion at the chromosomal level, while nucleic acid hybridization techniques have been used to probe the structure of the integrated viral DNA. Although these approaches are directed toward understanding the same phenomena, the use of different levels of study dictates that the data obtained by one approach may not be relevant to the data obtained from the other.

Cells from human SV40-transformed lines have been used in fusions with mouse cells and hybrids have been tested for expression of viral antigens. In early experiments of this type, Weiss was unable to correlate loss of T antigen (T-ag) with the loss of any specific human chromosome and concluded that the virus might integrate at more than one site (11). Croce and colleagues (12–18) have performed similar experiments with three different transformed human lines. They correlated T-ag expression with human chromosome 7 in two cases and chromosome 17 in another. Biochemical studies directed toward the question of specificity of viral integration have, in general, focused on nonpermissive transformed host cells. This is due to the fact that the induction of viral replication found in a transformed semipermissive host such as human cells obscured the detection of integrated copies. Botchan *et al.* (19) and Ketner and Kelly (20) have used site-specific restriction enzymes to define (*i*) the points on the viral chromosome involved in attachment to cellular DNA and (*ii*) the proximal chromosomal sequences with respect to these restriction sites. Both groups have concluded that SV40 viral insertion into rodent chromosomes cannot be mediated by a simple site-specific recombination event.

In this report we present the results of experiments that have utilized a combination of genetic and biochemical approaches to determine the chromosome site of integration of SV40 in a human cell line that has not heretofore been studied. Hybrid cell lines were obtained between B82, a murine cell line deficient in thymidine kinase (TK), and GM637, a human fibroblast cell line transformed with SV40. The hybrids were analyzed for SV40 T-ag, V antigen (V-ag), and integrated viral DNA. In every T-ag positive hybrid cell line only one inserted viral DNA sequence could be detected. Detailed chromosome and isozyme analysis allowed us to infer that this SV40 genome is integrated into human chromosome 8. While more independent transformed cell lines will have to be examined, it now seems quite clear, despite previous claims to the contrary (12–18), that SV40 insertion into human DNA is not chromosome specific.

MATERIALS AND METHODS

Cells. GM637 cells were derived by SV40 transformation of skin fibroblasts from an apparently normal woman. They were obtained from A. Greene, Institute for Medical Research, Camden, NJ. Though these were isolated as a mass population, they are probably clonal in origin because of the presence of common marker chromosomes in all cells (data not shown). Mouse B82 cells were also obtained from the same source. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with penicillin and streptomycin and 10% fetal bovine serum (Flow Laboratories) and 20 μM glutamine (GIBCO).

Cell Fusion. GM637 in passage 64 and B82 at 2×10^6 cells each were plated in a T-25 flask and incubated at 37° overnight. Fusions were performed by a method described by Davidson *et al.* (21). Briefly, the medium was completely removed and 3 ml of 50% (wt/vol) polyethylene glycol, molecular weight 1000, was added to the cell sheet. After a 2-min exposure the polyethylene glycol was removed and the cell sheet was rapidly washed three times with fresh medium. The cells were trypsinized 24 hr later and plated into T-25 flasks containing DMEM supplemented with 0.1 mM hypoxanthine/0.4 μM aminopterin/16 μM thymidine (HAT) and 10 μM ouabain. Colonies appeared in 2–3 weeks and were isolated by the use of stainless steel cloning cylinders. Cell lines were maintained in DMEM/HAT.

Subcloning and Back Selection. Secondary cell lines were derived by plating approximately 100 cells/T-25 flask. Well-

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Abbreviations: SV40, simian virus 40; T-ag, T antigen; V-ag, V antigen; TK, thymidine kinase; DMEM, Dulbecco's modified Eagle's medium; HAT, hypoxanthine/aminopterin/thymidine; P_i/NaCl, phosphate-buffered saline; MDH, malate dehydrogenase.

separated colonies were isolated and grown in DMEM/HAT. These were obtained from primary cell lines GB20 and GB7d. To obtain cells resistant to 5-bromodeoxyuridine (BrdUrd), cells were grown in nonselective medium for one to two passages and transferred to medium containing BrdUrd at 30 μg/ml. After 48 hr the culture was exposed to UV light for 1 hr. Pools of resistant cells from individual flasks were maintained separately. These cells were obtained from GB20 and GB7d and maintained in DMEM. All of the BrdUrd-resistant cells were sensitive to HAT medium.

