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Excision of Viral DNA from Host Cell DNA After Induction of Simian Virus 40-Transformed Hamster Cells

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Simian virus 40-transformed hamster cells were induced to produce infectious virus by treatment with mitomycin C or γ-irradiation. A portion of the simian virus-40 DNA, which is integrated into the host cell genome in uninduced cells, was recovered in a pool of relatively low-molecular-weight DNA early after induction treatment in the absence of DNA replication. The data indicate that excision of the viral genome occurs subsequent to the induction stimulus.

Simian virus 40 (SV40)-transformed cells usually contain only a few copies of the virus genome per diploid quantity of cell DNA (2, 9). In the transformed cell lines in which the association of viral and cellular DNA has been investigated, viral DNA has been shown to be covalently linked to cellular DNA (10). In general, infectious virus is not released spontaneously from SV40-transformed rodent cells and must be induced by various techniques (1, 4). We have been studying clones of virogenic SV40-transformed hamster kidney cells that can be induced to produce infectious virus after treatment with such agents as UV or γ-irradiation, mitomycin C, or bromodeoxyuridine and visible light. We have utilized these lines to study the mechanism of induction, in particular, the early events in the induction process (5, 6). One of the earliest events may be excision of the viral genome from its integrated state in host cell DNA, as is the case in the induction of lambda DNA from the *Escherichia coli* genome during induction (11). In this paper, we present data which indicate that the SV40 genome is excised from its integrated state subsequent to the induction stimulus.

The cell line studied, THK 22E, C1 1-1, AP_1 (clone E), has been described in detail (6). It is a clone isolated from a line of SV40-transformed inbred hamster kidney cells. All the cells of this clone contain the SV40 T antigen, but no V antigen has been detected by immunofluorescence studies. Clone E cells contain 0.5 to 1.5 SV40 genomes per diploid quantity of cell DNA as determined by C_0t analysis. Since the DNA content of clone E cells is approximately twice that of diploid hamster cells, actually 1.0 to 3.0 SV40 genomes per cell are present. Clone E cells occasionally produce trace amounts of virus spontaneously. However, virus yields of approximately 10^4 to 10^6 PFU/10^6 cells are obtained upon induction with mitomycin C or γ-irradiation.

Experiments illustrated in Fig. 1 and 2 were performed to examine the association of viral DNA with host cell DNA in clone E cells. Cellular DNA was subjected to velocity sedimentation in alkaline sucrose gradients; ^{32}P-labeled SV40 DNA was added to a parallel gradient as a marker (Fig. 1). SV40 DNA content was measured in the fractions of the gradient containing high-molecular-weight cellular DNA and compared with the SV40 DNA content of total, unfractionated cellular DNA that was extracted from cells cultured in the same experiment (Fig. 2). SV40 sequences were determined by following the kinetics of reannealing of ^{32}P-labeled SV40 DNA in the presence of unlabeled cellular DNA; the technique was performed by the method of Gelb et al. (2), and the results were calculated and expressed according to Ozanne et al. (9). From the degree of acceleration of the annealing reaction achieved by a given amount of cellular DNA, values of 1.6 SV40 genomes/cell were obtained in this experiment for both clone E total cellular DNA and fractionated high-molecular-weight cellular DNA. These results indicate that in clone E cells the SV40 DNA is linked to cellular DNA by alkali-stable bonds.

Excision of the SV40 viral genome from the integrated state was determined by following the movement of SV40 DNA from its association with high-molecular-weight host cell DNA to a pool of low-molecular-weight DNA after induction. Clone E cells were treated with either mitomycin C or γ-irradiation and were harvested at different times after induction. High-molecular-weight cellular DNA was separated from a pool of low-molecular-weight