T-ag Assay. Cells were grown on glass coverslips until they are 50–80% confluent. The medium was removed and after washing with phosphate-buffered saline (P_i/NaCl) the cells were fixed with 10% (vol/vol) formalin in P_i/NaCl for 10 min. They were then treated with Ca^{2+}- and Mg^{2+}-free P_i/NaCl for 1 min, 50% (vol/vol) acetone for 3 min at −20°, 100% acetone (−20°) for 5 min, 50% acetone for 3 min, and P_i/NaCl for 1 min. The coverslips were placed on top of moist filter paper in a petri dish and 20 μl of hamster antiserum to T-ag (Flow Laboratories) diluted 1:10 was added. Following incubation for 1 hr at 37° the coverslips were rinsed three times in P_i/NaCl. After excess P_i/NaCl was drained, 20 μl of fluorescein-labeled goat antiserum to hamster IgG diluted 1:10 was added to each coverslip and incubated at 37° for 1 hr. The coverslips were rinsed in P_i/NaCl and H_2O and mounted cell side down on clean glass slides with Elvanol. The slides were allowed to dry overnight and were observed with the aid of a Zeiss photomicroscope III equipped with an epiillumination system.

V-ag Assay. The methods for this assay are identical to T-ag assay except the primary serum used was an anti-SV40 neutralizing serum obtained from Flow Laboratories.

Viral DNA Assay. Total cellular DNA was obtained by the methods described by Botchan et al. (19). DNA was hydrolyzed with *Bal* I, an endonuclease isolated from *Brevibacterium albidum* (22). For blotting the DNA was fractionated by agarose electrophoresis. Following denaturation *in situ* the DNA was transferred to a nitrocellulose filter by the method described by Southern (23). SV40 DNA labeled to a high specific activity by ^{32}P was obtained by the methods of Maniatis *et al.* (24). The conditions of hybridization and autoradiography have been described (19).

Assay of Mitochondrial Malate Dehydrogenase. Rabbit antiserum against purified human mitochondrial malate dehydrogenase (MDH-2) was obtained as described (25). Cell extracts were tested against this antiserum by Ouchterlony double diffusion. Details of this method have been described by Shimizu *et al.* (26).

Enzyme Assays. Cell extracts were subjected to starch and cellulose acetate gel electrophoreses and stained for the enzymes listed in Table 1 by the methods described by Nichols and Ruddle (27). These enzymes represent 14 different human chromosomes.

Chromosome Analysis. Air-dried metaphase chromosome preparations were made according to standard procedures. The slides were stained with Atebrin (28), and chromosomes were photographed on H & W VTE pan film (H & W, St. Johnsbury, VT), using a Zeiss Axiomat microscope equipped with epi-illumination optics. Chromosome analysis was conducted on photographic prints.

RESULTS

In assigning SV40 integration to a human chromosome, ideally, one should determine the number of integrated viral copies present in the parental cell line and account for all of them in the hybrid cell lines. In general and with GM637 in this case, this is technically quite difficult because of the spontaneous induction of viral DNA replication in human cells (there are on the average 100 copies of SV40 DNA/cell in GM637; M. R. Botchan, J. K. McDougall, and J. Sambrook, unpublished data). To address ourselves to the question of the pattern of SV40 integration in GM637 as distinct from the free viral DNA, total high molecular weight DNA from GM637 was separated from free viral DNA by a two-step purification procedure (*i*) sucrose gradient fractionation (Fig. 1*A*) followed by (*ii*) an agarose electrophoresis separation (Fig. 1*B*). The high molecular weight DNA obtained from the agarose gels were then hydrolyzed with *Bal* I, and SV40 DNA was detected by blot hybridization as described in *Materials and Methods*. Fig. 1*C* shows the results of this experiment: only one band containing SV40 DNA was seen in the high molecular weight preparation from GM637. This same band was detected in eight other hybrid cell DNA preparations (Fig. 1*C* and Fig. 4). These results show that (*i*) at the DNA level no rearrangements occurred upon fusion with mouse B82 cells and selection of hybrid cells and (*ii*) the parent line possessed only one integrated locus of SV40 DNA per cell.

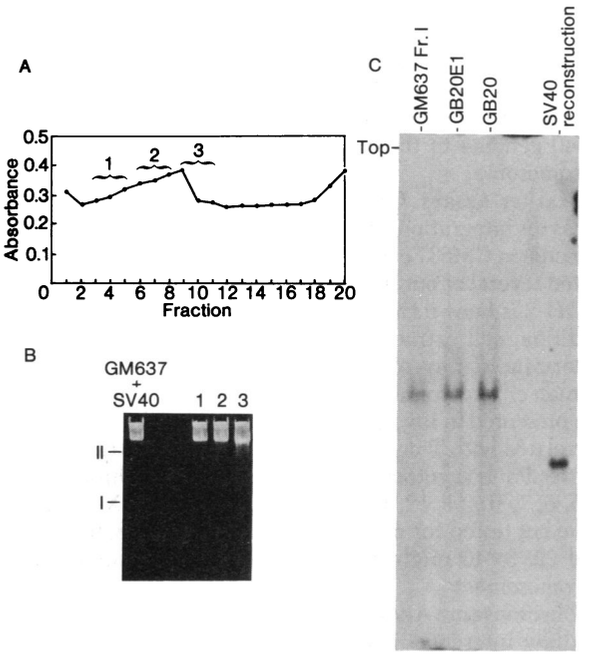

FIG. 1. Comparison of integrated SV40 DNA in parental cell line GM637 and two hybrid cell populations. Forty micrograms of total high molecular weight GM637 cellular DNA was separated from free SV40 DNA in the preparation by centrifugation through a neutral 5–20% sucrose gradient, containing 10% (vol/vol) glycerol, 0.1 M NaCl, 0.01 M Tris·HCl at pH 8, and 0.001 M EDTA. The sample in 100 μl was centrifuged through a 12-ml gradient in a Spinco SW 41 rotor at 41,000 rpm for 3 hr. Fractions were collected from a hole punched in the bottom of the tube. The A_{260} reading of each fraction is shown in *A*. Fractions were pooled and assayed for SV40 DNA by blot hybridization as described in *Materials and Methods*. The peak of free SV40 form I DNA was seen in fractions 17, 16, and 15. The high molecular weight cell DNA was further separated from free SV40 DNA by electrophoresis through a 1.4% agarose gel. *B* shows the ethidium bromide-stained pattern of the DNA from pooled fractions 1, 2, and 3. Roman numerals I and II point to the positions of free supercoiled and nicked circular SV40 DNA in this gel. The DNA from fraction I was eluted from the agarose gel and cleaved with the restriction enzyme *Bal*. The fragments were separated by electrophoresis through a 0.7% agarose gel, and the SV40-containing fragment in the DNA was detected by blot hybridization. *C* shows the autoradiographic detection of this hybridization. Five micrograms of total cellular DNA from hybrid cell clones GB20E1 and GB20 were hydrolyzed with the restriction enzyme *Bal* and run in parallel with GM637 fraction 1 DNA. The slot marked "SV40 reconstruction" contained 5 pg of nicked circular SV40 DNA.

Croce and colleagues, using three different SV40-transformed cells as human parents in cell fusions, have reported correlation of SV40 T-ag with human chromosome 7 in two cell lines and with chromosome 17 in another. To determine if such correlations could be made when GM637 was used as the human parent, we performed the following experiments.