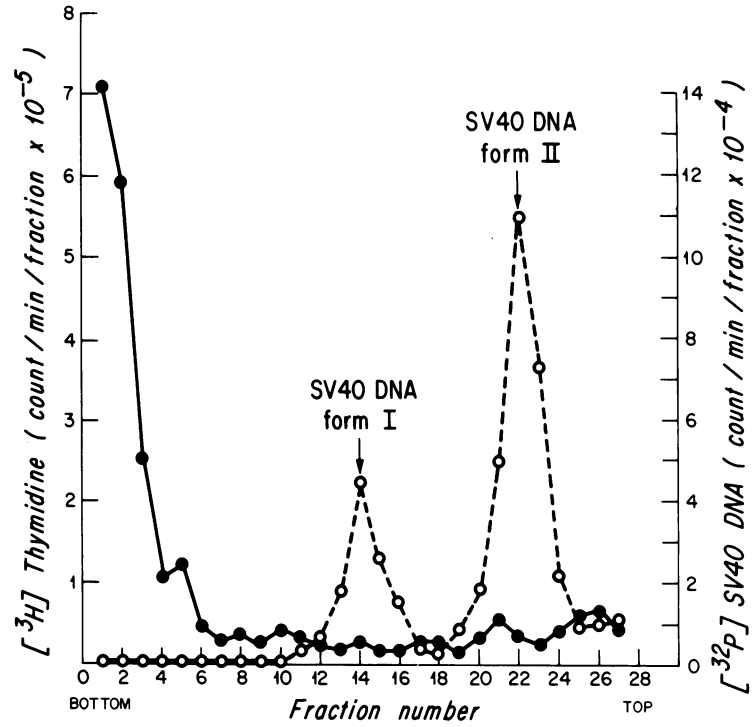

FIG. 1. *Fractionation of cellular DNA on alkaline sucrose gradients. Clone E cells were seeded in Falcon plastic plates (150 by 22 mm) and grown in Eagle minimal essential medium (Gibco) with a fourfold concentration of vitamins and amino acids, supplemented with 10% fetal bovine serum, 2 mM glutamine, and antibiotics. [^{3}H]thymidine, 0.05 μCi/ml (specific activity, 20 Ci/mmol; New England Nuclear Corp.) was included in the medium for 24 h. At the end of the labeling period, the cells were harvested with a solution containing 0.01 M Tris-hydrochloride, pH 7.4, and 0.4% sodium dodecyl sulfate. The resulting lysate was layered on top of an alkaline sucrose gradient (10 to 30%) in 0.3 N NaOH-0.01 M EDTA-0.5 M NaCl. After storage overnight at 4 C, the gradients were centrifuged (17,500 rpm, 16 h, SW25.1 rotor, Spinco model L centrifuge). To a parallel gradient, ^{32}P-labeled SV40 marker DNA (specific activity, 10^6 counts/min per μg, prepared as described in reference 6) was added. At the end of centrifugation, the radioactivity of an aliquot of each fraction was assayed, and the bottom three to five fractions were pooled, neutralized, and extensively dialyzed against 0.01 × SSC (0.0015 M NaCl plus 0.00015 M sodium citrate) containing 0.01 M EDTA; this fractionated high-molecular-weight cellular DNA was used for determination of SV40 DNA content by C_0t analysis. Symbols: ●, [^{3}H]thymidine (cellular DNA); ○, ^{32}P-labeled SV40 DNA marker.*

DNA, including free viral DNA, by the Hirt extraction procedure (3), and the amount of SV40 DNA sequences in the Hirt pellet and supernatant fractions was determined by measuring the rate of reassociation of ^{32}P-labeled SV40 DNA in the presence of unlabeled DNAs extracted from the Hirt fractions. Experiments were carried out to ensure the efficiency of the Hirt fractionation procedure in separating integrated from free viral DNA in clone E cells. In uninduced control cells, virtually all the high-molecular-weight cell DNA, containing the covalently bound SV40 DNA, was recovered in the Hirt pellet. When purified ^{32}P-labeled SV40 DNA was added to cultures of clone E cells at the time of harvest and the Hirt extraction was performed, approximately 86 to 93% of free labeled viral DNA was recovered in the Hirt supernatant fraction.