T-ag Expression. The parental mouse and human cells were tested for T-ag by indirect immunofluorescence. Every cell in the GM637 population was T-ag$^+$: B82 cells were T-ag$^-$. Eighteen independently derived hybrid cell lines and 14 secondary lines derived from a single primary cell line were tested for T-ag. Thirteen of the primary and all of the secondary cell lines were T-ag$^+$. Because of the HAT selection system all these hybrid cells are expected to retain the gene for human thymidine kinase (TK), which is on human chromosome 17. If the T-ag expression is syntenic with human TK, all the hybrids would be expected to be T-ag$^+$. The lack of correlation between T-ag and TK indicatedthat SV40 is not integrated in chromosome 17. To further test this possibility, we have isolated hybrid cell lines resistant to BrdUrd at 30 μg/ml. Of the nine such cell lines, derived from three independent primary hybrids, seven were T-ag$^+$. Because BrdUrd selects for cells that lack TK, these cells have lost human TK and thus human chromosome 17. The presence of T-ag in cells lacking chromosome 17 and its absence in some of the cells containing chromosome 17 show that the SV40 genome in these hybrid cells is not integrated in that chromosome.

Marker Assays. Croce has reported (12) that chromosome 7 has an integration site for SV40. To test whether this chromosome in GM637 carries an integrated SV40 genome, we have tested several of our hybrid cell lines for MDH-2. The gene for MDH-2 is known to be located on chromosome 7 (25, 29). In addition, cell extracts from these hybrids were also used to determine if any of 16 markers, representing 14 different human chromosomes, were present. Results from the analyses are presented in Fig. 2 and Table 1. None of the markers tested correlated with T-ag. These results indicate that the SV40 T-ag expression is asyntenic to markers located on chromosomes 1, 2, 5, 6, 7, 10, 11, 12, 14, 15, 17, 18, 19, 20, and X. Because we have not tested for markers representing 3, 4, 8, 9, 13, 16, 21, and 22, SV40 might be integrated in one or more of these chromosomes.

Chromosome Analysis. Enzyme data alone cannot be used to draw inferences about assignment of markers to specific human chromosomes. Low levels of specific human chromosome presence might be reflected by either a positive or negative enzyme pattern. In order to determine which human chromosome is necessary for T-ag expression, we undertook a detailed cytological analysis of several of our primary and secondary cell lines. GM637 is a heteroploid cell line, containing a mean number of 79 chromosomes. At least one normal copy

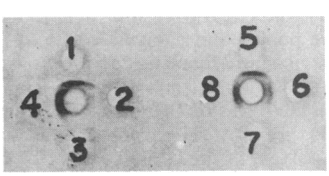

FIG. 2. Double immunodiffusion analysis of human mitochondrial NAD-linked malate dehydrogenase. The central wells contained 8 μl of the rabbit antiserum against human mitochondrial MDH that had been absorbed with mouse A9 cell extract. The numbered peripheral wells contained 8 μl each of the following test samples: 1, human HeLa cell extract; 2, human–mouse hybrid GB7d, which does not carry human chromosome 7; 3, mouse B82; 4, human–mouse hybrid GB10a, which carries human chromosome 7; 5, human HeLa; 6, human–mouse hybrid GB20A1, which carries human chromosome 7; 7, mouse A9; and 8, human–mouse hybrid GB20G2 (chromosome data not available).

Table 1. Correlations between SV40 T-ag expression and human biochemical markers

Enzyme	Chromosome	Concordant	Discordant
UGPP	1	4	7
MDH-1	2	2	9
Hex-B	5	3	5
ME-1	6	1	7
MDH-2	7	21	7
GOT	10	5	2
LDH-A	11	8	3
LDH-B	12	9	2
Pep-B	12	8	2
NP	14	10	1
MPI	15	4	6
Pep-A	18	8	2
GPI	19	2	9
ADA	20	0	8
G6PD	X	2	9

Concordant, T-ag and the human enzyme both present or absent; UGPP, UDPglucose pyrophosphorylase; Hex-B, hexosaminidase B; ME-1, malic enzyme 1; GOT, glutamic–oxaloacetic transaminase; LDH, lactate dehydrogenase; Pep, peptidase; NP, nucleoside phosphorylase; MPI, mannose phosphate isomerase; GPI, glucose phosphate isomerase; ADA, adenosine deaminase; G6PD, glucose-6-phosphate dehydrogenase.

of each of the chromosomes was present in most cells of this cell line (Fig. 3). Chromosome analysis of hybrid cell lines was conducted within one to two passages of T-ag and enzyme assays. The results of this analysis are presented in Table 2. The only chromosome that was present in all T-ag$^+$ cell hybrids was chromosome 8, with one exception. GB7d1, a BrdUrd-resistant subclone of GB7d, was T-ag$^+$ but did not contain an identifiable chromosome 8. Possible reasons for this discrepancy will be discussed later. Hybrids that did not contain chromosome 8 were T-ag$^-$. No correlations could be made between T-ag and any other intact or marker human chromosome. On the basis of these results we conclude that human chromosome 8 is necessary for T-ag expression in B82 × GM637 hybrids. The implication of this observation is that in GM637 this is the only human chromosome carrying an integrated SV40 genome.

DNA Analysis. Though it is reasonable to assume that chromosome 8 carries the integrated SV40 sequences, it would be useful to demonstrate it directly. The cytogenetic data predict that all hybrids that are T-ag$^+$ contain SV40 sequences integrated into chromosome 8.

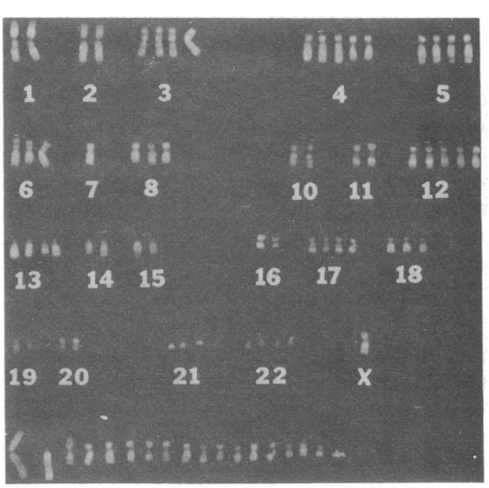

FIG. 3. GM637 karyotype; bottom line, marker chromosomes.

Table 2. T-ag expression and human chromosomal constitution of hybrid cell lines

Cell line	No. of cells	T-ag	Viral DNA	Chromosome 1	2	3	4	5	6	7	8	9	10	11	12	X	13	14	15	16	17	18	19	20	21	22
GB7d	32	+	NT				+	+	+		+				+						+				+	+
GB10a	24	−	NT		+		+			+					+	+					+				+	
GB20	26	+	+	+		+	+	+			+		+	+	+	+				+	+	+			+	
GB35c	17	−	−			+	+	+													+	+				
GB40a	17	−	−	+			+	+					+	+	+					+	+	+			+	
GB40c	29	+	NT	+		+	+				+		+	+	+	+				+	+	+				
GB42	25	+	NT				+	+			+			+							+					
GB44b	20	+	+	+	+	+	+				+		+	+	+	+	+		+	+	+	+			+	
GB20A1	33	+	NT	+		+	+			*	+		+	+	+	+				+	+	+				
GB20A2	31	+	NT	+		+	+				+		+	+	+	+				+	+	+			+	
GB20D1	22	+	NT	+	+	+	+				+		+	+	+	+				+	+	+			+	
GB20E2	26	+	NT	+		+	+				+		+	+	+					+	+	+				
GB20B1	17	+	NT	+	+	+	+				+			+	+	+				+	+	+		+	+	+
GB20C1	14	+	+	+		+	+				+		+		+	+				+	+	+			+	
GB20E1	8	+	+	+	+	+	+				+		+	+	+		+			+	+	+			+	
GB20F1	20	+	NT	+		+	+	+			+			+	+	+				+	+	+		+	+	
GB20G1	26	+	+	+	+	+	+				+		+		+	+	+			+	+	+			+	
GB20H1	16	+	NT	+		+	+				+		+	+	+	+	+		+	+	+	+			+	
GB202†	22	+	+	+		+	+	+			+		+		+	+						+				
GB7d1†	27	+	NT			+	+								+											

Chromosomes present at a frequency of 10% or more are considered positive. NT, not tested. * Chromosome 7 present in 9% of the cells. † These two cell lines are BrdUrd resistant.