Another factor to be considered in excision experiments relates to the amount of cell DNA found in the Hirt supernatant fraction. Some cellular DNA is always present in this fraction, and induction treatments increase the amount by causing DNA breakage. Therefore, one might also expect a proportionate increase in the amount of viral DNA in the Hirt supernatant fraction after induction. To monitor the effect of induction treatment on the size of cellular DNA, we determined the partitioning of cellular DNA between the Hirt pellet and supernatant fractions in each experiment by including cultures that had been labeled with [^{3}H]thymidine for 24 h prior to induction in

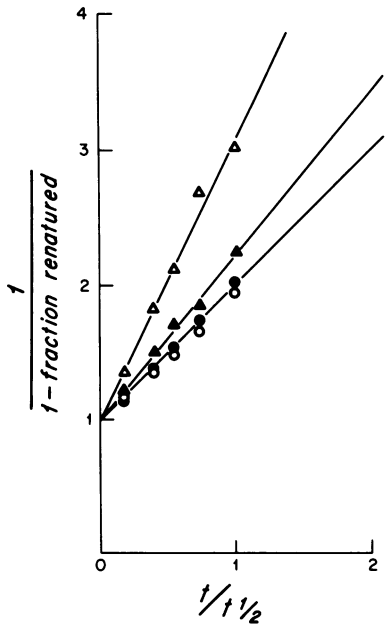

FIG. 2. *Reassociation kinetics of ^{32}P-labeled SV40 DNA in the presence of unlabeled total cellular DNA and fractionated high-molecular-weight cellular DNA from clone E. The mixtures of ^{32}P-labeled SV40 DNA and unlabeled DNAs were sonically treated, heat-denatured, and incubated at 68 C in medium containing 0.001 M EDTA-0.4 M sodium phosphate buffer (pH 6.8)-0.4% sodium dodecyl sulfate. Aliquots (0.2 to 0.3 ml) were removed at different time intervals, and the proportion of reassociated ^{32}P-labeled SV40 DNA was determined by chromatography on hydroxyapatite. The results are expressed in a standard second-order plot (12). The abscissa ($t/t^1/_2$) is the time of incubation of each mixture divided by the time required for 50% of ^{32}P-labeled SV40 DNA to reanneal in the presence of salmon sperm DNA. In experiments shown, 2 × 10^{-5} optical density at 260 nm (OD_{260}) units of ^{32}P-labeled SV40 DNA (specific activity, 10^6 counts/min per μg) per ml was incubated with one of the following: ○, 36 OD_{260} units of salmon sperm DNA per ml; ●, 5 OD_{260} units of salmon sperm DNA per ml; △, 36 OD_{260} units of total cellular DNA (extracted from clone E cells by the method of Marmur [8], with modifications described in reference 6) per ml; ▲, 5.3 OD_{260} units of fractionated high-molecular-weight cellular DNA (purified from bottom fractions of alkaline sucrose gradients, as described in Fig. 1) per ml.*

every experimental group. The amount of acid-precipitable, labeled DNA was determined in both the Hirt pellet and supernatant fractions, and the proportion of total cell DNA in the Hirt supernatant fraction was calculated. Our results are expressed in a number of ways, one of which indicates the amounts of SV40 DNA per milligram of total cell DNA present in either the supernatant or pellet fractions. If excision occurred, the supernatant fraction should show an enrichment for viral DNA sequences. However, if the presence of viral DNA in the Hirt supernatant resulted from random breakage of cell DNA, the relative amount of SV40 DNA in the supernatant fraction should be equal to or less than its relative content in the pellet fraction.

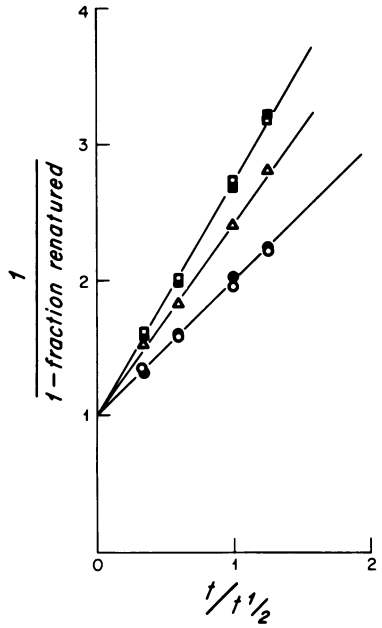

FIG. 3. *Reassociation kinetics of ^{32}P-labeled SV40 DNA incubated in the presence of unlabeled DNAs extracted from Hirt supernatant and pellet fractions of mitomycin C-induced and control cells. Clone E cells were maintained as described in the legend to Fig. 1. When subconfluent, a medium containing 0.5 μg of mitomycin C (Nutritional Biochemical Corp.) per ml was added to the cultures (zero time). A total of 10 μg of araC (cytosine-1-D-arabinofuranosyl hydrochloride; Sigma) per ml was added to the mitomycin C-treated cells at 7 h after initiation of induction or at the beginning of mitomycin C treatment. At the end of the incubation with mitomycin C (24 h), the cells were harvested and fractionated by the method of Hirt (3). DNA from the Hirt supernatant and pellet fractions was extracted essentially by the method of Marmur (8), with modifications described in reference 6. Control cells were treated similarly with medium lacking mitomycin C and/or araC. Reassociation kinetics were determined and the data were expressed as described in the legend to Fig. 2. ^{32}P-labeled SV40 DNA (2 × 10^{-5} OD_{260} units/ml) was incubated in the presence of the following unlabeled DNA samples: ○, 17 OD_{260} units of salmon sperm DNA per ml; ●, 10 OD_{260} units of DNA from Hirt supernatant of control cells per ml; △, 10 OD_{260} units of DNA from Hirt supernatant of induced cells per ml; □, 17 OD_{260} units of DNA from Hirt pellet of control cells per ml; ■, 17 OD_{260} units of DNA from Hirt pellet of induced cells per ml.*

Figure 3 shows the results of a representative experiment in which clone E cells were induced by mitomycin C. The rate of reassociation of ^{32}P-labeled SV40 DNA was the same in the presence of salmon sperm DNA as in the presence of DNA extracted from the Hirt supernatant fraction of uninduced cells, indicating that no virus-specific DNA could be detected in the latter. In contrast, reassociation of ^{32}P-labeled SV40 DNA was accelerated by the addition of DNA extracted from the Hirt supernatant fraction of mitomycin C-treated cells. The addition of DNAs purified from Hirt pellet fractions of both induced and uninduced cells accelerated the rate of reannealing of SV40 DNA to the same degree. The amount of SV40 DNA in the Hirt fractions was calculated from the degree of acceleration of ^{32}P-labeled SV40 DNA reassociation (see Table 1, experiment 4).

Several similar experiments are summarized in Table 1. The data are presented as the number of SV40 genomes present per cell (column 7) as well as the percentage of total viral DNA recovered in each of the Hirt fractions (columns 8 and 9). The table also shows the amount of SV40 DNA per-milligram of cellular DNA present in the Hirt fractions (columns 4 and 5) and the changes in the content of SV40 DNA in the Hirt supernatant fractions compared with Hirt pellet fractions (enrichment ratio, column 6).

The conditions of mitomycin C treatment used (0.5 μg/ml, 24 h of exposure) were those that were previously shown to induce maximum virus yields (6). In all the experiments, treatment with mitomycin C resulted in an enrichment of the Hirt supernatant fraction with SV40 DNA sequences (column 6). Treatment with mitomycin C alone (experiment 1)

TABLE 1. *Excision of SV40 DNA after mitomycin C induction*[a]

Expt no.	Treatment of cells	% of total cellular DNA present in Hirt supernatant fraction[b]	SV40 DNA (μg/mg of cell DNA × 10^4)[c] present in:		Enrichment ratio[d]	No. of SV40 genome copies/cell[e]	SV40 DNA (%) detected in:	
			Hirt supernatant	Hirt pellet			Hirt supernatant	Hirt pellet
1	Control	3.1	ND[f]	5.0		1.2	0	100.0
	Mitomycin C	6.6	42.0	9.1	4.6	2.9	20.7	79.3
2	Control	3.3	ND	4.0 ± 0.7		1.0	0	100.0
	Mitomycin C araC (7 to 24 h)	6.1	7.0 ± 3.0	4.0 ± 1.4	1.7	1.0	10.0	90.0
3	Control	2.9	ND	5.7		1.4	0	100.0
	Mitomycin C araC (7 to 24 h)	4.3	13.0	5.6	2.3	1.5	8.5	91.5
4	Control	3.5	ND	10.0 ± 2.0		2.1	0	100.0
	Mitomycin C araC (7 to 24 h)	6.9	16.0 ± 1.9	8.0 ± 1.6	2.0	2.0	10.0	90.0
5	Control	3.5	ND	9.1 ± 2.9		2.3	0	100.0
	Mitomycin C araC (7 to 24 h)	7.0	32.0 ± 4.6	8.0 ± 0.9	4.0	2.4	24.0	76.0
	Mitomycin C araC (0 to 24 h)	7.5	32.0 ± 4.6	10.0 ± 3.6	3.2	2.8	20.6	79.4