In order to determine that the SV40 DNA is covalently linked and that the pattern of integration in all T-ag$^+$ hybrids is identical, we have tested for the presence of SV40 sequences by the method of Botchan *et al.* (19) and Ketner and Kelly (20). Ten cell lines, five of which are primary and five secondary clones were tested. Representative results of these assays are presented in Fig. 4. The restriction enzyme used in this analysis does not cleave within the SV40 genome. Thus the integrated viral genome is expected to migrate to a position representing a higher molecular weight than SV40. This expectation is realized. All hybrids which showed a band of DNA which hybridized with labeled SV40 DNA were T-ag$^+$. T-ag negative hybrids showed no bands. All hybrids which are T-ag positive had a single band of DNA which hybridized with SV40 DNA and this migrated to the same position in all hybrids. These results indicate that all hybrids which are T-ag$^+$ have viral sequences integrated in the human genome and that the integration sites, in terms of flanking host sequences as defined by the cleavage by *Bal* I enzyme, are similar, if not identical. The presence of this inserted viral DNA correlated with the presence of chromosome 8.

V-ag. Human cells are semipermissive for SV40 replication, and tests for V-ag in GM637 showed that a small proportion (~1%) of the cells are V-ag$^+$. To determine if there is a chromosomal basis for the expression of V-ag, we studied its expression in cell hybrids. All hybrids that are T-ag$^-$ are also V-ag$^-$. Most of the hybrids that are T-ag$^+$ are also V-ag$^-$. In a few hybrids V-ag was expressed by a small proportion of the cells. Further investigations are necessary to determine the genetic basis for SV40 V-ag expression in these cell hybrids.

DISCUSSION

We have shown that, in a series of hybrids between B82 and GM637, SV40 T-ag expression is retained in some and lost in other hybrids. Chromosome analysis revealed that all hybrid cell lines that contained chromosome 8 were T-ag$^+$. All hybrid cell lines that did not retain an identifiable chromosome 8, with one exception, were T-ag$^-$. No correlations could be made between T-ag expression and any other human chromosome. We conclude that chromosome 8 in GM637 carries an integrated SV40 viral genome. The exception to the concordant expression of T-ag and chromosome 8 is the cell line GB7d1. Detailed examination of its chromosomal composition revealed a low level of human chromosome 8 and high frequencies of chromosome 12 and a modified chromosome 4. Other cell lines that contained these three chromosomes individually or in combinations were T-ag$^-$ if chromosome 8 was absent. Thus the most likely explanation for the exceptional behavior of GB7d1 is that it contains a modified or rearranged chromosome 8 carrying the SV40 genome.

We have established that SV40 has integrated at a single site in GM637 by two independent methods. First, we have shown that the high molecular weight DNA from GM637 shows a single band of DNA containing the SV40 genome after a blot hybridization experiment. All of the hybrid cell lines that contained SV40 DNA contained it in the region defined by the blots. Studies with other restriction enzymes, Pvu II and *Eco*RI, are consistent with this conclusion (data not shown). The second method is to examine a series of unreduced hybrids, among which collectively all human chromosomes are represented, and show that one specific chromosome correlates with SV40 gene expression. In this case the presence of human chromosome 8 correlates with SV40 T-ag expression.

Among the hybrids we have studied, all that are viral DNA positive are also T-ag$^+$ and contain a single copy of viral DNA. Thus, our DNA determinations support assignment of the SV40 integration site to a single chromosome by cytogenetic methods. These results also indicate that the viral genome does not excise and reintegrate into the mouse or the other parts of the human genome.

Croce and colleagues (12–18) have reported SV40 integration sites in human chromosomes 7 and 17. We failed to find correlations between these chromosomes and SV40 T-ag expression or DNA in our hybrids. The lack of correlation between SV40 DNA or T-ag and either MDH-2 and TK or chromosomes 7 and 17 rules out assignment of SV40 integration to either of these chromosomes in GM637. When our evidence and that presented by Croce and colleagues is taken together, it seems clear