[a] The cells were maintained and treated as described in the legends to Fig. 1 and 3, respectively. In experiments 2 to 5, araC (10 μg/ml) was added to the mitomycin C-treated cells at the indicated times after initiation of induction.

[b] Partitioning of cellular DNA between Hirt supernatant and pellet fractions was determined by labeling one to two plates in each experimental group with [^{3}H]thymidine (0.5 to 1.0 μCi/ml; specific activity, 20 Ci/mmol; New England Nuclear Corp.) for 24 h prior to the induction treatment. After 24 h, the labeled medium was removed, and the cells were induced as described above. At the end of incubation with mitomycin C, the cells were harvested and fractionated by the Hirt procedure, and the trichloroacetic acid-precipitable radioactivity in the Hirt supernatant and pellet fractions was determined.

[c] The numbers shown represent the mean from two to four independent determinations of SV40 DNA content performed at different times with ^{32}P-labeled DNA preparations of different specific activities. Where three to four determinations were performed, standard deviation from the mean is shown.

[d] Enrichment ratio is the content of SV40 DNA in the Hirt supernatant DNA (micrograms per milligram) divided by the content of SV40 DNA in the Hirt pellet DNA (micrograms per milligram), i.e., values in column 4 divided by values in column 5.

[e] Values shown as number of SV40 genomes per cell represent corrected values for the actual DNA content of clone E cells (6).

[f] ND, None detected. No SV40 DNA was detected. This means that the content of SV40 DNA in the Hirt supernatant fraction of the control cells was less than its content in the Hirt pellet fraction of control cells.

led to an increase in the total number of SV40 genome copies per cell, which indicates that minimal replication of SV40 DNA occurs in the first 24 h of treatment. To inhibit viral DNA replication, cytosine arabinoside (araC, 10 μg/ml) was included in the culture medium in all subsequent experiments. This dose of araC was found to cause >99% inhibition of incorporation of [^{3}H]thymidine into the DNA of clone E cells either exposed or unexposed to mitomycin C. In the presence of araC, no increase in total SV40 DNA content per cell was observed. In experiments 2 to 4, araC was added to the induced cells 7 h after the addition of mitomycin C. Experiment 5 shows that the same results were achieved whether araC was included in the medium from the beginning of mitomycin C treatment or added 7 h later. Movement of SV40 DNA from the Hirt pellet to the supernatant fraction could not be detected in the presence of araC alone.

Since treatment with mitomycin C requires prolonged exposure times (i.e., 24 h), the excision event could not be pinpointed in time. Therefore, γ-irradiation, which causes immediate and direct damage to DNA, was utilized in the next series of experiments. Results of a representative experiment (no. 1 in Table 2) are shown in Fig. 4. Whereas addition of DNA extracted from the Hirt supernatant fraction of unirradiated cells did not accelerate the rate of reassociation of ^{32}P-labeled SV40 DNA, indicating that this fraction did not contain detectable amounts of viral DNA, addition of DNA extracted from the Hirt supernatant fraction of irradiated cells increased the rate of reaction. When the amount of SV40 DNA present in the Hirt supernatant and pellet fractions was calculated, an enrichment of supernatant DNA for viral DNA sequences was observed in the absence of an increase in the total number of SV40 viral genomes per cell.