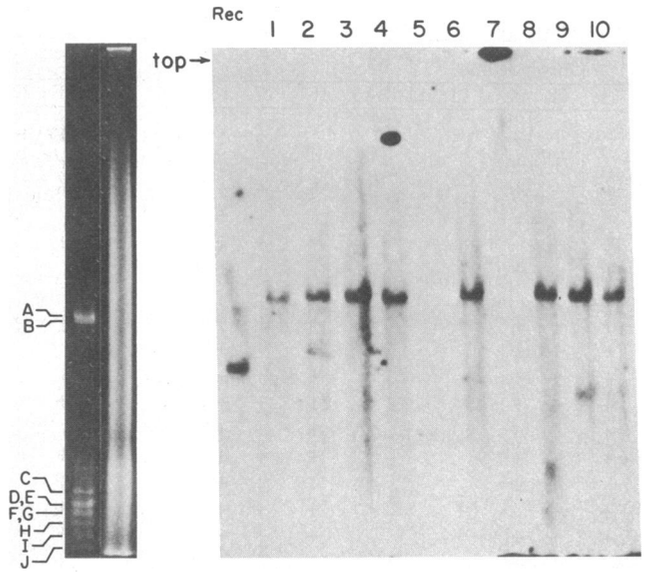

FIG. 4. Detection of integrated SV40 DNA sequences in hybrid cell genomes. Five micrograms of various cell DNAs were hydrolyzed with the *Bal* I restriction enzyme (22). After the incubation period the resultant fragments were separated by electrophoresis through a 0.7% agarose gel at 1.5 V/cm for 16 hr. After denaturation and transfer to nitrocellulose sheets, DNA on the sheets was hybridized with nick-translated SV40 DNA as described (19), except that the filter was pretreated in ten times concentrated Denhardt's solution, and the final hybridization solution had a final volume of 10 ml and contained 1 μg of radioactive SV40 DNA. The slot labeled "Rec" (reconstruction) contains 1 μg of adenovirus 2 DNA and 5 pg of SV40 DNA. This amount of SV40 DNA is equivalent to the mass of one viral genome in 5 μg of total cellular DNA, if each cellular genome contained one copy of the viral DNA. The variabilities in intensities of the bands across the gel were reproducible, and some cell lines clearly have slightly less viral DNA than others. This reflects the heterogeneity of the number of chromosomes 8 seen in the various hybrid populations. (*Right*) Autoradiogram of the nitrocellulose sheet after hybridization. (*Left*) Ethidium bromide stain pattern of the reconstruction slot showing the *Bal* I fragments of adenovirus 2 that were used as markers to calculate the molecular weight of the cell *Bal* I fragment that contains the SV40 genome and of the stained pattern of total hybrid cell DNA after cleavage with *Bal* I. Form II SV40 is detected in the reconstruction and a cell fragment of molecular weight approximately 5.5×10^6 in all the T-ag$^+$ hybrid cell DNAs. The cell samples were: 1, GB201; 2, GB202; 3, GB44b; 4, GB20C1; 5, GB40a; 6, GB34mp; 7, GB35c; 8, GB20E1; 9, GB20; and 10, GB20G1.

that SV40 can integrate at one of several sites in the human genome and that it is not chromosome specific. The presence of multiple sites for integration of SV40 in rodent cells has been shown (19, 20). All of these data indicate that the SV40 gene product(s) that is responsible for maintenance of transformation can be expressed from multiple chromosomal sites. One question left unanswered is whether the integration sites are multiple but unique or truly random.

The data presented in this paper and others (12–18) must be viewed in light of different levels of analysis employed. It is possible that recombination systems available for SV40 integration may require that only a small number of chromosomes are accessible for these events, while at the same time restraints imposed for insertion at the DNA sequence level may allow far more promiscuous recombination. Available evidence indicates that there is no chromosome or DNA sequence specificity in integration of SV40 genome into mammalian genomes.

Human cells are semipermissive for replication of SV40 (30). A small proportion of cells in a transformed line can produce viable SV40 particles. In GM637 about 1% of cells are V-ag$^+$. The viral DNA that is present inside these cells might be able to reintegrate at other potential sites such as the ones on 7 and 17, or if virus is released it might be able to superinfect other cells in the population and integrate at other available sites. However, our observations, both genetic and biochemical, indicate that all hybrids tested, and as such GM637, contain the virus at a single site. A simple explanation can be offered for this paradox. The cells that produce viable virus lyse, eliminating any traces of reintegration within them, and the virus that is released will be at too low a concentration to effectively superinfect cells. Alternatively, transformed cells that are survivors of initial viral infection may be resistant to viral absorption.

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