Table 2 presents results of several experiments in which we examined excision subsequent to γ-irradiation of clone E cells. In separate experiments, the cells were incubated for different lengths of time after irradiation, both in the presence and in the absence of araC. Movement of viral DNA from the Hirt pellet to the supernatant fraction in induced cells was observed 2.5 h after induction. Preliminary evidence indicates that no movement of viral DNA could be detected as early as 30 min postirradiation. Up to 10 h postirradiation, there was little difference between cells that were or were not incubated in the presence of araC after irradiation. By 10 h, an increase in the number of viral genomes present per cell was observed in cultures lacking araC, indicating that viral DNA replication occurred in induced cells. By 16 h,

TABLE 2. *Excision of SV40 DNA after γ-irradiation*[a]

Expt no.	Treatment of cells	% of total cellular DNA present in Hirt supernatant fraction[b]	SV40 DNA (μg/mg of cell DNA × 10^4)[c] present in:		Enrichment ratio[d]	No. of SV40 genome copies/cell[e]	SV40 DNA (%) detected in:	
			Hirt supernatant	Hirt pellet			Hirt supernatant	Hirt pellet
1	Control	2.1	ND[f]	10.0 ± 2.4		2.3	0	100.0
	2.5 h postirradiation	4.5	24.0 ± 5.3	8.8 ± 0.8	2.7	2.2	9.0	91.0
2	Control	2.0	ND	9.0		2.1	0	100.0
	4 h postirradiation	2.8	28.0	6.0	4.6	1.8	16.6	83.4
	4 hr postirradiation araC (0 to 4 h postirradiation)	3.8	49.0	5.5	8.8	1.9	26.3	73.7
3	Control	5.1	ND	10.4 ± 0.9		2.6	0	100.0
	10 h postirradiation	9.5	160.0 ± 18.8	7.2 ± 0.6	22.0	5.8	70.0	30.0
	10 h postirradiation araC (0 to 10 h postirradiation)	11.2	35.0 ± 7.9	7.8 ± 0.5	4.5	2.5	30.0	70.0
4	Control	1.7	ND	7.2		1.8	0	100.0
	16 h postirradiation	5.6	40.0	19.0	2.1	4.8	9.5	90.5
	16 h postirradiation araC (0–16 h postirradiation)	3.0	40.0	8.0	5.0	2.2	13.6	86.4

[a] The cells were treated as described in the legend to Fig. 4. Where indicated, araC in a final concentration of 10 μg/ml was added to the cultures immediately after irradiation. The cells were harvested at different times postirradiation and fractionated by the method of Hirt. The amount of SV40 DNA was determined as described in the legends to Fig. 2 and 4.

[b,c,d,e,f] See corresponding footnotes to Table 1.

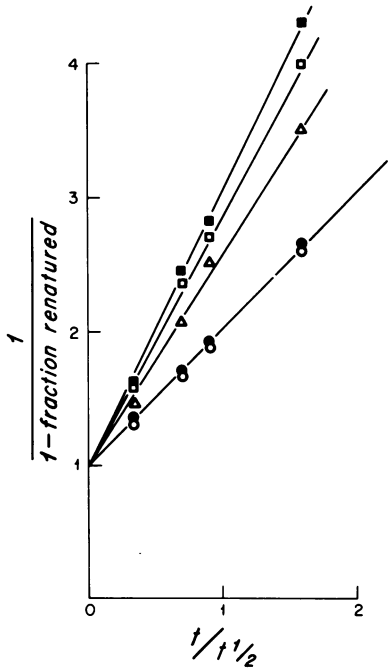

FIG. 4. *Reassociation kinetics of ^{32}P-labeled SV40 DNA incubated in the presence of unlabeled DNA from Hirt fractions of γ-irradiated and control cells. Clone E cells were maintained as described in the legend to Fig. 1. When subconfluent, the cultures were exposed to irradiation (25,000 rads; dose rate, 10,900 rads/min; ^{60}Co source) and were harvested at 2.5 h postirradiation. Control cells were not irradiated. Harvesting of the cells, partitioning of the host cell DNA between Hirt supernatant and pellet fractions, extraction of DNA, C_0t analysis, and calculations were performed as described in the legends to Fig. 2 and 3. ^{32}P-labeled SV40 DNA (3×10^{-5} OD_{260} units/ml) was incubated with the following unlabeled DNA samples: ○, 30 OD_{260} units of salmon sperm DNA per ml; ●, 10 OD_{260} units of DNA from Hirt supernatant of control cells per ml; △, 10 OD_{260} units of DNA from Hirt supernatant of irradiated cells per ml; □, 29 OD_{260} units of DNA from Hirt pellet of irradiated cells per ml; ■, 28 OD_{260} units of DNA from Hirt pellet of control cells per ml.*

much of the viral DNA in cultures lacking araC was present in the Hirt pellet fraction. Whether this DNA is associated with the high-molecular-weight host DNA and the nature of any such association are not known at present, but investigations are being carried out to clarify the issue. Thus, under our experimental conditions, excision occurred early after irradiation, but viral DNA replication was not detected until approximately 10 h postirradiation.

We have demonstrated that, early after the induction of virogenic SV40-transformed hamster cells, a portion of the viral DNA moves from its association with high-molecular-weight cellular DNA to a pool of relatively low-molecular-weight DNA. We propose that the observed enrichment of Hirt supernatant fractions for viral DNA in induced cells is due to an excision of viral DNA from its integrated state. It is unlikely that this effect is simply due to replication of SV40 DNA since it occurs to the same extent in the presence and absence of araC at early time periods postirradiation. In addition, at the time of excision, no increase occurred in the total number of SV40 genome copies per cell.

It is difficult at the present time to calculate the exact proportion of the cells in the population in which excision occurs. In experiments described herein, approximately 10 to 30% of the total viral DNA moved from the Hirt pellet to the Hirt supernatant fractions upon induction. We suspect that excision occurs in a greater proportion of cells than those which synthesize infectious virus (2 to 6%) by infectious center assay (6).

The excision event that we have described in SV40-transformed cells may also occur after induction of polyoma virus-transformed cells since the polyoma viral DNA is integrated into host cell DNA (7). It would be of interest to determine whether induction of RNA viruses from either untransformed or RNA virus-transformed cells leads to excision of proviral DNA.

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LITERATURE CITED

1. **Black, P. H.** 1968. The oncogenic DNA viruses: a review of in vitro transformation studies. Annu. Rev. Microbiol. 22:391-426.
2. **Gelb, L. D., D. E. Kohne, and M. A. Martin.** 1971. Quantitation of simian virus 40 sequences in African green monkey, mouse and virus-transformed cell genomes. J. Mol. Biol. 57:129-145.
3. **Hirt, B.** 1967. Selective extraction of polyoma DNA from infected mouse cell cultures. J. Mol. Biol. 26:365-369.
4. **Kaplan, J. C., P. H. Black, and H. Rothschild.** 1971. Induction of virogenic mammalian cells with chemical and physical agents, p. 143-158. *In* R. W. Begg (ed.), Proceedings, Canadian Cancer Conference 1971, vol. IX. University of Toronto Press, Toronto.
5. **Kaplan, J. C., L. F. Kleinman, and P. H. Black.** 1975. Cell cycle dependence of SV40 virus induction from transformed hamster cells by UV-irradiation. Virology 68:215-220.
6. **Kaplan, J. C., S. M. Wilbert, J. J. Collins, T. Rakusanova, G. B. Zamansky, and P. H. Black.** 1975. Isolation of SV40 transformed inbred hamster cell lines

heterogeneous for virus induction by chemicals or radiation. Virology 68:200–214.
7. **Manor, H., M. Fogel, and L. Sachs.** 1973. Integration of viral into chromosomal deoxyribonucleic acid in an inducible line of polyoma-transformed cells. Virology 53:174–185.
8. **Marmur, J.** 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208–218.
9. **Ozanne, B., P. A. Sharp, and J. Sambrook.** 1973. Transcription of simian virus 40. II. Hybridization of RNA extracted from different lines of transformed cells to the separated strands of simian virus 40 DNA. J. Virol. 12:90–98.
10. **Sambrook, J., H. Westphal, P. R. Srinivasan, and R. Dulbecco.** 1968. The integrated state of SV40 DNA in transformed cells. Proc. Natl. Acad. Sci. U.S.A. 60:1288–1295.
11. **Signer, E. R.** 1968. Lysogeny: the integration problem. Annu. Rev. Microbiol. 22:451–488.
12. **Wetmur, J. G., and N. Davidson.** 1968. Kinetics of renaturation of DNA. J. Mol. Biol. 31:349–370.